ULTRASTRUCTURE OF *LOBOSPHAERA RENIFORMIS* (WATANABE) KOMÁREK ET FOTT (= *CHLORELLALES*) FROM KING GEORGE ISLAND, SOUTH SHETLAND ISLANDS, ANTARCTICA

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ABSTRACT

Lobosphaera reniformis (Wat.) Kom. et Fott (= *Chlorella reniformis* Wat.) so far known only from Japan, and Papua Island, was for the first time found in Antarctica (King George Island, South Shetland Islands). In laboratory cultures a complete life cycle was obtained, and most of its stages were followed by the electron microscopy. Reproduction is by morphologically different autospores. In some large vegetative cells two Golgi apparatuses lying side by side were observed.

KEY WORDS: *Lobosphaera reniformis*, *Chlorellales*, Golgi apparatus, ultrastructure, Antarctica.

INTRODUCTION

The green alga described in this paper was found during a survey of epilithic algae carried out at the Arctowski research station by the XIII Polish Antarctic Expedition on King George Island. The same species was found again during investigation conducted on the moraines of the Ecology Glacier (XVI Polish Antarctic Expedition).

King George Island (located between lat. 61°50' and 62°15' S and long. 57°30' and 59°00' W.), the largest of the South Shetland Islands, is situated in the maritime Antarctic. The archipelago lies about 770 km south-west of Cape Horn and about 160 km north of Trinity Peninsula, the northernmost part of the Antarctic Peninsula (Fig. 1).

Lobosphaera reniformis was described by Watanabe in 1977 from the terrestrial habitat in Japan under the name *Chlorella reniformis* Wat. Some years later Watanabe collected this species also in the same habitat on Papua Island (New Guinea). Watanabe (1979) using a criterion of the presence or absence of pyrenoid transferred *Chlorella reniformis*, which was lacking pyrenoid, to the genus *Palmellococcus* Chodat 1894. Komárek and Fott (1983) in their monography of the order *Chlorococcales* included *Chlorella reniformis* in the genus *Lobosphaera* Reisinger.

Autospores from this material have various forms, which appears to be an important diagnostic feature for this species, and this is why we have undertaken detailed study on its structure.

MATERIALS AND METHODS

Small pieces of volcanic rocks colonized by epilithic algae were collected at the Rakusa Point at 29 December 1989, and on the moraines near the Ecology Glacier at 27 April 1992,

both places are in the vicinity of the Arctowski Station. The samples were stored deep-frozen at -20°C in sterile polythene plastic bags. Material was removed from the surface of the rock samples, and cultured on the standard Bristol agar medium, at 20°C under a 12/12 h light/dark cycle at 3000 lx provided by 40W fluorescent tubes.

For transmission electron microscopy the laboratory-grown cells were removed with a spatula from the surface of agar plates, and dispersed in fixative. The initial fixative was 2.6% glutaraldehyde and 0.05% ruthenium red in 0.067M phosphate buffer at pH 7.1 applied at room temperature for 1h. Three washes of 5 minutes each were done in buffer. The second fixative, applied also at room temperature, was 1.33% osmium tetroxide and 0.05% ruthenium red in buffer. Mixing of the osmium tetroxide and ruthenium red was done immediately prior to use. Such treatment immobilizes and stains a maximum amount of secreted mucilage enclosing the cells (Luft 1971). After several subsequent washes in buffer and dehydration in ethanol series, the cells were infiltrated overnight with a 1:1 mixture of propylene oxide and SPURR's hard medium (1969), and then embedded in the fresh SPURR's medium. The samples were polymerized at 60°C for 18 h. Silver- and gold-colored sections were cut with glass knives on a Reichert-Jung ultramicrotome, mounted on acetone-treated 700 mesh copper grids without support, and stained for 4 minutes in uranyl acetate (5% in 50% ethanol) followed by 2 minutes in lead citrate (Reynolds 1963). Observations and photographs were made with a Tesla BS 500 electron microscope.

RESULTS

Pure cultures of *Lobosphaera reniformis* (Wat.) Kom. et Fott 1983 (= *Chlorella reniformis* Wat. 1977), including all the developmental stages were obtained (Figs 2-7). Vegetative

Fig. 1. Map of King George Island with the Arctowski station.

cells are spherical, or almost spherical of the diameter 8-14 μm (Figs 2, 8-9). Reproduction is by the autospores of different shape. Autospores developed in the mother cell in the number not exceeding 8, are spherical, or subspherical of diameter 5-10 μm (Fig. 3, 11-13). Whereas autospores developed in the mother cell in larger numbers, above 8, are ellipsoidal, reniform or cylindrical, with dimensions: 2-7 μm wide, 4-12 μm long (Figs 4-5, 14-15). The latter types of autospores become in their ontogenic cycle spherical or subspherical vegetative cells. Cell walls of empty autosporangia

are often encountered. These walls show different state of disintegration (Figs 14, 16). Mature vegetative cells become either resting spores with the thick cell walls (Fig. 10) or they continue their developmental cycle producing autospores.

The chloroplasts are band-shaped, saucer-shaped or consisting of 2-3 parts separated by deep indentations. Their position is subparietal to parietal. Chloroplasts contain distinct starch grains but the pyrenoid both in autospores and vegetative cells is absent. The position of the Golgi apparatus, both in autospores and vegetative cells, is always perinuclear (Figs

Figs 2-7. *Lobosphaera reniformis* (drawings from the light microscope).

Fig. 3. Autosporangia with 8 and 4 autospores.

Figs 4-5. Autosporangia with 8 to 16 autospores.

Figs 6-7. Young vegetative cells of various shape.

Figs 8-20 *Lobosphaera reniformis* (transmission electron microscope).

Fig. 8. Spherical vegetative cell. G, Golgi apparatus; N, nucleus; m, mitochondria. x 10500.

Fig. 9. Subspherical vegetative cell. G, Golgi apparatus; N, nucleus; m, mitochondria. x 11000.

Fig. 10. Part of old vegetative cell showing thick cell wall. CW, cell wall; Ch, chloroplast. x 25500.

Fig. 11. Autosporangium showing 4 autospores (early stage). N, nucleus; cell wall of autosporangium (arrow). x 16000.

Fig. 12. Two autosporangia with fully developed 4 autospores. x 4500.

Fig. 13. Two fully developed autospores still inside the autosporangium. N, nucleus; cell wall of autosporangium (arrow). x 9000.

Fig. 14. Reniform autospore with the remnants of autosporangium cell wall. N, nucleus; cell wall of autosporangium (arrow). $\times 13500$.
Fig. 15. Elliptical autospore. N, nucleus; cell wall of autosporangium (arrow). $\times 9500$.
Fig. 16. Partially disintegrated autosporangium cell wall, note the internal direction of disintegration (arrow). $\times 8000$.

17-20). Arrangement of the Golgi apparatus in relation to the nucleus is not constant. In some sections the Golgi apparatus is seen in cross section (Figs 8, 17a, b, 18 a, b), whereas in others in tangential section (Fig. 20). In large adult cells there are occasionally two Golgi apparatuses lying beside each

other but not in the same plane. As a result of such a situation in the plane of the cross section one of the Golgi apparatuses is always sectioned somewhat obliquely (Figs 18-19). In the tangential section the oblique arrangement of two Golgi apparatuses is not so apparent (Fig. 20). Large nucleus is situated either centrally or laterally. The nuclear membrane facing two Golgi apparatuses is undulated and distinctly swollen (Figs 19-20). The nucleolus is located eccentrically and of relatively low electron density (Fig. 21).

DISCUSSION

Lobosphaera reniformis found on King George Island shows distinct similarity to *L. reniformis* (Wat.) Kom. et Fott (= *Chlorella reniformis* Wat.). The chloroplasts in the vegetative cells are band-shaped, saucer-shaped or they consists of two to three parts always devoid of pyrenoid. The reproduction is also by the autospores, which are morphologically different (spherical, elliptical, cylindrical and reniform). Apart from that, *Lobosphaera reniformis* is similar to *Chlorella reissigii* Wat. regarding both the shape of the chloroplast and the autospores. It differs from the latter by the lack of the pyrenoid. The lack of pyrenoid distinguishes *Lobosphaera reniformis* from *Chlorella subsphaerica* (Reisigl) Kom. et Fott (= *Pseudochlorella subsphaerica* Reisigl), however, regarding the shape of the autospores these two species show some similarity (Mrozińska 1991). Watanabe (1979) pointed out that the absence of the pyrenoid gives the sufficient grounds to segregate the genera *Lobosphaera* and *Chlorella*. The very characteristic feature for the genus *Chlorella* is that one of the autospores is always bigger than the rest, and remains inside the autosporangium considerably longer (Komárek and Fott 1983). Whereas in the genus *Lobosphaera* from the Antarctic the autospores within the autosporangium are more or less of the same size. The cell walls of empty autosporangia undergo a gradual disintegration, forming a network of electron-opaque fibrils. Similar fibrils in appearance and diameters were observed on the cells of *Chlorophyceae* (Massalski and Leppard 1979), and as part of empty cell walls in the water column of lakes (Leppard et al. 1977). Two Golgi apparatuses situated beside each other in *Lobosphaera reniformis*, were also observed in the living state in the species *Chlorella saccharophila* strain SAG 211-9 b (Tscheramak-Woess 1988). However, two Golgi apparatuses oriented in a parallel fashion in the form of double platelets, and 4 platelets after duplication observed in the living state in *Chlorella sphaerica* by Tscheramak-Woess (1988) were never found in *Lobosphaera reniformis*. Two Golgi apparatuses adjacent to the anterior projection of the nucleus were found in the zoospore of *Bumilleria sicula* Borzi, *Xanthophyceae* (Massalski and Leedale 1969). Schnepf & al. (1966) suggest that the position of two Golgi apparatuses is of taxonomical value. The undulated and swollen nuclear membrane facing two Golgi apparatuses may indicate a relationship between the nuclear membrane and the Golgi complex. Such relationship was also noted in *Tribonema vulgare* Pasher zoospores (Massalski and Leedale 1969), and in *Phaeophyceae* (Bouck 1965). The ultrastructure of the Golgi apparatus, obtained from the vegetative cells and the autospores can be used for further taxonomical investigation of the genus *Lobosphaera*.

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Fig. 17a. Fragment of cell with single Golgi apparatus. G, Golgi apparatus; N, nucleus. x 35000.

Fig. 17b. Fragment of another cell with single Golgi apparatus. G, Golgi apparatus; N, nucleus; m, mitochondrion. x 35000.

Figs 18a-b. Serial cross sections of two Golgi apparatus. G1-2, Golgi apparatus; N, nucleus; m, mitochondrion; arrow, undulated and swollen nuclear membrane. x 42000.

Fig. 19. Cross section of two Golgi apparatus. G1-2, Golgi apparatus; N, nucleus; m, mitochondrion; arrow, undulated and swollen nuclear membrane. x 42000.

Fig. 20. Tangential section of two Golgi apparatus. G1-2, Golgi apparatus; N, nucleus; x 35000.

Fig. 21. Fragment of cell with nucleus and nucleolus. N, nucleus; Nu, nucleolus; m, mitochondrion. x 42000.

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LITERATURE CITED

- BOUCK G.B. 1965. Fine structure and organelle associations in brown algae. *J. Cell Biol.*, 26: 523-537.
- KOMÁREK J., FOTT B. 1983. *Chlorophyceae* (Grünalgen), Ordnung: *Chlorococcales*. Das Phytoplankton des Süßwassers. Systematik und Biologie 7/1. In: Die Binnengewässer, Stuttgart, Schweizerbart-sche Verlagshandlung, 16: 1044 pp.
- LEPPARD G.G., MASSALSKI A., LEAN D.R.S., 1977. Electron-opaque microscopic fibrils in lakes: their demonstration, their biological derivation and their potential significance in the redistribution of cations. *Protoplasma* 92: 289-309.
- LUFT J.H., 1971. Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. *Anat. Rec.* 171: 347-368.
- MASSALSKI A., LEEDALE G.F., 1969. Cytology and ultrastructure of the *Xanthophyceae*. I. Comparative morphology of the zoospores of *Bumilleria sicula* Borzi and *Tribonema vulgare* Pasher. *Br. phycol. J.*: 4(2): 159-180.
- MASSALSKI A., LEPPARD G.G., 1979. Morphological examination of fibrillar colloids associated with algae and bacteria in lakes. *J. Fish. Res. Board Can.* 36(8): 922-938.
- MROZIŃSKA T., 1991. Aerophytic algae from North Korea. *Algological Studies* 58: 29-47.
- REYNOLDS E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.
- SCHNEPF K., KOCH W., DEICHGRABER G., 1966. Zur Cytologie und taxonomischen Einordnung von *Glaucocystis*. *Arch. Mikrobiol.* 55: 149-174.
- SPURR A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26: 31-43.
- TSCHERMAK-WOESS E., 1988. New and known taxa of *Chlorella* (*Chlorophyceae*): occurrence as lichen phycobionts and observations on living dictyosomes. *Pl. Syst. Evol.* 159: 123-139.
- WATANABE S., 1977. The genus *Chlorella* (*Chlorococcales*) from Japanese soils (3). *Journ. Jap. Bot.* 52(6): 169-178.
- WATANABE S., 1979. Green soil algae from Papua New Guinea. In: *Studies on Cryptogams of Papua New Guinea*. Syo Kurokawa (ed.). Academia Scientific Book Inc., Tokyo: pp. 87-102.

BADANIA NAD ULTRASTRUKTURĄ *LOBOSPHAERA RENIFORMIS* (WATANABE) KOMÁREK ET FOTT (*CHLORELLALES*) ZNALEZIONEJ NA WYSPIE KRÓLA JERZEGO, WYSPY POŁUDNIOWE SZETLANDY, ANTARKTYKA

STRESZCZENIE

Lobosphaera reniformis, dotychczas znana tylko z Japonii i Wyspy Papua, została również opisana z Antarktyki (Wyspa Króla Jerzego, Wyspy Południowe Szetlandy). Kompletne stadia cyklu rozwojowego uzyskano w hodowlach laboratoryjnych i większość z nich opracowano przy użyciu mikroskopu elektronowego. Stwierdzono, że reprodukcja dokonuje się poprzez morfologicznie zróżnicowane autospory. Dwa aparaty Golgiego, położone jeden obok drugiego, zaobserwowano w niektórych dużych komórkach wegetatywnych.

SŁOWA KLUCZOWE: *Lobosphaera reniformis*, *Chlorellales*, aparat Golgiego, ultrastruktura, Antarktyka.