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Characterization of a Eukaryotic Picoplankton Alga, Strain DGN-Z1, Isolated from a Soda Lake in Inner Mongolia, China

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ABSTRACT

Dagenoer Soda Lake is located on the Xilinhaote plateau in Inner Mongolia at an elevation of 1289 m. Blooms of one predominant kind of picoplankton algae were found in the lake throughout the year. A strain of this picoplankton alga, designated DGN-Z1, was isolated in axenic culture. Its cells are spherical or oval, 2-3 μm in diameter; it grows optimally at 0.5-1 M NaCl, and tolerates pH values from 7 to 12. Phylogenetic analysis based on sequence similarity of the 18S rRNA gene suggested that it belongs to the green algal species Picocystis salinarum.

INTRODUCTION

Planktonic organisms can be classified in different categories: bacterioplankton (mainly consisting of heterotrophic prokaryotes), phytoplankton (including cyanobacteria and eukaryotes), and zooplankton (formed by eukaryotic unicellular and multicellular organisms). In addition to this subdivision, organisms can be classified according to their size. Perhaps the simplest scheme is that of Dussart (1965) who divided plankton using a logarithmic size scale: macroplankton (200-2000 μm), microplankton (20-200 μm), and nanoplankton (2-20 μm). Sieburth et al. (1978) extended this classification scheme and added the terms picoplankton (0.2-2 μm) and femtoplankton (0.02-0.2 μm). The great importance of algal activity and the size of the picoplankton communities in the global primary production of aquatic ecosystems have been extensively documented in the literature (Craig 1985; Stockner 1988, 1989). In oligotrophic lakes, between 50 and 70% of the annual carbon fixation is attributed to organisms that pass through 1-2 μm pore size filters (Munawar & Fahnenstiel 1982, Callieri & Stockner 2002). Ten percent of the primary biomass in the sea was produced by the smallest free-living eukayote (Fouilland et al. 2004).

Figure 1–A bloom of picoplankton algae in Dagenoer soda lake covered with ice in winter.

MATERIALS AND METHODS

The Sample Collection Site

Dagenoer Soda Lake is located at 42°40'54"N, 115°50'37"E, in the Xilinhaote area of Inner Mongolia, Autonomous Region of China, at an altitude of 1297 m. The lake covers an area of approximately 2.1 km² and has a maximum depth of 1.1 m. The water has a salinity of 18.8% and a pH of 10. Four samples were collected in August, September, October and December 2003.
Isolation of an Axenic Culture of a Picoplankton Species

Clonal cultures were established by streaking water samples on 1% agar medium (Ds medium) which contains (in g l⁻¹): NaNO₃, 0.42; NaH₂PO₄·2H₂O, 0.156; NaHCO₃, 0.84; KCl, 0.074; MgSO₄·7H₂O, 1.23; CaCl₂·2H₂O, 0.044; 0.5 ml l⁻¹ of solution of 1% ferric citrate; and trace elements (in mg l⁻¹): H₃BO₃, 286; MnCl₂·4H₂O, 18.1; ZnSO₄·7H₂O, 2.2; CuSO₄·5H₂O, 0.79; g (NH₄)₆Mo₇O₂₄·4H₂O, 0.39. Cultures were incubated at room temperature under a 12 hours light–12 hours dark regime. Single colonies were picked and transferred to 5 ml of the same medium without agar in 20 mm capped glass test tubes. Cultures were established by serial restreaking on agar and isolating single colonies. The absence of bacteria was established by streaking on nutrient media (Lewin et al. 2000).

Scanning Electron Microscopy

Cells were harvested from a culture in the exponential growth phase, and collected by centrifugation at 4000 rpm for 10 minutes at 4°C. Then the cells were fixed with 2.5% glutaraldehyde, 1% osmium tetroxide, and 30 mM HEPES buffer (pH 7.2) at 4°C for 15 minutes. After rinsing twice with distilled water, they were dehydrated in an ethanol series (30%, 50%, 75%, 90%, 2×100%), and examined in a Hitachi S-570 electron microscope at 12 kV.

Figure 2–Strain DGN-Z1 observed in the scanning electron microscope.

Growth Experiments at Different Salinities and pH Values

Portions of 20 ml of exponentially growing cultures were transferred to flasks containing growth medium as described above, in which the salinity was varied between 0.5 and 5 M and pH between 7 and 12. Cultures were incubated at room temperature under a 12 hours light–12 hours dark regime, and the flasks were shaken three times a day. Algal growth was monitored by measuring OD₅₄₀ₙ₃ in a spectrophotometer.

Figure 3–Growth of strain DGN-Z1 at different salt concentrations.

DNA Extraction, and Amplification, Cloning, and Sequencing of the 18S rRNA Gene

Portions of cells (1 ml) were harvested from an exponentially growing culture by centrifugation (4°C, 4000 rpm, 10 min). The supernatant was discarded and the cells were incubated for 60 minutes in lysis buffer (500 µl TE buffer, 60 µl 10% SDS, 30 µl 20 mg ml⁻¹ proteinase K) in a water bath at 55°C. DNA was extracted after addition of 1 volume phenol-chloroform-isoamyl alcohol (25:24:1, pH 7.9) by brief vortexing, followed by centrifugation (12000 rpm, 10 min). The aqueous phase from each sample was mixed with 0.1 volume of 5 M NaCl and two volumes of 96% (vol/vol) ethanol and left overnight at -80°C. Each sample was centrifuged (12000 rpm, 30 min, 4°C), and the DNA pellet was washed with 70% ethanol. Finally, the extracted DNA was stored at -80°C in autoclaved deionized water. PCR was performed with the primers DMA1: 5’ to CGG GAT CCG TAG TCA TAT GCT TGT CTC 3’, DMA2: 5’ to CGG AAT TCC TTC TGC AGG TTC ACC 3’ (Olmos et al. 2000). The reaction was carried out in an Eppendorf tube with 50 µl solution containing 1 U of Taq polymerase, 1 x buffer B with 1.5 mM Mg²⁺, 10 nmol of deoxynucleoside triphosphates (Promega Cor.), 50 pmol of each primer, and approximately 50 ng of extracted template DNA. PCR reactions were performed in a thermocycler...
(Biorad iCycler) under the following conditions: hot start for 30 s at 94°C, 30 PCR cycles and 10 minute elongation at 72°C. Each single cycle consisted of a 30 s 94°C denaturing step, a 30 s annealing step at 55°C, and a 30 s elongation step at 72°C. PCR products were gel purified on 1% (wt/vol) low-melting-point agarose gels, cloned using the pGEM-T vector (Promega), and transformed according to manufacturer’s manual. Individual colonies were picked randomly for sequencing of the plasmid inserts. Plasmid DNA was sequenced using a ABI377 PE (Perkin Elmer) sequencing machine.

Phylogenetic Analysis

Sequence data were BLAST analyzed against the GenBank database (Altschul et al. 1997). Clone sequences were deposited in the GenBank. Sequences were aligned with their closest relatives and representative cultured and uncultured picoplankton species by using DNAMAN software. Phylogenetic trees were constructed from the alignment sequences by using Jukes-Cantor distance matrices for inferring the tree topology and neighbor joining and maximum-parsimony for bootstrap analysis (Saitou & Nei 1987).

RESULTS

One type of green picoplankton algae was dominant in Dagenoer Soda Lake throughout the year, even when the water was covered with ice in winter (Figure 1). A strain of this species, designated DGN-Z1, was isolated in axenic culture. Its cells are spherical or oval, and measure 2-3 μm in diameter (Figure 2). The optimal NaCl concentration for growth of strain DGN-Z1 was 0.5-1 M; no growth was obtained in 4 and 5 M (Figure 3). The isolate grew well at pH 7-12 (Figure 4).

The 18S rRNA gene sequence (GenBank accession number EU935604) was used for phylogenetic tree construction together with other picoplankton algae sequences such as AF153314 (Picocystis salinarum), EF440183 (Nanochloris sp. ant-2), Y15814 (Ostreococcus), AB183605 (Imantonia), EU106816 (Pelagomonas calceolata), EU106738 (Bolidamonas sp. RCC852), AF123596 (Bolidamonas mediterranea), and AY254857 (Florenciella parvula). The resulting phylogenetic tree is shown in Figure 5. The sequence similarity analysis and the phylogenetic analysis of the 18S rRNA gene suggested that the strain belongs to the species Picocystis salinarum.

DISCUSSION

Although autotrophic picoplankton can contribute high primary production and if of great ecological importance, little is known about the taxonomy of the organisms involved. Among the smallest eukaryotes are various autotrophic picoplanktonic chlorophytes, variously assigned to Bathycoccus Eikrem & Throndsen, Ostreococcus Courties & Chrétiennot-Dinet in Chrétiennot-Dinet et al., Chlorococcus Beijerinck, Choricystis (Skuja) Fott, Nanochlorum C. Wilhelm, Eisenbeis, A. Wild & R. Zahn.
Chlorophyceae, including *Picocystis salinarum* or salt lakes at a salinity of about 18%. The cell wall of composition (Lewin et al. 2000). It grows in saltern ponds features, notably in cell shape, cell wall and pigment described and named by Lewin et al. It has several unique isolated from a San Francisco Bay saltern and was (to which different from that of any species of the Trebouxiophyceae analyses. The species *Picocystis salinarum* was first isolated from a San Francisco Bay saltern and was described and named by Lewin et al. It has several unique features, notably in cell shape, cell wall and pigment composition (Lewin et al. 2000). It grows in saltern ponds or salt lakes at a salinity of about 18%. The cell wall of *Picocystis salinarum* has a sugar composition quite different from that of any species of the Trebouxiophyceae (to which *Nanochloropsis* and *Choricystis* belong) or the Chlorophyceae, including *Mychonastes* and some 40 strains of *Chlorella* assigned to nine species (Takeda & Hirokawa 1984; Takeda 1988a, 1988b, 1991, 1993; Krienitz et al. 1999), in which the fibrous components are mainly composed of glucose accompanied by mannose or glucosamine. Isolate DGN-Z1 appears to be cold-tolerant (Figure 1), and on the basis of its combined tolerance to high salt concentrations, high pH and low temperature it may represent a new type.

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