ABSTRACT

Rhizospheric bacteria were isolated from the rhizosphere of five legume plants viz., *Sesbania sesban, Leucaena leucocephala, Crotalaria junceae, Crotalaria pallida* and *Parkia roxburghii* growing in the four valley districts (Imphal East, Imphal West, Thoubal and Bishnupur) of Manipur. Root nodulating bacteria and endophytic bacteria were also isolated and characterized from the root nodules of four leguminous plants viz., *Sesbania sesban, Leucaena leucocephala, Crotalaria junceae* and *Crotalaria pallida* growing in the four valley districts. Twenty-six bacterial samples isolated from the rhizosphere using Burk,s nitrogen free media and twenty bacterial samples isolated from the root nodules using Yeast Extract Mannitol Agar medium were selected for further studies. These isolates were further studied for their morphological characters and biochemical characterization. Colony characteristic and motility were done for preliminary characterization of all the bacterial isolates. Gram staining was done for all the isolates following standard protocols. Several biochemical tests such as Catalase test, Starch hydrolysis test, Nitrate reduction test, Urease test, Oxidase test and Citrate test for all the selected bacterial isolates were performed to ascertain their biochemical characteristics. Antibiotics susceptibility was also determined against six antibiotics *viz.* Imipenem (IPM), oxacilin (OX), chloramphenicol (C), novobiocin (NV), ciproflocacin (CIP) and amoxyclav (AMC). Acetylene Reduction Assay was performed for all the bacterial isolates to determined their nitrogenase activity. Out the forty-six samples selected, thirty-five bacterial samples showed significant or some amount of of ARA (Acetylene Reduction Assay) or nitrogenase activity. The bacterial isolates were then screened for their ability to fix nitrogen by performing NifH gene amplification through Polymerase chain reaction (PCR). Considering the results obtained from ARA and NifH gene amplification, bacterial isolates were further selected for 16S rDNA gene amplification and sequencing for identification of the bacterial isolates. In few cases either of the two results was taken into consideration. Twelve bacterial isolates obtained from the rhizosphere and fourteen bacterial isolates obtained from the root nodules were selected for 16S rDNA analysis and sequencing. Phylogenetic analysis of the twelve bacterial isolates from rhizosphere confirmed their position as belonging to different organisms *viz., Bacillus spp., Enterobacter spp., Beijerinckia spp., Cedecea spp., Klebsiella spp.* and *Pseudomonas spp*. The sequences of all the above 12 strains were

deposited in GenBank under accession numbers: SS.T-1 (KM456220), SS.IW-3 (KM925076), CPS.B-1 (KM382276), CPS.B-2 (KM456219), CPS.IW-3 (KM925077), CPS.T-1 (KM925078), CJS.IE-1 (KM598638), LLS.T-1 (KM925075), LLS.IE(B)-1 (KM396262), PS.IE-2 (KM396263), PS.IW-1 (KM269071) and PS.T(B)-2 (KM598639). Phylogenetic analysis of the twelve bacterial isolates from rhizosphere confirmed their position as belonging to different organisms *viz. Mesorhizobium spp., Pseudomonas spp*., *Rhizobium spp., Pantoea spp., Bacillus spp., Bradyrhizobium spp., Enterobacter spp., Stenotrophomonas spp.* and *Cedecea spp.* The sequences of the said bacterial sequences deposited in GenBank along with their accession numbers are as follows: SN.T (KP331546), SN.IE-1 (KU355544), SN.B-1 (KX281718), CJN.T-1 (KP331547), CJN.B-1 (KU355542), CJN.IW-1 (KU355543), CJN.IE-2 (KX434625), CPN.B (KU935450), CPN.IW-1 (KU935451), CPN.T-1 (KU935452), CPN.T-4 (KU935453), LLN.B-1 (KU955582), LLN.T-1 (KU955583) and LLN.IE-1 (KX281719). From amongst the organisms obtained from both the sources, *Enterobacter cloacae, Beijerinckia fluminensis, Enterobacter asburiae, Bacillus subtilis, Enterobacter hormaechei, Bacillus altitudinis, Klebsiella oxytoca, Mesorhizobium huakuii, Pseudomonas azotoformans, Rhizobium huautlense, Stenotrophomonas maltophilia, Pantoea agglomerans, Bradyrhizobium japonicum* and *Mesorhizobium plurifarium* were identified as nitrogen fixers.