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Isolation and Characterization of Some Indian Hyphochytriomycetes

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Abstract

In the present study, three species of Hyphochytriomycetes were isolated, identified and described. They are Rhizidiomyces hirsutus Karling, Rhizidiomyces apophysatus Zopf and Rhizidiomyces bulbosus Karling. Among them, Rhizidiomyces bulbosus Karling species is being reported for the first time in India.

Keywords: Hyphochytriomycetes, *Rhizidiomyces* and *Rhizidiomyces bulbosus* Karling.

Introduction

Hyphochytriomycetes (Hyphochytriales) is a small group of chytrid-like organisms that contain about 23 known species[1]. They are strikingly similar in morphology and development to many of the true chytrids, but are distinguishable from them by the presence of single anterior tinsel-type flagellum on their zoospores. Fuller[2] included them in the class Hyphochytriomycetes of phylum Hyphochytriomycota. According to Berbee and Taylor[3] the Hyphochytriomycota, Labyrinthulomycota and Oomycota belong to the Kingdom Stramenopila. Hyphochytriomycota, consisting of a single order Hyphochytriales, has been classified into three families, namely Anisolpidiaceae, Rhizidiomycetaceae and Hyphochytriaceae on the basis of their thallus structure[4,5,2,6]. The Hyphochytriomycota are a small group of little known fungi with almost no economic importance; but molecular and ultrastructural evidences place them together with the biflagellate heterokont organisms such as Oomycota and Chromistan algae[7].

In India the study of Hyphochytriomycetes started as early as 1935, when Chaudhuri and Kochhar[8] reported *Rhizidiomyces apophysatus* from the oogonia of *Achlya klebsiana*. Few years later, Mundkur[9] reported the same species on *A. klebsiana.* However, the real start of research on Hyphochytriomycetes in India should be credited to J.S. Karling, a mycologist of USA who visited India in 1963 and described 6 species of Hyphochytriomycetes[10].

The purpose of this study was to throw light on description and information pertaining to habitats, substrates, and geographical locations of the following Hyphochytriomycetes isolated from fresh water and soil sources. Three species of *Rhizidiomyces* were isolated and illustrated in this paper. The detail of their life history, with particular emphasis on their delepmental stages on soild media is described. *Rhizidiomyces bulbosus* Karling species is newly recorded in India.

Material and Methods

Isolation: Baiting technique[11,12] was used for the recovery of Hyphochytriomycetes. Samples of water and soil were collected at random and taken to the laboratory. Each sample was divided into triplicates, which were introduced in separate Petri dishes and flooded with 40 mL of sterile deionized water. Each triplicate was baited with chitin (purified shrimp exoskeleton) and keratin (purified snake skin). All triplicates were incubated at ambient room temperature for two weeks. The baits were periodically examined under a microscope for about two weeks and when the isolates became visible on the baits, they were transferred to 1/4YpSs (yeast extract peptone soluble starch) agar medium containing 300 ppm penicillin G and 300 ppm streptomycin sulfate. All isolates were obtained in to pure form by a series of regular subculture carried out on 1/4YpSs agar. Stock culture of all the isolates was maintained on 1/4YpSs agar slants. The cultures were stored at 10°C and subcultured after every three months onto fresh media. All specimens were deposited at the Laboratory of Mycopathology and Microbial Technology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India.

Observation and identification: Thallus morphological features and developmental pattern of the isolates were examined using a light microscope on 1/4YpSs agar, PYG broth and on baits. The isolates were examined by light microscopy to assess range and variation in thallus structural features, including sporangial shape and size, *discharge* apparatus, number of discharge pores/ tubes, type of *zoospores discharge,* flagellation of the zoospore, possession of apophysis and morphology of rhizoidal system. The type of habitat and substrata from which each culture was originally isolated were analyzed. Identification and characterization of isolates were made with the help of Sparrow's `*Aquatic Phycomycetes*'[11] and Karling's `*Chytridiomycetarum Iconographia*'[13] and other relevant taxonomic literatures.

Results and Discussion

Taxonomic account of species: *Rhizidiomyces hirsutus*[14]: On 1/4YpSs agar: Thallus monocentric, eucarpic; sporangium *usually* spherical, variable in size, 40-130 µm in diameter, *multiple*, short, tubular rhizoidal main axis arising from the base or from several peripheral points of sporangium (figure 1. a and c.). On surface of mature sporangium wall with 2-18 or more hair-like appendages of up to some distance, which develop into an extrametrical well-developed, profusely branched rhizoidal system. Terminal branches were finely divided and sharply pointed at the tips (figure 1. a). Apophysis when present intra- or extramatrical and variable in size and shape. Zoosporangia with long exit tubes, usually 1-3 in numbers, elongate and tapering; the tubes contain numerous refractive globules and bodies of various sizes (figure 1. b and c). As the tubes elongated, they remain simple or branched once to several times and the tips of the broad branches often *enlarge* to form ovoid to subspherical swellings. The contents of zoosporangium emerge as an undifferentiated naked protoplasmic mass which develops a vesicular membrane and undergoes cleavage into zoospore rudiments (figure 1. d). The zoospores get librated by the rupture of vesicular membrane and get encysted to enlarge directly into the sporangium. Zoospores (4×8 µm) highly variable in shape; elliptical, oblong, oval, or spherical with several minute refractive globules and a stout anterior flagellum. Resting spores unknown. Color of colony white.

Specimens examined: Pond mud from Alfred Park, Allahabad, U.P., 23 June 2012. Isolated on shrimp chitin.

Distribution: India, Argentina, USA, Taiwan, Poland, New Zealand and Brazil

Mature sporangial wall with *multiple* elongated hair-like appendages is the main character of this species[15]. Many variations of thallus structure occur in axenic culture, which is the similar as described by Karling's[16]. This species is saprophytic in nature and occur mostly in ponds. It is one of the most widely distributed species of *Rhizidiomyces* and observed in most parts of the world.

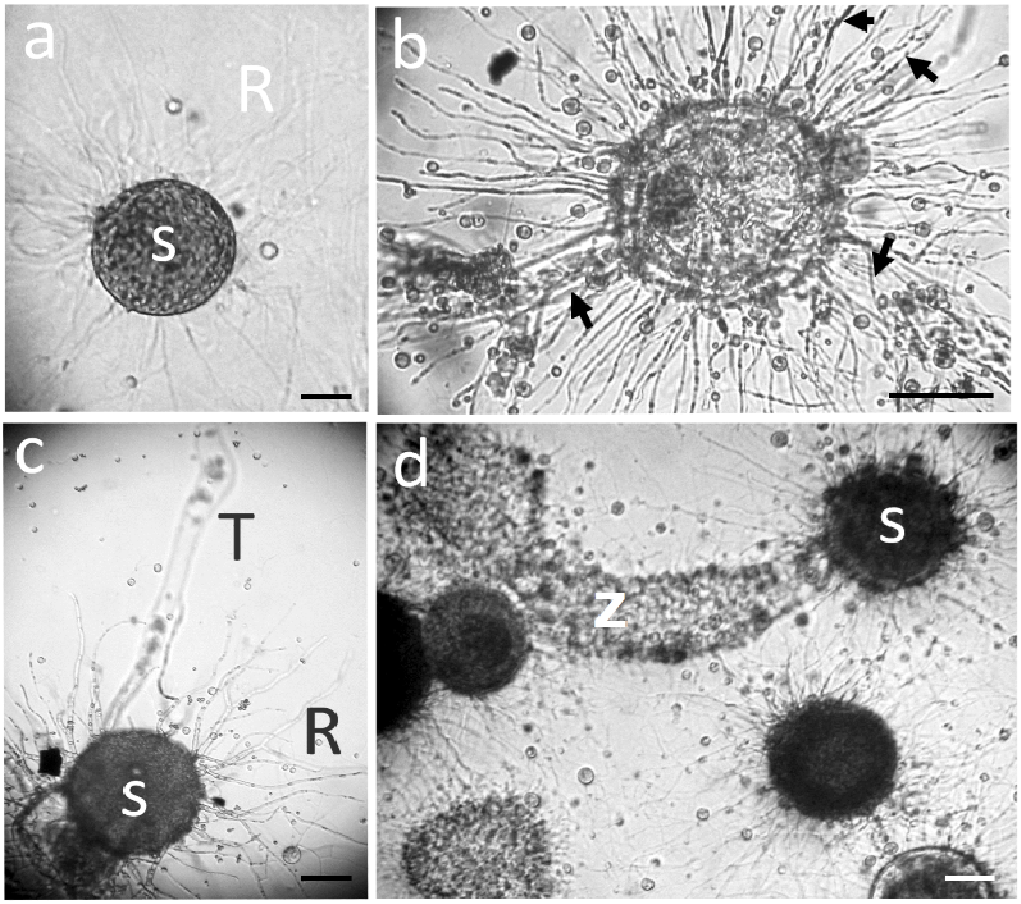

Figure-1

***Rhizidiomyces hirsutus* Karling. a. Maturing sporangium (S) with rhizoidal main axis (R) arising from the base or from several peripheral points of sporangium. b. Empty sporangia with 3 discharge tubes (arrows). c. Young sporangium (S) with long tapering discharge tube (T). d. Mature sporangium (S) with mass of quiescent spores (Z) liberated through the disruption of vesicular membrane. Bars = 20 µm for a, c and d; 50 µm for b**

Rhizidiomyces apophysatus[17]

On 1/4YpSs agar: Zoosporangia monocentric, eucarpic, gregarious, sessile, spherical when young but become oval or broadly pyriform, 20-60 µm, with a long tapering cylindrical discharge exit tube (figure 2. a); delimited from the intramatrical portion of the thallus by a cross wall at maturity. Intramatrical apophysis (3.5-5 µm) spherical to *ovoidal,* pyriform or broadly fusiform with extensively branched rhizoidal system arising from its base. The *first indication* of zoospore *discharge* from a *sporangium* was the appearance of clear papilla from the sporangium wall. When the tube reaches 5-15 µm in length, some of the protoplasm moves in to clear area and shows the movement of the protoplast from the sporangium into the vesicle, which results in the formation of wide single apical discharge pore at the apex of discharge tube (Fig. 2. a). On the completion of sporangium discharge, protoplast contents through the tube and the uncleaved protoplasm is all in the vesicle, similarly as described by Fuller[18]. This coenocytic, protoplasmic mass immediately cleaves to form swarming zoospore initials and discharged as a mass. After the discharge, the zoospores begin to move extrasporangial very slowly with a rocking motion. These movements of the zoospores increase and they swim away in all directions. Zoospores (3×6 µm) broadly elliptical and oblong with numerous small refractive globules. Resting spores unknown. Color of colony white.

Specimens examined: Pond mud from Alfred Park, Allahabad, U.P., 23 June 2012. Isolated on shrimp chitin.

Distribution: India, New Zealand, Argentina, Poland, USA and Brazil

Weekly parasitic on oogonia of *Saprolegnia ferax, S. asterophora*, and *Achyla polyandra* in Germany[17]; *A. mixta* in Switzerland[19]; *A. conspicua* in North Carolina[20,21,22,23,24] and New York[25], U.S.A.; *A. flagellata* in Japan[26] and U.S.A.[4]; and *A. klebsiana* in India[9]. Apparently, it is a facultative parasite which can be cultured readily on synthetic medium[22]. It also occurs on pollen grains when they are used to bait soil samples[27]. It is one of the most studied species of *Rhizidiomyces*.

Rhizidiomyces bulbosus[16]

On 1/4YpSs agar: Thallus epibiotic, monocentric, eucarpic, nonapophysate, spherical, 25–70 µm in diameter, with a hyaline smooth wall; usually developing 1 to 3 exit papillae at maturity. Rhizoids often irregular, ending bluntly, usually reduced and limited in extent, arising generally from the base of the sporangium, rarely from 2 to 3 places on the periphery. Exit papillae grow out and enlarge to become bulbous, subspherical, or broadly clavate, 8-20 µm in diam (figure 2. b).The bulbous exit papillae emerge from protoplasm which subsequently cleave into zoospores. The zoospore discharge as an initial burst and remains quiescent for 1-2 minutes. The released zoospores encysted at the apex of the exit papillae, where they developed in to sporangia forming heaped-up groups of sporangia on solid agar as described by Karling[16]. Zoospores small, hyaline with refractive globules, ovoid, 3-4.5 µm in length. Resting spores unknown. Color of colony white.

Specimens examined: Soil samples from Alfred Park, Allahabad, U.P., 25 July 2012. Isolated on snake skin.

Distribution: USA

Fairly smaller zoospores produced and development of 1 to 3, relatively short, bulbous, subspherical or broadly clavate exit papillae instead of long, tapering necks for the discharge of zoospores is the main character of this species[16]. Many variations of thallus structure occur in axenic culture. This species is saprophytic in nature and occurs mostly in soil.

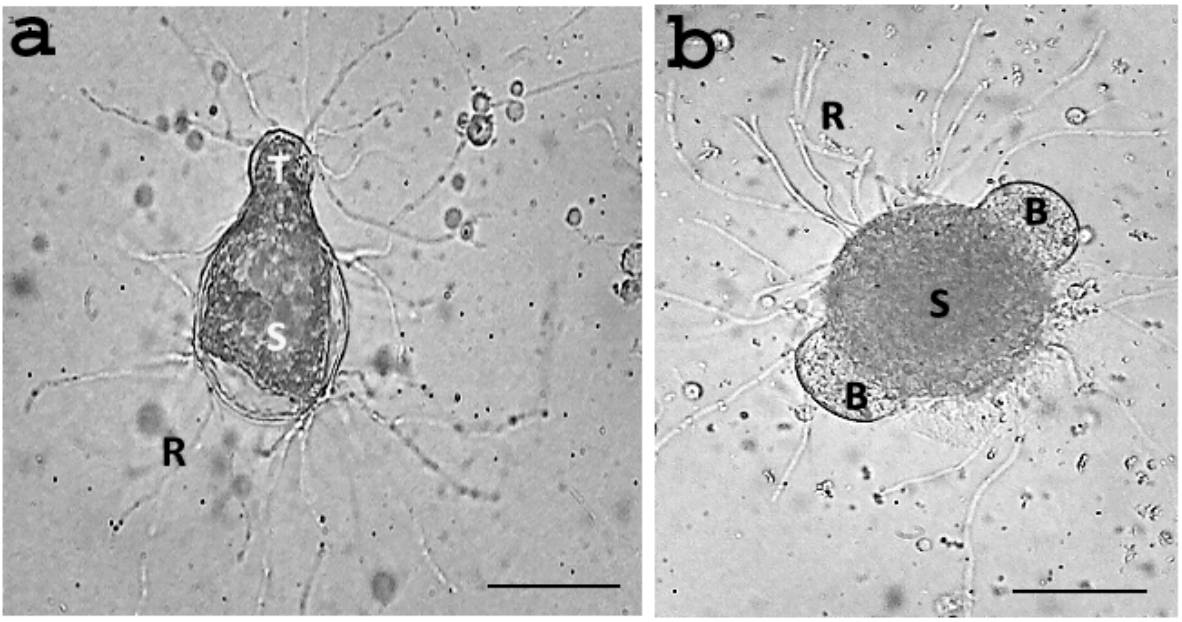

Figure-2

a. *Rhizidiomyces apophysatus* Zopf developing a long tapering cylindrical exit tube (T). b. *Rhizidiomyces bulbosus* Karling with two subspherical bulbous (B) Bars = 40 µm

Hyphochytriomycetes has been mentioned for the first time to India by Chaudhuri and Kochhar[8] and subsequently by Mundkur[9] and Karling[10], but despite being cosmopolitan and ubiquitous in its distribution, it never generated fascination among Indian scientists and researchers. Thus, the record of Hyphochytriomycetes of India is rather scanty as only six species have so far reported from India.

The results of the present study indicate that Hyphochytriomycetes are of fairly common occurrence in Indian aquatic and terrestrial habitats. *R. hirsutus* Karling, *R. apophysatus* Zopf and *R. bulbosus* Karling are isolated, described and illustrated for the first time on solid media in India. All of them are saprotrophic on broad range of substrate. The bulbous subspherical exit papillae with irregular rhizoids, possession of *fairly extensive rhizoidal system oriented on its base or sides* and unique pattern of discharge tubes formation are key characteristic features of *R. bulbosus* Karling, *R. hirsutus* Karling and *R. apophysatus* Zopf respectively.

Conclusion

The results of present study showed a taxonomic description and ecological/distributional data of three species of *Rhizidiomyces.* This study also provides a taxonomic key with figures for their identification using solely morphological and developmental characters on solid media. *R. bulbosus* Karling is mentioned for the first time in our country, being also the first record of it outside U.S.A. Thus, this investigation would certainly lead to better understanding of role, diversity and ecology of Hyphochytriomycetes of India.

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