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Isolation and characterization of rhizobia from leguminous plants

S. H. Wijesundera* and A. P. Halmillawewa

Department of Microbiology, Faculty of Science, University of Kelaniya, Sri Lanka
sandushi.wijesundera@gmail.com

Rhizobia play a significant role in agriculture. Their ability to fix atmospheric nitrogen through formation of root nodules in leguminous plants contributes in increasing legume yield. The present study was carried out with the aim of isolating rhizobial strains from legume root nodules and characterizing them to check their potential to be developed into biofertilizers. Tests were performed to detect plant growth promoting traits such as phosphate solubilization, nitrate reduction, abiotic stress tolerance, which contribute towards sustainable agriculture.

Four rhizobial strains, namely C1-n1 (from *Vigna unguiculata*), P3-n1 (from *Arachis hypogaea*), L1-n1 (from *Vigna unguiculata* ssp. *Sesquipedalis*) and L2-n1 (from *Vigna unguiculata* ssp. *Sesquipedalis*) were isolated. They were characterized based on their morphological and biochemical features. Preliminary identification of isolates was done by observing their growth on yeast extract mannitol agar with congo red as well as their reactions to Glucose peptone agar test, Hofers' alkaline test, and Lactose agar test. The isolated strain L2-n1, was identified as a slow growing alkaline producing one according to its reaction on bromo thymol blue medium, while others were recognized as acid producing fast growers. This result was congruent with their growth rates as well.

All isolates gave positive results for oxidase, catalase, urease, and nitrate reduction tests and a negative result for indole, methyl red, gelatine hydrolysis and starch hydrolysis test. Two strains (L1-n1 and P3-n1) gave a positive reaction to Voges-Proskauer test, while only L2-n1 gave a positive result for citrate test. No isolate was able to exhibit swarming motility and phosphate solubilization. Furthermore, the capability of these organisms to utilize six different carbon sources and produce mucus colonies was also tested. The degree of viscosity produced was compared visually. Strains grew well at 25 °C–38 °C range, while L1-n1 and P3-n1 were able to tolerate a temperature range of 6 °C – 55 °C, pH range of 5.0 - 9.0 and NaCl levels up to 5% (w/v). C1-n1 was able to tolerate a pH range of 3.0 - 9.0 and NaCl levels up to 2.5% (w/v), while L2-n1 showed a growth only at neutral pH and a NaCl level of 0.01% (w/v). Also majority of strains were able to tolerate the heavy metals tested (Cu, Cd & Pb) at different concentrations. Strain L1-n1 showed an antibacterial activity against *Staphylococcus aureus*. All isolates formed nodules during the plant infectivity assays which were performed using their original plant host species confirming their identity as rhizobia. Plasmids were not observed in any of the isolates after performing the Eckhardt gel electrophoresis. According to the above results, these isolates may not be useful as biofertilizers, as they do not exhibit plant growth promoting traits satisfactorily. However further studies are required to determine their nodulation and nitrogen fixing efficiencies.

Keywords: Rhizobia, Biofertilizers, Legume root nodules