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CHARACTERIZATION OF PHENOTYPIC CHANGES INVOLVED IN THE UPTAKE OF WEAKLY SOLUBLE HYDROCARBONS BY *PSEUDOMONAS AERUGINOSA*

by
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Dedicated to my parents

Nguyen, Trong-Thanh, MD

Pham, Ngoc-Dung, Eng

And my family

Do, Quang-Binh, PhD

Do, Thi-Minh-Ngoc

With love and gratitude

In science, you are free for imagination.

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ABSTRACT

Petroleum products like linear alkanes (e.g. hexadecane, octadecane, etc.) and polycyclic aromatic hydrocarbons (e.g. naphthalene, phenanthrene, etc.) are very important pollutants in the environment because of their abundance, toxicity, weak solubility, and persistence due to their poor bioavailability, the limitation of biodegradation pathways and a high tendency to the sorption to other hydrophobic organic matters. There are different types of treatments applied in the polluted environments, including chemical, physical and biological methods. The chemical and physical treatments can cause a secondary pollution, and are expensive. In contrast, the continuous exposure to the contaminants has led to the evolution of microorganisms that are able to grow on hydrocarbons, degrade and utilize the polluted hydrocarbons as their carbon sources, and this inspired the development of biological treatment methods. Thus, the ability of microbial-degrading hydrocarbons is a potential key element in the clean-up strategy of polluted sites. Many different microorganisms, including algae, fungi and bacteria, have been reported to possess the capability of degrading a wide variety of the polluted persistent organic compounds. Among these bacteria, different members of the *Pseudomonas* genus, which also possess the capacity to degrade weakly soluble hydrocarbons such as linear alkanes, polycyclic aromatic compounds (PAHs), etc., have already been isolated for studying mechanisms involving the effective biodegradation of polluted hydrocarbons by these microorganisms.

Pseudomonas aeruginosa bacteria are found in a wide variety of environments, such as soil, water or in association with plants and animals as well as inside of the human body. These bacteria can also grow on hydrophobic carbon substrates such as long-chain linear alkanes and PAHs. The ability to grow in various unfavorable conditions of these bacteria, such as on the petroleum-related products in the polluted environments, relies on their high adaptabilities. In response to the environmental stimuli, bacteria can use different second messengers to regulate various cellular processes for their survival and adaptive development.

At the time this project was started, it was known that the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (cyclic di-GMP or c-di-GMP) plays a key role in the bacterial biofilm development, including the *P. aeruginosa* species. c-di-GMP was shown to be overproduced in various *P. aeruginosa* rough small colony variants (SCVs) derived from different clinical isolates. This cell type possesses high abilities to grow and form biofilms in unfavorable conditions. The formation of rough SCVs of *P. aeruginosa* strain 57RP on dextrose in biofilm or in static liquid cultures was also observed in previous experiments in the laboratory of Prof. Richard Villemur. Strain 57RP and several other *P. aeruginosa* strains were also able to grow and form biofilm on hexadecane, other n-alkanes and PAHs used as a whole carbon source. We **hypothesized** that the 57RP rough SCVs, which shared some common phenotypic behaviors with the clinical rough SCVs, also overproduce c-di-GMP. However, to which extent this second messenger would influence the appearance of SCVs and the capacity of degrading linear alkanes and/or PAHs was still unclear. Several studies carried out on the growth of certain oil-degrading bacteria growing on different substrates revealed that hexadecane induced a large change in gene expression of biofilm cells compared to the cells growing on pyruvate {Sabirova, 2006 #10585}{Sabirova, 2011 #10578} or to the planktonic cells growing on acetate {Vaysse, 2009 #10584}. Many up-regulated proteins were found in different cell compartments such as the outer membrane, periplasm, inner membrane and cytoplasm. Question to which extent of different modulation of gene expression would be seen between the 57RP cells growing on hexadecane and the 57RP cells growing on other substrates, such as glucose, requires some proteomic studies to be carried out for a better understanding of the physiological adaptation of 57RP cells to the hydrophobic substrate.

The principal aim of this project was to characterize the phenotypic changes of a *P. aeruginosa* strain 57RP*,* previously isolated from a hydrocarbon-polluted site in Canada by our laboratory, and capable of using several weakly soluble hydrocarbons, such as hexadecane, as the sole carbon source for its growth. The present study was carried out to further investigate the previously suggested important role of c-di-GMP in the phenotypic switching of the bacterial cell states and characterize initially the components of the cell envelope of 57RP SCV grown on hexadecane.

The four main objectives of the present study were as follows:

1. **Isolate small colony variants (SCVs) of *P. aeruginosa* strain 57RP in the cultures with n-alkanes used as sole carbon sources.**
2. **Characterize the phenotypic changes of SCVs grown on hexadecane and octadecane.**
3. **Investigate the putative role of c-di-GMP in the phenotypic SCV formation and in the reversion to the wild-type phenotype.**
4. **Characterize the proteome profiles of SCV cells grown on hexadecane.**

The results obtained from the present study are summarized as follows.

Objective 1. *57RP, a slow growth strain with low cell hydrophobicities, also forms SCVs on octadecane.*

We confirmed that SCVs were formed by the *P. aeruginosa* strain 57RP during its growth on the weakly soluble liquid hexadecane (C_{16}). Besides that, this strain also formed SCVs on solid octadecane (C_{18}), indicating that 57RP was able to form SCVs on different n-alkanes used as sole carbon sources. SCVs were identified as the main growing cell type in these cultures. The SCVs reverted to the wild-type (WT) phenotype as the parent WT cells on a rich (TSA) agar medium.

In the case of 57RV, a rapid growth strain with high cell hydrophobicities, SCVs were also formed on hexadecane (MSM with 1% hexadecane shaking at 25^{o}C). This newly observed phenomenon was a manifestation of the natural potential for SCV formation of 57RV strain. This indicated that both strains 57RP and 57RV, with low and high cell hydrophobicities, respectively, were naturally capable of generating the phenotypic variants highly adaptive to selective stress conditions by phase variation mechanisms.

Objective 2. *Rhamnolipids are not an important factor for the growth ability of 57RP SCVs on hexadecane.*

We confirmed that SCV cells had a number of phenotypic traits that were different from those of their parent WT cells. The SCV cells were hyperpiliated with reduced motility compared to the WT cells. SCVs were able to grow without any initial long lag phase on n-alkanes as seen with the WT. The cell surface of SCVs was highly hydrophobic, allowing the SCV cells to adhere effectively to the hydrocarbons. The SCV cells were able to form biofilms at a much higher level compared to the parent WT cells. The observed phenotypic behaviors of SCV cells grown on hexadecane may represent a group of phenotypic characters typical for the small rough colony phenotype since they were also observed earlier with rough SCVs developing on dextrose when the same strain was cultivated as a biofilm or in static liquid cultures.

Besides the above mentioned confirmative data, we also demonstrated clearly that rhamnolipids are not an important factor for the growth ability of 57RP SCV on hexadecane (MSM with 1% hexadecane shaking at 25^{o}C) as this phenotypic variant produced extremely low amount of the extracellular biosurfactant on this hydrophobic hydrocarbon source.

Objective 3. *c-di-GMP is involved in the phenotypic switching.*

The main interest of Objective 3 was to investigate the relations between c-di-GMP production, a universal bacterial second messenger, and the formation of SCVs on hexadecane as well as the reversion of SCV to wild-type phenotype on a rich (TSA) medium. We found a positive correlation between the two coincidentally occurring events. First, high c-di-GMP levels were associated with SCV formation. Second, low c-di-GMP levels occurred with the SCV reversion to the wild-type phenotype. To further study the involvement of c-di-GMP in SCV formation, we have generated two integrant clones from 57RP WT cells. The first integrant, RP401, possessed an extra inducible gene encoding phosphodiesterase (PDE) for the hydrolysis of c-di-GMP. The second integrant, RP402,

possessed an extra inducible gene encoding diguanylate cyclase (DGC) for the synthesis of c-di-GMP. Expression of these two extra genes was under the control of an arabinose-inducible promoter. Results showed that the expression of the inducible extra DGC led obviously to an expected increase in the c-di-GMP production in the extra DGC-integrated cells, especially in the extra DGC-integrated SCVs. In contrast, the expression of the extra PDE in RP401 seriously affected the time of appearance and the number of the adaptive SVCs of 57RP401 grown on hexadecane.

The 57RP401 SCVs, occurred at the end of tested culture periods, and were morphologically similar to the common 57RP SCVs grown on hexadecane (MSM with 1% hexadecane shaking at 25^{o}C). A representative 401 SCV, randomly selected for further analyses, was also a c-di-GMP-dependent SCV and reverted to wild-type phenotype on TSA medium. This supported a previous suggestion of putative roles of c-di-GMP in phenotypic switching of bacterial cells. Also, for the first time, the important role of c-di-GMP in phenotypic switching, related to the formation and reversion to the wild-type phenotype of phenotypic SCVs, was initially characterized in a non-clinical *P. aeruginosa* strain grown on hexadecane. This is an original contribution of the present study to the current knowledge of the putative role of c-di-GMP in the general phenotypic switching of bacterial cells, including those of various *P. aeruginosa* strains.

Our results support that c-di-GMP plays an important role in the phenotypic ON/OFF switching (the SCV formation and reversion to wild-type phenotype) of the *P. aeruginosa* cells, respectively.

Objective 4. *57RP SCV cells grown on hexadecane synthesize unknown proteins and unusual* LipA *isoforms.*

In the comparative analysis of proteome profiles of SCV vs. WT cells, both grown in the same culture condition (MSM with 1% glucose shaking at 25^{o}C), we found noticable differences in the protein expression patterns between the two cell types. This observation suggests that gene expression was regulated (up/down) differently between these two cell

types. The proteome profiles of the cell membrane-binding and periplasmic proteins of the 57RP SCV grown on hexadecane (MSM with 1% hexadecane shaking at 25^{o}C) were further characterized. The characteristic cell components of 57R SCV grown on hexadecane include the presence of various proteins involved in the transport, antioxidant, nitrogen regulatory activities, etc.

In the further analyses of proteome profiles of SCV grown on hexadecane, we identified the unexpected presence of an unknown protein and three isoforms of secreted LipA. One of these three LipA isoforms was in the form of a lipase mature polypeptide and the other two were in the lipase premature form.

Presently, the underlying mechanisms for the appearance of these three LipA isoforms and the unknown protein, as well as their physiological role(s) in the adaptive abilities of the 57RP SCVs grown on hexadecane, remain to be determined.

Results obtained from the present study form the basis for the further, deeper investigations in the future to better understand the adaptive responses of *P. aeruginosa* cells to certain environmental stress conditions. These certainly include the investigations of the role of c-di-GMP, a bacterial general second messenger, in the phenotypic switching of bacterial cells to generate the environmental stress adapted variants. These also include, certainly, the further investigations of the not-yet-known underlying mechanisms for the phase variation-based, environment specific stimuli-induced generation of unusual protein isoforms, such as the three LipA isoforms formed in the 57RP SCVs that were adapted to grow well on hexadecane observed in the present study. The physiological role, if any, of the observed uncharacterized protein and the unusual LipA isoforms in the adaptive growth of the 57RP SCVs on hexadecane should also be an interesting topic for future investigations.

Key words: *Pseudomonas aeruginosa*, small colony variant (SCV), biofilm, c-di-GMP, lipase

RÉSUMÉ

Les produits pétroliers comme les hydrocarbures linéaires (par exemple de l'hexadécane, octadécane) et les hydrocarbures aromatiques polycycliques (par exemple le naphtalène, le phénanthrène) sont des polluants très importants dans l'environnement en raison de leur abondance, de leur toxicité, de leur faible solubilité, de leur persistance en raison de leur faible biodisponibilité, de la limitation des voies de biodégradation, et de la forte tendance à leur absorption à d'autres matières organiques. Il existe différents types de traitements appliqués dans les environnements pollués, y compris les méthodes biologiques, physiques et chimiques. Les traitements chimiques et physiques peuvent causer une pollution secondaire, et sont coûteux. L'exposition continue aux contaminants a conduit à l'évolution des micro-organismes qui sont capables de croître sur de tels hydrocarbures, comme sources de carbone, ce qui a inspiré le développement de procédés de traitement biologique. Ainsi, la capacité de dégradation microbienne des hydrocarbures est un élément clé dans la stratégie de nettoyage de sites pollués. De nombreux micro-organismes, y compris les algues, les champignons et les bactéries, ont été rapportés être capables de dégrader une grande variété de polluants organiques persistants. Différents membres du genre *Pseudomonas* possèdent la capacité de dégrader les hydrocarbures faiblement solubles tels que les alcanes linéaires et des HAPs.

Des souches appartenant à l'espèce *P. aeruginosa* ont été trouvées dans une grande variété d'environnements, tels que le sol, l'eau ou associés avec des plantes, des animaux ainsi que l'intérieur du corps humain. Ces bactéries peuvent également se développer sur des substrats carbonés hydrophobes tels que les alcanes linéaires à longue chaîne et les HAPs. L'adaptabilité de ces souches à dégrader ces produits récalcitrants résiderait dans la réponse aux stimuli de l'environnement. En effet, les bactéries peuvent utiliser différents seconds messagers pour réguler divers processus cellulaires pour leur survie et leur développement adaptatif.

Au moment où ce projet a été lancé, il était connu que le second bis-messager (3'-5')-guanosine cyclique dimère monophosphate (cyclique di-GMP ou c-di-GMP) joue un

rôle clé dans le développement de biofilm bactérien, y compris *P. aeruginosa*. Le c-di-GMP a été montré pour être surproduit dans diverses variantes de petites colonies rugueux de *P. aeruginosa* (SCVs) dérivées de différents isolats cliniques. Ce type de variantes bactériennes possède une forte capacité à croître et former un biofilm dans des conditions défavorables. La formation de SCVs rugueux chez de la souche 57RP de *P. aeruginosa* sur le dextrose dans le biofilm ou dans des cultures liquides statiques a également été observée dans des expériences antérieures dans le laboratoire du Pr. Richard Villemur. La souche 57RP et plusieurs autres souches de *P. aeruginosa* ont également été capables de se développer et de former un biofilm sur l'hexadécane, d'autres n-alcanes et des HAPs utilisés comme une source de carbone entière. Nous avons émis **l'hypothèse** que les SCVs rugueux 57RP, qui partageaient des comportements phénotypiques communs avec les SCVs rugueux cliniques, surproduisent aussi c-di-GMP. Cependant, dans quelle mesure ce second messager pourrait influencer l'apparition de SCV n'a pas été démontré. Plusieurs études réalisées sur la croissance de certaines bactéries dégradant l'huile sur différents substrats ont révélé que l'hexadécane induit un grand changement dans l'expression des gènes des cellules de biofilms par rapport aux cellules planctoniques qui poussent sur le pyruvate ou l'acétate. De nombreuses protéines, qui ont été exprimées à des niveaux plus élevés dans les SCV par rapport aux cellules WT, ont été trouvées dans différents compartiments cellulaires tels que la membrane externe, le périplasme, la membrane interne et le cytoplasme. La question de savoir quelle étendue de la modulation différente de l'expression des gènes serait observée entre les cellules 57RP croissantes sur l'hexadécane et les cellules 57RP cultivées sur d'autres substrats, comme le glucose, nécessitent des études protéomiques pour une meilleure compréhension de l'adaptation physiologique de 57RP au substrat hydrophobe.

L'objectif principal de ce projet était de caractériser les changements phénotypiques d'une souche *P. aeruginosa* (57RP), auparavant isolées d'un site pollué par des hydrocarbures au Canada par notre laboratoire. Cette souche est capable d'utiliser plusieurs hydrocarbures faiblement solubles tels que l'hexadécane comme seule source de carbone pour sa croissance. La présente étude a été réalisée pour étudier davantage l'importance de l'apparition des SCVs et de leur rôle dans la dégradation de ces hydrocarbures. Egalement,

nous avons voulu mettre en lumière l'implication du c-di-GMP dans le changement phénotypique et caractériser initialement les composants de l'enveloppe cellulaire de 57RP SCVs cultivées sur hexadécane.

Les quatre principaux objectifs de la présente étude sont les suivants:

1. **Former les variantes petites colonies (SCVs) de *P. aeruginosa* souche 57RP dans les cultures avec n-alcanes utilisés comme seule source de carbone.**
2. **Caractériser les changements phénotypiques de SCV cultivés sur l'hexadécane et l'octadecane.**
3. **Examiner le rôle putatif de c-di-GMP dans la formation de SCV phénotypique et la réversion à phénotype sauvage.**
4. **Caractériser les profils protéomiques de cellules SCV cultivées sur hexadécane.**

Les résultats obtenus à partir de la présente étude sont résumés comme suit.

Objectif 1. *57RP, une souche de croissance lente avec hydrophobie cellulaires faibles, forme aussi des SCVs sur octadécane.*

Nous avons confirmé que les SCVs ont été formées par la souche 57RP de *P. aeruginosa* au cours de sa croissance sur le hexadécane liquide faiblement soluble (C_{16}).

En outre, cette souche formait également SCV sur octadécane solide (C_{18}), indiquant que 57RP pourrait former des SCVs sur les différents n-alcanes utilisés comme seule source de carbone. Les SCVs ont été identifiés comme le principal type de cellule en croissance dans ces cultures. Le phénotype SCV est revenu au phénotype de type sauvage (WT) lors de cultures sur un milieu riche (TSA) d'agar.

Dans le cas de 57RV, une souche de croissance rapide avec hydrophobie cellulaire élevée, des SCVs a été formée également sur hexadécane (MSM avec 1% de hexadécane sous agitation à 25°C). Ce phénomène nouvellement observé était une manifestation du

potentiel naturel pour la formation de SCV de la souche 57RV. Ceci indique que les deux souches 57RP et 57RV, avec hydrophobies cellulaires basses et hautes, respectivement, étaient naturellement capable de générer des phénotypiques variantes hautement adaptatives aux sélectives conditions de stress par des mécanismes de variation de phase.

Objectif 2. *Rhamnolipides ne sont pas un facteur important pour la capacité de croissance de 57RP SCV sur l'hexadécane.*

Nous confirmé que les SCVs avaient un nombre de caractères phénotypiques différents de la souche sauvage. Les cellules des SCVs étaient hyperpiliées avec des activités mobiles réduites par rapport aux cellules de type sauvage. Les SCVs étaient capable de se développer rapidement sans phase de latence sur les n-alcanes. La surface des cellules de SCV était hautement hydrophobe, ce qui permet aux cellules d'adhérer efficacement aux hydrocarbures. Les comportements phénotypiques observés des cellules SCV cultivées sur hexadécane peuvent représenter un groupe de caractères phénotypiques typiques pour le petit phénotype de colonie rugueuse car ils ont également été observés plus tôt avec des SCVs rugueuses développées sur dextrose lorsque la même souche bactérienne a été cultivée sous forme de biofilm ou dans des cultures liquides statiques.

Outre les données confirmatifs mentionnées ci-dessus, nous avons aussi démontré clairement que rhamnolipides ne sont pas un facteur important pour la capacité de croissance de 57RP SCV sur l'hexadécane (MSM avec 1% de hexadécane sous agitation à 25°C) car cette variante phénotypique n'a presque pas produire le biosurfactant extracellulaire sur cette source d'hydrocarbure hydrophobe.

Objectif 3. *c-di-GMP implique dans la commutation phénotypique.*

L'intérêt principal de l'Objectif 3 était d'étudier les relations entre la production de c-di-GMP, un second messager bactérien universel, et la formation de SCV sur hexadécane

ainsi que la réversion de SCV à phénotype sauvage sur un milieu riche (TSA). Nous avons trouvé une corrélation positive entre les niveaux élevés de c-di-GMP et la formation de SCVs. Également, les niveaux bas de c-di-GMP ont corrélés avec la réversion de SCV au phénotype de type sauvage. Pour étudier davantage la participation de c-di-GMP dans la formation de SCV, nous avons généré deux clones de cellules intégrantes chez la souche sauvage. Le premier, RP401, possédait un gène inductible, codant pour la phosphodiestérase (PDE) impliqué dans l'hydrolyse du c-di-GMP. Le second, RP402, possédait un gène inductible, codant la cyclase diguanylate (DGC) pour la synthèse de c-di-GMP. Les résultats ont montré que l'expression de la DGC l'inductible supplémentaire conduit évidemment à l'augmentation attendue de la production c-di-GMP dans les cellules DGC intégrées supplémentaires, en particulier dans le DGC supplémentaire intégré SCVs. En revanche, l'expression de la PDE supplémentaire dans RP401 sérieusement affectait le temps d'apparition et le nombre des SCVs adaptatifs de 57RP401 cultivées sur hexadécane.

Les SCVs de 57RP401, sont apparu à la fin des périodes de culture testées, étaient morphologiquement similaires aux SCVs de 57RP communs cultivés sur hexadécane (MSM avec 1% de hexadécane sous agitation à 25°C). Le représentant 401 SCV, choisis au hasard pour d'autres analyses, était aussi un SCV c-di-GMP-dépendant et il révertait au phénotype de type sauvage sur le milieu TSA. Cette prise en charge d'une suggestion précédente des rôles putatifs de c-di-GMP dans la commutation phénotypique des cellules bactériennes. Aussi, pour la première fois, le rôle important de c-di-GMP dans la commutation phénotypique, liés à la formation et la réversion au phénotype de type sauvage des SCVs phénotypiques, a été initialement caractérisée dans une souche *P. aeruginosa* non-clinique cultivée sur hexadécane. Ceci est une contribution originale de la présente étude à la connaissance actuelle du rôle putatif de c-di-GMP dans la commutation phénotypique générale des cellules bactériennes, y compris celles de diverses souches de *P. aeruginosa.*

Nos résultats soutient que c-di-GMP joue un rôle important dans la commutation phénotypique de ON/OFF (comme la formation SCV et la réversion au type sauvage phénotypique) des cellules de *P. aeruginosa*, respectivement.

Objectif 4. *Cellules 57RP SCV cultivées sur hexadécane synthétisent également des protéines inconnues et isoformes de* LipA *inhabituelles.*

Dans l'analyse comparative des profils protéomiques de SCV contre les cellules de type sauvage (WT), cultivées dans les mêmes conditions de culture (MSM avec 1% de glucose sous agitation à 25°C), nous avons constaté des différences noticeables dans les profils d'expression des protéines entre les deux types de cellules. Cette observation signifie que l'expression des gènes a été réglementée (haut/bas) différemment entre ces deux types de cellules. Les profils protéomiques des protéines, comme les protéines membrane-liées et protéines périplasmiques de 57RP SCV cultivées sur hexadécane (MSM avec 1% de hexadécane sous agitation à 25°C), ont en outre été caractérisés. Les composants cellulaires caractéristiques de 57R SCV cultivées sur hexadécane incluent la présence de diverses protéines impliquées dans les activités de transportation, anti-oxydation, régulation de l'azote, etc.

Fait intéressant, même dans ces autres analyses des profils protéomiques du 57RP SCVs cultivées sur hexadécane, nous avons trouvé une présence vraiment inattendue de trois isoformes de LipA sécrétée et une protéine inconnue. L'un de ces trois isoformes de LipA était sous la forme de lipase polypeptide mature et les deux autres dans la lipase de forme prématurе.

Actuellement, les mécanismes sous-jacents de l'apparition de ces trois isoformes de LipA identifiés et la protéine inconnue, ainsi que leur(s) rôle(s) physiologique(s) dans les capacités d'adaptation des SCVs de 57RP cultivées sur hexadécane, restent en grande partie à déterminer.

Les résultats obtenus à partir de la présente étude constituent la base pour des investigations plus profondes dans l'avenir pour mieux comprendre les réponses adaptatives des cellules de *P. aeruginosa* à certains stress spécifiques liés à l'environnement. Principalement, les réponses phénotypiques liées aux mécanismes, que

ne sont pas encore claires, sous-jacentes de la participation de c-di-GMP, un second messager bactérienne générale, dans le phénotypique de commutation ON/OFF de cellules bactériennes (telles que la formation des SCVs et le retour à naturel phénotype); et, les mécanismes sous-jacents, aussi ne sont pas encore connus, pour les stimulé environnement-spécifiques induites générations, à base de la variation de phase, d'isoformes de protéines inhabituelles dans certains environnements stressants-adaptatives variantes phénotypiques de la *P. aeruginosa* espèce (comme les trois isoformes de LipA synthétisées dans les SCVs de 57RP cultivées sur hexadécane qui ont été observées dans la étude présente. Le rôle physiologique, le cas échéant, de la protéine observée, mais ne sont pas encore connus et les isoformes de LipA inhabituels dans la croissance adaptative des SCVs de 57RP sur hexadécane devrait également être un sujet non moins intéressant pour les futures enquêtes.

Mots clés : *Pseudomonas aeruginosa*, variante petite colonie (SCV), biofilm, c-di-GMP, lipase

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Abbreviations	Meaning
2-DE	Two-dimensional gel electrophoresis
BH	Bushnell-Haas Medium
c-di-GMP	3’-5’-cyclic diguanylic acid
cAMP	Cyclic adenosine 3’,5’-monophosphate
CF	Cystic Fibrosis
CFU	Colony Forming Units
cGMP	Cyclic guanosine 3’,5’-monophosphate (cGMP)
CUP	Chaperone Usher Pathway
DGCs	Diguanylate cyclases
DNA	Deoxyribonucleic Acid
EAL	Glutamic acid - Alanine - Leucine
EDTA	Ethylenediamine Tetra-acetic Acid
EPS	Extracellular Polymeric Substances
GGDEF	Glycine - Glycine - Aspartic acid - Glutamic acid - Phenylalanine
GMP	Guanosine monophosphate
GTP	Guanosine triphosphate

HPLC	High Pressure Liquid Chromatography
LB	Lysis Broth Medium
LPS	Lipopolysaccharide
MATH	Microbial Adhesion To Hydrocarbons
MATS	Microbial Adhesion To Silica
MS	Mass Spectrometry
MSM	Mineral salt medium
OD	Optical Density
PAHs	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PDEs	Phosphodiesterases
pGpG	5′-phosphoguanylyl-(3'-5')-guanosine
ppGpp	Guanosine tetraphosphate
pppGpp or (p)ppGpp	Guanosine pentaphosphate
PPi	Pyrophosphate
QS	Quorum Sensing
rpm	rotations per minute
RSCVs	Rough Small Colony Variants

SCVs	Small Colony Variants
SDS	Sodium dodecyl sulfate
SW	Siegmund-Wagner Medium
TFP	Type IV pili
TSA	Tryptic soy agar
TSB	Tryptic soy broth
TSE	Tris - Sucrose - EDTA
UV	Ultra Violet
WT	Wild Type

1 INTRODUCTION

1.1 Context and research question

Petroleum products like linear hydrocarbons (e.g. octadecane, hexadecane, etc.) and polycyclic aromatic hydrocarbons (e.g. naphthalene, phenanthrene, etc.) are very important pollutants in the environment because of their toxicity, insolubility and persistence. Furthermore, the cost for cleaning up the complex hydrocarbons-polluted regions following spills is considerable; for example, $US22.14/gallon in Canada and $US87.13/gallon in USA based on calculations from 1999 (Etkin, 2001). For example one tragic recent accident was the Deepwater Horizon oil spill in the Gulf of Mexico, Louisiana, USA on April 2010. The owner BP Group announced that approximately 4.9 million barrels (795 million liters) of oil had leaked into the gulf (https://www.toxicology.org/pm/Deepwater_oil_spill.pdf), with serious environmental and financial consequences, as BP has approximately $28 billion of damage claims and cleanup costs. These latter costs depend on; *inter alia*, the type of oil, the amount of oil, the sea conditions and the effectiveness of clean-up site etc. (White & Molloy, 2003). Therefore, finding an efficient and cost-effective pollution treatment technology is an important goal.

There are different types of treatments in polluted environments including chemical, physical and biological approaches. The chemical and physical treatments can cause secondary pollution, and are expensive while bioremediation is in many cases a cost-effective and environment friendly solution (reviewed in Dibble and Bartha (1979)). A long-term exposure of microbial communities to hydrocarbons leads to the selective enrichment of higher adaptive hydrocarbon-utilizing microorganisms as well as the hydrocarbon-catabolizing gene pool (Brooijmans *et al.*, 2009, Harayama *et al.*, 1999, Kostka *et al.*, 2011, Leahy & Colwell, 1990). Indeed, microbial degradation is a key module in polluted site clean-up strategy. Many different microorganisms including algae, fungi and bacteria harbor the capability of degrading a wide variety of persistent organic compounds. Among bacteria, members of the *Pseudomonas* genus possess the capacity to degrade weakly soluble hydrocarbons, such as linear alkanes and polycyclic aromatic compounds (PAHs). *Pseudomonas aeruginosa* is also a well-studied bacterium involved in

the formation of biofilm, and the production of biosurfactants, which are among the factors that would be involved in the solubilization of hydrocarbons, and the adherence of bacteria to hydrocarbons. In previous studies by Prof. Villemur and Prof. Déziel at the INRS-Institut Armand-Frappier, several strains of *P. aeruginosa* that could use aromatic and aliphatic hydrocarbons as sole carbon and energy sources were isolated (Déziel, 2001). Evidence was obtained that *P. aeruginosa* has to change its specific physiological characteristics to grow on these hydrophobic substrates. These characteristics include cell surface hydrophobicity, the ability to adhere to hydrocarbons, the production of biosurfactants, the capacity to generate phenotypic small colony variants (SCVs), and the development of a biofilm at the surface of the hydrocarbons. It was also observed that these strains have different degradation dynamics towards linear alkanes, whether in the liquid or solid phases. The spontaneous appearance of SCVs when certain strains of *P. aeruginosa* were cultivated on hydrocarbons as sole substrate indicated an important role of this colony type for growth on these substrates (Déziel, 2001).

At the time this thesis was initiated, it was known that the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (cyclic di-GMP or c-di-GMP) played a key role in bacterial biofilm development, including in *P. aeruginosa* (reviewed in Hengge (2009), Römling *et al.* (2013)). However, how this second messenger and the appearance of SCVs involved in the degradation of linear alkanes and/or PAHs was still unknown.

1.2 Hypothesis

Our **hypothesis** was that *P. aeruginosa*, isolated from a hydrocarbon-contaminated soil, as some other known clinical isolates of the same bacterial species, should use the same second messenger signaling system (c-di-GMP signaling) to initiate phenotypic switch to form environment-adaptive phase SCVs with typical phenotypic behaviors able to grow on n-hexadecane (C_{16}), a hydrophobic, poorly bioavailable substrate used as a sole carbon source and energy.

The ***principal aim*** of the project was to characterize the phenotypic changes of some strains of *P. aeruginosa* during growth on weakly soluble hydrocarbons through the study of mechanisms involving adhesion, SCV formation and second messenger signaling. Better understanding how microorganisms can adapt to particular environments should allow for the development or optimization of existing condition for biological treatments at contaminated sites.

1.3 Objectives of the thesis

The present study had the following objectives:

(1) Isolate small colony variants (SCVs) of *P. aeruginosa* strain 57RP during culture with n-alkanes used as sole carbon sources.
(2) Characterize the phenotypic changes of SCVs grown on hexadecane and octadecane
(3) Investigate the putative role of c-di-GMP in the phenotypic SCV formation and in the reversion to wild-type phenotype.
(4) Characterize the proteome profiles of SCV cells grown on hexadecane.

2 REVIEW OF THE LITERATURE

2.1 Hydrocarbons and bioremediation

2.1.1 n-Alkanes, Polycyclic Aromatic Hydrocarbons and bioavailability

Alkanes are saturated hydrocarbons that have the general formula C_nH_{2n+2} and possess a linear (Figure 2.1), a branched or a cyclic carbon chain. Many alkanes such as methane, and n-hexane are classified as dangerous agents for the environment and human health (Karakaya *et al.*, 1996). n-Alkanes, one of the main components in crude oil, with various carbon change lengths (C_8-C_{40}) are found frequently in the weathered oil petroleum contaminated sites (Kostka *et al.*, 2011). Their solubility in water decreases with the increase of carbon number in alkanes. For example, the water solubility of C_9 alkane is 0.22 mg/L, C_{10} is 0.052 mg/L (McAuliffe, 1969) and C_{16} is 0.009 mg/L, C_{18} is 0.0021 mg/L (Sutton & Calder, 1974).

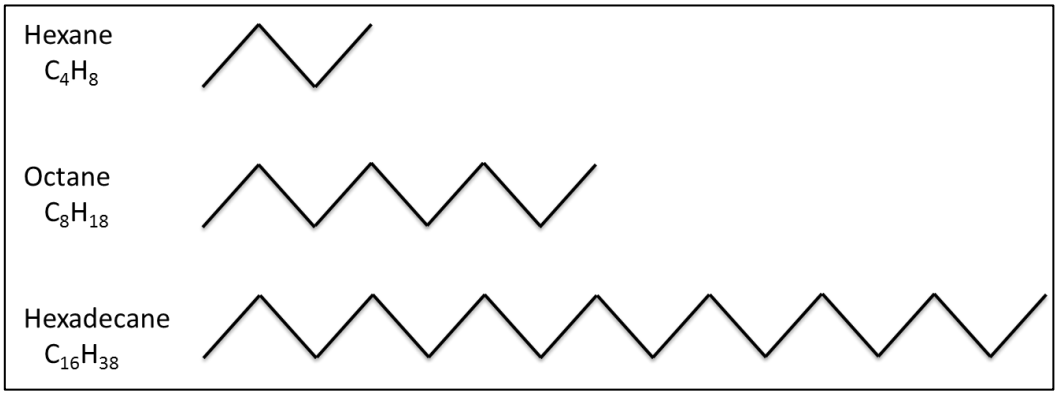


Figure 2.1 Chemical structure of selected n-alkanes (Bruice, 1998)

In addition to n-alkanes, other of components of crude oil includes Polycyclic Aromatic Hydrocarbons (PAHs). These are chemical compounds that contain more than one fused aromatic ring. PAHs are found widely in natural environments. Beside of their presence in petroleum fuels and coal products, their anthropogenic activities also cause PAH accumulation in the environment. The structural formula of selected PAHs is shown in figure 2.2. Research on PAHs attracts much attention, such as benzo [a] pyrene, because some of them cause toxic and carcinogenic effects on various organisms, including humans (Baird *et al.*, 2005). PAHs also have the ability to accumulate in fatty regions of animal bodies. They are considered as carcinogens because they can induce mouse skin tumors. PAHs can be modified in the liver, which generates reactive molecules that, when intercalates in DNA strands and can induce mutations (Baird *et al.*, 2005). PAHs are divided into two categories: the low molecular weight (LMW) PAHs with 2-3 aromatic rings (e.g. naphthalene and phenanthrene), and the high molecular weight (HMW) PAHs with 4 or more aromatic rings (e.g. pyrene) (Figure 2.2) (Samanta *et al.*, 2002, H Zhang *et al.*, 2004). Water solubility decreases with increasing molecular weight (Figure 2.2) (Juhasz & Naidu, 2000, Sutton & Calder, 1974).

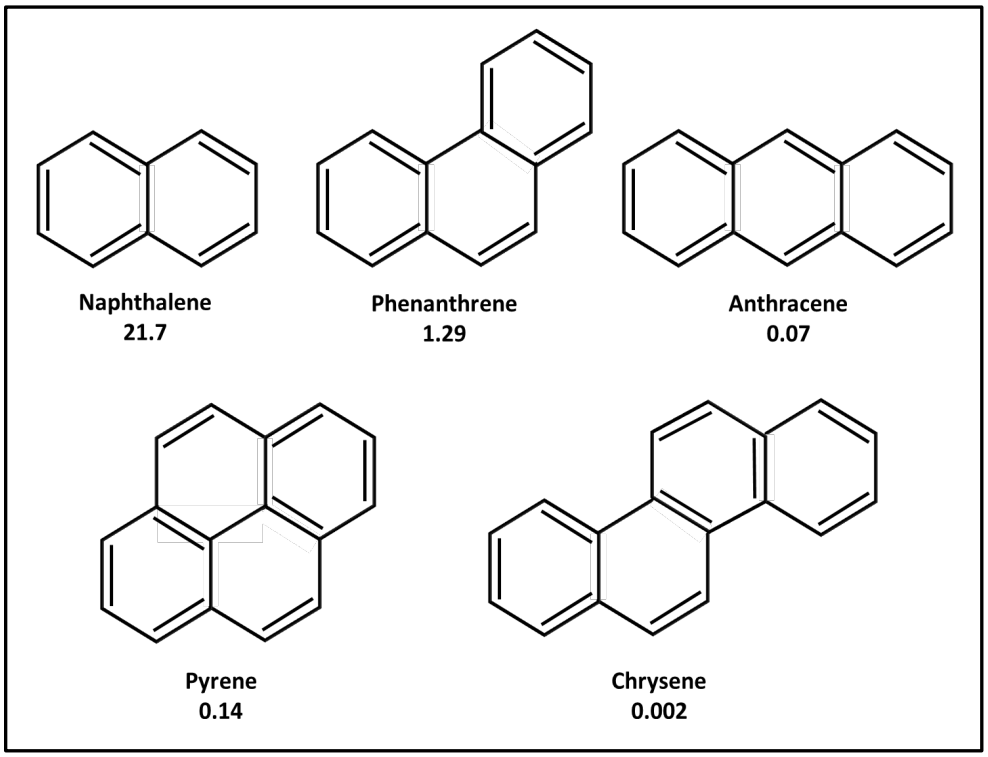


Figure 2.2 **Chemical structure and water solubility (mg/L) of selected PAHs (Juhasz & Naidu, 2000, Sutton & Calder, 1974).**

The hydrophobicity of long chain alkanes and PAHs render these compounds less available for microbial uptake and degradation, because bacteria acquire generally nutrients that are soluble in water. Furthermore, these molecules (especially PAHs) tend to absorb strongly to the environmental matrix such as soil particles. Despite of this limited bioavailability, there are microorganisms (*e.g. P. aeruginosa*) that have been isolated from polluted soils or sediments for their capacity to degrade these weakly soluble hydrocarbons (Déziel *et al.*, 2001, Déziel *et al.*, 1999, Déziel *et al.*, 1996, Harayama *et al.*, 1999). The precise mechanisms of uptake of such water insoluble molecules for their degradation are not well understood. Among these mechanisms are the attachment of microorganisms to the hydrocarbons, and the production of biosurfactants for their solubilization (De Kievit *et al.*, 2001, Johnsen & Karlson, 2004). Biosurfactants, produce by microorganisms, are surface active substances and have amphiphilic structure containing both hydrophilic and hydrophobic groups (reviewed in Pacwa-Płociniczak *et al.* (2011)). The water bioavailability of hydrophobic substances is increased by biosurfactants because of their ability to emulsify, form foam and disperse (JD. Desai & Banat, 1997). Classification of biosurfactants is based on physical and chemical characterization such as glycolipids; fatty acid, phospholipids and neutral lipids; lipopeptides and polymeric biosurfactant (reviewed in Pacwa-Płociniczak *et al.* (2011)). Among biosurfactants, rhamnolipids are glycolipids produced by *P. aeruginosa* (Déziel *et al.*, 1996, Ron & Rosenberg, 2002) (see section 2.4).

2.1.2 Bioremediation

Bioremediation processes use living organisms like microorganisms or both microorganisms and plants to degrade/remove toxic chemicals in contaminated water, air and soil. This approach is used to remove pollutants from contaminated environments, and/or to convert or transform these pollutants into less harmful compounds. Among pollutants, alkanes and PAHs are persistent compounds and are difficult to be degraded by the natural biota because of their poor bioavailability (Déziel, 2001, Johnsen *et al.*, 2005, Lafortune *et al.*, 2009). Bioremediation can be applied to treat soils, sediments, sludge and water (Ron & Rosenberg, 2002, Samanta *et al.*, 2002) contaminated with such

hydrocarbons. Furthermore, various environmental factors such as nutrients, pH, electron acceptor availability, etc. play a role in the process (reviewed in Choi *et al.* (2011).

Bioremediation is often the choice for treatment of different hydrocarbon-polluted sites due to its low cost and environment friendliness (reviewed in Choi *et al.* (2011). However, in addition to environmental conditions, the rate of bioremediation depends on the abundance and the type of microorganisms. Microorganisms such as fungi, *Actinomycetes* and other specific bacteria can be isolated from contaminated water, sludge or soils. For instance, after the oil spill breakup in Gulf of Mexico, Kostka and co-workers isolated many kinds of different hydrocarbon-degrading bacteria from beach sands, which were related to the genera *Alcanivora*x, *Marinobacter*, *Pseudomonas*, and *Acinetobacter* (Kostka *et al.*, 2011). To apply bioremediating activities in different polluted sites, the bacterial potential to degrade toxic chemicals were analyzed. For example, n-alkanes with chain length of C_{10}-C_{40} were used as sole carbon source by *Acinetobacter* sp. strain DMS 17874 (Throne-Holst *et al.*, 2007). In another study, *Rhodococcus rhodochrous* can use C_{12}-C_{20} (reviewed in Wentzel *et al.* (2007)) while *P. aeruginosa* strain RR1 can utilize long chain n-alkanes of C_{12}-C_{34} (reviewed in Wentzel *et al.* (2007)).

2.1.3 Alkane degradation by alkane hydroxylase

Alkane hydroxylase is a membrane bound enzyme involved in the initial step of the catabolism of linear hydrocarbons. This enzyme is an integral-membrane non-haem di-iron monooxygenase. It plays a role in hydroxylation of alkanes at the terminal position (Rojo, 2009). n-Alkanes are oxidized at the terminal methyl group to alcohol, aldehyde and to fatty acid at final step (Figure 2.3) (Harayama *et al.*, 1999). Acording to Beilen *et al.* (2007), alkanes are divided into three groups: short ($C_1 - C_4$), medium ($C_5 - C_{15}$) and long chain alkanes ($\geq C_{16}$). Alkane hydroxylases are divided into two groups. Type 1 is found widely in the genus *Pseudomonas,* and they can oxidize short and medium chain alkanes. Type 2 can oxidize long chain alkanes (Beilen & Funhoff, 2007). Among them, AlkB has been the most studied. AlkB is present in a wide variety of Gram negative as well as Gram-positive bacteria (Smits *et al.*, 2002, Whyte *et al.*, 2002).

The AlkB-type alkane hydroxylases were recognized as the enzymes that degrade alkanes with chain length of C_{10}-C_{20} in Gram-negative *Acinetobacter* strains (Throne-Holst *et al.*, 2007). Multiple alkane hydroxylase genes have been found in many bacteria. For instance, in *Rhodococcus* sp., Whyte *et al.* (2002) discovered 4 alkane monooxygenase genes in two different strains of *Rhodococcus* sp. (strain Q15 and NRRL B-16531) including *alkB1, alkB2, alkB3* and *alkB4.* Van Beilen *et al.* (2003) showed *alkB* homologs encoding functional enzymes sharing only 40% sequence identity with other AlkB. Normally, AlkB oxidized n-alkanes ranging from C_5-C_{16}, but more recent reports demonstrated that some alkane hydroxylases to oxidize longer alkanes (> C_{16}) (Amouric *et al.*, 2010, Quatrini *et al.*, 2008). While AlkB2 could oxidize alkanes shorter than C_{16}, AlkB1, AlkB3 and AlkB4 could oxidize longer chain alkanes up to C_{32} to C_{36} (Whyte *et al.*, 2002). AlkBa in *Rhodococcus ruber* SP2 oxidized of alkanes longer than C_{12} (Amouric *et al.*, 2009).

In *P. aeruginosa*, there are two known alkane hydroxylase genes (*alkB1* and *alkB2*) (Marín *et al.*, 2003, Winsor *et al.*, 2011). The *alkB1* gene was induced at late exponential phase by C_{10}-C_{24} alkanes, while AlkB2 activity peaked at early exponential phase (Marín *et al.*, 2003). The presence of alkane is necessary for expression of *alkB1* and *alkB2*, AlkB1 and AlkB2 were not detected when cultured on rich LB medium (Marín *et al.*, 2003).

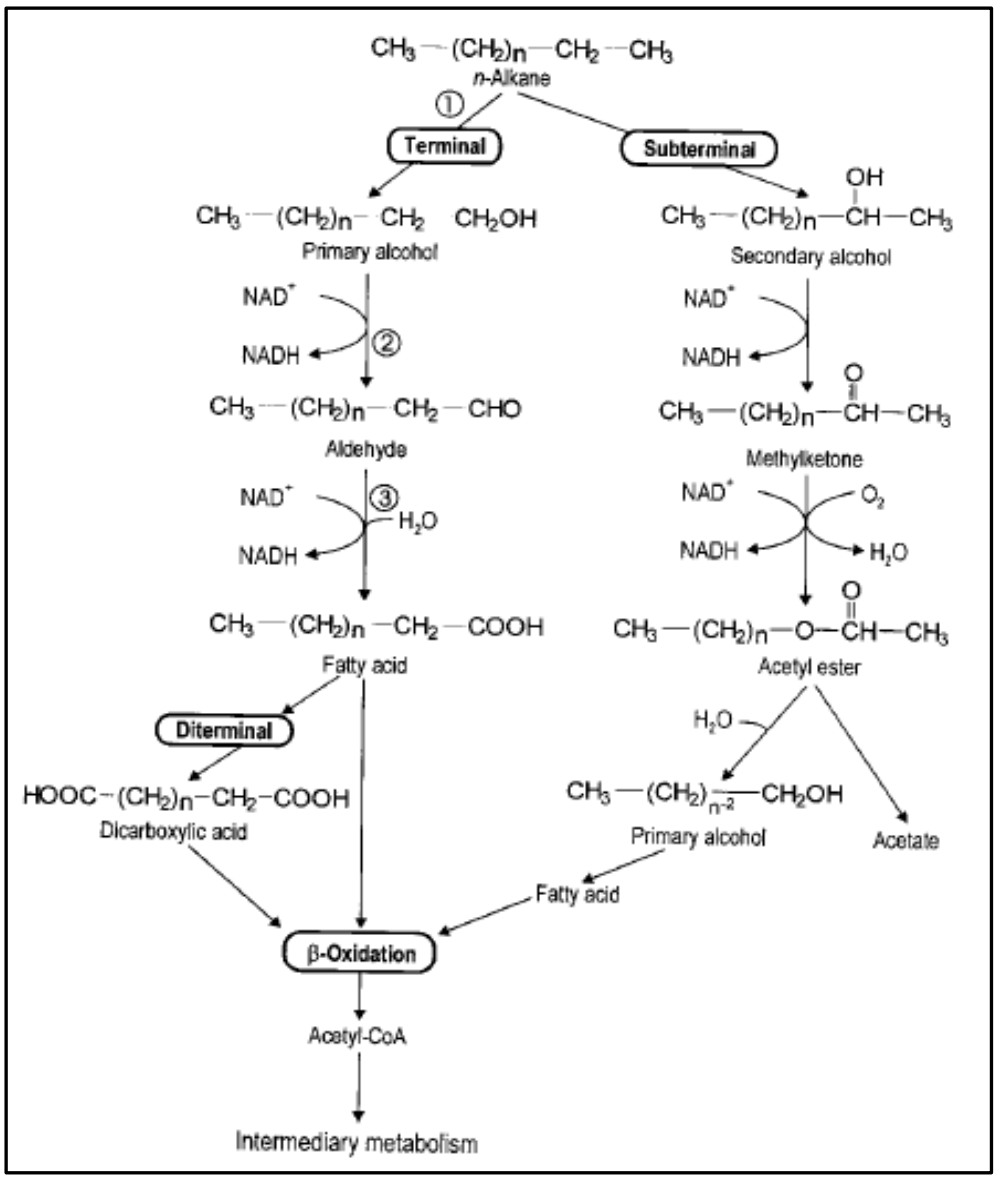


Figure 2.3 **Pathways of alkane degradation. (1)-n-alkane monooxygenase, (2)-alcohol dehydrogenase, (3)-aldehyde dehydrogenase (Harayama *et al.*, 1999) .**

2.2 Pseudomonas aeruginosa

2.2.1 General characterization

The γ-proteobacterium *Pseudomonas aeruginosa* is a Gram-negative bacillus found in diverse environments ranging from soil to various living hosts (reviewed in Kung *et al*. (2010)). Its size ranges from 0.5 to 0.8 μm by 1.5 to 3.0 μm (Iglewski, 1996). Its outer membrane is composed of lipopolysaccharides (LPS) which influence the cell surface hydrophobicity (Starr & Stolp, 1981). *P. aeruginosa* releases a variety of pigments such as pyocyanin (blue-green) and two siderophores which are pyoverdine and pyochelin (fluorescent yellow-green). The WT cells of this bacterial species possess a single flagellum and several cell-surface-scattered type IV pili (Iglewski, 1996) participate in the swimming and twitching motilities, respectively (Mattick, 2002). This free-living bacterium is, however, also an opportunistic pathogen. For example, chronic *P. aeruginosa* infection in people with cystic fibrosis leads to deteriorating lung function (Costerton *et al.*, 1999).

The *P. aeruginosa* strain PAO1 genome consists of one chromosome 6.2 Mbp with a G+C content of 66% and which contains more than 5500 predicted open reading frames (ORF) (Winsor *et al.*, 2011). Besides many genes encoding outer membrane proteins involved in environmental sensing, motility and adhesion, the genome of this bacterial species also contains a large number of genes involved in uptake and metabolism of different carbon sources for energy (reviewed in Kung *et al*. (2010)). For instance, some strains of *P. aeruginosa* can grow on n-alkane ranging from C_6 to C_{34}, (Wentzel *et al.*, 2007), such as hexadecane and octadecane (Bouchez-Naïtali & Vandecasteele, 2008, Déziel *et al.*, 2001, Obuekwe *et al.*, 2007). In the laboratory of Prof. Villemur, different *P. aeruginosa* strains (*e.g.* 57RP and 19SJ), isolated from oil-contaminated soils, have been found to utilize n-alkanes such as hexadecane, octadecane and PAHs such as naphthalene and phenanthrene as a sole carbon sources (Déziel, 1996, Déziel, 2001, Déziel *et al.*, 2001, Déziel *et al.*, 1996).

2.2.2 Motility

P. aeruginosa displays three main types of motility: swarming, swimming and twitching, which promote translocation and dissemination. The polar flagellum and type IV pili (TFP) are involved in these motilities.

2.2.2.1 Swarming

Swarming motility, first described by Henrichsen (1972), is a type of bacterial translocation where cellular tendrils move on solid or semi-solid surfaces. The *P. aeruginosa* swarming cells are morphologically characterized by hyperflagellation, expression of two polar flagella and cell elongation compared to the vegetative cells (Yeung *et al*., 2009). Besides flagella, swarming behavior requires the production of biosurfactants (Caiazza *et al.*, 2005, Déziel *et al.*, 2003, Tremblay *et al.*, 2007). Indeed, Déziel and co-workers showed that HAAs, an intermediate of rhamnolipid synthesis, found in the extracellular milieu, also contribute to swarming motility (Déziel *et al.*, 2003). In later work, Tremblay *et al.* (2007) demonstrated that rhamnolipids promoted swarming tendrils, a threadlike shape spreading of colony cells on surface of medium. Both flagella and rhamnolipids play an important role in swarming motility of *P. aeruginosa* cells (reviewed in Kearns (2010)). See section 2.4 for more on rhamnolipids.

2.2.2.2 Twitching

Twitching is a kind of bacterial translocation on solid surface, which is powered by the sequential extension and mediated attachment by intermittent TFP retraction. The movement of bacteria is dependent on TFP (Mattick, 2002). TFP are involved not only in the twitching motility but also in the initiation of biofilm formation in *P. aeruginosa* (Glick *et al.*, 2010, O'Toole & Kolter, 1998).

2.2.2.3 Swimming

Swimming motility is carried out by the single polar flagellum in aqueous

environments, contrary to twitching and swarming motilities, which are observed on surfaces. Contrary to swarming motility, rhamnolipids do not affect swimming motility (Tremblay *et al.*, 2007). Swimming and twitching are involved in chemotaxis towards a wide range of compounds (Barken *et al.*, 2008, Kato *et al.*, 2008).

2.2.3 Role of type IV pili in surface adherence and twitching motility

TFP are filamentous polar organelles typically of 5–7 nm in diameter and can extend to several μm in length. They are composed primarily of a single small protein subunit called PilA or pilin. TFP are involved in surface adherence of *P. aeruginosa,* and subsequently the colonization of the surface and development of biofilm (Anderson *et al.*, 2007, Kazmierczak *et al.*, 2006). They play an important role in attachment and in twitching motility. O'Toole and Kolter proposed that initial cell-surface interactions of *P. aeruginosa* are influenced by activities of flagella and pili (O'Toole & Kolter, 1998), and polar organelles are necessary for biofilm maturation, although flagella mutants can still form biofilms (Klausen *et al.*, 2003). In *P. aeruginosa*, nearly 40 genes have now been identified whose products are required for normal twitching motility. Besides those involved in the biogenesis and mechanical function of pili (such as *pilA*), there are several genes involved in controlling the production and function of the pili, and in responding to alterations in environmental conditions (Mattick, 2002).

2.3 Biofilms

2.3.1 Surface attached microbial communities

Biofilms are surface attached communities of living microorganisms densely encased in a self-produced matrix of extracellular polymeric substance (EPS), generally including extracellular DNA, proteins and polysaccharides (Allesen-Holm *et al.*, 2006, Harshey, 2003) called the biofilm matrix. Biofilms are found in a variety of habitats. They can be formed on both environmental abiotic surfaces, such as minerals, or at air-water interface, and biotic surfaces in the natural environment, such as other microbes, plants, external and internal surfaces of animals and the human body (reviewed in Karatan and Watnick (2009)). *P. aeruginosa* has become a model organism for the study of biofilm development (Figure 2.4) because this bacterial species is not only ubiquitous but also an opportunistic human pathogen with its high versatility and adaptation capabilities to different environments (reviewed in Valentini and Filloux (2016)).

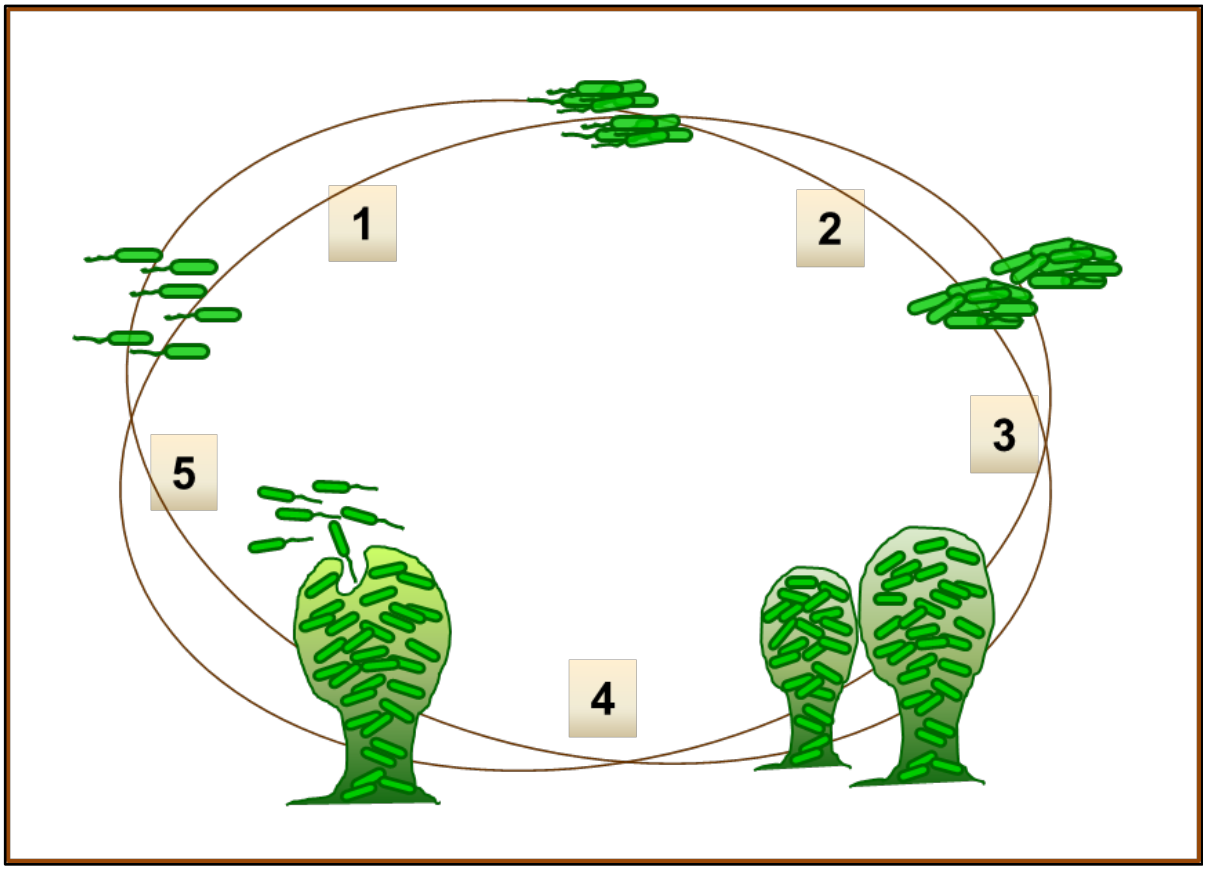


Figure 2.4 **Five stages of biofilm development: 1- Initial attachment, 2- Formation of microcolonies requiring twitching motility, 3- Formation of mushroom-type structure by Quorum Sensing, 4- Maturation, 5- Dispersal (According to Sauer et al. (2002), Costerton et al. (1999)).**

2.3.2 Biofilm formation

The development of a biofilm first involves the attachment of bacteria to a substratum. The second step typically involves the formation of microcolonies, which then grow and can form mushroom-type structures (typically observed in flow chamber cultures). Finally, bacteria detach from the surface and can then colonize other surfaces (Déziel, 2001, Sauer *et al.*, 2002). The initiation of biofilm formation seems to respond to environmental changes (Harshey, 2003, O'Toole, 2003, O'Toole & Kolter, 1998). Biofilm microcolonies will maintain their mushroom-shaped structure until deprivation of nutrients and will return to the planktonic state (free-floating, swimming state) (Barken *et al.*, 2008). The mushroom stalks are composed of non-motile bacteria in foci on the substratum, while mostly motile bacteria are associated with the cap when about to disseminate (Klausen *et al.*, 2003).

In nature, biofilms may exist in the form of communities of one or different types of microorganism. The later complex form of biofilms may consist of different strains or multiple species of microorganisms, including bacteria, archaea, protozoa, fungi and/or algae. In such a complex biofilm, different microbial groups may cooperate or compete with each other through their specialized metabolic functions in a given environment (reviewed in Nadell *et al.* (2009)).

2.3.3 Biofilm matrix of *P. aeruginosa*

Biofilm matrix comprises by polysaccharides, proteins and extracellular DNA (eDNA). There are differences between biofilm matrixes of microorganisms. In the biofilm matrix of *P. aeruginosa*, eDNA plays a key structural role in early stages in keeping cells together in biofilm formation and is released in the biofilm either by bacterial lysis or by the release of membrane vesicles (Allesen-Holm *et al.*, 2006). A young biofilm of *P. aeruginosa* PAO1 was destroyed when it was exposed to DNase I (Whitchurch *et al.*, 2002).

In *P. aeruginosa* biofilm matrix, Toyofuku and colleagues observed the presence of 178 proteins; 45 of which were found exclusively in the matrix fraction (Toyofuku *et al.*, 2012). Approximately 30% of the identified matrix proteins were outer membrane proteins, which are also typically found in outer membrane vesicles (OMVs). These authors demonstrated that some proteins associated with the biofilm matrix are derived from secreted proteins, others from lysed cells, while the large majority of the matrix proteins originate from OMVs and that the protein content of planktonic and biofilm OMVs is surprisingly different (Toyofuku *et al.*, 2012). However, the extracellular protein component of biofilm matrix may vary depending on the bacterial genotype, the specific environment and the development of some new highly adaptive subpopulations such as small colony variants.

Exopolysaccharides also play an important role in the biofilm formation and architecture at both early and late-stage biofilm development (Ghafoor *et al.*, 2011). A large quantity of exopolysaccharide produced by the attached bacteria not only promotes adherence but protects these bacteria from desiccation, and even helps these bacteria to acquire minerals and nutrients (Harshey, 2003). In *P. aeruginosa*, there are three types of exopolysaccharide including alginate, Psl and Pel (Ryder *et al.*, 2007).

Biofilm appears to be a good means of bacterial survival and growth in adverse conditions. For example, under the biofilm mode of growth, *P. aeruginosa* cells grown in the lung of cystic fibrosis condition patient are highly protected from the killing effect of antibiotics and the immune response (Costerton *et al.*, 1999). Further, biofilm state may also be useful in degrading hydrocarbons (Déziel *et al.*, 2001).

2.3.4 Environmental factors

The biofilm formation of microorganisms on a surface is affected by various environmental factors including nutrients, inorganic molecules, oxygen and nitric oxide, antimicrobials and surfactants (reviewed in Karatan and Watnick (2009)). A few of the

environmental factors affecting the biofilm formation of *P. aeruginosa* are explained below.

a) *Nutrients. P. aeruginosa* can grow on various nutritional sources, e.g. glucose, citrate, casamino acids and benzoate; however, its biofilm development is dependent on the carbon source. Under flow through culture conditions, when glucose was supplied as the carbon source the biofilm formed by *P. aeruginosa* was found to be heterogeneous with a mushroom shape (DG. Davies *et al.*, 1998, Stewart *et al.*, 1993), whereas upon supplied with citrate, casamino acids or benzoate the biofilm was uniform, flat and densely-packed (Heydorn *et al.*, 2000, Klausen *et al.*, 2003). However, a sudden increases in the concentration of several nutrients such as citrate, glucose, glutamate, succinate, or ammonium chloride induced biofilm dispersal (Sauer *et al.*, 2004).

b) *Inorganic molecules*. Iron is one of the inorganic molecules essential for most bacteria, including *P. aeruginosa.* Mutants of this microorganism that are defective in iron uptake are less able to form biofilms (Banin *et al.*, 2005).

c) *Oxygen depletion and nitric oxide*. In the deepest layers of the biofilm, bacterial cells usually suffer from the depletion of oxygen due to the consumption of cells that reside at the outer layers of the biofilm. For survival, these cells may require anaerobic metabolism, and anaerobic respiration can produce reactive nitrogen intermediates including nitric oxide (NO). The reactive nitrogen intermediates at high doses are harmful for DNA, proteins, and lipids. The NO stress, originating from either the endogenous anaerobic respiration or the exogenous supply, can induce biofilm dispersal in *P. aeruginosa* (Barraud *et al.*, 2006).

d) *Temperature and pH*. Hostacka and colleagues compared the effect of temperature and pH on the biofilm production of four strains of *P. aeruginosa.* These authors found that when grown at 37^{o}C, 3 of the 4 tested strains produced significantly less biofilm than that they produced at 30^{o}C (Hostacka *et al.*, 2010). The biofilm

production of the 4th strain grown at 37°C was slightly higher but not significantly than those grown at 30°C. These authors also found that in all tested media an increase in pH from 5.5 to 7.5 and 8.5 led to a higher biofilm production, e.g. the biofilm production at pH 8.5 was 139-244 % and at pH 7.5 136-164 % was higher than those observed at pH 5.5 (Hostacka *et al.*, 2010).

e) *Antimicrobials*. The antimicrobial compounds can also induce biofilm formation of *P. aeruginosa*. Hoffman and colleagues showed that the presence of subinhibitory concentrations of the aminoglycoside antibiotic tobramycin in the culture medium could induce biofilm formation of *P. aeruginosa* (Hoffman *et al.*, 2005).

f) *Surfactants*. (see section 2.4.3.3 Role of rhamnolipids in biofilm development).

2.4 Rhamnolipids

2.4.1 Biosurfactants

Rhamnolipids are surface-active agents that have properties of amphipathic molecules formed with both hydrophilic (rhamnose) and hydrophobic (lipid) moieties. These surface-active agents can be produced by various microorganisms living under different environmental conditions, especially at high cell densities. They are generally called biosurfactants (reviewed in Karatan and Watnick (2009)), since they have the property to reduce surface/interfacial tension.

Rhamnolipids produced by *P. aeruginosa* were identified over 70 years ago. It begun with Bergström and colleagues (1946) who found for the first time glycolipid compounds containing rhamnose and β-hydroxydecanoic acid in the culture of *Pseudomonas pyocyanea* on glucose (reviewed in Lang and Wullbrandt (1999)). The ratios of the two rhamnose and lipid components of glycolipids were established by Jarvis and Johnson (Jarvis & Johnson, 1949) on the product isolated from cultivation of *P. aeruginosa*

on glycerol, and the linkage between the two sugar units was established by Edwards and Hayashi (1965). Rhamnolipids produced by *P. aeruginosa* are frequently considered as the best characterized microbial biosurfactants (reviewed in Lang and Wullbrandt (1999)).

2.4.2 Rhamnolipid biosynthesis

2.4.2.1 Quorum sensing

Rhamnolipid synthesis is regulated by quorum-sensing (QS) circuits, which are utilized by various microorganisms to coordinate their behavior in a cell density-dependent manner. In *P. aeruginosa*, activities of the QS circuits are dependent on small molecules such as N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C_{12}-HSL) and N-butyryl-L-homoserine lactone (C_4-HSL) called autoinducers (reviewed in Karatan and Watnick (2009)). There are two AHL-based QS circuits LasI/R and RhlI/R, which produce and detect the autoinducers, and Rhl is under the control of Las system (Henke & Bassler, 2004) (Figure 2.6). In the presence of the cognate autoinducers, Las and Rhl pathways regulate quorum-sensing responses, activating a large number of genes. Among which are those involved in biofilm development, etc. (reviewed in Karatan and Watnick (2009)).

2.4.2.2 Rhamnolipid biosynthesis

Rhamnolipids are biosurfactants known to be produced by various *Pseudomonas* species, including *P. aeruginosa, P. chlororaphis, P. plantarii, P. putida* and *P. fluorescens*, of which *P. aeruginosa* is the predominant rhamnolipid producer (reviewed in Sekhon Randhawa and Rahman (2014)). Rhamnolipid production is regulated by RhlI/R of the QS circuits (reviewed in Henke and Bassler (2004)). Two main types of rhamnolipids usually produced by *P. aeruginosa* during growth on n-alkane are mono-rhamnolipid (mono-RL) and di-rhamnolipids (di-RL) (Hisatsuka *et al.*, 1971, Ito *et al.*, 1971) (Figure 2.5). In *P. aeruginosa,* biosynthesis of the two mono- and di-rhamnolipid is regulated by the action of three essential enzymes RhlA, RhlB and RhlC. The 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs) (Figure 2.5) are the precursors of rhamnolipids and are synthesized by RhlA (Cabrera-Valladares *et al.*, 2006, Déziel *et al.*,

2003, Zhu & Rock, 2008). Then, the HAAs are converted into mono-rhamnolipid via RhlB (rhamnosyltransferase 1) and to di-rhamnolipid by RhlC (rhamnosyltransferase 2) (Davey *et al.*, 2003, Déziel *et al.*, 2003, Soberon-Chavez *et al.*, 2005) (Figure 2.6). RhlA and RhlB are encoded by the *rhlAB* operon, while *rhlC* is elsewhere on the chromosome (Rahim *et al.*, 2001).

The production of glycolipids or rhamnolipids typically starts at the end of exponential growth phase (Muller *et al.*, 2010). However, nutritional components may affect the rhamnolipid production and it also depends on the strains used in experiments. For example, four *P. aeruginosa* strains used by Zhang and Miller (Y Zhang & Miller, 1994) produced rhamnolipids during growth in phosphate-limited proteose peptone-glucose-ammonium salts (PPGAS) medium but did not do so in mineral salt medium containing glucose or octadecane as the carbon source (Y Zhang & Miller, 1994). Other *P. aeruginosa* strains, such as the one (strain CCTCC AB93066) used by Zhong and colleagues (2008) produced rhamnolipids during growth in minimal salt medium (MSM) supplied with glucose as the carbon source (Zhong *et al.*, 2008) while Déziel (2001) observed the rhamnolipid production during growth of the 57RP strain on various carbon sources, including n-alkanes and PAHs (Déziel, 2001).

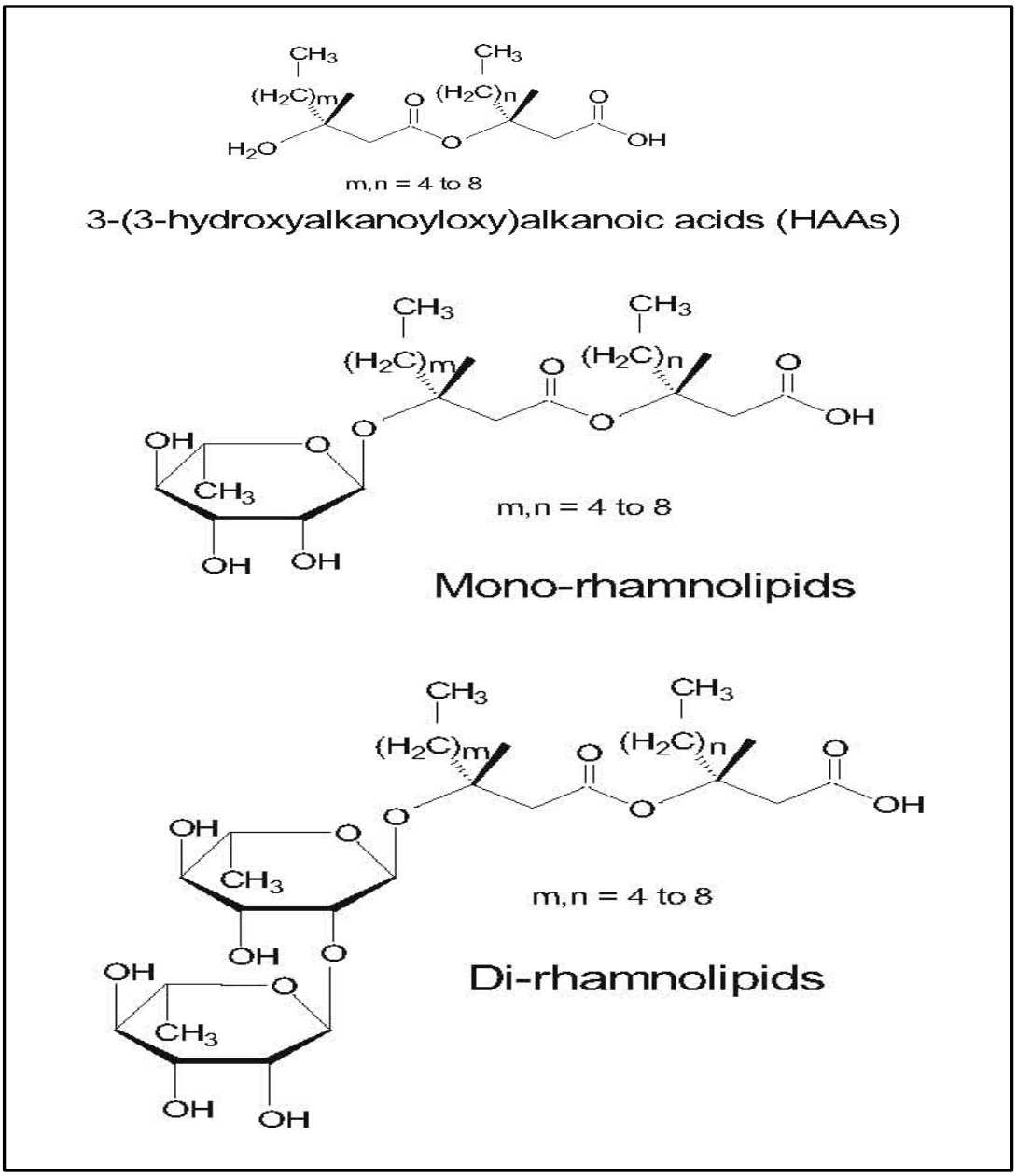


Figure 2.5 **Chemical structure of 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAAs), L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (mono-rhamnolipid) and L-rhamnosyl-L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (di-rhamnolipid) (Soberon-Chavez *et al*., 2005)**

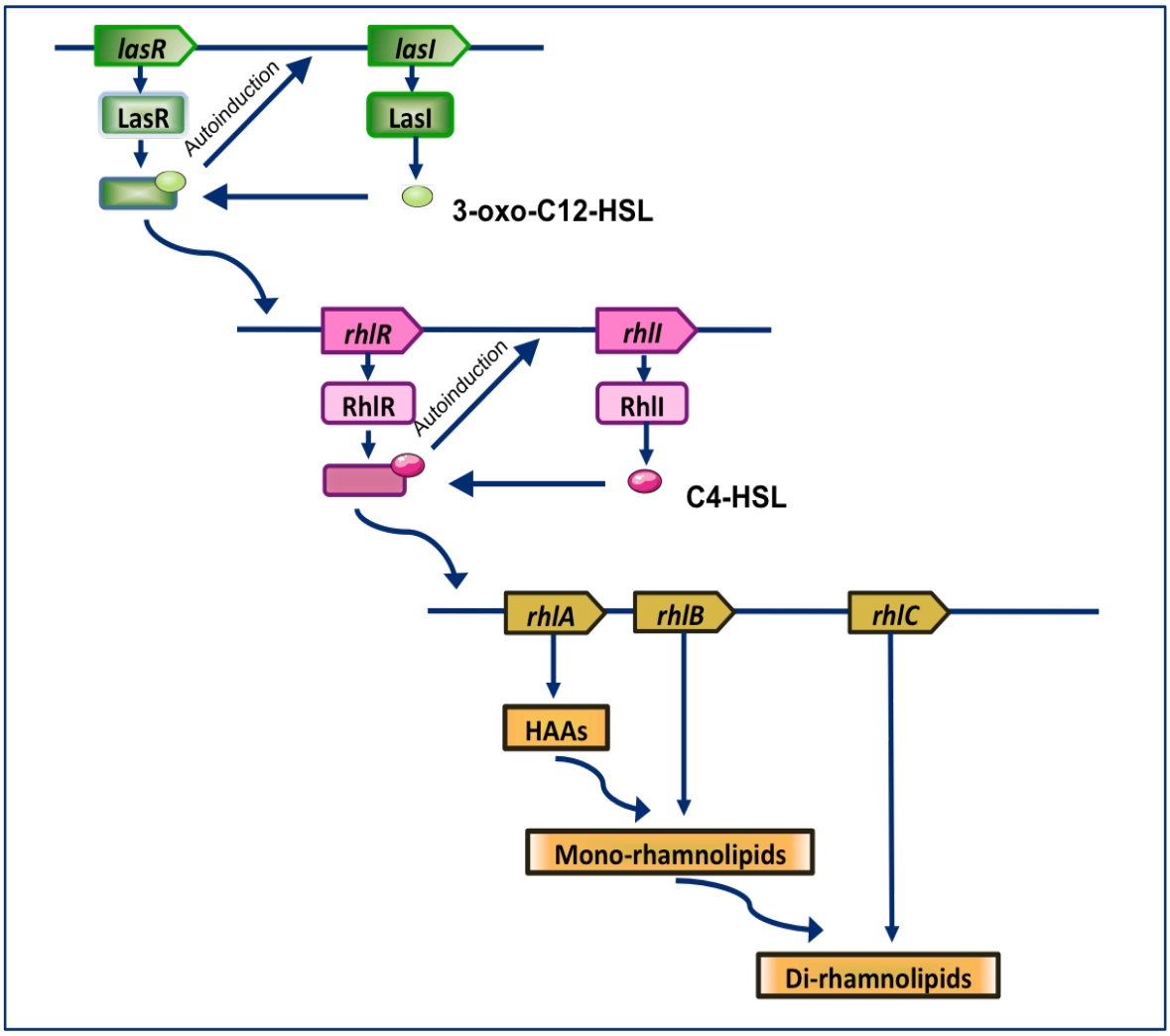


Figure 2.6 **Schematic diagram of rhamnolipid biosynthesis in *P. aeruginosa* controlled by QS system. N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-12-HSL) and N-butyryl-L-homoserine lactone (C4-HSL) (According to PN. Jimenez et al. (2012), Soberon-Chavez et al. (2005)).**

2.4.3 Roles of rhamnolipids

2.4.3.1 Role of rhamnolipids in the dispersion of hydrophobic substrates

Bacterial growth can be limited by the interfacial contact area when they grow on hydrocarbons. However, the limiting contact area can be overcome by cell-density-dependent mechanisms and by the dispersion or emulsification of the hydrophobic nutrients in the presence of biosurfactants. The dispersion of hydrocarbons by biosurfactants certainly enhanced the bioavailability of the initially hydrophobic nutrients to bacteria (Ron & Rosenberg, 2002). However, in certain *P. aeruginosa* strains an addition of rhamnolipids generates a negative effect on growth of some bacterial strains (Zhang & Miller, 1994).

Our main research model strain 57RP is a biosurfactant-producing strain and can adapt to grow on various hydrocarbon sources such as n-alkanes and PAHs (Déziel, 2001). Rhamnolipids might help to disperse hydrophobic hydrocarbons and to increase the contact surface between bacterial cells and substrates. However, the exact role of these biosurfactants in the growth ability of 57RP strain in culture with hydrophobic hydrocarbons, especially hexadecane, used as a sole carbon source, remains still unclear and will be investigated and discussed further in the present study.

2.4.3.2 Role of rhamnolipids in cell surface hydrophobicity

Zhang and Miller (1994) used four *P. aeruginosa* strains that produced rhamnolipids during growth in phosphate-limited proteose peptone-glucose-ammonium salts (PPGAS) but did not produce rhamnolipids during growth in the minimal salt medium containing either glucose or octadecane as the carbon source. These authors showed that the positive effect of added rhamnolipids on the increase of cell hydrophobicity and biomass production was found primarily with the two slow octadecane degraders that had low cell surface hydrophobicity (Y Zhang & Miller, 1994). In contrast, the addition of the exogenous biosurfactants showed almost no effect on the cell hydrophobicity and the biomass production of the other two fast octadecane degraders that had high cell surface

hydrophobicity. These authors also observed that rhamnolipid reduced the cell attachment to hydrocarbon of all four strains tested and the surfactant was not incorporated into the cells in both conditions with and without octadecane (Y Zhang & Miller, 1994). These authors suggested that the bioavailability of octadecane in the presence of rhamnolipids is controlled by both aqueous dispersion of octadecane and cell hydrophobicity (Y Zhang & Miller, 1994). In another study, Koch and colleagues (1991) isolated a transposon Tn5-GM-induced mutant 65E12 of *P. aeruginosa* PG201 that did not produce extracellular rhamnolipids in all conditions tested, and was also unable to take up ^{14}C-labelled hexadecane, and was unable to grow in media containing individual alkanes from C_{12} to C_{19}. However, when small amounts of purified rhamnolipids were added to the cultures, growth on these alkanes and uptake of [^{14}C]hexadecane of the mutant 65E12 were restored (Koch *et al.*, 1991). The above observed positive effect of an addition of extragenous rhamnolipids on the growth on hexadecane was further supported by studies of another *P. aeruginosa* strain (Bouchez-Naïtali & Vandecasteele, 2008).

The effects of rhamnolipids on the increase in the cell surface hydrophobicity in *P. aeruginosa* have been investigated in more detail. Al-Tahhan and colleagues (2000) observed the rhamnolipid-induced removal of lipopolysaccharide (LPS) from the cell surface of *P. aeruginosa* and changed the surface properties to be more hydrophobic. These authors concluded that rhamnolipid-induced LPS release is the probable mechanism of enhanced cell surface hydrophobicity (Al-Tahhan *et al.*, 2000). In a later study on the effect of rhamnolipids on cell surface properties of *P. aeruginosa*, Sotirova and colleagues (2009) found that exogenous addition of purified rhamnolipids at concentration above the critical micelle concentration (CMC) caused the reduction of total cellular LPS content and increased cell hydrophobicity. At concentrations below CMC, rhamnolipids did not affect the LPS component of the bacterial outer membrane, but caused changes in the outer membrane protein (OMP) composition of the bacterial cell (Sotirova *et al.*, 2009). In another study, Zhong and colleagues found the adsorption of mono-RL and di-RL on the cell surface of *P. aeruginosa* increased cell surface hydrophobicity (Zhong *et al.*, 2008). The authors also reported that the adsorption capacity of all cells to mono-RL was much stronger than to di-RL as the later surfactant was still released when its aqueous

concentration was already too high. The authors assumed that the adsorption was a result of the polar interaction between the rhamnolipid molecules and the cell surface chemical groups. Extention into the environment of the hydrophobic moiety of the rhamnolipid molecules may account for the increase in cell surface hydrophobicity at low concentrations of the surfactant (Zhong *et al.*, 2008).

Besides alkanes, saturated hydrocarbons, *P. aeruginosa* can also grow on other types of hydrocarbons used as the sole carbon source such as polycyclic aromatic hydrocarbons (PAHs). The two *P. aeruginosa* strains used in the present study are able to grow on these PAH substrates (Déziel, 2001, Déziel *et al.*, 1996). In a study on the evaluation of bacterial strategies to promote the bioavailability of PAHs, Johnsen and Karlson also found that *P. aeruginosa* produced extracellular surfactants in the cultures with phenanthrene supplied as the carbon source (Johnsen & Karlson, 2004).

Rhamnolipids exhibit both positive and negative effects on the interaction between the *P. aeruginosa* cells and the hydrocarbon substrate, including the increased dispersion of weakly soluble substrates, the enhancement of cell surface hydrophobicity, the induced release of LPS and decrease of OMPs, and the reduced adherence of cells to hydrocarbons (Y Zhang & Miller, 1994). However, not all *P. aeruginosa* strains produce rhamnolipids when grown on hydrocarbons. The hydrophobicity of the cell surface appeared to be an essential factor for the *P. aeruginosa* cells to be able to grow on the hydrophobic substrates such as hydrocarbons. The addition of exogenous rhamnolipids appeared to have positive effects on the ability of the *P. aeruginosa* with low cell hydrophobicity to degrade hydrocarbons. In contrast, the growth ability of the cells, with high cell hydrophobicities, may suffer from the additional rhamnolipids (Y Zhang & Miller, 1994).

2.4.3.3 Role of rhamnolipids in biofilm development

The general life cycle of bacterial biofilms, starting from the initial cell-to-surface attachment to the initiation of biofilm formation, development and its dispersal, is differently affected by rhamnolipids and this biosurfactant contributes substantially in the

active dispersal of biofilms to initiate a new biofilm life cycle (reviewed in (Kaplan, 2010), McDougald *et al.* (2012)). Results from research by Davey and colleagues (2003) on the biofilm development of *P. aeruginosa* have shown that rhamnolipids are involved in the different stages of biofilm development. For example, rhamnolipids can interfere with the attachment of bacterial cells to surfaces, which is the first step of biofilm formation (Davey *et al.*, 2003). The development of a young biofilm is also affected by rhamnolipids as the added rhamnolipids cause the dispersion of the biofilm. However, the mature biofilm is not affected by rhamnolipids, and, indeed, rhamnolipids contribute to the maintenance of the function of fluid channels by preventing the attachment of the other bacterial cells that may block the flow channels. One of the most significant roles of the rhamnolipid surfactant is that in the aged biofilms the self-produced rhamnolipids cause the dispersal of the aged biofilm cells. The rhamnolipid-detached cells could recolonize a new surface to exploit the nutrients still available in the environment and grow on (Davey *et al.*, 2003). Furthermore, rhamnolipids play a role in the detachment of cells from biofilms (Boles *et al.*, 2005).

2.4.3.4 Role of rhamnolipid on motility

Rhamnolipids or their HAAs precursors have different effects on swarming motility. Indeed, Tremblay and co-workers showed that di-RLs promoted swarming cells; in contrast, swarming motility was prevented by the presence of HAAs. In addition, mono-RLs play a role as wetting agent but are not involved in migrating swarms (Tremblay *et al.*, 2007). Another study showed that rhamnolipids are also involved in twitching motility (Glick *et al*., 2010). Pamp and Tolker-Nielsen (2007) presented genetic evidence that *P. aeruginosa rhlA* mutants, deficient in synthesis of biosurfactants, could not form microcolonies in the initial phase of biofilm formation. These *rhlA* mutants were also defective in migration-dependent development of mushroom-shaped multicellular structures in the later phase of biofilm formation (Pamp & Tolker-Nielsen, 2007).

2.5 Small colony variants

2.5.1 Small colony variant definition

Bacterial small colony variants (SCV) are adaptive phenotypes of naturally occurring subpopulations of bacteria that had been reported for the first time by Jacobsen and Mitteilungen in 1910 (reviewed in Proctor *et al.* (2006)). The SCVs are constituted of slow growing cells with atypical colony morphology, and distinctive phenotype traits. The most recognizable feature of the SCVs during growth on rich media is the formation of colonies, the size of which was significantly reduced as compared to that of wild-type bacteria, and the size reduction aspect implicates the name of the type of colony variants (Proctor *et al.*, 2006).

2.5.2 Common characteristics of *P. aeruginosa* SCVs

Besides the obvious size reduction, SCVs commonly share several other observable phenotypic features (Evans, 2015b), with a few representative examples of which are listed below.

a. High cell surface hydrophobicity and attachment (Déziel *et al.*, 2001, Drenkard & Ausubel, 2002, Haussler *et al.*, 2003).
b. High production of EPS and auto-aggregation (D'Argenio *et al.*, 2002, Malone *et al.*, 2010, Starkey *et al.*, 2009) and high capacity of forming biofilms (Déziel *et al.*, 2001, Drenkard & Ausubel, 2002, Haussler *et al.*, 2003).
c. Hyperpiliation and/or motility deficiencies (Déziel *et al.*, 2001, Haussler *et al.*, 2003, Wei *et al.*, 2011).
d. Increase in the c-di-GMP production. Inhibition the c-di-GMP synthesis or increase in degradation of the second messenger could induce phenotypic reversion (D'Argenio *et al.*, 2002, Drenkard & Ausubel, 2002, Haussler *et al.*, 2003, Malone *et al.*, 2010, Starkey *et al.*, 2009).

e. Ability to revert to the large size and different phenotypic characteristics of the wild type colony (Déziel *et al.*, 2001, Drenkard & Ausubel, 2002, Haussler *et al.*, 2003, Wei *et al.*, 2011), etc.

2.6 Phase variation

Bacterial cells, grown in the biofilm, including those derived from a clonal origin and living under a homogenous condition, often show a high degree of both genotypic and phenotypic variability representing a wide range of different physiological states. This wide range variability relates the physiological heterogeneity or bistability of the whole cell population. These phenotypic variations are based on different mechanisms, including the phase variation-induced changes in gene expression program, the transition of cell cycles, the response of cells to certain environmental stress-specific stimuli, and others (reviewed in Stewart and Franklin (2008), Veening *et al.* (2008)). However, an atypical phenotypic variation could also occur by spontaneous mutations to some specific gene(s), e.g. the c-di-GMP-metabolizing genes. The activities of these mutated genes would induce the phenotypic switching leading to the formation of various atypical phenotypic variants, including the c-di-GMP-dependent SCVs, too (see section 2.7.2.3).

SCV formation during growth on hexadecane, as the sole carbon source, and reversion to the wild phenotype during growth on rich media, has been reported in *P. aeruginosa* strain 57RP isolated from a crude-oil contaminated site (Déziel *et al.*, 2001, Déziel *et al.*, 1996). The authors suggested that the 57RP SCVs resulted from phase variation. They proposed that phase variation ensures the prior presence of phenotypic forms well adapted to initiate the formation of a biofilm as soon as environmental conditions are favorable (Déziel *et al.*, 2001). The opinion of SCV formation as a result of phase variation and environmental selection was also supported by results obtained from another research laboratory. Indeed, report of Drenkard and Ausubel indicated that 100% SCV reverted to WT phenotype when cultured on medium without antibiotic (Drenkard & Ausubel, 2002).

2.6.1 Phase variation definition

The phenomenon of phase variation was reported for the first time by Andrewes in 1922 (Andrewes, 1922) and is defined as the random switching of microbial phenotype in a reversible manner (reviewed in Henderson *et al.* (1999)).

One of the features that differentiate phase variation from point mutation is the variation rate. The rate of phase variation occurs on average 10^{-4} per cell per generation (Villemur & Déziel, 2005). In some cases, the variation rate can be as high as a change in 1 cell per 10 per generation but more often is on the order of 1 change per 10^3 cells per generation, depending on the gene, the bacterial species, and the regulatory mechanism involved (van der Woude & Bäumler, 2004). It is obvious that the phase variation rate is much higher than the rate of classical point mutation. For example, in *E. coli*, the spontaneous mutation rate was observed at 2.2×10^{-10} mutations per nucleotide per generation or 1.0×10^{-3} mutations per genome per generation (H. Lee *et al.*, 2012). However, in the complex case of spontaneous mutation-induced-phase-variation, the rate of the atypical variant formation (e.g. mutant SCV) and reversion was probably as high as the mutation rate of genes of bacterial cells grown in a given environment. At present, there is no information on the topic of switching from and to SCV state in the literature.

2.6.2 Mechanism of phase variation

There are various mechanisms known to be involved in the phase variation (reviewed in Bayliss (2009), Henderson *et al.* (1999), Villemur and Déziel (2005)), including:

a. ***Site-specific recombination.*** One of the site-specific recombination types is inversion. This is a change in the original orientation of DNA sequences that will affect the expression, the ON or OFF expression mode, of the gene located within or next to the switch, including the promoter of a gene located in the invertible element (Gally *et al.*, 1993, Wisniewski-Dyé & Vial, 2008).

The other type is insertion-excision, i.e. the translocation of a short DNA sequence, called an insertion sequence (IS) element, inserted into or excised from a target DNA sequence resulting in structural variation or restoration of the original sequence of DNA (Higgins *et al.*, 2007, van der Woude & Bäumler, 2004). The reversion by insertion-excision events in phase variation mediated by transposition targeting specific DNA sequences (van der Woude & Bäumler, 2004).

b. ***Gene conversion by homologous recombination.*** This is a genetic modification process under which nucleotide sequences are exchanged between two identical or similar molecules of DNA. Gene conversion is a result of homologous recombination that combined DNA sequences of an active copy of a gene with parts of its silent copies to result in a new gene product. An example of this type of genetic recombination was the observation of naturally occurring RecB allele variation that was the mechanism operating in the RecBCD pathway required for antigenic variation of pilin found in *Neisseria meningitidis* (Salvatore *et al.*, 2002).

c. ***Epigenetic modification - methylation.*** In this mechanism, structure of DNA sequence, the genotype, is not altered but the phenotype is altered. Methylation alters the binding of transcription factors, resulting in switches ON or OFF of gene expression and phenotypic variation. For example, the *agn43* gene, which encodes the outer membrane protein antigen 43 (Ag43) in *E. coli* contains a GATC sequence, overlapping with the binding site of the regulatory protein OxyR. When the GATC sequence is methylated by DNA-methylating enzyme deoxyadenosine methyltransferase (Dam), it prevents the OxyR protein from binding to the site, leading to the transcription of Ag43 gene (van der Woude & Henderson, 2008).

d. ***Nested DNA inversion.*** This type of phase variation is based on the movement of a promoter region from one copy of a gene to another in the genome. The movement of promoter region is carried out through homologous recombination in the conserved region at the 5' end of the other, usually silent, genes (Dworkin & Blaser, 1997).

e. ***Slipped strand mispairing (SSM).*** In this type of phase variation, a mispairing of short repeat sequences (SRS) is produced between the mother and daughter strand during replication or DNA repair, resulting in the change of number of repeat sequences. The common feature of SRS is that SRSs usually are 1 to 7 nucleotide-long and either homogeneous or heterogeneous repetitive DNA sequences (Wisniewski-Dyé & Vial, 2008). If SSM occurred in the promoter region, the result may be seen in the level of transcription, i.e. the activation or inactivation of gene expression (Torres-Cruz & van der Woude, 2003). If SSM occurred in the region outside of the promoter, the change in the number of short repeat sequences can affect the binding ability of regulatory proteins, such as activators or repressors; consequently, altering the expression of original genes (van der Woude & Bäumler, 2004). If SSM occurred in the coding region between the start and stop codons, known as translational slipped-strand mispairing, results in changes at the translational level, i.e. the quality of synthesized proteins is changed, due to change the length and/or the amino acid sequence resulting in nonfunctional proteins (Saunders *et al.*, 1998).

2.6.3 Roles of IS elements in generation of bacterial adaptive variants facing nutrient-stress conditions

The bacterial cells frequently face various nutrient-stressful conditions, such as the exhaustion of either easily accessible or difficultly accessible carbon sources. In response to nutrient stress, bacteria adopt various adaptation strategies for survival to overcome the facing nutrient-stress environments. One of the frequently observed operating strategies of bacteria for survival in nutrient stress conditions was the excision/insertion activity of various IS elements defined as DNA segments encoding necessary enzymes and other proteins mediating translocations of specific mobile DNA sequences within genomes of many bacteria (Kivisaar, 2010), including *Pseudomonas* (Mahillon & Chandler, 1998). Bacterial cells employ IS elements as the tools for genetic variation, phenotypic switching, promoting adaptation and survival (reviewed in Vandecraen *et al.* (2017)).

Some activities of IS elements relating the phenotypic switch, which was due to the activation or inhibition of gene expression generated by the inversion of a particular DNA sequence or insertion-excision of IS elements into or from specific chromosomal regions, have been recognized (Gally *et al.*, 1993, Higgins *et al.*, 2007, van der Woude & Bäumler, 2004, Wisniewski-Dyé & Vial, 2008).

2.7 Second messengers

2.7.1 Diverse second messengers are found in bacteria

Second messengers are nucleotide-based signals that play a role in relaying intracellular signals and regulating bacterial lifestyle. There are several kinds of second messengers including: (1) cyclic adenosine 3',5'-monophosphate (cAMP), (2) cyclic guanosine 3',5'-monophosphate (cGMP), (3) guanosine tetraphosphate or guanosine pentaphosphate ((p)ppGpp), (4) the recently intensely studied cyclic di-GMP (see Section 2.7.2), and most recently discovered (5) cyclic diadenosine monophosphate (c-di-AMP) present only in bacteria (Gomelsky, 2011, Pesavento & Hengge, 2009).

2.7.1.1 cAMP and cGMP

The cyclic nucleotides, cAMP and cGMP, are a common signaling molecule in both eukaryotes and prokaryotes (Kalia *et al.*, 2013).

In *P. aeruginosa*, there are two known adenylate cyclases involved in cAMP synthesis: the membrane-bound CyaB and the cytoplasmic CyaA adenylate cyclases. This second messenger is hydrolyzed by cAMP-specific phosphodiesterases (PN. Jimenez *et al.*, 2012). In addition, cAMP plays a role in regulating virulence factors, type III secretion systems (T3SS), and TFP in *P. aeruginosa* (Diaz *et al.*, 2011). cAMP binds to the receptor protein Vfr, involved in regulation of genes belonging to the Las QS system (PN. Jimenez *et al.*, 2012), and thus this second messenger is at the top of the quorum sensing hierarchy in *P. aeruginosa* (reviewed in PN. Jimenez *et al.* (2012)).

Like cAMP, cGMP is an other known signaling molecule. cGMP is synthesized by guanylate cyclases and hydrolyzed by cGMP specific PDEs (PN. Jimenez *et al.*, 2012). cGMP was found in *Escherichia coli*, *Bacillus licheniformis* and *Mycobacterium smegmatis* (Gomelsky, 2011, Linder, 2010). Although cGMP has been known the 1970s, study involved in this second messenger is scarce. Up to now, no study involving in cGMP in *P. aeruginosa* was published.

2.7.1.2 (p)ppGpp

Another second messenger, (p)ppGpp also known as the "alarmone" molecule, is an intracellular signal molecule produced in response to harsh environments, e.g., amino-acid starvation (Haseltine & Block, 1973), fatty acid limitation (Battesti & Bouveret, 2006), iron limitation (Vinella *et al.*, 2005), heat shock (Gallant *et al.*, 1977), glucose starvation (Potrykus & Cashel, 2008) and other stress conditions. This phenomenon is called stringent response (SR). There are two enzymes, RelA and SpoT, involved in ppGpp synthesis. pppGpp is synthesized from guanosine triphosphate (GTP) and adenosine triphosphate (ATP) by RelA, then this molecule is converted to ppGpp (Dalebroux & Swanson, 2012). SpoT is a synthase-hydrolase enzyme. Both pppGpp and ppGpp are generated by SpoT, but SpoT can also hydrolyses them to guanosine triphosphate (GTP) and pyrophosphate (PPi) or GDP and PPi (Dalebroux & Swanson, 2012). In *Vibrio cholera*, (p)ppGpp is also synthesized by the activity of SpoT and RelV, a novel (p)ppGpp synthase, under fatty acid and glucose starvation (Das *et al.*, 2009).

The best known role of (p)ppGpp in enhancing bacterial cell resilience in the fast changing environments is its impact on promoter selection by directing the RNA polymerase (RNAP) to certain genes required for survival, leading to the relocation of cellular resources toward amino acid synthesis, arresting growth and division of the stressed cell, etc. (Dalebroux & Swanson, 2012). The regulatory role of the nutrient stress-induced synthesis of (p)ppGpp can be seen in many aspects of microbial cellular processes, including growth, adaptation, secondary metabolism, survival, persistence, cell division, motility, biofilms, development, competence, and virulence (Potrykus & Cashel, 2008). In

E. coli, Boehm and colleagues (2009) found that biofilm induction involves upregulation of the polysaccharide adhesin poly-beta-1,6-N-acetyl-glucosamine (poly-GlcNAc) and two components of the poly-GlcNAc biosynthesis machinery, PgaA and PgaD. PAG-dependent biofilms appear to be regulated by ppGpp and c-di-GMP in opposite directions. In one direction, ppGpp inhibits biofilm formation, in the other one, biofilm formation is activated by c-di-GMP (Boehm *et al.*, 2009). In *Mycobacterium smegmatis*, a Gram positive bacterium, there is only one copy of a gene encoding for a protein with a GGDEF-EAL domain, responsible for c-di-GMP synthesis. This gene is *msdgc-1*, and its promoter induced by starvation or depletion of carbon sources like glucose or glycerol (Bharati *et al.*, 2013). The authors observed that *msdgc-1* utilizes SigA during the initial phase of growth, whereas near the stationary phase it uses SigB containing RNA polymerase for expression. Products of the *msdgc-1* bifunctional (both synthase: diguanylate cyclase [DGC] and hydrolase: phosphodiesterase [PDE]) protein are both c-di-GMP and pGpG. Bharati and colleagues (2013) found that when *msdgc-1* is deleted, the numbers of viable cells are ~ 10 times higher in the stationary phase when compared to that of wild type. The authors proposed that *msdgc-1* is involved in the regulation of cell population density (Bharati *et al.*, 2013). This observation indicates that c-di-GMP and/or pGpG produced by *msdgc-1* were not really necessary for survival of starved *M. smegmatis.* The inactivation of *msdgc-1* certainly contributed to increase the CTP and ATP pool required for (p)ppGpp synthesis at a proper level for signaling stringent response of carbon source-starved bacterial cells.

Recently, Brown and colleagues (2014) reported that nitrogen-starved *E. coli* synthesizes a global transcriptional regulator NtrC that activates the transcription of *relA,* a key gene responsible for the synthesis of ppGpp in the stressed bacterial cell. Results of these authors provided new insights into mechanism(s) relating the question of how a specific nutritional stress leads to the increasing the synthesis of ppGpp in bacteria (Brown *et al.*, 2014). The stringent response to nutritional stress, through the regulating activities of the starvation-induced ppGpp alarmone, should be considered when one is interested in the fate of the starved cells presented in a mix population that comprise both growing and

growth-resting cells, such as 57RP WT cells grown on hexadecane in the present study (see section 4.2)

In *P. aeruginosa*, the stringent response can activate quorum sensing (QS) and modulate cell density-dependent gene expression (van Delden *et al.*, 2001). The activation of the QS specific transcriptional regulators (LasR and RhlR) and their corresponding autoinducers. There are two main N-acyl-homoserine lactone (AHL) autoinducers, 3-oxo-C_{12}- HSL and C_4-HSL in *P. aeruginosa*. Autoinducers synthesis was prematurely induced by overexpression of *relA* during stringent response. The early expression of *lasR* and *rhlR* was also observed in a PAO1 *rpoS* (the stationary-phase sigma factor) mutant overexpressing *relA*. The premature production of AHL, which was observed during the stringent response induced by the amino acid analogue serine hydroxamate (SHX), was absent in a PAO1 *relA* mutant (van Delden *et al.*, 2001). The alarmone (p)ppGpp, produced from the activity of stress-induced RelA and SpoT proteins, signaling the stringent response leading to growth arrest, and the starvation-induced growth arresting cells usually become highly tolerant to antibiotics (Levin & Rozen, 2006). By analysis of starvation-inducing serine analog (SHX) responses to various antibiotics of an induced Δ*relA spoT* mutant and wild-type strain, Nguyen and colleagues (2011) found that the antibiotic tolerance of nutrient-limited and *P. aeruginosa* biofilm is mediated by activation of the SR-mediated tolerance to reduced levels of oxidant stress in bacterial cells (Nguyen *et al.*, 2011).

2.7.1.3 c-di-AMP

c-di-AMP is a signal molecule recently identified in *Listeria monocytogenes* (Woodward *et al.*, 2010). c-di-AMP is synthesized from two ATP molecules by diadenylate cyclase (DAC) and hydrolyzed to 5'-phosphorylated diadenosine nucleotide (pApA) by c-di-AMP phosphodiesterase (aPDE). In most bacteria, only 1-3 DACs are present (Corrigan & Grundling, 2013). In *Bacillus subtilis,* one DAC representative is DisA, which controlled sporulation checkpoint in response to broken

double stranded DNA (Witte *et al.*, 2008) and YybT is a PDE, which hydrolyzed c-di-AMP (Rao *et al.*, 2010). There is no information about c-di-AMP in *P. aeruginosa*.

2.7.2 c-di-GMP

2.7.2.1 c-di-GMP synthesis

Described for the first time in 1986 by the Benziman group, the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) (Figure 2.7) was recognized as an allosteric regulator of cellulose synthase in *Gluconoacetobacter xylinus* (Ross *et al.*, 1986). C-di-GMP is synthesized from 2 molecules of guanosine-5'-triphosphate (GTP) by diguanylate cyclase (DGCs) enzymes containing a conserved GGDEF amino acid domain. This molecule is also specifically hydrolyzed by phosphodiesterase (PDE) proteins containing the conserved EAL (Glu-Ala-Leu) amino acid domain, or HD-GYP (a subset of the larger HD family that possesses hydrolytic activities toward diverse substrates, including c-di-GMP) into 5′-phosphoguanylyl-(3'-5')-guanosine (pGpG) and two GMP molecules, respectively (reviewed in Römling *et al.* (2013)) (Figure 2.8). The activity of enzymes containing GGDEF domains increases the intracellular c-di-GMP level (D'Argenio & Miller, 2004, Hickman *et al.*, 2005, Hoffman *et al.*, 2005, Kulasakara *et al.*, 2006, Malone *et al.*, 2010) while the EAL enzymes are responsible for its breakdown (Hoffman *et al.*, 2005, Kulasakara *et al.*, 2006, Newell *et al.*, 2011). The enzymes responsible for c-di-GMP synthesis and degradation are key elements for the balance of the cellular c-di-GMP concentration.

In *P. aeruginosa*, the genome of strain PAO1 carries 41 open reading frames (ORFs) containing conserved amino acid domains, either of GGDEF, EAL, HD-GYP or hybrid GGDEF+EAL domains, which are putatively involved in the c-di-GMP-specific DGC and/or PDE activities (Hengge, 2009, Kulasakara *et al.*, 2006, Merighi & Lory, 2010). This includes 17 GGDEF (DGC), 16 GGDEF+EAL (hybrid proteins), 5 EAL (PDE), and 3 HD-GYP (PDE) ORFs. The genome of strain PA14 carries an additional ORF coding for EAL-conserved-domain protein (PDE), the EAL domain-containing PvrR

(Drenkard & Ausubel, 2002, Kulasakara *et al.*, 2006, Mikkelsen *et al.*, 2009).

The environmental or cellular alterations may affect the expression of certain DGC and PDE genes leading to an increase or decrease of the local and global c-di-GMP pool. The change of the local c-di-GMP pool may affect the regulation of certain signaling pathways (for example: control of activities of localized structural machineries such as flagella, type IV pili) whereas the change of the global c-di-GMP pool affect the coordination and cross talking between multiple pathways (e.g. signaling pathways involved in the biofilm development) (reviewed in Valentini and Filloux (2016)).

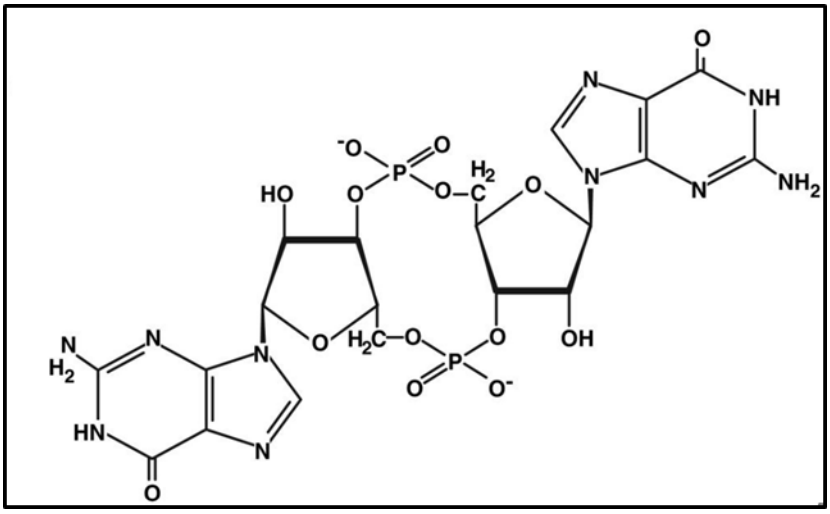


Figure 2.7 **Structure of c-di-GMP (Adapted from Hengge (2009)).**

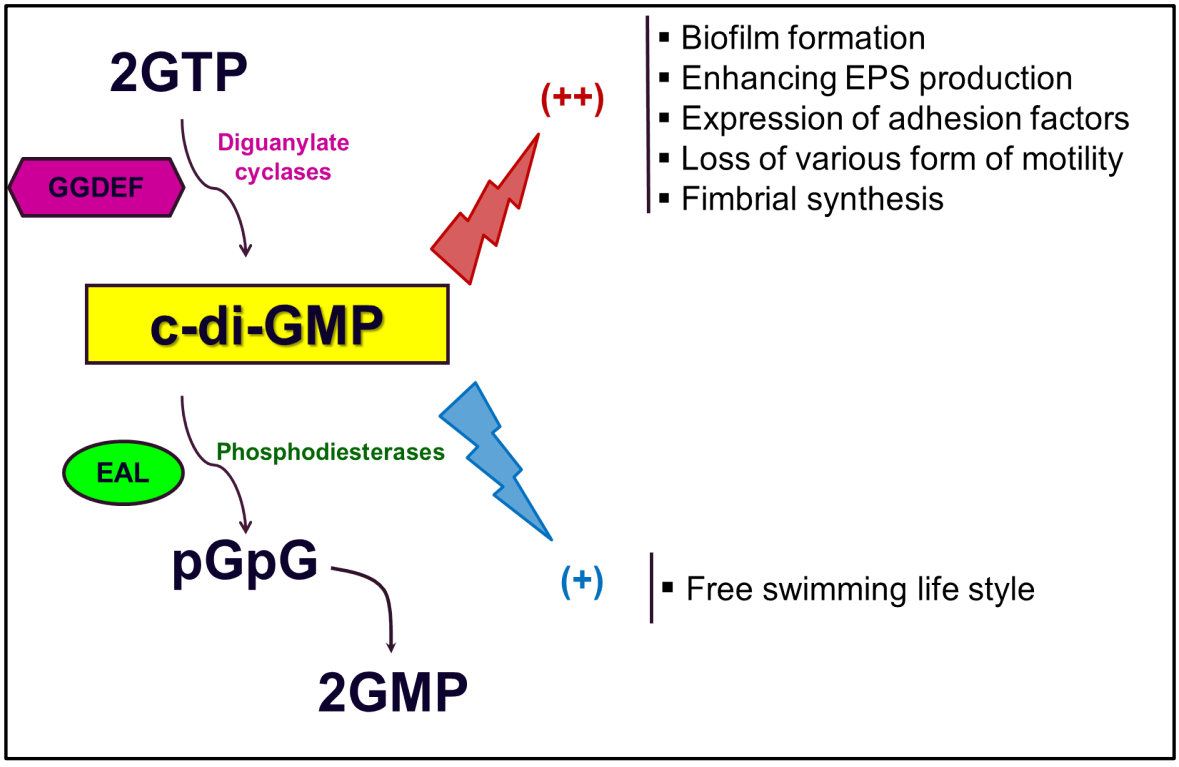


Figure 2.8 **Schematic diagram of c-di-GMP synthesis and its main role (Adapted from Haussler (2004), Hengge (2009), Malone *et al.* (2010)).**

2.7.2.2 Regulatory roles of c-di-GMP

A wide range of bacterial phenotypes is found to be affected by c-di-GMP, including motility-to-sessility transition, regulation of biofilms, regulation of cell cycle and differentiation, virulence, riboswitches, and RNA degradation (reviewed in (Römling *et al.*, 2013)).

In *P. aeruginosa*, several studies about c-di-GMP regulating gene expression have been reported and two models of c-di-GMP regulators are found: (1) regulators/effectors proteins and (2) riboswitches (Breaker, 2012, Sudarsan *et al.*, 2008, Weinberg *et al.*, 2007). In the first model, regulators/effectors include protein-protein interactions such as PilZ (Alm *et al.*, 1996), PelD (VT. Lee *et al.*, 2007), and transcriptional regulators like FleQ (Baraquet *et al.*, 2012, Hickman & Harwood, 2008). PilZ was one of the first c-di-GMP binding protein effectors identified. This protein is necessary for TFP biogenesis (Alm *et al.*, 1996). Furthermore, a number of proteins containing PilZ domains are known (Amikam & Galperin, 2006), such as Alg44 associated to alginate synthesis (Merighi *et al.*, 2007). When c-di-GMP binds to PilZ or proteins containing PilZ domain, these proteins change their structure and activate other genes (Benach *et al.*, 2007). Proteins containing either GGDEF or EAL amino acid domain or both domains interact with the PilZ domain (VT. Lee *et al.*, 2007) and the PilZ domain is considered as a sensor of c-di-GMP intracellular levels in *Pseudomonas* (Martínez-Granero *et al.*, 2014). Next, PelD, coded by the *pel* operon, is known as a c-di-GMP receptor. The *pel* operon, identified in a transposon screen in *P. aeruginosa* PA14, contains seven genes from *pelA* to *pelG*. These genes show sequence similarity with genes encoding sugar-processing enzymes. Biofilm formation will be defected if there is any insertion into or deletion of these genes (Friedman & Kolter, 2004, Sakuragi & Kolter, 2007). The experimental results of Lee and co-workers showed that PelD had ability to bind to c-di-GMP, and that the mutation of *pelD* c-di-GMP binding site resulted in a defect in production of exopolysaccharides (VT. Lee *et al.*, 2007). Thus PelD acts as an inducer of the *pel* operon. Hickman and Harwood showed that the transcriptional regulator FleQ acts as a regulator for Pel polysaccharide production in *P. aeruginosa* PAO1 (Hickman & Harwood, 2008). Upon binding c-di-GMP, FleQ promotes *pel* and *psl* transcription. In contrast, in the absence of c-di-GMP, a

complex, formed by the FleQ and the accessory ATP-binding FleN protein, which binds to two sites up- and down-stream of the *pel* promoter, bending the local DNA chain and so inhibits transcription (Baraquet *et al.*, 2012). In the case of flagellar gene expression, Baraquet and coworkers (2013) found that c-di-GMP competitively inhibits FleQ ATPase activity and interacts with the ATP-binding site on FleQ, causing down-regulation of flagella synthesis. The repression effect of c-d-GMP on flagellar synthesis can be further enhanced by the simultaneous presence of FleN (Baraquet & Harwood, 2013).

A riboswitch is a noncoding RNA fragment sensing small molecules and regulating downstream gene expression (Breaker, 2012, Weinberg *et al.*, 2007). A riboswitch sensing c-di-GMP has been identified upstream of genes coding for DGC and/or PDE proteins (Breaker, 2012). These riboswitches, containing the Genes for the Environment, for Membranes, and for Motility (GEMM) motif (Weinberg *et al.*, 2007), have two parts: (1) an aptamer, which contains an oligonucleic acid portion which allows binding to a specific molecule, and (2) an expression platform which changes structure after the ligand binds to the aptamer. When the c-di-GMP binds to the riboswitch, the ribosomal binding site is either changed or generated by self-splicing during translation (Breaker, 2012). However, until now, there is no riboswitch model demonstrated in *P. aeruginosa*.

2.7.2.3 Role of c-di-GMP in SCV formation

SCVs have been found in many genera of bacteria including many human pathogens (Proctor *et al.*, 2006). The second messenger c-di-GMP appears to be involved in the signaling related to the adaptive changes of bacteria (D'Argenio & Miller, 2004). Over the last decade, c-di-GMP has been extensively characterized for its function in controlling cellular behavior in bacteria. Understanding the role of c-di-GMP, a universal bacterial second messenger (Römling *et al.*, 2013), in the formation of SCVs is of both theoretical interest and practical importance.

In *P. aeruginosa*, formation of harsh environmental conditions promotes development of rough or wrinkled small colony morphotypes or variants (RSCVs) that can

be found as phase variants or mutants. For example, there were SCVs isolated from chronically infected cystic fibrosis (CF) patients (Haussler *et al.*, 2003, Malone *et al.*, 2010), from laboratory aging biofilms (Kirisits *et al.*, 2005), from transposon insertion mutants (D'Argenio *et al.*, 2002, Hickman *et al.*, 2005), or from mutants generated after sub-culturing in the presence of antibiotics (Wei *et al.*, 2011). In the other hand, RSCVs can also be found in the form as environmentally conditions-dependent phenotypic variants after in vitro exposure to antibiotics (Drenkard & Ausubel, 2002) as well as from biofilm cultures (Déziel *et al.*, 2001). In the last two studies, the variant-revertant phenotype switching fashion occurred at a fairly high frequency.

c-di-GMP is the enzymatic product of various GGDEF and/or of some GGDEF-EAL conserved domain-containing proteins (Römling *et al.*, 2013). c-di-GMP signaling is closely linked to or mediates SCV formation. One such protein is WspR (PA3702), a GGDEF domain containing (phosphate receiver) response regulator, which is a member of a family of signal transduction proteins and belongs to the chemosensory pathway in *P. aeruginosa* (D'Argenio *et al.*, 2002, Hickman *et al.*, 2005, Karatan & Watnick, 2009). Briefly, the chemosensory system operon of *P. aeruginosa* consists of genes encoding for WspA, WspB, WspC, WspD, WspE, WspF, and WspR, which are predicted to encode: a external stimuli detecting membrane-bound methyl-accepting chemotaxis protein (WspA), a methyltransferase (WspC), a histidine kinase response regulator (WspE), two supporting proteins WspB and WspD which coupled WspA to WspE, a methylesterase (WspF) and a GGDEF domain-containing response regulator (WspR); the methylation state of WspA is dependent on the opposing activities of WspC and WspF. WspE autophosphorylates and transfers the first external signal detected by WspA to WspR (by transferring phosphate to the phosphate receiver domain of the GGDEF domain-containing response regulator WspR). The phosphorylation causes a conformational change and the phosphorylated WspR displays its constitutive activity of DGC.

Inactivating mutations (by transposon insertional mutations) in WspF lead to the constitutive phosphorylation and activation of WspR, resulting in the production of intracellular signaling c-di-GMP molecules. The c-di-GMP production by phosphorylated

WspR protein was confirmed by *in vitro* activity assays (Hickman *et al.*, 2005). A close link between c-di-GMP production and the occurrence of RSCVs has been established in the *wspF* transposon insertion mutants (D'Argenio *et al.*, 2002, Hickman *et al.*, 2005) and was confirmed in other comparative studies using clinical isolates and laboratory-derived RSCVs (Starkey *et al.*, 2009). Furthermore, over-expression of an EAL domain-containing protein (PA2133) predicted to catalyze degradation of c-di-GMP reversed phenotypes of the *wspF* mutant and inhibited biofilm initiation by the reversed wild-type cells (Hickman *et al.*, 2005). In another study, the over-expression of the PA2133 protein also caused the phenotypic reversion to wild type phenotype of clinical (CF39s) and laboratory-derived (MJK8) RSCVs that normally had significantly higher levels of intracellular c-di-GMP than the corresponding wild-type strains (Starkey *et al.*, 2009). These authors also reported that among 50 RSCVs isolated from PAO1 biofilms, 34 (68%) had point mutations and frame shift mutations in the *wspF* alleles and were complemented by *wspF* (pWspF) supplied in *trans*. The rest were not complemented by *wspF* and appeared to harbor mutations in other loci. MJK8 was complemented by *wspF* but CF39s was not (Starkey *et al.*, 2009). Many genes were expressed differentially in RSCVs as compared to the corresponding wild-type (wt) strains. At the transcriptional level, it was observed that 358 and 472 genes were up-regulated and 202 and 256 genes were down-regulated in the laboratory *wspF* mutant and in clinical CF39 RSCV (CF39s), respectively (Hickman *et al.*, 2005, Starkey *et al.*, 2009). In other studies, a similar tendency of up- and down-regulation of gene expression in different RSCV strains isolated from PAO1 biofilms, including MJK8 RSCV, was also observed (Kirisits *et al.*, 2005). Among the up-regulated genes, there was a number of genes required for surface attachment, and biofilm formation, including *pel* and *psl* genes for exopolysaccharide production, genes controlled by quorum sensing and genes for regulatory proteins, etc. and the down-regulated genes included flagellar genes (Hickman *et al.*, 2005, Kirisits *et al.*, 2005, Starkey *et al.*, 2009).

It was interesting to note that different proteins having either GGDEF domain alone or GGDEF-EAL hybrid domains, both with or without response regulator domains, were co-expressed in several well analyzed RSCV strains that had higher levels of c-di-GMP than corresponding wild-type strains. In the transcript profile of the *wspF* mutant showing

constitutive activation of the WspR protein (PA3702), transcription of another gene (PA0169) coding for a GGDEF domain-containing protein, which increased 2.9-fold higher compared to PAO1 wild type cells (Hickman *et al.*, 2005). Co-activation of genes coding PA3702 and PA0169 proteins was also observed in another experiment carried out on the basis of elevated levels of c-di-GMP by activity of PA3702 protein. The expression of the PA0169 gene was observed in the transcript profile of PAO1-*wspF-pelA-pslBCD*, a triple mutant of PAO1 with elevated levels of c-di-GMP by active WspR (PA3702) and without auto aggregation, was 5.1-fold higher than the double mutant PAO1-*pelA-pslBCD* control strain that produced wild-type levels of c-di-GMP (Starkey *et al.*, 2009). In contrast, the clinical RSCV strain FC39s showed increased expression of genes encoding for 3 putative diguanylate cyclase/phosphodiesterase (GGDEF/EAL domains: PA0285, PA0861, PA2567) and one putative diguanylate cyclase (GGDEF domain: PA4843) all of which are different from the above-mentioned pair of genes PA3702-PA0169. Expression of PA0285 and PA4843 was 2.04 and 3.04 fold lower and PA0861 and PA2567 was 2.37 and 6.29 fold higher than the same genes expressed in FC39wt, respectively (Starkey *et al.*, 2009). Based on results from microarray analysis, Kirisits and colleagues (2005) reported that there was no change in expression of *wspR* in the RSCVs, including MJK8, compared to the wild-type strain, but did not rule out a WspR-mediated mechanism involving post-transcriptionally regulated activity (Kirisits *et al.*, 2005). In *Vibrio cholerae,* Massie and colleagues (2012) found no correlation between total intracellular c-di-GMP levels and biofilm formation or gene expression when considering all states (total intracellular c-di-GMP levels and biofilm formation or gene expression). However, individual diguanylate cyclase (DGC) showed a significant correlation between c-di-GMP production and c-di-GMP-mediated responses, and the rate of phenotypic change versus c-di-GMP concentration was significantly different between DGCs (Massie *et al.*, 2012). This observation may be applicable for certain DGCs in relation to SCV formation in *P. aeruginosa.*

Several other GGDEF domain-containing proteins were also found to be involved in SCV formation. Malone and colleagues (2010) reported that the *yfiBNR* operon is an SCV-related locus in *P. aeruginosa*. The *yfiBNR* operon encodes a tripartite signaling

module that regulates c-di-GMP levels in *P. aeruginosa*. YfiB is a PAL-like outer-membrane bound lipoprotein (PAL: peptidoglycan-associated lipoprotein), YfiR is a small periplasmic protein, and YfiN is an inner membrane-integral diguanilate cyclase (PA1120 – a GGDEF domain-containing protein) whose activity is tightly controlled by YfiR. Exopolysaccharide synthesis was identified as the downstream target for YfiBNR and YfiBNR with active YfiN protein mediating c-di-GMP dependent SCV formation in *P. aeruginosa*. Over-expression of the phosphodiesterase PA5295 (a GGDEF-EAL domain-containing protein) abolishes surface attachment of Δ*yfiR* mutant, without which the Δ*yfiR* transposon insertion mutant was auto-aggregative and hyper-adherent SCV (Malone *et al.*, 2010). YfiBNR functions via tightly controlled competition between allosteric binding sites on the three Yfi proteins and activating mutations were accumulated in all three components of the Yfi regulatory system during SCV formation *in vivo* (Malone *et al.*, 2012). Giardina and colleagues (2013) proposed a scheme of allosteric regulation of YfiN, in which, without cell wall stress and periplasmic redox stress, YfiR binds to the PAS domain of YfiN and inactivates its DGC activity. Under cell wall stress, YfiR binds to YfiB and under periplasmic redox stress YfiR detaches from the PAS domain of YfiN, causing HAMP (Histidine kinases, Adenylyl cyclases, Methyl binding proteins, Phosphatases) domain torsion and unlocking the GGDEF domain of YfiN. In both of the cases, the stress-induced YfiR-detached YfiN resumes its DGC activity resulting in the production of c-di-GMP (Giardina *et al.*, 2013). The YfiN protein (other name: TpbB) (PA1120) was found to be coexpressed with another membrane-bound c-di-GMP-synthesizing protein MorA (PA4601) in a clinical *P. aeruginosa* SCV 20265 isolated from a CF patient (Meissner *et al.*, 2007). Another case of GGDEF domain-containing proteins involving SCV formation was MucR (PA1727). This is a DGC-active GGDEF-EAL domain-containing protein of *P. aeruginosa*, over-expression of which also results in the formation of RSCVs with common characters of increased pellicle formation, auto-aggregation and formation of highly structured biofilms (Hay *et al.*, 2009).

In the case of SCVs occurring from phenotypic (phase) variation, there is not much information of involvement of DGC(s) in the SCV formation. However, Drenkard and Ausubel (2002) indirectly showed a close link between c-di-GMP production and RSCV

formation by introduction of a phenotype variant regulator PvrR into the RSCVs that appeared from PA14 cells after being exposed to kanamycin or other antibiotics. PvrR is an EAL domain-containing response regulator and homolog of which is PA3947 protein. Over-expression of *pvrR* resulted in the hydrolysis of intracellular c-di-GMP and total RSCV reversion to wild-type cells after being plated for 12h on LB agar (Drenkard & Ausubel, 2002). Moreover, the authors also observed that over-expression of *pvrR* in wild-type PA14 was reduced 6-fold in the frequency of resistant variants obtained after overnight plating on kanamycin plates compared to wild type. Beside the environmental effects, such as media, NaCl, and temperature on SCV formation, PvrR displayed a putative role in the regulation of phenotypic switching, primarily by inducing reversion from variants to wild-type phenotypes (Drenkard & Ausubel, 2002). c-di-GMP is hypothesized to play an important role in phenotypic switching in other *P. aeruginosa* strains in which SCVs appeared from phase variation at a high frequency in biofilms cultured without antibiotic stress. Furthermore, one such highly phase-variable strain is 57RP isolated from hydrocarbon-contaminated soil (Déziel *et al.*, 2001).

The concentration threshold of intracellular c-di-GMP is established by the expression of active c-di-GMP-metabolizing proteins. Beside the case of membrane-bound proteins, in several cases, cytoplasmic c-di-GMP-synthesizing proteins in their active form are not distributed evenly; instead, they are clustered in the cytoplasm. For example, in the GGDEF domain-containing response regulator WspR (PA3702), Güvener and Harwood (2007) found that the subcellular distribution of fluorescent protein-tagged WspR-YFP was dispersed when unphosphorylated (the inactive form) and formed bright cytoplasmic clusters when phosphorylated (the active form) (Guvener & Harwood, 2007). Due to the clustering of active WspR, the subcellular concentration of c-di-GMP depends on the diffusion to the surrounding area of c-di-GMP from individual synthesizing clusters localized in the cytoplasm. This reflects the spatiotemporal aspect of c-di-GMP signaling in the bacterial cell. A dynamic localization to the cell pole and local c-di-GMP synthesizing action after being phosphorylated by PleD, a two-component signaling protein with DGC activity required for polar differentiation, was also observed in *Caulobacter crescentus* (Paul *et al.*, 2004). Beside the clustering or dynamic localization of

phosphorylated GGDEF domain-containing response regulators, subcellular asymmetrical distribution of c-di-GMP can also be generated by the expression of some other EAL domain-containing proteins. For example, in both RSCV strain FC39s and wild type strain FC39wt isolated from the same cystic fibrosis (CF) sputum, Starkey and colleagues (Starkey *et al.*, 2009) observed the expression of one GGDEF domain-containing response regulator (PA4843), which requires phosphorylation for activation, and three other EAL domain-containing proteins (PA0285, PA0861, PA2567), among which PA0285, PA0861 are membrane-bound proteins. However, only FC39s displayed an elevated c-di-GMP level (Starkey *et al.*, 2009). Further, Christen and colleagues (2010) also observed a phenomenon of cell cycle-dependent fluctuation of c-di-GMP in *C. crescentus* and *P. aeruginosa*. c-di-GMP was asymmetrically distributed upon bacterial cell division, which may indicate an important regulatory step in extracellular organelle biosynthesis or function (Christen *et al.*, 2010). When EAL domain-containing proteins are overexpressed their corresponding PDEs will exert hydrolytic activity resulting in the reduction of the total intracellular quantity of c-di-GMP. In that case the concentration of c-di-GMP surrounding the specific downstream receptors dropped below a minimal threshold of the c-di-GMP signaling pathway together with the c-di-GMP-dependent regulatory system will be impaired. Pultz and coworkers (2012) showed that the response threshold of *Salmonella* and *Pseudomonas* PilZ domain proteins is determined by their binding affinities for c-di-GMP. The binding affinities for c-di-GMP of PilZ domains proteins of *S. enterica* serovar Typhimurium, YcgR, which controls flagella-based motility, and BcsA, the bacterial cellulose synthase, demonstrate 43-fold difference whereas *P. aeruginosa*, PA2960, PA3353, PA3542 [Alg44], and PA4324 proteins, span a 145-fold range. The lower-affinity proteins require a higher level of c-di-GMP for their activities than the higher-affinity proteins (Pultz *et al.*, 2012). Reversion to wild type of c-di-GMP-dependent RSCVs was observed when PDEs, such as PvrR, PA2133 or PA5295, were overexpressed in *P. aeruginosa* (Drenkard & Ausubel, 2002, Hickman *et al.*, 2005, Malone *et al.*, 2010, Starkey *et al.*, 2009).

Differential expression of GGDEF domain-containing proteins such as PA0169, PA1120, PA3702 and PA4843, or GGDEF-EAL domain-containing proteins PA0285,

PA0861 and PA2567 with or without response regulator domains has been identified in different PAO1 mutants forming RSCVs as mentioned above. All accumulated evidence points toward a multifunctional origin of the formation of SCVs in relation to c-di-GMP, and suggests a mechanism used by *P. aeruginosa* to adapt rapidly to new environments for its survival. Which of the c-di-GMP-metabolizing proteins, especially those with DGC activities, predominantly mediate RSCV formation based on more-or-less environmentally dependent phase variation is still an open question.

Phenotypic variation leads to the formation of phenotypic variants, including SCVs. In PA14, Drenkard and Ausubel (2002) observed that over-production of *pvrR* resulted in the hydrolysis of intracellular c-di-GMP and SCV reversion to the wild-type phenotype. These authors suggested a putative role for c-di-GMP in phenotypic switching, as high c-di-GMP levels are associated with the SCV formation and low levels of c-di-GMP with the reversion of SCVs to the wild-type phenotype (Drenkard & Ausubel, 2002). The role of c-di-GMP in the phenotypic switching of *P. aeruginosa* cells grown on hexadecane will be investigated and discussed further in the present study.

2.8 Involvement of c-di-GMP and QS in biofilm formation

Bacteria sense and respond to environmental cues by coordinating multiple signaling systems for regulation of adaptive, developmental processes. The regulatory connections of c-di-GMP signaling systems to QS systems have been demonstrated in various microorganisms, including *Vibrio cholerae*, *V. parahaemolyticus* and *Xanthomonas campestris*, (reviewed in Römling *et al.* (2013), Srivastava and Waters (2012)). In the above-mentioned examples, the interactions between QS and c-di-GMP signaling are mainly unidirectional, i.e. synthesis and/or hydrolysis of the second messenger are regulated by autoinducer concentration (Römling *et al.*, 2013), or c-di-GMP is epistatic to QS inputs (Srivastava & Waters, 2012). The general interactions are as follows. At low cell density, low levels of autoinducer(s) stimulate c-di-GMP synthesis and/or inhibit its hydrolysis and lead to biofilm formation. At high cell density, high levels

of autoinducer(s) inhibit c-di-GMP synthesis and/or stimulate its hydrolysis and lead to biofilm dispersal.

In *P. aeruginosa*, transcripts of various QS controlled genes, such as the transcriptional regulators LasR and RhlR, regulatory protein RsaL, autoinducer synthesis protein RhlI and LasI. were increased in the transcriptional profile of c-di-GMP-dependent RSCV mutants compared to their corresponding wild type cells (Hickman *et al.*, 2005, Kirisits *et al.*, 2005, Starkey *et al.*, 2009). The regulatory connections between QS and c-di-GMP signaling have also been observed in this bacterial species. Ueda and Wood (2009) demonstrated a connection between QS, c-di-GMP, Pel polysaccharide, and biofilm formation in *P. aeruginosa* through the tyrosine phosphatase TpbA. The authors showed that Las-mediated QS positively regulates expression of the *tpbA* (PA3885) gene. The TpbA protein responds to AHL signals and likely dephosphorylates PA1120, a GGDEF domain-containing protein (TpbB), which leads to reduce c-di-GMP concentrations. The reduction of c-di-GMP levels inhibits matrix exopolysaccharide formation, which leads to reduction of biofilm formation (Ueda & Wood, 2009). The TpbB or PA1120 protein is also designed as YfiN, the membrane-bound diguanylate cyclase of YfiBNR tripartite system, which has been identified as a key regulator of the SCV phenotype of *P. aeruginosa* cells (Malone *et al.*, 2012, Malone *et al.*, 2010).

The regulating interactions between QS and c-di-GMP signaling systems are illustrated in figure 2.9.

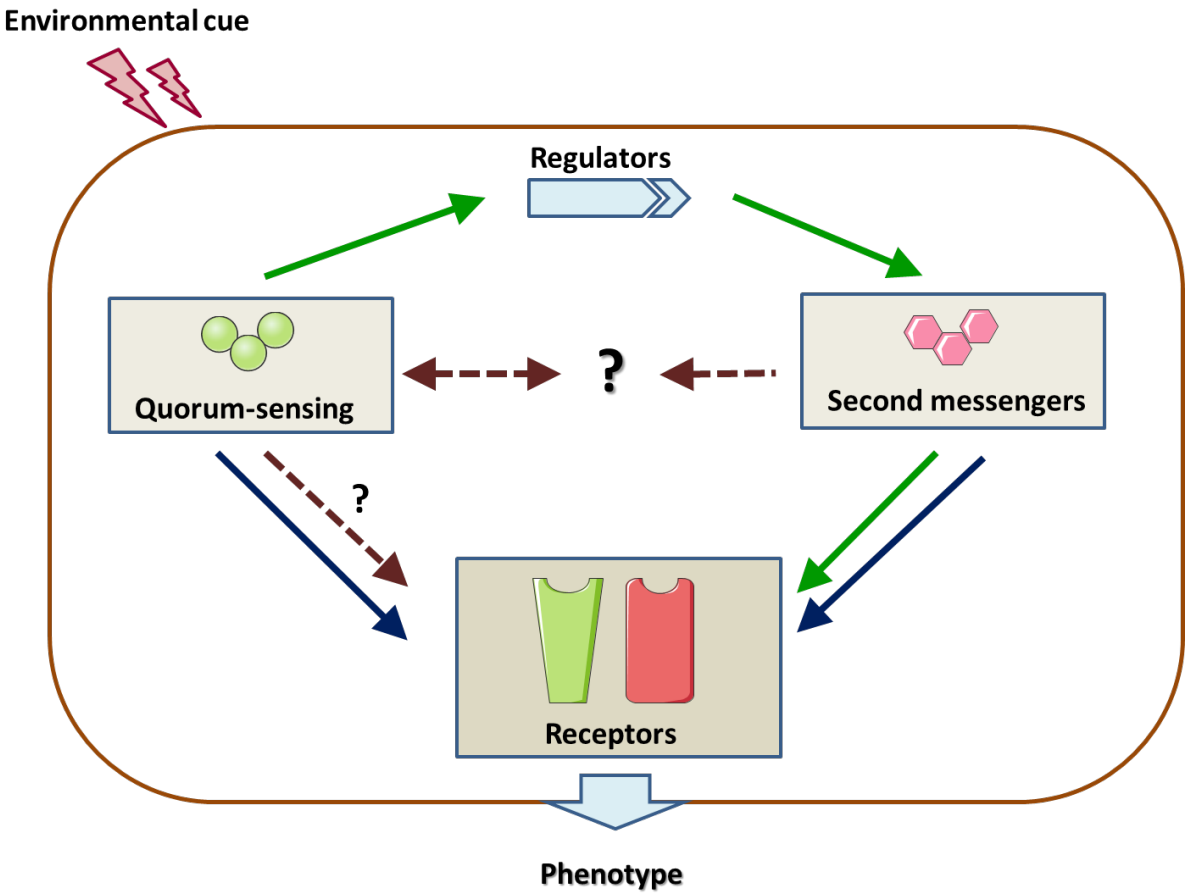


Figure 2.9 **Summary of signaling pathways for *P. aeruginosa* in adapting to environmental cues to produce specific phenotypes (Adapted from Kalia *et al.* (2013), Ueda and Wood (2009)). Green arrows indicate the evidenced relation between QS, regulators, second messengers and receptors. Navy arrows show the effect on bacterial phenotypes of both QS and second messengers. Brown arrows indicate unknown relations.**

2.9 Physiological adaptation of oil-degrading bacteria growing on hexadecane

Hexadecane (C_{16}), an n-alkane, is generally recognized as a hydrophobic, poorly bioavailable substrate among various petroleum hydrocarbons for different bacteria to use as a whole carbon source and energy. To be able to use such a difficultly accessible nutrient, bacteria should be able to undergo certain levels of modulation of gene expression to realize necessary physiological adaptations for survival. Several key factors involving the chemotaxis to alkanes, transport of alkanes across the cell membrane, terminal oxidation and degradation of alkanes were identified (reviewed in Wang and Shao (2013)). Examples for such necessarily adaptive responses include the modulation of expression of a large number of genes of two representative marine oil-degrading bacterial species growing on hexadecane, the *Alcanivorax borkumensis* (Sabirova *et al.*, 2011, 2006) and *Marinobacter hydrocarboclasticus* (Vaysse *et al.*, 2009). The adaptive modulation of gene expression, observed in biofilm cells of these bacteria growing on hexadecane, covers substantial changes in the protein component of three main cell compartments, such as the outer membrane (specific transporters of hydrophobic compounds, type VI secretion system), periplasm (presence of three different alkane-oxidizing systems) and cytoplasm (proteins/enzymes involving the redirection of carbon flux) as well as the specific expression of various stress-related genes compared to planktonic cells of the same species growing on other carbon sources such as pyruvate or acetate. At the present time, knowledge of physiological adaptation involving modulation of gene expression of *P. aeruginosa* strains growing on hexadecane is still very much limited.

2.10 Background of this work

Several *P. aeruginosa* strains have been isolated in our laboratories for their capacity to degrade hydrocarbons concomitant with the ability to produce biosurfactants (Déziel, 1996). Later on, two types of strains were noticed when they were grown on hexadecane (a C_{16} alkane): fast grower strains (R strain) and slow grower strains (L strain). In comparison to the L strains, such as 57RP, the R strains, such as 57RV, have a more

hydrophobic cell surface, form aggregates when cultured in liquid media, and generate a more abundant biofilm (Déziel, 2001). The L strain 57RP was able to form rough SCVs on dextrose when cultured as a biofilm or in static liquid cultures. The SCVs developed on dextrose have already been characterized for several phenotypic straits, including the cell surface morphology, cell hydrophobicity, motilities, adherence to surface, biofilm formation, production of biosurfactants as rhamnolipids (Déziel, 2001; Déziel *et al*., 2001). A period of latency was observed with the L strain 57RP before growth appeared when cultured with hexadecane, a hydrophobic, poorly bioavailable substrate used as whole carbon source and energy; this latency was not observed with the R strain 57RV (Déziel, 2001). The L strains generated SCVs before being capable of growing on hexadecane, which was proposed to explain the latency time. However, phenotypic behaviours of the SCVs formed on hexadecane were not well characterized yet as it was done with the SCVs formed on dextrose. The SCV formation was not observed in the cultures of strain 57RV. These observations were still to be confirmed.

We learned from the literature that there were several rough SCVs having been isolated from different clinical isolates of *P. aeruginosa* such as PAO1 and PA14. These RSCVs shared several phenotypic straits with those of the 57RP SCVs developed dextrose and were dependent on c-di-GMP. c-di-GMP is recognized as an intracellular second messenger used in signal transduction in a wide variety of bacteria. It is not known yet whether the 57RP SCVs are on so c-di-GMP-dependent variants? In addition, marine oil-degrading bacterial species such as *Alcanivorax borkumensis* (Sabirova *et al.,* 2011, 2006) and *Marinobacter hydrocarbonoclasticus* (Vaysse *et al.,* 2009), with which hexadecane induced a large change in cell physiology involving modulation of expression of a large number of genes. Proteomic analysis of biofilm cells developed on hexadecane, compared to planktonic cells grown on other carbon sources of the same species, the large change in protein component covered from the outer membrane to periplasm and cytoplasm. It is also not yet known that whether 57RP SCVs also shared some similar aspects of physiological adaptation when cultured on hexadecane?

In addition, a role of rhamnolipids in the degradation of weakly soluble hydrocarbons was investigated. One of the L strain, *P. aeruginosa* 57RP, was mutated in

the *rhlA* gene by insertional mutagenesis. This mutant, named 57RP-98, does not produce rhamnolipids. 57RP-98 has a slower growth rate on hexadecane than 57RP, suggesting the involvement of rhamnolipids in the growth on liquid alkanes. Importantly, 57RP is an L strain, and the rhamnolipid-negative mutant still generates SCVs when grown on hexadecane (Déziel, 2001). Taken together, this information supports a role for biofilm formation for growth of *P. aeruginosa* on hexadecane, however the role of rhamnolipids in this process remains unclear.

The background and questions mentioned above formed the basis to establish the four objectives as presented in section 1.3 of the present thesis.

3 MATERIALS AND METHODS

3.1 Bacterial strains and growth conditions

Bacterial strains. The *P. aeruginosa* strains used in the present study are listed in table 3.1. The 57RP strain used in this study was the main research strain. The 57RV and PA14 strains were used as comparative strains in certain experiments.

Table 3.1 List of *P. aeruginosa* strains used in this study.

Strain	Characterization	Type of growth on hydrocarbon	Reference
P. aeruginosa 57RP	Isolated from contaminated site, slow growth on hexadecane	L	Déziel (1996)
P. aeruginosa 57RV	Isolated from contaminated site, fast growth on hexadecane	R	Déziel (1996)
P. aeruginosa PA14	Isolated from a burn patient	Unknown	DG. Lee *et al.* (2006), Rahme *et al.* (1995)

L: Slow grower strain, R: Fast grower strain.

Culture condition. Three investigated *P. aeruginosa* strains were pre-cultured at 30^{o}C overnight on Tryptic Soy Agar (TSA) (BD Biosciences, Becton, Dickinson and Company, Sparks, MD, USA) plates from frozen stocks. One colony of each strain was then cultured in Tryptic Soy Broth (TSB) (BD Biosciences) shaken at 200 rpm for 16 hrs at the same temperature. The biomass of this culture was washed twice with mineral salt medium (MSM) sterilized at 121^{o}C for 20 min (MSM containing KH_2PO_4 0.7 g L^{-1}, Na_2HPO_4 0.9 g L^{-1}, $NaNO_3$ 2.0 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.4 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 0.1 g L^{-1} and $FeSO_4 \cdot 7H_2O$ 0.001 mg L^{-1}) at pH 6.7 (Siegmund & Wagner, 1991) before cultivation with n-alkane. The bacterial cell suspension was used to inoculate test tubes (16 x 150 mm, Pyrex) at an initial OD_{600} of 0.1 in 3-6 ml of MSM. A 1% (v/v) of hexadecane ($C_{16}H_{34}$) or octadecane ($C_{18}H_{38}$) (Sigma-Aldrich Canada Co., Oakville ON CANADA), used as sole

carbon source, was sterilized (121^{o}C for 20 min) separately and added into each test tube containing MSM before cultivation. The culture was carried out in triplicate and the negative control cultures without *P. aeruginosa* cells were included at the same time.

All experiments were performed at 25^{o}C in a horizontal gyratory shaking incubator at 200 rpm (Fisher Model G25, New Brunswick Scientific Edison, NJ USA) or in static conditions at room temperature.

3.2 Determination of bacterial growth by protein quantification

Bacterial cells from the whole content of culture tubes, including both cells that remained in the culture solution and those attached to tube wall, were harvested. Firstly, all the cell biomass attached to the tube wall was gathered into the aqueous culture solution using a sterile loop stick. Then, two hundred µl of EAC solution [EAC = Ethanol/Acetone/Chloroform (100:100:8) (IY Jimenez & Bartha, 1996)] was added into the aqueous medium phase and vortexed quickly for 20 sec. The whole cell content of the culture tubes was transferred into 1.5 ml microcentrifuge tubes. The cells were recovered by centrifugation at 7,000 x *g* for 10 min. The supernatant was removed and stored at -20^{o}C for further experimental purposes. The cell pellets were dispersed in 0.1N NaOH, vortexed and then heated at 70^{o}C for 60 min. These crude protein solutions were then serially diluted by sterilized water before being assayed with the Bradford reagent (Bio-Rad Kit). Each dilution was measured in triplicate wells. In each well, one hundred and sixty µl of each dilution was mixed with 40 µl of reagent in a well of a 96-well dish (Sarstedt Inc). The measurement at A_{595} was taken after 10-15 min of incubation by KC4 (BIO-TEK) or Infinite M1000 PRO (Tecan) spectrophotometers.

Bovine serum albumin (BSA) was used as standard protein for quantification. Five dilutions (0-50 µg/ml) of the BSA standard protein were prepared to build a standard curve and a linear regression. The assays were also performed in triplicate. The R^2 value was close to 1 (~ 0.98-0.99) and the equation was used to calculate unknown protein concentrations.

3.3 Motility assays

Motility assays, detailed below, were performed to test the ability of bacterial movement on semi-solid or solid surface. All motility assays were performed in triplicate plates to compare the motile activities of the SCVs and wild type strains.

3.3.1 Swimming

Five µl of bacterial cell suspension cultured in TSB overnight were inoculated on the center of the surface of the 0.3% agar in a Petri dishes containing 1% tryptone and 0.5% NaCl medium. Incubation was performed at 30^{o}C in plastic bags. The turbid zone formed by bacterial motile cells was measured after 16h. Swimming motility was assessed by measurement of the circular turbid zone formed by the bacterial cells migrating away from the inoculation point (Déziel *et al.*, 2001, Murray & Kazmierczak, 2008).

3.3.2 Swarming

The swarming plates were composed of M9 modified medium (containing 20 mM NH_4Cl, 12 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 1 mM $MgSO_4$, 1 mM $CaCl_2{\cdot}2H_2O$, 11 mM dextrose, 0.5% Casamino acids [Difco, BD Biosciences] at pH 7.0) with 0.5% Bacto-agar (BD Biosciences) (Tremblay & Deziel, 2008, Tremblay *et al.*, 2007). The agar plates were freshly prepared and dried for 1 h in a laminar hood before use (Tremblay & Deziel, 2008). The inoculation was performed by spotting five µl of bacterial suspension in the center of the plate (as described in section 3.3.1), and the plates were incubated at 30^{o}C for 24-38h.

3.3.3 Twitching

Five µl of the bacterial cell suspension were stabbed through a 3 mm thin layer of 1% agar to the bottom of the Petri dish. The spreading growth of bacteria between agar and polystyrene surface was observed after 24h of incubation at 30°C. The adherence of bacteria to the polystyrene surface was determined after removing the agar layer and staining the attached cells with 1% (w/v) of crystal violet in water (Déziel *et al.*, 2001, Haussler *et al.*, 2003).

3.4 Hydrophobicity assays

3.4.1 Microbial adhesion to hydrocarbon (MATH) test

The evaluation of microbial cell surface hydrophobicity was performed to compare the cell-surface hydrophobicity of the SCVs and the wild type cells by using microbial adhesive abilities to the hydrocarbon (MATH test). The original method was developed by Rosenberg *et al.* (1980) and improved by Déziel (2001). Fifteen to twenty ml of the bacterial cell suspension were harvested by centrifugation from overnight cultures in TBS medium at 30°C. After washing twice in phosphate-buffered saline (PBS) 25 mM, pH 7.4, the cells were dispersed in PBS and measured at OD_{600} (OD_b). A mixture of different volumes (0-700 µl) of hexadecane (Sigma Aldrich) and 1.5 ml of the bacterial cell suspensions were vortexed for 30-45 sec. OD_{600} (OD_a) of the aqueous phase was measured after equilibrating and separating the mixture into two phases in 30-45 min at room temperature (RT) (Déziel *et al.*, 2001, Haussler *et al.*, 2003). The percentage of adherence (A%) was calculated by formula (3.1) as described by Déziel *et al.* (2001).

$$A\% = \left(1 - \left(\frac{ODa}{ODb}\right)\right) x\ 100 \qquad (3.1)$$

3.4.2 Microbial adhesion to silica (MATS) test

The procedure was performed according to established MATH test procedures but fine granular silica sand (0-900 mg) (Fisher) was used instead of hexadecane, as described in Déziel (2001).

3.5 Biofilm quantification

A modified method of O'Toole and Kolter (1998) and Chavant *et al.* (2007) was used to determine the bacterial biofilm formation capacity of the SCVs and revertants in comparison with that of the wild type strain. Bacteria were cultured in polystyrene tubes (12 x 75 mm, Becton Dickinson Labware, Lincoln Park, NJ USA) in Bushnell-Haas medium (BD Biosciences) [Mg_2SO_4 0.2 g L^{-1}, $CaCl_2$ 0.02 g L^{-1}, KH_2PO_4 1 g L^{-1}, $(NH_4)_2HPO_4$ 1 g L^{-1}, KNO_3 1 g L^{-1} and $FeCl_3$ 0.05 g L^{-1} supplemented with 0.2% dextrose and 0.5% tryptone]. Every 2 hrs of culture, the whole cell contents were removed from a set of triplicate tubes, which were then washed 3-4 times under tap water. The attached cells to the tube wall were quantified by measurement at OD_{600} after staining the bacterial cells with crystal violet (1%) for 30-45 min and destaining in 2 ml of 95% ethanol. Two hundred µl of the destaining solution were put into 96 well microplate and measured by microplate reader Bio-Rad 680 at 595 nm or KC4 (BIO-TEK) or Infinite M1000 PRO (Tecan) spectrophotometers at 600 nm. The assays were repeated in triplicate measurements.

3.6 Transmission electron microscopy analysis

The transmission electron microscopy (TEM) technique was used to observe pili surrounding the bacterial cells. To perform this experiment, *P. aeruginosa* bacteria were cultured overnight on 1.5% agar plates. Ten µl of 3 M glucose solution was dropped directly onto one discrete colony and cells of this colony were dispersed for 1-2 min. A formvar-coated copper grid was deposited on the top of this cell suspension to allow the

cells to gently attach to the grid. The grid was rinsed in demineralized water for 1 min, and then these attached cells were stained with 1% aqueous phosphotungstic acid solution for 1 min. Samples were observed under a Hitachi H-7100 transmission electron microscope at the INRS-Institut Armand-Frappier.

3.7 Rhamnolipid analysis

To evaluate rhamnolipid production, *P. aeruginosa* cells were cultured in 3 ml of MSM medium supplemented with 1% hexadecane as sole carbon source in test tubes (16 x 150 mm, Pyrex) at 25°C on horizontal shaking incubators. After the initial 3-5 days and then each second day, a set of triplicate tubes was collected. The experimental period was performed for 15-19 days. The cell suspensions were centrifuged, the cell biomass in the pellets was measured by the total protein quantification method, and the supernatants were used for rhamnolipid quantification.

To analyze the rhamnolipid production levels, the collected supernatants were centrifuged 2 times at 16,000 x *g* for 30 min at 4°C to eliminate the residual cell debris and alkenes. These supernatants were then filtered through 0.2 µm PTFE filters (Chromatographic Specialties INC) and used directly for the HPLC analysis. All of the rhamnolipids produced were analyzed by the Quattro II (Waters, Mississauga, Ontario, Canada) triple quadruple mass spectrometer in negative electrospray ionization mode coupled to an HP 1100 (Agilent Technologies) high-performance liquid chromatograph (HPLC) equipped with a 10 cm (100 x 4.6 mm) C8 reverse-phase column (Kinetex). The mass range was scanned from the range of 130 to 800 Da. A 5.6 ppm of deuterium-labeled 4-hydroxy-2-heptylquinoline (HHQ-D4) was used as an internal standard (Lépine *et al.*, 2002). Twenty µl of the sample were injected to the HPLC. Total time for analysis was set up at 18 min with flow rates at 400 µl min^{-1}. Two mM of ammonium acetate buffer was used to make the decreasing gradients from 60% to 10% at the first min to the tenth min and the following 6 min for the washing column in the inlet method. Rhamnolipids were detected at m/z 503 (mono-rhamnolipid Rha-C_{10}-C_{10}) and 649 (di-rhamnolipid Rha-Rha-

C_{10}-C_{10}). Concentrations of rhamnolipids were calculated based on the integration of HHQ-D4 and the proper fragment ions and signal response of the standard rhamnolipids by using calibration curves.

3.8 Determination of c-di-GMP concentration

The investigated *P. aeruginosa* cells were cultured in the same conditions as described in the section 3.7. After the first 3-5 days and afterwards every second day, a set of triplicate tubes was collected and divided in two: one part was used for the total protein quantification and the other for the c-di-GMP extraction.

The intracellular c-di-GMP contents were extracted as described by Spangler (Spangler *et al.*, 2010) with some modifications. Cell samples were centrifuged at 16,000 x *g* for 30 min at 4^{o}C. The cell pellets were dispersed in 300 µl of the extraction solvent (ExSol: Acetonitrile/methanol/water [40:40:20 v/v/v]). After keeping on ice for 15 min, the extracts were heated at 95^{o}C for 10 min and then were centrifuged at 16,000 x *g* for 15 min. This step was repeated two times. The combination of these two extracts was evaporated under nitrogen gas at 40^{o}C, then dissolved in demineralized H_2O and kept at -20^{o}C until analysis.

To analyze the produced c-di-GMP, xanthosine 3′,5′-cyclic monophosphate (cXMP) (Sigma Aldrich) was used as an internal standard. Liquid chromatography with a 10 cm (50 x 1.00 mm) mini C18 reverse-phase column (Gemini) was used along with the Quattro II triple quadruple mass spectrometer for analysis in the positive electrospray ionization mode, which was coupled to an HP1100 HPLC by injecting 20 µl of sample. The Inlet method was performed for 34 min with the first 14 min maintenance at 100% of the solvent A (10 mM ammonium acetate buffer and 0.1% acetic acid), followed by using solvent B (10 mM ammonium acetate buffer and 0.1% acetic acid in acetonitrile) for column washing for 6 min at a flow rate of 100 µl min^{-1}. Quantifications were calculated by the integration of cXMP and the proper fragment ions, based on the signal responses of

the c-di-GMP standard (BioLog, Life Science Institute, Bremen, Germany) by using the calibration curves. The c-di-GMP was detected at 691 m/z.

3.9 Integration of inducible genes in strain 57RP

3.9.1 Chromosomal integration of the two inducible *pvrR* and PA14-72420 into the *P. aeruginosa* 57RP genome by conjugation

E. coli S17-1 strains containing plasmids pGGN05 and pGGN06 (Nicastro *et al.*, 2014) (see Table 3.2), respectively, provided by Dr. Gianlucca Nicastro (Instituto de Química, Universidade de São Paulo, São Paulo, Brazil) were used for the integrant creation (Figure 3.1). Briefly, studying gene (*pvrR* or PA14-72420) was cloned into mini-CTX2 vector between *Kpn*I-*EcoR*I sites inside the two Ω transcriptional terminators and then to be integrated at the *attB* specific site downstream of the tRNAser sequence of *P. aeruginosa* chromosome (Hoang *et al.*, 2000). These two strains were cultured on LB agar medium, supplemented with 12 μg ml^{-1} tetracycline (LB + Tet12) at 37^{o}C, until reaching the necessary biomass production levels (18-20 hrs). These *E. coli* cultured cells were collected from the agar plates and mixed well with cells of strain 57RP wild type on the same LB+Tet12 agar plate medium. After overnight incubation at 37^{o}C, the mixed cells were transferred onto LB agar plates supplemented with 125 μg ml^{-1} tetracycline and Triclosan 20 μg ml^{-1}. The growing colonies were collected and cultured on the TSB medium. To confirm that the *pvrR* and PA14-72420 genes were already integrated into the chromosome of the 57RP WT cells, several putatively integrated colonies were propagated and their biomass was collected for DNA extraction (section 3.9.2). This was followed by PCR amplification (section 3.9.3) using the *pvrR* and PA14-72420 specific primers (Table 3.2). Only the positive tested colonies, which contained the introduced c-di-GMP-metabolizing genes integrated in their chromosomes, were selected for further investigations.

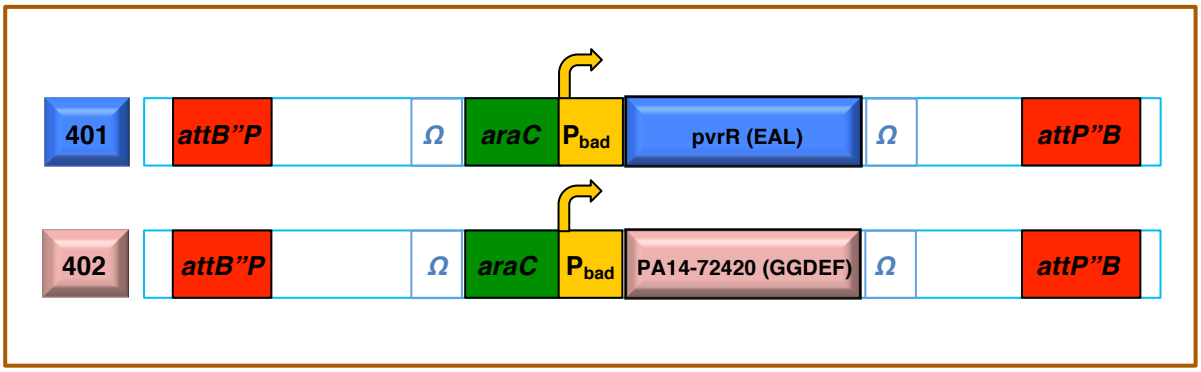


Figure 3.1 **Construct of arabinose-inducible (*araC*-P_{bad}) *pvrR* and PA14-72420 genes (Nicastro *et al.*, 2014) were cloned between KpnI-EcoRI sites inside the two Ω transcriptional terminators in mini-CTX2 vector to be integrated at the *attB* specific site downstream of the tRNAser sequence of *P. aeruginosa* chromosome (Hoang *et al.*, 2000). The *pvrR* gene encodes the EAL domain protein and the PA-14-72420 gene encodes the GGDEF domain protein (Winsor *et al.*, 2011). The empty boxes represent the plasmid backbone. *araC*: gene encodes the AraC protein, which regulates activity of the P_{bad}; P_{bad}: promoter regulates transcription of: *araB, araA*, and *araD* of the arabinose operon; *attB*: the bacterial attachment site; *attP*: the plasmid attachment site.**

Table 3.2 Strains, plasmids and primers used for the construction of the integrants

Name	Characterization	Reference
Strain		
P. aeruginosa 57RP	Wild type degrading hydrocarbon strain, isolated from contaminated soil	Déziel *et al.* (2001)
P. aeruginosa 57RP-401	*P. aeruginosa* strain 57RP carrying the *pvrR* gene under control of arabinose-inducible pBAD promoter in mini-CTX plasmid integrated at the *attB* cite of *P. aeruginosa* chromosome (3' noncoding region of tRNAser).	This study
P. aeruginosa 57RP-402	*P. aeruginosa* strain 57RP carrying the PA14-72420 gene under control of arabinose-inducible pBAD promoter in mini-CTX plasmid integrated at the *attB* cite of *P. aeruginosa* chromosome (3' noncoding region of tRNAser).	This study
PCR primer		
Pser-up*	5'-CGA GTG GTT TAA GGC AAC GGT CTT GA-3'	Hoang *et al.* (2000)
PvrR-R	5'-ATC AGG TCG AAC CTG TCC TG-3'	This study
PA72420-R	AGC GTC TCG CTA AAG CTC AG	This study
Plasmid		
pGGN05	2.5 kb *Kpn*I-*Spe*I fragment of *araC*-pBAD and *pvrR* coding region into *EcoR*I-*Kpn*I of Mini-CTX2**; Tc^{R}***	(Nicastro *et al.*, 2014)
pGGN06	3.6 kb *Kpn*I-*Spe*I fragment of *araC*-pBAD and PA14_72420 coding region into *EcoR*I-*Kpn*I of Mini-CTX2; Tc^{R}	(Nicastro *et al.*, 2014)

* Pser-up is a mini-CTX-specific primer. This Pser-up was used as forward primer to verify both *pvrR* and PA14-72420 genes. The length of PCR product should be 1637 bp and 2565 bp respectively.

** Mini-CTX2 site contains *attP* site for integration at chromosomal *attB* site; Tc^{R}

*** Resistance markers: Tc^{R}, tetracycline.

3.9.2 DNA extraction

Two ml of bacterial cultures were collected and centrifuged at 3,000 x *g* for 10 min. The cell pellets were washed with 500 µl of TEN (50 mM Tris-HCl pH 8.0, 100 mM ethylene-diamine-tetraacetic acid (EDTA), 150 mM NaCl) (Sambrook & Russell, 2001) and then dispersed in 500 µl TEN. The cell suspensions were transferred into 2 ml microtubes containing autoclaved glass beads (diameter 0.5 mm) and then homogenized for 30 sec at speed 6.0 in a FastPrep-24 Homogenizer (Biomedical, Solon, OH, USA). These homogenates were centrifuged at 16 000 x *g* at room temperature for 15 min. The aqueous phases were then extracted twice with Phenol/Chloroform/Isoamyl alcohol (25:24:1) solution and once with Chloroform/Isoamyl alcohol (24:1) solution. DNAs were precipitated overnight by 0.6-0.8 volume of isopropanol 100%. After centrifugation and washing with 70% ethanol, the DNAs were dissolved in deionized water for PCR amplification.

3.9.3 PCR amplification

To perform PCR amplification, DNA concentrations were measured by NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA). Fifty ng DNA were used as template for the PCR with 1X ThermoPol buffer of Taq DNA polymerase (M0267X BioLabs). PCR components were adjusted to reach the final concentrations of 1 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of each primer, BSA 0.5 µg μl^{-1}, 5% DMSO and 1 unit of Taq polymerase. The PCR conditions were performed by the initial denaturation at 94^{o}C for 5 min then followed by 30 cycles of the denaturation at 94^{o}C for 1 min, annealing at 62^{o}C for 45 sec and elongation at 72^{o}C for 2-3 min, depending on the length of the PCR-synthesizing DNA fragments (1637 bp for the fragment containing a part of *pvrR* gene and 2565 bp for PA14-72420 gene), and the final extension at 72^{o}C for 10 min. PCR products were verified by electrophoresis on 1% agarose and visualized by ultraviolet (UV) light using a Gel Imager System after staining with ethidium bromide.

3.9.4 Integrant cultivation

The integrants (RP401 and RP402) were cultured in 6 ml of MSM medium supplemented with 1% hexadecane, as a sole carbon source, as described above, and in the absence or presence of 0.2% (w/v) final concentrations of L-arabinose. The 57RP wild type strain and its derived SCVs were also cultured in the MSM medium supplemented with 1% hexadecane in the absence or in the presence of arabinose. The cell biomass was harvested for further analysis of the total protein concentrations and the c-di-GMP production levels from day 5 to day 15 of the cultivation, as described in sections 3.2 and 3.8.

3.9.5 Viable cell counting on agar plates

The viable cell count was performed by 10-fold serial dilutions of the cultures in MSM and subsequent plating onto TSA agar plates and incubation at 30^{o}C. The colony formation counting was performed after 24 hrs.

3.10 Proteome profile analysis by two dimensional gel electrophoresis

3.10.1 Protein extraction

The *P. aeruginosa* 57RP wild type strain cells were inoculated in MSM plus 1% glucose for 10 hrs at 25°C; the *P. aeruginosa* strain 57RP SCV cells were cultured in MSM medium supplemented with 1% hexadecane for 9 days at 25^{o}C. The resulting cell biomass was used as the materials for the 2D gel analysis experiments. Few modifications of the protein extraction methods (Quan *et al.*, 2013, Wei *et al.*, 2011) applied in the present study were described below.

(i) Outer membrane and periplasmic proteins. The bacterial cells in mid-log growth phase were harvested from 4-8 L cultures by centrifugation at 3,000 x *g* for 10 min. These cell pellets were gently dispersed in 0.1 volume of Tris-Sucrose-EDTA (TSE) buffer (200

mM Tris–Cl, pH 8.0, 500 mM Sucrose, 1 mM EDTA and 1 mM PMSF (phenylmethylsulfonyl fluoride)). After being kept for 1.5 hrs at 4^{o}C, these cell suspensions were centrifuged at 16,000 x *g* for 1 h at 4^{o}C. The cell pellets were kept for cytosolic protein extractions (see below). The supernatants were collected and ultra-centrifuged at 100,000 x *g* for 2 hrs at 4^{o}C, using the Ultracentrifuge (Optima L-100K Ultracentrifuge, COL 06K48, Beckman Coulter Inc., USA). (1) Parts of these pellets containing the outer membrane proteins were dried and dissolved in 0.1% SDS buffer for measurement of the protein concentrations by the Bradford assay method. The rest of the pellets were used for the Isoelectric focusing (IEF) analysis, using common buffer conditions (8M Urea, 2 M Thiourea, CHAPS 40 mM, 40mM DTT, 2% IPG 4-7), and for further analysis. (2) The expected periplasmic proteins in the ultra-centrifuged supernatant were precipitated with TCA (Trichloroacetic acid) to reach 25% final concentration at -20^{o}C. After washing twice with 80% acetone, the precipitated periplasmic proteins were dissolved in 0.1% SDS buffer and their concentrations were measured as above for outer membrane proteins.

(ii) Cytosolic proteins. The bacterial cell pellets obtained from previous separating steps, approximate of 200 ml of the cultured cell suspension, were washed twice with 100 mM Tris-HCl buffer (pH 8.0) and 10 mM Tris-HCl buffer (pH 8.0), respectively. Since the quality of the 2-D gel electrophoresis of cytosolic proteins relies on the purity of protein samples, the contamination of the macromolecular DNAs and the other non protein components should be removed from the protein samples of interest as much as possible (Do, 1995). The pellets were then dispersed in 4 ml of lysis buffer (8M Urea, 2M Thiourea, CHAPS 40 mM, 40mM DTT, 1 mM PMSF and DNAse 50 μg ml^{-1}) and sonicated by Sonifier 150 (Branson Ultrasonics Corporation, USA) three times for 30 sec. The unbroken cells and the cell debris were removed by centrifugation twice at 9,000 x *g* at 4^{o}C for 30 min. The supernatants then were mixed with TrisEDTA/Sucrose buffer (final concentration: 50 mM Tris-Cl pH 8.8, 5 mM EDTA pH 8.0, 450 mM sucrose and 0.2% β-mercaptoethanol) for 45 min at 4^{o}C. The homogenate was extracted twice with equal volume of phenol (water saturated with Tris-HCl pH 7.4). The phenol phases were collected by centrifugation (16,000 x *g*, 30 min at 4^{o}C) and pooled. The cytosolic proteins were precipitated by adding 5-10X volume of 100% methanol in the presence of 100 mM

NH_4OAc at -20°C for at least 2 hrs. After washing twice with 80% acetone and centrifuging at 3,000 x *g* for 15 min, the pellets were dissolved in 0.1% SDS buffer and measured concentration as previously described and dissolved in IEF buffer for the two dimensional gel electrophoretic analysis (2DE).

3.10.2 Two-dimensional gel electrophoresis

Approximately 100-130 µg of the extracted proteins in a total volume of 250 µl were applied on immobilized pH gradient IPG pH 4-7 gel strips (13 cm, GE HealthCare) for an in-gel hydratation under silicone oil for 16 hrs. The proteins were migrated on the IPGphor Isoelectric Focusing System (Pharmacia Biotech) at 20°C for a total of 5.5 hrs, including a gradient range 1000-8000 V for 3.5 hrs as recommended by the manufacturer's instructions. After migration, proteins in the strips were equilibrated with equilibration solution (6M Urea, 150 mM Tris-Cl pH 8.8, 2% SDS and 30% glycerol) containing 1% (w/v) dithiolthreitol for 15 min for reduction, and then again with the same component solution mentioned above containing 260 mM iodoacetamide for 15 min for alkylation. These strips were then transferred onto 12% acrylamide/bisacrylamide gels for the second dimension analysis. The electrophoresis was performed at 70V for 16 hrs by using the DCode Universal Mutation Detection System (BioRad) with 1X Tris-glycine buffer (25 mM Tris, 192 mM glycine, 0.1% SDS). The separated proteins on gels were visualized by silver nitrate staining. The proteins were fixed in gels with fixing solution (5% acetic acid, 50% ethanol) for 45 min. After washing 3 times with deionized water, the gels were stained with 0.1% silver nitrate for 30 min, and then developed in developing solution (2% sodium carbonate, 0.04% formaldehyde). The selected protein spots were cut out with a sterilized knife. The chosen protein spots were identified by recording all mass spectra on a hybrid linear ion trap-triple quadrupole mass spectrometer (Q-Trap, AB Applied Biosystems, MDS Sciex Instruments, California, USA). MASCOT (Matrix Science, London, UK) was used to create peak lists from MS and MS/MS raw data with *P. aeruginosa* protein banks by the proteomic service at the INRS-Institut Armand-Frappier. The NCBInr database was used to identify proteins from peptide identifications.

4 RESULTS

4.1 Formation of small colony variants

4.1.1 Growth of *P. aeruginosa* on hexadecane and octadecane

Three *P. aeruginosa* strains were tested for their ability to grow on hexadecane (liquid alkane) or octadecane (solid alkane) as the sole carbon source. Two culture conditions were tested with agitation and without agitation (static cultures). Two of these strains were isolated from a hydrocarbon-contaminated soil (strains 57RP and 57RV), and the third one, strain PA14, from a burn patient. *P. aeruginosa* PA14 grew poorly on these alkanes (Figure 4.1A). No further experiments were performed with this strain because of its poor growth on these alkanes. When cultured on hexadecane with agitation at 30^{o}C, strain 57RP had a lag phase of 2-3 days before exponential growth occurred. This lag phase was shorter or absent with octadecane (Figure 4.1B, C). The growth on solid octadecane of this strain in shaking conditions was faster than that in static conditions (Figure 4.1C).

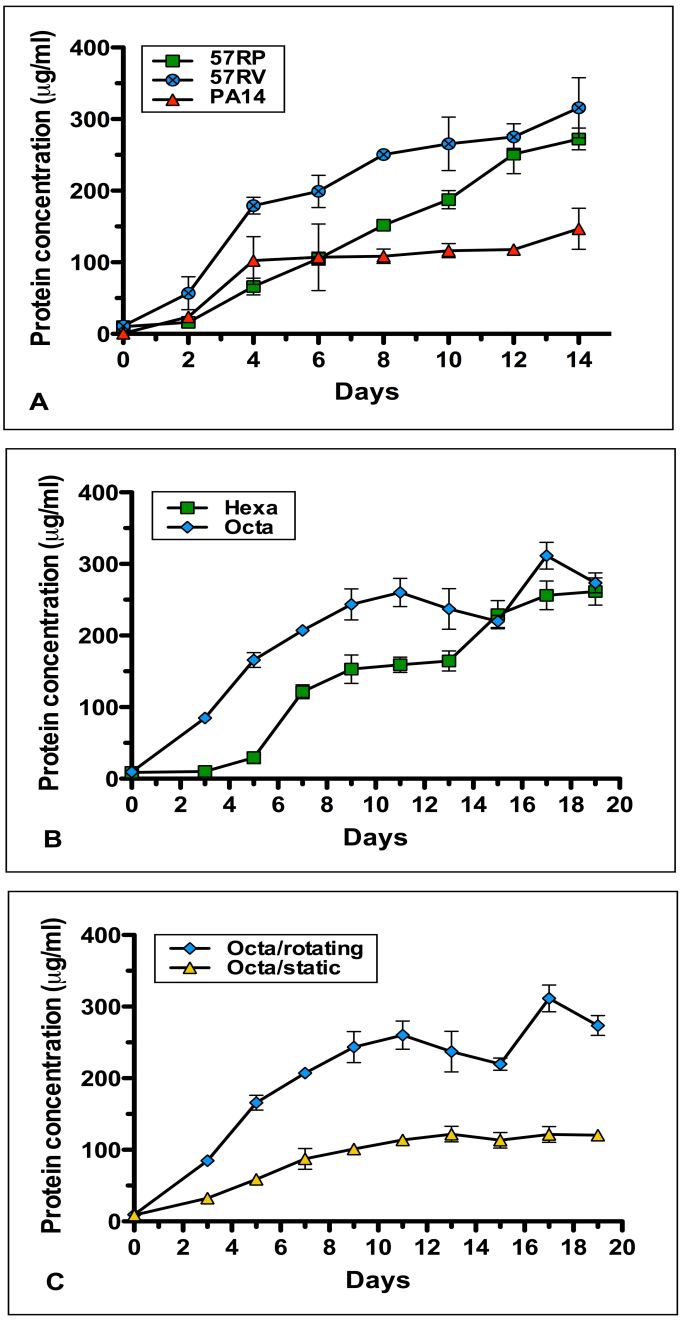


Figure 4.1 **Growth of different *P. aeruginosa* strains on MSM supplemented with 1% hexadecane (v/v) or octadecane in a rotator at 30°C for 14-19 days. (A) Growth of 57RP, 57RV and PA14 strains on hexadecane, (B) Growth of strain 57RP on liquid and solid alkanes, (C) growth of strain 57RP on octadecane in static and rotating conditions. Error bars represent the standard deviation from triplicate samples.**

4.1.2 SCVs generated on liquid and solid alkanes

A 5-7 day old culture of strain 57RP (Figure 4.2A) cultured on liquid hexadecane or solid octadecane was spread on TSA plates and incubated at 30°C. Several SCV-type colonies were observed (Figure 4.2B and D). These colonies were 1.5-2 mm in diameter after 48 hrs. They were small, rough and had a dry surface (Figure 4.2B, D). Some colonies were picked and cultured in a liquid rich medium (TSB) and then the cultures were spread again on TSA plates. Among the SCV colonies, several revertants (Rev) were observed (Figure 4.2C, E).

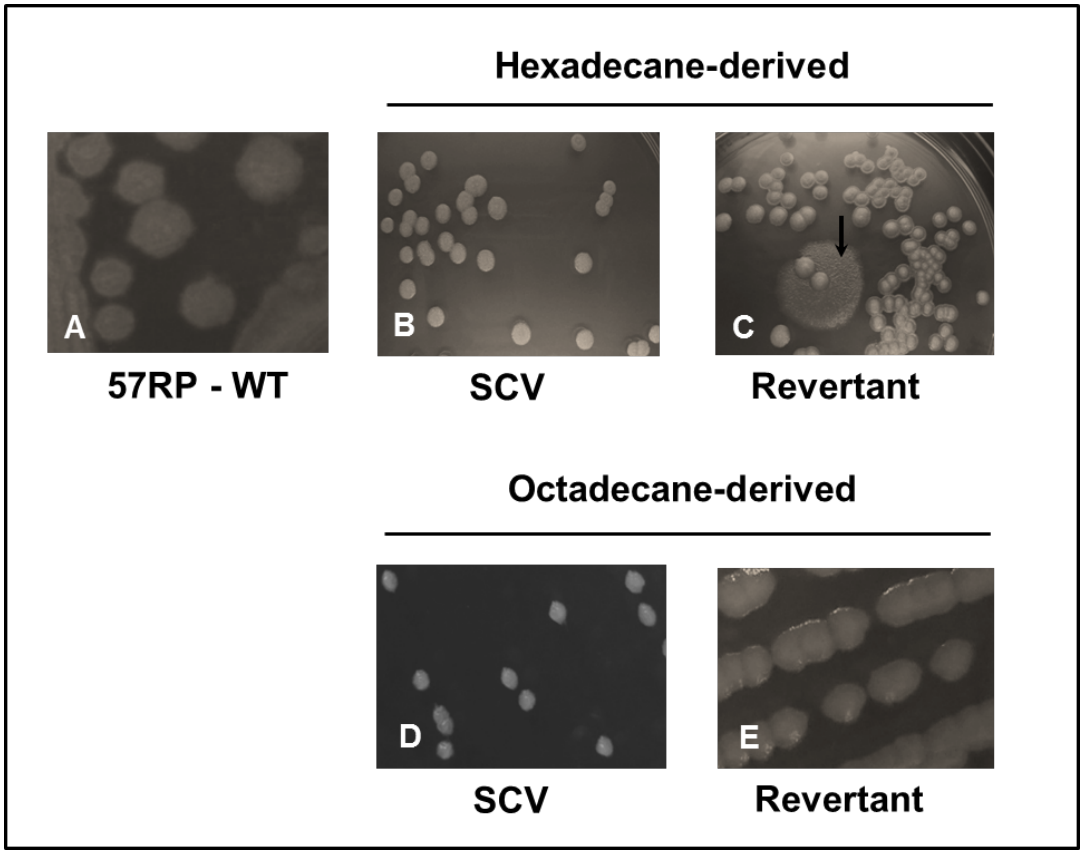


Figure 4.2 **Morphological phenotypes of strain 57RP on solid agar medium. A. Wild type; B, C. Small colony variants and revertant (arrow) derived from hexadecane and D, E. Small colony variants and revertants derived from octadecane.**

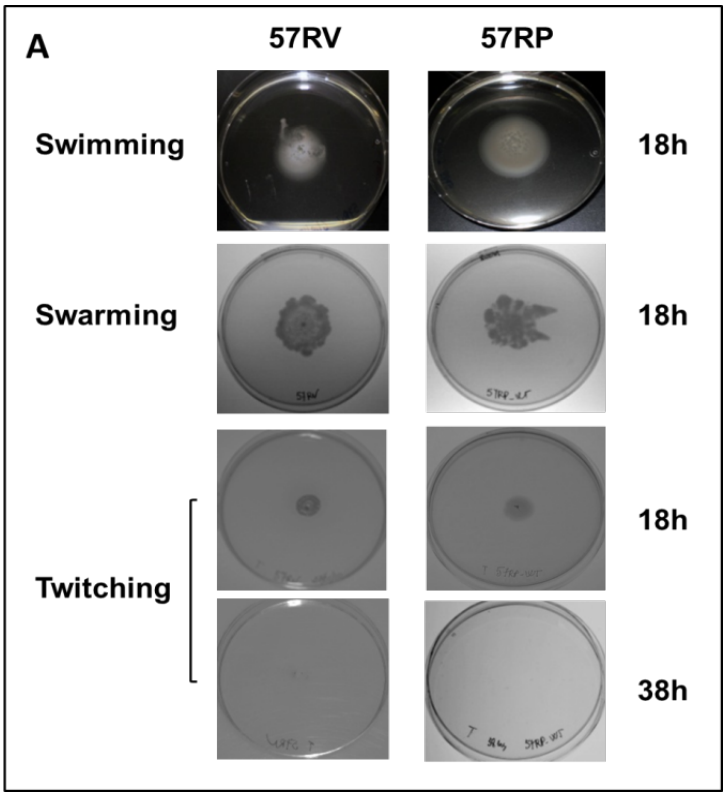
4.2 Physiological characteristics of strain 57RP SCVs

4.2.1 Motilities

The wild type strain 57RV (57RV WT), wild type strain 57RP (57RP WT), the representative 57RP SCVs and 57RP revertant strains were pre-cultured overnight in TSB medium then stab inoculated on swim plates (0.3% agar) or swarm plates (0.5% agar) or thin layer twitch plates (1% agar). Both 57RV and 57RP WT strains showed similar characteristics for the three motilities, including swimming, swarming and twitching. However, the two wild type strains also had individual traits such as the diameters of turbid zone on swimming plates for strain 57RV WT (around 24 mm) were smaller than 57RP WT (around 30 mm) (Figure 4.3).

As previously mentioned in the literature review (section 2.5.1), *P. aeruginosa* SCVs have reduced motilities, which include swimming, swarming and twitching (Déziel *et al.*, 2001, Haussler *et al.*, 2003). SCVs derived from strain 57RP in this study still maintained their motility defect as observed in Déziel (2001). This confirmed that the strains we used still have stable characters and will be good candidates for further studies. Three 57RP SCVs generated from liquid hexadecane (here named SCV1h, SCV2h and SCV3h) and three others generated from solid octadecane (here named SCV1o, SCV2o and SCV3o) were selected and examined for motility assays and biofilm assays. Two revertants (Rev), one from a SCV culture derived from hexadecane culture (Rev21h) and the other one from octadecane culture (Rev11o) were also tested.

The results showed that the diameters of turbid zones on swimming plates of 57RP WT and of both revertants were equivalent. These diameters were three times larger than that of SCVs derived from hexadecane (Figure 4.4A, B) and twice as large as that of SCV derived from octadecane. The swarming motility of the SCVs derived from both liquid and solid alkanes decreased compared to the WT and the Rev, while their adherence to the polystyrene surface in twitching motility increased (Figure 4.4A & 4.5A). All these results show that the three motilities were reduced in the 57RP SCVs derived from the two alkanes.



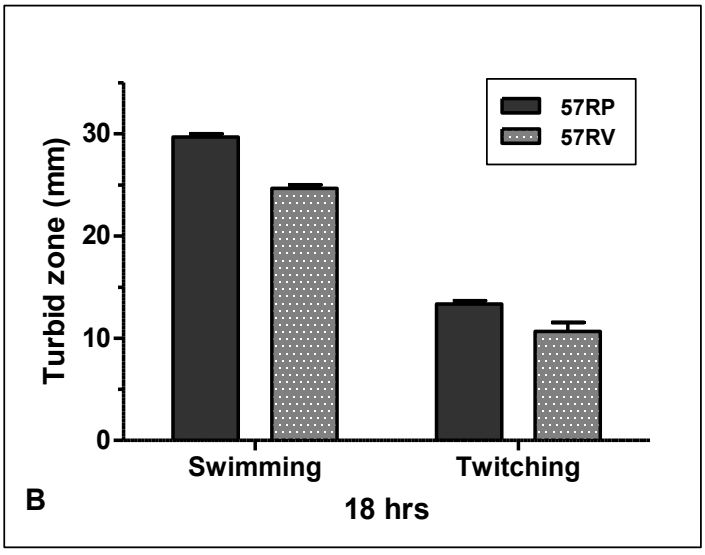


Figure 4.3 **A. Motilities of *P. aeruginosa* 57RV and 57RP strains on swimming plates (0.3% agar) for 18 hrs; swarming plates (0.5% agar) for 18 hrs; and twitching plates (1% agar) for 18h without removing agar and staining with crystal violet, and attachment of cells to the polystyrene Petri dish after staining with crystal violet in twitching motility for 38 hrs. B. Turbid zone of swimming and twitching motility after 18 hrs. Error bars represent the standard deviation from triplicate samples.**

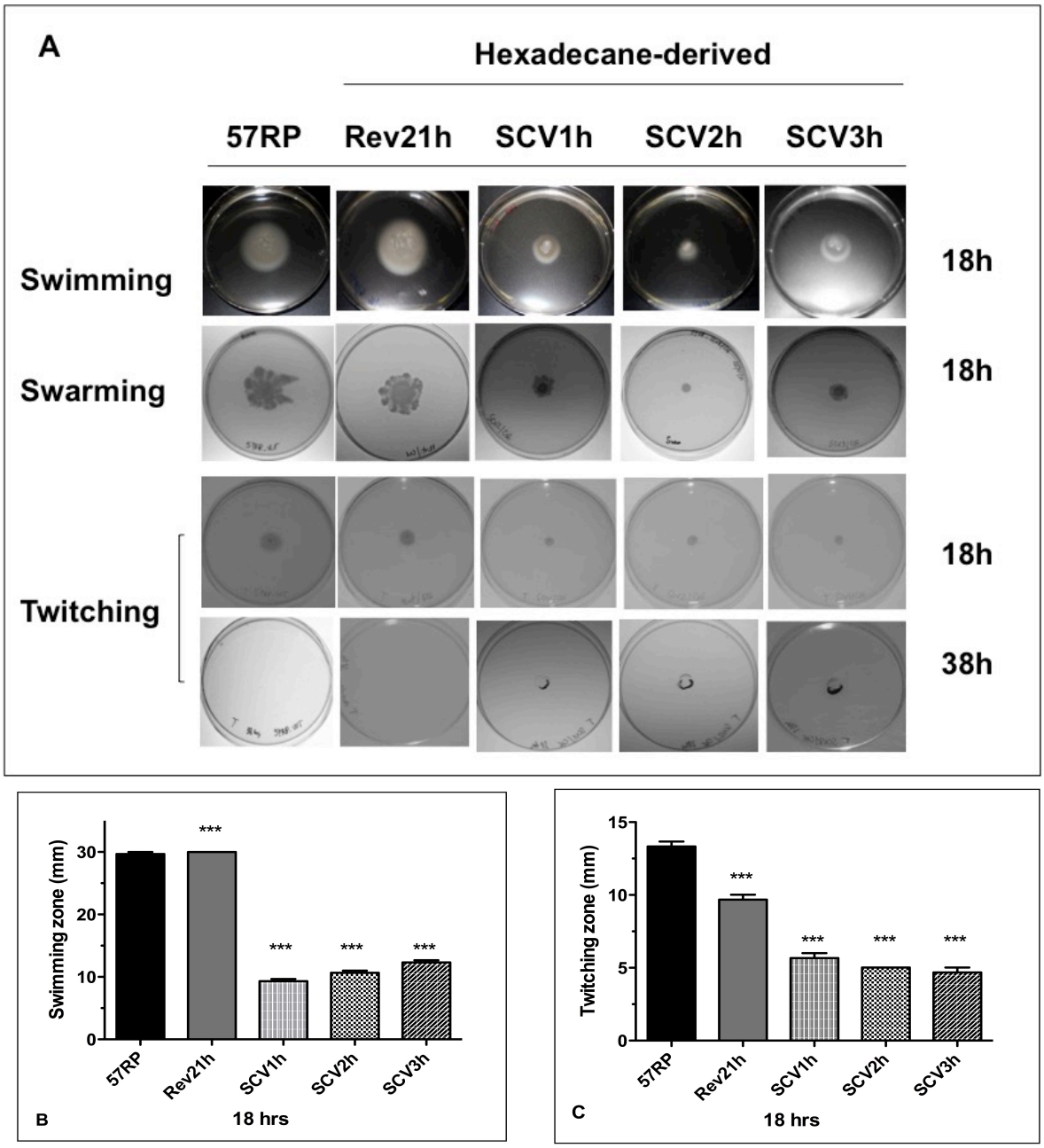


Figure 4.4 **A. Motilities of different strains derived from hexadecane: swimming plates (0.3% agar) for 18 hrs; swarming plates (0.5% agar) for 18 hrs; twitching plates (1% agar) for 18h without removing agar and staining with crystal violet, and attachment of cells to polystyrene Petri dish after staining with crystal violet in twitching motility for 38 hrs; B. Diameter of turbid zone in swimming motility after 18 hrs. C. Diameters of turbid zone in twitching motility after 18 hrs. Error bars represent the standard deviation from triplicate samples. Significance was determined by one-way analysis of variance followed by a Dunnett's Multiple Comparison Test for the difference between WT and individual SCVs, *** $P < 0.0001$.**

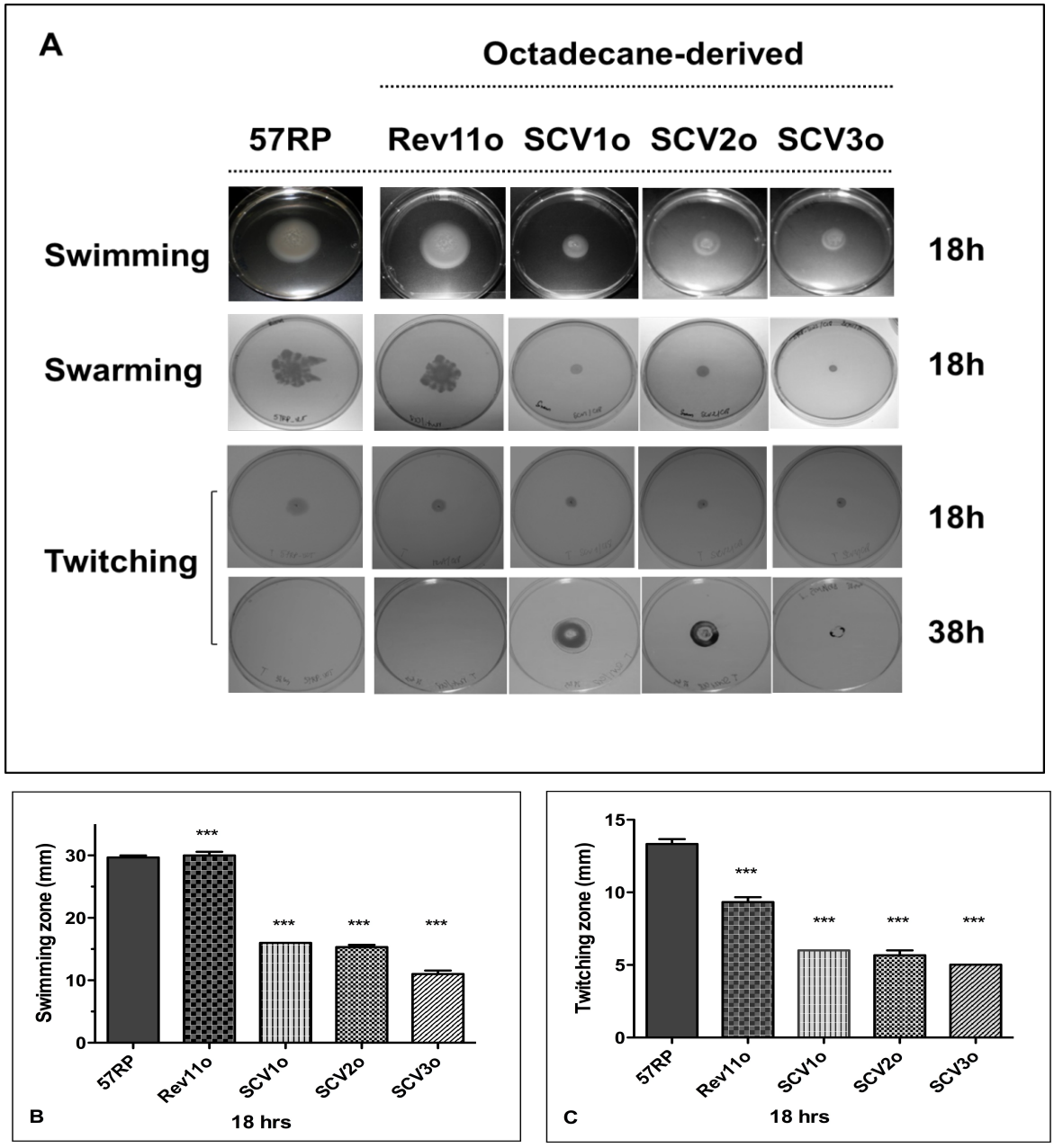


Figure 4.5 **A. Motilities of different strains derived from octadecane: swim plates (0.3% agar) for 18 hrs; swarm plates (0.5% agar) for 18 hrs; twitch plates (1% agar) for 18h without removing agar and staining with crystal violet, and attachment of cells to polystyrene Petri dish after staining with crystal violet in twitching motility for 38 hrs; B. Diameter of turbid zone in swimming motility after 18 hrs of SCVs and revertant derived from octadecane culture. C. Diameters of turbid zone in twitching motility after 18 hrs of SCVs and revertant derived from octadecane culture. Error bars represent the standard deviation from triplicate samples. Significance was determined by one-way analysis of variance followed by a Dunnett's Multiple Comparison Test for the difference between WT and individual SCVs, *** $P < 0.0001$.**

4.2.2 Biofilm formation

The next step was to verify the biofilm formation ability of the strains. The biomass of attached cells (biofilm) in the polystyrene tube assays was quantified for the 57RP WT, its derived SCV and Rev, and strain 57RV. Strain 57RV formed more biofilm (2-3 times) than 57RP WT (Figure 4.6). Biofilms derived from the 57RP SCVs, generated either from hexadecane or octadecane, were approximately 10 times thicker after 10 hrs than the ones derived from 57RP WT and the Rev (Figure 4.7 & 4.8). The 57RP WT and Rev strains showed similar results. As all the chosen SCVs generated from both liquid and solid alkanes showed similar behaviors in motilities and biofilm formation. One representative SCV, SCV2h generated from hexadecane cultures, SCV1o generated from octadecane cultures, and one revertant from these representative SCVs (Rev21h and Rev11o) were chosen for the further study of my project.

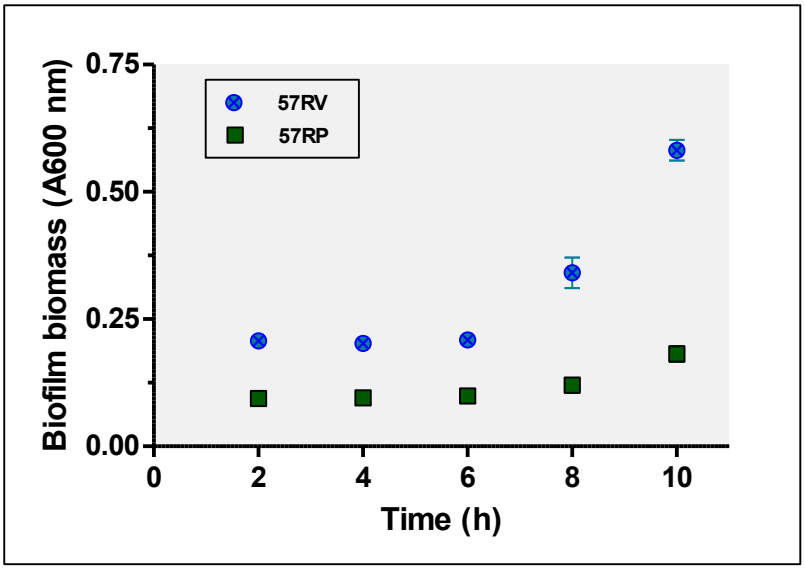


Figure 4.6 **Kinetics of biofilm formation of *P. aeruginosa* 57RP and 57RV in static conditions on Bushnell-Haass medium supplemented with 0.2% dextrose and 0.5% tryptone at 25°C during 10 hrs. Error bars represent the standard deviation of triplicate samples.**

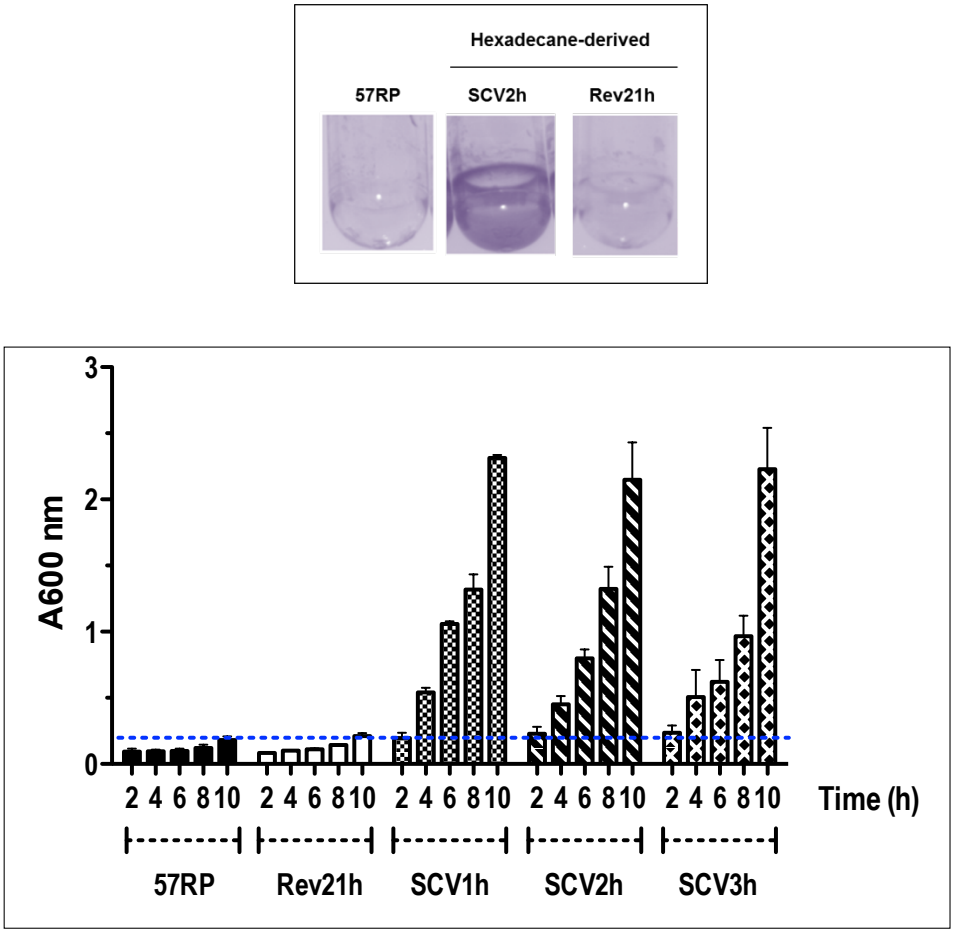


Figure 4.7 **Kinetics of biofilm formation of different hexadecane-derived phenotypes in static condition on Bushnell-Haass medium supplemented with 0.2% dextrose and 0.5% tryptone at 25^{o}C during 10 hrs. SCVs were generated from cultures performed on hexadecane. Error bars represent the standard deviation of triplicate samples.**

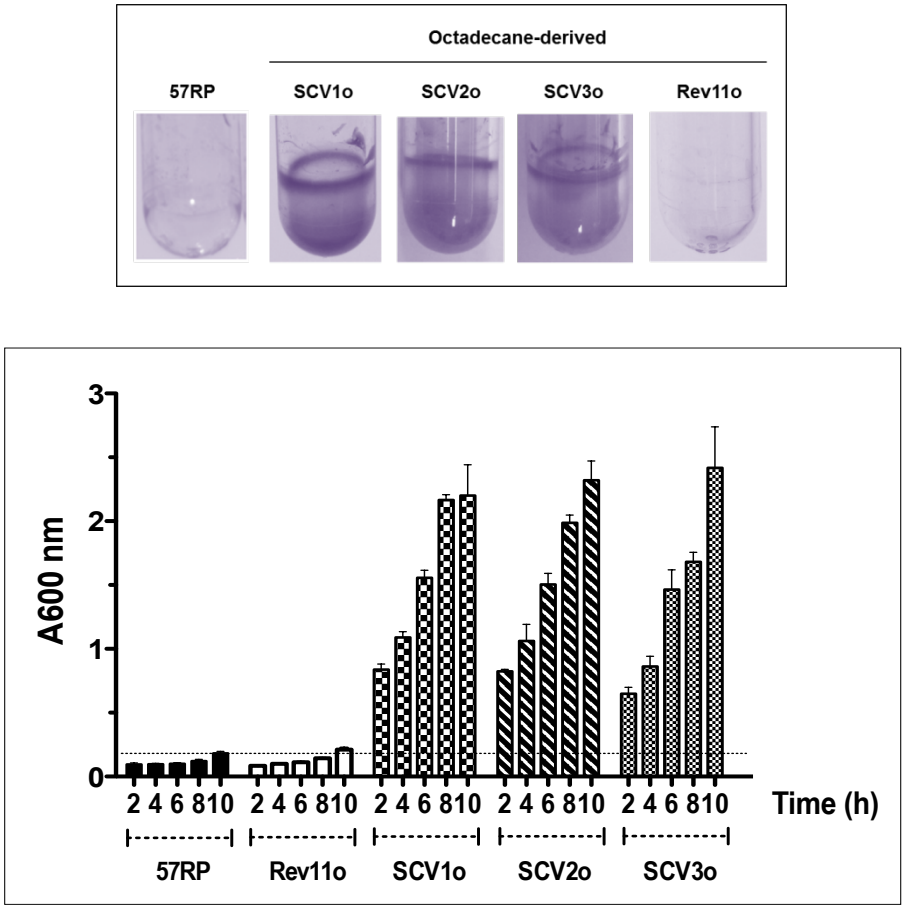


Figure 4.8 **Kinetics of biofilm formation of different phenotypes in static conditions on Bushnell-Haass medium supplemented with 0.2% dextrose and 0.5% tryptone at 25^{o}C during 10 hrs. SCVs were generated from cultures performed on octadecane. Error bars represent the standard deviation of triplicate samples.**

4.2.3 Surface hydrophobicity and cell adherence assays

The MATH assay was used to quantitatively assess the cell surface hydrophobicity of the 57RP WT, SCV2h, SCV1o, Rev21h and Rev11o strains. Sixty percent of the SCV2h and SCV1o cells were adherent to hexadecane, 20% more than that of the 57RP WT and the two revertants adherence (Figure 4.9A & C). These results suggested that cell surface of SCVs was more hydrophobic than the 57RP WT and the revertants. Similarly, when performing adherence experiments by the MATS assays, 80% of the SCV2h and SCV1o cells adhered to silica, while the percentage for the 57RP WT and the two revertants were less than 60% (Figure 4.9B & D). These results indicated that the high levels of adherence were distinctive characteristics of 57RP SCVs in comparison to the 57RP WT and the revertants. In addition, *P. aeruginosa* 57RV showed similar levels of cell surface hydrophobicity as well as adherence ability compared to the 57RP SCVs (Figure 4.9A & C), indicating that 57RV is a naturally highly adherent isolate. While 57RP WT is not.

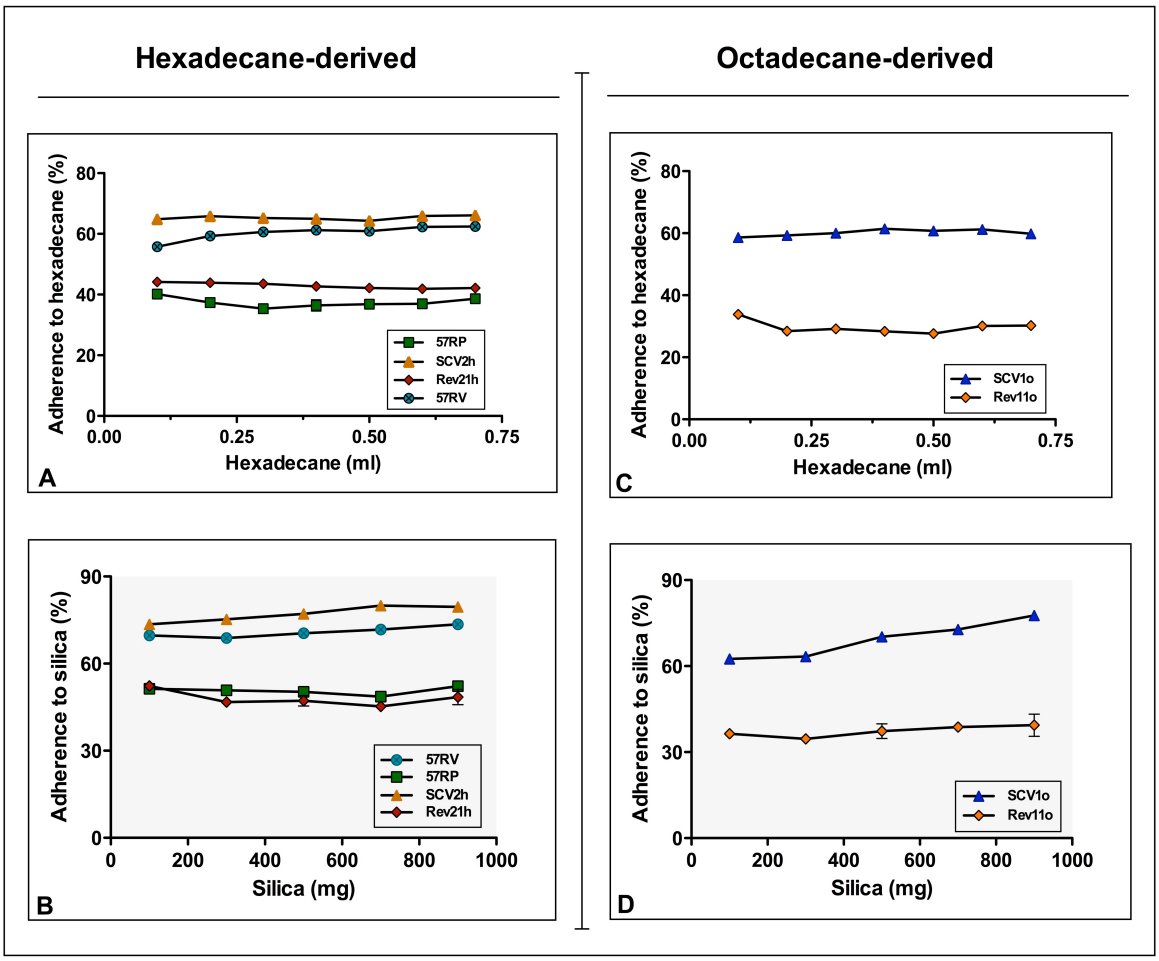


Figure 4.9 **MATH (panels A & C) and MATS (panels B & D) assays on the 57RP WT, SCV and Rev strains, and strain 57RV. A & B: The SCV and its revertant were derived from hexadecane cultures. C & D: The SCV and its revertant were derived from octadecane cultures. Experiments were repeated two times in triplicate tubes. Error bars represent the standard deviation of triplicate samples.**

4.2.4 High density of pili on cell surface of SCV

The fact that SCVs showed higher biofilm formation and reduced motilities then the 57RP WT strain suggested some changes in the production of pili. To confirm the isolates still had stable traits as previously observed (Déziel *et al.*, 2001) in our laboratory, the cells from the 57RP WT, SCV2h generated from hexadecane cultures and its Rev21h were cultivated on TSA plates and observed under transmission electron microscopy (Figure 4.10). Pili associated with SCV2h (Figure 4.10B) were more abundant than those observed on 57RP WT (Figure 4.10A) and Rev21h (Figure 4.10C). The pili formed on 57RP WT and Rev21h cells were observed only in the polar site and the pilus density was less abundant in comparison to SCV2h.

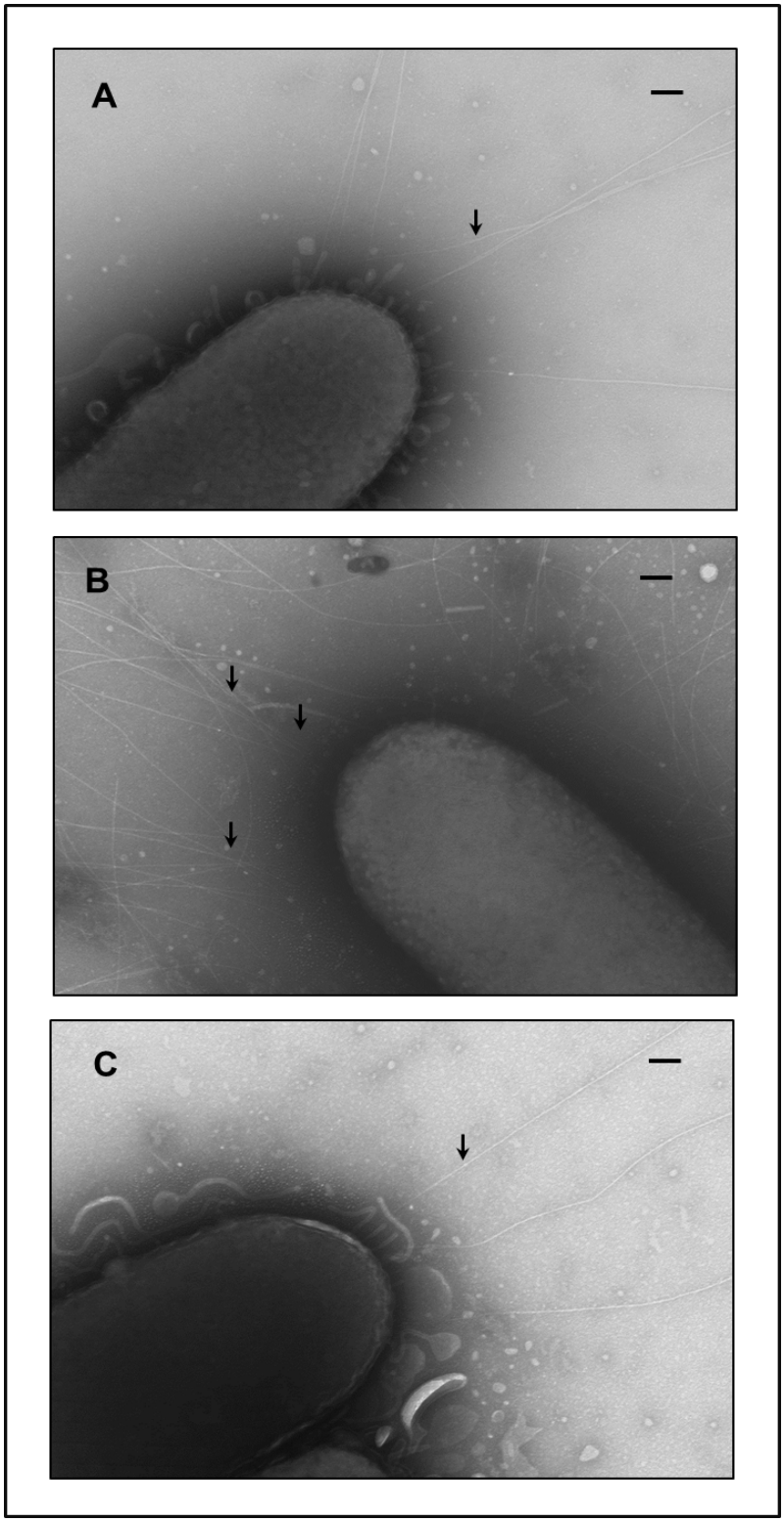


Figure 4.10 **Transmission electron microscopy images of a typical cell of 57RP-WT (A), SCV2h (B) and Rev21h (C) (print magnification 124 000x, bar ~ 100 nm). Arrows indicate pili. Bacteria were grown on TSA medium.**

4.2.5 Rhamnolipid production in SCV

Cultures of the 57RV, the 57RP WT, SCV2h and Rev21h strains with hexadecane were analyzed for rhamnolipid production. The long lag phase of about 9 days was observed with both 57RP WT and Rev21 whereas SCV2h grew almost without the initial lag phase (Figure 4.11A). However, the three cultures reached the similar high biomass levels after 14 days of culture. These results support the model that the active growth of SCVs occurred after the lag phase and contributed mainly to the increase in the biomass production of the initial 57RP WT and Rev21h cultures (Figure 4.11A & B).

Rhamnolipids produced by 57RP WT, SCV2h and Rev1h were similar in concentrations during 13 days (2-4 mg/L). Strain 57RV showed a similar level of rhamnolipids for the first 11 days. The rhamnolipid concentration of this 57RV strain increased to 5–7 mg/L at day 13-15 (Figure 4.11B), the start and progress of the exponential growth phase during which the appearance of 57RV SCVs was observed (data not shown).

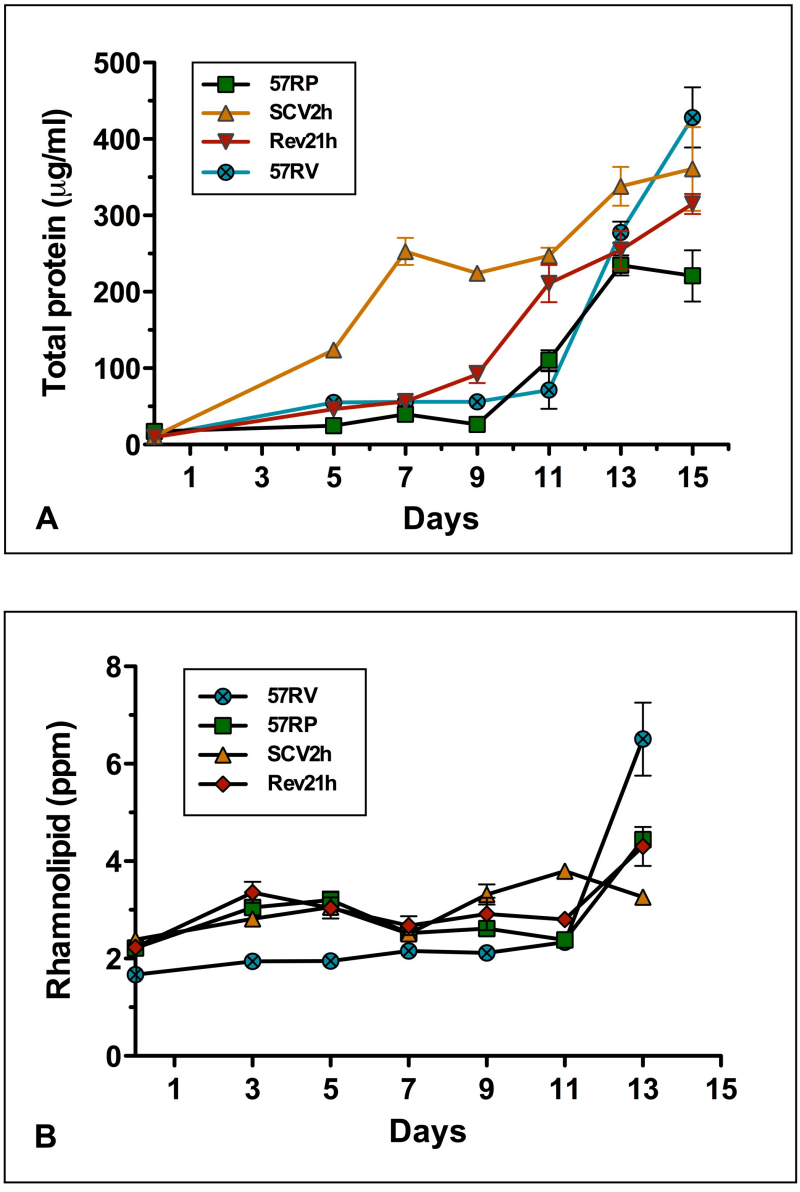


Figure 4.11 **The growth of different *P. aeruginosa* strains: 57RV, 57RP, SCV2h and Rev21h (A), and rhamnolipid production (B) on MSM supplemented with 1% hexadecane (v/v) at 25 ^{o}C under shaking conditions. Experiments were repeated two times in triplicate tubes. Error bars represent the standard deviation of triplicate samples.**

4.2.6 Analysis of c-di-GMP production

The c-di-GMP production levels were determined in the 57RP WT, SCV2h and Rev21h strains, and in strain 57RV, when they were cultured in the TSB rich liquid medium for 48 hours. Although all strains grew very well in this medium, c-di-GMP was not detected, suggesting that very low levels of c-di-GMP were produced in these cells (limit of detection of the method around 0.027 ppm as shown in figure 4.12). This was not unexpected since c-di-GMP production is associated with the sessile mode of growth.

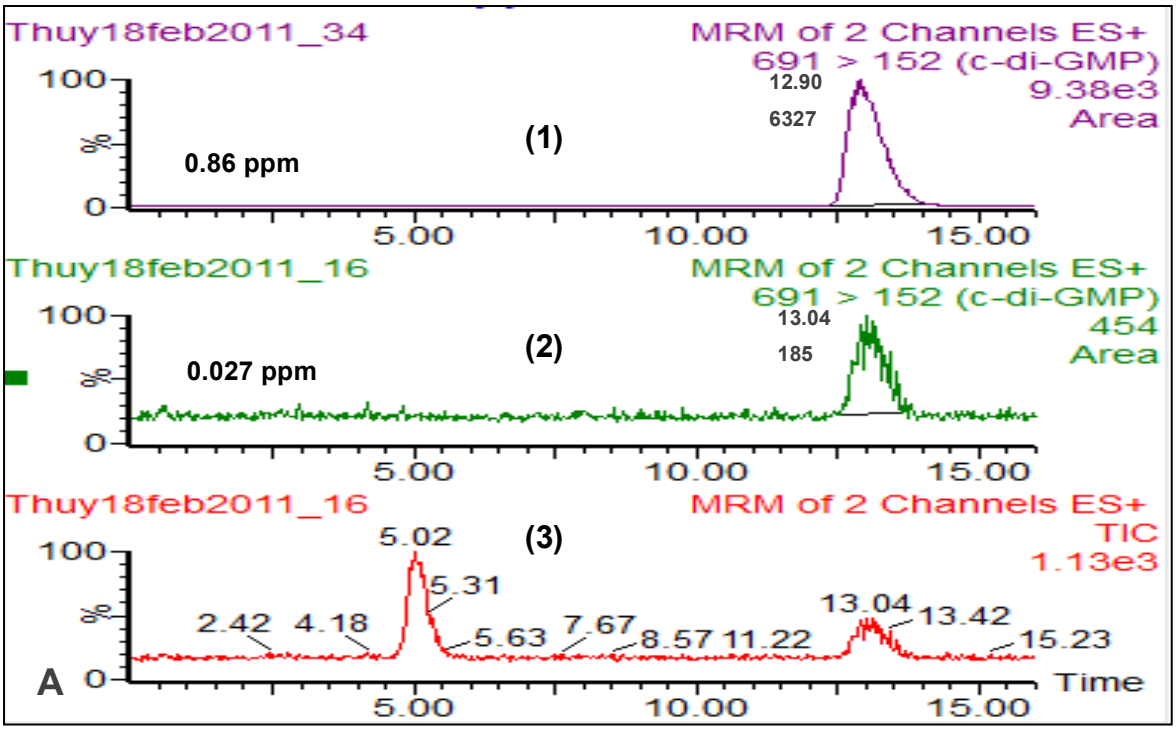


Figure 4.12 **Chromatograph from HPLC-MS analysis. (1) peak area of c-di-GMP standard of 0.86 ppm, (2) peak area of c-di-GMP standard of 0.027 ppm and (3) scanning peaks in multiple reaction monitoring (MRM) mode of internal standard cXMP and c-di-GMP standard.**

The c-di-GMP production levels were then measured every 2 days with the 4 strains cultivated in mineral salt medium supplemented with 1% hexadecane shaking at 25^{o}C. Since we knew that 57RP WT required the emergence of SCVs for growth on hexadecane and that SCVs seemed to represent the main biofilm forming phenotype, it was hypothesized that *P. aeruginosa* needs to form biofilm to effectively use hexadecane as a source of carbon and energy (Déziel, 2001). The c-di-GMP levels produced in the SCV2h cells were higher than the levels produced in the 57RP WT and Rev21h cells. The basic level of c-di-GMP in SCV2h was around 0.04 µg/mg protein for the first 7 days and increased afterwards to reach around 0.24 µg/mg protein after 13 days of growth. The c-di-GMP levels for the 57RP and Rev21h reached around 0.8 µg/mg proteins after 13 days. The c-di-GMP level in strain 57RV remained low during the whole culture time (Figure 4.13B).

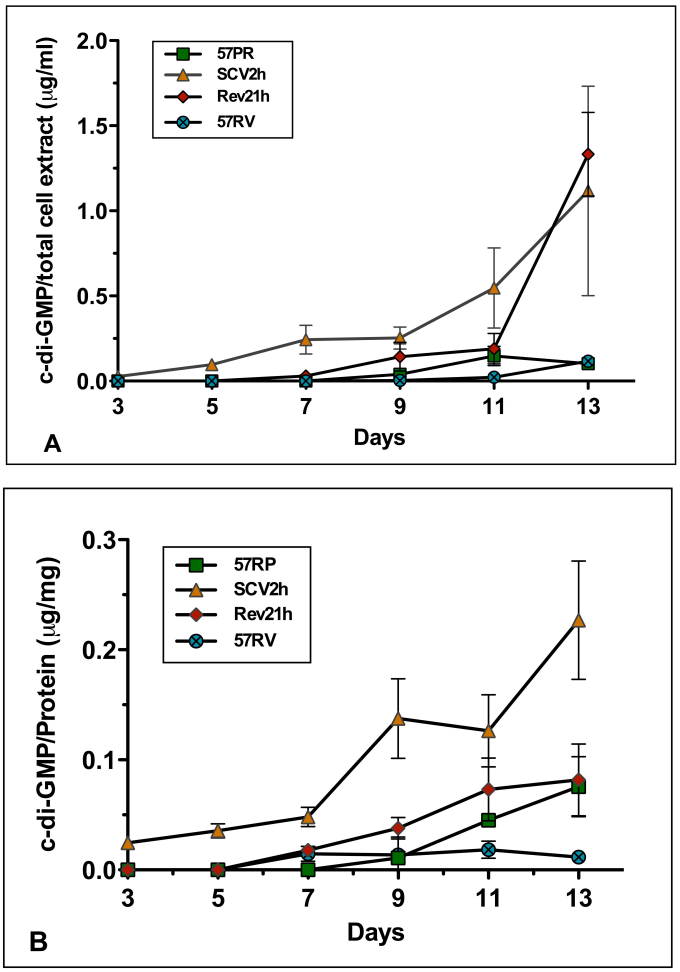


Figure 4.13 **(A) c-di-GMP production by different *P. aeruginosa* strains: 57RP WT, SCV2h, revertant Rev21h and 57RV and (B) correlation between c-di-GMP level and total protein of these strains, which were grown on MSM supplemented with 1% hexadecane at 25°C in shaking conditions. Experiments were repeated twice in triplicate tubes. Error bars represent the standard deviation of triplicate samples.**

4.3 Involvement of c-di-GMP in SCVs

4.3.1 Analysis of c-di-GMP in the RP401 and RP402 integrants

To assess the involvement of c-di-GMP in the formation of our SCVs, two genetic elements controlling either its synthesis or its degradation were integrated into the 57RP WT chromosome. The first genetic element was the *pvrR* (PA14-59790) gene, which encodes an EAL domain-containing protein, phosphodiesterase, involved in the degradation of c-di-GMP. The second genetic element was a gene that encodes a GGDEF domain-containing protein (PA14-72420) involved the synthesis of c-di-GMP. Both genes were under the control of the P_{BAD} promoter, which is inducible with arabinose. Two integrants, RP401 (EAL domain) and RP402 (GGDEF domain), were obtained and the presence of each of these genes in the chromosome was confirmed by PCR (Figure 4.14).

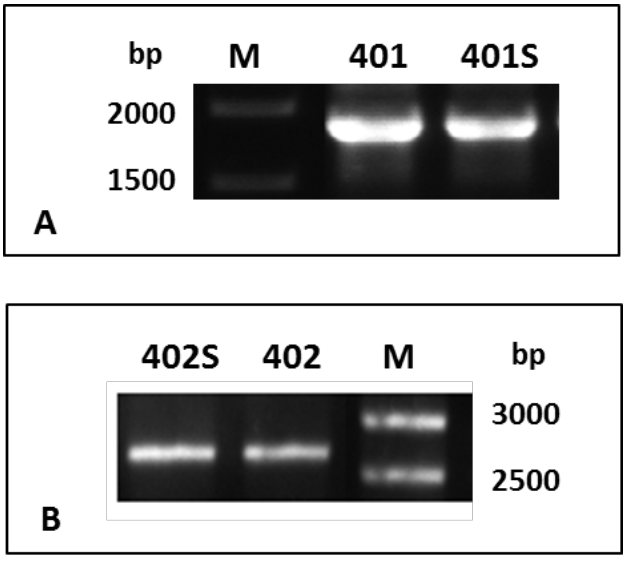


Figure 4.14 **Positive strains for the integration of the *pvrR* and PA14-72420 genes into the 57RP WT chromosome. One representative colony from each integrant was screened with specific PCR primers for the *pvrR* and the PA14-72420 genes. (A) PCR products of RP401 (~1637 bp) and its SCV (RP401S). (B) PCR product of RP402 (~2565 bp) and its SCV (RP402S). Marker (M) 5 kb (Fisher Bioreagent) was used as ladder a molecular weight ladder.**

Then, the impact of controlling the intracellular levels of c-di-GMP during growth on hexadecane of these two integrants was investigated. In the case of the RP401 integrant, the most noticeable result was the absence of observable growth throughout the culture period of the RP401 integrant cultured in the presence of arabinose. Arabinose stimulates the expression of the *pvrR* (EAL) gene and promotes the degradation of c-di-GMP (Figure 4.16A). However, the uninduced cultures of this RP401 integrant showed a similar growth level of the 57RP WT cultures. The RP402 integrant cultured in presence of arabinose, which stimulates the expression of the PA14-72420 (GGDEF) gene and promotes the synthesis of c-di-GMP, showed a higher level of the growth in MSM + hexadecane compared to the uninduced cultures (Figure 4.16A). The lag phase of this integrant was approximately two days shorter. Under the same conditions, the 57RP WT cultured in presence or absence of arabinose showed similar growth for the first 11 days with similar lag phase (Figure 4.16A). However, from day 13 to day 15, the 57RP WT cultured in the presence of arabinose reached higher levels (50%) than the culture without arabinose.

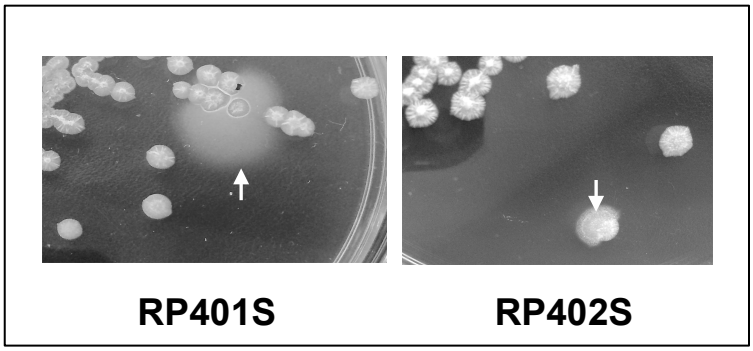


Figure 4.15 **Morphological phenotype of RP401S and RP402S on solid agar medium (TSA), arrows indicate revertants of these strains.**

No c-di-GMP could be detected in the RP401 integrant cultivated with arabinose (Figure 4.16B), which reflected the expression of *pvrR* and the consequent degradation of c-di-GMP. The c-di-GMP level in RP402 cultivated with arabinose was higher than that of RP402 grown in the absence of arabinose (Figure 4.16B) due to the expected expression of

the inducible extra c-di-GMP-synthesizing gene. The c-di-GMP levels in the RP401 and RP402 integrants cultivated in absence of arabinose were similar to those of the 57RP WT cultures (either in the presence or absence of arabinose) (Figure 4.16B).

SCVs were found in the RP401 and RP402 cultures cultivated on hexadecane either in presence or absence of arabinose. However, significant differences in the time of SCV occurrence have been observed after plating on TSA plates for RP402 (7-9 days) and RP401 (15 days) strains. The SCVs of both RP401 and RP402 integrants grown on hexadecane were similar in the morphological appearance, exhibiting small size with a wrinkled and dry surface, like the common SCVs derived from the 57RP WT grown on hexadecane (Figure 4.15). One representative SCV from each integrant was chosen: RP402S and RP401S. The formation of SCVs in the cultures of different strains is presented in table 4.1.

Table 4.1 **The formation of SCVs during the culture period on hexadecane with and without arabinose observed after plating on TSA medium**

Day	57RP WT		RP401		RP402	
	-A	+A	-A	+A	-A	+A
5	-	-	-	-	-	-
7	+	+++	-	-	+	±
9	+++	+++	++	-	++	+++
11	+++	+++	+++	-	+++	++++
13	+++	+++	+++	-	+++	+++
15	+++	+++	++	+	+++	++

(-) not detected, (±) appearance on only one of triplicate petri dishes, (+) appearance on all triplicate petri dishes. (+) 2-3 SCVs, (++) 6-7 SCVs, (+++) >10 and (++++) > 20 SCVs based on an average number of colonies counted on triplicate dishes.

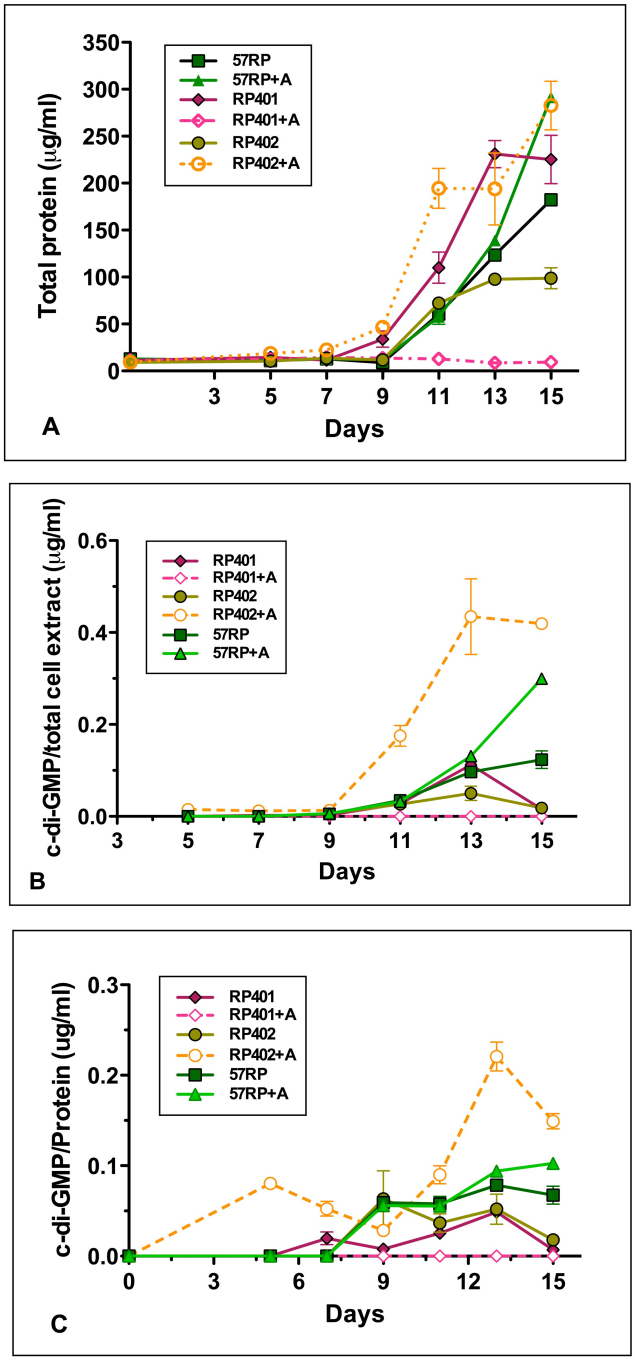


Figure 4.16 **57RP WT, RP401 and RP402 grown in MSM supplemented with 1% hexadecane in the absence or presence of 0.2% arabinose at 25°C in shaking conditions. (A). Growth expressed as total protein concentrations, (B). The c-di-GMP production levels in the presence and absence of arabinose. (C). Correlation between c-di-GMP levels and total proteins in the RP401 and RP402 integrants compared to 57RP WT. Error bars represent the standard deviation of triplicate samples.**

When cultivated with arabinose, the RP402 integrant formed more biofilm (by assays with the polystyrene tubes) than the 57RP WT and the RP401 integrant. In contrast, the 57RP WT and the RP401 and RP402 integrants had no significant difference in biofilm formation when cultivated for 10h in the absence of arabinose (Figure 4.17).

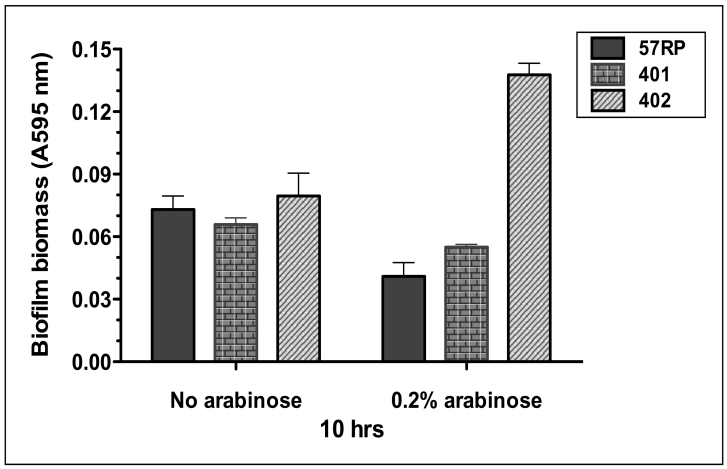


Figure 4.17 **Biofilm formation by RP401 and RP402 integrants. RP401 and RP402, 57RP WT were cultured on Bushnell-Hass medium supplemented with 0.2% dextrose and 0.5% tryptone at 25^{o}C for 10 hrs in polystyrene tubes in the absence or presence of 0.2% arabinose. The error bars represent the standard deviation of triplicate samples.**

4.3.2 Small colony variants derived from the RP401 integrant

The c-di-GMP levels were measured for the SCVs-derived from RP401 (RP401S). RP401S was cultivated in MSM plus 1% hexadecane in the presence or absence of arabinose. The same conditions were performed with the 57RP SCV2h. Both strains showed similar growth behavior (Figure 4.18A). The presence of arabinose stimulated the growth of RP401S and SCV2h strains.

Similar to SCV2h, 401S had no lag phase under tested conditions. Unexpectedly, with the presence of arabinose for the expression of the EAL domain protein (c-di-GMP degradation), RP401S grew better than without arabinose supplement (Figure 4.18A).

High c-di-GMP production in integrant SCVs. When cultured on hexadecane without arabinose, the c-di-GMP production levels of RP401S were low and similar to levels produced by SCV2h during the first 11 days of the culture. However, RP401S obviously increased its c-di-GMP production during the next days and stabilized during the last two days of the culture. SCV2h almost did not change its c-di-GMP production from day 11 to day 15 of culture. In contrast, when arabinose was added to the culture medium, RP401S sharply increased its c-di-GMP production levels from day 5 to day 13 then stabilized on day 15 of the culture. Arabinose also stimulated the c-di-GMP production of SCV2h compared to those levels produced in the culture conditions without arabinose. However, the c-di-GMP levels produced by SCV2h were lower than those produced by RP401S cultured in the same conditions from day 9 to day 15 of the culture. The highest levels of c-di-GMP production were observed on day 13 of the cultures and it was almost the beginning of stationary phase of growth for these strains (Figure 4.18C).

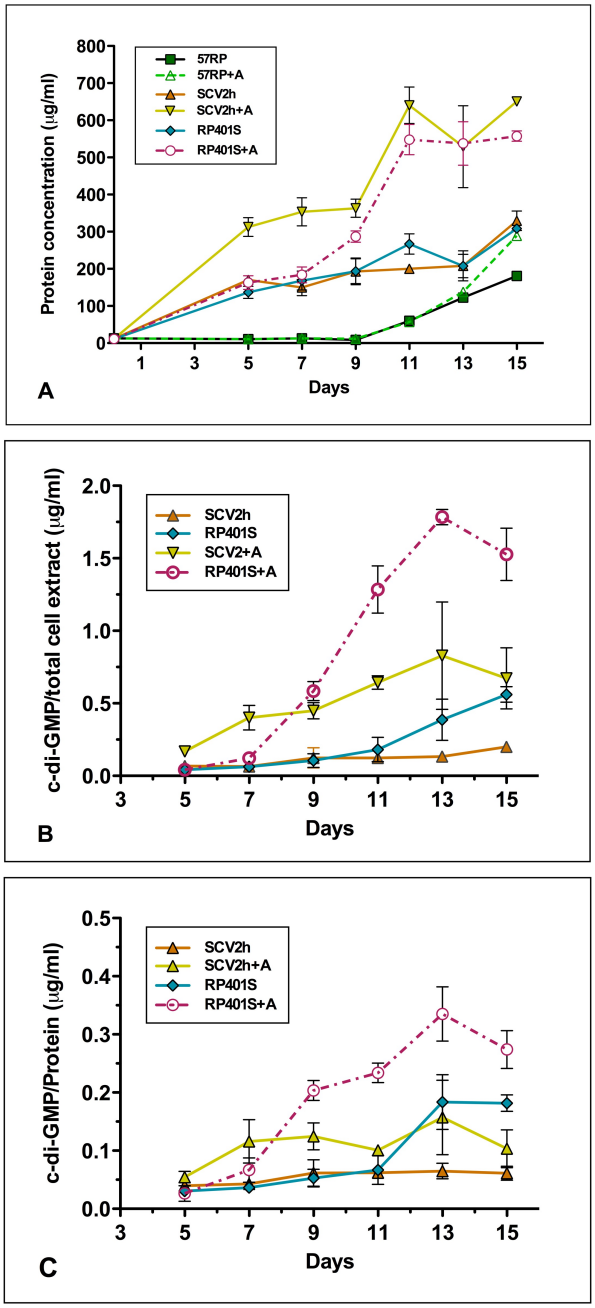


Figure 4.18 **Growth and c-di-GMP production of SCV2h and RP401S, which were cultivated in MSM supplemented with 1% hexadecane in the absence or presence of 0.2% arabinose. (A) The growth of SCV derived from WT strain 57RP (SCV2h) and the SCV derived from the integrant overexpressing the c-di-GMP degradation gene (RP401S). (B) c-di-GMP production. (C). Correlation of c-di-GMP production and biomass production. The error bars represent the standard deviation of triplicate cultured samples.**

High biofilm formation of integrant SCVs. In addition, RP401S showed meaningful differences in comparison to the 57RP WT strain in both conditions with or without arabinose. This high ability to form biofilm of RP401S displayed one of the main characteristics of small colonies like SCV2h generated from 57RP WT cells grown on hexadecane (Figure 4.19).

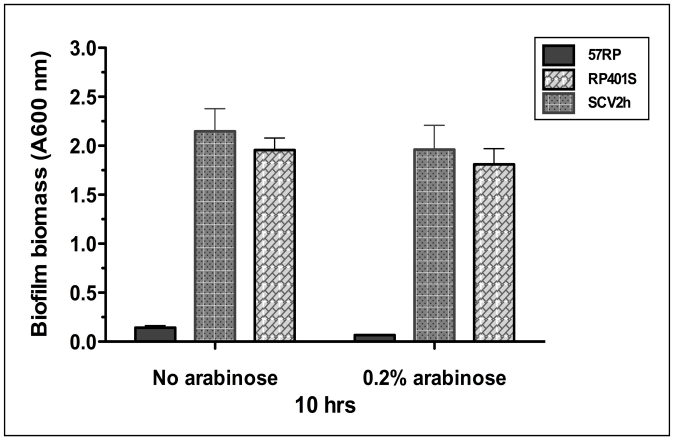


Figure 4.19 **Biofilm formation by RP401S, a small colony variant generated from its integrant containing the gene encoding for EAL domain protein in comparison with 57RP wild type and SCV generated from 57RP WT, which were cultured in Bushnell-Hass medium supplemented with 0.2% dextrose and 0.5% tryptone at 25^{o}C for 10 hrs in polystyrene tubes in the absence or presence of 0.2% arabinose. The error bars represent the standard deviation of triplicate cultured samples.**

4.4 Proteome analysis of SCV2h by 2-D gel electrophoresis

4.4.1 Total proteins

57RP WT and SCV2h were cultured in MSM supplemented with 1% glucose as a sole carbon source and their total protein extraction was performed at the beginning of the stationary phase. The DNAse-treated and phenol-extracted proteins were separated by 2-D gel electrophoresis (Figure 4.20). There were several proteins that showed an increase or decrease in production in SCV2h cells when compared to samples from 57RP WT. Some of these proteins were identified by mass spectrometry. Among the representative proteins with different functions there were the electron transfer flavoprotein (EtfA), the arginyl synthase (ArgS), the glutamine synthase (GlnA), the alginate oligosaccharide synthase (PA1787) and the pyruvate dehydrogenase (AceE) (Table 4.2).

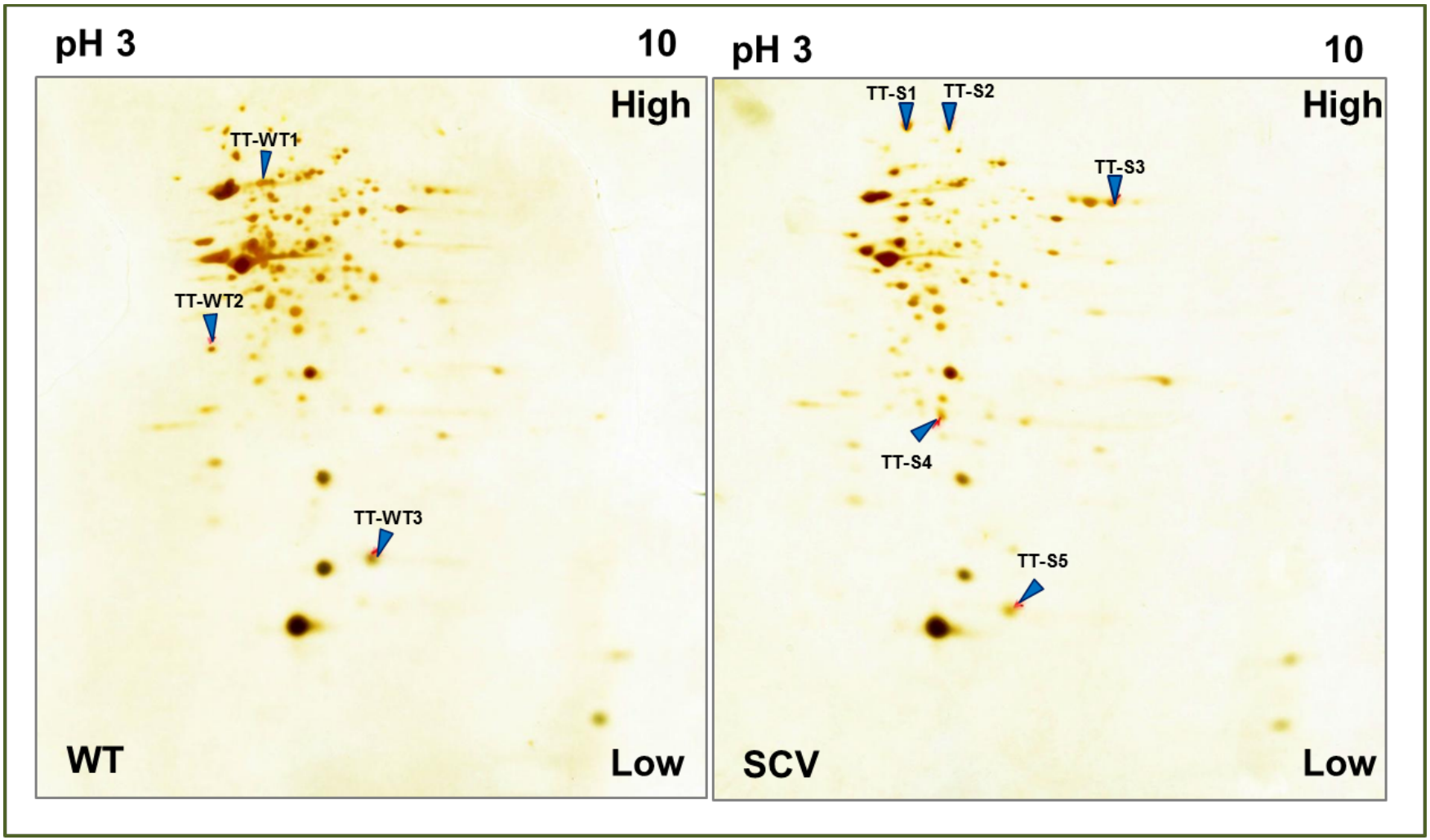


Figure 4.20 **2-D gel electrophoresis of total soluble proteins of 57RP-WT and SCV2h. The bacterial strains were grown on MSM supplemented with 1% glucose at 25^{o}C for 18 hrs.**

Table 4.2 **Identification of differentially expressed proteins between 57RP WT and SCV2h. The chosen protein spots are illustrated in figure 4.20 and identification was performed by Q-Trap mass spectra on a hybrid linear ion trap-triple quadruple mass spectrometer, peptide mass mapping with *P. aeruginosa* protein banks.**

Spot	Locus*	Mascot score	Gene	Compartment**	Predicted		Product/Annotation
					pI	Molecular weight (kDa)	
Up in 57RP wild type							
TT-WT1***	PA3637	498	*pyrG*	PP/CP‡3	5.38	59.6	CTP synthase
	PA5051	492	*argS*	PP‡2	5.24	65.2	Arginyl-tRNA synthetase
	PA4385	466	*groEL*	OM‡1,2	4.76	57.1	Heat-shock, chaperonin GroEL
	PA3471	424	*sfcA*	CP‡3	5.18	62.4	Malate dehydrogenase
	PA2935	366		Unknown	9.36	18.2	Electron transfer flavoprotein-ubiquinone oxidoreductase
	PA5119	307	*glnA*	OM‡1/CP‡2	4.95	51.9	Glutamine synthetase
	PA4123	182	*hpaE*	CP‡3	6.38	53	5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase
TT-WT2	PA2951	636	*etfA*	PP‡1	4.75	31.4	Electron transfer flavoprotein α-subunit; Induction β-oxidation

TT-WT3	PA3785	216		Unknown			Conserved hypothetical protein
	PA2951	212	*etfA*	PP‡1	4.75	31.4	Electron transfer flavoprotein α-subunit; Induction β-oxidation
Up in SCV2h							
TT-S1	PA1787	1736	*acnB*	OM‡1	5.01	93.6	Bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase
TT-S2	PA5015	1203	*aceE*	OM‡1	5.59	99.6	Pyruvate dehydrogenase subunit E1
TT-S3	PA0519	1020	*nirS*	PP‡1	8.21	62.7	Nitrite reductase precursor, cytochrome cd1 oxidase
TT-S4	PA5521	621		CP‡2	5.77	26.9	3-dihydroxy-2,3-dihydrophenylpropionate dehydrogenase
	PA1584	354	*sdhB*	IM‡1	6.92	26.2	Succinate dehydrogenase iron-sulfur subunit
TT-S5	PA1746	130		CP‡3/OM Vesicle‡1	6.63	18.2	Hypothetical protein, possible RNAse III
	PA2807	73		Unknown	7.90	22.5	Hypothetical protein, putative copper-binding protein

*PAO1 strain number. Annotation on www.pseudomonas.com, see Winsor *et al.* (2011).

**Compartment determined base on www.pseudomonas.com, (CP = Cytoplasm, PP = Periplasm, IM = Inner membrane, OM = Outer membrane; ‡1: Protein localization demonstrated in *P. aeruginosa*, ‡2: Protein localization demonstrated in other organisms, ‡3: Protein localization by computational prediction.)

***TT = Total protein

4.4.2 Outer membrane and periplasmic proteins

In order to further characterize the specific proteins, which are localized in the outer membrane (OM) and periplasm (PP) of the SCV cells growing on 1% hexadecane, the OM and PP proteins of the cultured SCVs were extracted separately. To facilitate the selection of protein spots representative of cell membrane and periplasmic proteins characteristic of 57RP SCV grown on hexadecane, the two similar protein fractions were also isolated from 57RP WT cells grown on glucose and resolved by 2-D gel electrophoresis. By comparing two 2-D gels with similar protein fractions, the coincident presence of various housekeeping proteins, which were expressed by the corresponding genes essential for cellular life (reviewed in Gil *et al.* (2004)) in both cell types though grown in different conditions, were recognized and avoided from the selection of representative protein spots for further analysis of SCV grown on hexadecane.

The mid-log phase time was chosen as the time to collect the biomass for protein extraction from the two WT and SCV samples. This occurred after 10-11 hrs with 57RP WT cells cultured on glucose in MSM at 25^{o}C (Figure 4.21) and after 9 days with SCV2h grown on hexadecane in MSM at 25^{o}C as observed previously (Figure 4.11A). Different protein spots of interest were isolated from the 2-D gels of SCV2h (Figure 4.22-4.24). The identification of several initially representative proteins by mass spectrometry is presented in Table 4.3. The identified proteins were from different categories and cell compartments (outer or inner membranes, periplasm as well as cytoplasm), including proteins involved in the transportation, anti-oxidation, regulation of nitrogen utilization, unknown proteins and, especially, the LipA isoforms.

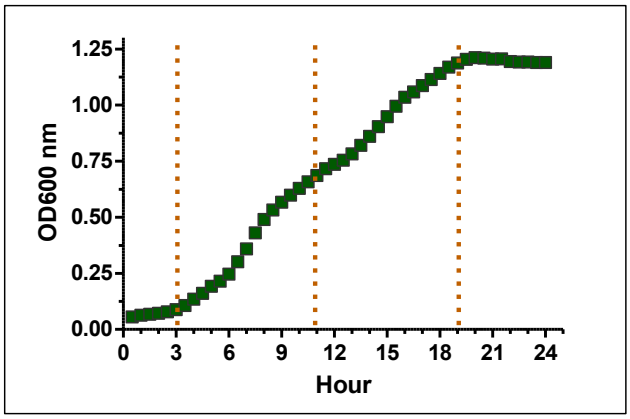


Figure 4.21 Growth of 57RP WT on MSM supplemented with 1% glucose at 25°C.

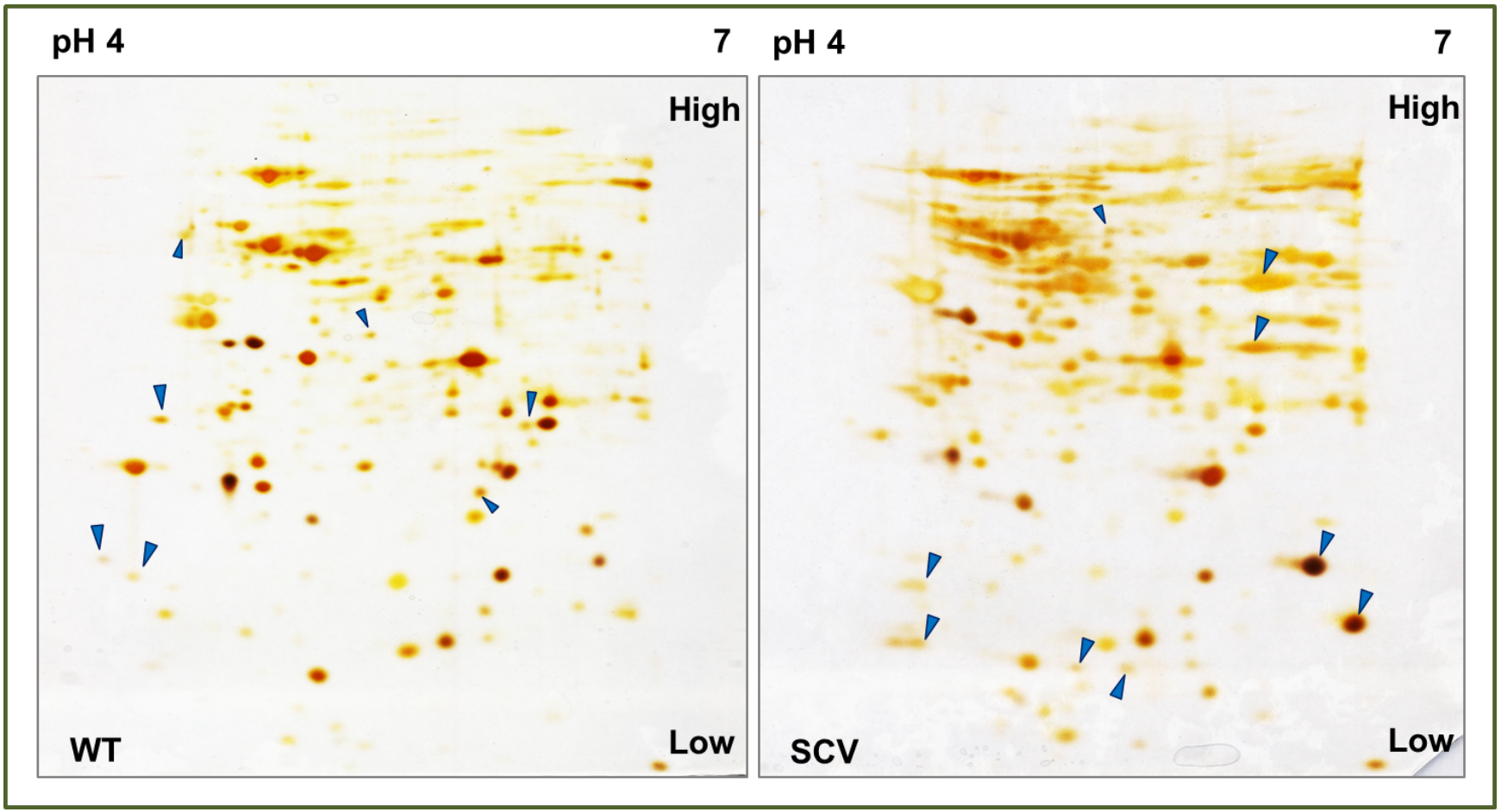


Figure 4.22 **2-D gel electrophoresis of the cytosolic proteins of 57RP-WT and SCV2h. The 57RP-WT was grown in MSM supplemented with 1% glucose for 11 hrs, and SCV2h in MSM + 1% hexadecane for 9 days at 25^{o}C. Arrows indicate protein spots of interest that were isolated for identification.**

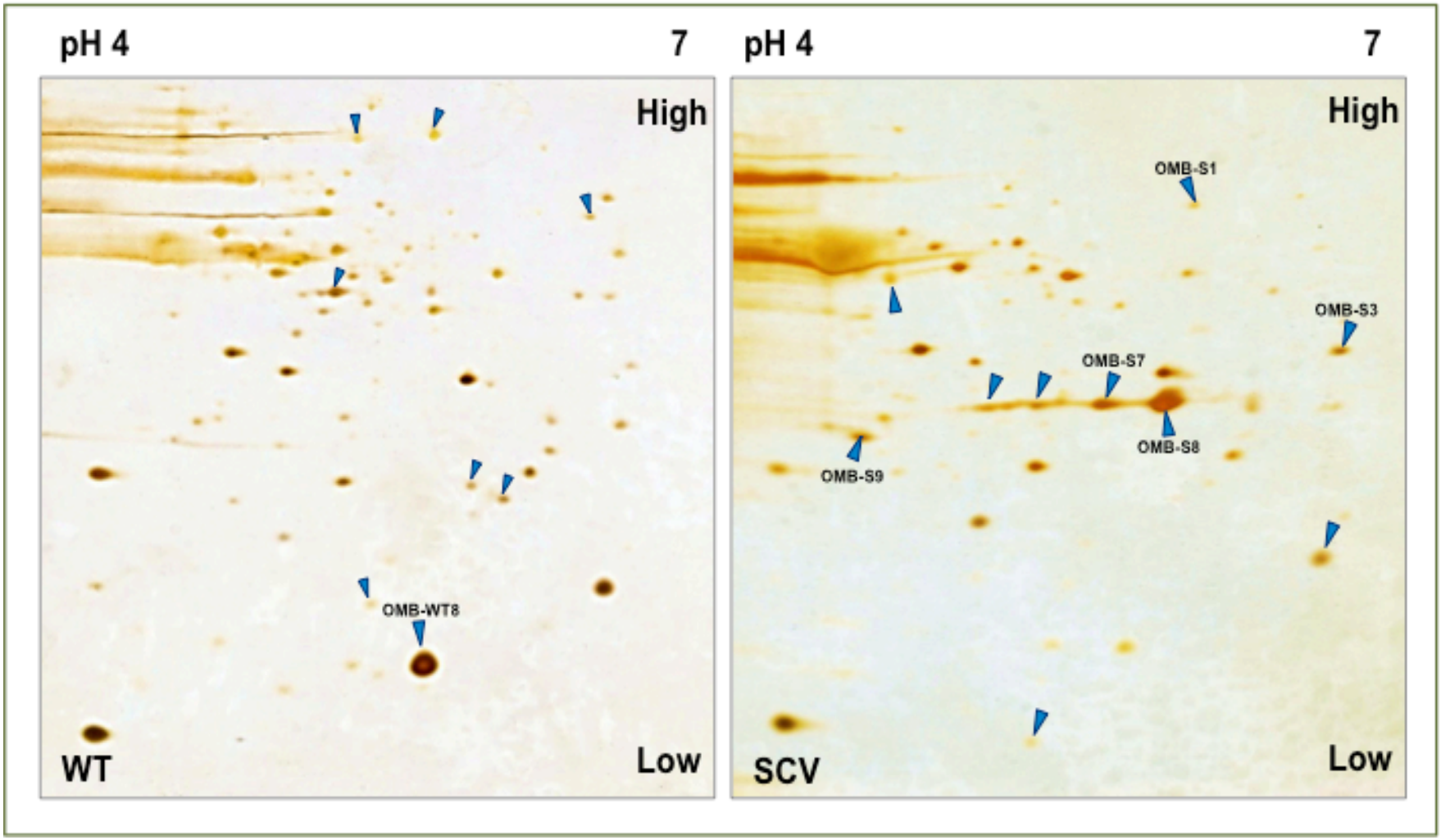


Figure 4.23 **2-D gel electrophoresis of the outer membrane proteins of 57RP-WT and SCV2h. The 57RP-WT was grown in MSM supplemented with 1% glucose for 11 hrs, and SCV2h in MSM + 1% hexadecane for 9 days at 25°C. Arrows indicating protein spots of interest were isolated for identification.**

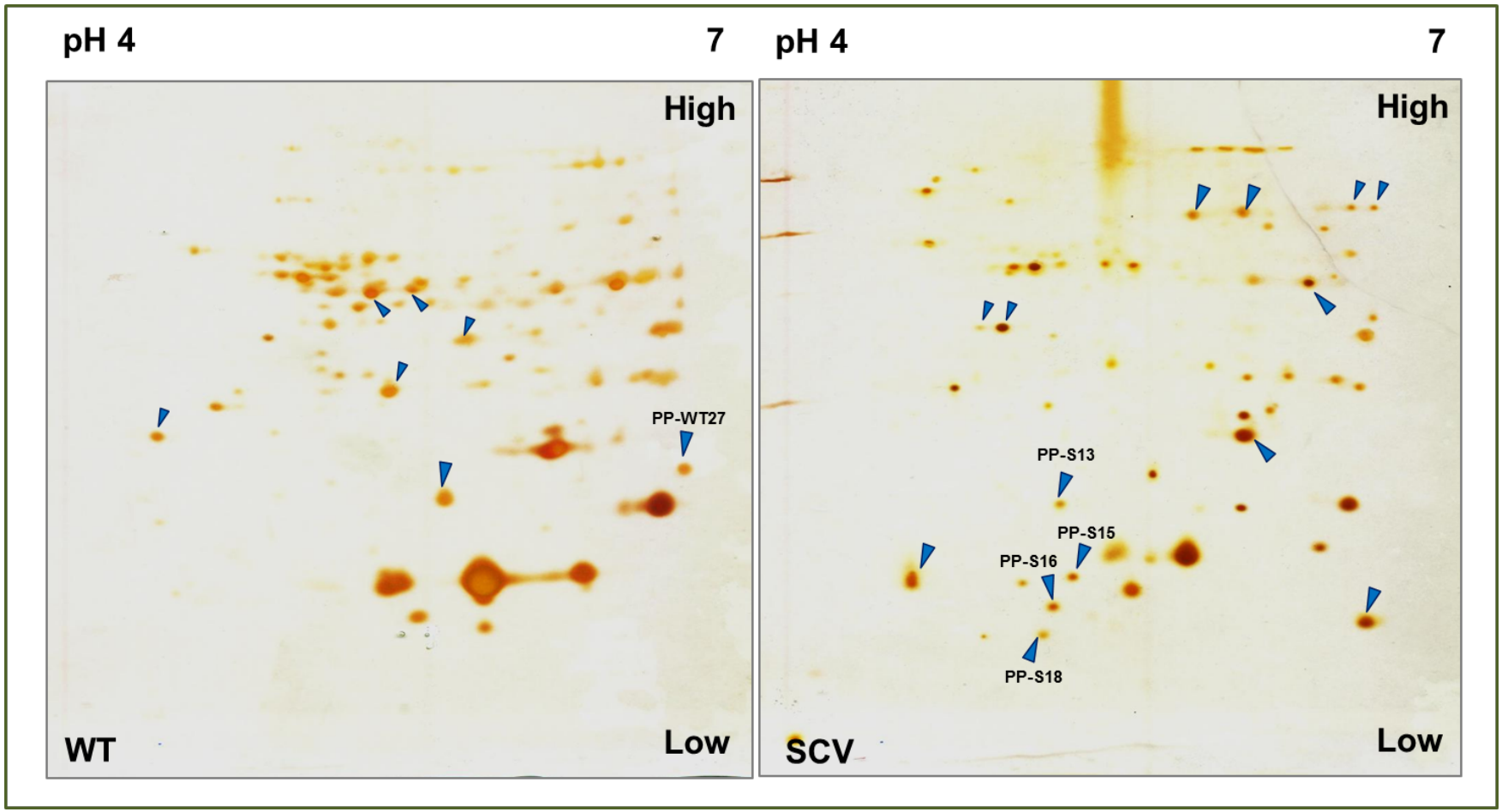


Figure 4.24 **2-D gel electrophoresis of the periplasmic proteins of 57RP-WT and SCV2h. The 57RP-WT was grown in MSM supplemented with 1% glucose for 11 hrs, and SCV2h in MSM + 1% hexadecane for 9 days at 25°C. Arrows indicating protein spots of interest were isolated for identification.**

Table 4.3 **Identification of representative proteins isolated from 2D-gels of SCV2h grown on hexadecane. The chosen protein spots are illustrated in figures 4.22-4.24 and identification was performed by Q-Trap mass spectra on a hybrid linear ion trap-triple quadruple mass spectrometer, peptide mass mapping with *P. aeruginosa* protein banks.**

Spot	Locus*	Mascot score	Gene	Compartment**	Predicted		Product/Annotation
					pI	Molecular weight (kDa)	
OMB-S1***	PA4589	341	*fadL*	OM‡2	6.26	49.7	Long-chain fatty acid transport protein; Homologous to *E. coli* FadL transporter; Probable outer membrane protein precursor in *P. aeruginosa*.
OMB-S3	PA3836	380		PP‡1	8.68	34.2	Putative ABC-type transport protein
OMB-S7	PA2862	60	*lipA*	OM‡1	6.88	32.7	Lactonizing lipase precursor (triacylglycerol lipase)
OMB-S8	PA2862	379	*lipA*	OM‡1	6.88	32.7	Lactonizing lipase precursor (3 amino acid different with OMB-S7)
OMB-S9	PA5153	84		PP	4.9	27.6	Amino acid (lysine/arginine/ornithine/histidine/octopine) ABC transporter periplasmic binding protein
OMB-S12	PA2862	82	*lipA*	OM‡1	6.88	32.7	Lactonizing lipase precursor (6 amino acid different with OMB-S7)

PP-S13****	PA2532	170	*tpx*	PP‡3	4.91	17.2	Thiol peroxidase,
PP-S15		< 60					No significant homology in Mascot database
PP-S16	PA5339	172		CP‡3	4.81	13.6	Putative endoribonuclease
	PA0388	109		Unknown	7.37	15.3	Hypothetical protein: putative homoserine O-acetyltransferase
PP-S18	PA5288	89	*glnK*	IM‡3	5.22	12.3	Nitrogen regulatory protein

*PAO1 strain number. Annotation on www.pseudomonas.com, see Winsor *et al.* (2011).

**Compartment determined base on www.pseudomonas.com. (CP = Cytoplasm, PP = Periplasm, IM = Inner membrane, OM = Outer membrane; ‡1: Protein localization demonstrated in *P. aeruginosa*, ‡2: Protein localization demonstrated in other organisms, ‡3: Protein localization by computational prediction.)

***OMB: From outer membrane fraction

****PP: From periplasmic fraction

5 DISCUSSION

5.1 P. aeruginosa 57RP: the selected research model strain

5.1.1 Growth modes on different alkanes

As it was mentioned previously (section 2.9), several *P. aeruginosa* strains have been isolated in the laboratory of Prof. R. Villemur for their capacity of degrading hydrocarbons and for their ability to produce biosurfactants (Déziel, 2001, Déziel *et al.*, 2001). Due to the long-term storage (more than 10 years) of these strains as frozen stocks under laboratory conditions, the first assays performed were necessary to confirm the physiological characteristics of the strains cultivated on alkanes that were previously observed (Déziel, 2001). Attention was drawn on the capacity of generating SCVs by these strains in response to the change in carbon sources to grow better in a specific cultivation environment. Firstly, to test the growth ability of these strains on long chain hydrocarbons, two *P. aeruginosa* strains, 57RP, a representative strain of slow growth with low cell hydrophobicities, and 57RV, a representative strain of fast growth with high cell hydrophobicities, were chosen for the present project. These "rapid" and "slow" phenotypes were noticed by Déziel in his thesis (2001). Strain PA14, a model *P. aeruginosa* strain was also used occasionally. However, *P. aeruginosa* PA14 grew poorly on alkanes, and this strain was not used further in subsequent experiments. The initial growth cultures on hexadecane and octadecane to differentiate the two investigated strains 57RP and 57RV were carried out in rotating conditions at 30^{o}C. After the initial confirmation of growth capacity on these hydrocarbons, growth cultures of all investigated strains and related cell types were carried out in gyratory shaking conditions (200 rpm) at 25^{o}C.

Slow growth strain 57RP. The preliminary results carried out on strain 57RP showed a lag phase during the first 3 days of cultivation on hexadecane and the growth was slightly increased on day 5 (Figure 4.1A). In addition, this strain could grow also on the solid alkane octadecane (Fig 4.1A & B), but no lag phase was observed under this condition. These different growth modes, depending on the state of carbon sources (liquid

vs. solid), were in accordance with previous results obtained in our laboratory (Déziel, 2001). Strain 57RP grew faster on octadecane in comparison with hexadecane in shaking conditions. This strain showed slower growth on octadecane in static conditions compared to the shaking cultures (Figure 4.1B). This is likely due to the fact that the contact surface between the cell surface and the solid substrate was higher in the shaking conditions than that in the static culture. As alkane metabolism is an oxygen-driven process (Rojo, 2009) and *P. aeruginosa* is an aerobic alkane-degrading species (reviewed in Wang and Shao (2013)) the shaking conditions would have increase the oxygen availability to the cultured cells compared to the static conditions.

Rapid growth strain 57RV. In the case of 57RV cultivated with 1% hexadecane in rotating culture conditions at 30^{o}C, no obvious lag phase was observed (Figure 4.1A) as compared to that of shaking culture conditions at 25^{o}C (Figure 4.11). Therefore, strain 57RV was used only in comparison to strain 57RP in certain cases in this study.

5.1.2 Common physiological characteristics

Based on a series of phenotypes previously investigated (Déziel 2001), several common physiological traits, including motilities (swimming, swarming and twitching), biofilm formation, cell hydrophobicity, adherence to surface, pili formation, and rhamnolipid production, were tested.

Motility. Strains 57RP and 57RV were tested for swimming, swarming and twitching motilities. In swimming, the turbid zone of strain 57RP was slightly larger than that observed for strain 57RV. For swarming, the tendrils of strains 57RP and 57RV did not reach to the edge of the Petri dish, in contrast with *P. aeruginosa* PA14 (data not shown). The twitching motility expansion zones of strains 57RP and 57RV were about equal. There was no significant cell attachment to polystyrene Petri dishes with either strains. These results also were in agreement with previous results (Déziel, 2001).

Biofilm formation. The level of biofilm generated by strain 57RP was much less abundant than that of strain 57RV. The results were in agreement with previously obtained results (Déziel *et al.*, 2001).

Adherence. Strain 57RV showed a stronger ability to attach to hydrocarbons than strain 57RP in the MATH assays. Furthermore, strain 57RV also showed stronger adherence to silica than strain 57RP in the MATS assays. These data were in agreement with the previous assessment (Déziel, 2001) that the cell surface of strain 57RV has a higher level of adherence by having a higher hydrophobicity membrane (adherence to hexadecane) and higher adherence to a substratum. The reason for this better adherence of fast strains vs. slow strains is not known.

Rhamnolipids. Rhamnolipids produced and released into the culture medium containing hexadecane by *P. aeruginosa* were also examined for both, the slow 57RP and the fast 57RV strains. The initially low and similar concentrations of extracellular rhamnolipids through the culture period of the 57RP WT, the 57RP SCV and the 57RP Rev cells indicate that 57RP cells did not require a new production of the biosurfactant for growth on hexadecane. As the 57RP strains were previously shown to be good rhamnolipid producers under the other conditions, such as SW1/10F mineral salts medium with 2% mannitol agitating at 30^{o}C (Déziel, 2001, Déziel *et al.*, 2001), the present culture conditions (MSM with 1% hexadecane shaking at 25^{o}C) were obviously not appropriate for a significant level of rhamnolipid production. For example, limiting iron, 30-37°C incubation, strongly aerated conditions, are the promoting factors for a maximal production of rhamnolipids (Abdel-Mawgoud *et al.*, 2011), and these growth factors were absent in the present study. The 57RV cells differentiated from the three 57RP cell types by a slight increase in the production of extracellular rhamnolipids (~4 ppm) that was observed only on the 13th day, about the middle of the exponential growth phase, after 11 days of their lag growth phase with no new production of the surfactant. Our results indicate that *P. aeruginosa* cells may or may not need to produce new extracellular rhamnolipids to be able to grow on hexadecane, depending on their genotypes, the selected phenotypic variants, the growth conditions used, etc.

5.1.3 *P. aeruginosa* 57RP generating SCVs

SCV formation on hexadecane. As expected (Déziel *et al.*, 2001), SCVs were observed with strain 57RP generally after 5-7 days of cultivation on hexadecane. Culturing these SCVs on rich medium (TSB) resulted in the occurrence of revertants.

SCV formation on octadecane. SCVs were also observed after 7-9 days of cultivation of strain 57RP on solid octadecane. The appearance of these SCVs on octadecane was not observed in previous studies (Déziel, 2001). There were a few investigations on the cultivation of *P. aeruginosa* on octadecane and no information on SCV formation had been provided (Hua *et al.*, 2013, Y Zhang & Miller, 1992, Y Zhang & Miller, 1994). The 57RP SCVs derived from hexadecane and octadecane cultures were small, rough and dry surface colonies. They could revert to wild type phenotype.

In the case of strain 57RV (strain used as an occasionally comparative strain to 57RP), at least 3 independent triplicate experiments were performed on hexadecane and the same results were obtained, which showed that strain 57RV also generated SCVs on this alkane (data not shown). This was a new observation with strain 57RV.

The above mentioned results suggested that capacity of forming SCVs under specific environmental conditions are a common characteristic of *P. aeruginosa* and, in our case, strain 57RP possesses the typical capacity of forming SCVs on both hexadecane and octadecane. In a more recent study, relating the growth and biodegradation of octadecane by *P. aeruginosa*, Hua and coworkers (2013) also investigated the uptake and transport of octadecane through the cell membranes into the cytoplasm. These authors did not provide any clear information on the formation and growth ability of the adaptive cell type(s), which might reflect the real interactions between the bacterial adapted cells and the actual environmental conditions. Zhang and Miller studied the effects of rhamnolipids on octadecane dispersion, cell hydrophobicity and biodegradation of several slow and rapid growth *P. aeruginosa* strain with low and high cell hydrophobicities, respectively, but these authors also did not report SCV formation during growth of the investigated strains (Y Zhang & Miller, 1992, Y Zhang & Miller, 1994).

Our observation of the SCV formation on solid and liquid alkanes of both strains, the fast strain with high hydrophobic cell surface (57RV) and the slow strain with low hydrophobic cell surface (57RP), contributes, in part, to explain a previously posed question: Do all *P. aeruginosa* strains form S (small) variants or only those that are naturally weakly adherent (Déziel, 2001)? The answer now is yes: both of the representative strains: the highly adhesive (57RV) and the less adhesive (57RP) *P. aeruginosa* strains could form highly adaptive SCVs and their observable occurrence was dependent on concrete growth condition(s) (for example: MSM culture medium with 1% hexadecane shaken at 25^{o}C used in the present study) that contribute to increase the phase variation rate, select and support the maintenance of the physiological state of the appearing SCVs to be observable. The observation that strain 57RV forms SCVs in the present study was in accordance with the current knowledge of the effects of different environmental stimuli, including the composition of culture medium, the growth temperature, etc. on the rate of appearance of phenotypic variants reported previously (Drenkard & Ausubel, 2002).

Results obtained from the above-mentioned tests with strain 57RP, confirm that strain 57RP still retained its common physiological characteristics even after more than 10 years of frozen preservation under laboratory conditions. Thus, *P. aeruginosa* strain 57RP was chosen as the research model strain for the main part of the study. Three SCVs colonies generated from each cultivation condition on either hexadecane or octadecane were collected and used for further experiments.

5.2 Common characteristics of SCVs

To investigate the common characters of SCVs, which were generated from strain 57RP in an established condition, several physiological characters of SCVs as mentioned above (section 5.1) were examined in comparison with their parent WT cells.

5.2.1 Motility

The three chosen 57RP SCVs generated from cultivation on hexadecane had a reduced level of swimming, swarming and twitching motilities. In swimming, the turbid zones of the SCVs were less than 3-fold of those zones of WT and revertants (Figure 4.3B), as expected (Déziel *et al.*, 2001). The swarming zones of the WT and the Rev21h were larger than these zones with the SCVs, which were small and concentrated on the center of Petri dishes (Figure 4.3A). No tendrils were observed in the swarming SCVs. Though twitching motility was reduced in all SCVs but the ability of cell attachment to polystyrene Petri dishes increased in comparison to WT and Rev (Fig 4.3A). Thus, all three selected SCVs have similar reduced motility phenotypes.

The same experimental procedures were performed with SCVs derived from octadecane cultivation. In swimming, turbid zones were less than twice in comparison with WT and Rev11o. Thus, the swimming ability of SCVs derived from octadecane was higher than SCVs derived from hexadecane. Similar to SCVs derived from hexadecane, all SCVs had a reduced swarming activity in comparison with the WT and its revertants. Interestingly, though all of the three selected SCVs also had a reduced twitching activity, the attachment of the cells to polystyrene Petri dishes was much stronger than that of SCVs derived from hexadecane cultures (Figure 4.4A). To summarize, all three SCVs derived from the cultivation with octadecane were less active in motilities than WT and revertants.

Swimming activities. Bacterial swimming relies on the activity of flagella, a motility organelle of flagellated bacteria, which enables movement and chemotaxis. *P. aeruginosa* has a single polar flagellum. In *P. aeruginosa,* motile activities of flagella and pili are important for bacterial movement, colonization of the surface and development of biofilm (Anderson *et al.*, 2007, Kazmierczak *et al.*, 2006). The bacterial flagellum is a complex apparatus composed of more than 20 different proteins. The flagellar basal body traverses the cell wall, whereas the curved hook connects the basal body to the whip-like flagella filament that protrudes several micrometers from the bacterial cell (Haiko & Westerlund-Wikström, 2013). Flagellar motile activities increase or decrease, depending on the planktonic and sessile lifestyle of the cell. Several factors contribute to the activity

of flagella. One of the proteins that participates in the regulation of flagellar gene transcription is FlhF. FlhF is one of three members of the signal recognition particle (SRP)-GTPase subfamily of SIMIBI-class nucleotide binding proteins (Murray & Kazmierczak, 2006, Schniederberend *et al.*, 2013). In *P. aeruginosa*, the loss of FlhF results in defective swimming and swarming motility of bacteria in comparison with their parent WT cells (Murray & Kazmierczak, 2006). In addition, swimming activities of bacteria are also regulated by other factors. For example, tyrosine phosphate TpbA (PA3885) displays its regulatory effects on biofilm formation (Ueda & Wood, 2009). Loss of TpbA decreased swimming, abolished swarming and attachment, although this does not affect production of rhamnolipids. The *tpbA* mutant had a wrinkly colony phenotype (SCV), formed a pronounced pellicle, had substantially more aggregation and had 28-fold more exopolysaccharide production (Ueda & Wood, 2009). In another study, investigating the GacS/GacA two-component regulatory system in *P. aeruginosa* (PA14), which regulates genes involved in virulence, secondary metabolism and biofilm formation, Davies and coworkers (2007) found that the loss-of-function mutation in GacS (*gacS*$^{-}$) was associated with hypermotility, reduced production of acylhomoserine lactones, impairing the biofilm maturation, and decreased antimicrobial resistance. However, like the other colony morphology variants described in the literature, SCVs that occurred in the biofilm of a GacS^{-} mutant strain were less motile than the wild-type strain and exhibited autoaggregating behaviors in broth culture (JA. Davies *et al.*, 2007). SCVs of *P. aeruginosa,* which developed in cystic fibrosis lung infection (Haussler *et al.*, 2003, Kirisits *et al.*, 2005), and the SCVs derived from the present study all exhibited reduced swimming activity in comparison with their WT and revertant cells.

Swarming activities. The swarming motile activity in *P. aeruginosa* requires flagella, and the self-production of biosurfactants to occur (Déziel *et al.*, 2003). *P. aeruginosa* cells require the production and release of RLs, including mono-RL and di-RLs, and HAAs which are synthesized with RhlA, RhlB and RhlC. Expression of *rhlA, rhlB* and *rhlC* is regulated by the QS system. HAAs act as wetting agents and provide chemotactic-like stimuli (Déziel *et al.*, 2003, Tremblay *et al.*, 2007). However, results gained from recent studies indicated that, *rhlAB* was more expressed in the swarm center

than at the tendril tips. The results implied that RLs were primarily produced by swarming cells in the center of a swarming colony (Tremblay & Deziel, 2010). The role of TFP in swarming is likely strain-dependent and still debatable while swarming motility was positively associated with the secretion of both proteases and type 3 exoenzymes (Murray *et al.*, 2010). In the present study, as well as in previous studies carried out on the same *P. aeruginosa* strains in our laboratory (Déziel, 2001), SCV cells swarmed at a lower velocity than the WT and revertant cells. It is possibly due to the presence of fimbrial adhesin of hyperpiliated SCV cells that made autoaggregation.

Twitching activities. Twitching movement is involved in the initiation of biofilm formation by *P. aeruginosa* (O'Toole & Kolter, 1998). In *P. aeruginosa*, approximately 40 genes are required for normal twitching motility such as *pilA* (Mattick, 2002). However, under certain conditions, for example in citrate minimal medium, Klausen and his coworkers suggested that flagella and TFP do not play a role in biofilm formation (Klausen *et al.*, 2003). Up to now, there are several models, which have been proposed to explain the process of biofilm formation. One of them supports the hypothesis that biofilm development depends on carbon source and is not necessary dependent on the participation of TFP (Klausen *et al.*, 2003). Indeed, the mutants, Δ*pilA* (*pilA* codes the TFP precursor), Δ*fliM* (*fliM* codes for the flagellar motor switch protein) and Δ*pilA*Δ*fliM* of *P. aeruginosa* PAO1, were still able to form biofilms with citrate minimal medium similar to that of wild type PAO1. Another model shows that bacteria use flagella to move in the surface fluid then form microcolonies and later form mushroom structures (Costerton *et al.*, 1999, Sauer *et al.*, 2002). Biofilm microcolonies will maintain their mushroom-shaped structure until the deficiency of nutrients occurs and will return to the planktonic form (free-floating, swimming state) (Barken *et al.*, 2008). In our study, the twitching zone of SCV cells, seen on the agar plate surface, was obviously smaller than the twitching zone of both WT and revertant cells, however, on the (upside-down) bottom surface it was larger than those of the other strains (Fig. 4.4 & 4.5). This phenomenon may be connected to a certain elevated level of attachment of SCV cells to both abiotic and biotic surfaces. TFP are not only used for twitching motility but also for the attachment of the bacterial cells to both abiotic and

biotic surfaces (Mattick, 2002). An assessment showed that TFP may contribute up to 90% of attachment of *P. aeruginosa* to human lung pneumocyte A549 cells (Hahn, 1997).

Besides, the expression of several genes involved in pili formation, the expression of other genes, such as *cupA* (Meissner *et al.*, 2007), was also reported to be associated with the formation of mutational SCVs. In PA14, expression of the *cup*D fimbrial genes are antagonistically controlled by the RocR, RcsB or the EAL-containing PvrR response regulators (Mikkelsen *et al.*, 2009). The active expression of the *cupD* genes was obviously associated with the small colony morphotype, increased biofilm formation and decreased motility. The presence of cup fimbriae, in addition to the TFP, on the cell surface of SCV supported the electronic observation that SCVs were hyperpiliated cell types compared to the WT Rev cells of the 57RP strain.

In the present study (Figure 4.10), as well as in a previous study (Déziel *et al.*, 2001), the SCVs derived from strain 57RP are hyperpiliated with a reduced twitching zone but with high levels of adherence to the Petri dish surface, compared to the wild-type and revertants. Both TFP and cup fimbriae of the SCV cells could attach not only to the abiotic surface but also to the surface of other cells in the population. The self-aggregation could have resulted in the reduction of twitching zone of SCV2h in comparison with the twitching zones of WT and revertant cells that showed no obvious adherence to the surface of Petri dishes. Though this observed phenomenon may explain in part the smaller swimming and swarming zone of SCVs in comparison with that of WT and Rev cells beside the reduction of flagellar gene expression observed in the *P. aeruginosa* SCVs (Kirisits *et al.*, 2005), it requires further investigations to define the contribution of the cup fimbriae and the TFP to the reduction of certain motile activities of SCV cells.

5.2.2 Biofilm formation of SCVs

The biofilm formation assays performed showed that the 57RP SCVs derived from hexadecane cultures produced higher levels of biofilm than the WT and the Rev21h

revertants. After 6 hrs of incubation, the SCVs biofilm was approximately 3 to 5-fold more abundant than the biofilm of WT and Rev21h, and 10 times more abundant after 10 hrs (Figure 4.7). The Rev21h showed a similar level of biofilm formation to that of the WT strain. The results are in agreement with other publications (Déziel *et al.*, 2001, Haussler *et al.*, 2003).

The same experiments were set up for all SCVs derived from cultivation on octadecane. After 10 hrs of incubation, the biofilm of SCVs increased 10 to 12-fold in comparison with those of WT and Rev11o. These results confirm the high capacity of SCVs to form biofilms, which derived from the cultivation on octadecane, as well as SCVs derived from cultivation on hexadecane.

SCVs having a higher capacity of forming biofilms in comparison to their WT are a common phenomenon in *P. aeruginosa* (Haussler *et al.*, 2003, Ikeno *et al.*, 2007, Kirisits *et al.*, 2005). In our case, the 57RP SCVs developed on hexadecane and octadecane formed biofilms much better than their WT and revertants strains.

The model is that a certain proportion of the 57RP cell population differentiates into SCV cells from the parental WT cells. Hyperpiliation, in addition to the other cell surface attached components like secreted proteins, etc., contribute to increase the capacity of attaching SCVs to the hydrophobic substrate to uptake nutrients for growth and biofilm development.

5.2.3 High hydrophobicity and adherence of cell surface in SCV

Two assays, MATH and MATS, were used to evaluate the cell surface hydrophobicity and its substrate surface adherence. Approximately sixty percent of SCV2h cells adhered to hexadecane, 20% more than that of WT or Rev21h in the MATH test. This result indicates that the cell surface of SCV2h was more hydrophobic than WT or Rev21h. On the other hand, the ability to adhere to silica in the MATS test of SCV cells reached 80%, approximately 30% higher than WT and Rev21h. These results were in accordance

with previous observations carried out on the same strain and suggest that, besides the contribution of TFP and putatively cup fimbriae, the cell hydrophobicities would play a substantial role in the increase in adherence of SCVs to various surfaces, both hydrophobic (e.g. hexadecane) and hydrophilic (e.g. silica) surfaces, that are present in the surrounding environment (Déziel *et al.*, 2001).

Similar results were obtained from the evaluation of cell surface hydrophobicity and cell adherence to silica with SCV1o derived from cultivations on octadecane. However, the capacity to adhere to silica of the SCV1o and its revertant was lower than that of SCV2h, which developed on hexadecane and its derived revertant.

There are very few reports on growth of *P. aeruginosa* on octadecane. Zhang and Miller (Y Zhang & Miller, 1992, 1994) studied the effect of *Pseudomonas*-produced rhamnolipids on octadecane dispersion, cell hydrophobicity and biodegradation. However, these authors gave no information on the SCV formation of the bacteria on octadecane. *P. aeruginosa* SCV cells were found to have elevated their cell surface hydrophobicity in comparison to their parent WT cells (Haussler *et al.*, 2003, Kirisits *et al.*, 2005). Our results are in accordance with these observations and indicate that 57RP SCV cells had a higher capacity of attaching to the hydrophobic substrate surface than their parental WT and Rev cells. Interestingly, the level of adherence of cell surface to silica (provided by the MATS assays) of SCV2h and its revertant Rev21h was also higher than that of SCV1o and its revertant Rev11o. It is possible they were isolated from two different culture conditions. SCV2h isolated from hexadecane culture condition and SCV1o isolated from octadecane culture condition.

In the case of the 57RV WT cells, the levels of both cell surface hydrophobicity and adherence to silica were very high and almost similar to those of 57RP SCV2h (Figure 4.9A, B). This observation indicates first that 57RV WT cells have a naturally high capacity of attaching to the hydrophobic substrates. This strain had a tendency of delaying the cell differentiation into SCV cells from the parent WT cells as the first SCVs were observed only from day 9-11 of cultivation on hexadecane. The occurrence of SCVs coincided also with the start of the exponential growth in 57RV culture with the tested

alkane (Figure 4.11A) like the case of strain 57RP cultured under the same conditions. Due to the fact that 57RV was not the main strain and was only occasionally used to compare with 57RP, the main investigated strain, the other specific traits, including c-di-GMP production, cell surface-associated proteins, extracellular rhamnolipid production, pili and motilities of 57RV WT and SCV were not analyzed in detail as was done with strain 57RP and its variants in the present study.

5.2.4 Pili are formed at high density on the cell surface of SCV

Type IV pili (Tfp) are involved in the initiation of biofilm formation (Costerton *et al.*, 1999, Kohler *et al.*, 2000). Beside the increase in expression of various TFP genes observed in SCV cells isolated from *P. aeruginosa* biofilms (Kirisits *et al.*, 2005), the SCV cells are commonly hyperpiliated and expressed both Tfp and *cupA* fimbriae genes (Haussler *et al.*, 2003, Meissner *et al.*, 2007) or *cupD* genes in PA14 SCVs (Mikkelsen *et al.*, 2009). Our results are in favor of the hypothesis that the SCV cells are able to form pili at much higher density than the WT and Rev cells. Indeed, SCV2h showed more abundant pili than WT and Rev21h in our case. Results obtained from our present study correspond to previous observations, which were taken place in our laboratory with strain 57RP (Déziel *et al.*, 2001).

The hyperpiliated SCVs have also been observed among isolates derived from the sputum of patients with cystic fibrosis (Hausler *et al.*, 2003). This phenotypic variant is highly adhesive and has an increased ability to form biofilms (reviewed in Evans (2015a)). In the airway epithelium, the type IV pili (Tfp) of *P. aeruginosa* are necessary and sufficient for maximal binding to host N-glycans at the apical surface of polarized lung epithelial cells, whereas the distinct host receptors of flagella are heparan sulfate (HS) chains of heparan sulfate proteoglycans (HSPGs) at the basolateral surface of the same polarized lung epithelium (Bucior *et al.*, 2012). In the present study, besides the obvious involvement of pili in the biofilm mode of growth whether the dense pili would play any

role in the adherence to hydrophobic alkanes of 57RP SCV remains unknown and requires further investigations.

5.2.5 Rhamnolipid production by SCVs

The 57RP SCV strain can grow well on hexadecane, which was used as a sole carbon source, with almost no new production of extracellular rhamnolipids. The very low rhamnolipid production in culture with hexadecane of both 57RP WT, 57RP SCV and 57RP Rev reflects the genotype-dependent character of 57RP strain in response to the environment-specific stimuli that were generated by culture conditions used in the present study.

On the basis of the current knowledge of the effect of extracellular rhamnolipids, either positive or negative, and the cell surface properties, either low or high cell hydrophobicities, on the ability of hexadecane uptake of various biosurfactant-producing or biosurfactant-non-producing strains (e.g. mutants) of *P. aeruginosa* (reviewed in Section 2.4.3 *Roles of rhamnolipids*), various strains of the species could be divided into several groups. One of the groups of biosurfactant-producing strains consists of strains having relatively high cell hydrophobicities and producing a certain amount of extracellular rhamnolipids during their growth on hexadecane. The hexadecane growth-adaptive cells (likely the SCV cells) of the 57RV strain (present study) could be a candidate for membership in this first group. The second group of the biosurfactant-producing strains consists of strains that do not produce extracellular rhamnolipids in their growth on hexadecane. These strains have low cell hydrophobicities and require an appropriate addition of the biosurfactant to the growth medium for better growth on hexadecane (Zhang & Miller, 1992, 1994). The third group consists of biosurfactant-producing strains, which do not produce rhamnolipids in the culture with hexadecane but have high cell hydrophobicities, and the addition of the exogenous rhamnolipids would impair their normally fast growth on hexadecane (Zhang & Miller, 1994). The 57RP SCV strain (present study) would likely belong to this third group. In the case of biosurfactant-non-

producing strains, there are two groups that consist of different types of mutants, including laboratory mutants. The first group consists of mutants that can grow slowly on various alkanes, including hexadecane, octadecane and eicosane, without production of rhamnolipids, such as the strain 57RP-98, a mutant derived from strain 57RP (Déziel, 2001). The second group consists of mutants that can grow on hexadecane only with an appropriate addition of exogenous rhamnolipids, such as the mutant 65E12, which is unable to grow in media containing individual alkanes from C_{12} to C_{19} (Koch *et al.*, 1997). However, this initial suggestion requires further investigations to be verified in the future.

Our results indicate that the growth of 57RP SCV cells on hexadecane relied primarily on the effective attachment to the hydrophobic hexadecane for carbon uptake by their high cell hydrophobicities that resulted from the natural phase variation-induced expression of specific genes and the environmental selection for the growth-adaptive variants. Rhamnolipids certainly help disperse hexadecane in the growth medium but do not appear to be an important factor for the growth ability of 57RP SCV with this hydrophobic carbon source. This is in agreement with the previous observation that the growth of fast degraders, which possess high cell hydrophobicities and do not produce rhamnolipids on octadecane, was impaired by the addition of isolated rhamnolipids to the culture medium supplemented with this alkane (Y Zhang & Miller, 1994). In addition, 57RP-98, a rhamnolipid-non-producing mutant derived from the strain 57RP, could grow on various alkanes (C_{16}-C_{20}) without any production of biosurfactant (Déziel, 2001).

5.2.6 c-di-GMP production

The levels of intracellular c-di-GMP in cells cultured with 1% hexadecane were significantly higher in the SCV2h cells than that of both WT and revertant cells. As the SCV2h cells, which grew without a lag phase on hexadecane, the c-di-GMP production in these cells was about 0.02 μg/mg protein during the first 3-5 days of cultivation on hexadecane, and reached 0.04-0.12 μg/mg protein on days 9-13 of cultivation. In contrast, a 7-9 day lag phase occurred for 57RP WT and Rev21h cultured on hexadecane in which

c-di-GMP was barely detected. After these lag days, c-di-GMP began to be produced at a significantly higher level in these two cell types and reached to about 0.08 µg/mg protein. This level of c-di-GMP was just slightly below the level that SCV2h cells produced during the first 3-5 days of cultivation and was detected just shortly after the first SCV cells occurred in these two cell populations. At the late exponential phase, the whole WT and Rev21h cells and their derived SCV cells accumulated c-di-GMP at low levels, only about 0.08 µg/mg protein on day 13 (Figure 4.13), which were very similar to levels produced by SCV2h on day 3-5 and much lower than the level that was observed in SCV2h cells at the day 13. Very low levels of c-di-GMP (under the limit of detection of 0.027 ppm) occurred in 57RP WT, SCV2h and Rev21h cultured in rich medium.

c-di-GMP has emerged to be one of the most important and universal bacterial second messengers since its discovery in 1987 as an allosteric activator of a bacterial cellulose synthase (Ross *et al.*, 1986). The signaling role of c-di-GMP has been found in many physiological aspects of bacteria. The role of c-di-GMP is very largely, from a key activator of the motility-to-sessile transition to the regulation of biofilm formation, regulation of cell cycle and differentiation regulation of gene expression via riboswitch "on/off" and binding to various downstream effector molecules, affecting RNA turnover, and virulence, and involving other regulatory systems (reviewed in Römling *et al.* (2013)). The cellular c-di-GMP-metabolizing (synthesizing and hydrolyzing) system senses and translates internal and external signals into c-di-GMP levels. The binding of diffusible intracellular c-di-GMP to diverse target molecules affects transcriptional, translational and post-translational functions resulting in regulation of multiple cellular processes (Mills *et al.*, 2011).

An obvious association of the expression of c-di-GMP-metabolizing genes, the increase in c-di-GMP production, the morphological surface dry and the rough (RSCV) formed colonies has been observed in *P. aeruginosa* (D'Argenio *et al.*, 2002, Hickman *et al.*, 2005, Starkey *et al.*, 2009). Meissner and coworkers reported that at 37^{o}C mutant SCV 20265 produced c-di-GMP almost 2.5-fold higher than the levels of c-di-GMP produced by the corresponding WT 20265 strain and 3-fold higher than c-di-GMP levels produced by

SCV 20265 carrying pUCP20-encoding PvrR (Meissner *et al.*, 2007). In another, the report of Malone and coworkers showed that clinical SCV strain Clin110 produced c-di-GMP ~30-fold higher than in PAO1 WT at 37^{o}C and ~13-fold higher than the levels of c-di-GMP produced by Clin163, a smooth colony mutant derivative of Clin110 (Malone *et al.*, 2012).

In our study, the observed differences in several common physiological aspects between the 57RP WT and SCV2h cells were in accordance with the previous observations described above. But since these SCVs were isolated under conditions very distinct from *in vivo* conditions where most SCVs have been isolated and reported, it was unclear whether hexadecane-derived SCVs would also overproduce c-di-GMP. Indeed, the SCV2h cells produced a significantly higher level of c-di-GMP compared to those of their parent 57RP WT and revertant cells. There was also a report on the SCV generation from WT cells cultivated on crude oil of different microbial species, which have been isolated from a crude oil-degrading microbial community, but no information on the c-di-GMP production by these cells has been described (Noordman & Janssen, 2002). To our knowledge until now, this is the first report on the c-di-GMP production activities in *P. aeruginosa* cells cultivated on hexadecane, especially in the culture-representative, highly adaptive SCV cells which were differentiated from their parent WT cells.

The first few SCVs in the 57RP WT cultured on hexadecane were generally observed after approximately 7 days. The frequency of SCV occurrence substantially increased in the following days of culture and was positively correlated with the increase in the c-di-GMP production after the lag growth period. These SCV cells, initially at very low density levels, obviously required a growth time long enough to increase their subpopulation to significantly contribute to the observable increase in the cell mass and c-di-GPM levels of the whole cultured cell population. At the same time, most of the initial cultured the 57RP cell population were normally not able to grow on hydrophobic hexadecane used as a sole carbon source in the mineral salts medium. The growth arrest of WT cells obviously caused the long lag growth phase observed during the first 7-9 days in the culture of the 57RP strain on hexadecane. Roles, if any, of c-di-GMP in switching of

phase variation of 57RP strain to form the hexadecane-growth-adaptive SCVs will be addressed in the following sections.

5.3 Physiological characters of DGC and PDE integrants

5.3.1 Controlling the intracellular concentration of c-di-GMP

In order to address step-by-step the question of the influence of the intracellular concentration of c-di-GMP for switching on the phase variation for SCV formation of strain 57RP on hexadecane, two integrant types have been generated (Figure 3.1, 4.14). The first integrant was RP401 that carried an inducible gene encoding for a phosphodiesterase (PDE) protein (PA14-PvrR, a homologue of PA3947 protein [an EAL domain-containing response regulator]) that catalyzes the degradation of c-di-GMP to linear diguanylate (l-di-GMP). The second integrant was RP402 that carried an inducible gene encoding a cytoplasmic diguanylate cyclase (DGC) protein containing GGDEF domain (PA14-72420, a homologue of PA5487 protein), which catalyzes the synthesis of c-di-GMP from two GTP molecules. Both of these extra genes were regulated by the P_{BAD} promoter putting the transcription of these genes under the control of arabinose in the culture medium.

5.3.2 Growth of integrants

The RP402 integrant cultivated on hexadecane and in the presence of arabinose grew with lag phase observed in the first 5 days, then its growth slightly increased on day 7 to 9, and reached to an exponential phase at day 9 to 15. In growth conditions without arabinose, RP402 grew slower with a long lag phase that was similar to that of WT cells.

In the case of RP401, a very contrasting difference in growth abilities of the integrant cells was observed in the cultivation conditions with and without arabinose

supplementation. In the absence of arabinose, growth of RP401 occurred after an initial 7 day long lag phase, while in the presence of arabinose, RP401 cells barely grew or just maintained the lag phase during the cultivation period.

The 57RP WT cells maintained a similar long lag phase until day 9 of cultivation before the start of exponential phase. In the later phase of growth, WT cells grew faster from day 13-15 in the presence of arabinose than they grew without arabinose supplementation (Figure 4.15A).

These data support a model where c-di-GMP levels explain the occurrence of SCVs and thus the length of the lag phase before exponential growth of strain 57RP can occur on hexadecane. Arabinose induced expression of the two extra but opposite c-di-GMP-metabolizing integrated genes resulted in the contrasting growth abilities between RP402 and RP401 integrants.

Arabinose is not a nutritive substrate for *P. aeruginosa* (Lequette & Greenberg, 2005) because this microbial species lacks genes (Winsor *et al.*, 2011) required for arabinose metabolism like *E. coli* (TA. Desai & Rao, 2010) so it can not use arabinose as a hydrocarbon source for growth and it can serve only as an inducer in these experiments. However, arabinose can serve as an intracellular compatible solute that at certain concentrations may more or less favor the growth of microbial cells in a given osmotic stress condition. Although hexadecane is a nutritive substance for *P. aeruginosa* cells, but it is hydrophobic in nature. Hydrophobic substances are known to induce water stress in microbial cells (Bhaganna *et al.*, 2010). However, bacteria could use certain sugar carbons as compatible solutes such as mannitol and other solutes to cope with osmotic stress. For example, *Pseudomonas putida* S12 grown in an osmotic stress medium containing minimal salts, glucose and NaCl (0.5 M) accumulated mannitol, Nα-acetylglutanylglutamine amide (NAGGN) and glutamate (Kets *et al.*, 1996). These authors also found that *P. aeruginosa* strain ATCC 17933, grown in the same osmotic stress medium as *P. putida* S12 did not accumulate mannitol but the other two compatible solutes: NAGGN and glutamate. Furthermore, Drenkard and Ausubel (2002) reported that environmental stimuli also have effects on the appearance of phenotypic variants demonstrating the *Pseudomonas* biofilm

formation and antibiotic resistance. These authors observed a 40-fold increase in the frequency of appearance of kanamycin-resistant variants (not just RSCV) obtained in LB media containing NaCl (85 mM) compared with the same medium without NaCl. The frequency of variants increased further, up to 200-fold, when the plates were incubated at 25^{o}C compared with 37^{o}C (Drenkard & Ausubel, 2002).

In our case, the growing cells of the 57RP strain, the SCV cells, in the exponential phase grew better on hexadecane in the presence of 0.2% arabinose than without its supplement (Figure 4.16A). Arabinose may serve as a compatible solute for 57RP-SCV to cope better with the osmotic stress generated by hexadecane on which they grew. It may also have some positive effects on the increasing the frequency of appearance of SCV cells that are capable of growing on hexadecane.

The growing cell type(s) of the two 57RP402 and 57RP401 integrants need to be identified and characterized further.

5.3.3 SCV formation of integrants

The RP401 and RP402 integrants also formed SCVs when cultured on hexadecane in both conditions with and without arabinose supplementation. In the presence of arabinose, RP402 cultures generated SCVs after 9 days, and after 11 days in RP401 cultures (Table 4.1). SCVs of these two integrants had a wrinkled and dry surface, small size and uneven shape similar to SCVs derived from 57RP WT cells grown on hexadecane. One of the verified integrants was chosen, named as 401S and 402S and used for further studies.

Data presented in Table 4.1 shows that though SCVs of the 57RP WT strain were detected on the 7th day of culture, addition of arabinose displayed a relatively positive effect on increasing the frequency of the occurrence of SCVs. In the culture of RP402, SCVs were also observed on day 7 in the absence of arabinose and at a more or less similar frequency relative to 57RP WT under the same culture conditions. Arabinose appeared to

have positive effects on the SCV formation of RP402 as the frequency of detected SCVs on the agar TSA medium was considerably higher compared with other cultures on day 11. The fluctuation in the appearance of the SCVs might be in part, due to the fact that SCV cells with abundant fimbrial structures on the cell surface are highly adhesive and they can stick together (Meissner *et al.*, 2007), leading to the reduction of the average number of observable SCVs developed on the TSA medium. The almost unclear differences in the number of observable SCVs developed on a rich medium with and without additional arabinose were likely due to the over production of putative fimbrial adhesin induced by an higher level of c-di-GMP (reviewed in Haussler, 2004) leading to an substantial increase in autoaggregation level of 402SCV cells on the arabinose-supplemented medium. The SCV formation of RP401 did not appear to be affected very much in the absence of arabinose. However, the formation and growth of SCVs in this integrant was dramatically affected by the presence of arabinose. Only a few SCVs were observed on day 15 in RP401 cultures, the last day of the comparative cultures. This was most likely due to the activity of the extra PDE protein whose expression was induced by arabinose and resulted in the dramatic reduction of c-di-GMP levels. Consequently, the formation of the c-di-GMP-dependent SCVs in these cultures was seriously affected.

In *P. aeruginosa*, the RSCV formation is commonly associated with an increase in intracellular c-di-GMP levels the reduction of which leads to reversion to wild-type morphology. However, the expression of a gene or a group of c-di-GMP-metabolizing genes, especially those already known to encode DGC proteins, in various reported mutant or transformants forming RSCV strains were not the same. For example, the expression of genes encoding GGDEF and/or GGDEF/EAL domain proteins, which were found in the transcriptional profiles of different mutation- and transformation-derived RSCV-forming strains, included WspR (PA3702) (D'Argenio *et al.*, 2002), YfiN (PA1120) (Malone *et al.*, 2012), MucR (PA1727) (Hay *et al.*, 2009), WspR (PA3702) and PA0169 (Hickman *et al.*, 2005), MorA (PA4601) and TpbB (PA1120) (Meissner *et al.*, 2007), and PA0285, PA0861, PA2567, PA4843 (Starkey *et al.*, 2009). In our case, the inducible PA5487 gene was integrated into the genome of strain 57RP402 for DGC protein synthesis in the presence of arabinose. In PA14, Drenkard and Ausubel (2002) found that antibiotic-

resistant phenotypic variants of *P. aeruginosa* with enhanced ability to form biofilms arise at high frequency both *in vitro* and in the lungs of CF patients. These authors also showed that overexpression of PvrR, an EAL domain-containing response regulator from plasmids (pED202) transferred to RSCVs, induced RSCV reversion to the wild type phenotype. Overexpression of this regulatory protein in wild type PA14 resulted in a 6-fold reduction in the frequency of resistant variants obtained after plating overnight cultures on kanamycin plates compared to the wild type. Drenkard and Ausubel proposed that PvrR is implicated primarily in inducing reversion from variant to wild-type phenotypes (Drenkard & Ausubel, 2002). Gene encoding PvrR protein was integrated into the genome of our 57RP401 strain. Kulasakara and coworkers (2006) found very high levels of enzymatic activities of several overexpressing proteins with DGC and PDE domains, including PA5487 (PA5487 gene in *P. aeruginosa* PAO1 is the same PA14-72420 gene in *P. aeruginosa* PA14) and PvrR, respectively.

In the present study, 57RP402 grew on hexadecane in the presence of arabinose with a shorter lag phase than the other strains. This observation reflects primarily that there were more SCV cells present in the cultured population due to the expression of the arabinose-inducible c-di-GMP-synthesizing gene that was integrated into the genome of 57RP402 cells. In the case of 57RP401 grown on hexadecane with arabinose, the long lag phase observed in the growth curve means that there were less SCV cells present in the cultured populations and that this is likely due to the expression of the integrated arabinose-inducible c-di-GMP-hydrolyzing gene in the 57RP401 cells. It also means that all of the non-growing cells, which dominated the initial populations cultured on hexadecane, suffered from carbon starvation during the culture period. Our results are in accordance with the observations previously reported in the literature in that the expression of c-di-GMP-synthesizing gene(s) play an important role in the SCV formation in *P. aeruginosa*. However, the c-di-GMP production levels of the 57RP401 and 57RP402 integrants still need to be verified. Both 57RP401S and 57RP402S reverted to the wild-type morphology upon plating on TSA medium.

5.3.4 c-di-GMP production of integrants

Plasmids encoding arabinose-inducible PvrR (pGGN05) and PA14-72420 (pGGN06) were developed by Nicastro and coworkers (Nicastro *et al.*, 2014). The enzymatic activities of PDE and DGC of the two inducible proteins PvrR and PA14-72420, respectively, in the presence of arabinose were confirmed in the cultures of different PA14 integrants. The authors reported that, for example, when cultured in M63 2% glucose medium at 37^{o}C with shaking the levels of c-di-GMP of the PvrR-integrated strain were reduced ~70% in the presence of 0.2% arabinose compared to the cultures without arabinose. In contrast, the PA14-72420-integrated strain cultured with 0.2% arabinose produced c-di-GMP levels almost 2-fold higher compared to the cultures without arabinose and ~6-fold higher c-di-GMP levels produced by the PvrR-integrated strain cultured with arabinose (Nicastro *et al.*, 2014). In our case, plasmids pGGN05 and pGGN06 were transferred by conjugation into the 57RP genome's *ctx* site to generate 57RP401 and 57RP402 strains, respectively, without removal of the plasmid backbone from the chromosome. The enzymatic activities of inducible proteins with the PDE and DGC domains were compared mainly in relation to the intracellular c-di-GMP levels and SCV formation of the two integral strains growing on hexadecane without and with arabinose supplementation.

In the cultures for both integrants on hexadecane without arabinose, the increase in c-di-GMP concentration, from undetectable to detectable amounts, was always associated with the observable increase of the total cell mass of the cultures. It was obviously based on the increasing contribution of the growing SCVs from an initially very small subpopulation of phenotypic variants, which occurred in the previous culture conditions, to the total cell mass of the present cultures on hexadecane (Table 4.1). This trend was more or less similar to that observed in the same cultures of 57RP WT cell populations. In the cultures on hexadecane with arabinose there were two opposite trends clearly observable relating the c-di-GMP production, growth of SCVs and expression of the two arabinose-inducible PDE and DGC proteins, respectively, in the 57RP401 and 57RP402 strains.

a. In the case of 57RP401: Expressions of the extra PDE proteins induced by arabinose resulted in a substantial prolongation of the lag phase of growth of the whole cultures on hexadecane. Almost all cells of the cultures, especially those phenotypic SCVs, which were normally formed in the previous cultures as seen in the cultures without arabinose, arrested their growth during the 15-day long period of culture on hexadecane. The first few SCVs were detected in the last day of culture upon plating on TSA medium (Table 4.1), however, no significant increase in the total cell mass was observed at that time (Figure 4.16). The c-di-GMP levels, if any, of these cultured cells were too low and below the technical sensitivity limit of the currently used measurement method. The significant contributions of SCV growing cells to the total cell mass and c-di-GMP levels were usually observed in the following days of the cultures as it was seen in the culture with hexadecane of 57RP WT. These observations indicated that the phase variation state of most, if not all, of the initially-to-be-formed SCVs was switched off due to the c-di-GMP-hydrolytic activities of the extra PDE induced by arabinose. The intracellular c-di-GMP levels in these cells might fall to zero or far below a minimal effective level, initially called "minimal effective concentration threshold" (MECT), which may happen in certain specific local pools, due to the membrane binding sites or clustering of activated c-di-GMP-synthesizing proteins, and the global concentration due to the nature of freely and fast dispersed of small c-di-GMP molecules in the cytoplasm (reviewed in Valentini and Filloux, 2016). This needs to be defined in the future studies, as at which level of c-di-GMP which SCVs were still be able to keep phenotypic variation state and grow on hexadecane. The appearance of 57RP401-SCVs in cultures with hexadecane and supplemented with arabinose may indicate that these cells have successfully exploited some yet unknown mechanism(s) to regain first the MECT of c-di-GMP to switch on and maintain their phase variation state.

b. In the case of 57RP402: Expression of the extra DGC induced by arabinose resulted in an obvious reduction (from 7-9 to 5 days) of the lag phase of growth of whole cultures on hexadecane. The observed shorter lag phase of growth reflected an involvement of the extra DGC in the induction of more 57RP402 cells becoming capable of growing on hexadecane with arabinose supplement, the rough SCVs (RSCVs), that occurred early in

the cultures compared to the cultures without arabinose. Activities of the inducible extra DGC led to the increase in the intracellular c-di-GMP levels in the integrant. The additional c-di-GMP contributed to the global concentration of this second messenger and helped certain cell subpopulation(s) other than the initial RSCVs to reach the MECT and to initiate the phenotypic switch to form new RSCVs on hexadecane in the presence of arabinose. Consequently, both growth and c-di-GMP levels of the 57RP402 cells cultured on hexadecane with arabinose were faster and higher compared with the culture conditions without arabinose. The high levels of c-di-GMP observed on day 5 of the cultures of 57RP402 may reflect the activities of the inducible extra-DGC proteins in the presence of arabinose in most of the cultured 57RP402 cells. The increasing c-di-GMP levels observed from day 7 until day 15 were produced mainly by RSCVs with the arabinose-inducing extra-DGC proteins, whose densities were low at the beginning but continuously increased until taking over the major cell mass of the cultures.

In the culture of *Alcanivorax borkumensis* SK2, a gram-negative marine oil-degrading bacterial strain, hexadecane, used as a sole carbon source, induced both up- and down-regulation of a number of genes when compared to growth with pyruvate (Sabirova *et al.*, 2011). A double-domain GGDEF-EAL protein gene (ABO-2433) responsible for the biosynthesis and hydrolysis of c-di-GMP, respectively, was found to be downregulated. At the same time, a HD-GYP domain protein gene (ABO-2132) responsible for the hydrolysis of c-di-GMP, was upregulated (Sabirova *et al.*, 2011). In our study, we do not know yet whether hexadecane has also induced similar up- and/or down-regulation of any c-di-GMP-metabolizing genes in the hexadecane-grown cultures of 57RP and integrants. The early fall of c-di-GMP/Protein in RP402, observed on day 7-9 of the hexadecane-grown culture with arabinose (Figure 4.16), might be linked to the upregulation and degrading activity of some EAL- and/or HD-GYP-domain proteins in the non-growing (WT) cells before the rise of c-di-GMP content produced by the growing RP402-SCVs. Further works should identify which among the 39-40 c-di-GMP-metabolizing genes already found in *P. aeruginosa* genome, whose proteins have various predicted functional and partner domains (reviewed in Ha *et al.*, 2014), are induced to be up- or down-regulated in response to hexadecane during the culture of 57RP and integrants.

Non-growing WT cells. Results obtained from the present experiments were in accordance with a number of previous observations relating the effect of the expressed c-di-GMP-synthesizing and the c-di-GMP-hydrolyzing proteins on the RSCV formation and reversion to wild-type phenotypes in *P. aeruginosa*. The induction of RSCV formation and variant reversion to the wild-type phenotype was closely associated with the activities of the highly expressed DGC and PDE proteins, respectively (Drenkard & Ausubel, 2002, Hay *et al.*, 2009, Hickman *et al.*, 2005, Malone *et al.*, 2010, Meissner *et al.*, 2007, Starkey *et al.*, 2009). The expected roles of c-di-GMP in the phenotypic switching ON or OFF appeared to be dependent on its intracellular concentrations as its levels increased or decreased compared to the MECT that may vary depending on both genotype of bacterial strains, cellular states and the environmental conditions used in the experiments, respectively. For example, Meissner and coworkers (2007) reported that the amount of c-di-GMP produced by revertants was only about one third compared to those levels produced by their relative SCVs due to the *in trans*-expression of PvrR proteins and more-or-less similar levels were produced by the WT cells in the same culture conditions (Meissner *et al.*, 2007).

Beside the good growth of c-di-GMP-dependent SCVs, observed in the cultures of 57RP and integrants on hexadecane without and with arabinose, there were many WT cells of the initial cultured population that were not able to grow in these culture conditions. On the one hand, the presence of non-growing or growth-arrested WT on hexadecane was manifested by a longer lag phase of growth of the whole initial cultured WT cell population. The growth-arrested cells could regain their normal growth abilities in a suitable growth condition. For example, beside the presence of SCVs, well-developed WT colonies were commonly seen at high densities upon plating the hexadecane WT- culture-derived cells on the TSA agar medium. On the other hand, it reflected the natural phenotypic variability, which often occurred at a high degree in the clonal populations of microbial cells under homogeneous conditions, the bistability caused by stochastic fluctuations in the cellular components, the epigenetic inheritance, etc. (Veening *et al.*, 2008). In the case of *Alcanivorax borkunensis* grown on n-alkanes, besides the upregulation of various predicted genes involved in the uptake, transport, and oxidation of

alkanes, biofilm formation, signal transduction and regulation, there was also an observable upregulation of several stress-related genes (Sabirova *et al.*, 2011). Presently, we do not know whether the activities of such or similar stress-related genes are also expressed in the alkane-cultures of *P. aeruginosa* strain 57RP or if the expression of these genes is just linked to a certain phenotypical cell state, such as SCVs observed in our study. Our opinion is that during the culture period, the non-growing WT cells suffered from a temporal starvation, arrested their growth and reacted to the carbon starvation by stringent response to promote survival (a hypothesis, proposed for further investigations in the future, is presented in Section 6.3 *Research scheme and perspective*).

The **stringent response** is signaled by (p)ppGpp, the nucleotide alarmone, that modulates gene expression by redirecting the RNA polymerase (RNAP) to certain genes leading to a substantial reallocation of cellular resources away from growth, replication, synthesis of ribosomal proteins, and stable RNAs, towards the expression of genes for synthesis of crucial amino acids and factors for stress resistance, and survival (Brown *et al.*, 2014, Potrykus & Cashel, 2008). During stress, the synthesis, stability or activity of proteins or regulatory RNAs of stressed bacteria are regulated by the alarmone ppGpp (Dalebroux & Swanson, 2012). To synthesize (p)ppGpp for stringent response signaling the starving cells should assure the availability of GTP inside the cell. It was reported that the activities of the promoter of *msdgc-1*, the signal gene encoding a bifunctional GGDEF/EAL domain protein involving c-di-GMP turnover in *Mycobacterium smegmatis*, is activated by carbon starvation (Bharati *et al.*, 2013). The carbon starvation-induced expression of genes involving the c-di-GMP turnover, which certainly contributes to assure the availability of GTP required for (p)ppGpp synthesis, might also function in starving *P. aeruginosa* cells. In fact, *P. aeruginosa* cell aggregates disperse upon carbon, nitrogen or oxygen limitation (Schleheck *et al.*, 2009). Though the glucose starvation-induced dispersal of *P. aeruginosa* was considered as a cAMP and energy dependent process, several c-di-GMP phosphodiesterases of the GGDEF-EAL-double domain proteins, such as BifA (PA4367) (Kuchma *et al.*, 2007), and DipA (PA5071) (Ha *et al.*, 2014) were found to inversely regulate biofilm formation. The increased PDE activity of DipA (PA5071) and reduction of c-di-GMP levels were essential for the dispersion of *P. aeruginosa* biofilm in

response to sudden changes in nutrient concentrations (Roy *et al.*, 2012). The considerable reduction of c-di-GMP levels during the 5-9th day observed in the cultures of 57RP402 on hexadecane with arabinose (Figure 4.16C) could be possibly, in part, due to the stringent response, leading to reprogramming of the expression of c-di-GMP-metabolizing genes with increased PDE activities and reduction of c-di-GMP levels and reduction of c-di-GMP levels of some major subpopulation(s) of the so called generally WT cells. Only some smaller cell subpopulation(s) may profit the contribution of inducible 57RP402 extra-c-di-GMP-synthesizing gene to the global c-di-GMP concentration to initiate the phenotypic switch to form new adaptive SCVs as demonstrated by a slight early increase in the biomass production of strain 57RP402 growing on hexadecane in the presence of arabinose (Figure 4.16A).

Major stress response. Stringent response is a major stress response of bacteria in reaction to various growth-restricting environments, including carbon starvation. However, bacteria also exploit potentials of different mutation-based mechanisms developed during evolution, as mechanisms based on the stationary phase mutagenesis, to accelerate adaptation to the nutrient-limiting conditions (Kivisaar, 2010). Some mutation-based mechanisms were established by transposition activities of the insertion sequence (IS) elements. IS elements are mobile genetic elements that are found in the genome of many bacteria, including *Pseudomonas* (Mahillon & Chandler, 1998). The IS transposition and excision rates, including the copy-and-paste, cut-and-paste and excision, may vary depending on the genotype of bacterial strains and the environmental conditions in which bacteria evolve (Sousa *et al.*, 2013). The activity of IS elements increases during stressful conditions, especially starvation including carbon source exhausted (reviewed in Vandecraen *et al.,* 2017), generating different types of mutations, including insertions, rearrangements such as deletions, inversions, duplications and translocations via recombination between homologous elements (Schneider & Lenski, 2004, Vandecraen *et al.*, 2017). Bacterial cells employ IS elements as the tools for genetic variation, phenotypic switching, promoting adaptation and survival. Some activities of IS elements leading to a phenotypic switch, due to the activation or inhibition of gene expression generated by the inversion of a particular DNA sequence or the insertion-excision of IS element into or from

specific chromosomal regions, have been reported (Gally *et al.*, 1993, Higgins *et al.*, 2007, van der Woude & Bäumler, 2004, Wisniewski-Dyé & Vial, 2008). In the cultures on hexadecane with arabinose of 57RP401, due to activities of the extra-PDE almost all cells remained in a growth-arrested state throughout the culture period. Just a few SCVs were detected on day 15 and the selected 57RP401 SCV reverted also to WT form upon being plated on TSA agar medium (Fig. 4.15). At the present time, we do not know yet the relations, if any, between the RSCVs of 57RP401 occurred in the cultures without and with arabinose and whether the appearance of the later SCVs resulted from the activities of IS elements during starvation or not. These questions require further investigations in the future.

As cited in the previous sections, several genes encoding GGDGEF and/or GGDEF/EAL domain proteins expressed in different mutation- and transformation-derived RSCV-forming strains of *P. aeruginosa* have been identified [WspR (PA3702) (D'Argenio *et al.*, 2002), YfiN (PA1120) (Malone *et al.*, 2012), MucR (PA1727) (Hay *et al.*, 2009), WspR (PA3702) and PA0169 (Hickman *et al.*, 2005), MorA (PA4601) and TpbB (PA1120) (Meissner *et al.*, 2007), and PA0285, PA0861, PA2567, and PA4843 (Starkey *et al.*, 2009)]. The identification of c-di-GMP-metabolizing genes expressed in our SCVs (e.g. by molecular techniques relating the mRNA detection, cloning and sequencing or DNA site-directed mutation) which appeared in the cultures on hexadecane without and with arabinose of three strains 57RP, 57RP401 and 57RP402, is necessary to estimate the role of the expressed genes in the phenotypic switch because both bacterial genotypes and culture conditions used in the present study are different from those previously reported in the literature.

5.3.5 High capacity for biofilm formation of integrant SCVs

In the culture without arabinose supplementation, the biofilm biomass of the three tested strains 57RP WT, 57RP401 WT and 57RP402 WT were almost similar (Figure 4.17). However, the presence of arabinose generated almost negative effects on the biofilm

formation of 57RP WT and 57RP401 WT cells but positive effects on 57RP402 WT cells. In the case of 57RP WT, the presence of arabinose in the testing Bushnell-Hass medium appeared to be not a supportive factor for biofilm formation compared to the previous culture condition without arabinose supplementation. An unclear yet question is that whether arabinose used in the testing culture condition did stimulate the induction of some native phosphodiesterase (PDE) leading to a decrease of its intracellular c-di-GMP together with an observable reduction of biofilm formation? The biofilm formation of 57RP401 WT was also reduced similarly to the case of 57RP WT cultured with arabinose. However, another expected effect of arabinose on 57RP401 WT cells was the induction of the extra PDE, a c-di-GMP-degrading enzyme, leading to the reduction of intracellular c-di-GMP concentration. The reduction of biofilm formation observed in the culture of 57RP WT and 57RP401 WT was in accordance with results obtained from recent studies describing the induction of biofilm dispersal of a PAO1 strain that harbors the arabinose-inducible *Escherichia coli* YhjH phosphodiesterase (Christensen *et al.*, 2013). However, results obtained from studies of Christensen and coworkers (Christensen *et al.*, 2013) indicated no significant difference in the biofilm formation between PAO1/pJN105, a control PAO1 strain that did not harbor the arabinose-inducible YhjH phosphodiesterase, and the PAO1/pP$_{BAD}$-yhjH, an arabinose-inducible strain. This was possibly due to the fact that strains, culture media, temperatures, incubation times, etc. used in the two studies were not the same and it reflected once again the genotype-specific and environment-dependent traits of the microbial phenotypic behaviors. In the case of 57RP402, the presence of arabinose stimulated its biofilm formation in parallel with an expected activation of the extra diguanylate cyclase (DGC), a c-di-GMP-synthesizing enzyme contributing to an increase of c-di-GMP concentration in the 57RP402 biofilm cells. In addition, c-di-GMP was also found to contribute to a complex regulation of biofilm formation via CsgD-like transcriptional regulators (Kader *et al.*, 2006, Newell *et al.*, 2011).

The SCVs of both 57RP (SCV2h) and integrant 57RP401 (57RP401S) expressed almost similar capacity for biofilm formation but obviously much higher than the 57RP WT cells in culture conditions with and without the supplement of arabinose (Figure 4.19). The SCV2h and 57RP401S produced substantial amounts of intracellular c-di-GMP, in

contrast, the second messenger of the 57RP WT cells was not detectable during its growth in the same culture conditions with and without arabinose (Figure 4.16, 4.18).

The observation mentioned above more-or-less demonstrated the important role of c-di-GMP in biofilm formation of *P. aeruginosa* (reviewed in Valentini and Filloux, 2016).

5.3.6 Rapid growth of integrant SCVs on hexadecane

The 401S strain, a SCV derived from RP401 integrant cells grown on hexadecane with arabinose, was chosen for further studies. When cultivated on hexadecane without arabinose supplementation, the growth curve of 401S was similar to that of SCV2h (Figure 4.18A). However, in the presence of arabinose, the SCV2h grew better than 401S during the first 7 days of cultivation. During the next days, between days 7-11, especially from days 9-11, 401S increased its growth as its biomass reached near to the biomass level of the SCV2h (Figure 4.18A). WT and SCV are differentiated from each other in both genetic and phenotypic adaptation aspects. After becoming SCV, the 57RP-401-SCV would respond to the environmental cues in a different way compared to the parent 401 "WT" integrant. The fast growth of SCV2h on hexadecane (Figure 4.11A) was positively correlated with its c-di-GMP production (Figure 4.13). Questions arose here, as to whether the increase in biomass of 401S growing on hexadecane with arabinose was also related to the increase in the c-di-GMP production in these cells, and will be further investigated and discussed in the following sections.

5.3.7 High capacity for c-di-GMP production of integrant SCVs

Data presented in Figure 4.18 showed that 401S was also a c-di-GMP-dependent SCV that could grow on hexadecane, and its capacity for c-di-GMP production depended on the culture conditions without or with arabinose supplementation. When cultivated on hexadecane without arabinose, 401S followed similar patterns of c-di-GMP production of

SCV2h and with an obvious higher level toward the end of the culture (Figure 4.18C). In the presence of arabinose, SCV2h generated higher levels of c-di-GMP production compared with SCV2h cultured without arabinose. Presently, reasons for the increase in c-di-GMP production in presence of arabinose of the SCV2h strain were not clear and it required further investigations for a better understanding. From day 7 toward day 15, the arabinose-supplied culture conditions induced strain 401S to accumulate much higher c-di-GMP levels compared to conditions without arabinose supplement. The parent RP401"WT" in the arabinose-induced cultures showed undetectable c-di-GMP amounts during its prolonged lag phase of growth (Figure 4.16).

Day 15 was the end of the exponential and the beginning of the stationary phase of growth of the 401S as well as the SCV2h grown on hexadecane with arabinose (Figure 4.18A). An observable decrease of c-di-GMP levels was seen in these two cultures and a similar c-di-GMP decreasing trend was also observed in the case of 57RP402 cultured in the same conditions and at the same culture age (Figure 4.16C). It might be due first to the high levels of carbon limitation, which occurred at the stationary phase of growth that put the biofilm cells in a starving situation. Starving cells react to carbon starvation via stringent response, reprogramming the expression of many genes, including the c-di-GMP-metabolizing genes. The increased PDE activities together with reduction of c-di-GMP levels were essential for the dispersion for new surface colonization (see previous section: c-di-GMP production of integrants). An increase in cell lysis may also contribute to the decrease of c-di-GMP during the stationary phase of growth.

Beside a putative role as intracellular compatible solutes against the osmotic stress generated by hexadecane in the medium, obvious positive effects of arabinose on the c-di-GMP production were seen in both 57RP402 (Figure 4.16C) and 57RP401s and, to a lesser extent, in 57RP-SCV2h cultures (Figure 4.18C). The high levels of c-di-GMP accumulated in 57RP402 were understandable because its cells harbored an inducible gene encoding extra-DGC proteins for c-di-GMP synthesis. The case of 401S, which harbored an inducible gene encoding extra-PDE proteins for c-di-GMP hydrolysis but also accumulated high levels of c-di-GMP, requires further investigations to better understand the not-yet-

known events (such as the reprogramming of gene expression because of ppGpp redirecting RNAP to certain genes due to the stringent response to temporal carbon starvation, the effects of (p)ppGpp on the stabilities and functions of expressed RNAs and proteins, the possibility of genome rearrangement to switch the ON-and-OFF state of affected genes or ORFs of in or surrounding DNA sequences due to the starvation-induced transpositions, insertion and/or excision activities of IS elements, mutations, etc. – as presented in more details in a previous section: c-di-GMP production of integrants) that could possibly happen in the temporary starving cells during a long lag phase of growth of the 57RP401 cultures with hexadecane and arabinose supplementation. Arabinose may also act as the external and internal signals that directly or indirectly induce an increase in DGC or decrease of PDE expression and activities or both since their various sensory domains are found in many c-di-GMP-metabolizing proteins of *P. aeruginosa* (Ha *et al.*, 2014). The putative role of arabinose, acting as a signal in certain cellular process, if any, may also be dependent on a MECT of arabinose available in the medium and inside the cell. This possibility also needs to be studied further.

5.4 Comparison of proteome profiles of P. aeruginosa 57RP and the SCV derivative

5.4.1 Differences in protein synthesis between 57RP SCV and 57RP WT

The phenotypic differences observed previously between 57RP SCV and 57RP WT were further characterized by total protein analysis. The total proteins of the two strains were extracted from their cells grown under the same culture conditions (MSM medium with 1% glucose shaking at 25^{o}C for 18h). However, the quality and stability of the cytosolic protein patterns separated by 2-D gel electrophoresis were achieved only after a previously effective removal of the contaminating macromolecular DNAs and the other non protein components from the extracted protein samples by DNase treatments and the phenol-chloroform re-extraction, respectively (Do, 1995). The representative proteins of the two strains were chosen on the commonly visual basis of their presence (up-regulated) on one sample gel but absence (down-regulated) on the other sample gel.

The 5 representative up-regulated proteins, selected from various specific protein spots of 57RP SCV, included TT-S1 (PA1787; PP/OM localization), TT-S2 (PA5015; PP/CP localization), TT-S3 (PA019; PP localization), TT-S4 (either PA5521; CP localization, or PA1584; IM localization) and TT-S5 (either PA1746; CP/OM vesicle or PA2807; unknown location). The two first identified proteins are the bi-functional aconitate hydratase 2/2-methylisocitrate dehydratase (product of *acnB* gene) and the Pyruvate dehydrogenase subunit E1 (product of *aceE gene*), respectively. These two proteins are the indispensable enzymatic components of the tricarboxylic acid or citric acid cycle (TCA), the final common oxidative pathway used by all aerobic organisms to generate energy through the oxidation of acetyl-CoA derived from carbohydrates, fats and proteins or amino acids (reviewed in Akram (2014)).

In the case of 57RP WT, among the three analyzed protein spots, the TT-WT1, TT-WT2 and TT-WT3, only the TT-WT2 was identified as the electron transfer flavoprotein alfa-subunit; Induction beta-oxidation (PA2951; *etfA*; PP). The other two protein spots need to be analyzed further as to a number of putative proteins that share high Mascot scores as well as close pH values and molecular mass (TT-WT1) or to the presence of a conserved hypothetical protein with no information concerning its pH value and molecular mass to compare with the other putative protein (TT-WT3).

The differences in protein spot quantity and quality observed in the 2-D gels indicated that there were certain differences in the gene expression between 57RP SCV and 57RP WT cells grown in the same culture conditions. Different phenotypic variants of the 57RP strain may respond to the same environmental stimuli by using distinct mechanisms.

5.4.2 Characteristic membrane and periplasmic proteins of 57RP SCV grown on hexadecane

Many essential cellular processes, including nutrient uptake, secretion of extracellular proteins, rhamnolipids and other products, depend on cell envelope components.

In order to know the cell envelope components of SCV, the outer membrane and periplasmic proteins of 57RP SCV, grown on hexadecane for 11 days at 25^{o}C, were extracted and used for the 2-D gel electrophoresis and identification. Since the 57RP WT cells were unable to grow on hexadecane, no protein samples from these cells could be collected from the cultures with hexadecane to compare directly with the protein samples derived from the 57RP SCV grown on hexadecane. However, to facilitate the selection of representative proteins, which were specifically expressed in the hexadecane-cultured SCV, the outer membrane and periplasmic proteins were also extracted from the 11h/25^{o}C glucose-cultured WT cells. These proteins were also resolved by 2-D gel electrophoresis and used to locate the protein spots of interest observed on gels of SCV samples.

Among the ten selected protein spots, the first identified outer membrane-bound protein (OMB-S1) of the SCV2h was FadL (PA4589) – a long-chain fatty acid transport protein homologous to the *E. coli* FadL transporter. As deduced from the DNA sequence, Black reported that the mature FadL of *E. coli* contains an abundance of hydrophobic amino acid residues that contribute to at least five regions of the protein with an overall hydrophobic character (Black, 1991). The other three single protein spots from SCV2h (OMB-S3, OMB-S9, PP-S13) have been identified as periplasmic (PP) proteins. Two of these three proteins (OMB-S3, OMB-S9) were predicted to be ABC (ATP-binding cassette) transport proteins. The OMB-S3 was identified as a putative ABC-type transport protein responsible for the uptake of amino acids, peptides or inorganic ions (PA3836) (Imperi *et al.*, 2009). The OMB-S9 was identified as the PA5153 protein, an amino acid (lysine/arginine/ornithine/histidine/octopine) ABC transporter periplasmic binding protein (Imperi *et al.*, 2009). The third periplasmic protein (PP-S13) was the Tpx, a thiol peroxidase (PA2532). This protein is part of an oxidative stress defense system and

functions as an antioxidant in reducing peroxides and inhibiting hydrogen peroxide response and protecting cell membranes. Tpx is also thought to be involved in the activation of certain transcriptional factors and signaling (reviewed in Rhee *et al.* (2005). In *P. aeruginosa* PAO1, a Δ*tpx* mutant became sensitive to H_2O_2, a reactive oxygen species (ROS), at a moderate concentration (0.5 mM) (Somprasong *et al.*, 2012). The presence and activity of this terminal oxidation protein supported more evidence for proteins involving in alkane hydroxylyzing systems, which found in *Alcanivorax borkumensis* (Sabinova et al., 2006). The PP-S18 was identified as an inner membrane binding nitrogen regulatory protein *glnK* (PA5288). The PP-S16 was more likely the putative cytoplasmic endoribonuclease (PA5339), which has pI 4.81, than the PA0388, which has pI 7.37, due to the position of PP-S16 protein spot was near to the middle of the 2-D pH 4-7 gel. The identification of the last four protein spots will be discussed in the next section (5.4.3).

The presence of the transport and oxidative stress defense system-related proteins, as above mentioned, reflects their important role in the uptake-secretion activities between the outer medium and the inner cytoplasm through the cell membranes as well as in the reduction of the risk of excessive ROS accumulated in the periplasm. Phase variation induced changes in gene expression required for the adaptive growth of 57RP SCV on hexadecane.

5.4.3 Unusual proteins synthesized by 57RP SCV grown on hexadecane

One of the last four up-regulated proteins, observed in SCV cells, was PP-S15 that had no homology with any proteins registered in the Mascot database. Currently, we do not know yet whether the ORF, from which the PP-S15 was derived, was newly established or activated due to the inversion of a DNA sequence that contained a promoter of other gene(s) or the excision of IS element(s) from a specific DNA sequence (reviewed in Section 2.6.2: Mechanism of phase variation) or as a result of horizontal gene transfer events (reviewed in Filloux (2010)). Our observations require further investigations to be more understandable.

The last three prominent protein spots (OMB-S7, OMB-S8, OMB-S12), have been identified as outer membrane proteins, PA2862. These proteins were lactonizing lipase precursor LipA proteins of *P. aeruginosa*. The LipA protein (EC 3.1.1.3) is involved in the hydrolysis of triglycerides from glycerol and three fatty acids into diacylglycerol carboxylate. Beside the lipase activity, LipA proteins also influence the regulation of pyoverdine production and expression of the sigma factor PvdS (Funken *et al.*, 2011). Previously, Déziel and coworkers (2001) observed an increase in pyoverdine production in the SCV cells compared to the WT cells of *P. aeruginosa* (57RP strain) when both cell types were cultured in the same King's B medium containing dextrose as carbon source for 16h at 37^{o}C. However, no information relating to the lipase production had been reported in that study. In *P. aeruginosa* PAO1, the open or closed active center of the enzyme depends on the structure of the α-helical lid (Nardini *et al.*, 2000). The open center occurs when contacted with the substrate. The activity of this enzyme can be mediated by the contact with a large range of hydrophobic substances (Stuer *et al.*, 1986). Moreover, the results of Kanwar and co-workers showed that, in *P. aeruginosa* sp. G6, the lipase was not produced when using glucose as a growth substrate (Kanwar *et al.*, 2002). These authors also compared the effect of various n-alkanes (C_{10} Decane – C_{20} Eicosane) on lipase production and found that hexadecane (C_{16}) was the best inducer of lipase production (e.g. 25±0.6 U/ml) (Kanwar *et al.*, 2002). However, these authors did not provide any information about the appearance of SCVs on hexadecane (Kanwar *et al.*, 2002). In *P. aeruginosa*, it is known (reviewed in Rosenau and Jaeger (2000)) that expression of the lipase bicistronic operon (lipase *lip*: LipA and foldase *lif*: LipH), which has two operators (P1 and P2), depends on the alternative sigma factor RpoN (σ^{54}) and is controlled by two two-component regulatory systems GacS/GacA (sensor histidine kinase/global activator) and LipQ/LipR (putative signal transducing sensor kinase/transcriptional activator). Expression of LipQ/LipR is possibly activated by the quorum sensing activator RhlR, whose expression is activated by GacA, or by some environmental or periplasmic signal(s) that remain unknown. LipR together with sigma factor RpoN activate the P1 promoter of the lipase operon (reviewed in Rosenau and Jaeger (2000)). According to Jaeger and coworkers (1994), the synthesis and secretion of lipases by bacteria is influenced by a variety of environmental factors like ions, carbon sources, or the presence of non-

metabolizable polysaccharides (Jaeger *et al.*, 1994). In the present experiments, hexadecane is not a substrate of lipase but serves as both an environmental hydrophobic carbon source signal and osmotic signal that might generate a lipid-like signal, resulting in the activation of the above mentioned two-component regulatory systems leading to the lipase synthesis and secretion.

Until now, there were not many reports on the subject of proteome profile analysis of SCVs generated from the cultivation of *P. aeruginosa* on hexadecane. In a study, entitled "Phenotypic and genome-wide analysis of an antibiotic-resistant small colony variant (SCV) of *P. aeruginosa*", Wei and coworkers (2011) observed a large number of genes differentially regulated in PAO-SCV cells compared to the wild-type PAO1 cells. There were 466 (~73%) up-regulated and 176 (~27%) down-regulated genes observed among the totally reported 642 differentially regulated genes. In a comparison of proteomes of WT and SCV, using 2-D gel electrophoresis of soluble proteins, these researchers identified several differentially produced proteins in SCV as up- or down-regulated gene products compared to its clonal wild-type PAO1. Among the 24 selected proteins, 8 up-regulated and 16 down-regulated gene products were reported by this research group (Wei *et al.*, 2011), none were identical to any of the 18 representative proteins observed in our present study, including 15 proteins of SCV2h. However, in the genome-wide transcriptional profile of PAO-SCV and PAO1 generated by microarray analysis of this research group, there were some up-regulated genes identical to our identified cell type-specific gene protein products. We do not know whether these mRNAs were also translated into proteins at a corresponding rate or not. For example, the expression of PA2862, PA3836 and PA5153, the three outer membrane proteins found in the 57RP-SCV cells grown on hexadecane, was found in the transcriptional profile of up-regulated genes in PAO-SCV compared to its clonal wild-type PAO1 at the late stationary phase of growth in the experiment of Wei and coworkers (2011). These authors also observed an up-regulated expression of PA1579 and PA2951 genes in the transcriptional profile of PAO-SCV; however, corresponding proteins of these genes were found specifically in the 57RP-WT cells grown on glucose.

The differences in gene expression between the PAO-SCV (Wei *et al.*, 2001) and SCV2h (the present study) obviously reflected the fact that these two SCV forms were generated from different strains of *P. aeruginosa* and developed on different culture conditions. The gentamicin-resistant mutant PAO-SCV was derived from the PAO1 strain (ATTC 15962). Both of the PAO1 strains (WT and SCV) were grown at 37°C in LB broth or on LB agar plates, iron poor casamino acids (CAA) medium or on the *Pseudomonas* agar medium (Difco Laboratories) (Wei *et al.*, 2011). In our present study, SCV2h was derived from 57RP WT grown on the MSM medium supplemented with 1% hexadecane as a sole carbon source. The SCV2h was either grown on glucose for 11 hrs for a total protein analysis, or on hexadecane for 9 days for the membrane-fractionated protein analysis. Even though these two phenotypic variants PAO1-SCV (Wei *et al.*, 2011) and 57RP-SCV (the present study) shared some more or less similarly up-regulated expression patterns of certain genes, they obviously differed from each other by the expression pattern of other specific genes. This certainly was related to their own evolving history and actual cultivation conditions.

The presence of the three LipA isoforms was an unusual event, likely reflecting a possible polymorphic aspect of the *lipA* gene production. About these three presently detected LipA isoforms of SCV2h, when compared to the peptide sequences, significant differences were found between the OMB-S7, OMB-S8 and OMB-S12 LipA. The OMB-S7 protein had three substitutions at the amino acid (aa) positions 156 (I), 202 (H) and 204 (V) compared to the V at position 156, Q at 202 and I at 204 of the corresponding LipA protein (PA2862) of PAO1 (Wohlfarth *et al.*, 1992), respectively. These three aa substitutions that occurred in the OMB-S7 protein of SCV2h shared two similar substitutions (at the two aa positions: 156, 204) found in SCV20265 (Eckweiler *et al.*, 2014), and all the same three substitutions (at aa positions: 156, 202, 204) were found in the natural variant TE3285 which was isolated from soil (Chihara-Siomi *et al.*, 1992).

The OMB-S12 protein of SCV2h also carried three distinct amino acid substitutions that occurred at positions: 125 (M), 126 (P) and 176 (A) compared to the aa pattern 125 (I), 126 (A) and 176 (T) found in the LipA sequence of PAO1, respectively. This was a

completely new pattern of amino acid substitutions compared to those substitutions already reported in the natural variant TE3285 isolated from soil (Chihara-Siomi *et al.*, 1992), the thermotolerant strain EF2 (Gilbert *et al.*, 1991), and SCV20265 (Eckweiler *et al.*, 2014). The aa substitution (Q) at position 33 reported in the natural variant EF2 compared to (K) in PAO1 was not observed in the protein variants OMB-S7 and OMB-S12 of SCV2h. Both of the two OMB-S7 and OMB-S12 proteins were still the in the form of pre-peptide of LipA which still bear the un-cleaved signal peptide.

OMB-S8, the third variant of LipA found in the SCV2h, was in the form of mature protein with its signal peptide cleaved off. OMB-S8 protein shared the whole peptide chain identical to the chain of the PAO1-PA2862 protein. This indicated that there were various LipA peptide variants that existed in the SCV2h cell population grown on hexadecane. One of the phenotypic variants was in the form of mature protein corresponding to the PAO1 LipA (PA2862). The other two were in the pre-protein forms, but one of which (OMB-7) was identical to the already known natural variant TE3285 (Chihara-Siomi *et al.*, 1992), the other was a completely new phenotypic variant (OMB-S12). This is an unexpectedly interesting observation of the existence of self-generated LipA isoforms in a *P. aeruginosa* strain, the 57RP-SCV grown on hexadecane.

Sommer and coworkers (1997) showed genetic and biochemical evidence that the *Streptomyces cinnamomeus* Tü89 secreted *lipA* encodes a proprotein of 275 amino acids (29,213 Da) with a pI of 5.35. The LipA signal peptide is 30 amino acids long, and the mature lipase sequence is 245 amino acids long (26.2 kDa) and contains six cysteine residues. Sequence similarity of the mature lipases (29% identity, 60% similarity) was observed mainly in the N-terminal 104 amino acids with the group II *Pseudomonas* lipases. In transgenic clones of *E. coli*, which carry the *S.cinnamomeus* Tü89 *lipA* gene, both processed and unprocessed forms of the transgenic LipA were secreted but only the processed form of this LipA (the mature form with the signal peptide cut off) was catalytically active (Sommer *et al.*, 1997). The two unprocessed LipA isoforms (OMB-7, OMB-12), observed in the present study, may not function as a processed active LipA but their presence on the outer membrane would contribute to the increase in cell

hydrophobicities, and with such higher cell hydrophobicities the 57RP SCV cells would adhere better to the hydrophobic hexadecane substrate. The inducible generation of certain protein isoforms may represent a flexible way of environmental adaptation abilities that is, certainly, only a part of the complex survival strategy of bacteria.

5.4.4 Not-yet-known mechanisms

At present, we do not know yet whether 3 is the final number of LipA isoforms produced in the SCV2h cells or not. It is still many other protein spots selected from the 2-D gel that have not been identified yet. In the case of existence of only three LipA isoforms, if the amino acid sequence of the three identified LipA isoforms reflected exactly the genetic information of their corresponding genes then the SCV2h has three *lipA* genes. There are several possibilities for the appearance of the three LipA isoforms, which were observed in the SCV2h cell population, to be considered. The simple case is that the spontaneous point mutations would cause the original population to separate into three subpopulations. The complex case would concern a combination of both gene duplications, for example by the activities of transposable DNA elements (reviewed in Darmon and Leach (2014)), and the spontaneous mutations. The cell population in the complex case comprises at least two subpopulations. It combines either the single gene or the double genes subpopulations, which produce their corresponding LipA isoforms, or the two double genes subpopulations but both share one same gene copy and produce their corresponding LipA isoforms. Another possibility is that the SCV2h has only one *lipA* gene and the original gene has not undergone both mutations and duplications. In this case, if the translation was exact, ensuring the synthesized peptide sequences corresponding to the mRNA molecules, due mainly to the proofreading and repair mechanisms, then a similar phenomenon of RNA and DNA sequence differences previously observed in cells of certain human tissues by Li and coworkers (Li *et al.*, 2011) would also have, somehow, happened with the *lipA* gene in the 57RP-SCV cells grown on hexadecane. Though these two observed unusual events are not comparable, they pose a question that whether the phase variation, resulted in the formation of special SCVs and biofilms in *P. aeruginosa*,

and the cell differentiation, resulted in the formation of specialized tissues in eukaryote, shares some common point(s) in the yet-unknown underlying mechanisms for the phenomenon of protein isoforms (present study) and of RNA and DNA sequence differences (Li *et al.*, 2011), respectively.

The mRNA editing, such as the adenine to inosine (A-to-I) or cytosine to uracil (C-to-U) deamination, is unknown in bacteria. The suggested phenomenon of mRNA and DNA sequence differences would have hardly happened in the SCV2h without production of some yet-unknown phase variation-related and the hexadecane-induced factors that would interfere with the otherwise normal transcription process leading to substitutions of specific bases in the mRNA. However, the protein isoforms could be generated also by the tRNA editing such as the A-to-I editing at the wobble position of tRNA anticodons (reviewed in Su & Randau, 2011). The four main wobble base pairs, as guanine-uracil (G-U), hypoxanthine-uracil (I-U), hypoxanthine-adenine (I-A), and hypoxantine-cytosine (I-C), had been proposed by Crick (Crick, 1966). The first prokaryotic RNA editing enzyme (TadA) has been identified from *Escherichia coli* by Wolf and coworkers in 2002 and this deaminase is sufficient for site-specific inosine formation at the wobble position (position 34) of tRNA(Arg2) (Wolf *et al.*, 2002). An extension (if any and happened by unknown reasons, hypothetically only) of the traditional Wobble position from the first to the second even to the third base position in the tRNA anticodon (corresponding to the third, second and the first base in mRNA codon) would make the base pairing altered to that of the standard base pairing rule and lead to the formation of various unknown protein isoforms from the modified translation.

Another possibility is that the specific charge of amino acids at the 3'-end of certain tRNAs can be altered and shifted from selective amino acids to preferential amino acids by the interference of some factor(s) like ribozymes. Morimoto and coworkers (2011) have selected from an RNA pool of random sequences and developed successfully flexizyme Fx3 (45-nucleotide-long sequence), an aminoacyl-tRNA synthetase (ARS)-like ribozyme that could charge various tRNAs with preferential amino acids (such as phenylalanine)

through very simple three-base-pair interactions between the ribozyme's 3'-end and the tRNA's 3'-end (Morimoto *et al.*, 2011).

Thus, any infidelity during in replication, transcription or translation would lead also to the formation of protein isoforms. The infidelity in replication leads to the formation of genetic mutants whereas the infidelities in transcription or translation lead to the formation of phenotypic variants. Which one of these infidel events was the main cause of the appearance of three LipA isoforms observed in the present study remains an unanswered question that requires further investigations to be carried out in the future. The presence of OMB-S7 and OMB-S12, corresponded exactly to the LipA of two natural variants TE3285 and PAO1, respectively, may reflect a possibility that, besides the spontaneous variations, there exist certain modes of adaptive variation that had been experienced during the long-term environmentally adaptive evolution of bacterial species in the past. Some such species evolution-experienced modes of adaptive variation are probably still inducible, by certain environmental stimuli, and expressed in certain bacterial strains, such as the 57RP SCV strain of *P. aeruginosa* grown on hexadecane. Verifications of this hypothesis require further investigations to be carried out in the future.

In the present study, results obtained from the identification of the 18 initially selected protein spots show that, to be able to grow on hexadecane, 57RP-SCVs have undergone a number of changes. The SCV formation-related changes in global gene expression did not only concern the down- or up-regulated expression of a number of already known specific genes but also the expression of some not-yet-known ORFs as well as the polypeptide isoforms of certain specific proteins, too.

Except the three unusual secreted LipA isoforms, the presence of representative proteins located in the outer membrane, periplasm, inner membrane and cytoplasm observed in the 57RP SCV growing on hexadecane, but not in the 57RP WT cells growing on glucose, may reflect some aspects of physiological adaptation involving modulation of gene expression as it was observed previously in biofilm cells growing on hexadecane compared to cells growing on pyruvate of *Alcanivorax borkumensis* (Sabirova *et al*., 2006,

2011) or to planktonic cells growing on acetate of *Marinobacter hydrocarboclasticus* (Vaysse *et al.*, 2009), respectively.

6 CONCLUSION AND PERSPECTIVE

6.1 Conclusion

The present study had four objectives: (1) Isolate small colony variants (SCVs) of *P. aeruginosa* strain 57RP during cultures with n-alkanes used as sole carbon sources; (2) Characterize the phenotypic changes of SCVs grown on hexadecane; (3) Investigate the putative role of c-di-GMP in the SCV formation and in the reversion to wild-type phenotypes; and (4) Characterize proteome profiles of SCV cells grown on hexadecane.

The results obtained from the present study are summarized as follows.

Objective 1. *57RP, a slow growth strain with low cell hydrophobicities, also forms SCVs on octadecane.*

We confirmed that 57RP, the main investigated *P. aeruginosa* slow growth strain with low cell hydrophobicities, was able to generate rough, dry small colony variants (RSCVs or briefly called SCVs) on the weakly soluble liquid hexadecane (C_{16}) as reported previously in the laboratory of Prof. Richard Villemur.

Besides that, this strain was also able to form SCVs on the solid octadecane (C_{18}) that was used as a sole carbon source. This new observation reflected that 57RP strain could form SCVs on various substrates, including different n-alkanes. The SCVs were identified as the main growing cell type in the cultures supplemented with the hydrophobic n-alkanes.

In the case of strain 57RV, the 57RV WT cells, which have cell hydrophobicities much higher than those of 57RP WT cells, the appearance of SCVs growing on hexadecane of this strain was a new observation compared to the previously obtained results with this rapid growth strain. This reflects a phenotypic variation-based adaptive response to certain environmental stress conditions used in the present study. Our observations were in accordance with the current knowledge that environmental conditions

substantially affect the phase variation rate of bacteria. The growth condition (MSM with 1% hexadecane shaking at 25^{o}C) used in the present study did obviously induce a substantial increase in the variation rate leading, consequently, to the observable appearance of 57RV SCVs formed on hexadecane and detected on the rich TSA medium plating plates. Both of the tested *P. aeruginosa* strains (57RP and 57RV) with low and high cell hydrophobicities, respectively, could naturally form the highly adaptive phenotypic variant cell type (SCV) in various environmental stress conditions.

Objective 2. *Rhamnolipids are not an important factor for growth of 57RP SCV on hexadecane and octadecane*.

We confirmed that the SCV cells had a number of phenotypic characteristics that were different from those characteristics of its parent WT cell. The SCV cells were hyperpiliated and able to grow fast without lag phase on n-alkanes compared to WT cells grown in the same culture conditions. These SCV cells also had reduced motile activities compared to parent WT cells. The SCVs reverted to wild-type (WT) phenotype on a rich (TSA) medium. The cell surface of SCVs was highly hydrophobic, allowing the SCV cells to adhere effectively to the hydrophobic hydrocarbons. The SCV cells were able to form biofilms at a much higher level compared to the parent WT cells. The observed general phenotypic traits of the two strains and their derived variants, which were used in the present study, are in accordance with the earlier observation of 57RP rough SCV formation on hexadecane and phenotypic characterization of those rough SCVs developed on dextrose more than 10 years ago. This reflected the usefulness of the frozen method for a long-term preservation of bacterial specimens in laboratory conditions.

Besides the above-mentioned experimentally confirmed information, we also demonstrated clearly that rhamnolipids are not an important factor for the 57RP SCV to grow fast without an initial lag phase on hexadecane. It was because of the fact that this phenotypic variant, though derived from the biosurfactant-producing parent strain, did not produce new extracellular rhamnolipids in the growth cultures with this n-alkane. This was

an first contribution of the present study to define the unimportant role of rhamnolipids in growth of the 57RP SCV on hexadecane. Instead of producing rhamnolipids to help dispersing hexadecane and so increasing the contact surface between the cultured cells and the weakly soluble hydrophobic substrate, the growth ability of this phenotypic variant relied primarily on the high cell hydrophobicities that should certainly have made the adherence of SCV cells to the hydrophobic substrate be more effective compared to the observed lower level of adherence to hexadecane of the 57RP parent WT cells with much lower cell hydrophobicities.

Objective 3. *The involvement of c-di-GMP in phenotypic switching*.

On the basis of various observations related to the involvement of the universal bacterial second messenger c-di-GMP in the life-style transition, from the planktonic to the sessile life cycle, as from the WT to SCV phenotypic variants, including the SCV formation observed in various clinical strains of *P. aeruginosa*, we hypothesized that SCV formation occurred in cultures of our laboratory nonclinical *P. aeruginosa* strains, such as 57RP SCVs grown on hexadecane, also resulted from an overproduction of c-di-GMP occurred within this phenotypic cell type.

We found a positive correlation between the two coincidentally occurring events, as the high c-di-GMP levels are associated with the SCV formation and low c-di-GMP levels with the SCV reversion to wild-type phenotype like the parent WT cells. To study further the involvement of c-di-GMP in the SCV formation, we generated two integrant clones from 57RP WT cells. These two integrants possessed genes encoding an extra PDE (RP401) and DGC (RP402), the two enzymes for hydrolysis and synthesis of c-di-GMP, respectively, under the control of an arabinose-inducible promoter. Results obtained from the comparative study showed that the effective c-di-GMP concentration appeared to be produced by certain not-identified-yet individual c-di-GMP encoding gene(s) among the 39-40 genes already known in *P. aeruginosa*. The expression of the extra DGC in RP402 obviously contributed to increase the intracellular c-di-GMP concentrations that, in turn,

induced phenotypic switching on in certain cells of some other stochastic cell subpopulations to form new SCVs in addition to those already formed by phase variation in the previous culture conditions. In contrast, the expression of the extra PDE in RP401 did seriously affect the time of appearance and the number of SCVs that usually occurred in the cultures of the parent 57RP strain with hexadecane as the cell mass of the cultures was not increased and just a few SCVs were detected on day 15 of the cultures.

We hypothesized that these growing RP401 SCVs were also the c-di-GMP-dependent SCVs that should have somehow either increased the c-di-GMP production to compensate the loss of c-di-GMP, due to the hydrolytic activities of the inducible extra PDE, or decreased the activities of the inducible extra PDE. In fact, a representative SCV clone (RP401S), derived from the inducible integrant RP401 grown on hexadecane in the presence of arabinose, did produce c-di-GMP at the levels more-or-less similar to and even accumulated c-di-GMP at the levels obviously higher than the levels produced by SCV2h (a representative 57RP-SCV), depending on the culture age and the absence or presence of arabinose in the culture conditions. These results support our hypothesis posed at the beginning of the present thesis that c-di-GMP plays an important role in the phenotypic switching on (SCV formation on alkanes) or off (reversion to wild-type phenotype in rich media), depending on levels higher or lower than some not-yet-identified minimal effective concentration threshold(s), respectively. This is the first time an important role of c-di-GMP in the phenotypic switching has been investigated and made considerably clear by the cultures of SCVs with hexadecane and by its reversion to wild-type phenotype on a rich (TSA) medium of a non-clinical strain of *P. aeruginosa*, the 57RP strain.

Objective 4. *57RP SCVs cells grown on hexadecane synthesize unknown proteins and generate* LipA *isoforms*.

In the comparative analysis of proteome profiles of SCV and WT cells of the 57RP strain, which were cultured in the same growth conditions (MSM with 1% glucose), we found certain differences in the protein expression pattern, either up- or down-regulated

expression, between these two cell types. The initially selected up-regulated proteins represented those proteins located in different cellular compartments, such as cytoplasm, inner and outer membranes and periplasm. This indicates that gene expression of these SCV and WT cells was up-/down-regulated differently in the same growth conditions.

Further analyses of proteome profiles of 57RP SCV cells grown on hexadecane showed that the cell membranes and periplasm of this cell type were characterized initially by the presence of various proteins involved in the transport, antioxidant, nitrogen regulatory activities, etc. In addition, several new and very interesting phenomena, relating to the appearance of an unknown protein and, especially, of the three outer membrane-bound protein isoforms were detected. The three outer membrane-bound protein isoforms have been identified as the secreted lipase (LipA) isoforms. For the first time, such an unusually physiological phenomenon was observed in a *P. aeruginosa* strain SCV cell population grown on hexadecane. Among these newly identified three LipA isoforms, there was one being in the mature polypeptide form and its amino acid sequence corresponded exactly to the amino acid sequence, at least, of the PAO1 LipA. The two other isoforms were in the premature polypeptide form of LipA. One of these two premature polypeptides was identical to the LipA of an already known *P. aeruginosa* natural variant (TE3285) and the other was a completely new LipA isoform.

At present, the underlying mechanisms for these new and unusual events and the physiological roles, if any, of the unusually synthesized proteins are largely unknown. The above-identified proteins represented only a part of a larger change essential for the formation of highly adaptive SCV cells capable of growing on n-alkanes. Further investigations are needed to be carried out in the future to provide a better understanding of the phase variation-based and the specific environmental stimuli-induced physiologically adaptive but unusual responses for survival and growth of the 57RP SCV cells cultured on hexadecane.

6.2 Proposal

To understand further the physiologically new and unusual events observed in the present study I propose several additional experiments to be carried out in the future investigations as follows:

a. Identify the c-di-GMP-metabolizing gene(s) involved in the SCV formation and reversion to wild-type phenotype of the 57RP strain grown in different conditions, primarily on hexadecane and TSA rich medium. Results obtained from these to-be-carried-out studies shall contribute to verify our previously suggested genotype-dependent and environment-specific characters of the adaptive gene expression-based on phase variation as observed in the 57RP SCV grown on hexadecane.

b. Identify the original LipA gene copy number of the 57RP WT strain and the nucleotide sequences of the corresponding gene(s) derived from 57RP SCV and 57RP Rev cells to see whether this gene(s) remained unchanged or some of which had undergone certain genetic variations. Compare the RNA sequences from WT, SCV and Rev, which were grown in the same growth conditions (e.g. MSM with glucose) and the SCV grown specifically on hexadecane in MSM, with the corresponding DNA sequences of the 57RP WT, 57RP SCV and the 57RP Rev cells, etc. Results obtained from these comparative studies, together with the already known information of amino acid sequences of the three newly identified LipA isoforms in the present study, should contribute to get further new insights into the currently not-yet-known underlying mechanisms for the appearance of polypeptide isoforms in bacteria such as the LipA isoforms observed in the 57RP SCV grown on hexadecane, a highly environmental stress adaptive phenotypic variant type of *P. aeruginosa* species.

c. Investigate the origin of ORF encoding the unknown protein PP-S15 (resulted either from a lateral gene transfer or from the phase variation-induced unusual events) and its role, if any, in the formation of LipA isoforms as well as in the environmental stress high adaptability of 57RP-SCVs grown on hexadecane.

6.3 Research scheme and perspective

6.3.1 Research scheme

Based on the results achieved from the present study, a schematic summary of the present research, relating the important role of c-di-GMP in the SCV formation, is introduced in Figure 6.1. This figure presents the formation and selection of highly adaptive phenotypic variants, the SCVs that were formed by phase variation in the previously cultured 57RP cell population prior to being exposed to hexadecane in the present culture MSM medium shaking at 25°C. The phenotype-specific characters of 57RP SCVs grown on hexadecane are also illustrated by the representative proteins, which were specifically found in different cellular compartments.

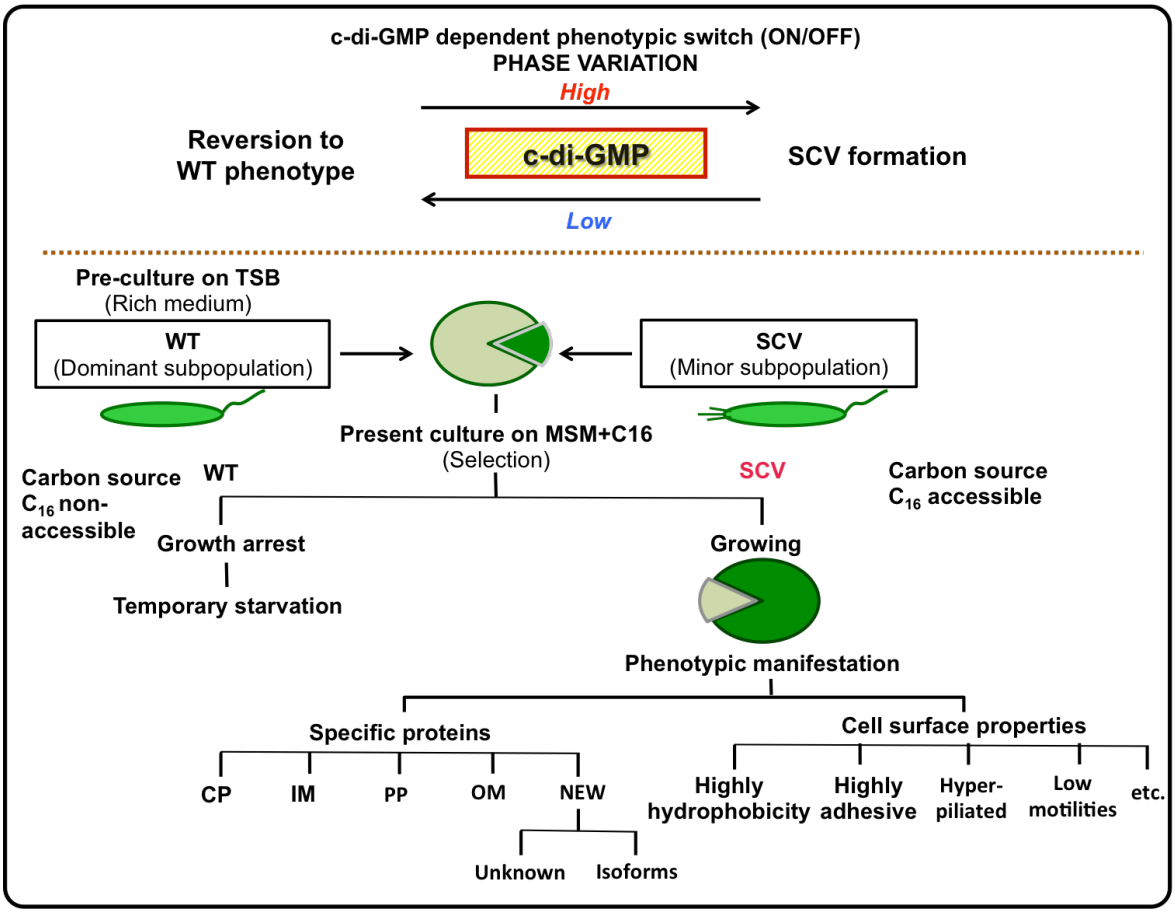


Figure 6.1 Schematic summary of present research

6.3.2 Perspective

I propose also another possible way that may lead to the formation of c-di-GMP-dependent SCVs from the 57RP WT cells grown on hexadecane (Figure 6.2). The 57RP WT cells, which dominated the cell population in the previous rich culture conditions, were not able to grow on the hexadecane that was used as a sole carbon source in the present culture conditions. Consequently, these major WT cells suffered from a temporal starvation and remained as the growth-arresting cells as well as and did not produce new c-di-GMP. Based on the current knowledge of stringent response in response to the carbon starvation of bacteria, I speculate that majority of the starving 57RP WT cells grown on hexadecane would also have synthesized the generally known alarmone (p)ppGpp and used it to reprogram their gene expression and changed their cellular processes for survival. In this case, the generally resultant growing SCV population may be comprised of two c-di-GMP-dependent SCV subpopulations, one of which was the subpopulation of phenotypic variants and the other was that of the genetic mutants as those previously identified clinical *P. aeruginosa* SCVs.

The proposed ways of the formation of the stress growth condition of highly-adapted phenotypic or genetic variants needs more time to be verified in the future. However, it will be really necessary to carry out, the investigations to better understand the role of the universal bacterial second messenger c-di-GMP in the phenotypic switching (On/Off) and its role in the phase variation-based, environment specific stimuli-induced expression of certain physiologically important genes, primarily, in the *P. aeruginosa* SCV cells that are highly adaptive to grow well in various environmental stress conditions.

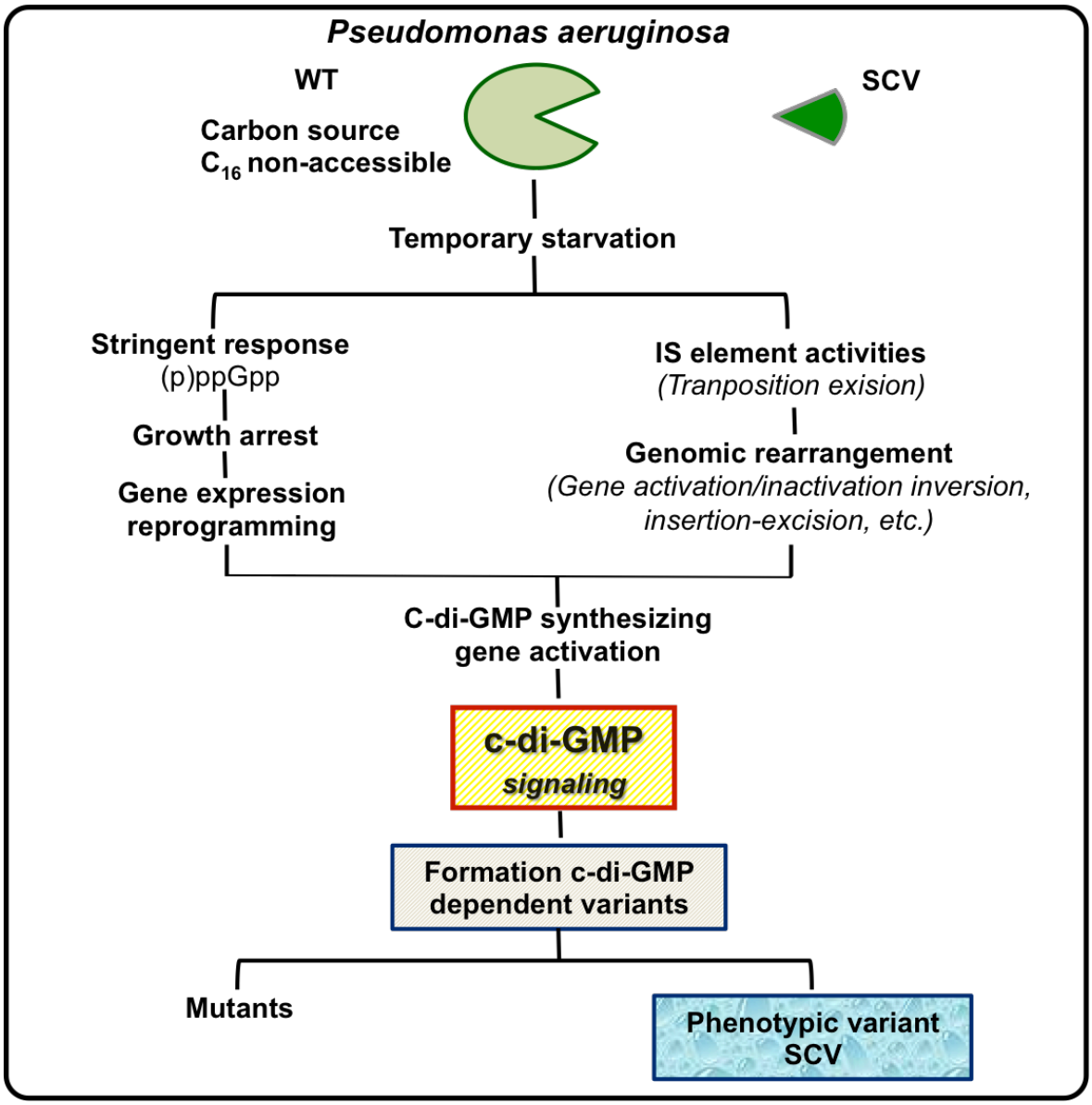


Figure 6.2 Proposal for future work

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8 APPENDIX

8.1 Scientific communications

Representative results of the Thesis have been presented in poster form at different national and international scientific conferences as follows:

1. Nguyen TT, Déziel E, Villemur R 2014. Involvement of c-di-GMP in small colony variant (SCV) formation and related protein appearance when *Pseudomonas aeruginosa* is grown on hexadecane. The International Union of Microbiological Societies In Montreal, QC, Canada, Jul. 27-Aug. 2, 2014.

2. Nguyen TT, Déziel E, Nicastro GG, Lépine F, Villemur R 2013. Characterization of small colonies of *Pseudomonas aeruginosa* cultured on liquid hexadecane. The Armand-Frappier Conference, Orford, Quebec, Canada, Nov. 14-16, 2013.

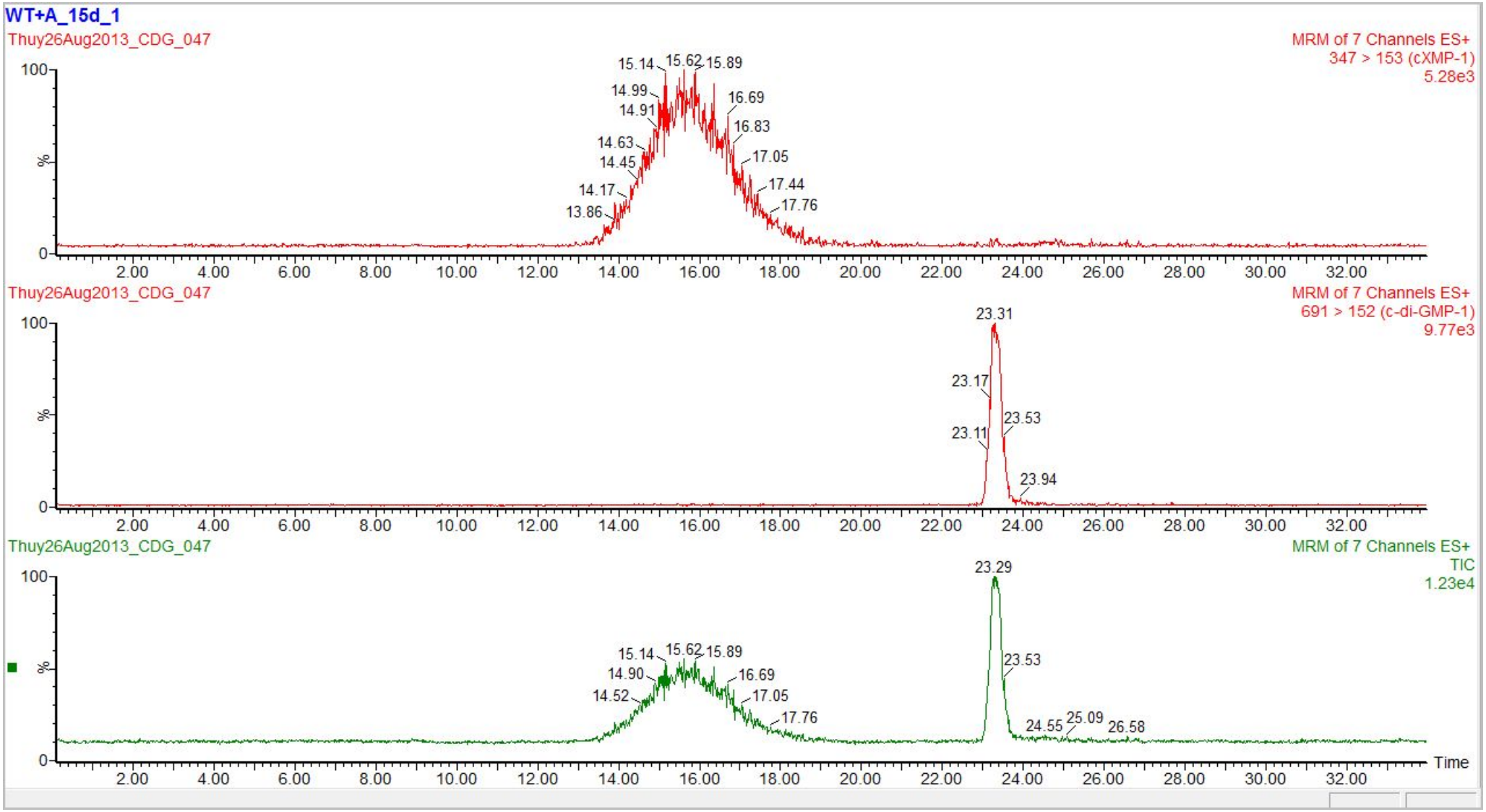
3. Nguyen TT, Déziel E, Lépine F, Villemur R 2012. Characterization of phenotypic changes involved in the uptake of weakly soluble hydrocarbon by *Pseudomonas aeruginosa* 57RP. The 6th international conference of The American Society for Microbiology on Biofilm, Miami, Florida, USA, Sep. 29 – Oct. 4, 2012.

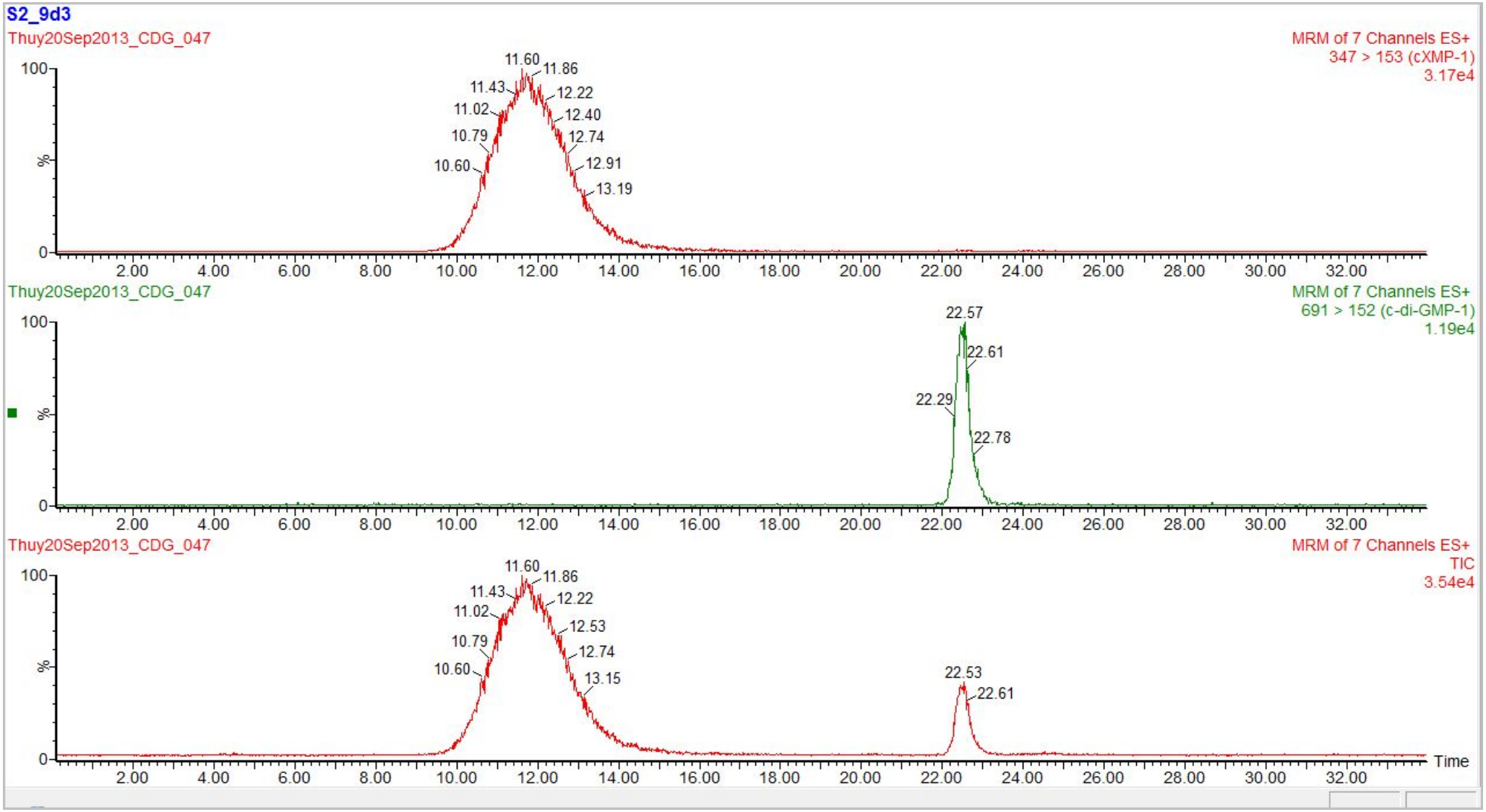
8.2 Manuscripts are in preparation

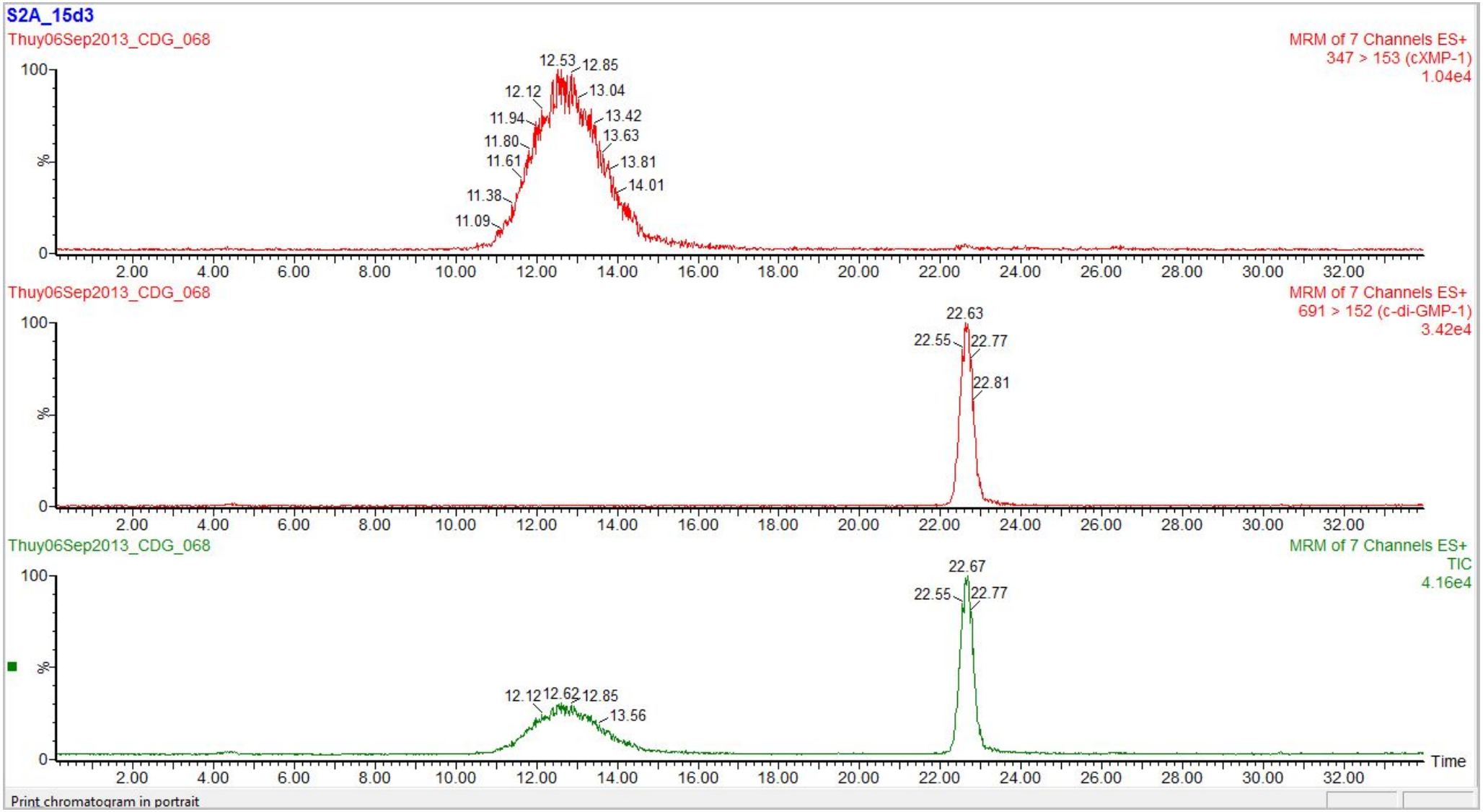
1. Role of c-di-GMP in the phenotypic switch on of the formation of small colony variants (SCVs) of *Pseudomonas aeruginosa* 57RP strain grown on hexadecane.

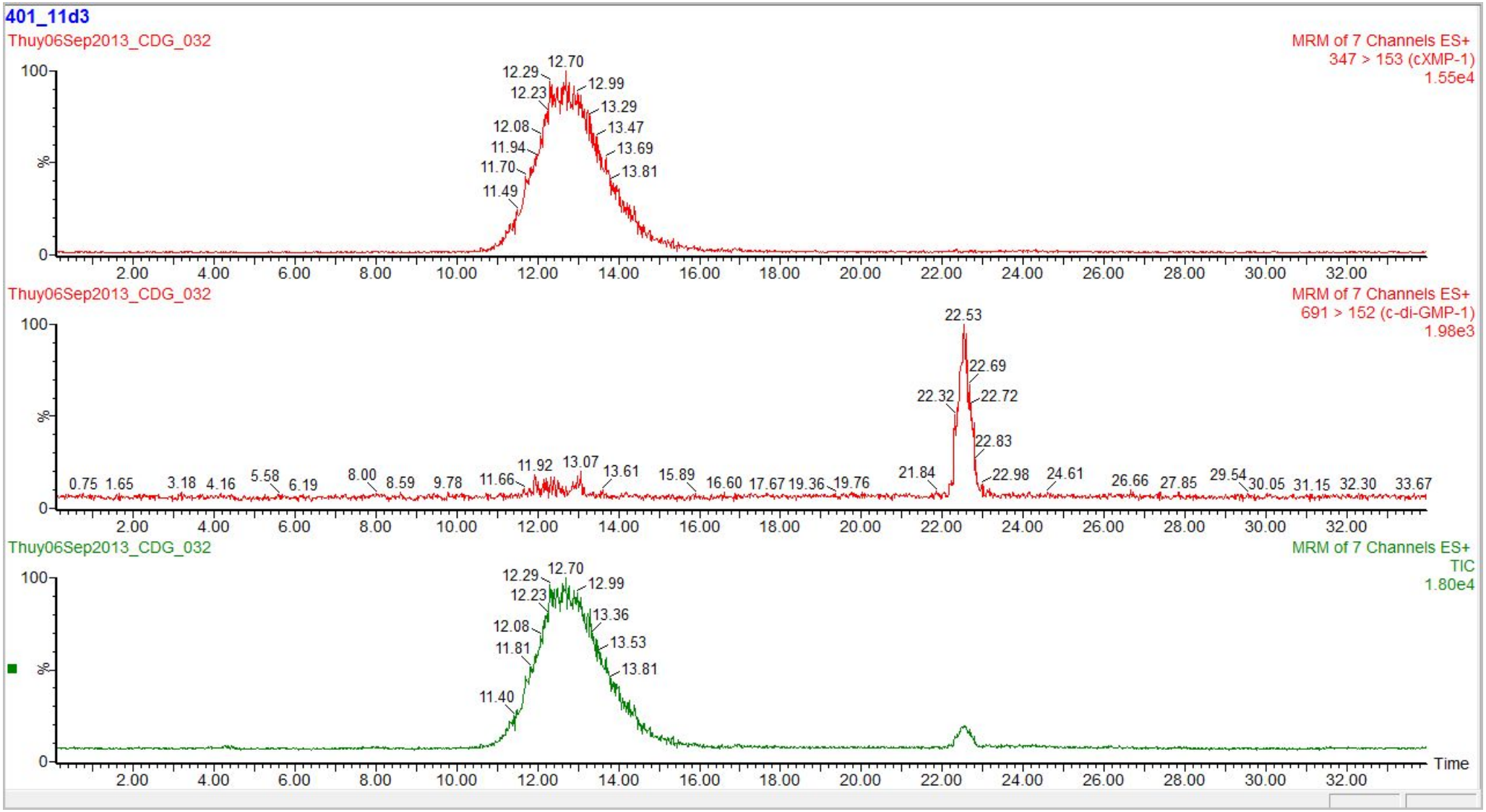
2. LipA isoforms are synthesized simultaneously in *Pseudomonas aeruginosa* 57RP SCVs grown on hexadecane.

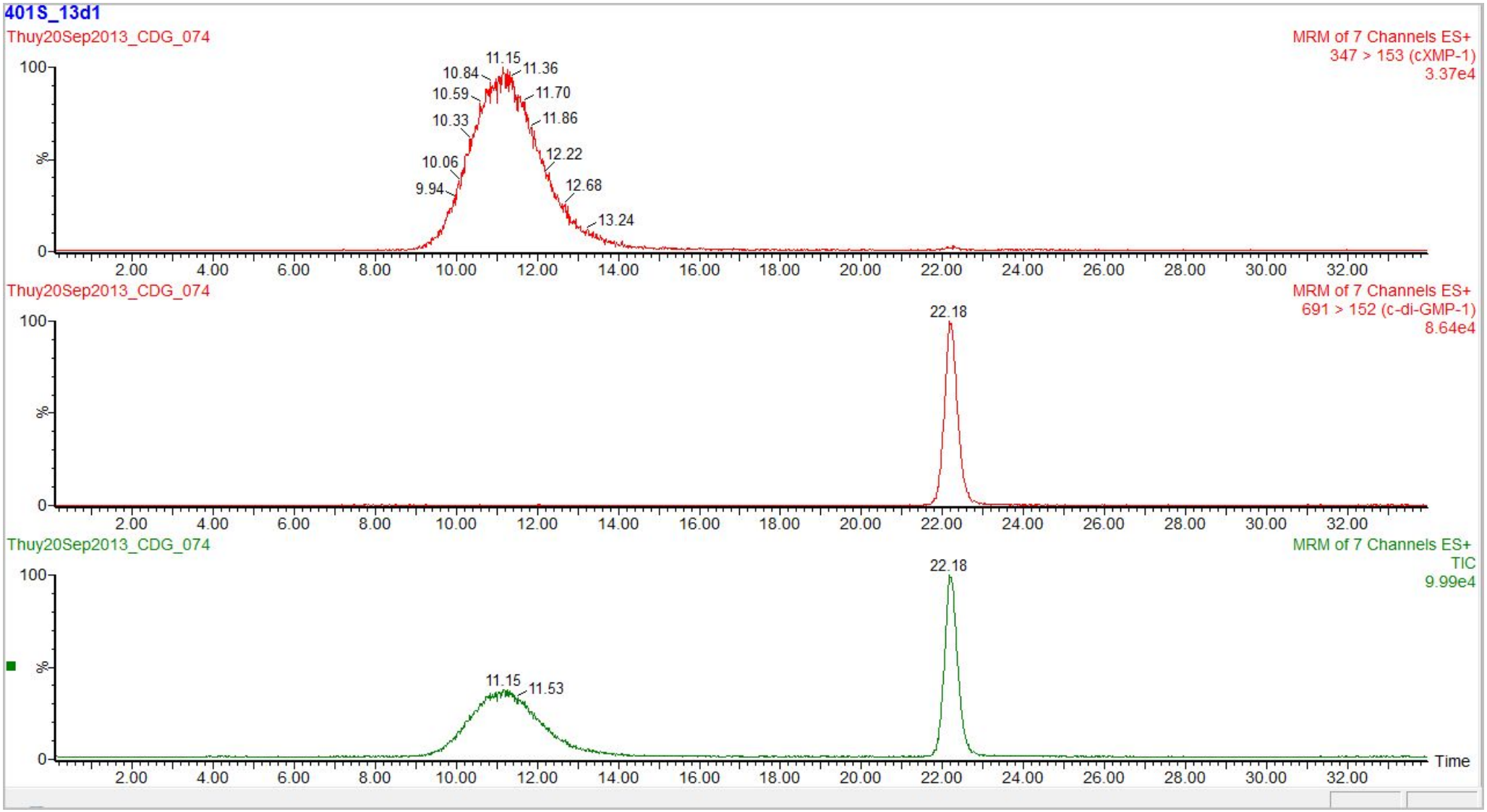
8.3 Selective chromatographs of some study strains


WT+A_15d_1
Thuy26Aug2013_CDG_047
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
5.28e3
15.14
15.62
15.89
14.99
14.91
16.69
16.83
14.63
14.45
17.05
17.44
14.17
17.76
13.86
Thuy26Aug2013_CDG_047
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
9.77e3
23.31
23.17
23.53
23.11
23.94
Thuy26Aug2013_CDG_047
MRM of 7 Channels ES+
TIC
1.23e4
23.29
23.53
15.14
15.62
15.89
14.90
16.69
14.52
17.05
17.76
24.55
25.09
26.58
%
100
0
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
Time


S2_9d3
Thuy20Sep2013_CDG_047
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
3.17e4
11.60
11.86
11.43
12.22
11.02
12.40
10.79
12.74
10.60
12.91
13.19
Thuy20Sep2013_CDG_047
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
1.19e4
22.57
22.61
22.29
22.78
Thuy20Sep2013_CDG_047
MRM of 7 Channels ES+
TIC
3.54e4
11.60
11.86
11.43
12.22
11.02
12.53
10.79
12.74
10.60
13.15
22.53
22.61
%
Time
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00


S2A_15d3
Thuy06Sep2013_CDG_068
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
1.04e4
12.53
12.85
12.12
13.04
11.94
13.42
11.80
13.63
11.61
13.81
11.38
14.01
11.09
Thuy06Sep2013_CDG_068
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
3.42e4
22.63
22.55
22.77
22.81
Thuy06Sep2013_CDG_068
MRM of 7 Channels ES+
TIC
4.16e4
22.67
22.55
22.77
12.12
12.62
12.85
13.56
%
Time
Print chromatogram in portrait


401_11d3
Thuy06Sep2013_CDG_032
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
1.55e4
12.29
12.70
12.23
12.99
13.29
12.08
13.47
11.94
13.69
11.70
13.81
11.49
Thuy06Sep2013_CDG_032
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
1.98e3
22.53
22.69
22.32
22.72
22.83
0.75 1.65 3.18 4.16 5.58 6.19 8.00 8.59 9.78 11.66 11.92 13.07 13.61 15.89 16.60 17.67 19.36 19.76 21.84 22.98 24.61 26.66 27.85 29.54 30.05 31.15 32.30 33.67
Thuy06Sep2013_CDG_032
MRM of 7 Channels ES+
TIC
1.80e4
12.29
12.70
12.23
12.99
13.36
12.08
13.53
11.81
13.81
11.40
%
100
0
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00
Time


401S_13d1
Thuy20Sep2013_CDG_074
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
3.37e4
11.15
10.84
11.36
10.59
11.70
10.33
11.86
10.06
12.22
9.94
12.68
13.24
Thuy20Sep2013_CDG_074
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
8.64e4
22.18
Thuy20Sep2013_CDG_074
MRM of 7 Channels ES+
TIC
9.99e4
22.18
11.15
11.53
%
Time
2.00
4.00
6.00
8.00
10.00
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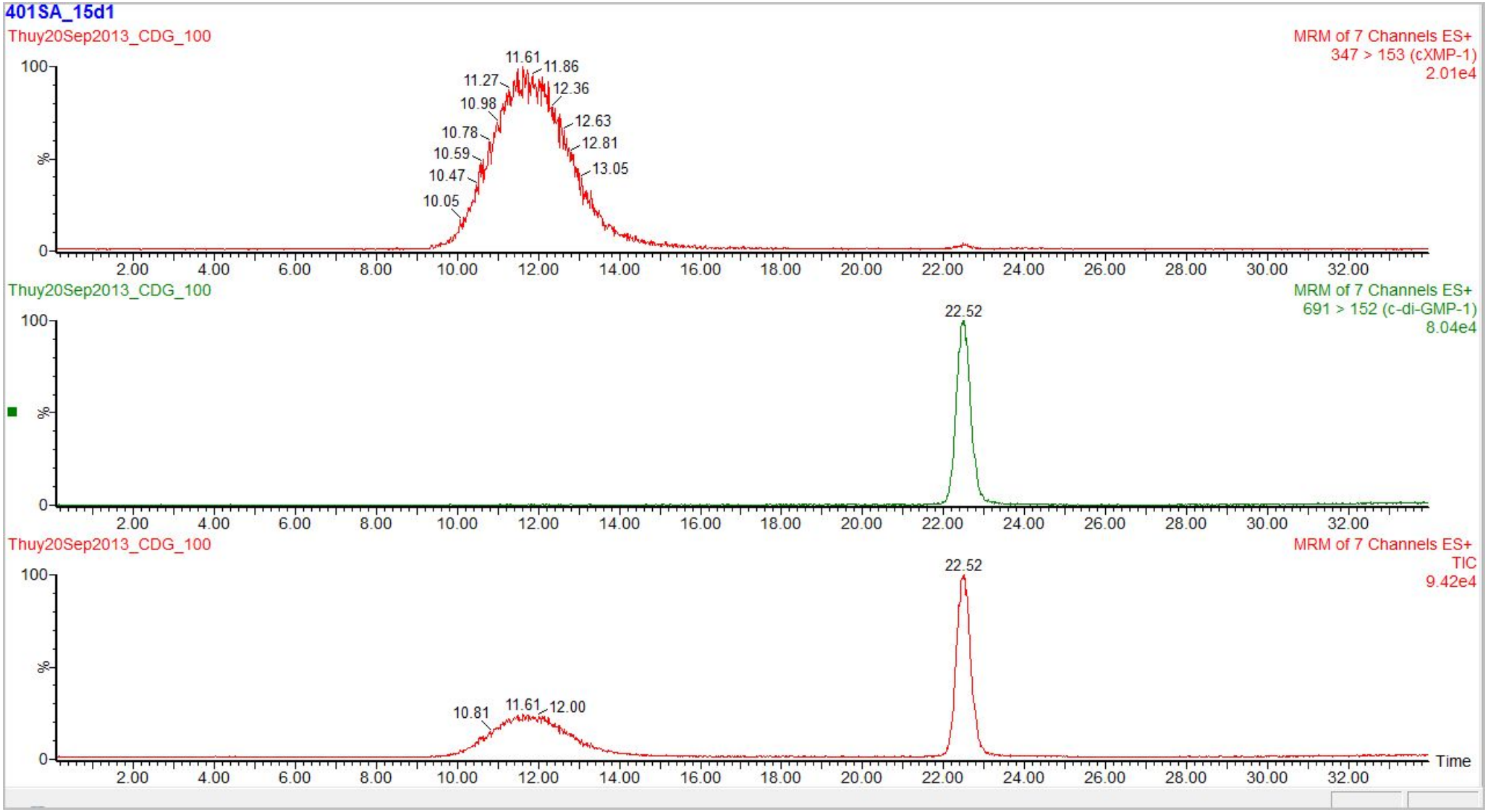
22.00
24.00
26.00
28.00
30.00
32.00


401SA_15d1
Thuy20Sep2013_CDG_100
MRM of 7 Channels ES+
347 > 153 (cXMP-1)
2.01e4
11.61
11.86
11.27
12.36
10.98
12.63
10.78
12.81
10.59
13.05
10.47
10.05
Thuy20Sep2013_CDG_100
MRM of 7 Channels ES+
691 > 152 (c-di-GMP-1)
8.04e4
22.52
Thuy20Sep2013_CDG_100
MRM of 7 Channels ES+
TIC
9.42e4
22.52
10.81
11.61
12.00
%
Time

8.4 Protein analysis

MATRIX SCIENCE MASCOT Search Results

Protein View: gi|15598833

pyrG gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	498
Nominal mass (M_r):	59581
Calculated pI:	5.42
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|116051634 from Pseudomonas aeruginosa UCBPP-PA14
- gi|152986406 from Pseudomonas aeruginosa PA7
- gi|218890138 from Pseudomonas aeruginosa LESB58
- gi|254236551 from Pseudomonas aeruginosa C3719
- gi|254242335 from Pseudomonas aeruginosa 2192
- gi|296387858 from Pseudomonas aeruginosa PAb1
- gi|313109041 from Pseudomonas aeruginosa 39016
- gi|355640019 from Pseudomonas sp. 2_1_26
- gi|386057376 from Pseudomonas aeruginosa M18
- gi|386067672 from Pseudomonas aeruginosa NCGM2.S1
- gi|392982639 from Pseudomonas aeruginosa DK2
- gi|24212158 from Pseudomonas aeruginosa PAO1
- gi|122260777 from Pseudomonas aeruginosa UCBPP-PA14
- gi|226733146 from Pseudomonas aeruginosa PA7
- gi|226733147 from Pseudomonas aeruginosa LESB58
- gi|9949796 from Pseudomonas aeruginosa PAO1
- gi|115586855 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126168482 from Pseudomonas aeruginosa C3719
- gi|126195713 from Pseudomonas aeruginosa 2192
- gi|150961564 from Pseudomonas aeruginosa PA7
- gi|218770361 from Pseudomonas aeruginosa LESB58
- gi|310881516 from Pseudomonas aeruginosa 39016
- gi|334843559 from Pseudomonas aeruginosa HB13
- gi|334844236 from Pseudomonas aeruginosa HB15
- gi|346055995 from Pseudomonas aeruginosa NCMG1179
- gi|347303682 from Pseudomonas aeruginosa M18
- gi|348036231 from Pseudomonas aeruginosa NCGM2.S1
- gi|354831539 from Pseudomonas sp. 2_1_26
- gi|375043802 from Pseudomonas aeruginosa MPAO1/P1
- gi|375051678 from Pseudomonas aeruginosa MPAO1/P2
- gi|384398589 from Pseudomonas aeruginosa PADK2_CF510
- gi|392318144 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598833 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 19%

Matched peptides shown in ***bold red***.

```
  1 MTRYIFVTGG VVSSLGKGIA SASLAAILEA RGLKITMLKL DPYINVDPGT
 51 MSPFQHGEVF VTQDGAETDL DLGHYERFVR TTMTQNNNFT TGRVYMDVLR
101 KERRGDYLGA TVQVIPHITD EIKRRIIKGA GDADVALVEI GGTVGDIESQ
151 PFLEAIRQLR VEIGAKRAML MHLTLVPYIA TAGETKTKPT QHSVKELRSI
201 GLQPDVLVCR SDHPIDVSSR RKIALFTNVE ERAVIALEDV DTIYRIPSVL
251 HAQGLDDIVV ERFGLECGQA DLSEWDRVVD AKLNPEREVT IAMVGKYMEL
301 LDAYKSLIEA MTHAGIQSRT KVNLRYIDSE DIEQQGTSLL EGVDAILVPG
351 GFGLRGVEGK ISTVQYAREN KIPYLGICLG MQVAVIEYAR NVLGWSDANS
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401 TEFDKSSGHP VVGLITEWQD ATGATEIRTE ASDLGGTMRL GAQECQLQTG
451 TLVHDCYAKD VIVERHRHRY EVNNNLLPQL EQAGLKISGR SGDGALVEVV
501 EAPEHPWFVA CQFHPEFTST PRDGHPLFSG FVNAALKYSG KA
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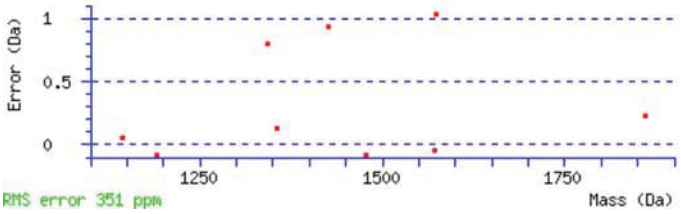
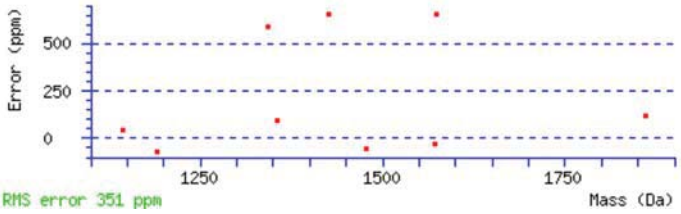
Unformatted sequence string: **542 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
195	4 – 17	714.3650	1426.7155	1425.7868	0.9287	0	55	0.23	1		R.YIFVTGGVVSSLGK.G
170	18 – 31	672.2838	1342.5531	1341.7616	0.7915	0	62	0.047	1		K.GIASASLAAILEAR.G
176	199 – 210	678.9332	1355.8519	1355.7231	0.1288	0	45	2.3	1		R.SIGLQPDVLVCR.S + Carbamidomethyl (C)
126	223 – 232	596.2802	1190.5458	1190.6295	-0.0837	0	70	0.0091	1		K.IALFTNVEER.A
127	223 – 232	596.2815	1190.5485	1190.6295	-0.0810	0	64	0.037	1		K.IALFTNVEER.A
214	233 – 245	739.3560	1476.6975	1476.7824	-0.0849	0	102	4.5e-06	1	U	R.AVIALEDVDTIYR.I
277	246 – 262	621.0856	1860.2349	1860.0105	0.2244	0	69	0.007	1	U	R.IPSVLHAQGLDDIVVER.F
118	297 – 305	573.3076	1144.6006	1144.5474	0.0532	0	46	2.3	1		K.YMELLDAYK.S
231	523 – 537	786.8887	1571.7629	1571.8096	-0.0467	0	45	2.3	1	U	R.DGHPLFSGFVNAALK.Y
233	523 – 537	525.2861	1572.8364	1571.8096	1.0267	0	48	1.1	1	U	R.DGHPLFSGFVNAALK.Y



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LOCUS       NP_252327               542 aa            linear   CON 29-OCT-2013
DEFINITION  CTP synthetase [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_252327
VERSION     NP_252327.1  GI:15598833
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 542)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 542)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 542)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 542)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 542)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
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  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
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   PUBMED   15908409
REFERENCE   6  (residues 1 to 542)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 542)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 542)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 542)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 542)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 542)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 542)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 542)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 542)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 542)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 542)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
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            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG07025.
            RefSeq Category: Reference Genome
                        COM: Community selected
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /strain="PAO1"
                     /db_xref="taxon:208964"
     Protein         1..542
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                     /EC_number="6.3.4.2"
                     /calculated_mol_wt=59487
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                     /note="CTP synthetase; Validated; PRK05380"
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     Region          3..255
                     /region_name="CTGs"
                     /note="CTP synthetase (CTPs) is a two-domain protein,
                     which consists of an N-terminal synthetase domain and
                     C-terminal glutaminase domain. The enzymes hydrolyze the
                     amide bond of glutamine to ammonia and glutamate at the
                     glutaminase domains and transfer nascent...; cd03113"
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                     /note="UTP binding site [chemical binding]"
                     /db_xref="CDD:239387"
     Region          289..532
                     /region_name="GATase1_CTP_Synthase"
                     /note="Type 1 glutamine amidotransferase (GATase1) domain
                     found in Cytidine Triphosphate Synthetase; cd01746"
                     /db_xref="CDD:153217"
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     Site            order(351,379)
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                     /db_xref="CDD:153217"
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                     /site_type="active"
                     /note="catalytic triad [active]"
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     CDS             1..542
                     /coded_by="complement(NC_002516.2:4070860..4072488)"
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                     to functionally studied protein)"
                     /transl_table=11
CONTIG      join(WP_003092366.1:1..542)
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MASCOT Search Results

Protein View: gi|15600244

argS gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	492
Nominal mass (M_r):	65158
Calculated pI:	5.35
Taxonomy:	**Pseudomonas aeruginosa PAO1**

This protein sequence matches the following other entries:

- **gi|107104151** from **Pseudomonas aeruginosa PACS2**
- **gi|254238242** from **Pseudomonas aeruginosa C3719**
- **gi|296391898** from **Pseudomonas aeruginosa PAb1**
- **gi|355643269** from **Pseudomonas sp. 2_1_26**
- **gi|386061224** from **Pseudomonas aeruginosa M18**
- **gi|23822207** from **Pseudomonas aeruginosa PAO1**
- **gi|9951342** from **Pseudomonas aeruginosa PAO1**
- **gi|126170173** from **Pseudomonas aeruginosa C3719**
- **gi|334837687** from **Pseudomonas aeruginosa HB15**
- **gi|347307530** from **Pseudomonas aeruginosa M18**
- **gi|354829774** from **Pseudomonas sp. 2_1_26**
- **gi|375041567** from **Pseudomonas aeruginosa MPAO1/P1**
- **gi|375046855** from **Pseudomonas aeruginosa MPAO1/P2**

Sequence similarity is available as **an NCBI BLAST search of gi|15600244 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 18%

Matched peptides shown in ***bold red***.

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  1 MKDTIRQLIQ QALDQLTADG TLPAGLTPDI QVENTKDRSH GDFASNIAMM
 51 LAKPAGMKPR DLAARLVEAI PAHEQLAKVE IAGPGFLNFF QDHVWLAASL
101 DRALADERLG VRKAGPAQRV VIDLSSPNLA KEMHVGHLRS TIIGDAVARV
151 LEFLGDTVIR QNHVGDWGTQ FGMLLAYLEE QPVDAEAELH DLEVFYRAAK
201 KRFDESPEFA DRARELVVKL QAGDPDCLRL WTRFNEISLS HCQKVYDRLG
251 VKLSMADVMG ESAYNDDLAQ VVADLTAKGL LTEDNGALCV FLEEFKNAEG
301 NPLPVIVQKA GGGYLYATTD LAAMRYRHNV LHADRVLYFV DQRQALHFQQ
351 VFEVARRAGF VPAGMELEHM GFGTMNGADG RPFKTRDGGT VKLIDLLEEA
401 ESRAYALVKE RNEQRAERGE EPFDEVQLRE IGRVVGIDSV KYADLSKHRT
451 SDYSFNFELM LSFEGNTAPY LLYACTRVAS VFRKLGQGRE QLGGKIVLEQ
501 PQELALAAQL AQFGDLINNV ALKGVPHLLC AYLYELAGLF SSFYEHCPIL
551 TAEDPAQKDS RLRLAALTGR TLEQGLELLG LKTLERM
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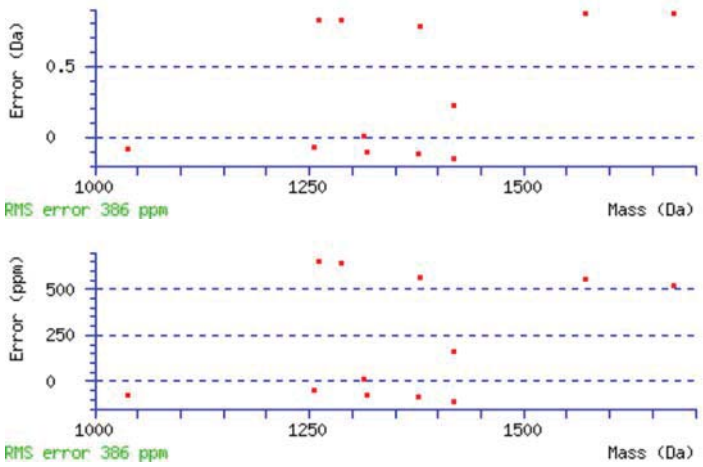
Unformatted sequence string: **587 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
190	66 – 78	709.8276	1417.6406	1417.7929	-0.1523	0	42	5.2	1	U	R.LVEAIPAHEQLAK.V
191	66 – 78	473.6806	1418.0200	1417.7929	0.2271	0	42	4.7	1	U	R.LVEAIPAHEQLAK.V
142	120 – 131	628.3337	1254.6529	1254.7183	-0.0654	0	42	5.6	1		R.VVIDLSSPNLAK.E
146	150 – 160	631.7721	1261.5297	1260.7078	0.8219	0	49	1.2	1		R.VLEFLGDTVIR.Q
180	297 – 309	689.8328	1377.6511	1377.7616	-0.1105	0	63	0.034	1		K.NAEGNPLPVIVQK.A

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
181	297 – 309	690.2774	1378.5402	1377.7616	0.7786	0	26	1.8e+02	3		K.NAEGNPLPVIVQK.A
90	336 – 343	520.2431	1038.4717	1038.5498	-0.0781	0	53	0.36	1	U	R.VLYFVDQR.Q
232	344 – 356	525.2382	1572.6928	1571.8209	0.8719	0	27	1.6e+02	2		R.QALHFQQVFEVAR.R
150	393 – 403	644.7557	1287.4968	1286.6718	0.8250	0	52	0.56	1		K.LIDLLEEAESR.A
250	416 – 429	559.2328	1674.6765	1673.8009	0.8756	1	44	3	1	U	R.AERGEEPFDEVQLR.E
160	419 – 429	659.7673	1317.5201	1317.6201	-0.1000	0	54	0.35	1	U	R.GEEPFDEVQLR.E
156	571 – 582	657.3949	1312.7752	1312.7602	0.0150	0	67	0.015	1		R.TLEQGLELLGLK.T

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DEFINITION  arginyl-tRNA synthetase [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_253738
VERSION     NP_253738.1  GI:15600244
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 587)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 587)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 587)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 587)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 587)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 587)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
```

```
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 587)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 587)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 587)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 587)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 587)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 587)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 587)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 587)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 587)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 587)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
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  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG08436.
            RefSeq Category: Reference Genome
                        COM: Community selected
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /calculated_mol_wt=65069
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                     /note="Arginyl tRNA synthetase N terminal dom; smart01016"
                     /db_xref="CDD:214975"
     Region          119..388
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                     /note="catalytic core domain of arginyl-tRNA synthetases;
                     cd00671"
                     /db_xref="CDD:185675"
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     Site            134..137
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                     /note="HIGH motif"
                     /db_xref="CDD:185675"
     Site            383..386
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                     /note="KMSK motif region"
                     /db_xref="CDD:185675"
     Region          433..587
                     /region_name="Anticodon_Ia_like"
                     /note="Anticodon-binding domain of class Ia aminoacyl tRNA
                     synthetases and similar domains; cl12020"
                     /db_xref="CDD:264512"
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                     /db_xref="CDD:153408"
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                     /site_type="other"
                     /note="anticodon binding site"
                     /db_xref="CDD:153408"
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                     /note="Product name confidence: class 2 (High similarity
                     to functionally studied protein)"
                     /transl_table=11
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MASCOT Search Results

Protein View: gi|576779

GroEL [Pseudomonas aeruginosa]

Database:	NCBInr
Score:	466
Nominal mass (M_r):	57036
Calculated pI:	5.04
Taxonomy:	Pseudomonas aeruginosa

Sequence similarity is available as an NCBI BLAST search of gi|576779 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 19 %

Matched peptides shown in ***bold red***.

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  1 MAAKEVKFGD SARKKMLVGV NVLADAVKAT LGPKGRNVVL DKSFGAPTIT
 51 KDGVSVAKEI ELKDKFENMG AQLVKDVASK ANDAAGDGTT TATVLAQAIV
101 NEGLKAVAAG MNPMDLKRGI DKATVAIVAQ LKELAKPCAD TKAIAQVGTI
151 SANSDESIGQ IIAEAMEKVG KEGVITVEEG SGLENELSVV EGMQFDRGYL
201 SPYFVNKPDT MAAELDSPLL LLVDKKISNI REMLPVLEAV AKAGRPLLIV
251 AEDVEGEALA TLVVNNMRGI VKVAAVKAPG FGDRRKAMLQ DIAILTGGTV
301 ISEEVGLSLE GATLEHLGNA KRVVINKENT TIIDGAGVQA DIEARVLQIR
351 KQIEETTSDY DREKLQERLG KLAGGVAVIK VGAATEVEMK EKKARVEDAL
401 HATRAAVEEG VVPGGGVALV RALQAIEGLK GDNEEQNVGI ALLRRAVESP
451 LRQIVANAGD EPSVVVDKVK QGSGNYGFNA ATGVYGDMIE MGILDPAKVT
501 RSALQAAASI GGLMITTEAM VAEIVEDKPA MGGMPDMGGM GGMGGMM
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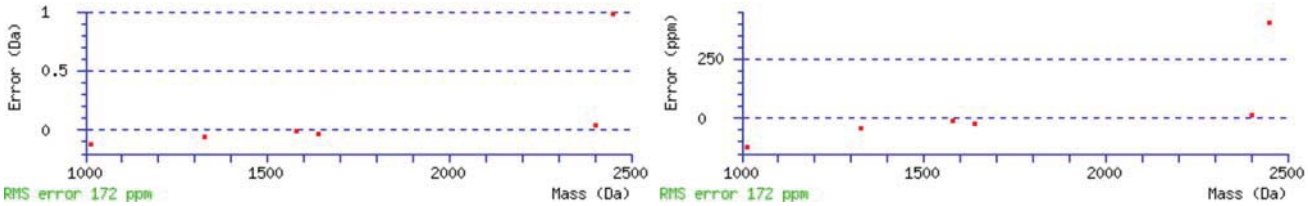
Unformatted sequence string: **547 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
165	16 – 28	664.8579	1327.7013	1327.7534	-0.0521	0	74	0.0035	1	U	K.MLVGVNVLADAVK.A
328	81 – 105	801.0917	2400.2532	2400.2132	0.0400	0	79	0.00049	1		K.ANDAAGDGTTTATVLAQAIVNEGLK.A
80	123 – 132	507.2593	1012.5040	1012.6281	-0.1240	0	57	0.16	1	U	K.ATVAIVAQLK.E
234	405 – 421	790.4366	1578.8587	1578.8730	-0.0143	0	83	0.00039	1		R.AAVEEGVVPGGGVALVR.A
331	422 – 444	818.1069	2451.2989	2450.3129	0.9860	1	85	0.00014	1	U	R.ALQAIEGLKGDNEEQNVGIALLR.R
243	453 – 468	820.9105	1639.8064	1639.8417	-0.0354	0	88	0.0001	1		R.QIVANAGDEPSVVVDK.V

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DEFINITION  GroEL [Pseudomonas aeruginosa].
ACCESSION   AAA53369
VERSION     AAA53369.1  GI:576779
DBSOURCE    locus PAU17072 accession U17072.1
KEYWORDS    .
SOURCE      Pseudomonas aeruginosa
  ORGANISM  Pseudomonas aeruginosa
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (sites)
  AUTHORS   Farinha,M.A., Mockett,R., Went,C.J., Jardine,S., Naczynski,L.M. and
```

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            Kropinski,A.M.
  TITLE     Physical mapping of several heat-shock genes in Pseudomonas
            aeruginosa and, the cloning of the mopA (GroEL) gene
  JOURNAL   Unpublished
REFERENCE   2  (residues 1 to 547)
  AUTHORS   Kropinski,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-NOV-1994) Andrew M. Kropinski, Queen's University,
            Microbiology & Immunology, Kingston, Ontario K7L 3N6, Canada
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
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                     /note="chaperonin GroEL; Reviewed; PRK12849"
                     /db_xref="CDD:183791"
     Region          4..523
                     /region_name="GroEL"
                     /note="GroEL_like type I chaperonin. Chaperonins are
                     involved in productive folding of proteins. They share a
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                     I is seven-fold and they are found...; cd03344"
                     /db_xref="CDD:48161"
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                     /note="ring oligomerisation interface [polypeptide
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     Site            order(141,186,193,375,409..410)
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MASCOT Search Results

Protein View: gi|15598667

malate dehydrogenase [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	424
Nominal mass (M_r):	62381
Calculated pI:	5.34
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|296388011 from Pseudomonas aeruginosa PAb1
- gi|386057561 from Pseudomonas aeruginosa M18
- gi|392982789 from Pseudomonas aeruginosa DK2
- gi|81783645 from Pseudomonas aeruginosa PAO1
- gi|9949615 from Pseudomonas aeruginosa PAO1
- gi|334837024 from Pseudomonas aeruginosa HB15
- gi|334837703 from Pseudomonas aeruginosa HB13
- gi|346059296 from Pseudomonas aeruginosa NCMG1179
- gi|347303867 from Pseudomonas aeruginosa M18
- gi|375043433 from Pseudomonas aeruginosa MPAO1/P1
- gi|375049186 from Pseudomonas aeruginosa MPAO1/P2
- gi|384398739 from Pseudomonas aeruginosa PADK2_CF510
- gi|392318294 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598667 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 20%

Matched peptides shown in ***bold red***.

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  1 MTETAKRPLY VPHAGPSLLE MPLLNKGSAF STQERIDFNL QGLLPHNIET
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101 IYTPTVGQAC QEFSKIYRTH RGLFISYPDR ERIDDILRSA TKNNVKIVVV
151 TDSERILGLG DQGIGGMGIP IGKLSLYTAC GGISPAYTLP VVLDVGTNNP
201 DLLNDPMYMG WRHERVSGAQ YEEFVDLFIQ AIKRRWPNVL LQFEDFAQTN
251 AMPLLERYKD ELCCFNDDIQ GTAAVAVGTL LAACKAKGEK LSEQTVTFVG
301 AGSAGCGIAE QIIAAMQLEG LDEAQARRRI FMVDRWGLLT DDMSNLLDFQ
351 HRLAQKRADL GAWGGQQGDD LALLEVIRNA RPTVLIGVSG QRGLFSEEVI
401 RELHSHCKQP LVMPLSNPTS RVEATPQEIL NWTDGQALVA TGSPFQPVQV
451 GDKRIPIAQC NNAYIFPGIG LGVIAARANR VTEGMLMAAA NALANCSPIV
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551 FWFPRYRAYR RTSF
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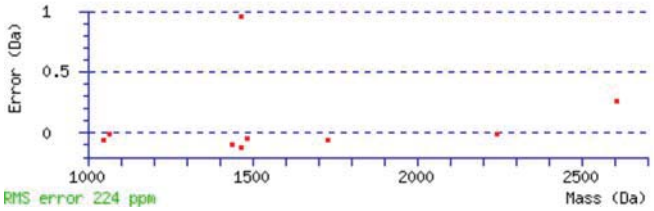
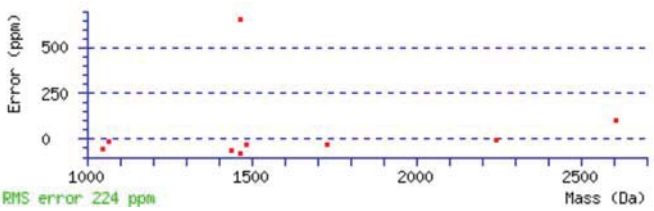
Unformatted sequence string: 564 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
318	7 – 26	749.0869	2244.2387	2244.2452	-0.0065	0	81	0.00038	1	U	K.RPLYVPHAGPSLLEMPLLNK.G
340	36 – 57	870.5321	2608.5746	2608.3133	0.2613	0	62	0.025	1	U	R.IDFNLQGLLPHNIETIEEQTER.A
261	58 – 71	866.8548	1731.6951	1731.7522	-0.0571	0	95	1.9e-05	1	U	R.AYSQYNLCNTDLDR.H + Carbamidomethyl (C)
216	77 – 88	742.3392	1482.6638	1482.7103	-0.0465	0	38	12	1		R.SIQDNNETLFFR.L
98	122 – 130	534.2738	1066.5330	1066.5447	-0.0117	0	35	27	2	U	R.GLFISYPDR.E
209	379 – 392	734.3633	1466.7121	1466.8318	-0.1196	0	22	4.1e+02	5	U	R.NARPTVLIGVSGQR.G
210	379 – 392	490.2718	1467.7935	1466.8318	0.9617	0	36	19	3	U	R.NARPTVLIGVSGQR.G
93	393 – 401	525.2559	1048.4973	1048.5553	-0.0580	0	49	1	1	U	R.GLFSEEVIR.E
199	409 – 421	720.3398	1438.6651	1438.7602	-0.0951	0	28	1.1e+02	1	U	K.QPLVMPLSNPTSR.V



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ACCESSION   NP_252161
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DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 564)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 564)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 564)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 564)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 564)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 564)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 564)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 564)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 564)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 564)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
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            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 564)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 564)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 564)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 564)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 564)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 564)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
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            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06859.
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                        UPR: UniProt Genome
            Method: conceptual translation.
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MASCOT Search Results

Protein View: gi|15598149

electron transfer flavoprotein-ubiquinone oxidoreductase [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	366
Nominal mass (M_r):	59891
Calculated pI:	5.51
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|107102503 from Pseudomonas aeruginosa PACS2
- gi|218890849 from Pseudomonas aeruginosa LESB58
- gi|254235928 from Pseudomonas aeruginosa C3719
- gi|254241663 from Pseudomonas aeruginosa 2192
- gi|386058074 from Pseudomonas aeruginosa M18
- gi|392983320 from Pseudomonas aeruginosa DK2
- gi|76364185 from Pseudomonas aeruginosa PAO1
- gi|9949050 from Pseudomonas aeruginosa PAO1
- gi|126167859 from Pseudomonas aeruginosa C3719
- gi|126195041 from Pseudomonas aeruginosa 2192
- gi|218771072 from Pseudomonas aeruginosa LESB58
- gi|334838661 from Pseudomonas aeruginosa HB13
- gi|346058766 from Pseudomonas aeruginosa NCMG1179
- gi|347304380 from Pseudomonas aeruginosa M18
- gi|375043223 from Pseudomonas aeruginosa MPAO1/P1
- gi|375046889 from Pseudomonas aeruginosa MPAO1/P2
- gi|384397155 from Pseudomonas aeruginosa PADK2_CF510
- gi|392318825 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598149 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 21%

Matched peptides shown in ***bold red***.

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  51 HILSGAVFEP RALNELFPDW KELGAPLNTP VTGDDIYVLK SAESATKVPN
 101 FFVPKTMHNE GNYIISLGNL CRWLAQQAEG LGVEIYPGFA AQEALIDENG
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 551 M
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Unformatted sequence string: **551 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

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Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
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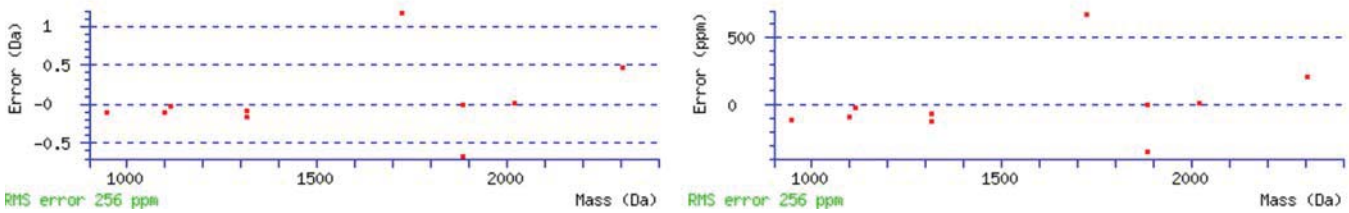
110	183 – 191	558.7491	1115.4836	1115.5070	-0.0234	0	39	11	1		K.YTLFAEGCR.G + Carbamidomethyl (C)
300	201 – 218	674.6751	2021.0035	2020.9854	0.0181	1	67	0.011	1	U	K.KYNLDSEADAQHYGIGIK.E
279	417 – 432	471.8267	1883.2776	1883.9451	-0.6676	0	32	35	1	U	K.IPVTLHDDKPDYACLK.K + Carbamidomethyl (C)
280	417 – 432	628.9887	1883.9444	1883.9451	-0.0008	0	32	36	1	U	K.IPVTLHDDKPDYACLK.K + Carbamidomethyl (C)
325	454 – 473	577.9108	2307.6140	2307.1495	0.4645	0	21	3.5e+02	3	U	K.LSSVFLSNTNHEEDQPIHLK.L
158	484 – 494	658.2580	1314.5014	1314.6568	-0.1554	0	51	0.66	1	U	K.NLPLYDEPAQR.Y

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            [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_251643
VERSION     NP_251643.1  GI:15598149
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 551)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 551)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 551)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 551)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 551)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 551)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 551)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
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REFERENCE   8  (residues 1 to 551)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 551)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 551)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
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            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 551)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 551)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 551)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 551)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
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  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
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  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 551)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 551)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06341.
            RefSeq Category: Reference Genome
                        COM: Community selected
                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
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                     /strain="PAO1"
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                     and conversion]; COG0644"
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                     /note="NAD(P)-binding Rossmann-like domain; pfam13450"
                     /db_xref="CDD:205628"
     Region          409..507
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                     /note="Electron transfer flavoprotein-ubiquinone
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                     /db_xref="CDD:218485"
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                     /note="Product name confidence: class 2 (High similarity
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                     /transl_table=11
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Mascot: http://www.matrixscience.com/

MASCOT Search Results

Protein View: gi|15600312

glnA gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	307
Nominal mass (M_r):	51912
Calculated pI:	5.14
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|107104215 from Pseudomonas aeruginosa PACS2
- gi|116053266 from Pseudomonas aeruginosa UCBPP-PA14
- gi|152987398 from Pseudomonas aeruginosa PA7
- gi|218894218 from Pseudomonas aeruginosa LESB58
- gi|254238175 from Pseudomonas aeruginosa C3719
- gi|254244000 from Pseudomonas aeruginosa 2192
- gi|296391969 from Pseudomonas aeruginosa PAb1
- gi|313110160 from Pseudomonas aeruginosa 39016
- gi|355643338 from Pseudomonas sp. 2_1_26
- gi|386061291 from Pseudomonas aeruginosa M18
- gi|386063361 from Pseudomonas aeruginosa NCGM2.S1
- gi|392986796 from Pseudomonas aeruginosa DK2
- gi|76363163 from Pseudomonas aeruginosa PAO1
- gi|9951417 from Pseudomonas aeruginosa PAO1
- gi|115588487 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126170106 from Pseudomonas aeruginosa C3719
- gi|126197378 from Pseudomonas aeruginosa 2192
- gi|150962556 from Pseudomonas aeruginosa PA7
- gi|218774446 from Pseudomonas aeruginosa LESB58
- gi|310882562 from Pseudomonas aeruginosa 39016
- gi|334836630 from Pseudomonas aeruginosa HB13
- gi|334836660 from Pseudomonas aeruginosa HB15
- gi|346060530 from Pseudomonas aeruginosa NCMG1179
- gi|347307597 from Pseudomonas aeruginosa M18
- gi|348031920 from Pseudomonas aeruginosa NCGM2.S1
- gi|354829843 from Pseudomonas sp. 2_1_26
- gi|375041793 from Pseudomonas aeruginosa MPAO1/P1
- gi|375048306 from Pseudomonas aeruginosa MPAO1/P2
- gi|384397267 from Pseudomonas aeruginosa PADK2_CF510
- gi|392322301 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15600312 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 17%

Matched peptides shown in ***bold red***.

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 51 DGSSIAGWKG IEASDMILMP DDSTAVLDPF TEEPTLILVC DIIEPSTMQG
101 YERDPRNIAK RAEEYLKSTG IGDTVFVGPE PEFFIFDEVK FKSDISGSMF
151 KIFSEQASWN TDADIESGNK GHRPGVKGGY FPVPPVDHDH EIRTAMCNAL
201 EEMGLVVEVH HHEVATAGQN EIGVKFNTLV AKADEVQTLK YCVHNVADAY
251 GKTVTFMPKP LYGDNGSGMH VHMSISKDGK NTFAGEGYAG LSETALYFIG
301 GIIKHGKALN GFTNPSTNSY KRLVPGFEAP VMLAYSARNR SASIRIPYVS
351 SPKARRIEAR FPDPAANPYL AFAALLMAGL DGIQNKIHPG DAADKNLYDL
401 PPEEAKEIPQ VCGSLKEALE ELDKGRAFLT KGGVFTDEFI DAYIELKSEE
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451 EIKVR**TFVHP LEYDLYYSV**

Unformatted sequence string: **469 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
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272	178 – 193	612.3007	1833.8803	1833.8799	0.0004	0	30	63	1		K.GGYFPVPPVDHDHEIR.T
273	178 – 193	459.4875	1833.9209	1833.8799	0.0410	0	34	23	1		K.GGYFPVPPVDHDHEIR.T
220	308 – 321	757.8153	1513.6161	1512.7209	0.8952	0	45	2.5	1		K.ALNGFTNPSTNSYK.R
221	308 – 321	757.9356	1513.8567	1512.7209	1.1358	0	49	1	1		K.ALNGFTNPSTNSYK.R
259	323 – 338	861.0308	1720.0470	1719.9018	0.1452	0	76	0.0018	1		R.LVPGFEAPVMLAYSAR.N
114	407 – 416	565.7599	1129.5052	1129.5801	-0.0749	0	28	1.6e+02	4	U	K.EIPQVCGSLK.E + Carbamidomethyl (C)
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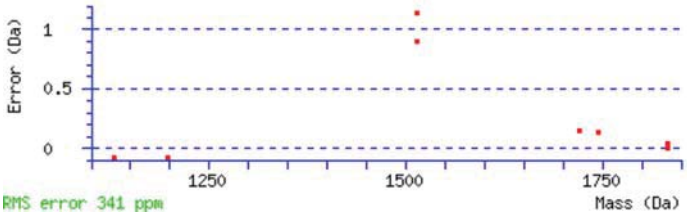

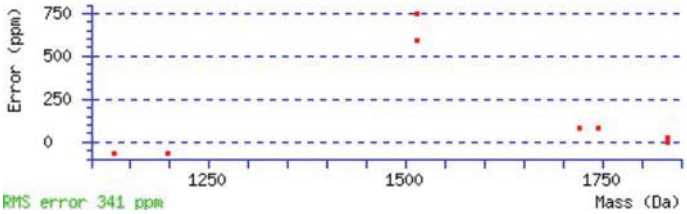


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DEFINITION  glutamine synthetase [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_253806
VERSION     NP_253806.1  GI:15600312
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 469)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 469)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 469)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 469)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
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REFERENCE   5  (residues 1 to 469)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 469)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 469)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 469)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 469)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 469)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 469)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 469)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 469)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 469)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 469)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
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REFERENCE   16 (residues 1 to 469)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG08504.
            RefSeq Category: Reference Genome
                        COM: Community selected
                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
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Mascot: http://www.matrixscience.com/

MATRIX SCIENCE MASCOT Search Results

Protein View: gi|82393828

2-hydroxymuconic semialdehyde dehydrogenase [Pseudomonas sp. KB35B]

Database:	NCBInr
Score:	182
Nominal mass (M_r):	51526
Calculated pI:	5.33
Taxonomy:	Pseudomonas sp. KB35B

Sequence similarity is available as an NCBI BLAST search of gi|82393828 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas29.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 9 %

Matched peptides shown in ***bold red***.

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  1 MKEIKHFING AFVGSASGRT FEDINPSNGQ VIGHVHEAGR AEVDAAVKAA
 51 RAALKGPWGK LSVAERAEIL HRVADGITAR FDEFLEAECL DTGKPKSLAS
101 HIDIPRGAAN FKVFADLLKN VGNEAFEMAT PDGAGAINYA VRWPKGVIGV
151 ISPWNLPLLL MTWKVGPALA CGNTVVVKPS EETPLTATLL GKVMQAAGVP
201 AGVYNVLHGF GGDSAGAFLT EHPDVDAYTF TGETGTGEVI MRAAAKGVRQ
251 VSLELGGKNA GIVFADCDME KAIEGTLRSA FANCGQVCLG TERVYVERPI
301 FDEFVARLKG GAESLVIGPP DDASSNFGPL VSLKHREKVL SYYQQALDDG
351 ASVITGGGVP DMPAHLAGGA WVQPTIWTGL SDDSAVVTEE IFGPCCHIRP
401 FDTEEEAIEL ANSLPYGLAS AIWTENSSRA HRVAGQIEAG VVWVNSWFLR
451 DLRTAFGGSK QSGIGREGGV HSLEFYTELK NICVKL
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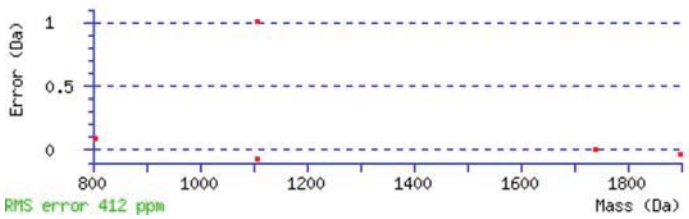
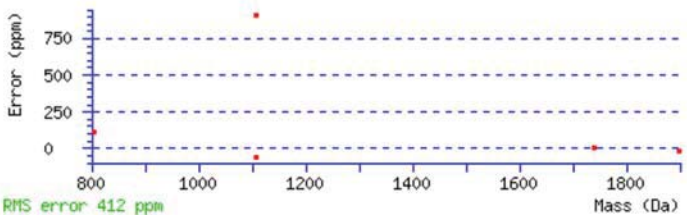
Unformatted sequence string: **486 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
287	81 – 96	633.6216	1897.8431	1897.8768	-0.0337	0	68	0.0088	1		R.FDEFLEAECLDTGKPK.S + Carbamidomethyl (C)
107	97 – 106	554.7768	1107.5390	1107.6036	-0.0647	0	39	10	1		K.SLASHIDIPR.G
108	97 – 106	370.5459	1108.6160	1107.6036	1.0123	0	35	24	1		K.SLASHIDIPR.G
43	113 – 119	403.2909	804.5673	804.4745	0.0927	0	32	72	1	U	K.VFADLLK.N
262	294 – 307	580.6420	1738.9041	1738.9043	-0.0001	0	43	3.3	1	U	R.VYVERPIFDEFVAR.L



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DEFINITION  2-hydroxymuconic semialdehyde dehydrogenase [Pseudomonas sp.
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ACCESSION   ABB72209
VERSION     ABB72209.1  GI:82393828
DBSOURCE    accession DQ265742.1
KEYWORDS    .
SOURCE      Pseudomonas sp. KB35B
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  ORGANISM  Pseudomonas sp. KB35B
            Bacteria; Proteobacteria.
REFERENCE   1  (residues 1 to 486)
  AUTHORS   Kim,Y.M., Park,K., Kim,W.C., Shin,J.H., Kim,J.E., Park,H.D. and
            Rhee,I.K.
  TITLE     Cloning and characterization of a catechol-degrading gene cluster
            from 3,4-dichloroaniline degrading bacterium Pseudomonas sp. KB35B
  JOURNAL   J. Agric. Food Chem. 55 (12), 4722-4727 (2007)
   PUBMED   17497880
REFERENCE   2  (residues 1 to 486)
  AUTHORS   Kim,Y.-M. and Rhee,I.-K.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-OCT-2005) Department of Agricultural Chemistry,
            College of Agriculture and Life Sciences, Kyungpook National
            University, 1370 Sangyeok-dong, Buk-gu, Daegu 702-701, Korea
COMMENT     Method: conceptual translation supplied by author.
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                     /db_xref="CDD:132260"
     Region          23..484
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                     /note="Human aldehyde dehydrogenase family 8 member
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                     /db_xref="CDD:143412"
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                     /note="NAD binding site [chemical binding]"
                     /db_xref="CDD:143412"
     Site            order(155,254,285,288)
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                     /note="catalytic residues [active]"
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MASCOT Search Results

Protein View: gi|15598147

etfA gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	636
Nominal mass (M_r):	31404
Calculated pI:	4.98
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|107102501 from Pseudomonas aeruginosa PACS2
- gi|116050953 from Pseudomonas aeruginosa UCBPP-PA14
- gi|218890851 from Pseudomonas aeruginosa LESB58
- gi|254235926 from Pseudomonas aeruginosa C3719
- gi|254241661 from Pseudomonas aeruginosa 2192
- gi|313108135 from Pseudomonas aeruginosa 39016
- gi|355641316 from Pseudomonas sp. 2_1_26
- gi|386058076 from Pseudomonas aeruginosa M18
- gi|386066980 from Pseudomonas aeruginosa NCGM2.S1
- gi|392983322 from Pseudomonas aeruginosa DK2
- gi|81540583 from Pseudomonas aeruginosa PAO1
- gi|9949048 from Pseudomonas aeruginosa PAO1
- gi|115586174 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126167857 from Pseudomonas aeruginosa C3719
- gi|126195039 from Pseudomonas aeruginosa 2192
- gi|218771074 from Pseudomonas aeruginosa LESB58
- gi|310880772 from Pseudomonas aeruginosa 39016
- gi|334838659 from Pseudomonas aeruginosa HB13
- gi|346058764 from Pseudomonas aeruginosa NCMG1179
- gi|347304382 from Pseudomonas aeruginosa M18
- gi|348035539 from Pseudomonas aeruginosa NCGM2.S1
- gi|354830914 from Pseudomonas sp. 2_1_26
- gi|375043225 from Pseudomonas aeruginosa MPAO1/P1
- gi|375046891 from Pseudomonas aeruginosa MPAO1/P2
- gi|384397153 from Pseudomonas aeruginosa PADK2_CF510
- gi|392318827 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598147 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas98.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 66%

Matched peptides shown in ***bold red***.

```
  1 MAILVIAEHN NAALAAATLN TVAAAKAIGG DIHVLVAGQN VAAVAEAAAK
 51 VEGVSKVLVA DNAAYAHQLP ENVAPLIAEL GKNYSHVLAP ATTNGKNFLP
101 RVAALLDVDQ ISEIVEVVSP DTFKRPIYAG NAIATVQSSA AVKVITVRTT
151 GFDAVAAEGG SAAVEQVSGP ADAGKSAFVG EELAKSDRPE LTAAKIVVSG
201 GRGMQNGDNF KILYALADKL GAAVGASRAA VDAGFVPNDM QVGQTGKIVA
251 PQLYIAVGIS GAIQHLAGMK DSKVIVAINK DEEAPIFQVA DYGLVADLFD
301 AVPELEKAV
```

Unformatted sequence string: 309 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
345	2 – 26	608.7263	2430.8760	2430.3594	0.5166	0	60	0.048	1	U	M.AILVIAEHNNAALAAATLNTVAAAK.A
346	2 – 26	811.3057	2430.8953	2430.3594	0.5359	0	95	1.4e-05	1	U	M.AILVIAEHNNAALAAATLNTVAAAK.A
335	27 – 50	749.5024	2245.4852	2244.2226	1.2626	0	45	1.6	1		K.AIGGDIHVLVAGQNVAAVAEAAAK.V
361	57 – 82	906.3166	2715.9279	2715.4595	0.4683	0	53	0.18	1		K.VLVADNAAYAHQLPENVAPLIAELGK.N
362	57 – 82	1359.2090	2716.4035	2715.4595	0.9440	0	67	0.0065	1		K.VLVADNAAYAHQLPENVAPLIAELGK.N
363	57 – 82	680.1939	2716.7466	2715.4595	1.2871	0	35	11	1		K.VLVADNAAYAHQLPENVAPLIAELGK.N

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
354	102 – 124	829.9688	2486.8846	2486.3156	0.5690	0	75	0.0013	1	U	R.VAALLDVDQISEIVEVVSPDTFK.R
297	125 – 143	639.7907	1916.3502	1916.0479	0.3023	0	75	0.002	1		K.RPIYAGNAIATVQSSAAVK.V
298	125 – 143	639.8102	1916.4087	1916.0479	0.3607	0	57	0.1	1		K.RPIYAGNAIATVQSSAAVK.V
299	125 – 143	640.0495	1917.1268	1916.0479	1.0788	0	44	2.3	1		K.RPIYAGNAIATVQSSAAVK.V
300	125 – 143	640.0777	1917.2112	1916.0479	1.1633	0	56	0.15	1		K.RPIYAGNAIATVQSSAAVK.V
301	125 – 143	640.0830	1917.2271	1916.0479	1.1791	0	62	0.033	1		K.RPIYAGNAIATVQSSAAVK.V
302	125 – 143	640.0917	1917.2533	1916.0479	1.2054	0	62	0.04	1		K.RPIYAGNAIATVQSSAAVK.V
303	125 – 143	640.1064	1917.2972	1916.0479	1.2493	0	66	0.015	1		K.RPIYAGNAIATVQSSAAVK.V
304	125 – 143	640.1248	1917.3526	1916.0479	1.3047	0	71	0.0044	1		K.RPIYAGNAIATVQSSAAVK.V
347	149 – 175	821.7883	2462.3431	2462.1561	0.1869	0	58	0.061	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
348	149 – 175	821.7972	2462.3699	2462.1561	0.2138	0	67	0.0083	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
349	149 – 175	821.8489	2462.5248	2462.1561	0.3686	0	80	0.0004	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
350	149 – 175	822.0783	2463.2132	2462.1561	1.0570	0	72	0.0028	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
351	149 – 175	822.0801	2463.2185	2462.1561	1.0623	0	50	0.47	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
352	149 – 175	822.1514	2463.4325	2462.1561	1.2764	0	44	1.7	1		R.TTGFDAVAAEGGSAAVEQVSGPADAGK.S
115	176 – 185	525.8422	1049.6698	1049.5393	0.1305	0	26	2.1e+02	3		K.SAFVGEELAK.S
117	176 – 185	525.8615	1049.7084	1049.5393	0.1691	0	23	4.1e+02	10		K.SAFVGEELAK.S
73	212 – 219	453.8935	905.7724	905.5222	0.2502	0	34	36	1		K.ILYALADK.L
293	229 – 247	953.0732	1904.1318	1903.9098	0.2219	0	57	0.12	1		R.AAVDAGFVPNDMQVGQTGK.I
294	229 – 247	953.1235	1904.2324	1903.9098	0.3226	0	57	0.11	1		R.AAVDAGFVPNDMQVGQTGK.I
295	229 – 247	635.7561	1904.2465	1903.9098	0.3367	0	71	0.0043	1		R.AAVDAGFVPNDMQVGQTGK.I
296	229 – 247	953.5331	1905.0517	1903.9098	1.1419	0	68	0.0097	1		R.AAVDAGFVPNDMQVGQTGK.I
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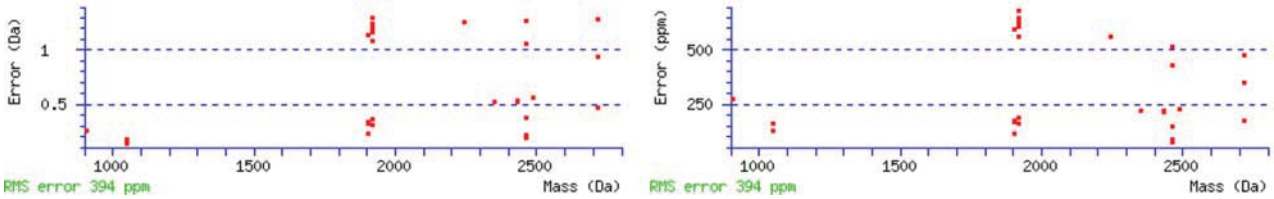


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DEFINITION  electron transfer flavoprotein subunit alpha [Pseudomonas
            aeruginosa PAO1].
ACCESSION   NP_251641
VERSION     NP_251641.1  GI:15598147
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 309)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 309)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 309)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 309)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 309)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 309)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
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  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 309)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 309)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 309)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 309)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
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            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 309)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 309)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 309)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06339.
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                        COM: Community selected
                        UPR: UniProt Genome
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            Method: conceptual translation.
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                     to functionally studied protein)"
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Mascot: http://www.matrixscience.com/

MASCOT Search Results

Protein View: gi|15598980

hypothetical protein [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	216
Nominal mass (M_r):	16990
Calculated pI:	6.28
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|218889931 from Pseudomonas aeruginosa LESB58
- gi|254236689 from Pseudomonas aeruginosa C3719
- gi|254242476 from Pseudomonas aeruginosa 2192
- gi|386057220 from Pseudomonas aeruginosa M18
- gi|392982488 from Pseudomonas aeruginosa DK2
- gi|9949957 from Pseudomonas aeruginosa PAO1
- gi|126168620 from Pseudomonas aeruginosa C3719
- gi|126195854 from Pseudomonas aeruginosa 2192
- gi|218770154 from Pseudomonas aeruginosa LESB58
- gi|334835763 from Pseudomonas aeruginosa HB13
- gi|346055794 from Pseudomonas aeruginosa NCMG1179
- gi|347303526 from Pseudomonas aeruginosa M18
- gi|375043650 from Pseudomonas aeruginosa MPAO1/P1
- gi|375051389 from Pseudomonas aeruginosa MPAO1/P2
- gi|384396870 from Pseudomonas aeruginosa PADK2_CF510
- gi|392317993 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598980 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas9A.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 22%

Matched peptides shown in ***bold red***.

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  1 MRTSFFAAAA LLAVSAFAQA HEYNAGQLHI EHPWALALPP TSPNGAAYFV
 51 VQNHGKENDT LLGADTPRAA SAEVHEHVHK NGMMSMQKVD SVDVAPGKDL
101 RFAPGGYHLM LMGLKQPLVA GERFPLTLHF RKAGDVPVEI VVESKAPAEQ
151 GGHEQHGH
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Unformatted sequence string: 158 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
213	102 – 115	512.6206	1534.8398	1533.7836	1.0562	0	29	91	1	U	R.FAPGGYHLMLMGLK.Q
100	124 – 131	515.7939	1029.5732	1029.5760	-0.0027	0	52	0.7	3	U	R.FPLTLHFR.K
101	124 – 131	344.2472	1029.7198	1029.5760	0.1438	0	40	11	3	U	R.FPLTLHFR.K
197	132 – 145	735.4277	1468.8408	1468.8137	0.0271	1	77	0.0013	1	U	R.KAGDVPVEIVVESK.A
167	133 – 145	671.3837	1340.7529	1340.7188	0.0341	0	58	0.12	1	U	K.AGDVPVEIVVESK.A
168	133 – 145	671.3854	1340.7562	1340.7188	0.0375	0	50	0.72	1	U	K.AGDVPVEIVVESK.A
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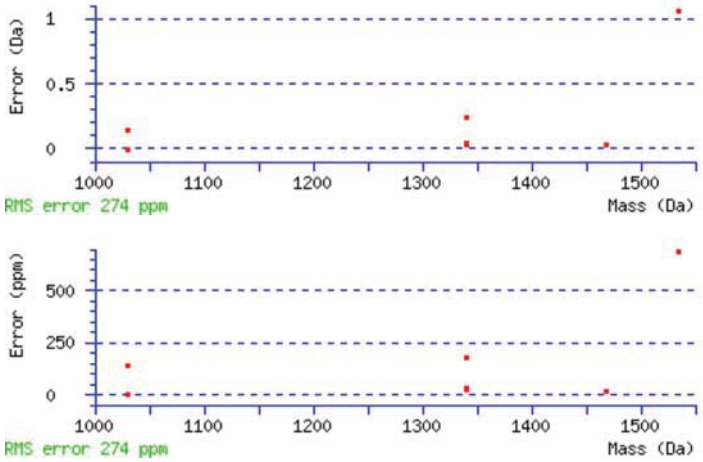


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DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 158)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 158)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 158)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 158)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
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REFERENCE   5  (residues 1 to 158)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 158)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 158)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
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   PUBMED   15608211
REFERENCE   8  (residues 1 to 158)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 158)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 158)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 158)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 158)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 158)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 158)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 158)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 158)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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                        COM: Community selected
                        UPR: UniProt Genome
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            Method: conceptual translation.
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MASCOT Search Results

Protein View: gi|15598147

etfA gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	212
Nominal mass (M_r):	31404
Calculated pI:	4.98
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|107102501 from Pseudomonas aeruginosa PACS2
- gi|116050953 from Pseudomonas aeruginosa UCBPP-PA14
- gi|218890851 from Pseudomonas aeruginosa LESB58
- gi|254235926 from Pseudomonas aeruginosa C3719
- gi|254241661 from Pseudomonas aeruginosa 2192
- gi|313108135 from Pseudomonas aeruginosa 39016
- gi|355641316 from Pseudomonas sp. 2_1_26
- gi|386058076 from Pseudomonas aeruginosa M18
- gi|386066980 from Pseudomonas aeruginosa NCGM2.S1
- gi|392983322 from Pseudomonas aeruginosa DK2
- gi|81540583 from Pseudomonas aeruginosa PAO1
- gi|9949048 from Pseudomonas aeruginosa PAO1
- gi|115586174 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126167857 from Pseudomonas aeruginosa C3719
- gi|126195039 from Pseudomonas aeruginosa 2192
- gi|218771074 from Pseudomonas aeruginosa LESB58
- gi|310880772 from Pseudomonas aeruginosa 39016
- gi|334838659 from Pseudomonas aeruginosa HB13
- gi|346058764 from Pseudomonas aeruginosa NCMG1179
- gi|347304382 from Pseudomonas aeruginosa M18
- gi|348035539 from Pseudomonas aeruginosa NCGM2.S1
- gi|354830914 from Pseudomonas sp. 2_1_26
- gi|375043225 from Pseudomonas aeruginosa MPAO1/P1
- gi|375046891 from Pseudomonas aeruginosa MPAO1/P2
- gi|384397153 from Pseudomonas aeruginosa PADK2_CF510
- gi|392318827 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15598147 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas9A.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 22 %

Matched peptides shown in ***bold red***.

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  1 MAILVIAEHN NAALAAATLN TVAAAKAIGG DIHVLVAGQN VAAVAEAAAK
 51 VEGVSKVLVA DNAAYAHQLP ENVAPLIAEL GKNYSHVLAP ATTNGKNFLP
101 RVAALLDVDQ ISEIVEVVSP DTFKRPIYAG NAIATVQSSA AVKVITVRTT
151 GFDAVAAEGG SAAVEQVSGP ADAGKSAFVG EELAKSDRPE LTAAKIVVSG
201 GRGMQNGDNF KILYALADKL GAAVGASRAA VDAGFVPNDM QVGQTGKIVA
251 PQLYIAVGIS GAIQHLAGMK DSKVIVAINK DEEAPIFQVA DYGLVADLFD
301 AVPELEKAV
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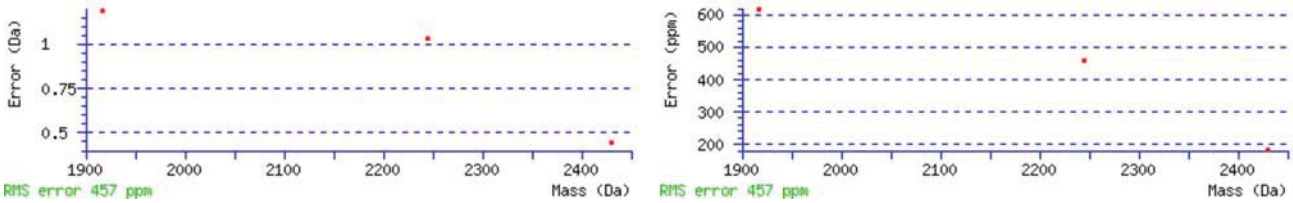
Unformatted sequence string: 309 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
257	2 – 26	811.2749	2430.8029	2430.3594	0.4435	0	83	0.0002	1	U	M.AILVIAEHNNAALAAATLNTVAAAK.A
254	27 – 50	749.4254	2245.2545	2244.2226	1.0319	0	58	0.081	1	U	K.AIGGDIHVLVAGQNVAAVAEAAAK.V
245	125 – 143	640.0858	1917.2355	1916.0479	1.1875	0	71	0.0046	1	U	K.RPIYAGNAIATVQSSAAVK.V

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DEFINITION  electron transfer flavoprotein subunit alpha [Pseudomonas
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ACCESSION   NP_251641
VERSION     NP_251641.1  GI:15598147
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 309)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 309)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 309)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 309)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 309)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 309)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 309)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 309)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 309)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 309)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
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            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 309)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 309)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 309)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 309)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06339.
            RefSeq Category: Reference Genome
                        COM: Community selected
                        UPR: UniProt Genome
            Method: conceptual translation.
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Mascot: http://www.matrixscience.com/

MATRIX SCIENCE MASCOT Search Results

Protein View: gi|15596984

acnB gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	1736
Nominal mass (M_r):	93569
Calculated pI:	5.22
Taxonomy:	**Pseudomonas aeruginosa PAO1**

This protein sequence matches the following other entries:

- **gi|107101218** from **Pseudomonas aeruginosa PACS2**
- **gi|116049735** from **Pseudomonas aeruginosa UCBPP-PA14**
- **gi|218892261** from **Pseudomonas aeruginosa LESB58**
- **gi|313110479** from **Pseudomonas aeruginosa 39016**
- **gi|355644131** from **Pseudomonas sp. 2_1_26**
- **gi|386059322** from **Pseudomonas aeruginosa M18**
- **gi|386065628** from **Pseudomonas aeruginosa NCGM2.S1**
- **gi|392984743** from **Pseudomonas aeruginosa DK2**
- **gi|81541220** from **Pseudomonas aeruginosa PAO1**
- **gi|9947768** from **Pseudomonas aeruginosa PAO1**
- **gi|115584956** from **Pseudomonas aeruginosa UCBPP-PA14**
- **gi|218772487** from **Pseudomonas aeruginosa LESB58**
- **gi|310882866** from **Pseudomonas aeruginosa 39016**
- **gi|334844320** from **Pseudomonas aeruginosa HB13**
- **gi|346057457** from **Pseudomonas aeruginosa NCMG1179**
- **gi|347305628** from **Pseudomonas aeruginosa M18**
- **gi|348034187** from **Pseudomonas aeruginosa NCGM2.S1**
- **gi|354829298** from **Pseudomonas sp. 2_1_26**
- **gi|375044255** from **Pseudomonas aeruginosa MPAO1/P1**
- **gi|375049860** from **Pseudomonas aeruginosa MPAO1/P2**
- **gi|384398046** from **Pseudomonas aeruginosa PADK2_CF510**
- **gi|392320248** from **Pseudomonas aeruginosa DK2**

Sequence similarity is available as **an NCBI BLAST search of gi|15596984 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas9F.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 43%

Matched peptides shown in ***bold red***.

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  51 NRVPPGVDEA AYVKAGFLSA IVKGEATSPL IDKQRAAELL GTMQGGYNIA
 101 TLVELLDDAE LANTAAEQLK HTLLMFDAFH DVAERAKKGN AAAKSVLQSW
 151 ADGEWFKAKP EVPEKLTLTV FKVPGETNTD DLSPAPDAWS RPDIPLHALA
 201 MLKMARDGIE PVQPGSVGPL KQIEAVKAKG FPVAYVGDVV GTGSSRKSAT
 251 NSVLWFFGDD IPFVPNKRAG GFCFGTKIAP IFYNTMEDAG ALPIEFDCTN
 301 LAMGDVIDVY PYEGKVVRHD SGEVVTTFEL KTPVLLDEVR AGGRIPLIVG
 351 RGLTEKARAE LGLGASDLFR KPEAPADSGK GFTLAQKMVG RACGLPEGQG
 401 VRPGTYCEPK MTTVGSQDTT GPMTRDELKD LACLGFSADL VMQSFCHTAA
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 501 SHTRFPIGIS FPAGSGLVAF AAATGVMPLD MPESVLVRFK GKLQPGITLR
 551 DLVHAIPYYA IQQGLLTVEK KGKKNIFSGR ILEIEGLNDL TVEQAFELSD
 601 ASAERSAAGC TIKLPEQAIA EYLKSNITLL RWMIGEGYGD PRTLERRAQA
 651 MEAWLAKPEL LEADKDAEYA AVIEIDLADV KEPVLCAPND PDDARLLSSV
 701 QGRKIDEVFI GSCMTNIGHF RAAGKLLDKV KGGIPTRLWL APPTKMDAHQ
 751 LTEEGYYGIY GKAGARMEMP GCSLCMGNQA RVQTGSTVVS TSTRNFPNRL
 801 GDATDVFLAS AELAAVSSIL GKLPTVEEYM AYAKDIDSMA ADVYRYLSFD
 851 QIAEFREAAA NAKIPVVQA
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Unformatted sequence string: **869 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
364	13 – 35	1173.7848	2345.5551	2345.2954	0.2597	0	48	0.72	1		R.AAQGVVPQPLNAEQTAGLVELLK.N
314	36 – 52	964.6549	1927.2953	1926.9687	0.3267	0	78	0.00097	1		K.NPPAGEEEFLLDLITNR.V
315	36 – 52	643.7622	1928.2648	1926.9687	1.2961	0	38	9.1	1		K.NPPAGEEEFLLDLITNR.V
158	53 – 64	622.8094	1243.6042	1243.6449	-0.0406	0	37	17	1		R.VPPGVDEAAYVK.A
159	53 – 64	622.8693	1243.7240	1243.6449	0.0791	0	56	0.24	1		R.VPPGVDEAAYVK.A
160	53 – 64	622.8734	1243.7323	1243.6449	0.0874	0	59	0.11	1		R.VPPGVDEAAYVK.A
60	65 – 73	453.3766	904.7387	904.5382	0.2005	0	64	0.044	1		K.AGFLSAIVK.G
298	121 – 135	901.4511	1800.8876	1800.8617	0.0259	0	58	0.11	1		K.HTLLMFDAFHDVAER.A
299	121 – 135	601.4281	1801.2625	1800.8617	0.4008	0	65	0.019	1		K.HTLLMFDAFHDVAER.A
258	145 – 157	777.0065	1551.9984	1551.7358	0.2626	0	63	0.032	1	U	K.SVLQSWADGEWFK.A
51	166 – 172	411.3452	820.6759	820.5058	0.1701	0	30	98	3		K.LTLTVFK.V
241	207 – 221	746.9067	1491.7988	1491.7933	0.0055	0	50	0.69	1	U	R.DGIEPVQPGSVGPLK.Q
242	207 – 221	747.0164	1492.0183	1491.7933	0.2249	0	56	0.2	1	U	R.DGIEPVQPGSVGPLK.Q
282	230 – 246	834.5345	1667.0543	1666.8315	0.2228	0	76	0.0018	1		K.GFPVAYVGDVVGTGSSR.K
223	319 – 331	731.3722	1460.7299	1460.7147	0.0151	0	74	0.0028	1	U	R.HDSGEVVTTFELK.T
105	332 – 340	521.3122	1040.6098	1040.5866	0.0232	0	55	0.26	1		K.TPVLLDEVR.A
106	332 – 340	521.3221	1040.6296	1040.5866	0.0429	0	59	0.12	1		K.TPVLLDEVR.A
107	332 – 340	521.7311	1041.4476	1040.5866	0.8610	0	51	0.8	1		K.TPVLLDEVR.A
163	359 – 370	624.9425	1247.8705	1247.6510	0.2195	0	61	0.063	1	U	R.AELGLGASDLFR.K
334	392 – 410	1038.5397	2075.0648	2074.9565	0.1084	0	29	59	1		R.ACGLPEGQGVRPGTYCEPK.M + 2 Carbamidomethyl (C)
335	392 – 410	693.0201	2076.0385	2074.9565	1.0820	0	45	1.4	2		R.ACGLPEGQGVRPGTYCEPK.M + 2 Carbamidomethyl (C)
225	459 – 470	734.8431	1467.6717	1467.7293	-0.0576	0	30	66	1		K.THHTLPDFIMTR.G
226	459 – 470	490.3660	1468.0762	1467.7293	0.3469	0	44	2.6	1		K.THHTLPDFIMTR.G
227	459 – 470	490.6755	1469.0047	1467.7293	1.2754	0	35	22	1		K.THHTLPDFIMTR.G
276	489 – 504	828.9092	1655.8039	1655.7937	0.0101	0	58	0.11	1		R.MLLPDTVGTGGDSHTR.F
277	489 – 504	553.2820	1656.8242	1655.7937	1.0305	0	40	7.1	1		R.MLLPDTVGTGGDSHTR.F
278	489 – 504	553.3180	1656.9323	1655.7937	1.1385	0	50	0.7	1		R.MLLPDTVGTGGDSHTR.F
355	551 – 570	1136.1583	2270.3021	2270.2310	0.0710	0	41	3.7	3		R.DLVHAIPYYAIQQGLLTVEK.K
356	551 – 570	757.9404	2270.7994	2270.2310	0.5684	0	56	0.11	3		R.DLVHAIPYYAIQQGLLTVEK.K
176	614 – 624	637.9504	1273.8863	1273.6918	0.1946	0	64	0.037	2	U	K.LPEQAIAEYLK.S
177	632 – 642	640.7982	1279.5818	1279.5656	0.0163	0	51	0.64	1		R.WMIGEGYGDPR.T
178	632 – 642	640.8060	1279.5975	1279.5656	0.0319	0	36	19	1		R.WMIGEGYGDPR.T
265	682 – 695	784.9443	1567.8740	1567.6937	0.1804	0	73	0.0038	1		K.EPVLCAPNDPDDAR.L + Carbamidomethyl (C)
327	705 – 721	666.0312	1995.0717	1994.9343	0.1375	0	59	0.062	1		K.IDEVFIGSCMTNIGHFR.A + Carbamidomethyl (C)
66	738 – 745	463.3462	924.6779	924.5433	0.1346	0	38	13	3		R.LWLAPPTK.M
67	738 – 745	463.3479	924.6812	924.5433	0.1379	0	38	11	1		R.LWLAPPTK.M
322	746 – 762	987.9158	1973.8171	1973.8829	-0.0658	0	60	0.061	1		K.MDAHQLTEEGYYGIYGK.A
323	746 – 762	658.9800	1973.9182	1973.8829	0.0353	0	66	0.013	1		K.MDAHQLTEEGYYGIYGK.A
293	767 – 781	871.5175	1741.0204	1740.6874	0.3330	0	103	3e-06	1		R.MEMPGCSLCMGNQAR.V + 2 Carbamidomethyl (C)
352	800 – 822	1124.6763	2247.3381	2247.1998	0.1383	0	143	2.5e-10	1	U	R.LGDATDVFLASAELAAVSSILGK.L
353	800 – 822	750.1485	2247.4237	2247.1998	0.2239	0	77	0.0009	1	U	R.LGDATDVFLASAELAAVSSILGK.L
214	823 – 834	707.9677	1413.9208	1413.6850	0.2358	0	47	1.7	1		K.LPTVEEYMAYAK.D
164	835 – 845	628.2721	1254.5296	1254.5550	-0.0255	0	78	0.0013	1		K.DIDSMAADVYR.Y
165	835 – 845	628.3580	1254.7014	1254.5550	0.1464	0	43	4.1	1		K.DIDSMAADVYR.Y
166	835 – 845	628.3880	1254.7614	1254.5550	0.2063	0	55	0.23	1		K.DIDSMAADVYR.Y
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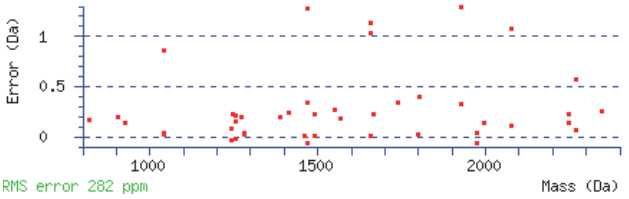

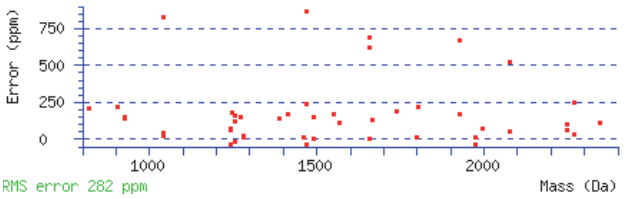


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KEYWORDS    RefSeq.
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SOURCE Pseudomonas aeruginosa PAO1
ORGANISM Pseudomonas aeruginosa PAO1
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (residues 1 to 869)
AUTHORS Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
Hancock,R.E. and Brinkman,F.S.
TITLE Pseudomonas Genome Database: facilitating user-friendly,
comprehensive comparisons of microbial genomes
JOURNAL Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
PUBMED **18978025**
REFERENCE 2 (residues 1 to 869)
AUTHORS Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
TITLE Defining the Pseudomonas aeruginosa SOS response and its role in
the global response to the antibiotic ciprofloxacin
JOURNAL J. Bacteriol. 188 (20), **7101-7110** (2006)
PUBMED **17015649**
REFERENCE 3 (residues 1 to 869)
AUTHORS Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
TITLE Cystic fibrosis sputum supports growth and cues key aspects of
Pseudomonas aeruginosa physiology
JOURNAL J. Bacteriol. 187 (15), **5267-5277** (2005)
PUBMED **16030221**
REFERENCE 4 (residues 1 to 869)
AUTHORS Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
TITLE A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
displays enhanced virulence and antimicrobial resistance
JOURNAL J. Bacteriol. 187 (14), **4908-4920** (2005)
PUBMED **15995206**
REFERENCE 5 (residues 1 to 869)
AUTHORS Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
Iglewski,B.H. and Passador,L.
TITLE Effect of anaerobiosis and nitrate on gene expression in
Pseudomonas aeruginosa
JOURNAL Infect. Immun. 73 (6), **3764-3772** (2005)
PUBMED **15908409**
REFERENCE 6 (residues 1 to 869)
AUTHORS Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
TITLE Screening for quorum-sensing inhibitors (QSI) by use of a novel
genetic system, the QSI selector
JOURNAL J. Bacteriol. 187 (5), 1799-1814 (2005)
PUBMED **15716452**
REFERENCE 7 (residues 1 to 869)
AUTHORS Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
Ching,W.K., Hancock,R.E. and Brinkman,F.S.
TITLE Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
community-based, continually updated, genome annotation
JOURNAL Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
PUBMED **15608211**
REFERENCE 8 (residues 1 to 869)
AUTHORS Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
Iglewski,B.H.
TITLE Microarray analysis of Pseudomonas aeruginosa quorum-sensing
regulons: effects of growth phase and environment
JOURNAL J. Bacteriol. 185 (7), 2080-2095 (2003)
PUBMED **12644477**
REFERENCE 9 (residues 1 to 869)
AUTHORS Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
TITLE Identification, timing, and signal specificity of Pseudomonas
aeruginosa quorum-controlled genes: a transcriptome analysis
JOURNAL J. Bacteriol. 185 (7), 2066-2079 (2003)
PUBMED **12644476**
REFERENCE 10 (residues 1 to 869)
AUTHORS Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
TITLE Complete genome sequence of Pseudomonas aeruginosa PA01, an
opportunistic pathogen
JOURNAL Nature 406 (6799), 959-964 (2000)
PUBMED **10984043**
REFERENCE 11 (residues 1 to 869)
CONSRTM NCBI Genome Project
TITLE Direct Submission
JOURNAL Submitted (06-OCT-2010) National Center for Biotechnology
Information, NIH, Bethesda, MD 20894, USA
REFERENCE 12 (residues 1 to 869)
AUTHORS Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
CONSRTM Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
TITLE Direct Submission
JOURNAL Submitted (08-SEP-2010) Department of Molecular Biology and

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            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 869)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 869)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 869)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 869)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
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            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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Mascot: http://www.matrixscience.com/

MASCOT Search Results

Protein View: gi|15600208

aceE gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	1203
Nominal mass (M_r):	99501
Calculated pI:	5.56
Taxonomy:	**Pseudomonas aeruginosa PAO1**

This protein sequence matches the following other entries:

- **gi|107104115** from **Pseudomonas aeruginosa PACS2**
- **gi|116053163** from **Pseudomonas aeruginosa UCBPP-PA14**
- **gi|218894113** from **Pseudomonas aeruginosa LESB58**
- **gi|254244101** from **Pseudomonas aeruginosa 2192**
- **gi|386061187** from **Pseudomonas aeruginosa M18**
- **gi|392986692** from **Pseudomonas aeruginosa DK2**
- **gi|12231033** from **Pseudomonas aeruginosa PAO1**
- **gi|9951302** from **Pseudomonas aeruginosa PAO1**
- **gi|115588384** from **Pseudomonas aeruginosa UCBPP-PA14**
- **gi|126197479** from **Pseudomonas aeruginosa 2192**
- **gi|218774341** from **Pseudomonas aeruginosa LESB58**
- **gi|347307493** from **Pseudomonas aeruginosa M18**
- **gi|375041626** from **Pseudomonas aeruginosa MPAO1/P1**
- **gi|375050122** from **Pseudomonas aeruginosa MPAO1/P2**
- **gi|384401817** from **Pseudomonas aeruginosa PADK2_CF510**
- **gi|392322197** from **Pseudomonas aeruginosa DK2**

Sequence similarity is available as **an NCBI BLAST search of gi|15600208 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masA3.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 28%

Matched peptides shown in ***bold red***.

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  51 TTPYRNTIPV THEARMPGDL FMERRIRSLV RWNALAMVMR ANKHDPDLGG
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 151 RISEEQLENF RQEVDGNGLS SYPHPWLMPD FWQFPTVSMG LGPIQAIYQA
 201 RFMKYLESRG FIPAGKQKVW CFMGDGECDE PESLGAISLA GREKLDNLIF
 251 VINCNLQRLD GPVRGNAKII QELEGVFRGA EWNVNKVIWG RFWDPLFAKD
 301 TAGLLQQRMD EVIDGEYQNY KAKDGAYVRE HFFGARPELL EMVKDLSDEE
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 701 GIIKGMYLLE EDKKEAAHHV QLLGSGTILR EVEEAAKLLR NDFGIGADVW
 751 SVPSFNELRR DGLAVERWNR LHPGQKPKQS YVEECLGGRR GPVIASTDYM
 801 KLYAEQIRQW VPSKEYKVLG TDGFGRSDSR KKLRNFFEVD RHWVVLAALE
 851 ALADRGDIEP KVVAEAIAKY GIDPEKRNPL DC
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Unformatted sequence string: **882 residues** (for pasting into other applications).

Sort peptides by ◡ Residue Number ◯ Increasing Mass ◯ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
279	42 – 55	784.4448	1566.8751	1566.8042	0.0709	0	61	0.057	1		R.SGTQLPYAITTPYR.N
280	42 – 55	784.4758	1566.9370	1566.8042	0.1328	0	60	0.07	1		R.SGTQLPYAITTPYR.N
129	66 – 74	548.2386	1094.4627	1094.4889	-0.0262	0	47	1.7	1		R.MPGDLFMER.R
127	82 – 90	546.2984	1090.5823	1090.5416	0.0408	0	47	1.8	1		R.WNALAMVMR.A
189	152 – 161	632.7790	1263.5434	1263.6095	-0.0661	0	70	0.0078	1	U	R.ISEEQLENFR.Q
190	152 – 161	632.7894	1263.5642	1263.6095	-0.0453	0	74	0.0031	1	U	R.ISEEQLENFR.Q
366	219 – 242	886.3957	2656.1654	2655.1404	1.0250	0	56	0.092	1		K.VWCFMGDGECDEPESLGAISLAGR.E + 2 Carbamidomethyl (C)
331	243 – 258	663.7399	1988.1980	1988.0513	0.1467	1	62	0.039	1		R.EKLDNLIFVINCNLQR.L + Carbamidomethyl (C)
311	245 – 258	866.4831	1730.9516	1730.9138	0.0378	0	59	0.074	1		K.LDNLIFVINCNLQR.L + Carbamidomethyl (C)
312	245 – 258	578.3049	1731.8929	1730.9138	0.9791	0	62	0.038	1		K.LDNLIFVINCNLQR.L + Carbamidomethyl (C)
174	269 – 278	602.3474	1202.6802	1202.6659	0.0143	0	53	0.41	1		K.IIQELEGVFR.G
285	309 – 321	802.4331	1602.8516	1602.6872	0.1644	0	65	0.021	1		R.MDEVIDGEYQNYK.A
150	345 – 353	567.7842	1133.5539	1133.5240	0.0298	0	37	20	3		K.DLSDEEIWK.L
152	345 – 353	567.8280	1133.6415	1133.5240	0.1174	0	34	42	2		K.DLSDEEIWK.L
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277	430 – 443	783.4007	1564.7868	1564.7773	0.0095	0	44	2.6	1		K.LPFYKPEEGSAEAK.Y
278	430 – 443	522.6223	1564.8450	1564.7773	0.0678	0	35	20	1		K.LPFYKPEEGSAEAK.Y
337	476 – 494	686.4524	2056.3352	2055.9718	0.3635	1	55	0.18	1		K.AMLDGSGDREISTTMAFVR.I
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160	485 – 494	577.8445	1153.6744	1153.5801	0.0942	0	63	0.036	1		R.EISTTMAFVR.I
134	509 – 518	554.7582	1107.5019	1107.6288	-0.1269	0	31	73	1		R.IVPIVPDEAR.T
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136	509 – 518	554.8327	1107.6509	1107.6288	0.0221	0	33	38	1		R.IVPIVPDEAR.T
120	519 – 527	538.2246	1074.4346	1074.4627	-0.0281	0	41	7.5	1		R.TFGMEGMFR.Q
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348	741 – 759	1062.0889	2122.1633	2122.0120	0.1513	0	92	3.5e-05	1		R.NDFGIGADVWSVPSFNELR.R
204	779 – 789	649.3601	1296.7056	1296.5768	0.1288	0	58	0.12	1		K.QSYVEECLGGR.R + Carbamidomethyl (C)
168	791 – 801	591.2901	1180.5657	1180.5798	-0.0141	0	56	0.21	1		R.GPVIASTDYMK.L
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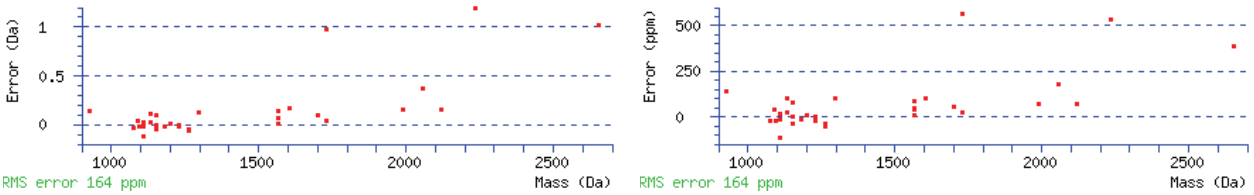


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VERSION     NP_253702.1  GI:15600208
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 882)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 882)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 882)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
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REFERENCE 4 (residues 1 to 882)
AUTHORS Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J., Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
TITLE A cystic fibrosis epidemic strain of Pseudomonas aeruginosa displays enhanced virulence and antimicrobial resistance
JOURNAL J. Bacteriol. 187 (14), **4908-4920** (2005)
PUBMED **15995206**
REFERENCE 5 (residues 1 to 882)
AUTHORS Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G., Iglewski,B.H. and Passador,L.
TITLE Effect of anaerobiosis and nitrate on gene expression in Pseudomonas aeruginosa
JOURNAL Infect. Immun. 73 (6), **3764-3772** (2005)
PUBMED **15908409**
REFERENCE 6 (residues 1 to 882)
AUTHORS Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M., Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
TITLE Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector
JOURNAL J. Bacteriol. 187 (5), 1799-1814 (2005)
PUBMED **15716452**
REFERENCE 7 (residues 1 to 882)
AUTHORS Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D., Ching,W.K., Hancock,R.E. and Brinkman,F.S.
TITLE Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating community-based, continually updated, genome annotation
JOURNAL Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
PUBMED **15608211**
REFERENCE 8 (residues 1 to 882)
AUTHORS Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and Iglewski,B.H.
TITLE Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment
JOURNAL J. Bacteriol. 185 (7), 2080-2095 (2003)
PUBMED **12644477**
REFERENCE 9 (residues 1 to 882)
AUTHORS Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
TITLE Identification, timing, and signal specificity of Pseudomonas aeruginosa quorum-controlled genes: a transcriptome analysis
JOURNAL J. Bacteriol. 185 (7), 2066-2079 (2003)
PUBMED **12644476**
REFERENCE 10 (residues 1 to 882)
AUTHORS Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P., Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R., Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
TITLE Complete genome sequence of Pseudomonas aeruginosa PA01, an opportunistic pathogen
JOURNAL Nature 406 (6799), 959-964 (2000)
PUBMED **10984043**
REFERENCE 11 (residues 1 to 882)
CONSRTM NCBI Genome Project
TITLE Direct Submission
JOURNAL Submitted (06-OCT-2010) National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA
REFERENCE 12 (residues 1 to 882)
AUTHORS Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
CONSRTM Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
TITLE Direct Submission
JOURNAL Submitted (08-SEP-2010) Department of Molecular Biology and Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby, British Columbia V5A 1S6, Canada
REMARK protein update by submitter
REFERENCE 13 (residues 1 to 882)
AUTHORS Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
CONSRTM Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
TITLE Direct Submission
JOURNAL Submitted (02-OCT-2008) Department of Molecular Biology and Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby, British Columbia V5A 1S6, Canada
REMARK protein update by submitter
REFERENCE 14 (residues 1 to 882)
AUTHORS Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
CONSRTM Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
TITLE Direct Submission
JOURNAL Submitted (05-JUL-2006) Department of Molecular Biology and Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby, British Columbia V5A 1S6, Canada
REMARK Sequence update by submitter
REFERENCE 15 (residues 1 to 882)
AUTHORS Ung,K.S., Hancock,R.E. and Brinkman,F.S.
CONSRTM Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
TITLE Direct Submission
JOURNAL Submitted (04-FEB-2003) Department of Molecular Biology and

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            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 882)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
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            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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                        UPR: UniProt Genome
            Method: conceptual translation.
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Mascot: http://www.matrixscience.com/

MASCOT Search Results

Protein View: gi|2781088

Chain A, Oxydized Nitrite Reductase From Pseudomonas Aeruginosa

Database:	NCBInr
Score:	1020
Nominal mass (M_r):	60141
Calculated pI:	7.40
Taxonomy:	Pseudomonas aeruginosa

This protein sequence matches the following other entries:

- gi|2781089 from Pseudomonas aeruginosa
- gi|4929916 from Pseudomonas aeruginosa
- gi|4929917 from Pseudomonas aeruginosa
- gi|4930068 from Pseudomonas aeruginosa
- gi|4930069 from Pseudomonas aeruginosa
- gi|5542315 from Pseudomonas aeruginosa
- gi|5542316 from Pseudomonas aeruginosa
- gi|5542317 from Pseudomonas aeruginosa
- gi|5542318 from Pseudomonas aeruginosa
- gi|5542319 from Pseudomonas aeruginosa
- gi|5542320 from Pseudomonas aeruginosa
- gi|22218647 from Pseudomonas aeruginosa
- gi|22218648 from Pseudomonas aeruginosa

Sequence similarity is available as an NCBI BLAST search of gi|2781088 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masA4.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 40%

Matched peptides shown in ***bold red***.

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  1 KDDMKAAEQY QGAASAVDPA HVVRTNGAPD MSESEFNEAK QIYFQRCAGC
 51 HGVLRKGATG KPLTPDITQQ RGQQYLEALI TYGTPLGMPN WGSSGELSKE
101 QITLMAKYIQ HTPPQPPEWG MPEMRESWKV LVKPEDRPKK QLNDLDLPNL
151 FSVTLRDAGQ IALVDGDSKK IVKVIDTGYA VHISRMSASG RYLLVIGRDA
201 RIDMIDLWAK EPTKVAEIKI GIEARSVESS KFKGYEDRYT IAGAYWPPQF
251 AIMDGETLEP KQIVSTRGMT VDTQTYHPEP RVAAIIASHE HPEFIVNVKE
301 TGKVLLVNYK DIDNLTVTSI GAAPFLHDGG WDSSHRYFMT AANNSNKVAV
351 IDSKDRRLSA LVDVGKTPHP GRGANFVHPK YGPVWSTSHL GDGSISLIGT
401 DPKNHPQYAW KKVAELQGQG GGSLFIKTHP KSSHLYVDTT FNPDARISQS
451 VAVFDLKNLD AKYQVLPIAE WADLGEGAKR VVQPEYNKRG DEVWFSVWNG
501 KNDSSALVVV DDKTLKLKAV VKDPRLITPT GKFNVYNTQH DVY
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Unformatted sequence string: **543 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
306	6 – 24	647.3419	1939.0038	1938.9548	0.0490	0	76	0.0014	1	U	K.AAEQYQGAASAVDPAHVVR.T
288	25 – 40	864.3869	1726.7592	1725.7152	1.0440	0	50	0.65	1	U	R.TNGAPDMSESEFNEAK.Q
315	140 – 156	663.0350	1986.0831	1985.0946	0.9886	1	41	4.9	1	U	K.KQLNDLDLPNLFSVTLR.D
302	141 – 156	929.0482	1856.0819	1856.9996	-0.9177	0	57	0.13	1	U	K.QLNDLDLPNLFSVTLR.D
192	174 – 185	665.8884	1329.7622	1329.7041	0.0581	0	92	5.5e-05	1	U	K.VIDTGYAVHISR.M
193	174 – 185	444.2858	1329.8356	1329.7041	0.1315	0	26	2.2e+02	1	U	K.VIDTGYAVHISR.M
194	174 – 185	444.2903	1329.8491	1329.7041	0.1450	0	50	0.76	1	U	K.VIDTGYAVHISR.M
195	174 – 185	444.6508	1330.9306	1329.7041	1.2265	0	59	0.092	1	U	K.VIDTGYAVHISR.M
61	192 – 198	417.3565	832.6985	832.5171	0.1815	0	36	30	1		R.YLLVIGR.D
132	202 – 210	552.8038	1103.5930	1103.5685	0.0245	0	49	1.3	1		R.IDMIDLWAK.E
271	268 – 281	544.6462	1630.9167	1630.7410	0.1757	0	27	1.5e+02	1		R.GMTVDTQTYHPEPR.V

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
312	282 – 299	658.7672	1973.2798	1973.0734	0.2064	0	74	0.0023	1		R.VAAIIASHEHPEFIVNVK.E
313	282 – 299	658.7826	1973.3260	1973.0734	0.2525	0	64	0.024	1		R.VAAIIASHEHPEFIVNVK.E
314	282 – 299	494.5512	1974.1758	1973.0734	1.1024	0	38	7.8	3		R.VAAIIASHEHPEFIVNVK.E
337	381 – 403	796.5711	2386.6915	2386.1805	0.5110	0	55	0.14	1		K.YGPVWSTSHLGDGSISLIGTDPK.N
272	412 – 427	544.6938	1631.0596	1630.9043	0.1553	1	67	0.013	1		K.KVAELQGQGGGSLFIK.T
254	413 – 427	752.3611	1502.7077	1502.8093	-0.1016	0	93	4.2e-05	1		K.VAELQGQGGGSLFIK.T
255	413 – 427	752.3810	1502.7474	1502.8093	-0.0619	0	89	8.6e-05	1		K.VAELQGQGGGSLFIK.T
256	413 – 427	752.3904	1502.7662	1502.8093	-0.0430	0	98	1.3e-05	1		K.VAELQGQGGGSLFIK.T
287	432 – 446	575.2844	1722.8314	1721.8009	1.0305	0	44	2.8	1		K.SSHLYVDTTFNPDAR.I
159	447 – 457	603.8156	1205.6166	1205.6656	-0.0490	0	82	0.00056	1		R.ISQSVAVFDLK.N
160	447 – 457	603.8308	1205.6471	1205.6656	-0.0185	0	90	7.6e-05	1		R.ISQSVAVFDLK.N
161	447 – 457	603.9253	1205.8360	1205.6656	0.1704	0	80	0.00091	1		R.ISQSVAVFDLK.N
162	447 – 457	604.2727	1206.5308	1205.6656	0.8652	0	66	0.022	1		R.ISQSVAVFDLK.N
163	447 – 457	604.3069	1206.5992	1205.6656	0.9336	0	29	97	2		R.ISQSVAVFDLK.N
303	463 – 479	930.4932	1858.9719	1858.9465	0.0254	0	80	0.00069	1		K.YQVLPIAEWADLGEGAK.R
268	489 – 501	527.2808	1578.8206	1578.7579	0.0627	1	39	9.7	1	U	K.RGDEVWFSVWNGK.N
216	533 – 543	700.3380	1398.6615	1398.6204	0.0410	0	38	11	1	U	K.FNVYNTQHDVY.-
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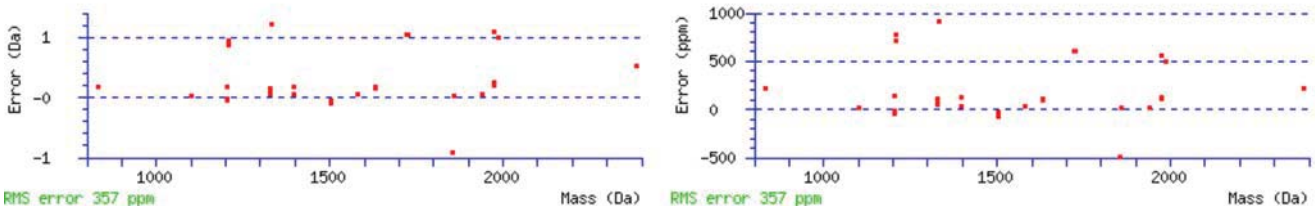


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DEFINITION  Chain A, Oxydized Nitrite Reductase From Pseudomonas Aeruginosa.
ACCESSION   1NIR_A
VERSION     1NIR_A  GI:2781088
DBSOURCE    pdb: molecule 1NIR, chain 65, release Oct 13, 2012;
            deposition: Jun 17, 1997;
            class: Nitrite Reductase;
            source: Mmdb_id: 6859, Pdb_id 1: 1NIR;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Pseudomonas aeruginosa
  ORGANISM  Pseudomonas aeruginosa
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 543)
  AUTHORS   Nurizzo,D., Silvestrini,M.C., Mathieu,M., Cutruzzola,F.,
            Bourgeois,D., Fulop,V., Hajdu,J., Brunori,M., Tegoni,M. and
            Cambillau,C.
  TITLE     N-terminal arm exchange is observed in the 2.15 A crystal structure
            of oxidized nitrite reductase from Pseudomonas aeruginosa
  JOURNAL   Structure 5 (9), 1157-1171 (1997)
   PUBMED   9331415
REFERENCE   2  (residues 1 to 543)
  AUTHORS   Nurizzo,D., Tegoni,M. and Cambillau,C.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-JUN-1997)
COMMENT     1 Nitrite Reductase.
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MASCOT Search Results

Protein View: gi|391846

dehydrogenase [Pseudomonas putida]

Database:	NCBInr
Score:	621
Nominal mass (M_r):	27476
Calculated pI:	5.58
Taxonomy:	Pseudomonas putida

Sequence similarity is available as an NCBI BLAST search of gi|391846 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masAA.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 41%

Matched peptides shown in ***bold red***.

```
  1 MGNQQVVSIT GAGSGIGLEL VRSFKSAGYC VSALVRNEEQ EALLCNEFKD
 51 ALEIVVGDVR DHAINEKLIK QTIARFGHLD CFIANAGIWD YMLSIEEPWE
101 KISSSFDEIF DINVKSYFSG ISAALPELKK TNGSVVMTAS VSSHAVGGGG
151 SCYIASKHAV LGMVKALAYE LAPEIRVNAV SPGGTVTSLC GPASAGFDKM
201 HMKDMPGIDD MIKGLTPLGF AAKPEDVVEP YLLLASRKQG KFITGTVISI
251 DGGMALGRK
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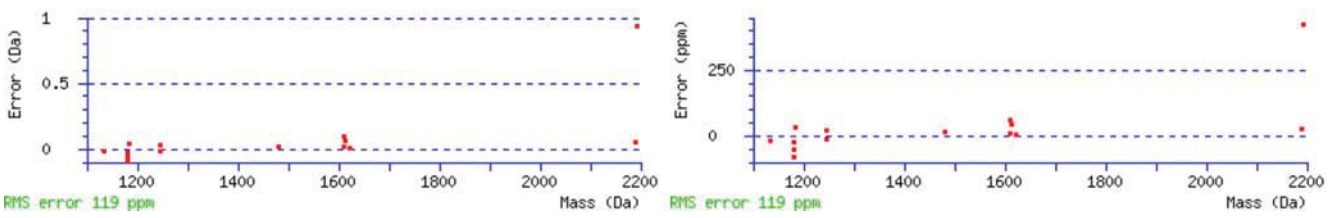
Unformatted sequence string: 259 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
133	26 – 36	591.7526	1181.4907	1181.5863	-0.0956	0	78	0.0014	1	U	K.SAGYCVSALVR.N + Carbamidomethyl (C)
134	26 – 36	591.7689	1181.5232	1181.5863	-0.0631	0	79	0.00096	1	U	K.SAGYCVSALVR.N + Carbamidomethyl (C)
135	26 – 36	591.7868	1181.5591	1181.5863	-0.0272	0	76	0.0021	1	U	K.SAGYCVSALVR.N + Carbamidomethyl (C)
230	37 – 49	812.3754	1622.7362	1622.7246	0.0117	0	87	0.00012	1	U	R.NEEQEALLCNEFK.D + Carbamidomethyl (C)
136	50 – 60	593.3466	1184.6786	1184.6401	0.0385	0	71	0.0076	1		K.DALEIVVGDVR.D
228	102 – 115	807.4407	1612.8669	1612.7984	0.0684	0	98	1e-05	1	U	K.ISSSFDEIFDINVK.S
214	116 – 129	741.9077	1481.8008	1481.7766	0.0242	0	46	1.7	1		K.SYFSGISAALPELK.K
226	116 – 130	805.9500	1609.8854	1609.8715	0.0139	1	70	0.0068	1		K.SYFSGISAALPELKK.T
227	116 – 130	537.6640	1609.9701	1609.8715	0.0986	1	46	1.5	1		K.SYFSGISAALPELKK.T
147	166 – 176	623.3379	1244.6613	1244.6764	-0.0151	0	53	0.47	1	U	K.ALAYELAPEIR.V
148	166 – 176	623.3605	1244.7065	1244.6764	0.0301	0	55	0.26	1	U	K.ALAYELAPEIR.V
270	177 – 199	1096.5648	2191.1151	2191.0580	0.0571	0	75	0.0016	1		R.VNAVSPGGTVTSLCGPASAGFDK.M + Carbamidomethyl (C)
271	177 – 199	731.6718	2191.9936	2191.0580	0.9356	0	76	0.0015	1		R.VNAVSPGGTVTSLCGPASAGFDK.M + Carbamidomethyl (C)
122	204 – 213	567.7526	1133.4907	1133.5097	-0.0190	0	39	14	1	U	K.DMPGIDDMIK.G

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DEFINITION  dehydrogenase [Pseudomonas putida].
ACCESSION   BAA20393
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VERSION     BAA20393.1  GI:391846
DBSOURCE    accession AB004059.1
KEYWORDS    .
SOURCE      Pseudomonas putida
  ORGANISM  Pseudomonas putida
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 259)
  AUTHORS   Noboru,T., Toshiya,I., Takashi,S., Kazuhiro,Y., Masamichi,K.,
            Yue-Wu,W., Masao,F. and Hohzoh,K.
  TITLE     The molecular analysis of NAH7-type cluster located on the
            chromosomes of Pseudomonas aeruginosa PaK1 and Pseudomonas putida
            OUS82
  JOURNAL   Unpublished
REFERENCE   2  (residues 1 to 259)
  AUTHORS   Takizawa,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-MAY-1997) Noboru Takizawa, Okayama Univesity of
            Science, Applied Chemistry; 1-1 Ridai-cho, Okayama, Okayama 700,
            JAPAN (Tel:086-252-3161(ex.4635), Fax:086-252-6891)
COMMENT     D16629: submitted (19-May-1997).
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                     /strain="OUS82"
                     /db_xref="taxon:303"
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     Region          1..259
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                     /note="2,3-dihydroxy-2,3-dihydrophenylpropionate
                     dehydrogenase; Provisional"
                     /db_xref="CDD:235739"
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                     /note="cis-biphenyl-2,3-dihydrodiol-2,3-dehydrogenase
                     (BphB)-like, classical (c) SDRs; cd05348"
                     /db_xref="CDD:187606"
     Site            order(11,13..17,35..36,40,57..59,85..87,117,138..140,153,
                     157,182..185,187..189)
                     /site_type="other"
                     /note="NAD binding site [chemical binding]"
                     /db_xref="CDD:187606"
     Site            order(113,140,153,157)
                     /site_type="active"
                     /db_xref="CDD:187606"
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                     /transl_table=11
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MASCOT Search Results

Protein View: gi|15596781

sdhB gene product [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	354
Nominal mass (M_r):	26138
Calculated pI:	6.59
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|116049529 from Pseudomonas aeruginosa UCBPP-PA14
- gi|218892459 from Pseudomonas aeruginosa LESB58
- gi|254234685 from Pseudomonas aeruginosa C3719
- gi|254239932 from Pseudomonas aeruginosa 2192
- gi|296390042 from Pseudomonas aeruginosa PAb1
- gi|313106597 from Pseudomonas aeruginosa 39016
- gi|355644632 from Pseudomonas sp. 2_1_26
- gi|386059526 from Pseudomonas aeruginosa M18
- gi|386065414 from Pseudomonas aeruginosa NCGM2.S1
- gi|392984951 from Pseudomonas aeruginosa DK2
- gi|9947547 from Pseudomonas aeruginosa PAO1
- gi|115584750 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126166616 from Pseudomonas aeruginosa C3719
- gi|126193310 from Pseudomonas aeruginosa 2192
- gi|218772685 from Pseudomonas aeruginosa LESB58
- gi|310879326 from Pseudomonas aeruginosa 39016
- gi|334834720 from Pseudomonas aeruginosa HB15
- gi|334836910 from Pseudomonas aeruginosa HB13
- gi|346057249 from Pseudomonas aeruginosa NCMG1179
- gi|347305832 from Pseudomonas aeruginosa M18
- gi|348033973 from Pseudomonas aeruginosa NCGM2.S1
- gi|354829142 from Pseudomonas sp. 2_1_26
- gi|375042388 from Pseudomonas aeruginosa MPAO1/P1
- gi|375048673 from Pseudomonas aeruginosa MPAO1/P2
- gi|384398250 from Pseudomonas aeruginosa PADK2_CF510
- gi|392320456 from Pseudomonas aeruginosa DK2

Sequence similarity is available as an NCBI BLAST search of gi|15596781 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masAA.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 30%

Matched peptides shown in ***bold red***.

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  1 MLQVSVYRYN PDKDAAPYMQ DFQVDTNGKD VMVLDVLALI KEQDEGFSYR
 51 RSCREGVCGS DGMNINGKNG LACITPLSAA GLKGGKLVIR PLPGLPVIRD
101 LVVDMSIFYK QYEKVKPFLQ NDTPAPAIER LQTPEEREKL DGLYECILCA
151 CCSTSCPSFW WNPDKFLGPA ALLQAYRFLA DSRDTKTEER LASLDDPFSV
201 FRCRGIMNCV NVCPKGLNPT KAIGHVRNML LQSGT
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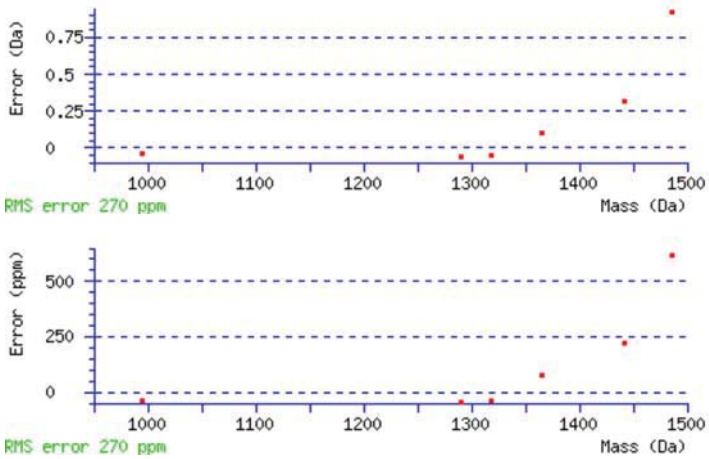
Unformatted sequence string: 235 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
88	1 – 8	498.2512	994.4878	994.5270	-0.0392	0	42	5	1		-.MLQVSVYR.Y

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
216	69 – 83	743.8671	1485.7196	1484.8021	0.9176	0	41	6.4	1	U	K.NGLACITPLSAAGLK.G + Carbamidomethyl (C)
199	87 – 99	481.7621	1442.2643	1441.9497	0.3147	0	45	2.5	1		K.LVIRPLPGLPVIR.D
170	166 – 177	660.3507	1318.6868	1318.7397	-0.0529	0	81	0.00067	1		K.FLGPAALLQAYR.F
182	191 – 202	683.9047	1365.7949	1365.6929	0.1021	0	75	0.0022	1		R.LASLDDPFSVFR.C
161	205 – 215	646.2697	1290.5249	1290.5883	-0.0634	0	71	0.0071	1		R.GIMNCVNVCPK.G + 2 Carbamidomethyl (C)

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DEFINITION  succinate dehydrogenase iron-sulfur subunit [Pseudomonas aeruginosa
            PAO1].
ACCESSION   NP_250275
VERSION     NP_250275.1  GI:15596781
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 235)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 235)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 235)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 235)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 235)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 235)
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  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 235)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 235)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 235)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 235)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 235)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 235)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 235)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 235)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 235)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 235)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
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            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG04973.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /note="2Fe-2S iron-sulfur cluster binding domain;
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                     /db_xref="CDD:257488"
     Region          145..218
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                     /db_xref="CDD:257853"
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Mascot: http://www.matrixscience.com/

MATRIX SCIENCE MASCOT Search Results

Protein View: gi|152987553

hypothetical protein PSPA7_3562 [Pseudomonas aeruginosa PA7]

Database:	NCBInr
Score:	130
Nominal mass (M_r):	18176
Calculated pI:	6.37
Taxonomy:	Pseudomonas aeruginosa PA7

This protein sequence matches the following other entries:

- gi|150962711 from Pseudomonas aeruginosa PA7

Sequence similarity is available as an NCBI BLAST search of gi|152987553 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masBC.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 16 %

Matched peptides shown in ***bold red***.

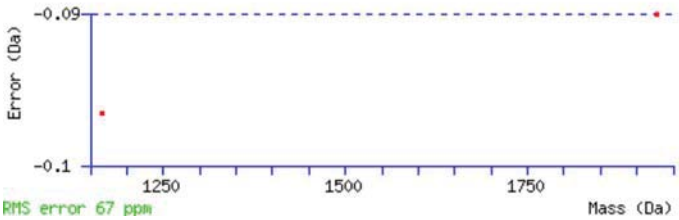
```
  1 MIREVEGDIL LSGAQVIAHG IAPQDHFDSG LALALRERWP SMVRDYRHAA
 51 HARAPEPGGI WVWAGVDEQG RTQCIVNLIT QGMLHSGRSA KPGKASLEDV
101 GHALRELARY VRSEGVSSLA LPCVATGVGG LAWSEVKPLV LRHLADLEIP
151 VILYEVYRKG VAADEKLA
```

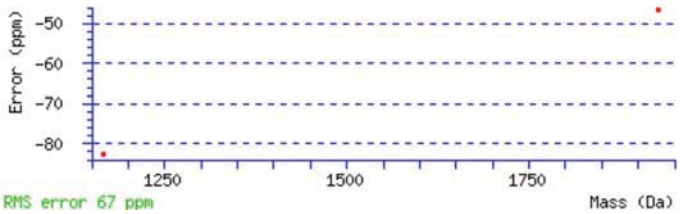
Unformatted sequence string: 168 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
230	72 – 88	643.3028	1926.8867	1926.9768	-0.0901	0	62	0.034	1	U	R.TQCIVNLITQGMLHSGR.S + Carbamidomethyl (C)
132	95 – 105	584.2612	1166.5078	1166.6044	-0.0965	0	68	0.012	1	U	K.ASLEDVGHALR.E



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LOCUS       YP_001348916            168 aa            linear   CON 10-JUN-2013
DEFINITION  hypothetical protein PSPA7_3562 [Pseudomonas aeruginosa PA7].
ACCESSION   YP_001348916
VERSION     YP_001348916.1  GI:152987553
DBLINK      Project: 58627
            BioProject: PRJNA58627
DBSOURCE    REFSEQ: accession NC_009656.1
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PA7
  ORGANISM  Pseudomonas aeruginosa PA7
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 168)
  AUTHORS   Roy,P.H., Tetu,S.G., Larouche,A., Elbourne,L., Tremblay,S., Ren,Q.,
            Dodson,R., Harkins,D., Shay,R., Watkins,K., Mahamoud,Y. and
            Paulsen,I.T.
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  TITLE     Complete genome sequence of the multiresistant taxonomic outlier
            Pseudomonas aeruginosa PA7
  JOURNAL   PLoS ONE 5 (1), E8842 (2010)
   PUBMED   20107499
  REMARK    Publication Status: Online-Only
REFERENCE   2  (residues 1 to 168)
  AUTHORS   Dodson,R.J., Harkins,D. and Paulsen,I.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-JUN-2007) The Institute for Genomic Research, 9712
            Medical Center Dr, Rockville, MD 20850, USA
REFERENCE   3  (residues 1 to 168)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-SEP-2006) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from ABR84736.
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..168
                     /organism="Pseudomonas aeruginosa PA7"
                     /strain="PA7"
                     /db_xref="taxon:381754"
                     /note="bacterial strain PA7 was obtained from Paul Roy,
                     University of Laval, Quebec, Canada"
     Protein         1..168
                     /product="hypothetical protein"
                     /calculated_mol_wt=18056
     Region          1..147
                     /region_name="Macro_Poa1p_like"
                     /note="Macro domain, Poa1p_like family. The macro domain
                     is a high-affinity ADP-ribose binding module found in a
                     variety of proteins as a stand-alone domain or in
                     combination with other domains like in histone macroH2A
                     and some PARPs (poly ADP-ribose...; cd02901"
                     /db_xref="CDD:239230"
     Site            order(8..9,22,29,31..32,125,127..129)
                     /site_type="other"
                     /note="ADP-ribose binding site [chemical binding]"
                     /db_xref="CDD:239230"
     CDS             1..168
                     /coded_by="complement(NC_009656.1:3690318..3690824)"
                     /note="Annotation generated by automatically transferring
                     the annotation from the manually curated GenBank accession
                     AE004091; identified by Glimmer2; putative"
                     /transl_table=11
CONTIG      join(WP_003152896.1:1..168)
```

MATRIX SCIENCE MASCOT Search Results

Protein View: gi|15598003

hypothetical protein [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	73
Nominal mass (M_r):	22443
Calculated pI:	7.18
Taxonomy:	**Pseudomonas aeruginosa PAO1**

This protein sequence matches the following other entries:

- **gi|9948891** from **Pseudomonas aeruginosa PAO1**

Sequence similarity is available as **an NCBI BLAST search of gi|15598003 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\masBC.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 6%

Matched peptides shown in ***bold red***.

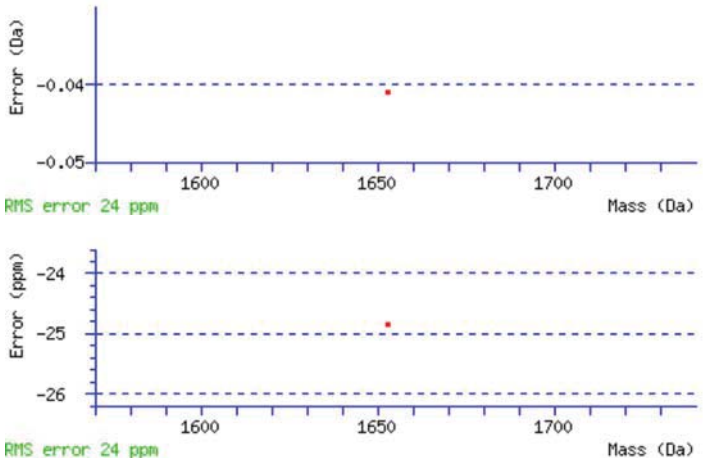
```
  1 MLPTASRQAQ RHRIGDINPM EIPRMFPRRL LPASLIVLGV LFGASAQASP
 51 AHGQAFGKPA QAAQASRSIE VVLGDMYFKP RAIEVKAGET VRFVLKNEGK
101 LLHEFNLGDA AMHAEHQKEM LEMQQSGMLT PTGMASMDHS QMGHGMADMD
151 HGRMMKHDDP NSVLVEPGKS AELTWTFTKA TRLEFACNIP GHYQAGMVGQ
201 LTVQP
```

Unformatted sequence string: **205 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
212	68 – 81	827.4165	1652.8185	1652.8596	-0.0411	0	73	0.0034	1	U	R.SIEVVLGDMYFKPR.A

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LOCUS       NP_251497               205 aa            linear   CON 20-MAR-2014
DEFINITION  hypothetical protein PA2807 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_251497
VERSION     NP_251497.1  GI:15598003
DBLINK      BioProject: PRJNA57945
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DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 205)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 205)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 205)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 205)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 205)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 205)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 205)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 205)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 205)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 205)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 205)
  CONSRTM   NCBI Genome Project
```

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  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 205)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 205)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 205)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 205)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 205)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06195.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..205
                     /organism="Pseudomonas aeruginosa PAO1"
                     /strain="PAO1"
                     /db_xref="taxon:208964"
     Protein         1..205
                     /product="hypothetical protein"
                     /calculated_mol_wt=22327
     Region          66..203
                     /region_name="Cupredoxin_like_2"
                     /note="Uncharacterized Cupredoxin-like subfamily; cd04211"
                     /db_xref="CDD:259873"
     Site            order(103,187,192,197)
                     /site_type="other"
                     /note="putative Type 1 (T1) Cu binding site [ion binding]"
                     /db_xref="CDD:259873"
     CDS             1..205
                     /coded_by="complement(NC_002516.2:3161599..3162216)"
                     /note="Product name confidence: class 4 (Homologs of
                     previously reported genes of unknown function, or no
                     similarity to any previously reported sequences)"
                     /transl_table=11
CONTIG      join(WP_010895638.1:1..205)
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MATRIX SCIENCE MASCOT Search Results

Protein View: gi|442646

Chain A, Crystal Structure Of Pseudomonas Aeruginosa Zinc Azurin Mutant Asp47asp At 2.4 Angstroms Resolution

Database:	NCBInr
Score:	111
Nominal mass (M_r):	13937
Calculated pI:	5.71
Taxonomy:	**Pseudomonas aeruginosa**

This protein sequence matches the following other entries:

- **gi|442647** from **Pseudomonas aeruginosa**
- **gi|442648** from **Pseudomonas aeruginosa**
- **gi|442649** from **Pseudomonas aeruginosa**

Sequence similarity is available as **an NCBI BLAST search of gi|442646 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas19.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 23%

Matched peptides shown in ***bold red***.

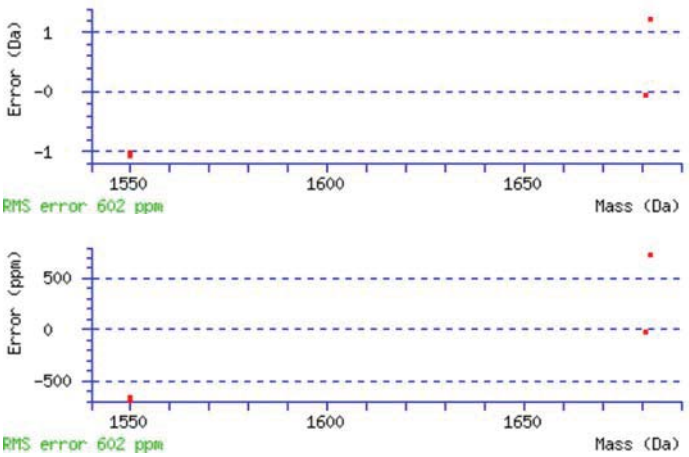
```
  1 AQCSVDIQGN DQMQFNTNAI TVDKSCKQFT VNLSHPGNLP KNVMGHDWVL
 51 STAADMQGVV TDGMASGLDK DYLKPDDSRV IAHTKLIGSG EKDSVTFDVS
101 KLKEGEQYMF FCTFPGHSAL MKGTLTLK
```

Unformatted sequence string: **128 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
318	28 – 41	775.8838	1549.7531	1550.8205	-1.0674	0	43	5.2	1	U	K.QFTVNLSHPGNLPK.N
319	28 – 41	775.9001	1549.7857	1550.8205	-1.0348	0	33	59	1	U	K.QFTVNLSHPGNLPK.N
338	86 – 101	841.4093	1680.8041	1680.8570	-0.0529	1	68	0.017	1	U	K.LIGSGEKDSVTFDVSK.L
339	86 – 101	561.7015	1682.0828	1680.8570	1.2257	1	30	94	1	U	K.LIGSGEKDSVTFDVSK.L

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LOCUS       1AZR_A                  128 aa            linear   BCT 10-OCT-2012
DEFINITION  Chain A, Crystal Structure Of Pseudomonas Aeruginosa Zinc Azurin
```

```
            Mutant Asp47asp At 2.4 Angstroms Resolution.
ACCESSION   1AZR_A
VERSION     1AZR_A  GI:442646
DBSOURCE    pdb: molecule 1AZR, chain 65, release Aug 27, 2007;
            deposition: Mar 4, 1993;
            class: Electron Transfer(Cuproprotein);
            source: Mmdb_id: 315, Pdb_id 1: 1AZR;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Pseudomonas aeruginosa
  ORGANISM  Pseudomonas aeruginosa
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 128)
  AUTHORS   Sjolin,L., Tsai,L.C., Langer,V., Pascher,T., Karlsson,G.,
            Nordling,M. and Nar,H.
  TITLE     Structure of Pseudomonas aeruginosai zinc azurin mutant Asn47Asp at
            2.4 A resolution
  JOURNAL   Acta Crystallogr. D Biol. Crystallogr. 49 (PT 5), 449-457 (1993)
   PUBMED   15299504
REFERENCE   2  (residues 1 to 128)
  AUTHORS   Sjolin,L., Tsai,L.C., Langer,V., Pascher,T., Karlsson,G.,
            Nordling,M. and Nar,H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAR-1993)
COMMENT     SEQRES.
FEATURES             Location/Qualifiers
     source          1..128
                     /organism="Pseudomonas aeruginosa"
                     /db_xref="taxon:287"
     Region          3..128
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                     /note="Azurin [Energy production and conversion]"
                     /db_xref="CDD:33053"
     Bond            bond(3,26)
                     /bond_type="disulfide"
     SecStr          3..11
                     /sec_str_type="sheet"
                     /note="strand 1"
     SecStr          13..16
                     /sec_str_type="sheet"
                     /note="strand 2"
     SecStr          18..23
                     /sec_str_type="sheet"
                     /note="strand 3"
     SecStr          27..37
                     /sec_str_type="sheet"
                     /note="strand 4"
     Het             join(bond(45),bond(46),bond(112),bond(117))
                     /heterogen="( CU,   1 )"
     SecStr          45..52
                     /sec_str_type="sheet"
                     /note="strand 5"
     SecStr          56..65
                     /sec_str_type="helix"
                     /note="helix 1"
     SecStr          81..87
                     /sec_str_type="sheet"
                     /note="strand 6"
     SecStr          91..99
                     /sec_str_type="sheet"
                     /note="strand 7"
     SecStr          108..112
                     /sec_str_type="sheet"
                     /note="strand 8"
     SecStr          121..128
                     /sec_str_type="sheet"
                     /note="strand 9"
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MASCOT Search Results

Protein View: gi|15599785

hypothetical protein PA4589 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	341
Nominal mass (M_r):	49692
Calculated pI:	5.89
Taxonomy:	**Pseudomonas aeruginosa PAO1**

This protein sequence matches the following other entries:

- **gi|514411731** from **Pseudomonas aeruginosa RP73**
- **gi|489214709** from **Pseudomonas aeruginosa**
- **gi|9950837** from **Pseudomonas aeruginosa PAO1**
- **gi|126170598** from **Pseudomonas aeruginosa C3719**
- **gi|404519602** from **Pseudomonas aeruginosa ATCC 14886**
- **gi|404532465** from **Pseudomonas aeruginosa ATCC 25324**
- **gi|404541594** from **Pseudomonas aeruginosa E2**
- **gi|514249701** from **Pseudomonas aeruginosa RP73**

Sequence similarity is available as **an NCBI BLAST search of gi|15599785 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas10.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 17%

Matched peptides shown in ***bold red***.

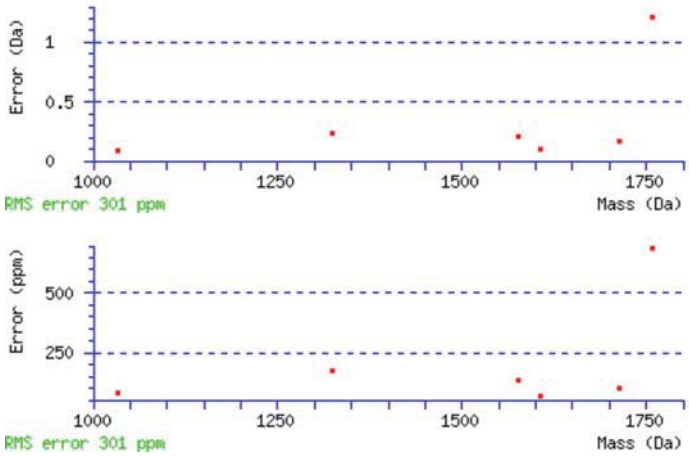
```
  1 MPLLCALIVG GLFGTSQAQA GGFMVPTTNT AGWGRAMAGG SLFPNDPSAA
 51 FNNPAAMAFI DKRIAQLTVN YADIDIKYNG DAYDYQGNPM TGGYQDGPGT
101 PELGTNDGGQ AGFGAWLPTG FLVVPINDRF AFGLSQVVPM GMRSTWDPNW
151 KGRDFAVDTK IETIGLTGSL SFKVNDNFSL GAGVIIQRTS GFVSQNLDLY
201 ASAANSPGMG GIPFPASNSS ALMRVKVDNT SPGFFAGAVW KPTDRDTLGF
251 AYHAKIRNKL KGHYNLYDHD GGLTEGAIEG GTPGLAYPGL DLRMGASASA
301 RLDIPAYASL DWVHQFNDRL SLGASATWTE WSSFQDLTLK SHGNTIVSIP
351 YTYRNTWTLA VGGDYKVTDQ WTMRAGVAYD QTPTHNATRD PRIPDGDRYF
401 ASLGAGYRFQ SMPELSIDAA YSRQFVKEVP LKTVNQDRLG GGRLDGRATS
451 KGQVFSLSAT YDF
```

Unformatted sequence string: **463 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
179	64 – 77	789.0391	1576.0636	1575.8508	0.2128	0	107	2.1e-06	1	U	R.IAQLTVNYADIDIK.Y
92	144 – 151	517.2822	1032.5498	1032.4665	0.0833	0	44	6.3	1		R.STWDPNWK.G
181	341 – 354	536.6459	1606.9158	1606.8103	0.1055	0	36	27	1	U	K.SHGNTIVSIPYTYR.N
141	355 – 366	662.9470	1323.8794	1323.6459	0.2335	0	55	0.35	1	U	R.NTWTLAVGGDYK.V
188	393 – 408	587.0285	1758.0637	1756.8533	1.2104	1	25	3.2e+02	2		R.IPDGDRYFASLGAGYR.F
185	409 – 423	857.9932	1713.9719	1713.8032	0.1687	0	73	0.0059	1	U	R.FQSMPELSIDAAYSR.Q

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LOCUS       NP_253279               463 aa            linear   CON 20-MAR-2014
DEFINITION  hypothetical protein PA4589 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_253279
VERSION     NP_253279.1  GI:15599785
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 463)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 463)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 463)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 463)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 463)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 463)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 463)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 463)
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  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 463)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 463)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 463)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 463)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 463)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 463)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 463)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 463)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG07977.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /note="Outer membrane protein transport protein
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                     /db_xref="CDD:251889"
     CDS             1..463
                     /coded_by="complement(NC_002516.2:5139050..5140441)"
                     /note="Product name confidence: class 3 (Function proposed
                     based on presence of conserved amino acid motif,
                     structural feature or limited sequence similarity to an
                     experimentally studied gene)"
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CONTIG      join(WP_003123403.1:1..463)
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MASCOT Search Results

Protein View: gi|15599031

hypothetical protein PA3836 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	380
Nominal mass (M_r):	34199
Calculated pI:	7.75
Taxonomy:	

This protein sequence matches the following other entries:

- gi|386067927 (no taxonomy information for this entry)
- gi|478477302 (no taxonomy information for this entry)
- gi|489183438 (no taxonomy information for this entry)
- gi|9950012 (no taxonomy information for this entry)
- gi|126168671 (no taxonomy information for this entry)
- gi|310881725 (no taxonomy information for this entry)
- gi|348036486 (no taxonomy information for this entry)
- gi|375043599 (no taxonomy information for this entry)
- gi|375051338 (no taxonomy information for this entry)
- gi|404347864 (no taxonomy information for this entry)
- gi|404525111 (no taxonomy information for this entry)
- gi|476649190 (no taxonomy information for this entry)
- gi|477549388 (no taxonomy information for this entry)
- gi|508142189 (no taxonomy information for this entry)
- gi|508146237 (no taxonomy information for this entry)
- gi|543878358 (no taxonomy information for this entry)
- gi|543884020 (no taxonomy information for this entry)

Sequence similarity is available as **an NCBI BLAST search of gi|15599031 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas12.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 26%

Matched peptides shown in ***bold red***.

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  1 MMQAYKNKWL PLSALALGMA LSFGANAEEQ KSVAVTAIVE HPALDAARDG
 51 VKEALQEAGY EDGKNLKWQY QSAQGNTGTA AQIARKFIGD KPDVIVGIAT
101 PSAQALVAAT KSIPIVFSTV TDPVGAHLTP SWEASGTNVT GVSDMLALDK
151 QIELIKKVVP GAKRIGMVYN PGEANSVVVV KELKELLPKM GLSLVEASAP
201 RSVDVSSAAR SLVGKVDAIY TNTDNNVVSA YEALVKVGND AKIPLIASDT
251 DSVKRGAIAA LGINYKEMGK QTGRMVVRIL KGEKPGEIKP ETSDNLQLFV
301 NPGAAQKQGV TLSDELLKSA AEVIK
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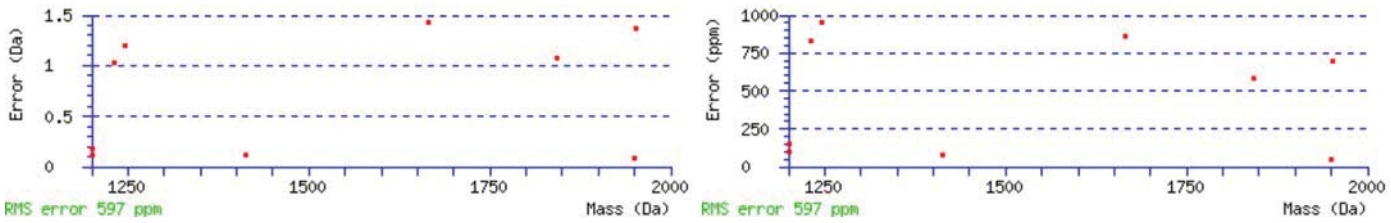
Unformatted sequence string: **325 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
204	53 – 67	556.0845	1665.2316	1663.8053	1.4264	1	15	3.7e+03	9	U	K.EALQEAGYEDGKNLK.W
240	68 – 85	976.0205	1950.0264	1949.9344	0.0920	0	111	7.3e-07	1		K.WQYQSAQGNTGTAAQIAR.K
241	68 – 85	651.4401	1951.2985	1949.9344	1.3641	0	68	0.013	1		K.WQYQSAQGNTGTAAQIAR.K
109	190 – 201	616.3412	1230.6679	1229.6438	1.0241	0	63	0.08	1	U	K.MGLSLVEASAPR.S
227	237 – 254	615.3589	1843.0548	1841.9734	1.0814	1	33	51	1	U	K.VGNDAKIPLIASDTDSVK.R
162	243 – 255	472.3062	1413.8969	1413.7827	0.1142	1	65	0.037	1	U	K.IPLIASDTDSVKR.G
121	255 – 266	416.6444	1246.9115	1245.7193	1.1922	1	27	2.8e+02	9		K.RGAIAALGINYK.E
104	308 – 318	601.8951	1201.7756	1201.6554	0.1201	0	66	0.037	1	U	K.QGVTLSDELLK.S
105	308 – 318	601.9231	1201.8317	1201.6554	0.1763	0	65	0.05	1	U	K.QGVTLSDELLK.S

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LOCUS       NP_252525               325 aa            linear   CON 20-MAR-2014
DEFINITION  hypothetical protein PA3836 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_252525
VERSION     NP_252525.1  GI:15599031
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 325)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 325)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 325)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 325)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 325)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 325)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 325)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 325)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 325)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
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   PUBMED   12644476
REFERENCE   10 (residues 1 to 325)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 325)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 325)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 325)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 325)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 325)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 325)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG07223.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /coded_by="NC_002516.2:4297250..4298227"
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MASCOT Search Results

Protein View: gi|15598058

lactonizing lipase [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	60
Nominal mass (M_r):	32703
Calculated pI:	6.37
Taxonomy:	

This protein sequence matches the following other entries:

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- gi|386066889 (no taxonomy information for this entry)
- gi|392983415 (no taxonomy information for this entry)
- gi|478478295 (no taxonomy information for this entry)
- gi|514410138 (no taxonomy information for this entry)
- gi|489181493 (no taxonomy information for this entry)
- gi|266475 (no taxonomy information for this entry)
- gi|9948950 (no taxonomy information for this entry)
- gi|45341 (no taxonomy information for this entry)
- gi|50882001 (no taxonomy information for this entry)
- gi|78192239 (no taxonomy information for this entry)
- gi|115586086 (no taxonomy information for this entry)
- gi|126167780 (no taxonomy information for this entry)
- gi|126194905 (no taxonomy information for this entry)
- gi|310880677 (no taxonomy information for this entry)
- gi|346058654 (no taxonomy information for this entry)
- gi|348035448 (no taxonomy information for this entry)
- gi|354830685 (no taxonomy information for this entry)
- gi|375041042 (no taxonomy information for this entry)
- gi|375051099 (no taxonomy information for this entry)
- gi|384398384 (no taxonomy information for this entry)
- gi|392318920 (no taxonomy information for this entry)
- gi|404346871 (no taxonomy information for this entry)
- gi|404534990 (no taxonomy information for this entry)
- gi|404536065 (no taxonomy information for this entry)
- gi|477550381 (no taxonomy information for this entry)
- gi|477559757 (no taxonomy information for this entry)
- gi|508137722 (no taxonomy information for this entry)
- gi|514248108 (no taxonomy information for this entry)
- gi|522637999 (no taxonomy information for this entry)
- gi|534389737 (no taxonomy information for this entry)
- gi|543883139 (no taxonomy information for this entry)

Sequence similarity is available as **an NCBI BLAST search of gi|15598058 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas9.tmp
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Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 6 %

Matched peptides shown in ***bold red***.

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 51 VDYWFGIPSA LRRDGAQVYV TEVSQLDTSE VRGEQLLQQV EEIVALSGQP
101 KVNLIGHSHG GPTIRYVAAV RPDLIASATS VGAPHKGSDT ADFLRQIPPG
151 SAGEAVLSGL VNSLGALISF LSSGSTGTQN SLGSLESLNS EGAARFNAKY
201 PQGIPTSACG EGAYKVNGVS YYSWSGSSPL TNFLDPSDAF LGASSLTFKN
251 GTANDGLVGT CSSHLGMVIR DNYRMNHLDE VNQVFGLTSL FETSPVSVYR
301 QHANRLKNAS L
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Unformatted sequence string: **311 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
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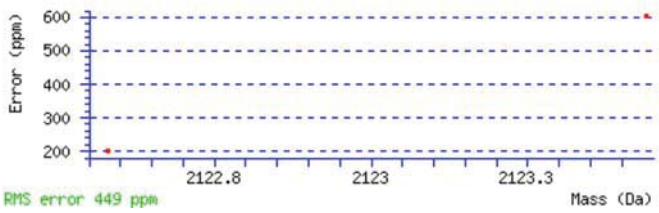
266	116 – 136	531.8664	2123.4363	2122.1535	1.2829	0	22	5.8e+02	7	U	R.YVAAVRPDLIASATSVGAPHK.G

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DEFINITION  lactonizing lipase [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_251552
VERSION     NP_251552.1  GI:15598058
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 311)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 311)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 311)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 311)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 311)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 311)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 311)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
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REFERENCE   8  (residues 1 to 311)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 311)
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  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 311)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
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  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 311)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
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REFERENCE   12 (residues 1 to 311)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 311)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 311)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 311)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
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REFERENCE   16 (residues 1 to 311)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06250.
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                        UPR: UniProt Genome
            Method: conceptual translation.
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     Region          <105..214
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                     /note="Product name confidence: class 1 (Function
                     experimentally demonstrated in P. aeruginosa)"
                     /transl_table=11
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Mascot: http://www.matrixscience.com/

MASCOT Search Results

Protein View: gi|15598058

lactonizing lipase [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	379
Nominal mass (M_r):	32703
Calculated pI:	6.37
Taxonomy:	

This protein sequence matches the following other entries:

- gi|116050865 (no taxonomy information for this entry)
- gi|386066889 (no taxonomy information for this entry)
- gi|392983415 (no taxonomy information for this entry)
- gi|478478295 (no taxonomy information for this entry)
- gi|514410138 (no taxonomy information for this entry)
- gi|489181493 (no taxonomy information for this entry)
- gi|266475 (no taxonomy information for this entry)
- gi|9948950 (no taxonomy information for this entry)
- gi|45341 (no taxonomy information for this entry)
- gi|50882001 (no taxonomy information for this entry)
- gi|78192239 (no taxonomy information for this entry)
- gi|115586086 (no taxonomy information for this entry)
- gi|126167780 (no taxonomy information for this entry)
- gi|126194905 (no taxonomy information for this entry)
- gi|310880677 (no taxonomy information for this entry)
- gi|346058654 (no taxonomy information for this entry)
- gi|348035448 (no taxonomy information for this entry)
- gi|354830685 (no taxonomy information for this entry)
- gi|375041042 (no taxonomy information for this entry)
- gi|375051099 (no taxonomy information for this entry)
- gi|384398384 (no taxonomy information for this entry)
- gi|392318920 (no taxonomy information for this entry)
- gi|404346871 (no taxonomy information for this entry)
- gi|404534990 (no taxonomy information for this entry)
- gi|404536065 (no taxonomy information for this entry)
- gi|477550381 (no taxonomy information for this entry)
- gi|477559757 (no taxonomy information for this entry)
- gi|508137722 (no taxonomy information for this entry)
- gi|514248108 (no taxonomy information for this entry)
- gi|522637999 (no taxonomy information for this entry)
- gi|534389737 (no taxonomy information for this entry)
- gi|543883139 (no taxonomy information for this entry)

Sequence similarity is available as **an NCBI BLAST search of gi|15598058 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas14.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 47%

Matched peptides shown in ***bold red***.

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 51 VDYWFGIPSA LRRDGAQVYV TEVSQLDTSE VRGEQLLQQV EEIVALSGQP
101 KVNLIGHSHG GPTIRYVAAV RPDLIASATS VGAPHKGSDT ADFLRQIPPG
151 SAGEAVLSGL VNSLGALISF LSSGSTGTQN SLGSLESLNS EGAARFNAKY
201 PQGIPTSACG EGAYKVNGVS YYSWSGSSPL TNFLDPSDAF LGASSLTFKN
251 GTANDGLVGT CSSHLGMVIR DNYRMNHLDE VNQVFGLTSL FETSPVSVYR
301 QHANRLKNAS L
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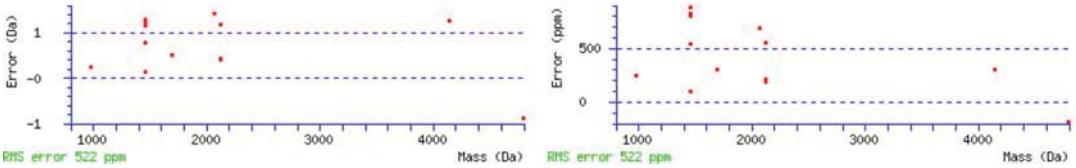
Unformatted sequence string: **311 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
386	64 – 101	1382.1270	4143.3592	4142.1019	1.2573	1	56	0.065	1	U	R.DGAQVYVTEVSQLDTSEVRGEQLLQQVEEIVALSGQPK.V
338	83 – 101	689.8510	2066.5311	2065.1055	1.4255	0	52	0.52	1	U	R.GEQLLQQVEEIVALSGQPK.V
261	102 – 115	729.4760	1456.9374	1456.7899	0.1475	0	85	0.00041	1		K.VNLIGHSHGGPTIR.Y
262	102 – 115	365.4025	1457.5809	1456.7899	0.7910	0	32	83	1		K.VNLIGHSHGGPTIR.Y
263	102 – 115	486.9933	1457.9582	1456.7899	1.1683	0	22	7.7e+02	10		K.VNLIGHSHGGPTIR.Y
264	102 – 115	487.0076	1458.0010	1456.7899	1.2111	0	31	1e+02	1		K.VNLIGHSHGGPTIR.Y
265	102 – 115	487.0313	1458.0721	1456.7899	1.2822	0	31	1.1e+02	1		K.VNLIGHSHGGPTIR.Y
343	116 – 136	708.5236	2122.5488	2122.1535	0.3954	0	46	2	1	U	R.YVAAVRPDLIASATSVGAPHK.G
344	116 – 136	708.5393	2122.5961	2122.1535	0.4426	0	42	4.8	1	U	R.YVAAVRPDLIASATSVGAPHK.G
345	116 – 136	708.7830	2123.3272	2122.1535	1.1737	0	45	2.8	1	U	R.YVAAVRPDLIASATSVGAPHK.G
112	137 – 145	491.3547	980.6948	980.4563	0.2385	0	43	6.6	1	U	K.GSDTADFLR.Q
389	146 – 195	1200.9004	4799.5725	4800.4417	-0.8692	0	26	41	1	U	R.QIPPGSAGEAVLSGLVNSLGALISFLSSGSTGTQNSLGSLESLNSEGAAR.F
307	200 – 215	850.1532	1698.2919	1697.7719	0.5199	0	71	0.0084	1	U	K.YPQGIPTSACGEGAYK.V + Carbamidomethyl (C)

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ACCESSION   NP_251552
VERSION     NP_251552.1  GI:15598058
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DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 311)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
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REFERENCE   2  (residues 1 to 311)
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            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
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   PUBMED   12644477
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   PUBMED   12644476
REFERENCE   10 (residues 1 to 311)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
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  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
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            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 311)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
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  REMARK    Sequence update by submitter
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  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
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            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
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            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
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            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG06250.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
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     source          1..311
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                     /db_xref="taxon:208964"
     Protein         1..311
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                     /calculated_mol_wt=32592
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                     /db_xref="CDD:245225"
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                     /transl_table=11
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MASCOT Search Results

Protein View: gi|15600346

amino acid ABC transporter substrate-binding protein [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	84
Nominal mass (M_r):	27544
Calculated pI:	5.13
Taxonomy:	

This protein sequence matches the following other entries:

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- gi|152987515 (no taxonomy information for this entry)
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- gi|392986832 (no taxonomy information for this entry)
- gi|478481604 (no taxonomy information for this entry)
- gi|514412270 (no taxonomy information for this entry)
- gi|489186744 (no taxonomy information for this entry)
- gi|9951454 (no taxonomy information for this entry)
- gi|115588521 (no taxonomy information for this entry)
- gi|126170075 (no taxonomy information for this entry)
- gi|126197344 (no taxonomy information for this entry)
- gi|150962673 (no taxonomy information for this entry)
- gi|218774481 (no taxonomy information for this entry)
- gi|310882613 (no taxonomy information for this entry)
- gi|346060494 (no taxonomy information for this entry)
- gi|347307633 (no taxonomy information for this entry)
- gi|348037359 (no taxonomy information for this entry)
- gi|354829639 (no taxonomy information for this entry)
- gi|375047274 (no taxonomy information for this entry)
- gi|375048464 (no taxonomy information for this entry)
- gi|384397301 (no taxonomy information for this entry)
- gi|392322337 (no taxonomy information for this entry)
- gi|403244855 (no taxonomy information for this entry)
- gi|404345640 (no taxonomy information for this entry)
- gi|404518771 (no taxonomy information for this entry)
- gi|404521572 (no taxonomy information for this entry)
- gi|404529536 (no taxonomy information for this entry)
- gi|404539527 (no taxonomy information for this entry)
- gi|404540589 (no taxonomy information for this entry)
- gi|452185198 (no taxonomy information for this entry)
- gi|453046665 (no taxonomy information for this entry)
- gi|476644328 (no taxonomy information for this entry)
- gi|476645068 (no taxonomy information for this entry)
- gi|477553690 (no taxonomy information for this entry)
- gi|477556982 (no taxonomy information for this entry)
- gi|500726915 (no taxonomy information for this entry)
- gi|508129742 (no taxonomy information for this entry)
- gi|508132183 (no taxonomy information for this entry)
- gi|514250240 (no taxonomy information for this entry)
- gi|522636447 (no taxonomy information for this entry)
- gi|532137077 (no taxonomy information for this entry)
- gi|534388136 (no taxonomy information for this entry)
- gi|537448730 (no taxonomy information for this entry)
- gi|543878109 (no taxonomy information for this entry)
- gi|543880478 (no taxonomy information for this entry)

Sequence similarity is available as **an NCBI BLAST search of gi|15600346 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas16.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.

Variable modifications: Carbamidomethyl (C)

Protein sequence coverage: 10%

Matched peptides shown in ***bold red***.

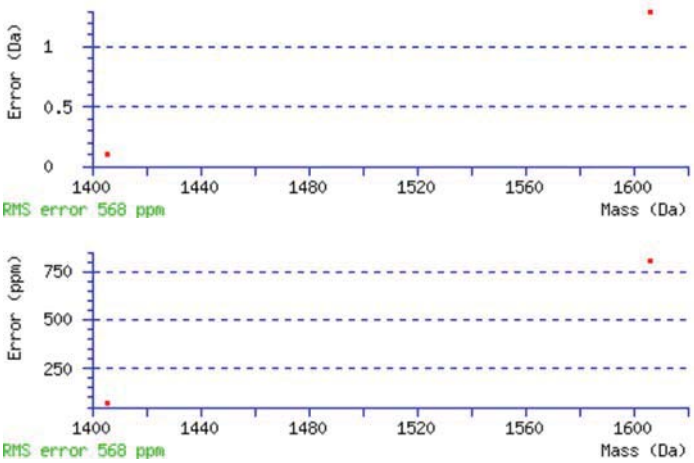
```
  1 MKNYKKILLA AAATLAFALD ASAADKLRIG TEGAYPPFNG IDASGQAVGF
 51 DLDIGKALCA KMKTECEVVT SDWDGIIPAL NAKKFDFIVA SMSITDERKQ
101 AVDFTDPYYT NKLQFVAPKS VDFKTDKDSL KGKVIGAQRA TIAGTWLEDN
151 MADVVTIKLY DTQENAYLDL SSGRLDGVLA DKFVQYDWLK SDAGKEFEFK
201 GEPVFDNDKI GIAVRKGDPL REKLNAALKE IVADGTYKKI NDKYFPFSIY
```

Unformatted sequence string: **250 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
270	224 – 238	536.3956	1606.1649	1604.8773	1.2876	1	48	2	2	U	K.LNAALKEIVADGTYK.K
232	240 – 250	703.9001	1405.7857	1405.6918	0.0939	1	36	29	1		K.INDKYFPFSIY.-




```
LOCUS       NP_253840               250 aa            linear   CON 20-MAR-2014
DEFINITION  amino acid ABC transporter substrate-binding protein [Pseudomonas
            aeruginosa PAO1].
ACCESSION   NP_253840
VERSION     NP_253840.1  GI:15600346
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 250)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 250)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 250)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 250)
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```
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 250)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 250)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 250)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 250)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 250)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 250)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 250)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 250)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 250)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 250)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
```

```
REFERENCE   15 (residues 1 to 250)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 250)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG08538.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..250
                     /organism="Pseudomonas aeruginosa PAO1"
                     /strain="PAO1"
                     /db_xref="taxon:208964"
     Protein         1..250
                     /product="amino acid ABC transporter substrate-binding
                     protein"
                     /calculated_mol_wt=27430
     Region          27..245
                     /region_name="PBPb"
                     /note="Bacterial periplasmic transport systems use
                     membrane-bound complexes and substrate-bound,
                     membrane-associated, periplasmic binding proteins (PBPs)
                     to transport a wide variety of  substrates, such as, amino
                     acids, peptides, sugars, vitamins and inorganic...;
                     cd00134"
                     /db_xref="CDD:238078"
     Region          27..245
                     /region_name="SBP_bac_3"
                     /note="Bacterial extracellular solute-binding proteins,
                     family 3; pfam00497"
                     /db_xref="CDD:249906"
     Site            order(35,73,98,142,181)
                     /site_type="other"
                     /note="substrate binding pocket [chemical binding]"
                     /db_xref="CDD:238078"
     Site            order(164,168,174)
                     /site_type="other"
                     /note="membrane-bound complex binding site"
                     /db_xref="CDD:238078"
     Site            order(207..208,209..211)
                     /site_type="other"
                     /note="hinge residues"
                     /db_xref="CDD:238078"
     CDS             1..250
                     /coded_by="NC_002516.2:5801315..5802067"
                     /note="Product name confidence: class 1: Function
                     experimentally demonstrated in P. aeruginosa."
                     /transl_table=11
CONTIG      join(WP_003096156.1:1..250)
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MASCOT Search Results

Protein View: gi|126330

RecName: Full=Lactonizing lipase; AltName: Full=Triacylglycerol lipase; Flags: Precursor

Database:	NCBInr
Score:	82
Nominal mass (M_r):	32717
Calculated pI:	6.37
Taxonomy:	Pseudomonas sp. 109

This protein sequence matches the following other entries:

- gi|216902 from Pseudomonas sp

Sequence similarity is available as an NCBI BLAST search of gi|126330 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas17.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 8 %

Matched peptides shown in ***bold red***.

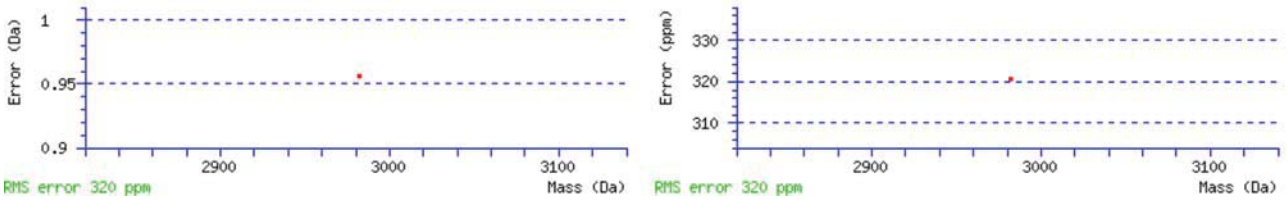
```
  1 MKKKSLLPLG LAIGLASLAA SPLIQASTYT QTKYPIVLAH GMLGFDNILG
 51 VDYWFGIPSA LRRDGAQVYV TEVSQLDTSE VRGEQLLQQV EEIVALSGQP
101 KVNLIGHSHG GPTIRYVAAV RPDLMPSATS VGAPHKGSDT ADFLRQIPPG
151 SAGEAVLSGL VNSLGALISF LSSGSAGTQN SLGSLESLNS EGAARFNAKY
201 PQGIPTSACG EGAYKVNGVS YYSWSGSSPL TNFLDPSDAF LGASSLTFKN
251 GTANDGLVGT CSSHLGMVIR DNYRMNHLDE VNQVFGLTSL FETSPVSVYR
301 QHANRLKNAS L
```

Unformatted sequence string: 311 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
187	275 – 300	995.1459	2982.4159	2981.4593	0.9566	0	82	0.00035	1	U	R.MNHLDEVNQVFGLTSLFETSPVSVYR.Q

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LOCUS       LIP_PSEU0               311 aa            linear   BCT 16-OCT-2013
DEFINITION  RecName: Full=Lactonizing lipase; AltName: Full=Triacylglycerol
            lipase; Flags: Precursor.
ACCESSION   P26877
VERSION     P26877.1  GI:126330
DBSOURCE    UniProtKB: locus LIP_PSEU0, accession P26877;
            class: standard.
            created: Aug 1, 1992.
            sequence updated: Aug 1, 1992.
            annotation updated: Oct 16, 2013.
            xrefs: D10166.1, BAA01035.1, A40943
            xrefs (non-sequence databases): ProteinModelPortal:P26877,
            SMR:P26877, GO:0005576, GO:0046872, GO:0004806, GO:0016042,
            InterPro:IPR000073, Pfam:PF00561, PROSITE:PS00120
KEYWORDS    Calcium; Direct protein sequencing; Disulfide bond; Hydrolase;
            Lipid degradation; Lipid metabolism; Metal-binding; Secreted;
            Signal.
SOURCE      Pseudomonas sp. 109
  ORGANISM  Pseudomonas sp. 109
            Bacteria; Proteobacteria.
```

```
REFERENCE   1  (residues 1 to 311)
  AUTHORS   Ihara,F., Kageyama,Y., Hirata,M., Nihira,T. and Yamada,Y.
  TITLE     Purification, characterization, and molecular cloning of
            lactonizing lipase from Pseudomonas species
  JOURNAL   J. Biol. Chem. 266 (27), 18135-18140 (1991)
   PUBMED   1917947
  REMARK    NUCLEOTIDE SEQUENCE [GENOMIC DNA], PROTEIN SEQUENCE OF 27-45, AND
            FUNCTION.
COMMENT     On Jun 10, 2005 this sequence version replaced gi:77805.
            [FUNCTION] Catalyzes the hydrolysis of triglycerides. Catalyzes the
            synthesis of macrocyclic lactones in anhydrous organic solvents.
            [CATALYTIC ACTIVITY] Triacylglycerol + H(2)O = diacylglycerol + a
            carboxylate.
            [COFACTOR] Binds 1 calcium ion per subunit (By similarity).
            [SUBCELLULAR LOCATION] Secreted.
            [SIMILARITY] Belongs to the AB hydrolase superfamily. Pseudomonas
            lipase family.
FEATURES             Location/Qualifiers
     source          1..311
                     /organism="Pseudomonas sp. 109"
                     /db_xref="taxon:72584"
     gene            1..311
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     Protein         1..311
                     /gene="lipL"
                     /product="Lactonizing lipase"
                     /EC_number="3.1.1.3"
                     /note="Triacylglycerol lipase"
                     /UniProtKB_evidence="Evidence at protein level"
     Region          1..308
                     /gene="lipL"
                     /region_name="LipA"
                     /note="Predicted acetyltransferases and hydrolases with
                     the alpha/beta hydrolase fold [General function prediction
                     only]; COG1075"
                     /db_xref="CDD:224001"
     Region          1..26
                     /gene="lipL"
                     /region_name="Signal"
                     /experiment="experimental evidence, no additional details
                     recorded"
     Region          27..311
                     /gene="lipL"
                     /region_name="Mature chain"
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /note="Lactonizing lipase. /FTId=PRO_0000017746."
     Region          45..287
                     /gene="lipL"
                     /region_name="Abhydrolase_1"
                     /note="alpha/beta hydrolase fold; pfam00561"
                     /db_xref="CDD:249959"
     Site            108
                     /gene="lipL"
                     /site_type="active"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="Nucleophile (By similarity)."
     Bond            bond(209,261)
                     /gene="lipL"
                     /bond_type="disulfide"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="By similarity."
     Site            235
                     /gene="lipL"
                     /site_type="metal-binding"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="Calcium (By similarity)."
     Site            255
                     /gene="lipL"
                     /site_type="active"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="Charge relay system (By similarity)."
     Site            277
                     /gene="lipL"
                     /site_type="active"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="Charge relay system (By similarity)."
     Site            279
                     /gene="lipL"
                     /site_type="metal-binding"
                     /inference="non-experimental evidence, no additional
                     details recorded"
                     /note="Calcium (By similarity)."
```

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Site            283
                /gene="lipL"
                /site_type="metal-binding"
                /inference="non-experimental evidence, no additional
                details recorded"
                /note="Calcium (By similarity)."
Site            287
                /gene="lipL"
                /site_type="metal-binding"
                /inference="non-experimental evidence, no additional
                details recorded"
                /note="Calcium; via carbonyl oxygen (By similarity)."
```

MASCOT Search Results

Protein View: gi|15596776

hypothetical protein PA1579 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	345
Nominal mass (M_r):	22097
Calculated pI:	7.71
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|218892464 from Pseudomonas aeruginosa LESB58
- gi|386059531 from Pseudomonas aeruginosa M18
- gi|386065409 from Pseudomonas aeruginosa NCGM2.S1
- gi|478479652 from Pseudomonas aeruginosa B136-33
- gi|514409034 from Pseudomonas aeruginosa RP73
- gi|489195072 from Pseudomonas aeruginosa
- gi|20978846 from Pseudomonas aeruginosa PAO1
- gi|9947542 from Pseudomonas aeruginosa PAO1
- gi|126166611 from Pseudomonas aeruginosa C3719
- gi|126193306 from Pseudomonas aeruginosa 2192
- gi|218772690 from Pseudomonas aeruginosa LESB58
- gi|347305837 from Pseudomonas aeruginosa M18
- gi|348033968 from Pseudomonas aeruginosa NCGM2.S1
- gi|354829147 from Pseudomonas sp. 2_1_26
- gi|375042393 from Pseudomonas aeruginosa MPAO1/P1
- gi|375048668 from Pseudomonas aeruginosa MPAO1/P2
- gi|403246690 from Pseudomonas aeruginosa CIG1
- gi|404349942 from Pseudomonas aeruginosa PAO579
- gi|404521644 from Pseudomonas aeruginosa ATCC 14886
- gi|404528583 from Pseudomonas aeruginosa ATCC 700888
- gi|404539366 from Pseudomonas aeruginosa ATCC 25324
- gi|404543963 from Pseudomonas aeruginosa E2
- gi|451756237 from Pseudomonas aeruginosa 18A
- gi|453046935 from Pseudomonas aeruginosa PA21_ST175
- gi|476641005 from Pseudomonas aeruginosa str. Stone 130
- gi|476642378 from Pseudomonas sp. P179
- gi|477551738 from Pseudomonas aeruginosa B136-33
- gi|477561428 from Pseudomonas aeruginosa PA45
- gi|500731216 from Pseudomonas aeruginosa VRFPA02
- gi|508136197 from Pseudomonas aeruginosa PAK
- gi|508138920 from Pseudomonas aeruginosa MSH-10
- gi|514247004 from Pseudomonas aeruginosa RP73
- gi|522604247 from Pseudomonas aeruginosa HB13
- gi|532133754 from Pseudomonas aeruginosa VRFPA03

Sequence similarity is available as **an NCBI BLAST search of gi|15596776 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas6D.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 37%

Matched peptides shown in ***bold red***.

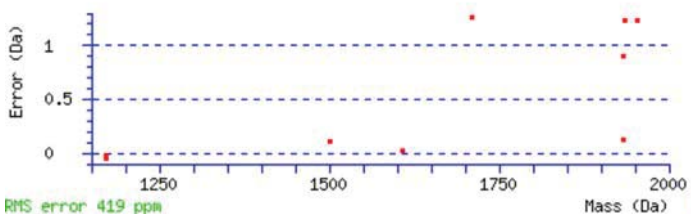
```
  1 MRGILRMTVL AAAVFGVAGN ALAEDWKLAK DEDGVKVYLS SVQGSKYKAY
 51 RGVTDIKADV ATIEALQEDV KGSCKWIHAC AEMKLLKQEG ADAWTYSKID
101 MPWPVTGRDV VIHVTTEKTA DGTVIRHLKA DPTYIPEEKG QIRVPKLVGE
151 WKLQPKGQGV TEVTYQVETE PGGSIPSWLA NSFVVDAPLN TLKGLRSAAE
201 KR
```

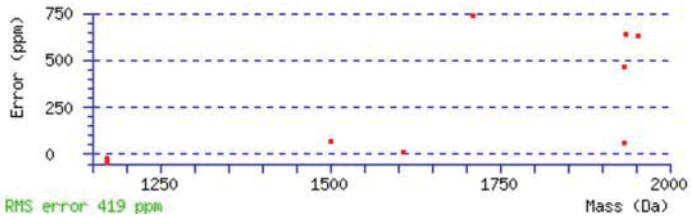
Unformatted sequence string: **202 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
288	31 – 46	571.3793	1711.1161	1709.8472	1.2689	1	37	19	1	U	K.DEDGVKVYLSSVQGSK.Y
251	58 – 71	751.4434	1500.8723	1500.7671	0.1052	0	67	0.024	1	U	K.ADVATIEALQEDVK.G
313	58 – 75	645.3621	1933.0643	1932.9462	0.1181	1	35	30	1	U	K.ADVATIEALQEDVKGSCK.W + Carbamidomethyl (C)
314	58 – 75	967.9304	1933.8462	1932.9462	0.8999	1	45	3	1	U	K.ADVATIEALQEDVKGSCK.W + Carbamidomethyl (C)
315	58 – 75	645.7329	1934.1768	1932.9462	1.2306	1	52	0.57	1	U	K.ADVATIEALQEDVKGSCK.W + Carbamidomethyl (C)
271	85 – 98	805.4264	1608.8383	1608.8147	0.0236	1	75	0.0035	1	U	K.LLKQEGADAWTYSK.I
164	99 – 108	586.2740	1170.5335	1170.5856	-0.0521	0	51	1.1	1	U	K.IDMPWPVTGR.D
165	99 – 108	586.2866	1170.5586	1170.5856	-0.0269	0	44	5	1	U	K.IDMPWPVTGR.D
320	109 – 126	652.4380	1954.2923	1953.0531	1.2392	1	63	0.042	1	U	R.DVVIHVTTEKTADGTVIR.H



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DEFINITION  hypothetical protein PA1579 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_250270
VERSION     NP_250270.1  GI:15596776
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 202)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 202)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 202)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 202)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 202)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
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  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 202)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
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   PUBMED   15716452
REFERENCE   7  (residues 1 to 202)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 202)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 202)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 202)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
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  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 202)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 202)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 202)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 202)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 202)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 202)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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            Method: conceptual translation.
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MASCOT Search Results

Protein View: gi|15597728

thiol peroxidase [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	170
Nominal mass (M_r):	17223
Calculated pI:	5.16
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|116050476 from Pseudomonas aeruginosa UCBPP-PA14
- gi|218891489 from Pseudomonas aeruginosa LESB58
- gi|386058569 from Pseudomonas aeruginosa M18
- gi|386066469 from Pseudomonas aeruginosa NCGM2.S1
- gi|392983912 from Pseudomonas aeruginosa DK2
- gi|478478666 from Pseudomonas aeruginosa B136-33
- gi|514409826 from Pseudomonas aeruginosa RP73
- gi|489180469 from Pseudomonas aeruginosa
- gi|11134977 from Pseudomonas aeruginosa PAO1
- gi|9948589 from Pseudomonas aeruginosa PAO1
- gi|115585697 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126167458 from Pseudomonas aeruginosa C3719
- gi|126194341 from Pseudomonas aeruginosa 2192
- gi|218771715 from Pseudomonas aeruginosa LESB58
- gi|310880124 from Pseudomonas aeruginosa 39016
- gi|346058238 from Pseudomonas aeruginosa NCMG1179
- gi|347304875 from Pseudomonas aeruginosa M18
- gi|348035028 from Pseudomonas aeruginosa NCGM2.S1
- gi|354830300 from Pseudomonas sp. 2_1_26
- gi|375044800 from Pseudomonas aeruginosa MPAO1/P1
- gi|375045476 from Pseudomonas aeruginosa MPAO1/P2
- gi|384400575 from Pseudomonas aeruginosa PADK2_CF510
- gi|392319417 from Pseudomonas aeruginosa DK2
- gi|403247509 from Pseudomonas aeruginosa CIG1
- gi|404346522 from Pseudomonas aeruginosa PAO579
- gi|404522695 from Pseudomonas aeruginosa ATCC 14886
- gi|404534363 from Pseudomonas aeruginosa ATCC 700888
- gi|404534528 from Pseudomonas aeruginosa CI27
- gi|404544739 from Pseudomonas aeruginosa ATCC 25324
- gi|404546014 from Pseudomonas aeruginosa E2
- gi|451755808 from Pseudomonas aeruginosa 18A
- gi|453044949 from Pseudomonas aeruginosa PA21_ST175
- gi|476650622 from Pseudomonas aeruginosa str. Stone 130
- gi|477550752 from Pseudomonas aeruginosa B136-33
- gi|477560443 from Pseudomonas aeruginosa PA45
- gi|500730025 from Pseudomonas aeruginosa VRFPA02
- gi|508137316 from Pseudomonas aeruginosa PA14
- gi|508141478 from Pseudomonas aeruginosa MSH-10
- gi|508143454 from Pseudomonas aeruginosa PAK
- gi|514247796 from Pseudomonas aeruginosa RP73
- gi|522606951 from Pseudomonas aeruginosa HB13
- gi|522630662 from Pseudomonas aeruginosa HB15

Sequence similarity is available as an NCBI BLAST search of gi|15597728 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas5D.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 29%

Matched peptides shown in ***bold red***.

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  51 IFPSVDTPTC ATSVRKFNVE AGKLANTVVL CISADLPFAQ KRFCGAEGLE
 101 NVVNLSTLRG REFLENYGVA IASGPLAGLA ARAVVVLDEQ NKVLHSELVG
 151 EIADEPNYAA ALAAL
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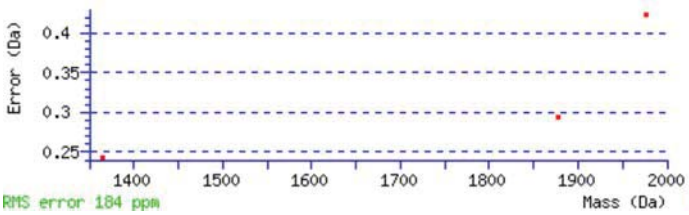
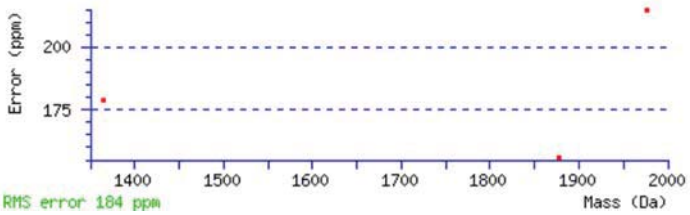
Unformatted sequence string: **165 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
172	8 – 20	683.4816	1364.9487	1364.7048	0.2439	0	49	1.6	1	U	K.GNPVNVDGQLPQK.G
261	48 – 65	989.2212	1976.4278	1976.0037	0.4240	0	33	49	1		K.VLNIFPSVDTPTCATSVR.K + Carbamidomethyl (C)
253	93 – 109	940.1192	1878.2238	1877.9305	0.2933	0	92	6.7e-05	1	U	R.FCGAEGLENVVNLSTLR.G + Carbamidomethyl (C)



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ACCESSION   NP_251222
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DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 165)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 165)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 165)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 165)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 165)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 165)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
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REFERENCE   7  (residues 1 to 165)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
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REFERENCE   8  (residues 1 to 165)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 165)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 165)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
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            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 165)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 165)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 165)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 165)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 165)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 165)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
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  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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                     /transl_table=11
CONTIG      join(WP_003089950.1:1..165)
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MASCOT Search Results

Protein View: gi|15600532

hypothetical protein PA5339 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	172
Nominal mass (M_r):	13601
Calculated pI:	5.10
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|116053486 from Pseudomonas aeruginosa UCBPP-PA14
- gi|218894442 from Pseudomonas aeruginosa LESB58
- gi|386061516 from Pseudomonas aeruginosa M18
- gi|386068995 from Pseudomonas aeruginosa NCGM2.S1
- gi|392987062 from Pseudomonas aeruginosa DK2
- gi|478481798 from Pseudomonas aeruginosa B136-33
- gi|514412444 from Pseudomonas aeruginosa RP73
- gi|489187203 from Pseudomonas aeruginosa
- gi|9951658 from Pseudomonas aeruginosa PAO1
- gi|115588707 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126169898 from Pseudomonas aeruginosa C3719
- gi|218774671 from Pseudomonas aeruginosa LESB58
- gi|310879484 from Pseudomonas aeruginosa 39016
- gi|346060305 from Pseudomonas aeruginosa NCMG1179
- gi|347307822 from Pseudomonas aeruginosa M18
- gi|348037554 from Pseudomonas aeruginosa NCGM2.S1
- gi|354829605 from Pseudomonas sp. 2_1_26
- gi|375042075 from Pseudomonas aeruginosa MPAO1/P1
- gi|375049991 from Pseudomonas aeruginosa MPAO1/P2
- gi|384399534 from Pseudomonas aeruginosa PADK2_CF510
- gi|392322567 from Pseudomonas aeruginosa DK2
- gi|403244751 from Pseudomonas aeruginosa CIG1
- gi|404345829 from Pseudomonas aeruginosa PAO579
- gi|404518517 from Pseudomonas aeruginosa ATCC 14886
- gi|404520612 from Pseudomonas aeruginosa ATCC 700888
- gi|404527157 from Pseudomonas aeruginosa ATCC 25324
- gi|404529290 from Pseudomonas aeruginosa CI27
- gi|404540336 from Pseudomonas aeruginosa E2
- gi|451755236 from Pseudomonas aeruginosa 18A
- gi|453043169 from Pseudomonas aeruginosa PA21_ST175
- gi|476636785 from Pseudomonas aeruginosa str. Stone 130
- gi|476644878 from Pseudomonas sp. P179
- gi|477553884 from Pseudomonas aeruginosa B136-33
- gi|477557172 from Pseudomonas aeruginosa PA45
- gi|500726694 from Pseudomonas aeruginosa VRFPA02
- gi|508129346 from Pseudomonas aeruginosa PAK
- gi|508131024 from Pseudomonas aeruginosa PA14
- gi|508131988 from Pseudomonas aeruginosa MSH-10
- gi|514250414 from Pseudomonas aeruginosa RP73
- gi|522608168 from Pseudomonas aeruginosa HB13
- gi|522635797 from Pseudomonas aeruginosa HB15
- gi|532136997 from Pseudomonas aeruginosa VRFPA03

Sequence similarity is available as an NCBI BLAST search of gi|15600532 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas67.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 43%

Matched peptides shown in ***bold red***.

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1 MTKTVIHTDN APAAIGTYSQ AIKAGNTVYV SGQIPLDPKT MELVEGFEEQ
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  51 TVQVFENLKA VIEASGGYLH DVAKLNIFLT DLSHFAKVNE IMGRYFTQPY
 101 PARAAIGVAA LPKGAQVEMD AIVVLE
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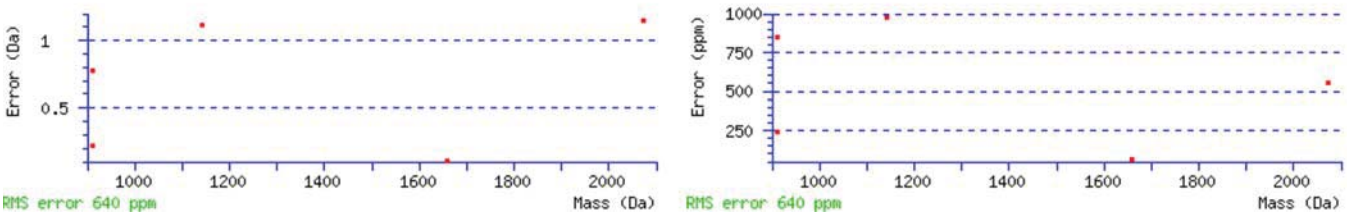
Unformatted sequence string: **126 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
342	4 – 23	691.4153	2071.2240	2070.0746	1.1494	0	48	1.2	1	U	K.TVIHTDNAPAAIGTYSQAIK.A
313	24 – 39	553.6662	1657.9769	1657.8675	0.1094	0	52	0.74	1	U	K.AGNTVYVSGQIPLDPK.T
194	95 – 103	572.3449	1142.6753	1141.5556	1.1197	0	19	1.8e+03	4	U	R.YFTQPYPAR.A
107	104 – 113	455.8997	909.7848	909.5647	0.2201	0	52	0.76	2	U	R.AAIGVAALPK.G
108	104 – 113	456.1762	910.3379	909.5647	0.7732	0	31	1.1e+02	4	U	R.AAIGVAALPK.G

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DEFINITION  hypothetical protein PA5339 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_254026
VERSION     NP_254026.1  GI:15600532
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 126)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 126)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 126)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 126)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 126)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 126)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 126)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
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            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 126)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 126)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 126)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 126)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 126)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 126)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 126)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 126)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 126)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG08724.
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                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
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MASCOT Search Results

Protein View: gi|15595585

hypothetical protein PA0388 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	109
Nominal mass (M_r):	15293
Calculated pI:	6.60
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|218889129 from Pseudomonas aeruginosa LESB58
- gi|386056456 from Pseudomonas aeruginosa M18
- gi|514407991 from Pseudomonas aeruginosa RP73
- gi|489191610 from Pseudomonas aeruginosa
- gi|9946241 from Pseudomonas aeruginosa PAO1
- gi|126196865 from Pseudomonas aeruginosa 2192
- gi|218769352 from Pseudomonas aeruginosa LESB58
- gi|346059643 from Pseudomonas aeruginosa NCMG1179
- gi|347302762 from Pseudomonas aeruginosa M18
- gi|375040775 from Pseudomonas aeruginosa MPAO1/P1
- gi|375047548 from Pseudomonas aeruginosa MPAO1/P2
- gi|403250270 from Pseudomonas aeruginosa CIG1
- gi|404348731 from Pseudomonas aeruginosa PAO579
- gi|404527357 from Pseudomonas aeruginosa ATCC 14886
- gi|404550652 from Pseudomonas aeruginosa ATCC 25324
- gi|453045766 from Pseudomonas aeruginosa PA21_ST175
- gi|476646401 from Pseudomonas aeruginosa str. Stone 130
- gi|476647785 from Pseudomonas sp. P179
- gi|477555981 from Pseudomonas aeruginosa PA45
- gi|500727043 from Pseudomonas aeruginosa VRFPA02
- gi|508131366 from Pseudomonas aeruginosa MSH-10
- gi|514245961 from Pseudomonas aeruginosa RP73
- gi|522606635 from Pseudomonas aeruginosa HB13

Sequence similarity is available as an NCBI BLAST search of gi|15595585 against nr.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas67.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C)

Protein sequence coverage: 10%

Matched peptides shown in ***bold red***.

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  1 MRRFTAFVLG LCLSLPVLAE QVQRFGDLDV HYNVFNSSFL QPNVASAVGL
 51 VRSKAQGVIN VVPMEKGKPV EAAVTGSAKD LTGKVIPLEF RRVSEEGAIY
101 NLAQFPISQR ETLVFTIKVE AKGEPAQTFS FNKEIFPDE
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Unformatted sequence string: 139 residues (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
294	53 – 66	500.6621	1498.9644	1498.8177	0.1466	1	48	1.7	1	U	R.SKAQGVINVVPMEK.G
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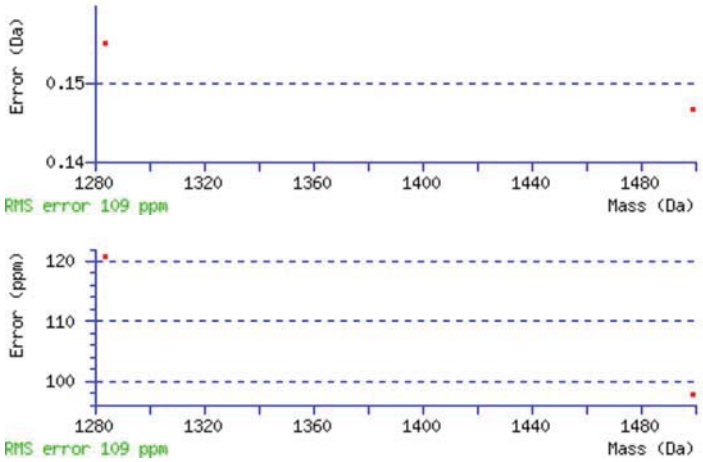


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LOCUS       NP_249079               139 aa            linear   CON 20-MAR-2014
DEFINITION  hypothetical protein PA0388 [Pseudomonas aeruginosa PAO1].
ACCESSION   NP_249079
VERSION     NP_249079.1  GI:15595585
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 139)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 139)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 139)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 139)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 139)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 139)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 139)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
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   PUBMED   15608211
REFERENCE   8  (residues 1 to 139)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
REFERENCE   9  (residues 1 to 139)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 139)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 139)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 139)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 139)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 139)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 139)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 139)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
            Lagrou,M., Garber,R.L., Goltry,L., Tolentino,E.,
            Westbrock-Wadman,S., Yuan,Y., Brody,L.L., Coulter,S.N.,
            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG03777.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
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                     /note="Domain of unknown function (DUF4426); pfam14467"
                     /db_xref="CDD:222767"
     CDS             1..139
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                     /note="Product name confidence: class 4 (Homologs of
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                     similarity to any previously reported sequences)"
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MASCOT Search Results

Protein View: gi|15600481

nitrogen regulatory protein P-II 2 [Pseudomonas aeruginosa PAO1]

Database:	NCBInr
Score:	89
Nominal mass (M_r):	12279
Calculated pI:	5.41
Taxonomy:	Pseudomonas aeruginosa PAO1

This protein sequence matches the following other entries:

- gi|116053436 from Pseudomonas aeruginosa UCBPP-PA14
- gi|146280897 from Pseudomonas stutzeri A1501
- gi|146305300 from Pseudomonas mendocina ymp
- gi|152984472 from Pseudomonas aeruginosa PA7
- gi|218894391 from Pseudomonas aeruginosa LESB58
- gi|330501230 from Pseudomonas mendocina NK-01
- gi|333898577 from Pseudomonas fulva 12-X
- gi|339492616 from Pseudomonas stutzeri ATCC 17588 = LMG 11199
- gi|386019194 from Pseudomonas stutzeri DSM 4166
- gi|386061466 from Pseudomonas aeruginosa M18
- gi|386068944 from Pseudomonas aeruginosa NCGM2.S1
- gi|392422818 from Pseudomonas stutzeri CCUG 29243
- gi|392986971 from Pseudomonas aeruginosa DK2
- gi|397685603 from Pseudomonas stutzeri DSM 10701
- gi|431928844 from Pseudomonas stutzeri RCH2
- gi|478481747 from Pseudomonas aeruginosa B136-33
- gi|514412397 from Pseudomonas aeruginosa RP73
- gi|489187068 from Pseudomonas aeruginosa
- gi|9951602 from Pseudomonas aeruginosa PAO1
- gi|19033135 from Pseudomonas stutzeri A15
- gi|115588657 from Pseudomonas aeruginosa UCBPP-PA14
- gi|126169946 from Pseudomonas aeruginosa C3719
- gi|126197203 from Pseudomonas aeruginosa 2192
- gi|145569102 from Pseudomonas stutzeri A1501
- gi|145573501 from Pseudomonas mendocina ymp
- gi|150959630 from Pseudomonas aeruginosa PA7
- gi|218774620 from Pseudomonas aeruginosa LESB58
- gi|310879425 from Pseudomonas aeruginosa 39016
- gi|327479166 from Pseudomonas stutzeri DSM 4166
- gi|328915516 from Pseudomonas mendocina NK-01
- gi|333113842 from Pseudomonas fulva 12-X
- gi|338799988 from Pseudomonas stutzeri ATCC 17588 = LMG 11199
- gi|346060356 from Pseudomonas aeruginosa NCMG1179
- gi|347307772 from Pseudomonas aeruginosa M18
- gi|348037503 from Pseudomonas aeruginosa NCGM2.S1
- gi|354829555 from Pseudomonas sp. 2_1_26
- gi|359370884 from Pseudomonas psychrotolerans L19
- gi|375042025 from Pseudomonas aeruginosa MPAO1/P1
- gi|375048560 from Pseudomonas aeruginosa MPAO1/P2
- gi|379065416 from Pseudomonas stutzeri ATCC 14405 = CCUG 16156
- gi|384397438 from Pseudomonas aeruginosa PADK2_CF510
- gi|390985006 from Pseudomonas stutzeri CCUG 29243
- gi|392322476 from Pseudomonas aeruginosa DK2
- gi|395807159 from Pseudomonas stutzeri DSM 10701
- gi|399113279 from Pseudomonas pseudoalcaligenes CECT 5344
- gi|400346456 from Pseudomonas mendocina DLHK
- gi|403244819 from Pseudomonas aeruginosa CIG1
- gi|404345779 from Pseudomonas aeruginosa PAO579
- gi|404518595 from Pseudomonas aeruginosa ATCC 14886
- gi|404520879 from Pseudomonas aeruginosa ATCC 700888
- gi|404527277 from Pseudomonas aeruginosa ATCC 25324

- gi|404529240 from Pseudomonas aeruginosa CI27
- gi|404540285 from Pseudomonas aeruginosa E2
- gi|409119598 from Pseudomonas sp. Chol1
- gi|409779661 from Pseudomonas stutzeri KOS6
- gi|431827131 from Pseudomonas stutzeri RCH2
- gi|451755367 from Pseudomonas aeruginosa 18A
- gi|452009836 from Pseudomonas stutzeri NF13
- gi|452183740 from Pseudomonas aeruginosa VRFPA01
- gi|453043220 from Pseudomonas aeruginosa PA21_ST175
- gi|475321707 from Aegilops tauschii
- gi|476636837 from Pseudomonas aeruginosa str. Stone 130
- gi|476644929 from Pseudomonas sp. P179
- gi|476727815 from Pseudomonas sp. HPB0071
- gi|477553833 from Pseudomonas aeruginosa B136-33
- gi|477557122 from Pseudomonas aeruginosa PA45
- gi|500726763 from Pseudomonas aeruginosa VRFPA02
- gi|508129295 from Pseudomonas aeruginosa PAK
- gi|508131074 from Pseudomonas aeruginosa PA14
- gi|508132039 from Pseudomonas aeruginosa MSH-10
- gi|514250367 from Pseudomonas aeruginosa RP73
- gi|516322090 from Pseudomonas stutzeri B1SMN1
- gi|522608117 from Pseudomonas aeruginosa HB13
- gi|522635958 from Pseudomonas aeruginosa HB15
- gi|532136955 from Pseudomonas aeruginosa VRFPA03

Sequence similarity is available as **an NCBI BLAST search of gi|15600481 against nr**.

Search parameters

MS data file:	C:\DOCUME~1\ANASTA~1\LOCALS~1\Temp\mas6C.tmp
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	**Carbamidomethyl (C)**

Protein sequence coverage: 19%

Matched peptides shown in ***bold red***.

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 51 YVVDFLPKVK IDVAIADDQL DRVIEAITKA ANTGKIGDGK IFVVNLEQAI
101 RIRTGETDTD AI
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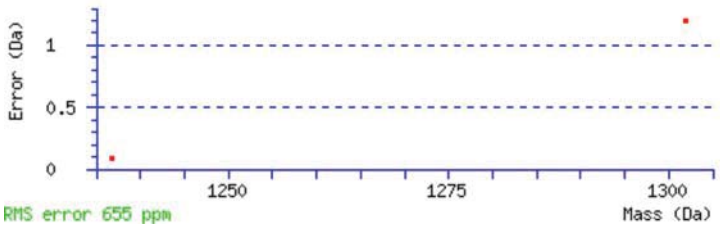
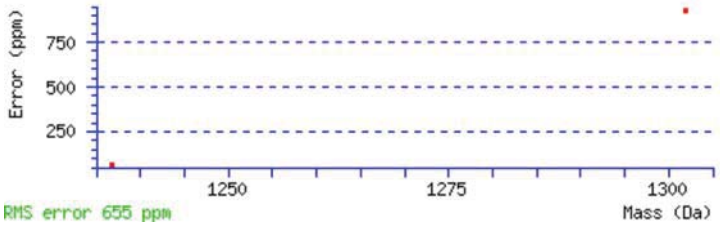
Unformatted sequence string: **112 residues** (for pasting into other applications).

Sort peptides by ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

Show predicted peptides also

Query	Start – End	Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
160	48 – 58	619.3683	1236.7221	1236.6390	0.0831	0	46	3.2	1		R.GAEYVVDFLPK.V
178	91 – 101	651.9847	1301.9549	1300.7503	1.2046	0	43	7	6	U	K.IFVVNLEQAIR.I



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DEFINITION  nitrogen regulatory protein P-II 2 [Pseudomonas aeruginosa PAO1].
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VERSION     NP_253975.1  GI:15600481
DBLINK      BioProject: PRJNA57945
DBSOURCE    REFSEQ: accession NC_002516.2
KEYWORDS    RefSeq.
SOURCE      Pseudomonas aeruginosa PAO1
  ORGANISM  Pseudomonas aeruginosa PAO1
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (residues 1 to 112)
  AUTHORS   Winsor,G.L., Van Rossum,T., Lo,R., Khaira,B., Whiteside,M.D.,
            Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas Genome Database: facilitating user-friendly,
            comprehensive comparisons of microbial genomes
  JOURNAL   Nucleic acids Res. 37 (DATABASE ISSUE), D483-D488 (2009)
   PUBMED   18978025
REFERENCE   2  (residues 1 to 112)
  AUTHORS   Cirz,R.T., O'Neill,B.M., Hammond,J.A., Head,S.R. and Romesberg,F.E.
  TITLE     Defining the Pseudomonas aeruginosa SOS response and its role in
            the global response to the antibiotic ciprofloxacin
  JOURNAL   J. Bacteriol. 188 (20), 7101-7110 (2006)
   PUBMED   17015649
REFERENCE   3  (residues 1 to 112)
  AUTHORS   Palmer,K.L., Mashburn,L.M., Singh,P.K. and Whiteley,M.
  TITLE     Cystic fibrosis sputum supports growth and cues key aspects of
            Pseudomonas aeruginosa physiology
  JOURNAL   J. Bacteriol. 187 (15), 5267-5277 (2005)
   PUBMED   16030221
REFERENCE   4  (residues 1 to 112)
  AUTHORS   Salunkhe,P., Smart,C.H., Morgan,J.A., Panagea,S., Walshaw,M.J.,
            Hart,C.A., Geffers,R., Tummler,B. and Winstanley,C.
  TITLE     A cystic fibrosis epidemic strain of Pseudomonas aeruginosa
            displays enhanced virulence and antimicrobial resistance
  JOURNAL   J. Bacteriol. 187 (14), 4908-4920 (2005)
   PUBMED   15995206
REFERENCE   5  (residues 1 to 112)
  AUTHORS   Filiatrault,M.J., Wagner,V.E., Bushnell,D., Haidaris,C.G.,
            Iglewski,B.H. and Passador,L.
  TITLE     Effect of anaerobiosis and nitrate on gene expression in
            Pseudomonas aeruginosa
  JOURNAL   Infect. Immun. 73 (6), 3764-3772 (2005)
   PUBMED   15908409
REFERENCE   6  (residues 1 to 112)
  AUTHORS   Rasmussen,T.B., Bjarnsholt,T., Skindersoe,M.E., Hentzer,M.,
            Kristoffersen,P., Kote,M., Nielsen,J., Eberl,L. and Givskov,M.
  TITLE     Screening for quorum-sensing inhibitors (QSI) by use of a novel
            genetic system, the QSI selector
  JOURNAL   J. Bacteriol. 187 (5), 1799-1814 (2005)
   PUBMED   15716452
REFERENCE   7  (residues 1 to 112)
  AUTHORS   Winsor,G.L., Lo,R., Sui,S.J., Ung,K.S., Huang,S., Cheng,D.,
            Ching,W.K., Hancock,R.E. and Brinkman,F.S.
  TITLE     Pseudomonas aeruginosa Genome Database and PseudoCap: facilitating
            community-based, continually updated, genome annotation
  JOURNAL   Nucleic acids Res. 33 (DATABASE ISSUE), D338-D343 (2005)
   PUBMED   15608211
REFERENCE   8  (residues 1 to 112)
  AUTHORS   Wagner,V.E., Bushnell,D., Passador,L., Brooks,A.I. and
            Iglewski,B.H.
  TITLE     Microarray analysis of Pseudomonas aeruginosa quorum-sensing
            regulons: effects of growth phase and environment
  JOURNAL   J. Bacteriol. 185 (7), 2080-2095 (2003)
   PUBMED   12644477
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REFERENCE   9  (residues 1 to 112)
  AUTHORS   Schuster,M., Lostroh,C.P., Ogi,T. and Greenberg,E.P.
  TITLE     Identification, timing, and signal specificity of Pseudomonas
            aeruginosa quorum-controlled genes: a transcriptome analysis
  JOURNAL   J. Bacteriol. 185 (7), 2066-2079 (2003)
   PUBMED   12644476
REFERENCE   10 (residues 1 to 112)
  AUTHORS   Stover,C.K., Pham,X.Q., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S., Hufnagle,W.O., Kowalik,D.J., Lagrou,M.,
            Garber,R.L., Goltry,L., Tolentino,E., Westbrock-Wadman,S., Yuan,Y.,
            Brody,L.L., Coulter,S.N., Folger,K.R., Kas,A., Larbig,K., Lim,R.,
            Smith,K., Spencer,D., Wong,G.K., Wu,Z., Paulsen,I.T., Reizer,J.,
            Saier,M.H., Hancock,R.E., Lory,S. and Olson,M.V.
  TITLE     Complete genome sequence of Pseudomonas aeruginosa PA01, an
            opportunistic pathogen
  JOURNAL   Nature 406 (6799), 959-964 (2000)
   PUBMED   10984043
REFERENCE   11 (residues 1 to 112)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2010) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   12 (residues 1 to 112)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-2010) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   13 (residues 1 to 112)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2008) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    protein update by submitter
REFERENCE   14 (residues 1 to 112)
  AUTHORS   Winsor,G.L., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JUL-2006) Department of Molecular Biology and
            Biochemisry, Simon Fraser University, 8888 University Dr., Burnaby,
            British Columbia V5A 1S6, Canada
  REMARK    Sequence update by submitter
REFERENCE   15 (residues 1 to 112)
  AUTHORS   Ung,K.S., Hancock,R.E. and Brinkman,F.S.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (04-FEB-2003) Department of Molecular Biology and
            Biochemistry, Simon Fraser University, 8888 University Dr.,
            Burnaby, British Columbia V5A 1S6, Canada
REFERENCE   16 (residues 1 to 112)
  AUTHORS   Stover,C.K., Pham,X.-Q.T., Erwin,A.L., Mizoguchi,S.D., Warrener,P.,
            Hickey,M.J., Brinkman,F.S.L., Hufnagle,W.O., Kowalik,D.J.,
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            Folger,K.R., Kas,A., Larbig,K., Lim,R.M., Smith,K.A., Spencer,D.H.,
            Wong,G.K.-S., Wu,Z., Paulsen,I.T., Reizer,J., Saier,M.H.,
            Hancock,R.E.W., Lory,S. and Olson,M.V.
  CONSRTM   Pseudomonas aeruginosa Community Annotation Project (PseudoCap)
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2000) Department of Medicine and Genetics,
            University of Washington Genome Center, University Of Washington,
            Box 352145, Seattle, WA 98195, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence is identical to AAG08673.
            RefSeq Category: Reference Genome
                        UPR: UniProt Genome
            Method: conceptual translation.
FEATURES             Location/Qualifiers
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Mascot: http://www.matrixscience.com/