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SHORT COMMUNICATION

Methods used to reveal genetic diversity in the colony-forming prymnesiophytes *Phaeocystis antarctica*, *P. globosa* and *P. pouchetii*—preliminary results

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Abstract Previous work on the genetic diversity of *Phaeocystis* used ribosomal DNA and internal transcribed spacer (ITS) sequence analyses to show that there is substantial inter- and intraspecific variation within the genus. First attempts to trace the biogeographical history of strains in Antarctic coastal waters were based on a comparison of ITS sequences. To gain deeper insights into the population structure and bloom dynamics of this microalga it is necessary to quantify the genetic diversity within populations of *P. antarctica* from different locations (i.e., each of the three major gyres in the Antarctic continental waters) and to calculate the gene flow between them. Here we describe methods to quantify genetic diversity and our preliminary results for *P. antarctica* in comparison to two other colonial species: *P. globosa* and *P. pouchetii*. For this study of genetic diversity, two fingerprinting techniques were used. First, amplified fragment-length polymorphisms (AFLPs) were established as a pre-screening tool to assess clone diversity and to select divergent clones prior to physiological investigations. Second, the more-powerful microsatellite markers were established to assess population structure and biogeography more accurately. Results show differences in the AFLP patterns between isolates of *P. antarctica* from different regions, and that a wide variety of microsatellite motifs could be obtained from the three *Phaeocystis* species.

Keywords AFLP · Microsatellite marker · *Phaeocystis* · *P. antarctica* · *P. globosa* · *P. pouchetii*

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Introduction

The genus *Phaeocystis* was erected by Lagerheim (1893/1896) to accommodate the colonial alga *Tetraspora pouchetii* described by Hariot in Pouchet (1892). The newly combined species, *Phaeocystis pouchetii*, can be found in Arctic waters. Two other colonial species were described soon after that: *P. globosa* by Scherffel (1899, 1900) from temperate waters and *P. antarctica* by Karsten (1905) from the Antarctic. Kornmann (1955) expressed doubt that these species were separate and lumped up all colonial species into a single taxon *Phaeocystis pouchetii*. Despite physiological studies (Baumann and Jahnke 1986; Jahnke

and Baumann 1986, 1987; Jahnke 1989) that confirmed the separation of these taxa, it took a molecular study (Medlin et al. 1994) to end the controversy over the validity of the three colonial species. Since that time further molecular studies using other gene loci have confirmed the separation of these taxa (Lange et al. 2002) and other species have also been included in the genus (Zingone et al. 1999).

Phaeocystis is known to play an important role in ecology and biogeochemistry because it is distributed worldwide and forms massive blooms. Its blooms can fix a high amount CO_2 and produce a substantial amount of dimethylsulfoniopropionate (DMSP), which is the biological precursor of the climatically important trace gas of the atmosphere dimethylsulfide (DMS) (Smith et al. 1991; Stefels 1997; Arrigo 1999; Verity and Smetacek 1996). *P. antarctica* is widely distributed in the Southern Ocean where it is among the most abundant primary producers and is thus a major contributor to organic matter vertical fluxes. It is known from physiological studies from many phytoplankton species that there is a high variability among strains for every trait examined (Wood and Leatham 1992). Thus, a study of the genetic diversity and gene flow among *Phaeocystis* strains around the Antarctic is both timely and necessary. To pursue this we chose two techniques to assess the genetic diversity within and among *Phaeocystis* spp. One technique, amplified fragment-length polymorphisms, (AFLPs) provides a rapid means to screen the entire genome for polymorphic genetic loci as a pre-screening tool to select most divergent clones for physiological investigations, whereas analysis of microsatellite loci provides a more rigorous method by which population genetic statistics can be applied to assess the genetic diversity in populations and gene flow between them. Both of these techniques will be applied to each of the three colonial species, but we report here preliminary data to establish these techniques for *P. antarctica*.

Amplified fragment length polymorphism

The AFLP technique developed by Vos et al. (1995) was used as described in John et al. (2004). Genomic DNA of 48 *Phaeocystis* strains previously extracted in earlier studies (Medlin et al. 1994; Lange et al. 2002) was digested over night at 37°C with two restriction enzymes (*Eco*RI and *Mse*I, New England BioLabs, Frankfurt a. Main, Germany). This enzyme combination consists of a rare (*Eco*RI, six-base-pair recognition sequence) and a frequent (*Mse*I, four-base-pair recognition sequence) cutter. Subsequent to digestion, site-specific adapters were ligated to the ends of the restriction fragments (see Fig. 1).

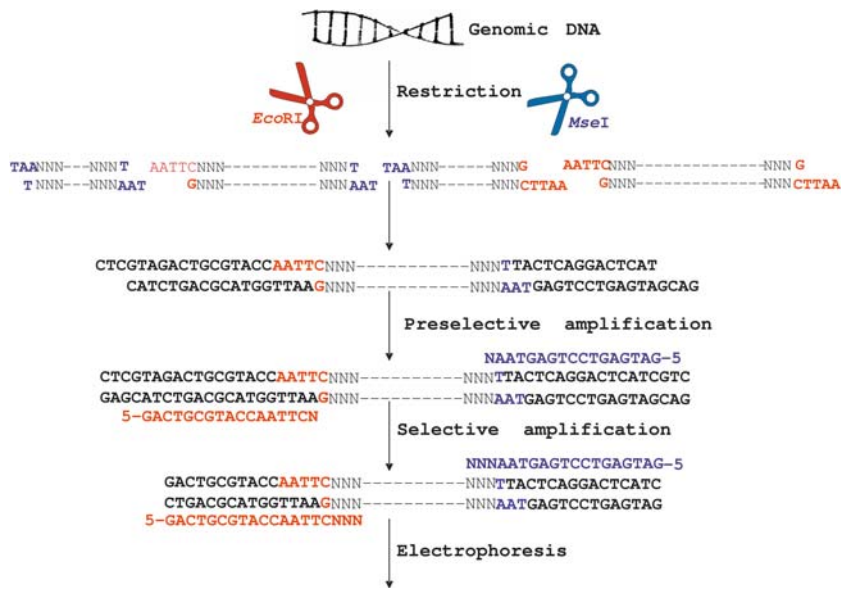

Fig. 1 Schematic diagram showing the construction of the AFLP fragments (from Müller 2005)