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FIRST REPORT OF *PYTHIUM OSTRACODES* DRECHSLER FROM PAKISTAN

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Abstract

During a taxonomic study on Oomycetous fungi from Sindh province of Pakistan, a *Pythium* species was isolated from soil collected from guava field in Hyderabad district, Sindh, Pakistan. Morphologically this species resembled *P. ostracodes* which is re-described and illustrated herein. It appeared to be the first report of *P. ostracodes* from Pakistan.

Introduction

Pythium Pringsheim is a complex genus which belongs to the class *Oomycetes* (order *Pythiales*, family *Pythiaceae*) containing over 120 described species that occupy a variety of terrestrial and aquatic habitats (Plaats-Niterink, 1981; Dick, 1990). Depending upon the environmental factors, most of the *Pythium* species can live as saprophytes in soil, on plant debris or act as pathogens mostly of young succulent plant tissues such as fresh fine roots of seedlings (Hendrix & Campbell, 1973; Plaats-Niterink, 1981; Krober, 1985).

A fair amount of studies on *Pythium* species have been carried out in different countries of the world (Plaats-Niterink, 1981; Dick 1990), whereas, little work has been done on the *Pythium* species occurring in Pakistan (Mirza & Qureshi, 1978; Malik & Khan, 1944; Ahmed, 1956; Shahzad & Ghaffar, 1993; Abdul-Haq & Shahzad, 1998; Lodhi *et al*., 2004 a,b). During the present investigation on Oomycetous fungi of Sindh, *P. ostracodes* was isolated from soil of guava field in Hyderabad district, Sindh province, Pakistan. The culture has been deposited at the Karachi University Culture Collection (KUCC) as KUCC-OOP-03036.

Materials and Methods

Collection of samples: Soil samples were collected from guava field at Hyderabad. The samples were collected at random from a depth of 0-5 inches of soil. Samples from a field were mixed to obtain a composite sample.

Isolation of fungi: Isolation was done by baiting technique (Harvey, 1925). Soil sample kept in a polyethylene bag was moistened by the addition of sterile water and mixed thoroughly to get a paste like consistency. With the help of a sterilized teaspoon, the soil was placed at one side in each of the three sterilized Petri plates and approximately 15 ml sterilized water was added. Two grass blades (3 cm long) were placed in each Petri plate, one near the soil and the other away from the soil. The Petri plates were incubated at room temperature. After 5-8 days a faint halo of fungal threads was observed on the baits. The baits were rinsed in sterilized water to remove soil particles, and placed into fresh sterilized Petri plates half-filled with sterile water and new fresh baits were added. After

2 days of incubation, the baits colonized by Oomycetous fungi were transferred on the corn-meal agar (CMA) medium amended with 100 ppm PCNB for purification. Corn-meal Dextrose Agar (CMDA), Potato Dextrose Agar (PDA), Water Agar, Corn-meal Agar (CMA), and Potato-Carrot Agar were used for maintenance of pure culture and study of colony characteristics.

Preparation of water culture for identification: Water culture of the fungus was prepared by adding an inoculum disc and a grass blade to sterile water in a Petri plate and incubating at 25ºC. When the sporangia, zoospore and sexual structures were produced, the fungus was identified after reference to Sparrow (1960), Plaats-Niterink (1981) and Dick (1990).

Biometric values: Biometric values viz., aplerotic index, ooplast index and wall index were determined after Shahzad *et al*., (1990) using the following formulae:

Aplerotic index (%) =

$$100\left\{\frac{\Sigma\frac{\left(\frac{op_1}{2}\right)^3}{\left(\frac{os_1}{2}-ot_1\right)^3}\cdots\frac{\left(\frac{op_{20}}{2}\right)^3}{\left(\frac{os_{20}}{2}-ot_{20}\right)^3}}{20}\right\}$$

Wall index (%) =

$$100\left[1-\left\{\frac{\Sigma\frac{\left(\frac{os_1}{2}-ot_1\right)^3}{\left(\frac{os_1}{2}\right)^3}\cdots\frac{\left(\frac{os_{20}}{2}-ot_{20}\right)^3}{\left(\frac{os_{20}}{2}\right)^3}}{20}\right\}\right]$$

Ooplast index (%) =

$$100\left\{\frac{\Sigma\frac{\left(\frac{os_1}{2}\right)^3}{\left(\frac{og_1}{2}\right)^3}\cdots\frac{\left(\frac{os_{20}}{2}\right)^3}{\left(\frac{og_{20}}{2}\right)^3}}{20}\right\}$$

where os= oospore diameter, og= oogonium diameter, op= ooplast diameter, ot= oospore wall thickness.

Taxonomic Description

Morphological characteristics: Main hyphae upto 6 µm. Sporangia terminal, proliferating, sub-globose, 26x30-36x40 µm. Discharge tube upto 5 x 6 µm. Encysted zoospores upto 11 µm. Oogonia smooth, intercalary, unilaterally intercalary occasionally terminal, (20-)32-35(-37) (av. 31) µm diam. Antheridia 1-2 per oogonium, monoclinous, occasionally diclinous, laterally applied to the oogonium. Oospores smooth, plerotic or nearly plerotic, (19-)31-34(-36) (av. 29.4) µm diam. Ooplast not differentiated from the rest of the oospore contents. Oospore wall (2-)3-4(-5) (av. 3.4) µm thick (Fig. 1).

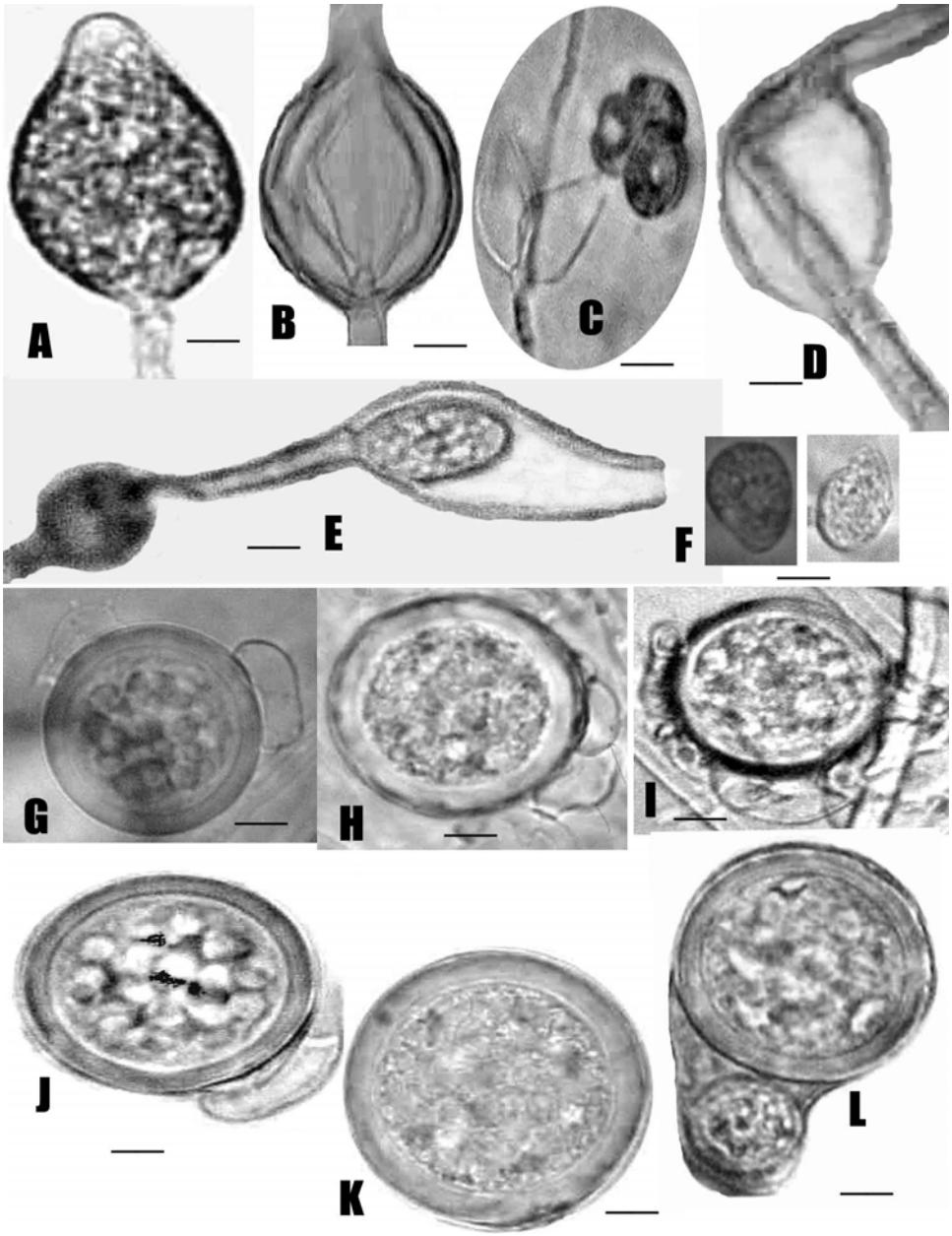

Fig. 1 Different morphological structures of *P. ostracodes.* A-E, Globose, proliferating, filled and empty sporangia; F, Zoospores; G-I, Smooth, globose oogonia with laterally applied antheridia with lateral attachment; K, Oogonia with plerotic oospore; L, bisporous oogonia.

Scale bar: A-B, D-F = 10µm; C = 20µm; G-L = 7µm.

Biometric values: Aplerotic index 86.6%, wall index 54.6%. The ooplast index was not determined because ooplast was not distinguishable from the rest of the oospore contents.

Colony characteristics: This isolate of *P. ostracodes* showed white aerial mycelium without any pattern on PDA and PCA, on CMA submerged growth without any pattern, on CMDA chrysanthemum pattern with superficial growth.

Daily growth rate at 25 ºC: 2.6 mm on CMA, 3.5 mm on PDA, 2.3 mm on PCA and 3.8 mm on CMDA.

Discussion

There are four species *viz., P. ostracodes, P. oedochilum, P. palingenes* and *P. helicoides* which are characterized by having proliferating sporangia, thick-walled large oospores and large antheridia applied laterally to the oogonia. Our isolate of *P. ostracodes* differs from *P. oedochilum* and *P. palingenes* by its mostly monoclinous antheridia and plerotic or nearly plerotic oospores. It differs from *P. helicoides* by its antheridia which do not entwine the oogonial stalk. Our isolate of *P. ostracodes* differs from the description given by Plaats-Niterink (1981) where the reported oogonial diameter [(14-) 35-38 (-43) (av. 35) µm] is slightly greater than the diameter of our isolate [(20-) 32-35 (-37) (av. 31) µm]. All other characters are more or less the same as described by Plaats-Niterink (1981). Therefore, the present isolate has been identified as *P. ostracodes. P. ostracodes* was first originally isolated from wheat in Texas (Drechsler, 1943). Takahashi *et al*., (1965) isolated *P. ostracodes* from rhizomes of lotus in Japan. No previous record of *P. ostracodes* from Pakistan is available (Mirza & Qureshi, 1978; Malik & Khan, 1944; Ahmed, 1956; Shahzad & Ghaffar, 1993; Abdul-Haq & Shahzad, 1998; Lodhi *et al*., 2004 a,b). This, therefore, appears to be the first record of *P. ostracodes* from Pakistan.

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