2 LITERATURE REVIEW CHAPTER 2

2.1 Review on Plant growth promotion activity of endophytic bacteria

Chemical fertilisers contaminate the environment and are harmful to the health of both humans and animals. An effective way to produce environmentally acceptable biofertilizer is with plant-based microorganisms. Microbe-based innovative methods are currently receiving increased attention as a way to lessen the adverse effects of traditional agricultural practices (Hassan, 2017). Agriculture needs to be improved in order to guarantee food safety for the world's expanding population. Endophytic bacteria are benign microorganisms that reside inside plant hosts and are recognised to aid in the development and growth of the host plants (Lotfalian et al., 2017). By fixing atmospheric nitrogen, absorbing phosphate, potassium, and zinc, creating compounds that chelate iron, and secreting different phytohormones like auxins, ethylene, gibberellins, and cytokines, endophytic bacteria have been shown to directly support plant growth. There are several plants from which endophytic bacteria were isolated previously which showed several plant growth promotion activities listed in Table 1. Bacterial endophytes can indirectly give the host plant resistance or tolerance to biotic and abiotic stresses by producing siderophores, releasing antimicrobial compounds, competing for resources and space, and altering the plant resistance response (Das & Das, 2024). Rhizospheric bacteria and bacterial endophytes both use processes that are known to promote plant growth. Such mechanisms are comparable. In agriculture, horticulture, silviculture, and environmental clean-up techniques, endophytic plant growth-promoting bacteria can function similarly to rhizospheric plant growth-promoting bacteria in plant growth and development (Santoyo et al., 2016).

Nitrogen is the most important nutrient that limits plant growth. Since plants cannot reduce ambient N2, they require exogenously fixed nitrogen for growth and development. The atmospheric N2 must first be transformed into ammonia before plants may use it (Gupta et al., 2012). There are several endophytic bacteria which help in nitrogen fixation. Similarly, phosphorus is essential for plant growth, but it is often unavailable in soil. It binds with calcium, iron, or aluminum, forming complexes. Plants can only absorb phosphorus as orthophosphate anions. In order to facilitate the uptake of nutrients like phosphorus, endophytic bacteria can increase their solubility and bioavailability in the rhizosphere (Ku et al., 2019). Phytohormones, or plant growth regulators, are chemicals that affect how plants grow and develop, even in small amounts. Indole-3-acetic acid (IAA) helps plants by promoting cell growth and differentiation. Endophytic bacteria produce key phytohormones like auxins, cytokinins, abscisic acid, ethylene, gibberellins, and jasmonates (Fouda et al., 2021).

Different plant growth promotion abilities, such as nitrogen-fixation, P-solubilization, siderophore, indole-3-acetic acid (IAA) synthesis, or 1-aminocyclopropane-1-carboxylate (ACC) deaminase, were demonstrated by *Methylobacterium* spp. and *Brevundimonas* spp. isolated from tea plants (Yan et al., 2018).

The bacterial endophytes that desert plants associate with allow them to withstand the severe environmental challenges that are characteristic of arid and semiarid environments. Nevertheless, little is known about the characteristics, roles, and variables influencing the connection of bacterial endophytes with desert plants. Endophytic bacteria have previously been isolated from two indigenous medicinal plants that grow naturally in the arid South Sinai region of Egypt: *Fagonia mollis* Delile and *Achillea fragrantissima* (Forssk) Sch. Bip. The bacterial endophytes that were isolated had the ability to solubilise phosphate with clear zones that ranged from 7.6 ± 0.3 to 9.6 ± 0.3 mm. Furthermore, the bacterial endophytes that were obtained enhanced the production of indole acetic acid (IAA) in broth media from 10 to 60 $\mu g \cdot mL^{-1}$ when the concentration of tryptophan increased from 1 to 5 $mg \cdot mL^{-1}$. *Bacillus* and *Brevibacillus* strains isolated from the leaves of both plants significantly improved plant growth and the amounts of nitrogen (N) and phosphorus (P) in the shoots (Alkahtani et al., 2020).

Endophytic bacteria were previously isolated from various sections of healthy *Musa acuminata* plant samples, such as leaves, shoots, and roots, that were gathered from different agricultural regions of Madhya Pradesh, India. Each isolate was examined for its ability to promote plant development through the synthesis of indole-3-acetic acid, phosphate solubilisation, nitrogen fixation, ammonia, hydrogen cyanide (HCN), and siderophores. Two strains of *Bacillus cereus* and one strain of *Enterobacter hormaechei* showed positive for all 5 characteristics. Two strains of *Enterobacter cloacae* showed negative for only HCN production test whereas positive for all other tests and one strain of *Enterobacter cloacae* showed negative HCN as well as ammonia production tests (Singh S. et al., 2022).

The endophytic bacterial strains that were recovered from the roots, leaves, and flowers of the medicinal plant *Calendula officinalis* from the experimental field of Agricultural University of Athens in Spata in 2017 were subsequently identified and categorised into the genera *Rhizobium*, *Bacillus*, *Pseudomonas*, and *Pantoea*. 91.7% of the *Bacillus* strains tested positive for the production of the plant growth hormone IAA, and half of them demonstrated positive results in solubilising P, producing siderophores, and urease. All of the *Pseudomonas* strains produced iron-chelating compounds and IAA and solubilised P (Tsalgatidou et al., 2023).

In order to enhance plant growth, endophytic bacteria were isolated from commercially significant tea clones grown in North-eastern India. The 106 endophytic bacteria that were obtained to belong to the phyla Proteobacteria, Firmicutes, and Actinobacteria were associated with 22 distinct genera and six significant clusters. In terms of PGP characteristics, the percentage of isolates that tested positive for the nitrogen fixation, phosphate solubilisation, production of ammonia, siderophore, indole acetic acid, and 1-aminocyclopropane-1-carboxylic acid deaminase was 78.3, 28.3, 95.3, 30.2, 86.8, 87.7, respectively (Hazarika et al., 2021).

The presence of plant growth-promoting characteristics such as the ability to fix nitrogen, solubilise inorganic phosphorus, and produce phytohormones, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and siderophores was assessed in bacterial endophytes that were isolated from the roots and leaves of *Miscanthus giganteus* plants. Isolation of leaf bacteria *Pantoea ananat* was the only one to exhibit ACC deaminase activity, produced siderophores, and phytohormones. In terms of producing phytohormones, the root bacterial isolate of *Pseudomonas libanensis* demonstrated the greatest outcomes (Lovecká et al., 2023).

From the leaves and stems of robust wild pistachio trees (*Pistacia atlantica* L.) in the Baneh and Marivan districts of Iran, endophytic bacteria were recovered. A total of 61 endophytic bacteria were isolated and categorised based on their phenotypic traits. The 16S rRNA gene was partially sequenced to further identify ten chosen isolates from each group. Bacteria belonging to the genera *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Pantoea*, and *Serratia* were identified. *Pantoea brenneri* and *Pseudomonas protegens* demonstrated positive results for phosphate solubilisation, siderophore synthesis, and nitrogen fixation activity in plant growth promotion activities. Phosphate solubilisation was demonstrated by *Stenotrophomonas maltophilia* isolated from the same plant. The only bacteria capable of producing hydrogen cyanide in small quantities was *Bacillus anthracis* (Etminani & Harighi, 2018).

In several nations, mostly in Latin America, sugarcane (*Saccharum* spp.) plays a significant economic role. Sugarcane leaves and stems from fields in Brazil's distilleries Japungu and Tabu were used to isolate endophytic bacteria. *Methylobacterium* spp. and *Brevibacillus agri* isolates were obtained exclusively in leaves, but *Herbaspirillum seropedicae* was found exclusively in stems. All endophytes produce IAA, and more than half of them solubilise phosphate and have ACC deaminase activity (Antunes et al., 2022).

The richness of metabolites in ginger rhizomes makes them extremely noteworthy, yet little is known about the physiological processes occurring in these tissues or the functional role of the associated microbes. Pseudomonas sp. isolated from *Zingiber officinale*'s rhizome demonstrated actions that promoted plant growth, such as producing siderophores and IAA, ACC deaminase etc (Jasim et al., 2014).

Cyperus esculentus L. var. sativus, commonly referred to as chufas, earth nuts, or tigernut, ranks second in abundance within the Cyperaceae family. According to recent research, *C. esculentus* L. is valuable for its high-quality oils that resemble olive oil and are packed with beneficial components found in leaf samples, such as flavonoids, lactones, coumarins and their glycosides, steroids and triterpenoids, and cardiac glycosides. *Franconibacter* sp. YSD YN2, an endophytic bacterium that was isolated from *Cyperus esculentus* L. var. sativus leaves, demonstrated good performance of PGP properties, including the ability to dissolve potassium, solubilise phosphate, and produce indole acetic acid and siderophores (Wang et al., 2022).

Cultivable bacterial endophytes were isolated from *Passiflora incarnata* during its vegetative stage. Many people utilise this tropical plant as a traditional herbal remedy.

58 bacterial endophytes were categorised into nine genera using the 16S rRNA gene's sequencing and phylogenetic analysis. The most prevalent genus was *Bacillus* (70.7%), followed by *Pantoea* (6.9%) and *Pseudomonas* (8.6%). According to PCR-based screening or biochemical assays, the majority of the isolates (94.8%) showed potential for at least one PGP feature such as nitrogen fixation, phosphate solubilization, indole-acetic-acid synthesis, and siderophore production (Cueva-Yesquén et al., 2021).

The flowering plant known as *Thymus vulgaris* L., or thyme (Family: Lamiaceae), is native to Northern Africa, the Mediterranean region, and some regions of Asia. The bacteria isolated from the root of *Thymus vulgaris* L., *Bacillus haynesii* T9r, *Citrobacter farmeri* T10r, *Bacillus licheniformis* T11r, *Bacillus velezensis* T12r, and *Bacillus velezensis* T13r, exhibited the nitrogen-fixation, phosphate solubilisation activity, ammonia, hydrogen cyanide (HCN), siderophores, and IAA production (Abdel-Hamid et al., 2021).

A member of the Lamiaceae family, *Teucrium polium* L. is a wild plant that grows natively in the Saint Katherine Protectorate, which is a region of Egypt's Sinai Peninsula. *Bacillus cereus* and *Bacillus subtilis* were isolated from *Teucrium polium* and their plant growth-promoting (PGP) properties were characterised. The bacterial endophyte's PGP activities that might either directly or indirectly support plant health and growth were assessed. With rising tryptophan levels in the media, Tp.1B [*Bacillus cereus* (JQ660645)] and Tp.6B [*Bacillus subtilis* (KP641618)] produced the highest IAA. Tp.1B [*Bacillus cereus* (JQ660645)], Tp.3B [*Bacillus cereus* (JQ660645)], Tp.6B [*Bacillus subtilis* (KP641618)], Tp.7B [*Bacillus subtilis* (KP641618)] showed differentially producing ammonia. Isolate Tp.1B [*Bacillus cereus* (JQ660645)], Tp.4B [*Bacillus subtilis* (KP641618)], Tp.6B [*Bacillus subtilis* (KP641618)], Tp.7B [*Bacillus subtilis* (KP641618)] showed phosphate solubilization activities (Hassan, 2017).

One well-known medicinal evergreen tree that grows in the Indian Himalaya's temperate regions is *Taxus wallichiana* Zucc. As a source of the anti-cancer medication "taxol," it is well known in medicine. Antimicrobial and antioxidant properties have also been identified. Enterobacter and Burkholderia were separated from the *T. wallichiana* plant. These endophytes showed several plant growth promotion activities which includes production of siderophore, HCN, ammonia, IAA and ACC deaminase abilities (Adhikari & Pandey, 2020).

The family Asteraceae, to which the genus Pulicaria belongs, is found, among other locations, in North Africa. Alkaloids, tannins, flavonoids, and phenolics are among the bioactive compounds found in this plant that have therapeutic uses as anti-inflammatory, anti-carcinogenic, antioxidant, antibacterial, and anti-aging agents. They also protect the heart and help prevent a number of chronic illnesses. The following endophytic bacteria were recovered and identified as being associated with the leaves of the native medicinal plant *Pulicaria incisa*: *Burkholderia cepacia*, *Acinetobacter radioresistant*, *Brevibacillus brevis*, *Bacillus cereus*, *Bacillus subtilis*, *Paenibacillus barengoltzii*, and *Agrobacterium fabrum*. In the presence or absence of tryptophan, these isolates demonstrated remarkable efficacy in the synthesis of IAA. In the presence of 5 mg mL^{-1} tryptophan after 10 days, the highest IAA productivity was seen for *B. cereus* BI-8 and *B. subtilis* BI-10, with values of 117 ± 6 and 108 ± 4.6 μg mL^{-1}, respectively. These two isolates showed great potential for producing ammonia and solubilising phosphate (Fouda et al., 2021).

According to an experiment carried out in 2021 by a team of Chinese scientists, the four endophytic bacteria that were isolated from the rhizome of *Pairs polyphylla* var. *yunnanensis*, a Chinese medicinal plant, were played an important role in enhancing the growth of *P. polyphylla* plants and could be employed as inoculants to produce a sustainable crop production system. These isolates also showed in vitro plant growth promotion activity like indole acetic acid production, phosphate solubilization and nitrogen fixation (Tao et al., 2022). Simillarly, all of the isolated strains of *Musa acuminata* have the capacity to affect host plant growth and may be employed as a successful bioinoculant to promote *M. acuminata growth* (Singh et al., 2022). Endophytic bacteria were recovered from the roots, stem, and leaves of *Calotropis procera*. The strain's ability to stimulate rice plant's growth and development was evaluated. A biochemical examination of certain isolated endophytic bacteria indicated that they could produce indole acetic acid (IAA), and they also demonstrated a noticeable increase in rice plant's growth and development (Hamayun et al., 2021).

The synthesis of bioactive substances by medicinal plants creates a selective environment that supports a variety of endophytic bacteria. Because they frequently adapt well to harsh environmental conditions, these endophytes are especially useful in agriculture for marginal soils. An untapped source of biotechnological potential for sustainable agriculture is represented by endophytic bacteria found in medicinal plants. With further study, these microorganisms may play a major role in environmentally friendly farming methods.

Table 1: List of plant growth promoting endophytic bacteria isolated from different tissues of medicinal plants

Medicinal plants	Tissue used in isolation	Isolated endophytic bacteria	Plant growth promotion characteristics	References
Miscanthus giganteus	Leaf	*Pantoea ananat*	ACC deaminase activity, produced siderophores, and phytohormones	Lovecká et al., 2023
Miscanthus giganteus	Root	*Pseudomonas libanensis*	Produced phytohormones	Lovecká et al., 2023
Sugarcane (*Saccharum* spp.)	Leaf	*Methylobacterium* spp. and *Brevibacillus agri*	IAA production	Antunes et al., 2022
Sugarcane (*Saccharum* spp.)	Stem	*Herbaspirillum seropedicae*	IAA production	Antunes et al., 2022
Zingiber officinale	Rhizome	*Pseudomonas* sp.	The ability to produce IAA,	

			ACC deaminase and siderophore	Joseph & Mini Priya, 2011
Cyperus esculentus L	Leaf	*Franconibacter* sp.	Dissolve potassium, solubilise phosphate, and produce indole acetic acid and siderophores	Wang et al., 2022
Thymus vulgaris L.	Root	*Bacillus haynesii* T9r, *Citrobacter farmeri* T10r, *Bacillus liforichenmis* T11r, *Bacillus velezensis* T12r, and *Bacillus velezensis* T13r	nitrogen-fixation, phosphate solubilization, Produces ammonia, hydrogen cyanide (HCN), siderophores, and IAA.	Abdel-Hamid et al., 2021
Teucrium polium		*Bacillus cereus* and *Bacillus subtilis*	IAA and ammonia production, phosphate solubilization	Hassan, 2017
Taxus wallichiana Zucc.	Root	Burkholderia	Siderophore, HCN, ammonia production, IAA and ACC deaminase	Adhikari & Pandey, 2020

			producing abilities.	
Taxus wallichiana Zucc.		Enterobacter	Siderophore, HCN, ammonia production.	Adhikari & Pandey, 2020
Pulicaria incisa	Leaf	*Bacillus cereus* and *Bacillus subtilis*	Production of IAA and ammonia, phosphate solubilisation.	Fouda et al., 2021
Pairs polyphylla var. *yunnanensis*	Rhizome		IAA production, phosphate solubilization and nitrogen fixation	Tao et al., 2022
Lonicera japonica		*Pseudomonas* sp.	phosphate solubilization, siderophore, indole acetic acid and hydrogen cyanide production, and fixation of atmospheric nitrogen.	Gupta et al., 2016
Echinacea purpurea		*Burkholderia* sp.	phosphate solubilization, siderophore, indole acetic acid and hydrogen	Gupta et al., 2016

			cyanide production, and fixation of atmospheric nitrogen.	
Musa acuminata	Root, stem, and leaves	*Bacillus cereus*, *Enterobacter hormaechei*	Phosphate solubilization, siderophore, indole acetic acid and hydrogen cyanide production, and fixation of atmospheric nitrogen.	Singh S. et al., 2022
		Enterobacter cloacae	Phosphate solubilization, siderophore, IAA production, and fixation of atmospheric nitrogen.	Singh S. et al., 2022
Berberis aristata		*Serratia marcescens*	Nitrogen fixation, production of IAA and siderophore	Sharma et al., 2024
Xanthium strumarium		*Bacillus cereus*	Production of IAA, phosphate and potassium solubiliser	Sharma et al., 2024

Xanthium strumarium		*Citrobacter freundii*	Production of ammonia and IAA, phosphate and potassium solubiliser	Sharma et al., 2024
Lessertia frutescens (L.)	Leaf	*Bacillus licheniformis* BaDB06, *Bacillus subtilis* LCDB-BP2,	Phosphate solubiliser	Tsipinana et al., 2024
Lessertia frutescens (L.)	Root	*Bacillus paralincheriformis* AJVR1, and *Kosakonia* sp. MBWS.8	Phosphate solubiliser	Tsipinana et al., 2024

2.2 Review on salt tolerance ability of endophytic bacteria

Worldwide, soil salinisation causes major problems for agriculture; thus, finding sustainable ways to help plant growth and development under high salt stress conditions has become an essential part of the research. Endophytic bacteria have gained much attention for their ability to confer salt tolerance to host plants. It is possible to mitigate the adverse effects of salt stress and enhance the plant growth by using salinity-tolerant endophytic bacteria which have ability to tolerate salt stress. *Vigna radiata* (L.) Wilczek, (mungbean) which is a very nutritious food, is considered the world's most important pulse crop. The production of legume grains was delayed by a number of abiotic stressors, including salt stress, which inhibits the activity of symbiotic bacteria. The *V. radiata* is extremely susceptible to salinity. Plants use a variety of techniques to decrease the salt stress which, includes osmosensing, antioxidant production, and ionic homeostasis maintenance. Thirteen endophytic bacteria were isolated and identified from the roots of mungbean, whose ability to withstand NaCl salts up to 4% was also evaluated. The salt tolerance ability was observed in six bacterial isolates: *Bradyrhizobium japonicum*, *Achromobacter denitrificans*, *Pseudomonas extremorientalis*, *Rhizobium pusense*, *Agrobacterium leguminum*, and *Serratia quinivorans* (Zahra et al., 2023). The stress-tolerant sections of several plants, including Lantana camara, Phoenix dactylifera, Hemerocallis fulva, Salvia rosmarinus, Commiphora wightii, and Abutilon indicum, were used to isolate 50 endophytic microbes. Two of the isolates showed the greatest resistance to salt stress, surviving up to 16% NaCl (Kaur & Karnwal, 2023).

75 endophytic bacteria from the roots of robust *Oryza sativa* plants which were cultivated in a salty environment in Bangladesh's southern coastal region. In the nutrient agar medium, isolates of four genomically different groups—*Enterococcus*, *Achromobacter*, *Bacillus*, and *Stenotrophomonas*—exhibited tolerance to NaCl ranging from 1.37 to 2.57 mol/L (Jhuma et al., 2021). From 24 samples of salt-tolerant rice seeds, 179 strains of endophytic bacteria were identified; nearly 95% of these bacteria showed tolerance to a 2% salt concentration (0.34 mol/L). Bacillus paralicheniformis was the isolate that was found to be tolerant of up to 15% NaCl (Wang et al., 2024). The capacity of endophytic bacteria that were isolated from the roots of *Oryza sativa* to encourage rice seed germination was determined. *Burkholderia cenocepacia* and *Pantoea dispersa* were the two isolates that demonstrated a 99.7% and 99.3% rice seed-promoting impact, respectively (Do et al., 2023).

Bacillus thuringiensis and *Bacillus pumilis* isolated from rhizome of *Curcuma longa* L. showed tolerance to 8 % of NaCl concentration. However, *Clavibacter michiganensis* and *Pseudomonas putida* demonstrated resistance to a 6% NaCl concentration (Kumar et al., 2016). 5% salt tolerance was demonstrated by endophytic bacteria that were isolated from *Glycosmis pentaphylla* leaves. However, identification of the isolates was not done. In salt tolerance ability test, all isolates showed tolerance to different concentration of NaCl (Das & Das, 2022).

Numerous beneficial microorganisms can be found in the rhizosphere, phyllosphere, and endophytically of mangroves. This makes them an excellent ecological home for isolating halotolerant endophytic bacteria with special traits. For the purpose of isolating endophytes, the leaves and roots of the mangrove trees *Acanthus ilicifolius* L. at Corangi Wildlife Sanctuary were chosen. After isolating eight endophic bacteria, salt tolerant ability was tested and it was found that the isolates tolerated salinity up to 8% NaCl and grew best on 3% NaCl nutrient agar (Deepika et al., 2023).

Salt-tolerant endophytic bacteria offer significant potential to enhance the sustainability of agriculture in saline environments. Salt-tolerant endophytic bacteria play a crucial role in mitigating the adverse effects of soil salinity on crops by promoting plant growth in stress environment.

2.3 Review on anti-microbial activity of endophytic bacteria

Global healthcare is severely hampered by infectious diseases, particularly in light of the rise in antibiotic resistance. New antimicrobial medication development and research are urgently needed to address this pressing problem. The secondary metabolites obtained from endophytic bacteria are particularly noteworthy as potential sources of antimicrobials in this context (Eshboev et al., 2024). Endophytic bacteria, which reside within the tissues of plants without causing harm, have garnered significant attention in recent years due to their potential in producing bioactive compounds. Medicinal plants, in particular, offer a unique niche for endophytes due to their production of secondary metabolites that may shape the bacterial community and enhance their antimicrobial potential. Endophytes produce antimicrobial compounds that are safe for the environment, poisonous to pathogens, and non-toxic to humans. Several new antibiotics that are effective against bacteria that are resistant to multiple drugs were developed by Endophytic *Streptomyces* sp. (Singh et al., 2017). Previously, it was reported that the endophytic bacterium *Pseudomonas viridiflava* produced the new lipopeptide Ecomycins. Also, pseudomycins, an antifungal compound was produced using *Pseudomonas syringae*. Antibiotics munumbicins A, B, C, and D which are identified recently showed a broad range of antimicrobial activities against bacteria, fungus, and a species of *Plasmodium* that cause disease in plants. These were obtained from *Streptomyces* NRRL 30562, which was isolated from a medicinal plant, *Kennedia nigriscans*. Munumbin B works well against acid-fast *Mycobacterium tuberculosis*, a multidrug-resistant (MDR) bacteria. In addition to having antimicrobial activity against both Gram-positive and Gram-negative bacteria, the munumbicins C and D also showed the ability to fight the most severe malaria-causing *Plasmodium falciparum*. The antibiotic Kakadumycins is derived from the endophytic bacterium *Streptomyces* (NRRL30566), which was isolated from a Grevillea tree. Similar to munumbicins, kakadumycin A has antibacterial activity and works well against *P. falciparum* (Bhore et al., 2013). Worldwide, studies are being conducted to isolate and examine the antimicrobial activities of endophytic bacteria from various plant species. Previously, there were several endophytic bacteria isolated from different plants showed anti-microbial activities listed in Table 2.

The *Cordia dichotoma*, a medicinal plant found in the Jammu region of India, which was used to isolate the bacterial isolates, which are classified into the genera *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Acidomonas*, *Streptococcus*, *Rastonia*, *Micrococcus*, *Staphylococcus*, and *Alcalignes*. Most of the *C. dichotoma*'s bacterial endophytes exhibited anti-microbial efficacy against *Bacillus subtilis*, and *Klebsiella pneumoniae* (Sharma & Mallubhotla, 2022).

In Iran, 23 different medicinal plants were used to solate endophytic bacteria and antimicrobial activities of the isolates were evaluated against some harmful bacteria. The findings revealed that 16 of these bacterial isolates showed the ability to inhibit the growth of some pathogenic bacteria which includes *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus mirabilis*, *Shigella flexneri*, and *Escherichia coli*. Among all, there were two bacteria, *Amycolatopsis tolypophora* isolated from the root of the medicinal plant *Stachys lavandulifolia* and *Bacillus thuringiensis* obtained from the root of *Physalis alkekengi* showed a notable antimicrobial activity to inhibit the growth of various strains of harmful bacteria. Furthermore, among the tested bacterial isolates, 13 exhibited significant antiviral activity (Beiranvand et al., 2017).

Endophytic bacteria were isolated from two widely used medicinal herbs in India's north eastern states, namely *Swertia chirata* (Chirata) and *Datura stramonium* (Datura). Isolates were studied for their anti-bacterial and anti-fungal activities. *Staphylococcus* sp. isolated from the root of Chirata showed anti-bacterial activity against *Xanthomonas oryzae* and anti-fungal activity against *Fusarium oxysporum*. *Bacillus sp*. isolated from the same plant also showed anti-bacterial activity against *Ralstonia solanacearum*.

Endophytic bacterial isolate, *Rhodococcus jialingiae* from Datura plant showed anti-bacterial activity against *Ralstonia solanacearum* (Devi et al., 2021).

A total of 24 bacterial endophytes were isolated from three medicinal plant namely *Cichorium intybus* L, *Pelargonium hortorum*, and *Portulaca oleracea*. Of which 10 numbers of endophytic bacteria displayed an average inhibition zone of more than 9.5 mm against *Staphylococcus aureus* and *Enterococcus faecalis* (Lotfalian et al., 2017).

Uzbekistan's native plant, *Ajuga turkestanica* (Lamiaceae), is utilised extensively in traditional medicine for both its aboveground and root parts. Twelve bacterial strains were chosen in order to produce crude extracts using ethyl acetate and methanol. In antimicrobial activity test, all strains showed inhibitory activity against gram-positive bacteria, *Bacillus subtilis*-5 and *Staphylococcus aureus*-91, gram-negative bacteria, *Escherichia coli*-221, and *Pseudomonas aeruginosa*-225 and *Candida albicans*-247. All extracts had the following range of action at the lowest inhibitory concentration: *B. subtilis* 12.5 μg/mL −50 μg/mL, *S. aureus* −3.122 μg/mL − 50 μg/mL, *P. aeruginosa* 12.5 μg/mL −50 μg/mL, and *E. coli* 6.25 μg/mL − 25 μg/mL. All strains of endophytic bacteria had the following inhibitory impact on plant phytopathogenic microbes: *Fusarium oxysporium*: 44% − 70%, 3–5 cm; *Fusarium proliferatum*: 27.8% − 56%, 4.1–7 cm. Finally, it was proposed that *B. mojavensis* and *B. amyloliquefaciens* could be a good biocontrol agent to give plants resistance to *F. oxysporium* and *F. proliferatum* disease as well as other phytopathogens (Mamarasulov et al., 2023).

Endophytic bacteria linked to the medicinal plant *Origanum heracleoticum* L. demonstrated the capacity to inhibit the growth of human opportunistic pathogens that are part of the *Burkholderia cepacia* complex (Bcc), albeit to varying degrees. The taxonomic attribution of the various inhibitory patterns was based on the genus level; the majority of active strains are members of the Gram-positive genera *Bacillus*, *Arthrobacter*, and *Pseudarthrobacter*. The maximum antibacterial activity was shown against the strains of *Klebsiella pneumoniae* and Coagulase-negative *staphylococci* which are the multi drug resistance microbes. Sulphurated compounds. The strong antibacterial action of *Arthrobacter* sp. OHL24 against *S. aureus* strains was caused by sulfur-containing substances (Semenzato et al., 2024).

A more sustainable commercial source of bioactive compounds can be found in endophytes that exhibit the capacity to manufacture the same molecules as their plant hosts. The synanthropic plant *Urtica dioica* L., often known as common stinging nettle, is used extensively in herbal medicine because of the variety of bioactive compounds it contains, such as polyphenols, which have anti-inflammatory, antioxidant, and anti-cancerous properties. All of the bacterial strains that were isolated from stinging nettles were able to produce polyphenols and biosurfactants (Marchut-Mikołajczyk, 2023). Previously, biosurfactants have demonstrated antimicrobial action against a number of harmful microorganisms (Lourenço et al., 2024).

Burkholderia gladioli and *Bacillus aryabhattai* isolated from the seed of three different Pakistani wheat varieties showed antimicrobial activity against *Klebsiella pneumonia*, *Escherichia coli*, and *Bacillus subtilis* (Shah et al., 2022). *Burkholderia* and *Bacillus* genus were previously mentioned as possible biocontrol agents against *Burkholderia glumae* THT. Endophytic bacteria isolated from rice, *Burkholderia vietnamiensis* TUR04-01, *B*. *vietnamiensis* TUR04-03, and *Bacillus aryabhattai* AMH12-02 shown antimicrobial activity against *B*. *glumae* (Valdez-Nuñez et al., 2020). *Bacillus atrophaeus* had previously been identified as a viable candidate for use as a biocontrol agent to give cotton and other crops resistance to *Verticillium* wilt disease and other phytopathogens. *Bacillus atrophaeus* and *Bacillus mojavensis*, which were isolated from the wild ethnomedicinal plant *Glycyrrhiza uralensis* (liquorice), exhibited a wide range of antifungal and antibacterial activity against the common bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteritidis*, and *Escherichia coli*, as well as fungal pathogens of tomato (*Fusarium oxysporum*, *Fulvia fulva*, *Alternaria solani*), cotton (*Fusarium oxysporum*, *Verticillium dahliae*), pomegranite (*Ceratocystis fimbriata*), and Tsao-ko (*Pestalotiopsis microspora* and *Fusarium graminearum*) (Mohamad et al., 2018).

Pseudomonas azotoformans NBRC12693, which was isolated from the roots of the therapeutic plant *Codonopsis pilosula*, of Weiyuan County, Gansu Province, China shown antimicrobial efficacy against *Bacillus subtilis* and *Staphylococcus aureus*. *P*. *azotoformans* demonstrated a significant minimum inhibitory concentration (MIC) of 250 µg/ml against S. aureus and 500 µg/ml against *B*. *subtilis* (Lodi et al., 2023). Additionally, *P. azotoformans* strain UICC B-91 was identified from the Indonesian endemic traditional medicinal plant *Nessia altissima* Blume. It showed antimicrobial activity against *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6583, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 25241, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 19659 and *Candida albicans* ATCC 10231 (Pratiwi et al., 2022). Previously, antimicrobial activity against *Shigella flexneri*, *Salmonella enterica* ser. *typhi*, and *Escherichia coli* was demonstrated by *Pseudomonas aeruginosa* isolated from *Ageratum conyzoides* Linn (Boonman et al., 2023).

Chryseobacterium sp., which was isolated from *Gunnera perpensa* L. rhizomes, had encouraging antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* (Mahlangu et al., 2024). In South Korea, a novel species of *Chryseobacterium* called *Chryseobacterium tagetis* sp. nov. was isolated from the root of the medicinal plant, *Tagetes patula*, and it showed anti-microbial activity against gram-negative *Xanthomonas campestris* (Chhetri et al., 2022).

In 2020, endophytic bacteria were isolated from a number of medicinal plants in Myanmar. Of these, two bacterial isolates from *Boscia variabilis* Collett & Hemsl. (Capparaceae) and one endophyte from *Catharanthus roseus* exhibited both antifungal and antibacterial activity against plant-pathogenic fungi and human-pathogenic bacteria, respectively. Two antagonistic strains from *B. variabilis* Collett & Hemsl. (Capparaceae) were identified by 16S rRNA sequencing as *Bacillus subtilis* sub sp. subtilis str. 168 and *Bacillus amyloliquefaciens* DSM7, respectively, while one strain from *C. roseus* was identified as *Bacillus amyloliquefaciens* DSM7. Different culture medium showed different production of bioactive compounds. Bacillus medium supplemented with 4% starch and 0.3% peptone was the most effective medium for producing the most bioactive compounds for *B. amyloliquefaciens* DSM7, while *B. subtilis* subsp. subtilis str. 168 produced the most antimicrobial compounds when incubated in the medium supplemented with 3% starch and 2% peptone. For B. amyloliquefaciens DSM 7, the ideal conditions for the maximum production of the antimicrobial compound were a medium pH of 6 at 35 °C after two days of incubation, while B. subtilis subsp. subtilis str. 168 secreted the highest concentration of the bioactive compounds at pH 7.5 and 35˚C a day later (Myo et al., 2020).

New antibiotics can be found in natural compounds derived from endophytic actinobacteria (EA). Previous research has examined the antibacterial activities of EA that was extracted from *Luffa cylindrica*. Each of the six strains exhibited antibacterial activity against at least one of the tested microorganisms, including *E. coli*, *P. aeruginosa*, and *S. aureus*. The strain with the strongest antibacterial activity against both gram-positive and gram-negative bacteria was S. praecox (Mahdi et al., 2022). *Azadirachta indica* A. Juss. endophytic actinomycetes were screened and assessed for their antimicrobial efficacy against a variety of harmful bacteria and fungi (Verma et al., 2009).

It was determined that the isolate *Bacillus subtilis* SCB-1 possesses antifungal properties against a variety of fungal diseases, including those belonging to the genera *Saccharicola*, *Cochliobolus*, *Alternaria*, and *Fusarium*. Its ability to bio-control fungal infections may be due to the bacterial isolate's production of volatiles and the strong antifungal chemical surfactin (Hazarika et al., 2019).

Numerous secondary metabolites are produced by endophytes to shield their host plants against disease development. Symbiotic, commensalistic, and mutualistic connections are formed by endophytes as they colonise the interior tissues of the host plant. Additional bioactive substances with antibacterial, anticancer, and anti-inflammatory qualities are produced by bacterial endophytes (Mahlangu et al., 2024). These compounds offer an alternative to traditional antibiotics, potentially overcoming mechanisms of resistance. Thus, endophytic bacteria could pave the way for sustainable strategies to address the global challenge of antimicrobial resistance.

Table 2: Endophytic bacteria isolated from different medicinal plant and their antimicrobial activity against different pathogenic microorganisms

Sl. No.	Endophytic bacteria	Host plant	Antimicrobial activity against pathogenic microbes	Bioactive compounds	References

1	*Bacillus thuringiensis* OM320575	*Cordia dichotoma*	*Bacillus subtilis*, *Klebsiella pneumoniae*	Dibutyl phthalate, eicosane, tetrapentacontane, heneicosane, and hexadecane,	Sharma & Mallubhotla, 2022
2	*B. mojavensis* and *B. amyloliquefaciens*	*Ajuga turkestanica*	*F. oxysporium* and *F. proliferatum*		Mamarasulov et al., 2023
3	*Arthrobacter* sp. OHL24	*Origanum heracleoticum* L.	*S. aureus*	Dimethyl disulfide	Semenzato et al., 2024
4	*Bacillus cereus* and *Bacillus mycoides*	*Urtica dioica* L		Biosurfactants and polyphenols	Marchut-Mikołajczyk et al., 2023
5	*Burkholderia gladioli* and *Bacillus aryabhattai*	Wheat varieties	*Klebsiella pneumonia*, *Escherichia coli*, and *Bacillus subtilis*		Shah et al., 2022
6	*Burkholderia vietnamiensis* TUR04-01, *B. vietnamiensis* TUR04-03,	Rice	*Burkholderia glumae*		Valdez-Nuñez et al., 2020

	and *Bacillus aryabhattai* AMH12-02				
7	*Bacillus atrophaeus*	*Glycyrrhiza uralensis*	*Verticillium* wilt disease		Mohamad et al., 2018
8	*Pseudomonas azotoformans* NBRC12693	*Codonopsis pilosula*	*Bacillus subtilis* and *Staphylococcus aureus*		Lodi et al., 2023
9	*Pseudomonas azotoformans* strain UICC B-91	*Nessia altissima* Blume	*Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6583, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 25241, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 19659 and *Candida albicans* ATCC 10231	Isobutyl amide alkaloid, monoterpenoid alkaloid, lycopodium alkaloid and cyclo-dipeptide alkaloid	Pratiwi et al., 2022

10	*Pseudomonas aeruginosa*	*Ageratum conyzoides* Linn	*Shigella flexneri*, *Salmonella enterica* ser. *typhi*, and *Escherichia coli*		Boonman et al., 2023
11	*Chryseobacterium* sp.	*Gunnera perpensa* L.	*Staphylococcus aureus* and *Klebsiella pneumoniae*		Mahlangu et al., 2024
12	*Chryseobacterium tagetis* sp.	*Tagetes patula*	*Xanthomonas campestris*		Chhetri et al., 2022
13	*Staphylococcus* sp.	Chirata plant	Anti- bacterial activity against *Xanthomonas oryzae* and anti-fungal activity against *Fusarium oxysporum*.		Devi et al., 2021
14	*Bacillus sp.*.	Chirata plant	Anti-bacterial activity against *Ralstonia solanacearum*		Devi et al., 2021
15	*Nocardia* sp.	*Azadirachta indica*	*Bacillus subtilis*		Verma et al., 2009

16	*Streptomyces*	*Azadirachta indica*	*Pseudomonas fluorescens*		Verma et al., 2009

2.4 Review on extracellular enzyme activity of endophytic bacteria

Endophytic bacteria have gained so much of importance because of their ability to produce a wide range of bioactive compounds and enzymes. Endophytic bacteria can be used for the mass production of some enzymes which have the applications in different fields which includes agricultural, industrial use etc. It is possible to use enzymes in place of hazardous chemicals. As time goes on, scientists are starting to find potential in the synthesis of microbial enzymes (Fadiji & Babalola, 2020). Using enzyme-mediated catalysis to produce biofuels is one of method that has gain a lot of interest worldwide. The production of different clean fuels, the enzymes now used in the manufacture of biohydrogen and biodiesel (Bangaru et al., 2022). Enzymes like proteases and lipases are used in the production of pharmaceuticals, including anti-inflammatory and antimicrobial agents (Meghwanshi et al., 2020). Enzymes like xylanases improve dough quality in baking, while pectinases are used in juice clarification and extraction processes (Sakina et al., 2023).

High catalytic activity microbial enzymes are employed in many industrial sectors due to their greater stability, lower cost, and ability to be produced in large quantities through fermentation processes. Previously it was reported that endophytic bacteria can produce cellulase, protease, amylase, pectinase, lipase, asparaginase, and other enzymes. Consequently, endophytic bacteria can be a novel source of enzymes with a variety of possible uses. In Van, Turkey, 128 endophyte bacterial isolates obtained from cultivated and wild grains were used to evaluate their ability to produce extracellular enzyme. The percentages of bacterial isolates to produced extracellular enzymes, lipases, proteases, amylases, cellulases, pectinases, and xylanases were 74.2%, 65.6%, 55.4%, 32%, 21.8%, and 7.8%, respectively. Using 16S rRNA gene sequencing, certain strains were identified from the isolates which includes the genera *Pseudomonas*, *Micrococcus*, *Paenibacillus*, *Streptococcus*, *Curtobacterium*, *Chryseobacterium*, and *Bacillus* (Dogan & Taskin, 2021).

Endophytic bacteria isolated from *Vigna radiata* and *Cajanus cajan* were found to produce hydrolytic enzymes. It was reported that 9 isolates of 47 endophytic bacteria produced all four enzymes: amylase, cellulase, protease, and pectinase. Among 9 isolates, *Bacillus cereus* NMP2, *Bacillus subtilis* NMP1, and *Bacillus licheniformis* MHN12 were identified as endophytic bacteria that produce hydrolytic enzymes (Namita et al., 2021). From the tissues of four distinct crops and two wild plants, including *Zea mays* L. (maize), *Vicia faba* L. (broad bean), *Secale cereale* L. (rye), *Triticum aestivum* L. (wheat), *Arctium lappa* L. (burdock), and *Equisetum arvense* L. (horsetail), 23 strains of endophytic bacteria were selected for the screening of hydrolytic enzyme production. At least one of the four enzymes—protease, cellulase, lipase, and esterase activities—was detected in 21 isolates. Nonetheless, eight isolates showed all distinct enzyme activity. The 23 endophytic bacterial isolates had protease, cellulase, lipase, and esterase activity in 65.22%, 52.16%, 86.96, and 82.60% of the strains, respectively (Woźniak et al., 2023).

The therapeutic plant *Lessertia frutescens* (L.) was used to isolate endophytic bacteria. The important hydrolytic enzymes amylase (86%), gelatinase (86%), protease (29%), lipase (43%), and d-nase (57%), were produced by isolates. *Bacillus subtilis* LCDB-BP2, which was isolated from the leaf of the *L. frutescens* plant, produced lipase, amylase, protease, and gelatinase. Results for the activities of amylase, gelatinase, lipase, and d-nase were positive for *Bacillus licheniformis* QT445 that was isolated from roots. *Bacillus subtilis* ZIM3 isolated from root showed amylase, protease, gelatinase, and d-nase activities (Tsipinana et al., 2024).

In India, one of the often-utilised herbs with therapeutic value is *Holostemma ada-kodien* Schult. Three endophytic bacteria were from the healthy rhizome of *H. ada-kodien*. In the hydrolytic enzyme production test, all three endophytic bacterial strains produced good results for cellulase, xylanase, and pectinase production, while showing negative results for lipase and amylase. All three endophytic bacteria were identified using 16S r DNA sequencing: *Bacillus pumilus*, *Micrococcus luteus* and *Bacillus pseudomycoides* respectively (Pushkaran et al., 2020).

The majority of medicinal supplements are found in the roots of *Codonopsis pilosula* (Franch.) Nannf. Asian nations including China, Japan, and Korea have been using the roots of this plant as food and medicine for thousands of years. Endophytic bacteria were isolated from the root of the *C. pilosula* and the isolates showed different enzymatic activities. Significant amylase activity was demonstrated by *Bacillus subtilis* JCM1465, which produced 6 U/ml of enzyme. Other endophytes, including *Bacillus velezensis* FZB42, *Bacillus altitudinis* strain 11–1-1, and *Paenibacillus terrae* strain IHBB 9910, produced 5.6, 5, and 4.5 U/ml of enzyme, respectively. *Bacillus velezensis* FZB42 had highly considerable cellulase enzyme activity, generating 8.9 U/ml, and protease activity, generating 0.3 U/ml (Lodi et al., 2023). Protease, cellulase, and xylanase activities were 28.6 U/ml, 1.4 ± 0.024 U/ml, and 0.4 ± 0.02 U/ml, respectively, while α-amylose activity was 14.2 U/ml for the isolate *Bacillus amyloliquefaciens* that was taken from the leaves of the medicinal plant *Kalanchoe daigremontiana* (Shokhiddinova & Normurodova, 2024).

Endophytic bacteria are invaluable for their ability to produce extracellular enzymes with diverse applications. Harnessing their enzymatic potential can lead to advances in sustainable agriculture, green technologies, and industrial processes. Continued research into their mechanisms and optimization of enzyme production will further expand their utility in various sectors.