Exposure to low UVA doses increases KatA and KatB catalase activities, and confers cross-protection against subsequent oxidative injuries in *Pseudomonas aeruginosa*

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Solar UVA radiation is one of the main environmental stress factors for *Pseudomonas aeruginosa*. Exposure to high UVA doses produces lethal effects by the action of the reactive oxygen species (ROS) it generates. *P. aeruginosa* has several enzymes, including KatA and KatB catalases, which provide detoxification of ROS. We have previously demonstrated that KatA is essential in defending *P. aeruginosa* against high UVA doses. In order to analyse the mechanisms involved in the adaptation of this micro-organism to UVA, we investigated the effect of exposure to low UVA doses on KatA and KatB activities, and the physiological consequences. Exposure to UVA induced total catalase activity; assays with non-denaturing polyacrylamide gels showed that both KatA and KatB activities were increased by radiation. This regulation occurred at the transcriptional level and depended, at least partly, on the increase in H2O2 levels. We demonstrated that exposure to low UVA produced a protective effect against subsequent lethal doses of UVA, sodium hypochlorite and H2O2. Protection against lethal UVA depends on *katA*, whilst protection against sodium hypochlorite depends on *katB*, demonstrating that different mechanisms are involved in the defence against these oxidative agents, although both genes can be involved in the global cellular response. Conversely, protection against lethal doses of H2O2 could depend on induction of both genes and/or (an)other defensive factor(s). A better understanding of the adaptive response of *P. aeruginosa* to UVA is relevant from an ecological standpoint and for improving disinfection strategies that employ UVA or solar irradiation.

INTRODUCTION

Bacteria respond to changes in the environment by regulating their gene expression in order to adapt to stressful conditions. The study of the mechanisms involved in bacterial adaptation to stress factors is a fundamental task in bacterial physiology. Solar UVA radiation (400–315 nm), the major fraction of UV radiation reaching the Earth’s surface, represents one of the main environmental stress factors for bacteria. Several lines of research have demonstrated that lethal effects of UVA are due to oxidative damage of proteins, lipids and DNA by the action of reactive oxygen species (ROS) (Chamberlain & Moss, 1987; Hu & Tappel, 1992; Girard et al., 2011). ROS are generated by the absorption of UVA by endogenous photosensitizers, such as flavoproteins, cytochromes and quinones, and include singlet oxygen, superoxide anions, hydroxyl radicals and H2O2 (Bäumler et al., 2012; Pezzoni et al., 2015). In order to investigate the mechanisms involved in the adaptation of bacteria to UVA, the global transcriptomic response of bacteria exposed to low doses of UVA was analysed in micro-organisms such as *Shewanella oneidensis*, *Escherichia coli*, *Nostoc punctiforme* and *Enterococcus faecalis* (Qiu et al., 2005; Berney et al., 2006a; Soule et al., 2013; Sassoubre et al., 2014). These studies revealed that activation of genes coding for enzymes responsible for ROS detoxification and DNA repair is a common response to UVA exposure. In the case of *Pseudomonas aeruginosa*, activation of genes involved in DNA repair, such as *recA* and *din*, by UVA has been reported (Kidambi et al., 1996). In addition,
a protective effect of exposure to sublethal UVA against the action of subsequent lethal doses was found to be dependent on *relA*, the main gene responsible for the synthesis of the transcriptional regulator ppGpp (Pezzoni et al., 2012). However, there is no information about the induction by UVA of genes involved in detoxification of ROS; thus, mechanisms associated to this adaptive stress response remain unknown.

*P. aeruginosa* is a bacterium present in terrestrial and aquatic environments, and an opportunistic human pathogen able to produce severe complications in immunocompromised individuals, patients with burn wounds and people suffering cystic fibrosis. It has multiple enzymic systems for defence against ROS. These include two superoxide dismutases (Fe-SOD and Mn-SOD) (Hassett et al., 1993) to decompose the superoxide anion to H₂O₂ and O₂, and four alkyl hydroperoxidases (AhpA, AhpB, AhpCF and Ohr) (Ochsner et al., 2000) and three monofunctional catalases (KatA, KatB and KatC) (Brown et al., 1995; Ma et al., 1999; Mossialos et al., 2006) to decompose peroxides to H₂O and O₂. KatA is the main catalase and has unique characteristics: it is unusually stable, and essential to H₂O₂ and UVA resistance, osmoregulation and virulence (Hassett et al., 2000; Costa et al., 2010; Pezzoni et al., 2014; Lee et al., 2005). KatA is highly expressed during all growth phases, but it is induced at the stationary growth phase and by increased levels of H₂O₂ (Brown et al., 1995; Heo et al., 2010). KatB is only detected in the presence of H₂O₂ or paraquat and is only partially involved in resistance to oxidative stress (Brown et al., 1995; Lee et al., 2005). However, KatC is induced by high temperature and dispensable in tolerance to H₂O₂ (Mossialos et al., 2006).

In order to better understand how *P. aeruginosa* deals with high UVA doses, we investigated whether exposure to sublethal doses of UVA is able to modulate KatA and KatB activities, and the effect of this photostress on the response to subsequent oxidative injuries. Taking into account the relevance of *P. aeruginosa* as a pathogen, the results presented in this study should be considered in disinfection strategies that employ UVA or solar irradiation as killing agents, such as photocatalytic treatments or solar disinfection of drinking water (SODIS).

**METHODS**

**Bacterial strains and growth conditions.** The *P. aeruginosa* strains used in this work were PAO1 (WT), and its isogenic derivatives PW8190 (katA::I*s/lacZ*) and PW8769 (katB::I*s/lacZ*); transposon I*slacZ*::hah is inserted in-frame in both mutants so that they can be used as reporter strains in expression assays by measuring β-galactosidase activity. Mutants were obtained from the University of Washington Genome Center (Jacobs et al., 2003). 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 bring the volume up to 1000 ml in distilled water); for solid medium, agar was added at 15 g l⁻¹. Kanamycin (150 μg ml⁻¹) was added to primary cultures of complemented katA pkatA and katB pkatB strains; all other cultures were obtained in the absence of antibiotics.

**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 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 aluminium anodised reflectors to enhance the fluence rate on the section to be irradiated.

**Growth under sublethal UVA irrradiation.** Mid-exponential cultures (OD₅₆₀ 0.3) were diluted to OD₅₆₀ 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 W m⁻² at the level of the free surface of the suspension, whilst 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 OD₅₆₀. The fluorescence employed may be encountered normally in the environment (Hoerter et al., 2005).

**Chemiluminescence assays.** Production of photoemissive species was followed by means of a liquid scintillation system in the out-of coincidence mode (Cadenas & Sies, 1984). For this purpose, 5 ml 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 c.p.m. per OD₅₆₀ unit.

**Total catalase activity assay.** Cultures grown under UVA or in the dark were harvested at OD₅₆₀ 0.3 by centrifugation, suspended in ice-cold 50 mM potassium phosphate buffer, pH 7, sonicated in an ice-water bath and clarified by centrifugation at 12 000 g for 10 min at 4 °C. Catalase activity was monitored in the supernatant by following the decomposition of 10 mM H₂O₂ according to Aebi (1984). One unit of activity was that which decomposed 1 μmol H₂O₂ min⁻¹ (mg protein)⁻¹. Protein content was determined by the Lowry method (Lowry et al., 1951).

**Catalase native PAGE analysis.** KatA and KatB activities were analysed in non-denaturing polyacrylamide gels, according to the method of Wayne & Diaz (1986). Briefly, 10 μg protein per sample from extracts obtained for total catalase activity assays was loaded in 5 % non-denaturing polyacrylamide gels and run at 35 V/15 mA. Gels were soaked in distilled water for 5 min, followed by 10 min incubation in 4 mM H₂O₂ at room temperature. The H₂O₂ solution was replaced by distilled water and incubated for an additional 5 min. The distilled water was replaced by a solution containing 1 % (w/v) ferric chloride and 1 % (w/v) potassium ferricyanide. When the gel turned dark green, the ferric chloride/potassium ferricyanide solution was replaced with distilled water and the gel was photographed.

**β-Galactosidase assays.** Strains PW8190 (katA–lacZ) and PW8769 (katB–lacZ) were grown under UVA or in the dark to OD₅₆₀ 0.3 (either in LB or in LB in the presence of ROS scavengers), and β-galactosidase activity was measured in cells treated with SDS and chloroform (Miller, 1972). Specific activities were expressed in Miller units referred to OD₅₆₀. The scavengers used were: 1 μg bovine catalase ml⁻¹ or 1.2 % sodium pyruvate for H₂O₂ (McDonald et al., 1983; Khangraeng & Reed, 2005), 1 mg mannitol ml⁻¹ for hydroxyl radical (Cai et al., 2014) and 1 % DMSO for superoxide anion (McDonald et al., 1983).

**Quantitative real-time (qRT)-PCR.** Total RNA from the PAO1 strain grown under UVA or in the dark (OD₅₆₀ 0.3) was extracted by
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 katA mRNA, assays were performed using the primers katAleft (5′-ATGCGTTCATACCGAGCA-3′) and kathright (5′-AGTGCTAACTGATGCCAGCA-3′), designed for this study on the basis of the genome sequence of the PAO1 strain (Stover et al., 2000). For quantification of kath RNA, two sets of primers were employed: sense (5′-GACGAGAATCTGAAGAG-3′) and antisense (5′-CTCTCGTGCTC-GGTATGC-3′), which have been employed previously (Chang et al., 2005; Small et al., 2007), and kathBleft (5′-GGGGGCAACCCAGG-3′) and kathBright (5′-CGTGAGGAGAAATCGAG-3′), designed for this study. The 16S rRNA gene was used as reference for normalization of expression levels of target genes in each condition by employing the primers 5′-AGCTTGTGCTTGGATTGCAGC-3′ and 5′-AAGGGGCGATCTGAGTCGTGAC-3′. The cycling conditions for katA and 16S rRNA genes were as follows: denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 25 s, 62 °C for 15 s and 72 °C for 15 s, with fluorescence acquisition at 80 °C in single mode. The same conditions were used for kathB amplification, except for the annealing temperature at 56.5 °C. Relative changes in the expression of individual genes between the treated and control conditions were obtained through the relative standard curve method (Larionov et al., 2005).

Construction of complementing plasmids. Complementing plasmid pkatA was described in a previous study (Pezzoni et al., 2014). In order to complement the kath mutation, plasmid pkatB was constructed as follows. Genomic DNA of PAO1 was amplified by PCR by using Q5 High-Fidelity DNA Polymerase (New England Biolabs) and the primers leftkatA (5′-GGAGCAGTCTCAATGACGGCCTC-3′) and rightkatB (5′-AAGGCTGCAAGAATCCAC-3′), designed for this study. The PCR amplification product (2190 bp), containing the full-length fragment of kath and its promoter region (Brown et al., 1995), was ligated into pGEM-T Easy vector (Promega) by previous A-tailing treatment. One of these fractions was irradiated from above at a fluence of 25 W m⁻², they suffered a growth delay compared with control cultures maintained in the dark (Fig. 1a); viable cell count was not affected by the treatment (data not shown). The difference in growth between control and UVA-treated cultures of catalase mutants was significant (P<0.05) throughout the experiment (katA strain) or between 100 and 240 min (katB strain). In the case of the WT, although a growth delay was observed in the UVA-exposed culture compared with the control, this difference was not significant. To evaluate in vivo whether the growth delay could be associated to oxidative damage, the ultraweak chemiluminescence procedure was employed (Tilbury & Quickenden, 1988; Pizarro, 1995). A strong peak of light production was observed in all three strains at the beginning of the exposure, indicating oxidative damage (Fig. 1b), but no difference was observed between strains (Fig. 1b).

Effect of sublethal UVA doses on cell growth and oxidative damage in P. aeruginosa

In order to analyse whether low UVA doses were able to induce catalase activity and the physiological consequences of this phenomenon, we first analysed the growth and the extent of oxidative damage of the WT PAO1 and deficient catalase derivatives ΔkatA and ΔkatB exposed to different UVA fluence rates (results not shown). A condition that did not alter cell viability significantly but produced certain oxidative damage was employed throughout this study (Fig. 1). When strains were cultured in complete medium under UVA delivered at a fluence rate of 25 W m⁻², they suffered a growth delay compared with control cultures maintained in the dark (Fig. 1a); viable cell count was not affected by the treatment (data not shown). The difference in growth between control and UVA-treated cultures of catalase mutants was significant (P<0.05) throughout the experiment (katA strain) or between 100 and 240 min (katB strain). In the case of the WT, although a growth delay was observed in the UVA-exposed culture compared with the control, this difference was not significant. To evaluate in vivo whether the growth delay could be associated to oxidative damage, the ultraweak chemiluminescence procedure was employed (Tilbury & Quickenden, 1988; Pizarro, 1995). A strong peak of light production was observed in all three strains at the beginning of the exposure, indicating oxidative damage (Fig. 1b), but no difference was observed between strains (Fig. 1b).

Induction of KatA and KatB activities by exposure to low UVA doses

Total and individual catalase activities were analysed in the WT and its derivatives katA and katB grown under UVA or in the dark. Cells were analysed at the exponential growth phase to avoid the induction of katA expression at the stationary growth phase in order to highlight the effect of the radiation (Brown et al., 1995). Fig. 2(a) shows that exposure to UVA significantly induced total catalase activity (P<0.05) in the three strains compared with...
control cells. UVA exposure increased total catalase activity ~25% in the WT and the katB strain; this activity could be detected in the katA strain only under UVA. Comparison between irradiated katA and katB strains, which only produce KatB or KatA, respectively, demonstrated that both catalases were induced by the treatment, with KatB activity being substantially lower (about sixfold) than KatA activity. We then analysed KatA and KatB activities in non-denaturing polyacrylamide gels by making use of their different mobility when they are run in the native form. KatA is a heteromultimer consisting of two 56 kDa monomers and one 45 kDa monomer (Ma et al., 1999); KatB is a tetrameric enzyme composed by four 57 kDa monomers (Brown et al., 1995). Electrophoresis in non-denaturing gels confirmed that growth under UVA increased both KatA and KatB activities, and that KatB activity was not detected under non-inducing conditions (Fig. 2b). These results suggested that exposure of P. aeruginosa to sublethal UVA radiation produces a significant increase in KatA and KatB activities – a possible defensive mechanism against subsequent oxidative injuries.

**Induction of katA and katB transcription by exposure to low UVA doses: role of H2O2**

To understand the mechanism underlying the higher KatA and KatB activities by exposure to UVA radiation, transcription levels of katA and katB were analysed by qRT-PCR in irradiated and control cultures of the WT. The level of katA mRNA increased significantly by about fivefold (P<0.05) under UVA radiation compared with the control assay, whereas no increase in katB mRNA was observed due to the treatment (Fig. 3). In addition to the katB primers successfully employed by other authors (Chang et al., 2005; Small et al., 2007), another set of primers designed for this work was tested to perform qRT-PCR assays, with the same result. It was reported that transcription of katA and katB genes is induced in response to increased H2O2 levels by activation of the transcriptional regulator OxyR (Ochsner et al., 2000; Heo et al., 2010). The role of H2O2, superoxide anion and hydroxyl radical in the regulation of katA and katB by UVA was then analysed by using the reporter strains katA–lacZ and katB–lacZ and specific ROS scavengers. Fig. 4(a) shows that the production of β-galactosidase of the strain carrying the katA fusion increased significantly (P<0.005) by about threefold in irradiated cultures compared with control cultures in plain LB. The presence in the culture medium of the H2O2 scavengers catalase and sodium pyruvate inhibited the production of β-galactosidase in cultures grown under UVA; on the contrary, the presence of DMSO (superoxide anion scavenger) or mannitol (hydroxyl radical scavenger) did not modify significantly the value observed in the culture without scavengers (Fig. 4a). In the case of the katB fusion, a
significant twofold induction \((P<0.05)\) was seen in irradiated cultures compared with the control condition (Fig. 4b). As seen for \(katA\), both \(H_2O_2\) scavengers inhibited expression of \(\beta\)-galactosidase of irradiated cultures compared with cultures grown under UVA without scavengers or in the presence of DMSO or mannitol (Fig. 4b).

In summary, our results indicated that regulation of KatA and KatB activities by exposure to sublethal UVA occurs at the transcriptional level and depends, at least in part, on the increase of endogenous \(H_2O_2\) concentration.

**Protection against subsequent oxidative stress factors by exposure to sublethal UVA doses: role of \(katA\) and \(katB\)**

To evaluate whether exposure to low UVA doses can trigger cross-protection against subsequent oxidative injuries, the WT was grown under low UVA doses or in the dark and then challenged with lethal doses of UVA, sodium hypochlorite or \(H_2O_2\). Catalase-deficient derivatives were submitted to the same treatments to evaluate the role of \(katA\) and \(katB\) in this phenomenon.

Fig. 5(a) shows that growth under sublethal UVA significantly increased the viability of the WT \((P<0.005)\) and the \(katB\) strain \((P<0.05)\) challenged with lethal UVA doses; this protection was not observed in the \(katA\) strain, demonstrating that this phenomenon depends on the \(katA\) gene. Growth under sublethal UVA significantly increased the resistance of the WT and the \(katA\) strains against sodium hypochlorite \((P<0.05)\); on the contrary, the treatment did not affect the response of the \(katB\) strain, indicating that protection depends on \(katB\) induction (Fig. 5b). Finally, growth under UVA induced protection to \(H_2O_2\) in the three strains in spite of their different sensitivity levels (Fig. 5c), suggesting that both genes are important but not essential, at least individually, for the UVA-mediated \(H_2O_2\) protection. Complementation assays with plasmids carrying the WT \(katA\) and \(katB\) alleles confirmed the roles of \(katA\) and \(katB\) in these phenotypes (Figs 5a–c). Taken as a whole, these results indicated that exposure to low UVA doses confers tolerance to subsequent lethal doses of different oxidative agents by mechanisms that involve induction of \(katA\) and \(katB\) genes.
DISCUSSION

In the environment, P. aeruginosa can be exposed to low UVA doses that are unable to affect its cell viability significantly. However, ROS levels high enough to induce the expression of defensive systems may be reached. ROS include the oxidizing agent H$_2$O$_2$, which is also generated by aberrant electron flow during aerobic respiration or by phagocytic cells during infections. To defend itself from H$_2$O$_2$, P. aeruginosa decomposes it into H$_2$O and O$_2$, largely by the action of KatA and KatB catalases. In this work, we demonstrate for the first time to the best of our knowledge that the genes coding for both enzymes are

![Graph showing Miller units for different conditions.](image)

**Fig. 4.** Reporter strains (a) katA–lacZ and (b) katB–lacZ were grown under sublethal UVA radiation or in the dark (control), in plain LB and in LB in the presence of catalase (Cat), sodium pyruvate (Pyr), DMSO or mannitol (Man). Samples were taken at OD$_{650}$ 0.3 and β-galactosidase activity was quantified. Data are presented as mean ± SE of at least three independent assays. *P<0.05; **P<0.005.

![Graph showing survival fraction for different conditions.](image)

![Graph showing sodium hypochlorite inhibition diameter for different conditions.](image)

![Graph showing H$_2$O$_2$ sensitivity for different conditions.](image)

**Fig. 5.** The PAO1 strain, the katA and katB mutants, and their complemented derivatives katB pkatB and katB pkatB, were grown under sublethal UVA (pre-UVA) or in the dark (control) and then exposed to lethal doses of (a) UVA, (b) sodium hypochlorite or (c) H$_2$O$_2$. (a) The total UVA dose employed for lethality assays was 216 kJ m$^{-2}$ (fluence rate of 20 W m$^{-2}$ for 180 min). After exposure, samples were taken to assess cell viability. (b) Sodium hypochlorite sensitivity was assessed by a diffusion assay by placing 8 µl 0.6 % bleach onto filter paper discs. Data are presented as mean ± SE of at least three independent experiments. *P<0.05; **P<0.005. (c) H$_2$O$_2$ sensitivity was measured by spotting serial dilutions of cell suspensions onto LB plates and LB plates containing 200 µM H$_2$O$_2$. Images of representative experiments are shown.
activated during its adaptation to sublethal UVA radiation and that this induction is accompanied by an increase in the corresponding enzymic activities. The extent of the growth delay produced by exposure to low doses of radiation could be a good indicator of response to UVA: the longer the growth delay (katA > katB > WT), the greater the degree of sensitivity to the radiation. Although the treatment did not produce significant cell death, chemiluminescence peaks, attributed to photon emission by excited carbonyl groups and singlet O₂ dimers arising from the decomposition of membrane lipid peroxides (Tilbury & Quickenden, 1988), indicate that cells suffered oxidative damage. Previous results demonstrating the essential role of KatA in the protection against UVA in planktonic cells and biofilms of P. aeruginosa (Costa et al., 2010; Pezzoni et al., 2014) indicate that induction of the katA gene by the radiation could constitute an adaptive mechanism to face with higher UVA doses. The essential role of KatA may be associated with the unique properties of this enzyme: it has normally high activity levels (Brown et al., 1995), is resistant to several agents (Hassett et al., 2000), active in the extracellular medium (Hassett et al., 2000) and has high affinity for NADPH, a strong reducing agent (Su et al., 2014). Although, as observed previously (Pezzoni et al., 2014), katB has been demonstrated not to be relevant in the defense against lethal UVA doses, its induction could contribute in this regard under other experimental conditions.

Unlike P. aeruginosa, catalase has a minor role in protection against lethal doses of UVA in other bacteria (Sammartano et al., 1986; Eisenstark & Perrot, 1987; Kramer & Ames, 1987). However, other components of the antioxidative response as well as genes involved in DNA repair participate in the defense against UVA. Mutant strains of Salmonella enterica lacking alkyl hydroperoxide reductase (ahp) or defective in glutathione synthesis (gsh) have high UVA sensitivity (Kramer & Ames, 1987), and a mutant strain of E. coli defective for both Fe-SOD and Mn-SOD has greater sensitivity to UVA compared with the WT strain (Hoerter et al., 1989). In addition, mutant strains of E. coli deficient in the excision repair system (uvr) are highly sensitive to UVA (Webb & Brown, 1976; Shennan et al., 1996), and similar results were obtained in strains of Salmonella and E. coli defective for RecA, the protein responsible for expression of genes involved in the SOS response (Eisenstark, 1970; Webb & Brown, 1976). The RpoS protein, the alternative sigma transcription factor involved in general stress responses during the stationary growth phase, is also involved in UVA defense both in enterobacteria and Pseudomonas syringae (Miller et al., 2001; Maatouk et al., 2004; Berney et al., 2006b). Studies of global gene expression during UVA adaptation in bacteria such as E. coli, Shewanella oneidensis and Enterococcus faecalis showed activation of the genes coding for antioxidant enzymes ahpCF, ahpC and sodA (Qiu et al., 2005; Berney et al., 2006a; Sassoubre et al., 2014); however, induction of genes coding for main catalases was not a common element in these studies. Microarray data of E. coli adapted to UVA revealed no effect on katG and repression of katE, the genes coding for HPI and HPII catalases, respectively (Berney et al., 2006a). This information is consistent with adaptive studies demonstrating a slight increase in HPI activity and a decrease in HPII activity by growth under sublethal UVA doses (Hoerter et al., 2005). In the case of Shewanella oneidensis, some minor catalase genes seem to be induced by UVA (Qiu et al., 2005), but the gene coding for the only functional catalase, KatB (Jiang et al., 2014), is not affected by the treatment. Thus, to the best of our knowledge, the induction of catalase by UVA and its relevant role in defense against lethal doses of UVA appears to be unique to P. aeruginosa.

The results presented in this study demonstrate that both katA and katB genes are induced by UVA exposure, but their behavior was somewhat different. Whilst katA clearly showed induction by the two techniques used (five-fold by qRT-PCR and three-fold by using a transcriptional fusion), a twofold activation of katB was seen only with the corresponding reporter strain. The absence of induction of katB by the qRT-PCR assay could be explained by the fact that this analysis was based on one time point; as expression can vary temporally, it is possible that a minor katB mRNA peak occurred at a different time and it could not be detected. On the contrary, the reporter assay involves all of the previous history of the cells during UVA exposure before sampling; as a consequence, this method is probably more sensitive to show katB activation by UVA compared with qRT-PCR.

Although significant H₂O₂ levels have never been measured in micro-organisms exposed to UVA (Hartman, 1986; Kramer & Ames, 1987), our data employing ROS scavengers and reporter strains support the hypothesis that H₂O₂ is a major product of exposure to lethal UVA doses (Tyrrell, 1985; Hartman, 1986; Kramer & Ames, 1987; Khaengraeng & Reed, 2005). In addition, it is demonstrated that induction of katA and katB depends, at least in part, on the H₂O₂ levels generated by UVA exposure, suggesting an OxyR-mediated response. OxyR is a central regulator of the oxidative stress response in P. aeruginosa. When it is oxidized by H₂O₂, it undergoes a conformational change and acquires DNA-binding capacity, allowing transcription of genes involved in oxidative stress defense, such as katA, katB, ahpB and ahpCF, amongst others (Wei et al., 2012).

This study demonstrates that the effect of UVA on KatA and KatB activities is accompanied by cross-protection phenomena. A relA-dependent protection against high UVA doses by pre-exposure to low UVA doses has been described previously for P. aeruginosa (Pezzoni et al., 2012). Under the conditions employed in our work, we demonstrated that this adaptive response depends on katA activation. In addition, induction of protection to the routinely used antimicrobial sodium hypochlorite by UVA exposure was demonstrated for the first time, to the
best of our knowledge. In this case, the phenomenon depends on katB induction. A previous study analysing the transcriptomic response of P. aeruginosa to sodium hypochlorite-induced oxidative stress revealed an increase in katB expression in the presence of this agent (Small et al., 2007). This finding and our results demonstrate the importance of katB gene in the response to bleach. Thus, this is the first report of a predominant role of KatB over KatA in defence against a stress factor. This result is quite surprising as a previous study comparing the roles of katA and katB in oxidative stress, osmotic stress and virulence demonstrated that katB always plays a secondary role compared with katA (Lee et al., 2005). Why katB is more relevant than katA in the defence against sodium hypochlorite is an open question and an interesting subject for further studies. It has been demonstrated that hypochlorous acid produces a rapid inactivation of catalase by modification of its haem group (Mashino & Fridovich, 1984; Krych-Madej & Gebicka, 2015). Thus, a mechanism that might explain the predominant role of KatB in the defence against bleach is a higher sensitivity of KatA to direct inhibition by this agent, in spite of its unusual high stability (Hassett et al., 2000). We also observed protection against lethal H2O2 levels by previous UVA exposure – a cross-protection phenomenon described in E. coli (Tyrrell, 1985). In this case, protection does not depend on KatA or KatB, at least individually. It is also possible that other inducible antioxidative factors, e.g. the OxyR-dependent alkylhydroperoxide reductases AhpC and/or AhpCF, could be induced by UVA exposure and to contribute to H2O2 resistance.

Different disinfection strategies, including SODIS and photocatalytic treatments (Gamage & Zhang, 2010; McGuigan et al., 2012), have been developed by taking advantage of the lethal effects produced by UV radiation. The results presented in this paper are relevant to improving them. SODIS consists of placing drinking water in transparent plastic or glass bottles, which are exposed to the sun. The germicidal effect is based on the combined effect of thermal heating by sunlight and UV radiation, mainly UVA. In photocatalytic treatments, the UVA light is applied in the presence of a photocatalyst, mainly TiO2, enhancing the bactericidal effect. This is a valuable disinfection technique against a wide range of harmful micro-organisms and represents a viable alternative to traditional disinfection methods such as chlorination, which can produce harmful products. On the basis of our results, it is clear that it is necessary to be sure that the doses of radiation are strong enough to produce irreversible cell death in order to avoid cross-protection against other antibacterial agents.

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REFERENCES


