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NOTE

A Marine Picophytoplankter from Korea: *Pycnococcus provasolii* Guillard

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INTRODUCTION

Our knowledges of the distribution and biology of picoplanktonic organisms have increased over the last forty years. Since Sieburth *et al.* (1978) presented the size range of plankton, a lot of studies on the picoplankters have appeared. Takahashi and Hori (1984) found *Chlorella*-like coccoid green alga and cyanobacteria in the western North Pacific Ocean at the depth of the subsurface chlorophyll maximum layer. Recently some marine picophytoplankton have been isolated in the western Pacific Ocean (Miyashita *et al.*, 1993, Ikemoto *et al.*, 1995; Hasegawa *et al.*, 1996). They have been identified on the basis of the composition of pigments and ultrastructural features.

The genus *Pycnococcus* is the first coccoid prasinophyte which does not have the typical prasinophycean morphology. It does not show the scales in the cell surface nor flagella with an apical depression (Guillard *et al.*, 1991). The present sample has been misidentified as a species of the chlorococcalean picoplankter in the previous report (Chung and Kang, 1995). With additional data of pigment analyses, the present specimen is identified as *Pycnococcus provasolii* Guillard (Guillard *et al.*, 1991). Here we first report the presence of this species in the Korea Strait and the East Sea with its ultrastructural characteristics and pigment composition.

MATERIALS AND METHODS

Isolation

Field samples were collected on May 12, 1994 in the Western Channel of the Korea Strait (Chung and Kang, 1995) and Oct. 15, 1995 in the East Sea (41°10' N 135°10' E). The samples were isolated by a serial dilution method in f/2-Si media. The isolated specimens are maintained at 20°C under cool white fluorescence light of the irradiance of 50 $\mu E \cdot m^{-2} \cdot sec^{-1}$, and 12 : 12 (L : D) photoperiod in the same media.

Pigment analysis

Pigment analysis of the isolated picophytoplanktonic species was performed by HPLC (Shimadzu systems, Sasa *et al.*, 1992). The type specimen (CCMP 1203) was used to identify the peaks of pigments for reference.

Electron microscopy

Fixed cells with 3% glutaraldehyde in seawater were collected on a membrane filter (0.2 μm pore size) and then embedded in agar. The embedded agar was cut into small cubes. Small agar cubes were post-fixed in 1% OsO_4 and dehydrated in ethanol. After change in propylene oxide, blocks were embedded in epoxy resin (Spurr, 1969) and cured.

The blocks were cut with a diamond knife using an ultramicrotome into thin sections, then mounted onto copper grids (formvar coated hole grid or 400mesh Cu Grid). After staining with uranyl acetate and lead citrate sections were examined and photographed with a JEOL 1000 CX TEM.

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RESULTS AND DISCUSSION

Pigment composition

Pigment composition, which includes chlorophylls *a* and *b*, Mg-2,4-divinyl-phaeoporphyrin a5 monomethyl ester (Mg-2,4-D), and prasinoxanthin, is close to that observed in the Mamiellales (Prasinophyceae). The peaks of both present specimen and CCMP 1203 were identical as shown in Fig. 1.

Electron Microscopy

The cell is solitary, nonmotile and coccoid shaped (Plate I. Fig A). Unlike *Prasinoderma coloniale*, the cell does not make colonies (Hasegawa *et al.*, 1996). Cell surface is smooth and unornamentally scaleless. Sometimes irregularly shaped cells appear. This specimen is different from *Prasinococcus capsulatus* which makes gelatinous matrix (Miyashita *et al.*, 1993).

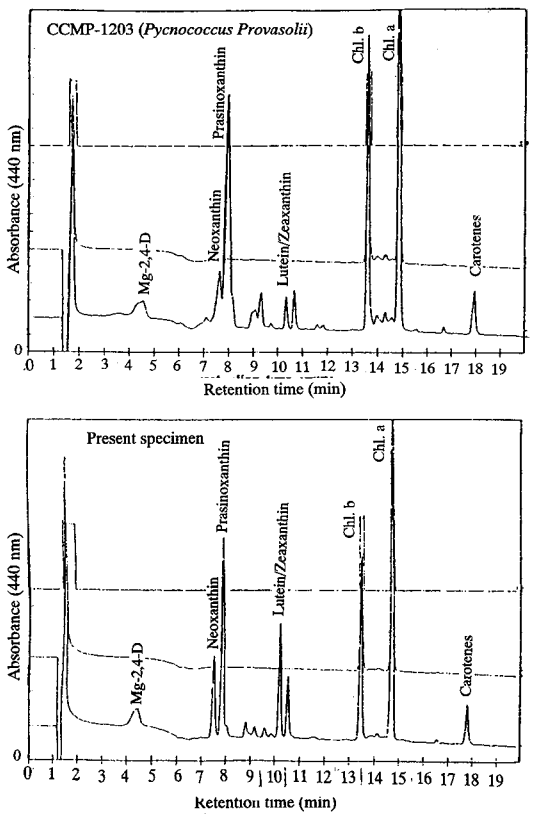

Fig. 1. HPLC separation chromatogram of pigment.

The motile cells have not been found in this study. The cell does not have basal bodies and centrioles. It is speculated that it may be undescribed cyst phase of a known micromonadophytes (Guillard *et al.*, 1991). Although the original descriptions include the motile feature with flagellum, it should be reexamined. Recently the mode of the cell division is described through the video monitoring. It took place in a few second. It may be binary cell division mode. One of the two daughter cells is squeezed out from the parent cell, while the other remains within the parent cell wall. However they do not show additional layers of parental cell wall like *Prasinoderma coloniale* (Hasegawa *et al.*, 1996). The cell possesses a nucleus with a nucleolus. There is a 2-3 lobed chloroplast with pyrenoids, a mitochondrion and Golgi bodies. There are conspicious pyrenoids in a chloroplast. The pyrenoid is covered with cap-like (c) structure and surrounded by starch sheath (s) (Pl. I, Fig. B). Thylakoids do not penetrate into the pyrenoid. The outer double membrane of the mitochondrion protruded into the chloroplast in the region of the pyrenoid as in the original description. This mitochondrial protrusion pushes the chloroplast outer double membrane into the pyrenoid matrix. Sometimes the end of protrusion may be bilobed at the end of protrusion like *Prasinococcus capsulatus* (Miyashita *et al.*, 1993) (Pl. I, Fig. C). There is 'operculum-like' region in the cell wall which is different from the collar-like region in *Prasinococcus capsulatus* (Miyashita *et al.*, 1993) (Pl. I, Figs. D & E). Coiled material discharged from the cell is found in an opening region of the cell wall (Pl. II, Fig. A: arrow).

Deformation of cell shape

In unialgal culture system, some structural deformation appeared. It is speculated that there may be some relationship with bacteria. There is numerous fine slender threadlike structure between an algal cell and a bacterium (Pl. II, Fig. B). Sometimes protrusions which have close contact with the ad-

jacent bacteria are made (Pl. II, Fig. C). The shape of projection is simple but some have modified disc-like structure at the base of attachment (Pl. II, Fig. D). The deformed irregularity of cell shape is often recognized in these cases. The reason of deformational irregularity of cell shape by bacteria is being under investigation.

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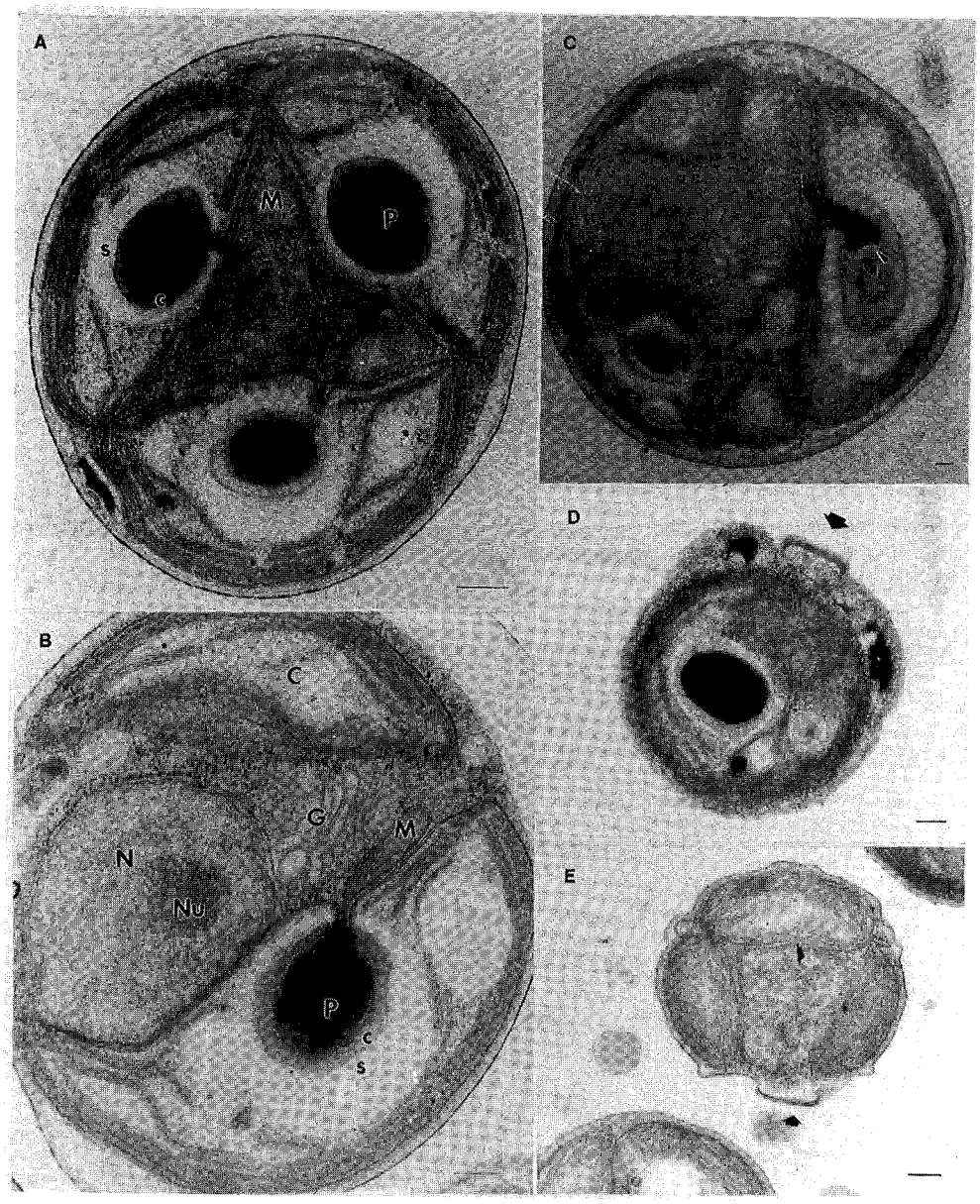

Plate I. *Pycnococcus provasolii* Guillard (Electron micrographs, bar = 200 nm).

Fig. A. Part of the cell showing the structure of three chloroplast lobes which contain pyrenoids with surrounding cap-like region (c) and starch sheath (s). Median section showing mitochondrion (M).

Fig. B. Median section showing nucleus (N) with nucleolus (Nu), mitochondrion (M), bilobed chloroplast which contains pyrenoid with surrounding cap-like region and starch sheath. Sections indicating mitochondrial envelope and chloroplast envelope in the pyrenoid matrix, which protrude into chloroplast.

Fig. C. Section showing bilobed end of mitochondrial protrusion (arrowhead).

Fig. D. & E. Section showing 'operculum-like' region on the cell wall (arrow).

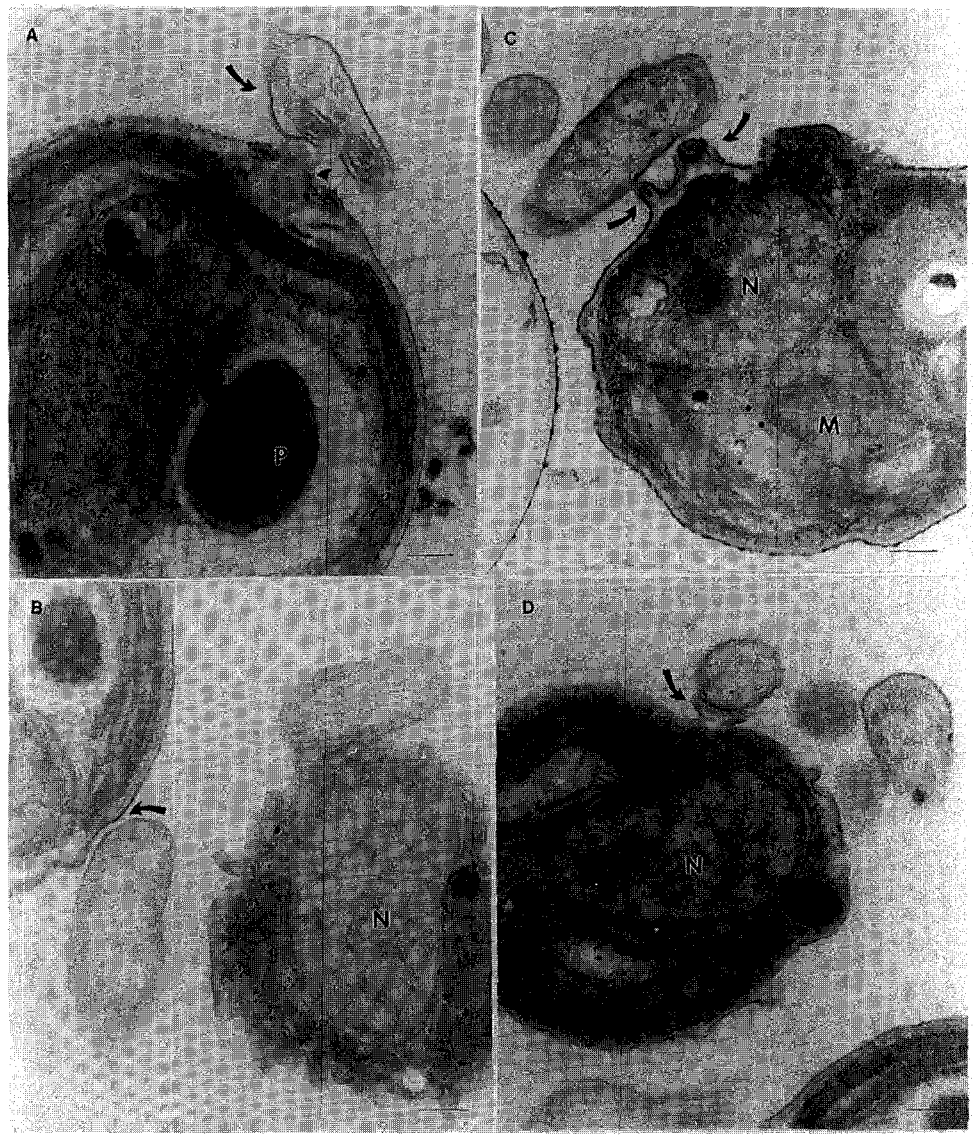

Plate II. *Pycnococcus provasolii* Guillard (Electron micrographs, bar = 200 nm)
Fig. A. Section showing the discharge of coiled material (arrow) through an opening in the cell wall (arrow head).
Fig. B. Numerous slender thread-like connection (arrow) and attachment between algal cells and bacteria.
Fig. C. Section showing deformed cell with bacterial attachment on the protrusion (arrows).
Fig. D. Section showing deformed cell with the disc-like attachment protrusion to a bacterium (arrow).