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EXPERIMENTAL ARTICLES

Survival and Expression of DNA Repair Genes in Marine Bacteria *Pseudomonas pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 in Response to Environmental Stressors[1]

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Abstract—A comparative response of marine bacteria *Pseudomonas pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 under pH stress and UV radiation (UVR) revealed that both their survival pattern and repair mechanism are species specific. In case of *P. pseudoalcaligenes* NP103, the survival was maximum at pH 8, which decreased with decline in pH of the medium. Whereas, in *P. aeruginosa* N6P6, maximum survival was observed at pH 7. On exposure to UVR at different doses (25–200 mJ/cm^2) and increasing concentrations of Na^+ (1–6%), considerable differences in the recovery (2% for *P. pseudoalcaligenes* NP103 and 3% for *P. aeruginosa* N6P6) from UVR induced damage was observed. The qRT-PCR analysis of DNA repair genes (*recA* and *uvrA*) of marine bacteria subjected to different pH conditions showed significant ($P < 0.05$) up-regulation of both genes at pH 6, indicating higher degree of DNA damage at low pH. Furthermore, exposure of UVR irradiated cell suspensions to visible light exhibited greater photo-reactivating capacity in *P. pseudoalcaligenes* NP103 as compared to *P. aeruginosa* N6P6. The present findings indicate that pH and UVR exposure have crucial role in dictating the light dependent and independent DNA repair pathway in marine bacteria. Further, we speculate that both these repair response to the environmental stressors varies with bacterial species.

Keywords: DNA repair, *Pseudomonas, recA*, viable cell count, survival, *uvrA*

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INTRODUCTION

DNA is the nature's most supreme long-term genetic information storage structure, which contains the genetic directives. The elegant simplicity of the DNA double helix belies the intricate pathways that have progressed to replicate, modify and preserve the integrity of the genome (Lenhart et al., 2012). DNA in the living cell is subjected to many chemical alterations. If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected. This damage must be repaired quickly and efficiently to sustain the integrity of the genome. Cells contain a number of proteins and specific DNA repair systems that helps in maintaining its correct structure (Janion, 2008). The cellular responses to DNA damage include processes that deal with its consequences (e.g. tolerance and apoptosis) as well as direct correction of the damage by DNA repair mechanisms, which may require activation. In response to UV damage, bacteria have been reported to show different repair pathways, including photoenzymatic repair, nucleotide excision repair and recombination repair. The photoenzymatic repair is dependent on the activation of the photolyases or DNA repair enzymes upon visible light exposure (Rupert, 1962). Photolyases are universally present and functional in many species including bacteria, fungi, plants and animals and are concerned in repairing the damage occurred due to UVR exposure (Selby and Sancar, 2006). Nucleotide excision repair is regulated by UvrABC endonuclease enzyme complex, consisiting of four Uvr proteins, UvrA, UvrB, UvrC and DNA helicase II. Whereas, the recombination repair, is the major repair mechanism in bacteria, and is mediated by RecBCD pathway. If there is a major damage in the bacterial DNA and inhibit DNA replication, the repair mechanism involved is called SOS repair system (Žgur-Bertok, 2013).

Marine bacteria contribute around 90% of the cellular DNA in oceanic environments. The importance of preservation of genomic integrity of marine bacteria is attributed to the fact that these minute creatures are key players of marine ecosystems and biogeochemical cycles (Joux et al., 1999). Anthropogenic factors have significant impact on these microbes, potentially altering their diversity, function and community

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dynamics. Among different anthropogenic factors, solar UV radiation (UVR) (290–400 nm) and pH are two critical stressors and any alterations in the diversity, function and community dynamics of microbes may pose serious threats and disrupt ecological balance (Zenoff et al., 2006; Dash et al., 2013). Several studies regarding the detrimental effects of UV-A (320–400 nm) causing indirect damage to DNA, lipids and proteins through aggravating reactive oxygen intermediates and UV-B (290–320 nm) causing direct and indirect damage have been undertaken earlier (Joux et al., 1999; Dash et al., 2013). Marine bacteria are easily susceptible to UVR induced photodamage due to their haploid genomes with functional redundancy. Additionally, pH drop has profound effects on microbial processes as most of the marine bacteria are adapted to alkaline pH (Padan et al., 2005). Low pH depurinates DNA, which not only melts the double helix, but also removes the information from the strands. At very low pH, the phosophodiester backbone of DNA hydrolyzes, further reducing the DNA to nucleotides and nucleosides (Imamura et al., 1999). In spite of such deleterious effects of environmental stressors, bacteria survive by intrinsic DNA repair mechanisms. However, little is known regarding the responses and diverse repair mechanisms of marine bacteria. In the present work, effect of pH on survival and expression of *uvrA* and *recA* involved in UVR induced DNA damage repair have been studied in marine bacteria under the genus *Pseudomonas*.

MATERIALS AND METHODS

Bacterial Strains

Pseudomonas pseudoalcaligenes NP103 and *P. aeruginosa* N6P6 were isolated from water samples collected from Paradip port (20°17.542′ N and 086°42.996′ E) of Odisha coast, India. The isolates were identified by 16S rRNA gene sequencing following Mangwani et al., (2014). The partial 16S rRNA gene sequence of *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 has been submitted to NCBI GenBank under accession no JX273778 and KJ461700. The cultures have been deposited to BCCM under the deposition no. BCCM/LMG 28190 and BCCM/LMG 28185. Both the isolates were able to tolerate up to 6% of NaCl and in absence of Na^+ growth was inhibited which confirmed the true marine nature of these isolates.

UVB Irradiation and Growth Conditions

Overnight grown bacterial culture was diluted to 1 : 100 in Luria Bertani (LB) broth (Himedia, India). 25 mL of this diluted suspension was transferred to a 100 mL flask and incubated at 37°C under constant shaking. At regular time interval, the growth was measured in terms of absorbance (~600 nm) and CFU/mL (by spread plate method). The growth was monitored over the period of 24 h.

Cell mass from 10 mL of log phase bacterial culture was harvested by centrifugation (6000 rpm, 10 min). The culture supernatant was discarded and cell pellet was resuspended in 10 mL of Phosphate buffer saline (PBS). Each cell suspensions were transferred into sterile petri dish (Tarson, India) and were irradiated following Joux et al. (1999). The petri dish with lid was removed and exposed to UVR (254 nm) by 20W UV-B lamp (Philips). The UV dose (in milli Joules per centimetre square) was calculated by multiplying the intensity and exposure time in seconds (25–200 mJ/cm^2). The UVR exposed culture was diluted to 1 : 100 in LB broth medium and incubated at 37°C for 24 h under constant rotary agitation. Over the period of 24 h, growth was measured in terms of absorbance (~600 nm). The tolerance of marine bacteria to UVR was also measured at different pH (6, 7, 8 and 9) and NaCl concentration (6, 4, 3, 2 and 1%).

Photoreactivation and Dark Repair

To determine the effect of visible light on the survival of marine bacteria, viable cell count was carried out by spread plate method after UVR exposure. For this, log phase culture was serially diluted and spread on to LB agar plate. After that, the plates were exposed to UV dose (25–200 mJ/cm^2). The contribution of dark and light repair in UV-B induced cultures was studied separately by covering one set of UV-B exposed cultures in petri dish by aluminium foil and incubating at 37°C for 24 h in dark chamber without agitation. Another set of UV-B exposed cultures were subjected to visible light treatment (20 W white bulb, Philips) at an intensity of 0.026 W m^{-2}. The cell suspensions were continuously shaken at 30 rpm during photoreactivation and viable cell count was performed to determine colony forming units (CFU/mL).

DNA Damage Study: Comet Assay

The DNA damage upon UVR exposure was studied by neutral comet assay following Solanky and Haydel (2012). The details steps are described below.

Preparation of Culture

2 mL log phase bacterial culture was centrifuged to collect cell mass. The cell mass was washed twice with PBS. The cell pellet was resuspended in 2 mL of PBS. The cell suspension was exposed to different UV doses (25–200 mJ/cm^2). 2 μL of UVR exposed cells were mixed with 200 μL of 0.5% low melting agarose (in PBS).

List of primers used in the present study

Target organisms	Target Gene	Oligonucleotide sequence (5'–3')	Amplicon size
P. pseudoalcaligenes NP103	*recA*	F-TGGAACCCAAGCTGTTCTCC	162 bp
		R-AGTAGTAGACGTTGCGCCG	
	uvrA	F-CGTGAGGATGGTAATGGACGA	157 bp
		R-GATATCCAGCCCCAGCGAAC	
	16S	F-GTCTTCGGATTGTAAAGCAC	284 bp
		R-GCTACACAAGGAAATTCCAC	
P. aeruginosa N6P6	*recA*	F-AACTGGTCGCGAATGGTCTT	163 bp
		R-AGATCATCGATCTGGGCGTG	
	uvrA	F-TGGAACCCAAGCTGTTCTCC	162 bp
		R-AGTAGTAGACGTTGCGCCG	
	16S	F-GTCTTCGGATTGTAAAGCAC	284 bp
		R-GCTACACAAGGAAATTCCAC	

Slide Preparation and Microgel Formation

Clear glass slides (2.5 × 7.5 cm) were coated with a thin layer of 1% agarose (in PBS) by brief dripping. The precoated slides were allowed to solidify for 15 min (1st layer). After the formation of 1st layer, 100 μL of UVR exposed culture in low melting agarose was dispensed symmetrically over the precoated agarose slides (2nd layer). A 3rd layer (comprised of 0.5% low melting agarose, 5 μg/mL RNAse A, 1 mg/mL lysozyme and 0.25% N-lauroylsarcosine) was then made over the 2nd layer. The coated slides were kept at 4°C for 10 min to allow solidification of agarose layers. The slides were then incubated at 37°C for 30 min. The embedded cells were lysed by immersing coated slides into a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM TRIS, 1% sodium laurylsarcosine and 1% Triton X-100, pH 10) for 1 h at 30°C, followed by Proteinase K digestion in digestion buffer (2.5 M NaCl,10 mM EDTA,10 mM TRIS, 0.5–0.6 mg/mL of Proteinase K at pH 7.4) for 2 h at 37°C.

Electrophoresis and Staining

After enzymatic digestion, slides were electrophoresed in an electrophoresis buffer (300 mM sodium acetate, 100 mM TRIS, pH 9) for 50 min at 20 V. After electrophoresis, slides were immersed in 1 M ammonium acetate (in ethanol) for 20 min. Further, slides were immersed in absolute ethanol and 70% ethanol for 30 min and 10 min respectively. The slides were then allowed to dry.

Dry slides were pretreated with 5% DMSO solution and 10 mM NaH_2PO_4. While the slides were wet, DNA was stained with 50 μL of 1 μM Propidium iodide. The stained slides were observed under Fluorescence Microscope (Olympus, 1X71) at 40X magnification.

Quantitative Real-time PCR

Total RNA from UV-treated and untreated bacterial culture was extracted using TRIzol reagent (Invitrogen, USA) following Dash et al. (2014). For the first strand cDNA synthesis, 1 μg of total RNA from each group was treated with 1 unit of DNase I (Invitrogen, USA) and reverse transcription was carried out using oligo-dT primer and RevertAid first strand cDNA synthesis kit (Fermentas, USA).

Quantitative real-time PCR analysis of the target genes (i.e. *uvrA* and *recA*) and housekeeping gene (16S rRNA gene) was performed in Realplex real-time PCR system (Eppendroff, Germany). Amplification of target gene(s) was carried out in 10 μL reaction volume, containing 5 μL of 2X lightCycler® 480 SYBR Green I master mix (Invitrogen, USA), 1.0 μL of cDNA, 0.5 μL of 0.75 μM of each forward and reverse primer and 3 μL of PCR grade water. PCR amplifications were performed in triplicates (list of primers illustrated in table and PCR program in supplementary file). For specificity of RT-PCR, the reaction carried out without cDNA was used as negative control. Relative levels of target gene expression were calculated using the $2^{-\Delta\Delta CT}$ method (Pfaffl, 2001; Dash et al., 2014).

Statistical Analysis

All data are expressed as mean ± standard deviation (SD) of triplicate experiments. Student's t test was performed to signify the effect of pH and UVR on *recA* and *uvrA* relative gene expression. $P \leq 0.05$ was considered significant.

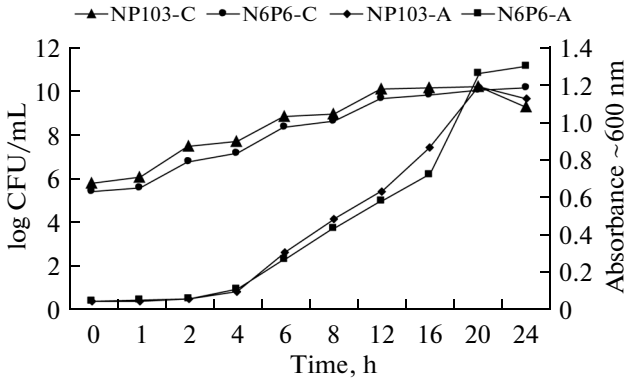

Fig. 1. Growth cuve for *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 (C is log CFU/mL, A is absorbance ~600 nm). In terms of log CFU/mL stationary phase was observed after 12 h of growth.

RESULTS

Tolerance of Marine Bacteria to UVR

The growth of *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 was monitored in terms of optical density at 600 nm and viable cell count after 24 h. In both bacterial strains, the stationary phase was observed after 12 h of growth (Fig. 1). The response of marine bacteria *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 after UVR exposure was monitored in terms of turbidity measured spectrophotometrically. In both the strains, a decrease in growth with increase in UV dose was observed (Fig. 2). Moreover, increase in UV dose resulted into a prolonged lag phase.

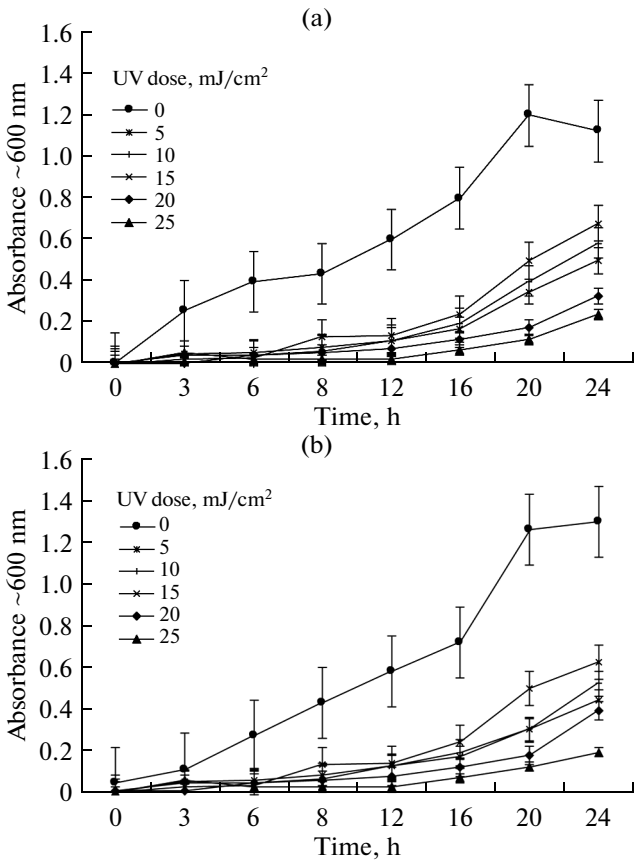

Fig. 2. Growth of marine bacteria after UVR exposure at different UV dose (mJ/cm^2) (a) *P. pseudoalcaligenes* NP103, (b) *P. aeruginosa* N6P6. With increase in UV dose, bacteria showed decreased in growth and prolonged lag phase.

Effect of pH on Tolerance to UVR

The effect of pH on tolerance to UVR was monitored spectrophotometrically. After UVR exposure, growth was monitored at different pH. *P. pseudoalcaligenes* NP103 grown at pH 7 showed maximum recovery as evidenced from 92.5 and 65% survival observed after 25 mJ/cm^2 and 50 mJ/cm^2 of UVR treatment respectively, as compared to growth on pH 6, pH 7 and pH 9 (Fig. 3a). *P. aeruginosa* N6P6 too showed maximum recovery from damage after UVR exposure at pH 7 with maximum survival of 77 and 61.5% after 25 and 50 mJ/cm^2 of UVR exposure respectively (Fig. 3b).

Effect of NaCl on Tolerance to UVR

The effect of UVR exposure on the growth of *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 was observed at different concentrations of NaCl (6% to 1%). The optimum growth was observed at 2% NaCl in *P. pseudoalcaligenes* NP103 (Fig. 4a). For *P. aeruginosa* N6P6, maximum growth was observed at 3% NaCl (Fig. 4b). The higher NaCl concentration has inhibitory effect on the growth of both UVR exposed and unexposed culture.

Effect on Light on Survival After UVR Exposure

The percentage survival upon UVR exposure was calculated by viable count method. After UVR exposure, the plates were incubated in both light and dark conditions. The % survival in both the strains incubated at visible light was around 0.005% even after brief exposure to UVR (25 mJ/cm^2). In both the strains, the survival at dark incubation was slightly more than that of light incubation. However, with increase in UV dose, the % survival upon incubation in light was increased. *P. pseudoalcaligenes* NP103 was able to grow even after 200 mJ/cm^2 of UV exposure (Fig. 5a). Whereas, in *P. aeruginosa* N6P6, complete loss of viability was observed beyond 100 mJ/cm^2 UVR exposure (Fig. 5b).

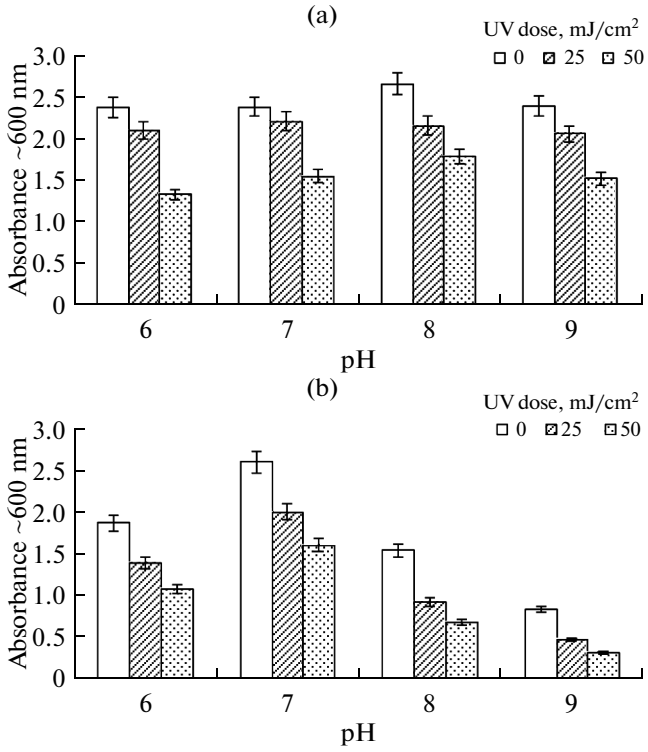

Fig. 3. Growth of marine bacteria after UVR exposure at different pH (a) *P. pseudoalcaligenes* NP103 (b) *P. aeruginosa* N6P6. *P. pseudoalcaligenes* NP103 showed maximum growth at pH 8. For *P. aeruginosa* N6P6 optimum growth was observed at pH 7.

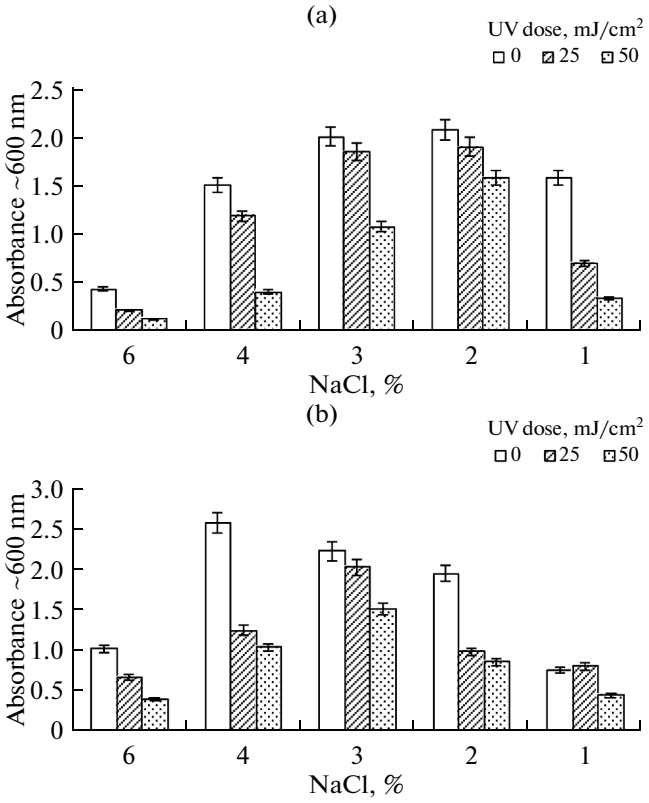

Fig. 4. (a) Effect of NaCl on tolerance to UVR (a) *P. pseudoalcaligenes* NP103 (b) *P. aeruginosa* N6P6. Beyond optimum level, NaCl has inhibitory effect on the growth of both UVR exposed and unexposed culture. *P. pseudoalcaligenes* NP103 showed maximum recover from UVR damage at 2%. For *P. aeruginosa* N6P6, growth and recovery from UVR damage was optimum at 3% NaCl.

Comet Assay for DNA Damage Detection

DNA damage by UVR was studied by neutral comet assay. The comet was observed under fluorescence microscope after staining with Propidium Iodide. A round head like structure was observed in control of both *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 (Fig. 6a, 6b). Even though there was significant decline in the growth of marine bacteria after 200 mJ/cm^2 UV dose, but substantial DNA damage was lacking (absence of tail). The tail size increased with increase in UV dose. The tail length was maximum at after 12000 mJ/cm^2 of UV exposure, which corresponds to heavy DNA damage in both the strains of marine bacteria (Fig. 6).

recA and uvrA Expression

Effect of pH on *recA* and *uvrA* expression was studied by qRT-PCR. Bacterial strains were grown at different pH (pH 6–9). At stationary phase of growth cell mass was harvested and exposed to 50 mJ/cm^2 of UVR. Expression of both *recA* and *uvrA* was higher at pH 6 for *P. pseudoalcaligenes* NP103 after UVR exposure. Expression of *recA* increased by 55 fold at pH 6 after UVR exposure. Whereas, expression of *uvrA* increase by 19 fold at pH 6 (Fig. 7a). There was a significant increase in *uvrA* expression ($P < 0.001$) upon UV exposure at pH 6. However, there was no significant increase in its expression at pH 8 and pH 9 ($P > 0.05$). In contrary, *recA* expression varied significantly at all the tested pH ($P < 0.01$). At pH 9, *recA* and *uvrA* expression increased by 9.4 and 6.8 fold respectively. At pH 6 and pH 9, expression of *recA* was more than *uvrA*. However, at pH 7 and pH 8, the expression of *uvrA* was higher than *recA* (Fig. 7). In *P. aeruginosa* N6P6, a significant increase of *uvrA* expression was observed only at pH 6 ($P < 0.05$) and its expression was increased by 90.59 fold (Fig 7b). The *recA* expression at pH 6 and pH 9 was 37.93 and 30.38 fold respectively. However, no significant increase in *recA* and *uvrA* expression was observed at pH 7 and pH 8 ($P > 0.05$).

For both the bacterial strains, the relative expression of *recA* and *uvrA* was very high at pH 6 after UVR exposure (Fig. 8). The fold change in *recA* expression after UVR exposure at pH 6, pH 8 and pH 9 was 222.2, 6.69 and 77.6 respectively. Whereas, *uvrA* expression after UVR exposure at pH 6, pH 8 and pH 9 was 31.4, 11.9, 25.4 respectively (Fig. 8a, 8b). Although the

expression of *recA* and *uvrA* was affected by change in pH, but as compared to pH 7 the significant effect of alkaline pH on their expression was lacking ($P > 0.05$).

In *P. aeruginosa* N6P6, *recA* expression at pH 6 in non-UVR treated culture was 15 fold more than that at pH 7. Whereas, at pH 8 and pH 9 fold change in its expression was 4.1 and 6.9 respectively (Figs. 8c, 8d). High expression of *uvrA* in non-UVR treated culture at different pH indicates the sensitivity of *P. aeruginosa* N6P6 to pH shift and probable role of DNA damage. In UVR treated *P. aeruginosa* N6P6 culture, the fold change in expression of *recA* at pH 6, pH 8 and pH 9 was 305.6, 2.9 and 83.6 respectively. The change in its expression was significant at pH 6 and pH 9. The relative fold change in *uvrA* expression was 2517, 8.9, and 151.2 at pH 6, pH 8 and pH 9 respectively (Figs. 8c, 8d), which affected significantly at pH 6 only ($P < 0.01$). The variance in *recA* and *uvrA* expression at different pH indicates that these genes are involved in pH and UV stress.

DISCUSSION

The ensuing impact of changing pH on marine life has become one of the most influential issues. Besides pH stress, the deleterious effects of UV radiations causing DNA damage in marine bacteria have also been recorded. These changes play an important role in the overall functioning of marine bacteria. A comprehensive study on the effect of change of pH, UVR and NaCl concentration on marine bacteria was carried out. Seawater pH is typically limited to a range of 7.5–8.4. pH 8 is the optimum pH for bacterial growth in marine environment. A further increase (or decrease) in pH reduces the growth rate in the bacteria (Krause et al., 2012). It was observed that the recovery from UVR by *P. pseudoalcaligenes* NP103 was highest at pH 7 (measured in terms of % survival). Whereas, for *P. aeruginosa* N6P6, optimum growth after UVR exposure was found at pH 7. The finding suggests significance of growth on pH for survival upon UVR exposure. Similarly, in marine environment, microbes are subjected to fluctuating surface water pH and solar radiation. Looking at the current scenario of global warming, it can be expected that fluctuations in surface ocean water pH and solar radiation may affect the microbial community structure as response varies from organisms to organisms (Arrieta et al., 2000). Joux et al. (1999) also studied the response of different marine bacteria toward UVR in terms of survival. From their study they concluded that UVR can induce cyclobutane pyrimidine dimers (CPDs) and survival from damaged varies among bacterial isolates.

In the present study, the decrease in salt concentration, up to certain level caused increase in growth of the bacteria. Growth was maximum at NaCl concentration of 3% in *P. aeruginosa* N6P6 whereas, in *P. pseudoalcaligenes* NP103, it is found to be 2% which is in accordance with Stanley and Morita (1968).

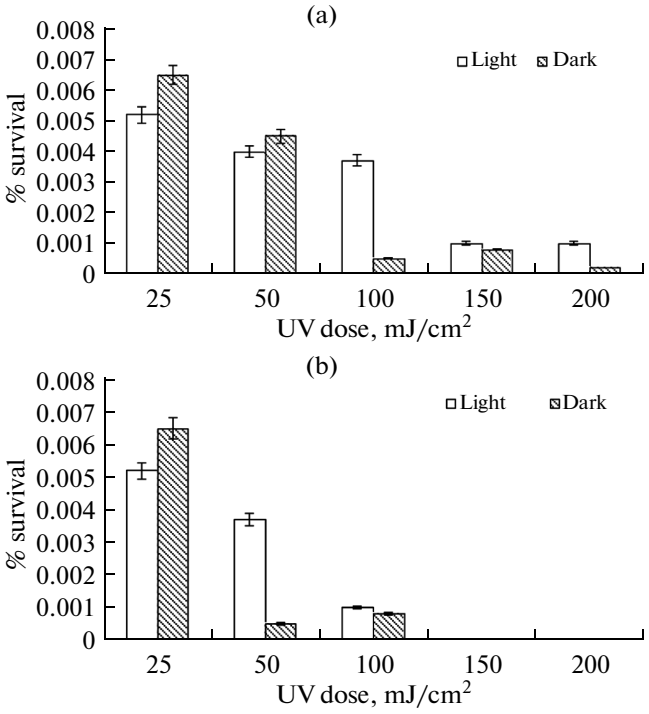

Fig. 5. (a) Effect of light on survival after UVR exposure (a) *P. pseudoalcaligenes* NP103 (b) *P. aeruginosa* N6P6. With increase in UV dose, an increase in the % survival under light was observed.

However, salt concentration beyond 4% has inhibitory effect on both UVR exposed and unexposed bacterial culture. Bacteria have damage response mechanisms to increase their chance of survival in response to the UVR and other damage causing agents. Several methods are used to find out the amount of damage in the bacterial cells. In the present study, DNA damage was confirmed by comet assay in UVR exposed bacterial cultures at the dose greater than 200 mJ/cm^2. The tail was observed in UVR exposed bacterial culture and heads were seen in control slides in the comet assay. The findings reflect that with an increase in UVR exposure damage increases. Exposure to UVR can induce the formation of mutagenic DNA lesions such as cyclobutane- pyrimidine dimers. However, longer exposure to UVR generates multiple adducts and cause DNA strand breaks. Comet assay is a rapid and sensitive method for measuring DNA strand breaks (Sinha and Hader, 2002; Lee and Steinert, 2003; Collins, 2004).

The presence of genes involved in repair of DNA damage are lost and gained in the several bacterial species as a response to a change in habitat or DNA damaging agents (Eisen and Hanawalt, 1999). Photolyase is the oldest UV damage repair protein which must have evolved in the first organisms that inhabited regions where they were exposed to UV light (Thompson and Sancar, 2002). Tolerance of two marine *Pseudomonas* strains to UVR under different growth

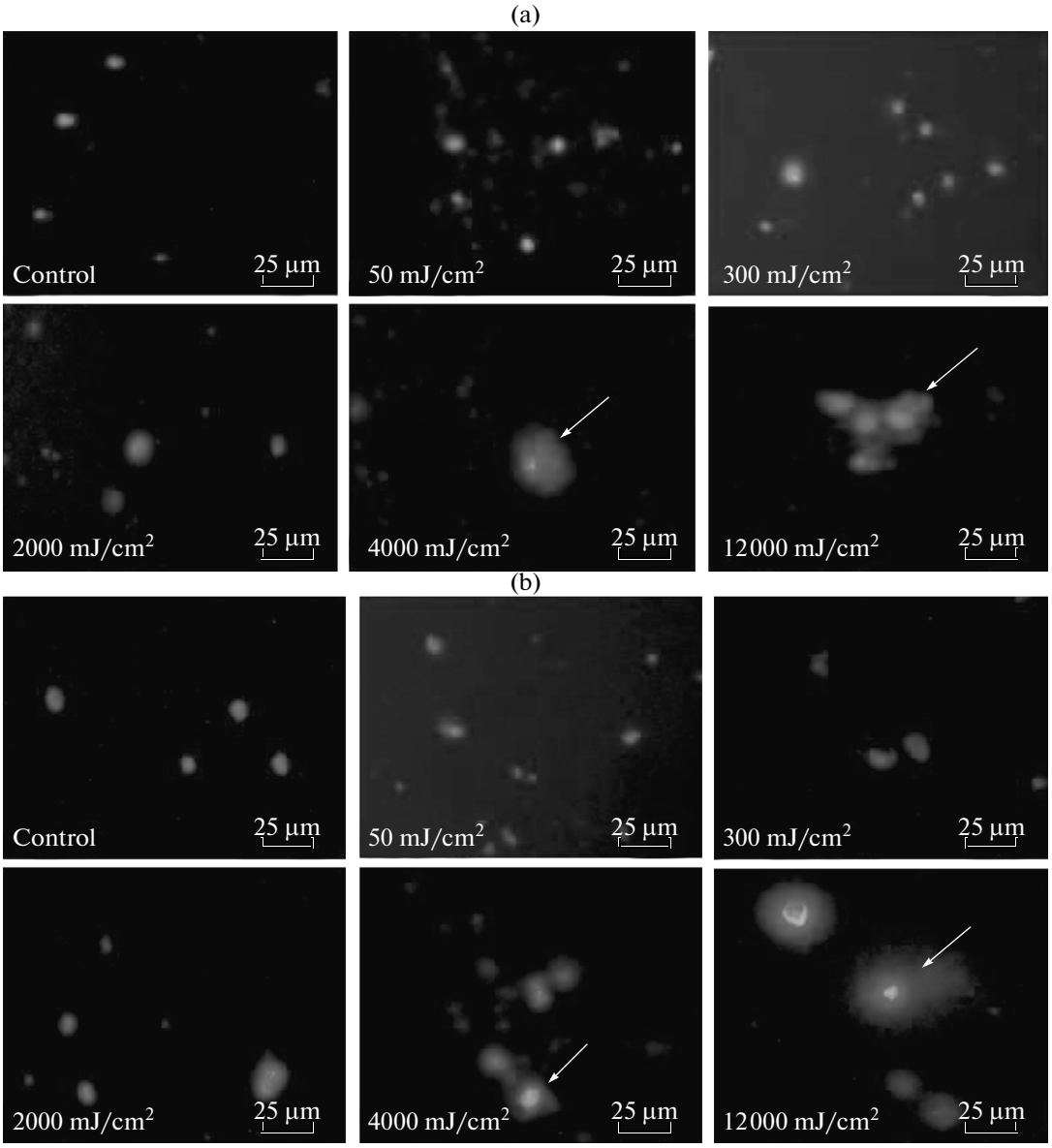

Fig. 6. DNA damage study by neutral comet assay (a) *P. pseudoalcaligenes* NP103, (b) *P. aeruginosa* N6P6. The tail (pointed with an arrow) size increased with increase in UV dose. The tail length was maximum after 30 min of UVR exposure, which corresponds to heavy DNA damage. The tail indicates the migrated DNA due to strand fragmentation.

conditions has been studied. Both *P. pseudoalcaligenes* NP103 and *P. aeruginosa* N6P6 showed reduction in growth in response to increase in UV dose. The percentage survival on exposure to visible light after UVR treatment was also studied. It was observed that initially dark repair mechanism was more active and with passage of time photorepair becomes significant. Not all organisms possess photolyases but almost all, with the possible exception of some archaea, have excision repair mechanisms (Crowley and Hanawalt, 1998).

Any change in integrity of DNA can result in the induction of pathways to restore its original conformation. The activation of any repair pathway is relied upon the type of error and growth state of cell. Exposure to UVR to sublethal dose results in adducts formation, which induces SOS response. Survival depends chiefly, upon the activation of repair mechanism to deal with resulting damages. NER and recombination repair pathway are major pathways involved in repair of UVR induced DNA damage (Mangoli et al., 2014). Variance in the expression of *recA* and *uvrA* at different pH indicates the sensitivity and survival is also dependent on H^+ concentration.

The expression of *recA* mRNA elevated upon UVR exposure. Booth et al. (2001) reported an upturn in the RecA protein expression in *Vibrio nartriegens* with increase in UVR induced DNA damage. They suggested that *recA* expression is a suitable monitoring

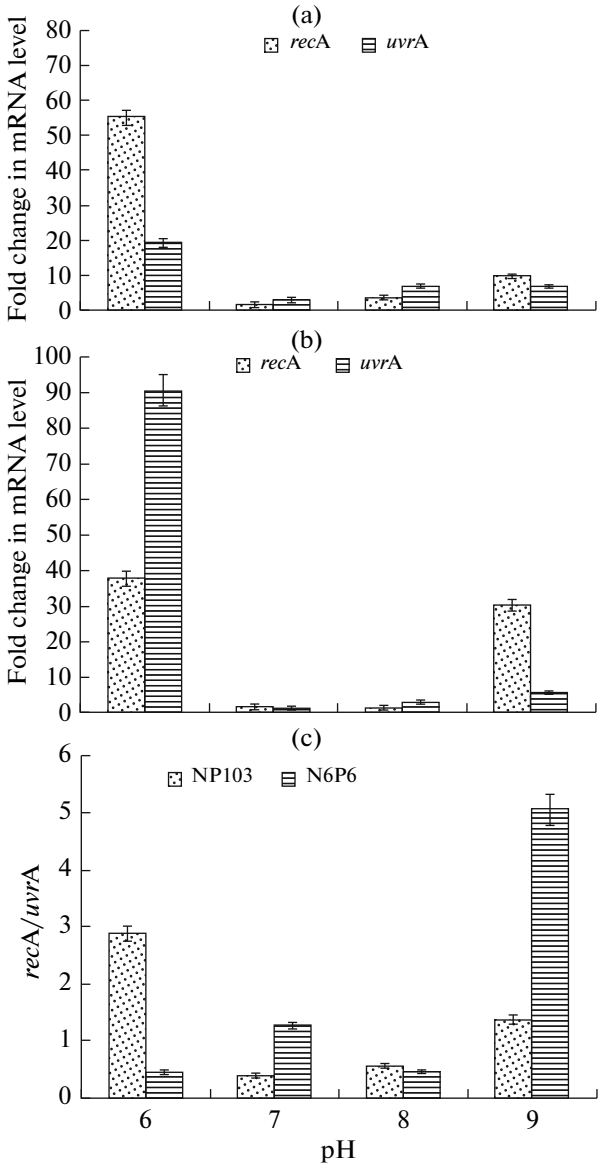

Fig. 7. *recA* and *uvrA* expression upon UVR exposure at different pH (a) *P. pseudoalcaligenes* NP103. (b) *P. aeruginosa* N6P6. (c) Ratio of *recA* to *uvrA* fold change. The fold change in *recA* and *uvrA* mRNA level at different pH after UVR exposure is expressed in terms of *recA* and *uvrA* expression in UVR unexposed bacterial culture.

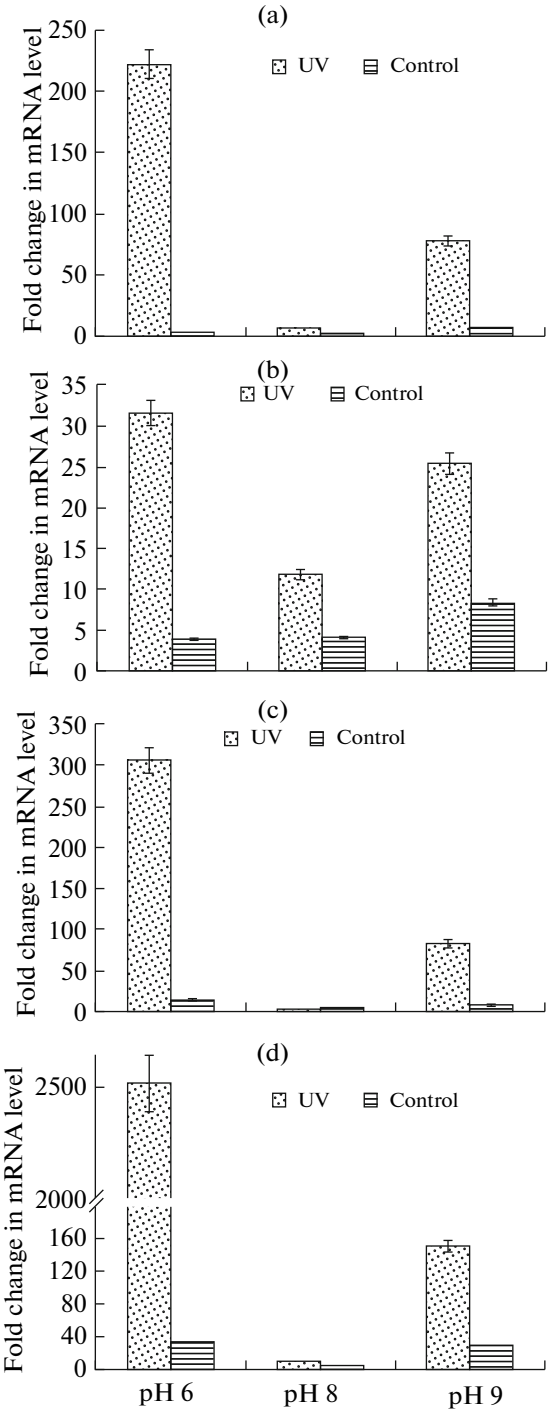

Fig. 8. Fold change in *recA* and *uvrA* expression at different pH in UVR exposure (UVR) and unexposed (Control) in bacterial culture. The fold change in mRNA level is expressed with respect to target gene level at pH 7. *recA* (a) and *uvrA* (b) expression in *P. pseudoalcaligenes* NP103. *recA* (c) and *uvrA* (d) expression in *P. aeruginosa* N6P6.

system for UVR induced DNA damage and repair in marine environment. *recA* is also a key regulator of UVR induced DNA damage repair pathways, which is highly conserved genetic element and universally present in eubacteria. *uvrA* encodes for protein, which is a component of NER pathway. *uvrA* along with *uvrB* and *uvrC* gene product form a protein complex, which functions in locating and excision of bulky DNA lesions (Jaciuk et al., 2011). *uvrA* and NER pathway is reported to be involved in acid-induced damage repair in some bacteria (e.g. *Streptococcus mutans* and *Lactobacillus helveticus*). Thus, the induction of NER repair pathway is also involved during survival under pH stress (Jaciuk et al., 2011). Upon UVR exposure the expression amplified exceedingly. The higher expression of uvrA at pH 6 in non-UV treated cells in marine bacteria *P. pseudoalcaligenes* NP103 and *P. aeruginosa*

N6P6 indicates similar kind of mechanism for survival under stress. Similar trend was observed in *recA* expression. Thus, from the above study it can be concluded that survival of bacteria to UVR is affected by growth conditions. The genus *Pseudomonas* is one of the most heterogeneous and dominant groups of bacteria having diverse distribution. *P. aeruginosa* is commonly present in coastal marine environment and used for various environmental applications (Dash et al., 2013; Chakraborty and Das, 2014). *P. pseudoalcaligenes* from marine origin has been reported earlier (Romanenko et al., 2005). *P. aeruginosa* has been extensively studied for its DNA damage and repair pathways (Ochsner et al., 2000). However, there are limited studies on *P. aeruginosa* of marine origin with respect to the effect of environmental stressors on DNA damage. Moreover, to the best of our knowledge, *P. pseudoalcaligenes* has not been documented for DNA damage repair studies. Thus, the findings of the present work provide a comprehensive insight into the differential response of marine bacteria to environmental stressors at species level.

In the present study, response of *Pseudomonas* spp. to UVR was evaluated under different growth conditions. The survival of bacteria has been affected considerably by pH and Na^+ concentration. Moreover, the expression level of DNA repair genes (*recA* and *uvrA*) was affected significantly by pH changes. In particular, low pH (i.e. 6) caused increase in the expression of *recA* and *uvrA* even in absence of UVR, indicating the added impairments rooted by pH shifts on marine bacteria. However, acidic pH and UVR stress synergistically elevated the expression of DNA repair genes. Thus, understanding the effects of low pH and UVR exposure on the tiny single celled bacteria may pave the way to estimate the future ocean scenario under the increased anthropogenic pressure, climate change and ocean acidification events.

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