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Natural chemicals produced by marine microalgae as predator deterrents can be used to control ciliates contamination in microalgal cultures

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ABSTRACT

Large-scale production of microalgae for biofuels is often hampered by contamination of cultures with predators that feed on microalgae. An important group of predators are ciliates. Some species of marine microalgae in natural ecosystems are known to produce chemicals that act as deterrents against predators. In this study, we tested whether these chemicals (trans,trans-2,4-decadienal, dimethyl sulfoniopropionate (DMSP), glycine betaine and proline) as well as a chemical analogue (methyl 3-(methylthio)propionate or MMP) can be used to control contamination of cultures of the microalga *Chlamydomonas* by the predatory ciliate *Sterkiella*. All chemicals were capable of rapidly eradicating the ciliates from a contaminated *Chlamydomonas* culture, but at a higher dose also had a negative effect on the microalga. For each chemical an optimal dose was determined at which ciliates were controlled and losses in microalgal biomass productivity were minimized (0.13 mM decadienal, 4.75 mM DMSP, 10 mM MMP, 250–300 mM proline and 250–300 mM glycine betaine). In the case of DMSP, MMP and proline, biomass productivity was even the same as that of a non-contaminated culture. The chemicals were also effective against other ciliates (*Stylonychia notophora*, *Oxytricha* sp. and 2 different *Paramecium* species). These chemicals therefore have potential to be used as natural pesticides to control contamination of microalgal cultures by ciliates. Of all chemicals tested, DMSP and MMP are the most promising because they are effective at a relatively low dose and have a limited negative effect on microalgal productivity.

1. Introduction

Microalgae have received significant interest in the past decade as a source of biomass for the production of biofuels [1–3]. In the past years, production of microalgae has moved from the laboratory to large-scale outdoor cultures. These large-scale production systems often experience large losses in productivity due to contamination of the culture by predators, parasites and competing microalgae [4–6]. Among these contaminants, ciliates that feed on microalgae are a particularly wicked problem. They achieve very high growth rates and as a result can cause a crash of the culture within a timeframe of only a few days [7].

Contamination of large-scale microalgal cultures by ciliates can be controlled by either preventive or curative measures. Prevention of contamination requires sterilisation of the culture medium as well as the air used to sparge the cultures because ciliates or their resting stages can be present in water as well as in air [8,9]. This is a challenge because production of microalgae requires large volumes of water (200–2000 L kg^{-1} dry biomass) and cultures need to be continuously sparged with large volumes of air to remove excess oxygen. Even if the initial abundance of ciliates in the culture medium is as low as only 1 individual per m^3 of culture, culture crashes may still occur within a couple of weeks [10]. Some curative measures have been proposed to control ciliates during a culture crash. Quinine sulfate at a concentration of 10–20 mg L^{-1} can be used to treat microalgal cultures that are infected by ciliates [11,12]. The supply of quinine, however, is limited as quinine has to be isolated from the bark of wild chinchona trees. Although copper sulfate reduce ciliate numbers in microalgal cultures, this chemical also has a negative impact on the microalgae themselves [12]. A recent study demonstrated that high concentrations of CO_2 (330–2000 mg L^{-1}, pH < 4.5) reduces numbers of the ciliate *Paramecium* in microalgal cultures [13]. Other predators of microalgae such as rotifers can be controlled by addition of the pesticide rotenone or by raising the free ammonia concentration (by addition of NH_4^+ in combination with an increase in pH), but these measures have no influence on ciliates [14,15].

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Microalgae in natural ecosystems have evolved chemical defense mechanisms to fend off predators [16]. Several species of marine microalgae such as *Phaeocystis* spp. or *Emiliania huxleyi* produce dimethylsulfoniopropionate (DMSP) and this chemical acts as a deterrent to predators including ciliates [17]. Marine microalgae also accumulate the amino acid derivative glycine betaine, which is chemically similar to DMSP. Glycine betaine was shown to inhibit feeding of the predatory dinoflagellate *Amphidinium longum* on microalgae [17]. When marine diatoms are consumed by natural predators, polyunsaturated fatty acids present in cell membrane are oxidized to the aldehyde trans,trans-2,4-decadienal [18,19]. This was shown to reduce the feeding rate as well as the reproduction of marine copepods that feed on these diatoms [18,20]. Another study has shown that proline reduces feeding by ciliates on marine microalgae [21].

The aim of this study was to evaluate whether chemicals that are produced as feeding deterrents by marine microalgae in natural environments may be used to control ciliate contamination in freshwater microalgae cultures. We used the freshwater microalga *Chlamydomonas* and the hypotrich ciliate *Sterkiella* as model systems. In addition to three chemicals that are known to inhibit predators of marine microalgae (DMSP, glycine betaine, trans,trans-2,4-decadienal and proline), we also included methyl 3-(methylthio)propionate (MMP). Because MMP is a chemical analogue of DMSP [22,23], we hypothesized that it might have a similar activity. We evaluated whether these chemicals could eradicate *Sterkiella* from *Chlamydomonas* cultures and were able to secure microalgal productivity. In addition, we also tested the effect of the chemicals on four other ciliate species (*Stylonychia notophora*, *Oxytricha* sp. and 2 different *Paramecium* species).

2. Materials and methods

2.1. Cultivation of microalgae and ciliates

The chlorophyte microalga *Chlamydomonas reinhardtii* SAG 77.81 and the hypotrich ciliate *Sterkiella* sp. were used as model systems to evaluate the potential of five chemicals to control ciliate contamination in microalgal cultures. *Chlamydomonas reinhardtii* is a widely studied microalgal species. Hypotrich ciliates are a group of ciliates that commonly invade large-scale microalgal cultures ([11,6]). *Chlamydomonas* cultures were maintained in 2 L batch cultures in Wright's Cryptophyte (WC) medium [24] in a temperature-controlled room (20 ± 2 °C) at a light intensity of 80 μmole $m^{-2} s^{-1}$ and a light-dark cycle of 16:8. Growth of *Chlamydomonas* cells was monitored spectrophotometrically at optical density 750 nm (OD750) [25]. Optical density was calibrated against gravimetric dry weight measurements (using Whatman glass microfiber filters, grade GF/F) and against cell abundance (determined microscopically using a Bürker counting chamber).

The ciliate *Sterkiella* was isolated from a rainwater storage reservoir, Meulebeke, Belgium. Water from the reservoir was inoculated to a *Chlamydomonas* culture. After a few days a ciliate species emerged in the culture that consumed *Chlamydomonas*. The ciliate was isolated using a fine Pasteur pipette, washed several times in sterile WC medium and then transferred to a fresh *Chlamydomonas* culture. It was identified as *Sterkiella* based on morphological identification (Fig. 1) and partial 18S ribosomal DNA sequencing (GenBank accession number: MF375457). *Sterkiella* cultures were maintained in 6-well plates in 3 mL volume cultures. The cultures were transferred every 2 days to a fresh *Chlamydomonas* culture: 0.5 mL of the ciliate suspension was transferred to a well containing 2 mL of WC medium and 0.5 mL of a stationary phase *Chlamydomonas* culture (5×10^6 *Chlamydomonas* cells mL^{-1}). Exponentially growing *Sterkiella* cultures with an abundance of about 500 cells mL^{-1} were used in all experiments. The ciliates were further concentrated by a 10 μm nylon mesh to obtain a density of 2000 cells mL^{-1} before addition to the experimental treatments.

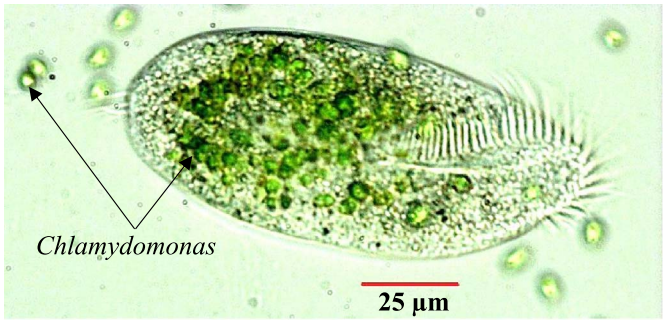

Fig. 1. Microscopic photograph of the hypotrich ciliate *Sterkiella* and the *Chlamydomonas* cells on which it feeds.

2.2. Evaluation of ciliate inhibition by natural chemicals

Five chemicals were tested for growth inhibition of ciliates. DMSP was obtained from Toronto Research Chemicals, Ontario, Canada while trans,trans-2,4-decadienal (hereafter decadienal), glycine betaine, MMP and proline were obtained from Sigma-Aldrich, Belgium. A stock solution of the chemicals was prepared on the day of the experiments. DMSP (1 M) was dissolved in distilled water. Glycine betaine (1 M) and proline (1 M) were added in relatively high volumes, so these chemicals were dissolved in WC medium. Due to their low solubility in water, decadienal (0.487 M) and MMP (1 M) were dissolved in methanol. This resulted in addition of a maximum of 0.06% methanol for decadienal and 0.87% methanol for MMP in the experimental treatments at the highest concentration tested. Preliminary experiments had demonstrated that addition of up to 2% methanol to the cultures had no significant effect on either *Chlamydomonas* or *Sterkiella*.

Exploratory experiments were carried out to determine the concentration range at which the chemicals resulted in inhibition of the ciliate *Sterkiella*. These experiments showed that the chemicals not only eradicated ciliates but also had a negative effect on the microalga *Chlamydomonas*, at higher concentrations. Therefore, a range of concentrations was tested to find an optimal concentration at which *Sterkiella* was eradicated while *Chlamydomonas* was not affected. For each chemical, five different concentrations were tested in addition to a control treatment that received no chemicals (Table 1).

Experiments were carried out in 100 mL volume cultures that were aerated with sterile-filtered air with gentle stirring (magnetic stirrer, 10 rpm). Each treatment was prepared in triplicate. These experiments were carried out in the same conditions (light, temperature) as for the cultivation of the *Chlamydomonas* stock culture (see above). The initial concentrations of microalgal prey and ciliate predator were 6.26×10^6 *Chlamydomonas* cells mL^{-1} (corresponding to OD750 of 0.3) and 25 *Sterkiella* cells mL^{-1}. Growth of *Chlamydomonas* was monitored spectrophotometrically (OD750) while *Sterkiella* abundance was determined microscopically in a 1 mL subsample that was preserved with formaldehyde (5%). The cultures were monitored after 0.5, 24, 48 and 72 h.

Table. 1
Concentration ranges used for each chemical in the experiments.

	Control	C 1	C 2	C 3	C 4	C 5
Decadienal (mM)	0	0.065	0.13	0.195	0.26	0.325
DMSP (mM)	0	4.5	4.75	5	5.25	5.5
MMP (mM)	0	1	5	10	15	20
Proline (mM)	0	200	250	300	350	400
Glycine betaine (mM)	0	200	250	300	350	400

DMSP: dimethylsulfoniopropionate; MMP: methyl 3-(methylthio)propionate; C1, C2, C3, C4, C5: concentration 1, 2, 3, 4, 5.