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Short Communication

Selection and evaluation of *Rhizobial* strains of *Vigna radiata* L. beneficial to biological nitrogen fixation

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This study aimed to select suitable strains that can be used as inoculants to enhance legume production and simultaneously reduce the use of inorganic fertilizers. Evaluations of the selected strains of *Vigna radiata* on the basis of polysaccharide (exopolysaccharides and lipopolysaccharides) produced reinforced evidence that *Rhizobial* strains and its mutant nodulate promote growth differently under stressed environments. Strains performance evaluation demonstrated that the R0132 (1106):*Tn*5 inoculant of *Sinorhizobium fredii* (Formerly, *Rhizobium fredii*) is a prime candidate as a commercial inoculant. It benefited growth of *V. radiata* and it was more easily cultured on solid and liquid media than any of the other strains tested including R0132 (1112):*Tn*5, R0132 (0097):*Tn*5 and R0132 (1106):*Tn*5. Mutant R0132 (1106):*Tn*5 exhibited superior growth promoting ability under extreme environmental condition; therefore it has potential to be used in India.

Key words: *Rhizobial* mutant, biological N_2 fixation.

INTRODUCTION

The chemical agriculture though intensive has yielded fruitful results in terms of improving total productivity but over the years it adversely affected the environment. Overuse of chemical fertilizers has seriously deteriorated the soil health. An efficient fertilizer, capable of fixing atmospheric nitrogen, can play an important role in reducing chemical 'N' fertilizer. *Rhizobium* has long been regarded as an important microorganism because of its symbiotic nature and ability to fix nitrogen. *Rhizobium*-legume symbiosis is of great ecological and agricultural importance, being a major contributor to natural or biological N_2 fixation and allows legumes to grow in the absence of mineral or organic nitrogen in the soil and holds great promise for small holder farmers, since it is a component of foods.

The outcome of the current efforts on the molecular analysis of *Rhizobium* strain will be the construction of improved strain which can more successfully infect chosen legumes and contain the desired properties of promptness of nodulation in an alien-leguminous plant. A variety of nitrogen fixation. This indicates that nod genes of rhizobia reports exists with regard to infection, nodulation and involves in the production of lipopolysaccharides (LPS) and exopolysaccharides (EPS) or Nod factors are responsible for the establishment of symbiosis between *Rhizobium*-legume, located on pSym of *Rhizobium* (Giuntini et al., 2005). A compatible Nod factor is not the only requirement for effective nodulation. Bacterial cell surface components such as LPS, cyclic-β- glucans, EPS, capsular proteins, and K-antigens are also recognized by the plant, and help determine host specificity (Mathis et al., 2005).

This study shows that specific strain R0132 (1106):Tn5 of *Sinorhizobium fredii* can act as complimentary entophytes to Green gram or Mung bean, although they can depress or stimulate plant growth depending on environmental conditions (Selvaraj and Iyer, 1984).

MATERIALS AND METHODS

The certified seeds of *Vigna radiata* was procured from IARI, New Delhi. The undamaged clean and uniform seeds selected and surface sterilized by dipped the seed in 95 percent ethanol for a few seconds. After washing with sterile water, these were rinsed in 0.1

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Table 1. Correlation among nodule efficiency, extra cellular polysaccharide, Leghaemoglobin, soluble protein and nitrogenase activities of *Rhizobia* wild types and their mutants.

Isolate designation	Nodule efficiency			Extracellular Polysaccharides (µg/mg FW of CW)		Leghaemoglobin (µg/mg of nodules)	Soluble protein (µg/mg of nodules)	Tolerance			Antibiotic Resistance		ARA (µmol h^{-1} mg^{-1} FW of nodules)
	Nodule no.	Nodule weight (mg)						pH	Salt (mM)	Temp (°C)	Str^n	Kan^n	
		Fresh	Dry	EPS	LPS								
R0132 (Wild Type)	37	261.30	35.40	101.20 (M)	102.90	10.23	52.36	7.6	500	32	Str^{250}	-	3.21
R0132 (1106)::*Tn*5	41	293.63	38.32	150.00 (M)	72.30	18.23	53.20	7.6	300	40	Str^{250}	Kan^{50}	10.36
R0132 (0097)::*Tn*5	32	250.31	33.34	122.23	144.70	11.61	48.90	7.6	400	32	Str^{250}	Kan^{50}	6.44
R0132 (1112)::*Tn*5	42	281.25	39.00	141.30	88.40	18.47	51.28	7.6	750	32	Str^{250}	Kan^{50}	8.34

(M) = In presence of mannitol.

percent mercuric chloride and acidified with 5 ml/L concentrated HCI for about 2 min. Seeds were washed again several times with sterile distilled water and sown at different location of C.C.S. University, Meerut. The field was irrigated whenever required. The nodulation usually started about 9-10 days after emerging of the seedling.

Bacterial strains

Rhizobium strain designated as R0132, isolated from the root nodules of locally grown V. radiata plants.

Transposon (Tn5) mutagenesis (Mutagenesis of Rhizobium sp R0132)

Culture of *Escherichia coli* WA 803 (pGS9) was obtained from IARI New Delhi. Antibiotic was procured form sigma corporation for U.S.A and other media and chemicals were procured from this media private Limited. *Rhizobium* sp R0132 was grown to log phase in LB at 30°C, rpm 200. To raise WA 803, Kan 50 and Clm10 was added (subscripts indicate concentration in µg/ml). One ml each culture spun at 5000 rpm for 5 min and re-suspended in 1.0 ml 10 $MgSO_4$. Equal volumes (200 µl) of these two washed suspensions were mixed, centrifuged at 5000 rpm for 5 min and re-suspended in 100 µl, 10 mM, $MgSO_4$ this was spotted for patch-mating on LA surface and incubated for 8 h at 30°C. The cells were re-suspended later in 2.5 ml of 10 m M $MgSO_4$, plated on M9 medium with Kanamycin (50 µl/ml) and incubated at 30°C for 24 h. Individual R0132:*Tn*5 exconjugants streaked for analysis on HAM Agar.

RESULTS AND DISCUSSION

Regarding polysaccharides production, it has been observed that nodulation parameters showed the positive correlation with EPS and negative to LPS. Correlation has also been observed when the amount of EPS is compared to nodulation parameters. Mutant R0132 (1106):*Tn*5, and R0132 (1112):*Tn*5 secretes large amount of EPS 146.9 and 145.2, respectively, capable in formation of 41 and 42 nodule per plant with nodule fresh weight of 281.25 and 293.63 µg per mg nodule, respectively.

It has also been observed that mutant that produced smaller amount of EPS 82.2 and is capable in formation of only 22 nodules per plant shows that EPS, positively affected the nodulation as higher EPS producing mutants gave higher nodulation while lower range of EPS production mutant exhibited lower nodulation. Some isolates also showed negative correlation between amount of EPS and nodule number per plant such as that produce smaller amount of EPS 102.8 and 94.6 and were capable of formation of 32 and 38 nodules per plant, respectively.

It is observed that plant inoculated with mutant isolate R0132 (1106):*Tn*5 and R0132 (1112):Tn5 that produced large amount of exopolysaccharides, possess more nodular weight in comparison of plant inoculated with the other rhizobial mutant. Plants inoculated with mutant R0132 (1112):*Tn*5 produced higher EPS (145.2) and possess 293.63 mg nodule fresh weight per plant. On the other hands plant inoculated with R0132 (1106): *Tn*5, produced 146.9 EPS and possess 281.25 mg nodule fresh weight per plant.

Similarly, nodule dry weight also showed positive correlation with exopolysaccharides in these mutants. Whereas parent isolate R0132 showed significant variation. From the observation it has been clear that isolate R0132 (1106):*Tn*5 and R0132 (1112):*Tn*5 contributed to more nodule as well as dry weight. On other hand, it shows negative correlation of nodule efficiency with lipopolysaccharides producing mutants. As higher LPS production mutants showed lower nodule number and weight while lower LPS production mutants showed higher nodulation efficiency (Table 1).

REFERENCES

Giuntini E, Mengoni A, Bazzicalupo M, De Filippo C, Cavalieri D, Aubin-Harth N, Landry CR, Becker A (2005). Large-scale genetic variation ofthe symbiosis-required megaplasmid pSymA revealed by comparative genomic analysis of *Sinorhizobium*

meliloti natural strains. BMC Genomics, p. 6.
Mathis R, de Rycke R, D' Haeze W, Van Maelsaeke E, Anthonio E, Van Montagu M, Holsters M, Vereecke D, Van Gijsegem F (2005). Lipopolysaccharides as a communication signal for progression of legume endosymbiosis. Proceedings of the National Academy of Sciences of the United States of America, 102 (7): 2655-2660.
Selvaraj G, Iyer VN (1984). Transposon Tn5 specifies streptomycin resistance in *Rhizobium* spp. J. Bacteriol. 158(2): 580–589.