

## MOLECULAR CHARACTERIZATION AND DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF BACTERIAL ENDOPHYTES FROM *OCIMUM SANCTUM* LINN. (LAMIACEAE)

### Abstract

Endophytic microorganisms of medicinal plants have gained great importance, and new evidence points to their significant ecological and biotechnological importance. Endophytes are indeed a group of organisms, such as fungi, bacteria, or actinomycete, which live in plant tissues. Although many endophytes have mutually beneficial relationships with plants, not all endophytes are symbiotic. Some endophytes can damage plants under certain conditions and cause more harm instead of providing benefits. Antimicrobial activity is attributed to the presence of endophytes in the plant. This study focuses on the isolation, characterization, and screening of endophytes associated with *Ocimum sanctum* leaves and stem. The aim is to evaluate the antibacterial properties of the endophytic bacteria. Genomic DNA extraction, PCR analysis, and sequence analysis were performed to characterize the molecular features of the isolated endophytes. Research results confirm the use of *Ocimum sanctum* against pathogenic microorganisms. It also highlights the importance of isolating and identifying antimicrobials from endophytic bacteria as a valuable approach in the search for new natural products. A total of 30 endophytic species were isolated from the leaves and stem of *Ocimum sanctum* and screened for their antimicrobial activity using antibiotic discs containing Thrombomycin, Chloramphenicol, and Streptomycin. Additionally it was also determined that these species produce enzymes such as cellulase, amylase, pectinase, and protease. Overall, this study demonstrates the potential of endophytic bacteria associated with aromatic shrubs such as *Ocimum sanctum*. Its use as a source of antimicrobial emphasizes the importance of further research in this area.

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## I. INTRODUCTION

Obviously, *Ocimum sanctum* L (Tulsi) is a highly valuable resource in the isolation of endophytic bacteria due to its ethno-botanical history and medicinal properties (Cohen 2014). Endophytes are microorganisms that live in tissues of plants and play an important role in the development of the medicinal properties of plants. Endophytic microorganisms have been found to colonize healthy plant tissues and occur in many genera and species in the plant host (Guo et al. 2008, Hallman et al. 1997). These microorganisms are capable of producing many biologically active compounds such as antimicrobials, immune suppressants, anti-inflammatory drugs, anticancer compounds and plant growth hormones (Petrini 1986). They have a wide range of medical, agricultural, and commercial uses (Liarzi et al. 2016). Isolation and identification of these compounds from endophytes led to the discovery of new natural products with vast potential in many fields (Selim et al., 2016). Additionally, endophytes also produce extracellular enzymes such as amylase, cellulase, protease, lipase, and pectinase to build defense mechanisms against plant attacks, which play an important role in many industries (Hawar 2022). Endophytic microorganisms have been reported to exist in plants from diverse surroundings, including geothermal soils, aquatic, marine, beaches, tropical, atmospheric, xerophytic, desert, Antarctic, rainforests, mangrove swamps and forests (Strobel et al. 2002, Suryanarayanan & Murali, 2006). The nature and abundance of endophytes are influenced by the many environmental conditions like soil, temperature, and humidity. The interaction between endophytes and plants is mainly controlled by the genes of the pathogen and the host plant and is also controlled by the environment. Studying endophytic organisms is important for understanding their interactions with plants (Petrini 1991, Wilson 1995). Plants that live in a unique environment, compete with other organisms or require the utmost protection to survive are competitive hosts for endophytes. The study of endophytic bacteria and their possible applications holds great promise for further developments in many fields.

## II. MATERIALS AND METHODS

**Plant Material:** Endophytic bacteria were isolated from healthy and mature leaves growing at the Union Christian College Aluva, Kerala, India. The leaves were carefully stored in clean plastic bags, taken to the laboratory, and used for further experiments.

**Isolation of endophytic bacteria from *Ocimum sanctum*:** Surface sterilization is the first and necessary step in isolating endophytes to remove endophytic bacteria from the surface and ensure that only endophytic bacteria are isolated. This process involves applying an oxidizing agent or a general antiseptic for few second to minutes, followed by rinsing several times with sterile solutions. The surface sterilization process, isolation, and purification of endophytic bacteria from *Ocimum sanctum* plant materials can be performed by employing the several steps.

**Pre-Treatment:** The leaves and stems of *Ocimum sanctum* were washed thoroughly under tap water to remove any soil particles and most of the microbial surface epiphytes. It's important to ensure that the plant material is clean before further processing or analysis.

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**Surface Sterilization:** Leaves and stems of *Ocimum sanctum* are collected fresh and washed in slowly flowing tap water for 15 min to remove residues and contaminants from the plant surface. The plant material was then washed in Tween 20 solution (1 drop in 200 mL sterile water) for 1 min. Rinse plant material three times with sterile water after Tween 20 treatment. This is done to ensure that traces of Tween 20 are eliminated completely. The final step of the surface sterilization process is to use a disinfectant such as 2% sodium hypochlorite and 70% ethanol. Treat plant material with sodium hypochlorite for 1 min to kill any remaining surface microorganisms. Then rinse with sterile water to remove the sterilant. After sodium hypochlorite treatment, the plant material was treated with 70% ethanol. Ethanol acts as an additional disinfectant, eliminating any remaining surface microorganisms. Rinse the plant again with sterile water to remove any residual ethanol. By performing these steps, the surface sterilization process is designed to effectively eliminate surface microbes while minimizing damage to tissues and endophytic bacteria within.

**Cultivation of Endophytic Bacteria:** Nutrient agar medium was used to isolate endophytic bacteria. Since nutrient agar contains no substances that inhibit the growth of endophytic fungi, the antifungal agent nystatin (30 µg/mL) was added to the culture medium. The presence of nystatin in the culture medium helps prevent the growth of endophytic fungi and ensures that only endophytic bacteria can grow and be isolated.

**Isolating, Purifying, and Subculturing Endophytic Bacteria:** After the process of surface sterilization of the plant material, let it dry. Using a sterile techniques, remove the surface of the stems using a sterile scalpel in the laminar flow unit. The leaves are cut into several pieces and were placed in a nutrient medium supplemented with an antifungal agent. Incubate the plates at  $28 \pm 2^\circ\text{C}$  to get large number of endophytic bacteria. The After 24 hrs of incubation morphologically different bacterial colonies were selected and streaked on nutrient agar plates to obtain pure cultures. The selected isolates were then subcultured on nutrient agar slants, and these endophytic isolates were stored at  $4^\circ\text{C}$  until further use.

**Characterization of Endophytic Bacteria:** Characterization of endophytic isolates was based on morphological and phenotypic features of colonies such as microscopic features, gram staining, endospore staining, motility, catalase, oxidase activity, and molecular techniques. Selected endophytes was inoculated into fresh agar slants and stored at  $4^\circ\text{C}$  or at  $-80^\circ\text{C}$  in 20% glycerol vials for long term use. From the 30 isolates obtained, 10 isolates labeled as TH1, TH2, TH3, SH1, SH2, SH3, HW1, HW2, HW3 and HW4 were selected for further characterization studies. The molecular characteristics of these isolates were examined to better understand their properties and potential applications.

### III. SCREENING OF ENDOPHYTIC BACTERIA

**Enzyme Assays:** Pure cultures of endophytic bacteria were screened for production of enzymes such as amylase, cellulase, pectinase, and protease. For cultivating endophytic bacteria, use minimal media containing the following: 0.3g beef extract, 0.5 g peptone, 0.5g sodium chloride,

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2g agar, and 100 ml distilled water. Bacterial colonies were spotted on minimal agar medium and incubated under conditions suitable for enzyme production. After incubation, check the plate to see if a clear zone has formed around the bacteria.

**Amylase Activity:** Isolates were analyzed for amylolytic activity by testing starch hydrolysis based on the solubilization of starch. The culture is inoculated onto starch agar plates containing 0.3g beef extract, 0.05g gastric enzyme digest, 1g starch, 2g agar in 100 ml distilled water and incubated at 37°C for 48 hrs. A positive result is indicated by the appearance of a clear hydrolysis area after filling the plate with iodine solution for 30 s. \]

**Cellulolytic Activity:** Screening of bacteria for cellulolytic activity based on the area from CMC (carboxymethylcellulose) agar plates using the Congo red method. The isolates were inoculated into CMC agar medium containing 0.5g carboxymethylcellulose, 0.1g NaNO<sub>3</sub>, 0.1g K<sub>2</sub>HPO<sub>4</sub>, 0.1g KCl, 0.05g MgSO<sub>4</sub>, 0.05g beef extract, 0.1g glucose, 2g agar and 100 ml distilled water.. After incubation at 37°C for 48 hrs, flood the plate with Congo red solution (0.1%) and let it sit for 5 min. discard the Congo red solution and wash the plate with 1M NaCl solution for 15-20 min. Observe for area of clearance indicating a positive result, i.e utilization of cellulose.

**Pectinase Activity:** Isolates were inoculated onto Pectinase Screening Agar Medium (PSAM containing 1 g pectin, 0.1g beef extract, 0.5g peptone, 0.2g CaCO<sub>3</sub>, 0.2g NaCl, and 2g agar dissolved in 100 ml distilled water. The inoculated plates were then incubated at 37°C for 48 hrs. After incubation, isolates which use pectin as a carbon source form a zone of pectinase screening agar medium.

**Protease Activity:** Isolates were inoculated onto Casein agar plate containing 0.015g beef extract, 0.5g NaCl, 2g agar, and 1g casein in 100 ml distilled water. The inoculated plates were then incubated at 37°C for 24 hrs. After incubation, iodine solutions were added to the plates and clear areas were observed for casein degrading protease producing bacteria.

**Antimicrobial Activity:** Antagonistic activity of isolates was screened using the agar disc diffusion method and recorded the zone of clearance. Antimicrobial assay was carried out using Muller Hinton agar. Use a sterile cotton swab to spread the isolate onto the agar plate to create a homogeneous culture. After incubating at 37 °C for 24 hrs, use a scale to measure the diameter (mm) of the inhibition zone around each well. Antimicrobial assay was performed in triplicates.

#### **IV. MOLECULAR CHARACTERIZATION OF ENDOPHYTIC BACTERIA**

**DNA Extraction:** DNA extraction was performed by centrifuging a 2 ml suspension of bacterial cells at 15,000 g at 4°C for 10 min. The resulting pellet was then resuspended in 500 µl of TNE buffer containing 10 mM Tris, pH 8.0, 1 mM EDTA, and 0.15 mM NaCl, and centrifuged again at 15,000 g for 10 min at 4 °C. This pellet was incubated in 500 µl of lysis buffer containing 0.05 mM Tris-HCl, pH 8.0, 0.05 mM EDTA, 0.1 mM NaCl, 2% SDS, 0.2% PVP, and 0.1% mercaptoethanol (0.1%). Next, a solution of proteinase K (20 mg/ml) was added and the mixture was initially incubated at 37°C for one hour followed by two hours at 55 °C. Subsequent

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extraction was carried out using the well-established phenol-chloroform method. The pellet was deproteinized by adding an equal volume of a solution containing phenol (balanced with Tris at pH 8.0), chloroform, and isoamyl alcohol (in a ratio of 25:24:1). To achieve separation, the layers of phenol and aqueous solution were centrifuged at 15,000g for 15 min at a temperature of 4°C. The aqueous phase was transferred into a new tube, and this process was repeated again. Subsequently, an equal volume of a mixture containing chloroform and isoamyl alcohol (24:1) was added and gently mixed by inversion. The aqueous phase was separated by centrifuging at 15,000 g for 15 min at 4°C to separate. This was then transferred to a fresh tube, precipitated by adding an equal volume of chilled absolute ethanol and incubated at -20°C overnight. The obtained DNA precipitate was centrifuged at 15,000 g for 15 min at 4°C and the pellet was subsequently washed with 70% ice-cold ethanol. This process was repeated with another centrifugation step, followed by discarding the supernatant and leaving the tube open until the pellet dried. Finally, the DNA pellet was dissolved in 100 µl of molecular grade water. The purity of the extracted DNA was analyzed spectrophotometrically and visualized using agarose gel for electrophoresis.

**PCR Amplification:** Amplification of the 16S rRNA gene was performed using universal primers 16S F (GAG TTT GAT CCT GGC TCA) and 16S R (ACG GCT ACC TTG TTA CGA CTT). The process was carried out in DNA thermal cycler (Biorad). The amplification profile involved an initial denaturation step at 95 °C for 5 min, followed by annealing at 58°C for 30 s and extension at 68°C for 2 min. This cycle was repeated 34 times followed by final extension process, at 68°C for 10 min. The PCR products were then separated on a 1% agarose gel.

**Sequencing and Analysis:** Sequencing reaction was done using ABI PRISM 3700 Large Dye Sequencer. The sequences were compiled, analysed, and screened for vector regions using the “VecScreen” system of National Centre for Biotechnology Information (NCBI). After screening, the sequences were aligned with homologous sequences obtained from the GenBank database through the BLAST algorithm of NCBI which compare nucleotide for inferring functional and evolutionary relationships. The 16S rRNA nucleotide sequences were then uploaded to the submission wizard of the NCBI submission portal and assigned accession numbers.

**Phylogenetic Analysis:** Phylogenetic analysis of 16S rRNA gene sequences was studied using Molecular Evolutionary Genetic Analysis (MEGA 5.10) software. To construct the phylogenetic tree, the Unweighted paired group method with arithmetic mean (UPGMA), a type of hierarchical clustering algorithm to combine sequences based on their distance was used. Finally, significant information is obtained through the tree drawn by this user friendly software.

**Results:** Thirty species of endophytic bacteria were isolated from leaves and stems. Pure endophyte cultures were prepared and subcultured on nutrient agar slants for further studies. These tests also demonstrated the ability of the isolated endophyte to utilize starch, cellulose, pectin, and casein. Of these, enzyme production was seen in 10 isolates labeled TH1, TH2, TH3, SH1, SH2, SH3, HW1, HW2, HW3, and HW4, and these isolates were subjected to molecular analysis and 16S rRNA sequencing. This molecular analysis, combined with enzyme production

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data, may provide insight into the diversity of endophytic bacteria associated with *Ocimum sanctum* and potential biotechnological applications.

By screening for antimicrobial activity, the isolates with potential antimicrobial properties were identified for further study. Of the ten isolates, *Microbacterium* sp. strain UCCB140, *Micrococcus* sp. strain UCCB141, *Neobacillus bataviensis* strain UCCB142, *Paenibacillus* sp. strain UCCB144, *Rhizobium* sp. strain UCCB145, *Staphylococcus wameri* strain UCCB146, *Methylobacterium* sp. strain UCCB147 and *Bacillus cereus* strain UCCB148 were found to be sensitive to the sterile antibiotics Thrombomycin, Chloramphenicol and Streptomycin. The zone of inhibition was recorded and was used as an indicator of the antimicrobial activity of the isolated bacteria. The larger the inhibition zone, the more potent the antibiotic is against the standard antibiotic.

Degradation of specific substrates (amylase of starch, cellulase of cellulose, pectin, pectinase, protease of casein) by secreted enzymes was represented by the interstitial zone. The larger the clear area, the more enzyme produced by the endophyte. This analytical method helps identify endophytic bacteria that can produce the desired enzymes in high yield and can also be used in many industrial applications such as food, textiles, and biotechnology.

Out of the ten endophytic bacterial isolates the strains named *Bacillus* sp. strain UCCB139, *Micrococcus* sp. strain UCCB141, *Neobacillus bataviensis* strain UCCB142, *Paenibacillus* sp. strain UCCB144, *Rhizobium* sp. strain UCCB145, *Staphylococcus wameri* strain UCCB146, *Methylobacterium* sp. strain UCCB147, *Bacillus cereus* strain UCCB 148 hydrolysed starch and secreted amylase enzyme. *Microbacterium* sp. strain UCCB140, *Agrobacterium larrymoorei* strain UCCB143 and *Paenibacillus* sp. strain UCCB144 can utilize pectinase enzyme. The strains *Bacillus* sp. strain UCCB139, *Neobacillus bataviensis* strain UCCB142, and *Micrococcus* sp. strain UCCB141 can utilize the enzyme cellulase. The strains *Neobacillus bataviensis* strain UCCB142, *Agrobacterium larrymoorei* strain UCCB143 and *Paenibacillus* sp. strain UCCB144, *Rhizobium* sp. strain UCCB145, *Staphylococcus wameri* strain UCCB146, *Methylobacterium* sp. strain UCCB147 can utilize casein and produce protease enzyme.

The DNA of these ten endophytic bacteria was extracted and molecular characterization was performed the extracted DNA samples were analysed by PCR technique and nucleotide sequencing. These sequences were compared with the BLAST algorithm using the NCBI website. The GenBank database was matched, and the phylogenetic tree was drawn. The results were deposited in the Genbank and accession numbers such as MH192999, MH198277, MH198278, MH193371, MH198279, MH198280, MH193373, MH198281, MH198282 and MH193385 as given in Table 1 were obtained.

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**Table 1:** Endophytic bacterial strains from *Ocimum sanctum*

Sl.No	Code	Strain	Genbank Accession no.
1	TH 1	<i>Bacillus</i> sp. strain UCCB 139	MH192999
2	TH 2	<i>Microbacterium</i> sp. strain UCCB 140	MH198277
3	TH -3	<i>Micrococcus</i> sp. strain UCCB 141	MH198278
4	SH 1	<i>Neobacillus bataviensis</i> strain UCCB 142	MH193371
5	SH 2	<i>Agrobacterium larrymoorei</i> strain UCCB 143	MH198279
6	SH 3	<i>Paenibacillus</i> sp. strain UCCB 144	MH198280
7	HW 1	<i>Rhizobium</i> sp. strain UCCB 145	MH193373
8	HW 2	<i>Staphylococcus warneri</i> strain UCCB 146	MH198281
9	HW 3	<i>Methylobacterium</i> sp. strain UCCB 147	MH198282
10	HW 4	<i>Bacillus cereus</i> strain UCCB 148	MH193385

## V. DISCUSSION

*Ocimum sanctum* leaves, roots, and stems are rich in endophytic bacterial diversity. Isolation and screening of endophytic bacteria from pure leaves have significant potential in medicine, agriculture, and industry (Singh & Verma 2010). Endophytes are rich in bioactive metabolites. Endophytic microorganisms are bacteria that live in plants, especially in leaves, roots, and stems. Endophytes do not harm plants. The ability of endophytes to produce a variety of secondary metabolites has provided scientists with many interesting compounds and elements that can be developed as new drugs.

Isolation techniques are often used together to sterilize the tissue, covering small sterile tissue by softening the tissue, and streaking on nutrient agar or vacuum house or pressure extraction. Theoretically, biocides should kill plant pathogens without affecting tissues and endophytic organisms (Ezeobiora et al. 2021). However, this goal is difficult to achieve because the conditions required to kill bacteria at the end of the site can be fatal to some endophytic bacteria, and over time they can enter individual plant tissues.

In general, the need for new antibiotics arises from the advancement of existing antibiotics (Reygaert 2018). This problem extends beyond antibiotic therapy. For example, agricultural microbes are also known to increase resistance to commonly used antibiotics. Endophytes are chemical synthesizers in plants. It is believed that endophytes impair the immune system by producing secondary metabolites with antimicrobial activity (Sathish et al. 2012).

30 different endophytes were isolated from fresh leaves of *Ocimum sanctum* in 3 different locations. Pure endophyte cultures were prepared and subcultured on nutrient agar slants for

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further studies. The Kirby Bauer disk diffusion method is widely used in antimicrobial resistance testing, and this method was used to determine the antimicrobial activity of 10 endophytic bacteria isolated from *the Ocimum sanctum*. Overall, in vitro antimicrobial results showed that the highest resistance was observed against *Staphylococcus warneri* strain UCCB146 and *Bacillus cereus* strain UCCB148. These studies provide insight into the formation and diversity of endophytes. Many natural products obtained from endophytes have been proven to have antibacterial, antifungal, antiviral, antibacterial, and antifungal properties and are important bioactive natural products (Fadiji et al. 2020). Most endophytic bacteria produce many new antibiotics such as Ecomycins, Pseudomycins, Munumbicins, and Kakadumycins (Christina et al. 2013). These drugs inhibit the growth of pathogenic bacteria and fungi.

The ability of isolated endophytic bacteria to utilize starch, cellulose, pectin, and casein has been discovered. This study shows that ten types of endophytes produce large amounts (high activity) of cellulase, amylase, pectinase, and protease. Clear zone around the colonies indicates a positive result and shows that the isolated bacteria can produce different enzymes that have many applications in industries such as food processing, textiles, pharmaceuticals, and juice (Haile et al. 2022 ).

It is suggested that enzymes obtained from endophytes isolated from *Ocimum sanctum* plants have similar properties (Tiwari et al. 2013). This can only be confirmed when these compounds are purified and tested in animal models.

Molecular characterization were obtained by extracting the DNA of 10 endophytic species and the DNA was amplified using PCR technique and a phylogenetic tree was constructed. Molecular techniques have revolutionized the field of microbiology and have greatly aided in the isolation and characterization of bacteria. Due to the wide range of functions of endophytes, their functional properties can be complex. However, advanced technologies hold great promise in facilitating this research. Molecular techniques, including advanced techniques and specific molecular and biochemical tests, play an important role in the isolation, identification, and activity of microbial endophytes. These techniques lead to a better knowledge of the different roles and possible uses of endophytic organisms in agriculture (Shah et al. 2021).

There are only a few reports on various types of endophytic bacteria and fungi in medicinal plants. This suggests that there is much to investigate and understand the diversity of endophytic microorganisms associated with Indian medicinal plants (Gouda et al. 2016 ). The findings in this study indicate that isolation and identification studies of bioactive compounds may become significant in the search for new natural products.

## VI. CONCLUSION

Despite the diverse approaches used to isolate and characterize endophytes vary, they all follow similar principles. The procedure usually involves sample preparation, which involves collecting tissues and sterilizing their surfaces to remove external contaminants (Kafur & Basheer 2011). Identification of specific organisms often involves molecular techniques such as



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Polymerase Chain Reaction and sequencing of genes or regions, as well as phylogenetic analysis. This allowed the researchers to find the taxonomic position and relatedness of the isolated endophytes. In general, the isolation and characterization of endophytes must carefully consider the specific species examined and the limitations of the culture. Advances in molecular techniques have provided new ways to study previously benign endophytes, expanding our understanding of their wide variety and utility.

The title of this article is "Molecular Characterization and Determination of antimicrobial properties of endophytes obtained from *Ocimum sanctum*". (Lamiaceae) awareness has been raised about the importance of endophytes as a good source of antibiotics, enzymes, and secondary metabolites, and new resources have been found to explore their diversity and functions that endophytes cannot access. According to our results, endophytic bacteria exhibited broad-spectrum antibiotics and produced the highest enzyme activity based on their morphological characteristics along with molecular analysis.

Isolation and analysis of endophytic bacteria from leaves have important applications in medicine, agriculture, and industry. Endophytes are rich in bioactive metabolites. Endophytic microbes are organisms that inhabit the plant leaves, roots, and stems. Endophytes can produce a range of secondary metabolites, providing researchers with numerous leads for compounds of pharmaceutical significance and possible development as new drugs. Endophytes are a promising source of bioactive compounds continuously isolated, characterized, and explored for the finding of best bioactive compounds that are employed in medicine, agriculture and various industries (Gupta et al. 2023).

Endophytes are an alternative source of drugs, which may conserve biodiversity and drug resistance. They are thought to stimulate the immune system against invading bacteria by producing secondary metabolites with antimicrobial activity. Endophytic bacteria such as *Microbacterium sp.*, *Paenibacillus sp.*, *Bacillus sp.*, *Neobacillus bataviensis*, *Micrococcus sp.*, *Staphylococcus warneri*, *Methylobacterium sp.*, and *Bacillus cereus* respond sensitivity to antibiotics sterile disks Thrombomycin, Chloramphenicol and Streptomycin. Bioactive compounds produced by endophytes should be the basis for the research and development of new antibiotics, anticancer medicines and treatments for various diseases in humans, animals, and plants (Singh et al., 2017). These compounds have the potential to provide an alternative treatment and solve the growing problem of drug resistance. To further analyse and understand the phylogenetic relationships and evolution of different endophytic organisms, many researchers have conducted studies on the amplification of specific genes and comparisons with known studies. Evolutionary relationships between different endophytic bacteria can be analysed using specific genes, which are often used as molecular markers to study bacterial diversity and phylogeny (Mishra et al. 2017).

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**Conflict of Interest:** The authors declare that there is no conflict of interest.

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## REFERENCES

- [1] Cohen, M. M. (2014). Tulsi-*Ocimum sanctum*: A herb for all reasons. *Journal of Ayurveda and Integrative Medicine*, 5(4), 251.
- [2] Guo, B., Wang, Y., Sun, X., & Tang, K. (2008). Bioactive natural products from endophytes: a review. *Applied Biochemistry and Microbiology*, 44, 136-142.
- [3] Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F., & Kloepper, J. W. (1997). Bacterial endophytes in agricultural crops. *Canadian journal of microbiology*, 43(10), 895-914.
- [4] Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. *Microbiology of the Phyllosphere*, 175-187.
- [5] Liarzi, O., Bucki, P., Braun Miyara, S., & Ezra, D. (2016). Bioactive volatiles from an endophytic *Daldinia* cf. *concentrica* isolate affect the viability of the plant parasitic nematode *Meloidogyne javanica*. *PLoS one*, 11(12), e0168437.
- [6] Selim, K. A., El-Beih, A. A., AbdEl-Rahman, T., & El-Diwany, A. I. (2012). Biology of endophytic fungi. *Current Research in Environmental & Applied Mycology*, 2(1), 31-82.
- [7] Hawar, S. N. (2022). Extracellular enzyme of endophytic fungi isolated from *Ziziphus spina* leaves as medicinal plant. *International Journal of Biomaterials*, 2022.
- [8] Strobel, G., Ford, E., Worapong, J., Harper, J. K., Arif, A. M., Grant, D. M., & Chau, R. M. W. (2002). Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. *Phytochemistry*, 60(2), 179-183.
- [9] Suryanarayanan, T. S., & Murali, T. S. (2006). Incidence of *Leptosphaerulina crassiasca* in symptomless leaves of peanut in southern India. *Journal of Basic Microbiology*, 46(4), 305-309.
- [10] Petrini, O., Andrews, J. H., & Hirano, S. S. (1991). Microbial ecology of leaves. *Spring-Verlag, New York*, 179-197.
- [11] Wilson, D. (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos*, 274-276.
- [12] Singh, V., & Verma, O. (2010). *Ocimum sanctum* (tulsi): Bio-pharmacological activities. *Webmed Central Pharmacol*. 1:WMC001046 10.9754/journal.wmc.2010.00104.
- [13] Ezeobiora, C. E., Igbokwe, N. H., Amin, D. H., & Mendie, U. E. (2021). Endophytic microbes from Nigerian ethnomedicinal plants: a potential source for bioactive secondary metabolites-a review. *Bulletin of the National Research Centre*, 45(1), 1-10.
- [14] Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS microbiology*, 4(3), 482.
- [15] Sathish, L., Pavithra, N., & Ananda, K. (2012). Antimicrobial activity and biodegrading enzymes of endophytic fungi from eucalyptus. *International Journal of Pharmaceutical Sciences and Research*, 3(8), 2574.
- [16] Fadji, A. E., & Babalola, O. O. (2020). Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. *Frontiers in Bioengineering and Biotechnology*, 8, 467.
- [17] Christina, A., Christopher, V., & Bhore, S. J. (2013). Endophytic bacteria as a source of novel antibiotics: an overview. *Pharmacognosy reviews*, 7(13), 11.
- [18] Haile, S., Masi, C., & Tafesse, M. (2022). Isolation and characterization of pectinase-producing bacteria (*Serratia marcescens*) from avocado peel waste for juice clarification. *BMC microbiology*, 22(1), 1-15.

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- [19] Tiwari, R., Awasthi, A., Mall, M., Shukla, A. K., Srinivas, K. S., Syamasundar, K. V., & Kalra, A. (2013). Bacterial endophyte-mediated enhancement of in planta content of key terpenoid indole alkaloids and growth parameters of *Catharanthus roseus*. *Industrial Crops and Products*, 43, 306-310.
- [20] Shah, D., Khan, M. S., Aziz, S., Ali, H., & Pecoraro, L. (2021). Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. *Microorganisms*, 10(1), 21.
- [21] Gouda, S., Das, G., Sen, S. K., Shin, H. S., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in microbiology*, 7, 1538.
- [22] Kafur, A., & Basheer Khan, A. (2011). Isolation of endophytic actinomycetes from *Catharanthes roseus* (L.) G. Don leaves and their antimicrobial activity. *Iranian Journal of Biotechnology*, 9(4), 302-306.
- [23] Gupta, A., Meshram, V., Gupta, M., Goyal, S., Qureshi, K. A., Jaremko, M., & Shukla, K. K. (2023). Fungal Endophytes: Microfactories of Novel Bioactive Compounds with Therapeutic Interventions; A Comprehensive Review on the Biotechnological Developments in the Field of Fungal Endophytic Biology over the Last Decade. *Biomolecules*, 13(7), 1038.
- [24] Singh, M., Kumar, A., Singh, R., & Pandey, K. D. (2017). Endophytic bacteria: a new source of bioactive compounds. *3 Biotech*, 7, 1-14.
- [25] Mishra, V. K., Passari, A. K., Leo, V. V., & Singh, B. P. (2017). Molecular diversity and detection of endophytic fungi based on their antimicrobial biosynthetic genes. *Molecular Markers in Mycology: Diagnostics and Marker Developments*, 1-35.