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Colonial and Morphological Characteristics of various fungi Species Isolated from soil in Bangalore city

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ABSTRACT
Fungi play an important role in environment and human welfare. For instance, some fungi are edible, some produce antibiotics and they are used in a wide range of industries. Fifteen cultures were examined for characters of morphology. The variables used in this study were macromorphological characters: colony diameter, obverse and reverse colony colour, and the presence or absence of exudates. Moreover, microscopic characteristics of the fungal isolates were examined using an optical microscope. The colony appearance of each fungal isolate was characterized on different agar media: CYA, YES, and MEA. Based on colony macromorphology, as well as the structure of conidiophores, it was revealed that fungal isolates obtained from YES and CYA belong to Penicillium sp and Penicillium crustosum have longest conidia.
Key words: *Cladosporium cladosporioides, Penicillium sp, Penicillium crustosum, Soil.*

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INTRODUCTION

Fungi are the most important microorganisms for the decomposition of organic matter due to their degradation abilities. When large amounts of extracellular enzymes, essential for the degradation of substrates, such as lignocellulose, which are produced the decomposition of this organic matter can occur [1]. Fungi are important eukaryotic microorganisms that affect humans being and the majority of living in different ways. Soil fungi play an important role in the degradation and decomposition of organic debris [2]. In adding up, fungi are used in industrial and food fermentation and they exist commonly in different types of soils, indoor and outdoor air, food, agro wastes and water.

Therefore fungi are found almost everywhere. Somewhere else molecular, biochemical and physiological methods are important for the systematic of these species, morphological properties are commonly used for identification [3]. A number of species belonging to these genera have been isolated and identified in this study were carried out in India.

Petri dishes were first examined under a dissecting microscope and then under a high resolution light microscope to determine the colonial features and the morphological structures of the fungi. During determination of the morphological structures, a modified mounting medium, Lacto-Cotton Blue, as proposed [4] was used.

Soil as a basin for a wide variety of filamentous fungi has been recognized for a long time. The shapes of fungal colonies exhibit salient diversity depending on the substrate conditions as well as on the fungal species. Although the shapes and the surface textures of colonies provide useful information to determine the species or to monitor the state of growth, colony patterning looks to be highly sensitive to the environmental factors.

This research explores the colony morphology of *Cladosporium cladosporioides, Penicillium crustosum* and *Penicillium sp* based on a morphology diagram that was made by a colony model. The aim of the present study is to determine of colony morphology potential of *Cladosporium cladosporioides, Penicillium crustosum* and *Penicillium sp* microfungi isolated from soil in Karnataka State (India). Fungal cultures were isolated, purified, and classified based on characters of colony morphology and microscopic features.

MATERIALS AND METHOD

Soil sampling

Samples of soil were collected from six different sites within three areas in Karnataka state of india: Banashankari (3 sites), Herbal (2 sites), and Medawar (1 sites). At each site, samples of soil were taken at depths of 10-40 cm, packed into labeled sterilized polyethylene bags using a sterilized spatula, and stored at 4 °C until examination for further examination.

Fungi isolation

The fungi were originally isolated by plating collected samples on Potato Dextrose Agar (PDA) during 7 days. Individual colonies of filamentous fungi were picked up and purified by streaking on agar medium. The fungal isolates were kept on agar medium at 4°C. Petri dishes were first examined under a high resolution light microscope to determine the colonial features and the morphological structures of the fungi. During determination of the morphological structures, a modified mounting medium, Lacto-Cotton Blue as described [4] and confirmed by National Fungal Culture Collection of India (NFCCI), Pune.

Morphological examinations of the fungi examined are listed in Table 1. The fungi were grown for 7 day as three-point inoculations on Czapek agar (CYA) Yeast extract: 0.5%; Sucrose: 3%; K_2HPO_4: 0.1% Czapek: 0.001%; Agar: 1.5g. Malt extract agar (MEA) Malt extract: 3%, Peptone: 0.3%, Agar: 2g and Yeast extract agar (YES) Yeast extract: 2%, Sucrose: 15%, $MgSO_4.7H_2O$: 0.05%, (1% $ZnSO_4.7H_2O$, 5% $CuSO4.5H_2O$) solution in 100 ml, Agar: 2 g at 25 °C. After 5–7 days of incubation at 25°C in the dark, resulting colonies were counted and transferred to appropriate identification media [5]

Morphological and microscopic characterization of fungi

A total of 15 fungal cultures were isolated from the collected soil samples. The morphological properties showed variability between the different cultures and nutrient media used. All the isolates were also subjected to microscopic analysis for their characterization and identification. In addition, the cultures obtained had different obverse and reverse colony colors. In general, almost all fungal isolates had white to cream obverse and pale reverse colony color on YES medium. Colonies on CYA medium were white to cream, yellow, glaucous, or dark green on the obverse, whereas on the reverse they were pale, yellow, orange, fawn, and dark brown. On YES medium both obverse and reverse colony colors of isolates varied from white and cream to dark green and from pale to beige, respectively. Most isolates on MEA medium had obverse colony colors of white to cream, yellow, or dark green, with the colony reverse being yellow, orange or fawn. These are consistent with the observations [6].

Almost all the fungal cultures obtained did not produce exudates on MEA and YES media. The presence of uncolored, yellow, brown, and dark brown exudates was observed on CYA and MEA media. Some *Penicillium sp* is known to produce distinct exudate droplets yellow in *Penicillium chrysogenum* and dark brown in *Penicillium venetum* [7].

Among the fungal isolates obtained there were examples with both simple and branched conidiophores with metulae, phialides and conidia, features which are observed amongst *Penicillium sp* [6].

RESULTS AND DISCUSSION

Results of this research have demonstrated that some soil in Karnataka state can be considered as a valuable natural source of filamentous fungi from *Cladosporium cladosporioides, Penicillium crustosum* and *Penicillium sp.* In contrast, the identification of most groups of filamentous fungi including *Cladosporium cladosporioides, Penicillium sp* and *Penicillium crustosum* continue to be based on their morphology. The gross appearance of colonies developed on agar media recommended for this particular fungal (CYA, MEA and YES) is considerable importance in identification. Colony diameters of the isolates varied on different media are: 20-45mm (YES), 15-80 mm (CYA) and 15-34 mm (MEA) Fig.1. Although some researchers conducted on *Aspergillus sp* with a similar design was reported by some studies [8].

In this study we used morphological method with three differential culture media for identification of 3 important fungi species. A culture time of 5 to 7 days is generally required for identification of these fungal species. Using differential media like CYA, YES and MEA with macroscopic and microscopic characteristics of fungal growth on these culture media enabled us to discriminate three fungi species. Further studies would be helpful in clarifying the media and conditions that are most effective for the recovery and identification of fungi.

Pitt [6] reported that *E.egyptiacum* is a relatively rare soil fungus. Although a comparatively rare species, *P. novae-zeelandiae* is widely found in soils and decaying vegetation.

E. egyptiacum differ from other related species by some distinguishing features; it forms cleisthotecia which are pale, and when grown on CYA sometime produce a brownish orange pigment in the reverse [6]. Samson [9] reported that the main characteristic of *P. ramosus* is the typically branched and erect synnemata, measuring 2.5-5.0 cm in length in natural habitat.

Fungal cultures have different obverse and reverse colony colours. Almost, fungal isolates have white to cream obverse and pale reverse colony colour on CYA, MEA and YES with *Penicillium sp* and *Penicillium crustosum* except *Cladosporium cladosporioides* which have dark colour on obverse and reverse colony colour. Colonies on CYA, MEA and YES with *Penicillium sp* and *Penicillium crustosum* are white to cream, yellow and dark green colour on obverse whereas in reverse pale, yellow, orange, light brown, and dark brown. Fig.1.

The colony reverse is usually pale to yellowish or brownish. Presence of uncoloured, yellow, brown, and dark brown exudates were observed only on CYA and MEA. According to literature data, some *Penicillium sp* indeed are able to produce distinct exudates droplets, for instance, yellow in *Penicillium chrysogenum* and dark brown in *Penicillium venetum*

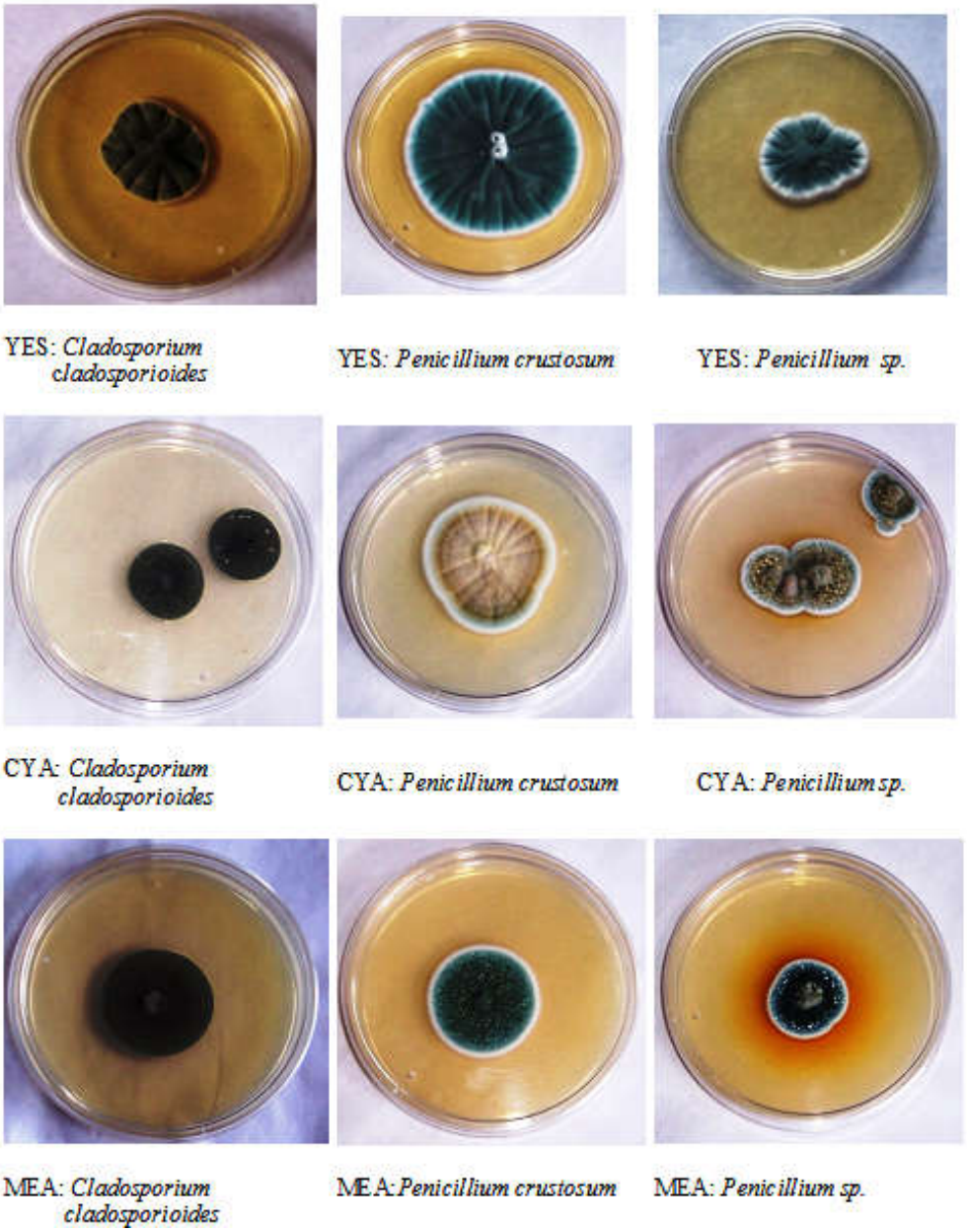

Fig. 1 – Morphological characteristics of examined fungal isolates on different agar media. YES: Yeast Extract Sucrose Agar; CYA: Czapek Yeast Extract Agar; MEA: Malt Extract Agar.

Tablet 1. Comparison of cultural and morphological characteristics between different media and fungi.

	CARACTERISTICS OF FUNGI FILAMENTOUS
YES	*Cladosporium cladosporioides:* Black, yellow to pale in medium. Absence of white mycelia at the margin
	Penicillium crustosum: Greenish black, white mycelia at the margin about 1-2 mm, white foam in the center.
	Penicillium sp. Greenish to black, white mycelia at margin around 2-3mm, irregular radial form
CYA	*Cladosporium cladosporioides*: Black, white droplet of exudates, white to cream in the medium, absence of white mycelia at margin.
	Penicillium crustosum: Brownish to white in the center, white mycelia at the margin 2-4 mm
	Penicillium sp. Grayish colour, irregular radial, golden yellow or droplet of exudates, grayish at the margin.
MEA	*Cladosporium cladosporioides:* Black, brown to black in media, absence of mycelia
	Penicillium crustosum: Greenish with white droplet, white ring at the margin
	Penicillium sp.
	Greenish to black, white mycelia at the margin, white droplet, yellow golden in the media.

The isolate demonstrated cultural and micro morphological features which are similar to those of *Penicillium raistrickii.* Other characteristics were pretty different from those of *P. raistrickii*, such as the production of colonies with velutinous texture, the production of a dark yellowish green diffusible pigment in all culture media and the absence of true sclerotia in the mycelium.
The isolate also exhibited some affinity with other species in the subgenus *Furcatum* such as *Penicillium paxillii* (*e.g.* long, smooth stipes and metulae with enlarged apices) and *Penicillium herquei* (*e.g.* colony reverses of green colors and production of dark yellowish green diffusible pigment in culture media) [10].

CONCLUSION

Mycelia were white with heavy sporulation yielding grayish green colonies on all media. Colonies were radially sulcate and velutinous, with clear exudate, and produced a yellow to orange on CYA and MEA by *Penicillium sp*. This study demonstrates that some soils in Karnataka state can be considered as a valuable natural source of filamentous fungi. Isolated fungal cultures can be recommended for further studies in terms of determination of their ability for statins production.

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