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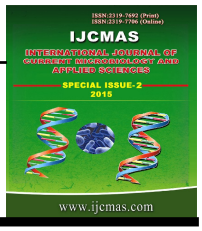


Original Research Article

Study on Production of Bioactive Compounds and Plant Promoting Ability of Endophytes Isolated from *Rosa sp.* and *Mangifera indica*

Tanmayee R. Dasari, Suchita M. Inamdar and Kshitija V. Pawar*

Department of Microbiology, Modern College of Arts, Science and Commerce, Shivajinagar, Pune-05, India

**Corresponding author*

K e y w o r d s

Endophytes, *Rosa sp.*, *Mangifera indica*, Antimicrobial activity, IAA, Bioactive compounds

A B S T R A C T

Endophytes are microorganisms which inhabit inner parts of plant tissue, without causing any disease symptoms. The present study was design to isolate and characterize the endophytes present in commercially important plants like Rose plant (Rosaceae) and Mango tree (Anacardiaceae). Samples from mango tree (leaves) and Rose plant (stem) were collected from healthy plants in the month of December. Endophytes (Bacteria, Fungi) were isolated on Potato dextrose agar, Nutrient agar and Tryptic soy agar. Characterization of isolates was carried out on the basis of morphological and biochemical analysis. Total, 8 bacterial and 5 fungal isolates of endophytes were obtained from Rose stem. In case of mango leaves, 2 bacterial and 5 types of fungal endophytes were obtained. The production of bioactive compounds by fungal endophytes was screened and further its efficiency was analysed by performing antimicrobial activity. The plant promoting activity of bacterial endophytes was checked on Wheat by performing Pot experiment.

Introduction

Endophytes are the micro-organisms which live within the plant tissue without causing any harm to the plants (Jena and Tayung, 2013). Generally, endophytes show symbiotic or mutualistic relationship with their respective host plant but due to their diverse nature they can also be opportunistic pathogens. Plant endophytic fungi had been recognized as an important source of natural bioactive compounds with applications in many industries such as agriculture, food, medicine etc. Many scientists have their interest in studying fungal endophytes as potential producers of bioactive compounds. Previous studies reveal that, some endophytes have ability to produce antibiotics, antimycotics and anticancer compounds (Dandu Anitha *et al.*, 2013). Some endophytes also have the ability to improve bioremediation which leads to the improvement of soil fertility.

Mangifera indica tree and Rosa plant are commonly cultivated in many tropical and sub-tropical regions of the world. Mango (*Magnifera indica*) belongs to the family

Anacardiaceae, is economically and commercially important tree. Previous studies have reported presence of endophytes in mango plant which includes, *Bacillus coagulans, Bacillus megatarium, Bacillus pumilus* and *Bacillus subtilis, Alternaria alternate, Phomopsis mangiferae*, etc. (Joona *et al.*, 2013).

The rose plant belongs to the family Rosaceae. It was previously found that *Bacillus* species, *Methylococcus* species, *Acinetobacter* species, *Planococcus* species, *Acetobacter* species, *Micrococcus* species inhabit in rose plant as endophytes (Bahig El-Deeb *et al.*, 2015). There is less information available about bacterial and fungal communities which are associated with different parts of rose plant.

According to previous discoveries, 1.5 million fungal species were estimated on our planet. Of these only about 10% have been discovered and nearly 1% is examined for their production of secondary metabolites (Sogra Fathima Musavi and Raj Mohan Balakrishnan, 2014).

The purpose of present study is to isolate and characterize endophytes from healthy rose stem and mango leaves for detection of plant growth promoting capabilities of isolated endophytes such as production of IAA and other bioactive compounds.

Materials and Methods

Sample collection

Healthy and mature plant was chosen for sampling purpose. The samples were collected in month of December from Agriculture College, Pune-05. The mango leaves and rose stem were cut with sterile blade and further samples were proceeding at laboratory for isolation of endophytes. Sampling and isolation procedures were carried out thrice and the microbial colonies obtained all three times showing similar characteristic were considered as endophytes.

Isolation of endophytes

Rose stem

For isolation of endophytes, the rose stems were rinsed in distilled water to remove dust and debris. After washing stems were cut into 2-3cm long pieces under aseptic condition. These pieces of stem were surface sterilized by 90% ethanol for 30 seconds and then immersed into sodium hypochloride (NaOCl) solution for 3minutesto remove the epiphytic organisms. Then these were rinsed in sterile distilled water for 4-5 times. Then it kept on sterile filter paper for surface drying. The longitudinal cut is made on stems by using sterile scalpel and the inner surface were placed downward on Nutrient agar (NA), Potato dextrose agar (PDA) and Triptic soy agar (TSA) plates. These plates were incubated at 30ºC for 48 hours, to obtain bacterial and fungal endophytes.

Mango leaves

The healthy leaves of mango were taken and surface sterilized by following same procedure as above. Then isolation of endophytes from leaves was carried out by two different methods.

In first method, after surface sterilization mango leaves were cut into 0.5mm ×1-2 cm pieces by using sterile scalpel under aseptic condition. Cell sap exuded from cut region of leaves was inoculated by placing the cut leaves region on NA, PDA and TSA. In second method, surface sterilized leaves were macerated with sterile saline by using mortal and pastel. This extract was streaked

on NA, PDA and TSA plates. Plates were incubated at 30ºC for 48 hours to obtain endophytes from veins and other tissue of leaves.

The isolates were further streaked to obtain the pure cultures. The bacterial endophytes were characterized on the basis of morphological, biochemical analysis and by referring Bergey's Manual of Determinative Bacteriology (9th edition). The fungal colonies were analysed with respect to their average diameter, coloration of the mycelium, sporulation, and coloration of the medium. Fungal mycelia were observed by following staining with lactophenol cotton blue.

Production of bioactive compounds

The fungal isolates (MLPF 1 to MLPF 5) were studied for production of bioactive compounds. Pure culture of each fungal isolate was inoculated into separate 250 ml of Erlymeyer flask containing potato dextrose broth (PDB). The flasks were incubated on shaker for 15 days at room temperature. After incubation, it is filtered through Whatmann filter paper having 0.45µm pore size for the removal of fungal growth. As it was known that fungal endophytes produced bioactive compounds, the efficiency of these bioactive compounds was checked by following antimicrobial assay.

Antimicrobial assay

The filtrates obtained from fungal isolates (MLPF 1 to MLPF 5) were checked for their antimicrobial activity against four bacteria that is Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi. Agar well diffusion method was performed in which 0.1ml cell suspension of each test organism (containing approximately 1 × 108 CFU/ml cell density) was spread on Muller Hinton agar plates. Then, 6mm wells were made on seeded media using sterile cork borer. 30µl of endophytic filtrate was loaded into each respective well, along with one negative control. Then plates were incubated at 30°C for 24 hours. After incubation plates were observed for zones of inhibition and their diameter was measure

IAA production

10 bacterial isolates (RSNB1 to RSNB8, MLNB1 and MLNB2) were grown in 10 different flasks containing Luria broth supplemented with 1mg/ml tryptophan. After 48 hours of incubation the culture was centrifuged at 10,000 rpm for 15minutes and IAA production of each bacterial isolate was checked by using Salkovaski's reagent.

Pot experiment

The bacterial isolates (RSNB1 to RSNB8, MLNB1 and MLNB2) were selected to carry out pot experiment. Wheat grains from local market were brought and grains of similar size were chosen. Total 30 grains were surface sterilized. Pot experiment was performed in triplicates for each isolate with one control each. 5ml cell suspension of each bacterial endophyte (containing approximately 1 × 108 CFU/ml cell density) was thoroughly mixed with 2 gram of cocco peat. Then, thin coat of this mixture was made on each wheat grain.

All the endophyte-inoculated seeds and control seeds were transferred in plastic pots containing sterile soil. Before putting into the plastic pots, soil was autoclaved at 121°C for three times after every 24 hours. All seedlings were cultured into sunlight and regularly watered with distilled water.

Results and Discussion

A total of 15 endophytes were obtained from these plant parts, out of which 5 fungal and 2 bacterial endophytes were obtained from mango leaves while 8 bacterial endophytes were obtained from rose stem.

Ten endophytic bacterial isolates were obtained from the rose stem and mango leaves. These were further screened for gram nature, motility and morphological characterization. The biochemical results showed that 8 out of 10 isolates were able to produce acid and utilize glucose, lactose and fructose but could not utilize sucrose. All 10 isolates were able to reduce nitrate to nitrite and could produce the catalase enzyme.

On the basis of morphological, biochemical characteristics and by referring, Bergey's Manual of Determinative Bacteriology (9th edition) RSNB 4, RSNB 5, RSNB 6 and RSNB 7 may belongs to the genus *Acinetobacter*, RSNB 8 may belong to genus *Cyclobacterium* while RSNB 1, RSNB 2, RSNB 3, MLPB 1 and MLPB 2 may belongs to genes Cellulomonas.

Five fungal isolates were obtained from mongo leaves. These were further screened for its morphological characterization by lactophenol blue staining method. On the basis of morphology and by referring Biology of fungi (1st edition) and Handbook of soil fungi MLPF1 may belongs to genus Aspergillus, MLPF2 and MLPF4 may belongs to genus *Fusarium*, MLPF3 may belongs to genus *Penicillium* and MLPF5 may belongs to genus *Alternaria*.

Antibacterial activity of bioactive compounds obtained from fungal isolates

The endophytic fungi were grown to produce bioactive compounds and filtrate obtained from each fungal isolates was further screen to determine its antimicrobial activity by performing agar well diffusion method against bacterial pathogenic strains.

The MLPF 1 showed maximum inhibition zone against Staphylococcus aureus, MLPF 2, MLPF 4 and MLPF 5 showed maximum inhibition zone against *Salmonella typhi*, while MLPF 3 showed maximum inhibition zone against *Escherichia coli* (Table no. 1, Figure 1).

Plant growth promoting endophytic bacteria

The RSNB-2, RSNB-6 and MLNB-2 showed comparatively higher IAA production while RSNB-5 and RSNB-8 showed the least production of IAA than others (Figure 2). All bacterial isolates showed different plant promoting activity. The isolate RSNB 2, RSNB 6 and MLNB 2 showed the maximum plant growth promoting ability than others which was seen in pot experiment (Figure 3). Endophytes are ubiquitous in nature and it had been found that every plant species studied to date have colonization of endophytes. It may inhabit in all available tissues of plant such as leaves, bark, roots, xylem etc. (Saikkonen *et al.*, 1998; Chapela and Boddy, 1988; Fisher et al.1993). In present study endophytes were isolated from leaves of mango and stems of rose. There is less information available about bacterial and fungal communities which are associated with different parts of rose plant; therefore we included rose plant in our study. The various parts of mango tree are used to treat many diseases such as diarrhoea, dysentery, anaemia, asthma, hypertension etc. because of the different constituents of plants which includes polyphenolics, mangiferin, tannin, gallic acid and its derivatives.

Table.1 Diameter of zone of inhibition of bioactive compounds obtained from fungal endophytes against bacteria

Sr. No.	Fungal Isolates	Diameter of Zone of inhibition in (mm)			
		E. coli	*S. aureus*	*S. typhi*	*P.aeuroginosa*
1	MLPF 1	14	17	14	No zone
2	MLPF 2	12	12	16	No zone
3	MLPF 3	15	13	14	No zone
4	MLPF 4	11	11	12	No zone
5	MLPF 5	11	No zone	14	No zone

Figure.1 Antimicrobial activity of bioactive compounds: A) MLPF 3 showing zone of inhibition against *E. coli*, B) MLPF 3 showing zone of inhibition against *S. typhi*, C) MLPF 1 showing zone of inhibition against *S. aureus*, D) MLPF 1 showing zone of inhibition against *E. coli*

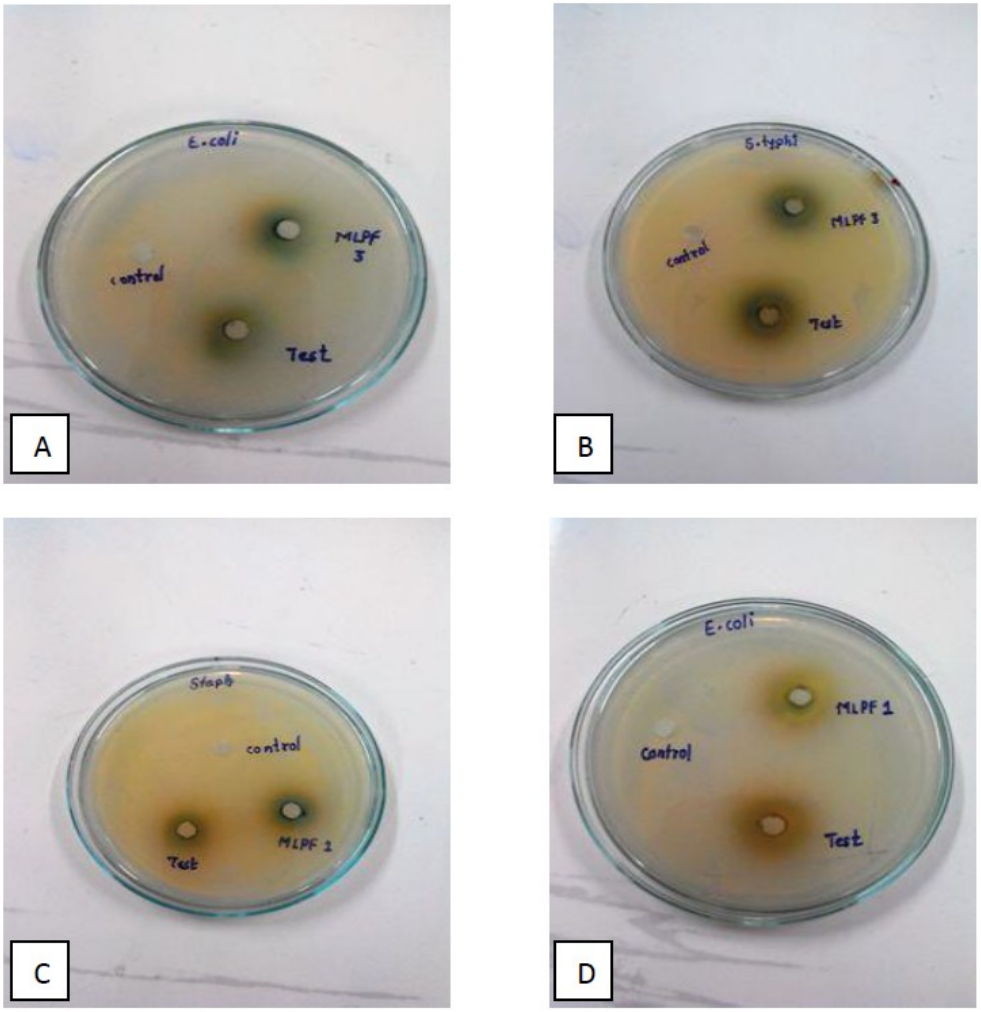
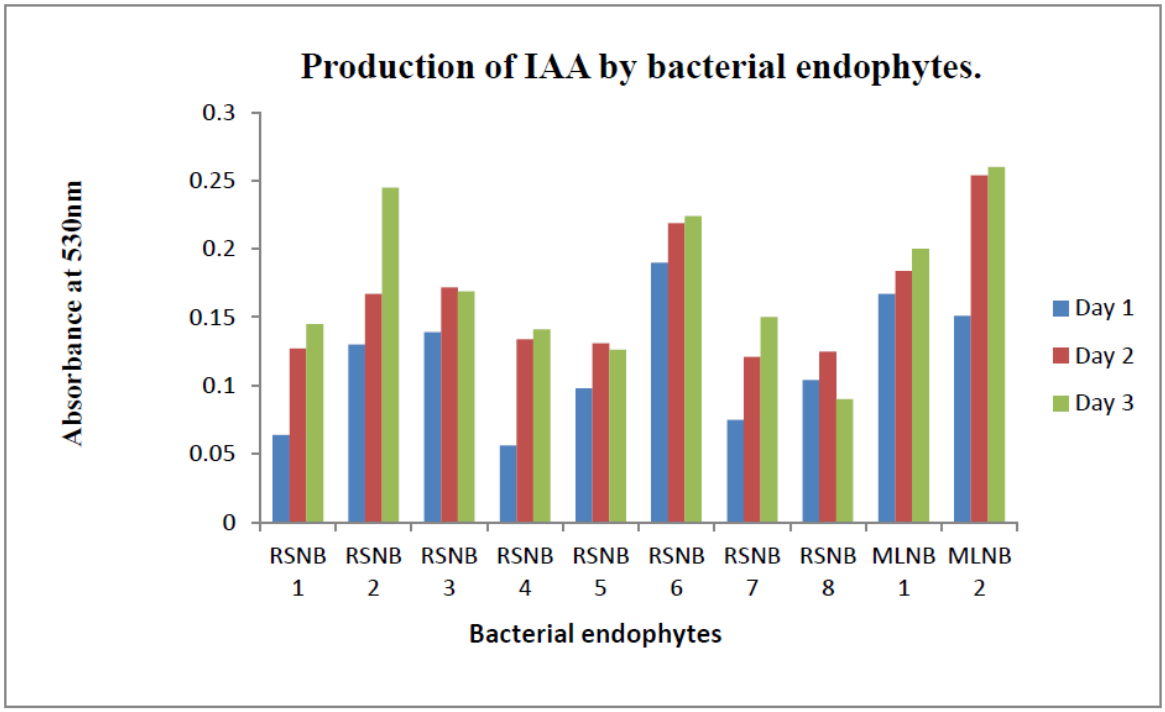


Figure.2 Production of IAA by bacterial endophytes



RS: Rose stem; N: Nutrient agar; B: bacteria,
ML: Mango leaves; N: Nutrient agar; B: bacteria

Figure.3 Pot experiment: bacterial endophytes RSNB 2 and RSNB 6 showing plant growth promoting activity on Wheat seedlings

The mangiferin and gallic acid are mainly found in leaves of *Mangifera indica*. The species reported as endophytes from mango includes *Bacillus coagulans, Bacillus megatarium, Bacillus pumilus and Bacillus subtilis, Alternaria alternate, Phomopsis mangiferae,* etc. Scientists have previously discovered that endophytes colonization gets influenced by season because depending on season, amount of plant constituents also differs. Hence, endophytes discussed in this paper may predominate during the month of December. In present study, isolated bacterial endophytes were screened based on morphology and biochemical analysis which concluded that they may belong to *Acinetobacter, Cyclobacterium* and *Cellulomonas* genera, which is not reported before. But still further 16S rRNA sequencing is required for its confirmation. These bacteria were screened for production of IAA to understand the mutualistic relationship of these isolates with its host plant. Further to analyse its relationship with non-host plants, pot experiment was performed on cereal like wheat (test plant). From obtained result, it was clear that, endophytes which showed maximum IAA production (RSNB 2, RSNB 6 and MLNB 2) also showed highest plant growth (consisting highest plant promoting activity). Hence, these isolates can further be analysed and can be used as a biofertilizers. There is an increasing need for new bioactive compounds that can be used in medicine, industry and agriculture. Endophytes are the microorganisms which have great potential as sources for new bioactive compound. Total 5 fungal isolates were obtained from mango leaves which were screened for production of bioactive compounds. Further, efficiency of those bioactive compounds was checked by performing antimicrobial assay against four human pathogens. MLPF 1, MLPF 2 and MLPF 3 showed maximum zone of inhibition against *S. aureus*, *S. typhi* and *E. coli*, respectively. Based on the results, it can be concluded that, these endophytes may provide protection to its host from various plant pathogens by producing bioactive compounds. These bioactive compounds are also useful for humans, as they have antimicrobial activity against human pathogens. The isolated fungal endophytes showed zone of inhibition against *E. coli*, *S. typhi* and *S. aureus* but these are sensitive to *P. aeuroginosa*. Therefore, these fungal endophytes can be used in medicinal and pharmaceutical industries after further sequencing and confirmation. Therefore there is a need of further studies of these isolated endophytes for better production of particular bioactive compound and for enhancement of the plant growth promoting ability of isolates.

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