Algae
Volume 20(4): 295-298, 2005

[Note]

Ornamented Resting Spores of a Green Alga, *Chlorella* sp., Collected from the Stone Standing Buddha Statue at Jungwon Miruksazi in Korea

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The growth of subaerial microalgae on historic buildings or various cultural properties causes discoloration and physico-chemical deterioration of the surfaces. We collected a subaerial chlorophyte, *Chlorella* sp., from the stone Standing Buddha statue at Jungwon Miruksazi, which is a national treasure of Korea, and found dormant, thick-walled spores with regular pentagonal ornamentation along with the vegetative *Chlorella* cells. The morphology of *Chlorella* resting spores was compared to that of the other green algal resting cells. The ornamented spores and smooth-walled vegetative cells revived in 2 weeks in a liquid freshwater medium and started reproduction by autospores. To our knowledge, the ability of *Chlorella* to form ornamented dormant spores in drought condition was not previously recorded. The ornamentation of spores would supplement taxonomic characteristics of this genus.

Key Words: biodeterioration, *Chlorella*, drought resistance, resting spore

INTRODUCTION

The occurrence of subaerial microalgae on man-made stone objects such as roofs, building facades, and monuments, causes considerable damage to surfaces over time, including aesthetically unacceptable discoloration known as patinas and incrustations (Gaylarde and Morton 1999; Tomaselli *et al.* 2000) and physico-chemical deterioration (*e.g.* Schlichting 1975; Gaylarde and Gaylarde 2000; Schuman *et al.* 2005). These problems become particularly important when dealing with historic buildings or various cultural properties (*e.g.* Kim *et al.* 2001; Klochkova *et al.* in press). Particularly, members of Chlorococcales, including *Chlorella*, *Chlorococcum*, *Scenedesmus*, and *etc.* are known to cause etching of the minerals (Watanabe 1977; Welton *et al.* 2003).

By any standards, anthropogenic stone surfaces are extreme growth environments and the inhabiting algae must be specialized to repeated dehydration, intense UV irradiation, and fluctuations of temperature. Therefore, studies on their physiology are important, including adaptation to stress by resting stage formation and later retrieval in response to fluctuating environments. However, many studies were not conducted regarding the ability of subaerial algae from such habitats to tolerate stress conditions. Adhikary and Satapathy (1996) reported that the predominant organism on the rock surfaces of Indian temples, the blue-green alga *Tolypothrix byssoidea* (Hass.) Kirchn., retained low levels of activity, as indicated by triphenyl tetrazolium chloride hydrolysis, even under extremely arid and hot conditions. The thick mucilaginous sheath probably protected this alga from desiccation and high light intensity. Recently, Klochkova *et al.* (in press) reported on a *Chlorococcum* species from the stone walls of Miruksazi stupa in Korea, which developed dormant warty spores and thus survived several years of desiccation.

In the present paper we describe a desiccation-tolerant subaerial green alga *Chlorella* sp. The ornamented resting spores of this species were collected from the stone Standing Buddha statue at Jungwon Miruksazi in Korea.

MATERIALS AND METHODS

Jungwon Miruksazi was designated as Korean

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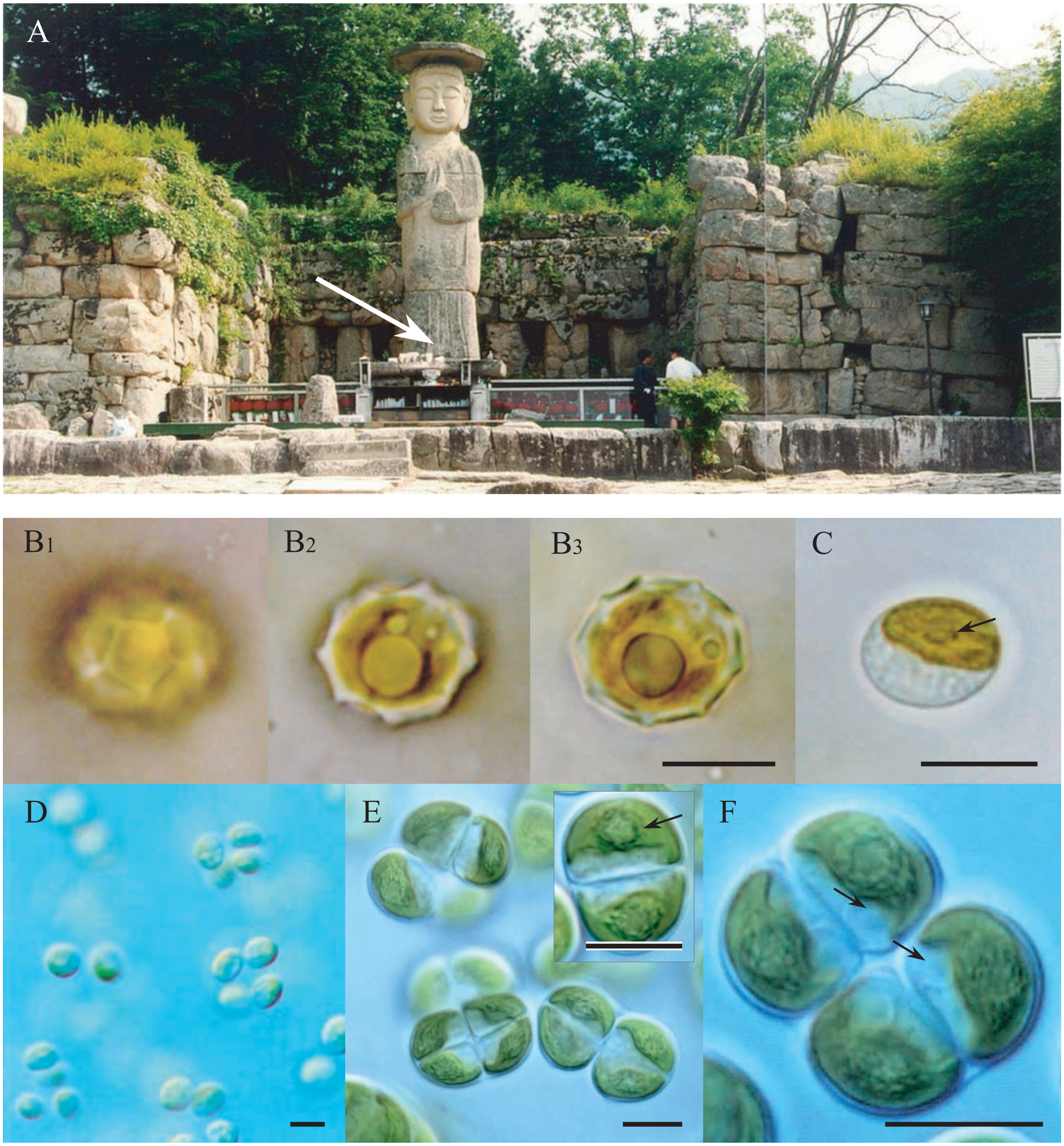

Fig. 1. Photograph of Jungwon Miruksazi and morphology of resting spores and vegetative cells of *Chlorella* sp. collected from the stone statue (Fig. A, arrow). **A.** Incrustations caused by algae and lichens can be seen with the naked eye. **B-D.** Field materials of *Chlorella* sp. **B.** Through-focus images of the resting spore showing surface view (Fig. B_1) and middle focal plane (Figs B_2 and B_3). **C.** Vegetative cell without ornamentation (arrow points to pyrenoid). **D.** Cell groups. **E-F.** Progeny cells were derived in freshwater medium. **E.** Undulate chloroplast margins and pyrenoid associated with numerous starch grains (inset, arrow) can be seen. **F.** Enlarged photograph of autospores. Arrows points to nuclei inside the cells. Scale bars = 10 μm.

National Historic site No. 317 (Fig. 1A; http://www.lifeinkorea.com/Travel2/273#standing). In June 2002, scrapings were made on the stone surface of the statue's base and were placed in a plastic container. During that time, the spot where samples were collected was dry under direct sunlight. The algal biofilm on statue appeared dark brown-green in color. Algal cultures were established in a liquid ATCC Medium 625 (http://wwwcyanosite.bio.purdue.edu) of pH 7.5, containing $NaNO_3$ 496 mg, K_2HPO_4 39 mg, $MgSO_4$ 75 mg, $CaCl_2$ 36 mg, $FeC_6H_5O_7$ (ferric citrate) 6 mg, $C6H8O_7.H_2O$ (citric acid monohydrate) 6 mg, EDTA 1 mg, $Na_2SiO_3.9H_2O$ 59.0 mg, ddH_2O 1 liter, in 90 × 15-mm Petri dishes. Once progeny cells were released, they were transferred to 250 ml glass flasks and grown on a shaking incubator (Vision Scientific Co., Ltd) at 25° C, 15 μmol photons m^{-2} s^{-1} cool-white fluorescent lighting and 12:12 h light-dark regime.

RESULTS AND DISCUSSION

In the scrapings, the cells of *Chlorella* sp. were most abundant (Figs 1B-D) and *Phormidium* sp., *Klebsormidium klebsii* (G.M. Smith) Mattox *et* Blackwell, two species of pennate diatoms, bacteria and filamentous fungi were present (not shown). The vegetative cells of *Chlorella* were single or in 2- or 4-celled groups (Figs 1C and D), thin- and smooth-walled, 6.5-10 μm wide and 7-12 μm long, symmetrical, with light yellowish-brown or yellowish-green chloroplast with pyrenoid (Fig. 1C). Sporangia with autospores were not observed. Mucilage surrounded the cell groups and probably contributed to the algal survival mechanism against desiccation because of its physico-chemical property allowing the retention of water (*e.g*. Schumann *et al*. 2005). *Chlorella* does not have a mucilage cover, indicating that other organisms (*e.g*. bacteria, fungi, diatoms) might have excreted it.

Dormant, thick-walled spores were present among the vegetative cells of *Chlorella* (Fig. 1B). The spores had regular pentagonal ornamentation on the outer surface and were 9-10 μm wide and 10-14 μm long, with light yellowish-brown chloroplast (Fig. 1B).

When cultured in a liquid freshwater medium both ornamented spores and smooth-walled vegetative cells revived in 2 weeks and started reproduction by autospores, 2 or 4 in each sporangium (Figs 1E and F). In cultured progeny cells the chloroplast was grass green, with undulate margins and a pyrenoid associated with numerous starch grains (Figs 1E and F). The nucleus was located laterally next to the chloroplast (Fig. 1F). No mucilaginous sheath surrounded individual cells or cell groups.

Free-living *Chlorella* has been recorded from different aquatic habitats and is associated with soil and subaerial surfaces (John and Tsarenko 2002; Shubert 2003). It often occurs on anthropogenic surfaces, including stone monuments and stone walls of buildings, either painted or unpainted (*e.g*. Schlichting 1975; Joshi and Mukundan 1997; Gaylarde and Gaylarde 2000; Schuman *et al*. 2005). Therefore, it should be tolerant to harsh environmental conditions, including fluctuations of temperature and repeated cycles of drying and wetting. The regular pentagonal ornamentation of *Chlorella* resting spores looks similar to that of the hypnozygote of *Chlorococcum echinozygotum* Starr (Chung 1993). Some other green algal resting cells are known to develop spiny or warty cell surface, for example hypnozygotes of *Chlamydomonas nivalis* (Bauer), *Volvox* sp., Zygnemataceae (Graham and Wilcox 2000), and akinetes of a soil alga *Chlorosarcina longispinosa* Chantanachat *et* Bold (Chantanachat and Bold 1962).

Klochkova *et al*. (in press) described dormant warty spores of a terrestrial green alga *Chlorococcum* sp. isolated from the stone walls of the inner chamber of Miruksazi stupa in Korea. Those spores formed in permanent darkness and drought condition were able to survive for over 5 years and to revive in freshwater medium. By comparison, the resting spores of *Chlorella* formed in natural light-dark condition, but they were exposed to high temperature and seasonal water shortage. Thus, the environments that triggered resting spore formation were different to each other, but desiccation seemed to be the major factor. To our knowledge, the ability of *Chlorella* to withstand desiccation by forming ornamented dormant spores was not previously recorded (*e.g*. Graham and Wilcox 2000; John and Tsarenko 2002; Shubert 2003) and supplements the taxonomic characteristics of this genus.

ACKNOWLEDGEMENT

This work is a partial result of the National Research Laboratory grant from KISTEP to G.H.K.

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Received 14 October 2005
Accepted 11 November 2005