International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Isolation of Temperature and Acid Tolerant Strains of *Rhizobium* sp. on Pulse Crops

Uma Sankareswari R.[1], K. Ilamurugu[2]

[1]Assistant Professor, Department of Agricultural Microbiology, AC&RI, Madurai, TamilNadu, India

[2]Professor, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-3

Abstract: ***The present experiments were carried out during 2004 - 2007 at Dept. of Agrl. Microbiology, Tamil Nadu Agricultural University, Coimbatore. The UV mutants of five Rhizobium isolates were screened according to high survival percentage calculated after 5 min exposure. Further it was subjected to high temperature (35, 40, 45 and 50°C) exposure and acid pH (5.5 and 6.0) and its survivability was estimated. The best performing nitrogen fixing Rhizobium strains viz., CO 5, COG 15, TNAU 14, COS 1 and CRR 6 were selected for the development of temperature and acid tolerance study. In order to know the variation, these strains were subjected to UV irradiation treatment for 5 – 60 minutes which resulted a total of 51 mutants. Among them, five mutants (MB1, MG1, MS1, MC1 and MGn1) were selected and subjected to temperature (35 to 50°C) and pH (5.5 and 6.0) stress and 40 isolates were obtained and its survivability of CRR 6 Rhizobium strain was found to be increased initially (0.40% for 5 min) and then decreased gradually (0.16% at 60 minutes) which was followed by other strain of Rhizobium. The population (8.21 to 8.36 log $_{10}$cfu ml^{-1}) of UV mutant MB1 was increased to significant level for 24 – 72 h of incubation at 35°C and pH 5.5. When the temperature increased from 35 to 45°C, there was a gradual decline in the population level (8.21 to 7.72 log $_{10}$cfu ml^{-1}) at pH 5.5. At 50°C, the population was increased (7.30 log $_{10}$cfu ml^{-1} to 7.60 log $_{10}$cfu ml^{-1})upto 48 h of incubation beyond which the multiplication of rhizobia reduced further.***

Keywords: UV mutants, Rhizobium, survival percentage, high temperature, acid pH

1. Introduction

In scientific and intensive agriculture, *Rhizobium* spp. play a vital role because of their ability to fix atmospheric nitrogen in symbiosis with leguminous plants and this contributes to the nourishment of the plants during their life period and after their death, they enrich the soil with combined formation of nitrogen and promoting the nitrogen fixing nodules. Today, the need for effective utilization of this phenomenon is increasingly felt as it substitutes the costly commercial nitrogenous fertilizers. For the successful exploitation of this phenomenon, the legume rhizobia symbiosis must be efficient [17].

Nitrogen fixation depends on the physiological state of the host plant [25] and on the effectiveness and environmental fitness of the microsymbiont as well as on the interaction between the two partners. The nodule formation and nitrogen fixation of the symbiotic process are affected by stress conditions which might be considered as limiting factors. The most important factors are pH stress and temperature is considered as prime factors because it limits nodulation and nitrogen fixation and thus limiting rhizobial survival and persistence in soil. Thereby the exchange of molecular signals between rhizobia and their host was affected and reduced the nodulation process [9]. [12] reported that the high soil temperatures in tropical and subtropical areas are a major problem for biological nitrogen fixation of legume crops. High root temperatures strongly affect bacterial infection and nitrogen fixation in several legume species, including soybean, guar, peanut, cowpea and beans. Critical temperatures for nitrogen fixation are 30°C for clover and pea, for soybean, guar, peanut and cowpea; it ranges between 35 – 40°C.

Thus *invitro* evaluation of strains under stress might be a useful method in finding rhizobial isolates adapted to different environments, where extreme temperatures and pH limit symbiotic nitrogen fixation [19].

In the present study, the effect of stress (temperature and pH) was evaluated on rhizobial strains to identify the temperature and acid tolerant *Rhizobium* strains suitable to Semi-Arid Tropics regions (SAT).

2. Materials and Methods

Rhizobial cultures

The standard rhizobial cultures used in this study were COS 1, CRR 6, COG 15, TNAU 14 and CO 5 were obtained from the culture collection center of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.

Maintenance of rhizobial strains

The standard *Rhizobium* strains were maintained on yeast extract mannitol agar (Annexure I) slants at 4ºC (Vincent, 1970). Yeast extract mannitol broth was inoculated with the rhizobial strains and incubated at 28ºC (pH 6.8 –7.0) for 3 days in an orbital shaker and this culture was used for the study of development of temperature and acid tolerant strains. The details were given below in Table 1.

Table 1: Details of rhizobial strains

S. No	Rhizobial strains	Strain no.	Host species	Source
1.	*Bradyrhizobium* sp.	COS 1	*Glycine max*	Dept.of Agricultural Microbiology, TNAU, Coimbatore.
2.	*Rhizobium* sp.	CRR 6	*Vignaunguiculata*	
3.	*Rhizobium* sp.	COG 15	*Vignaradiata*	
4.	*Rhizobium* sp.	TNAU 14	*Arachis hypogea*	
5.	*Rhizobium* sp.	CO 5	*Vigna mungo*	

Mutagenesis by UV irradiation

Five standard rhizobial cultures were taken and exposed to UV radiation in laminar air flow chamber (2600 A°) for time periods varying from 5 min to 60 min. Samples were collected at 5 min interval. For each culture, control (without UV radiation exposure) was maintained aside in the dark.

After given UV radiation, they were kept in dark for stabilization of thymine – thymine (T -T) dimers. A 0.1 ml of the UV treated bacterial suspension was then inoculated in 25 ml petri plates containing YEM media. These were incubated for 48 h at 28°C for colony formation and the colony count was recorded. The survival percentage (%) was calculated by using the given formula.

$$\text{Survival (\%)} = \frac{\text{100 x Colony count obtained for time (t)}}{\text{Colony count obtained for time (t) without UV radiation exposure}}$$

Method of high temperature exposure and acid pH

UV mutants of five *Rhizobium* isolates were screened according to high survival percentage calculated after 5 min exposure. Further it was subjected to high temperature (35, 40, 45 and 50°C) exposure and acid pH (5.5 and 6.0) and its survivability was estimated. The procedure was given below.

1) Taken five UV mutant *Rhizobium* isolates inoculated into test tube containing 10 ml of yeast extract mannitol broth (YEM) maintained at two different pH 5.5 and 6.0 using 0.1 N HCl.
2) Then it was incubated at high temperature exposure (35, 40, 45 and 50°C) maintained in an incubator for a period of 3 – 4 days compared with the control (culture maintained at 28°C).
3) After the turbidity of the isolates had noticed, taken 0.1 ml of bacterial suspension for every 24h interval upto 96h, then inoculated in petriplates containing YEM media and kept for incubation at room temperature for colony formation. Population count was recorded and expressed as the logarithm of the number of viable cells g^{-1} and the data were analyzed statistically.
4) Single colonies were picked up from each plate incubated at high temperatures (35 to 50ºC) and subcultured in YEM broth for 4 days. These isolates were transferred to YEM agar slants and stored at 4ºC, then used for further studies.

By adopting the appropriate procedure, 40 isolates were obtained at particular higher temperature exposure (35 to 50ºC) and pH (5.5 – 6.0) and the isolates were named accordingly as given in table below.

First alphabet S – soybean, G – green gram, B – blackgram, Gn – groundnut and C – cowpea
Second alphabet a – 35°C, b – 40°C, c- 45°C and d - 50°C
Third alphabet p1 – pH 5.5 and p2 - pH 6.0

Table 2: Details of isolates obtained

S. No	UV mutant strain	Temperature	pH	No. of isolates	Name designated
1.	MS1	35	5.5	1	Sap1
		35	6.0	1	Sap2
		40	5.5	1	Sbp1
		40	6.0	1	Sbp2
		45	5.5	1	Scp1
		45	6.0	1	Scp2
		50	5.5	1	Sdp1
		50	6.0	1	Sdp2
2.	MG1	35	5.5	1	Gap1
		35	6.0	1	Gap2
		40	5.5	1	Gbp1
		40	6.0	1	Gbp2
		45	5.5	1	Gcp1
		45	6.0	1	Gcp2
		50	5.5	1	Gdp1
		50	6.0	1	Gdp2
3.	MB1	35	5.5	1	Bap1
		35	6.0	1	Bap2
		40	5.5	1	Bbp1
		40	6.0	1	Bbp2
		45	5.5	1	Bcp1
		45	6.0	1	Bcp2
		50	5.5	1	Bdp1
		50	6.0	1	Bdp2
4.	MGn1	35	5.5	1	Gnap1
		35	6.0	1	Gnap2
		40	5.5	1	Gnbp1
		40	6.0	1	Gnbp2
		45	5.5	1	Gncp1
		45	6.0	1	Gncp2
		50	5.5	1	Gndp1
		50	6.0	1	Gndp2
5.	MC1	35	5.5	1	Cap1
		35	6.0	1	Cap2
		40	5.5	1	Cbp1
		40	6.0	1	Cbp2
		45	5.5	1	Ccp1
		45	6.0	1	Ccp2
		50	5.5	1	Cdp1
		50	6.0	1	Cdp2
Total no. of isolates		40			

3. Results and Discussion

Exposure of rhizobial strains to UV irradiation Mutation studies

The five rhizobial strains (CO 5, COG 15, TNAU 14, COS 1 and CRR 6) were subjected to ultraviolet irradiation (UV) treatment for 5 – 60 min to obtain clones of rhizobia. The strains *viz*., COS 1, CRR 6 and CO 5 could able to adapt to UV treatment upto maximum of 60 min whereas TNAU 14 and COG 15 could adapt only upto 35 and 40 min, respectively. There was a gradual decline in survival of rhizobia when compared to the control which were not subjected to treatment. One clone was randomly selected from each UV exposure and was named according to the host species. As a result, 51 mutants were obtained after UV treatment.The present data revealed that long exposure of UV irradiation on five rhizobial strains (COS 1, CO 5, COG 15, TNAU 14 and CRR 6) for 5 to 60 min interval, recorded a decrease in rhizobial population when compared to the control (not subjected to UV irrradiation) and corroborated with the earlier findings of [11], who reported that the population of cells must be permitted to undergo a limited

number of cell divisions on agar plates and in the conditions must be changed so that the mutant clones can continue growing to form visible colonies.The present results coincide with the findings of [8] who reported the effect of exposure of bacterial suspensions to UV radiation. The sensitivity to UV radiation of several microorganisms of different habitats (*Rhizobium meliloti, Rhodobacter sphaeroides, Escherichia coli,* and *Deinococcus radiodurans*) had been studied by them and the results revealed that *D. radiodurans* was an extremely resistant bacterium, *Rhizobium meliloti* was more resistant than *R. sphaeroides*, and *E. coli* was the most sensitive bacterium tested.

Survival percentage

The survival percentage of CRR 6 *Rhizobium* strain was found to be increased initially (0.40% for 5 min) and then decreased gradually (0.16% at 60 minutes) which was followed by COS 1 and CO 5 (0.43 to 0.11%; 0.43 to 0.07%) whereas the survival percentage of COG 15 and TNAU 14 was determined as 0.43 and 0.37% for 5 min treatment (Table 3).

Based on survival percentage, isolates exposed for a period of 5 min to UV irradiation were selected as mutants (MB 1, MG 1, MGn 1, MS 1 and MC 1) to carry out further experiments.

Table 3: Survival percentage of Rhizobium strains after UV exposure

S.No.	Rhizobial strains	Survival percentage (%)												
		Time interval (min)												
		0	5	10	15	20	25	30	35	40	45	50	55	60
1.	COS 1	100	0.43	0.43	0.34	0.29	0.26	0.23	0.20	0.20	0.17	0.17	0.14	0.11
2.	CRR 6	100	0.40	0.40	0.35	0.29	0.27	0.24	0.24	0.24	0.21	0.21	0.19	0.16
3.	TNAU 14	100	0.37	0.37	0.34	0.31	0.26	0.24	0.24	0.21	-	-	-	-
4.	COG 15	100	0.43	0.37	0.34	0.28	0.28	0.25	0.22	-	-	-	-	-
5.	CO 5	100	0.43	0.36	0.36	0.32	0.32	0.29	0.29	0.25	0.22	0.18	0.09	0.07

Influence of UV mutant *Rhizobium* strains exposed to temperature and acidic stress

UV mutant MB 1 was exposed to a range of temperature *viz*., 35, 40, 45 and 50°C and pH of 5.5 and 6.0 for 24 – 96 h of incubation to know their tolerance limit. The results showed that the population (8.21 to 8.36 log $_{10}$cfu ml^{-1}) of UV mutant MB1 was increased to significant level for 24 – 72 h of incubation at 35°C and pH 5.5. When the temperature increased from 35 to 45°C, there was a gradual decline in the population level (8.21 to 7.72 log $_{10}$cfu ml^{-1}) at pH 5.5. At 50°C, the population was increased (7.30 log $_{10}$cfu ml^{-1} to 7.60 log $_{10}$cfu ml^{-1}) upto 48 h of incubation beyond which the multiplication of rhizobia reduced further. The interaction between the hours of incubation, temperature and pH had significant effect on the survival of rhizobial mutants (Fig 1).

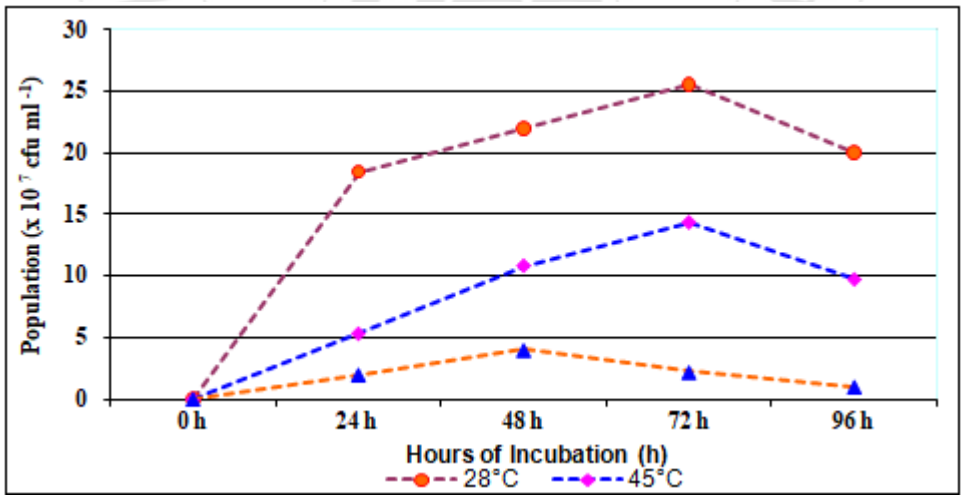

Figure 1: Effect of temperature (28 - 50°C) and pH (5.5) on UV mutant MB 1

In the laboratory, the rhizobial strains grown on the low pH media (5.5) possessed greater acid tolerance than isolates from the higher pH media. After exposure to temperature stress (35^{o}C to 50^{o}C), the production of discrete colonies at low pH indicated the presence of the bacterial population of cells that could tolerate hydrogen ion stress. The laboratory procedure developed to identify acid tolerant accessions produced discernible differences between strains, and did not appear to be critically reliant on the level of inoculation.

[6] reported that the level of growth at a given pH was largely a function of the number of viable cells in the inoculum. [5] considered that once a sufficient number of cells had been applied to the medium at low pH, selective substrate utilization in the poorly buffered environment could raise the pH to a level which permitted the onset of growth.

In the present study, the use of a strong buffer appears to have overcome this phenomenon, and enabled strains to be categorized as either acid tolerant or intolerant without meticulous attention to either the level of inoculation or the

final pH of the medium. The present results revealed that most of the colonies which appeared "dry" colony type grew particularly well on low pH (5.5) growth media when compared with high pH media (6.0). The present results revealed that UV mutant MB 1 and MG 1 adapted well to acid conditions on acidified media as well as temperature tolerant (35°C to 50°C). On contrary, [14] reported that, mung bean rhizobia growth in an acidified medium was uselessly imprecise indicator of acid tolerance.

The present data revealed that the growth of rhizobial population was slightly lower at pH 5.5 when compared to pH 6.0 and the strains able to nodulate the host. These results confirm with the recent findings of [18] who reported that the growth rate of *Bradyrhizobium* SEMIA 6144 was decreased by 50 per cent when grown in medium maintaining acid pH 5.5 compared to the neutral pH (6.8 – 7.0) and the strains able to nodulate the peanut roots even the growth and viability decreased in culture medium at low pH. [2] reported that *Bradyrhizobium* isolates had the ability to survive in yeast extract mannitol broth at low pH 4.0.

When the UV mutant MG 1 was exposed to 45°C, the population increased (7.65 log $_{10}$cfu ml^{-1} to 8.02 log $_{10}$cfu ml^{-1}) upto 72 h of incubation and then declined gradually. At 50°C, the rhizobial population increased (7.36 log $_{10}$cfu ml^{-1} to 7.43 log $_{10}$cfu ml^{-1}) upto 48 h of incubation. In general, the population of rhizobia increased to significant level upto 48 h of incubation at 50°C beyond which, it declined gradually (Table 4).

Table 4: Effect of temperature (28 - 50°C) and pH (5.5 – 6.0) on UV mutant MG 1

S.No	Hours of incubation	Population (x 10^7 cfu ml^{-1})									
		28°C		35°C		40°C		45°C		50°C	
		pH 5.5	pH 6.0	pH 5.5	pH 6.0	pH 5.5	pH 6.0	pH 5.5	pH 6.0	pH 5.5	pH 6.0
1.	0	-	-	-	-	-	-	-	-	-	-
2.	24	15.4 (8.19)	16.6 (8.22)	14.0 (8.15)	13.5 (8.13)	7.5 (7.88)	9.2 (7.96)	4.5 (7.65)	5.4 (7.73)	2.3 (7.36)	2.7 (7.43)
3.	48	18.5 (8.27)	20.2 (8.31)	17.3 (8.24)	15.4 (8.19)	12.2 (8.09)	13.8 (8.16)	10.4 (8.02)	11.2 (8.05)	2.7 (7.43)	3.9 (7.59)
4.	72	22.2 (8.35)	24.3 (8.39)	20.5 (8.31)	21.3 (8.33)	16.5 (8.22)	16.5 (8.22)	13.5 (8.13)	13.6 (8.13)	1.8 (7.26)	1.9 (7.28)
5.	96	12.5 (8.10)	18.5 (8.27)	15.3 (8.18)	16.2 (8.21)	9.3 (7.97)	9.2 (7.96)	8.5 (7.93)	8.9 (7.95)	1.2 (7.08)	1.4 (7.15)

Particulars	SEd	CD (0.05%)
Hours of incubation	0.279	0.553
Temperature	0.279	0.553
pH	0.176	0.350
Hours of incubation x Temperature	0.623	1.236
Temperature x pH	0.394	0.782
Hours of incubation x pH	0.394	NS
Hours of incubation x Temperature x pH	0.881	1.748

Log values are represented in Paranthesis

The interaction between the hours of incubation, temperature and pH had significant effect on the survival of rhizobial mutants. When the UV mutant MGn 1 was exposed to 45°C and pH 5.5, the population increased (7.70 log $_{10}$cfu ml^{-1} to 8.15 log $_{10}$cfu ml^{-1}) significantly upto 72 h of incubation compared to the control (28°C). At 50°C, the rhizobial population decreased. The interaction between the hours of incubation, temperature and pH did not show any significant variation on the survival of rhizobial mutants. When the UV mutant MS 1 was exposed to 45°C, the population increased (7.61 log$_{10}$cfu ml^{-1} to 8.15 log $_{10}$cfu ml^{-1}) at pH 5.5 for 24 - 72 h of incubation compared to the reference culture which was grown at 28°C. The interaction between the hours of incubation, temperature and pH showed significant effect on the survival of rhizobial mutants. When the UV mutant MC1 was exposed to 45°C, the population increased (7.60 log $_{10}$cfu ml^{-1} to 8.09 log $_{10}$cfu ml^{-1}) at pH 5.5 for 24 - 72 h of incubation compared to the control maintained at 28°C. The interaction between the hours of incubation, temperature and pH showed significant effect on the survival of rhizobial mutants.

Comparatively at pH 6.0, all the UV mutant MB 1*Rhizobium* sp. had shown slight increase in their growth (Fig 2).

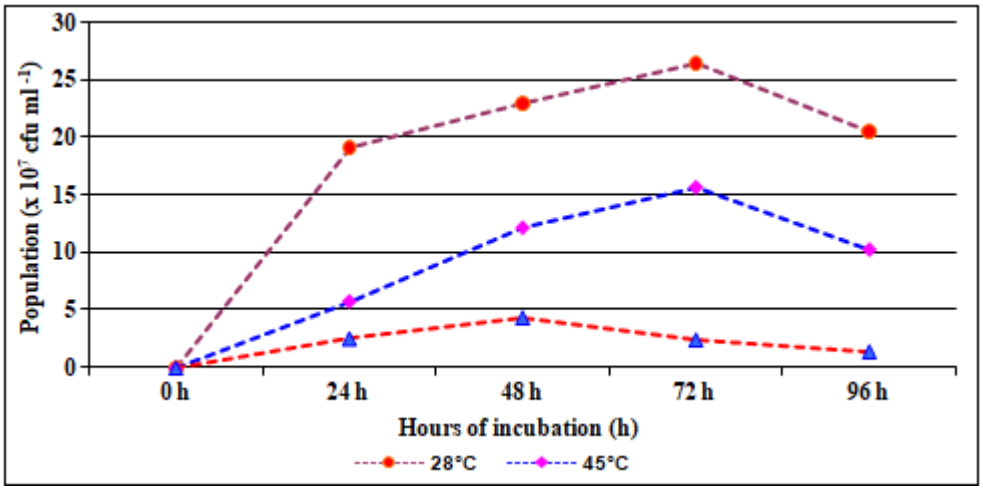

Figure 2: Effect of temperature (28 - 50°C) and pH (6.0) on UV mutant MB 1

4. Temperature tolerance of *Rhizobium* strains

At normal temperature of 28°C, the growth of UV mutant MB1 rhizobial cells had increased from 8.26 to 9.35 $\log_{10}$cfu ml^{-1} for 24 – 96 h of incubation at pH 5.5. At 35°C exposure, in general, the growth was reduced. Further, the population of rhizobia gradually decreased for a period of four days incubation at 40, 45 and 50°C. These results were in accordance with the earlier findings of [21] who reported that ten inoculant strains of *Rhizobium* spp. showed a gradual decline in population during 8 weeks of incubation at 37°C, while exposure to 46°C was lethal to all strains in less than 2 weeks. The same findings were reported by [12] that several heat-tolerant nitrogen fixing bean nodulating *Rhizobium* strains which grew at 40°C had better growth. [10] reported that eight temperature tolerant isolates of cowpea *Rhizobium* spp. for blackgram were developed by *invitro* screening and by isolating from high temperature zones and these isolates could be able to perform better at high temperature of 42°C. There was no variation observed due to high temperature at plasmid level.

In the present study, all the five UV mutant *Rhizobium* sp. (COS 1, CRR 6, COG 15, CO 5 and TNAU 14) were capable of surviving under high temperature (35 - 50°C) and pH (5.5 and 6.0) stress. These results confirm the earlier findings of [7] who reported that some free-living rhizobia (saprophytic) were capable of surviving under drought stress or low water potential. Also confirms with the recent findings of [3] who reported that growth of five isolates obtained from extreme environments (temperature and pH) in sand dune legumes of the South West Coast of India and it could adapt to tolerate 30 - 40°C and pH 5 – 6. [26] reported that the heat tolerant rhizobia are likely to found in environments affected by temperature stress and the rhizobia had maximum growth at temperature of 44.2°C.

[1] and [21] suggested that the Kenyan isolates grew normally at 35°C and over 50 per cent could tolerate 40°C. Hence 40°C was chosen as the value above which rhizobia were considered as temperature tolerant. The above findings are consistent with the present data which revealed that all the five UV mutant *Rhizobium* sp. were able to tolerate upto temperature 35 to 50°C in liquid culture and YEM agar plates. This result also confirmed the earlier findings of [19], who reported that total loss in rhizobia viability was observed in chickpea, lentil and bean inoculants when they were exposed to ambient temperatures of 44°C but these *R. phaseoli* isolates survived and multiplied on YEM agar plates at 45 – 47°C. *Rhizobium japonicum* strain had also been reported to survive in liquid culture at 48.7°C [13]. [16]; [15] reported that *Mesorhizobium ciceri* and *M. mediterraneum* adapted to maximum temperature of 40°C.

[20] reported that the mutant strains of *Rhizobium trifoli* incapable of nodulating pea seedlings and thereby could not form nodules on the host. Such variant forms apparently occur spontaneously in these strains at a low frequency which could be significantly increased by irradiation with ultraviolet, x - rays and fast neutrons. Also suggested that this loss of infectiveness in nodule may be attributed partly to reduction in infective ability since the average number of nodules formed per plant of clover or pea is appreciably lower than for comparable inoculation by strains of nonmutant *R. triofolii* or *R. leguminosarum* respectively. Cultural characteristics of mutant strains resembled with those of the non mutant *R.trifoli* strains. These findings disagree with the present results reported by [24] that the mutant strains of *Rhizobium* exposed to higher temperature of 35 - 50°C and acid stress conditions were able to nodulate their specific host such as blackgram, greengram, groundnut, soybean and cowpea when compared with the parent strain (wild type) growing in growth pouches for 30 days. [4] reported that two moderately acidophilic chickpea mesorhizobia had positive correlation between the symbiotic effectiveness at low pH and the acid tolerance of rhizobial isolates. [23] reported that the strains of *Bradyrhizobium japonicum* grown in sterile growth pouches were screened for tolerance to acid stress conditions maintained in culture medium and found that the strains able to nodulate the host plant. *Rhizobium japonicum* mutant strains could be able to nodulate the host plant (soybean) after 28 days incubation [22].

5. Acknowledgements

I thank my Dr.K.Ramasamy, Professor (Agrl.Microbiology), Tamil Nadu Agricultural University, oimbatore and Dr.K. Ilamurugu, Professor (Agrl.Microbiology) for encouraging me then and there to write up research article aswellas given

good suggestions regarding the completion of article.

References

[1] Alexander, M. 1982. Ecology of *Rhizobium*. *In*: Biological Nitrogen Fixation Ecology, Technology and Physiology. (ed.) M. Alexander, Plenum Press, London. pp. 39-50.

[2] Appunu, C. and B. Dhar. 2006. Symbiotic effectiveness of acid tolerant *Bradyrhizobium* strain with soybean in low pH soil. Afr. J. Biotech., 5(10): 842 – 845.

[3] Arun, B. and K.R. Sridhar. 2005. Growth tolerance of rhizobia isolated from sand dune legumes of the South West Coast of India. Engineering in Life Science, 5(2): 134 – 138.

[4] Brigido, C., A.Alexander, M. Laranjo and S.Oliveira. 2007. Moderately acidophilic mesorhizobia isolated from chickpea. Lett. Appl. Microbiol., 44(2): 168 -174.

[5] Bromfield, E.S.P. and D.G. Jones. 1980. Studies on acid tolerance of *R. trifolii* in culture and soil. J. Appl. Bacteriol., 48: 253-264.

[6] Cunningham, S.D. and D.N. Munns. 1984. The correlation between extracellular polysaccharide production and acid tolerance in *Rhizobium*. Soil Sci. Soc. Am. J., 48: 1273-1276.

[7] Furhmann, J., C. B. Davey, and A. G. Wollum. 1986. Desiccation tolerance in clover rhizobia in sterile soils. Soil Sci. Soc. Am. J. 50:639-644.

[8] Gascon, J., A. Oubina, A. Perez – Lezaune and J. Urmenta. 1995. Sensitivity of selected bacterial species to UV radiation. **Current Microbiol., 30 (3)** : 195 - 205

[9] Hungria, M. and M.A.T. Vargas.2000. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crop Res., 65:151–164.

[10] Harikumar, V.S., T.G. Vimalakumari and S. Gunasekaran. 2003. Effect of high temperature on biochemical and genetic constituents of cowpea *Rhizobium* spp. for blackgram (*Vigna mungo*). Advances in agricultural biotechnology (Abstr.).

[11] Luria, S. and M. Delbruck. 1943. Mutations of bacteria from virus sensitive to virus resistance. **Genetics, 28**; 491 – 511.

[12] Michiels, J., C. Verreth, and J. Vanderleyden.1994. Effects of temperature stress on bean nodulating *Rhizobium* strains. Appl. Environ. Microbiol., 60:1206-1212.

[13] Munevar, F. and A.G. Wollum. 1982. Response of soybean plants to high root temperature as affected by plant cultivar and *Rhizobium* strain. Agron. J., 74: 138-142.

[14] Munns, D. N., H. H. Keyser, V. W. Fogle, J. S. Hohenberg, T. L. Righetti, D. L. Lauter, M. G. Zaruog, K. L. Clarkin, and K.W. Whitacre. 1979. Tolerance of soil acidity in symbiosis of mung bean with rhizobia. Agron. J., 71:256-260.

[15] Nour, S.M., J.C. Cleyet – Marel, P. Normandand and M.P. Fernandez. 1995. Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of *Rhizobiummediterraneum* sp. nov. Int. J. Syst. Bacteriol., 45: 640 – 648.

[16] Nour, S.M., M.P. Fernandez, P. Normandand, J.C. Cleyet – Marel. 1994. *Rhizobiumciceri* sp. nov., consisting of strains that nodulate chickpea (*Cicer arietinum* L.) Can.J. Microbiol., 40: 345 – 354.

[17] Obaton, M. 1972. Vne source de proteins peu exploites la symbiose fixatrice d'azoteentirelesslegumineuses of less*Rhizobium*. PlantesVivirieres, 180-184.

[18] Ponsone, L., A. Fabra and S. Castro. 2004. Interactive effects of acidity and aluminium on the growth, lipopolysaccharide and glutathione contents in two nodulating peanut rhizobia. Symbiosis, 36: 193 – 204.

[19] Rodrigues, C.S., M. Laranjo and S. Oliveira. 2006. Effect of heat and pH stress in the growth of chickpea Mesorhizobia. Current Microbiology, 53: 1-7.

[20] Schwinghamer, E.A. 1962. Studies on induced variation in the rhizobia. III. Host range modification of *Rhizobium trifolii* W. by Spontaneous and Radiation Induced mutation. American Journal of Botany, 49(3): 269-277.

[21] **S**omasegaran, P., V. G. Reyes, and H. J. Hoben. 1984. The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage and distribution. Can. J. Microbiol. 30:23-30.

[22] Stacey, G., A.S. Paau, K. Date Noel, R.J. Maier, L.E. Silver and W.J. Brill.1982. Mutants of *Rhizobium japonicum* defects in nodulation. Arch. Microbiol., 138 (3) : 219 – 224.

[23] Taylor, R.W., M.L.Williams and K.R.Sistani.1991. Nitrogen fixation by soybean – *Bradyrhizobium* combinations under acidity, low phosphorus and high aluminium stresses. Plant and Soil, 131: 293 – 300.

[24] Uma Sankareswari, R.2007. Studies on the development of temperature and acid tolerant *Rhizobium* sp. on Blackgram(Unpublished doctoral dissertation/thesis),Tamil Nadu Agricultural University,Coimbatore,Tamil Nadu.

[25] Zahran, H.H. 1999. *Rhizobium* - legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev., 63:968–989.

[26] Zhang, X.P., M. Karsistoo, R. Harper and K. Lindstrom. 1991. Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. Int. J. Syst. Bacteriol., 41: 104 – 113.