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# **RE-DESCRIPTION OF PYTHIUM ADHAERENS SPARROW**

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### Abstract

A *Pythium* species with filamentous non-inflated sporangia, smooth oogonia of moderate size, aplerotic oospores and diclinous antheridia was isolated and identified from Pakistan. This species very closely resembles *P. adhaerens* Sparrow. The species is re-described and illustrated.

# Introduction

*Pythium adhaerens* was first isolated and described by Sparrow (1931) from green alga *Rhizoclonium hieroglyphicum* in USA. Liu (1977) also reported this species from sugarcane in Malaysia. *P. adhaerens* was found to be pathogenic on sugar beet, maize, pea, tomato and cucumber (Sparrow, 1932). Plaats-Niterink (1981) recognized it as a valid taxon in her monograph of the genus *Pythium* and stated that 'no material available' for studying the organism. Plaats-Niterink fully depended on the description given by Sparrow (1931).

During a taxonomic study on Oomycetous fungi from Sindh province of Pakistan, a *Pythium* species was isolated from soil collected from onion field at Aderolal, District Sanghar, Sindh, Pakistan. Morphologically this species resembled *P. adhaerens* with few differences. *P. adhaerens* is, therefore, re-described and illustrated herein.

## **Materials and Methods**

Soil and water samples were collected at random from a depth of 0-5 inches from onion fields at Aderolal, district Sanghar in Sindh province of Pakistan. Samples from a field were mixed to obtain a composite sample. Oomycetous fungi were isolated using 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 sterilized teaspoon, the soil was placed at one side in each of three sterilized Petri plates and 10-15 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. Dicot leaves, hemp seeds, sesame seeds and young cucumber stems were also used as baits. The Petri plates were incubated at room temperature. After 5-8 days, when a faint halo of fungal threads were observed on the baits, the baits were rinsed in sterilized water to remove soil particles, 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 for purification.

Water culture of fungi was prepared by adding a 1 cm<sup>2</sup> inoculum block and grass blades to sterile water in a Petri plate and incubating it at 25°C for the production of sporangia, zoospore and sexual structures. Identification up to species level was made after reference to Sparrow (1960) Plaats-Niterink (1981) and Dick (1990).

Growth pattern and radial growth rate were observed on CMA, potato carrot agar (PCA), potato dextrose agar (PDA) (Plaats-Niterink, 1981) and CMA containing 20 g Dextrose  $L^{-1}$  (CMDA) at 25 °C.

Biometrics values viz., aplerotic index, ooplast index and wall index were determined after Shahzad *et al.*, (1990). The culture has been deposited at the Karachi University Culture Collection (KUCC) as KUCC-OOP-03009.

#### **Re-Description of** Pythium adhaerens Sparrow

(Figs. 1 and 2)

This isolate of *P. adhaerens* showed submerged colony growth with a very light chrysanthemum pattern on PCA, CMA and PDA but a prominent chrysanthemum pattern with superficial mycelial growth was observed on CMDA. Sporangia were filamentous, non-inflated, not differentiated till the formation of discharge tube and vesicle. Abundant zoospore discharge occurs in the liquid culture after washing followed by cold shock. The diameter of zoospores cyst was 8-11 (av. 9.5)  $\mu$ m. Discharge tubes up to 100  $\mu$ m in length. Oogonia smooth, globose, mostly intercalary, rarely terminal, (15-) 20-23 (-26)  $\mu$ m diam. Oospores are extremely aplerotic, (13-) 16-19 (-22)  $\mu$ m in diam. Antheridia 1-5 per oogonium, mostly diclinous rarely monoclinous, branched, occasionally intercalary and making narrow apical contact with the oogonium. Sometimes more than one antheridial stalk originate from the one-parent hyphae and encircle the oogonium. Aplerotic index 54%, wall index 40.5% and ooplast index was 29%. Oospores wall thin, 1-2 (av. 1.5)  $\mu$ m. Daily growth rate 25 mm on CMA, 27 mm on PDA, 28 mm on PCA and 24 mm on CMDA at 25 °C.

### Discussion

The group of *Pythium* species which have filamentous non-inflated sporangia, smooth intercalary oogonia of moderate size, aplerotic oospores and diclinous and monoclinous antheridia includes *P. adhaerens, P. angustatum* Sparrow, *P. diclinum* Tokunaga, *P. capillosum* Paul and *P. capillosum* var. *helicoides* Paul. *P. angustatum* has monoclinous antheridia mostly making broad apical contact with the oogonium, sac like oogonia, occasionally with 2-4 oospores per oogonium. These characters make it different from our isolate. Similarly, mostly terminal oogonia, less number of antheridia per oogonium i.e. 1-2, un-branched antheridial stalk and thick walled oospores of *P. diclinum* also make it distinct from our isolate. *P. capillosum* and *P. capillosum* var. *helicoides* were characterized by occasionally papillate oogonia, frequent encircling of oogonia by antheridia in a way to running along the oogonial wall through most of the oogonial surface or completely encircling it, or frequent entwining of oogonial stalk by antheridia. These characters clearly differentiate these organisms from our isolate.

According to the original description of *P. adhaerens* given by Sparrow (1931) the oogonia were terminal or intercalary, 11-25 (av. 17.5)  $\mu$ m in diam., antheridia 1-4 per oogonium, born on a single stalk or rarely on distinct stalks, diclinous; Oospores aplerotic, 7-22 (av. 14.5)  $\mu$ m in diam., wall 2-2.5  $\mu$ m thick. However, in our isolate the intercalary oogonia are abundant rarely terminal with diameter of (15-) 20-23 (-26)  $\mu$ m. Antheridia were mostly diclinous but occasionally monoclinous, antheridial branches 1-5 in numbers. These morphological characters more or less coincide with the characters reported by Sparrow (1931) and it is difficult to designate it as a new species. Therefore, the present isolate has been identified as *P. adhaerens*.



Fig. 1 Sporangia and zoospores of *P. adhaerens*. A & B, Sporangia with discharge tube and vesicle; C, zoospores in a vesicle; D, zoospores dispersal; E & F, encysted and germinating zoospores. Scale bar: A, B, D,  $E = 30 \mu m$ ; C &  $F = 10 \mu m$ 



Fig. 2. Sexual structures of *P. adhaerens*. A & B, intercalary oogonia; C, aplerotic oospores; D, bisporous oogonia; D & F, diclinous antheridia making narrow apical contact; G & H, encircling of the oogonium by antheridial stalk.

Scale bar: A = 50  $\mu$ m; B, F & G = 20  $\mu$ m; C, D & H = 10  $\mu$ m; E = 25  $\mu$ m

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# 456