***PERSPECTIVE* PAPER**

# The end of the reign of a ''master regulator'': A defect in function of the LasR quorum sensing regulator is a common feature of *Pseudomonas aeruginosa* isolates

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**ABSTRACT (150 words)**

*Pseudomonas aeruginosa*, a bacterium known to cause infections in immunocompromised individuals, regulates several of its virulence functions using three interlinked quorum sensing (QS) systems (*las*, *rhl*, and *pqs*). Despite its presumed importance in regulating virulence, dysfunction of the *las* system regulator LasR occurs frequently in strains isolated from various environments, including clinical infections. This newfound abundance of LasR-defective strains calls into question existing hypotheses regarding their selection. Indeed, current assumptions concerning factors driving the emergence of LasR-deficient isolates and the role of LasR in the QS hierarchy must be reconsidered. Here, we highlight that LasR is not the primary master regulator of QS in all *P. aeruginosa* genetic backgrounds. We also revisit and complement current knowledge on the ecology of LasR-dependent QS in *P. aeruginosa*, review the hypotheses explaining the putative adaptive benefits of selecting against LasR function, and discuss the implications of this renewed understanding.

## Current understanding of *Pseudomonas aeruginosa* quorum sensing

The bacterium *Pseudomonas aeruginosa* is a versatile opportunistic pathogen found mostly in environments related to human activity such as soil, water, and hospital settings (1). *P. aeruginosa* causes infections in immunocompromised individuals and people living with cystic fibrosis (CF) (2-4). To adapt to diverse and dynamic environments, this bacterium uses quorum sensing (QS), an intercellular communication system. Through the production and detection of small diffusible autoinducers, QS coordinates the expression of numerous key cellular functions at the population level, including virulence, in a cell density-dependent manner (5).

*P. aeruginosa* has three interdependent QS systems, known as *las*, *rhl*, and *pqs*. Based on observations of prototypical strains such as PA14 and PAO1, the *las* system is generally considered to be at the top of the QS regulatory cascade (6, 7). This system comprises the LasI synthase, which produces the autoinducer *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-$C_{12}$-HSL). This signal molecule subsequently binds to its cognate LuxR-type transcriptional regulator, LasR. Once activated by its ligand, LasR regulates the transcription of several target genes, such as *lasB* encoding for the LasB elastase, as well as *lasI*, which codes for the LasI synthase, completing a positive feedback loop that is characteristic of many QS systems (8-10). LasR activation also induces the transcription of the *rhlI* and *rhlR* genes, which constitute the *rhl* QS system (6, 7, 10-12). The RhlI synthase produces the *N*-butanoyl-L-homoserine lactone ($C_4$-HSL) autoinducer signal, which can bind to another LuxR-type transcriptional regulator, RhlR, activating the transcription of several genes, including those implicated in the production of virulence factors, including pyocyanin (*phz1* and *phz2* operons) and rhamnolipids (*rhlAB* and *rhlC*) (11, 13-15). The third QS system, *pqs*, is regulated by MvfR (also known as PqsR), a LysR-type transcriptional regulator. The *pqs* system relies on the production of 4-hydroxy-2-alkylquinolines (HAQs) by the enzymes encoded by the *pqsABCDE* operon (16, 17). Indeed, the PqsABCD enzymes synthesize 4-hydroxy-2-heptylquinoline (HHQ), which can be converted into the *Pseudomonas* quinolone signal (PQS; 3,4-dihydroxy-2-heptylquinoline) by the genetically unlinked PqsH monooxygenase (16, 18, 19). HHQ and PQS can both act as autoinducing ligands of MvfR, leading to the transcriptional activation of the *pqs* operon (18, 20).

The interconnection of all three systems is widely recognized. LasR activation positively regulates the expression of *rhlR*, *mvfR* and *pqsH*, while RhlR activation represses *pqs* operon activity (10, 15, 17, 21-24). Additionally, PqsE interacts with RhlR through an incompletely defined chaperone-like function to activate the *rhl* system and heighten the expression of some target genes (12, 25-28). These examples highlight the complex regulation that characterizes *P. aeruginosa* QS. However, while most of the

research that informed this interconnected but hierarchical model of QS was performed in a few, well-defined laboratory strains, the frequent detection of LasR-defective strains still able to produce QS-regulated factors has revealed certain inconsistencies regarding the central role of the LasR regulator within the QS hierarchy. Here, we will thoroughly investigate this issue, unveiling new hypotheses regarding the relative positions of LasR, RhlR and MvfR (PqsR) within the *P. aeruginosa* QS hierarchy.

**LasR-defective strains are generally prevalent in both chronic and acute clinical environments**

Over time, numerous studies have highlighted the natural occurrence of *P. aeruginosa* strains with impaired LasR activity. Initial reports of LasR-defective variants reflected their detection within microbial populations that had evolved for years within the chronically infected lungs of people with CF, which was unexpected given the requirement of QS-controlled factors for full expression of bacterial virulence *in vitro* (29). However, multiple reports have identified LasR-defective strains in CF respiratory cultures, and the selection of these strains by the CF lung environment is now a well-accepted dogma. The emergence of *lasR* mutants has been associated with increased inflammatory markers, increased neutrophilic inflammation, and deteriorated pulmonary function (30-36). These variants have also been implicated in pathogenesis in corneal ulcers (37). Moreover, we recently identified a high prevalence of LasR-defective strains in non-clinical environments as well, such as sinks, contaminated soils and animal products (4). While a defect in LasR activity has seldom been reported in strains isolated from acute infections, a recent study from O'Connor *et al*. (2022) identified *P. aeruginosa* isolates carrying *lasR* mutations commonly present within many environments, although the impact of these mutations on LasR activity *per s*e was not investigated (37-39).

Based on the emerging picture of a widespread prevalence of LasR-defective strains in various environmental niches, we hypothesized that defects in LasR activity could also be found in acute clinical contexts. To evaluate the prevalence of LasR-defective strains isolated from both chronic and acute infections, we assessed LasR activity in 92 *P. aeruginosa* strains from diverse sources, including burn wounds, keratitis, urinary tract infections, bronchitis, CF lungs, chronic obstructive pulmonary disease (COPD), and others (**Table S1**) (40). LasR activity was evaluated using a previously published method relying on unbiased phenotypic profiling based on the quantification of QS-regulated metabolites such as HAQs and pyocyanin (4). Based on this model, we found that 41% of the 92 isolates from our panel of clinical strains exhibited impaired LasR activity (**Fig. 1**). This data is consistent with our previous findings, where 40% of isolates from a panel of 176 *P. aeruginosa* environmental strains were found to be LasR-defective (4). These results also support the conclusions of O'Connor *et al*. (2022), as well as our

hypothesis that LasR-defective strains are common in all clinical contexts and are not restricted to CF clinical isolates, as previously assumed (38) due to a predominant focus of prior analyses on CF isolates. Here, we found the prevalence of defects in LasR function to be similar among isolates from various environments, including when comparing CF and non-CF sources.

In the literature, the prevalence of *lasR* mutants among *P. aeruginosa* isolates from CF patients has been estimated to be from 20 to 25% as defined using genotypic techniques (34, 41, 42). However, different studies have used a variety of methods to estimate the proportion of LasR-defective strains, hampering direct comparison of results. Relying solely on the identification of mutations in the *lasR* coding sequence, as is usually reported, could misidentify LasR-defective strains, given its complex regulation and the unpredictable relationship between coding sequence and protein function (4, 34, 43). Indeed, a great diversity of mutations in the *lasR* gene has been identified, not all of which completely abrogate function (34). Additionally, some mutations in regulatory elements could also modify LasR expression or activity (4, 34). Examining a single phenotypic trait could also bias LasR-defective strain identification given the complex regulation of many phenotypes. Our approach, based on phenotypic profiling rather than examining only one trait, allows for unbiased identification of all isolates with a defect in LasR activity (4). Therefore, we refer to LasR-defective strains rather than *lasR* mutants.

Taken together with previous studies, the data presented here further highlight that LasR-defective strains occur commonly, regardless of their clinical or environmental origin (4, 34). As a result, we must reconsider prior assumptions, including the notion that chronically colonized CF lungs specifically provide a selective pressure promoting the loss of LasR function (31, 32).

**Factors driving the emergence of LasR-defective strains remain elusive**

Despite decades of research, the precise drivers for the emergence of LasR-defective strains remain elusive. LasR function seems especially prone to be lost, and the *lasR* gene might even be considered a hotspot for various types of genetic variations (4, 34, 38). One particularly popular hypothesis suggests that LasR-defective cells arise as “cheaters”, taking advantage of neighboring cells with a functional LasR that provide “public goods”, such as exoproteases (e.g. LasB elastase), to the entire population. By exploiting this strategy, LasR-defective variants reduce the population metabolic cost associated with the expression of these proteins when they are essential for bacterial growth. However, this behavior has only been observed under specific *in vitro* conditions (44-48). While such cheating behavior could potentially extend to different contexts and environments beyond the CF lung, providing an interesting

explanation for the emergence of these variants in multiple niches, evidence suggests that this cannot be the sole explanation, and that *lasR* mutants are not always "cheaters" (49). It is important to consider that LasR-defective strains can also arise in various conditions that do not implicate "public goods" (50). Therefore, other factors must contribute to the emergence of LasR variants.

As stated above, LasR-defective strains have been typically associated with the chronically infected lungs of people with CF (29, 31, 51). Extensive investigations have explored the advantages conferred by defective LasR function in this specific environment, revealing their relatively high fitness in the presence of specific amino acids, such as phenylalanine, which is especially abundant in CF secretions (31, 52). Relative to wild-type strains, these variants exhibit altered metabolism, including lower oxygen consumption and enhanced nitrogen utilization, providing them with a competitive edge over their wild-type counterpart (51, 53). Furthermore, LasR-defective strains exhibit relative resistance to specific antimicrobials and enhanced tolerance to alkaline stress, resulting in protection from cell lysis (31, 32, 54-56). These findings support the notion that diminished LasR activity confers substantial advantages in conditions known to be common in CF lungs. However, the growing evidence for the prevalence and abundance of LasR-defective variants in diverse infections and environmental contexts disproves any specificity for the CF lung and warrants expanding current models of their emergence. For instance, it was proposed that diversification of *P. aeruginosa* populations in the CF lung could favour long-term survival to multiple unpredictable stresses (57). This phenomenon could also apply outside of the CF lung. Accordingly, the effects of LasR impairment on growth on different carbon sources was suggested to explain the emergence of LasR-defective variants in the context of the CF lung, but such mechanisms could likely arise in many other environmental settings, as growth conditions and carbon sources differ (58).

Alternatively, we might consider the emergence of LasR-defective variants as beneficial for a population that contains them. Supporting this model, controlled evolution experiments performed *in vitro* demonstrated the tendency of LasR-defective clones to rapidly emerge, with their proportion frequently stabilizing at about 50% of the total population (44, 46, 50, 54, 59). For instance, swarming colonies of *P. aeruginosa* with higher proportions of LasR-defective cells tend to have fitness advantages over those without LasR variants (50). The population dynamics of mixed populations of *P. aeruginosa*, comprising wild-type and LasR-defective strains, could partially explain the prevalence of the latter in diverse environments. In natural settings, *P. aeruginosa* forms biofilms, which are bacterial communities known to be composed of diverse physicochemical niches. Jeske *et al*. (2022) showed that LasR function is

especially prone to be lost in a biofilm context (39). Within these biofilms, factors produced by strains with a functional LasR could influence the behaviour of surrounding LasR-defective strains within specific niches. Similarly, LasR-defective strains can modulate the behavior of LasR-functional strains by affecting, for instance, QS signaling and function (60, 61). Therefore, the concomitant mixed presence of both LasR-functional and LasR-defective strains appears to be beneficial to the overall population.

**LasR-defective clinical isolates could be acquired directly from surrounding environments**

The same high prevalence of LasR-defective strains in both clinical and environmental contexts suggests potential adaptative benefits associated with modulating LasR activity in populations of *P. aeruginosa*, irrespective of the environment. Therefore, it is possible that LasR-defective strains isolated from infections originated from environmental sources, as previously proposed, rather than emerging *in situ* (4, 37). Two distinct studies provided evidence supporting this notion, as the *lasR* gene of some *P. aeruginosa* isolates already exhibited mutations at initial detection in CF respiratory samples, consistent with direct acquisition from environmental sources (47, 62). While *P. aeruginosa* lineages have been observed to lose LasR function over time during an infection, the discussion above indicates that such events are not necessarily specifically selected by the CF lung environment (29, 63-66).

**Presence of naturally occurring LasR-impaired function is not necessarily synonymous with loss of QS**

A widely held notion is that LasR occupies the top position in the QS regulatory hierarchy (43, 67). An alternative hypothesis is that the presence of this hierarchy is not a universal feature of *P. aeruginosa* and reflects studies conducted primarily with one strain, PAO1, which could itself be considered as an outlier regarding its QS mechanisms (68). We now know that isolates with defective LasR activity can still exhibit robust QS-dependent regulation of virulence factors (4, 34, 69-71). Since LasR upregulates the *rhl* and the *pq*s QS systems in studied laboratory-adapted strains, it has been generally assumed that a defect in LasR activity should result in deficient QS regulation, and thus reduced transcription of target genes and production of virulence factors. However, LasR-defective strains with a functional *rhl* QS system, referred to as ''RAIL'' (RhlR Active Independently of LasR) strains, have been identified. Such strains have been isolated from CF lungs, as well as from diverse environments (4, 34, 69-71). Using the same method as previously mentioned, we found that about half of the LasR-defective clinical strains from our panel (**Fig. 1**) possess LasR-independent RhlR activity (**Fig. 2**), in accordance with previously published findings (4). Hence, an impaired LasR protein does not necessarily lead to diminished production of virulence factors. Thus, the dogma based on the study of prototypical strains stating that LasR is the conserved “master regulator” of the QS regulatory cascade should be reconsidered. Beside

regulating known RhlR-dependent virulence factors such as pyocyanin, RhlR can activate virulence factors typically regulated by LasR, such as various exoproteases (4, 34, 72, 73). Together, these observations and concepts highlight the crucial role of RhlR, accentuating its importance as a QS regulator in *P. aeruginosa*. However, the precise regulatory mechanisms involved in LasR-independent RhlR regulation remain elusive. One mechanism might involve PqsE, encoded by the last gene of the *pqs* operon. PqsE plays a major role in promoting RhlR activity, at least in prototypical strains (12, 25, 28, 74, 75), underscoring the importance of MvfR and the *pqs* system for maintaining the full suite of QS regulation. Since an important subset of naturally evolved *P. aeruginosa* strains activate QS and produce virulence factors without relying on LasR, we need to better understand the nature of the ecological pressure sustaining its regulatory activity in some lineages.

**Inhibiting *rhlR*-dependant targets for improved quorum sensing inhibition**

*P. aeruginosa* is naturally tolerant of, and resistant to, a wide range of antibiotics, limiting the efficacy of treating infections by this bacterium (76). To address this issue, anti-virulence therapies have emerged as a promising approach for the development of new drugs, since they offer distinct advantages. Unlike antibiotics that directly target the survival of the bacteria, anti-virulence therapies aim to inhibit non-essential virulence factors without affecting viability. This approach theoretically reduces the development of resistance since no selective pressure for survival is applied. In *P. aeruginosa*, QS represents an interesting target, since it modulates the expression of multiple virulence factors unrelated to bacterial survival (77-80). Rationally, many anti-virulence therapies target *P. aeruginosa* LasR or the production of 3-oxo-$C_{12}$-HSL, since the *las* system has been considered to be on top of the QS regulation cascade (81-83). Unfortunately, the high prevalence of LasR-defective strains and the clear absence of QS hierarchy in some strains suggests that LasR is not an ideal target. Instead, we propose that researchers working on anti-QS therapies should consider focusing on other QS targets, such as the *rhl* or *pqs* system, which can still be active in the absence of LasR and seem better conserved. There have been very few reports of strains lacking RhlR or MvfR activity, perhaps because these regulators are indispensable for the proper functioning of QS (38, 84-86). Thus, targeting *rhl*-dependent QS, such as production/function of $C_4$-HSL or PqsE, could be interesting approaches in the control of *P. aeruginosa* infections and their clinical consequences. Studying the QS ecology in strains from a broader range of origins (clinical and environmental) should allow for better target selection.

**CONCLUSION**

Loss of LasR function is common among *P. aeruginosa* isolated from any source, including both environmental and clinical settings, and regardless of the acute or chronic nature of the infections. Furthermore, despite the absence of LasR activity, the *rhl* system is still functional in a subset of LasR-defective strains, assuring QS functionality and the expression of survival and virulence factors.

Based on available knowledge, current hypotheses, and accumulating data, we propose that LasR-defective strains isolated from infection-related settings could be acquired directly from the environment, where genomic diversification occurs based on differences in regulation in various niches. We also propose that LasR is not as essential for functional QS as it has been commonly believed and that there may be a larger variety of QS architectures in *P. aeruginosa* than previously thought. Based on the rarity of isolates with completely deficient RhlR activity, and the relatively high frequency of LasR deficiency, we suggest that RhlR plays a more central role in QS regulation than LasR. Additionally, RhlR activity depends on MvfR-dependent regulation through the expression of *pqsE* via the *pqs* operon. More investigation on the importance of this partnership, until now mainly investigated in prototypical strains, throughout a larger panel of strains is needed, given the possible importance of the *pqs* system in maintaining QS activity in *P. aeruginosa.* Accordingly, to better understand the diversity of QS, future studies should focus on *P. aeruginosa* obtained from a broader range of environments and consider the dynamics of cocultures with naturally co-isolated strains.

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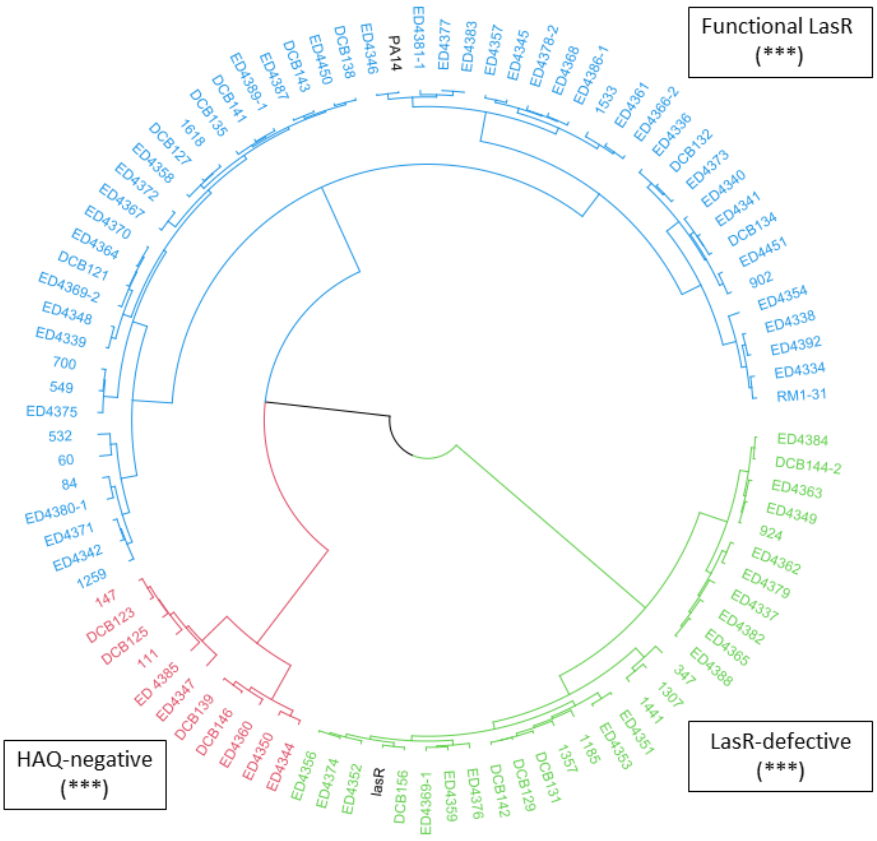
## FIGURE LEGENDS

**Figure 1: Clustering analysis performed on a panel of 92 strains of *P. aeruginosa* isolated from acute and chronic infections.** Clustering analysis is based on selected variables from a previous study. Briefly, HAQ concentrations and pyocyanin production were measured in King's A medium at two different time points (4). Strains PA14 (LasR-functional, top) and PA14 *lasR*::Gm (LasR-defective, bottom, "lasR") were included in the analysis as references. Three robust clusters were identified. One of these clusters comprises LasR-defective strains, whereas another includes strains with a functional LasR protein. The third cluster comprises strains which produce negligible levels of HAQs. Further analyses are required to confirm the functionality of LasR in this subset of isolates, as described before (4). Raw data used to generate this analysis is presented in **Table S2**. Statistical analyses were made as previously described using R software (4, 87). Robustness (*) represents the proportion of clustering runs in which a pair of isolates appeared together in some cluster, given that they were clustered together in at least one run, averaged over all such pairs (***: 80-89%).

**Figure 2: Clustering analysis performed on a subset of LasR-defective isolates of *Pseudomonas aeruginosa* shown in Figure 1.** Clustering analysis was based on chosen variables from a previous study. Briefly, HAQs concentrations, pyocyanin production and activity of a *rhlA-gfp* reporter were measured in King's A medium at two different time points using the same methods as previously described (4, 88). RhlR Active Independently of LasR (RAIL) strain E90 was included in the analysis as a reference. Raw data used to generate this analysis is presented in **Table S2**. Statistical analyses were made as previously described using R software (4, 87). Robustness (*) represents the proportion of clustering runs in which a pair of isolates appeared together in some cluster, given that they were clustered together in at least one run, averaged over all such pairs. (****: >90%).


Functional LasR
(***)
LasR-defective
(***)
HAQ-negative
(***)
PA14
lasR
ED4381-1
ED4377
ED4383
ED4357
ED4345
ED4378-2
ED4368
ED4386-1
1533
ED4361
ED4366-2
ED4336
DCB132
ED4373
ED4340
ED4341
DCB134
ED4451
902
ED4354
ED4338
ED4392
ED4334
RM1-31
ED4384
DCB144-2
ED4363
ED4349
924
ED4362
ED4379
ED4337
ED4382
ED4365
ED4388
347
1307
1441
ED4351
ED4353
1185
1357
DCB131
DCB129
DCB142
ED4376
ED4359
ED4369-1
DCB156
ED4356
ED4374
ED4352
ED4344
ED4350
ED4360
DCB146
DCB139
ED4347
ED 4385
111
DCB125
DCB123
147
1259
ED4342
ED4371
ED4380-1
84
60
532
ED4375
549
700
ED4339
ED4348
ED4369-2
DCB121
ED4364
ED4370
ED4367
ED4372
ED4358
DCB127
1618
DCB135
DCB141
ED4389-1
ED4387
DCB143
ED4450
DCB138
ED4346

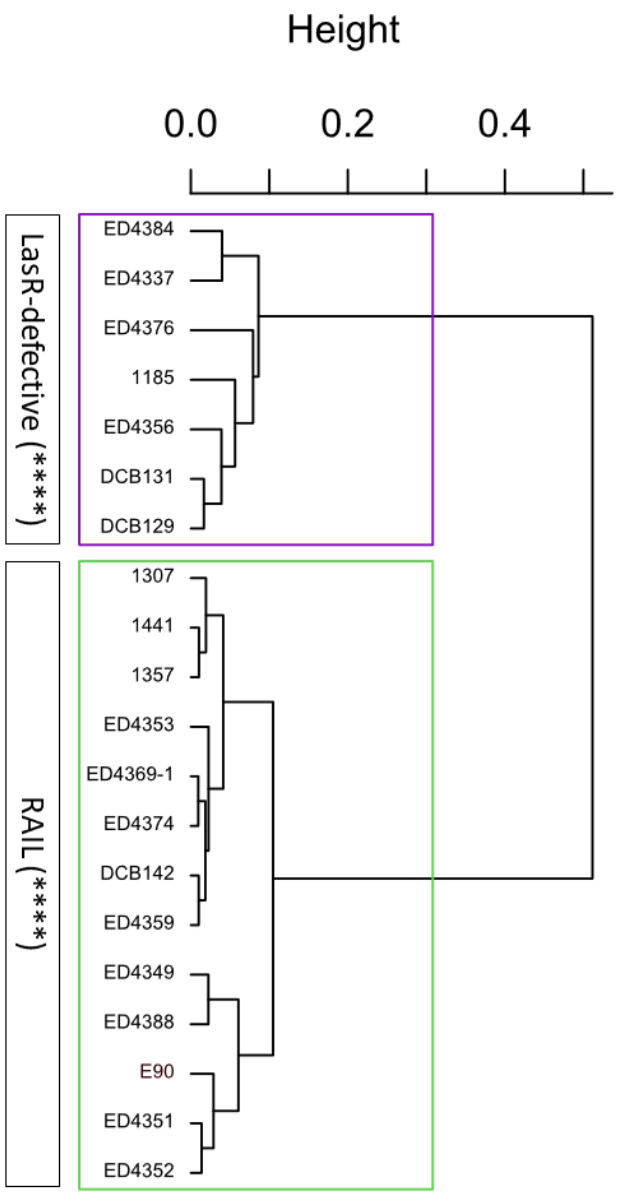

Height
0.0
0.2
0.4
LasR-defective (****)
RAIL (****)
ED4384
ED4337
ED4376
1185
ED4356
DCB131
DCB129
1307
1441
1357
ED4353
ED4369-1
ED4374
DCB142
ED4359
ED4349
ED4388
E90
ED4351
ED4352

Table S1 : List of isolates and their origin

| Isolate | Original ID | Origin | Human pathology | Reference |
|---|---|---|---|---|
| ED4334 | AUS111 | Brisbane, Australia | Urinary tract infection | Kidd *et al.* 2012 (1) |
| ED4451 | AUS263 | Brisbane, Australia | Urinary tract infection | Kidd *et al*. 2012 (1) |
| ED4336 | AUS344 | Brisbane, Australia | Urinary tract infection | Kidd *et al.* 2012 (1) |
| ED4450 | AUS430 | Brisbane, Australia | Urinary tract infection | Kidd *et al*. 2012 (1) |
| ED4337 | JJ692 | Minneapolis, USA | Urinary tract infection | Wolfgang *et al.* 2003 (2) |
| ED4338 | S54485 | Seattle, USA | Urinary tract infection | Wolfgang *et al.* 2003 (2) |
| DCB144-2 | | Ivory Coast | Urinary tract infection | This study |
| DCB146 | | Ivory Coast | Urinary tract infection | This study |
| DCB156 | | Ivory Coast | Urinary tract infection | This study |
| ED4339 | HM293 | Liverpool, UK | Intestinal cancer | Freschi *et al*. 2015 (3) |
| ED4340 | HM299 | Liverpool, UK | Intestinal cancer | Freschi *et al*. 2015 (3) |
| ED4341 | HM300 | Liverpool, UK | Intestinal cancer | Freschi *et al*. 2015 (3) |
| ED4392 | HM301 | Liverpool, UK | Intestinal cancer | Freschi *et al*. 2015 (3) |
| ED4342 | HM306 | Liverpool, UK | Intestinal cancer | Freschi *et al*. 2015 (3) |
| ED4366-2 | 934436V | Unknown | Bronchiectasis | De Soyza *et al.* 2014 (4) |
| ED4367 | AUS489 | Brisbane, Australia | Bronchiectasis | Kidd *et al.* 2012 (1) |
| ED4368 | AUS491 | Brisbane, Australia | Bronchiectasis | Kidd *et al.* 2012 (1) |
| ED4369-1 | AUS496-1 | Brisbane, Australia | Bronchiectasis | Kidd *et al.* 2012 (1) |
| ED4369-2 | AUS496-2 | Brisbane, Australia | Bronchiectasis | Kidd *et al.* 2012 (1) |
| ED4370 | AUS499 | Brisbane, Australia | Bronchiectasis | Kidd *et al.* 2012 (1) |
| DCB131 | | Ivory Coast | Ear infection | This study |
| DCB132 | | Ivory Coast | Ear infection | This study |
| DCB134 | | Ivory Coast | Ear infection | This study |
| DCB135 | | Ivory Coast | Ear infection | This study |
| DCB138 | | Ivory Coast | Ear infection | This study |
| DCB139 | | Ivory Coast | Ear infection | This study |
| DCB141 | | Ivory Coast | Ear infection | This study |
| DCB142 | | Ivory Coast | Ear infection | This study |
| DCB143 | | Ivory Coast | Ear infection | This study |
| ED4381-1 | AUS471 | Brisbane, Australia | Ear infection | Kidd *et al.* 2012 (1) |
| ED4382 | AUS134 | Brisbane, Australia | Ear infection | Kidd *et al.* 2012 (1) |
| ED4383 | AUS439 | Brisbane, Australia | Ear infection | Kidd *et al.* 2012 (1) |
| ED4384 | AUS440 | Brisbane, Australia | Ear infection | Kidd *et al*. 2012 (1) |
| DCB121 | | Ivory Coast | Pleuropulmonary condition | This study |
| DCB123 | | Ivory Coast | Pleuropulmonary condition | This study |
| DCB125 | | Ivory Coast | Pleuropulmonary condition | This study |
| DCB127 | | Ivory Coast | Pleuropulmonary condition | This study |

| | | | | |
|---|---|---|---|---|
| DCB129 | | Ivory Coast | Pleuropulmonary condition | This study |
| RM1 | | Laval, Canada | COPD | This study |
| ED4344 | 57P31PA | USA | COPD | Freschi *et al*. 2015 (3) |
| ED4345 | PA-W13 | Nottingham, UK | Wound | Freschi *et al*. 2015 (3) |
| ED4346 | AUS210 | Brisbane, Australia | Wound | Kidd *et al.* 2012 (1) |
| ED4347 | AUS407 | Brisbane, Australia | Wound | Kidd *et al.* 2012 (1) |
| ED4348 | So098 | Sofia, Bulgaria | Wound | Pirnay *et al.* 2009 (5) |
| ED4349 | A13 | Paris, France | Wound | Pirnay *et al.* 2009 (5) |
| ED4350 | A22 | Paris, France | Wound | Pirnay *et al.* 2009 (5) |
| ED4351 | PA-W31 | Nottingham, UK | Wound | Freschi *et al*. 2015 (3) |
| ED4352 | PA-W39 | Nottingham, UK | Wound | Freschi *et al*. 2015 (3) |
| ED4353 | PA-W42 | Nottingham, UK | Wound | Freschi *et al*. 2015 (3) |
| ED4354 | PA-W46 | Nottingham, UK | Wound | Freschi *et al*. 2015 (3) |
| ED4356 | PA-W8 | Nottingham, UK | Ulcer | Freschi *et al*. 2015 (3) |
| ED4357 | PA-W9 | Nottingham, UK | Ulcer | Freschi *et al*. 2015 (3) |
| ED4358 | PA-W11 | Nottingham, UK | Ulcer | Freschi *et al*. 2015 (3) |
| ED4359 | PA-W20 | Nottingham, UK | Ulcer | Freschi *et al*. 2015 (3) |
| ED4360 | A17 | Paris, France | Leg Ulcer | Pirnay *et al.* 2009 (5) |
| ED4361 | PA-W47 | Nottingham, UK | Burn | Freschi *et al*. 2015 (3) |
| ED4362 | Mi162 | USA | Burn | Pirnay *et al.* 2009 (5) |
| ED4363 | Lo049 | London, UK | Burn | Pirnay *et al.* 2009 (5) |
| ED4364 | Aa249 | Aachen, Germany | Burn | Pirnay *et al.* 2009 (5) |
| ED4365 | PA-W10 | Nottingham, UK | Burn | Freschi *et al*. 2015 (3) |
| ED4371 | 39145 | UK | Keratitis | Stewart *et al.* 2011 (6) |
| ED4372 | 39016 | UK | Keratitis | Freschi *et al*. 2015 (3) |
| ED4373 | 39177 | Manchester, UK | Keratitis | Freschi *et al*. 2015 (3) |
| ED4374 | 152504sp2 | Portugal | Pneumonia | Freschi *et al*. 2015 (3) |
| ED4375 | AUS422 | Brisbane, Australia | Pneumonia | Kidd *et al.* 2012 (1) |
| ED4376 | AUS275 | Brisbane, Australia | Bacteraemia | Kidd *et al.* 2012 (1) |
| ED4377 | AUS462 | Brisbane, Australia | Bacteraemia | Kidd *et al.* 2012 (1) |
| ED4378-2 | AUS301-1 | Brisbane, Australia | Bacteraemia | Kidd *et al*. 2012 (1) |
| ED4379 | AUS150 | Brisbane, Australia | Bacteraemia | Kidd *et al.* 2012 (1) |
| ED4380-1 | AUS307 | Brisbane, Australia | Bacteraemia | Kidd *et al.* 2012 (1) |
| ED4385 | 15108-1 | Spain | Acute infection | Freschi *et al*. 2015 (3) |
| ED4386-1 | 13121-1 | France | Acute infection | Freschi *et al*. 2015 (3) |
| ED4387 | PA-W2 | UK | Spine pressure sore | Freschi *et al*. 2015 (3) |
| ED4388 | F2 | London, UK | Hyponatremia | Martin *et al.* 2013 (7) |
| ED4389-1 | B1(P2356) | Bangkok, Thailand | Primary Ciliary Diskinesia | Freschi *et al*. 2015 (3) |
| 60 | 278S180511BSL_PA2 | Montreal, Canada | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 84 | IST27 | Lisbon, Portugal | Cystic Fibrosis | Leitão *et al.* 1996 (8) |
| 111 | PA54A | Sherbrooke, Canada | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 147 | PAC33B | Sherbrooke, Canada | Cystic Fibrosis | Freschi *et al*. 2015 (3) |

| | | | | |
|---|---|---|---|---|
| 347 | AUS077 | Brisbane, Australia | Cystic Fibrosis | Kidd *et al.* 2011 (9) |
| 532 | AUS717 | Brisbane, Australia | Cystic Fibrosis | Kidd *et al*. 2013 (10) |
| 549 | AMT0020-84 | Seattle, USA | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 700 | C5311 | Vancouver, Canada | Cystic Fibrosis | Pirnay *et al.* 2009 (5) |
| 902 | S2239 | Dunedin, New Zealand | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 924 | U0284 | Hobart, Australia | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 1185 | PA508 | Montreal, Canada | Cystic Fibrosis | Beaulac *et al*. 1996 (11) |
| 1259 | AA2 | Germany | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 1307 | 5987 | Québec, Canada | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 1357 | AL6 | Munich, Germany | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 1441 | VD329 | McMasterville, Canada | Cystic Fibrosis | Ouellet *et al.* 2014 (12) |
| 1533 | 13 | Rovereto, Italy | Cystic Fibrosis | Freschi *et al*. 2015 (3) |
| 1618 | SMC1596 | Lebannon, USA | Cystic Fibrosis | (13) |

**Table S2 : Concentrations of HAQs (HHQ, PQS and HQNO), AHLS and pyocyanin quantification . All data is normalized by total protein concentration in sample.**
**Expression of rhlA-gfp reporter (RFU/OD600).**

| Souches | PYO_6h | PYO_24h | HHQ_6h | HHQ_24h | PQS_6h | PQS_24h | HQNO_6h | HQNO_24h | 3-oxo-C12-HSL_6h | C4-HSL_6h | *rhlA*-GFP | Final classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ED4334 | 0.010 | 0.027 | 2.987 | 9.751 | 0.501 | 6.592 | 0.959 | 1.237 | | | | Functional LasR |
| ED4451 | 0.128 | 0.228 | 1.456 | 0.614 | 0.152 | 0.994 | 0.331 | 0.322 | | | | Functional LasR |
| ED4336 | 0.227 | 1.075 | 15.479 | 14.096 | 0.450 | 7.353 | 0.562 | 0.807 | | | | Functional LasR |
| ED4450 | 0.038 | 0.056 | 1.269 | 0.089 | 0.230 | 0.902 | 0.189 | 0.169 | | | | Functional LasR |
| ED4337 | 0.024 | 0.108 | 0.213 | 5.987 | 0.211 | 0.478 | 0.025 | 0.825 | | | 443.547 | LasR-defective |
| ED4338 | 0.017 | 0.058 | 1.670 | 3.230 | 0.303 | 5.587 | 0.234 | 1.378 | | | | Functional LasR |
| DCB144-2 | 0.030 | 0.029 | 0.897 | 7.956 | 0.027 | 0.483 | 0.060 | 0.277 | | | | LasR-defective |
| DCB146 | 0.056 | 0.963 | 0.006 | 0.028 | 0.015 | 0.143 | 0.001 | 0.000 | 0.003 | 0.064 | | LasR-defective, HAQ-negative |
| DCB156 | 0.017 | 0.305 | 1.760 | 15.028 | 0.025 | 3.229 | 0.016 | 0.122 | | | | LasR-defective |
| ED4339 | 0.091 | 0.083 | 2.257 | 0.993 | 0.360 | 1.355 | 0.478 | 0.469 | | | | Functional LasR |
| ED4340 | 0.057 | 0.189 | 1.052 | 0.989 | 0.268 | 0.974 | 0.181 | 0.435 | | | | Functional LasR |
| ED4341 | 0.052 | 0.118 | 1.437 | 1.239 | 0.119 | 1.844 | 0.287 | 0.519 | | | | Functional LasR |
| ED4392 | 0.086 | 0.157 | 1.374 | 1.924 | 0.099 | 3.937 | 0.144 | 0.524 | | | | Functional LasR |
| ED4342 | 0.095 | 0.060 | 0.794 | 0.064 | 0.281 | 1.064 | 0.229 | 0.207 | | | | Functional LasR |
| ED4366-2 | 0.031 | 0.224 | 1.427 | 1.078 | 0.214 | 2.656 | 0.084 | 0.471 | | | | Functional LasR |
| ED4367 | 0.036 | 0.019 | 1.090 | 0.030 | 0.442 | 0.334 | 0.284 | 0.047 | | | | Functional LasR |
| ED4368 | 0.048 | 0.213 | 1.093 | 1.811 | 0.315 | 6.338 | 0.145 | 0.366 | | | | Functional LasR |
| ED4369-1 | 0.023 | 0.273 | 0.322 | 2.687 | 0.057 | 0.711 | 0.038 | 0.424 | | | 1865.114 | LasR-defective, RhlR-active |
| ED4369-2 | 0.080 | 0.078 | 1.841 | 0.274 | 0.058 | 1.356 | 0.116 | 0.210 | | | | Functional LasR |
| ED4370 | 0.071 | 0.130 | 2.437 | 0.485 | 0.255 | 1.737 | 0.356 | 0.312 | | | | Functional LasR |
| DCB131 | 0.077 | 0.785 | 2.488 | 11.881 | 0.055 | 2.659 | 0.141 | 0.594 | | | 795.692 | LasR-defective |
| DCB132 | 0.156 | 0.855 | 4.022 | 2.710 | 0.064 | 2.346 | 0.275 | 0.472 | | | | Functional LasR |
| DCB134 | 0.096 | 0.437 | 1.976 | 3.010 | 0.163 | 2.470 | 0.321 | 0.758 | | | | Functional LasR |
| DCB135 | 0.056 | 0.057 | 1.556 | 0.162 | 0.361 | 0.640 | 0.093 | 0.075 | | | | Functional LasR |
| DCB138 | 0.083 | 0.065 | 1.509 | 0.118 | 0.208 | 1.076 | 0.123 | 0.209 | | | | Functional LasR |
| DCB139 | 0.031 | 0.792 | 0.003 | 0.014 | 0.009 | 0.154 | 0.000 | 0.001 | 0.002 | 0.039 | | LasR-defective, HAQ-negative |
| DCB141 | 0.090 | 0.054 | 1.510 | 0.099 | 0.524 | 1.047 | 0.171 | 0.120 | | | | Functional LasR |
| DCB142 | 0.059 | 0.553 | 2.377 | 7.133 | 0.014 | 2.032 | 0.080 | 0.649 | | | 4095.298 | LasR-defective, RhlR-active |
| DCB143 | 0.077 | 0.191 | 1.518 | 0.116 | 0.212 | 0.772 | 0.180 | 0.177 | | | | Functional LasR |
| ED4381-1 | 0.042 | 0.105 | 1.175 | 0.222 | 0.589 | 3.401 | 0.062 | 0.063 | | | | Functional LasR |
| ED4382 | 0.056 | 0.024 | 0.695 | 12.888 | 0.423 | 0.124 | 0.031 | 0.718 | | | | LasR-defective |
| ED4383 | 0.049 | 0.051 | 2.106 | 0.395 | 0.442 | 3.810 | 0.184 | 0.116 | | | | Functional LasR |
| ED4384 | 0.069 | 0.037 | 3.831 | 26.349 | 0.067 | 0.811 | 0.203 | 0.754 | | | 1856.629 | LasR-defective |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DCB121 | 0.048 | 0.040 | 1.044 | 0.151 | 0.138 | 0.901 | 0.222 | 0.121 | | | | Functional LasR |
| DCB123 | 0.062 | 0.036 | 0.022 | 0.010 | 0.113 | 0.062 | 0.000 | 0.001 | 0.000 | 0.000 | | LasR-defective, HAQ-negative |
| DCB125 | 0.049 | 0.043 | 0.007 | 0.024 | 0.081 | 0.138 | 0.001 | 0.000 | 0.147 | 0.088 | | Functional LasR, HAQ-negative |
| DCB127 | 0.075 | 0.100 | 2.075 | 0.373 | 0.222 | 0.657 | 0.186 | 0.253 | | | | Functional LasR |
| DCB129 | 0.091 | 0.509 | 3.729 | 10.960 | 0.027 | 2.033 | 0.197 | 0.588 | | | 768.2022 | LasR-defective |
| RM1-31 | 0.025 | 0.088 | 0.896 | 3.466 | 0.108 | 3.357 | 0.053 | 0.415 | | | | Functional LasR |
| ED4344 | 0.059 | 0.156 | 0.003 | 0.000 | 0.013 | 0.047 | 0.000 | 0.000 | 0.131 | 0.105 | | Functional LasR, HAQ-negative |
| ED4345 | 0.023 | 0.062 | 1.107 | 0.523 | 0.147 | 3.740 | 0.051 | 0.157 | | | | Functional LasR |
| ED4346 | 0.092 | 0.159 | 1.215 | 0.203 | 0.524 | 2.030 | 0.120 | 0.172 | | | | Functional LasR |
| ED4347 | 0.042 | 0.027 | 0.005 | 0.013 | 0.026 | 0.248 | 0.000 | 0.001 | 0.197 | 0.102 | | Functional LasR, HAQ-negative |
| ED4348 | 0.046 | 0.025 | 2.762 | 1.344 | 0.334 | 1.712 | 0.277 | 0.338 | | | | Functional LasR |
| ED4349 | 0.025 | 0.254 | 2.876 | 15.104 | 0.037 | 1.318 | 0.236 | 0.407 | | | 3460.466 | LasR-defective, RhlR-active |
| ED4350 | 0.074 | 0.319 | 0.003 | 0.002 | 0.006 | 0.038 | 0.000 | 0.000 | 0.192 | 0.057 | | Functional LasR, HAQ-negative |
| ED4351 | 0.024 | 0.567 | 2.921 | 7.788 | 0.025 | 4.237 | 0.103 | 0.369 | | | 2321.154 | LasR-defective, RhlR-active |
| ED4352 | 0.058 | 0.733 | 1.534 | 10.486 | 0.021 | 6.161 | 0.052 | 0.559 | | | 3143.739 | LasR-defective, RhlR-active |
| ED4353 | 0.029 | 0.608 | 3.302 | 6.647 | 0.056 | 5.668 | 0.100 | 0.419 | | | 4460.897 | LasR-defective, RhlR-active |
| ED4354 | 0.108 | 0.943 | 0.207 | 3.968 | 0.017 | 5.734 | 0.005 | 0.123 | | | | Functional LasR |
| ED4356 | 0.057 | 1.579 | 1.270 | 12.463 | 0.040 | 4.469 | 0.062 | 0.497 | | | 908.556 | LasR-defective |
| ED4357 | 0.051 | 0.102 | 1.441 | 0.321 | 0.274 | 4.608 | 0.177 | 0.260 | | | | Functional LasR |
| ED4358 | 0.100 | 0.084 | 1.149 | 0.166 | 0.273 | 0.433 | 0.203 | 0.111 | | | | Functional LasR |
| ED4359 | 0.054 | 0.116 | 1.067 | 5.268 | 0.066 | 1.400 | 0.081 | 0.885 | | | 2551.094 | LasR-defective, RhlR-active |
| ED4360 | 0.016 | 0.389 | 0.004 | 0.005 | 0.056 | 0.104 | 0.000 | 0.000 | 0.001 | 0.000 | | LasR-defective, HAQ-negative |
| ED4361 | 0.072 | 0.318 | 1.824 | 0.923 | 0.665 | 3.691 | 0.211 | 0.962 | | | | Functional LasR |
| ED4362 | 0.054 | 0.119 | 0.066 | 5.414 | 0.016 | 0.315 | 0.001 | 0.026 | | | | LasR-defective |
| ED4363 | 0.129 | 0.422 | 1.625 | 13.163 | 0.053 | 1.514 | 0.067 | 0.443 | | | | LasR-defective |
| ED4364 | 0.133 | 0.149 | 3.394 | 0.785 | 0.348 | 2.295 | 0.304 | 0.409 | | | | Functional LasR |
| ED4365 | 0.042 | 0.032 | 0.525 | 8.554 | 0.032 | 0.182 | 0.009 | 0.306 | | | | LasR-defective |
| ED4371 | 0.050 | 0.082 | 0.997 | 0.376 | 0.552 | 2.353 | 0.410 | 0.420 | | | | Functional LasR |
| ED4372 | 0.071 | 0.057 | 0.951 | 0.021 | 0.278 | 0.230 | 0.217 | 0.058 | | | | Functional LasR |
| ED4373 | 0.221 | 0.775 | 7.702 | 5.280 | 0.286 | 5.238 | 0.544 | 0.771 | | | | Functional LasR |
| ED4374 | 0.079 | 0.959 | 1.416 | 12.892 | 0.094 | 4.675 | 0.046 | 0.450 | | | 9145.778 | LasR-defective, RhlR-active |
| ED4375 | 0.053 | 0.157 | 2.148 | 0.450 | 0.323 | 0.402 | 0.046 | 0.062 | | | | Functional LasR |
| ED4376 | 0.027 | 0.046 | 2.944 | 20.263 | 0.255 | 6.950 | 0.459 | 1.538 | | | 877.858 | LasR-defective |
| ED4377 | 0.049 | 0.051 | 2.106 | 0.395 | 0.442 | 3.810 | 0.184 | 0.116 | | | | Functional LasR |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ED4378-2 | 0.049 | 0.034 | 1.779 | 1.440 | 0.325 | 4.926 | 0.099 | 0.124 | | | | Functional LasR |
| ED4379 | 0.051 | 0.051 | 0.285 | 30.005 | 0.026 | 0.668 | 0.004 | 0.080 | | | | LasR-defective |
| ED4380-1 | 0.230 | 0.101 | 0.836 | 0.653 | 0.520 | 1.098 | 0.413 | 0.593 | | | | Functional LasR |
| ED4385 | 0.011 | 0.066 | 0.005 | 0.014 | 0.241 | 0.031 | 0.000 | 0.000 | 0.189 | 0.079 | | Functional LasR, HAQ-negative |
| ED4386-1 | 0.015 | 0.014 | 0.545 | 0.518 | 0.117 | 3.678 | 0.102 | 0.181 | | | | Functional LasR |
| ED4387 | 0.043 | 0.151 | 1.226 | 0.084 | 0.227 | 0.744 | 0.121 | 0.118 | | | | Functional LasR |
| ED4388 | 0.038 | 0.190 | 0.693 | 13.862 | 0.061 | 0.554 | 0.002 | 0.282 | | | 1947.333 | LasR-defective, RhlR-active |
| ED4389-1 | 0.115 | 0.070 | 1.058 | 0.151 | 0.332 | 0.766 | 0.108 | 0.173 | | | | Functional LasR |
| 60 | 0.090 | 0.251 | 0.101 | 0.017 | 0.154 | 0.098 | 0.124 | 0.147 | | | | Functional LasR |
| 84 | 0.084 | 0.096 | 0.211 | 0.802 | 0.679 | 1.192 | 0.606 | 0.212 | | | | Functional LasR |
| 111 | 0.009 | 0.065 | 0.008 | 0.010 | 0.063 | 0.057 | 0.001 | 0.001 | 0.000 | 0.001 | | LasR-defective, HAQ-negative |
| 147 | 0.019 | 0.023 | 0.006 | 0.006 | 0.034 | 0.024 | 0.001 | 0.001 | 0.000 | 0.002 | | LasR-defective, HAQ-negative |
| 347 | 0.035 | 0.025 | 16.667 | 35.302 | 0.030 | 0.073 | 0.653 | 2.299 | | | | LasR-defective |
| 532 | 0.109 | 0.508 | 1.699 | 1.393 | 0.307 | 1.327 | 2.079 | 5.452 | | | | Functional LasR |
| 549 | 0.405 | 1.888 | 54.543 | 12.628 | 0.562 | 7.254 | 1.458 | 2.144 | | | | Functional LasR |
| 700 | 0.030 | 0.106 | 11.589 | 1.691 | 0.771 | 2.306 | 0.376 | 0.516 | | | | Functional LasR |
| 902 | 0.190 | 1.047 | 2.626 | 0.964 | 0.212 | 1.422 | 0.214 | 0.597 | | | | Functional LasR |
| 924 | 0.032 | 1.727 | 11.905 | 55.513 | 0.113 | 4.342 | 0.101 | 1.020 | | | | LasR-defective |
| 1185 | 0.017 | 0.643 | 8.329 | 15.380 | 0.041 | 3.922 | 0.000 | 0.001 | | | 1683.102 | LasR-defective |
| 1259 | 0.059 | 0.049 | 1.752 | 0.280 | 0.611 | 1.233 | 0.591 | 0.399 | | | | Functional LasR |
| 1307 | 0.138 | 0.038 | 3.361 | 5.134 | 0.153 | 0.730 | 0.453 | 1.305 | | | 11139.456 | LasR-defective, RhlR-active |
| 1357 | 0.021 | 1.118 | 2.956 | 6.033 | 0.069 | 1.190 | 0.049 | 0.241 | | | 5754.809 | LasR-defective, RhlR-active |
| 1441 | 0.101 | 0.446 | 3.304 | 4.704 | 0.453 | 1.058 | 0.327 | 0.514 | | | 5531.655 | LasR-defective, RhlR-active |
| 1533 | 0.029 | 0.159 | 0.949 | 0.799 | 0.311 | 4.921 | 0.312 | 0.895 | | | | Functional LasR |
| 1618 | 0.163 | 0.125 | 3.991 | 0.481 | 0.778 | 1.430 | 0.540 | 0.680 | | | | Functional LasR |
| PA14 | 0.152 | 0.159 | 1.337 | 0.245 | 0.673 | 2.485 | 0.125 | 0.137 | 0.104 | 0.056 | | Functional LasR |
| PA14 lasR::Gm | 0.057 | 0.615 | 0.858 | 10.037 | 0.077 | 2.028 | 0.013 | 0.132 | 0.005 | 0.025 | | LasR-defective |
| E90 | 0.233 | 2.158 | 2.602 | 6.620 | 0.027 | 5.952 | 0.482 | 2.715 | | | 3699.276 | LasR-defective, RhlR-active |

Grey box indicates no data

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