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Random Screening of fungal species from the different sources of Soil in Kalaburagi

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Abstract

Fungi from the sources of soil of Kalaburagi region was investigated at some different locations from February to June 2015 the fungi were isolated by using soil dilution and soil plate method. The mycelia growth rate, colony character and sporulation of different types of fungal isolates grown on the Potato Dextrose Agar (PDA), were observed after seven days of incubation at 25±1°C. The colony diameter, culture characteristics (texture, surface and reverse coloration, zonation) and sporulation of selected test fungi were greatly influenced by the type of growth medium used, On the basis of cultural and microscopic characteristics, the isolated cultures were identified as, Aspergillus Rhizopus, Mucor, Fusarium sp. etc. different genus were obtained from twenty different sites of soil samples.

Keywords: Aspergillus, PDA, culture media sporulation Dimorphic etc.

Introduction

Fungi are eukaryotic organisms with membrane bound nucleus, well differentiated apparatus and a cell wall, hence not typical eukaryotic organisms. They are much larger than bacteria, the vegetative cells being 2-10 µm in diameter (Prescott *et al*., 1999). Most fungi are non-motile throughout their lifecycle although spores are carried a great distance by wind. Growth of mycelium substitutes for mortality, bringing the organism into contact with new food sources and different mating strains (Sendron and Araro, 1999). All fungi are heterotrophic and most of them are saprophytes. Some can also be parasites on living animals or plants although very few fungi absolutely require a living host (Kathleen, 2005). Most fungi are dimorphic, meaning they exist in two forms; they have unicellular and yeast like forms in their host but when growing saprophytically in soil or lab medium, they have filamentous forms. Almost all fungi that exhibit dimorphism are pathogenic to man (Sendron and Araro, 1999). They replicate sexually by fusion of gametes and asexually by spore formation, and exist in macroscopic or microscopic forms (Prescott *et al.*, 1999). The present study was undertaken to isolate different fungi from different sources of soil in Kalaburagi region.

Materials and Methods

Samples collection: Fungi were isolated from soil, which were collected from different parts of

kalaburagi city, such as dairy farm, poultry farm, sewage soil, near lack site, garden/parks/nursery, agricultural field, near road side, residential colonies and market site etc. A fungal colony was first grown on the Potato dextrose agar medium and its morphology was studied using standard cover-slip technique and lactophenol cotton blue staining procedure. The cover slip was inserted in tilted position in the petriplate itself and the culture was allowed to grow for some time. Then the cover slip was taken out with the help of forceps and put inverted on slide containing a drop of lactophenol cotton blue stain and visualized under microscope at 40 X magnification. Thin mycelia of fungal isolates were also spread on the glass slide and teased with needles followed by addition of a drop of lactophenol stain. The stained and air-dried slides were further examined under microscope at 40 X magnification. The fungi were identified on the basis of mycelia and spore characteristics.

Isolation of fungi: In case of soil, the collection site of sample were cleaned of all the superficial deposit such as; stone, grass, litter *etc*. and a pit of 15x15 cm was drug. The soil was loosened inside the pit and collected in sterile bags, which were brought to the laboratory. Previously prepared PDA containing petriplates was exposed at different corners of the general wards of the hospitals for 5-10 minutes. These petriplates were then transported to the laboratory and kept in incubator up to 7 days at 37 C for the growth of fungi.

Direct Plate Method (Warcup, 1950): In this procedure a small amount of soil sample (0.005 to 0.015 grams was taken from the Main sample by means of a sterile nichrome needle with a flattened tip and it was dropped into the bottom of a sterile plate, agar medium was poured and particles were distributed throughout the medium by shaking and rotating the plate.. After this, isolation were made from the plates, different fungal species were picked up with the help of sterile needle and then streaked into the slant, containing Potato Dextrose Agar (PDA) medium.

Medium used for the isolation: Here we used Potato Dextrose Agar Medium (PDA) for the isolation of each type of fungi. PDA contains:-

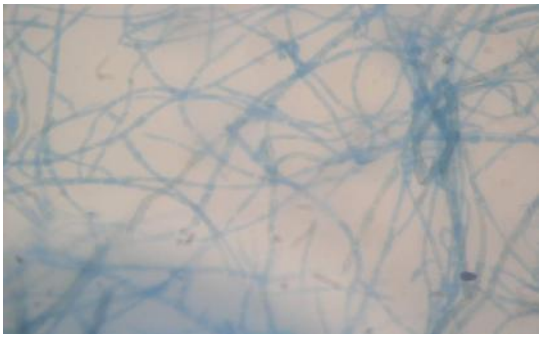
Potato extract - 200 ml
Dextrose - 15 gram
Distilled water - 1000 ml

Identification of fungi: The isolated fungi were identified to the genus level, by staining with Lacto phenol cotton blue stain in compound microscope.

Types of presumptive isolated fungi from soil sources.

(*Aspergillus, Pencillium, Rhizopus, Mucor, Fusarium*) etc.

Photograph A: *Aspergillus* sp (globose conidiophores)



Photograph B: *Mucor* slide of stained with Lactophoenol cotton blue.

Morphological characters of isolated fungi:

Genus fungi isolated = **a) Aspergillus b) Rhizopus c) Fusarium**

A.For the identification of ***Aspergillus* sp**

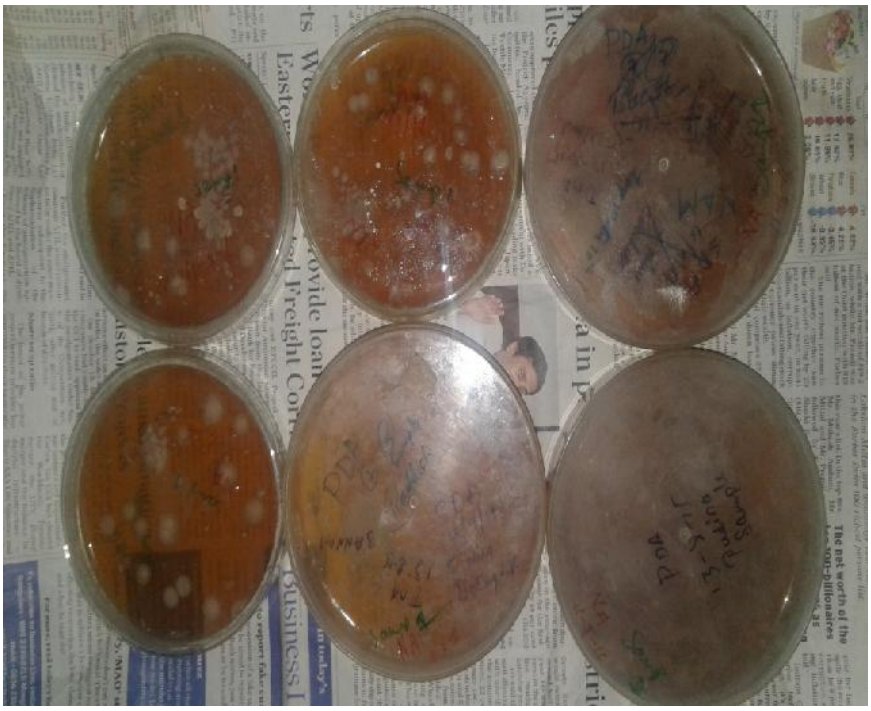
Media type = PDA (Potato dextrose agar) Colony character = (texture = Velvety thick and surface color = creamish thick Reverse color Zonation = Radially furrowed on the reverse.

B.For the identification of ***Rhizopus* sp**

Media type = PDA (Potato dextrose agar) Colony character = (texture = powdery and surface color = Olivaceous black with sterile white margin. Colour zonation = Radially furrowed.

C. For the identification of ***Fusarium* sp**

Media type = PDA (Potato dextrose agar) colony character = (texture = Floccose and Surface color = Magenta pink. Color zonation = With concentric zones of dark and light reddish colouration



Photograph – 1 Isolation of fungi on PDA Plates.

Photograph -2: PDA slants of different types of fungi:

Results and Discussion

Table 1: Morphological characters of fungal species.

Fungal Species	Sample No.	Sampling SITE.	Media type	Colony character	Surface colour	Colour zonation
Aspergillus	2,4,8,9.	Garden soil, cement dust site,	Potato dextrose agar	Velvety thick	Cremish thick reverse	Radially furrowed on the reverse
Rhizopus	5, 7 14 19.	Garden soil, Lake soil, road site soil	Potato dextrose agar	Powdery	Black with sterile margin	Radially furrowed
Fusarium	6,11,15 16.	Garden soil, Cement site, soil near road side.	Potato dextrose agar	Floccose	Magneta pink	Concentration zone of dark and light reddish colouration

During the investigation period it was fond that fungal floras were isolated from various sources of soil samples. *Aspergillus* , *Fusarium*, *Mucor Rhizopus*, *Trichoderma* species were observed, During the investigation period (Between February-May) monthly seasonal variation were also observed. Minimum (6) fungal species were found in the April month of summer season due to high temperature, dry winds and percentage of humidity is very low in the environment which is not favorable for fungal growth. All of these species have been reported as commonly isolated from different sources of soil samples by Deming (2002) and Onofri *et al* (2007). It is reported that the fungal isolates obtained in their study were mainly *Aspergillus* species, while others were *Trichoderma*, *Penicillum*, *Rhizopus* and *Rhodotorula* species which were all able to utilise hydrocarbon as carbon source. Elisane *et al*, (2008), who also isolated four strains from the contaminated soil. Sharma (2010) isolated same fungi at Darjeeling tea garden soil and Sharma *et al* (2011) reported some same fungi from Lachung soil. Fungal biofertilizers, which have been used to improve plant growth by enhancing phosphorus absorption in plants, are phosphate solubilizing microorganisms. The Commonly widespread fungi are *Penicillium*, *Aspergillus*, *Chaetomium* and *Trichoderma* species. There are a number of biofertilizers available in the market.

However Applications are based on their ability to supply and mobilize plant nutrients, control plant diseases and promote plant growth and development. The use of fungi as biofertilizers is not new, as most of these have been developed in the last two decades. There are Numerous reports stating the success in promoting plant growth as biofertilizers. Fungal biofertilizers help to minimize the use of synthetic chemical fertilizers. This is beneficial since synthetic chemical probably compounds have detrimental effects on humans and the environment (Sarma *et al*. 2012; 2014). Fungal biofertilizers are presently used on a very small scale as compared to chemical compounds.

Conclusion

Fungal biodiversity of regional epidemiology is a significant factor in environment and to be continuously monitored so that the reservoir of such fungi in the environment to be isolated and identified. We conclude that the environment be continuously monitored so the reservoirs of such fungi in the environment confirm the need for further documented studies to evaluate the presence of micro fungal analysis study for future work.

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