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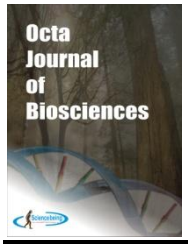

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Research Article

Isolation & Characterization of Rhizobia and their Effect on *Vigna radiata* Plant

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Abstract: *Rhizobia* is Gram negative bacteria that fix nitrogen , bacteria colonize plant cell with root nodules and commonly found in pulse . In present study rhizobia isolated from root nodules of vigna radiata and characterized morphologically, biochemical test were to as certain its physiology under normal conditions , three bacterial strain (Rp1 , Rp2, Rp3) were tested for their effect on root, Shoot and no. of nodules of vigna radiata plant in green house condition. Comparatively in all three strains Rp1 strain was found to most effective in positively Enhancing the growth of the plant in all parameters.

Key words: *Rhizobia*, bacteria, *Vigna radiate, Plant*

INTRODUCTION

Soil-inhabiting bacteria viz. Rhizobia that form symbiotic relationships with plant legumes species in root nodules. The bacteria fix nitrogen from the atmosphere to form ammonia, which is assimilated by the plant. The Rhizobia fix substantial quantities of nitrogen symbiotically between 80 to 150 kg N ha^{-1} in 90 days (Giller *et al.*, 1987 Toomsan, 1990). Kernel nitrogen is either directly derived from nitrogen fixation as indicated by a maximized acetylene reducing activity at pod filling (Williams *et al.*, 1990) or indirectly derived through metabolisation and translocation of plant nitrogen (Bray, 1983). A global inventory of the process of nitrogen for agriculture crop production indicated that biological nitrogen fixation is predominant; approximately 175 million metric tons per year of nitrogen (gaseous) is fixed biologically (Burns and Hardy, 1975). Brockwell and Bottomley (1995) concluded that particular N_2 fixation by legumes, is an ecologically efficient substitute for fertilization of crops and pasture with inorganic Nitrogen. Now a days rhizobial inoculant have some other quality with addition to nitrogen fixing capacity with enhanced nodulation, such as production of plant growth promoting hormones like Indole acetic acid (IAA), secretion of siderophores and solubilization of phosphates etc (Chakraborty and Purkayastha, 1984; Modi *et al.*, 1985; Halsall, 1993; Bashan and Holguin, 1997).

MATERIALS AND METHODS

Isolation &Chracterization of Rhizobia

Root nodules were collected from the young and healthy seedling of *Mung plant* from farmer's field at different location of Dehradun district, Uttranchal state. Mung plants were uprooted carefully so as to get intact are obtained. Initially, detached nodules were washed under running tap water to remove the adhering soil particles from nodule surface. Nodules were dipped in 0.1% of Mercuric Chloride ($HgCl_2$) solution for 30 seconds and later washed successively ten times with sterilized distilled water to remove the traces of toxic $HgCl_2$, surface sterilized nodules transferred in test tube containing 5ml of sterilized distilled water. These nodules crushed with the help of sterilized rod to obtained a milky suspension of bacteriods. These were streaked on Yeast Extract Mannitol Agar Media , and further identify by gram's staning method , and for characterization of bacteria all biochemical test were performed .

To study of rhizobial strain effect on vigna radiata plant

We performed pot experiments of plant in green house in sterile condition ,

Length and waight of root & shoot **:** we have measured root & shoot length in cm. and after taken dry weight of root & shoot we measured in g. unit

Root nodules:- Root nodules were counted in no. of nodules / strain

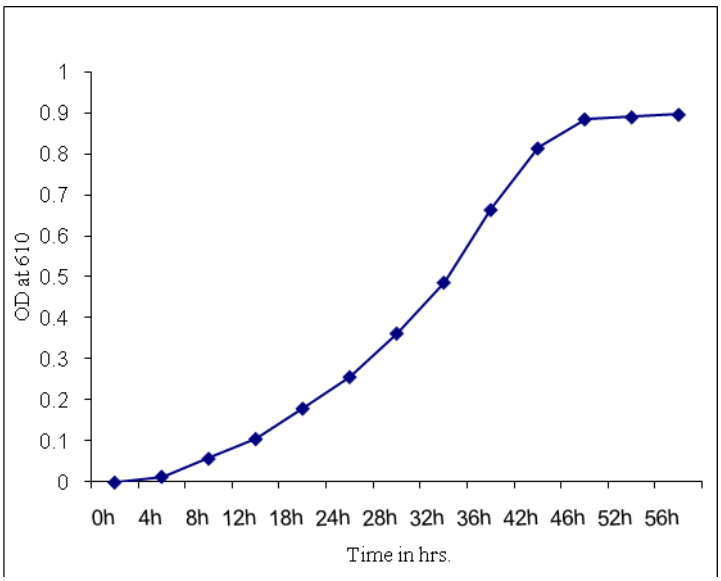
Root colonization study of Rhizobium bacteria :- Rhizobia bacteria isolate from crushed nodules of experimental plant and these milky suspension streaked on YEM agar media , rhizobial bacterial population has been count in colony forming unit (cfu) / strain of plant.

RESULTS

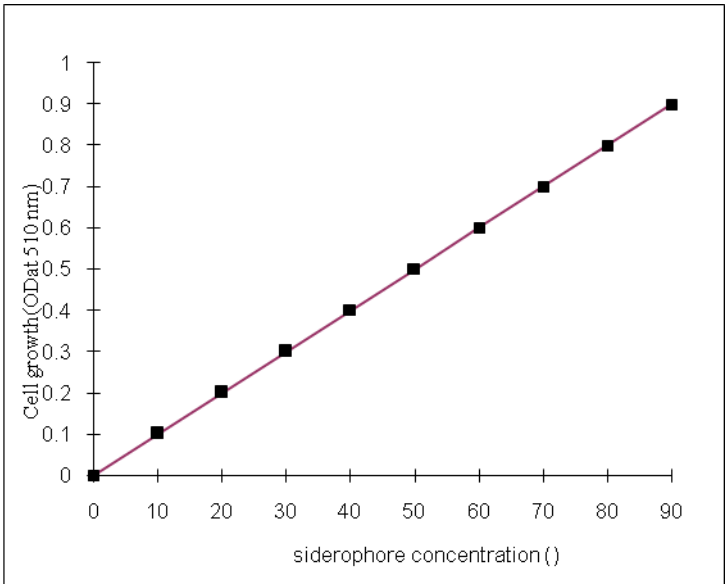
Isolation & Identification of bacteria:- Root nodulating bacteria were isolated from the nodule of *Mung* plant . all strains were Gram- negative, did not absorb red colour when cultured in YEMA containing congo red dye. We have performed biochemical test for characterization of rhizobia bacteria, all tested were found positive, the results of biochemical test shown in Table 1.

Effect of Rhizobial strain on plant:-We have measured effects of bacteria on plant after 10, 20 & 30 days respectively, in this duration we saw different changes in root, shoot, and in nodules of plants. We have taken three strain RP1, RP2 ,RP3 and one control. In first 10 days we have observed increase in the length of root and shoot , the no. of nodules also increased , in RP1 strain compare to other strains and control. After 20 & 30 days again RP1 strain showed good results rather than two strains and control also. Results shown in the Table- 5,6,7.

Colony formation in root of plant:-Mung plant show higher colonization of bacteria in roots .In this method plant treated by previous three strain at the regular interval of 10, 20 and 30 days. As after 20 days we were observed RP2 show lower colony forming strain and RP1 show higher colonization in root. Next in 30 days we measured results and data has been found in this order RP1< RP2< RP3< in bacterial population.



Graph 1 : Generation time of *Rhizobium* Strain



Graph 2: Standard curve of Cathacholic Sidrophore Production

Table 1: Characterization of Rhizobium strains isolated from Mung

Morphology	Rhizobium		
	RP1	RP2	RP3
Gram stain Morphology Biochemical activity	-ve Rods	-ve Rods	-ve Rods
Growth on GPA	-	-	-
Catalase activity	+	+	+
Urea hydrolysis	+	+	-
Growth in .4% Agar (Motility)	+	+	+
Acid reaction in litmus milk	-	-	-
Citrate utilization	-	-	-
Genration time (hr)	3.5	3.6	3.5
Growth in 8% KNO3	+	+	-
Gelatin hydrolysis	-	-	-
NO3 reduction	-	-	-
Oxidase activity	+	+	+
Ability to produce H2S Growth on HAM	-	-	-
Acid production	+	+	+
Starch hydrolysis	-	-	-

Table 2: Production of IAA HCN Siderophore and Phosphate solubilization by Rhizobium strains

Test	Rhizobium		
	RP1	RP2	RP3
IAA production	+++	++	+
HCN production	++	+	_
Ammonia Production	+	_	+
Siderophore Production	++	+	_
Phosphate Solubilization	+	+	_

Table 3: Carbon-Source utilization by *Rhizobium* spp.

C- Source	**Rhizobium**		
	RP1	**RP2**	**RP3**
Glucose	+	+	+
Mannitol	+	+	–
Sucrose	+	+	+
Lactose	+	+	+

Table 4: Nitrogen-Source utilization by *Rhizobium* spp.

C- Source	Rhizobium		
	RP1	RP2	RP3
Glucose+Yeast extract	+	+	+
Mannitol+Yeast extract	+	+	+
Sucrose+Yeast extract	+	+	+
Lactose+Yeast extract	+	+	+

Table 5: Effect of *Rhizobium* on plant growth of *Mung* after 10 days

Treatment	Length (C.m)	Length (C.m)	Dry Weight (Gm.)	Dry Weight (Gm.)	Nodules
	Root	Shoot	Root	Shoot	(No.)
Rhizobium RP-1	4.4	15.9	0.013	.027	38
Rhizobium RP-2	4.1	15.3	0.009	.025	35
Rhizobium RP-3	4.0	15.1	0.008	.023	34
Control	3.2	10.3	0.003	.014	0

Table 6: Effect of *Rhizobium* on plant growth of *Mung* after 20 days

Treatment	Length (C.m)	Length (C.m)	Dry Weight (Gm.)	Dry Weight (Gm.)	Nodules
	Root	Shoot	Root	Shoot	(No.)
Rhizobium RP-1	4.7	29.8	0.016	.039	63
Rhizobium RP-2	4.3	26.3	0.014	.037	62
Rhizobium RP-3	4.1	24.2	0.011	.035	58
Control	3.5	18.1	0.006	.025	0

Table 7: Effect of *Rhizobium* on plant growth of *Mung* after 30 days

Treatment	**Length (C.m)**	**Length (C.m)**	**Dry Weight (Gm.)**	**Dry Weight (Gm.)**	**Nodules**
	Root	Shoot	Root	Shoot	(No.)
Rhizobium RP-1	4.9	30.0	0.022	.049	124
Rhizobium RP-2	4.4	28.1	0.019	.047	113
Rhizobium RP-3	4.2	25.8	0.016	.045	100
Control	3.8	21.1	0.010	.035	0

Table 8 : Root colonization study of *Rhizobium* on the growth of plant after 20 days

Treatment	**Population (cfu ($x10^5$))**
Rhizobium + RP1+ Sterilized soil + Moong	**1.8**
Rhizobium + RP2+ Sterilized soil + Moong	**1.3**
Rhizobium + RP3+ Sterilized soil + Moong	**1.6**

Table 9 : Root colonization study of Rhizobium on the growth of plant after 30 days

Treatment	**Population (cfu ($x10^5$))**
Rhizobium + RP1+ Sterilized soil + Moong	2.5
Rhizobium + RP2+ Sterilized soil + Moong	2.2
Rhizobium + RP3+ Sterilized soil + Moong	2.1

DISCUSSION

All the *Rhizobium* strains were isolated from nodules of *Mung* strains RP-1, RP-2, RP-3 showed circular, pin head type small sized colonies on CRYEMA (Cango Red Yeast Extract Mannitol Agar), and secreted high mucilaginous compounds around the colonies as just mention else where (Arora et al., 2001; Deshwal et al., 2003). Such strains also showed that their generation time were always lower than 3.7 h as also evident by the characteristics of family of rhizobiaceae *Bergey's manual of determinative bacteriology* (Holt *el al*., 1994) for fast growing strains of *Rhizobium*. Elkan, (1992) reported that root nodulating bacteria have been differentiated on the basis of growth on defined substrate, as fast growers and slow growers and further, reported that fast growing bacteria have less than 6 h in selective broth medium. Similar observations have been reported by many researcher (EI-Sheikh and Wood, 1990; Carson *et al.*,2000; Arora *et al.*, 2001). CAS dye with $FeCl_3$ formed blue colour and formation of orange halo around the bacterial colony and decolouration of CAS assay solution occurred when supernatant added to it. It is due to removal of Fe from CAS indicator complex, confirmed that *Rhizobium* strains RP-1, RP-2 were positive for siderophore production. Similar reports were found by number of workers (Schwyn and Neilands, 1987; Carson *et al*., 1992; El Barraho *et al*., 1997; Arora *et al*., 2001*et al*.,). Suneja *et al*., (2000) observed that blue colour of CAS solution due binding of iron with CAS dye and when iron remove form CAS complex showed decolouration in *Rhizobium ciceri*. Such reports have given by Van Rossum *et al*., (1994) in *Bradyrhizobium* sp. and Carson *et al*., (2000)

Treatment of V. radiata seeds with rhizobacteria exhibiting ACC-deaminase activity significantly enhanced the root length (up to 50%) and number of roots (up to 47%), over water treated control(Ahmad et al., 2008) . In our study revealed with treatment seeds of plant with rhizobacteria promote the growth of root length(up to 49%).

CONCLUSION

This study has demonstrated the effect of rhizobia bacteria on given specimen plant and bacteria formed higher population on roots of plant . The rhizobium bacteria to have found plant growth promoting characters, which show positive result on growth of root, shoot length of plant and root surface colony forming activity.

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