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Isolation and Characterization of Bacteria that Promote Plant Growth (PGPB) from the Rhizosphere and Endosphere of Ficus Carica

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ABSTRACT

In order to promote sustainable agriculture, environmental preservation, and biotechnological developments, it is crucial to isolate and characterize rhizospheric and endospheric bacteria. The fruit of Ficus carica, commonly known as fig, belongs to the Moraceae family. The Ficus genus contains a diverse range of phytochemicals, including phenolics, polyphenols, flavonoids, tannins, anthocyanins, coumarins, volatile compounds, glycosides, saponins, carotenoids, alkaloids, triterpenoids, and vitamins. Due to this rich phytochemical profile, fig fruit holds significant value in the medical field. The goal of the study was to separate, screen, and describe (PGPB) bacteria that promote plant growth from the rhizosphere and endosphere of Ficus carica. These bacteria included those that produce phosphate solubilizers, gibberellic acid (GA), and indole acetic acid (IAA). Samples of rhizospheric soil and Ficus carica roots were gathered in order to isolate microorganisms. The study's goal is to determine whether bacterial isolates can create gibberellic acid (GA), indole acetic acid (IAA), and solubilize phosphate. We also characterized the isolates based on their cultural, morphological, and biochemical traits. Among the 15 isolates collected, five in particular showed a significant capacity for gibberellic acid (GA) synthesis, making them potential candidates for further study in agricultural applications to boost crop growth. Five isolates (IS1, IS7, IS8, IS9, and IS15) showed phosphate solubilization, suggesting their ability to transform insoluble forms of phosphate into plant-bioavailable forms. Interestingly, five of these phosphate-solubilizing isolates (IS1, IS7, IS8, IS9, and IS15) may also generate Indole Acetic Acid (IAA) and Gibberellic Acid (GA), making them extremely intriguing. While all isolates show promise, IS1 and IS15 appear to be the most adaptable in terms of promoting overall plant growth due to their high amounts of Indole Acetic Acid (IAA), Gibberellic Acid (GA), and phosphate solubility. The study's findings have the potential to assist a wide range of sectors by promoting more ecological and economically viable methods

Keywords: *Ficus carica, PGPB, IAA, GA, Rhizospheric and Endospheric microorganisms*.

1. INTRODUCTION

Botanical name: *FICUS CARICA*

Common name : Fig

Kingdom : Plantae

Family: Moraceae

Genus :*Ficus* (Badgujar *et al*., 2014)

Ficus (Moraceae) is one of the most diverse angiosperm genera, with approximately 800 tree, epiphyte, and shrub species. (Singh *et al*., 2011) Ficus L., popularly known as "Fig," is considered a keystone plant in tropical rain forests because its fruits are consumed year-round by insects, birds, and other organisms. (Chaudhary *et al*., 2012) A new study examined the medicinal benefits of figs, which are a popular fruit consumed both fresh and dry across the world. (Pourghayoumi *et al*., 2012). Fresh figs are eaten while dried fruits are employed as in vitro antioxidants. The fruits were commonly used in ancient medicine because to their multiple health benefits, which included antipyretic, tonic purgative, diuretic, astringent, carminative, laxative, stimulant against throat diseases, emmenagogue, emollient, antitussive, and resolvent characteristics. Along with its anti-inflammatory and anti-paralyzing properties, it also helps with nosebleeds and promotes hair growth **(**Kuthuru *et al*., 2022*).* In traditional medicine, fig leaves (Ficus carica L.) are frequently used as a treatment or prophylactic for various health issues (reducing blood sugar and triglyceride levels, cardiovascular disorders, etc.). Most people just call it "Fig." In the traditional medical system, F. carica leaves, fruits, and roots are used to treat a variety of illnesses, including gastrointestinal (colic, indigestion, loss of appetite, and diarrhoea), respiratory (sore throats, cough, and bronchial issues), inflammatory, and cardiovascular disorders. (Mawa *et al*., 2013) Firstly, Kloepper and

Schroth (W. Kloepper, M.N. Schroth) have given the term "rhizobacteria" to those soil bacteria that competitively colonized in plant roots and promoted growth and development of host plants with inhibition of plant diseases. Plants grow in intimate, continuing contact with microorganisms in the rhizosphere—the area surrounding roots. Plant growth-promoting rhizobacteria (PGPR) are microorganisms that live in the rhizospheres of several plants and benefit the host plant in a variety of ways. (Saeed *et al*., 2021) The effects of PGPR are contingent upon various ecological and environmental parameters, including plant species, age, developmental stage, and soil type. (Noumavo *et al*., 2016). Via a number of processes, including phosphate solubilization, nitrogen fixation, and the production of phytohormones, PGPR can increase the availability and absorption of nutrients(Arora *et al*., 2012). Plant growth promoting bacterium (PGPB) are free-living soil bacteria found in rhizosphere soil. They contribute to the fixation of atmospheric nitrogen, for example. Azospirillum bacteria are Gram-negative and have several ways to fix nitrogen through partnerships with non-leguminous plants (cereals and grasses) from varied geographical origins (Mahbouba *et al*., 2013), siderophores production(Das *et al*., 2019),mineral solubilization (Tilak *et al*., 2005) and synthesis of phytohormones (Chopade *et al*., 2008). PGPB promotes plant development through direct mechanisms such as plant hormone manufacture (auxins and cytokinins), stress-induced ethylene reduction, nitrogen fixation, and phosphate solubilization. Indirectly, they promote growth by exhibiting antagonistic activity against phytopathogenic microorganisms, facilitated by the synthesis of siderophores, antibiotics, enzymes, fungicidal molecules, and niche competition (Gamalero and Glick, 2011). Bacterial endophytes in dried Ficus carica fruit are poorly documented, and previous research has solely focused on root-associated endophytes that promote plant growth.

2. LITERATURE REVIEW

A comprehensive literature review indicated that Ficus carica, a sacred medicinal plant, is used to cure anemia, bronchitis, diabetes, fever (jaundice), hemorrhoids, inflammation, liver disorders, infectious diseases and other maladies globally.(Badgujar *et al*., 2014). PGPB/PGPR offer cost-effective and healthy alternatives to chemical fertilizers, antibiotics, herbicides, and pesticides, with the potential to promote agro-ecological sustainability. However, it is vital to recognize that PGPR having a favorable effect on one plant species may not have the same effect on others. Several bacterial and fungal strains, such as Bacillus, Pseudomonas, or Penicillium, that emit organic acids or phosphatases are capable of solubilizing phosphorus and are thus potential as PGPR (Zhao *et al*., 2020). Previous studies have demonstrated the high effectiveness of Streptomyces CMU-MH021 isolate against RKNs and fungal plant diseases. Additionally, it produced IAA and a siderophore. This strain will be propagated and tested in greenhouses with economically relevant crops. This strain may benefit multicrop and multiplant pathogen management in agricultural fields in the future (Ruanpanun *et al*., 2010). A previous study concluded that bacterial isolates identified as Pseudomonas spp. and Azotobacter spp. had potential for gibberellic acid production and could be further explored for their utilization for plant growth-promoting capacity (Desai, 2017).

3. METHODOLOGY

Soil samples were taken from three separate Ficus carica plants at Varunji and Gharewadi Karad, Maharashtra, India. Mature *Ficus carica* plants were selected, and soil samples adhering to their roots were collected as rhizospheric samples. Plant tissues, specifically roots, were aseptically harvested as endophytic samples.

A. Isolation of Rhizospheric and endophytic microorganisms of *Ficus carica*:

i. Isolation of Rhizospheric Bacteria :

Soil samples were collected under sterile circumstances from healthy plant rhizospheres. Samples were collected near the roots of Ficus carica or another plant of interest and transported in sterile containers for processing. The collected soil samples were serially diluted. 100 µL of each dilution was plated into appropriate growth media, such as nutritional agar. The samples were equally distributed using a sterile glass spreader. The plates were then incubated at 30°C for 24 to 72 hours. Following incubation, unique colonies were studied and chosen based on their morphological traits including form, size, and color. Individual colonies were selected and streaked onto fresh agar plates to produce pure cultures. To establish that the isolated microorganisms were rhizospheric, control samples were prepared from soil taken away from the plant roots. The absence of growth on control plates suggested that rhizospheric bacteria had been successfully isolated. Pure cultures were kept on agar slants at 4°C. (Aneja, 2003)

ii. Isolation of endophytic microorganisms of Ficus carica

Healthy plants were chosen for the isolation of endophytic bacteria. Plant roots were gathered under sterile circumstances. Ficus carica samples were stored in sterile bags and processed within 24 hours. Soil and debris were removed from the gathered plant tissues by carefully washing them with tap water. Surface sterilization of the tissues was carried out. :The roots were immersed in 70% ethanol for 1-2 minutes. The roots were then transferred to a 1-2% sodium hypochlorite solution for 2-5 minutes and then were rinsed in sterile distilled water 3-4 times to remove any traces of the sterilizing agents. Under sterile conditions, the sterilized plant roots were cut into small pieces (1-2 cm). The roots were crushed in a sterile mortar

and pestle or blender with sterile phosphate-buffered saline (PBS) to release endophytic bacteria. The macerated tissue extract was then placed in sterile tubes. The macerated extract was serially diluted by adding 1 mL to 9 mL of sterile water, followed by subsequent dilutions. Plate 100 µL from each dilution onto nutrient agar. A sterilized glass spreader was used to evenly distribute the sample. The plates were incubated at 30°C for 24-72 hours. Following incubation, unique bacterial colonies were identified and chosen based on their form, size, and color. Individual colonies were selected and streaked on fresh agar plates to obtain pure cultures.

To establish that the isolated bacteria were actual endophytes (not surface pollutants), a control experiment was carried out with the sterilized tissues' final rinse water. The absence of growth on control plates demonstrated that the bacteria had been successfully surface sterilized, confirming their endophytic origin. Pure cultures were stored on agar slants at 4°C until ready for use. (Aneja, 2003)

B. Screening of Isolates for IAA, GA Production, and Phosphate Solubilization

i. Screening of the isolates for IAA Production :

Isolates were inoculated into a 100 mL sterile King's broth and incubated for 48 hours. At 300°C, in a rotary shaker at 100 rpm. After incubation, culture samples were centrifuged to obtain cell-free supernatants. To 1 mL of the supernatant, add 2 mL of Salkowski's reagent. The combination was left to react for an hour in the dark. The presence of Indole Acetic acid was identified by the appearance of a pink to red color in the reaction mixture (Zhao et al., 2020). *Screening for GA Production:*

The GA production was determined using a bioassay, where the culture supernatant was applied to germinating seeds (*Phaseolus mungo*). Enhanced germination and elongation of the seedlings indicated the presence of GA. (Nedunchezhiyan *et al*., 2023)

ii. Screening for Phosphate Solubilization:

The isolates' phosphate solubilization ability was assessed by inoculating them on Pikovskaya's agar and incubating them at 30°C for 24 to 72 hours. The plates were next examined for the presence of a visible halo zone surrounding the colonies. A distinct zone suggested that phosphate had been solubilized. Each isolate was assessed for IAA, GA generation, and phosphate solubilization. (Pikovskaya R.E., 1948)

C. Secondary Screening of the isolates for the production of IAA & GA :

i. Indole Acetic Acid (IAA) Assay

A 100 mL potato dextrose broth was inoculated with isolates that tested positive for IAA generation, and they were then cultured for 48 hours. at 30°C in a 100rpm rotary shaker. Following incubation, cell-free supernatants were extracted from culture samples by centrifugation. 1 milliliter of the supernatant was mixed with 2 milliliters of Salkowski's reagent. For one hour, the combination was left to react in the absence of light. Indole acetic acid was present in the reaction mixture because of the pink to red tint that emerged. The UV spectrophotometer was used to measure the color's intensity at 530 nm. IAA was run using the normal set. By plotting the standard concentration of IAA against O.D., the concentration of IAA generated by the isolates was ascertained. (Ruanpanun *et al*., 2010)

ii. Gibberellic Acid (GA) Assay :

The isolates' ability to produce gibberellin was evaluated by a screening procedure. In particular, rhizospheric isolates were added to 250 ml conical flasks containing 100 ml of nutrient media. For 48 hours, the culture flasks were incubated at 35°C. The bacterial culture was centrifuged for 15 to 20 minutes at 10,000 rpm after 48 hours. 3.75 N HCl was used to bring the culture supernatants' pH down to 2.5. The liquid-liquid (ethyl acetate and NaHCO3) extraction procedure were then used to extract the culture supernatants.Using a UV spectrophotometer set to 254 nm, the quantity of gibberellic acid (GA3) in the ethyl acetate phase was determined in comparison to a control flask. A test tube containing equal parts of the cell-free extract and ethyl acetate was shaken vigorously for ten minutes in order to estimate GA3 using the DNPH (2,4-Dinitrophenyl hydrazine) technique. Three times, this procedure was carried out. After letting the ethyl acetate evaporate at ambient temperature, the remaining ingredients were dissolved in pure alcohol. After adding 1 milliliter of DNPH to this suspension, it was incubated for five minutes at 100°C before being cooled in a water bath. Five milliliters of 10% potassium hydroxide were added to this, and it was left to stand until a red wine hue appeared. After that, 15 milliliters of sterile distilled water were added, and the mixture was ultimately diluted to a 1:2 ratio. The UV-VIS Spectrophotometer was used to measure the color intensity at 430 nm. Various aliquots of standard gibberellic acid (0.8 mg/ml) were made with 100% alcohol and assessed in a similar way for the standard curve. (Desai, 2017)

iii. Phosphate Solubilization Assay

The diameter of the clear halos surrounding microbial colonies on Pikovskaya's agar plates was evaluated in order to assess the isolates' capacity to solubilize phosphate. On Pikovasky's agar, aliquots of isolates that tested positive for phosphate

solubilization were spot injected, and they were then incubated for 72 hours at 30 degrees Celsius. The colony's surrounding halo zone was measured and recorded. (Ponmurugan *et al*., 2006)

D. Study of Cultural, Morphological & Biochemical characteristics of the isolates

The colony characterization of well isolated colonies was recorded as their The suspension of well isolated colony was prepared and was used to study its gram nature by gram staining and motility by hanging drop technique. The biochemical tests performed were Endospore staining, starch hydrolysis, Voges-Proskauer (VP), Oxidase test, and sugar fermentation test. Identification of isolates obtained in pure cultures were characterized by morphology, colony characteristics, and various biochemical tests recommended in the Bergey's Manual of Determinative Bacteriology. (sakazaki *et al*., 1963)

4. RESULTS AND DISCUSSION

Results of Isolation & screening of IAA, GA Producing and Phosphate Solubalizing Microorganisms.

Table no. 3: Results of Primary Screening of the isolates for IAA / GA production and Phosphate solubilisation ability are represented in Table 3.1.

Table 3.1 Isolation of IAA, GA Producing and Phosphate Solubilizing Microorganisms.

Isolate	Type	IAA production	GA Production	Phosphate Solubilization
IS1	**Rhizosphere**	+	+	+
IS2	**Rhizospheric**	+	+	-
IS3	**Rhizospheric**	+	+	-
IS4	**Rhizospheric**	+	+	-
IS5	**Rhizospheric**	+	+	-
IS6	**Rhizospheric**	+	+	-
IS7	**Endospheric**	+	+	+
IS8	**Rhizospheric**	+	+	+
IS9	**Endospheric**	+	+	+
IS10	**Rhizospheric**	+	+	-
IS 11	**Rhizospheric**	+	-	-
IS 12	**Rhizospheric**	+	+	-
IS13	**Rhizospheric**	+	+	-
IS 14	**Rhizospheric**	+	+	-
IS 15	**Rhizospheric**	+	+	+

(+) Indicate Positive, (–) Indicate Negative

All 15 isolates demonstrated the ability to produce IAA, indicating their potential role in promoting root elongation, cell division, and overall plant growth.

With the exception of IS6 and IS11, GA production was seen in the majority of the isolates. GA is essential for encouraging blooming, stem elongation, and seed germination. IS1, IS7, IS8, IS9, and IS15 are among the isolates from both rhizospheric and endospheric habitats that showed a great ability for GA production. As such, they are good candidates for more research in agricultural applications to improve crop development. Phosphate solubilization was exhibited by five isolates (IS1, IS7, IS8, IS9, IS15), demonstrating their ability to convert insoluble forms of phosphate into bioavailable forms for plants. For plants to flourish, phosphate is an essential macronutrient and It may be dissolved by microbes, which helps to increase soil fertility and nutrient availability. Interestingly, five of these phosphate-solubilizing isolates (IS1, IS7, IS8, IS9, IS15) are also capable of both IAA and GA production, making them particularly promising.

Table no. 4: Results of secondary screening of the isolates for IAA, GA production are represented in Table no. 4.1

Table no. 4.1: Result of Quantitive analysis of IAA produced by promising isolate

Isolate	**Amount of IAA produced (µg/ ml)**	**Amount of GA produced (ug/mL)**	**Zone of diameter of halo zone around the colony Phosphate solubilization**
IS1	34	0.45	4.5
IS7	22	0.65	3.2
IS8	23	0.39	4.9
IS9	22	0.43	2.1
IS15	19	1.5	2.9

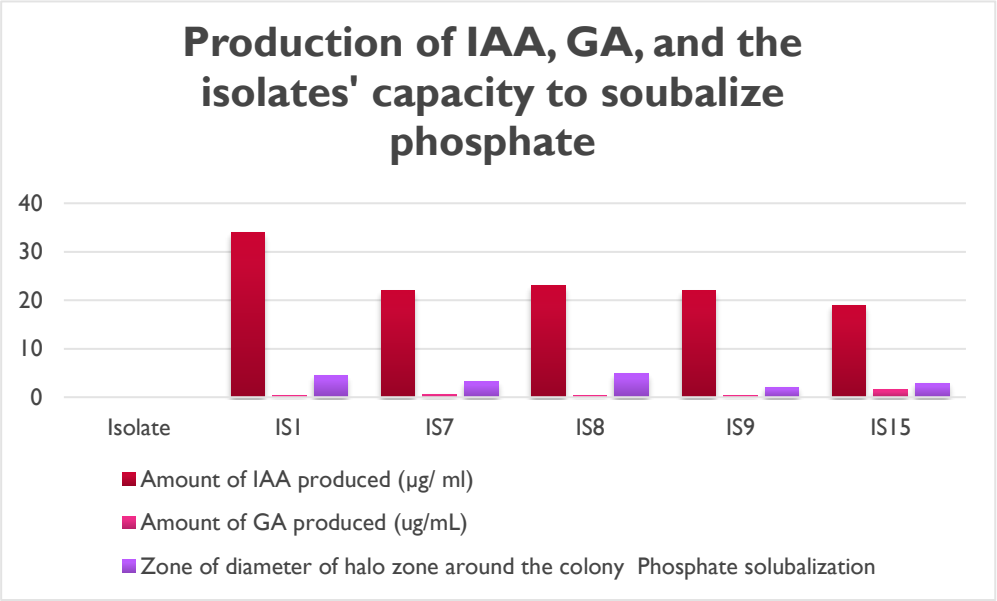

Figure 1: IAA, GA Production, Phosphate solubilizing ability of the Isolates

The highest amount of IAA (34 µg/mL) was created by IS1, followed by IS8 (23 µg/mL) and IS7 (22 µg/mL), suggesting that they might also favorably influence root development. IS9 and IS15 produced slightly lower levels of IAA (22 µg/mL and 19 µg/mL, respectively), though still within a range that suggests significant plant growth promotion.

Gibberellic Acid (GA) is another important plant hormone, contributing to processes such as seed germination, stem elongation, and flowering. The isolates also demonstrated variability in GA production: IS15 produced the highest amount of GA (1.5 µg/mL), indicating that it could be particularly effective in promoting stem elongation and possibly early flowering in plants. IS7 (0.65 µg/mL) and IS1 (0.45 µg/mL) exhibited moderate levels of GA production, IS9 (0.43 µg/mL) and IS8 (0.39 µg/mL) produced the lowest amounts of GA.

One important metric of how well these isolates can make phosphate accessible to plants and enhance nutrient absorption is their capacity to solubilize phosphate, as indicated by the diameter of the halo zone surrounding the colony IS8 exhibited the largest halo zone (4.9 mm), IS1 also showed a substantial halo zone (4.5 mm), indicating that it might significantly improve the availability of phosphate. IS7 and IS15 demonstrated moderate solubilization with halo diameters of 3.2 mm and 2.9 mm, respectively, suggesting they have a fair ability to solubilize phosphate. IS9 produced the smallest halo zone (2.1 mm), suggesting that its ability to solubilize phosphate is somewhat limited compared to the other isolates. The results indicate that IS8 and IS1 have the strongest phosphate solubilizing capabilities, which could be particularly beneficial in phosphorus-deficient soils.

Table 4.2: Results of Identification of promising isolates based on their cultural, Morphological and biochemical characteristics.

Isolate	Tentative Identification
IS1	*Azotobacter spp*
IS7	*Bacillus subtilis*
IS8	*Serattia marscens*
IS9	*Pseudomonas aeruginosa*
IS15	*Micrococcus luteus*

Several studies have revealed that *Azotobacter*, *Bacillus*, and *Pseudomonas* species belong to the class of microorganisms known as plant growth-promoting rhizobacteria.

Bacillus species are the most abundant bacteria in the plant rhizosphere. According to (Hashem *et al*., 2019) They extend the life of plants, shield them from diseases and stress, and release metabolites and hydrolytic enzymes (proteases, β-glucanases, and cellulases) to encourage plant growth. *Bacillus* is noted for its ability to manufacture a variety of secondary metabolites, hormones, cell-wall-degrading enzymes, and antioxidants, thereby boosting the plant's defense against pathogen attacks. *Azotobacter* species (by the synthesis of physiologically active compounds, the generation of phytopathogenic inhibitors, nitrogen fixation in soil, and the balancing of nutrient intake) may create favorable impacts on crop growth and output. (Sumbul *et al*., 2020) *Serratia spp.* strains, as endophytic and rhizospheric microorganisms, when interacting with plants, not only contribute positively to stimulating plant growth, increasing yields, and improving soil quality normally, but it can also be utilized in environments exposed to various abiotic and biotic stresses. (Verma *et al*., 2019) Additionally, *P. aeruginosa*, *P. cepacia*, and *P. fluorescens* have anthelmintic, antibacterial, antiviral, cytotoxic, and antitumor characteristics that can combat phytopathogens. (Bhavya and Geetha 2021)

Fig 2: Results of Qualitative assay of IAA production.

5. CONCLUSION

Isolate IS1 exhibited the highest IAA production (34 µg/mL) and strong phosphate solubilization (4.5 mm halo), along with moderate GA production (0.45 µg/mL), making it a highly effective plant growth-promoting agent. **IS15** demonstrated the highest GA production (1.5 µg/mL) but lower IAA (19 µg/mL) and moderate phosphate solubilization (2.9 mm), indicating its potential in promoting stem and shoot growth. **The maximum phosphate solubilization (4.9 mm) was seen in isolate IS8** and moderate IAA production (23 µg/mL), though it had relatively low GA output (0.39 µg/mL). **Isolate IS7** produced moderate levels of all traits, making it a well-rounded candidate, while **Isolate (IS9)** showed moderate IAA production (22 µg/mL) but lower GA production (0.43 µg/mL) and phosphate solubilization (2.1 mm), suggesting it may have more

specialized growth-promoting functions. The data indicate that while all isolates are capable of IAA production. While all isolates exhibit promising characteristics, **Since IS1 and IS15 have significant amounts of IAA, GA, and phosphate solubilization capabilities, they seem to be the most adaptable in terms of promoting plant growth overall.** These isolates would make excellent candidates for more research into creating inoculants or biofertilizers to increase crop output. However, more research should be done on the molecular characterization of the isolates, product measurement, and production optimization.

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