DIVERSIFICATION OF SOME FORAGE LEGUMES RHIZOBIA ISOLATED IN THAILAND

N. Teaumroong¹, K. Teamtaisong, A. Nantagij², P. Wadisirisuk², S. Kotepong² and N. Boonkerd¹

1 - School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima 30000

2 - Soil Microbiology Research Group, Soil Science Division, Department of Agriculture, Bangkok, Thailand

Atmospheric N_2 was able to be biologically fixed through forage legumes and provided not only enough N for their growth but also released into soil. Therefore, rhizobia varied in their effectiveness infixing N_2 to be more benefit from N_2 fixation, it is important to select high effective strains of forage legume rhizobia to inoculate seeds before planting. In this study effective rhizobia strains in four tropical forage legumes were selected according to their N₂-fixing efficiency and characterized in both terms genotypic and phenotypic. Soil samples from North and Northeast of Thailand were collected for isolating rhizobia by using forage legumes as trapping host plants. Desmanthus virgatus, Stylasanthes hamata, Chamaecrista rotundifolia, Centrosema pubescens and Centrocema pascuorum (cavalcade) were choosen as host plants for isolation of rhizobial strains from their nodules. The high efficiency of N₂ fixing strains were deteced by ARA assays. In order to investigate their physiological properties, colour reaction on YMA + BTB medium, IAA production, antibiotic resistance profiles and 19 different substrates utilization (APIXYM-test) were achieved, while to determine their genomic fingerprints, nif and nod genes were employed and RAPD-, REP-PCR for distinguishing the strains also be conducted. Characterization of 24 strains of D. virgatus rhizobial strains, the results indicated that most of them were fast-grower group while other plant hosts rhizobia comprised of both fast-grower and slow-grower. In addition by using antibiotic resistant it was cleary that most of strains were susceptible to erythromycin. Moreover, among these strains also found that neither α -monosidase nor α -fucosidase were produced from these strains. On the other hand, several enzymes involved in carbohydrate degradation were found such as α galactosidase, β -galactosidase, β -glucoronidase, α -glucosidase and N-acetyl-glucosamidase. Moreover, some S. hamata rhizobial strains exhibited the enzyme activities profiles with the same patterns as most bradyrhizobial strains. In case of D. virgatus rhizobia, one of them seemed to be the Rhizobium tropici and the rest were closely related in intraspecies level. This was confirmed by cross nodulation between rhizobial strains and plant hosts such as *Phaseolus vulgaris* and *D. virgatus* prior to detected with direct-nodule PCR approach. However, when distinguish the other plant hosts rhizobia by using random primers the results suggested that in each plant host, great diversity of rhizobia were found.

References

- 1. Boonkerd N, Promsiri S (1993) Kasetsart J. 27, 292-302.
- 2. Nuntagit A et al (1997) J. Gen. Appl. Microbiol. 43, 183-187.
- 3. Pankhurst CE (1977) Can. J. Microbiol. 23, 1026-1033.

Acknowledgement

This work was supported by NRCT-JSPS and SUT.

196