
Collection and Identification of Micromycetes from Rawalakot Azad Kashmir, Pakistan

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Abstract A study to find out the most promising Micromycetes of Azad Jammu Kashmir were carried out and reported the presence of eight species from soil, air and roots of plants on MEA, PDA, CZAPek Dox Agar. Reported species were as follows:- *Penicillium aethiopicum*, *P. atramentosum*, *Nomuraea viridulus*, *Paecilomyces herbarum*, *P. varioti*, *Mattirolella crustosa*, *Byssoschlamys nivea* and *B. fulva*.

Keywords: Micromycetes, Azad Kashmir, Biotrophs, Exenic cultures, Sporulation, Permanent slide.

Introduction

Fungi are ubiquitous and important because of their presence in all climates and on all substrates. Most of them can be easily cultured though some require special media to grow but the Biotrophs fail to grow axenic cultures. For proper growth and Sporulation of a fungus specific requirements (nutrients, temperature, moisture, light and relative humidity etc.) of a fungus are to be fulfilled (Akar *et al.*, 2006). Few serious and systemic efforts have been made to survey Aspergilli of world. Many species of *Aspergillus* were investigated for the first time (Raper and Thom, 1949). Gardezi (1998) reported thirty four species of fungi from Azad jammu and Kashmir, Pakistan. Gardezi *et al.* (1998) reported two species of *Aspergillus flavus* and *Metarrhizium anisoplia* from Azad jammu and Kashmir Pakistan. Gardezi (2006) reported forty species of fungi from Azad Kashmir Pakistan.

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Materials and methods

Sample collected (1994-1998) from different localities of Azad Jammu and Kashmir in separate sterile cellophane or paper bags were placed in a refrigerator and cultured on agar media as early as possible. Different types of media were prepared for isolation and sporulation. Specimens of diseased plant material if not sporulating were incubated in a moist chamber to induce sporulation before isolation. Diseased pieces of roots, stems and rotten fruits were surface sterilized with 10% Sodium hypochlorite (NaClO) for 1-3 minutes, washed in sterile water and placed in Petri dishes on sterilized agar media. These specimens were incubated at about 26 °C. Permanent slides of Micromycetes were prepared by cleaning and staining techniques (Bruzzese and Hasan, 1983).

Isolation from soil were made by direct soil planting methods (Mirza *et al.*, 1979) and incubated in dark at 26 °C for 48 to 72 hours. After 24 hours of incubation, fungal colonies start growing. Inoculum from margin of developing young colonies was transferred to fresh plates for purification and further cultural studies. All pure cultures were deposited in department of Plant Pathology, The University of Poonch, Rawalakot.

Results and discussions

Penicillium eathiopicum Frisvad, Mycologia (6) 837-861, 1989.

Colonies on MEA at 24 °C growing rapidly, 25-30 mm in 7 days and 50-65 mm in 14 days, deeply floccose up to 5 mm after 7 days and often touching the side Petri dishes after 14 days, later collapsing and becoming somewhat funiculate with fairly abrupt, even circular margin without becoming pale pink, drops sparse tiny, colorless or lacking reverse at first, then gradually turning pale yellow from center to outwards, sometimes shading in pale orange or buff conidial structures which is very simple consisting of single phialides or whorl of phialides occurring irregularly along aerial hyphae, phialides or whorl of phialides conidiophores when present 7-15 µm long and 1.5-2 µm, at base tapering to a long tube 0.5 µm diameter, usually bent sharply, conidia cylindrical with rounded or pointed end 3-4 × 1-2 µm, in chains which are twisted but seldom tangled whit mostly 40 × 50 µm long occasionally up to 90 µm. Isolates of this species have been identified as *P. viridicatum* *P. crustosum*, *P. chrysogenum*, *P. expansum* and *P. verrucosum* var. *corymbiferum*. This species was reported from tropical soil (Frisvad and Filtenborg, 1989) cereals (Denmark) having rough stipe's on MEA and first time from cereals of Rawalakot Azad Jammu & Kashmir, Pakistan.

***Penicillium atramentosum* Thom. Bull.**

Colonies on Czapek Dox Agar Media 24 °C growing moderately well 25-30 mm, in 7 days and 47 -68 mm in 14 days, 2-4 mm deep forming at first a loosely floccose, cushion becoming more densely matted and eventually funiculose with maximum branches and phialides from lower nodes, conidiophores arising from aerial hyphae 15 – 45 µm long or from the mycelium close to substrate and then longer up to 400 µm, smooth septate, 7-2 µm in diameter, phialides whorls of up to 12 – 15 µm long almost cylindrical basal portion 1.2 – 2 µm diameter and long tip 5- 7 µm diameter conidia cylindrical to fusiform 4- 6 x 1 – 1.5 µm in length almost straight chains, which seldom become tangled abortive heads are common. Especially in stack cultures, consisting of swollen curled and twisted hyphae in place of normal phialides or faint and sweet reverse white at first becoming clear pale yellow or yellow patches of greenish yellow finely zoned with grayish violet blue grey, conidial structures varying from single phialides to whorls with a treatment whorls of phialides and with a few or gradually turning white at first becoming very pale blue gray drops nil. *P. atramentosum* is quite common in soil of Netherland, U. K. and found in red pepper, hay cucumber, carrot, spoiled bread (Denmark) and camembert cheese Frisvad and Filtenborg (1989). *P. atramentosum* is similar to *P. chrysogenum* microscopically but different from latter, more common species by good growth on MEA, dark conidia, good relationship with *P. excalicum*, by Raper and Thom (1949).

***Nomuraea viridulus* Tzean. Hsieb Cheng et Wu. Mycologia 84: 781- 786, 1992.**

Colonies on MEA growing slowly ,18-20 mm in 14 days consisting of atough matted basal felt with a thin floccose at first Pure white, eventually developing a pale pink tinged, sometimes with a green tint showing through from the reversed, drops large, sparse, colourless to faint yellow or lacking, reverse at first white becoming olive green to dark green, often more or less zoned and shading to white or yellow at margin with colour not diffusing, conidial structures elongated to compact varying in complexity from single detached phialides to ends with at terminal whorls of phialides and whorls of branches and phialides from lower nodes. Conidiophores fluxuous bearing irregular verticals and metulae, appressed whorls of phialides arising from aerial hyphae fairly short or from the basal felt and then up to 150 X 1-2 µm diameter, often swollen at the tip to 3-5 µm septate, smooth, phialides 12-16 X 4

µm long with basal portion cylindrical 1.5-2.5 µm diam, tapering at at just over half to long to a narrow tube 0.5-1 µm, .Conidia broadly elliptical finally roxg zoned 3-4 x 2-3 µm hyaline under the microscope but very pale pink in mass in chains which are usually less than 100 µm long divergent, straight or indulate, seldom tangled and complex.

The distinguishing characters of *N. viridulus* consist of erect fluxuous bearing irregular verticals and metulae, appressed whorls of phialides, phialides without collula and huge cylindrical, ellipsoidal, usually slightly curved or allantoid conidia Tzean *et al.* (1992) .

***Paecilomyces herbarum* Agnes, Brown and Smith. Trans . Brit. Mycol. Sco.40:17-89, 1957.**

Colonies on Czapek, agar broadly spreading with medium rate of growth, slow growing 7mm diameter, in 7 days, thin, wet, transparent, not showing any sign of sporing, after 2 dirregularly branched, verticillate, 2µm diam, phialides verticals 1-2µm diameter, at base 2-2.6 µm diameter, rough toward the base. Conidia irregularly ellipsoid, frequently, roughened 6-14µm. In its natural habitat on old stems of herbaceous plant, forms a thin funiculose mycelium close to the substrate with funiculose aerial tufts, sporing structure very similar to those produced on PDA but phialides occasionally longer up to 20 µm. Conidia germinate very slowly. Spores from a three months old culture on PDA source directly on the surface of fresh PDA, showed no sign of germination or even erswelling after 24 hours. After 24 hours most of the conidia had enlarged slightly in width and a high percentage had produced germ tube, even after 24 hours some of the conidia were just showing first signs of germination. The germ tubes are formed from any part of the surface of the spores, but most of polar. Spores produced two germ tubes each, by germinating successively Agnes *et al.* (1957).

Paecilomyces varioti Bainier. Saccardo, Sylloge 20, 1911, Sylloge 22, 1913. Colonies on Czapek growing moderately rapidly, becoming 10-20mm, deep, consisting of a dense matted basal felt with floccose funiculose overgrowth, with margin either narrow and abrupt or broad, ochraceous, shading through pale yellow to white at the margin, becoming deeper in colour. Odour nill after 6-7 days sweet aromatic, fruiting bodies variable, ranging from solitary phialides to complex heads with several stages of branching usually irregular, consisting whorls of phialides mixed with secondary branches, conidiophores arising from aerial hyphae often lacking 60 – 70 x 2-7 µm, phialides up to 7 in whorl cylindrical basal portion, tapering, long slender tube 2 µm, in diam but sharply, variable 7- 16 µm x 1-2 µm in diam at the broadest part. Conidia smooth, ellipsoid to cylindrical 2-6 µm x 1-3 µm.

This species is common and cosmopolitan in occurrence on a wide variety of substrate, occasional strains, comparatively restricted or remain very pale colored, Agnes *et al.* (1957). *P. varioti* has closely related species are *P. divaricatum*, *P. mandshuricum*, *Corollium dermatophagum* and *Eidamia catenulate* described by Raper and Thom (1949) who had seen it in situ isolates.

Mattirolella crustosa Thorni and Kimbrough Mycologia 74: 242 -249, 1982. Colonies on Czapek, s agar at 24 °C growing fairly rapidly, 20 -23 mm, in 7 days and 40- 45mm, in 14 days varying in depth from 2mm to the limits of the Petri dishes, loose floccose becoming denser in age, white, tinged with yellow in center, drops nil, odour nil, reserve white with a central spot of clear yellow, sporing structure simple, consisting of single phialides borne directly on aerial hyphae, or of loose branching systems which are seldom recognizably pectinate, conidiophores 30- 40 µm, long and 1-2 µm, diam. Slightly swollen at apex, smooth, septate, phialides very variable, sometimes of the *Paecilomyces* type, tapering gradually from base to apex, 10 -19 µm x 1-1.6 µm, at widest part, conidia variable, subglobose, elliptical, or cylindrical 2- 3.2 µm x 1.6 -2 µm, in short chains, but with the spores lying broad side on to one another, or more or less diagonal or slanting down completely to form irregular spores ball. Colonies on malt agar similar but growing somewhat less dense, spores mostly irregular masses, rarely in chains.

This species seem to form a link between *Paecilomyces* and *Cordyceps* since the phialides may be typical of genus and the species may be either in chains or in balls. *M. crustosa*, composed basal layer of sporodochium entirely of haustorial mother cells, Khan and Kimbrough (1974). Thorne and Kimbrough (1982) saw *M. crustosa* (Thax.) Khan and Kimbrough, attached to the exoskeleton of host termites. Colla (1929) described originally that *M. sylvestris* penetrated in single layer of exoskeleton cells of *Rhinotermes marginalis* (L) inducing hypertrophy in epidermis.

***Byssochlamys ninea* Westling 1909 . p. 134 Taf. 4**

Colonies on PDA spreading rapidly, covering a Petri dish in 7 days, consisting at first of a loosely floccose to funiculose mat, becoming a densely matted felt with some loosely funiculose tufts, dying down in age, appearing almost granular with margin thin arachnoid during the growing period, white to fast off -white, drops lacking at first, then faintly mouldy, reverse white becoming faintly yellowish, conidial structures sparse, but when present similar to those on malt agar, sporodochium numerous, conglomerate, globose to subglobose, 7.5-10.6 µm x 6.5- 8.5 µm. This species is reported from the soil of Rawalakot on 7.8.1998. Westling (1909) isolated it from the specimens of the *Geastrum* and described it as perfect stage of *Paecilomyces* (Agnes *et al.*,

1957) Bainier (1907) emphasized on *Paecilomyces*, *Aspergillus*, *penicillium*, relationship and their distinct features.

***Byssochlamys fulva* Olliner et. Smit 1933, P197, Pl. 602.**

Colonies on Malt agar at 30°C similar to colonies of Czapek, s Dox Agar Medium but growing rather more rapidly and somewhat deeply floccose when young, conidial structures usually evident, the more complex ones consisting of several whorls of phialides, or mixed phialides and branches borne at successive nodes along the conidiophores, but all types produce from these comparatively complex heads to single phialides borne directly on trailing hyphae, conidiophores when present 16- 300 µm long and 2-4 µm diam., somewhat branched, phialides with swollen base and tapering at about ½ the length to a long thin tube about ½ µm in diam, very variable in length 8-19 µm and 1.6- 3 µm diam. At the broadest part conidia smooth, broad ellipsoid, often with one end slightly flattened 9.8 µm x 2.4 - 6 µm. This species reported from Rawalakot on 7. 8. 1998. This species was described by Olliver and Rendle (1934). Hull (1939) fairly common organism in orchard soil of England and serious problem in fruit preserving industry recorded. According Raper and Thom (1949) is not reported from any country except as *B .musticola* from USSR.

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