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ISOLATION AND IDENTIFICATION OF *PENICILLIUM* SPP., FROM THE RIVER INDUS BED AT KOTRI

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Abstract

The mycoflora from the bed of river Indus at Kotri was investigated at three locations viz., Right Bank, Center and Left Bank from June 2004 to May 2005. Twenty four soil samples were collected from surface, 10, 20, and 30 cm depth. The fungi were isolated by using soil dilution and soil plate method. Of the 73 strains of fungi isolated 10 species of *Penicillium* viz., *P. caesicolum* (1.81%), *P. commune* (1.81%), *P. chrysogenum* (14.73%), *P. funiculosum* (28.36%), *P. lilacinum* (4.33%), *P.notatum* (12.53%), *P. sclerotiorum* (2.52%), *P. tardum* (26.47%), *P. vinaceum* (5.51%) *and P. roseo-purpureum* (1.89%) were identified. Greater number of species were isolated on soil plate method than on dilution plate method. Higher number of species were recovered from left bank as compared to right bank while in center isolates were in low frequency.

Introduction

Soil is a complex and dynamic environment in which the biological activity is mostly governed by microorganisms. The beneficial effects of soil microorganisms are manifold and range from nitrogen fixation and organic matter decomposition to breakdown of metabolic by-products and agrochemical, enhancing the bioavailability of nitrates, sulphates, phosphates and essential metals (Bridge & Spooner, 2001). Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth & Bisby, 1995) The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits, e.g., the isolation and identification of the soil fungus *Penicillium* led to a large pharmaceutical industry of antibiotics (Diana, 1994). It is estimated that there are 1.5 million fungal species on earth, of which only about 70,000 have been described up to now (Hawksworth & Rossman, 1997). The present research is an attempt to study the mycoflora diversity from various depths at three locations of river Indus at Kotri. Apparently no report is available for fungi recorded from this site (Sultan *et al*., 1997). This paper concentrates mainly on species of *Penicillium.*

Description of the research area: The study area is located at longitude 68.°22'E, latitude 25.°22'N. Air temperature ranges between 9.3°C to 40.4°C. There are significant extremes of rainfall in the basin. According to Climatic Normals of Pakistan (1961-90) the mean annual rainfall over the Indus plains at kotri is 178 mm. The rainy months are late June through September and the driest months are November through March, when the average monthly rainfall rarely exceeds 30 mm. The soil texture ranges from coarse to fine with 85% in the moderately fine categories, mostly suitable for irrigated agriculture. The medium size of soil gradually reduces downstream so that heavier texture is more common. The pH value generally ranges from 8 to 8.50. Most of the vegetation in the drainage basin and the alluvial valley consists of thorn-scrub forests and

desert with the exception of the northern part of the basin, which is dominated by alpine steppe vegetation (Coleman, 2004).

Material and Method

The soil samples assayed in this investigation were obtained from three sites viz Right Bank, Left Bank and Center of the bed of River Indus. Vertical samples were taken from surface, 10, 20 and 30cm depths with disinfected screw-cap vials. Vials were applied perpendicularly to the vertical surface of the profile. Two samples were collected at each depth. The samples were stored in sterilized polythylene bags until they reach the laboratory. The samples were processed using the soil dilution plate (Waksman, 1922) and soil plate method (Warcup, 1950).

Soil dilution plate Method: The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.5ml volumes were pippeted onto Potato Dextrose Agar and incubated at 26°C for three days.

Soil Plate Method: About 0.005 g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (45°C) agar medium (PDA) was added, which was then rotated gently to disperse the soil particles in medium. The plates were then incubated at 26°C for three days.

Fungal colonies were counted and screening for *Penicillium* species was made from mixed isolates and subcultured on MEA. Sub culturing was continued until a pure isolate was obtained. Identification was performed according to Raper & Thom (1949) and Gilman (1945).

Result and Discussion

A total of 10 species were obtained from 24 soil samples. From the three sites, Right bank, Center and Left bank, a total of 1269 colonies of *Penicillium* spp., were isolated of which 696 (approximately 55%) consisted of *Penicillium funiculosum* (28.37) and *P. tardum* (26.42). *P. chrysogenum* (14.74%) and *P. notatum* (12.52%) were recovered in a moderate frequency followed by *P. caseicolum* (1.82%) and *P. commune* (1.82%), *P. roseo-purpurum* (1.89%), *P. sclerotiorum* (2.52%), *P. lilacinum* (4.33%), *P. vinaceum* (5.25%) (Table 1).

Rate of occurrence of species isolated in terms of percentage from each of the three sites is given in table 1

Name of isolates	Right bank	Center	Left bank
Penicillium caseicolum	1.58	-----	0.24
Penicillium commune	0.16	0.08	1.58
Penicillium chrysogenum	2.84	2.44	9.46
Penicillium funiculosum	4.33	2.76	21.28
Penicillium lilacinum	1.49	0.95	1.89
Penicillium notatum	3.62	2.60	6.30
Penicillium roseo-purpurum	0.08	0.79	1.02
Penicillium sclerotiorum	0.79	-----	1.73
Penicillium tardum	22.54	0.95	2.99
Penicillium vinaceum	1.42	1.26	2.84

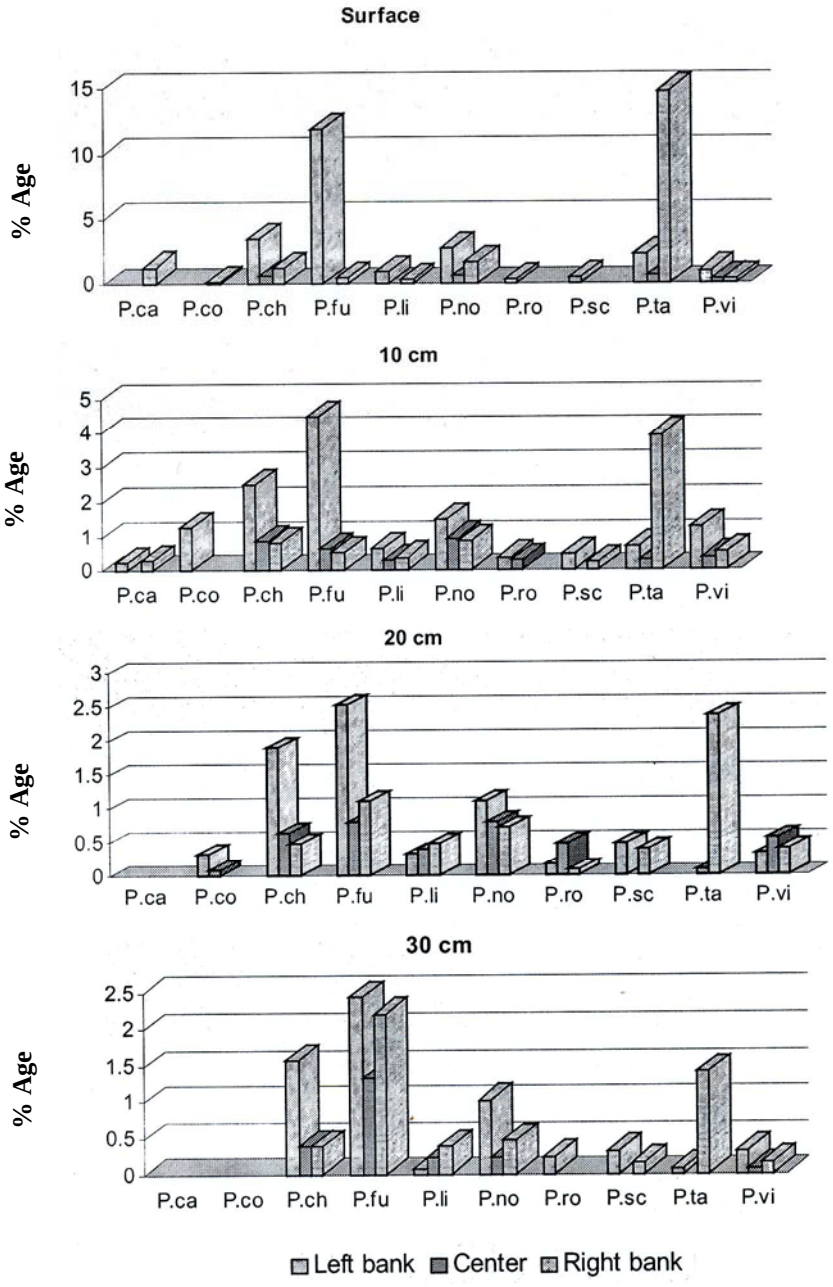

Fig. 1. Distribution and frequency of isolation of *Penicillium* sp., at different depth.

P.ca =*P. caseicolum*, P. co =*P. commune*, P. ch =*P. chrysogenum*, P. fu = *P. funiculosum*, P. li = *P. lilacinum*, P. no =*P. notatum*, P. ro =*P. roseo-purpurum*, P. sc = *P. sclerotiorum*, P. ta = *P. tardum*, P. vi = *P. vinaceum*.

From the samples of Right bank, *P. tardum* was the predominant fungus isolated and accounted for 22.54% of the total fungi isolated from right bank whereas from samples of left bank, *P. funiculosum,* was the predominant fungus and accounted for 21.28%. No one fungus predominated from the center part of the bed; instead of *P. notatum* (2.60), *P. chrysogenum* (2.44) and *P. funiculosum* (2.76) isolated with approximately equal frequency. *P. scleroitorum* and *P. caseicolum* were at isolated from this site.

The study revealed that the total number of species isolated decreased with increased sampling depth. A greater number of species and colonies were isolated on soil plates than on dilution plates and were recovered from left bank (49.33%) where the soil is rich in organic matter and vegetation as compared to right bank (38.85%) while in center, isolates were lowest (11.83) in frequency and variation.

No previous report on the prevalence and distribution of this genus in the soil of River Indus is found. This preliminary survey reflects the high prevalence of soil fungi in bed of river Indus. A detailed survey covering many more samples to ascertain the distribution of soil fungi in river Indus with particular emphasis on the ecological factors determining the prevalence in various soil types and habitats is essential.

References

Ainsworth, G.C. and G.R. Bisby. 1995. *Dictionary of the Fungi eight edition.* Commonwealth Mycological Institute Kew, Surrey pp 445.

Bridge, P., and B.Spooner. 2001. *Soil fungi: diversity and detection. Plant Soil*, 232: 147-154.

Coleman, J. 2004. *Indus River Delta, Pakistan, Asia*. In drainage basin of the Indus River system World Delta 15.

Diana, W.F. 1994. *Soil biodiversity: its importance to ecosystem processes*. Report of a workshop held at the natural history museum, London, England.

Gilman, J.C. 1957. *A manual of soil fungi*. The Iowa State University Press. Iowa USA.

Hawksworth, D.L. and A.Y. Rossman. 1997. Where are all the undescribed fungi? *Phytopathology*, 87: 888-891.

Raper, K.B. and C. Thom. 1949. *Manual of Penicillia.* Williams and Wilkins Co. Balitimore, USA.

Sultan, A., S.H. Iqbal and A.N. Khalid. 1997. *Fungi of Pakistan.* Dept of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore Pakistan. 248pp.

Waksman S.A. 1922. *A method of counting the number of fungi in the soil. J. Bact*., 7. 339-341.

Warcup, J.H. 1950. *The soil plate method for isolation of fungi from soil. Nature*, 166: 117-118.

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