

## ENUMERTION OF ENDOPHYTIC BACTERIA FROM *SOLANUM TRILOBATUM* L.

\*Bhuvaneswari, S.<sup>1</sup>, Madhavan, S.<sup>2</sup> and Panneerselvam, A.<sup>3</sup>

<sup>1</sup>PG Department of Microbiology, Shrimati Indira Gandhi College, Trichy-620 002. Tamil Nadu, India.

<sup>2</sup>PG Department of Botany, Mannai Rajagopalaswami Government Arts College, Mannargudi, Tamil Nadu, India.

<sup>3</sup>PG and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur, Tamil Nadu, India.

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\*Correspondence for  
Author:  
Bhuvaneswari, S.,  
PG Department of  
Microbiology, Shrimati Indira  
Gandhi College, Trichy-620  
002. Tamil Nadu, India.

### ABSTRACT

Endophytic bacteria are beneficial microbes that reside in living plant tissues, mainly in the intercellular space and inside vascular tissues, without either doing harm to the host or providing any benefit to other microbial residents. Endophytes can be useful for prospection of bioactive compounds that may have medical and pharmaceutical applications. The aim of the present work was to investigate the bacterial endophytes from the medicinal plant *Solanum trilobatum* L. Totally 15 bacterial endophytes were isolated by using Nutrient agar and LB medium. Among these, twelve isolates were Gram positive and three isolates were Gram negative. The bacterial endophytes were partially characterized by biochemical test. Isolated bacterial endophytes might be useful to its respective host *Solanum trilobatum*

L. and might be producing economically and pharmaceutically important bioactive compounds.

**Keywords:** Endophytic bacteria, medicinal plant, *Solanum trilobatum*, bioactive compounds

### INTRODUCTION

Endophytic bacteria are defined by their ability to colonize plant tissues without causing symptoms or morphological changes (Strobel *et al.*, 2004). Generally Endophytic bacteria are

minor inhabitants than rhizosphere bacteria or bacterial pathogens. Endophytic bacteria in particular plant host are not limited to a single species but comprise several genus and species. Endophytic bacteria have been isolated from both Monocotyledonous and Dicotyledonous plants, ranging from woody tree species to herbaceous crop plants. Studies on the interaction between plants and Endophytic microorganism show that these organisms perform different and important ecological functions they cut to promote plant growth (Ahmad *et al.*, 2008) and produce biologically active secondary metabolites many of which are important for biotechnology. Bacterial endophytes can produce novel natural products found in their host plant (Mehanni and Safwat, 2010).

*Solanum trilobatum* Linn (Family: Solanaceae) is one of the important medicinal plant, more commonly available in Southern India. *S. trilobatum* is an extensively used Indian traditional medicine to cure numerous diseases viz., tuberculosis, respiratory problems and bronchial asthma (Govindhan *et al.*, 2004). *S. trilobatum* contains chemical compounds like Sobatum,  $\beta$ -solamarine, solasodine, solaine, glycoalkaloid and diosogenin. Sobatum the partially purified petroleum ether extract of *S. trilobatum* was reported to be very effective in tumor reduction (Mohanan and Devi, 1996). It also possess antiulcerogenic activity (Amir and Kumar, 2004) and ovicidal activity (Rajkumar and Jebanesan, 2005). Hence in the present investigation, the bacterial endophytes were isolated and identified from *S. trilobatum* L..

## MATERIALS AND METHODS

### Collection of plant

*Solanum trilobatum* L. plant (Fig.1) was collected from Mannargudi, Thiruvarur Dt., Tamilnadu, India. The botanical identity was confirmed with Herbarium centre, Dept of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Aut.), Poondi, Thanjavur, Tamilnadu, India.

### Scientific classification of *Solanum trilobatum* L.

**Class:** Magnoliopsida

**Order:** Solanales

**Family:** Solanaceae

**Genus:** *Solanum*

**Species:** *trilobatum* L.

**Vernacular name in Tamil:** Thoodhuvalai



**Fig. 1.** *Solanum trilobatum* L.

### **Surface sterilization**

Fresh healthy leaves were taken and washed with running tap water to soil particles and were surface sterilized by sequential immersion in 70% ethanol for 5 minutes and a solution of sodium hypochlorite (0.9% available chlorine) for 20 minutes. Then, leaves were washed three times in sterile distilled water to remove surface sterilizing agents before being seeded in 10% NaHCO<sub>3</sub> (Sodium Bicarbonate) solution to disrupt the plant tissues and to inhibit the growth of fungi (Cao *et al.*, 2003). Surface sterility test was performed for each of the sample to ensure the elimination of surface micro organism.

### **Isolation of endophytic bacteria**

Nutrient agar (NA) (Beef extract - 3 g/l, peptone - 5 g/l, sodium chloride - 5 g/l, agar - 15 g/l,) and Luria Bertani (LB) agar medium (Tryptone - 10g/l, yeast extract - 5g/l, sodium chloride - 5g/l, agar -15g/l) was used for isolation of endophytic bacteria. The leaves were divided into small fragments (1-3cm) with sterile surgical blades under aseptically transferred to the NA and LBA medium. Then the plates were incubated at 37°C for 2 to 4 days. Endophytic bacterial cultures growing on selective media were isolated, purified and maintained on slopes of nutrient agar for further study.

### **Identification of endophytic bacteria**

The selected endophytic bacterial strains were identified by morphological, biochemical characterization. In morphological characterization, macroscopic and microscopic features of the selected isolates were studied, additionally an array of biochemical test including catalase, oxidase, indole, methyl red, voges proskauer, triple sugar iron, citrate utilization,

urease, nitrate reduction and carbohydrate fermentation test were performed the standard protocols (Smibert and Krieg, 1995; Sneath, 2001).

## RESULT AND DISCUSSION

Endophytes from medicinally important plants are of a great interest, especially in understanding their potential medicinal properties and to explore their potential applications (Mehanni and Safwat, 2010; Qin *et al.*, 2011).

In the present study, totally 15 endophytic bacteria were isolated from leaves of *Solanum trilobatum* L by using Nutrient Agar and Luria Bertani agar medium (Plate 1). The key of endophyte isolation is the efficacy of surface sterilization. The routine surface disinfection techniques were commonly used before isolation of endophytes (Larran *et al.*, 2001.). To isolate from inner tissues of plant the sterilized distilled water which had been used to wash the sterilized leaves was cultured on nutrient agar plate. If there were no colonies formed within 2-3 days, it was disinfected thoroughly and the isolated bacteria were proven endophytic bacteria.

The endophytic bacterial isolates were identified by morphological, biochemical characterization such as gram staining, motility, catalase, oxidase, indole, methyl red, voges proskauer, triple sugar iron, citrate utilization, urease, nitrate reduction and carbohydrate fermentation test. The results were depicted in table 1 and Plate 2 & 3.

Likewise, bacterial endophytes have been reported from various medicinal plants; for examples, *Piper nigrum*, *Gynura procumbens*, *Strobilanthes crispata*, *Vernonia amygdalina* and *Aquilaria* species (Aravind *et al.*, 2009; Bhore *et al.*, 2010; Bhore and Tiong, 2012 and Bhore *et al.*, 2013). However, this is the first study to elucidate diverse types of bacterial endophytes in *Solanum trilobatum* L. Our research findings could be useful, as a foundation for further research on endophytic bacteria for its economically and pharmaceutically important bioactive compounds.

Morphological and Biochemical Characterization of Endophytic Bacteria from *Solanum trilobatum* L.

S. NO	Gram staining	Motility	Indole	MR	VP	Catalase	Oxidase	Citrate	Nitrate reduction	TSI	Urease	Carbohydrate fermentation	Endophytic bacteria
1.	+ve coccus	Non motile	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Fermentative microbes & alkaline	-ve	+ve	<i>Deinococcus radiopugnans</i>
2.	+ve cocci	Non motile	-ve	-ve	+ve	-ve	+ve	-ve	+ve	Acid butt & gas production	+ve	+ve	<i>Melissococcus pluton</i>
3.	+ve coccus	Non motile	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Fermentative microbes	+ve	-ve	<i>Saccharococcus thermophilus</i>
4.	-ve cocci	Non motile	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Gas production, Fermentative	-ve	+ve	<i>Brucella melitensis</i>
5.	+ve cocci	Non motile	-ve	+ve	+ve	+ve	+ve	-ve	+ve	Alkaline slant	-ve	+ve	<i>Stomatococcus mucilaginosus</i>
6.	+ve cocci	Non motile	-ve	+ve	+ve	-ve	+ve	+ve	+ve	Fermentative microbes & alkaline	-ve	+ve	<i>Streptococcus pyogenes</i>
7.	+ve cocci	Non motile	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Alkaline slant	+ve	+ve	<i>Staphylococcus aureus</i>
8.	+ve cocci	Non motile	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Alkaline slant	+ve	-ve	<i>Salinococcus roseus</i>
9.	+ve cocci	Non motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	Alkaline slant	+ve	-ve	<i>Gemella haemolysans</i>
10.	-ve Rod	Non motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	Alkaline slant & Acid butt	+ve	+ve	<i>Klebsiella pneumoniae</i>
11.	-ve Rod	Non Motile	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Alkaline slant & Acid butt	+ve	+ve	<i>Buttiauxella agrestis</i>
12.	+ve cocci	Non motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	Alkaline slant & Acid butt	+ve	+ve	<i>Pediococcus damnosus</i>
13.	+ve Rod	Motile	-ve	-ve	+ve	+ve	-ve	+ve	+ve	Gas Production & Acid butt	-ve	+ve	<i>Bacillus subtilis</i>
14.	+ve coccus	Non motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	Alkaline slant & Acid butt	+ve	+ve	<i>Coprococcus eutactus</i>
15.	+ve coccus	Non motile	-ve	-ve	-ve	-ve	+ve	+ve	+ve	Alkaline slant & Acid butt	+ve	+ve	<i>Aerococcus viridans</i>

MR-Methyl Red; VP-Voges Proskauer; TSI-Triple Sugar Iron; + Positive; - Negative

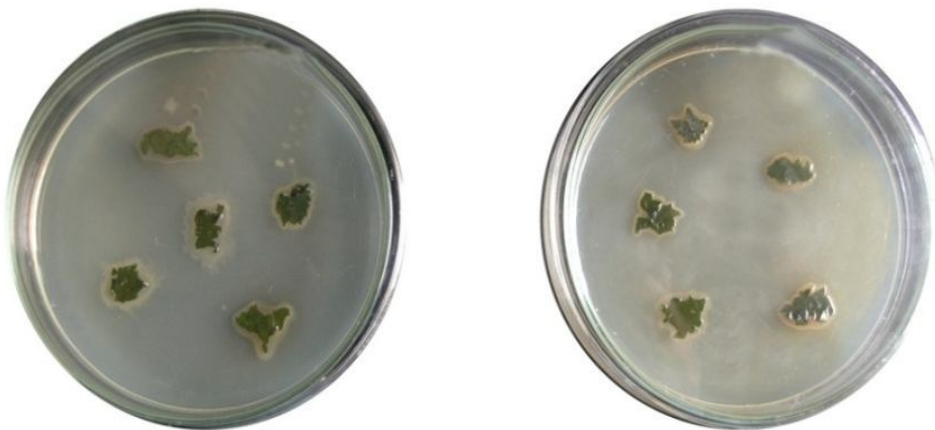
Plate - 1

Isolation of endophytic bacteria from *Solanum trilobatum* L.

Master plates

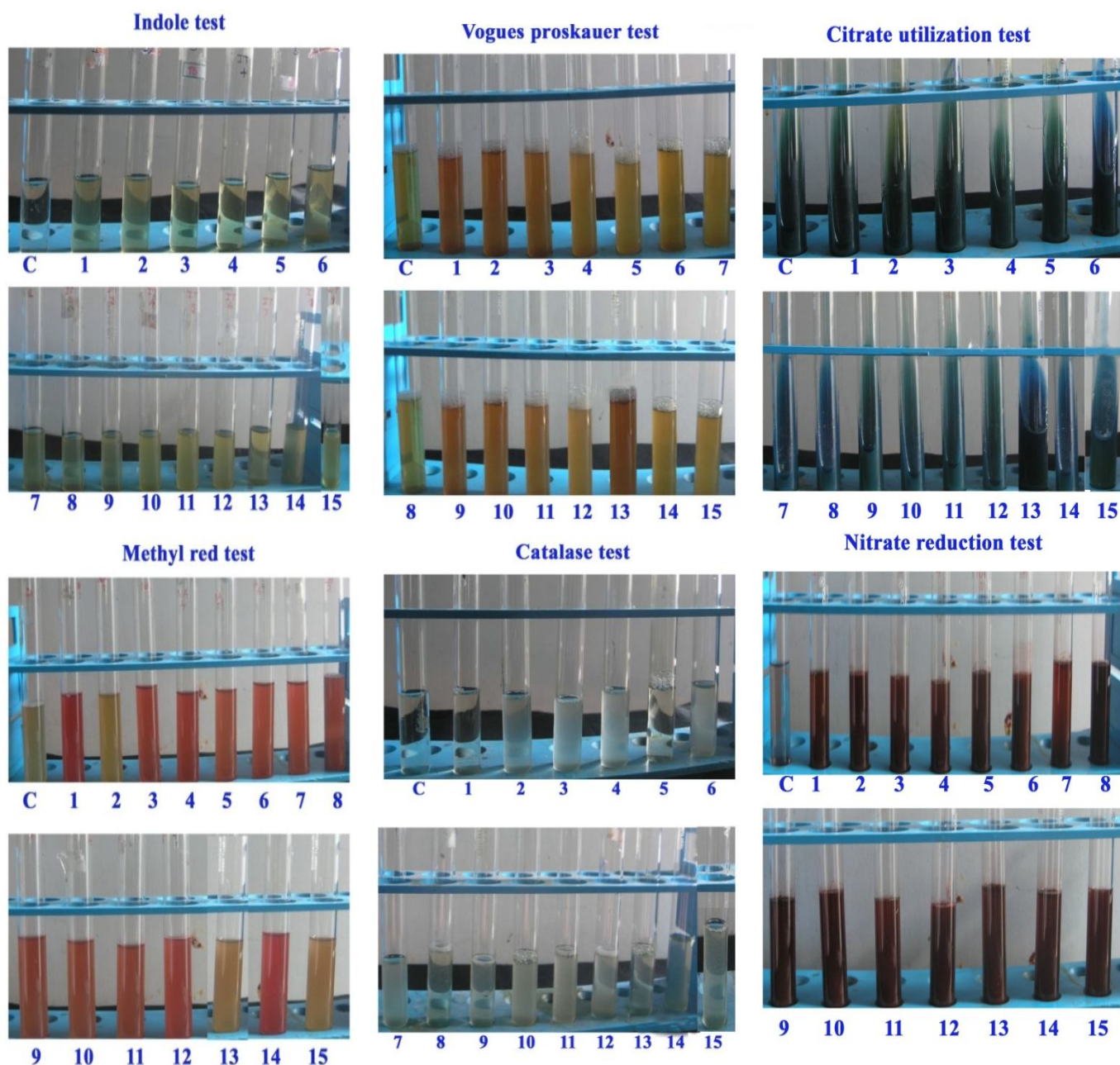


Nutrient agar Medium



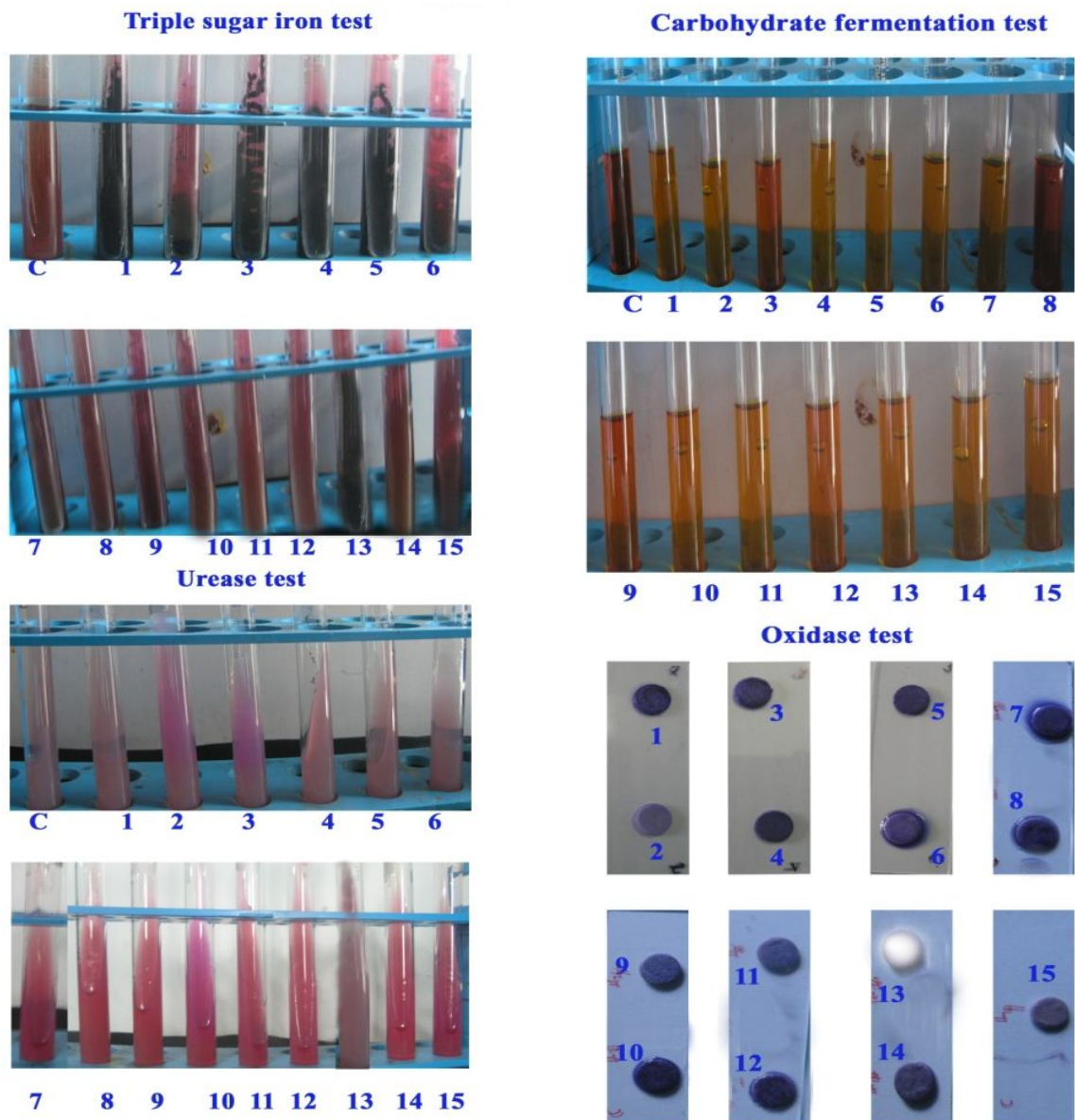
Luria Bertani (LB) agar medium

## Plate 2. Biochemical characterizations of isolated bacterial endophytes



1. *Deinococcus radiopugnans*, 2. *Melissococcus pluton*, 3. *Saccharococcus thermophilus*,  
 4. *Burcella melitensis*, 5. *Stomatococcus mucilaginosus*, 6 - *Streptococcus pyogenes*,  
 7 - *Staphylococcus aureus*, 8 - *Salinococcus roseus* 9 - *Gemella haemolysans*, 10 -  
*Klebsiella pneumoniae*, 11 - *Buttiauxella agrestis*, 12 - *Pediococcus damnosus* 13 -  
*Bacillus subtilis*, 14 - *Coprococcus eutactus*, 15 - *Aerococcus viridans*

## Plate 3. Biochemical characterization of isolated bacterial endophytes



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