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Kong Fanjing

*R&D Center for Saline Lake and Thermal Deposits, Chinese Academy of Geological Sciences, Beijing*

Jia Qinxian

*R&D Center for Saline Lake and Thermal Deposits, Chinese Academy of Geological Sciences, Beijing*

Er Jia

*Key Lab of Saline Lake Resources and Environments, Ministry of Lands and Resources, Beijing*

Zheng Mianping

*R&D Center for Saline Lake and Thermal Deposits, Chinese Academy of Geological Sciences, Beijing*

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

Kong Fanjing<sup>1,2,3</sup>, Jia Qinxian<sup>1,2,3</sup>, Er Jia<sup>2</sup> & Zheng Mianping<sup>1,2,3</sup>

<sup>1</sup>R&D Center for Saline Lake and Thermal Deposits, Chinese Academy of Geological Sciences, Beijing 100037 China; <sup>2</sup>Key Lab of Saline Lake Resources and Environments, Ministry of Lands and Resources, Beijing 100037, China; <sup>3</sup>Institute of Mineral Resources, Chinese Academy of Geological Sciences, Beijing 100037, China

Corresponding author:

Kong Fanjing

Key Lab of Saline Lake Resources and Environments, Ministry of Lands and Resources, and R&D Center for Saline Lake and Thermal Deposits, Chinese Academy of Geological Sciences, Beijing 100037, China

E-mail: kfjbj2002@yahoo.com.cn

## 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  $\mu\text{m}$  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  $\mu\text{m}$ ), microplankton (20-200  $\mu\text{m}$ ), and nanoplankton (2-20  $\mu\text{m}$ ). Sieburth et al. (1978) extended this classification scheme and added the terms picoplankton (0.2-2  $\mu\text{m}$ ) and femtoplankton (0.02-0.2  $\mu\text{m}$ ). 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  $\mu\text{m}$  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 eukaryote (Fouilland et al. 2004).

Planktonic organisms can further be subdivided based on their physiological properties and their taxonomic affiliation (Stockner et al. 1978; Malone 1980). In the past decade, molecular biology method have been applied to the analysis of the diversity of small eukaryotes in the ocean,

and the diversity of small plankton was shown to be very high (López-García et al. 2001; Moon-van der Staay et al. 2001; Moreira & López-García 2002).

In this paper we report the isolation and characterization of a small phytoplankton species from Dagenoer Soda Lake, an inland soda lake located in the Xilinhaote area of Inner Mongolia, Autonomous Region of China. The primary production of plankton in this lake is very high (Huo et al. 2005). One type of small green plankton is dominant throughout the year, even when the lake is covered with ice (Figure 1). We here document the physiological properties of the organism as well as its phylogenetic affiliation, based on molecular methods.



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

## MATERIALS AND METHODS

### The Sample Collection Site

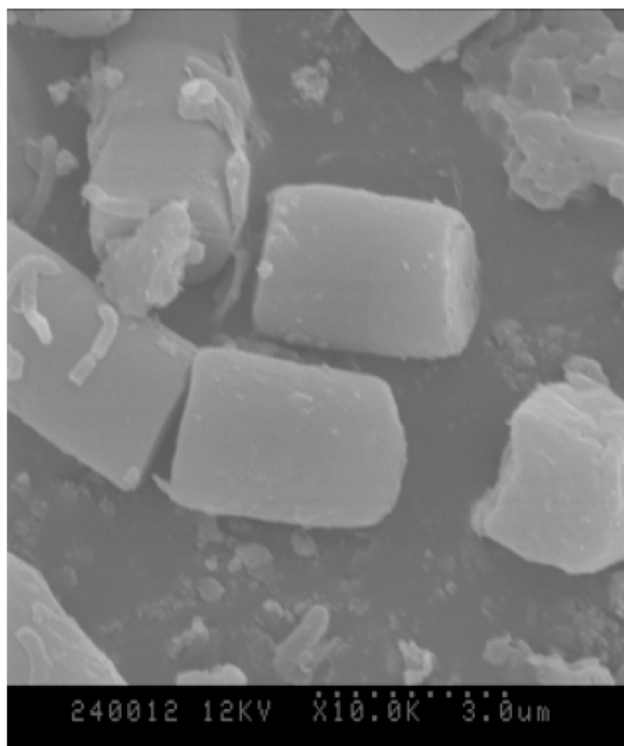
Dagenoer Soda Lake is located at 42°40'54"N, 115°50'37"E, in the Xilinhaote area of Inner Mongolia, Autonomous Region of China, at an altitude of 1297 m. The lake covers an area of approximately 2.1 km<sup>2</sup> and has a maximum depth of 1.1 m. The water has a salinity of 18.8% and a pH of 10. Four 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<sup>-1</sup>): NaNO<sub>3</sub>, 0.42; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 0.156; NaHCO<sub>3</sub>, 0.84; KCl, 0.074; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.23; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.044; 0.5 ml l<sup>-1</sup> of solution of 1% ferric citrate; and trace elements (in mg l<sup>-1</sup>): H<sub>3</sub>BO<sub>3</sub>, 286; MnCl<sub>2</sub>·4H<sub>2</sub>O, 18.1; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 2.2; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.79; g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, 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

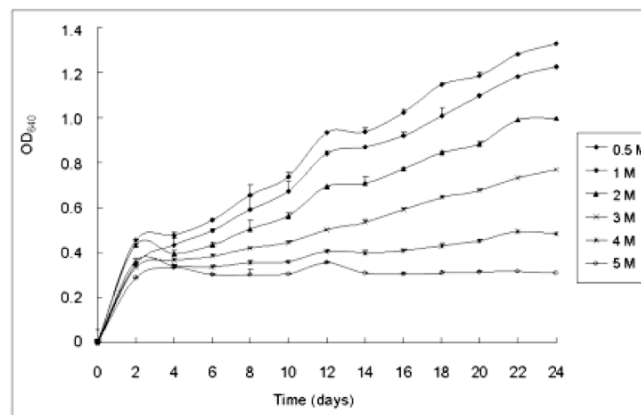
Cells were harvested from a culture in the exponential growth phase, and collected by centrifugation at 4000 rpm for 10 minutes at 4°C. Then the cells were fixed with 2.5% glutaraldehyde, 1% osmium tetroxide, and 30 mM HEPES buffer (pH 7.2) at 4°C for 15 minutes. After rinsing twice with distilled water, 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 OD<sub>540nm</sub> 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<sup>-1</sup> 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 Mg<sup>2+</sup>, 10 nmol of deoxynucleoside triphosphate (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).

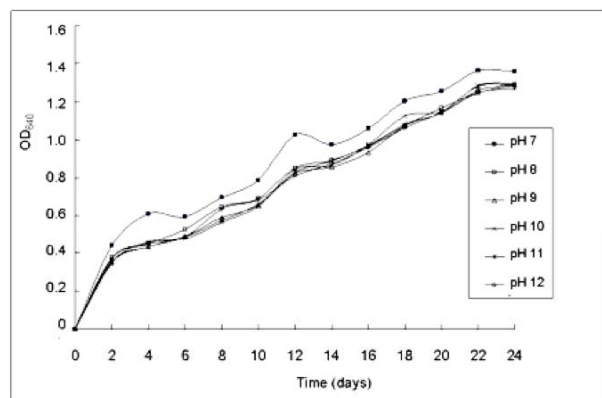


Figure 4—Growth of strain DGN-Z1 at different pH values.

### 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*.

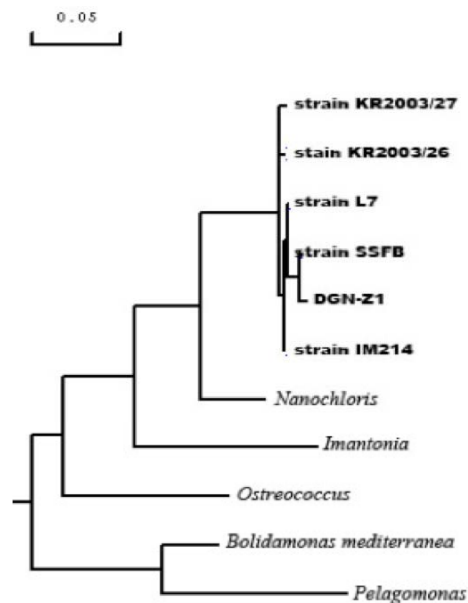


Figure 5—Phylogenetic tree based on 18S rRNA gene sequences, showing the phylogenetic position of strain DGN-Z1. Sequences used: five strains of *Picocystis salinarum*: DQ267705 (strain KR2003/27), DQ267704 (strain KR2003/26), AF153313 (strain L7), AF153314 (strain IM214), AF125167 (strain SSFB); in addition EF440183 (*Nanochloris* sp. ant-2), Y15814 (*Ostreococcus*), AB183605 (*Imantonia*), EU106816 (*Pelagomonas calceolata*), EU106738 (*Bolidamonas* sp. RCC852), AF123596 (*Bolidamonas mediterranea*), AY254857 (*Florenciella parvula*), and EU935604 (DGN-Z1).

### 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., *Pycnococcus* Guillard in Guillard et al. (Eikrem & Throndsen 1990; Guillard et al. 1991; Courties et al. 1994, 1998), *Chlorella* Beijerinck, *Choricystis* (Skuja) Fott, *Nanochlorum* C. Wilhelm, Eisenbeis, A. Wild & R. Zahn

(Andreoli et al. 1978; Huss & Sogin 1990; Krienitz et al. 1996; Huss et al. 1999), and *Mychonastes*. Simpson & van Valkenburg (Kalina & Puncochgova 1987; Hanagata 1998; Krienitz et al. 1999). In Dagenoer Soda Lake, the predominant tiny alga represented by isolate DGN-Z1 may be *Picocystis salinarum*, based on 18S rRNA phylogenetic 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 *Nanochlorum* 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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