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Isolation, Morphological Characterization and Antibacterial Activity of Endophytic Bacteria from Medicinal Plants of Muthathi Wild Life Sanctuary, Mandya, Karnataka, India

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Abstract

Medicinal plants play a vital role in providing crucial health care to human populations. These plants are very good source of endophytic bacteria with their potential to produce the bioactive compounds having antibacterial activity against human pathogens. Endophytes are microorganisms that are existing in living tissues of several plant tissues and these are chemical synthesizers have been founding regular association without apparently causing any symptom of diseases. The purpose of this study was to isolate the endophytic bacteria along with their initial characterization from few selected medicinal plants and to evaluate antibacterial activities against human pathogens. A total of 84 endophytic bacteria were isolated and identified by morphological characterization from the leaves of few selected medicinal plants of Cauvery Wild Life Sanctuary (Muthathi), Mandya. These isolates were subjected to antibacterial activity against eight clinically significant pathogenic bacteria. Out of this, 8 bacterial endophytes exhibited the antibacterial activity against *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens* by using standard protocol of agar well diffusion method. *Aloe vera (L)* Burm.f, *Cassia auriculata* L, *Madhuca longifolia* J.F.Macbr, *Origonum majorana* L., *Sesbania grandiflora* L, *Vitex Negundo* L, *Withania Somnifera* L Dunal, plants isolates were inhibit the effectiveness antibacterial activity with zone of inhibition. Thus, this study exposed bacterial endophytes with the capability of producing antimicrobial elements of pharmacological importance.

Keywords

Antibacterial activity, Cauvery Wild Life Sanctuary, Endophytic Bacteria, Medicinal Plants, Morphological characterization, Muthathi.

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INTRODUCTION

Plants are still considered as one of the most significant sources of biologically active compounds in natural products research. Many plant species have been consumed globally in traditional healing and have been studied extensively for their pharmacological properties (1). Majority of medicinal plants are the important part of the human society to combat diseases. In developing country

like India, medicinal plants are the important source for people to fight against numerous ailments and disorders. Various plant species are explored by indigenous civilizations for their medicinal applications and are traditionally used by traditional societies for their preliminary health treatment (2).

Endophytes are defined as microorganisms which occupy inside of healthy plant tissues and are now considered as universal symbionts of plants from their amazingly common revealing from many plant species (3). Endophytic bacteria are a class of microbes which resides within the interior tissues of plants without causing harm to the host plants (4). Many studies have emphasized endophytes from medicinal plants and their application in different regions (5). Recently, many known, as well as new endophytic bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant, etc., have been identified (6).

Endophytes are actively or passively generating the physiological changes in the plant cell (7). They are longer in growth advancement over the rhizobacteria outstanding to better adaptations against abiotic or biotic pressures (8). Bacterial endophytes stimulate host plant growth through straight mechanisms by producing phytohormones IAA, gibberellins, cytokinins (9), phosphate solubilisation (10), N2 fixation (11) or indirectly by production of antibiotics (12), siderophores (13) and lytic enzymes against the pathogens (14). Many endophytes constitute the common soil bacteria (Pseudomonas, Burkholderia and Bacillus) that produce diverse range of secondary metabolites, antibiotics and volatile organics to counters the deleterious effects of pathogens by mechanisms in line with the PGPR (15, 13).

Several important features and medicinal properties of plants may be due to endophytes residing in plants. Numerous studies have also revealed that endophytes synthesize bioactive compounds which help to promote plant growth and increase resistance in plant against their pathogens (16, 17). The medicinal plants that produce bioactive compounds have associated with endophytes that produce the same natural compounds (Tan and Zou, 2001). From the microbial sources the isolation of bioactive compounds is easier and more economical for large-scale production than plant sources. Antimicrobial activities have been demonstrated in a variety of metabolites biosynthesized by plant endophytes (19).

In the current study, an attempt has been made to isolate the endophytic bacteria from the selected medicinal plants of Muthathi Wild Life Sanctuary, Mandya and evaluated their antibacterial activity against few human bacterial pathogens. These endophytic bacteria may be the source of the eventual antimicrobial medicines.

MATERIALS AND METHODOLOGY

Sample Collection:

For the isolation of endophytic bacteria plant samples were collected from Muthathi Wild Life Sanctuary. Fresh and healthy leaves were collected in separate polythene bags, labelled, transported to the laboratory and stored at 10°C. Samples were collected on the basis of medicinal properties of plants so that they give rise to bacteria with some medical importance.

Isolation of Endophytic Bacteria:

The endophytic bacteria were isolated by the following method observed by Suresha and Jayashankar, 2018 (20). The healthy plant leaves were surface sterilized as per the protocol described by (21) with some minor modifications. Samples were cut into 4×5mm long segments. To remove epiphytic micro-organism and dust, samples were surface sterilized by dipping in ethanol (70%) for 1-2min, followed by a sodium hypochlorite (NaOCl) solution (4% available chlorine) for 1min and then rinsed in ethanol (70%) for nearly 1-2min. After that, it was finally rinsed in distilled water. Sterilized the plant leaves were dried under sterile condition on placing over sterilized blotting paper. Then it is placed on nutrient agar medium plate and incubated at 37ºC for 24hrs. After incubation period of time the plates were observed for the growth of endophytic bacteria. Plates were observed periodically and when colony appeared out from leaf segments, they were sub cultured and brought to pure culture in NA slants and stored at 4ºC. All isolated endophytic bacteria were maintained in the refrigerator.

Morphological Identification of Endophytic Bacteria:

Standard taxonomic key included for the morphological characterization of the isolated endophytic bacteria from leaves of few selected medicinal plants. The results were showed that, out of 84 bacterial endophytes had round, irregular and punctiformin shape, margins were undulate, lobate and filamentous in edge. Flat, umbonate, raised and convex elevation with smooth surfaces. The color of the colony was white, cream and yellow of those isolated bacterial cultures from the leaves of the medicinal plants were found. Gram staining was used to determine the morphology of bacteria and distinguish between of both Gram-positive bacteria

and Gram-negative bacteria, observed under microscope with cocci-shaped, motile and circular with entire margins (22).

Selected Organisms to Evaluate the Antibacterial Activity:

Three gram-positive bacteria: *Bacillus subtilis, Listeria monocytogenes* and *Staphylococcus aureus.* Five gram-negative bacteria: *Pseudomonas aeruginosa, E. coli, Klebsiella pneumonia, Proteus mirabilis* and *Shigella sp.* were obtained from the GENESPY Service Pvt Ltd., Mysore were grown in Brain Heart Infusion (BHI) media for 24h at 37 °C under constant shaking (150rpm) and stored at 4 °C.

Test Culture and Growth Condition:

The given bacterial cultures were grown fresh in Luria Bertani (LB) media at 37°C for 24h. The culture was centrifuged at 10,000rpm for 10min to collect the cell free supernatant (CFS) and filter sterilized which was then used for antibacterial assay.

Antibacterial Activity using Agar Well Diffusion Assay:

The *in vitro* antibacterial assay was performed by Agar Well Diffusion method with some minor modifications (23). The bacterial human pathogens were used for antibacterial assay like *S. aureus, B. subtilis, L. monocytogens, E. coli, K.pneumoneae, P. aeruginosa, P. mirabilis* and *Shigella.* BHI agar plates were prepared by inoculating 1% of freshly grown pathogenic culture. Wells of 4mm in diameter was made in the plate by using sterile cork borer. Then, 70µl of given CFS was added in each well. The sample was allowed to diffuse for 20min at 4°C. Later, plates were incubated at 37°C for 24-48h. After incubation, the zone of inhibition was measured in mm and recorded. Antibiotic chloramphenicol was used as positive control (24). The antibacterial activity of CFS was evaluated by formation of zone of inhibition, which was measured and expressed in mm.

RESULTS

Tab 1. Growth of Endophytic Bacteria Isolated from Different Medicinal Plants on Nutrient Agar media and their Morphological Characteristics with Gram Staining

Isolates/Plant samples	Shape /Form	Colour of Colony	Margin /Edge	Opacity	Elevation	Texture /Surface	Appearance	Gram Staining/ Cell form
C-1	Circular	Cream	Undulate	Opaque	Flat	Smooth	Present	+ ve, cocci
C-2	Irregular	Cream	Undulate	Opaque	Umbonate	Smooth	Present	+ve, cocci
C-3	Punctiform	Yellowish cream	Lobate	Transparent	Flat	Smooth	Present	+ve, cocci
C-4	Punctiform	Cream	Undulate	Transparent	Umbonate	Smooth	Present	+ve, cocci chain
C-5	Irregular	Cream	Undulate	Opaque	Convex	Smooth	Present	+ve, cocci chain
C-6	Irregular	Cream	Filamentous	Opaque	Raised	Smooth	Present	-ve, cocci
C-7	Circular	Cream	Undulate	Opaque	Convex	Smooth	Present	-ve, cocci chain
C-8	Irregular	Cream	Filamentous	Transparent	Flat	Smooth	Present	+ve, cocci
C-9	Irregular	Cream	Lobate	Transparent	Raised	Smooth	Present	+ve, cocci
C-10	Irregular	Yellowish cream	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci chain
C-11	Irregular	Light cream	Undulate	Transparent	Flat	Smooth	Present	-ve, cocci
C-12	Circular	Yellow	Entire	Transparent	Convex	Smooth	Shiny	+ve, cocci
C-13	Irregular	Yellowish	Undulate	Transparent	Convex	Smooth	Present	+ve, cocci chain
C-14	Circular	Yellowish cream	Entire	Transparent	Raised	Smooth	Present	+ve, cocci chain
C-15	Irregular	White	Filamentous	Transparent	Pulvinate	Smooth	Present	+ve, cocci chain
C-16	Circular	Cream	Entire	Opaque	Flat	Smooth	Shiny	+ve, cocci
C-17	Irregular	Cream	Undulate	Transparent	Flat	Smooth	Present	+ve, cocci
C-18	Circular	Cream	Entire	Transparent	Convex	Smooth	Present	+ve, cocci
C-19	Punctiform	Orange	Undulate	Transparent	Convex	Smooth	Present	+ve, cocci
C-20	Circular	White	Entire	Transparent	Flat	Smooth	Present	+ve, cocci

C-21	Punctiform	Cream	Undulate	Opaque	Flat	Smooth	Shiny	-ve, cocci chain
C-22	Punctiform	Cream	Entire	Transparent	Flat	Smooth	Shiny	+ve, cocci
C-23	Punctiform	Cream	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci
C-24	Circular	White	Entire	Transparent	Convex	Smooth	Present	+ve, cocci
C-25	Punctiform	Cream	Undulate	Opaque	Raised	Smooth	Shiny	+ve, cocci
C-26	Irregular	Cream	Undulate	Opaque	Raised	Smooth	Shiny	+ve, cocci
C-27	Irregular	Cream	Undulate	Opaque	Raised	Smooth	Shiny	+ve, cocci
C-28	Irregular	Yellowish cream	Undulate	Transparent	Flat	Smooth	Present	+ve, cocci
C-29	Circular	Yellowish cream	Entire	Transparent	Convex	Smooth	Present	-ve, cocci
C-30	Punctiform	White	Undulate	Transparent	Convex	Smooth	Present	+ve, cocci
C-31	Circular	Cream	Entire	Opaque	Flat	Smooth	Shiny	+ve, cocci
C-32	Irregular	Yellow	Entire	Opaque	Raised	Smooth	Shiny	+ve, cocci
C-33	Punctiform	White	Filamentous	Opaque	Flat	smooth	Shiny	+ve, cocci
C-34	Circular	White	Entire	Transparent	Raised	Smooth	Present	+ve, cocci
C-35	Punctiform	Yellow	Entire	Transparent	Flat	Smooth	Present	+ve, cocci
C-36	Irregular	Orange	Entire	Transparent	Raised	Smooth	Present	+ve, cocci
C-37	Irregular	Yellowish cream	Undulate	Transparent	Flat	Smooth	Present	+ve, cocci
C-38	Irregular	Yellow	Undulate	Opaque	Raised	Smooth	Shiny	+ve, cocci
C-39	Irregular	Cream	Undulate	Opaque	Flat	Smooth	Shiny	+ve, cocci
C-40	Punctiform	Cream	Entire	Transparent	Flat	Smooth	Present	+ve, cocci cluster
C-41	Circular	Cream	Entire	Transparent	Raised	Smooth	Present	+ve, cocci
C-42	Irregular	Yellowish cream	Undulate	Opaque	Flat	Smooth	Present	-ve, cocci cluster
C-43	Punctiform	Orange	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci
C-44	Punctiform	Cream	Filamentous	Opaque	Flat	Smooth	Present	-ve, cocci chain
C-45	Irregular	Cream	Filamentous	Opaque	Raised	Smooth	Present	+ve, cocci cluster
C-46	Irregular	Cream	Filamentous	Opaque	Flat	Smooth	Present	+ve, cocci
C-47	Punctiform	Cream	Entire	Transparent	Raised	Smooth	Present	+ve, cocci
C-48	Circular	White	Undulate	Transparent	Flat	Smooth	Present	-ve, cocci cluster
C-49	Irregular	Cream	Undulate	Transparent	Convex	Smooth	Present	-ve, cocci
C-50	Irregular	White	Undulate	Transparent	Convex	Smooth	Present	+ve, cocci
C-51	Irregular	White	Undulate	Transparent	Raised	Smooth	Present	+ve, cocci chain
C-52	Irregular	Cream	Undulate	Transparent	Raised	Smooth	Present	-ve, cocci
C-53	Irregular	Cream	Undulate	Transparent	Raised	Smooth	Present	-ve, cocci chain
C-54	Irregular	Cream	Lobate	Transparent	Umbonate	Smooth	Present	+ve, bacilli
C-55	Circular	Yellowish cream	Entire	Transparent	Convex	Smooth	Present	+ve, cocci
C-56	Circular	White	Entire	Transparent	Convex	Smooth	Present	+ve, cocci
C-57	Irregular	Cream	Undulate	Transparent	Raised	Smooth	Present	+ve, cocci chain
C-58	Irregular	White	Undulate	Transparent	Raised	Smooth	Shiny	-ve, cocci
C-59	Circular	Yellowish cream	Entire	Transparent	Flat	Smooth	Shiny	-ve, cocci

C-60	Irregular	Cream	Undulate	Transparent	Convex	Smooth	Present	-ve, cocci chain
C-61	Irregular	Pale yellow	Rhizoid	Opaque	Raised	Smooth	Present	+ve, cocci
C-62	Irregular	Yellow	Undulate	Transparent	Flat	Smooth	Present	-ve, cocci chain
C-63	Circular	Yellowish cream	Entire	Transparent	Convex	Smooth	Shiny	+ve, cocci
C-64	Irregular	Cream	Undulate	Opaque	Convex	Smooth	Present	-ve, cocci chain
C-65	Irregular	White	Undulate	Transparent	Raised	Smooth	Present	-ve, cocci
C-66	Punctiform	Cream	Undulate	Opaque	Flat	Smooth	Present	-ve, cocci
C-67	Punctiform	White	Entire	Transparent	Raised	Smooth	Shiny	+ve, cocci
C-68	Circular	Yellowish cream	Undulate	Transparent	Flat	Smooth	Shiny	+ve, cocci
C-69	Circular	White	Entire	Transparent	Flat	Smooth	Present	+ve, cocci chain
C-70	Punctiform	Cream	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci
C-71	Irregular	White	Undulate	Transparent	Flat	Smooth	Present	+ve, cocci
C-72	Circular	Cream	Undulate	Transparent	Convex	Smooth	Present	-ve, cocci
C-73	Irregular	White	Undulate	Transparent	Raised	Smooth	Present	+ve, cocci
C-74	Punctiform	Pale white	Undulate	Opaque	Flat	Smooth	Present	-ve, cocci
C-75	Circular	Yellowish cream	Entire	Transparent	Convex	Smooth	Shiny	+ve, cocci
C-76	Punctiform	Cream	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci
C-77	Punctiform	Cream	Undulate	Opaque	Raised	Smooth	Present	+ve, cocci chain
C-78	Irregular	White	Undulate	Transparent	Flat	Smooth	Present	-ve, cocci
C-79	Irregular	Cream	Undulate	Transparent	Raised	Smooth	Present	+ve, cocci
C-80	Irregular	Cream	Filamentous	Transparent	Raised	Smooth	Present	+ve, cocci cluster
C-81	Circular	White	Entire	Transparent	Convex	Smooth	Present	+ve, cocci
C-82	Punctiform	Cream	Entire	Transparent	Flat	Smooth	Shiny	+ve, cocci
C-83	Punctiform	Cream	Undulate	Opaque	Flat	Smooth	Shiny	+ve, cocci
C-84	Circular	White	Rhizoid	Transparent	Convex	Smooth	Present	+ve, cocci

Table 2: List of Antibacterial Activity of Isolated Bacterial Cultures

Culture code	Zone of inhibition(mm)							
	S.aureus	***E.coli***	***B.subtilus***	***P.aeruginosa***	***Shigella***	***K.pneumoniae***	***P.mirabilis***	***L.monocytogens***
C-1	-	-	-	-	-	-	-	-
C-2	-	-	-	-	-	-	-	-
C-3	-	-	-	-	-	-	-	-
C-4	-	-	-	-	-	-	-	-
C-5	-	-	-	-	-	-	-	-
C-6	++	++	++	++	++	++	++	++
C-7	-	-	-	-	-	-	-	-
C-8	-	-	-	-	-	-	-	-
C-9	++	++	+	++	+++	+++	+	++
C-10	-	-	-	-	-	++	-	-
C-11	-	-	-	-	-	-	-	-
C-12	+	+++	+++	+	++	+	+++	+
C-13	-	-	-	-	-	-	-	-
C-14	-	-	-	-	-	-	-	-
C-15	-	-	-	-	-	-	-	-
C-16	+++	++	+	++	+	+	+++	+

C-17	-	-	-	+	-	-	-	-
C-18	-	-	-	-	-	-	-	-
C-19	-	-	-	-	-	-	-	-
C-20	-	-	-	-	-	-	-	-
C-21	-	-	-	-	-	-	-	-
C-22	-	-	-	-	-	-	-	-
C-23	+++	+	+++	+++	+	+	+++	+
C-24	-	-	-	-	-	-	-	-
C-25	-	-	-	-	-	-	-	-
C-26	-	-	-	-	-	-	-	-
C-27	-	-	-	-	-	-	-	-
C-28	-	-	-	-	-	++	-	-
C-29	-	-	-	-	-	-	-	-
C-30	++	+++	+	+	+++	+	+	+++
C-31	-	-	-	-	-	-	-	-
C-32	-	-	-	-	-	-	-	-
C-33	-	-	-	-	-	-	-	-
C-34	-	-	-	-	-	-	-	-
C-35	-	-	-	-	-	-	-	-
C-36	+++	++	+	+	+	+++	+	++
C-37	-	-	-	-	-	-	-	-
C-38	-	-	-	-	-	-	-	-
C-39	-	-	-	-	-	-	-	-
C-40	-	-	-	-	-	-	-	-
C-41	-	-	-	-	-	-	-	-
C-42	-	-	-	-	-	-	-	-
C-43	-	-	-	-	-	-	-	-
C-44	-	-	-	-	-	-	-	-
C-45	-	-	-	-	-	-	-	-
C-46	-	-	-	-	-	-	-	-
C-47	-	-	-	-	-	-	-	-
C-48	-	-	-	-	-	-	-	-
C-49	-	-	-	-	-	-	-	-
C-50	-	-	-	-	-	-	-	-
C-51	-	-	-	-	-	-	-	-
C-52	-	-	-	-	-	-	-	-
C-53	-	-	-	-	-	-	-	-
C-54	-	-	-	-	-	-	-	-
C-55	+++	+++	+++	+++	+++	+++	+++	+++
C-56	-	-	-	-	-	-	-	-
C-57	-	-	-	-	-	-	-	-
C-58	-	-	-	-	-	-	-	-
C-59	-	-	-	-	-	-	-	-
C-60	-	-	-	-	-	-	-	-
C-61	-	-	-	-	-	-	-	-
C-62	-	-	-	-	-	-	-	-
C-63	-	-	-	-	-	-	-	-
C-64	-	-	-	-	-	-	-	-
C-65	-	-	-	-	-	-	-	-
C-66	-	-	-	-	-	-	-	-
C-67	-	-	-	-	-	-	-	-
C-68	-	-	-	-	-	-	-	-
C-69	-	-	-	-	-	-	-	-
C-70	-	-	-	-	-	-	-	-

C-71	-	-	-	-	-	-	-	-
C-72	-	-	-	-	-	-	-	-
C-73	-	-	-	-	-	-	-	-
C-74	-	-	-	-	-	-	-	-
C-75	-	-	-	-	-	-	-	-
C-76	-	-	-	-	-	-	-	-
C-77	-	-	-	-	-	-	-	-
C-78	-	-	-	-	-	-	-	-
C-79	-	-	-	-	-	-	-	-
C-80	-	-	-	-	-	-	-	-
C-81	-	-	-	-	-	-	-	-
C-82	-	-	-	-	-	-	-	-
C-83	-	-	-	-	-	-	-	-
C-84	-	-	-	-	-	-	-	-
Chloramphenicol	23	28	22	23	20	23	20	20

Inhibition Zone: - : No activity, +: Weak activity indicates the clear zone 5~9mm, ++: Moderate activity indicates the clear zone 10~ 12mm, +++: High activity indicates the clear zone 13~16mm and indicates the clear zone >16mm.
Note: C1 to C84 are the bacterial cultures isolated from selected medicinal plants.

Table 3: Broad Spectrum Activity of some Isolated Endophytic bacteria

Culture code	Zone of inhibition(mm)							
	S.aureus	*E.coli*	*B.subtilus*	*P.aeruginosa*	*Shigella*	*K.pneumoniae*	*P.mirabilis*	*L.monocytogens*
C-6	++	++	++	++	++	++	++	++
C-9	++	++	+	++	+++	+++	+	++
C-12	+	+++	+++	+	++	+	+++	+
C-16	+++	++	+	++	+	+	+++	+
C-23	+++	+	+++	+++	+	+	+++	+
C-30	++	+++	+	+	+++	+	+	+++
C-36	+++	++	+	+	+	+++	+	++
C-55	+++	+++	+++	+++	+++	+++	+++	+++

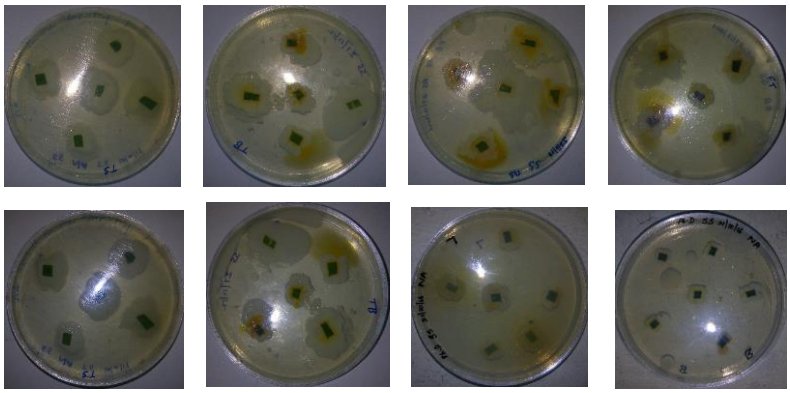

Fig 1: Leaf Segments of Medicinal Plants Inoculated in Nutrient Agar Medium to the Growth of Endophytic Bacteria and their Morphological Characteristics.

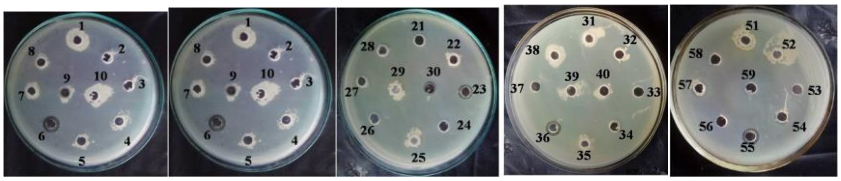

Fig 2: Antibacterial Activity of Endophytic Bacteria showing Zone of Inhibition against Bacterial Human Pathogens.

Endophytic Bacterial Cultures Isolated from Medicinal Plants Showing the Antibacterial Activity against Human Pathogens

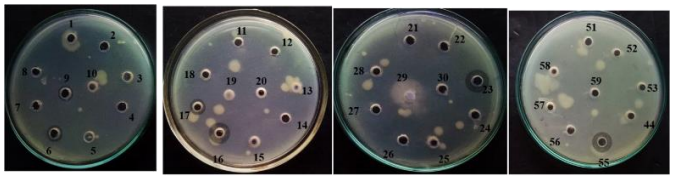

Fig a: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Pseudomonas aeruginosa.*

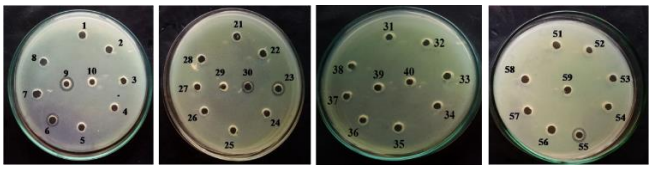

Fig b: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Listeria monocytogens*

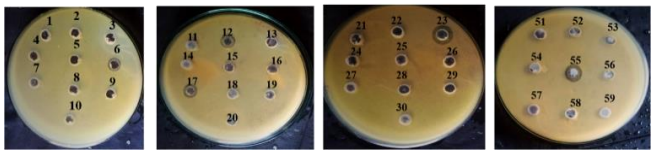

Fig c: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Proteus mirabilis*

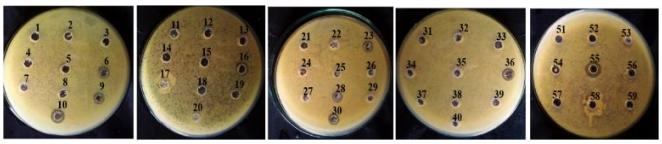

Fig d: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Klebsiella pneumoniae*

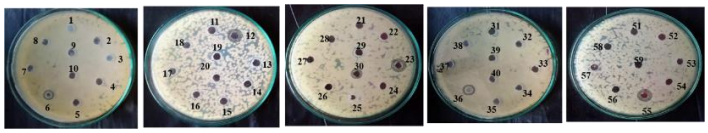

Fig e: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Bacillus subtilis*

Fig f: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Shigella species*

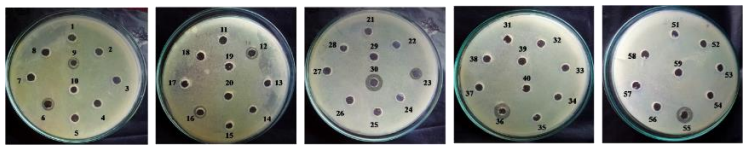

Fig g: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Escherichia coli.*

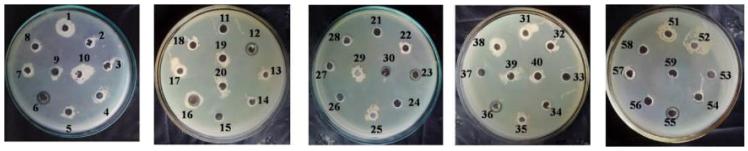

Fig g: Plates showing the antibacterial activity of endophytic bacteria with the zone of inhibition against *Staphylococcus aureus*

A total of 84 endophytic bacteria were isolated from the 28 different plants of Muthathi Wild Life Sanctuary (MWLS), Mandya (**Table 1**). In the present research, results were based on the evaluation of secondary metabolite produced in stationary condition as well as directly diffused through agar wells. Screening of bacteria was done on the basis of their antibacterial activity against clinically significant eight human bacterial pathogens; *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens* by using standard protocol of agar well diffusion method. The range of antibacterial activity was expressed in diameter of inhibition zones (mm), shown in **(Table 2)**. The isolated bacterial cultures were namely C6, C9, C12, C16, C23, C30, C36 and C55 showed maximum zone of inhibition against all eight bacterial strains, but best results seen by C6, C9 and C55. On the other hand C12, C16, C23, C30 and C36 exhibited good antibacterial activities with zone of inhibition against all eight pathogenic bacteria.

DISCUSSIONS

About, 84 cultures of endophytic bacteria were isolated from selected medicinal plants of Muthathi Wild Life Sanctuary. Endophytic bacteria are found in virtually every plant on earth (17). Different plant parts such as root, stem and nodule (25), leaves, stems and root (26) can also be used for isolation of endophytic bacteria. The initial identification of the bacterial isolates was done based on various morphological features of isolated endophytic bacteria. The colony characteristics of endophytic bacteria had round, irregular and punctiform in shape, margins were undulate, lobate and filamentous in edge. Flat, umbonate, raised and convex elevation with smooth surfaces. The color of the colony was white, cream and yellow of those isolated bacterial cultures from the leaves of the medicinal plants were found and have also find Gram-positive and Gram-negative bacteria was summarised in the **(Table 1)**.

As summarized in the results, antibacterial activity of endophytic bacteria was observed by the presence of zone of inhibition produced by endophytic bacteria against pathogenic bacteria. All the isolates from endophytic bacteria were screened for the antibacterial activity against*S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens*. The overall antibacterial results shown that maximum activity was observed against *E. coli, S. aureus* and *K. pneumoneae.* A total of 84 cultures were isolated from 28 medicinal plants and were taken for the determination of antibacterial activity against the clinically important bacterial pathogens. Out of which 8 isolates, showed the extensive spectrum of contrasting antibacterial activity against test pathogens with zone of inhibition and standard antibiotic was summarized in **(Table 2).**

Sushma and Jayashankar, (27) observed, the endophytic bacterial cultures C38, C46, C52, C57 and C82 showed the highest antibacterial activity against *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens*, and similarly C5, C13, C17, C23 and C39 Suresha and Jayashankar (20), reported the potential antibacterial activity with the highest zone of inhibition against *E. aeruginosa, K. pneumoniae, Lactobacillus sp. P. mirabilis, P. aeruginosa, S. aure*us and *S. mutan*s. The antibacterial activity was also observed by Maryam Beiranvand et al., (28) of endophytic actinobacteria from the isolates EB4 and EB7 showed inhibitory activity against *Bacillus cereus.* Pal et al., (29) reported the antimicrobial activity of the bacterial endophytes of *Passiflora foetida* indicating the inhibitory effect of the majority of the isolates against E. coli, S. aureus, and K. pneumoneae. Endophytic Bacteria from the Medicinal plant of *Andrographis paniculata* showed promising antimicrobial activity against few human pathogens *S. aureus*, *E. coli*, *S. typhi*, *Proteus* sp, *Pseudomonas* sp and *Klebsiella* sp (30). This study is also almost similar to the above authors.

CONCLUSIONS

In the present study, a total of 84 bacterial isolates were isolated and the endophytic bacteria present in leaves of selected medicinal plants of Muthathi Wild Life Sanctuary, Mandya. Only 8 bacterial endophytes, exhibited maximum antibacterial activity against *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens*. Thus, it can be decided from the present study, the endophytic bacteria isolated from selected medicinal plants have antibacterial properties against few human pathogens. Hence, they have pharmacologically important and it will leads for the innovation of novel medicines.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Alvin A, Miller KI, Neilan BA. Exploring the potential of endophytes from medicinal plants as sources of anti-mycobacterial compounds. Microbiol Res. (2014), 169(7):483-95.
2. Khan, A. V, Ahmed, Q. U, Mir, M. R, Shukla, I, Khan, A. A. Antibacterial efficacy of the seed extracts of Melia azedarach against some hospital isolated human pathogenic bacterial strains. Asian Pacific Journal of Tropical Biomedicine, (2011), 452-455.
3. Petrini O; Cambridge, UK, Cambridge University Press (1986), 175–18.
4. Schulz B, Boyle C, Eds. *What are Endophytes: Microbial RootEndophytes*. Springer-Verlag: Berlin; (2006).
5. Garcia, A., Rhoden, S.A., Rubin-Filho, C.J., Nakamura,C.V. and Pamphile, J.A. (2012) Diversity of foliar endophytic fungi from the medicinal plant *Sapindus saponaria* L. and their localization by scanning electron microscopy. Biol. Res., 45: 139-148.
6. Strobel GA, Daisy B. Bio prospecting for Microbial Endophytes and Their Natural Products. Microbiology and Molecular Biology Reviews 2003; 67:491-502.
7. Conrath U, Beckers GJM, Flors V, Garcı´a-Agustı´n P, Jakab G, Mauch F Priming: getting ready for battle. Mol Plant Microbe Interact (2006), 19:1062–1071.
8. Pillay VK, Nowak J. Inoculum's density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (Lycopersicon esculentum L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol (1997), 43:354–361.
9. Lee S, Flores-ncarnacion M, Contreras-Zentella M, Garcia-Flores L, Escamilla JE, Kennedy C Indole-3-acetic acid biosynthesis is deficient in Glucon acetobacter diazotrophicus strains with mutations in cytochrome C biogenesis genes. J Bacteriol (2004), 186:5384–5391.
10. Wakelin S. Phosphate solubilisation by Penicillium spp. closely associated with wheat roots. Biol Fertil Soils (2004), 40:36–43.
11. Compant S, Reiter B, Sessitsch A, Nowak J, Cle´ment C, Ait Barka E. Endophytic colonization of Vitis vinifera L. by a plant growth promoting bacterium, Burkholderia sp. strain PsJN. Appl Environ Microbiology (2005), 71:1685–1693.
12. Ezra D, Castillo UF, Strobel GA, Hess WM, Porter H, Jensen JB, Condron MAM, Teplow DB, Sears J, Maranta M, Hunter M, Weber B, Yaver D. Coronamycins, peptide antibiotics produced by a verticillate Streptomyces sp. (MSU-2110) endophytic on Monstera sp. Microbiology (2004), 150:785–793.
13. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D. Endophytic bacteria and their potential applications. Crit Rev Plant Science (2002), 21:583–606.
14. Chernin L, Chet I. Microbial enzymes in bio control of plant pathogens and pests. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology and applications. Marcel Dekker, New York, (2002) pp. 171–225.
15. Kloepper JW, Rodriguez-Kabana R, Zehnder GW, Murphy J, Sikora E, Fernandez C. Plant root-bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. Australia's J Plant Pathology (1999), 28:27–33.
16. Rosenblueth, M., E. Martines-Romero. 2006. Bacterial endophytes and their interaction with hosts. APS, (2006), 19, 827-837.
17. Ryan, R., P, Germaine, K, Franks, A, Ryan, D, J, Dowling, D. N. 2008. Bacterial endophytes: Recent developments and applications. FEMS Microbial. Letter, (2008), 278, 1-9.
18. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Natural Product Reports 2001; 18:448–59.
19. Strobel GA, Dirksie E, Sears J, Markworth C. Volatile antimicrobials from a novel endophytic fungus. Microbiol 2001; 147:2943-50.
20. Suresha S and M. Jayashankar. Antimicrobial Activity of Endophytic Bacteria Isolated from few Plants of Muthathi Wild Life Sanctuary, Mandya, Karnataka. International Journal of Pharmaceutical Sciences and Research. Received on 31 August, 2018; accepted (IJPSR/RA-10852/08-18) on 27 October; published in IJPSR, 2019; Vol.10 (5): 1000-06.
21. Gond S, Verma V, Kumar A, Kumar V, Kharwar R. Study of endophytic fungal community from different parts of *Aegle marmelos* Correae (Rutaceae) from Varanasi (India). World Journal of Microbiology and Biotechnology (2007); 23: 1371-1375.
22. Shilpa Sharma and Shikha Roy. "Isolation and Identification of a novel Endophyte from a plant *Amaranthus spinosus".* International Journal of Current Microbiology and Applied Sciences (2015) 4(2): 785-798.
23. Lorian V. Antibiotics in laboratory medicine. Williams and Wilkins, Baltimore, 1996.
24. Xie J, Zhang R, Shang C, GuoY. Isolation and Characterization of a Bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits Antimicrobial activity against domestic Animal pathogens. Afr J Biotechnology (2009); 8 (20)5611e9.
25. Hung, P.Q. and Annapurna, K. Isolation and characterization of endophytic bacteria in soybean (Glycine sp.). Omonrice, (2004) 12: 92-101.
26. Sobral, J.K., Araujo, W.L., Mendes, R., Kleiner, A.A.P. and Azevedo, J.L. Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosate herbicide. Plant Soil (2005) 273(1):91-99.
27. Sushma M, M Jayashankar and Vinu AK. Antibacterial activity of endophytic bacteria isolated from few medicinal plants of Br hill, Karnataka. Journal of Pharmacognosy and Phytochemistry (2018) 7(5): 2338-2342.
28. Maryam Beiranvand, Mansour Amin, Abdolrazag Hashemi-Shahraki, Bizhan Romani, SajadYaghoubi, Parisa Sadeghi; Antimicrobial activity of endophytic bacterial populations isolated from medical plants of Iran. Iranian Journal of Microbiology Volume 9 Number 1 (February-2017) 11-18.
29. Pal, A., Chattopadhyay, A. and Paul, A.K. Diversity and antimicrobial spectrum of endophytic bacteria isolated from *Paederia foetida* L. Int. J. Curr. Pharm. Res, (2012) 4(3):123-127.
30. Arunachalam, Gayathri P. Studies on Bio prospecting of Endophytic Bacteria from the Medicinal plant of *Andrographis paniculata* for their antimicrobial activity and antibiotic Susceptibility pattern, PG and Research Department of Microbiology, Sri Sankara Arts and Science College, Enathur, Kanchipuram. (2010) 2:4. ISSN-0975-1491.