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Investigation of the dinoflagellate community of Lake Tovel by genetic analysis of environmental samples

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Introduction

Dinoflagellates constitute a diverse and ubiquitous assemblage in marine and freshwater ecosystems. They play an important part in aquatic food web as their nutritional modes are auto-, mixo- or heterotrophic. Hence, the dinoflagellate assemblage comprises both primary producers and predators. Due to recent advances in molecular biology (particularly DNA sequence determination) the taxonomy of dinoflagellates is currently under major revision (SAUNDERS et al. 1997, DAUGBJERG et al. 2000). Phylogenetic studies on dinoflagellates published in the last decade include mostly marine species (FLØ JØRGENSEN et al. 2004, GAST et al. 2004). Community studies of marine dinoflagellates based on environmental DNA samples are few in number, whereas DNA studies on freshwater dinoflagellate communities are virtually in the making.

The experimental site chosen for this study is Lake Tovel, an Italian alpine lake famous for its past red summer blooms caused by a dinoflagellate identified as *Glenodinium sanguineum* March (MARCHESONI 1941). Previous publications based on material collected in Lake Tovel have focused on *Glenodinium sanguineum* while essentially ignoring the many other dinoflagellates known to exist in the lake. Recent limnological surveys have revealed the number of dinoflagellate taxa present in Lake Tovel has increased and shifted species composition during the last 50 years (FLAIM et al. 2003, 2004). Given the inherent difficulty of properly identifying naked dinoflagellates by light microscopy, especially from fixed samples, and due to the difficulty of establishing *in vitro* cultures, we applied a molecular approach directly on environmental samples to further describe the dinoflagellate community inhabiting the lake. Recently, several molecular techniques proved to be good tools for natural community investigation, especially on marine prokaryotes (DIEZ et al. 2001). Here we used restriction analysis length polymorphisms (RFLP) of PCR products combined with a clone library construction. This approach has previously been applied for screening of eukaryotic communities based on SSU (Small Subunit Unity) rDNA (ROMARI & VAULOT 2004). However, this gene fragment is not useful for investigations on dinoflagellate diversity due to its high level of genetic conservation.

In order to investigate the dinoflagellate community in Lake Tovel, we developed an approach based on PCR-RFLP of environmental samples using a group-specific primer designed to amplify the LSU (Large Subunit Unity) rDNA of dinoflagellates only (HANSEN & DAUGBJERG 2004).

Key words: Lake Tovel; dinoflagellate community study; environmental samples; LSU rDNA; RFLP.

Methods

Lake Tovel is situated in Trentino province (northern Italy) at an altitude of 1178 m a.s.l. The lake is divided in two basins: a north-eastern one (z_{max} = 39 m) and a much smaller south-western (z_{max} = 5 m) basin called Red Bay because of its past red blooms. The lake is usually frozen from December to April and is classified as Oligotrophic, with phosphorus as the limiting nutrient (CORRADINI et al. 2001).

Water samples from the centre of Red Bay were collected at 0–1 m with the Ruttner bottle, promptly filtered in the lab with a 5 µm SVPP filter (Millipore) and frozen at –20 °C. The following samples are considered here: 19 August 2003 (summer), 22 October 2003 (autumn), 18 November 2003 (winter-last sampling), 11 May 2004 (spring-ice melting).

Total genomic DNA was extracted from SVPP filters using the CTAB method (DOYLE & DOYLE 1987) but with a few modifications as outlined in D'ANDREA et al. (subm.). Extracted DNA was amplified, first using primers D1Rf and dino-specific (HANSEN & DAUGBJERG 2004) and then re-amplified in semi-nested using the forward primer D3Af (DAUGBJERG et al. 2000). PCR reactions (total reaction volume 25 µl) were performed with HotMaster Taq (Eppendorf) with 200 µM of dNTPs and 0.5 µM of each primer.

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PCR conditions are outlined in D'ANDREA et al. (subm.). The amplified PCR fragments were checked on a 1% agarose gel stained with ethidium bromide. PCR products were then cloned on a PCR TOPO-XL vector (Invitrogen) and 100 colonies grown on LB agar plates were picked up and reamplified. Products of reamplification were digested with restriction enzymes Taq I and Sau 3A I, Cfr 13 I, Alu I. Reaction conditions were as suggested by the manufacturer (Amersham Biosciences) and fragments were separated on 2% agarose gel stained with ethidium bromide.

Results

To assess diversity of the dinoflagellate community as revealed by each restriction enzyme, some samples were digested with the four enzymes. Fig. 1 shows patterns found for some colonies isolated from the October sample and treated with Taq I or Cfr 13 I.

Taq I proved to be the most informative enzyme because it permitted identifying more ribotypes than others: in 100 colonies from October, sample Taq I distinguished 7 profiles instead of 5 recognised by Alu I and 4 by Sau 3A I and Cfr 13 I. Taq I phylogenetic assignments proved to be the least ambiguous because they were usually repeated with at least 2 other enzymes.

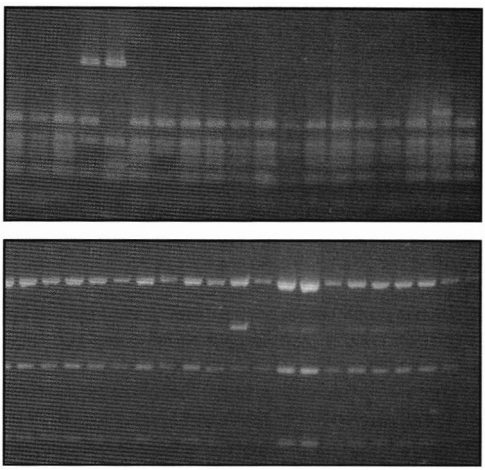

Fig. 1. Agarose gel showing a comparison of the ribotypes revealed by 19 colonies isolated from the Oct'03 sample after digestion with the enzymes Taq I(up) and Cfr 13 I(down).

Ribotype differences are significant only within the dominant pattern "E", which is divided into 8 subgroups when considering the 4 enzymes. However in the August sample (data not shown) this heterogeneity was less evident: within the Taq I dominant pattern "A", only 2 subgroups were recorded by Cfr 13 I and Sau 3A, while Alu I did not distinguish any subgroup. For these reasons we chose Taq I for preliminary rapid screening by RFLP on samples collected over the year in Red Bay.

Fig. 2 illustrates the relative occurrence of Taq I ribotypes in environmental samples from different seasons as percentage of colonies showing a particular profile: 10 patterns designated as A– L, were recorded considering all the samples analysed.

Taq I ribotypes were interpreted as separate genera or groups of similar species (D'ANDREA et al. subm.); when using an enzyme pool we can reach a species distinction or intraspecific separation.

Seasonal changes in the community are evident and reflect the natural succession of species caused by encystment and excystment or by proliferation of one species in a particular period. In August we obtained the highest biodiversity of the four samples considered, in fact, almost all patterns were found even if in low quantities.

We found a dominant pattern which changed over the months: A in summer (89% of colonies), E in autumn and winter, F in spring. Presence of a dominant ribotype agreed with microscopical observations of live material but was probably overestimated because of the competitive nature of PCR, which amplifies more frequently with more concentrated DNA molecules and can fail to amplify less abundant ones (DÍEZ et al. 2001). The emerging trend suggested an August maxima of almost all ribotypes with one dominant; in October and November some species disappeared or were less abundant while one autumnal-winter ribotype (E) became dominant and a new ribotype (L) was revealed; at ice melt a spring group (F) was dominant while the summer ribotype A began to increase in abundance.

This temporal distribution tends to reflect seasonality of dinoflagellates and turbulence in

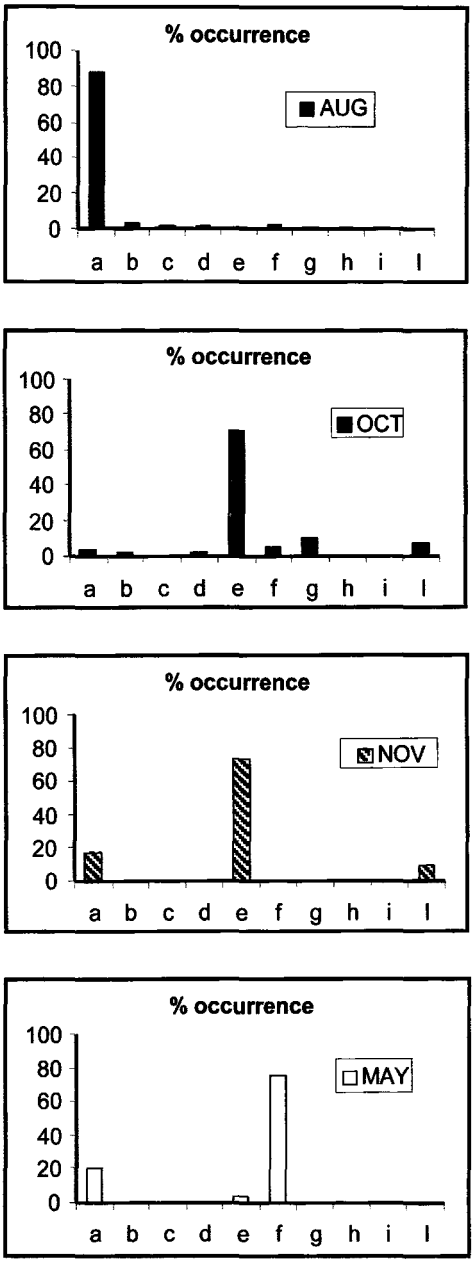

Fig. 2. Relative occurrence of Taq I ribotypes in Red Bay environmental samples collected over the year: percentage of the colonies analysed showing each pattern (A to L).

Red Bay (FLAIM et al. subm.). Despite the impossibility of seeing all low density groups, our approach reflected the community trend expected from live observations and allowed identification of more patterns than the morphotypes observed by light microscopy. A finer identification of dinoflagellates present in the lake was achieved, overcoming difficulties in distinguishing similar species by gross morphological features seen with light microscopy.

This work can be regarded as one of the first attempts to use PCR-RFLP of LSU rDNA for freshwater dinoflagellate community studies on environmental samples. We show this molecular approach is an easy and reliable tool for screening of seasonal variability among the dinoflagellate group.

Comparison of LSU sequences of different ribotypes with the published database is in progress to see if any environmental sequence is similar to an already sequenced dinoflagellate. However we would expect to discover that some environmental ribotypes represent unknown dinoflagellates, peculiar to this habitat.

The study of spatial and temporal distribution of dinoflagellates, which starts with this work, will also be useful for long-term monitoring of these organisms in other Trentino lakes.

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