CONCLUSIONS CHAPTER-7

Endophytic bacteria can be a solution to various issues in the health sector, such as medication resistance, and in the agriculture sector, such as increased environmental pollution and health risks for both humans and animals due to the use of chemical fertilisers. Several endophytes that were isolated from various plants previously demonstrated antimicrobial and anti-cancer properties in addition to their ability to promote plant development. Since numerous endophytic bacteria have been shown to be able to produce extracellular enzymes including amylases, pectinases, proteases, and lipases, among others, they can also be used in other industries, such as the food and beverage, textile, and leather sectors. A wide variety of economically significant endophytic bacteria are found in medicinal plants. Although, many medicinal plants have not yet been thoroughly investigated, even though endophytic bacteria are present in almost all plants. In this study, endophytic bacteria were isolated, characterised and identified from three different medicinal plants, including *G. pentaphylla*, *H. auriculata* and *P. thyrsiformis* from different locations of the Kokrajhar district, Assam. Surface sterilisation is crucial for the isolation of endophytic bacteria as it ensures both the preservation of endophytic bacteria and the elimination of epiphytic bacteria. Here, endophytic bacteria from three medicinal plants have been successfully isolated using the optimised surface sterilisation approach, where the different concentrations of sodium hypochlorite are used for different durations along with 70% ethanol. Following isolation, these endophytic bacteria were characterised through morphological features, Gram staining, SEM analysis, antibiotic sensitivity to some common antibiotics, and some biochemical analysis. In this study, gram-positive bacteria are more common than gram-negative bacteria. 10 gram-positive isolates and 6 gram-negative isolates were found among the 16 endophytic bacteria. Almost all isolates are rod shaped except PTL-4 which is circular in shape.

A total of 16 endophytic bacterial strains were identified, belonging to diverse phyla, including Firmicutes, Proteobacteria, and Actinobacteria, and genera such as *Pseudomonas*, *Bacillus*, *Agrobacterium*, *Solibacillus*, *Lysinibacillus*, and *Kocuria*. The findings of this study contribute to the identification of endophytic bacteria – *Kocuria assamensis*, *Prescottella equi* and *Alkalicoccobacillus gibsonii* – among 16 that have been

reported for the first time as endophytic bacteria. Also, this is first report on the isolation of endophytic bacteria from the tissues of *G. pentaphylla* as well as *P. thyrsiformis*.

The isolated endophytic bacteria exhibited diverse plant growth-promoting (PGP) activities, including the production of IAA, ammonia, and phosphate solubilisation. Out of sixteen, eleven isolates showed positive results for the IAA production ability. Highest production was observed in HAR-5 (258.6 ± 2.05 µg/ml) followed by HAL-1 (121 ± 1.63 µg/ml), PTL-3 (81 ± 1.24 µg/ml), GPL-3 (80.16 ± 2.89 µg/ml), GPS-4 (76.66 ± 2.49 µg/ml), GPL-1 (76.33 ± 1.24 µg/ml), GPL-2 (75.83 ± 1.31 µg/ml), PTS-2 (67 ± 1.24 µg/ml), PTL-4 (45 ± 0.47 µg/ml), GPR-5 (31.66 ± 2.49 µg/ml), PTS-1 (15 ± 0.81 µg/ml) in presence of 400 µg/ml of L-tryptophane. For ammonia almost, all isolates showed ability to produce ammonia except PTS-1 and PTR-5. In this study, seven isolates showed the ability to solubilise calcium phosphate. Highest clearing zone was observed for PTS-1 (3.50 ± 0.11 mm) isolates followed by PTR-6 (2.66 ± 0.28 mm), PTR-5 (2.1 ± 0.12 mm), PTS-2 (2 ± 0.05 mm), GPR-5 (1.6 ± 0.12 mm), HAS-3 (1.59 ± 0.06 mm) and GPL-3 (1.4 ± 0.08 mm) when growing in Pikovskaya's agar medium containing calcium phosphate. The diverse plant growth-promoting traits of the isolated endophytic bacteria suggest their potential as biofertilisers in sustainable agriculture. Their ability to produce IAA, ammonia, and solubilise phosphate can enhance plant growth, improve nutrient availability, and reduce dependence on chemical fertilizers. With a minimum tolerance of 3% NaCl and a maximum tolerance of 10% NaCl, all isolates demonstrated remarkable resistance to high salt concentrations. The isolates exhibited varying degrees of NaCl tolerance: PTS-2 tolerated up to 10%, GPS-4 up to 8%, GPL-1, GPL-2, GPL-3, and HAS-3 up to 7%, HAL-1 and PTR-6 up to 6%, GPR-5, HAL-2, PTR-5, PTL-4, and PTL-3 up to 5%, PTS-1 up to 4%, and HAS-4 up to 3%. In agriculture, these isolates can be used to boost plant growth when salt stress is present. In antibiotic sensitivity test, some isolates, such as PTS-1, PTR-5, GPL-2, GPS-4, GPR-5, HAL-1, HAL-2, HAS-3, HAS-4, and HAR-5 exhibited resistant (R) to multiple antibiotics, suggesting a strong ability to withstand certain treatments. Others, like PTS-2, PTL-3, PTL-4, PTR-6, GPL-1, and GPS-3 demonstrated high sensitivity to multiple antibiotics, as evidenced by large inhibition zone diameters across several antibiotics. Intermediate responses were also observed in some cases, indicating partial susceptibility. The isolates which showed resistant to multiple antibiotics can be used for bioremediation treatment to reduce

environmental pollution. These isolates are also crucial for biotechnological research because these isolates can use in extraction of antibiotic-resistant genes.

The hydrolytic enzyme-producing abilities of 16 endophytic bacterial isolates were evaluated for amylase, protease, lipase, cellulase, pectinase, and xylanase activities. Protease was the most commonly produced enzyme, detected in 13 isolates, while amylase was the least common, found in only six isolates. Some isolates, like PTR-6 and HAL-1, exhibited broad enzyme production, whereas others, such as PTL-3 and HAL-2, showed limited enzymatic activity. These isolates can be used in the food and beverage, textile, leather, and pharmaceutical industries for mass production of such enzymes.

Among the isolates, PTS-1 exhibited the strongest antimicrobial activity against five pathogenic bacteria: *S. aureus* (MTCC 737), *E. coli* (MTCC 443), *B. subtilis* (MTCC 441), *E. aerogenes* (MTCC 2822), and *P. aeruginosa* (MTCC 1688). Following PTS 1, PTS-2 and PTL-3 exhibited antibacterial activity against three of six pathogenic bacteria. Then, GPL-1, GPL-2, GPS-4 and GPR-5 showed inhibitory activity against two of six pathogenic bacteria. Antimicrobial activity against at least one pathogenic bacterium was demonstrated by PTL-4, PTR-6, GPL-3, HAL-1, HAL-2, HAS-3, and HAR-5. These isolates can be used in the production of antimicrobial drugs whereas thorough study is needed.

In conclusion, the work makes a substantial contribution to the development of a biofertiliser that can be used in agriculture in place of chemical fertilisers. Also, the isolates can be used in agriculture to fight abiotic stress like salt stress, as the isolates can help the plant to grow in the soil which contains high salt concentration. The findings also contributed to introducing *K. assamensis*, *P. equi* and *A. gibsonii* as endophytic bacteria for the first time.