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BIO-PROSPECTING OF ENDOPHYTIC BACTERIA FROM MEDICINAL PLANT

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ABSTRACT

Plants are generally associated with diverse microorganisms. Endophytic organisms are those that colonize the plant internal tissue showing no external sign of infection or negative effect on there. Endophytic microbes from medicinal plants are good source of functional metabolites. Endophytic microorganisms can be derived from any part of the plant like bark, leaves, flowers, fruits, roots, seeds etc. In the present study, efforts have been made to isolate and physiological activity of endophytic bacteria inhabiting leaves of medicinal plants such as Kasarali (*Catharanthus roseus* L.), Thuthuvalai (*Solanum trilobatum* L.) and Tulsi (*Ocimum sanctum* L.) which are growing in the Trichy region. The density of endophytic populations recovered in nutrient agar medium, which varied from 4.26 x 10^5 to 1.34 x 10^5 $CFUg^{-1}$ per fresh weight. Colonization frequency and isolation rates 42.22, 37.78, 46.67 % and 21.05%, 17.65% and 23.81% in kasarali, thuthuvalai and tulsi respectively. Among the 12 isolates, KA-1, KA-2, KA-3, TV-5, TV-7, TL-8, TL-9 AND TL-11 were identified as *Bacillus* sp. KA-4, TV-6 and TL-10 were identified as *Pseudomonas* sp and TL-12 as *Klebsiella* sp. Among the 12 isolates, 9 isolates had amylolytic, lipolytic and proteolytic activity with different zone of clearance. Among the 12 isolates, 11isolates had cellulolytic activity with different zone of clearance. Among the 12 isolates, nine isolates showed antibacterial activity against either gram-positive or gram-negative bacteria. The endophytic bacterial extracts were more effective in gram-positive bacteria than gram-negative bacteria. The MIC of ethyl acetate of endophytic bacterial isolates were ranged from 3.13 to 50 mg/ml. Endophytes isolated form

medicinal plants may be beneficial to the host. The endophytic microorganisms are a very promising source for production of bioactive compounds.

KEYWORDS: *Solanum trilobatum* L., *Catharanthus roseus* L., *Ocimum sanctum* L.

INTRODUCTION

Natural products from endophytes are reported to have broad spectrum of biological activities, such as antibiotics, antipathogens, immunosupressants, anticancer, antioxidant, enzymes such as lipase, amylase, protease, cellulose (Huang *et al*. 2007). Endophytic microbes from medicinal plants are good source of functional metabolites (Huang *et al*., 2008). Endophytes are able to increases host fitness and competitive abilitity, by increasing nutrientional uptake, resistance to seed predators, seed germination success, tolerance to heavy metals, high salinity and good growth rate through biochemical pathways such as phytohormone indole 3 acetic acid (IAA) from fungal endophytes(Rudgers *et al*., 2004).

Endophytic microbes associated with traditionally used medicinal plants particularly of the tropics could be a rich source of functional metabolites(Tejesvi *et al*., 2007). Endophyte also produces extracellular hydrolyases to establish a resistance mechanism against plant invasion which includes some of the extracellular enzymes like cellulases, proteinase, lipases and esterases(Zhang *et al*., 2006). . The biologically active natural products from endophytes are excellent resources for medicine, agriculture and industry (Guo *et al*., 2008). Metabolites produced by fungal endophyte can be a good source of novel natural antioxidant compounds (Wu *et al*., 2007). Plant pathogenic fungi were also control by bacterial endophytes.

Coronamycin characterize a complex peptide antibiotic with activities against pythiaceaus fungi, human fungal pathogen *Cryptococcus neoformans* and also against the malarial parasite (Ezra *et al*., 2004). Endophytic fungi are a promising source of novel compounds. About 51% of biologically active substances from fungal endophytes. (Strobel *et al.*,2001).

Endophytes found to improve the ecological adaptability of hosts by enhancing their tolerance to environmental stresses and resistance to phytopathogens. Endophytic fungi are able to protect their host plant from drought conditions (Clay and Schardl, 2002).

MATERIALS AND METHODS

Collection of Sample

Mature healthy plant leaves were collected and identified by experts from Department of Botany, Government Arts College Namakkal. Samples were immediately brought to laboratory and were used within 8 h. Healthy plants were selected growing in different regions of Trichy during winter seasons for 2015. Endophytic flora was isolated from leaves of Kasarali (:*Catharanthus roseus* L.), Thuthuvalai (*Solanum trilobatum* L.) and Tulsi (*Ocimum sanctum* L.)

Enumeration of endophytic bacterial population

Standard Plate Count Method

The endophytic bacteria were enumerated by modifying the isolation procedure described by Gyaneshwar *et al.* (2001). One gram of leaf sample was macerated by in 9 ml of sterile water. From this, 1.5 ml of aliquot was centrifuged at 1,300 rpm at 4°C for 10 min. The supernatant was serially diluted up to 10^{-5} and each dilution was transferred (1 ml) to nutrient agar plates with three replications and incubated for four days at 27°C.

After appropriate incubation period calculate the number of colonies showing different morphology from respective medium per plate and record by using digital colony counter. Total viable counts are calculated from the following formula.

Total viable count = Average number of colonies x size of aliquot x dilution factor.

Processing of sample for isolation of Endophytes

The leaves samples from selected medicinal plants were taken and cut into bits (1-2cm). These samples were washed in running tap water to remove soil particles and adhered debris, and finally washed with distilled water.

Samples were immersed in 70% ethanol for 1-3 min and 4% aqueous solution of sodium hypochlorite 1.5 min, l min with 70 % ethanol again and finally rinsed 4-5 times with sterile distilled water (**Kharwar** *et al*., 2008).

Isolation of Endophytic bacteria

The leaf pieces were aseptically inoculated on nutrient agar with 50mg/l Cyclohexamide and incubated at 30°C for 24-96 hrs. Pure endophytic cultures were observed for growth of

bacterial colonies surrounding the leaf sections and maintained on fresh nutrient agar medium (**Fernando** *et al.,* 2005).

Calculation of colonization frequency

Colonization frequency (CF) was calculated as described by **Hata and Futai** (1995). Colonization frequency (%) of an endophyte species was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed **X** 100

Identification of Endophytic bacteria

The bacterial isolates were characterized morphologically and biochemically by following Bergey's Manual of Systematic Bacteriology (Sneath, 1986). Endophytes in the pure culture were preserved on the slant at 6°C and each tube was labelled with code number of the host plant and isolate code with date of isolation.

Amylolytic activity

The isolates were tested for amylase activity by employing zone clearing technique (Gomes *et al.,* 2002) using starch agar medium. The development of blue color indicated the presence of starch, while the areas around the hydrolytic bacteria appeared clear.

Lipolytic activity

The 24 hours cultures of isolates were spot inoculated on the lipase screening medium. The plates were incubated at 37ºC for 3 days. Colonies which produced lipase formed clear zones around itself in the medium due to hydrolysis of tween 80, the only carbon source in the medium (Haba *et al.,* 2000).

Proteolytic activity (Riffel and Brandelli, 2006)

The 24 hours culture of isolates was spot inoculated on Milk agar plates. The plates were incubated at 30ºC for 3 days. The diameter of the zones around the colonies was measured in terms of millimeters.

Cellulolytic activity (Ariffin *et.al,* 2006)

The 24 hours culture of isolates was spot inoculated on carboxy methyl cellulose containing media and incubated for 3 days. After incubation, plates were flooded with 0.2 aqueous congo red and destained with 1M NaCl for 15 min. Clear zone surrounding the active colony indicated cellulase activity.

Fermentation and extraction

Erlenmeyer flasks (250 ml) containing autoclaved at 121°C for 15 min. After this, a loop full of preserved bacteria was inoculated into nutrient broth and was incubated 37°C for 2 days at 150 rpm and 25°C. Following incubation, the fermentation broths were then filtered through two-folds of cheese cloths. The filtrates were extracted twice with equal volumes of ethyl acetate. The organic solvent extracts were evaporated in a rotary evaporator and then stored at 4°C until used (Kwon et al. 2007). The ethyl acetate extracts of endophytes were individually tested against a gram negative and gram positive human pathogenic bacteria.

Evaluation of antimicrobial activity of endophytic organisms by Disc diffusion assay

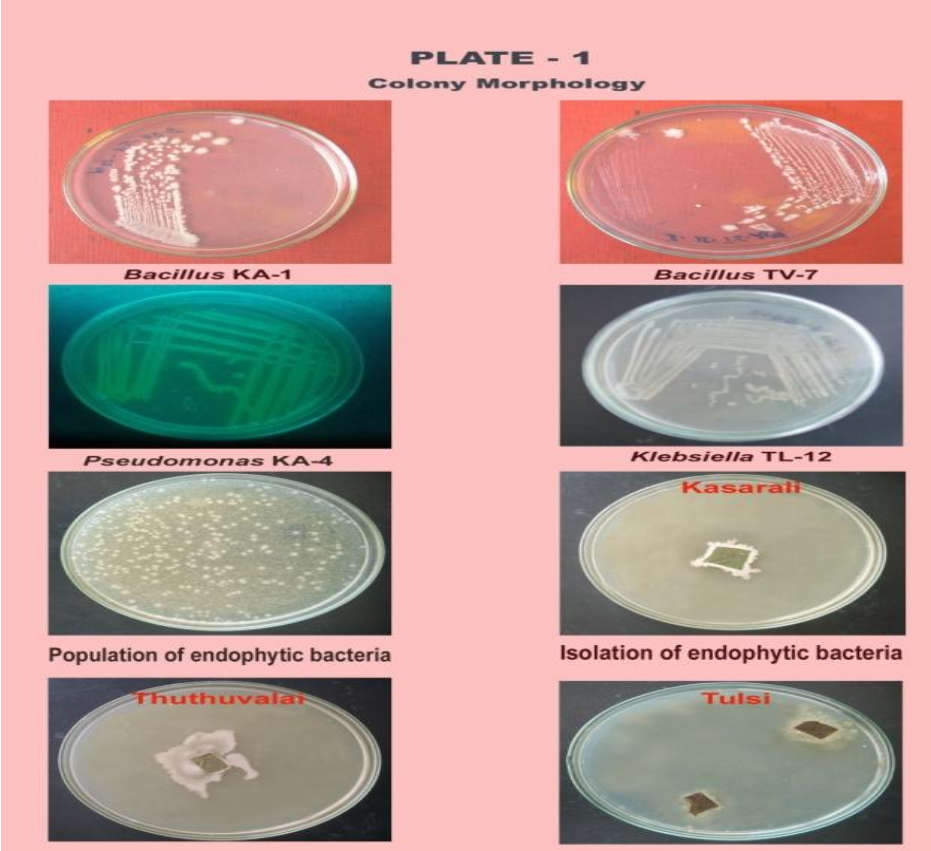
Antibacterial activity was evaluated using the disc diffusion assay with Pre-warmed Mueller-Hinton agar plates seeded with 24 h old culture of test bacteria such as *Staphulococcus aureus* and *Proteus mirabilis*. Crude extract dissolved in ethyl acetate (1 mg/ml) and 20 µl extract was impregnated onto sterile paper discs (6 mm diameter) and placed onto the surface of inoculated agar plates. Plates were incubated at 37°C for 24 hrs.

Antibacterial activity was expressed as the diameter of the inhibition zone in mm (millimeter) produced by the extracts across the disc (Radji *et al*., 2011). Antibacterial activity was determined with zone of inhibition in mm excluding the disk with extracts. The microbroth dilution test was performed to determine minimum inhibitory concentrations (MICs) using the procedure as described by Jorgensen *et al.* (1999).

RESULTS

Population of endophytic bacteria in the leaves of selected medicinal plants

From the analysis, the density of endophytic populations recovered in nutrient agar medium, which varied from 4.26 x 10^5 to 1.34 x 10^5 $CFUg^{-1}$ per fresh weight Thuthuvalai leaves harboured the maximum number of bacterial density followed by tulsi and kasarali (Plate1)

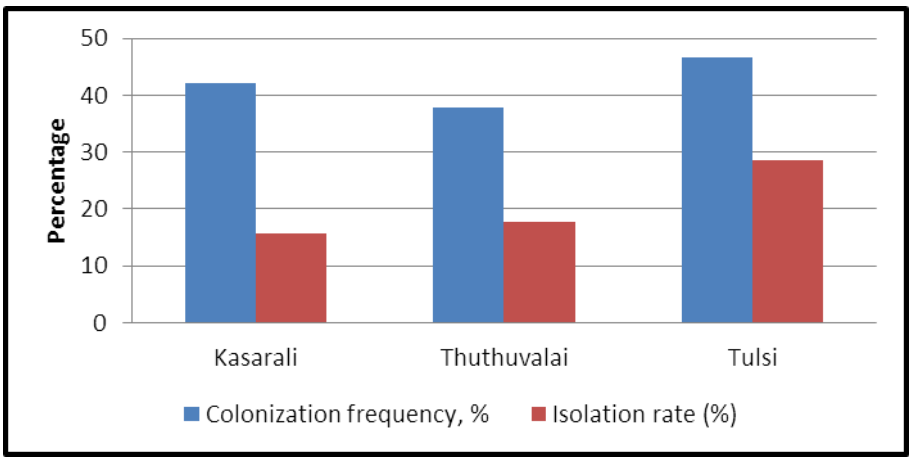


Isolation of endophytic bacteria

A total of 19 bacterial endophytes were isolated in pure form from 45 segments of kasarali leaves, 17 isolates from thuthuvalai leaves and 21 isolates from tulsi leaves which colonization frequency were 42.22, 37.78 and 46.67 % respectively. The isolation rates were 21.05%, 17.65% and 23.81% in kasarali, thuthuvalai and tulsi respectively (Fig1)

Figure-1

Colonization frequency and isolation rate of endophytic bacteria in the Leaves of medicinal plants



Microscopic and morphological characteristics of endophytic bacterial isolates.

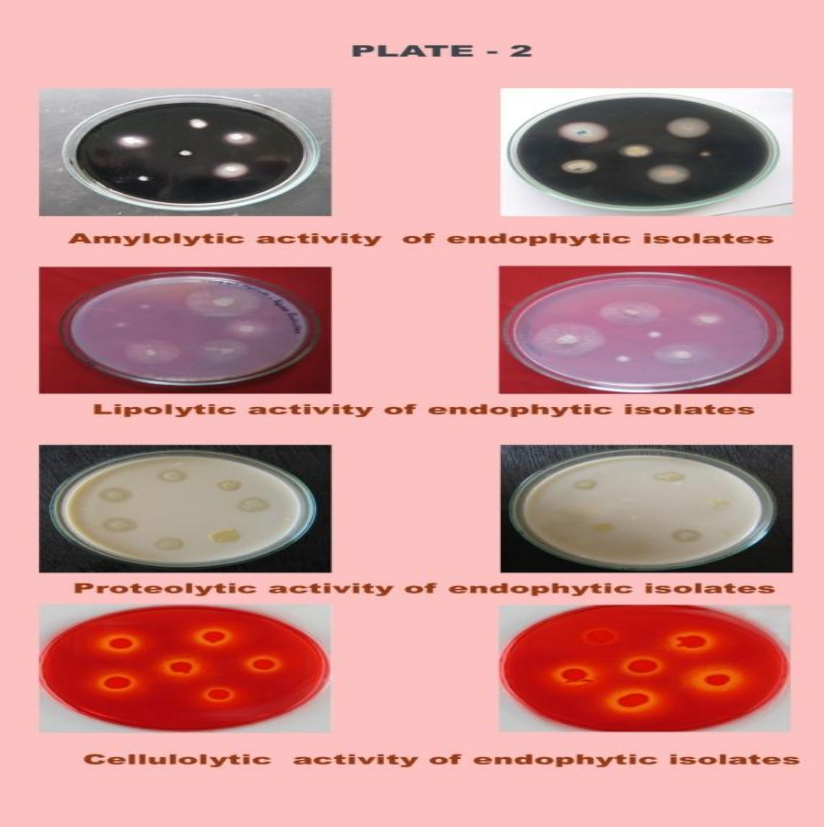
In kasarali, thuthuvalai& tulsi leaves,both rods with gram positive and gram negative , non-endospore forming motile bacteria, spore forming motile bacteria were isolated. Twelve isolates were identified based on gram staining, spore staining, motility and catalase and oxidase test, which were presented in table- 1 Among the 12 isolates, KA-1, KA-2, KA-3, TV-5, TV-7, TL-8, TL-9 AND TL-11 were identified as *Bacillus* sp. which designated as *Bacillus* with bearing respective code. KA-4, TV-6 and TL-10 were identified as *Pseudomonas* sp. with respective code while TL-12 as *Klebsiella* sp. with bearing TL-12.

Table-1: Identification of endophytic bacterial isolates

S.No	Isolates name	Catalase	Oxidase	Bacterial genus	Designation of isolates
1	KA-1	+	+	*Bacillus* sp.	*Bacillus* KA-1
2	KA-2	+	+	*Bacillus* sp.	*Bacillus* KA-2
3	KA-3	+	+	*Bacillus* sp.	*Bacillus* KA-3
4	KA-4	+	+	*Pseudomonas* sp.	*Pseudomonas* KA-4
5	TV-5	+	+	*Bacillus* sp.	*Bacillus* TV-5
6	TV-6	+	+	*Pseudomonas* sp.	*Pseudomonas* TV-6
7	TV-7	+	+	*Bacillus* sp.	*Bacillus* TV-7
8	TL-8	+	+	*Bacillus* sp.	*Bacillus* TL-8
9	TL-9	+	+	*Bacillus* sp.	*Bacillus* TL-9
10	TL-10	+	+	*Pseudomonas* sp.	*Pseudomonas* TL-10
11	TL-11	+	+	*Bacillus* sp.	*Bacillus* TL-11
12	TL-12	-	+	*Klebsiella* sp.	*Klebsiella* TL-12

Enzymatic activity of endophytic bacterial isolates.

Among the 12 isolates, 9 isolates had amylolytic activity with different zone of clearance. The isolate *Bacillus* TL-8 had the maximum zone of clearance (22mm in diameter) followed by *Bacillus* TL-8 (20mm), *Bacillus* KA-2 (18mm), *Bacillus* TV-7 (17mm), *Bacillus* KA-3 (16mm), *Bacillus* KA-1 and *Bacillus* TL-11 (14mm), *Pseudomonas* KA-4 (12mm) and *Pseudomonas* TL-10 (8mm). Among the 12 isolates, 11isolates had cellulolytic activity with different zone of clearance. The maximum cellulolytic activity was observed with *Bacillus* TL-11 (19mm) followed by *Pseudomonas* TL-10 (16mm), *Bacillus* KA-2, *Bacillus* TV-5 and *Bacillus* TL-9 (14mm), *Pseudomonas* TV-6 and *Bacillus* TL-8 (12mm), *Klebsiella* TL-12 (11mm) and *Bacillus* KA-1 (10mm). The remaining isolates had less cellulolytic activity. (plate2)



Among the 12 isolates, 9 isolates had lipolytic activity with different zone of clearance. The maximum activity was observed with *Bacillus* TL-11 (19mm), *Bacillus* KA-3 (16mm), *Bacillus* TV-5 and *Bacillus* TL-9 (14mm), *Bacillus* KA-1, *Pseudomonas* KA-4, *Pseudomonas* TV-6 and *Bacillus* TL-8 (12mm). The maximum proteolytic activity was observed with *Klebsiella* TL-12 (16mm), *Bacillus* KA-1 (15mm), *Pseudomonas* TV-6, *Bacillus* TV-7 and *Bacillus* TL-9 (14mm). The remaining isolates showed less protease production ability. (table2)

Table-2: Enzymatic activity of endophytic bacterial isolates (Zone of clearance, diameter in mm)

S.No	Designation of isolates	Amylolytic activity	Cellulolytic activity	Lipolytic activity	Proteolytic activity
1	*Bacillus* KA-1	14	10	12	15
2	*Bacillus* KA-2	18	14	0	12
3	*Bacillus* KA-3	16	9	16	0
4	*Pseudomonas* KA-4	12	8	12	0
5	*Bacillus* TV-5	0	14	14	11
6	*Pseudomonas* TV-6	**0**	12	12	14
7	*Bacillus* TV-7	17	0	0	14
8	*Bacillus* TL-8	20	12	12	0
9	*Bacillus* TL-9	22	14	14	14

10	*Pseudomonas* TL-10	8	16	0	10
11	*Bacillus* TL-11	14	19	19	12
12	*Klebsiella* TL-12	0	11	0	16

Antibacterial activity of endophytic bacterial extract against human pathogens

Among the 12 isolates, nine isolates showed antibacterial activity against either gram-positive or gram-negative bacteria. The endophytic bacterial extracts were more effective in gram-positive bacteria than gram-negative bacteria. *Pseudomonas* TL-10 extract showed the maximum inhibition of Staphylococcus aureus (18mm) followed by *Bacillus* TV-5 (16mm), *Bacillus* KA-2 (14mm), *Bacillus* KA-1 (12mm), *Pseudomonas* KA-4 and *Bacillus* TL-9 (11mm), *Bacillus* KA-3 (10mm), *Bacillus* TL-8 (9mm) and *Bacillus* TV-7 (7mm). *Bacillus* KA-1 showed the maximum inhibition of *Proteus mirabilis* (15mm) followed by *Bacillus* TL-8 (14mm), *Bacillus* KA-3 and *Pseudomonas* KA-4 (13mm), *Bacillus* TV-7 (11mm), *Bacillus* KA-2 and *Bacillus* TL-9 (10mm)(table3).

Table-3: Antibacterial activity of endophytic bacterial extract against human pathogens

S.No	Designation of isolates	Zone of inhibition (diameter in mm)	
		Staphulococcus aureus	***Proteus mirabilis***
1	*Bacillus* KA-1	12	15
2	*Bacillus* KA-2	14	10
3	*Bacillus* KA-3	10	13
4	*Pseudomonas* KA-4	11	13
5	*Bacillus* TV-5	16	0
6	*Pseudomonas* TV-6	0	**0**
7	*Bacillus* TV-7	7	11
8	*Bacillus* TL-8	9	14
9	*Bacillus* TL-9	11	10
10	*Pseudomonas* TL-10	18	0
11	*Bacillus* TL-11	0	0
12	*Klebsiella* TL-12	0	0

Minimum inhibitory concentration of endophytic bacterial extract against human pathogens

Minimum inhibitory concentrations of endophytic bacterial extract against human pathogens were tabulated in table-7 and plate-3. The MIC of *Bacillus* TV-5 and *Pseudomonas* TL-10 crude extract showed 3.13 mg/ml against *Staphulococcus aureus*, followed by *Bacillus* KA-2 (6.25mg/ml), *Bacillus* KA-1, *Bacillus* KA-3 and *Pseudomonas* KA-4 (12.5 mg/ml), *Bacillus* TL-9 (25 mg/ml), *Bacillus* TV-7 and *Bacillus* TL-9 (50mg/ml). The MIC of

Bacillus KA-1 and *Bacillus* TL-8 crude extract showed 6.25 mg/ml against *Proteus mirabilis*, followed by *Bacillus* KA-2, *Bacillus* KA-3, *Pseudomonas* KA-4 and *Bacillus* TL-9 (25mg/ml) and *Bacillus* TV-7 (5mg/ml).(plate3,Table4)

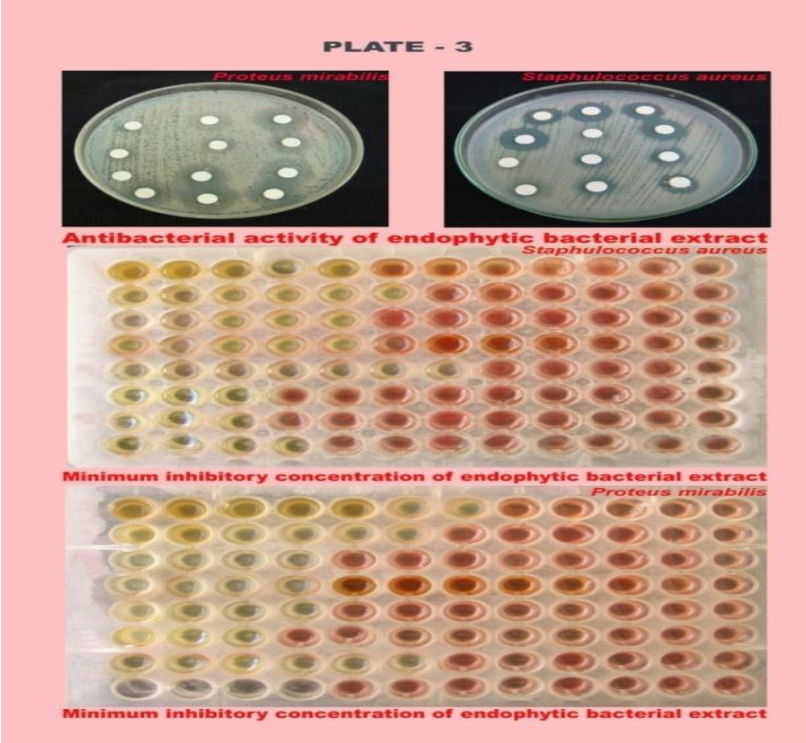


Table-4: Minimum inhibitory concentration of endophytic Bacteria.

S.No	Designation of isolates	MIC (mg/ml)	
		Staphulococcus aureus	***Proteus mirabilis***
1	*Bacillus* KA-1	12.5	6.25
2	*Bacillus* KA-2	6.25	25
3	*Bacillus* KA-3	12.5	25
4	*Pseudomonas* KA-4	12.5	25
5	*Bacillus* TV-5	3.13	0
6	*Bacillus* TV-7	50	50
7	*Bacillus* TL-8	50	6.25
8	*Bacillus* TL-9	25	25
9	*Pseudomonas* TL-10	3.13	0

DISCUSSION

Various investigators reported endophytic microbes from various plant exists in different ecosystems. It is not worthy that of the nearly 3, 00,000 plant species that exists on earth each individual plant is host to one or more endophytes. Only a few these plants have ever been completely studied relative to their endophytic biology. Consequently the opportunity to find

new and interesting microorganism among myriads of plants in different settings and ecosystems is great (Strobel and Daisy, 2003) The present investigation was attempted for bioprospecting of endophytic bacteria from selected medicinal plants such as Kasarali (*Catharanthus roseus* L.), Thuthuvalai (*Solanum trilobatum* L.) and Tulsi (*Ocimum sanctum* L.) were collected at Trichy region, Tamilnadu. Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). In the present investigation, the density of endophytic populations recovered in nutrient agar medium, which varied from 4.26×10^5 to 1.34×10^5 $CFUg^{-1}$ per fresh weight. Thuthuvalai leaves harboured the maximum number of bacterial density followed by tulsi and kasarali. As a relative unexploited reservoir of bioresources, some endophytes have been demonstrated to be excellent producer of extracellular enzymes such as proteolytic, hydrolytic, kerationolytic and lignolytic enzymes (Aysha *et al*., 2006; Mandyam *et al*., 2010). In the present investigation, Among the 12 isolates, 9 isolates had amylolytic activity with different zone of clearance. The isolate *Bacillus* TL-8 had the maximum zone of clearance (22mm in diameter). Cellulolytic activity was observed in seven bacterial isolates and three fungal isolates (Jalgaonwala and Mahajan, 2011). The maximum cellulolytic activity was observed with *Bacillus* TL-11 (19mm) followed by *Pseudomonas* TL-10 (16mm). The Lipolytic activity was observed for isolates B. pumilus, B. megaterium, and *Pseudomonas* sp. (Jung *et al*. 2003). In the present investigation, Among the 12 isolates, 9 isolates had lipolytic activity with different zone of clearance. The maximum activity was observed with *Bacillus* TL-11 (19mm) and *Bacillus* KA-3 (16mm).

The presence of proteolytic activity in *Klebsiella* TL-12 (16mm), *Bacillus* KA-1 (15mm), *Pseudomonas T*V-6, Bacillus TV-7 and *Bacillus* TL-9 (14mm). Up to now most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents antiviral, antidiabetic agents and other bioactive compounds by their different functional roles (Guo *et al*., 2008)., Among the 12 isolates, nine isolates showed antibacterial activity against either gram-positive or gram-negative bacteria. The endophytic bacterial extracts were more effective in gram-positive bacteria than gram-negative bacteria. Jonathan et al. (2008) reported that the minimum inhibitory concentration (MIC) of the extracts ranged between 2.75 and 15.75mg/ml. The lowest MIC (2.75mg/ml) was found with the extract of *Marasmius*

jodocodo against E. coli. In the present investigation, the MIC of ethyl acetate of endophytic bacterial isolates was ranged from 3.13 to 50 mg/ml.

CONCLUSION

The diversity of endophytes obtained from healthy plant tissues suggested that an even broader flora of endophytes might be found across diverse plant species. It is clearly understood that endophytes isolated form medicinal plants may be beneficial to the host. The endophytic microorganisms are a very promising source for production of bioactive compounds. Further investigations are suggested in order to classify the microorganisms and exploit the potential of the substance produced to inhibit pathogenic microorganisms.

REFERENCES

1. Ariffin H, Abdullah N, UmiKalsom MS, Shirai Y, Hassan MA. Production and characterisation of cellulase by *Bacillus pumilus* EB3. *Int. J. Eng. Technol*, 2006; 3: 47-53.
2. Aysha JI, Edweis CB, and Jose DG. Enzyme activity of endophytic bacterial isolates of *Jacaranda deurrens* Cham (Carobinha-do-Campo).Brazilian. *Archives of Biology and Biotechnology*, 2006; 49: 3; 353-359.
3. Clay K, and Schardl C. Evolution origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist*, 2002; 160: 99-127.
4. Ezra D, Uvidello F, Castillo, Strobel GA, Hess WM, Porter H, Jensen JB,Margaret AM, Condron DB,Teplow JS, Maranta M, Maranta MH, Weber B. and Yaver D. Cronomycins, peptide antibiotics produced by a verticillate *Streptomyces* Sp (MSU-2110) endophytic on *Monstera* Sp *Microbiology*, 2004; 150: 785-793.
5. Fernando EV, Pava-Ripolli M, Posada F. and Buyer JS.Endophytic bacteria *Coffea arabica* L. *J. Basic Microbiol*, 2005; 45(5): 371–380
6. Gyaneshwar P, James E.K., Natarajan, M., Reddy, P.M., Reinhold-Hurek, B. & Ladha, J.K. Endophytic colonization of rice by a diazotrophic strain of Serratia marcescens. *Journal of Bacteriology*, 2001; 183: 2634-2645.
7. Guo BY, Wang X, Sun, and Tang K. Bioactive natural products from endophytes: A review. *Appl. Microbiol. Biotechnol*, 2008; 44(2): 136-142.
8. Gomes DJ, Hasan MF & Rahman MM. Screening for α-amylase producing thermophilic fungi recovered from natural decomposing lignocellulosic materials. *Dhaka Univ J Biol Sci*, 2002;11(1): 39-48.

9. Huang WY, Cai YZ, Hyde KD, Corke H, Sun M. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity*, 2008; 33: 61-75.
10. Hata K, Futai K. Endophytic fungi associated healthy pine needles and needles infested by pine needle gall midge *Thecodiplosis japonensis. Can. J.Bot*, 1995; 73: 384-390
11. Jalgaonwala RE, Mohite BV, Mahajan RT. Evaluation of Endophytes for their antimicrobial activity from Indigenous medicinal Plants belonging to North Maharashtra region India. Int J *Pharmaceut Biomed Res, 2010*; 1(5): 136-41
12. Jorgensen JH, Turnidge JD, Washington JA .Antibacterial susceptibility test: dilution and disk diffusion methods. Manual of Clinical Microbiology. Washington, 1999; D.C: ASM Press pp. 1526-156.
13. Jonathan SG, Kigigha LT and Ohimain E. Evaluation of the Inhibitory Potentials of Eight Higher Nigerian Fungi against Pathogenic Microorganisms. *African Journal of Biomedical Research*, 2008; 11: 197 – 202.
14. Kobayashi DY, and Palumbo JD. Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon, C.W. and White, J.F. (Eds.) Microbial endophytes. Marcel Dekker, Inc., *N.Y., New York*, 2000; 199-233.
15. Kharwar RN, Verma VC, Kumar A, Gond S.K, Harper KJ, Hess WM, Lobkovosky E., Ma C, Ren Y, Strobel GA. Javanicin, an antibacterial naphthaquinone from an endophytic fungus of neem, *Chloridium* Sp. *Current Microbiol*, 2008; 58: 233-238.
16. Rudgers JA, Kaslow JM, and Clay K. Endophytic fungi alter relationshipsbetween diversity and ecosystem properties. *Ecology Letters*, 2004; 7: 1; 42-51.
17. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products .*Mol.biol*.Rev, 2003; 67: 491-502.
18. Strobel GA. Microbiology gift from rain forest. Can.J.*Plant Pathol*, 2002; 24: 1;14-20..
19. Wu-YH, Yi-Z.C, Jie-X, Harlod C and Meisun. A potential Antioxidant Resources: Endophytic fungi from medicinal plants .*Economic Botany*, 2007; 61:1;14-30;6:893-903.
20. Zhang XX, George A, Bailey MJ. and Rainey PB. The histidine utilization (hut) genes of *Pseudomonas fluorescens* SBW25 are active on plant surfaces, but are not required for competitive colonization of sugar beet seedlings. *Microbiology*, 2006; 152: 1867–1875.