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ISOLATION OF *Penicillium marneffei* FROM THE MANGO FRUIT OF WALKING MANGO TREE

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ABSTRACT

***Penicillium marneffei* is an opportunistic dimorphic fungus causing infection in both immunocompetent and immunocompromised patients. Infection by *Penicillium marneffei* is an important emerging public health problem, especially among travelers and inhabitants in SE China and SE Asia infected with human immunodeficiency virus (HIV). However, the natural habitat and transmission mode of the etiologic agent remains unclear in HIV patients. In this study, mango fruit was collected from walking mango tree. *P. marneffei* was identified by microscopic and morphological analysis. The constituent recovery of this fungus from the fruit suggests that it could be Endophytic in this dicot host.**

KEYWORDS : Endophytic, Human pathogenic fungi, HIV, Etiological agent, Dicot host

Invasive fungal infections are frequent in patients with cancer (Armstrong, 1973). *Penicillium marneffei* is known to be endemic in S E Asia. It causes infections of RE system in humans in immunocompetent & more often in immunocompromised individuals especially in AIDS patients. As a result of recent increase of HIV infection.(Sirisanthana et al.,1998) *P.marneffei* has become one of the principal new emerging fungal pathogens (Jayanetra et al., 1984; Supparatpinyo et al.,1994).

The typical manifestations of *P. marneffei* infection in HIV-infected individuals include fever, anemia, weight loss, skin lesions, generalized lymphadenopathy, and hepatomegaly . (Mootsikapun et al., 2006).

The primary treatment with amphotericin B and itraconazole and secondary prophylaxis with itraconazole are very effective regimens . Patients who do not receive timely and appropriate antifungal treatment have poor outcomes.

Penicillium marneffei a dimorphic fungus is a rare opportunistic pathogen. (Chan and Woo , 1990).At present it is considered to be the third most frequent opportunistic pathogen after tuberculosis and cryptococcosis in endemic areas. (Woo et al., 2003) Recognition of this rare disease is important because it is amenable to treatment.(Viviani and Trtorano, 1998).

Walking Mango Tree (*Mangifera indica*, Family: Anacardiaceae); is located in Gujarat, India. This Tree has moved about 200 meters from its original place in more than two centuries and is continuing its walk.

The mango tree, which finds mention in the list of 50 heritage trees of Gujarat, has several unique features not seen elsewhere in the world. Its branches grow parallel to the ground from the main stem.

Roots develop from a part of the branch that touches the ground, which develops in the form of a stem and the original stem dries off.

The branch keeps on growing parallel to the ground from the new stem and new roots appear in the same pattern. This process has continued for several hundred years, perhaps over a thousand years.

The mango tree may have been planted by early Parsi settlers about 1,300 years ago. The age, however, still remains unverified. It was reported by the locals that the bark acts as a clotting agent and prevent infection. (Gautama et al., 2013).

The fruit of this tree turns into orange-red color when ripen. The pH of the fruit is 2.4. The number of varies year-year. Being acidic, this fruit is not consumed by any person, but only for the sake of tasting it, people taste it.

The main intension to carry out this work was to understand its suitable pH for its growth and for the pigmentation. To determine the suitable Carbon and Nitrogen source's. To understand their growth stages.

The constituent recovery of this fungus from the fruit suggests that it could be Endophytic in this dicot host. If found to be effective, their natural habitat and transmission mode of the etiological agents in HIV patients can be

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understood much better.

MATERIALS AND METHODS

Plant Material

The mango fruit was collected from walking mango tree and stored under 4ºC.

Media

(i) Potato Dextrose Agar (PDA)

(ii) Czapek's Dox

(iii)Sabouraud Dextrose Agar

(iv) Martin Rose Bengal Agar (MRBA)

Methodology

(i) Serial Dilution

Standard protocols were followed on Serial dilution method (10-1, 10-2, 10-3, 10-4, 10-5 concentration range).1 gram of the mango pulp was added into 10 ml of sterile water (Stock solution). 1ml of the stock solution was diluted into 10ml of sterile water and serially diluted further. Later 1 ml of the each diluted solution was pipette out into the petriplates containing the fungal media.

Each plate was wrapped with cling wrap to avoid any contamination and to maintain the pure colony growth. The plates were incubated around 30ºC for their growth.

Same method was followed for Carbon & Nitrogen sources. Every 1 gram of the source was added into 10 ml of the autoclaved water (Stock solution). 1ml of the stock solution was diluted in 10ml of autoclaved water & serially diluted. Then 1ml of the broth culture was inoculated into the above solutions. This was incubated around 30ºC for their growth.

Carbon Sources

- Citric acid
- Dextrose
- Glycerol
- Lactose
- Mannitol
- Succinic acid
- Sucrose

Nitrogen Source

- Ammonium chloride
- Peptone

(ii) Slide Culture

1ml of the media was placed on a autoclaved glass side and a spore of *P.marneffei* was placed onto it and cover slip was kept upon it. This was incubated around 30ºC for their growth and the observations were recorded.

(iii) pH

The media with different pH were prepared to find out the growth and pigmentation for *P.marneffei* ranging from 2.5-8.5.

RESULTS

The colony growth and the red pigmentation were clearly observed on the day 4 after pipetting onto the fungal media. Permanent slides were prepared and identified based on their microscopic and macroscopic features.

DISCUSSION

The colonies of *Penicillium* other than *Penicillium marneffei* are rapid growing, flat, filamentous, and velvety, woolly, or cottony in texture. The colonies are initially white and become blue green, gray green, olive gray, yellow or pinkish in time. The plate reverse is usually pale to yellowish.

Penicillium marneffei is thermally dimorphic and produces filamentous, flat, radially sulcate colonies at 25°C. These colonies are bluish-gray-green at center and white at the periphery. The red, rapidly diffusing, soluble pigment observed from the reverse is very typical. At 37°C, *Penicillium marneffei* colonies are cream to slightly pink in color and glabrous to convoluted in texture.

For species other than *Penicillium marneffei*, septate hyaline hyphae (1.5 to 5 µm in diameter), simple or branched conidiophores, metulae, phialides, and conidia are observed. Metulae are secondary branches that form on conidiophores. The metulae carry the flask-shaped phialides. The organization of the phialides at the tips of the conidiophores is very typical. They form brush-like clusters which are also referred to as *penicilli*. The conidia (2.5-5µm in diameter) are round, unicellular, and visualized as unbranching chains at the tips of the phialides. In its filamentous phase, *Penicillium marneffei* is microscopically similar to the other *Penicillium* species. In

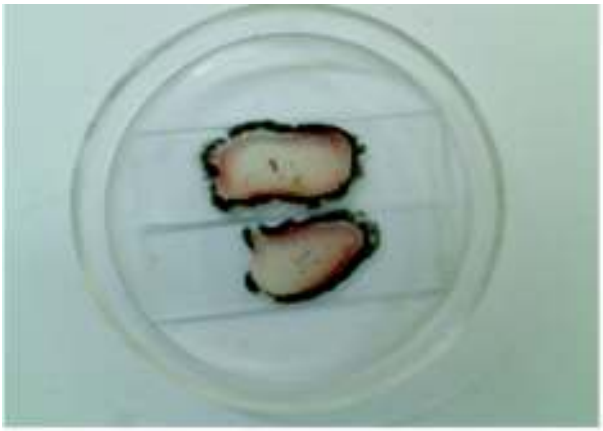


Figure 1 : Slide Culture of *P. marneffei*

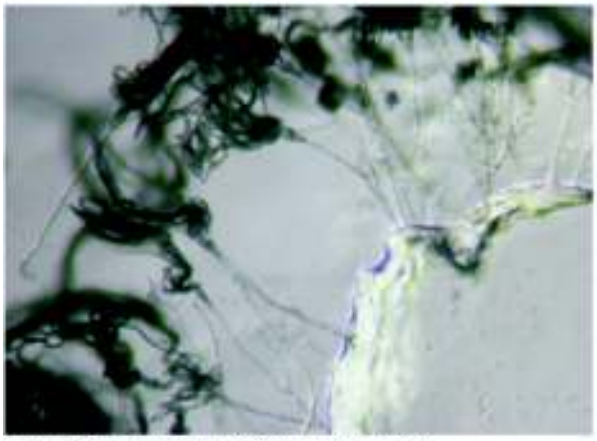


Figure 1 : Slide Culture of *P. marneffei* at 60x

Figure 1 : Slide Culture of *P. marneffei* at 100x

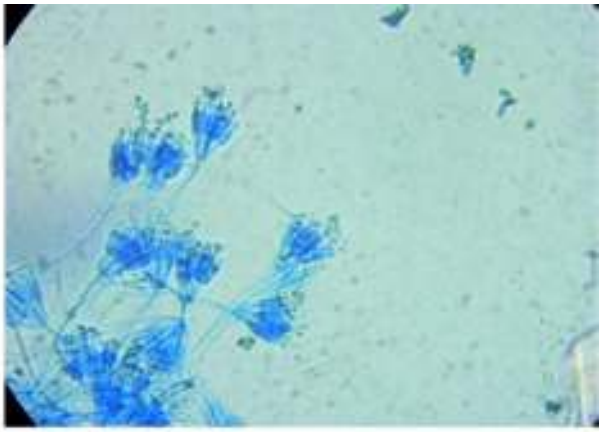


Figure 4 : Microscopic view at 10x

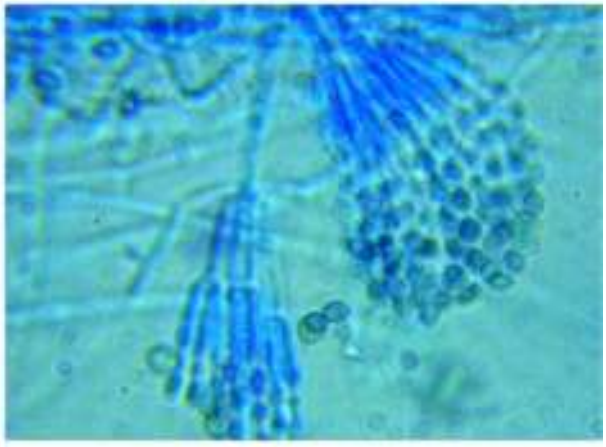


Figure 5 : Microscopic view at 100x

Figure 6 : Microscopic view at 100x

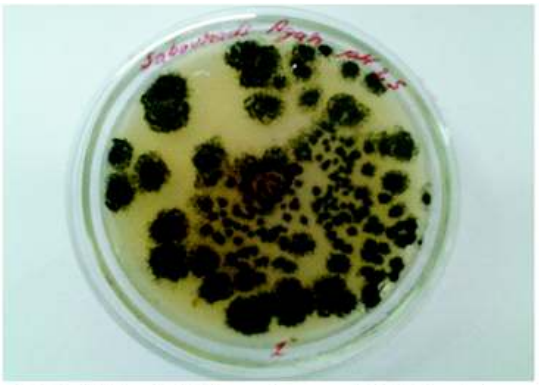


Fig.7: Culture in Sabourads agar pH 3.5 -front view

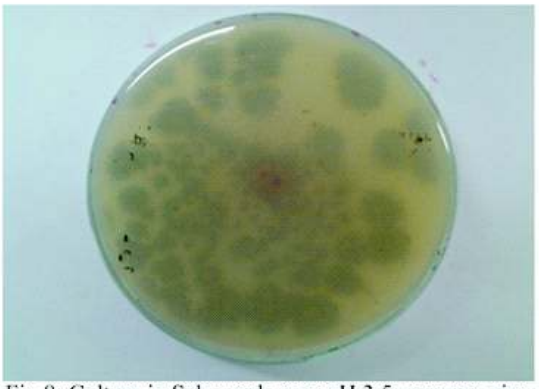


Fig.8: Culture in Sabourads agar pH 3.5 -reverse view

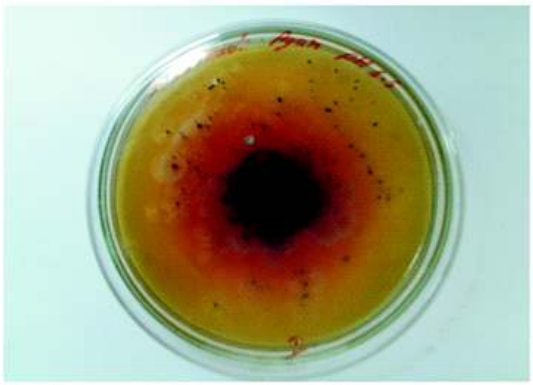


Fig.9: Culture in Sabourads agar pH 6.5-top view

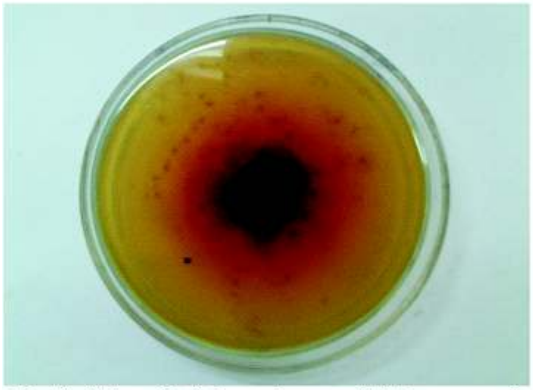


Fig.10: Culture in Sabourads agar pH 6.5- reverse view

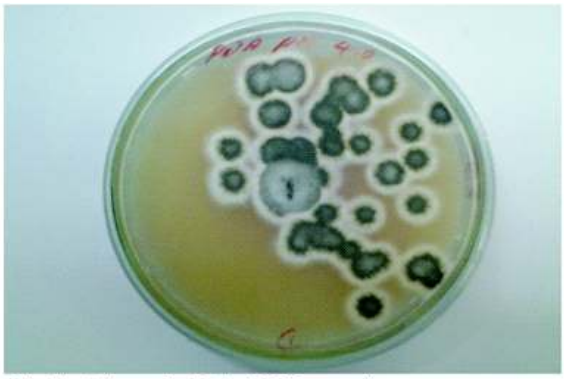


Fig.11: Culture in PDA pH 3.5-top view

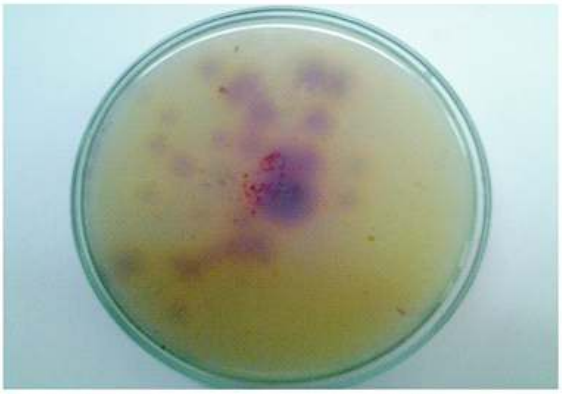


Fig.12: Culture in PDA pH 3.5-reverse view

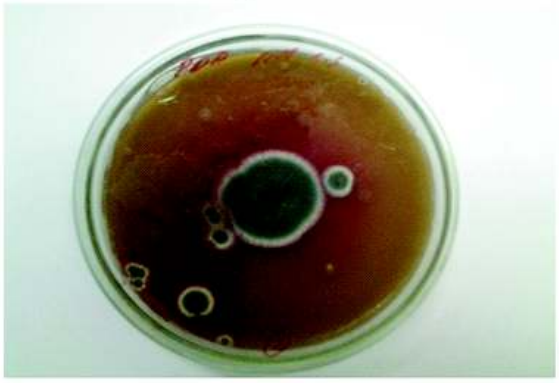


Fig.13: Culture in PDA pH 6.5-top view

Fig.14: Culture in PDA pH 6.5-reverse view

Fig.15: Citric acid- Carbon source

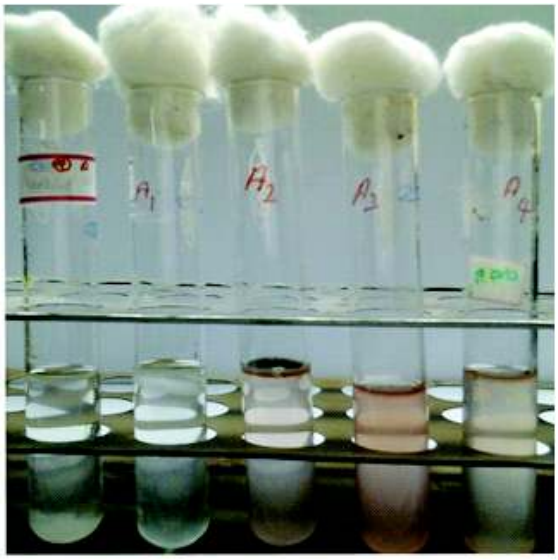
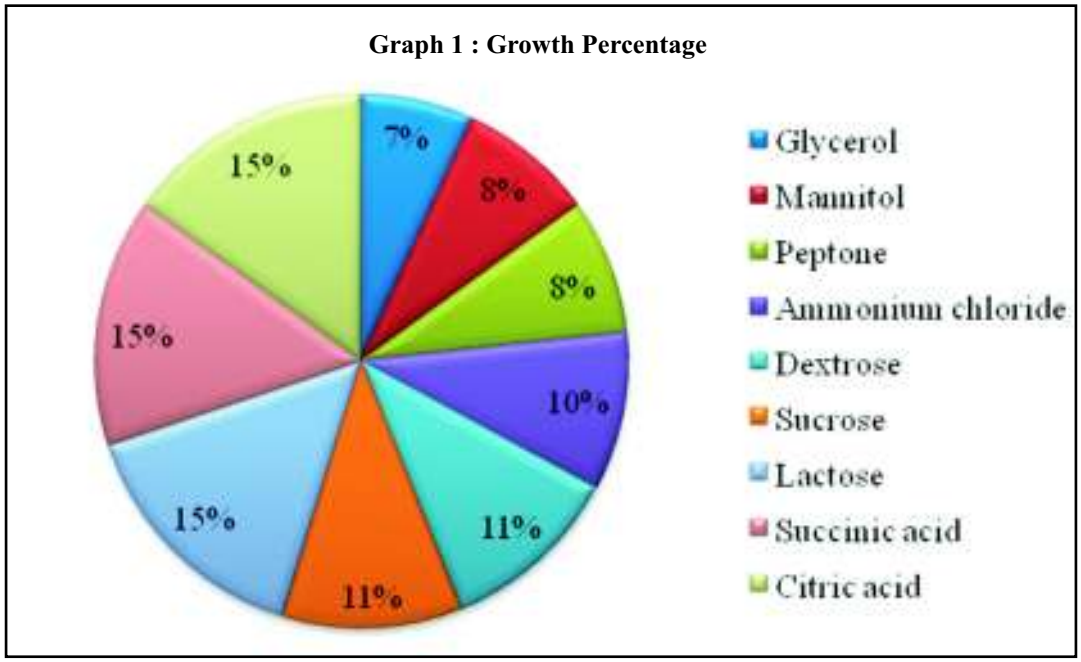


Fig.16: Succinic acid- Carbon source



its yeast phase, on the other hand, *Penicillium marneffei* is visualized as globose to elongated sausage-shaped cells (3 to 5 µm) that multiply by fission.

Penicillium marneffei is easily induced to produce the arthroconidial yeast-like state by subculturing the organism and incubating at 35°C, in which after a week, yeast-like structures dividing by fission and hyphae with arthroconidia are formed.

P.marneffei has never been isolated from plant parts so far. We isolated this fungus while working upon isolation of various fungi present in this tree. We could isolate 129 types of fungi belonging to 10 genera, but were successful in identifying only 81. Among these wide types of fungi, *P.marneffei* was one among it. The interesting thing about this fungus is that it's found more abundantly in people suffering from HIV/AIDS. Doesn't mean that it is absent in our body, but is highly activated in those infected ones. The connection between the fungi and virus is

Graph 2 : Suitable pH and Media for Growth and Pigmentation

unknown so far. So the information regarding this fungus was studied upon the isolations of blood samples from infected people and not from any plant material. So our study reveals some information about its adaptation, which could lead to our further studies upon establishing the relation between virus and the fungi.

The slide culture (Figure, 1) reveals its clusters of long chain of conidiophores (Figure, 3), which gets disturbed while preparing the slides (Figure, 4-6). Whit the slide culture we can see the growth of this fungi from the initial stage to the final stage (Figure, 2).

The pH study reveals the suitable pH for its growth and for the pigmentation. We found out that at pH 3.5 the growth of these fungi was suitable in PDA than any other pH (Figure, 11-12). The pigmentation was more seen at pH 6.5 in PDA (Figure, 13-14). The growth of the fungi was seen to be more effective in Sabouraud's Dextrose agar on 8th day after incubation (Figure, 7-10). But the same effect was shown in PDA within 4th day after incubation. So PDA was much suitable media for us to carry the further study.

Though the literature says that Brain Heart Infusion Agar (BHIA) is most suitable media for its growth, but it not the same on our study. Even after 15 days after incubation in BHIA, the growth & pigmentation was not observed. So PDA was most suitable media for the study followed by Sabouraud agar. Czapek's Dox and BHIA did not give an effective result (Graph, 2).

We checked upon totally 9 different salts for Carbon and nitrogen sources, among which 2 were nitrogen and the remaining 7 for carbon sources. The growth in Peptone and ammonium chloride was not much efficient. It had a growth about 8% and 11% respectively. On carbon sources, they showed 15% growth in Succinic acid, Citric acid and lactose (Figure,15-16). By undertaking this study, it reveals that the growth is more luxuriant on carbon sources more than the nitrogen sources. Here serial dilution method was followed to see at which dilution they grow. It was observed that at 10-2 dilution the growth was abundant than rest of the dilutions. So citric acid and succinic acid are the suitable carbon source for *P.marneffei*.

CONCLUSION

This study reveals the presence of a human pathogenic opportunistic fungus present in this mango fruit. Their Endophytic association needs to be further studied. Their manifestation in this acidic environment also needs to be worked out.

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