Note

Potential biological sources of long chain alkyl diols in a lacustrine system

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Long chain alkyl diols (LCDs) have been detected in a range of marine and lacustrine environments, as well as in several algal cultures. However, the identity of the producers, their preferred ecological niche and seasonality are uncertain. We applied a gene-based approach to determine the identity and abundance of Eustigmatophyceae 18S rRNA genes and compared the data with the distribution of LCDs in the water column of Lake Challa (East Africa). Gene-based analysis revealed three known and two novel Eustigmatophyceae groups. Maxima in the number of gene copies and LCD concentration coincided at 9 m water depth, signifying Eustigmatophyceae as important producers of LCDs. In addition, seasonal changes in LCD abundance in sedimenting particles revealed several blooms of LCD producers over the annual cycle.

1. Introduction

Long chain alkyl diols (LCDs) consist of an alkyl chain with OH groups at C-1 and at a mid-chain position. LCDs with 28–32 carbons and OH groups at C-1,13 and C-1,15 have been found in Eustigmatophyceae cultures of marine (Nannochloropsis sp., Eustigmatophyceae sp.; Volkman et al., 1992) and freshwater species (Vischeria sp., Eustigmatos sp.; Volkman et al., 1999). Other sources are some members of the Proboscia diatom genus (Sinninghe Damsté et al., 2003) and the alga Apedinella radians of the Dictyochophyceae phylum, both of which produce 1,14-diols (Rampen et al., 2012). LCDs have been found in marine and lacustrine sediments (e.g. Versteegh et al., 1997). Recently, Rampen et al. (2012) proposed the long chain diol index (LDI) as a marine paleotemperature proxy based on the C30 1,15-diol abundance relative to the C28 1,13-diol and C30 1,13-, 1,15-diols. Eustigmatophyceae are generally considered to be major producers of LCDs in lakes but the identity of lacustrine LCD producers, their preferred niche in the water column and their seasonality is uncertain. Such information could improve the predictive power of the LDI proxy.

We have developed a genetic-based approach to identify and quantify the abundance of potential LCD producers, based on the 18S rRNA gene of members of the Eustigmatophyceae and its comparison with the distribution, abundance and seasonality of LCDs in a lake system.

2. Study site and sampling

Lake Challa is a permanently stratified crater lake on the southeastern flank of Mt. Kilimanjaro (East Africa). Suspended particulate matter (SPM) was collected at 5 and 10 m intervals throughout the water column in early February 2010 (see Buckles et al., 2013 for details and conditions at the time of sampling); here we focus on samples from between 0.5 and 24 m depth, i.e. within and just below the photic zone. A mid-lake sediment trap at 35 m depth provided monthly samples of settling particles between August 2009 and August 2010.

3. Material and methods

3.1. DNA methods

DNA was extracted from SPM filtered on GF/F 0.7 μm filters as described by Buckles et al. (2013). Primer pair Eust287F (5’-CGA CRA MTC ATT CAA GYT TCT GCC-3’), Eust810R (5’-CCA TGC TAR TGT ATT CAS GGC CT-3’) was designed manually, and tested computationally and in PCRs. Gradient PCR was performed with melting temperature (Tm) ranging from 52–63 °C with genomic DNA extracted from different algal cultures (optimal Tm 58 °C). Quantitative PCR (qPCR) using the Eust287F/810R primer pair was performed at Tm 61 °C and 45 cycles following the conditions described by Buckles et al. (2013). A phylogenetic tree was inferred from the Neighbour-joining method and distances computed with the Jukes-Cantor method. Sequences NCBI accession numbers are KF765160 – KF765375.
3.2. Lipid methods

Filters from the SPM and the sediment trap were hydrolyzed (cf. de Leeuw et al., 1983) by refluxing for 1 h with 1 N KOH in MeOH (96%). After cooling, the solvent was acidified with 2 N HCl/MeOH (1:1; v/v) to pH 2 and transferred to a separatory funnel. The filters were extracted using MeOH/H2O (1:1 v/v; 1 ×). MeOH and dichloromethane (DCM; 3 ×). Solvent was collected in a separatory funnel containing ca. 25 ml bidistilled H2O. The DCM layer was separated from the H2O/MeOH layer and the remaining H2O/MeOH layer extracted (3 ×) with DCM. The extracts were combined and rotary evaporated to near dryness. The resulting extract and the residual filters were hydrolyzed with acid (3 h reflux, 2 N HCl/MeOH, 1:1; v/v) and neutralized with 1 N KOH in MeOH (96%). Filters were extracted as above, while for the extracts, 3 ml bidistilled H2O was added and the lipids extracted using DCM (4 ×). All extracts were combined, dried under N2, eluted in DCM over a pipette column containing Na2SO4, dried under N2, methylated in DCM using CH2N2 in Et2O and dried under N2. An internal standard (C22,7,16-diol) was added to the extract and each extract was fractionated into apolar and polar fractions using a glass pipette column with activated Al2O3 and eluted with hexane/DCM (9/1; v/v) and DCM/MeOH (1/1; v/v). Each polar fraction was silylated prior to gas chromatography-mass spectrometry (GC–MS). LCD analysis was as per Rampen et al. (2012).

4. Results and discussion

4.1. Eustigmatophyceae and LCD diversity and abundance

To determine eustigmatophyte diversity in Lake Challa SPM, clone libraries were generated by cloning 18S rRNA gene fragments generated by the primers Eust287F/Eust810R. Sequences from 0.5, 9 and 19 m water depth all clustered into five distinctive phylogenetic groups (Fig. 1). No clustering of sequences according to depth was observed as those recovered from the three depths were distributed throughout the tree. Group 1 sequences were closely related to those of the Goniochloridaceae family (Pribyl et al., 2012),
while groups 4 and 5 sequences clustered with sequences of the Monodopsidaceae and Eustigmataceae families. Sequences falling in groups 2 and 3 diverged from sequences of cultured representatives, supporting their assignment to one or more unknown Eustigmatophyceae families. Quantification of Eustigmatophyceae gene copies showed a distinctive peak at 9 m depth (Fig. 2A). The most abundant LCDs in the February SPM samples were C32 1,15 (138 ng/l), C30 1,15 (54 ng/l) and C34 1,17-diols (23 ng/l). Of these, the C34 1,17-diols may be produced by the novel Eustigmatophyceae groups 4 and 5 sequences clustered with sequences of the Eustigmatophyceae with group 2 and 3 sequences, since these diols have been found in lake samples (Versteegh et al., 1997; Zhang et al., 2011), but have not been detected in freshwater eustigmatophyte cultures (Volkman et al., 1999). Maximum LCD abundance was at 9 m water depth, suggesting an important role of eustigmatophytes as LCD producers. Seasonal variation in LCD distributions suggested that successive LCD-producing blooms were due to different eustigmatophyte algae or changes in the LCDs produced by a unique algal population in evolving abiotic conditions.

5. Conclusions

Application of a 18S rRNA gene-based method has revealed the presence of both known and novel groups of Eustigmatophyceae in Lake Challa. Maximum abundance of Eustigmatophyceae gene sequences coincided with maximum LCD abundance at 9 m water depth, suggesting an important role of eustigmatophytes as LCD producers. Seasonal variation in LCD distributions suggested that successive LCD-producing blooms were due to different eustigmatophyte algae or changes in the LCDs produced by a unique algal population in evolving abiotic conditions.

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