

The relationship between plants and microbes is a dynamic, intricate process that has existed for as long as plant colonisation on Earth. Plants are frequently invading both useful and dangerous microorganisms, primarily bacteria and fungi, in both natural and agricultural environments (Dolatabadian, 2020). Different chemical signaling routes are used by microorganisms and plants to interact with one another. Plants undergo many biological reactions as a result of these interactions, including the production of secondary metabolites (Chamkhi et al., 2021). Through complex chemical communication within the rhizosphere, plant roots interact with microbes to form biofilms that benefit the host plant. In the case of plant growth-promoting rhizomicrobes/bacteria (PGPR), this process results in the priming of defense, or induced resistance, in the plant host (Mhlongo et al., 2018). Positive interactions like symbiotic or non-symbiotic associations and negative interactions like competition or parasitism associations can be used to categorize plant-microbial relationships (Chamkhi et al., 2021). Microorganisms that inhabit the outside of plants are referred to as epiphytes, and those that inhabit their interiors are called endophytes (Nadarajah & Abdul Rahman, 2021). Reservoirs of bioactive substances, endophytes are a category of endosymbiotic microorganisms that live inside plant tissues (Das & Das, 2022).

**1.1 General introduction:**

De Bary (1866) first defined an endophyte as "any organism that grows within plant tissues," setting it apart from an epiphyte, which is an organism that lives on the surface of plants (Gouda et al., 2016). Endophytes are microorganisms that live inside the plant tissues without exhibiting any symptoms or causing diseases. Endophytes are found in all or most plants. These organisms are mainly transmitted via seeds, and as soon as the seeds germinate, they start to help in plant growth and development (White et al., 2019). Endophytic microbes are basically categorised in three groups depending on their survival methods use inside the hosts- obligatory endophytes (OE), facultative endophytes (FE), passive endophytes (PE). OE are those that are unable to multiply outside of plant tissue and are typically transferred by seeds. FE are live in the soil and frequently enter the plant roots when they get the opportunity. PE are microorganisms

that do not primarily colonise plant tissues, but rather do so in response to external stimuli like wounds on root hairs. (Fadiji & Babalola, 2020).

Endophytic bacteria have several applications in different fields like agriculture, medicine, and other fields as they have ability to enhance plant growth and development, increase tolerance to biotic and abiotic stresses, and produce bioactive compounds that may have medicinal value (Wu et al., 2021). Endophytic bacteria have been isolated and characterised from of different plant hosts, such as agronomic crops, prairie plants, plants that can tolerate harsh environments, and wild and perennial plants (Afzal et al., 2019). These microorganisms can be isolated from almost all tissues like- stems, roots, flowers, leaves, and seeds etc. Certain stages of plant growth are when bacterial colonisation takes place, and even at the seed stage, there might already be a stable endophytic bacterial community (Wu et al., 2021). Seed bacterial endophytes have some characteristics which include the capacity to colonise internal plant parts, such as the reproductive organs. The most interesting characteristic of the seed endophytes is that they have the ability transfer vertically and preserve themselves in the host plants (Walitang et al., 2018). There are several reports on several endophytic taxa which have the ability to colonise the inoculated plants, leading to an increase in their height and biomass. These taxa include *Rahnella*, *Gluconobacter*, *Pantoea*, *Azoarcus*, *Burkholderia*, and *Pseudomonas* (Kandel et al., 2017). Endophytic bacteria and their hosts have a dynamic and flexible relationship in which the microorganisms respond to little variations in the host plant's development by changing their gene expression or by producing different metabolites. These microorganisms have the ability to tolerate harsh environments which makes them good source of some new biologically active compounds. They have played a crucial role in the production of environmentally friendly and sustainable medicine (Ek-Ramos et al., 2019). Furthermore, it has been found that the richness, composition, network, and function of microbial communities differ significantly among plant species due to the highly selective environments of the rhizosphere. Rhizosphere is the soil area which surrounds the plant roots (Guo et al., 2022). A number of external factors can influence the composition of endophytic bacterial communities, such as soil conditions, latitude, longitude, altitude, and season. (Wu et al., 2021). From the roots of the plants to the stems and leaves, endophytic bacterial populations and variances often decrease (Walitang et al., 2018).

Typically, endophytic microorganisms can enter into the plants through root zone. The ability of bacteria to colonise the host endophytically is regulated by a number of bacterial characteristics. Endophytic bacteria must recognise specific compounds in the root exudates in order to start the complex colonisation process, which usually starts at the roots. For their own ecological benefit, plants produce these root exudates to interact with helpful microbes (Afzal et al., 2019). The interactions between the plant and soil microorganisms occur at the rhizosphere. Substrate in root exudate may facilitate early communication between bacterial endophytes and their host plants, hence aiding in the colonisation of endophytes. Some root exudates that help in attracting bacterial endophytes are organic acids, proteins, and amino acids etc. Oxalate's function in attracting the advantageous bacterial strain *Burkholderia phytofirmans* PsJN by host plants has been demonstrated in earlier studies (Kandel et al., 2017). Root hairs, root fractures, or wounds caused by nematode or microbial activity are the primary entry routes for bacterial colonization. Through their root hairs, endophytes like *Pseudomonas putida* and *P. fluorescens* colonized olives. Root colonisation also occurs through intercellular spaces in the cortex and epidermis (Chaudhary et al., 2022).

Bacterial endophytes can be isolated from a variety of plant tissues, including the roots, stems, leaves, seeds, fruits, tubers, ovules, and nodules etc (Afzal et al., 2019). Endophytic bacteria mainly colonise in the gaps between the cells due to the abundance of carbohydrates, amino acids, and other nutrients, though some of them can also colonise inside cells. Colonisation is also promoted by plant receptors which is associated with signals linked to phytohormones such as salicylic acid (SA), auxin, and jasmonic acid (JA) (Pinski et al., 2019). The composition of the endophytic bacteria can be influenced by the genotype of the plant. Due to the difference in the genetic makeup, endophytic bacteria can colonise inside plant tissues, but rhizobacteria cannot (Mushtaq et al., 2023). Endophytic bacteria colonise host plants through several stages, including the identification of root exudates, moving closer to the plant, attaching to the root surface, and producing biofilms that infect the root and colonise various tissues inside the plants. The biomolecules which involve in these stages have the ability to change both the host plant's and endophytic bacteria's gene expression. Several bacterial genes were found to be associated with plant-bacteria interaction and were isolated from different endophytic bacteria of different plants, including *bpl.2* (Bphyt\_4275), *bpl.1* (Bphyt\_0126), *pcol*, *rpff*

(Smal\_1830), *cesT* (2553406074), *ppkA* (azo3888), *hrpE* (Hrubri\_2433), *hrcN* (Hrubri\_2444), *pta*, *als*, *cheA*, *tlp1*, *mot3*, *fliC3* (azo2704), *rfbB* (Hsero\_4410), *rfbC* (Hsero\_4411), *rfbD* (AMK58\_RS28935), etc (Pinski et al., 2019). However, no specific gene or gene family has been found to properly explain the relationship between endophytic bacteria and plant (Mushtaq et al., 2023).

Both aboveground and underground plant tissues can have endophytic bacteria, which aid in the growth and development of the host plant both under normal and stressful conditions. The endophytic bacterial population has evolved to colonise many organs in a systemic and selective manner. Previously it was observed that the *Spiranthes spiralis* seeds preserved their core microbial community, indicating a possible vertical transfer of the microbiota. Additionally, the decrease in species richness and variety in the aerial vegetative organs may indicate a filtering effect of the host plant (Alibrandi et al., 2020). Diversity in bacterial communities were observed in the different tissues of the same plant from different places. Previously, endophytic bacteria were isolated from a medicinal plant, *Mirabilis himalaica*, from five different locations in Tibet, China. And it was reported that the two phyla that predominated in all of the samples were Proteobacteria and Actinobacteria, with the dominating genera varying according to the tissues. Also, according to Redundancy analysis, there is a substantial correlation between the endophytic bacterial community and soil organic matter, pH, accessible phosphorus, total phosphorus, and total nitrogen. The relative abundances and composition of endophytic bacteria were therefore evidently determined by the climatic type, ecosystem type, geographic location, and tissues (Zhang et al., 2023). Even the past cultivation of the soil has had a significant impact on the diversity of endophytic bacteria (Correa-Galeote et al., 2018). It has also been reported that plant variability is a major factor in microbial community diversity, composition, network, and function because of the difference in highly selective set-up in the rhizosphere. When differences in soil bacterial communities under different types of *Phyllostachys edulis* (bamboo) were explored, it was previously shown that bacterial communities were affected by the compartment niches (Guo et al., 2022).

The composition of the endophytic bacterial community was influenced by the host plant's growing environment. Previously it has been reported that *Populus euphratica*

growing in a high-saline condition exhibits low species diversity, particularly in sap tissue (Yue et al., 2022). Additionally, the host plant's age can also influence the endophytic bacterial diversity (Asraful Islam et al., 2010). Even within the same cultivars, the endophytic bacterial microbiota in grapevine shoot xylems differed according to the cultivar and the grapevine-growing location, and the microbiota changed according to the shoot growth stage (Hamaoka et al., 2022). The diversity of endophytic bacterial communities can also be influenced by the host plant's stem length (Wang et al., 2022).

When compared to the autumn season, mulberry (*Morus L.*) plants that grow in the spring have more bacterial operational taxonomic units (OTUs),  $\alpha$ -diversity, and bacterial community complexity (Ou et al., 2019). In the case of *Cinnamomum camphora* (L.) Presl., the highest diversity was observed during the early winter in comparison to the summer and spring seasons (Elmagzob et al., 2019). Thus, it was established that the diversity of endophytic bacterial populations is also significantly influenced by the season.

## **1.2 Surface sterilisation methods:**

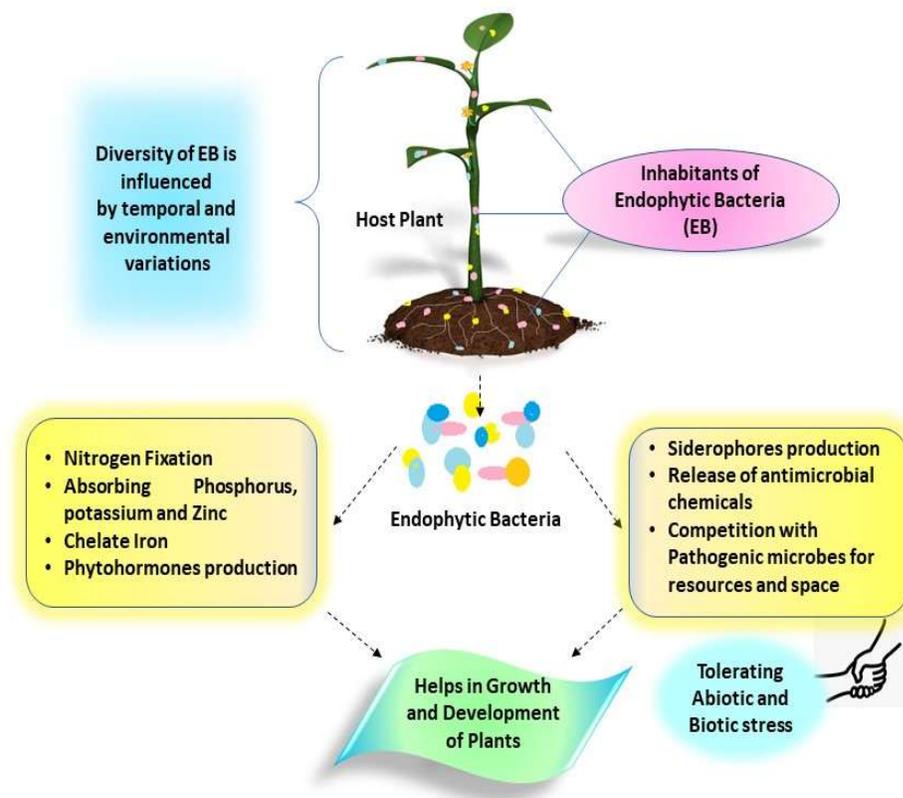
Surface sterilisation is the most crucial stage in the isolation of endophytic bacteria, as it determines whether endophytic bacteria will grow and whether epiphytic microbes will be removed. Ensuring complete surface sterilisation with little damage to the endophytic diversity requires careful consideration of the sterilant solution, concentration, and exposure duration. Surface sterilants that are frequently utilised include formaldehyde (40%), ethanol (70%–90%), sodium hypochlorite (2%–10%), and mercuric chloride (0.1%). Pre-treatment with surfactants like Tween 80, Tween 20, and Triton X-100 also increased the efficiency (Sahu et al., 2022). Surface sterilization is a necessary step prior to high-throughput sequencing (HTS) and plant endophyte tissue separation; however, it has an impact on the diversity and composition of endophytic bacteria. It was previously reported that both the pre-treatment duration and the concentration of sodium hypochlorite had a substantial impact on the diversity of bacterial endophytes found in the leaves and stems of the tea plant. And it was reported that pre-treatment with 0.5% NaClO for 8 minutes and 2.0% NaClO for 3 minutes was appropriate for the leaves and stems of the tea plant (Yu et al., 2022). To isolate endophytic bacteria, different concentrations and periods of ethanol and sodium hypochlorite solution were

used under 16 sterilization conditions for the leaf and 40 sterilization conditions for the bulb of *Allium sativum*. The findings of the experiment demonstrated that 70% ethanol (6 minutes), 2% sodium hypochlorite (1 minute), and 70% ethanol (30 seconds) are effective for sterilizing the leaf's surface, and 70% ethanol (6 minutes), 3% sodium hypochlorite (1 minute), and 70% ethanol (30 seconds) are effective for sterilizing the bulb of *Allium sativum* (Srivastava et al., 2024). Selecting the right growth media is essential since it has a direct impact on the quantity and kind of endophytic bacteria that are extracted from plant tissue. Different culture media, such as nutrient agar (NA), tryptic soy agar (TSA), and water agar (WA), are generally used to isolate endophytic bacteria (Srivastava et al., 2024). Endophytic bacteria can also be isolated using Luria Bertani (LB) Agar medium (Singh R. et al., 2022). Prior research on the isolation of endophytic bacteria from leaf and stem explants of the medicinal plant *Tinospora cordifolia* showed that LB agar performed better than nutrient agar (Duhan et al., 2020).

### **1.3 Plant growth promotion activities of endophytic bacteria:**

According to the Food and Agricultural Organisation, there will be 9 billion people on Earth by 2050; therefore, crop yields need to increase to keep up with demand. In order to boost crop productivity, chemical fertilisers are primarily used in agriculture. However, using chemical fertilisers in agriculture has several drawbacks, including environmental pollution, long-term changes in the soil ecology, physiochemical composition, declining agricultural productivity, and various health risks. Numerous interactions between plants, soil, and microbes have a significant impact on plant productivity and soil health (Chaudhary et al., 2022). In the field of sustainable agriculture, plant growth-promoting rhizobacteria, or PGPR, play a significant role. The functions of PGPR include the regulation of hormonal and nutritional balance, the induction of resistance against plant diseases, and the solubilization of minerals to facilitate their simple uptake by plants (Vejan et al., 2016). In the future, using natural symbionts like bacterial endophytes may help make farming more environmentally friendly by lowering the requirement for chemical fertiliser inputs during crop plant growth and development (Kandel et al., 2017). Endophytic bacteria either directly or indirectly aid in the growth and development of plants (Fig 1). They can directly benefit host plants by enhancing nutrient absorption and regulating phytohormones linked to

growth as well as stress. By using hydrolytic enzymes, antibiotics, nutrient limitation, and strengthening plant defences against pests and diseases, endophytic bacteria can indirectly enhance plant growth and development (Afzal et al., 2019). Strains of *Bacillus* and *Brevibacillus* were often obtained from the leaves of two therapeutic plants, *Achillea fragrantissima* (Forssk) Sch. Bip. and *Fagonia mollis* Delile. It was shown that various plant growth-promoting activities of these endophytic bacterial species could potentially replace the use of artificial fertilisers in agriculture (Alkahtani et al., 2020).



**Fig 1: Plant growth promotion activities of endophytic bacteria**

Phosphorus (P) is regarded as an essential macronutrient whose availability may restrict plant growth. It can be found in both organic and inorganic forms in soils. The pH and type of the soil typically affect the phosphorus's adsorption and precipitation. Phosphorus can be retained in acidic soils by weak oxides of iron, aluminium, and

oxyhydroxides. On the other hand, because of the presence of calcium, precipitation happens in alkaline soils (Matos et al., 2017). The microbial processes that lead to the solubilisation of P involve the generation of organic acid anions, which include oxalic acid, gluconic acid, malic acid, citric acid, salicylic acid, and benzoic acid (Varga et al., 2020). Endophytic bacteria primarily solubilise insoluble phosphate by reducing pH as a result of organic acid production. Plants can absorb and utilise ortho-phosphate, which is produced when endophytic bacteria break down phosphate complexes in the soil by releasing organic acids into it (Das & Das, 2024). A selective medium used to determine phosphate solubilization activity is Pikovaskaya's medium. Isolates were initially inoculated onto the medium and incubated at 28 °C for a period of 5 to 7 days. A clearing zone around the colony indicates a positive result for phosphate solubilization activity (Anand et al., 2016). The ability to solubilise phosphate is also assessed using National Botanical Research Institute (NBRI) media, which contain insoluble phosphate (Amri et al., 2023). *Bacillus subtilis* IALR1033, *Bacillus safensis* IALR1035, *Pantoea agglomerans* IALR1325, *Pseudomonas psychrotolerans* IALR632 and *Pantoea vagans* IALR611 are a few examples of phosphate-solubilising endophytic bacteria (Mei et al., 2021).

For plants to grow and develop properly, one of the essential components needed is nitrogen (N). The nitrogen gas (N<sub>2</sub>) that is present in the earth's atmosphere is used primarily by plants as nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium ion (NH<sub>4</sub><sup>+</sup>), which are fixed via a biological process called N<sub>2</sub> fixation. Endophytic bacteria such as *Burkholderia*, *Rhizobium*, *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Frankia*, *Enterobacter*, and *Azospirillum* have been identified as N<sub>2</sub> fixing bacteria (Rana et al., 2023). The most significant enzyme involved in nitrogen-fixation is termed nitrogenase, and it is made up of two components that are often referred to as molybdenum ferritin and ferritin. The genes encoding the ferritin are nifH, while the genes encoding the molybdenum ferritin are nifD and nifK (Yan et al., 2018). The endophytic nitrogen-fixing bacterium *Klebsiella variicola* DX120E was inoculated with sugarcane cultivars in order to evaluate the effects on enzymatic activity and biomass related to nitrogen and carbon metabolism. It was reported that the activity of the enzymes related to nitrogen metabolism and gluconeogenesis increased (Qin et al., 2022). Nitrogen fixing ability of endophytic bacteria can be determined by various methods which includes, culturing of endophytic

bacteria in N-free media like Ashby's mannitol agar, Nfb agar (Yan et al., 2018), molecular based detection, acetylene reduction assay, through Kjeldal method estimation of nitrogen can also be done (Renugadevi et al., 2024),  $^{15}\text{N}$  isotopic dilution assay (Puri et al., 2018).

One of the most significant growth regulators is phytohormones, which are well-known for their significant effects on plant metabolism and for stimulating the plant's defence mechanisms in the face of stress. Abiotic stressors, however, change the endogenous concentrations of phytohormones, including salicylic acid (SA), auxins, gibberellins, jasmonic acid, and abscisic acid (ABA), which prevents plant growth (Egamberdieva et al., 2017). The phytohormones produced by endophytic bacteria are auxins, cytokinins, abscisic acid, ethylene, gibberellins, strigolactones, brassinosteroids, and jasmonates. Among all, indole-3-acetic acid (IAA) primarily promotes the elongation and differentiation of plant cells (Eid et al., 2021). The IAA production ability of *Enterobacter hormaechei* and *Bacillus aryabhatai*, which were isolated from the root of *Chrysopogon zizanioides* (L.) and the leaf of *Bruguiera cylindrica* (L.), was shown to be 246.00 and 195.55  $\mu\text{g}/\text{mL}$  in 1,000  $\mu\text{g}/\text{mL}$  of L-tryptophan at pH 6 for a 48-hour period (Khianngam et al., 2023). Endophytic bacteria *Paecilomyces formosus* LHL10 and *Sphingomonas sp.* LK11 have been seen to produce gibberellins (GAs) and indole-3-acetic acid (IAA) (Bilal et al., 2018). IAA production was observed in broth media with an increase from 10 to 60  $\mu\text{g}\cdot\text{mL}^{-1}$  with an increase in tryptophan concentration from 1 to 5  $\text{mg}\cdot\text{mL}^{-1}$ , exhibited by endophytic bacteria isolated from the leaves of two medicinal plants, *Fagonia mollis* Delile and *Achillea fragrantissima* (Forssk) Sch. Bip (Alkahtani et al., 2020). Furthermore, some endophytic bacteria can produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that breaks down the compound ACC, which is the direct precursor of ethylene in all higher plants, hence lowering levels of the phytohormone ethylene (Santoyo et al., 2016a). It was reported that *Bacillus subtilis* LK14, which was isolated from the bark of *Moringa peregrina* Forssk, produced ACC deaminase of  $448.3 \pm 2.91$  nM  $\alpha$ -ketobutyrate  $\text{mg}^{-1} \text{h}^{-1}$  (Khan et al., 2016). Sixteen of the twenty-six endophytic bacteria isolated from *Pisum sativum* plant root nodules shown the capacity to generate ACC deaminase (Maheshwari et al., 2019). The effectiveness of methylotrophic bacteria that produce ACC deaminase in improving rice root cell viability by limiting salt-induced apoptosis and postponing senescence was previously assessed by

tracking the physiological, biochemical, and genetic traits of the plants. Furthermore, it was noted that the interactions between microbes and plants are crucial for mitigating stress, with *Methylobacterium* spp. controlling ethylene emission, which is essential for increasing plant biomass by preventing apoptosis (Roy Choudhury et al., 2023).

Siderophores are secondary metabolites of low molecular weight that chelate iron and are produced by many types of microbes. They are useful for scavenging iron-limited environments. Endophytic bacteria can produce siderophores, which supply plants with iron and promote plant growth (Maheshwari et al., 2019). Global research attention is also now being directed on endophyte-assisted phytoremediation, an innovative method for remediating soil heavy metals (HMs). Previous studies have described the ability of endophytic bacteria, including *Burkholderia*, *Pseudomonas*, *Pantoea*, and *Herbaspirillum*, to withstand Cd toxicity and produce hydroxamate siderophores (Li et al., 2023).

Endophytes have antagonistic effect against disease-causing plant pathogens in addition to reducing the damage produced by phytopathogens. By directly assisting in the synthesis and release of secondary metabolites or antimicrobial agents, such as siderophores, hydrolytic enzymes, and antibiotics, which can help prevent or lessen pathogen invasion, or indirectly by competing with the pathogen for resources and available space, endophytes support pathogen defence (Kamran et al., 2022). *Bacillus halotolerans*, isolated from the roots of *Lilium davidii* var. unicolor, proven to have antagonistic activity against plant pathogens, including *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Fusarium oxysporum* (Gao et al., 2022). Both *Stenotrophomonas* and *Bacillus* sp. can inhibit the embryonic stages of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, which causes pine wilt disease (Ponpandian et al., 2019). Enterobacter endophytic strains obtained from *Mimosa pudica* nodules exhibited nematocidal activity against *Panagrellus redivivus* and *Nacobbus aberrans* (Sachman-Ruíz et al., 2022). In agricultural situations, endophytic bacteria may prove to be a viable alternative to biocontrol.

Inhibiting plant pathogens is not the only thing that endophytic bacteria can accomplish; they also showed antimicrobial activity against the microbes that can infect humans. *Bacillus halotolerans* isolated from the root of *Codonopsis pilosula*

demonstrated antifungal and antibacterial properties against *Candida albicans* and *Escherichia coli*, respectively. Significant anti-bacterial action was demonstrated by *Pseudomonas azotoformans* at 250 µg/ml against *Staphylococcus aureus* and 500 µg/ml against *Bacillus subtilis* and extremely minimal effect against *E. coli* (Lodi et al., 2023). Drug discovery for a variety of disorders, including as cancer and infections, may be greatly aided by endophyte-derived secondary metabolites. After being initially obtained from *Streptomyces thioluteus*, polyether aureothin was first described as a cytotoxic medication (Zotchev, 2024).

Many sectors of the industry use microbial enzymes with high catalytic activity because they are more affordable, more stable, and readily manufactured in large quantities using fermentation techniques. Several extracellular hydrolytic enzymes, such as lipases, proteases, amylases, cellulases, pectinases, and xylanases, can be produced by endophytic bacteria, which include the *Pseudomonas*, *Micrococcus*, *Paenibacillus*, *Streptococcus*, *Curtobacterium*, *Chryseobacterium*, and *Bacillus* genera that have been isolated from grains plants. Detergent agents, leather processing, xenobiotic chemical degradation, food processing, pharmaceuticals, biofuels, and other enzyme-related technologies are among the industrial sectors impacted by the discoveries of these enzymes (Dogan & Taskin, 2021). One hydrolytic enzyme that helps prevent pathogens from invading plants is cellulase (Ketankumar, 2021). Researchers have also discovered that endophytic bacteria produce several bioactive compounds with significant medicinal value. Several new antibiotics that are effective against bacteria that are resistant to multiple drugs were developed by endophytic *Streptomyces* sp. The antimicrobial compounds generated by endophytes are safe for the environment, poisonous to pathogens, and non-toxic to humans (Singh et al., 2017).

In addition to providing all of these benefits, endophytic bacteria also aid in the host plant's ability to withstand various environmental challenges. Global crop production is increasingly confronted with the challenge of abiotic stress. Drought, salinity, temperature, and nutrient shortage are the most frequent abiotic stresses. *Burkholderia phytofirmans* strain PsJN, an endophytic bacterium, has been reported to modify photosynthesis and the metabolism of carbohydrates in response to chilling stress, improving the cold tolerance of grapevine plants (Kamran et al., 2022). High salt

concentrations up to 16% of NaCl were reported to be tolerated by endophytic bacteria. It has also been discovered that endophytic bacteria can withstand heavy metals like lead, cadmium, etc (Kaur & Karnwal, 2023). Drought tolerance up to -1.5 MPa water potential was demonstrated by *Acinetobacter* sp. Eo3, *Pseudomonas* sp. Ni5, *Bacillus safensis* Ni7, and *Stenotrophomonas* sp. C3, which were isolated from five distinct xerophytic plants (Juby et al., 2023). Therefore, using endophytic bacteria to mitigate various abiotic challenges in agriculture can be a great alternative.

Endophytic bacteria can be isolated from almost all plants, but those found in medicinal plants are of utmost significance. Medicinal plants are the reservoir of numerous bioactive compounds. These plants are used worldwide, either directly or indirectly, to cure a variety of diseases (Das & Das, 2022). Medicinal plant endophytes have the ability to replicate the compounds made by their host plants and are crucial in the synthesis of bioactive compounds (Srivastava et al., 2024). Numerous industries, including agriculture, medicine, and other fields, benefit from the use of endophytic bacteria because they can enhance plant development, increase tolerance to biotic and abiotic challenges, and produce metabolites that may have therapeutic value (Wu et al., 2021). North East India supports approximately 50% of India's hotspot, which is also one of the 'biodiversity hotspots' of the world. The Northeast contains a wide variety of therapeutic plants that grow in the sparse to dense forests found in alpine to tropical climates (Dutta et al., 2023).

#### **1.4 Descriptions of sample plants:**

*Glycosmis pentaphylla* (Retz.), a medicinal plant, is native to tropical and subtropical areas of India, Australia, China, Java, Sumatra, Borneo, Bangladesh, Myanmar, Bangladesh, Indonesia, Malaysia, Thailand, Vietnam, and the Philippines (Khandokar et al., 2021). Shrub or small tree with a height of around 1.5–5.0 m, *G. pentaphylla* is a species of plant in the Rutaceae family (Fig 2) (Aye et al., 2019). The plant is widely used as a traditional medicine to treat a wide range of conditions, such as rheumatism, urinary tract infections, fever, cough, chest pain, anaemia, jaundice, liver disorders, inflammation, bronchitis, pain, bone fractures, toothaches, gonorrhoea, diabetes, cancer, and other chronic illnesses (Khandokar et al., 2021). Different parts of *G. pentaphylla* showed different medicinal properties. An extract from *G. pentaphylla*'s

leaves and stems demonstrated cytotoxic, antibacterial, and antioxidant properties, as well as hepatoprotective action against the hepatotoxic effects of paracetamol in Swiss albino mice (Aye et al., 2019). Previous research on *G. pentaphylla* whole plant ethanol extract showed that the plant has antioxidant, analgesic, and antibacterial properties. Against bacterial strains such as *S. aureus*, *S. dysenteriae*, *S. paratyphi*, and *S. typhi*, it shown antibacterial activity (Kumar Sarkar et al., 2013). The two compounds, arborine and skimmianine, with the lowest MIC and MBC values against Multi Drug Resistant *Staphylococcus aureus*, were isolated from the leaves of *G. pentaphylla* (Murugan et al., 2020). The fruits of *G. pentaphylla* have also been reported to contain alkaloids, including 3-O-methoxyglycocitrine II, noracronycine, 1-hydroxy-3-methoxy-10-methyl-9-acridone, and kokusaginine. When tested against the oral cancer CAL 27 cell line, the volatile oil fraction extracted from *G. pentaphylla* fruit exhibited anticancer activity (Teja et al., 2024).



**Fig 2: Image of the medicinal plant *G. pentaphylla***

Jaundice, anaemia, rheumatism, and face inflammations can all be effectively treated with the roots. 7H-Furo (3,2-G) (1)Benzopyran-7-one,2,3 – dihydro – 2 – (1-Hydroxylmethylethyl) – (s) – (10.96%), Stimast-5-en3-yl-9-octadecenoate (9.06%), Gama.-Sitosterol (8.84%), 5-hydroxypipelic acid (6.23%), (-)-Guaiol (5.58%), 1H-Indole-3-Ethanamine, 5 Methoxy-N, 1- Dimethyl (5.81%), and Gama.-Sitosterol (8.84%) are all reported to be present in the roots of *G. pentaphylla* (Chamundeeswari et al., 2014).

Many people use the stems as a brush to clean their teeth. The antibacterial activity of the methanolic extract obtained from the leaves and stems of *G. pentaphylla* was observed, with the maximum zone of inhibition recorded for *E. coli* ( $23.67 \pm 0.76$  mm) and *Salmonella paratyphi* ( $15.33 \pm 0.76$  mm). Both the extracts showed significant cytotoxic and antioxidant activity (Howlader et al., 2011).



**Fig 3: Image of the medicinal plant *P. thyrsiformis***

*Phlogacanthus thyrsiformis* is endemic to the sub-tropical Himalayas, with extensions into Bhutan, the upper Gangetic plains, Bihar, Assam, North Bengal, Arunachal Pradesh, and Manipur. It can also be found growing as undergrowth in damp, shaded areas in some sub-Himalayan regions Sal forests of Assam. It is an evergreen shrub belonging to the Acanthaceae family that reaches a height of 2.4 metres (Fig 3). Its blooms are up to 30 cm long, elongated thyrsoid panicles with a tubular, curved corolla that is orange or brick red in villous areas. Its leaves are 13 to 35 cm long. *P. thyrsiformis* is considered holy plant in the Meitei population of Manipur, where it is primarily found in domestic gardens (Phurailatpam et al., 2014). Many folk medicines have been reported to contain different parts of this plant to treat a variety of conditions, including fever, the antidote to the pox, skin conditions like sores, scabies, jaundice, liver and spleen diseases, indigestion, acidity, gastritis, pharyngitis, chronic leucorrhoea, cough and cold, chronic

bronchitis, asthma, and rheumatism (Saikia et al., 2018). It is used by tribes in upper Assam, North East, INDIA to treat helminthiasis. Its leaves have reportedly been used to treat gout, fever, rheumatism, and allergies. Additionally, its leaf extract produced some bioactive substances which identified for the first time, such as  $\beta$ -sitosterol, lupeol, and botulin, were produced by its leaf extract (Deori et al., 2023). It shown antimicrobial efficacy against *Salmonella enteric* (MTCC 1164) and *Salmonella typhimurium* (MTCC 3231). Additionally, it demonstrated anticancer action against HeLa cells, which are cervical cancer cells (Kumar et al., 2017). In addition to being widely used in northeastern Indian cuisine, the flowers of *P. thyriformis* are also frequently employed as a traditional remedy for liver and kidney stones (Das et al., 2017). The flowers of the plant were found to include flavonoids, saponins, tannins, phenols, steroids, and terpenoids in a previous study (Deori et al., 2023). *P. thyriformis* flower aqueous extracts demonstrated possible anti-urolithiatic properties. A study on kidney stone inhibition was conducted utilising an aqueous extract of *P. thyriformis* flowers and its biofabricated silver nanoparticles on struvite stones and calcium oxalate stones. The extract and nanoparticles thereby successfully decreased the size of struvite stones in vitro and removed calcium oxalate stones in Wistar rats in vivo (Das et al., 2017). Additionally, a toxicity assessment revealed that *P. thyriformis* extract has no harmful effects on rats or mice (Deori et al., 2023). Thus, it is safe to use in traditional medicine.

*Hygrophila auriculata* Heine is a wild herb usually found in wet areas along riverbanks, ditches, and rice fields in India. It is a member of the Acanthaceae family. India, Sri Lanka, Burma, Malaysia, and Nepal are among the countries where the plant is frequently found. This sub-shrub typically grows beside waterways in swampy areas. It has a reddish-brown stem. The leaves are whorled, with the larger, lanceolate, scalerous outer pair with minutely dentate, subsessile edges and sharp, straight or curved thorns (Fig 4). Bract and bracteoles are leafy, and flowers are arranged in axillary whorls. In traditional medicine, the plant's roots, seeds, and aerial portions are frequently used to cure conditions like jaundice, hepatic blockage, rheumatism, inflammation, pain, urinary infection, edoema, gout, malaria etc (Hussain et al., 2010). In addition, this plant is well-known for its hypoglycemic, antibacterial, anticancer, anti-diabetic, and radical scavenging properties. Lupeol, butelin, terpenoids, flavonoids, and fatty acids are some of this plant's key components (Sultana et al., 2018). The ethanolic extract of the leaves



**Fig 4: Image of the medicinal plant *H. auriculata***

exhibited active anti-microbial activity against *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium canis*, and *Trichophyton mentagraphytes*, while the stem exhibited activity against *Candida albicans*, *Mycobacterium canis*, and *Trichophyton mentagraphytes* (Hussain et al., 2010). Additionally, at 500  $\mu\text{g/mL}$  concentration, it also demonstrated anti-microbial activity with a maximal zone of inhibition of 16 mm for *Proteus vulgaris*. *H. auriculata* leaf extract exhibited antioxidant activity (Anusha & Rajkumar Immanuel, 2019). With a range of 8–16 mm zone of diameter, the entire plant extract demonstrated anti-microbial efficacy against *Aspergillus niger*, *Escherichia coli*, and *Klebsiella pneumoniae*. It was also reported to contain phytochemicals like alkaloids, flavonoids, tannins, and saponins. Some bioactive compounds isolated from *H. auriculata* which includes Butane, 1, 1- diethoxy- 3-methyl- ; Pentane,1,1-diethoxy-; 3,3 –Diethoxy-2-Butanone; Propane,1,1,3-triethoxy-; 1,1,3- Triethoxybutane ; Benzene, [Ethoxy (1-Propenyloxy; Nonane, 3,7-Dimethyl-; Diethyl Phthalate; Octadecanoic acid, 2-oxo-methyl ester; Isopropyl myristate; 2- Hexadecan-1-ol, 3,7,11,15-tetram; 2,6,10-trimethyl, 14- Ethylene- 14-Pe; 2,6,10- trimethyl, 14-Ethylene-14-Pe; 7-Octadecyne,2-methyl-; Heptadecanoic acid, Ethyl ester; Phytol Isomer; Phytol,acetat; Tridecanol, 2-ethyl-2-

methyl-; Squalene; 1,2-Benzenedicarboxylic (Jebamalai et al., 2021). *H. auriculata* has a number of therapeutic properties that have been used historically to treat a variety of illnesses. Its leaf is used to cure arthralgia, lumbago, Prameha, cough, and anal fistula. Its root is used to treat calculus and jaundice, while its seed is used to treat blood problems. Its veggie is beneficial for anaemia. The decoction of its root and entire portion is beneficial for rheumatoid arthritis (Sarvananda L, 2018).

### **1.5 Research gap:**

1. Despite the fact that endophytic bacteria are found in almost all plants, many medicinal plants have not yet been properly explored.
2. The composition of endophytic bacteria can be influenced by environmental factors as well as soil conditions; in this case, research on the endophytic bacteria found in several medicinal plants in Kokrajhar areas is still ongoing.
3. Many isolated endophytic bacteria have not yet been functionally characterised.

### **1.6 Objectives:** The main objectives of this present study are:

1. To isolate the endophytic bacteria associated with the leaf, stem, and root of medicinal plants like *Glycosmis pentaphylla*, *Hygrophila auriculata*, and *Phlogacanthus thyrsiformis*.
2. To study the morphological, microscopic and biochemical characterisation of the isolated endophytic bacteria.
3. Molecular identification of the isolates.
4. To study a number of plant growth promotion activities of the isolated endophytic bacteria.
5. To screen the ability of isolated endophytic bacteria to produce extracellular enzymes.
6. To evaluate anti-bacterial activities of isolated endophytic bacteria.