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 the field of molecular biology (particularly DNA sequence determination) the taxonomy of dinoflagellates is currently under major revisions (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 that the number of dinoflagellate taxa present in Lake Tovel has increased and there was a shift in species composition during the last 50 years (FLAIM et al. 2003, FLAIM et al. 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 (DÌEZ et al. 2001). Here we have 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 elucidate 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).

Keywords

Lake Tovel, dinoflagellate community study, environmental samples, LSU rDNA, RFLP.

Methods

Lake Tovel is situated in the 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 it is classified as oligotrophic with phosphorus as the limiting nutrient (CORRADINI et al. 2001).

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

Total genomic DNA was extracted from the SVPP filters using the CTAB method (DOYLE & DOYLE 1987) but with a few modification as outlined in D'ANDREA et al. (submitted). 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 25µl) were performed with the HotMaster Taq (Eppendorf) with 200µM of dNTPs and 0.5µM of each primer. The PCR conditions are outlined in D'ANDREA et al. (submitted). 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 one hundred colonies grown on LB agar plates were picked up and reamplified. The products of reamplification were digested with the restriction enzymes Taq I and Sau 3A I, Cfr 13 I, Alu I. The reaction conditions were as suggested by the manufacturer (Amersham Biosciences) and the fragments were separated on 2% agarose gel stained with ethidium bromide.

Results

In order to assess the diversity of the dinoflagellate community as revealed by each restriction enzyme, some samples were digested with the four enzymes. Fig.1 shows the 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 then the 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 of the other enzymes.

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 is 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 choose Taq I for a preliminary rapid screening by RFLP on the samples collected over the year in the Red Bay.

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

Taq I ribotypes are interpreted as separate genera or groups of similar species (D'ANDREA et al. submitted); when using an enzymes 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 the proliferation of one species in a particular period. In August we obtained the highest biodiversity of the four samples considered, in fact almost all the patterns were found even if in low quantities.

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

This temporal distribution tends to reflect the seasonality of dinoflagellates and turbulence in the Red Bay (FLAIM et al. submitted, Verh. Internat. Verein. Limnol.). Despite the impossibility of seeing all of the low density groups, our approach reflected the community trend expected from live observations and allowed to identify more patterns than the morphotypes observed by light microscopy. A finer identification of the 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 that this molecular approach is an easy and reliable tool for the screening of the seasonal variability among the dinoflagellate group.

The comparison of LSU sequences of different ribotypes with the published database is in progress in order 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 the spatial and temporal distribution of dinoflagellates, which starts with this work, will also be useful for a long term monitoring of these organisms in other Trentino lakes.

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FIGURES

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).

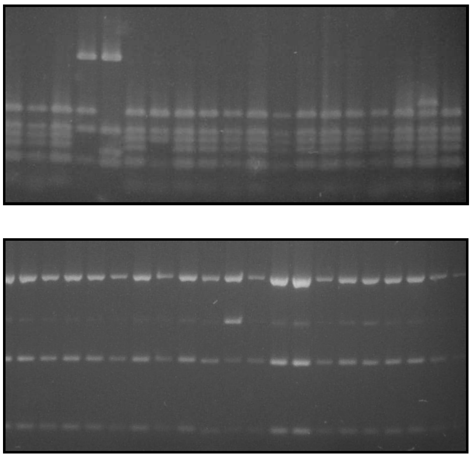

Fig. 2.

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).