

NATTAPORN SUNTHORNVICHARANA : RESTRICTION FRAGMENT LENGTH POLYMORPHISM
(RFLP) IN NITROGEN FIXING BACTERIA Bradyrhizobium japonicum. THESIS
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The objective of this study was to classify Bradyrhizobium japonicum strains by RFLP which utilized southern blot hybridization with nif structural genes and common nod genes and to determine the relationship between the RFLP groups and their efficiency in nodulation and nitrogen fixation. Twenty three strains of B. japonicum were used in the RFLP analysis and tested on soybean SJ.5 cultivar. It was found that enzyme nitrogenase activities from nodules as measured by ARA of 23 strains were not significantly different. Nodulation by different strains were, however, different. Majority of strains formed 30-43 nodules per plant, some strains formed medium of 24-26 nodules only one strain formed the lowest number of 20 nodules per plant. It was also found that the 23 rhizobial strains formed two types of nodule pattern. The strains THA2, THA5 and USDA 117 formed nodules scatter on lateral root while the rest of 20 strains formed nodules mainly on tap root.

Restriction patterns using EcoRI, HindIII, PstI, BamHI could classify 23 strains into 9 groups. Among the 9 groups, strain USDA76, USDA94, USDA142 and TAL 432 were grouped separately; eg, USDA76 in group 6, USDA94 in group 7, USDA142 in group 8 and TAL 432 in group 9. Hybridization with nif probe on EcoRI and PstI digests one could group 23 strains into 4 and 5 groups respectively. However, when nod ABC and D probe was used to hybridize with total DNA digested with BamHI the rhizobial strains except USDA 35, USDA184, and TAL 377, could be classified into 8 groups.

From these results, no relationship between nitrogenase activity and RFLP grouping by nif HDK probe to the effectiveness in nitrogen fixation of rhizobial strain on SJ.5 soybean was shown. Furthermore, there was no relationship of RFLP grouping by nod ABC and D probe and nodulation detected. The probable explanation may due to the probe used were not sensitive to identify effectiveness in rhizobia.

It was found that there was relationship of grouping between restriction pattern and ELISA analysis. RFLP grouped by nod ABC and D probe were classified and results were more different than the one of nif HDK grouping ; however, they resulted to serological grouping by ELISA (Boonkerd,N.,et.al.,1989)