The Pharma Innovation Journal 2022; 11(1): 1450-1452

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 1450-1452

www.thepharmajournal.com
Received: 03-10-2021
Accepted: 06-12-2021

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Isolation and identification of microflora from the rhizosphere of banyan tree

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Abstract
The Banyan (*Ficus benghalensis*) is the National tree of India which belongs to Family Moraceae. The banyan tree has more importance in Hindus. It is used as the medicinal as well as ornamentle plant. It is immortal tree because can grow and survive for centuries. The beneficial microbiome present in the rhizospheric soil. In some plants rhizospheric activities stimulate the microflora which helps in disease management as well as plant development. The soil sample were collected from the rhizosphere of Banyan tree which were deforestred during widening of road from the location of Gangakhed and Pathri Road of Parbhani District. The microflora from these was isolated on PDA and NA medium by Serial dilution method. *Trichoderma harzianum, Trichoderma hamatum, Bacillus subtilis, Trichoderma asperellum, Trichoderma kiningi, Pseudomonas fluoresence, Trichoderma longibactarum* and *Pseodomonas striata* were identified.

Keywords: Isolation, identification, microflora, rhizosphere, banyan

Introduction
In Indian tradition *Ficus benghalensis* L. (Banyan tree), *Ficus riligiosa* L. (Pipal tree) and *Ficus recemosa* L. (Umbar tree) are worshiped because each and every part these trees is useful in human life. From the reviews it was observed that the rhizospheric soil contains huge number of beneficial microflora which helps these plants for their growth and development of these trees.

It was long known that the rhizosphere activities of some plant stimulates beneficial microorganisms which helps in plant growth and plant disease management. is present in plant The rhizosphere is the zone where the huge amount microorganism are present which also contains sloughed-off cells and chemicals released by roots provide ready food source for growth. Microbes can make nutrients and minerals in the soil available to plants, produce hormones that spur growth and stimulate plant immune system.

The rhizosphere is a hot spot of microbial interactions as exudates of released by plant roots are a main food source for microorganisms and a driving force for their population density and activities (Raaijmakers *et al*., 2009) [8].

Now a days the scientist working in agriculture field are using the soil from the root zone of banyan tree for management of soil borne diseases in fruit crops. Therefore, it was thought to exploit the rhizosphere of banyan tree for the isolation of effective strains of biocontrol agents.

Material and Methods
Collection of Soil Samples
Soil samples were collected from the road sides from the rhizosphere of banyan tree which were deforested during the widening of road. The soil samples were taken from the depth of 15 cm around the rhizosphere area of banyan tree. The samples were collected and carried in the laboratory of Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Agricultural University, Parbhani. For isolation of different micro-organisms.

Isolation of Micro-Organisms
These soil samples were air dried in shade and isolation of microflora were done by using serial dilution technique. Potato Dextrose Agar and Nutrient Agar medium were used for isolation and growth. Isolation was carried out under aseptic conditions. Dilution 10^{-3}, 10^{-4} and 10^{-5} were used for fungus isolation on PDA and dilution 10^{-7}, 10^{-8}, 10^{-9} for isolation of bacteria on NA. Also King's B media and Trichoderma selective medium were used for isolation.

Identification of Microorganisms

A. Identification of Fungal isolates

The fungal isolates which was isolated from rhizosphere of banyan were identified on the basis of their cultural and morphological characteristics and confirmed the fungus by microscopic observation.

B. Identification of Bacterial isolates

The bacterial isolates which was isolated from rhizosphere of banyan were identified on the basis of their cultural and morphological characteristics and by performing biochemical tests. Also confirmed the bacteria by microscopic observation.

a) Gram Staining

The Gram-reaction of each isolate was determined by the following procedure. First a loop full of the bacterium suspension was smeared on the clean glass slide, air fixed by gentle heating on flame of the spirit lamp. Aqueous Crystal violet solution (0.5) was spread over this smear for 30 second and then washed with running tap water for a minute and then flooded with Grams iodine solution for one minute and rinsed in a tap water. Later decolorized with 95% of ethanol until colour runoff, washed with water and treated with Safranin as counter stain about 10 seconds, washed with water, air/blot dried and observed under research microscope.

b) KOH test (Potassium hydroxide)

A drop of 3 per cent potassium hydroxide was placed on clean glass slide and to this a drop 48 hrs old bacterial culture was mixed with clean inoculation loop and stirred for 10 seconds and observed for slime threads. when raised the wire loop, if strands of viscid material seen, then the bacterium is gram negative and if strands was not seen, then the bacterium is gram positive.

c) Catalase oxidation test

A loop full of 48 hrs old culture of the test bacterium was placed on the clean glass slide, and to this a drop of 3 per cent hydrogen peroxide (H_2O_2) was mixed and allowed to react for few minutes and observed for the production of gas bubbles.

Result and Discussion

It was revealed from the data presented in Table-3 that *Trichoderma* spp. was mostly present in collected soil samples. *Trichoderma harzianum* was isolated from the soil sample which was collected from Gangakhed road of Parbhani District. *Trichoderma hamatum* was isolated from the another soil sample collected from the location of Gangakhed road from Parbhani Tahsil of Parbhani District. *Trichoderma asperellum* and *Trichoderma koningii* was isolated from the soil sample which was collected from the location of Gangakhed road which comes under Gangakhed Tahsil of Parbhani District. *Trichoderma longibrachiatum* and *Aspergillus niger* were isolated from the soil sample collected from the location of Pathri road which comes under Parbhani Tahsil of Parbhani District.

Bacillus subtilis was isolated from the rhizospheric soil sample collected from the location of Gangakhed road which comes under Parbhani Tahsil of Parbhani District. *Pseudomonas fluorenscens* was isolated from soil sample collected from the location of Pathri road which comes under Parbhani Tahsil of Parbhani district. *Pseudomonas striata* was isolated from the soil sample collected from the location of Pathri road which comes under Pathri Tahsil of Parbhani District.

Table 1: Details of isolates isolated from the rhizosphere of banyan tree

Sr. No.	Sample Code	Isolate Code	Identified Isolate
1.	PBN1	ISO-1	*Trichoderma harzianum*
2.	PBN2	ISO-2	*Bacillus subtilis*
		ISO-3	*Trichoderma hamatum*
3.	GGK3	ISO-4	*Trichoderma asperellum*
		ISO-5	*Trichoderma koningi*
4.	PBN4	ISO-6	*Pseudomonas fluorescens*
5.	PBN5	ISO-7	*Trichoderma longibrachiatum*
		ISO-8	*Aspergillus niger*
6.	PTR6	ISO-9	*Pseudomonas striata*

Identification of Microflora

Identification of Fungus

Table 2: Cultural characteristics of fungal isolates

Sr. No.	Isolate code	Name of Isolate	Colony Growth rate(cm/day)	Colony Colour	Reverse Colony Colour	Culture Smell	Colony edge
1.	ISO-1	*T. harzianum*	8-9 cm in 3days	Green to dark green	Colourless	Malt	Wavy
2.	ISO-3	*T. hamatum*	8-9cm in 3 days	Green	Colourless	Coconut	Wavy
3.	ISO-4	*T. asperellum*	8-9 in 3 days	Dirty green	Dark greenish	Coconut	Smooth
4.	ISO-5	*T. koningii*	7-8 in 3days	White to green	Colourless	Malt	Effuse
5.	ISO-7	*T. logibrachiatum*	8-9 in 3days	White to green	Colourless	Malt	Effuse
6.	ISO-8	*Aspergillus niger*	8-9 in 3 days	Initial white then turns black with conidial production	Blackish		Wavy

Table 3: Morphological characteristics of fungal isolates

Sr. No.	Isolate Code	Mycelial form	Mycelial colour	Conidiation	Conidiophore	Conidia	Phialade	Chlamydospore
1.	ISO-1	Floccus to Arachnoide	Watery white	Ring like zone	Highly branched regular 2-3 µm L	Subgolobus 3.6-4.5µm	Globus 8-15×2-3µm^2	Not observed
2.	ISO-3	Floccuse to Arachnoid	Watery white	Concentric Rings	Regularly branched	Green, ellipsoidal, smooth	Ellipsoidal to ovoidal	Sub globus to globus
3.	ISO-4	Floccus to Arachnoid	Watery white	Ring like zones	Ball like structure	Obovoid green3.6-4.5µm	Sigmoid or hooked 8-14×2.4-3µm^2	Not observed
4.	ISO-5	Floccuse to Arachnoid	Watery white	Ring like zones	Regularly branched			Not observed
5.	ISO-7	Floccuse to	Watery	Circular	Regularly		Radiate around the	Not

		Arachnoide	white	zones	branched		observed entire vesicle	
6.	ISO-8	Resembles the structrure of plant	Black	Concentric rings	Smooth and colourless	Biserite heads are radiate, cells biserste, brown and smooth walled		

Table 4: Cultural, morphological and biochemical characteristics of Bacteria isolated from banyan rhizosphere

Sr. No.	Isolate code	Isolate identified as	Cell Size	Colony shape	Colour	Elevation	Surface	Margin	Pigmentation	Gram reaction	Shape	KOH Test	Catalase Test
1.	ISO-2	*Bacillus substilis*	0.25-1.0×4-10 µm^2	Circular	White or Slightly yellow	Convex	Rough, Opaque	Irregular	Brown	Positive	Rod	Negative	Positive
2.	ISO-6	*Pseudomonas fluorescens*	0.5×2.0-2.5µm^2	Round	Yellowish green	Convex	Smooth, shiny	Irregular	Yellowish green	Negative	Rod	Positive	Positive
3.	ISO-9	*Pseudomonas striata*	0.5-1.0 ×1.5-5.0µm^2	Round	Colourless		Shiny but not mucoide	Smooth margin	Colourless	Negative	Rod	Positive	Positive

The results of the present studies are matched with the results of earlier worker. Panaiyadian and Chellaia (2011) [7] isolated microorganisms from rhizospheric soils of Pachamalai Hills, Tamil Nadu, India. An attempt to isolate and to identify the soil microbial populations from the rhizosphere located at Pachamalai forest area.

Ikediugwu and Monday (2012) [3] isolated the rhizospheric microorganisms of rubber plants (*Hevea brasiliensis*) by using the soil dilution plate method, while the rhizoplane microorganisms were determined by serial washing of root lengths and plating on PDA plates.

Soesanto *et al.,* (2011) [12] aimed at determining morphological and physiological features of *Pseudomonas fluorescens (*P60). The morphology was characterized visually and microscopically by colony growth such as colony form, color, edge, and grams reaction.

Todorova and Kozhuharova (2010) [14] identified as *Bacillus subtilis* on the basis of their morphological, cultural and physiology- biochemical properties.

Kumar and Sharma (2011) [5] studied the growth pattern of *Trichoderma* isolates and the conidial wall pattern and shape rough and sub-globose in *T. harzianum*, while in case of *T. viride* smooth and globose to abovoid.

The results of the present studies are in conformity with the results of the previous workers *viz.*, Kannan and Sureendar (2009) [4], Kumar and Sharma, (2011) [5], Akinola *et al.,* (2012) [2], Ru and Di (2012) [9], Zang Ru *et al.* (2012) [9], Wro and Damle (2013) [15], Ahmed *et al.,* (2014) [1] Shahid *et al.,* (2014) [10], Sonawane *et al.,* (2014) [13] and Manasa *et al*., (2017) [6]. Sharma *et al*., (2017) [11].

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