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PHYLOGENETIC ANALYSIS OF GENETIC VARIABILITY AMONG ROOT NODULE BACTERIA IN DIFFERENT SPECIES OF LEGUMINOUS PLANTS

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Abstract

Biological N2 fixation by Rhizobium play major role in agricultural soils. The major N2-fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of low-N soils. Legumes such as peas, beans, lentils, soybeans, alfalfa and clover help to feed the meat-producing animals of the world as well as humans. Crop yields are greatly improved in modulated plants. Legumes can also grow well in poor soils where there is not enough fixed nitrogen to support other types of plants. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the next generation of plants. Since NIFH gene is one of the most conserved domains in root nodule bacteria especially Rhizobium species, for identifying molecular variability, it was used in this study. Total genomic DNA from collected samples were isolated, NIFH gene were amplified and digested using AluI restriction enzyme to identify the polymorphism. AluI are of first-rate and accomplish restriction enzyme digestion and unfailing restriction end nucleases.

Introduction

The **cowpea**, *Vigna unguiculata* (fig.1.) is an annual herbaceous legume from the genus *Vigna*. It requires very few inputs, as the plants root nodules are able to fix atmospheric nitrogen, making it a valuable crop for resource poor farmers and well-suited to intercropping with other crops. Adzuki bean (*Vigna angularis)* seed is an important source of protein, starch, mineral elements, and vitamins. In Chinese Medicine, adzuki beans have commonly been used to treat diuretic functions, and other disease such as dropsy and beriberi. Its medical application has been reported also in Korea (Yoshida *et al*., 2009). Given these characteristics, adzuki bean is widely used in a variety of foods (e.g., paste in pastries, desserts, cake, porridge, adzuki rice, jelly, adzuki milk, ice cream) for at least a billion people. Rhizobium spp. is unable to reproduce, once a functioning nodule is formed. (Prescott *et.al*, 1996). The presence of bacteria (Agrobacterium radiobacter) other than Rhizobium in root nodules was first reported by Sturz (Sturz *et al.*, 1997).

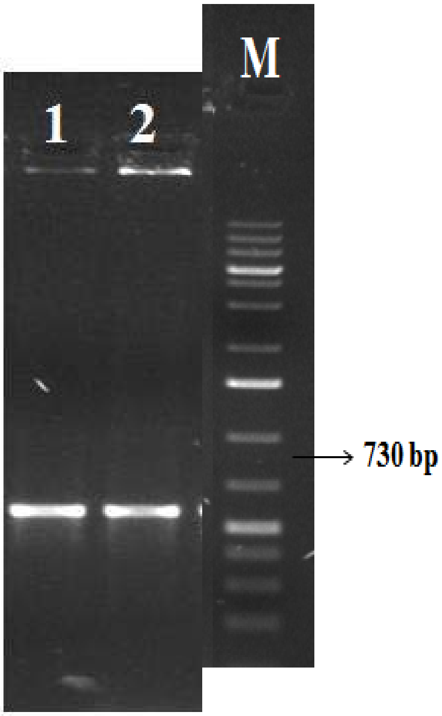
Aims and Objectives

1. To isolate and culture the bacteria from root nodules of different species of leguminous plants.
2. To isolate good quality genomic DNA from the bacteria isolated from root nodules.
3. To amplify the **NIFH** gene region of the bacterial DNA isolated from the root nodule.
4. To identify the bacterial species with the help of **NIFH gene** sequence

Methodology & Results

As part of the study, Root nodules from different pea species were collected from different fields to study the molecular variability of root nodule bacteria associated with them. Healthy nodules were selected, crushed and serially diluted and plated on Yeast extract mannitol agar plates. After 2 days of incubation at 280C, single pink colonies were selected from each plate (each root nodule), grown in Yeast extract mannitol broth, and total bacterial genomic DNA were isolated from each colony. From these isolated DNA, conserved region specific PCR was done to determine molecular level variation among the samples. Since NIFH is one of the most conserved domain in root nodule bacteria species, for identifying molecular variability, it was used in this study. Total genomic DNA from collected samples were isolated, NIFH gene were amplified and digested using AluI restriction enzyme to identify the polymorphism.

Gel image of PCR product for NIFH (730 bp) primer for variation analysis. Lane 1- Cowpea (*Vigna unguiculata*), lanes 2 adzuki bean (*Vigna angularis*)



The sequence obtained after sequencing PCR was used as a query sequence and subjected to a nucleotide blast search in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi)keeping default conditions. A request ID BZ144V0W016 was obtained for the same.. The query sequence showed an identity of 100%, Query coverage of 100% and an E value 0 to accession number: KC478681. Hence the query sequence was identified as ***Rhizobium leguminosarum.***

Conclusion

Thus, we can conclude that the most conserved domain i.e. NIFH gene is also showing similarity within species. It was observed that the sample have similarity even though it lies within the same group. Also, we can conclude that, by this similarity in NIFH gene, the atmospheric nitrogen fixation by these bacteria also same and the influence of this will affect the legumes also. As an assessment through this study, we can state that during the course of time, mutations or recombination’s happened in the conserved domain so that it is changed resulting in the observed polymorphism among these samples. Further investigation is necessary to study the extent of variation and to identify the presence of recombination events in these bacteria. It was observed that in samples 1, 2 bacterial DNA isolated from root nodules of cow pea and adzuki beans, only a single AluI site present as it yields only 2 bands after digestion. This indicates similarity within these samples. Even within pea species itself, this similarity is clearly seen.

References

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