1130

ISOLATION AND TYPING OF NITROGEN FIXING BACTERIA ASSOCIATED WITH RICE

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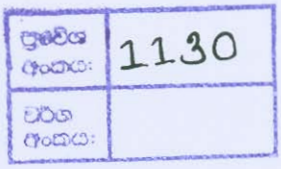

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ABSTRACT

A study was undertaken to isolate and characterize nitrogen fixing bacteria associated with rice with a long term goal of developing biofertilizer for rice cultivation. Rice varieties were sampled from RRDI, Bathalagoda. *In situ* nitrogenase activity of plant parts were determined by Acetylene Reduction Assay (ARA). Bacterial population sizes were determined by ARA based MPN technique. Nitrogen fixing potential of bacterial isolates was determined by ARA and *nif*H-PCR. All strains were genotyped using 16S rRNA-RFLP. Results showed that ARA activity of non-surface sterilized plant parts were higher and ranged from 8.85 to 157.46 nmol of C_2H_4 h^{-1} g^{-1} dry weight. Highest ARA activity (157.46 nmol of C_2H_4 h^{-1} g^{-1} dry weight) was observed in shoots of Sudu heenati traditional variety. MPN of non-surface sterilized plant parts ranged 10^5-10^7 CFU g^{-1} dry weight. MPN of the shoots were comparatively high. Surface sterilization reduced the MPN in all plant parts. Isolation gave a total of 163 strains. ARA activity of isolated strains ranged from $<0.17\times10^{-10}$ to 0.24×10^{-6} μ mol C_2H_2 $cfu^{-1}h^{-1}$. Highest ARA activity (0.11×10^{-6} μ mol C_2H_2 $cfu^{-1}h^{-1}$) was shown by a strain isolated from shoot of Sudu heenati variety. *nifH*-PCR gave expected band at 370 bp with all strains. 16S rRNA-RFLP of 159 strains, revealed 21 RFLP patterns and 40, 16S rRNA genotypes. Results indicate that roots and shoot surface of local rice varieties are populated with high numbers of active nitrogen fixing bacteria and are potential sites for colonization. Shoot was most successful site for colonization. Rhizoplane bacteria may be more prevalent in submerged plant parts. Presence of *nifH* amplicon in all isolated strains indicated their potential for fixing nitrogen from air. 16S rRNA-RFLP revealed existence of 40, 16S rRNA genotypes associated with tested varieties of rice.

Key words: Acetylene Reduction Assay, diasotrophs, N_2 – fixation, *nif*H, 16S rRNA –RFLP analysis