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Notes and Short communications

Divergences in response to UV radiation between polar and non-polar eukaryotic microorganisms.

Short title: UV-radiation tolerance in polar *Euplotes*

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Abstract Ultraviolet radiation exerts detrimental effects on terrestrial and marine ecosystems, in particular in polar regions where elevated solar radiation is caused by stratospheric ozone reduction. We explored the sensitivity to ultraviolet radiation of two polar species of the ciliate *Euplotes*, *Euplotes focardii* and *Euplotes nobilii*, in comparison with their close phylogenetic relatives inhabiting mid-latitude and tropical waters. Results showed that the two polar species face UV radiation more efficiently than their non-polar relatives, however by adopting different mechanisms. While *E. focardii* survives for longer times to UV radiation, *E. nobilii* recovers faster from UV-induced damages.

Introduction

Among the environmental factors influencing the temporal and spatial distribution of aquatic microorganisms, a dominant role is played by the intensity of ultraviolet (UV) radiation and depth of UV penetration into the water column. These factors are particularly detrimental to microbial communities [4, 16, 25, 26] because UV-radiation may cause cell-damage on several cell targets, either directly through absorption of the hazardous short-wavelength (280-320 nm) UV-B radiation, or indirectly through the generation of reactive oxygen species induced by the longer wavelength (320-400 nm) UV-A radiation [1, 15, 19, 24]. Polar organisms are particularly exposed to UV-induced cellular damage, especially those of the Antarctic regions where the continuous decline in stratospheric ozone tends to substantially increase the levels of UV-radiation [5, 6, 11, 20].

In polar ecosystems, species of the ubiquitous ciliate *Euplotes* are a very common component of marine microbial communities and their isolates can easily be adapted to grow in stable laboratory cultures [12, 13]. We used two sets of these cultures, one representative of *E. focardii*, a species endemic to Antarctic waters [22], and the other one representative of *E. nobilii*, which is distributed in both Arctic and Antarctic waters [7, 21], in order to compare polar species of *Euplotes* with their non-polar relatives in terms of their capacity to withstand UV-radiation. Based on the knowledge of phylogenetic trees of *Euplotes* species generated by matching of the small subunit (SSU)-rRNA nuclear gene sequences [8, 23], *E. quinquecarinatus* and *E. raikovi* were chosen as non-polar relatives of *E. focardii* and *E. nobilii*, respectively. From this comparison we obtained clear evidence that the polar species of *Euplotes* are either more resistant to UV exposure (*E. focardii*), or can more easily repair the UV-induced cell damages (*E. nobilii*).

Materials and Methods

Strain origin and cultivation

Collection sites of the *Euplotes* strains used in this study are presented in Table 1. The *E. focardii* and *E. nobilii* cultures were maintained at 4 °C and the cultures of *E. quinquecarinatus* and *E. raikovi* at 23 °C. All were fed with the green algae *Dunaliella tertiolecta* grown in defined salt artificial seawater (32‰, pH 8.0). Cells were washed free of food and re-suspended in fresh sea water for 2 days before being used in experiments.

Irradiation experiments

Irradiation experiments were carried out in 96-well plates using aliquots of 10,000 cells/well in 100 µl of seawater and fluorescent lamps (UVP Inc., Upland, CA, USA) of 365 nm and 15 W for UV-A, and 302 nm and 15 W for UV-B. Any other light source was excluded throughout irradiation. The distance between the plates and the lamps was set to 10 cm in order to expose cells at UV-A and UV-B to average intensities of 3.90 mW/cm^2 and 4.00 mW/cm^2, respectively. The UV outputs were monitored with a UVX Digital Radiometer (UVP Inc., Upland, CA, USA), supplied with a 365 (UVX-36) and 310 (UVX-31) nm detector placed at the same distance from the UV source as the plates.

Cell viability assays

The cell viability was assayed according to a previously described standard protocol [2]. After the UV irradiation, cells were incubated for 60 min in the presence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (10 µl/well from a 5 mg/ml solution), which is reduced to formazan by viable cells. The reaction was stopped through the addition of DMSO to dissolve the insoluble purple formazan, quantified by absorbance at 570 nm using a Microplate Reader 680 XR

Spectrophotometer (Bio-Rad, Hercules, CA, USA). The statistical significance of the data was evaluated by combining ANOVA and Newman-Keuls Multiple Comparison post-test.

Results and Discussion

The sensitivity to UV-radiation of the two polar species, *E. focardii* and *E. nobilii*, was first compared with the sensitivity of the two non-polar species, *E. quinquecarinatus* and *E. raikovi*, by exposing equivalent cell samples to UV-A or UV-B radiation in temperature-controlled chambers (the polar species at 4 °C and the non-polar at 23 °C), and measuring the cell viability at increasing intervals of UV exposure. This exposure ranged from 10 min (23.32 kJ m^{-2} UV dose) to 8 h (1119.36 kJ m^{-2} UV dose) for UV-A, and from 3 min (7.20 kJ m^{-2} UV dose) to 5 h (720.00 kJ m^{-2} UV dose) for UV-B. As shown in Fig. 1, viability in all four species progressively decreased after UV exposure. However, their survival rates appeared to be markedly different. *E. focardii* was much more resistant than any other species to both UV-A and UV-B radiation; its viability was approximately 70% at 3 h of UV-A exposure, remaining above 65% even at 8 h of UV-A exposure, and was still above 50% at 30 min of exposure to the more harmful UV-B radiation. In contrast, *E. quinquecarinatus* (the *E. focardii* non-polar relative) was the least resistant species, as the majority of cells were no longer viable after 3 h of UV-A and 30 min of UV-B radiation. However, no significant variations in survival rates were detected between *E. nobilii* and *E. raikovi*: the values of viability of both species were about 40% at 3 h of UV-A exposure, and 20-30 % at 30 min of UV-B exposure.

In a second set of experiments, we assessed the cell capacity to recover from UV-induced damage by exposing cells to the sub-maximal/maximal doses of UV-A or UV-B radiation (3 h equivalent to 419.76 kJ m^{-2} for UV-A, and 1 h equivalent to144.00 kJ m^{-2} for UV-B) and comparing the cell viability immediately after UV exposure and at 6 h of recovery in the dark. As

shown in Fig. 2, while UV-B radiation was indistinctly lethal to all four species, UV-A radiation was lethal to all species except *E. nobilii*. Contrary to viability rates in *E. focardii* and the two other non-polar species, which did not recover from the negative effects of the UV-A radiation, the *E. nobilii* viability rate nearly doubled at 6 h of rescue in the dark by increasing from 34% to 60% in the Antarctic strains and from 44% to 83% in the Arctic ones.

The conclusive evidence that arises from these results is that the polar species of *Euplotes* withstand UV radiation much more efficiently than their phylogenetically non-polar relatives, albeit by apparently adopting different strategies. While *E. focardii* appears to be strongly protected against direct UV radiation and unable to recover from UV damage, *E. nobilii* seems to be poorly protected against UV radiation and counterbalances this scarce protection with a remarkable capacity to quickly repair UV damage. We suggest that these differences between *E. focardii* and *E. nobilii* in withstanding UV radiation reflect differences in the adaptive evolution of these two species to the polar environment.

E. focardii is a strictly psychrophilic species endemic to the Antarctic waters [22], characterized by an unusually reduced spectrum of transcriptional responses of its heat-shock protein (*hsp-70*) genes [10]. This suggests a close correlation between the ancient adaptation of *E. focardii* to the UV-radiation in Antarctica and the evolution of a specific defense mechanism based on the constitutive synthesis of photo-protective compounds such as the sunscreen mycosporine-like amino acids [9]. Enzymes of the shikimate pathway responsible for the production of these amino acids are ancient in the evolution of eukaryotes [14], and the finding of these enzymes are widely distributed in aquatic organisms [3], including ciliates [14, 17, 18], credits the hypothesis that *E. focardii* relies on them (rather than on the activity of the *hsp-70* genes) to prevent the deleterious effects of UV radiation.

Differently from *E. focardii*, *E. nobilii* is not endemic to Antartica, but it is a bipolar species represented by Antarctic and Arctic populations characterized by a promptly inducible *hsp-70* gene

activity [10]. A strengthening of this activity, and presumably of other genes encoding anti-oxidant enzymes, would thus well explain the *E. nobilii* unusual capacity to rapidly repair UV-induced damage.

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Figure 1 Effects of UV radiation on cell viability. Cells were treated with UV-A (**a**) or UV-B (**b**) for increasing times before MTT assay. Data represent the mean ± SEM of 4 independent experiments, each one in eight replicates, and are expressed as percent of control, taking as 100% the value of reduced MTT absorbance in equivalent samples of not-irradiated cells of the respective species. §$P < 0.001$.

Figure 2 Recovery from UV-radiation damage. Cells were treated with UV (light columns), or with UV followed by a period of dark (gray columns) before MTT assay, as indicated. Data represent the mean ± SEM from 3 independent experiments, each one in eight replicates, and are expressed as percent of control, taking as 100% the value of reduced MTT absorbance in not-irradiated cells of the respective species. *$P < 0.001$.

Table 1 Geographic origin and strain denomination of *Euplotes* species used in this study.

Species	Strain	Collection Site	Latitude/Longitude
E. nobilii	Ed.Po-6	Edmonson Point (Antarctica)	S 74°19'/E 165°07'
	5QAA15	Qaanaaq, Greenland (Denmark)	N 77°28'/W 69°20'
E. focardii	95	Terra Nova Bay (Antarctica)	S 74°42'/E 164°06'
E. quinquecarinatus	HAB1	Hapuna Beach (Hawaii, U.S.A.)	N 19°59'/W 155°49'
E. raikovi	LPSA5	Lampedusa Island, Sicily (Italy)	N 35°30'/E 12°36'