International Journal of Applied and Natural Sciences(IJANS)
ISSN 2319-4014
Vol. 2, Issue 1, Feb 2013, 17-22
© IASET

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

COMPARATIVE VIABILITY OF TWO *RHIZOBIUM* STRAINS IN SOILS

M. A. SALEH

Microbiology Laboratory, Department of Genetic Engineering and Biotechnology,
University of Rajshahi, Rajshahi-6205, Bangladesh

ABSTRACT

Viability of two *Rhizobium* strains (Rvm307 and Rca220) in four different soil samples *i.e*. soils of Kakonhat, Rajshahi; Ishwardi, Pabna; Bogra and Rajshahi university campus were studied under sterile and natural condition. After introduction of *Rhizobium* in soil, the abundance of *Rhizobium* decreased gradually with the increased incubation period. The rate of decline of Rvm307 is relatively slow in comparison to Rca220. The survivality of *Rhizobium* was lower in natural soil sample than in sterile sample. In the soil of Bogra,both the strains showed the higher survival rate than other three samples and the strain Rvm307 showed better performance than other strain in all the conditions,.

KEYWORDS: Natural Soil, *Rhizobium*, Sterile Soil, Viability

INTRODUCTION

Rhizobia are economically important soil bacteria. Bioinoculant of efficient, effective and competent *Rhizobium* stains are often used as bio-fertilizer for maintaining soil fertility (Subba Rao, 1977). A suitable inoculam strain must be able to survive under a wide range of field condition (Vincent, 1965). Soil is a complex matrix that is difficult to manipulate to control environmental factors and to prefect interaction with the indigenous microbial community (Young & Burns, 1993).Soil type affect the ability of introduced organisms to colonize the rhizosphere or root soil interface (Kluepfel, 1993).Survival of *Rhizobium* probably related to soil quality, a term used to define the physical, chemical and biological factors in soil related to fertility and sustainable production (Parker et al., 1977). Bangladeshi soils show great variant in their quality. Depending on the agro ecological variation of soils, the land of Bangladesh is divided into 25 agro ecological zones (Saheed,1992).Because of variation in nutrient status, the viability of *Rhizobium* in soil of different areas of Bangladesh may be variable. So, investigation should be made to be sure and to detect suitable nutrient status for the viability of *Rhizobium.*

MATERIALS AND METHODS

Two *Rhizobium* strains Rvm307 and Rca220 were collected from the soil science division of Bangladesh Agricultural Research Institute (BARI),Joydevpur, Gazipur. Four different soil samples were also used in the present investigation. The samples were collected from different parts of Bangladesh. One was collected from Rajshahi University campas and others from Kakonhat, Rajshahi; Bogra and Ishwardi, Pabna. The soil samples were also analyzed in the laboratory of Soil Research Development Institute (SRDI) with the kind help of scientific officer and lab attendant. The samples were first air dried separately for one week to prepare them for analysis. Visible waste was removed from the soil samples and discarded.

The soil samples were passed through the grinder and subsequently, a 2mm stainless steel sieve. The ground sieved soil samples were transferred to a plastic container with screw cap and then labeled properly .The soil samples, subsequently were used for analysis of pH and nutrient contents of soil by the methods directed by SRDI. For one strain, the four soil samples were taken separately in 20 gm quantity in 8 petridishes (two for each) and labeled properly. Among

them, four samples (one for each) were sterilized in an autoclave (121^0C, 20 minutes). Other four petridishes were remaining natural. This procedure was repeated for another strain. Sterility was tested by plating on nutrient agar.

The inoculum added to these samples was grown at 29^0C in YEM broth on a rotary shaker operating 75 rpm and the cells in the late logarithmic phase were collected by centrifugation at 7000 rpm for 20 minutes and the biomass resuspended in sterile saline to get desired density of cells. The sterile soil samples were mixed with 10 ml *Rhizobial* suspension with sterile glass rod. Inoculated soil sample were incubated at 28^0C. At regular time interval (5 days) approximately 1gm soil from each treatment was subjected to viable count on LB agar medium containing 100 µg/ml Penicillin (for Rvm307) and 250 µg /ml gentamycin (for Rca220) by drop plate method of Miles and Misra (1938).

RESULTS

The result of the soil samples analysis are presented in table1.From that table it has been that,the soil of Bogra is the most rich in nutrient content among the soil samples because out of 10 analyzed nutrients five(Ca,P,K,Fe & S) are present in highest amount in that sample. On the other hand, the soil of University Campus is the poorest in nutrient contents because, out of 10 analyzed nutrients five (Mg, P, Cu, Fe, Zn) are present in the lowest amount in that sample.

Table 1: Chemical Analysis of Four Soil Samples

Constituents		Soil Samples			
		Kakonhat, Rajshahi	Bogra	Ishwardi, Pabna	University, Campus
pH		5·3	5·7	8·1	8·5
Total nitrogen (%)		0·07	0·19	0·09	0·05
Mili equivalent per 100gm	Calcium	1·6	2·5	28·5	13·3
	Magnesium	0·8	0·5	1·6	0·38
	Potassium	0·47	o·40	0·35	0·44
Microgram per gram soil	Phosphorus	30	82·6	41·9	10·2
	Sulfur	24	27·3	1·4	14·3
	Copper	2·1	1·43	2·48	0·18
	Iron	21	192·9	136	20·1
	Manganese	13·6	3·1	26·3	8·4
	Zinc	3·99	1·13	2	0·53

In the present investigation, the population of *Rhizobium* in four different soil samples at different incubation periods were counted. It was observed that,the rate of survivality decreased gradually with the increased incubation period.

The population of *Rhizobium* was maintained very well up to 35 days. The highest no. of *Rhizobial* population was observed in sterile soil of Bogra and it was 12×10^9 cfu/g and 8×10^9 cfu/g of soil for Rvm307 and Rca220 respectively after 5 days of incubation.For natural soil sample, it was 9×10^9 cfu/g and 6×10^9 cfu/g of soil.

After 35 days of incubation,the lower survival rate was observed in Rajshahi university campus soil sample.In that case, it was17×10^6 cfu/g and 11×10^6 cfu/g in sterile soil sample and 10×10^6 cfu/g and 9×10^6 cfu/g in natural soil sample for Rvm307 and Rca220 respectively.

From the figure 1 and 2,it was also observed that the viability of *Rhizobium* was more in sterile soil sample than natural soil sample and Rvm307 showed better performance in all conditions.

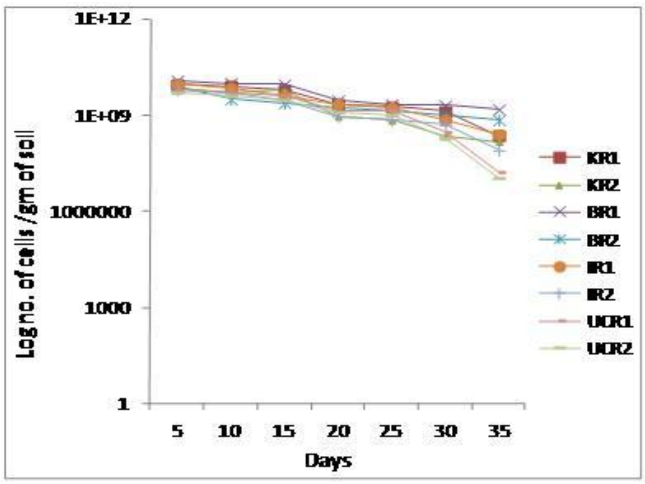

Figure 1: Population of *Rhizobium* in Sterile Soil Sample (where R1 and R2 indicates Rvm307 and Rca220 Respectively & K, B, I and UC Indicates Soil of Kakonhat, Bogra, Ishwardi and University Campus)

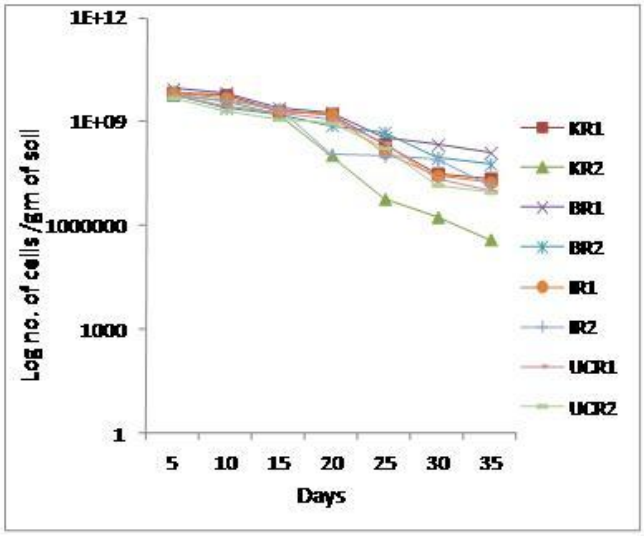

Figure 2: Population of *Rhizobium* in Natural Soil Sample (where R1 and R2 indicates Rvm307 and Rca220 Respectively & K, B, I and UC Indicates Soil of Kakonhat, Bogra, Ishwardi and University Campus)

DISCUSSIONS

Soil reaction is the most important characteristics influencing the physical and chemical properties of soil. Plant growth and micro organisms activity depend upon soil reaction and possible condition of the soil *i,e.* soil acidity, neutrality and alkalinity. Soil management practice, which build up organic matter content and arrest pH decline e.g. limiting are likely to create soil condition that encourage survival, persistence and higher population of *Rhizobium* in soil.The survivality of *Rhizobium* depends on their ability to complete favourly with indigenous soil *Rhizobia* and subsequently from a large proportion of nodule (Elkins et al,.1976)

Some strains of a species of *Rhizobia* survive better than others in soils. In the present investigation, the strain Rvm307 always showed better survivality than Rca220 which is consistent with the results of Bromfield and Jones (1980).The moisture condition influences the survival of microorganism in soil (Heldin and Newton, 1948). The counts of bacteria in soil are closely related to the moisture content. Through survival of the organisms has been reported by several

workers to be quite prolonged (Richmond, 1926).In the present investigation, it was seen that with the increased incubation period there was decrease in the moisture content which was accompanied by decreased count of the *Rhizobial* population.

Each soil system has its own distinctive biological space with regard the level of microbial biomass and enzyme activity (Nannipieri et al., 1983) and bacteria introduced into sterilized soil reach a certain population level independent of inoculums density (Postma et al.,1990). It was therefore suggested that the final population size in sterilized soil represent the capacity of the soil in terms of available habitable space, moisture and substrate for maintenance of the bacteria. In this experiment, the survivality of *Rhizobium* was clearly affected by soil quality. Survivality of *Rhizobium* was higher in soil samples of Bogra and Kakonhat, Rajshahi, than Ishwardi, Pabna and University Campus soil sample. The differences in survivality could not be explained by the biological space alone, nutritional factors might be also responsible.

Soil sample of Bogra was very rich in certain nutrient such as phosphorus and iron which are known to stimulate the *Rhizobial* growth (Subba Rao, 1999). This might be the case of higher survivality of *Rhizobium* in that sample. Although the soil sample of Kakonhat, Rajshahi was also rich in nutrient content, the lower survivality of the strains might be due to toxicity of higher manganese contents in acidic pH (Blamey et al., 1983;).The toxicity of manganese can be overcomed by higher calcium content (Rios et al., 1968) but the soil of Kakonhat, Rajshahi was also deficient in calcium content. The calcium deficient cells become swollen and vacuolated. The highest manganese content was found in soil sample of Ishwardi, Pabna but the higher calcium content might overcome its toxicity. The lowest survivality of *Rhizobium* in soil sample of University Campus was probably because of its poor nutrient contents.

Survivality of the *Rhizobium* was lower in natural soil sample than in sterile soil sample. Competetion with more efficient indigeneous strains for nutrient and space might be an important cause of lower survivality of strain in natural soil sample than in sterile sample (Saha and Haque,2002). Predation might be another factor which decreased the survivality of *Rhizobium* in natural soil sample. Fungicides, herbicides and other plant protectants might have toxic effect to *Rhizobia.* The inhibitory or stimulatory effects of soil micro organisms such as bacteria, fungi and actinomycetes on *Rhizobium* are known. It was reported that the survivality of *Rhizobium leguminosarum* in natural soil was greatly affected by certain protozoa, fungi and bacteriophage (Chonkar and Subba Rao, 1966; Subba Rao, 1999).

ACKNOWLEDGEMENTS

The author express his sincere gratitude to the ministry of science and technology,the people's Republic of Bangladesh for financial support.

REFERENCES

1. Blamey, P.F.C., Edwards,D.G. and Asher,C.J.1983.Effects of aluminium, OH: Al and P:Al molar ratios and ionic strength on soyabean root elongation in solution culture. Soil Sci.136: 197-207.
2. Bromfield, E.S.P., and D.H.Jones. 1980. Studies on acid tolerance of Rhizobium trifolii in culture and soil.J.Appl.Bacteriol.48:253-264.
3. Chonkar,P.K.and Subba Rao, N.S.1966.Fungi associated with legumes root nodules and their effect on rhizobia. Canad.J.Microbial.58: 71-76.
4. Elkins, D.M, Hamilton G.,Chan C.K.Y, Briskovich M.A.& Vandeventer, J.W.1976. Effect of cropping history on soyabean growth and nodulation.Soil Rhizobia Agronomy Journal, 68:513-517.

5. Hedlin, R.A. and Newton, S.D.1948. Some factors influencing the growth and survival of Rhizobium in humans and soil cultures.Canad.J.Res.26: 174-186.
6. Kluepfel,D.A.1993. The behavior and tracking of bacteria in the rhizosphere.Annual Review of phytopathology.31:441-472.
7. Miles,A.A. and Misra,S.S.1938.The eastimation of the batericidal power of Blood.J.Hyg.Camb.38:732
8. Nannipieri,P.,Muccini,L. and Ciardi,C.1983.Microbial biomass and enzyme activites:production and persistence. Soil. Biol.Biochem.15: 679-685.
9. Parker,C.A., Trinick, M.J. and Chatel, D.L.1977.Rhizobia and rhizosphere in habitatns.In:A Treatise on dinitrogen fixation.(Eds. Hardy, R.and Gibson, A.H.)Wiley, New York.p.311.
10. Postma, J., Hok-A-Hin, and Oudo Vashaar, J.H.1990. Influence of the inoculum density on the growth and survival of Rhizobium leguminosarum biovor trifolii introduced into sterile and nonsterile loamy sand and silt loam. FEMS. Microbio.Ecol .73: 49-58.
11. Richmond, T.E.1926. Longivity of the legume nodule organism.J.Amer. Soc. Sic, 4 : 414-416.
12. Rios, V., Martini, A. and Tejeira, R.1968. Efecto del encalado sobre la acidez y el contenido de aluminio y hierro extraible en nueve suelos de Panama.Turrialba . 18,139-146.
13. Saha,A.K and Haque,M.F.2002.Effect of soil quality on the viability of GFP marked Rhizobium.Univ.J.Zool. Rajshahi Univ.Vol.21.pp.59-61
14. Saheed,S.M.1992.Use of soil resources data for agricultural development planning in Bangladesh.BPATC,Savar,Dhaka. Subba Rao, N.S.1977.Biofertilizer in agriculture.2nd edition, Oxford and IBH Publishing Co. New Delhi.p.208
15. Subba Rao, N.S.1999.Rhizobium and legume root nodulation .In:Soil Microbiology .Oxford and Publishing Co.Pvt.Ltd.New Delhi.p.407
16. Vincent,J.M.1965.Environmental factors in the fixation of nitrogen by the legume.Soil nitrogen(Agronomy series monograph no.10)American society of Agronomy.p.435
17. Young,C.S. and Burns,R.G. 1993. Detection, Survival and activity of bacteria added to soil. In:Soil Biochemistry. Marcel Dekker Inc.New York.p.263