ABSTRACT

Endophytic bacteria are those that multiply intracellularly or intercellularly in their host plants at least once during their life cycle without showing any obvious symptom of disease. Plant-associated bacteria represent a vast and untapped source of unique phytochemical compounds, biofertilisers, and plant growth promoters that can be used as sustainable and natural alternatives to agrochemicals. Numerous issues in agriculture and health can be resolved by endophytic bacteria, including reducing environmental pollution brought on by the long-term use of chemical fertilisers and producing medications that will aid in the fight against drug resistance. In addition to their capacity to stimulate plant growth, a number of endophytes (which were previously isolated from a variety of plants) have shown antibacterial and anti-cancer properties. The ability of many endophytic bacteria to produce extracellular enzymes, such as lipases, amylases, pectinases, cellulase, xylanase and proteases, has been demonstrated. These enzymes can be used in various industries, including the food and beverage, textile, and leather sectors. There are several different types of endophytic bacteria that are economically important and can be isolated from medicinal plants. Although many medicinal plants have not yet been fully studied. With these considerations, the present study was carried out to isolate, characterise and identify endophytic bacteria present in some medicinal plants, like *Glycosmis pentaphylla*, *Hygrophila auriculata* and *Phlogacanthus thyrsiformis*, collected from different locations of the Kokrajhar district, Assam. For the isolation of endophytic bacteria, surface sterilisation plays an important role. In this study, surface sterilisation was done by using 70% ethanol and sodium hypochlorite in different concentrations and for different durations. The conformation of proper surface sterilisation was done by performing a sterility test. Here, 16 endophytic bacteria have been successfully isolated from the leaf, root and stem of three medicinal plants using the optimised surface sterilisation method. A total of 5 endophytic bacteria were isolated from the different tissues of *G. pentaphylla*: GPL-1, GPL-2 and GPL-3 from the leaves; GPS-4 from stems and GPR-5 from roots. Also, from *H. auriculata*, a total of 5 endophytic bacteria were isolated: HAL-1 and HAL-2 from leaves; HAS-3 and HAS-4 from stems; HAR-5 from roots. Whereas from *P. thyrsiformis*, 6 endophytic bacteria were isolated: PTS-1 and PTS-2 from stems; PTL-3 and PTL-4 from leaves; PTR-5 and PTR-6 from roots. Morphological, microscopic and biochemical characterisation of the isolates

was done using some standard protocol. For microscopic analysis, both Gram staining and scanning electron microscopy analysis were performed. Of the 16 isolates, 10 exhibited Gram-positive nature and 6 exhibited Gram-negative nature. Under microscope, almost all isolates were rod-shaped except PTL-4, which is circular in shape. In biochemical characterisation, almost all isolates showed positive for the catalase test and negative for the indole test. Almost all isolates showed positive for the oxidase test except PTL-3, PTL-4, PTR-6, HAL-2, HAS-3 and HAR-5, whereas GPL-2 is slightly positive as the purple colour is produced after some seconds (delayed production). All the isolates showed positive for the citrate test except PTR-6. In the methyl red test, all isolates showed negative results except HAL-2, which showed a positive result. Out of sixteen, eleven isolates showed positive and five showed negative for the Voges-Proskauer test. Antibiotic sensitivity tests were also performed on the isolates against a number of standard antibiotics. Isolates PTS-1, PTR-5, GPL-2, GPS-4, GPR-5, HAL-1, HAL-2, HAS-3, HAS-4 and HAR-5 showed resistant to ampicillin, whereas PTS-2, PTL-3, PTL-4, PTR-6, GPL-1, and GPL-3 showed susceptibility to ampicillin. All the isolates showed resistant to penicillin. For gentamicin, almost all isolates were susceptible except GPR-5 and HAS-3, which showed resistant to gentamicin. Except for GPR-5, all isolates showed susceptibility to ciprofloxacin. Against cefotaxime, PTS-1, GPL-2, GPS-4, GPR-5, HAL-1, HAL-2, HAS-3, HAS-4, and HAR-5 were resistant; PTR-6 showed intermediate resistant, whereas isolates PTS-2, PTL-3, PTL-4, PTR-5, GPL-1, and GPL-3 showed susceptibility to cefotaxime.

For molecular identification, the genomic DNA was extracted from all 16 bacterial isolates using the CTAB method with some modifications. After that, PCR amplification was done for all isolates using the universal primer for the 16S rDNA gene, namely, 27F (forward primer) and 1492R (reverse primer). Amplified products were checked in 1.5% agarose gel, and then sequencing was performed. After getting the sequence, identification was done by performing BLAST followed by constructing a phylogenetic tree using MEGA 11 software. Then, the endophytic bacteria were identified from three medicinal plants, which are as follows: *Pseudomonas oryzihabitans* strain HAL1DD (HAL-1), *Proteus mirabilis* strain HAL2DD (HAL-2), *Stenotrophomonas geniculata* strain HAS3DD (HAS-3), *Agrobacterium cavarae* strain HAS4DD (HAS-4), *Lysinibacillus macrolides* strain HARDD (HAR-5) from *H.*

auriculata; *Alkalicoccobacillus gibsonii* strain GPDD1 (GPL-1), *Bacillus cereus* strain GP2DD (GPL-2), *Bacillus subtilis* strain GP3DD (GPL-3), *Bacillus cereus* strain GP4DD (GPS-4), *Bacillus australimaris* strain GPRDD (GPR-5) from *G. pentaphylla*; *Pseudomonas aeruginosa* strain DD3 (PTS-1), *Agrobacterium larrymoorei* strain DDBU3 (PTS-2), *Solibacillus silvestris* strain DDBU6 (PTL-3), *Kocuria assamensis* strain DDBU9 (PTL-4), *Alkalicoccobacillus gibsonii* strain PTR1DD (PTR-5), *Prescottella equi* strain PTR2DD (PTR-6) from *P. thyrsiformis*. All the sequences were submitted to NCBI GenBank for accession numbers.

Some plant growth promotion activities, such as phosphate solubilisation, production of ammonia, indole-3-acetic acid (IAA), and salt tolerance ability of the isolates, were carried out. Seven of the sixteen isolates exhibited positive results in the phosphate solubilisation activity. The highest solubilisation index was observed in PTS-1 isolates, followed by PTR-6, GPR-5, PTR-5, GPL-3, PTS-2, and HAS-3. For ammonia production, almost all isolates showed positive results except PTS-1 and PTR-5. Eleven isolates out of 16 had shown positive results for their capacity to produce IAA in the presence of 400 µg/ml of L-tryptophan. The test was performed spectrophotometrically by comparing with standard IAA. Highest production was observed in HAR-5 (258.6 ± 2.05 µg/ml), which is 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), and PTS-1 (15 ± 0.81 µg/ml). A salt tolerance (1 to 10 % NaCl) ability test was also performed for the isolates. The highest tolerance to NaCl was observed in PTS-2, which is 10%, followed by GPS-4 (8%), HAS-3, GPL-1, GPL-2, and GPL-3 (7%). And all other isolates showed at least tolerance to 4% NaCl. Overall, all the isolates showed high tolerance to NaCl concentrations.

The ability of the isolates to produce extracellular enzymes such as lipase, cellulase, amylase, protease, pectinase, and xylanase was also evaluated using a standard protocol. Protease production was demonstrated by the majority of isolates, with the exception of PTL-4 and HAS-3. PTL-4, PTR-5, GPL-3, HAL-1, HAL-2, and HAS-3 showed positive for amylase production. HAL-1, HAL-2, HAR-5, GPL-1, GPL-2, GPS-4, PTL-4, and PTR-6 all showed positive lipase production. Positive activity for cellulase

production was demonstrated by PTS-1, PTR-6, GPL-2, GPL-3, GPR-5, HAL-1, and HAS-4. Xylanase production was shown by nine isolates, which include PTS-1, PTS-2, PTL-4, PTR-6, GPL-1, GPS-4, GPR-5, HAS-4, and HAR-5. For pectinase activity, PTS-1, PTS-2, PTR-6, GPL-1, GPL-2, HAL-1, HAS-3, and HAR-5 showed positive results.

The antimicrobial activity of the isolates was evaluated against six pathogenic bacteria: *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Enterobacter aerogenes* (MTCC 2822), *Klebsiella pneumoniae* (MTCC 109), and *Pseudomonas aeruginosa* (MTCC 1688). PTS-1 demonstrated broad-spectrum antimicrobial activity against most of the tested pathogens, excluding *K. pneumoniae*. Whereas, PTS-2 showed anti-microbial activity against *E. coli*, *B. subtilis* and *P. aeruginosa*. PTL-3 also selectively inhibited *S. aureus*, *B. subtilis*, and *P. aeruginosa*. GPL-1, GPL-2 GPS-4, and GPR-5 exhibited antibacterial activities against at least two pathogenic bacteria. Conversely, PTL-4, PTR-6, GPL-3, HAL-1, HAL-2, HAS-3, and HAR-5 demonstrated antibacterial activity against at least one of the six tested microorganisms.

The current study concludes that *Prescottella equi*, *Kocuria assamensis*, and *Alkalicoccobacillus gibsonii* are three of 16 bacteria that have been identified as endophytic bacteria for the first time. The first report on the isolation of endophytic bacteria from the medicinal plants *P. thyrsiformis* and *G. pentaphylla* is the second significant finding of our study. Phosphate solubilisation, IAA, and ammonia production are the three traits that PTS-2, GPL-3, and GPR-5 show positive in plant growth promotion activity. Ten of the sixteen isolates tested positive for at least two activities. In this study, all the isolates showed high tolerance to NaCl; these isolates can be a solution to salt stress management in agricultural systems. The extracellular enzyme activity of the isolates suggested that they could be used in industry to produce the enzymes on a large scale. In the antimicrobial activity, all the isolates from *P. thyrsiformis* and *G. pentaphylla* and HAL-1, HAL-2 and HAS-3 from *H. auriculata* showed antimicrobial activity against different pathogenic bacteria. Some of the isolates can be used in the production of antimicrobial drugs as well as in the production of functional biofertilisers to use in the agriculture system. However, more research in this area is needed to examine

both its toxicity and capacity to generate bioactive molecules. From these isolates, several new bioactive compounds may be discovered in future studies.