Research Article

# Plant Growth Promotion in *Solanum lycopersicum* through Endophytic bacteria Isolated from Flower of *Samanea saman*

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## ABSTRACT

**Aim:** Plant Growth Promotion in *Solanum lycopersicum* through Endophytic bacteria isolated from Flower of *Samanea saman* and antifungal activity against *Fusarium oxysporum*. **Materials and Methods:** Endophytes were isolated from the Rain Tree Flower samples collected from Sadakathullah Appa College Campus, Rahmath nagar, Tirunelveli, Tamil Nadu, India and assessed for their protective activity against *Fusarium oxysporum*, which causes wilt disease. The flower samples washed in tap water for removing dirt particles and it is soaked in 70% ethanol for 3 min; further washed with sodium hypochlorite solution (2.5% available Chlorine) for 5 min and cleaned well with sterile distilled water. Flower sample were crushed with a sterile mortar and pestle and then plated on nutrient agar media. The plates were incubated for 3 to 5 days at 28°C for bacterial growth. bacterial strains was tested antagonism activity against *Fusarium oxysporum* using Potato dextrose agar. The actively growing fungal (*F. oxysporum*) here mycelial disc (5 mm) was inserted at one side and a loop of endophytic bacteria was streaked in a line of about 6 cm in length. One control plate was also kept for observation. Those plates were incubated at 28±2°C for 7 days and after inhibition zone of inhibition was measured. **Results and Discussion:** In the present study, a total of 2 endophytes SRFB1 and SRFB2 were isolated from the Rain Tree Flower samples, among them, endophyte SRFB1 showed significant results on shoot and root length, fresh and dry weight, respectively, when compared with the control. One of the isolates considered as efficient endophytic isolate as it possessed plant growth promotion and SRFB1 showed positive results against *Fusarium oxysporum*. **Conclusion:** The present study shows that the endophytes isolated from the flower samples can be commercially formulated as effective bio control agent against soil-borne fungal pathogens which affects plants. In future those formulated endophytes can be served as a good replacement against chemical fertilizers which cause had effect to agriculture.

**Keywords:** *Endophytes, Solanum lycopersicum, Fusarium oxysporum, Samanea saman,* Plant Growth Promotion.

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## INTRODUCTION

Chemical fertilizers, manures and insecticides are widely used techniques for increasing crop yields. Which mostly harm the environment by nitrate leaching into groundwater, phosphorus and nitrogen running off the surface and eutrophication in aquatic environments.[1] Efforts have been made to reduce the usage of chemical pesticides and fertilizers by substituting (or combining) with environmentally friendly products, including beneficial microorganisms which leads to environmental sustainability.[2-4] The microorganisms and plants have mutually beneficial relationships.[5] Which are crucial to food and agricultural safety. The microorganisms

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reside inside the plant may live in the phyllosphere and rhizosphere, known as endophytes.[6] The plant endophytic bacteria coexist in symbiotic relationships with host plant that promote the growth and protection from biotic and abiotic challenges. Endophytic bacteria lives in the xylem and root cortex of the plant involved in exchange of nutrients and enzymes.[7,8] Endophytes invade the tissues of the hosts, multiply without posing a threat to the host's health and promotes the growth of the plant. Endophytic bacteria stimulate the plant growth and health through phytostimulation, biofertilization and biocontrol methods.[9] Tomatoes (*Solanum lycopersicum*) are the most important significant agricultural crop which are high in minerals like potassium, iron, etc., and plants with phytochemicals like lycopene and vitamin C. those plants has phenolic components such as flavonoids, hydroxycinnamic acid, chlorogenic acid, homovanillic acid and ferulic acid. Tomato (*Solanum lycopersicum* L.) is an essential part of the daily diet, eaten both as fresh, unprocessed fruit and in a variety of processed forms.[10] So a nutrient-dense food that may be eaten raw or cooked and can contribute significantly to a balanced diet.[11] The fungus *Fusarium oxysporum,* causes wilt disease, in tomato crops with great susceptibility due to the pathogen's extensive host range and soil persistence. This is one of the most critical diseases impacting tomatoes globally.[12] *Fusarium* wilt causes significant losses in susceptible tomato varieties, particularly when soil and air temperatures are high during the warm season.[13] The pathogen causes a significant yield loss, ranging from 25% to 55%, in optimal infection conditions, yield loss can be as high as 90%.[14] The pathogen can be managed through soil disinfection using methyl bromide, chloropicrin, or metham sodium, as well as systemic fungicides like benomyl, thiabendazole and thiophanate. However, sustainable fungicide uses in managing *Fusarium oxysporum* is challenging due to the development of resistant isolates and their detrimental effects on the natural environment, agroecosystems and human health.[15] The management of this organism using chemicals was highly challenging, costly and harmful to the environment. It is necessary to go for a substitute or alternate eco-friendly device. As a result, substitute strategies must be devised and evaluated. The present study was designed to isolate the endophytic bacteria from the flowers of Rain tree (*Samanea saman*) and examine its growth promotion and protection against fungal pathogen (*Fusarium oxysporum*) in the *Solanum lycopersicum* and evaluate its efficiency.

## MATERIALS AND METHODS

### Collection and isolation of endophytes

Rain Tree Flower (*Samanea saman*) Samples were collected from Sadakathullah Appa College Campus, Tirunelveli, Tamil Nadu, India.

The flower samples were washed in tap water for removing dirt particles and it is soaked in 70% ethanol for 3 min, further washed with sodium hypochlorite solution (2.5% available Chlorine) for 5 min and cleaned well with sterile distilled water. Flower samples were crushed with a sterile mortar and pestle and then plated on nutrient agar media. The plates were incubated for 3 to 5 days at 28ºC for bacterial growth.[16]

### Morphological Characterization of isolates

The isolated endophytic bacteria were cultured in NA agar plates using the quadrant streak method and incubated for 3 days. After 3 days of incubation, different characteristics of colonies, such as size, colony diameter, colour, form, surface, elevation and margin were recorded.

### *In vitro* antibiosis test

#### *Dual culture technique*

In this method, the bacterial strains was tested for antagonism activity against *Fusarium oxysporum* using Potato dextrose agar. The actively growing fungal (*F. oxysporum*) here mycelial disc (5 mm) was inserted at one side and a loop of endophytic bacteria was streaked in a line of about 6 cm length. One control plate was also kept for observation. Those plates were incubated at 28±2ºC for 7 days and after inhibition zone of inhibition was measured. The fungal hypha growth was measured, and abnormalities seen in zone of inhibition was recorded and compared with control plates.

### *In vivo* screening-Endophytes on plant growth promotion

#### *Sterilization of seed*

Tomato seeds were soaked in 70% (v/v) ethanol for 2 min and immersed 0.2% (v/v) sodium hypochlorite and after washed with sterile distilled water for surface sterilization.[17] The seeds were dried overnight under sterile conditions.

### Seed bacterization

Endophytic isolate SRFB1 and SRFB2 were grown on nutrient agar for 24 hr at 28±2ºC, culture were scrapped from the plates and finally suspended in sterile 1% Carboxymethylcellulose (CMC) to a concentration of

approximately $10^7$ CFU/mL. 1 g of surface sterilized tomato seeds were soaked in endophytic bacterial suspension with CMC for 30 min and after that it was kept for drying overnight in sterile plates. Seeds treated with 1% CMC to take as the control.

### Plant growth assay

Bacterized seeds were transferred to plastic pots (5 seeds per pot) containing non-sterilized soil and cow dung in the ratio of 4:1 and kept in a greenhouse. A control pot without bacterized seeds was also maintained. The pots were kept at 26±2ºC, RH of 90% observed regularly. These pots were photoperiods of 16 hr for 60 days. Every day the plants were watered and observed keenly. The measurement of plant shoot and root length, fresh and dry weights were noted and recorded for 10th and 20th intervals (Figure 1).

## RESULTS

### Isolation of endophytic bacteria

Rain tree flowers were collected from Sadakathullah Appa College Campus, Tirunelveli, Tamil Nadu, India. Sterilized and further 2 endophytic bacteria were isolated from those flowers. The isolated bacteria were named SRFB1 and SRFB2 and kept for further Studies (Figure 2).


**Figure 1: Plant growth promotion study.**

### Morphological Characterization of PGPE isolates

Morphological characteristics of the colonies of each isolate were examined on Nutrient agar (Table 1).

### *In vitro* antagonistic activity

The endophytic isolates were screened for their efficiency in inhibiting plant pathogen *F. oxysporum*. Among the isolates SRFB1 and SRFB2, SRFB1 showed the highest clear zone of inhibition of (12 mm) against *F. oxysporum* (Table 2). SRFB1 was a potent antagonist against *Fusarium oxysporum* (Figure 3).

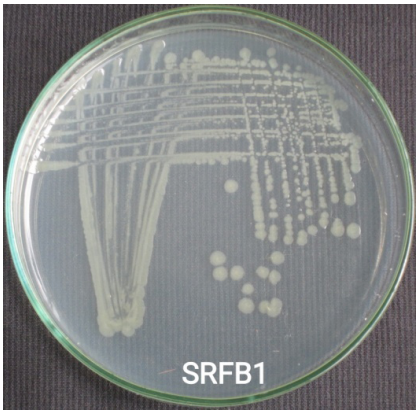


**Figure 2: Endophyte SRFB1 Isolated from Rain tree flower.**

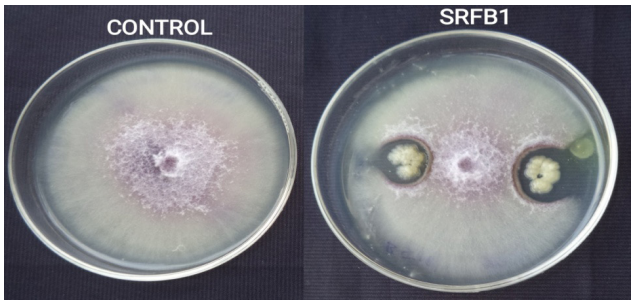


**Figure 3: Antifungal Activity of Endophyte SRFB1 against *Fusarium oxysporum*.**

### *In vivo* Plant growth promotion studies

The isolated endophytic bacteria SRFB1 showed the maximum shoot length and root length 20.6 cm and 11.52 cm when compared with control in the 20th day, respectively. The SRFB1 showed a significant difference in shoot length and root length when compared to control in the 10 and 20 days (Figure 4). The highest Biomass, 1.318 g was observed in SRFB1 treated tomato plant. The dry weight also showed a higher weight of 0.462 g than the control (Figure 5).

**Table 1: Morphological Characterization of PGPE isolates.**

| Sl. No. | Strain | Size | Colony diameter | Colour | Form | Surface | Elevation | Margin | Opacity |
|---|---|---|---|---|---|---|---|---|---|
| 1 | SRFB1 | Large | 3 mm | White | Circular | Smooth | Flat | Entire | Opaque |
| 2 | SRFB2 | Large | 4 mm | Yellowish white | Irregular | Dry | Flat | Undulate | Opaque |

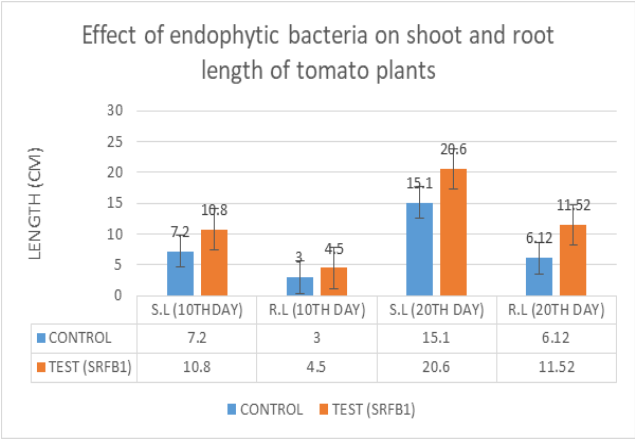


**Figure 4: Effect of endophytic bacteria on shoot and root length of tomato plants.**

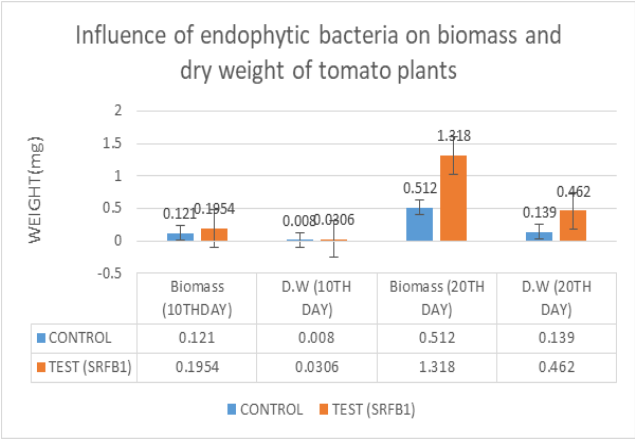


**Figure 5: Influence of endophytic bacteria on biomass and dry weight of tomato plants.**

**Table 2: Antifungal Activity of Endophytic isolates against *Fusarium oxysporum*.**

| Sl.No | Strain code | Zone of Inhibition (mm) |
|---|---|---|
| 1 | SRFB1 | 12 |
| 2 | SRFB2 | - |

## DISCUSSION

In this study, we report the isolation of 2 strains thus, *in vitro* antifungal test[18,19] and green house study[20] investigating the use of a fungal antagonist to suppress or eradicate pathogens. We isolated culturable bacterial endophytes from Rain tree flower and explored their potentiality as PGPB. The bacterial community associated with the pods of rain tree has been previously studied.[21] However, this is the first study describing the presence of bacteria in the flower of rain tree. In the early stages, bacterial endophytes are crucial for sustaining plant health, helping with nutrient mobilization and promoting plant growth.[22] Therefore, identifying and analyzing bacterial endophytes from flowers may help choose strains that have the potential to increase tomato plant growth. This is the first report of endophytes from rain tree flower that promote plant growth; although certain distinct bacterial species have been shown in the past to facilitate rice seedling growth, endophytes isolated from maize rhizospheres has recently improved rice growth and yield by changing the root architecture.[23] Previous research has shown that inoculating plants with microorganisms that promote plant growth can have positive effects on their growth. For example, inoculating *Zea mays* L. with *Pseudomonas tolaasii* IEXb and *Pseudomonas koreensis* SP28 has been shown to stimulate plant height and shoot weight.[24] Kumar *et al.* reported that various endophytic strains of Bacillus and Pseudomonas demonstrated positive results in plant growth-promoting trait analyses.[25] Fang *et al.*, reported that the strain *Pseudomonas aurantiaca* JD37 exhibited excellent colonization potential and promoted maize growth.[26] Our study also reported similar observations following seed treatment with endophytic bacteria in tomato plants. In this study, Tomato plants inoculated with PGPB endophytic bacterial strain SRFB1 showed a greater increase in shoot and root length, as well as in fresh and dry weight, compared to the control. Therefore, this study represents an ecological method for detecting the plant growth-promoting features of seed endophytes interacting with tomato plants during the early phases of plant development. Fungal pathogens are preferred as test organisms for antagonistic confrontation assay due to their wide host range, prolific growth and ability to cause major economic loss in various crops.[27] The biocontrol of fungal phytopathogens is an indirect mechanism by which symbiotic bacteria can promote plant growth. Bacterial seed endophytes from the genera *Pseudomonas, Pantoea, Paenibacillus* and *Bacillus* have shown antifungal activity against some phytopathogenic fungi such as *Curvularia* sp., *Fusarium oxysporum*, *Pythium ultimum*, *Rhizoctonia solani* and *Pyricularia grisea*.[28] Radhakrishnan *et al.*, focused on the endophytic bacterium showed significant antifungal activity against *Fusarium oxysporum*.[29] Our study also made similar observations regarding the antifungal activity of endophytic bacteria against *Fusarium oxysporum*. In this study, we found that *SRFB1* inhibited the growth of the phytopathogenic fungus *Fusarium oxysporum* (Table 2). The results of this study have shown that strains SRFB1 have the ability to promote plant growth and operate as biocontrol agents.

## SUMMARY

Biological control agents used as crucial tools in agricultural biotechnology for reducing the use of chemical pesticides. In this study the two endophytic bacterium associated with the rain tree flower (*Samanea saman*) were isolated characterized and applied for the growth of tomato plants. The research focuses on improving tomato plants, testing efficacy, in green house conditions and selecting the potent isolates for PGPR in crop colonization may have a wide range of hosts. One of these strains namely SRFB1 has been shown to progressively promote plant growth in tomato plants. This one strain has the potential to be used as biofertilizer, biostimulant and biocontrol agents; *In vitro* tests demonstrated that the bacterial endophyte SRFB1 performs well against the fungal pathogen *Fusarium oxysporum*, which support for further research, in discovering novel species that can promote these protective effects. Therefore, this study suggests that endophyte-inoculated plants may have increased resistance to pathogens such as *F. oxysporum*. The strain SRFB1 may be selected for the development of biostimulants for enhancing tomato plant growth. This is one of the pioneer studies in the field of agricultural biotechnology.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**NA:** Nutrient Agar; **PDA:** Potato Dextrose Agar; **PGP:** Plant Growth Promotion; **SRFB:** Sadakathulla Appa College Rain tree Flower Bacteria; **CMC:** Carboxymethylcellulose.

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