**Molecular characterization and determination of antibacterial activity of bacterial endophytes from *Ocimum sanctum* Linn. (Lamiaceae)**

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**Abstract**

 Endophytic microbes of medicinal plants have gained tremendous importance with emerging evidence of their immense ecological and biotechnological relevance. Endophytes are indeed a group of organisms such as fungi, bacteria, or actinomycetes that live inside the internal tissues of plants. While many endophytes have a mutually beneficial relationship with the plant, not all endophytes are symbiotic. Some endophytes can become pathogenic to the plant under certain conditions, causing harm instead of providing benefits. The antimicrobial activity is attributable to the presence of endophytic bacteria in the plant. The study focused on isolating, characterizing, and screening endophytic bacteria associated with leaves and branches of *Ocimum sanctum* Linn. (Lamiaceae). The aim was to assess the antimicrobial activity of the isolates against standard antibiotics. Genomic DNA extraction, PCR analysis, and sequence analysis were performed to molecularly characterize the isolated endophytic bacteria. The results of the study justified the use of *Ocimum sanctum* L. against human pathogenic bacteria. It also highlighted the importance of isolating and identifying antimicrobials from endophytic bacteria as a valuable approach in the search for novel natural products. A total of 30 endophytic strains, were isolated from the host plant and their antibacterial activity was evaluated using sterile antibiotic discs containing Thrombomycin, Chloramphenicol, and Streptomycin. Additionally, the production of enzymes such as cellulase, amylase, pectinase and protease by these strains was determined. Overall, the study shed light on the potential of endophytic bacteria associated with medicinal plants like *Ocimum sanctum* L. as a source of antimicrobial compounds and emphasized the importance of further research in this field.

**Introduction**

 Certainly, *Ocimum sanctum* L. (Tulsi) is a highly valuable resource for isolating endophytic microbes due to its ethno-botanical history and medicinal properties (Cohen 2014). Endophytes, which are microorganisms that reside within plant tissues without causing disease, play a crucial role in the development of medicinal properties in plants. Endophytic bacteria and fungi have been found to colonize healthy plant tissues, and they consist of multiple genera and species within a single plant host(Guo et al. 2008, Hallman et al. 1997). These microorganisms have the potential to produce a wide range of bioactive compounds, including antibiotics, antifungals, antivirals, immunosuppressants, antioxidants, anticancer agents, and plant growth hormones (Petrini 1986). These compounds have various applications in medicine, agriculture, and industry (Liarzi et al. 2016). The isolation and identification of these bioactive compounds from endophytes can lead to the discovery of novel natural products with significant potential in different fields (Selim et al. 2016). Furthermore, endophytes also produce extracellular hydrolytic enzymes, such as cellulases, proteases, lipases, and pectinases, to establish a resistance mechanism against plant invasion, which have important roles in various industries (Hawar 2022). Endophytic microorganisms have been reported in plants from diverse environments including tropic, temperate, aquatic, oceans, xerophytic, deserts, Antarctic, geothermal soils, rainforests, mangrove swamps and coastal forests (Strobel et al. 2002, Suryanarayanan & Murali, 2006). The nature and abundance of endophytes can be influenced by environmental conditions such as soil, temperature and humidity. The interaction between endophyte and plant is mainly controlled by the genes of both organism and host plant which are further modulated by the environment. The study of endophytic microorganisms is crucial for understanding their interaction with their host plants (Petrini 1991, Wilson 1995). Plants in unique environments that fight to compete with other living organisms or require great resistance as possible to survive are probable candidates to host endophytes. The exploration of endophytes and their potential applications holds great promise for further advancements in various fields.

**Materials and methods**

**Plant Material**

The endophytic bacteria were isolated from healthy fresh and mature leaves of *Ocimum sanctum* growing in the premise of Union Christian College Aluva, Kerala, India. The leaves were carefully kept in clean plastic bags, brought to the laboratory and used for further experimental purposes.

**Isolation of endophytic bacteria from *Ocimum sanctum***

 Surface sterilization is the initial and mandatory step for the isolation of endophytes to eliminate any surface microbes and ensure that only the endophytic microorganisms are isolated. This process involves treating the plant tissues with an oxidant or general sterilizing agent for a specific period of time, followed by multiple rinses with sterile solutions.

The surface sterilization process, isolation and purification of endophytic bacteria from *Ocimum sanctum* plant tissue can be performed by employing the following steps:

**Pre-treatment**

 The leaves and stems of *Ocimum sanctum* were washed separately under tap water to remove adhering soil particles and the majority of microbial surface epiphytes is a part of pre-treatment.

**Surface sterilization**

Freshly collected leaves and stems of *Ocimum sanctum* was washed under slow running tap water for 15 min to remove any debris or contaminants from the surface of the plant. The plant material was then washed in a solution of Tween 20 (1 drop in 200 mL sterile distilled water) for 1 min. After the Tween 20 treatment, the plant material was rinsed three times with sterile distilled water. This was done to ensure the removal of any traces of Tween 20. The final step in the surface sterilization process involves the use of sterilizing agents such as 2% sodium hypochlorite, 70% ethanol. The plant material was treated with sodium hypochlorite for 1min to kill any remaining surface microbes. It was then rinsed with sterile distilled water to remove the sterilizing agent. Following the sodium hypochlorite treatment, the plant material was treated with 70% ethanol. Ethanol acts as a further disinfectant to eliminate any remaining surface microbes. The plant material is rinsed with sterile distilled water again to remove any traces of ethanol. By employing these steps, the surface sterilization process aims to effectively eliminate surface contaminants while minimizing any potential harm to the plant tissue and the endophytic microorganisms within.

**Cultivation of endophytic bacteria**

 To isolate endophytic bacteria Nutrient agar media was used. Since there is no component in nutrient agar which can suppress the growth of endophytic fungi, it is necessary to supplement the media with an antifungal agent, nystatin. It was added to the nutrient agar media at a concentration of 30 μg/mL. The presence of nystatin in the media helps prevent the growth of endophytic fungi, ensuring that only endophytic bacteria can grow and be isolated.

**Isolation, purification, and subculture of endophytic bacteria**

 After surface sterilizing the plant material, it was allowed to dry. Using aseptic techniques, the surface of the stems is removed using a sterile scalpel in the laminar airflow cabinet. The leaves are cut into pieces and each piece of plant material is placed on a nutrient agar medium supplemented with antifungal agents. The nutrient agar plates with plant tissues are sealed using parafilm tape and incubated at 28±2°C in order to recover the maximum possible colonies of bacterial endophytes. The observation is made for 48 hrs. After 24 hrs from the bacterial cultures, morphologically different bacterial colonies were selected and repeatedly streaked on fresh nutrient agar plates to get pure cultures. All selected isolates were subcultured in nutrient agar slants and finally, all the purified endophytic bacteria were maintained at 4°C till further use.

**Characterization of endophytic bacteria**

 The characterization of the endophytic bacterial isolates was based on the morphological and phenotypic characteristics of the colony such as microscopic features, gram reaction, endospore staining, motility, catalase, oxidase activity, as well as molecular techniques.  The selected endophytic bacteria are transferred to new agar slants, and stored at 4°C or in 20% glycerol vials at -80oC for long-term use. Among the 30 isolates obtained, 10 isolates labelled as TH1, TH2, TH3, SH1, SH2, SH3, HW1, HW2, HW3, HW4 were chosen for the further characterization studies. Molecular characteristics of these isolates were performed to gain a better understanding of their properties and potential applications.

**Screening of Endophytic bacteria for the production of enzyme**

The pure cultures of the endophytic bacteria were screened for high-yield production of amylase, cellulase, pectinase and protease production. For the cultivation of endophytes, minimal media containing: Beef extract 0.3g, Peptone 0.5g, NaCl 0.5g, Agar 2g, Distilled water 100ml was used. The endophytic bacteria were spotted on the minimal media plates and incubated under suitable conditions for enzyme production. After incubation, the plates were examined for the formation of clear zones around the bacterial colonies.

**Amylase Activity**

The amylolytic activity of the isolates was screened by the starch hydrolysis test on the basis of the zone of solubilisation on Starch agar. Cultures were inoculated on Starch agar plates containing Beef extract 0.3g, Peptic digest 0.05g, Starch 1g, Agar 2g in 100ml of distilled water and incubated at 37oC for 48 hrs. After incubation plates were flooded with iodine solution for 30 seconds, a clear zone of hydrolysis indicates positive results.

 **Cellulolytic activity**

 The cellulolytic activity of the strains was screened on the basis of the zone of clearance on CMC (Carboxymethylcellulose) agar plates by the method Congo red test (Teather and Wood, 1982). Isolated strains were inoculated onto CMC agar plates containing 0.5 g carboxymethylcellulose, 0.1g NaNO3, 0.1g K2HPO4, 0.1g KCl, 0.05g MgSO4, 0.05g beef extract, 0.1g glucose, 2g agar in 100 ml of sterile distilled water and incubated at 37oC for 48 hrs. After incubation, the plates were flooded with Congo red solution (0.1%) and allowed to stand for 5 minutes. The Congo red solution was then discarded, and the plates were washed with 1M NaCl solution for 15-20 min. The clear zone was observed around the colony indicating a positive result, i.e. utilization of cellulose.

**Pectinase activity**

The isolated microbes were grown on Pectinase Screening Agar Medium (PSAM). Pectinase agar medium contains 1gm pectin, 0.1g beef extract, 0.5g peptone, 0.2g CaCO3, 0.2g NaCl, 2g agar in 100ml sterile distilled water. The inoculated plates were then incubated at 37oC for 48 hrs. After incubation the strains which are able to utilize pectin as a source of carbon develop a zone on pectinase screening agar medium.

**Protease activity**

The isolated strains were inoculated on Casein Agar plates containing 0.015g beef extract, 0.5g NaCl, 2g agar, 1g casein in 100 ml distilled water. Inoculated plates were then incubated at 37oC for 24 hrs. After incubation the plates were flooded with iodine solution and strains which degrade casein in the medium produce protease enzyme show a zone of clearance.

**Screening for antimicrobial activity**

Antagonistic activity of the isolates was screened against the standard antibiotic discs by agar disc diffusion method and zone of inhibition was recorded. The assay is carried out in Muller Hinton Agar. Broth cultures of the endophytic bacteria were swabbed onto the agar plates using sterile cotton swabs in order to create a uniform lawn culture. After 24 hrs of incubation at 37oC in an incubator, the diameter of the inhibition zone around each well was measured millimeter by using a scale. The antimicrobial assay was carried out in triplicates.

**Molecular characterization of Endophytic bacteria**

**DNA extraction**

To extract the DNA, 2 ml of bacterial cell suspension (18 hrs old bacterial cell suspension grown in Luria Bertani broth) was centrifuged at 15,000 *g* for 10 min at 4°C. The pellet was collected and resuspended in 500µl of TNE buffer (10mM Tris-Cl, pH 8.0, 1 mM EDTA, 0.15 mM NaCl) and centrifuged again at 15,000 *g* for 10 min at 4°C. Subsequently, the pellets were resuspended in 500 µl lysis buffer (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) (Lee et al., 2003) and 10µl of proteinase K (20mg/ml) was added and incubated initially for 1 hr at 37°C and then for 2 hrs at 55°C. Further extraction was carried out by the phenol-chloroform method. The sample was deproteinated by adding an equal volume of phenol (Tris- equilibrated, pH 8.0), chloroform and isoamyl alcohol mixture (25:24:1). The phenol and the aqueous layers were separated by centrifugation at 15,000*g* for 15 min at 4°C. The aqueous phase was carefully pipetted out into a fresh tube and the process was repeated once more. Following this, an equal volume of chloroform: isoamyl alcohol (24:1) mixture was added, mixed by gentle inversion and centrifuged at 15,000 *g* for 15 min at 4°C to separate the aqueous phase which was then transferred to a fresh tube. Then the DNA was precipitated by incubation at -20°C overnight after adding an equal volume of chilled absolute ethanol. The precipitated DNA was collected by centrifugation at 15,000 *g* for 15 min at 4°C and the pellet was washed with 70% ice-cold ethanol. Centrifugation was repeated once more the supernatant was decanted and the tubes were left open until the pellet dried. The DNA pellet was dissolved in 100µl Milli-Q (Millipore) grade water. The isolated DNA was quantified spectrophotometrically (Abs260) and the purity of the DNA was assessed by calculating the ratio of absorbance at 260 nm and 280 nm (Abs260/Abs280). Electrophoresis was done using 1% agarose gel.

**PCR amplification of the extracted DNA**

Amplification of the 16S rRNA gene was performed according to Reddy et al. (2000) using universal primers 16S F (GAG TTT GAT CCT GGC TCA) and 16S R (ACG GCT ACC TTG TTA CGA CTT). The amplification was performed using a DNA Thermal cycler (Biorad). The reaction mixture (final volume 25 µl) contained 2.5 µl 10 X buffer, 1 µl 10 pmol each of oligonucleotide primer, 1.5 µl DNA template, 2.5 µl 2.5 mM each deoxynucleoside triphosphate, 1 µl Taq polymerase, and the remaining volume was made up with sterile Milli-Q water. The amplification profile consisted of initial denaturation at 95°C for 5 min followed by 34 cycles of denaturation at 94°C for 20s, annealing at 58°C for 30s and extension at 68°C for 2 min followed by a final extension at 68°C for 10 min. The PCR product was separated on 1 % agarose gel prepared in 1X TAE buffer and stained with ethidium bromide.

 **Sequencing and analysis**

Nucleotide sequencing was performed using ABI PRISM 3700 Big Dye Sequencer. Sequenced DNA data were compiled, analysed and screened for vector regions using ‘VecScreen’ system accessible from the National Centre for Biotechnology Information (NCBI). After removing the contaminating vector regions, the sequences were matched with homologous sequence obtained from the GenBank database using the BLAST algorithm available from the NCBI website (http://www.ncbi.nlm.nih.gov). These 16S rRNA nucleotide sequences were later deposited in the submission wizard, of the NCBI submission portal and their accession numbers were obtained.

**Phylogenetic analysis**

 Most phylogenetic trees are built from molecular data, DNA or protein sequences. In this study the phylogenetic analysis of the 16S rRNA gene sequence was done by Molecular Evolutionary Genetics Analysis (MEGA 5.10) software and the tree was constructed by using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), a hierarchical clustering algorithm that creates a phylogenetic tree by iteratively grouping sequences based on their pairwise distances. This is user-friendly software for mining online databases building sequence alignments and phylogenetic trees. Construction of a phylogenetic tree from the aligned sequences by the UPGMA method of MEGA permits the selection of the most suitable substitution model. Finally, the constructed tree was presented to reveal the relevant information.

**Results**

 Thirty endophytic bacteria were isolated from healthy leaves and stems of *Ocimum sanctum.* These bacterial endophytes were made into pure culture and these were subcultured onto nutrient agar slant for further studies. The ability of these endophytic bacterial isolates to utilize starch, cellulose, pectin and casein was also experimented. Among these,10 isolates labelled as TH1, TH2, TH3, SH1, SH2, SH3, HW1, HW2, HW3, and HW4 exhibited enzyme production and these isolates were then analyzed molecularly and 16S rRNA sequencing was performed. This molecular analysis, coupled with enzyme production data, can provide valuable insights into the diversity and potential biotechnological applications of the endophytic bacteria associated with *Ocimum sanctum*.

 By screening for antimicrobial activity, the isolates with potential antimicrobial properties were identified for further study. Of the ten isolated strains, *Microbacterium sp.* strainUCCB140, *Micrococcus* *sp*. strain UCCB141, *Neobacillus bataviensis* strainUCCB142, *Paenibacillus sp.* strain UCCB144, *Rhizobium* *sp*. strain UCCB145, *Staphylococcus wameri* strain UCCB146, *Methylobacterium sp*. strainUCCB147 and *Bacillus cereus* strain UCCB148 showed sensitivity against sterile antibiotic discs Thrombomycin, Chloramphenicol and Streptomycin. The diameter of the inhibition zone was recorded and was used as an indicator of the antimicrobial activity of the isolates. A larger inhibition zone indicates stronger antimicrobial activity against the standard antibiotics.

 The degradation of the specific substrate (starch for amylase, cellulose for cellulase, pectin for pectinase, and casein for protease) by the secreted enzymes is represented by the zone of clearance. The larger the clear zone, the higher the enzyme production by the endophytic bacteria. This screening process helps to identify the endophytic bacteria with high-yield production of the desired enzymes, which may be further utilized for various applications in industries such as food, textile, and biotechnology.

Out of the ten endophytic bacterial isolates the strains named *Bacillus sp.* strainUCCB139, *Micrococcus* *sp*. strain UCCB141, *Neobacillus bataviensis* strainUCCB142, *Paenibacillus* *sp.* strainUCCB144, *Rhizobium* *sp.* strainUCCB145, *Staphylococcus wamer*i strain UCCB146, *Methylobacterium sp.* strainUCCB147, *Bacillus cereus* strain UCCB 148 hydrolysed starch and secreted amylase enzyme. *Microbacterium sp.* strainUCCB140, *Agrobacterium larrymoorei* strainUCCB143 and *Paenibacillus* *sp.* strainUCCB144 have the ability to utilize pectinase enzyme. The strains *Bacillus sp.* strainUCCB139, *Neobacillus bataviensis* strainUCCB142, and *Micrococcus* *sp*. strain UCCB141 have the ability to utilize the enzyme cellulase. The strains *Neobacillus bataviensis* strainUCCB142, *Agrobacterium larrymoorei* strainUCCB143 and *Paenibacillus sp.* strain UCCB144, *Rhizobium* *sp.* strainUCCB145, *Staphylococcus wamer*i*.* strain UCCB146, *Methylobacterium sp.* strainUCCB147 has the ability to utilize casein and produce protease enzyme.

Molecular characterization was performed by DNA extraction of these ten endophytic strains and the extracted DNA samples were amplified by performing PCR and nucleotide sequencing after removing all the contaminants these sequences were matched with homologous sequence obtained from the GenBank data base using the BLAST Algorithm from NCBI website and the phylogenetic tree was drawn. The sequences obtained were submitted in the Genbank and corresponding accession numbers were obtained such as MH192999, MH198277, MH198278, MH193371, MH198279, MH198280, MH193373, MH198281, MH198282 and MH193385 as given in Table 1.

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

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

**Discussion**

 The leaves, roots and stems of *Ocimum sanctum* is rich in endophytic bacterial diversity. Isolation and screening of endophytic microbes from leaves of *Ocimum sanctum* have important potential in medicine, agriculture and industries (Singh & Verma 2010). Endophytes are rich in bioactive metabolites. Endophytic microbes are those organisms that inhabit interior of the plants especially leaves, roots and stems. Endophytes does not harm the host plant. Endophytes have the ability to produce a range of secondary metabolites, providing researchers with numerous leads for compounds of pharmaceutical significance and possible development as new drugs.

 The most commonly used isolation procedures combine surface sterilization of the plant tissue, plating small sterilized segments onto nutrient agar and also by the maceration of the plant tissue and streaking onto nutrient agar, or vacuum or pressure extraction techniques. Theoretically, the sterilizing agent should kill any microbe on the plant surface without affecting the host tissue and the endophytic microorganisms (Ezeobiora et al. 2021). Though, this is challenging to achieve because the conditions required to kill the last microbe on the surface may already be lethal for some endophytic microorganisms and over time the agent may penetrate the plant tissue.

The need for new antimicrobial agents, in general, comes from the increasing rates of resistance to existing antibiotics (Reygaert 2018). This problem extends beyond the clinical application of antimicrobial drugs, such as agricultural microorganisms are also known to have acquired resistance to commonly used antimicrobial chemicals. Endophytes are the chemical synthesizers inside the plant. Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites bearing antimicrobial activity (Sathish et al. 2012).

Thirty different bacterial endophytes were isolated from leaves of *Ocimum sanctum* of three different sites. These bacterial endophytes were made into pure culture and these were subcultured onto nutrient agar slant for further studies. Kirby Bauer Disc diffusion method has been widely adopted for antimicrobial susceptibility testing and here we adopted this method for determining the antimicrobial effect of ten endophytic bacteria isolated from *Ocimum sanctum*. The overall in vitro antibacterial results shown that maximum sensitivity was observed against *Staphylococcus wamer*i strain UCCB146 and *Bacillus cereus* strain UCCB148. The studies have provided valuable insights into the occurrence and diversity of culturable endophytes. Many natural products produced by endophytes have proven to be antibacterial, antifungal, antidiabetic, antioxidants, immunosuppressives and great source of bioactive natural products (Fadiji et al. 2020). The majority of endophytic bacteria produced different kinds of novel antibiotics like Ecomycins, Pseudomycins, Munumbicins, Kakadumycins (Christina et al. 2013). These compounds inhibit the growth of pathogenic bacteria and fungi.

 The ability of these endophytic bacterial isolates to utilize starch, cellulose, pectin and casein were found out. Present study indicates that ten endophytes produce good amount (high activity) of cellulase, amylase, pectinase and protease enzyme. The presence of a clear zone around the colonies indicates a positive result. This suggests that the isolated strains have the potential to produce different enzymes, which have various applications in industries such as food processing, textile, pharmaceutical and fruit juice production (Haile et al. 2022).

The conjecture that the enzymes from the endophytic bacteria isolated from the *Ocimum sanctum* plant will have similar properties (Tiwari et al. 2013). This can be confirmed only after purification, characterization and testing of these compounds in animal models.

Molecular characterization were performed by DNA extraction of these ten endophytic strains and the extracted DNA samples were amplified using PCR technique and nucleotide sequences were obtained and the phylogenetic tree was constructed. Molecular techniques have revolutionized the field of microbiology and greatly assisted in isolating and characterizing microorganisms. Functional characterization of endophytes can be challenging due to their diverse functionality. However, cutting-edge high-throughput techniques offer promising solutions to simplify this aspect of the study. Molecular techniques, along with high-throughput approaches and specific molecular and biochemical assays, play a crucial role in the comprehensive isolation, identification, and functional characterization of microbial endophytes. These techniques contribute to a better understanding of the diverse roles and potential applications of endophytes in sustainable agriculture (Shah et al. 2021).

Only few reports showed the diversity of endophytic bacteria and fungi from the medicinal plants. This indicates that there is still much to explore and understand about the diversity of endophytic microorganisms associated with medicinal plants in India(Gouda et al. 2016). The results obtained in this study leads to the justification that the studies on isolation and identification of the bioactive compounds can be an important and crucial approach to search for novel natural products.

**Conclusion**

Despite the diverse approaches used to isolate and characterize endophytic organisms, they all follow a similar rationale. The process typically involves sample preparation which involves collecting plant tissues and surface sterilizing them to remove any external contaminants (Kafur & Basheer 2011). Identification of specific species often involves molecular techniques such as PCR amplification and sequencing of specific genes or regions, as well as phylogenetic analysis. This allows researchers to determine the taxonomic position and relatedness of the isolated endophytes. Overall, the isolation and characterization of endophytic organisms require careful consideration of the specific species being studied and the limitations of cultivation methods. Advances in molecular techniques have provided new avenues for studying previously unculturable endophytes, expanding our understanding of their diversity and potential applications.

This paper entitled “Molecular characterization and determination of antibacterial activity of bacterial endophytes from *Ocimum sanctum* linn. (lamiaceae)was undertaken realizing the importance of endophytes to be a good source of antimicrobial agents, enzymes and secondary metabolites and also to find out the new possibilities for exploring the diversity and functional potential of inaccessible endophytes. From our findings, it is understood that endophytic bacteria showed broad spectrum antibiotic sensitivity and the highest enzyme producers were characterized based on its morphological features combined with molecular analysis.

Isolation and screening of endophytic microbes from leaves of *Ocimum sanctum* have important potential in medicine, agriculture and industries. Endophytes are rich in bioactive metabolites. Endophytic microbes are those organisms that inhabit in terroir of the plants especially leaves, roots and stems. Endophytes have the ability to produce a range of secondary metabolites providing researchers with numerous leads for compounds of pharmaceutical significance and possible development as new drugs. Endophytes can be a promising source of bioactive compounds, and should be continuously isolated, characterized, and investigated for the discovery of lead bioactive compounds which can be employed in agriculture, medicine, and industries (Gupta et al. 2023).

Endophytes can be an alternate source of drugs which will help to conserve biodiversity and drug resistance. They are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites bearing antimicrobial activity. Endophytic bacterial strains such as *Microbacterium, Paenibacillus sp, Bacillus sp, Neobacillus bataviensis, Microccus sp, Staphylococcus wameri, Methylobacterium, Bacillus cereus* are sensitive against sterile antibiotic discs Thrombomycin, Chloramphenicol and Streptomycin. The bioactive compounds produced by endophytes have shown promise as starting points for the discovery and development of novel antibiotics, anticancer drugs, and therapeutics for various diseases in humans, animals, and plants (Singh et al. 2017). These compounds have the potential to provide alternative treatment options and address the growing problem of drug resistance. To further classify and understand the phylogenetic relationships and evolution between different groups of endophytic bacteria, studies that involve amplifying and comparing specific genes with known functions are conducted by many researchers. Evolutionary relationships between different endophytic bacteria species can be assessed using specific genes and these genes are often used as molecular markers to study the diversity and phylogeny of bacteria (Mishra et al. 2017).

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**Data availability:** All the data obtained in the study are incorporated in the manuscript.

**Ethics statement:** This article does not contain any study involving human participants or animals.

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