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Short research note

Zoospore adhesion and germination upon non-toxic substances in *Pseudulvella* sp. (Chlorophyta)

María Virginia Sánchez Puerta, Patricia I. Leonardi & Eduardo J. Cáceres
Laboratorio de Ficología y Micología, Dpto. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina
E-mail: mvsp@wam.umd.edu

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Abstract

One of the most significant processes in the life history of an alga is the colonization of a new substratum. In the present study, we evaluate whether different organic compounds, such as agar, gelatine, chicken albumin, glycerine and polylysine, promote zoospore recruitment and germination in a periphytic, fresh-water green microalga of the genus *Pseudulvella* (Chlorophyta). Given the low adhesion capacity of its zoospores a series of experiments were conducted in order to find a substance and its optimal concentration that increases zoospore recruitment and allows us to follow the processes of settlement, attachment and germination of zoospores. Polylysine significantly increased the number of zoospores attached with no significant effect on the germination rate. The minimum effective concentration of polylysine for improving zoospore settlement was 0.1%.

Introduction

Algal spores are capable of detecting and responding to environmental conditions that favour their subsequent survival (Fletcher & Callow, 1992). The best-known responses are to surface topography (thigmotactic response) and light (phototactic response; Christie & Shaw, 1968; Evans & Christie, 1970; DeNicola & McIntire, 1990). Lesser known influences include surface chemistry and surface energy (Müller & Luthe, 1981; Griffith, 1985). The surface properties of potential fouling substrata have not received much attention although work in other fields has stressed their importance (Baier, 1970). Fewer investigations have been conducted on substances that increase algal adhesion, most of them being focused on marine macroalgae (Connor, 1986; Santelices & Bobadilla, 1996). Santelices & Aedo (1999) took advantage of the surface properties to raise recruitment of algal spores. They did experiments on the marine macroalgae *Ulva rigida* C. Agardh, *Mazzaella laminarioides* (Bory) Fredericq and *Lessonia nigrescens* Bory, which revealed that artificial substrata coated with polylysine retained significantly more spores than either untreated surfaces or other organic substances. *Pseudulvella* Wille is a genus of green microalgae, with disk shaped thalli that grow epiphytically on different plants and on several inorganic substrata. The life history of this genus has been only poorly studied (Snow, 1899; Wille, 1909; Setchel & Gardner; 1920 a,b; Philipose, 1947; Yarish, 1975), essentially due to the low adhesion capacity of its zoospores in culture and its low growth rate. Therefore, we carried out a series of experiments testing different organic non-toxic compounds in relation to algal adhesion. The aim of the experiments was to find a substance that increases zoospore recruitment that would allow the study of the processes of settlement, attachment and germination in *Pseudulvella*. Thus we report quantitative aspects of zoospore settlement and we propose the optimal concentration of an adhesion-promoting substance.

Thalli of *Pseudulvella* sp. were kept in an aquarium under controlled conditions of temperature (25 $\pm$ 2 $^{\circ}$ C) and artificial illumination (12:12 h LD photoperiod). Formation of zoospores took place after

48–96 h of darkness followed by a photoperiod of 12 h of light.

Experiment 1

Rectangular glass slides (2.5 × 2.7 cm) were coated with different organic compounds: 5% chicken albumin, 1% agar, 2% and 10% gelatine, 99% glycerine and 0.1% polylysine while the control slides were uncoated. To evaluate the zoospore retaining capacity of the different compounds, twenty replicate slides per treatment were placed at random in the aquarium for 5 days at the beginning of the light period. After that, the number of settled zoospores was estimated by examining 30 randomly chosen microscopic fields (about 1 mm^2 each) of each slide surface under a Nikon Eclipse inverted microscope at a magnification of ×200.

In order to determine the differences among treatments, the mean values of settled zoospores per slide were analyzed using a one-way ANOVA (analysis of variance) followed by a Tukey's test (Sokal & Rohlf, 1969). Germination rates were estimated employing the same methodology as above. For this purpose, 6 slides per treatment were incubated for 48 h in another aquarium under controlled conditions of temperature (25±2 °C) and photoperiod (12 h of light). These data were also analyzed using a one-way ANOVA, followed by a Tukey's test.

Polylysine retained significantly more zoospores than the other treatments (Fig. 1, $F = 10.46$, $p = 1.74E\text{-}9$). The number of settled zoospores adhered to glass slides coated with albumin, agar, gelatine (2 and 10%) or glycerine was not significantly different from the number of zoospores settled on uncoated controls (Fig. 1). Figure 2 shows the percentage of germination of settled zoospores, which varied among the different compounds, ranging from 34% to 71%, most of them being less than 50%. Germination rate of zoospores settled on albumin at 5% was significantly higher than the rate obtained with the other treatments (Fig. 2, $F = 7.88$, $p = 2.04E\text{-}5$). The germination rate of the zoospores settled on polylysine (0.1%) did not differ significantly from uncoated controls (Fig. 2).

Experiment 2

To establish the minimum effective concentration of polylysine, six replicate glass slides coated with polylysine in various concentrations (0.1%, 0.01% 0.001% and 0.0001%) and controls were tested. A one-way ANOVA revealed that the four data sets were significantly different from each other. The number of settled zoospores adhered to glass slides coated with polylysine at 0.1% was significantly higher ($F = 7.48$, $p < 0.001$) than the other concentrations. Therefore, the minimum effective concentration of polylysine for improving zoospore settlement was 0.1% (Fig. 3).

Polylysine concentration 0.1% entrapped 2 times as many zoospores as the uncoated control slides and the other treatments, which responded all in an equal less effective way. Similar results were observed in the green macroalga *Ulva rigida*, while the effect of these treatments was variable in *Mazzaella laminarioides* and *Lessonia nigrescens* (Santelices & Aedo, 1999).

The germination rates were, notwithstanding, less than 50% for control slides and slides coated with agar (1%), polylysine (0.1%), gelatine (10%) and glycerine (99%). Comparable germination rates were reported for propagules of *Mazzaella laminarioides* and *Lessonia nigrescens* retained by slides with the same treatments (Santelices & Aedo, 1999) and slides with pedal mucus of different species of gastropods and control slides (Santelices & Bobadilla, 1996). In contrast, a significantly higher percentage of zoospore germination in *Pseudulvella* sp. was observed with albumin, as it was the case in *Lessonia nigrescens* and *Ulva rigida,* whereas in *Mazzaella laminarioides* the germination rate was very low (Santelices & Aedo, 1999).

In conclusion, polylysine 0.1% increased significantly the recruitment rate of *Pseudulvella* zoospores. Consequently, even though with this treatment the germination rate of zoospores was low (nearly 50%), the higher number of settled zoospores allowed us to follow a major number of germination processes than with the other treatments and therefore it was more effective to accomplish this purpose.

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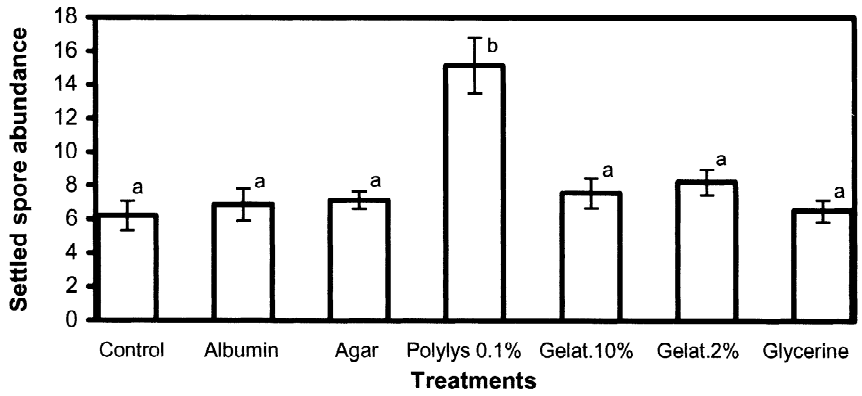

Figure 1. Experiment 1. Abundance (number/mm^2) of algal propagules retained by slides coated with different compounds and uncoated slides. Bars represent standard errors. Columns with different letter are significantly different ($p < 0.01$).

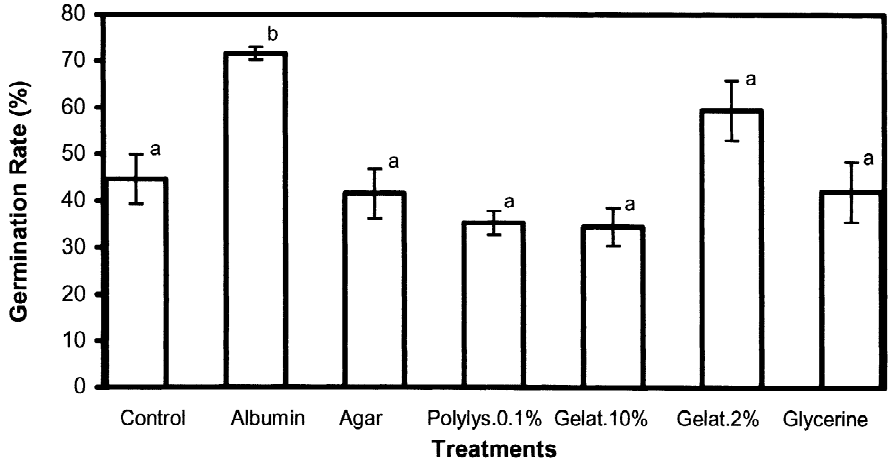

Figure 2. Experiment 1. Germination rate (%) of algal propagules retained by uncoated slides and slides coated with different compounds. Bars represent standard errors. Columns with different letter are significantly different $p < 0.01$).

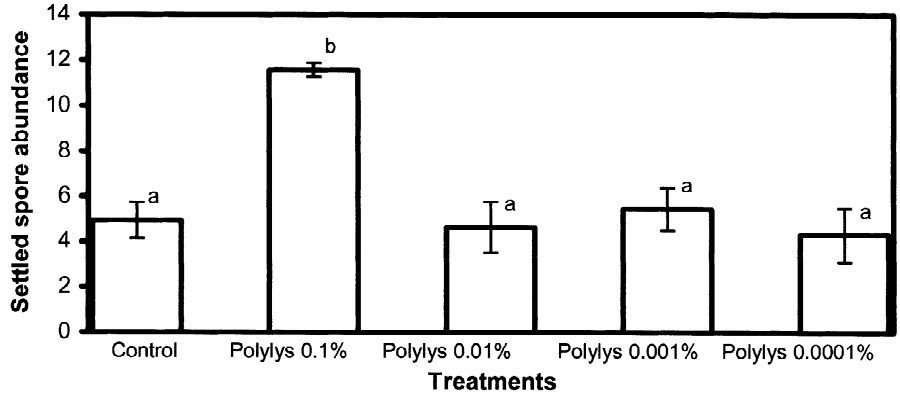

Figure 3. Experiment 2. Abundance (number mm^2) of algal propagules retained by slides coated with polylysine at different concentrations and uncoated slides. Bars represent standard errors. Columns with different letter are significantly different ($p < 0.01$).

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