Journal of Photochemistry and Photobiology B: Biology 103 (2011) 234–242

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Interspecies variation in survival and growth of filamentous heterotrophic bacteria in response to UVC radiation

Manuela Filippini [a,*], Claudio Ortelli [b], Miroslav Svercel [a], Homayoun C. Bagheri [a]

[a] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[b] Institute of Finance, University of Lugano, Via Giuseppe Buffi 13, CH-6900 Lugano, Switzerland

ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form 14 March 2011
Accepted 28 March 2011
Available online 7 April 2011

Keywords:
UVC radiation
Filamentous bacteria
Cytophagaceae
Growth
Resistance

ABSTRACT

Ultraviolet radiation is an important environmental constraint on the evolution of life. In addition to its harmful effects, ultraviolet radiation plays an important role in generating genetic polymorphisms and acting as a selective agent. Understanding how prokaryotes cope with high radiation can give insights on the evolution of life on Earth. Four representative filamentous bacteria from the family *Cytophagaceae* with different pigmentation were selected and exposed to different doses of UVC radiation (15–32,400 J m^{-2}). The effect of UVC radiation on bacterial survival, growth and morphology were investigated. Results showed high survival in response to UVC for *Rudanella lutea* and *Fibrisoma limi*, whereas low survival was observed for *Fibrella aestuarina* and *Spirosoma linguale*. *S. linguale* showed slow growth recovery after ultraviolet exposure, *R. lutea* and *F. limi* showed intermediate growth recovery, while *F. aestuarina* had the fastest recovery among the four tested bacteria. In terms of survival, *S. linguale* was the most sensitive bacterium whereas *R. lutea* and *F. limi* were better at coping with UVC stress. The latter two resumed growth even after 2 h exposure (~10,800 J m^{-2}). Additionally, the ability to form multicellular filaments after exposure was tested using two bacteria: one representative of the high (*R. lutea*) and one of the low (*F. aestuarina*) survival rates. The ability to elongate filaments due to cell division was preserved but modified. In *R. lutea* 10 min exposure reduced the average filament length. The opposite was observed in *F. aestuarina*, where the 5 and 10 min exposures increased the average filament length. *R. lutea* and *F. limi* are potential candidates for further research into survival and resistance to ultraviolet radiation stress.

1. Introduction

Much of life on Earth is dependent on solar energy. However, radiation in the ultraviolet (UV) range causes damage to DNA and is thus often lethal to organisms [1]. This damage is wavelength dependent. UVA (320–400 nm) causes only indirect damage to DNA, proteins and lipids through reactive oxygen intermediates. UVB (280–320 nm) and UVC (100–280 nm) cause both indirect and direct damage because of the strong absorption by DNA molecules at wavelengths below 320 nm [2]. The consequence is the production of cyclobutane pyrimidine dimers (CPD) causing distortion of the DNA molecule, which might cause malfunctions in cell replication and lead to cell death [3]. Bacteria are particularly vulnerable to UV damage because their small size limits effective cellular shading, and their genetic material comprises a significant portion of their cellular volume [4]. Moreover, UV adsorbing compounds (mycosporine-like amino acids and scytonemin) that confer some protection to eukaryotic organisms and cyanobacteria are not widespread in most bacterioplankton [5]. The documented prevalence of pigmentation in UV-resistant species of bacteria suggests the use of carotenoids as UV screening and antioxidative agents [6,7]. However, several studies (e.g., [8,9]) found that pigmentation had little effect on UV resistance. In addition to pigmentation, bacteria have several repair mechanisms in response to UV-induced damage. Repair mechanisms are usually classified into dark repair (DR) and photoreactivation. The latter occurs only in the presence of certain wavelengths of visible light.

Studies on UV can be grouped into two categories: those on ecologically more relevant radiations UVA and B, and those on UVC. UVA and B reach the Earth's surface and their effect on the ecology of microorganisms has been studied in detail in aquatic systems [10]. Even though UVC is absorbed by oxygen and ozone in the Earth's atmosphere, the study of solar ultraviolet radiation is important because of the role of UVC as a major constraint on biological evolution [11]. The effects of solar UV radiation are thought to have been prevalent before the spread of terrestrial life for at least two billion years, until the stratospheric ozone layer developed [12]. Therefore, it is likely that early in their history, living organisms developed a number of

* Corresponding author. Tel.: +41 44 635 47 36; fax: +41 44 635 68 87.
E-mail address: manuela.filippini@ieu.uzh.ch (M. Filippini).

1011-1344/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2011.03.018

mechanisms for protection against the destructive effects of UVC [13].

Jagger [14] suggested that the amount of solar radiation present in an organism's environment dictates its level of UV resistance. Therefore bacteria that evolved after the ozone layer's formation should, in principle, not be resistant to UVC (given that they have not been exposed to it). However, differences in resistance have been observed (e.g., [15,16]). This could either indicate that during their evolutionary history bacteria were somehow exposed to UVC, leading to the evolution of protection that has been subsequently maintained, or that unspecific mechanisms used for other stress factors are protecting them from damaging radiations. Studies on UVC resistant bacteria have been primarily done on spore-forming bacteria (e.g., *Bacillus*). However, recently Ghosh et al. [17] demonstrated that a variety of non-spore-forming bacteria are able to survive extreme conditions. Therefore, as already suggested by Cockell et al. [18], it is important to also take into account resilient non-spore-forming microbes.

Members of the phylum *Bacteroidetes* are widely distributed in different environments and constitute one of the most abundant picoplankton groups [19]. Furthermore, they play an important role in the global carbon cycle; they have the ability of degrading polysaccharides and other macromolecules [20]. Among this phylum, the *Cytophagaceae* family has been the least studied. The primary objective of this study is a phenomenological characterization of the effects of UVC on survival, growth and morphology of filamentous heterotrophic bacteria from this family. We used type strains of four genera showing different pigmentation. This allows us to test the potential effect of existing pigmentation and to compare species specific responses.

2. Materials and methods

2.1. Bacterial strains

Four non motile filamentous heterotrophic bacteria belonging to the phylum *Bacteroidetes*, family *Cytophagaceae*, were used: (i) *Rudanella lutea*, an orange bacterium isolated by Weon et al. [21] (DSM 19387^T) from air, (ii) *Spirosoma linguale*, a yellow bacterium isolated by Larkin [22] (DSM 74^T) from a laboratory water bath, (iii) *Fibrella aestuarina* BUZ2^T [23]) and (iv) *Fibrisoma limi* BUZ3^T [24], both isolated from mud samples from the North Sea coast of Germany (with pink and orange pigments, respectively). Pigments of each bacterium were extracted in the dark in 99% EtOH for 20 min and the absorption of the cells-free coloured supernatant measured with a spectrometer (SpectraMax 384 Plus, Molecular Devices, USA).

2.2. UVC irradiation procedure and survival curve

Prior to the experiments, bacteria were grown on R2A (DSMZ 830) and/or SM (DSMZ 7), plates for 4 days in the dark at 28 °C. Cell suspensions were prepared by adding cells to 0.2% NaCl until reaching an OD_{600nm} of 0.080 ± 0.010, which is approximately 5×10^6 CFU ml^{-1}. This density should prevent shading between cells [8]. Cell suspensions were dispersed onto a Petri dish, approx. 0.5 cm depth of the liquid, and placed open on ice to prevent heating. Plates were placed 60 cm below a G30T8 germicidal lamp (Jiangsu Juguang, China) having a maximum emission peak at 254 nm and with an estimated intensity of 1.5 J m^{-2} s^{-1} (at 60% of the lamp's maximal intensity at manufacture, Zimmermann Messtechnik AG personal communication). Intensity was assumed uniform and to reduce any possible difference due to position, the plates were always placed at a fixed position. For the short UVC experiment, the cell suspensions were exposed for 0 s (unexposed samples), 10 s, and 1, 3, 5 and 10 min. A subsample of the 10 min UVC exposed suspension was illuminated for an additional 20 min under a 5000 k daylight white fluorescent lamp (T8-15W supplied by ESCO, Europe, 1klux intensity) to allow photoreactivation. The unexposed samples (0 s) were kept in the dark on ice and were used as control. These samples were packaged and handled in the same manner and at the same temperature as the exposed cell suspensions. After each exposure (0 s, 10 s, 1, 3, 5, 10 min and 10 min plus 20 min white light (10 min + P)), 100 μl of each cell suspension was serially diluted in 0.2% NaCl and 2 × 100 μl was spread on SM plates. Plates were incubated in the dark at 28 °C to allow bacterial growth and the colony forming units (CFU) were counted after 3–5 days. All handling was done in the dark to prevent photoreactivation. These procedures were also applied to the second experiment where bacteria were exposed to longer UVC periods (0 s, 30 min, 1, 2, 3 and 6 h). In the same way as explained above, cell suspensions were dispersed onto a Petri dish which was subsequently placed open on ice and finally irradiated. Cell survival after each treatment was calculated by dividing the CFU count after each UVC exposure by the CFU of the unexposed controls (0 s UVC) and was expressed as a percentage. To compare strain resistances to radiation, D-values were extrapolated from the best fit curve of the survival data with the higher R^2 values (linear or exponential). D_{37} is defined as the radiation dose required to inactivate 63% of a bacterial population, whereas D_{10} is the radiation dose which inactivates 90% of the bacterial population [8]. UVC dose was calculated by multiplying the lamp intensity by the exposure time in seconds.

2.3. Growth curve and morphology

Control or exposed samples (0.9 ml) were added to 10 ml of SM broth and incubated in the dark on a shaker (200 rpm) at 28 °C. The conditions approximate optimal conditions for these bacteria. The bacterial recovery was followed in the dark and hence the efficiency of light independent DNA repair mechanisms were investigated. The growth for the short UVC experiment (UVC exposure ranging from 10 s to 10 min) was determined by measuring optical density (OD) over time. Briefly, 200 μl of samples was transferred on a microtiter plates and the increase in OD_{600nm} over time was read with a spectrometer (SpectraMax 384 Plus, Molecular Devices, USA). After OD measurement, samples were fixed with formaldehyde (2% final concentration) and stored at 4 °C until filament morphology was examined by light microscopy, which was completed within one day after fixation. Light microscopy was done using an Olympus BX51 microscope. Five to 15 μl were placed on a microscope slide and several pictures were taken with a digital camera. The length of approximately 150 filaments on at least three different pictures was measured manually with the soft imaging system CellF (Olympus, Germany). For the long UVC exposure experiment (ranging from 30 min to 6 h) only qualitative results in terms of "growth/no growth" were determined.

2.4. Statistics

Survival and growth experiments were repeated at least three times. No significant difference between experiments was determined (p-value < 0.05), therefore the results for each experiment type were pooled together. Analysis of variance (ANOVA) was performed at the significant level of $p < 0.05$ using SPSS 17.0 to test the effect of the UVC exposure on survival, growth curves and filament lengths. Survival results, expressed as percentage of the untreated control, were arcsine-transformed, whereas OD measurements were logarithm transformed prior to statistical analyses. Means were separated using Tukey's test ($p < 0.05$). In order to compare growth curves for each bacterium in different UVC