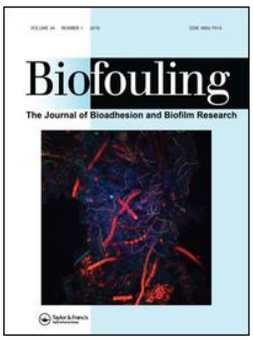

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Exposure to low doses of UVA increases biofilm formation in *Pseudomonas aeruginosa*

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ABSTRACT

The establishment of bacterial biofilms on abiotic surfaces is a complex process regulated by multiple genetic regulators and environmental factors which are able to modulate the passage of planktonic cells to a sessile state. Solar ultraviolet-A radiation (UVA, 315-400) is one of the main environmental stress factors that bacteria must face at the Earth's surface. The deleterious effects of UVA are mainly due to oxidative damage. This paper reports that exposure to low UVA doses promotes biofilm formation in three prototypical strains of *Pseudomonas aeruginosa*, a relevant opportunistic human pathogen. It demonstrates that exposure of planktonic cells to sublethal doses of UVA can increase cell surface hydrophobicity and swimming motility, two parameters known to favor cell adhesion. These results suggest that UVA radiation acts, at least in part, by promoting the first stages of biofilm development.

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KEYWORDS
Pseudomonas aeruginosa; ultraviolet-A; biofilm; hydrophobicity; swimming motility

Introduction

Biofilms are the prevailing lifestyle of bacteria in most natural and artificial environments. They consist of microbial communities embedded in a matrix of extracellular polymeric substances (EPS) composed mainly of proteins, DNA and polysaccharides (Branda et al. 2005; Flemming and Wingender 2010). The biofilm matrix plays an important role in multiple processes, including cell attachment, cell-to-cell interconnection, protection of bacterial cells against stress factors, and exchange of genetic material (Molin and Tolker-Nielsen 2003; Friedman and Kolter 2004; Jackson et al. 2004; Ma et al. 2009; Yang et al. 2009).

Biofilm develops via a series of stages beginning with the surface attachment of planktonic bacteria followed by the formation of microcolonies and the subsequent development of differentiated structures (Costerton et al. 1995; O' Toole and Kolter 1998; Lawrence and Neu 1999). The cycle ends when biofilms cells disperse (Sauer et al. 2004). On the one hand, biofilm formation and dispersion are regulated by external factors such as the medium composition, the carbon substrate availability and the environmental stress (De Kievit et al. 2001; Sauer et al. 2004). Environmental stress is often responsible for the generation of oxidative stress, which has been related to biofilm formation (Gambino and Cappitelli 2016). Wei et al. (2012) showed that hydrogen peroxide promotes the biofilm lifestyle in *Pseudomonas aeruginosa* by the activation of the oxidative stress response protein OxyR. Quorum sensing (QS), the mechanism employed by bacteria to regulate gene transcription in response to population size (Fuqua et al. 2001), enhances the oxidative response in *P. aeruginosa* (Hasset 1999; Costa et al. 2010) and is important in the development of a well-structured biofilm (De Kievit and Iglewski 1999). On the other hand, some bacterial properties, such as motility and cell hydrophobicity, are also involved in biofilm formation and maturation (De Weger et al. 1987; Grant et al. 1993; Gilbert et al. 1991; van Loosdrecht et al. 1987; Wang et al. 2011).

It has been demonstrated that biofilm cells are more resistant than planktonic cells to adverse conditions, including nutrient limitation, desiccation, and low pH (Rinaudi and Giordano 2010) as well as exposure to antibiotics, biocides (Mah and O' Toole 2001; Nickel et al. 1985; Hassett et al. 1999) and ultraviolet radiation (Elasri and Miller 1999; Pezzoni et al. 2014). Solar ultraviolet A radiation (UVA, 315-400 nm), the major fraction of ultraviolet radiation reaching the Earth's surface, is one of the main stress agents that bacteria face in their environment (Webb 1977; Fernández and Pizarro 1996; 1999). It has been

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Supplemental data for this article can be accessed here.

widely demonstrated that lethal UVA effects are produced by oxidative damage caused by reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical. ROS are generated by the absorption of the light by endogenous photosensitizers, e.g. flavoproteins, cytochromes and quinones, in presence of oxygen (Baümler et al. 2012; Pezzoni et al. 2015). The lethal action has been attributed mainly to oxidative lesions at membranes level (Moss and Smith 1981; Chamberlain and Moss 1987; Bosshard et al. 2010). On the other hand, exposure to low (non-lethal) doses of UVA induces several sublethal effects such as a transient inhibition of bacterial growth without significant cell death (Jagger 1981) due to a direct effect on certain tRNAs (Favre et al. 1985; Ramabhadran and Jagger 1976), oxidative disturbance of bacterial membranes (Pizarro 1995) and loss in phage susceptibility (Day and Muel 1974). Transcriptomic analyses in microorganisms exposed to low doses of UVA revealed a general antioxidative response (Berney et al. 2006; Qiu et al. 2005; Soule et al. 2013; Sassoubre et al. 2014). In addition, it was demonstrated in *P. aeruginosa* that low doses of UVA induce the production of N-butanoyl-L-homoserine lactone (C4-HSL), one of the main signals of QS (Costa et al. 2010). In a later work, exposure of *P. aeruginosa* to low UVA fluences was reported to have a protective effect against subsequent lethal doses that depended on the induction of the stringent response (Pezzoni et al. 2012). It has recently been observed that total catalase activity in *P. aeruginosa* increases significantly under low UVA doses due to an induction in the expression of *katA* and *katB* genes (Pezzoni et al. 2016).

The aim of this study was to explore the effect of sublethal UVA doses on biofilm formation of three prototypic strains of *P. aeruginosa*, a relevant bacterium found in aquatic and terrestrial environments and opportunistic human pathogen. The study found that biofilm formation is promoted when *P. aeruginosa* is exposed to UVA. This could be ascribed, at least in part, to increased cell attachment, since UVA modifies cell surface hydrophobicity and swimming motility, two key factors in cell adhesion. Biofilms resistance and the difficulty in eradicating them are important issues that require attention due their negative effects on health and industry. This study may thus contribute to understanding the behavior of *P. aeruginosa* biofilms and provide useful information for improving techniques using solar radiation or UVA as killing agents.

Material and methods

Bacterial strains and growth conditions

The *Pseudomonas aeruginosa* strains used in this study were PAO1 and PA14, isolated from burn wounds (Holloway 1955; Rahme et al. 1995), and the collection strain ATCC 27853, isolated from blood (Medeiros et al. 1971). Bacterial cultures were routinely grown at 37 °C in complete LB broth (10 g tryptone, 5 g yeast extract and 5 g NaCl, bringing the volume up to 1,000 ml in distilled water); for solid medium, agar was added at 15 g l^{-1}.

Irradiation source

Cell suspensions were irradiated using a bench with two Philips TDL 18W/08 tubes (>95% of UVA emission at 365 nm). The incident fluence under the 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 sublethal UVA irradiation and biofilm formation

Overnight cultures were diluted to OD_{650} 0.05 in LB medium and divided into two 15 ml fractions, each of which was placed in a glass beaker (4.5 cm internal diameter) containing glass slides placed vertically at the bottom to allow the development of biofilm at the air-liquid interface (ALI) (Friedman and Kolter 2004). The beakers with the slides 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 W m^{-2} at the level of the free surface of the suspension, while the other was covered with a black plastic sheet (dark control). The fluence employed may be encountered normally in the environment (Hoerter et al. 2005).

Chemiluminescence assays

Photoemissive species production was followed by means of a liquid scintillation system in the out-of-coincidence mode (Cadenas and Sies 1984). Aliquots were taken during bacterial growth 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.

Biofilm analysis

Amount of living bacterial cells

Biofilm cell concentration was determined by counting the number of colony forming units (CFU) on LB plates. Slides containing the biofilms grown under UVA radiation or in darkness were removed at the specified times and washed with distilled water to remove any remaining suspended cells. The bacterial biomass was then scraped from the glass in saline solution (0.1 M NaCl in water) with a sterile plastic spatula and homogenized by vigorous vortexing. Appropriate dilutions of these suspensions were plated on LB solid medium. Plates were incubated in the dark immediately after irradiation and the colonies were counted after 24 h at 37 °C.

Total biomass

Total biofilm mass was evaluated by staining with crystal violet. Slides carrying biofilms were washed letting sterile distilled water (about 10 ml) drop down gently on them to remove unattached cells. Slides were then stained with 0.1% (wt vol^{-1}) aqueous crystal violet solution for 30 min. The crystal violet attached to the slides was dissolved in a mixture of 96% ethanol and 30% acetic acid (1:1). Absorbance at 575 nm was measured in the resulting solution.

Biofilm visualization

Biofilm was visualized and cell membrane integrity determined by staining with the fluorescent stains SYTO 9 and propidium iodide (PI), following Smith and Hunter (2008). PI is a red dye that only enters cells with permeabilized cytoplasmic membrane, and SYTO 9 is a green dye that stains all types of cells. Thus, green cells are alive and red cells are dead. The slides carrying the biofilms were removed, washed with distilled water, immersed in a solution containing 3.5 µM SYTO 9 and 20 µM PI in distilled water, and incubated in the dark for 15 min at room temperature. Biofilm images were visualized with an Epifluorescence Microscope Olympus BX51. The surface covered by red (dead) cells in these images was calculated using the Image J software (Rasband 1997).

Isolation and chemical analysis of the biofilm matrix

The process of biofilm harvesting and matrix fractionation was based on previous studies (Chang et al. 2007; Tapia et al. 2009; Messiaen et al. 2014). Briefly, the slides were removed from the glass beakers and washed once with distilled water. The biofilms were carefully scraped from the glass surfaces and suspended in 0.1 M NaCl. The cells were dispersed by vigorous stirring for 5 min at room temperature and then separated by centrifugation for 30 min at 4 °C. The absence of cells in the supernatant (matrix fraction) was confirmed by plating on solid LB medium. The viable cell number obtained in the cell fraction was similar to that observed in biofilm assays without matrix separation, indicating that no significant cell lysis was generated by the procedure. Content of alginate, proteins, DNA and total polysaccharides was evaluated in the matrix fraction. DNA and proteins were quantified without previous separation; total polysaccharides were separated by precipitation with ethanol at −20 °C and centrifugation (Chang et al. 2007) before quantification. Alginate content was determined using the carbazole assay (Knutson and Jeanes 1968; Ma et al. 1998), with sodium alginate (BDH) as standard. Protein content was determined by Lowrý's method (Lowry et al. 1951), using bovine albumin (Sigma) as standard. Extracellular DNA was quantified by evaluating the absorbance at 260/280 nm using a Nanodrop 2000 (Thermo Scientific NanoDrop®). Total polysaccharides were determined by the phenol/sulfuric acid method (Dubois et al. 1956), using glucose (Merck) as standard.

Motility assays

To evaluate motility, samples of cell suspensions exposed to UVA radiation or maintained in the dark were taken at the specified times and plated onto different media depending on the type of motility assayed. Plates were incubated at 37°C for 24 h, and halo diameters measured. Swimming motility was evaluated on tryptone plates (1% tryptone, 0.5% NaCl) containing 0.3% agar as previously described (Wiegand et al. 2008). Swarming motility was evaluated on LB broth plates added with 1% agar and glucose 5 g l^{-1}. Twitching motility was evaluated on LB broth plates solidified with 1% agar. Twitch plates were briefly dried and bacteria were stab inoculated with a sharp toothpick to the bottom of the Petri dish.

Microbial adhesion to hydrocarbons assays

The microbial adhesion to hydrocarbons (MATH) test was adapted to investigate cell hydrophobicity (Rosenberg et al. 1980; Rosenberg 2006). Control and irradiated bacterial cell suspensions were mixed with toluene at a ratio of 1:1 (volume) and incubated for 30 min at 30 °C. After 30 min, the loss in absorbance (OD_{600}) of the aqueous phase relative to that of the initial cell suspension was measured and hydrophobicity was calculated as percentage of cells adhering to toluene.

Statistical analysis

The significance of each treatment was evaluated by an unpaired two-tailed Student's *t*-test with confidence levels at >95% (i.e. $p < 0.05$ was considered as significant).

Results

Effect of sublethal UVA doses on cell growth

In this work, three representative *P aeruginosa* strains were evaluated. The strains PA01 and PA14 present similar levels of biofilm formation (Kukavica-Ibrulj et al. 2008; Bielecki et al. 2010; Colvin et al. 2012). The strain ATCC 27853, previously tested in the authors laboratory (Degiorgi et al. 1996; Fernández and Pizarro 1999) was used due to decreased alginate production (unpublished results). In order to determine the conditions to study the effect of sublethal UVA on biofilm formation, the three strains were exposed to different UVA fluence rates and the growth was analyzed spectrophotometrically. Fluence rate of 25 Wm^{-2} produced a growth delay without significant alteration of cell viability (results not shown) and was chosen as the sublethal UVA dose used in this work; this fluence rate was employed in a previous study of gene expression in the PAO1 strain (Pezzoni et al. 2016).

When strains were cultured under UVA delivered at a fluence rate of 25 Wm^{-2}, they experienced a slight growth delay compared to control cultures maintained in the dark (Figure 1a). To evaluate whether the growth delay was associated to oxidative damage, the ultraweak chemiluminescence procedure was employed (Tilbury and Quickenden 1988; Pizarro 1995). As shown in Figure 1b, a peak was observed at the beginning of the exposure, indicating oxidative damage. Control cells maintained in the dark did not show relevant changes in chemiluminescence values.

UVA promotes biofilm formation in *P. aeruginosa*

The growth and development of biofilms of PAO1, PA14 and ATCC 27853 strains in static systems exposed to UVA radiation was studied. Two stages were analyzed: the attachment phase, after incubation for 30–60 minutes (Ono et al. 2014) and the mature biofilm, after 24h under the same conditions (Vanysacker et al. 2013).

Figure 2a shows the effect of exposure to low UVA doses on the number of viable cells in the biofilms. A significant increase in viable cell count was observed in all three strains, both in initial attachment and in the mature biofilm, compared to the controls in the dark.

Biofilm biomass on control and treated slides was evaluated by staining with crystal violet, a common method to determine biofilm biomass without disrupting the biofilm (Christensen et al. 1985), which stains not only cells but also any biological material adhering to the surface. In PAO1 and ATCC 27853 there was a significant increase in crystal violet quantification both in the attachment phase (60 min) and in mature biofilms (24 h) (Figure 2b). In PA14, although there were higher crystal violet measurements in biofilms grown under UVA, the increases were not statistically significant (Figure 2b).

The influence of UVA on biofilm formation was then evaluated by epifluorescence microscopy using dye indicators of cell viability. For this purpose, mature biofilms (24 h; UVA and control) were stained with red fluorescent PI coupled to green fluorescent SYTO 9, and biofilm formation and cell viability were evaluated without biofilm disruption; biofilms images at 30 and 60 min showed little observable mass (results not shown). For 24 h biofilms, no significant difference was observed in viability values between strains and treatments (Figure S1). However, there was an increase in biofilm biomass grown under UVA in all three strains (Figure S1).

UVA affects the matrix composition of *P. aeruginosa* biofilms

The effect of UVA exposure on the matrix composition was then studied in the biofilms of the three strains at different stages (Figure 3). In the PAO1 strain, there was a significant increase in the concentration of proteins, DNA and total polysaccharides during initial attachment (mainly at 60 min) in the biofilm matrix formed under UVA. The same trend was observed in mature biofilms, with the difference between treated and controls being more significant. Alginate concentration only increased significantly in mature biofilms.

In strain PA14, the results were strikingly different. In the attachment phase (30 and 60 min), UVA radiation only caused a significant increase in matrix protein content. Conversely, in the mature biofilm, the only matrix component to increase significantly under UVA was polysaccharides.

In strain ATCC 27853, the results were similar to those for PAO1 except for alginate, which was undetectable at 30 min, but detected later in small amounts, with no significant difference between treatments, perhaps due to its low concentration in the matrix of this strain.

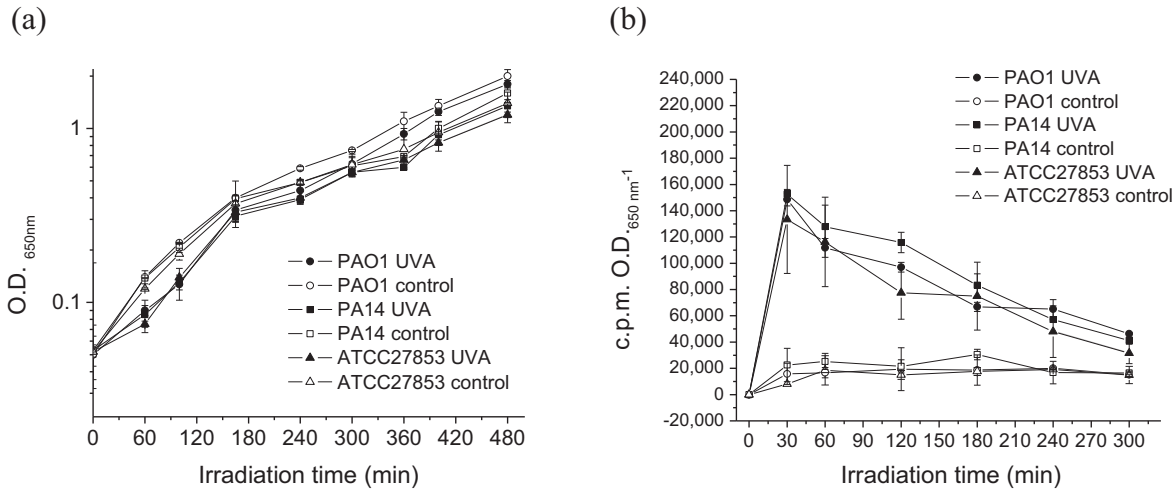

Figure 1. Effect of sublethal UVA doses on (a) cell growth and (b) chemiluminescence values of PAO1, PA14 and ATCC27853. Cells were grown under UVA (fluence rate 25 Wm^{-2}) or in the dark (control). Aliquots were taken at different times and OD_{650} and chemiluminescence were measured. Error bars represent the SDs of at least three independent experiments.

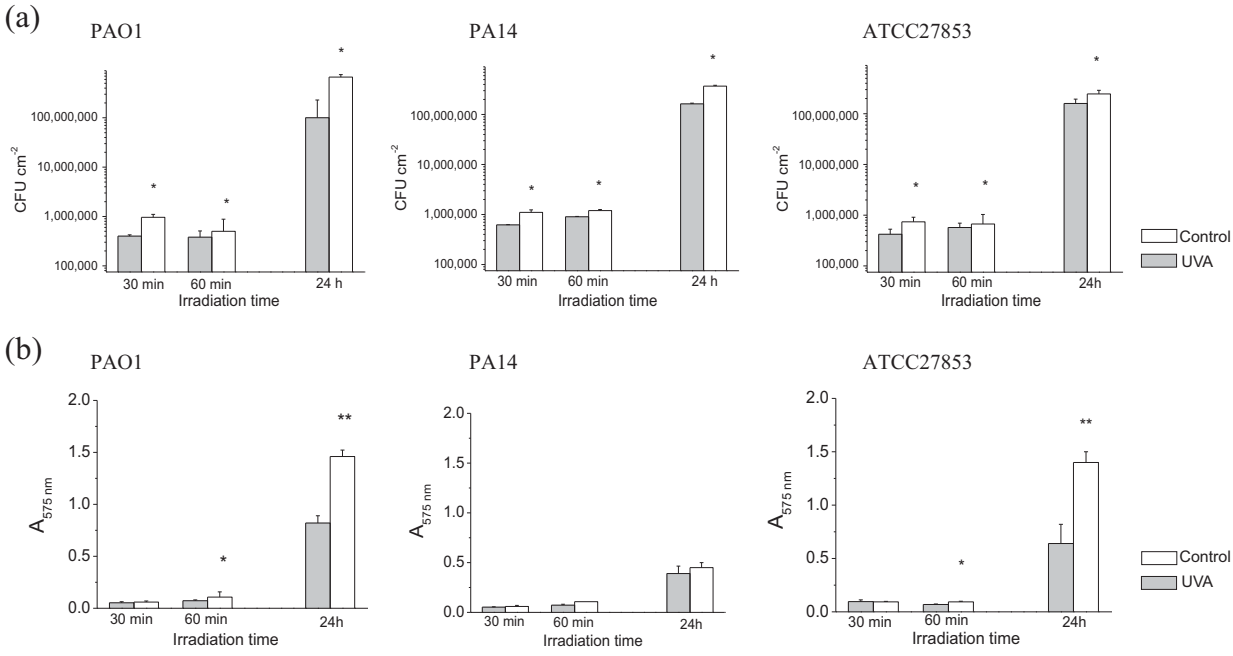

Figure 2. Effect of sublethal UVA doses on biofilm formation. Biofilms were grown under UVA or in the dark (control) for 30 min, 60 min, or 24 h. The number of CFU attached to the slides was quantified by the plate count method (a). The whole biofilm mass was quantified by crystal violet staining by measuring absorbance at 575 nm (b). Each value is the mean of three independent tests. Error bars indicate SDs. $^{*}p < 0.05$, $^{**}p < 0.005$.

UVA enhances swimming motility of *P. aeruginosa* cells

The effect of UVA on the motility phenotype (swimming, swarming and twitching) of planktonic cells was assessed. As shown in Figure 4, there was a significant difference in the swimming motility diameter in the three strains mainly after UVA radiation for 30 min, which could contribute to initial attachment to solid surfaces under UVA exposure.

On the contrary, when swarming and twitching motilities were analyzed, no difference was observed between UVA and control cells in any of the strains (results not shown).

UVA increases surface hydrophobicity of *P. aeruginosa* cells

Sabra et al. (2003) demonstrated that under oxidative conditions some changes occur in the outer membrane

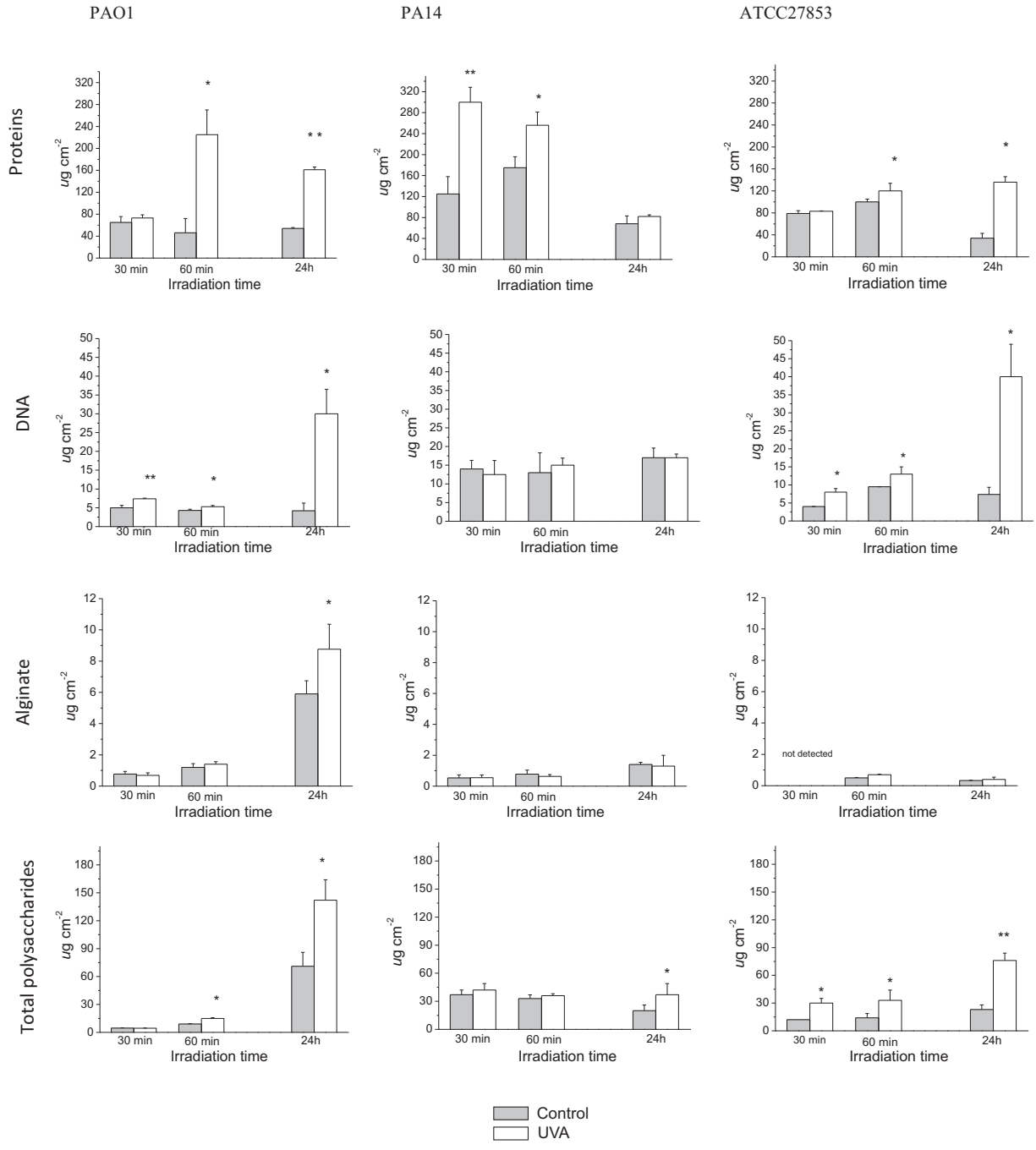

Figure 3. Effect of sublethal UVA doses on matrix composition. After 30 min, 60 min or 24 h incubation under UVA or in the dark (control), biofilm matrices were isolated and extracellular polymeric substances (proteins, DNA, alginate and total polysaccharides) were quantified. Each value is the mean of three independent tests. Error bars indicate SDs. $^{*}p < 0.05$, $^{**}p < 0.005$.

resulting in changes in cell hydrophobicity, a known factor to induce cell adhesion. Then, the effect of UVA on cell hydrophobicity was analyzed by assaying microbial adhesion to hydrocarbons. For this purpose, control and irradiated cells were collected at different times and hydrophobicity was calculated as described in Materials and methods. Figure 5 shows that exposure to UVA significantly increased cell hydrophobicity, especially in the PAO1 strain.

Discussion

Regulation of biofilm formation in *P. aeruginosa* is a complex process. Multiple genetic regulators such as the two component systems Roc1 and GacAS/LadS/RetS, QS, sigma factor RpoS and the second messenger bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) participate in the control of biofilm development (Heydorn et al. 2002; De Kievit 2009;

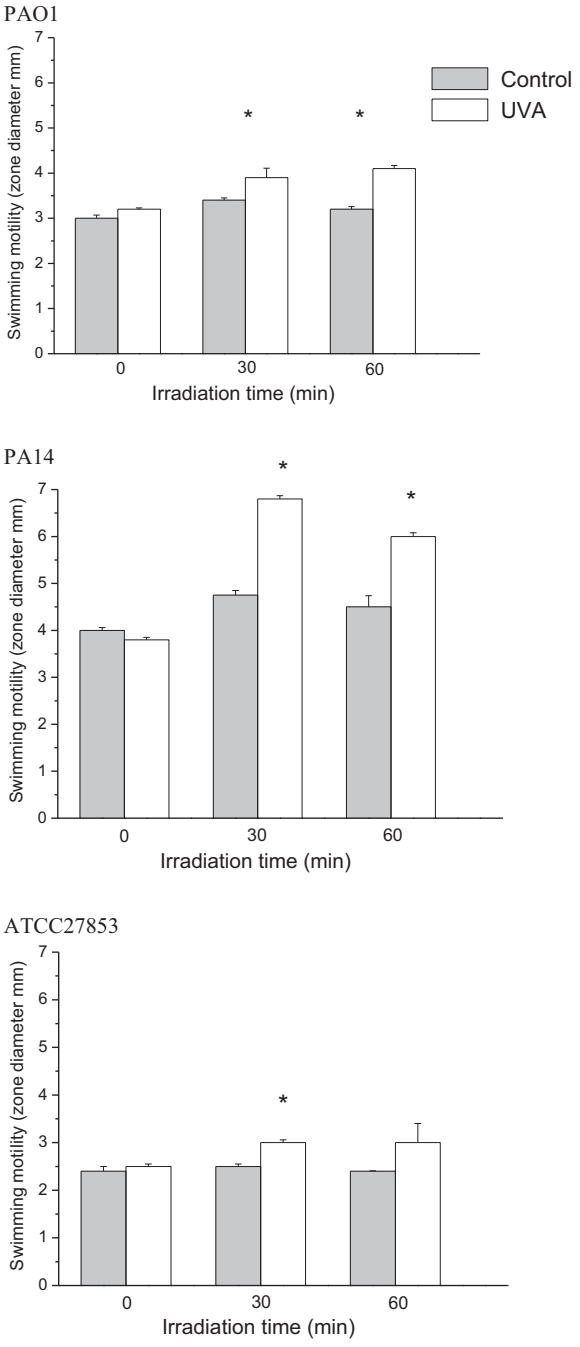

Figure 4. Effect of sublethal UVA doses on swimming motility. Planktonic cells were collected and stabbed into a swimming plate after 0, 30 and 60 min incubation under UVA or in the dark (control). Plates were incubated overnight at 37 °C and the swimming phenotype was evaluated by halo diameter measurements. Each value is the mean of three independent tests. Error bars indicate SDs. $*p < 0.05$.

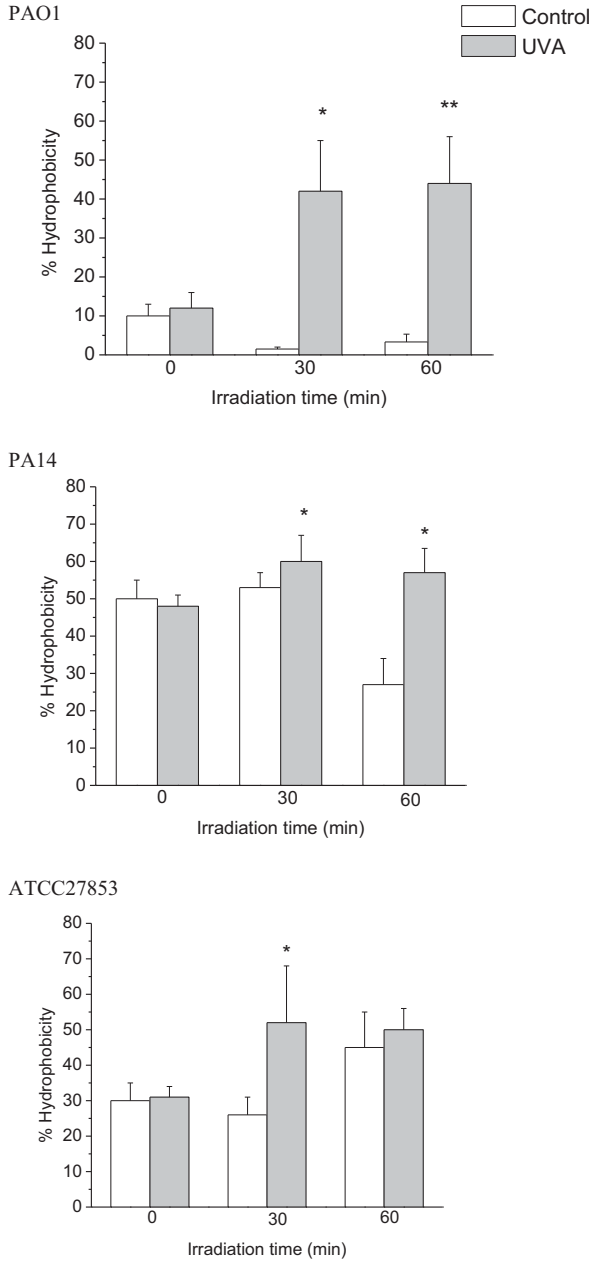

Figure 5. Effect of sublethal UVA doses on cell hydrophobicity. Planktonic cells exposed to 0, 30 and 60 min incubation under UVA or maintained in the dark (control), were collected and hydrophobicity was measured using the MATH test. Each value is the mean of three independent tests. Error bars indicate SDs. $*p < 0.05$, $**p < 0.005$.

Mikkelsen et al. 2011; Francis et al. 2017). In addition, environmental factors such as temperature, nutrients, pH, microbial communities, interaction with host, oxidative stress and antimicrobials modulate the passage of cells to the sessile state (Garret et al. 2008, Di Bonaventura et al. 2007, Gambino and Cappitelli 2016, Kaplan 2011). The present study shows that low doses of UVA radiation, an oxidative stress factor present in nature, are able to increase biofilm formation in *P. aeruginosa*. To determine whether this phenomenon is common throughout this species, the common laboratory strains PAO1 and PA14, isolated from burn wounds, and the collection strain ATCC 27853, isolated from blood, were assayed. Despite their differences, such as the type of matrix

polysaccharides and the expression of genes involved in cellular adhesion and biofilm maturation (Colvin et al. 2012; Cao et al. 2017), the response to UVA was similar in all three strains.

Although the mechanism by which UVA promotes biofilm formation is still unknown, the current study shows that two parameters involved in cell adhesion are enhanced by UVA exposure: swimming motility and cell surface hydrophobicity. The study of motility is relevant because various studies have suggested that it is essential to *P. aeruginosa* biofilm formation (Singh et al. 2002; Klausen et al. 2003a; 2003b; Shrout et al. 2006). Swimming motility is a mode of bacterial movement powered by rotating flagella and occurs when individual cells move in liquid environments (Mah and O'Toole 2001). Swarming is defined as rapid multicellular movement of bacteria on a surface, also powered by rotating flagella (Kearns 2010), and twitching motility is surface motility powered by the extension and retraction of type IV pili, which confers slow movements (Mah and O'Toole 2001). The results shown in this study revealed that only swimming motility increases under UVA exposure. Correlation between biofilm formation and swimming motility has been demonstrated previously in a study with clinical isolates of *P. aeruginosa* (Fonseca et al. 2004); it should be noted that in this study, the authors also report a relationship between biofilm formation and twitching. It has been demonstrated that swimming motility is regulated by the QS subsystem Rhl in *P. aeruginosa* (O' Toole and Kolter 1998). Previously, Costa et al. (2010) reported that low UVA doses increase the levels of C4-HSL, the signal able to bind the RlhR receptor to activate Rhl-dependent genes. It is therefore possible that the increase in swimming motility observed under UVA is caused by QS induction by UVA.

In addition to swimming motility, it has been reported that increased hydrophobicity of the cell surface promotes initial attachment of bacteria to surfaces (Gilbert et al. 1991; van Loosdrecht et al. 1987; Wang et al. 2011). Here, we demonstrate that exposure to sublethal UVA produces an increase in the hydrophobicity of *P. aeruginosa* cells. Previously, it was reported that microorganisms can switch between hydrophobic and hydrophilic phenotypes in response to changes in environmental conditions such as temperature, nutrient composition and growth phases (Borecká-Melkusová and Bujdaková 2008; Bujdaková et al. 2013). Davies (2000) concluded that the differences in this parameter result from the properties conferred by outer membrane molecules such as lipopolysaccharides. Some evidence shows that the presence of lipopolysaccharides on a cell surface tends to make bacteria more hydrophilic and the loss or change in these structures makes the cell more hydrophobic in nature (Al-Tahhan et al. 2000; Norman et al. 2002). Under oxidative conditions, some changes were observed in lipopolysaccharide formation that produce changes in hydrophobicity resulting in increased adherence capacity (Sabra et al. 2003). Further studies are necessary to determine whether the oxidative damage produced by UVA exposure modifies lipopolysaccharides, making the cells more hydrophobic, and the nature of this change.

The analysis of biofilm matrix produced under UVA radiation is interesting since the matrix acts as a barrier against chemical and/or physical injuries (Brown and Gilbert 1993; Hall-Stoodley et al. 2004; Flemming and Wingender 2010), increasing resistance of biofilm cells (Brown and Gilbert 1993). The increase in matrix proteins in biofilms grown under UVA could be relevant due to their important functions in adherence, interaction with other matrix molecules and matrix stability (Mann and Wozniak 2011). On the other hand, in mature biofilms grown under UVA, DNA increased significantly in strains PAO1 and ATCC 27853. Extracellular DNA plays an important role during biofilm development and is the primary cell-to-cell interconnecting compound in mature biofilms (Nemoto et al. 2003). Regarding exopolysaccharides, the opportunistic pathogen *P. aeruginosa* has the capacity to mainly synthesize three types implicated in biofilm formation: alginate, Psl and Pel. Alginate provides structural stability (Hentzer et al. 2001; Nivens et al. 2001) and its production is associated with increased adhesion to solid surfaces (Boyd and Chakrabarty 1995). Several authors have suggested that alginate has a protective function against oxidative stress because it acts as a scavenger of free radicals (Simpson et al. 1989). Psl and Pel serve as key structural components of the biofilm matrix (Colvin et al. 2012). Psl is produced during planktonic growth, mediating initial attachment to surfaces and contributing to microcolony formation (Ma et al. 2009), while Pel is an essential polymer for the formation of a pellicle at the air-liquid interface (Friedman and Kolter 2004). In a recent paper, Chua et al. (2016) studied the role of these two exopolysaccharides in response to oxidative stress generated by hydrogen peroxide and conclude that Psl is more important than Pel in conferring ROS resistance. PAO1 and ATCC 27853 are capable of producing both exopolysaccharides, while PA14 only produces

Pel, since the three genes of the *psl* operon are deleted in this strain. In mature biofilms of all three strains, there was an increase in matrix polysaccharides of biofilms grown under UVA, which might be due to the increase in Pel and Psl in PAO1 and ATCC 27853 and Pel in PA14; the increase in alginate production observed in PA01 strain upon UVA exposure suggests that alginate plays an important role in biofilm formation under these conditions.

In summary, this work demonstrates that exposure to sublethal UVA doses induces biofilm formation in *P. aeruginosa*, a versatile bacterial species that produces robust biofilms resistant to diverse antibacterial agents and is very difficult to eradicate. For these reasons, *P. aeruginosa* biofilms have enormous impact on industry and human health (Nickel et al. 1985; Willcox et al. 2001; Rajasekar et al. 2010; Mansouri et al. 2012). UVA is an important environmental factor regulating natural communities. In recent years, various disinfection strategies have been proposed using solar or artificial UV radiation, such as SODIS (SOlar DISinfection of natural waters) and photocatalytic treatments (Gamage and Zhang 2010; McGuigan et al. 2012). Taking into account the results presented in this paper, it is important to ensure that the radiation used in the disinfection treatments results in lethal effects in order to prevent the induction of adaptive responses such as biofilm formation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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