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Transposon Induced Nitrogenase in Rhizobium japonicum Infecting Vigna radiata

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The present study established a transposon mutagenesis procedure for Rhizobium japonicum forming symbiotic association with Mung bean (Vigna radiata). Suicide plasmid pko3 introduce the transposon Tn3 in to Rhizobium japonicum via Escherichia coli mediated conjugation and successfully generated 800 mutants with frequency of 3.7 × 10-6. These 800 mutants along with rifampicin resistant mutant and wild strain were screened for nodulation, which showed 100 mutants with pink coloured nodulation to mung bean plant. Out of 100 mutants 10 were promising for nitrogen fixation. The mutant AVR040 showed nitrogenase activity 12.4 µmol/h/mg fresh weight of nodule which is more than wild 9.61µmol/h/mg fresh weight of nodule, while AVR063 showed higher nitrogenase activity 19.4 µmol/h/mg fresh weight of nodule than AVR040.

Rhizobium, a soil inhabitant is a gram negative, motile, rod shaped bacterium that establishes a symbiotic association with the host legume by forming nodules on the surface of root. For the establishment and functioning of Rhizobium-legume symbiosis, coordinated expression of the several symbiotic genes of both partners is required.A transposon is a DNA fragment which can transpose from one site to another in the genome. One of the applications of the transposon is to mutate a gene (mutagenesis) and determine the physical location of genes of interest. Mung bean (*Vigna radiata*) is an important legume and a wellknown economic crop in tropical and subtropical countries. It is often included in crop rotation to replenish nitrogen and improve soil fertility. Mung bean is used in several food products. It is rich in vitamin and minerals which are necessary for human body.

Different species of *rhizobium* are able to nodulate and fix nitrogen with *Vigna radiata* such as *Bradyrhizobium japonicum*, *Bradyrhizobium liaoningense*, *Rhizobium fredii*, etc. To ensure an optimum rhizobial population in the rhizosphere, seed inoculation of legumes with an efficient rhizobial strain is necessary, this helps to improve nodulation, nitrogen fixation and yield of leguminous crops.The increased use of chemical fertilizers in agriculture, which constitutes the largest human interference in the nitrogen cycle, has prompted concerns regarding profound pollution impacts such as increased emissions of nitrogen oxides, soil acidification and water eutrophication. Fortunately the fixed nitrogen provided by biological nitrogen fixation is less prone to leaching and volatilization as it is utilized in situ, therefore this biological process contributes an important input for agriculture in a sustainable manner. The object of the present invention is to enhance nitrogen fixing ability of Rhizobium infecting *Vigna radiata* by transposon mutagenesis which will compensate in the requirement of chemical fertilizer.In the present study, slow growing rhizobium strain was isolated from root nodules of mung bean (*Vigna radiata*) and transposon Tn3 was used to generate mutants. It has been seen that there is increase in nodulation and nitrogen fixation of mung bean as compared with the parent strain.

Conclusion:

The present study concluded that the strain *Rhizobium japonicum* AVR (063)::Tn3 showed higher nitrogenase activity as compared with the other mutants and wild type. This strain also benefited growth of *Vigna radiata* with increase in nitrogen fixation, nodule number and plant biomass; thereby it has potential to be used as inoculants to mung bean plant.