Photochemistry and Photobiology, 20**, **: *–*

Research Article

Homeophasic Adaptation in Response to UVA Radiation in *Pseudomonas aeruginosa*: Changes of Membrane Fatty Acid Composition and Induction of *desA* and *desB* Expression

Magdalena Pezzoni[1]*, Marleen De Troch[2], Ramón A. Pizarro[1] and Cristina S. Costa[1]

[1]Departamento de Radiobiología, Comisión Nacional de Energía Atómica, San Martin, Argentina
[2]Marine Biology Research Group, Ghent University, Ghent, Belgium

Received 12 August 2021, accepted 22 October 2021, DOI: 10.1111/php.13548

ABSTRACT

In bacteria, exposure to changes in environmental conditions can alter membrane fluidity, thereby affecting its essential functions in cell physiology. To adapt to these changes, bacteria maintain appropriate fluidity by varying the composition of the fatty acids of membrane phospholipids, a phenomenon known as homeophasic adaptation. In *Pseudomonas aeruginosa*, this response is achieved mainly by two mechanisms of fatty acid desaturation: the FabA–FabB and DesA–DesB systems. This study analyzed the effect of ultraviolet-A (UVA) radiation—the major fraction of solar UV radiation reaching the Earth's surface—on the homeophasic process. The prototypical strain PAO1 was grown under sublethal UVA doses or in the dark, and the profiles of membrane fatty acids were compared at early logarithmic, logarithmic and stationary growth phases. In the logarithmic growth phase, it was observed that growth under sublethal UVA doses induced the expression of the desaturase-encoding genes *desA* and *desB* and increased the proportion of unsaturated fatty acids; in addition, membrane fluidity could also increase, as suggested by the indices used as indicators of this parameter. The opposite effect was observed in the stationary growth phase. These results demonstrate the relevant role of UVA on the homeophasic response at transcriptional level.

INTRODUCTION

The bacterial membrane is very important to bacterial survival because it constitutes a barrier between cells and the environment, responding to external changes (1). The main components of bacterial membranes are phospholipids, which are responsible for their fluidity, function and structure (2,3). Exposure to changes in temperature, osmolarity, pH, oxidative stress and organic compounds, among other factors, can alter membrane fluidity, affecting its functions as barrier and energy transducer, and its role in the process of cell division (4). In order to adapt to these changes, bacteria maintain appropriate fluidity by varying the length, the saturation degree, the *cis/trans* monounsaturated (*cis/trans* MUFA) ratio and the branching of phospholipids acyl chains, as well as the proportion of cyclopropane fatty acids (CFAs) (5–8). This phenomenon, known as homeoviscous or homeophasic adaptation (5,9,10), involves activation of gene expression and/or protein activity to maintain optimal cell viability (4). For example, in *Pseudomonas* strains, the ratio between unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs) changes when cells are exposed to diverse membrane-altering conditions (low temperatures, ethanol, hydrocarbons, toluene) in order to adjust membrane fluidity to the new condition (11–17). The switch between planktonic and sessile states also involves changes in the composition of membrane phospholipids, with the consequent modification of membrane fluidity, indicating that membrane composition is regulated not only by cell stressors but also by lifestyle (18,19). In *Pseudomonas aeruginosa*, homeophasic adaptation is achieved mainly by two mechanisms of fatty acid desaturation. The main synthetic pathway of UFA synthesis is through FabA and FabB enzymes, which introduce double bonds in nascent acyl chains (20). In addition, two oxygen-dependent Δ9 desaturases, DesA and DesB, collaborate with the Fab system in UFA synthesis (21). DesA is a Δ9 desaturase that introduces double bonds into existing FA chains esterified to lipids, at the position sn-2. It is assumed that DesA collaborates with the FabAB pathway under aerobic conditions (21). DesB is another oxygen-dependent Δ9 desaturase that inserts a double bond into full length SFAs, suggesting that this enzyme selectively desaturates exogenous FAs (21). DesB is also involved in adaptation to osmotic stress and in pathogenic processes (22,23).

Pseudomonas aeruginosa is a versatile bacterium present in terrestrial and aquatic environments, and an opportunistic human pathogen. It has complex regulatory genetic mechanisms which explain its great adaptive ability to face environmental changes such as ultraviolet-A (UVA, 400–315 nm) radiation. UVA, the main fraction of solar ultraviolet radiation reaching the Earth's surface, is one of the main environmental stress factors for *P. aeruginosa*. It has been shown that the lethal effects of UVA on bacteria are mainly due to oxidative damage of the bacterial membrane (24,25). This damage is produced by the action of reactive oxygen species (ROS) generated by the absorption of light by endogenous photosensitizers (*e.g.*, flavoproteins, cytochromes, quinones) in presence of oxygen (26,27). It includes lipid peroxidation, increased saturation of fatty acids (FAs), and protein carbonylation and aggregation (28–30). The lethal effects

*Corresponding author email: pezzoni@cnea.gov.ar (Magdalena Pezzoni)

of UVA radiation have been exploited for disinfection purposes in strategies such as SODIS (Solar DISinfection of natural waters) and photocatalytic treatments (31,32).

On the other hand, it has also been demonstrated that exposure of bacteria to low UVA doses produces adaptive effects such as a transient inhibition of bacterial growth, called growth delay, proposed as an adaptive mechanism against the lethal and mutagenic effects of higher doses of UVA and solar radiation (33,34). In order to better understand bacterial adaptive responses to this radiation, global transcriptomic responses to UVA have been studied in several microorganisms (35–38). These studies revealed that activation of genes that encode enzymes responsible for ROS detoxification and DNA repair is a common response to UVA exposure. In the case of *P. aeruginosa*, the first results on adaptive responses to UVA refer to activation of genes involved in DNA repair, such as *recA* and *din* (39). It has been reported that exposure to low UVA doses provides a protective effect against subsequent high UVA doses. This phenomenon was found to depend on *relA*, the main gene responsible for the synthesis of the master transcriptional regulator ppGpp (40). In addition, low UVA exposure has also been found to activate transcription of the catalase-encoding genes *katA* and *katB*. Their products, KatA and KatB, are involved in the detoxification of hydrogen peroxide, a toxic compound involved in UVA damage (41). Recent studies report the induction of biofilm formation by exposure to sublethal UVA doses (42). This depends on the activation of quorum sensing genes (43) and constitutes a clear adaptive response, given the greater resistance to UVA of biofilms compared to planktonic cells (44).

In order to learn more about the adaptive responses of bacteria to UVA, we studied the role of UVA in the homeophasic process in *P. aeruginosa* by analyzing changes in FA composition of the cell membrane and the associated genetic response. This knowledge would be useful to help to understand the general adaptive response of *P. aeruginosa* to high UVA doses, both in the natural environmental and during the application of antibacterial techniques using UVA.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *P. aeruginosa* strain used in this study was the wild-type PAO1 (45). Bacterial cultures were routinely grown at 37°C with shaking in complete LB broth (10 g tryptone, 5 g yeast extract and 5 g NaCl, bringing the volume up to 1000 mL in distilled water). For solid medium, agar was added at 15 g L^{-1}.

Irradiation source. Cell suspensions were irradiated using two Philips TDL 18W/08 tubes (95% of UVA emission at 365 nm). The incident fluence under our experimental conditions was measured at the surface of the suspensions with a 9811.58 radiometer (Cole-Parmer Instruments). The UVA tubes were mounted on aluminum anodized reflectors to enhance the fluence rate on the section to be irradiated.

Growth under UVA irradiation. Mid-exponential cultures (OD_{650} 0.3) were diluted to OD_{650} 0.05 in LB medium and divided into two 30 mL fractions, each of which was placed in a glass beaker (4.5 cm internal diameter). The beakers were placed in a multichamber coupled to a thermocycler bath so that the temperature of the suspensions was maintained at 37°C. One of the fractions was irradiated from above at a fluence rate of 25 Wm^{-2} at the level of the free surface of the suspension, while the other fraction was covered with a black plastic sheet (dark control). The cell suspensions were stirred continuously with a magnetic bar. Cell growth of irradiated and control suspensions was followed by measuring OD_{650}. The applied fluence may be encountered normally in the environment (46).

Chemiluminescence assays. Production of photoemissive species was followed by means of a liquid scintillation system in the out-of-coincidence mode (47). This phenomenon has been attributed to photon emission by excited carbonyl groups and singlet O_2 dimers arising from the decomposition of membrane lipid peroxides (48), which in turn are associated with an increase in reactive oxygen species generated by UVA radiation (24). For this purpose, 5 mL aliquots were taken during bacterial growth, every 30 min for the first hour and then every 60 min, and quickly transferred to the scintillation system, equipped with photomultipliers sensitive in the blue region up to 600–650 nm (Tri-Carb 1500; Packard Instruments). Chemiluminescence values were expressed as counts per minute (cpm) per OD_{650} unit.

FA analysis. FA analysis was performed by the FAME (fatty acid methyl esters) analysis technique. For this purpose, 10 mL aliquots of logarithmic (OD_{650} 0.1 and 0.3) and 24 h stationary cultures grown under UVA or in the dark were centrifuged for 10 min at 21.000 g and the cell pellets were frozen at −80°C and lyophilized. FAME were prepared from these samples using a direct transesterification procedure with 2.5% (v:v) sulfuric acid in methanol as described by De Troch *et al*. (49). The internal standard, the FA nonadecanoic (19:0, Fluka 74208, 5 μg) was added prior to the transesterification procedure. FAME were extracted twice with hexane. The hexane was evaporated and the residue was dissolved in 200μL of this solvent. FA composition was analyzed using a gas chromatograph (GC, HP 7890B; Agilent Technologies, Diegem, Belgium) equipped with a flame ionization detector (FID) and connected to an Agilent 5977A Mass Selective Detector (Agilent Technologies, Diegem, Belgium). The GC was further equipped with a PTV injector (CIS-4, Gerstel, Mülheiman der Ruhr, Germany). A 60 m × 0.25 mm × 0.20 μm film thickness HP88 fused-silica capillary column (Agilent Technologies) was used for the gas chromatographic analysis, at a constant Helium flow rate (2 mL min^{-1}). The injected sample was split equally between the MS and FID detectors at the end of the GC column using an Agilent capillary flow technology splitter. The oven temperature program was as follows: at the time of sample injection, the column temperature was 50°C for 2 min, then gradually increased at 10°C min^{-1} to 150°C, followed by a second increase at 2°C min^{-1} to 230°C. The injection volume was 2 μL. The injector temperature was held at 30°C for 0.1 min and then ramped at 10°Cs^{-1} to 250°C and held for 10 min. The transfer line for the column was maintained at 250°C. The quadrupole and ion source temperatures were 150°C and 230°C, respectively. Mass spectra were recorded at 70 eV ionization voltage over the mass range of 50–550 mz^{-1} units. Analysis of the chromatograms was done with Agilent MassHunter Quantitative Analysis software (Agilent Technologies, Diegem, Belgium). The signal obtained with the FID detector was used to generate quantitative data of all the compounds. Peaks were identified based on their retention times, compared with external standards as a reference (Supelco 37 Component FAME Mix; Sigma-Aldrich, Overijse, Belgium) and by the mass spectra obtained with the Mass Selective Detector. Quantification of FAME was based on the area of the internal standard (19:0) and on the conversion of peak areas to the weight of the FA by a theoretical response factor for each FA (50,51).

Membrane fluidity indices. Three indices based on FA composition were employed to indirectly infer changes in membrane fluidity: UFAs/SFAs ratio, *cis*MUFAs/*trans*MUFAs ratio and Membrane Viscosity Index. The latter takes into account the saturation degree, the *cis*/*trans* ratio and the proportion of CFAs (MVI = SFAs +*trans* MUFAs/*cis* MUFAs + CFAs) (4). Higher values of the two first indices suggest higher membrane fluidity (7,8); on the contrary, higher values of MVI suggest lower membrane fluidity (4).

Quantitative real-time (qRT)-PCR. Total RNA from the PAO1 strain grown under UVA or in the dark was extracted at the logarithmic (OD_{650} 0.1 and 0.3) and stationary growth phases (24 h cultures) using a Total RNA Extraction kit (RBC Biosciences). After treatment with DNase I, cDNA was obtained using random hexamers (Promega) and avian myeloblastosis virus reverse transcriptase (Promega) following the manufacturer's instructions. qRT-PCR was performed using a LightCycler (DNA Engine; MJ Research) and Real-Time PCR Mix (EvaGreen qPCR Mix Plus, no Rox). For quantification of *desA* mRNA, assays were performed using the primers desAFw (5′ GAAGAGCTGCA CAACAACCA 3′) and desARv (5′ GGAAGCGGTTGTTGAGGATC 3′); for quantification of *desB* mRNA, assays were performed using the primers desBFw (5′ CTGGGCAAGATCCTCGAGAA 3′) and desBRv

(5′ GATATGGTTGTGGGTGTGCC 3′). *desA* and *desB* primers were designed using the Primer3 program (Whitehead Institute for Biomedical Research). *fabA* mRNA was quantified by using the primers fabAFw (5′ CCGGGTAACGCGCAACT 3′) and fabARv (5′ CCGACATCGCTGAT GTGAAC 3′), which have been employed previously (52). The 16S rRNA gene was used as reference for normalization of expression levels of target genes in each condition by employing the primers 5′ AGCTTGCTCCTTGATTCAGC 3′ and 5′ AAGGGCCATGATGACT TGAC 3′ (38). The cycling conditions for *fabA*, *desA*, *desB* and 16S rRNA genes were as follows: denaturation at 95°C for 5 min, 40 cycles at 95°C for 20 s, 56°C for 15 s and 72°C for 15 s, with fluorescence acquisition at 80°C in single mode. Relative changes in the expression of individual genes between the treated and control condition were obtained through the relative standard curve method (53).

Statistical analysis. All samples were analyzed in triplicate. Data are represented as means ± standard errors (SE). The significance between treatments was evaluated by an unpaired two-tailed Student's *t*-test with confidence levels > 95% (*i.e.*, $P < 0.05$ was considered significant).

RESULTS

Effect of UVA on cell growth and oxidative damage

In order to analyze the effect of UVA on the homeophasic adaptation process, we first evaluated the growth and the extent of oxidative damage of the wild-type strain PAO1 exposed to different UVA fluence rates (results not shown). A condition that did not alter cell viability significantly but produced certain oxidative damage was further employed throughout this study (Fig. 1). When the PAO1 strain was cultured in LB medium under UVA delivered at a fluence rate of 25 Wm^{-2}, it suffered a small growth delay compared to the control culture maintained in the dark (Fig. 1a). However, the viable cell count was not affected by the treatment (data not shown). To evaluate *in vivo* whether the growth delay could be associated with oxidative damage, the ultraweak chemiluminescence procedure was employed. A strong peak of light production was observed at the beginning of the exposure and the chemiluminescence values were clearly higher in UVA treated cells, suggesting oxidative cell damage by these UVA doses (Fig. 1b). This condition was considered suitable for the study of homeophasic adaptation.

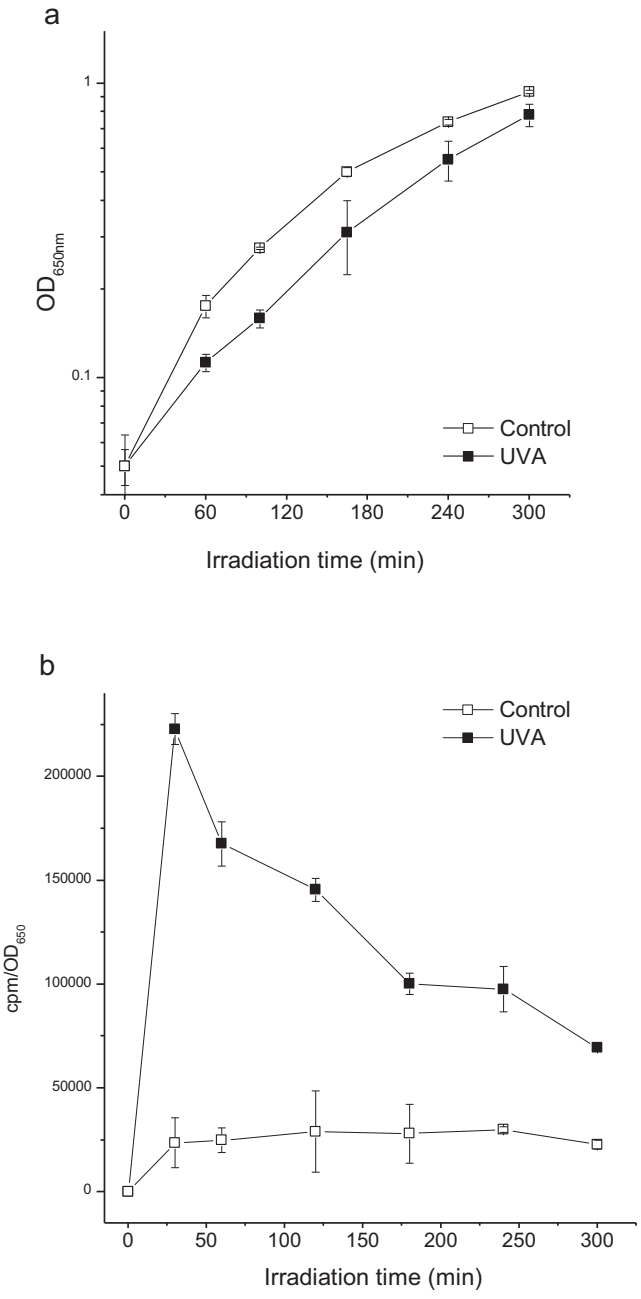


Figure 1. Effect of exposure to sublethal UVA doses (fluence rate 25 Wm^{-2}) on the growth (a) and chemiluminescence values (b) of the PAO1strain. Control cells were grown under similar conditions but kept in the dark.

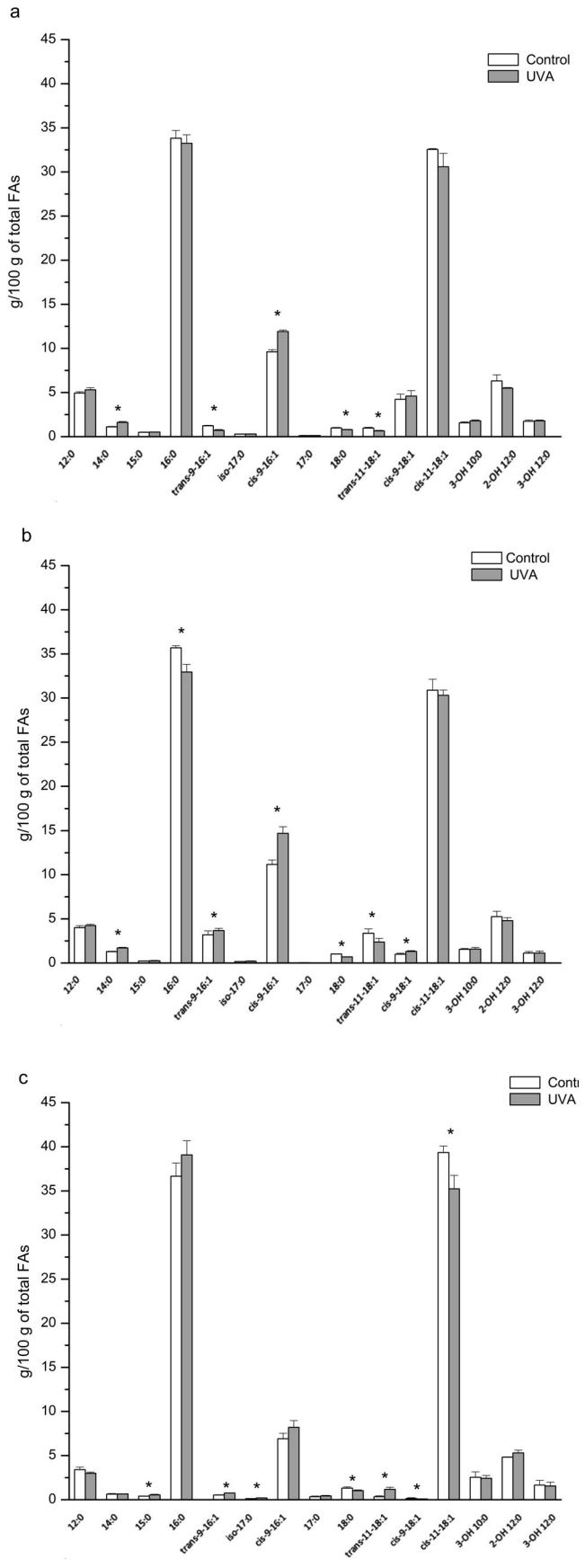
Effect of UVA on membrane FA composition

The FA profiles of cells grown under the conditions of UVA exposure described above and their respective controls are shown in Fig. 2. Three growth stages were analyzed: early logarithmic (OD_{650}0.1, Fig. 2a), logarithmic (OD_{650}0.3, Fig. 2b) and stationary (24 h cultures, Fig. 2c). In all growth stages studied, the main FAs were palmitic acid (16:0), cis vaccenic acid (cis-11-18:1), and to a lesser extent, palmitoleic acid (cis-9-16:1). At the logarithmic growth phase, the most relevant change due to the treatment was the increase in the UFA cis-9-16:1. This effect was more marked at OD_{650} 0.3, where, furthermore, there was a slight increase in the UFA trans-9-16:1, and a decrease in the analogous SFA 16:0 (Fig. 2b). These effects were not observed in the stationary growth phase (Fig. 2c).

In order to perform a global analysis, FAs were grouped into different classes: (Fig. 3). Comparative analysis between these groups revealed a significant increase in UFAs and decrease in SFAs at the logarithmic phase (OD_{650} 0.3, Fig. 3b), an increase in SFAs at the stationary phase (Fig. 3c), and a reversion of the proportion of *trans*MUFAs between early logarithmic and stationary growth phases (Fig. 3a and c). Surprisingly, CFAs were not detected, even in the stationary phase, when they are expected.

Different indices based on FA composition have been used as indicators of membrane fluidity, which is a key parameter for dealing with membrane stressors. Figure 4 shows the effect of

Figure 2. Membrane FA profiles in PAO1 cells harvested at logarithmic (OD_{650} 0.1 and OD_{650} 0.3) and stationary growth phases (a–c), grown under UVA exposure (fluence rate 25 Wm^{-2}) or in the dark. Values are presented as the mean ± SE of at least three independent assay, in g per 100 g of total FAs. *$P < 0.05$



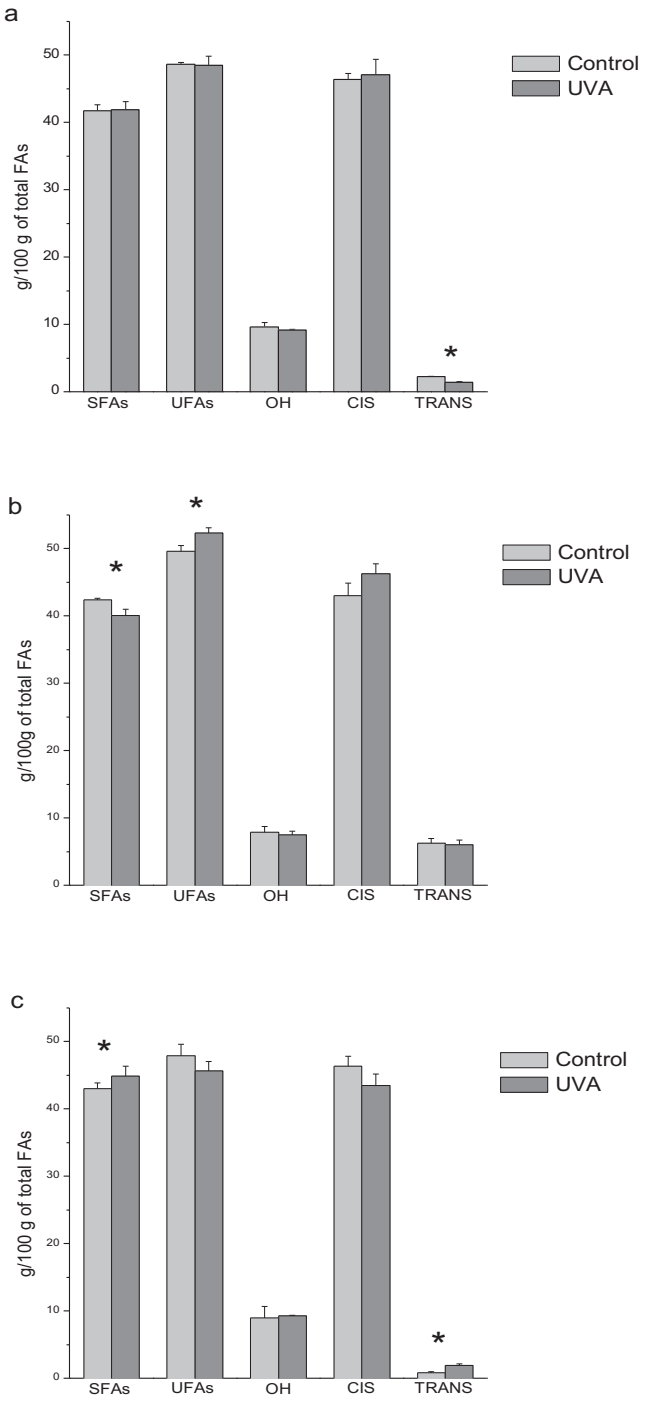


Figure 3. Comparison of FA classes in PAO1 cells grown under UVA exposure (fluence rate 25 Wm^{-2}) or in the dark, until OD_{650} 0.1 (a), OD_{650} 0.3 (b) and stationary growth phase (c). SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; OH, hydroxyl fatty acids; CIS, *cis*-monounsaturated fatty acids; TRANS, *trans*-monounsaturated fatty acids.*$P < 0.05$.

exposure to sublethal UVA during cell growth on membrane fluidity indices. The UFAs/SFAs ratio (Fig. 4a) and the MVI index (Fig. 4c) suggest that UVA increased membrane fluidity at the logarithmic growth phase (OD_{650} 0.3). This effect was only observed at the early growth phase (OD_{650} 0.1) when the *cis* MUFAs/*trans*MUFAs ratio was used as fluidity index (Fig. 4b).

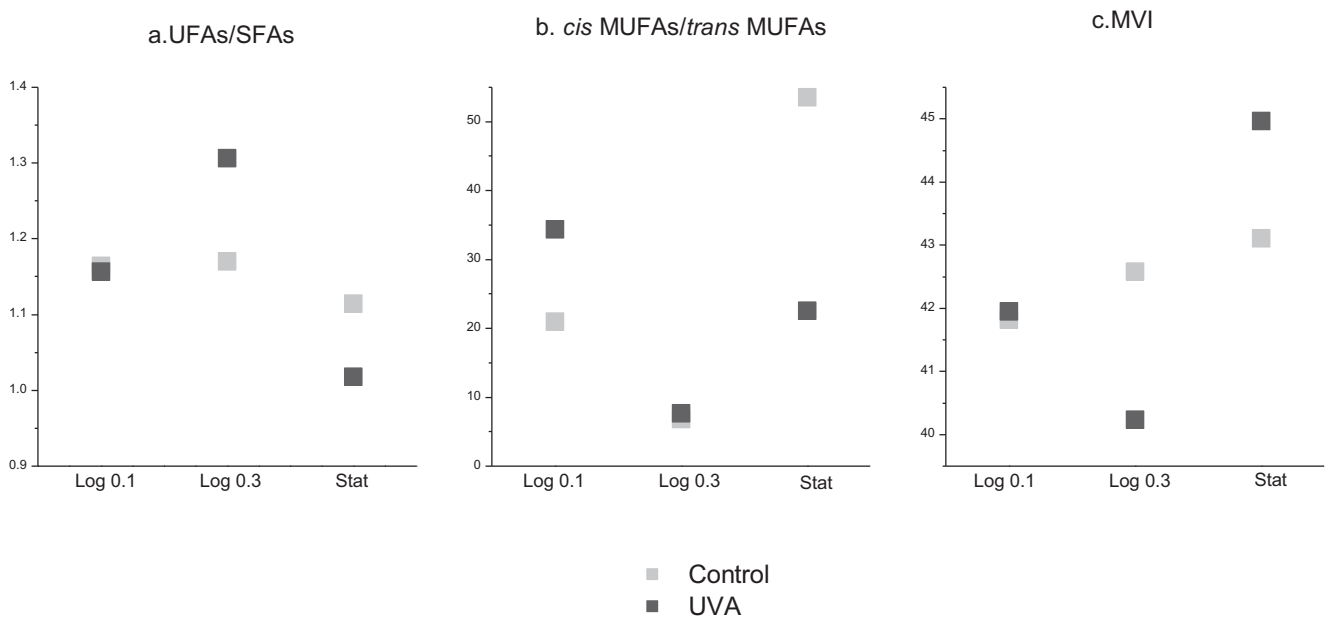


Figure 4. Membrane fluidity indices UFAs/SFAs (a), *cis*MUFAs/*trans*MUFAs (b) and MVI (c) of PAO1 cells grown under UVA exposure (fluence rate 25 Wm^{-2}) or in the dark, until OD_{650} 0.1 (Log 0.1), OD_{650} 0.3 (Log 0.3) and stationary growth phase (Stat).

On the other hand, at the stationary growth phase, the three indices suggested lower membrane fluidity in UVA-treated cells compared to the controls (Fig. 4a–c).

Effect of UVA on expression of *fabA*, *desA* and *desB* genes

To explain the changes observed on membrane FA composition after UVA exposure, we conducted qRT-PCR assays to analyze the effect of UVA on the expression of three *P. aeruginosa* desaturase-encoding genes: *fabA*, *desA* and *desB*. Figure 5 shows that at the logarithmic growth phase there was significant induction of *desA* (OD_{650} 0.1 and 0.3) and *desB* (OD_{650} 0.1); no change in *fabA* expression was seen (Fig. 5a and b). On the contrary, at the stationary growth phase, the expression of the three desaturase genes was downregulated by the radiation compared to control cells (Fig. 5c).

DISCUSSION

Studies on bacterial adaptive mechanisms to UVA radiation may provide better understanding of the global response to this environmental stressor. "Learning from nature" can provide new insights into bacterial stress response and lead to the development of better antibacterial applications. UVA, as the main component of solar UV radiation, is a stress agent with strong effects on bacteria (29,54). As a result, its use has been proposed in diverse disinfection technologies (31,32,55).

This study demonstrated in *P. aeruginosa* that homeophasic process constitutes a relevant adaptive mechanism triggered by UVA radiation. The study was performed with bacteria grown under continuously delivered sublethal doses of UVA radiation, which did not affect cell viability but produced the necessary damage to trigger an adaptive response. Like other authors, we employed the ultra-weak chemiluminescence method as an indicator of oxidative damage (48,56). Although this is not a very specific methodology, previous results obtained by our group under identical irradiation conditions demonstrated the consistency between chemiluminescence values and the manifestation of oxidative stress indicators, such as the hydrogen peroxide-dependent *katA* and *katB* expression and induction of catalase activity (41).

To the best of our knowledge, this study demonstrates for the first time that in *P. aeruginosa*, growth under low UVA doses induces the expression of *desA* and *desB* desaturase genes during the logarithmic growth phase, with a consequent increase in the proportion of UFAs. It should be noted that changes in the expression of these genes exceed those obtained by FAME analysis, perhaps due to restrictions under our working conditions in the membrane composition of *Pseudomonas*. As suggested by Guerzoni *et al*. in *Lactobacillus helveticus* exposed to sublethal doses of oxidative stress agents, a protective effect in actively growing cells could be ascribed to the increase in oxygen consumption related to higher desaturase activity, thus lowering the oxygen availability for ROS generation (57).

A contrary tendency was observed after 24 h of continuous incubation under UVA exposure. At this growth stage, the desaturase genes *fabA*, *desA* and *desB* were clearly under-expressed compared to the dark controls. Thus, in contrast to what was observed at the logarithmic growth phase, a higher SFA proportion was detected in irradiated cells. It should be noted that during the stationary growth phase, the bacteria face new stress factors such as nutrient limitation and chemical stressors. Among other changes, the proportion of SFAs increases, membrane fluidity decreases, and cells reprogram their gene expression pattern in order to survive the new situation (17,19,58). These changes seem to be stronger when high total doses of UVA are accumulated, and more defenses must be deployed. In this regard, results similar to ours were reported in a study where the changes in

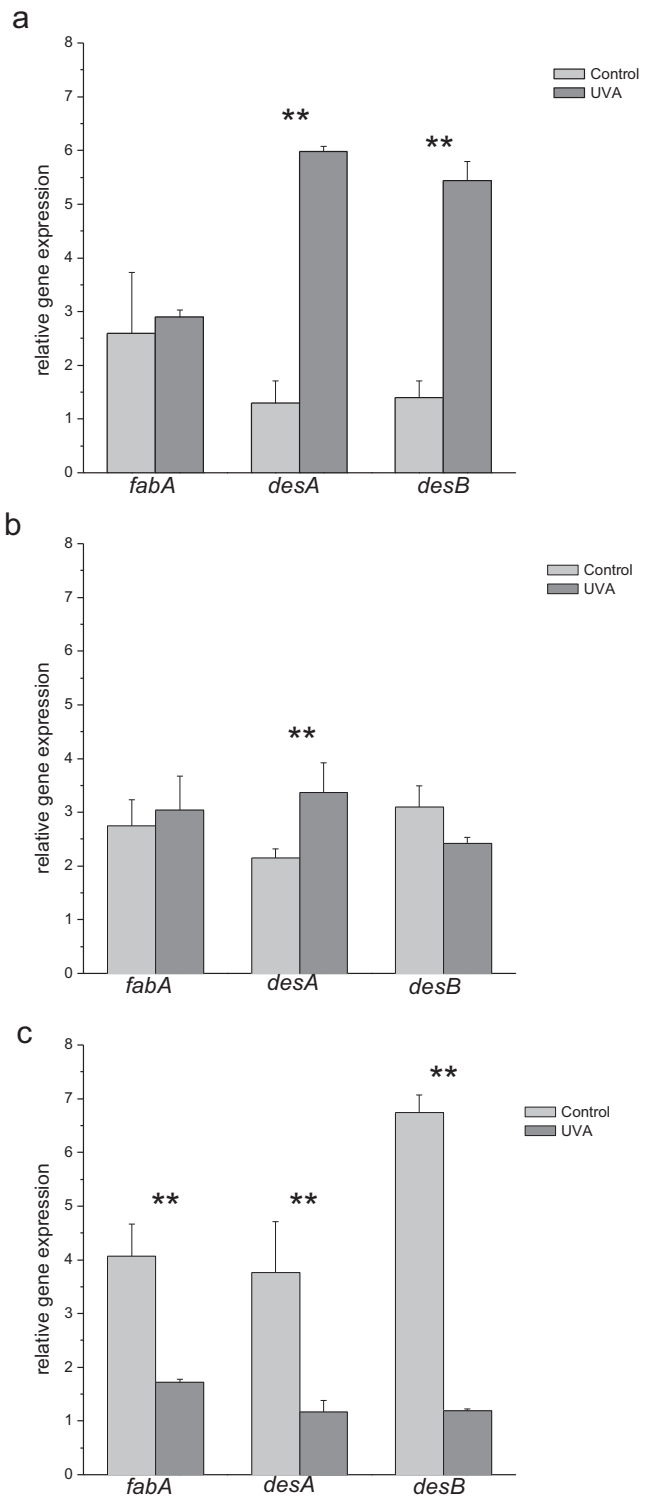


Figure 5. qRT-PCR of *fabA*, *desA* and *desB* genes from the PAO1 strain grown under sublethal UVA radiation (fluence rate 25 Wm^{-2}) or kept in the dark until OD_{650} 0.1, OD_{650} 0.3 and stationary growth phase. The 16S rRNA gene was used as reference for normalization in each condition. Data are presented as mean ± SE of at least three independent experiments with three replicates. $^{**}P < 0.005$.

membrane FA composition of *P. aeruginosa* cells adapted to ultraviolet-C radiation (UVC, 280–100 nm) were analyzed (59). UVC is used in germicidal lamps and its energy is directly absorbed by the genetic material, although alterations in membrane were also associated with UVC damage (60). In this study, it was observed that adaptation to UVC also involves an increase in UFAs compared to SFAs only in the logarithmic growth phase (59). It has been reported that the increase in UFAs is toxic under exposure to strong oxidative agents such as continuous UVA radiation (61, 62). Further studies are needed, taking into account the growth phase, the UVA fluence rate and the UVA total doses, among other factors, in order to gain more in-depth understanding of the role of membrane FA composition in the response to UVA radiation.

As a consequence of UVA exposure, an increase of membrane fluidity has been suggested in the logarithmic phase, while the opposite effect was seen in the stationary growth phase, according to data obtained from membrane fluidity indices based on FA composition. It has been largely demonstrated that changes in the composition of membrane FA may modify the membrane fluidity; however, it should be noted that the use of membrane fluidity indices is insufficient to assess changes in this parameter (4). The membrane fluidity may also depend on the presence of other membrane constituents such as carotenoids (63), which are known to be synthesized by bacteria as *P. aeruginosa*, among others (64). Then, further measurements of bacterial membrane fluidity by appropriate physicochemical methods (65) should be performed to confirm these observations.

In order to assess the impact of changes in the composition of membrane FAs on gene regulation, it was demonstrated in *P. aeruginosa* that membrane fluidity, in addition to its structural and functional role, is a signal for induction of adaptive mechanisms, given its ability to regulate gene expression (4, 66). The changes in *desA* and *desB* regulation observed in this study could be triggered by UVA-promoted changes in membrane composition and fluidity, which in turn could be the signal for the activation or repression of their transcriptional regulators.

In summary, this study showed the potential of FA profile as an indicator of stress response in bacteria facing low UVA doses, and the ability of UVA to regulate homeophasic adaptation processes at the transcriptional level. It would be interesting to conduct further studies on the mechanisms involved in the regulation of desaturase gene expression by UVA in order to gain better understanding of this adaptive phenomenon.

Acknowledgements—The excellent technical assistance of Ms. P. Pereyra Schuth (Comisión Nacional de Energía Atómica, Argentina) and Dr. Bruno Vlaeminck (Marine Biology Research Group, Ghent University) is gratefully acknowledged. M.P.is a researcher at Consejo Nacional de Investigaciones Científicas yTécnicas (CONICET, Argentina).

Financial support for this research was received from Comisión Nacional de Energía Atómica (Argentina) and Agencia Nacional de Promoción Científica y Tecnológica (Argentina, PICT 0598). The research leading to the results presented in this publication was also conducted using infrastructure funded by EMBRC Belgium – FWO agreement 20151029-03 and Hercules agreement 20140910-03. The FA analyses were supported by Special Research Fund of Ghent University (BOF-UGent) in the form of the starting grant 'Energy transfer at the basis of marine food webs in a changing world' awarded to M. D. T.

REFERENCES

1. Russel, N. J., R. I. Evans, P. F. terSteeg, J. Hellemons, A. Verheul and T. Abee (1995) Membranes as a target for stress adaptation. *Int. J. Food Microbiol.* **28**(2), 255–261.
2. Raetz, C. R. and W. Dowhan (1990) Biosynthesis and function of phospholipids in *Escherichia coli*. *J. Biol. Chem.* **265**(3), 1235–1238.
3. Denich, T. J., L. A. Beaudette, H. Lee and J. T. Trevors (2003) Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. *J. Microbiol. Meth.* **52**(2), 149–182.
4. Baysse, C. and F. O'Gara (2007) Role of membrane structure during stress signalling and adaptation in *Pseudomonas*. In *Pseudomonas* (Edited by J. L. Ramos and A. Filloux), pp. 193–1224. Springer.
5. Sinensky, M. (1974) Homeoviscous adaptation-a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl Acad. Sci.* **71**(2), 522–525.
6. Diefenbach, R., H. J. Heipieper and H. Keweloh (1992) The conversion of *cis* into *trans* unsaturated fatty acids in *Pseudomonas putida* P8: evidence for a role in the regulation of membrane fluidity. *Appl. Microbiol. Biotechnol.* **38**(3), 382–387.
7. Suutari, M. and S. Laakso (1992) Unsaturated and branched chain-fatty acids in temperature adaptation of *Bacillus subtilis* and *Bacillus megaterium*. *Biochem. Biophys. Acta.* **1126**(2), 119–124.
8. Loffeld, B. and H. Keweloh (1996) *cis/trans* isomerization of unsaturated fatty acids as possible control mechanism of membrane fluidity in *Pseudomonas putida* P8. *Lipids* **31**(8), 811–815.
9. Zaritsky, A., A. H. Parola, M. Abdah and H. Masalha (1985) Homeoviscous adaptation, growth rate, and morphogenesis in bacteria. *Biophys. J.* **48**(2), 337–339.
10. Hartig, C., N. Loffhagen and H. Harms (2005) Formation of *trans* fatty acids is not involved in growth-linked membrane adaptation of *Pseudomonas putida*. *Appl. Environ. Microbiol.* **71**(4), 1915–1922.
11. Loffhagen, N., C. Hartig and W. Babel (2004) *Pseudomonas putida* NCTC 10936 balances membrane fluidity in response to physical and chemical stress by changing the saturation degree and the *trans/-cis* ratio of fatty acids. *Biosci. Biotechnol. Biochem.* **68**(2), 317–323.
12. Doumenq, P., M. Acquaviva, L. Asia, J. P. Durbec, Y. Le Dreau, G. Mille and J. C. Bertrand (1999) Changes in fatty acids of *Pseudomonas nautica*, a marine denitrifying bacterium, in response to n-eicosane as carbon source and various culture conditions. *FEMS Microbiol. Ecol.* **28**(2), 151–161.
13. Dubois-Brissonnet, F., C. Malgrange, L. Guerin-Mechin, B. Heyd and J. Y. Leveau (2001) Changes in fatty acid composition of *Pseudomonas aeruginosa* ATCC 15442 induced by growth conditions: consequences of resistance to quaternary ammonium compounds. *Microbios* **106**(414), 97–110.
14. Kim, I. S., J. H. Shim and Y. T. Suh (2002) Changes in membrane fluidity and fatty acid composition of *Pseudomonas putida* CN-T19 in response to toluene. *Biosci. Biotechnol. Biochem.* **66**(9), 1945–1950.
15. Kiran, M. D., J. S. Prakash, S. Annapoorni, S. Dube, T. Kusano, H. Okuyama, N. Murata and S. Shivaji (2004) Psychrophilic *Pseudomonas syringae* requires *trans*-monounsaturated fatty acid for growth at higher temperature. *Extremophiles* **8**(5), 401–410.
16. Mrozik, A., Z. Piotrowska-Seget and S. Labuzek (2004) Changes in whole cell-derived fatty acids induced by naphthalene in bacteria from genus *Pseudomonas*. *Microbiol. Res.* **159**(1), 87–95.
17. Munoz-Rojas, J., P. Bernal, E. Duque, P. Godoy, A. Segura and J. L. Ramos (2006) Involvement of cyclopropane fatty acids in the response of *Pseudomonas putida* KT2440 to freeze-drying. *Appl. Environ. Microbiol.* **72**(1), 472–477.
18. Benamara, H., C. Rihouey, T. Jouenne and S. Alexandre (2011) Impact of the biofilm mode of growth on the inner membrane phospholipid composition and lipid domains in *Pseudomonas aeruginosa*. *Biochem. Biophys. Acta.* **1808**(1), 98–105.
19. Chao, J., G. M. Wolfaardt and M. T. Arts (2010) Characterization of *Pseudomonas aeruginosa* fatty acid profiles in biofilms and batch planktonic cultures. *Can. J. Microbiol.* **56**(12), 1028–1039.
20. Hoang, T. T. and H. P. Schweizer (1997) Fatty acid biosynthesis in *Pseudomonas aeruginosa*: cloning and characterization of the *fabAB* operon encoding b-hydroxyacylacyl carrier protein dehydratase (FabA) and b-ketoacyl-acyl carrier protein synthase I (FabB). *J. Bacteriol.* **179**(17), 5326–5332.
21. Zhu, K., K.-H. Choi, H. P. Schweizer, C. O. Rock and Y.-M. Zhang (2006) Two aerobic pathways for the formation of unsaturated fatty acids in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **60**(2), 260–273.
22. Kim, S., J. Ha, H. Lee, S. Lee, J. Lee, Y. Choi, H. Ho, Y. Yoon and K.-H. Choi (2019) Role of *Pseudomonas aeruginosa* DesB in adaptation to osmotic stress. *J. Food Prot.* **82**(8), 1278–1282.
23. Schweizer, H. P. and K.-H. Choi (2011) *Pseudomonas aeruginosa* aerobic fatty acid desaturase DesB is important for virulence factor production. *Arch. Microbiol.* **193**(3), 227–234.
24. Chamberlain, J. and S. H. Moss (1987) Lipid peroxidation and other membrane damage produced in *Escherichia coli* K1060 by near-UV radiation and deuterium oxide. *Photochem. Photobiol.* **45**(5), 625–630.
25. Bosshard, F., M. Bucheli, Y. Meur and T. Egli (2010) The respiratory chain is the cell's Achilles' heel during UVA inactivation in *Escherichia coli*. *Microbiology* **156**(7), 2006–2015.
26. Baümler, W., J. Regensburger, A. Knak, A. Felgenträger and T. Maisch (2012) UVA and endogenous photosensitizers – the detection of singlet oxygen by its luminescence. *Photochem. Photobiol. Sci.* **11**, 107–117.
27. Pezzoni, M., M. Meichtry, R. A. Pizarro and C. S. Costa (2015) Role of the *Pseudomonas* quinolone signal (PQS) in sensitizing *Pseudomonas aeruginosa* to UVA radiation. *J. Photochem. Photobiol., B* **142**, 129–140.
28. Bosshard, F., K. Riedel, T. Schneider, C. Geiser, M. Bucheli and T. Egli (2010) Protein oxidation and aggregation in UVA-irradiated *Escherichia coli* cells as signs of accelerated cellular senescence. *Environ. Microbiol.* **12**(11), 2931–2945.
29. Santos, A. L., V. Oliveira, I. Baptista, I. Henriques, N. C. M. Gomes, A. Almeida, A. Correia and Â. Cunha (2013) Wavelength dependence of biological damage induced by UV radiation on bacteria. *Arch. Microbiol.* **195**(1), 63–74.
30. Santos, A. L., C. Moreirinha, D. Lopes, A. C. Esteves, I. Henriques, A. Almeida, M. Rosário, M. Domingues, I. Delgadillo, A. Correia and Â. Cunha (2013) Effects of UV radiation on the lipids and proteins of bacteria studied by mid-infrared spectroscopy. *Environ. Sci. Technol.* **47**(12), 6306–6315.
31. McGuigan, K. G., R. M. Conroy, H.-J. Mosler, M. du Preez, E. Jaswa and P. Fernandez-Ibañez (2012) Solar water disinfection (SODIS): a review from bench-top to roof-top. *J. Hazard. Mater.* **235–236**, 29–46.
32. Gamage, J. and Z. Zhang (2010) Applications of photocatalytic disinfection. *Int. J. Photoenergy* **2010**(1), 11. https://doi.org/10.1155/2010/764870.
33. Jagger, J. (1981) Near UV radiation effects on microorganisms. *Photochem. Photobiol.* **34**(6), 761–768.
34. Caldeira de Araujo, A. and A. Favre (1986) Near ultraviolet DNA damage induces the SOS responses in *Escherichia coli*. *EMBO J* **5**, 175–179.
35. Berney, M., H.-U. Weilenmann and T. Egli (2006) Gene expression of *Escherichia coli* in continuous culture during adaptation to artificial sunlight. *Environ. Microbiol.* **8**(9), 1635–1647.
36. Soule, T., Q. Gao, V. Stout and F. Garcia-Pichel (2013) The global response of *Nostoc punctiforme* ATCC 29133 to UVA stress, assessed in a temporal DNA microarray study. *Photochem. Photobiol.* **89**(2), 415–423.
37. Sassoubre, L. M., M. M. Ramsey, M. S. Gilmore and A. B. Boehm (2014) Transcriptional response of *Enterococcus faecalis* to sunlight. *J. Photochem. Photobiol. B: Biol.* **130**, 349–356.
38. Qiu, X., G. W. Sundin, L. Wu, J. Zhou and J. M. Tiedje (2005) Comparative analysis of differentially expressed genes in *Shewanella oneidensis* MR-1 following exposure to UVC, UVB, and UVA radiation. *J. Bacteriol.* **187**(10), 3556–3564.
39. Kidambi, S. P., M. G. Booth, T. A. Kokjohn and R. V. Miller (1996) *recA*-dependence of the response of *Pseudomonas aeruginosa* to UVA and UVB irradiation. *Microbiology* **142**(4), 1033–1040.
40. Pezzoni, M., R. A. Pizarro and C. S. Costa (2012) Protective effect of low UVA irradiation against the action of lethal UVA on *Pseudomonas aeruginosa*: role of the *relA* gene. *J. Photochem. Photobiol. B: Biol.* **116**, 95–104.
41. Pezzoni, M., P. M. Tribelli, R. A. Pizarro, N. I. Lopez and C. S. Costa (2016) Exposure to low UVA doses increases KatA and KatB catalase activities, and confers cross-protection against subsequent oxidative injuries in *Pseudomonas aeruginosa*. *Microbiology* **162**(5), 855–864.

42. Pezzoni, M., R. A. Pizarro and C. S. Costa (2018) Exposure to low doses of UVA increases biofilm formation in *Pseudomonas aeruginosa*. *Biofouling* **34**(6), 673–684.
43. Pezzoni, M., R. A. Pizarro and C. S. Costa (2020) Role of quorum sensing in UVA-induced biofilm formation in *Pseudomonas aeruginosa*. *Microbiology* **166**(8), 735–750.
44. Pezzoni, M., R. A. Pizarro and C. S. Costa (2014) Protective role of extracellular catalase (KatA) against UVA radiation in *Pseudomonas aeruginosa* biofilms. *J. Photochem. Photobiol., B* **131**, 53–64.
45. Holloway, B. W. (1955) Genetic recombination in *Pseudomonas aeruginosa*. *J. General Microbiol.* **13**(3), 572–581.
46. Hoerter, J. D., A. A. Arnold, D. A. Kucczynska, A. Shibuya, C. S. Ward, M. G. Sauer, A. Gizachew, T. M. Hotchkiss, T. J. Fleming and S. Johnson (2005) Effects of sublethal UVA irradiation on activity levels of oxidative defense enzymes and proteins oxidation in *Escherichia coli*. *J. Photochem. Photobiol. B: Biol.* **81**(3), 171–180.
47. Cadenas, R. and H. Sies (1984) Low-level chemiluminescence as an indicator of singlet molecular oxygen in biological systems. In *Methods in Enzymology* (Edited by L. Parker), pp. 221–231. Academic Press, London.
48. Tilbury, R. N. and T. L. Quickenden (1988) Spectral and time dependence studies of the ultraweak bioluminiscence emitted by the bacterium *Escherichia coli*. *Photochem. Photobiol.* **47**(1), 145–150.
49. De Troch, M., P. Boeckx, C. Cnudde, D. Van Gansbeke, A. Vanreusel, M. Vincx and M. J. Caramujo (2012) Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. *Mar. Ecol. Prog. Ser.* **465**, 53–67.
50. Ackman, R. G. and J. C. Sipos (1964) Application of specific response factors in the gas chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. *J. Am. Oil Chem. Soc.* **41**, 377–378.
51. Wolff, R. L., C. C. Bayard and R. J. Fabien (1995) Evaluation of sequential methods for the determination of butterfat fatty acid composition with emphasis on *trans*-18:1 acids. Application to the study of seasonal variations in French butters. *J. Am. Oil Chem. Soc.* **72**, 1471–1483.
52. Subramanian, C., C. O. Rock and Y. M. Zhang (2010) DesT coordinates the expression of anaerobic and aerobic pathways for unsaturated fatty acid biosynthesis in*Pseudomonas aeruginosa*. *J. Bacteriol.* **192**(1), 280–285.
53. Larionov, A., A. Krause and W. Miller (2005) A standard curve based method for relative real time PCR data processing. *BMC Bioinformatics* **6**, 62.
54. Madronich, S., L. O. Björn and R. L. McKenzie (2018) Solar UV radiation and microbial life in the atmosphere. *Photochem. Photobiol. Sci.* **17**(20), 1918–1931.
55. Coohill, T. P. and J. L. Sagripanti (2009) Bacterial inactivation by solar ultraviolet radiation compared with sensitivity to 254 nm radiation. *Photochem. Photobiol.* **85**(5), 1043–1052.
56. Pizarro, R. A. (1995) UVA oxidative damage modified by environmental conditions in *Escherichia coli*. *Int. J. Radiat. Biol.* **68**(3), 293–299.
57. Guerzoni, M. E., R. Lanciotti and P. S. Cocconcelli (2001) Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. *Microbiology* **147**(8), 2255–2264.
58. Jaishankar, J. and P. Srivastava (2017) Molecular basis of stationary phase survival and applications. *Front Microbiology* **8**, 2000.
59. Ghorbal, S. K. B., A. Chatti, M. M. Sethom, L. Maalej, M. Mihoub, S. Kefacha, M. Feki, A. Landoulsi and A. Hassen (2013) Changes in membrane fatty acid composition of *Pseudomonas aeruginosa* in response to UV-C radiations. *Curr. Microbiol.* **67**(1), 112–117.
60. Pradenas, G. A., B. A. Paillavi, S. Reyes-Cerpa, J. M. Pérez-Donoso and C. C. Vásquez (2012) Reduction of the monounsaturated fatty acid content of *Escherichia coli* results in increased resistance to oxidative damage. *Microbiology* **158**(5), 1279–1283.
61. Seel, W., D. Baust, D. Sons, M. Albers, L. Etzbach, J. Fuss and A. Lipski (2020) Carotenoids are used as regulators for membrane fluidity by *Staphylococcus xylosus*. *Sci. Rep.* **10**, 330.
62. Ram, S., M. Mitra, F. Shaha, S. R. Tirkeya and S. Mishra (2020) Bacteria as an alternate biofactory for carotenoid production: A review of its applications, opportunities and challenges. *J. Funct. Foods* **67**, 103867.
63. Fonseca, F., C. Pénicaud, E. E. Tymczyszyn, A. Gómez-Zavaglia and S. Passot (2019) Factors influencing the membrane fluidity and the impact on production of lactic acid bacteria starters. *Appl. Microbiol. Biotechnol.* **103**, 6867–6883.
64. Baysse, C., M. Cullinane, V. Dénervaud, E. Burrowes, J. M. Dow, J. P. Morrissey, L. Tam, J. T. Trevors and F. O'Gara (2005) Modulation of quorum sensing in *Pseudomonas aeruginosa* through alteration of membrane properties. *Microbiology* **151**(8), 2529–2542.
65. Kim, D.-K., S.-J. Kim and D.-H. Kang (2017) Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts. *Food Res. Int.* **97**, 280–287.
66. Regensburger, J., T. Maisch, A. Knak, A. Gollmer, A. Felgentraeger, K. Lehner and W. Baeumler (2013) UVA irradiation of fatty acids and their oxidized products substantially increases their ability to generate singlet oxygen. *Phys. Chem. Chem. Phys.* **15**(40), 17672–17680.