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Studies of Rhizobacteria from the Rhizosphere of *Murraya koenigii* for Plant Growth Promoting and Antibacterial Activities

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Abstract

Rhizosphere is the area of intense microbiological activity. Plant growth promoting rhizomicroflora inhabits rhizosphere of plants and enhances plant growth through production and release of metabolites. Medicinal plants support a great diversity of microflora in their rhizosphere including PGPR. *Murraya koeinigii* is one of the important medicinal plants. The leaves improve functioning of the small intestine. The leaves, the bark & the roots of *Murraya koenigii* spreng are used as a tonic and a stomachic. The present study is intended to isolate plant growth promoting rhizobacteria from the rhizosphere soil of *Murraya koeinigii* near Solapur region. In the present study total 13 bacterial isolates were obtained from the rhizosphere soils of *Murraya koenigii.* All the isolates were screened for plant growth promoting activities viz. NH_3, HCN, siderophore and IAA production and PO_4 solubilization. The percentage of NH_3, HCN, siderophore and IAA production and PO_4 solubilization were 53, 66.6, 57.1, 92.30 and 57.1% respectively. Antimicrobial activity of isolates from *Murraya koenigii* showed significant results. Isolate MK 9 showed antimicrobial activity against all tested pathogens, *Xanthomonas citri, Xanthomonas axonopodis, Aspergillus niger* and *Fusarium oxysporum.* Isolates MK2, MK3, MK5 and MK6 and MK11 showed significant zone diameters against all tested pathogens except *X. citri* and MK4 showed inhibition of *Xanthomonas axonopodis.* Evaluation of plant growth promotion was carried out by treatment of seeds with selected bacterial isolates. There was significant increase in root length, shoot length & fresh weight with MK9 compared to control and significant increase in shoot length and fresh weight with MK3.

Keywords
Rhizosphere, PGPR, medicinal plants, *Murraya koenigii*

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INTRODUCTION:

Plant rhizosphere soil is a unique biological niche with a diverse microflora of bacteria, fungi, protozoa and algae and these microorganisms have played significant role in nutrition by a high input of organic materials derived from the plant roots and root exudates that are necessary for microbial growth [1].

Rhizosphere is characterized by greater microbiological activity than the soil away from the plant roots. It is a selective habitat for microorganisms. The intensity of such activity depends on the distance to which exudations from the root system can migrate. India is a natural, invaluable storehouse of medicinal plant diversity of great importance for human beings [2]. Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. The medicinal plants are considered rich sources of ingredients, which are used in drug development and synthesis [3].

The *Murraya koenigii* is commonly found in outer Himalayas. It is small or medium sized tropical or subtropical tree, most famous for its aromatic leaves that provide curry spice. Curry leaves are extensively used in southern India and Srilanka but are also of some importance in Northern India [4].

The leaves are the edible part and they are shiny, dark green, aromatic and slightly bitter in taste. These leaves are one of the ingredients of Indian curries. Curry leaves improve functioning of the stomach and small intestine and promote their action. The leaves, the bark & the roots of *Murraya koenigii* spreng can be used as a tonic and a stomachic. The bark and the roots are used as a stimulant. They are also used externally to cure eruptions and the bites of poisonous animals. The green leaves can be eaten raw for curing dysentery and infusion of washed leaves stops vomiting [4].

There are plenty reports on studies on the plant extracts against human pathogens and plant pathogens. In literature, there is no report about PGPR and antimicrobial activity of microorganisms from rhizosphere soils of *Murraya koeinigii* (MK). In the present study PGP activities and antimicrobial activities of bacteria against bacterial and fungal plant pathogens is studied.

MATERIALS AND METHODS:

Isolation of microorganisms:

Bacteria were isolated from soil sample by serial dilution technique using nutrient agar by incubating at28 ± 2°C for 24 hours. Isolates were characterized on the basis of morphological, cultural and biochemical characters.

The bacterial isolates were identified on the basis of their cultural, morphological and biochemical characteristics. Bacterial isolates were studied for various enzymatic activities viz. Amylase, caseinase, catalase, oxidase and urease. All isolates were screened for PGP activities viz. NH_3, HCN, Siderophore and IAA production and PO_4 solubilization.

NH_3 production:

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10ml peptone water in each tube separately and incubated for 48-72 hours at 28 ± 2ºC. Nesseler's reagent (0.5ml) was added in each tube. Development of brown to yellow color was a positive test for ammonia production [5].

HCN production:

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lock [6]. Nutrient agar was amended with 4.4 gm glycine per liter and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5 % picric acid solution was placed in the top of the plate and plates were sealed with paraffin wax and incubated at 28± 2ºCfor 4 days. Development of orange to red color indicated HCN production.

Siderophore production:

All isolates were assayed for siderophore production on Chrome azurol S agar medium described by Schwyn and Neilands [7]. Chrome azurol S agar medium plates were prepared, and spot inoculated with bacterial and actinomycetes isolates separately and incubated at 28±2ºC for 48 -72 hrs. Development of yellow - orange halo around the growth was considered as positive for Siderophore production.

IAA Production:

IAA production was detected by the modified method described by Bric *et al* [8]. Each isolate was inoculated to 5ml of nutrient broth containing 1 mg/ml concentration of tryptophan and incubated for 72 hrs at 28 ± 2ºC. After 3 days the broth was centrifuged at 3000 rpm for 30 minutes. The cell free supernatant was collected and used for detection of IAA production.

One ml supernatant was mixed with 2ml of Salkowski reagent (2ml of 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) and tubes were incubated. Development of pink colour indicated IAA production.

Phosphate Solubilization:

The isolates were screened on Pikovskaya's agar plates individually to examine phosphate solubilization as described by Gaur [9]. The isolates were streaked on Pikovskaya's agar plates individually to examine their ability to solubilize Tri calcium phosphate. The isolates showing a clear zone of solubilization around growth indicated PO_4 solubilization.

Antimicrobial activity:

Antifungal and antibacterial activity against plant pathogens of bacterial isolates was studied by agar

well diffusion method [10]. The bacterial isolates tested for their antifungal activity were fully grown in nutrient broth media. Test fungi were grown on potato dextrose agar. The spores were scraped and suspended in 10ml of sterile normal saline solution. Diluted spore suspension (0.1ml) of the fungi was spread on Muller Hinton agar. Wells of 10 mm diameter were punched into the agar medium and filled with 200µl of bacterial culture. The plates were incubated at 28 ± 2ºC for 5-6 days for antifungal and 24 hrs for antibacterial activity. The antifungal and antibacterial activity was evaluated by measuring the zone of inhibition against test fungi and bacteria.

RESULTS AND DISCUSSION:

In the present study 13 bacterial isolates were obtained from the rhizosphere soils of *Murraya koenigii.* The Isolates were characterized on the basis of morphological, cultural and biochemical characters. The bacterial isolates were identified by using PIBWin software [11]. Some bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristics. Bergey's manual of determinative bacteriology was used as a reference to identify the isolates (Table 1). Total 13 bacterial isolates obtained from rhizospheric soil of *Murraya koenigii* were tested for their enzymatic activities and Percentage of isolates showing amylase, caseinase, catalase, oxidase and urease were reported as 61.53, 76.92, 76.92, 38.46 and 100 % respectively (Fig 1, photo plate 1& 2).

Table 1: Identification of bacterial isolates and their Id score by using PIBWin MICRO IS software

Sr.No	Isolate No	Id Score	Bacterial isolate identified as
1	MK 1	**	*Bacillus*
2	MK 2	0.94225	*Bacillus pumilis*
3	MK 3	**	*Bacillus*
4	MK 4	0.94225	*Bacillus pumilis*
5	MK 5	**	*Paenibacillus*
6	MK 6	**	*Bacillus*
7	MK 7	PIBWin	*Micrococcus species*
8	MK 8	**	*Bacillus*
9	MK 9	**	*Bacillus*
10	MK 10	0.84441	*Taxon 21*
11	MK 11	0.84441	*Taxon 21*
12	MK12	0.98339	*Micrococcus luteus 3*
13	MK 13	**	*Micrococcus*

**** Up to genus level.**

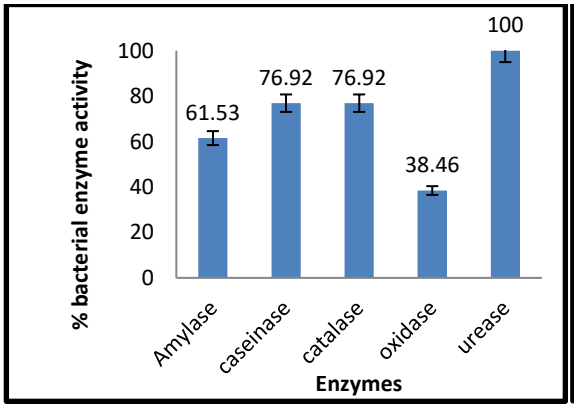


Fig 1. Percentages of Enzyme activity

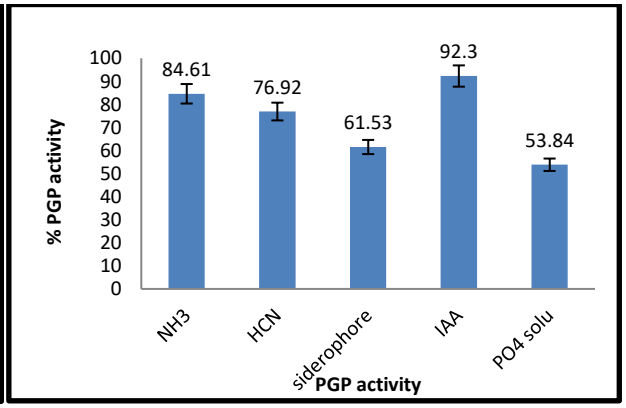


Fig 2 Percent PGP activity of isolates of bacterial isolates

PP 1 amylase activity of isolates **PP 2 Caseinase activity of isolates**

Fig 3. Graphical presentation of Indole Acetic Acid (IAA) µg/ml production by bacterial isolates

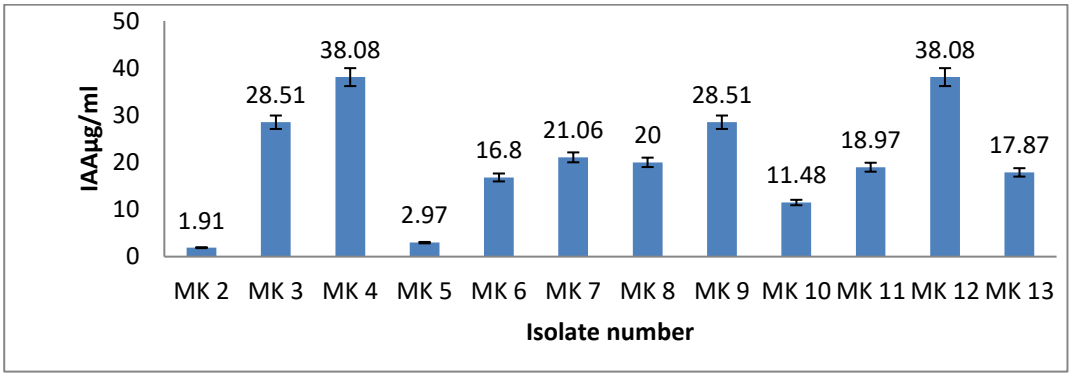


Table 2. Isolate wise amount of IAA (µg /ml) produced by rhizobacteria

Sr.No	Isolate number	IAA(µg/ml) produced
1	MK1	0
2	MK2	1.91±0.15
3	MK3	28.51±0.24
4	MK4	38.08±0.18
5	MK5	2.97±0.13
6	MK6	16.80±0.27
7	MK7	21.06±0.11
8	MK8	20±0.22
9	MK9	28.51±0.24
10	MK10	11.48±0.36
11	MK11	18.93±0.08
12	MK12	38.08±0.24
13	MK13	17.87±0.17

Table 3: Antimicrobial activity against plant pathogens:

	Zone diameter in mm against test organisms			
Isolate number	***X. citri***	***X. axonopodis***	***Fusarium oxysporum***	***Aspergillus***
MK2	-	30.1±0.854	25.86±2.031	31±1
MK 3	-	26.33±0.611	27.3±3.182	28.8±1.31
MK 4	-	29.23±0.68	-	
MK 5	-	26.3±0.608	20.56±0.418	27.33±1.527
MK 6	-	20.63±0.55	11.93±0.901	15.2±0.721
MK 9	22.4±1.24	25.66±3.785	15±7.81	21±0.901
MK 11	-	25.26±0.642	-	-

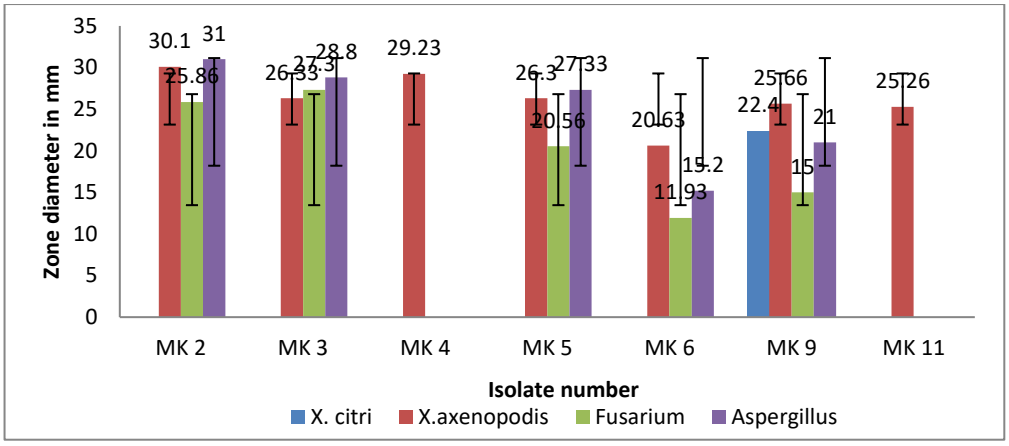
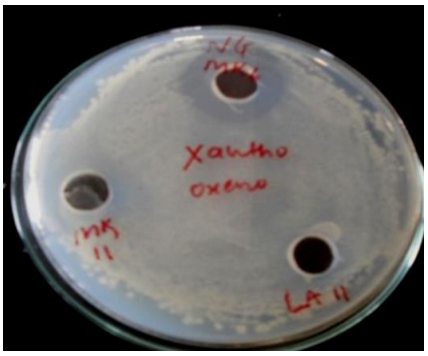


Fig 4. Antimicrobial activity of bacterial isolates from *Murraya koenigii*

PP 3 Antimicrobial activity of MK9 against *Xanthomonas axenopodis*



PP 4 Antimicrobial activity of MK4 against *Xanthomonas axenopodis*

PP 5 Antimicrobial activity of MK9 against *Asp. niger*

Pot experiment

Increase in shoot length due to treatment with bacterial isolate

PP 5 Treatment with MK9 isolate

PP 6 Increased root length and shoot length due to MK 9

Tamilarasi *et al.,* [12] enumerated diversity of rhizosphere bacteria, actinomycetes and fungi from the rhizosphere and non rhizosphere soil of 50 different medicinal plants including *Azadirachta indica, Calotropis gigantea, Murraya koenigii* in and around Bharathiar University and studied for enzymatic activities and found that 91.26% isolates produced amylase ,73.6 % isolates produced caseinase and 90.3 % isolates produced urease.

All the isolates were screened for plant growth promoting activities viz. NH_3, HCN, IAA and siderophore production and PO_4 solubilization. The results showed that not all isolates except MK3 and MK9 possessed all 5 PGP activities. The percentage of NH_3, HCN, siderophore and IAA production and PO_4 solubilization were 53%, 66.6%, 57.1%, 92.30% and 57.1% respectively (Fig.2). Malleswari and Bagyanarayana [13] reported similar results for IAA production. Ahmed [14] obtained bacterial and actinomycetes isolates from 11 medicinal plants and all the isolates were screened for plant growth promoting traits like IAA. The collected isolates possessed multiple PGP activities.

Quantification of IAA produced was also carried out colorimetrically. The results are presented in table 2 and amount of IAA produced was calculated from standard graph. The range of IAA produced by the isolates was 1.91 - 38.085 µg /ml (Fig.3).

Antibacterial and antifungal activity of isolates from *Murraya koenigii* showed significant results. Isolate MK 9 showed antimicrobial activity against all tested pathogens, *Xanthomonas citri, Xanthomonas axonopodis, Aspergillus niger* and *Fusarium oxysporum.* Isolates MK2, MK3, MK5 and MK6 showed significant zone diameters against *Xanthomonas axonopodis* (Table 3, Fig. 4), *Aspergillus niger* and *Fusarium oxysporum*. Isolate MK 11 also showed antibacterial activity against *Xanthomonas axonopodis* and antifungal activity against *Fusarium oxysporum.* MK 4 showed significant inhibition of *Xanthomonas axonopodis.* Only one isolate MK 9 showed antibacterial activity against *Xanthomonas citri*.

Malleswari and Bagyanarayana [15] studied antagonistic activity of the bacterial isolates in terms of inhibition zone diameters as an indicator if reduction of growth of plant pathogen *Macrophomina phaseolina.*

Jalgaonwala *et al.,*[16] isolated total seventy eight bacterial endophytes and one hundred forty two fungal endophytes from aerial and underground parts of selected medicinal plants like *Pongamia glabra, Curcuma longa, Murraya koeinigii, Azadirachta indica and E.globules*. The results showed that fifteen bacterial isolates and fourteen fungal isolates possess antifungal and antibacterial activities.

Pot experiment with green gram seeds and PGP bacterial isolate

Evaluation of plant growth promotion was carried out by treatment of seeds with selected bacterial isolates, sowing the seeds and studying various parameters like those that root length, shoots length, fresh weight, and dry weight [17]. There was significant increase in root length with MK9 compared to control and significant increase in shoot length with MK3 and MK9 as compared to control. There was significant increase in fresh weight with MK9 and slight increase in fresh weight with MK3.

CONCLUSION:

Many bacterial isolates from *Murraya koeinigii* showed significant results for plant growth promoting activities and antibacterial and antifungal activity against bacterial and fungal plant pathogens. These potential isolates can be used as biofertilizer to improve plant growth and bio controlling agents.

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