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Screening capacity of UV-absorbing compounds in spores of Arctic Laminariales

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Abstract

The functional significance of phlorotannins as ultraviolet radiation screens in brown algae is presented. Spectral analysis of zoospore suspensions of the three Arctic Laminariales *Saccorhiza dermatodea*, *Alaria esculenta* and *Laminaria digitata* showed strong absorption in the UV waveband, characteristic of phlorotannins. An induction in the synthesis of the UV-absorbing compound in zoospore suspensions of *S. dermatodea* and *A. esculenta* was observed as an increase in absorbance in the UV region after 8 h exposure to the whole light spectrum. Transmission of UVR was also negatively correlated with zoospore density in both these species but not in *L. digitata*. 'Biofilters' constructed from UV-transparent acrylic sheet, containing zoospore suspensions or solutions of phloroglucinol showed varying capacity to protect zoospore cultures from the lethal effects of ultraviolet radiation. Phloroglucinol protects the zoospores from damage by screening out the much harmful shorter UV-B spectra (280–290 nm). Cultured spores of *A. esculenta* and *L. digitata* after exposure to the whole light spectrum covered by filters containing phloroglucinol showed high rates of germination, unlike controls covered by seawater-only filters that showed 100% mortality. Biofilters containing zoospore suspensions act as buffers and showed variable UV-protection properties on the germination of its conspecies. At highest zoospore density ($\sim 4 \times 10^6$ spores ml^{-1}), zoospores were observed to screen UV radiation maintaining viability among shielded spores in all species investigated. The protective function of zoospore film is, however, density-dependent in *L. digitata*. At lower spore density, UV-screening function in *S. dermatodea* and *A. esculenta* is attributed to their capacity to accumulate and release UV-absorbing compounds into the medium. Ultraviolet radiation transmission by zoospore suspensions of *Saccorhiza* and *Alaria* decreased during exposure to the whole light spectrum which is consistent with the earlier observation of enlarged phenolic vesicles following UVR exposure. The increase in vesicle size and the corresponding increase in UV-absorbing capacity may contribute to greater tolerance of UVR exposure in both species.

Keywords: Absorption spectra; *Alaria esculenta*; *Laminaria digitata*; Phenolics; *Saccorhiza dermatodea*; UV radiation; Zoospores

1. Introduction

An increasing awareness of the environmental stress caused by increased levels of UVR attributable to global stratospheric ozone depletion has stimulated interest in the impact of elevated levels on marine macroalgae.

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UV-B radiation (UVBR) represents a very significant ecological impact that can potentially threaten the survival of species and thereby the health and diversity of marine coastal ecosystems. UVBR causes a range of deleterious effects in algae including damage to photosystem II, thylakoid membranes, microtubules, DNA and the formation of superoxide radicals (Franklin and Forster, 1997). The early life stages are particularly susceptible, and UVBR has been shown to inhibit germination, photosynthesis and survival of spores and zygotes of various species of brown algae (Dring et al., 1996; Huovinen et al., 2000; Swanson and Druehl, 2000; Wiencke et al., 2000, 2004; Makarov and Voskoboinikov, 2001; Flores-Moya et al., 2002; Altamirano et al., 2003; Schoenwaelder et al., 2003; Henry and Van Alstyne, 2004; Roleda et al., 2005, 2006). In plants, UVBR induces genes of the phenylpropanoid pathway that lead to the synthesis of phenolics such as flavonoids, lignin and tannins, compounds that, like phlorotannins, absorb UVBR and have broad defence-related functions (Jordan, 1996, 2002). The UV-screening function of phenolic compounds in higher plants is widely accepted; indeed Rozema and co-workers (1997, 2002) argued that this function made phenolics critical to the success of plant life on land.

Phlorotannins are polymers of phloroglucinol, unique to the Phaeophyceae. They occur in cells in vesicles known as physodes and are also deposited in cell walls (Schoenwaelder and Clayton, 1998a, 1999). Their functional significance has been the subject of some debate for well over a century (see Ragan, 1976; Ragan and Glombitza, 1986; Schoenwaelder, 2002a). However, it is only in the past few years that the probability that phlorotannins have multiple roles has been acknowledged (Arnold and Targett, 2002, 2003). Their strategic disposition in brown algal thalli, concentrated in cells of the outer epidermal layers (Tugwell and Branch, 1989; Schoenwaelder, 2002a), is consistent with a generalist role in defence against herbivores. Numerous experimental studies have examined the importance of phlorotannins as chemical defence agents, and have confirmed their effectiveness against a range of invertebrate herbivores and some species of fish (Targett and Arnold, 1998; Amsler and Fairhead, 2006).

Experimental evidence to support the UV-protective role of phlorotannins in brown algae is rather more preliminary. Swanson and Druehl (2002) showed that seawater containing phlorotannin exudates of *Macrocystis* increased survivorship of germinating *Laminaria groenlandica* spores exposed to UVBR. Schoenwaelder et al. (2003) linked higher numbers of physodes in *Fucus spiralis* embryos with a greater tolerance to elevated levels of UV-A radiation (UVAR) and UVBR, compared with more susceptible *Fucus* spp. In addition, they used phloroglucinol filters to screen out UVR and allow normal development of embryos of susceptible *Fucus serratus*. Embryos of *F. gardneri* are rather susceptible to UVR but UV-tolerance was developed and is related to the increase in phlorotannin concentrations during their maturation (Henry and Van Alstyne, 2004).

Recently it was demonstrated that zoospores of Arctic *Alaria esculenta* and *Saccorhiza dermatodea* are less sensitive to UVR or have a better capacity to recover from UVR-induced stress than zoospores of species from deeper water, *Laminaria digitata*, *L. saccharina* and *L. solidungula* (Wiencke et al., 2004). During the experiments, enlargement of physodes in *A. esculenta* and *S. dermatodea* was observed and inferred a possible protective function of these enlarged compartments against UVR. The aims of the present study were to measure the UV-absorbing properties of zoospore suspensions of species of Arctic Laminariales and to investigate their potential capacity to protect spores from the damaging effects of UVR. Accumulation and extrusion of UV-absorbing compounds were also investigated in zoospore and gametophyte stages. In addition we tested whether phenolics, in the form of the commercially available phloroglucinol has an effective screening capacity to protect the delicate zoospores from damage caused by UVR.

2. Methodology

2.1. Algal material

Fertile sporophytes of *S. dermatodea* (Pyl.) J. Ag., *A. esculenta* (L.) Grev., and *L. digitata* (Huds.) Lamour., were collected between May and June 2004 by SCUBA divers in Kongsfjorden at Prins Heinrichøya or Blomstrandhalvøya close to Ny Ålesund (Spitsbergen, 78°55′N, 11°56′E), Svalbard, Norway. Blades with sori were abscised from three different individuals per species, cleaned of epiphytes, blotted with tissue paper and kept in darkness in a moist chamber at 0 °C overnight up to a maximum of 2 days. To induce rapid release of zoospores, sori were immersed in 5–10 ml filtered (0.2 μm pore size) seawater at ± 15 °C and exposed to natural light close to a window. The initial zoospore density was counted by the use of a Neubauer chamber (Brand, Germany). Stock suspensions were diluted with filtered seawater to give spore densities appropriate for each experiment. Due to the extent of the experimental work, fertile sporophytes were collected several times and sori were processed separately. A dilution series was made from a known zoospore suspension (e.g. 100%, 80% 60%, 40% 20%). Spores were also used