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SHORT COMMUNICATIONS

Green Microalgae Isolated from Associations with White Sea Invertebrates

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Endophotosymbionts (cyanobacteria, microalgae, or their functionally active chloroplasts) were found in mollusks, sponges, corals, anemones, freshwater hydra, worms, and ascidians [1–2]. Associations of colonial hydroids with epibiotic microalgae and cyanobacteria were previously described [3–5]. It is known that isolation of animal microsymbionts is seldom successful. The information on the isolation of phototrophic microrganisms associated with invertebrates of the high latitude seas, especially the White Sea, is limited and mainly refers to cyanobacteria [4].

The goal of the present work was to study the microalgae isolated from associations with the White Sea invertebrates and to characterize their morphology, ultrastructure, and the composition of pigments and fatty acids.

Microalgae analyzed in the study were isolated from the benthic animals collected in the region of the Moscow State University White Sea Biological Station (66°34′N, 33°08′E) in the Kandalaksha Bay of the White Sea. Microalgae were isolated from the invertebrates with green zones containing red autoluminescent bodies within their tissues or covers. Isolation and microscopy were carried out as described [4, 5]. Fatty acid and pigment analysis was carried out according to the method in [6]; molecular genetic analysis was carried out as described in [7].

Nineteen cultures of eukaryotic unicellular algae without rigid shells were isolated: isolate 1Es66E from the sponge *Eumastia sitiens* (Schmidt, 1870); isolates 1Hp86E, 1Hp86E-1, and 1Hp86E-2 from the sponge *Halichondria panicea* (Pallas, 1766); isolates 1Pm66B and 1aPm66B from the throchophore larvae of the polychaete *Phyllodoce maculata* (L., 1767); isolates 1Lf66E, 2Lf67E, and 3Lf87E from the hydroid *Laomedea flexuosa* (Alder, 1857); isolates 3Dp86E-1, 4Dp86E, 9Dp66E, and 11Dp66E from the hydroid *Dynamena pumila* (L., 1758); isolates 1Gl67B, 2Gl67E, and 4Gl87E from the hydroid *Gonothyraea loveni* (Allman, 1859); isolates 2Cl66E and 3Cl67E from the hydroid *Coryne lovenii* (M. Sars, 1846); isolate 1Sm66E from the hydroid *Sertularia mirabilis* (Verrill, 1873).

Seven microalgal isolates described in this study (1Es66E, 1Hp86E, 1Hp86E-1, 1Hp86E-2, 1Pm66B, 3Dp86E-1, and 2Cl66E) were unicellular, without flagella, reproducing by autospores (2–8 within one sporangium and even more in case of 3Dp86E-1). The former two isolates had oval cells, the latter five, spherical cells. The cell wall consisted of two layers with the ultrastructure corresponding to the polysaccharide inner and sporopollenin outer layers of the green algae cell wall [8]. In five latter isolates, the outer layer contained the epistructures visible by electron microscopy: tubes of diverse (sometimes unique) structure, villi, rosettes, warts, etc. (Figs. 1a, 1b). The microalgae had chloroplasts with two-membrane envelopes and thylakoids in stacks (Fig. 1c). In 1Es66E and 1Hp86E, the chloroplasts were located close to the cell wall, occupied a lesser part of the cytoplasm, had compact form, and did not contain pyrenoids. In the isolates 1Hp86E-1, 1Hp86E-2, 1Pm66B, 3Dp86E-1, and 2Cl66E, the chloroplasts were of lobar shape, occupied the major part of the cytoplasm, and contained pyrenoids lacking intrapyrenoid thylakoids (Fig. 1d). All the investigated microalgae abounded in storage compounds: lipid globules, starch grains, polyphosphate- or phenolic-like granules, etc.

The pigment composition of the microalgae was typical of *Chlorophyta*, including chlorophylls *a* and *b*, lutein, β-carotene, neoxanthin, as well as the violaxanthin cycle components, violaxanthin, antheraxanthin, and zeaxanthin. Absorption spectra of the suspensions of these microalgae therefore contained the chlorophyll absorption bands (maximum at 678 nm) and the wide band caused by common absorption of chlorophylls and carotenoids in the blue part of the visible spectrum (Fig. 2). The dominant fatty acids were long-chain saturated acids and unsaturated fatty acids with carbon chains of 14 to 20 atoms and one to three double bonds.

According to the described properties, these microalgae belonged to the phylum *Chlorophyta*. The isolates 1Hp86E-1, 1Hp86E-2, 1Pm66B, 3Dp86E-1,

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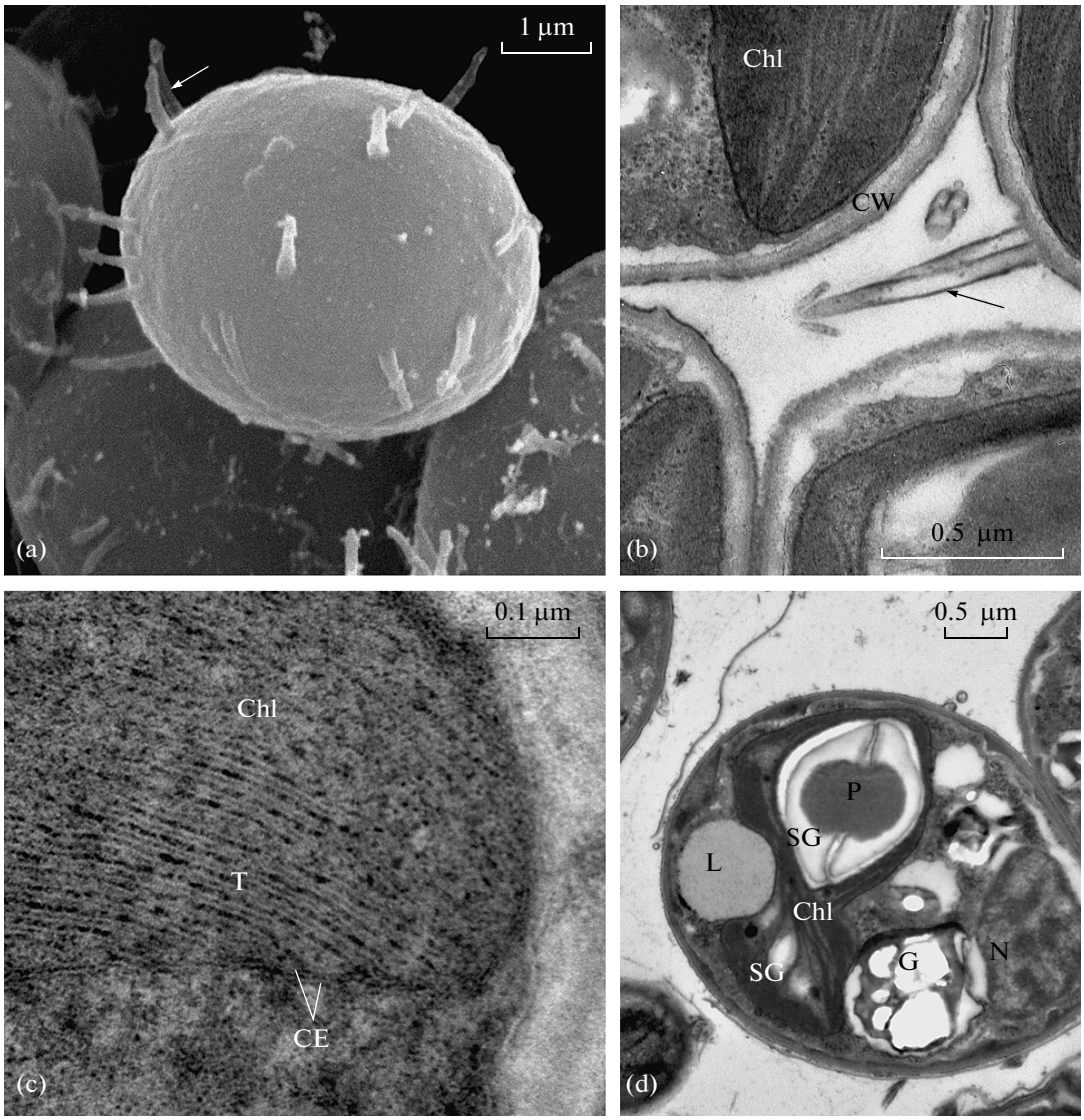

Fig. 1. Structure of the microalga isolated from the hydroid *Dynamena pumila* (3Dp86E-1 isolate): cell morphology (a), organization of the surface structures (b), part of a chloroplast (c), and the cell ultrastructure (d). G, granule of the storage product; SG, starch grain; CW, cell wall; L, lipid globule; CE, chloroplast envelope; P, pyrenoid; T, thylakoids; Chl, chloroplast; N, nucleus. Arrows indicate the tubes at the surface.

and 2Cl66E possessed the properties of representatives of the order *Sphaeropleales*, family *Scenedesmaceae*. The epistructures observed in these microalgae were similar to those described for the genus *Desmodesmus*, subfamily *Desmodesmoideae*, family *Scenedesmaceae* [9]. Characteristics of the epistructures, as well as the DNA sequence analysis, have diagnostic significance for taxonomy of the genus *Desmodesmus* and closely related organisms [10]. Comparative analysis of the nucleotide sequence of the 18S rRNA gene of the isolate 1Hp86E-2 indicated its close relation to the organisms of the family *Scenedesmaceae*. The highest similarity was shown for the 18S rRNA gene sequence from an unclassified organism, *Scenedesmaceae* sp. Tow 9/21 P-14w. Among the close relatives of 1Hp86E-2 were microalgae of the genera *Scenedesmus* and *Desmodesmus*, which was withdrawn from the former genus [11]. The isolates 1Es66E and 1Hp86E also possessed the properties characteristic for the green algae, order *Sphaeropleales*; however, unlike the five microalgae listed above, they had no epistructures and their chloroplasts did not contain pyrenoids.

Thus, unicellular green algae associated with invertebrates of high-latitude seas were characterized for the first time. Abundance of the intracellular storage polymers and lipids in the isolated microalgae suggests an important role of these organisms as potential producers of valuable substances.

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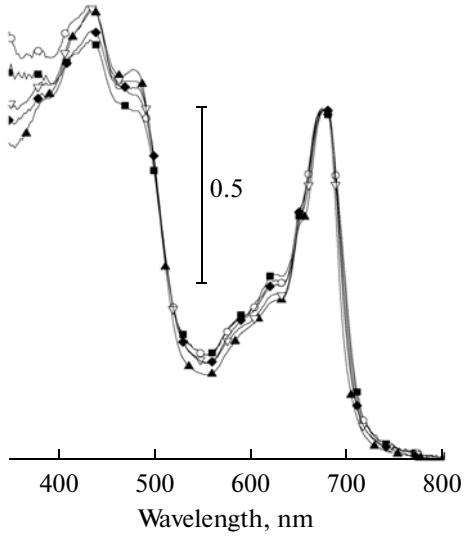

Fig. 2. Typical absorption spectra of the microalgal cell suspensions: 1Hp86E-2 (solid squares), 3Dp86E-1 (circles), 1Es66E (solid triangles), 2Cl66E (turned triangles), and 1Pm66B (solid rhombs) normalized against the red chlorophyll maximum.

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