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Morphology and possible host range of *Rhizophydium algavorum* sp. nov. (Chytridiales) - an obligate parasite of algae

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Summary

Culture of *Rhizophydium* (Chytridiales) was isolated from a little pond near St.-Petersburg and described as representative of a new species – *Rh. algavorum*. It is an obligate parasite of algae with a very wide possible host range. From 137 strains of chlorococcalean algae examined 33 are sensitive, they represent 20 species of 5 genera. Xanthophycean alga *Tribonema gayanum* is sensitive as well. Parasite forms sessile sphaerical sporangia. Zoospores escape from the sporangium upon the single wide lateral papilla.

Key words: *Rhizophydium*, new species, algal parasite, possible host range, Chlorococcales.

Introduction

Many chytrids have been recorded as the parasites of freshwater algae including the representatives of Chlorococcales (Sparrow 1960). Green chlorococcalean algae are very common in eutrophic waters, many species are important planktonic primary producers in aquatic ecosystems and influence water quality of their habitat (Tsarenko & Krienitz 1997). Some forms are of evident practical significance (Borowitzka & Borowitzka 1988). Data concerning fungal parasites of Chlorococcales are very limited (Sparrow 1960, Golubeva 1995), these algae are not mentioned in the list of chytrids occurring in Leningrad region (Golubeva 1982). During our survey of chytrids occurring on chlorococcalean algae in waters of Russian Federation, we isolated seven strains of parasites which, according to Sparrow (1960) and Golubeva (1995), can be considered as the representatives of genus *Rhizophydium* (Gromov et al.,1997). The morphology, cultural characters, and possible host range of the isolates vary. One strain, *Rhizophydium* X–34 CALU (Collection of Algae in Leningrad University), is obligatory parasitic, it has very wide possible host range, including the representatives of different Chlorococcales genera, and even xanthophycean alga *Tribonema gayanum* Pash. We can not attribute this form to any known *Rhizophydium* species, and describe it as a new species – *R. algavorum* sp. nov. The description of morphology of this organism, its possible host range, and the character of growth in cultures of algae, as well as formal description of the species, is presented in this communication.

Material and Methods

The culture of parasite was isolated from a little pond near St.-Petersburg by inoculation of *Chlorococcum minutum* Starr CALU 746 culture (0.5L) in N 1 medium (Gromov and Titova 1991) with a sample of water (0.5L) filtered through a piece of gause. After 2 weeks of incubation, infected cells appeared in the culture, and the material was used for the inoculation of algae grown on the surface of No 1 medium containing agar – 1.5%, glucose – 0.1%, peptone – 0.1%, yeast extract – 0.1%, benzyl penicillin – 5 x 10^3 unit mL^{-1} ("organic medium"). The parasite produces brown plaques on the lawn of algae. Material from a plaque was used for the inoculation of axenic algal culture in liquid organic medium without penicillin. All cultures were incubated at 25^0 C under continuous illumination of 25 mmol photon m^{-2} s^{-1} supplied by 40 W cool-white fluorescent tubes. Live and heat-killed algae were used in inoculation experiments. Algae used included the strains from CALU collection (Gromov and Titova 1991). The lawn of live algae on N 1 medium with agar was inoculated with a drop of infected culture. Dispersal of brown zone from the spot of inoculation gives evidence

of algal sensitivity to the parasite. The material from brown zone was examined under the microscope. Growth of the fungus on heat-killed algae and on pollen grains was examined in liquid medium N 1. Algae were killed by placing the tube culture on water bath at 80^0 C for 10 min. Cells of sensitive strains of *Ankistrodesmus*, *Chlorella* and *Chlorococcum* were used in these experiments. A possibility of colony formation by the parasite on organic media was examined with an organic medium of the composition shown above, and medium YPD (0.1% yeast extract, 0.1 % Bacto-peptone, 1.0 % dextrose) recommended by Barr (1969).

For scanning electron microscopy (SEM), cells were fixed for 40 min in 2% glutaraldehyde in 0.05 M sodium cacodylate (pH 7.2), rinsed in distilled water, placed on a cover slip, dehydrated in ethanol, critical-point dried, and coated with gold.

For transmission electron microscopy (TEM), the material was fixed at room temperature with 2.5% glutaraldehyde in 0.05 M cacodylate buffer, dehydrated in ethanol, and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Hitachi 300 S electron microscope at 80 kV.

Results

Rhizophydium CALU X–34 produces monocentric, eucarpic, epibiotic, spherical, sessile, inoperculate sporangium up to 13 mm in diameter. Endobiontic part is represented by a rhizoidal system. Zoospores are usually elongated, moderately amoeboid, 2–3 mm thick, about 5 mm long; the flagellum is about 15 mm long including 5 mm whiplash (Fig. 1,a); a conspicuous oil globule is visible in light microscope. Encysted zoospore is round (Fig.2,c). Zoospore, swimming or encysted, contains a nucleus with nuclear cap, mitochondria (Fig.1,b,c), and dark inclusions associated with mitochondria (Fig.1b). Microtubules visible on Fig.1,c near the nucleus originate from the retracted flagellum, or represent cytoplasmic microtubules. The described pattern does not contradict the *Rhizophydium* zoospore ultrastructure type as formulated by Barr and Hadland-Hartmann (1978). The sporangium is formed by enlargement of zoospore. Sporangium wall is thin and smooth (Fig.1, c–f). In some sporangia, the proximal part is a bit narrowed (Fig. d), but real extramatrical stalk is absent. The system of dividing tubular rhizoids is fairly developed, it originates from a single stem (Fig.1,c,e,f). Rhizoidal axis is not thicker than the remainder of the rhizoids (Fig.1,e,f). Rhizoids of the ripening sporangium contact with remnants of the host content, which usually consist of one or several reddish masses (not shown). Sporangium wall usually persists after the discharge of zoospores (Fig.2,e,f). Zoospores escape from the sporangium upon the deliquescence of a single, wide, usually lateral, but sometimes sub-apical, papilla (Fig. 2,e,f). The way of zoospore discharge here is non-vesicular (Barr 1975); zoospores do not discharge into the gelatinous vesicle, but do so through the pore forming a loose mass, and swim away. The contents of ripe sporangium is separated from the rhizoidal system by a thin crosswall (Fig.2,e). Resting spore was not observed in culture. The fungus grows on algae cultivated in solid media, and forms brown plaques on the lawn of algal cells. Practically all algal cells in the plaque are destroyed by the parasite. In liquid media, depending on a host, all algae are killed, or, after a period of active development of infection, the parasites begin to die, and soon disappear. In this case some algal cells survive, and produce secondary growth of the culture. In any case the re-inoculation of fresh algal culture every 2–4 weeks is needed to keep the culture of parasite safe. Only a limited development of parasites could be observed in the dark. *Rhizophydium* X–34 does not grow on organic media without algae and does not form free-lying sporangia in organic media with algae. It does not grow on pollen grains and on the cells of algae killed by heat. It is obligatory parasitic.

Sensitive algae are: *Ankistrodesmus braunii* Brunnth. CALU–33,CALU–80; *A. nannoselene* Skuja CALU–204: *Chlorella ellipsoidea* Gerneck CALU–2; *C. pyrenoidosa* Chick CALU–164, CALU–168, *C. zofingiensis* Donz CALU–190; *Chlorococcum perforatum* Arce et Bold CALU–481; *C. minutum* Starr CALU–746; *C. sphacosum* Archibald et Bold CALU–847; *C. isabelience* Archibald et Bold CALU–941; *Kirchneriella obesa* (West) Schmidle CALU–28, CALU–447; *Scenedesmus acuminatus* (Lagerh.) Chodat CALU–411; *S. acutus* Meyen CALU–65, CALU–144, CALU–494, *S. basilensis* Chodat CALU–203; *S. bijugatus* (Turp.) Kutz. CALU–12, CALU–116; S. *dimorphus* Kutz. CALU–198; *S. dispar* Breb. CALU–417; S. *naegelii* Chodat CALU–199; *S. obliquus* (Turp.) Kruger CALU–13, CALU–66, CALU–220, CALU–358, CALU–392, CALU–400, CALU–424, CALU–433; *S. obtusiusculus* Chodat CALU–446.

From 137 strains of chlorococcalean algae examined 33 are sensitive, they represent 20 species of 5 genera; 2 examined strains of *Kirchneriella obesa* are sensitive; some cultures of *Ankistrodesmus*, *Chlorella*, *Chlorococcum* and *Scenedesmus* are resistant; resistant are all 6 examined cultures of *Scenedesmus quadricauda* (Turp.) Breb. The sensitivity of chlorococcalean algae to the parasite is of the strain, but not of the genus or species character. The xanthophycean alga *Tribonema gayanum* Pash., examined as a possible host, proved sensitive as well.

Algae from other taxonomic groups have not been examined.

The cultures of some species of *Rhizophydium* algal parasites were examined by Barr and Hickman (1967) and by Barr (1970, 1973, 1975), but fungi examined could grow saprophytically.

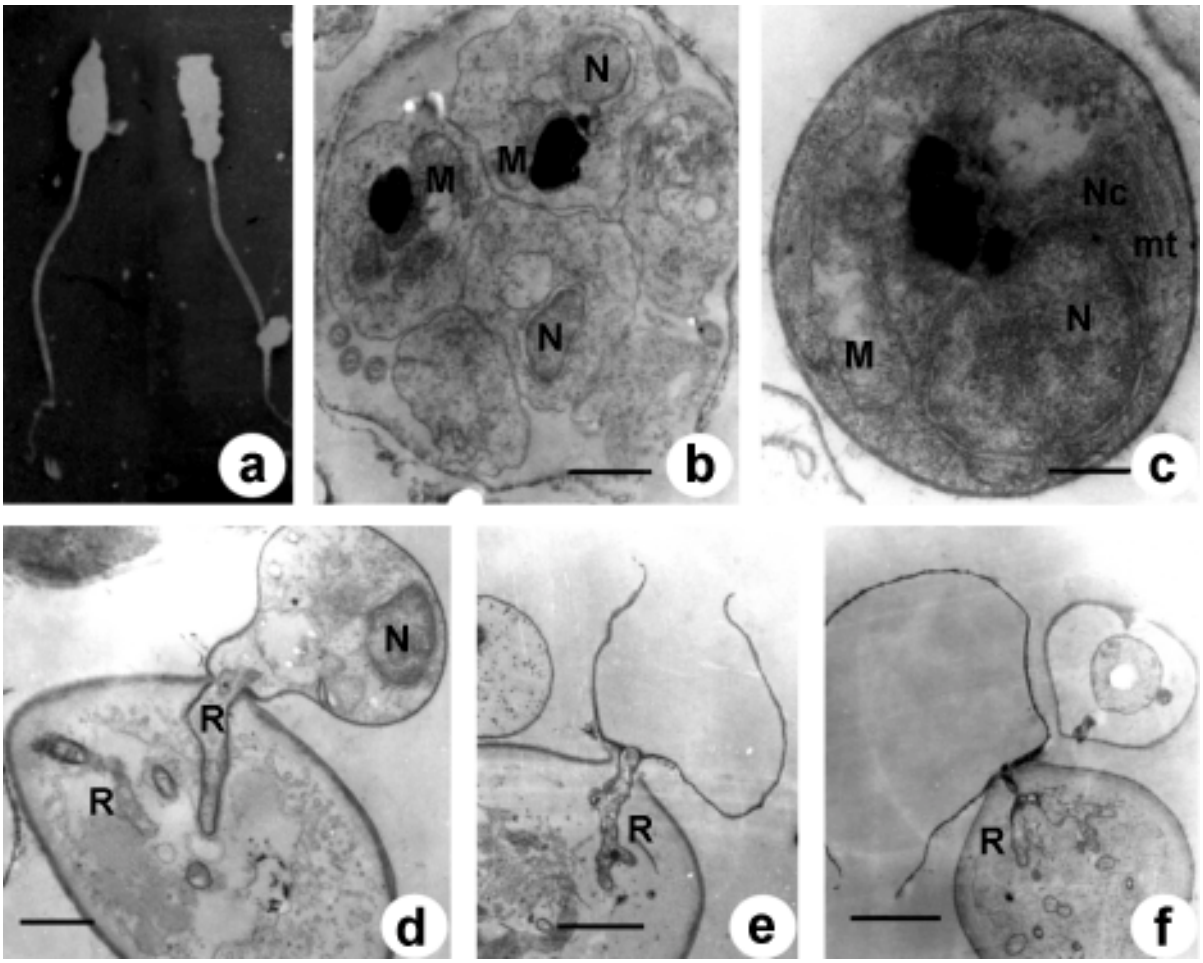

Fig. 1. Morphology and ultrastructure of *Rhizophydium* X–34. **a.** Zoospores, SEM. **b.** Zoospores in discharging sporangium, TEM. **c.** Encysted zoospore, TEM. **d.** Young sporangium, dividing rhizoids are visible, rhizoids are tubular, TEM. **e, f.** Empty sporangia on the destroyed cells of *Chlorococcum minutum*. Rhizoidal system is visible, TEM. M – mitochondrion, MT – microtubules, N – nucleus, NC – nuclear cap area, R – rhizoids. Scale bars: a – 2 μm, b–d – 1 μm, e, f – 3 μm.

Discussion

Details of the morphology are essential for a specific definition of *Rhizophydium* strains. Our organism has a single prominent lateral discharge papilla. It is characteristic of *Rhizophydium punctatum* Golub. (Golubeva 1995), but this fungus grows on pollen grains, its zoospores are round. Wide lateral papilla is characteristic of *R. canterae* Sparrow and *R. karlingii* Sparrow(Sparrow, 1960) – the parasites of green algae, although not of chlorococcalean ones (Sparrow 1960). *R. karlingii* possesses from one to three discharge papillae, whereas strain X–34 always one. The endobiotic part of *R. karlingii* consists of a knob – or peglike structure 4 mm in diameter, zoospores are nearly spherical, strain X–34 forms thin rhizoids (Fig.1,d–f), its zoospores are elongated (Fig.1,a). In *R. canterae*, zoospores lack a conspicuous globule, while in our strain it is always visible in viable zoospores. *R. canterae* is parasitic on sporelings of *Oedogonium* sp. Strain X–34 can not be considered as a member of the species mentioned from the point of view of its morphology. It is evident that a possible host range can not be used for a specific determination of this organism. At the same time, the obligatory parasitic type of life is of evident significance, as well as a very wide range of possible hosts. We are considering this organism as representative of a new species.

Diagnosis: *Rhizophydium algavorum* sp. nov.

Zoosporangia sessilia, sphaerica, 12 mm diam., membrana tenui hyalina incolorata, poro unico laterale. Rhizoideis tenues ramosae, fere isodiametrici ab uno axi basi sporangii abeuntes. Zoosporae elongatae, 5 mm longa, 2 mm diam, globulo unico refractivo, flagello praeditae, 15 mm longa.

Obligatorius parasitica in algae, Chlorococcales et *Tribonema gayanum*.

Typus: Russia, Petropoli, stagnum. Typus cultura X–34 in CALU collectio Universitatis Petropolitanum.

Zoosporangium sessile, sphaerical, about 12 mm in diameter, sporangial wall is thin, smooth, colourless. Single lateral discharge pore. The system of dividing tubular rhiz-

oids is fairly developed, it originates from a single stem. Rhizoidal axis is not thicker than the remainder of the rhizoids. Zoospores elongated 5 mm long, 2 mm thick, with a single refractive globule. Back flagellum 15 mm long.

Obligatory parasitic on algae, Chlorococcales and *Tribonema gayanum*.

Type: Diagnosis is based on isolate X–34 in CALU collection of St.-Petersburg University obtained from pond near St.-Petersburg, Russia.

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