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ORIGINAL PAPER

Haemolytic activity of live *Phaeocystis pouchetii* during mesocosm blooms

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Abstract Chemical defence is a potential mechanism contributing to the success of *Phaeocystis* species that repeatedly dominate the phytoplankton in coastal areas. Species within the genus *Phaeocystis* have long been suspected of imposing negative effects on co-occurring organisms. Recently a number of toxins have been extracted and identified from *Phaeocystis* samples, but it is not clear if they do enhance the competitive advantage of *Phaeocystis* species.

In the present study the cytotoxic impact of live *Phaeocystis pouchetii* to human blood cells in close proximity, regardless of the nature of the responsible mechanism, was quantified using a bioassay. Haemolytic activity was measured during blooms of *P. pouchetii* in mesocosms. These environments were chosen to mimic natural conditions including chemically mediated interactions that could trigger defensive and/or allelopathic responses of *Phaeocystis*.

Haemolytic activity correlated with *P. pouchetii* numbers and was absent during the preceding diatom bloom. Samples containing live *P. pouchetii* cells showed the highest activity, while filtered sea water and cell extracts were less haemolytic or without effect. Dose-response curves were linear up to 70% lysis, and haemolysis in samples containing live *P. pouchetii* cells reached EC_{50} values comparable to known toxic prymnesiophytes (1.9 * 10^7 cells l^{-1}). Haemolytic activity was enhanced by increased temperature and light. The results indicate that unprotected and thus presumably vulnerable cells present in a *P. pouchetii* bloom may lyse within days.

Keywords Allelopathy · Chemical defence · Prymnesiophyte · PUFA · PUA

Introduction

Phaeocystis is an algal genus which regularly dominates vernal blooms in coastal regions all over the world, especially in temperate and higher-latitude waters. These almost monospecific

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blooms (Lancelot et al. 1987) have a great impact on the local marine food web because they produce the bulk of the primary production in springtime (Arrigo et al. 1999; DiTullio et al. 2000; Schoemann et al. 2005, introduction of this issue). There is likely a combination of mechanisms behind the bloom forming capacity of this genus. For example, *Phaeocystis* is able to take advantage of eutrophication (Lancelot et al. 1987; Cadée and Hegeman 2002), resulting in high biomass production. Blooms are typically dominated by the colonial form of these species and adjustment of colony size could be a response to escape grazing pressure, thereby reducing population losses [Jacobsen and Tang 2002; Tang 2003, see however Nejstgaard et al. (this issue) for a discussion on this mechanism]. Envelopment of the cells by a colonial mucous layer could be another mechanism to reduce losses because only the motile cells seem to be susceptible to viral lysis (Brussaard et al. 2005, this issue).

Also, *Phaeocystis* has been suspected for a long time of having a negative effect on co-occurring organisms. Penguins died after consumption of krill that fed on *P. antarctica* (Sieburth 1960, 1961). Schools of herring seem to avoid *Phaeocystis* blooms (Savage 1930), and mass mortality of caged fish occurred during a *P. globosa* bloom in the China Sea (Huang et al. 1999). Cod larvae died in the presence of natural densities of *P. pouchetii* (Eilertsen and Raa 1995; Aanesen et al. 1998) and negative effects of *Phaeocystis* were recorded on the bryozoan *Electra pilosa* (Jebram 1980). Bacterial consumption rates of acrylate in field samples increased when the > 20 μm fraction containing *P. globosa* colonies was removed (Noordkamp et al. 2000). And then there is the so-called legend of *Phaeocystis* unpalatability (Huntley et al. 1987) that says that healthy colonies are not consumed due to some sort of chemical deterrence (Estep et al. 1990). The observed negative effects of *Phaeocystis* presence on other organisms may be a key to its bloom forming capacity; chemical deterrence could be a way to reduce grazing (Nejstgaard et al., this issue) as well as inhibiting competitors (allelopathy), thereby increasing fitness.

Up to now, three toxic components that could be involved in chemical deterrence have been identified in *Phaeocystis* species: acrylate, a polyunsaturated aldehyde, and a haemolytic glycolipid. (1) Acrylate is produced by *Phaeocystis* (Guillard and Hellebust 1971; Sieburth 1960) upon enzymatic cleavage of dimethylsulphoniopropionate (DMSP, Stefels and Dijkhuizen 1996) and accumulates in mM concentrations in the colonial mucous layer (Noordkamp et al. 1998). During growth, however, acrylate is unlikely to cause harmful effects on nearby living cells because it is not excreted from the colonies (Noordkamp et al. 2000). Additionally, the concentration of acrylate present in the water column is not expected to exceed the 4 μM observed in senescent cultures (Noordkamp et al. 2000). This is much lower than the mM range of $L(E)C_{50}$ values reported for marine organisms (Sverdrup et al. 2001). Therefore, acrylate is not a likely component to be involved in allelopathy. Acrylate produced by *Phaeocystis* could, however, have a negative impact on grazers (and their consumers) when *Phaeocystis* cells accumulate in their guts. In these acidic environments the high concentrations of acrylate will be in the protonated toxic form (below pH 4.25). A grazing-activated chemical defence system based on the conversion of DMSP into DMS and acrylate upon cell damage was already described for another prymnesiophyte, *Emiliania huxleyi* (Wolfe et al. 1997).

(2) The isolation and identification of an unsaturated aldehyde from *P. pouchetii* (Hansen et al. 2004) may indicate a line of defence that was recently revealed for diatoms (Paffenhöfer et al. 2005, and references therein). Membrane lipids are converted into mildly toxic polyunsaturated fatty acids (PUFAs) by a grazing-activated enzymatic conversion. In the presence of reactive oxygen species (ROS) PUFAs may be converted into highly toxic polyunsaturated aldehydes (PUAs). In laboratory tests these PUAs negatively affect copepod fecundity and egg-hatching, and induce apoptosis in sea urchin embryos and cytotoxicity in human cell lines (Pohnert and Boland 2002). Precursors for PUAs, such as the PUFA eicosapentaenoic acid (EPA), are abundantly present in *P. globosa* (Hamm and Rousseau 2003). Although these haemolytic PUFAs (Arzul et al. 1998; Fu et al. 2004) provide essential nutrition