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ORIGINAL ARTICLE

Effect of γ-irradiation on membrane fatty acids and peptidoglycan's muropeptides of *Pantoea agglomerans*, a plant pathogen

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Abstract

Aims: The aim of this study was to evaluate the effect of γ-irradiation on the fatty acids (FA) and muropeptides content of two strains of an *Enterobacteriacea*: *Pantoea agglomerans*.

Methods and Results: *Pantoea agglomerans* strains ATCC 49174 and RL1 isolated from irradiated carrots were used for this study. Radiation treatments (1 and 3·5 kGy) were performed to study the radiotolerance. Total lipids were obtained by multiple extractions using methanol/chloroform (2 : 1) and were quantified by GC. Muropeptides were purified by successive enzymatic digestions and analysed using a reverse phase C_{18} column in high performance liquid chromatography. A significant ($P \leq 0{\cdot}05$) modification of the bacterial wall was noticed for the membrane FA composition and the muropeptides.

Conclusion: Effects of irradiation on the bacterial membrane are noticeable and could play an important role on the cellular response and ability to survive this harsh environment.

Significance and Impact of the Study: To our knowledge, it is the first study to demonstrate the effects of ionizing irradiation on the modification of the FA and one of the few to confirm its effects on the muropeptides of the peptidoglycan.

Introduction

Gamma-irradiation has long been used for decontamination and/or sterilization of food products to improve product safety and shelf life. Even if low-dose irradiation (≤3 kGy) is effective in destroying most bacteria, it does not prevent the growth of certain radiation resistant strain such as *Deinococcus radiodurans* which requires higher irradiation doses up to 20–40 kGy to destroy this micro-organism. The mechanisms by which prokaryotes become resistant to ionizing-radiation are not known and this resistance cannot be explained as an adaptation to environmental radiation. Natural sources of ionizing radiation on earth emit at very low levels (Rainey *et al.* 2005) making it impossible to generate the acute doses to which these organisms show resistance. Other than it's DNA repairing abilities, it has been suggested that the extreme radioresistance of *D. radiodurans* was due to other mechanisms such as carotenoid pigments production, accumulation of Mn^{2+}, intervention of enzymatical systems (i.e. superoxide dismutase) which contribute to compensate for the stress generated by reactive oxygen species (ROS) produced by ionizing radiation and the metabolism (Imlay 2003).

These ROS produced by irradiation are generally superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$) and can affect the whole cell by inducing damages to its DNA and other important molecules such as the proteins and lipids (Ghosal *et al.* 2005). The ROS can induce lipid peroxidation and reduce the membrane fluidity (Cabiscol *et al.* 2000). Some molecules have the ability to diminish the harmful effects of the ROS such as

anti-oxidative enzymes (i.e. peroxidase, catalase and superoxide dismutase) or nonenzymatic systems (i.e. vitamins A and E, accumulation high level of Mn^{2+}). One of these protective mechanism against irradiation deleterious effects is the induce of a 'viable but nonculturable' (VBNC) state on common microbiological media (Oliver 2005). Work of Lacroix and Lafortune (2004) has observed a resistant bacterium in a probable VBNC state that was identified as *Pantoea agglomerans*. *P. agglomerans* is an *Enterobacteriaceae* like *Escherichia coli*, but is up to five times more radiotolerant (Le-Tien *et al.* 2006). Nowadays, ionizing radiations studies seem to show that protein oxidation is the main cause to the inability to recover and repair damages observed after such a stress (Daly *et al.* 2007). It is of interest to note that unsaturated fatty acids (UFA) have the ability to scavenge the ROS (Imlay 2003). In this context, as reported by Di Pasqua *et al.* (2006), bacteria are able to modify the FA composition of their membrane when challenged by environmental stress such as variation of temperature, pH, osmotic pressure and ethanol concentration. Although, bacterial cytoplasmic membranes can compensate for modified growth conditions by a process known as homeoviscous adaptation which adapt the membrane so it can retain it's fluidity as the environment changes (Signoretto *et al.* 2000). Exposition to UV radiations has also produced modifications of the membrane FA (Gupta *et al.* 2008). Some enzymes known as FA desaturases have been proposed for such modification. It was also demonstrated that these enzymes are not produced under normal conditions, but environmental stress induce their production (Aguilar and de Mendoza 2006).

The bacterial walls are composed of an important structure responsible for the resistance to the osmotic stress and typical shape. This structure is named peptidoglycan or murein. The murein is made of multiple glycans strands, composed of alternating β1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) residues, cross-linked by small peptides to form a three dimensional sacculus surrounding the cytoplasmic membrane. Between different bacterial species the structure of the glycan chains does not seem to change, but the muropeptides responsible for the cross-link show many variations when the micro-organism is submitted to different growth condition such as temperature shift. The muropeptides are composed of more than 50 different types in *E. coli* when it is exposed to various growth condition (Vollmer and Bertsche 2008). Other studies have demonstrated the effect of γ-irradiation on the composition of the muropeptides of *E. coli*. Irradiation does not disrupt the membrane integrity, but the peptides responsible for the connections between the glycans chains are modified (Caillet *et al.* 2005). Thus, it was hypothesized that the modification of membrane FA, muropeptides and bacterial enzymes induction after radiations treatments play a significant role in the radioresistance and in the recovery of bacteria during the VBNC state.

The objective of this study was to evaluate the effect of gamma radiation on the FA content of *P. agglomerans strain* from American Type Culture Collection ATCC 49174 (used as a nonirradiated control) and strain RL1 isolated from irradiated carrots to highlight the relation between the modification of the FA composition of the bacterial membrane and the radioresistance. The degree of unsaturation of the FA composing the bacterial membrane was also evaluated to demonstrate the global effects of the modifications.

Materials and methods

Micro-organisms and growth condition

Pantoea agglomerans strain ATCC 49174 (American Type Culture Collection, Manassas, VA, USA) and strain RL1, previously isolated from irradiated carrots at 7 kGy by (Lacroix and Lafortune 2004) and identified by comparing the sequence of the 16 S rDNA with DNA sequences from the National Center for Biotechnology Information (NCBI) database using the standard nucleotide–nucleotide homology search Basic Local Alignment Search Tool (Blast, http://www.ncbi.nlm.nih.gov/Blast.cgi). Bacteria were subcultured in tryptic soy broth (1·0%, v/v) (Difco Laboratories, Detroit, MI, USA) at 30°C for 24 h from the stock culture maintained at −80°C in TSB containing glycerol (20%, w/v). Prior to the experiment, 1 ml of culture was incubated through one cycle of 24 h at 30°C in TSB to obtain a working culture containing approximately 10^9 CFU ml^{-1}. The bacterial culture was centrifuged at 2000 ***g*** for 15 min at 4°C and washed with NaCl (0·85%, w/v) and then resuspended in 1 l of TSB and incubated for 24 h preceding the irradiation treatments.

Irradiation

The bacterial cultures (50 ml) were irradiated at a mean dose of 1 kGy to create damaged cells and with a mean dose of 3·5 kGy to kill cells. An underwater irradiator (UC-15A) (MDS Nordion international Inc., Kanata, ON, Canada) equipped with a ^{60}Cobalt source was used to deliver radiations at a mean dose rate of 16·2 kGy h^{-1}. This irradiator was certified by the National Institute of Standards and Technology (Gaithersburg, MD, USA) and the dose rate was established using a correction for decays of source. Amber Oersoex 3042D (Atomic Energy Research Establishment; Harwell, Oxfordshire, UK) was

used to validate the doses distributions. The radiation treatment was carried out at the Canadian Irradiation Centre (Laval, QC, Canada) at room temperature (20°C).

Lipids extraction

Total lipids were extracted as described by Evans (Evans *et al.* 1998) with slight modification. Practically, the bacterial cultures were centrifuged at 2500 ***g*** for 15 min and resulting bacterial pellet was washed twice using NaCl (0·85%, w/v). The pellet was then resuspended in 5 ml of sterile distilled water and a volume of 9·5 ml of methanol/chloroform (2 : 1, v/v) was added. The mixture was shaken 1 min on a mechanical vortexer and left untouched for 2 h. Thereafter, the mixture was centrifuged at 2500 ***g*** for 5 min. The supernatant (SN1) was retained by decantation. The pellet was resuspended in 12 ml of methanol/chloroform/water (2 : 1 : 0·8; v/v/v) and the extraction was operated as described previously for SN1 to obtain supernatant 2 (SN2). The supernatants (SN1 and SN2) were combined and a volume of 7 ml of chloroform/water (1 : 1) was added. The mixture was allowed to separate in two phases and the lower phase containing the lipids was harvested. The chloroform was then evaporated under a stream of nitrogen and the resulting precipitate was resuspended in hexane. The resulting solution was stored at −20°C until further analysis.

Methylation of fatty acid

Lipid samples were *trans*-methylated for analysis of their acyl groups using fatty acyl methyl ester (FAME) form using a modified method provided by Supelco guideline T496125B (Supelco; Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). A sample of 1 ml of total lipid extract was combined in a sealed glass screw cap with 2 ml of Boron Trifluoride (BF_3) in methanol (14%, w/w) containing 250 mg of sodium sulfate. Then the solution was heated at 60°C for 10 min. After cooling down at room temperature, 1 ml of MiliQ water (Millipore, Billerica, MA) and the same volume of hexane was added into vessel. The upper phase was retained and dried under a stream of nitrogen. The FA extract was resuspended in a minimal volume of hexane. The sample was stored at −20°C until further analysis.

Gas liquid chromatography

The FAME were analysed using a Varian 3400 gas chromatograph (Varian Inc., Palo Alto, CA, USA) equipped with a flame ionization detector fitted with a fused silica capillary column Equity-1 30 m × 0·25 mm ID × 0·25 μm of film thickness (Supelco). A volume of 1 μl of sample was injected using the following operation temperature program: injector at 270°C; detector at 290°C; initial oven at 70°C for 1 min.; ramp 1, 15°C min^{-1} to 150°C; ramp2; 10°C min^{-1} to 280°C and hold for 15 min. The FAME peaks were identified by comparison with those of a standard FAME solution (Supelco, 37 component FAME standards). The peaks were integrated with the VARIAN STAR CHROMATOGRAPHY WORKSTATION ver.5 (Varian Inc.). The areas of the peaks of interest were added together and individual peaks were expressed as a percentage of this total.

Cell wall preparation

Bacterial cell walls were prepared as described by (Signoretto *et al.* 2000) to analyse the muropeptides. A volume of 1 l of bacterial culture in stationary growth phase was quickly chilled in an ice-ethanol bath until the temperature dropped below 10°C. Cells were harvested by centrifugation for 15 min at 2500 ***g*** at 4°C and the resulting pellet was washed in phosphate buffer (10 mmol l^{-1}, pH 7·2) at the same temperature. The pellet was then transferred into sodium dodecyl sulfate (SDS, 4%, w/v) solution maintained at 100°C and was incubated for 30 min under stirring. The cell walls were concentrated and washed with distilled water. Cell walls were then broken using a cell disrupter (FastPREP, model FP 120; Qbiogene, Inc., Carlsbad, CA, USA) containing glass beads (0·2 mm) and regulated at speed 4 for 60 s. The suspension was then centrifuged at 5000 ***g*** for 2 min to remove the glass beads and the unbroken cells. The resulting supernatant containing the cell walls was centrifuged for 30 min at 30 000 ***g*** at 4°C.

Preparation of peptidoglycan

The peptidoglycan preparation was done as described by (Signoretto *et al.* 2000). Bacterial walls were treated at 37°C in 100 mmol l^{-1} Tris–HCl (pH 7·5) with α-amylase (100 μg ml^{-1}; *EC 3·2·1·1*; Sigma). After 2 h, DNase (10 μg ml^{-1}; *EC 3·1·21·1*; Sigma) and RNase (50 μg ml^{-1}; *EC 3·1·27·5*; Sigma) were added with 20 mmol l^{-1} $MgSO_4$ and incubated for 2 h. The suspension was then treated with trypsin (100 μg ml^{-1}; *EC 3·4·21·4*; Sigma) in the presence of 10 mmol l^{-1} $CaCl_2$ for 16 h. The enzymes in SDS (1%, w/v) were inactivated by heating at 100°C for 15 min. Cell walls were collected, washed and freeze-dried. Cell walls were resuspended in 100 mmol l^{-1} $(NH_4)_2CO_3$ and treated with alkaline phosphatase (50 μg ml^{-1}; *EC 3·1·3·1*; Sigma) for 16 h at 37°C. The enzymes were inactivated by heating at 100°C for 5 min, the peptidoglycan was collected by centrifugation for

60 min at 45 000 ***g*** at 4°C and washed twice with distilled water. The pure peptidoglycans were stored at −20°C.

Preparation and separation of the muropeptides by high performance liquid chromatography (HPLC)

Muropeptides were obtained as described by (Glauner 1988). The peptidoglycan (1 mg ml^{-1}) was digested for 16 h at 37°C in presence of muromidase (20 μg ml^{-1}: *EC 3·2·1·17*; Sigma) dissolved in 12·5 mmol l^{-1} phosphate buffer (pH 5·5) containing sodium azide (0·02%, w/v). Clarification of the suspension indicated complete digestion of the peptidoglycan. The samples were heated at 100°C for 5 min and then centrifuged for 5 min in an Eppendorf centrifuge. The resulting supernatant was diluted with an equal volume of 0·5 mol l^{-1} borate buffer (pH 9·0) and then immediately reduced with 15 mg of sodium borohydride for 15 min at room temperature. The pH value of the solution was adjusted to 2·0 with *ortho*-phosphoric acid to stop the reaction. The samples were stored at −20°C. The HPLC analyses were performed using a PROSTAR 230 (Varian Inc.) equipped with a ternary pump delivery system, a Rheodyne injection valve (Waters, Ltd., Dorval, QC, Canada) and a ProStar 330 diode-array UV/Vis detector (Varian). Integration and data elaboration were performed with the STAR CHROMATOGRAPHY WORKSTATION software (Varian). A Lichrosorb RP18 column (5 μm, 250 by 4 mm; Varian) was used. All solvents were filtered on 0·45-μm pore-size filter disk (Millipore Canada, Ltd., Etobicoke, ON, Canada) and degassed by filtration under vacuum. A gradient elution was carried out using the following solvent systems: (i) mobile phase A includes methanol (5%, v/v) in 100 mmol l^{-1} phosphate buffer (pH 2·5) and 0·3 ppm sodium azide; (ii) mobile phase B contains methanol (30%, v/v) in 100 mmol l^{-1} phosphate buffer (pH 2·5) and 0·3 ppm sodium azide. The column was eluted over 150 min at a flow rate of 0·5 ml min^{-1} with a linear gradient, starting 10 min after injection, from 5% to 30% of the same composition of mobile phase. A column oven (Waters) was used to maintain the temperature of the column at 52°C. For each sample, a volume of 200 μl was injected after filtration through a 0·45-μm pore-size filter disk. The eluted compounds were read by spectrophotometry at 206 nm. Peaks were separated in 11 fractions which the first ten consisted of 10 min intervals of the run and the eleventh one was comprised of the remaining area after a 100 min till the end of the run.

Statistical analysis

An analysis of variance and Duncan's multiple-range tests were employed to analyse statistically all results. Student's *t*-test was utilized with the analysis of variance and paired comparisons. Differences between means were considered significant at $P \leq 0{\cdot}05$. STAT-PACKETS STATISTICAL ANALYSIS software (SPSS Base 10.0; SPSS, Inc., Chicago, IL, USA) was used for the analysis. The study was done in two replicates and for each replicate, three samples were injected into the columns.

Results

Modifications were investigated by GLC analysis of FAMEs extracted from the cells after an exposure to γ-irradiation. This experiment was carried out in parallel to investigate whether there was a difference between the strain which has never been submitted under the radiation stress and the strain RL1 isolated from carrots after irradiation at 7 kGy (Lacroix and Lafortune 2004). The FA composition of *P. agglomerans* obtained from the strain ATCC 49174 and the strain RL1 are shown in Figs. 1 and 2. Data analysis has shown that there were significant changes ($P \leq 0{\cdot}05$) in the FA composition of the bacterial membrane for the two strains irradiated at 1 and 3·5 kGy in comparison with the control (nonirradiated). It is of interest to note that irradiation at low doses induced less modifications.

For the strain ATCC 49174, the irradiation treatment modified the FA by diminishing the percentage of the UFA in the membrane. At 1 kGy, the saturated fatty acids (SFA) with 18 carbons chain length (C_{18}) increased at the expense of the C_{16} SFA. At 3·5 kGy, a decreasing of the concentration of the UFA were noticed. This decrease was because of the lowering of the C_{16} containing one insaturation ($C_{16:1}$), the lowering of the C_{18} containing one insaturation ($C_{18:1}$) and the increase of the C_{18} SFA.

For the strain RL1, the radiations had a contrary effect on the lipids. The composition in FA of this strain showed an increase of the UFA following the treatments.

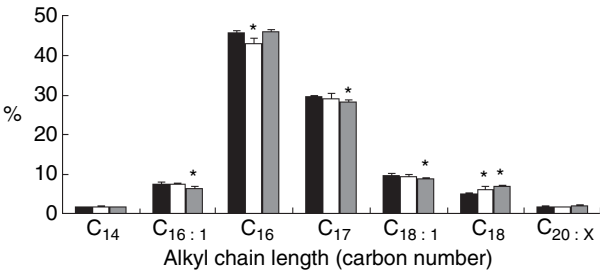

Figure 1 Fatty acids composition of the membrane of *P. agglomerans* strain ATCC 49174. (■) Control (nonirradiated), (□) 1 kGy irradiated, (▨) 3·5 kGy irradiated, $C_{20:X}$, includes all variants of the 20 carbons UFA, *Significantly different ($P \leq 0{\cdot}05$) as compared with the control (nonirradiated).

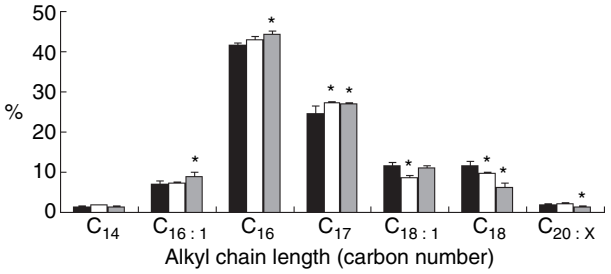

Figure 2 Fatty acid composition of the membrane of *P. agglomerans* strain RL1. (■) Control (nonirradiated), (□) 1 kGy, (▨) 3·5 kGy, $C_{20:X}$, includes all variants of the 20 carbons UFA. *Significantly different ($P \leq 0{\cdot}05$) as compared with the control (nonirradiated).

At 1 kGy the shift seemed to take place in the length of the FA chain itself. The concentration of the C_{18} FA diminished while de C_{17} FA increased. At 3·5 kGy, the shift to a smaller carbon chain seemed to take place, but in a smaller proportion. The C_{18} and $C_{20:X}$ decreased, but this time the C_{16} and the C_{17} were increased. Also, the $C_{18:1}$ concentration was not affected and the $C_{16:1}$ was augmented.

The FA ratio (saturated/unsaturated) present in *P. agglomerans* ATCC 49174 membrane (a) and RL1 (b) are shown in Fig. 3. This proportion demonstrates more easily the relation between the effects of γ-irradiation and the saturation level of the FA. An increasing ratio would indicate an increase of the SFA and/or a reduction of the UFA. A reduction of the ratio would indicate the decrease of the SFA and/or an increase of the UFA. For both strains significant modifications ($P \leq 0{\cdot}05$) seemed to take place at 3·5 kGy. However, no significant ($P > 0{\cdot}05$) effect was observed on samples treated at 1 kGy. For the strain ATCC 49174 C_{16}, C_{18} and total UFA ratios were significantly diminished ($P \leq 0{\cdot}05$). While for the strain RL1 the C_{16}, C_{18} and total UFA ratios increased significantly ($P \leq 0{\cdot}05$).

The fractions of the muropeptides composition of *P. agglomerans* strain ATCC 49174 and strain RL1 are shown in Fig. 4. In Fig. 4, each fraction (1–10) represents a 10 min interval during the analysis of the muropeptides using HPLC and the eleventh one is the sum of all peptides eluted between 100 and 150 min. According to (Glauner 1988), peaks obtained with retention times (RT) between 0 and 55 min are generally monomers, with RT between 55 and 83 min are dimers, those with RT between 84 and 105 min are trimers, and those with RT more than 105 min are oligomers. Many significant differences ($P \leq 0{\cdot}05$) were noticed at various irradiation doses for both bacterial strains. For the strain ATCC 49174 treated at 1 kGy, oligomers seemed to be higher in percentage to the expense of the smaller peptides. At 3·5 kGy, moderate changes to the overall composition were observed, but the shift to greater size peptides was still noticeable. With regard to the strain RL1 treated at 1 kGy, few changes were observed. At 3·5 kGy, a tendency in the diminution of the trimers was observed, whereas every other type were increased.

Discussion

Biological membranes are essential for the cell integrity, providing a barrier between the inside and outside environments for the cell (Pedersen *et al.* 2006). These barriers act as support to different proteins which are involved in several different cell functions such as signal transduction, solute transport, protein targeting and trafficking, etc. (Edidin 2003). A number of studies suggest that membranes can sense extreme environmental changes and particularly, the presence of ROS in the media. As reported by several works (Tatzer *et al.* 2002; Shigapova *et al.* 2005), the degree of fatty acyl desaturation of membrane lipids is considered to be a critical factor in membrane fluidity. This phenomenon is closely related to the membrane remodelling which is a cellular response to environmental stresses able to protect the cells from toxic ROS. The relation between fatty acyl desaturation and bacterial adaptation was reported for a number of strains

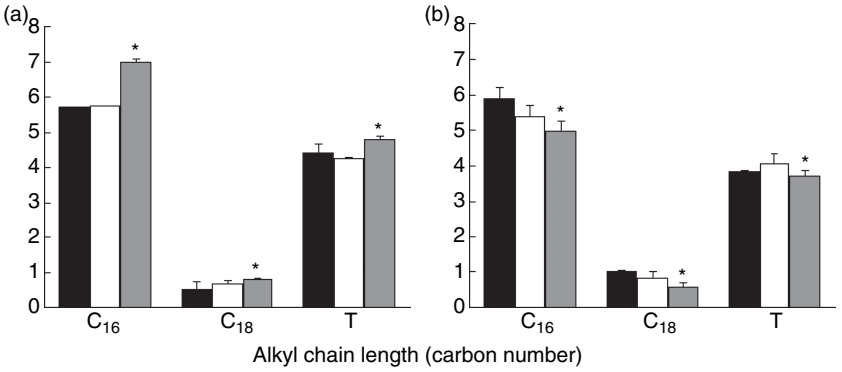

Figure 3 Fatty acids ratio (saturated/unsaturated) for strain *P. agglomerans* ATCC 49174 (a) and strain RL1 (b). (■) Control (nonirradiated), (□) 1 kGy, (▨) 3·5 kGy, T total fatty acids. *Significantly different ($P \leq 0{\cdot}05$) as compared with the control (nonirradiated).

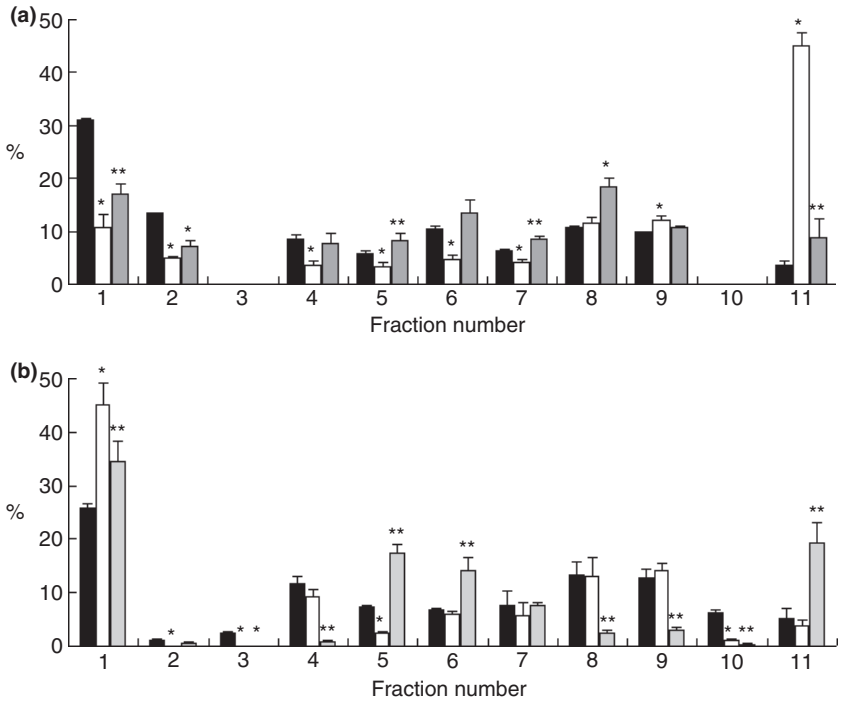

Figure 4 Effect of gamma radiation on the muropeptide composition of *P. agglomerans* strain ATCC 49174 (a) and strain RL1 (b). *Significantly different ($P \leq 0{\cdot}05$) as compared with the control (nonirradiated). **Significantly different ($P \leq 0{\cdot}05$) as compared with the control (nonirradiated) and irradiated at 1 kGy. The whole run has been separated in 10 fractions. The first 10 fractions represent the first 10 min intervals and the 11th one represents the total area from a 100 min till the end.

exposed to heat, cold or oxidative damage (Guerzoni *et al.* 2001; Shigapova *et al.* 2005). Generally, this implies that membrane lipids play an essential role in microbial adaptation under different environmental changes.

The *P. agglomerans* bacterial strains ATCC 49174 and the strain RL1 were used to investigate the effects of γ-irradiation on the cellular membrane FA and the muropeptides of the peptidoglycan. According to the obtained results, irradiation treatments influence the composition of the bacterial wall. However, modifications of the membrane by γ-irradiation were not important when compared with those treated with antimicrobial agents such as essential oils (i.e. thymol) targeting directly on the membrane (Di Pasqua *et al.* 2007). It was also noticed that modifications by irradiation of membrane FA are generally dose dependant. Probably, more energy applied to the bacteria resulted in an augmentation in the number of modifications of the FA. It could be hypothesized that the increase of certain types of FA is related to an overproduction by the bacteria. For the strain RL1, the desaturation of the FA could be related to an increase or activation of an enzyme activity. According to Aguilar and de Mendoza (2006) stress can induce such activity. It is worth to note that UFA can scavenge the ROS and an augmentation in the concentration of UFA could result in favour to the radiotolerance. The change in the length of the FA could be the direct consequence of the bacterium remodeling its membrane composition. This mechanism of defense toward environmental stresses could be the bacterial response to the destructive effect of the ROS. Although, the hypothesis based on the activation of a specific defense mechanism could be well supported as it is known that bacteria have the ability to modify it membrane composition to adapt itself to its environment (Zhang and Rock 2008). In the present study, results obtained were demonstrated that after irradiation, modification of UFA was significantly increased ($P \leq 0{\cdot}05$) for the RL1. In this case, the explanation could be based on the mechanism (as mentioned above) designed to ameliorate the environmental changes on the physical state of the cell membrane through membrane FA desaturation. Consequently, the involvement of enzymes as desaturases seems essential in this phenomenon. Moreover, numerous reports were demonstrated that the desaturases are the key enzymes in carotenoid biosynthesis pathway (Xu *et al.* 2007). It is of interest to mention that the carotenoids are not involved in the DNA repair process directly, but participated in the protective mechanism of bacteria cells as ROS scavengers. The carotenoids can quench harmful ROS generated from water radiolysis to prevent oxidative damage to proteins (including DNA repair proteins), enzymes and membrane lipid peroxidation (Stahl *et al.* 1998). Previous works done in our laboratory with *P. agglomerans* strains ATCC 49174 and RL1 have demonstrated that γ-irradiation treatments can induce the production of carotenoids in bacteria (Dussault *et al.* 2008). Although, the induce production did not resulted in a greater radioresistance. As carotenoid production rely

on many enzymatic reactions, the previous finding support the fact that overall enzymatic activity was increased by ether the augmentation of the number of enzyme units or by the modification of the enzyme itself.

In comparing with the modifications of the membrane FA, the alteration of the peptidoglycan's muropeptides did not seemed to be dose dependant. For the strain ATCC 49174 treated at 1 kGy, the muropeptides had a larger size. As linking between peptides of the murein is enzymaticaly regulated, the modifications made by the irradiation treatment could be related to the increasing activity of the transpeptidases, the enzyme responsible for the creation of crosslink between muropeptides. Although, at 3·5 kGy, bigger peptides, in length, could be observed as compared with the nonirradiated control. For the strain RL1, irradiation at 1 kGy seemed to inhibit the formation of the crosslinking bridges. At 3·5 kGy, the increase of the peptide length was noticed as well as in the ATCC strain. The length increase was greater for the strain RL1 than the ATCC. This difference could give an advantage toward radioresistance. Stronger murein could provide a longer period before the cell loose its integrity under radiation exposure and this would allow the bacteria to repair its cell components. In conclusion, DNA is the most fatal target concerning the damages done by γ-irradiation, but its effect on the bacterial membrane it noticeable and could play an important role on the cellular response and ability to survive this harsh environment.

Acknowledgements

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