ISSN Online: 2349-7785

Growth Response and Protein Profile of Region Specific *Rhizobium* Strains Isolated from *Vigna mungo* from Different Localities of Odisha State, India at Different Temperature, pH, Salinity and Iron

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Received 22 October 2015; accepted in revised form 18 November 2015

Abstract: Six strains of *Rhizobium* were isolated from *Vigna mungo* and their growth response to various environmental variables like different temperatures, pH, salinity (NaCl) and iron (Fe-citrate) was studied. *Rhizobium* sp.UU-25 was the most tolerant species to high (45ºC) and low temperature (4ºC), high salinity concentration (0.5M) but was sensitive to lower pH (pH 5). *Rhizobium* sp. UU-24 also grew at a wider temperature range (45ºC to 4ºC) alkaline pH (pH 10) and at higher concentration of iron (250 µg/ml) but was the very sensitive to a little increase in salinity. UU-27 grew at lower pH (pH 4), non-saline conditions and low iron concentration of 10 µg/ml in the media. UU-28 grew well only in the presence low iron (10 µg/ml) and was sensitive to lower temperature (4ºC) and acidic pH (pH 5) of the media. *Rhizobium* strain UU-23 was most sensitive to higher temperature (35ºC), alkaline pH (pH 10), but was tolerant to higher level of salinity (1M). Based on their growth response to these stresses the *Rhizobium* strains UU-23, UU-25 and UU-27 were selected to study the modification of their protein pattern upon exposure to all these stresses for 72h. Absence of NaCl in the medium lead to over production of a 130 KDa protein in all the three *Rhizobium* strains and also of one chaperonin (67 kDa protein) in the strain UU-25 and UU-27 was detected. In addition, one 45 kDa protein was repressed and one 62 kDa protein was over produced in these three organisms when exposed to 100 µg/ml or above concentration of Fe-citrate.

Key words: *Rhizobium*, growth response, protein profile, pH, temperature, salinity, iron.

Introduction

Modern agriculture is largely based on the use of nitrogenous chemical fertilizers which is not only opposite to sustainable agriculture but also affect the soil fertility and reduced the number of soil microflora [20,21]. Legumes are important in agriculture being a chief source of protein and also beneficial for soil fertility due to their capability of biological nitrogen fixation because of *Rhizobium* endosymbionts [19]. Hence inoculation of efficient strains of rhizobia is important when a legume is introduced in a region. However, in many cases the limiting factors of the soil restrict the establishment of inoculated strains. The yield of leguminous crop not only depend on the rhizobial partners in the symbiosis but also on environmental and soil factors operating in a given location which are not congenial to support their growth and establishment [14]. These factors are soil pH, temperature of soil during the cultivation cycle as well as the following dry season. Further, soils of eastern Indian salinity and iron are very high which

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are also the limiting factors for successive of *Rhizobium* inoculation. *Rhizobium* inoculants were reported as highly sensitive to slight change in the agroclimatic conditions especially with respect to soil reaction, moisture condition and temperature [9]. It has been noticed that *Rhizobium* strain brought from outside and inoculated in a new region often fail to establish due to the change of agroclimatic conditions from where it was isolated [1]. Thus it is necessary to isolate and screen the native *Rhizobium* strains for development of biofertilizer, which can adapt well to local soils upon inoculation and produce effective nodules for fixing nitrogen.

In the present work, different strains of *Rhizobium* were isolated from local soils and assessed for their growth and protein profiles under different environmental conditions like at low and high pH, temperature, salinity (NaCl) and iron (Fe) for selection of stress compatible strains to be used as biofertilizer.

Materials and methods

Collection of plant samples

Thirty days old selected *Vigna mungo* plants were collected from different cultivated fields of Ganjam District in the southern region of Odisha state, India. With effective and pinkish nodules were kept in ice box containing slush ice and transported to the laboratory.

Isolation of *Rhizobium* strains and growth conditions

The well-formed, healthy and pinkish nodules on the roots were carefully cut out with a sterile razor blade. The nodules were immersed in 95 % (v/v) ethanol for 10 sec, sterilized for 5 minutes in 0.1 % acidified Mercuric chloride ($HgCl_2$-1g L^{-1}, Conc. HCl-5ml L^{-1}) and washed six times with sterile distilled water to get rid of the chemical **(Chen and Lee 2001)**. Each nodule was crushed in a small aliquot of sterile distilled water using a sterile glass rod. Small aliquot was plated on Yeast-Extract Mannitol Agar medium (YEMA) and incubated at 28±2^{0}C for 4-7 days [4].

Confirmation of *Rhizobium* isolates

Distinct colonies were picked up and transferred to agar slants for further purification. Confirmation of the Rhizobia was ascertained by streaking on YEMA medium supplemented with congored (0.25 g/100 ml to obtain final concentration of 25 ppm.), Bromothymol blue test and EPS production [11,23]. The rhizobial colonies appear as white and translucent [25]. One week old Rhizobial colonies kept on YEM agar media (1.5 % agar) were used for preparation as inoculants. For this purpose loop of the respective colonies were inoculated in sterile YEM medium in liquid broth [7]. Totally six strains of *Rhizobium* were isolated. Those were UU-23 (from Maniakati), UU-24 (from Surada), UU-25 (from Gobindapur), UU-26 (from Paduraisuni), UU-27 (from Lathipada) and UU-28 (from Asurabandha) and routinely maintained on YEMA slants at 4^{o}C.

Response of rhizobia strains under different pH, temperature, salinity and iron

Growths of these six *Rhizobium* isolates (UU-23, UU-24, UU-25, UU-26, UU-27 and UU-28) were studied, UU stands for (Utkal University strain number) at various pH levels (pH 5-10), temperatures (4-45^{o}C), salinity (0.0025-1M) and iron (0-300 µg/ml). These strains were grown by inoculating uniform amount of culture suspension into experimental tubes. Corning hard glass test tubes (18 x 200 mm) plugged with non-absorbent cotton wool was used for culturing. Ten ml of culture including the inoculums were incubated for up to 72 h with agitation in a rotary shaker. Triplicates were set for each set of experiments and mean of 3 closely concordant determinations were calculated and presented in the text. Growth was measured through absorbance of the culture suspension at 600 nm in a UV-vis Spectrometer (Hitachi-U2000).

SDS-PAGE of protein

SDS-PAGE protein profile of three different *Rhizobium* strains UU-23, UU-25 and UU-27 isolated from *Vigna mungo* and grown for 3 days at different pH (pH-6, pH-7, pH-7.8, pH-9), three different temperature (25, 30, 35^{o}C), salinity [NaCl of zero, 0.0025M(control), 0.025M, 0.25M] and Fe-citrate (zero, 50 µg/ml, 100 µg/ml, 200 µg/ml) was examined. Protein content was

determined following Lowry *et al*. [17] and equal amount of protein was loaded to the gel and SDS-PAGE was performed. The cell pellets were washed once in 10 mM Tris HCl, pH-7.6 and suspended in 200 µl of a 1:1 mixture of 10 mM Tris HCl, pH-7.6 and sodium dodecyl sulphate (SDS). SDS sample buffer contained 10 % (v/v) glycerol, 5 % (v/v) 2-mercptoethanol, 3 % (w/v) SDS and 62 mM Tris HCl, pH-7.6. The cells were immersing in boiling water for 5 min and then immediately placing them in ice water. The cells were then mixed vigorously and then were stored at -20°C. Proteins were electrophoresed on 12 % polyacrylamide SDS linear slab gel in a vertical system overlaid with 5 % staking gel. 12 % resolving gel contained Tris HCl- pH-8.8, 30 % acrylamide, 10 % SDS (Sodium dodecyl sulphate), 10 % APS (Ammonium persulphate) and 12 µl of TEMED (N, N, N, Tetramethyl ethylene diamine). 5 % Staking gel contained Tris/HCl pH-6.8, 30 % acrylamide, 10 % SDS, 10 % APS and 8 µl TEMED. Electrophoresis buffer contained 25 mM Tris-Glycine (pH-8.3) and 20 % SDS. Equal amounts of proteins were loaded in to each well. Electrophoresis was carried out using a Biotech power pack (Yarcard, India) with a power supply 100 mv/20 milliampere 1h, followed by 30 milliampere 3 h. The gel was stained with 0.1% Coomassie Brilliant Blue (G-250) for 24 h and was destained with normal distilled water for 1- 2 days. Molecular weight of the proteins was determined by the comparison of their mobilities with those of marker protein. Proteins of known molecular weight (Merck protein standard mixture IV, 1.15791.0001, batch K 90925591 containing cytochrome C-12.3, myoglobin-16.9, carbon anhydrase- 30.0, ovalbumin-42.7, albumin-66.2, ovotransferin-78.0 kDa) was used as marker. Photograph of the gel was taken in a Bio-Rad Geldoc system and analyzed using 1D analysis software.

Results

Growth response of Rhizobium species from Vigna mungo under various environmental variables

Growth pattern of six *Rhizobium* strains from *Vigna mungo* cultured at various temperatures e.g. 4, 25, 28, 30, 35 and 45°C, pH (5, 6, 7, 7.8, 9 & 10), NaCl (ranging from nil to 1M) and iron as citrate (nil to 300 µg/ml) was examined. Unless otherwise stated the cultures were maintained at 28°C and pH 7.8 throughout the growth period. Maximum growth of all the strains was obtained at 30°C with little change in temperature between 25-35°C. Growth of the strains UU-23, UU-26 and UU-28were considerably affected at 45°C, however, UU-24, UU-25 and UU-27 showed almost similar growth at the temperature range of 25-30°C with little less at lower temperature (Fig-2). Similarly all these isolates grew well at pH-7.8 and increase or decrease of pH of the culture showed detrimental effect on their growth. *Rhizobium* strains UU-24, UU-26 and UU-28 were quite tolerant to pH ranging from 7 to 9 (Fig-1-B). All these isolates grew well in presence of 0.025M NaCl (Control). Upon increase of the NaCl concentration in the media except for the strain UU-23 and UU-27 up to 0.1 M, the growth of all other strains decreased in presence of higher concentration of NaCl (Fig-1-C). Growth of all the isolates though were affected in absence of NaCl, none of them could tolerate up to 1M NaCl, and in many, e.g. strains UU-27 and UU-28 even growth was drastically reduced in presence of 0.5 M NaCl (Fig-1-C). Since Odisha soil is rich in iron tolerance of the *Rhizobium* isolates from *Vigna mungo* to increase in iron concentration is of immense importance. The results showed that the *Rhizobium* strains UU-24, UU-25 and UU-26 tolerated and grew well in presence of up to 10µg/ml of iron citrate (Fig-1-D). With further increase in iron concentration the growth of all the strains were adversely affected. The adverse effect of iron was comparatively less pronounced in strains UU-27, UU-24, UU-25 and UU-26 in presence of up to 200 µg of iron/ml. However, with further increase up to 300 µg/ml growths of all the strains was decreased up to 80-90% (Fig-1-D).

Comparative analysis of the growth pattern of all the six strains of *Rhizobium* from *Vigna mungo* to lower and higher pH (pH 6 and 9), temperature (25 and 45°C), salinity (zero and 0.25M NaCl), and iron (0.05 and 0.25 mg/ml of Fe-citrate) was analyzed and given in table 1. It was found that considerable variation exists between these

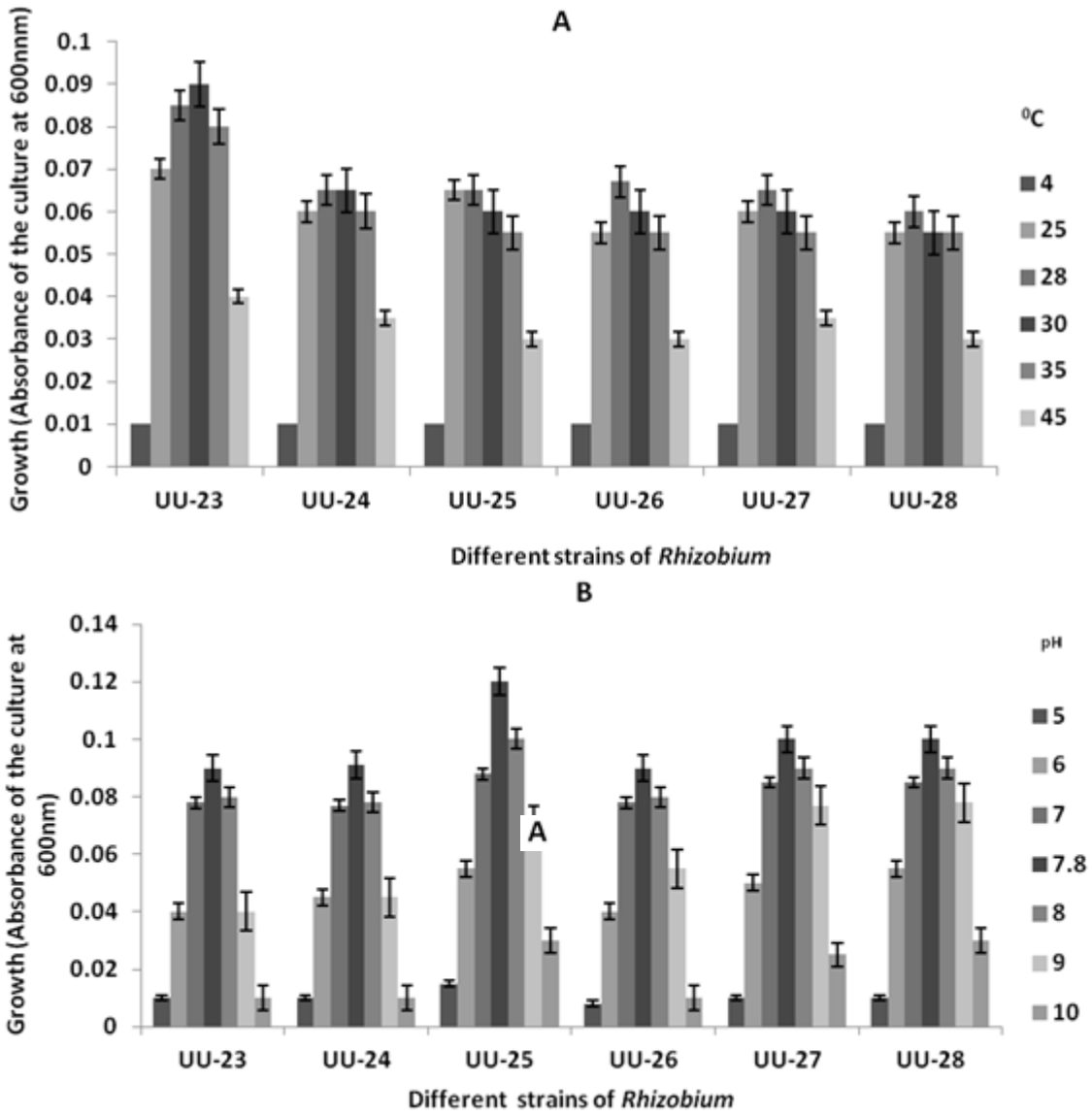

Fig. 1. A-B. Growth of different strains of *Rhizobium* isolated from *Vigna mungo* from different (A) temperature (^{o}C) & (B) pH. Cultures were incubated for 120h at 28±2^{o}C. Initial inoculum of the culture suspension at 600 nm was 0.02. UU-23, Maniakati; UU-24, Surada; UU-25, Gobindapur; UU-26, Paduraisuni; UU-27, Lathipada; UU-28, Asurabandha

Table 1. Comparative growth response of six strains of *Rhizobium* sp. isolated from *Vigna mungo* to low and high pH, temperature, salinity (NaCl) and iron (Fe-citrate)

Temperature	Low (25^{o}C)	UU-26< UU-28< UU-23< UU-27< UU-24< UU-25
	High (45^{o}C)	UU-23< UU-26< UU-24< UU-28< UU-27< UU-25
pH	Low pH (6)	UU-25< UU-28< UU-24< UU-23< UU-26< UU-27
	High pH (9)	UU-23< UU-27< UU-25< UU-28< UU-24< UU-26
Salinity (NaCl)	Zero	UU-23< UU-25< UU-26< UU-28< UU-27< UU-24
	High (0.25 M)	UU-27< UU-24< UU-26< UU-28< UU-23< UU-25
Iron (Fe-Citrate)	Low (0.05 mg/ml)	UU-26< UU-24< UU-23< UU-25< UU-27< UU-28
	High (0.25 mg/ml)	UU-28< UU-23< UU-25< UU-27< UU-26< UU-24

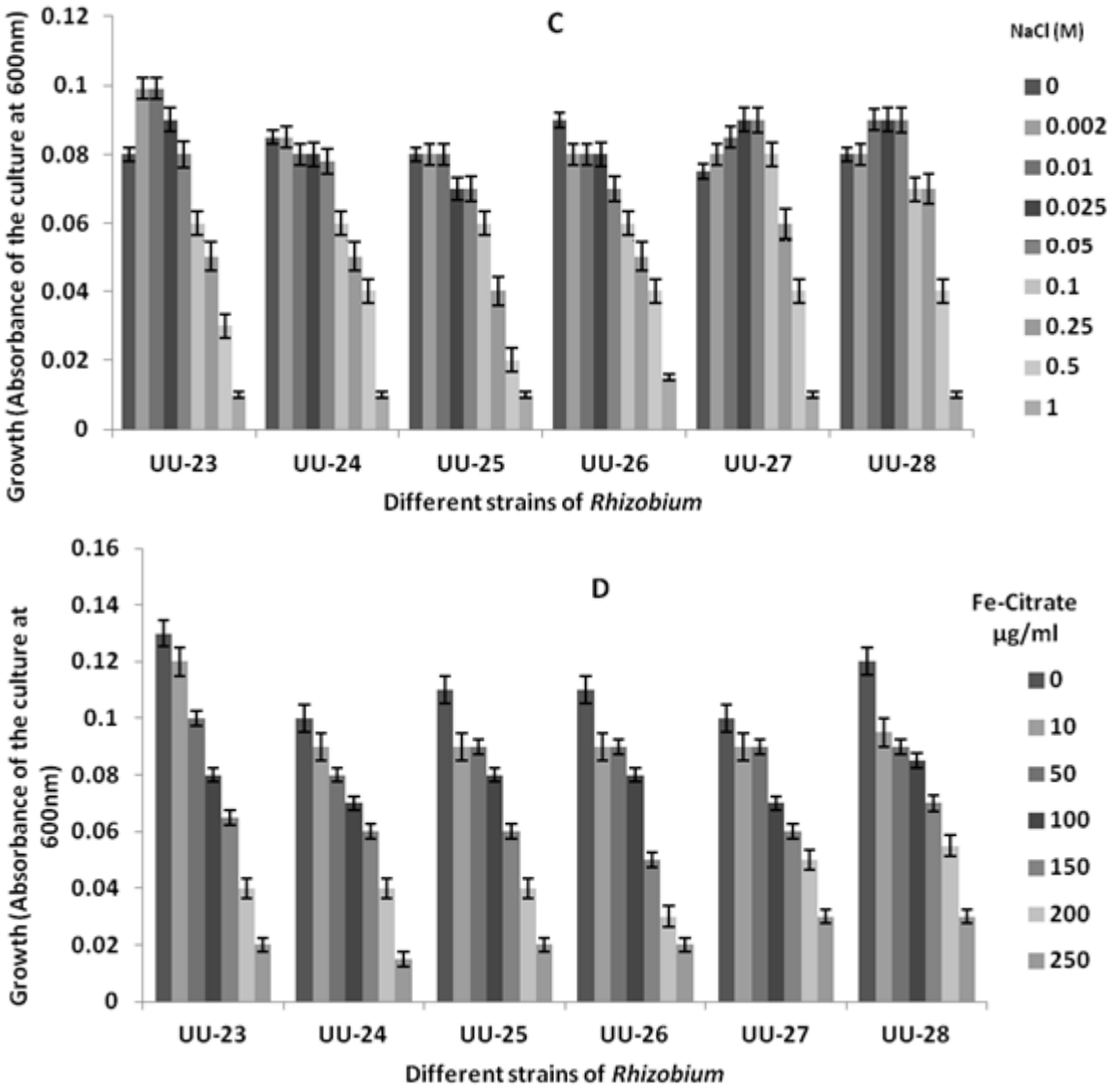

Fig. 1. C-D. Growth of different strains of *Rhizobium* isolated from *Vigna mungo* from different (C) Salinity (as NaCl) & (D) iron (as Fe-citrate). Cultures were incubated for 120 h at 28±2°C. Initial inoculum of the culture suspension at 600 nm was 0.02. UU-23, Maniakati; UU-24, Surada; UU-25, Gobindapur; UU-26, Paduraisuni; UU-27, Lathipada; UU-28, Asurabandha

organisms on the basis of their resistantance to several of the environmental variables. The strain UU-25 from *Vigna mungo* was found to be most tolerant species to high and low temperature, high salinity but was sensitive to lower pH. Next to this strain UU-24 tolerated maximum to lower temperature, alkaline pH and to higher concentration of iron but was the very sensitive to grow at little increase in salinity. UU-27 was tolerant to lower pH and to low iron concentration in the media. Strain UU-28 grew well only in the presence of low iron and was sensitive to lower temperature, acidic pH, high iron concentration. Strain UU-23 was most sensitive to higher temperature, alkaline pH, non-saline condition, and was tolerant to higher level of salinity (NaCl).

Basing on these results on the relative tolerance of six strains of *Rhizobium* from *V. mungo*, three strains UU-23, UU-25 and UU-27 were selected and changes in their protein profile in response to various environmental variables was analyzed (Table 1).

Modification of protein synthesis

SDS-PAGE protein profile of three different *Rhizobium* strains UU-23, UU-25 and UU-27 (regional isolates) from *Vigna mungo* grown for 3 days at different pH (pH-6, 7, 7.8 and 9), three different temperatures (25, 30 and 35°C), salinity [zero and 0.0025M (control), 0.025 and 0.25M NaCl], iron (zero, 50, 100, 200 µg/ml of Fe-citrate) was examined and the results are presented in Fig-2-A-D. Modification of protein synthesis with respect to the treatment the strains were subjected to was analyzed. When a particular kDa protein was absent upon exposure to a particular stress in comparison to control is expressed as repression of protein. Similarly when a specific protein band appeared in the banding pattern upon exposure to a particular stress is expressed as induction of protein. Also when a specific kDa protein band became prominent in comparison to control is presented as over production of protein and presented in table 2. With change in pH in the *Rhizobium* strain UU-23 two proteins e.g. 37 and 50 kDa were repressed in the cultures at pH 6 and pH 7, and in addition one 28 kDa protein was over produced at pH 6. At pH 9 two proteins 37 and 55 kDa were over produced even when the organism was found to be least tolerant to high pH. Similarly in *Rhizobium* UU-27, one 50 kDa was induced and a 45 kDa protein was over produced when the organism was grown at lower and higher pH than neutral, and in addition two more proteins, one chaperonin-55 KDa and one high molecular weight protein-130 kDa were over-produced at pH-9 (Fig-2, Table-2). In Rhizobium strain UU 25, however, none of the proteins were repressed, induced or over produced showing that there was no appreciable change the protein synthesis when subjected to different pH in this organism **(**Fig 2-A, Table 2).

Protein synthesis pattern of the three *Rhizobium* species from *Vigna mungo* subjected to three different temperatures 25, 30 and 35°C is given in Fig. 2-B and table 2. In *Rhizobium* UU-23 strain one molecular chaperonin, 62 kDa and one high molecular weight protein, 130 kDa were over produced when subjected to 35°C. In strainUU-23, two more proteins e.g. 16 and 45 kDa and in strain UU-27 three more proteins e.g. 16, 45 and 67 KDa were over produced at comparatively higher temperature. In strains UU-25 and UU-27, however, a 62 KDa protein and 45 KDa proteins were repressed in respective strains when grown at 25°C (Fig. 2-B, Table 2).

Modification of protein pattern in these three *Rhizobium* strains when grown in the absence of NaCl and also in presence of 0.025 and 0.25 M NaCl in the media is given in Fig. 2-C and Table 2. None of the proteins were neither induced nor repressed with change of NaCl concentration in the growth medium. Also there was no over-production of any proteins when they were grown in presence of NaCl. However, absence of NaCl in the medium lead to over production of a 130 KDa protein in all the three strains and also of one chaperonin, 67 kDa in strain UU-25 and strain UU-27. Growth response to salinity showed that the strains UU-25 grew well in absence of NaCl where as the strain UU-27 was most sensitive to 0.25 M NaCl. Hence these results showed that over-production of the same proteins in both the organisms in the NaCl free media have no correlation with that of their growth. Analysis of these results showed that induction, repression or over production of proteins in the three strains of *Rhizobium* from *V. mungo* occurring at different localities of a region in response to pH and temperature variables is not uniform and is strain specific. Further, the protein induced, repressed or overproduced in the three strains of *Rhizobium*, i.e. UU-23, UU-25 and UU-27 was quite different. Also one 45 kDa protein was repressed and one 62 kDa protein was over produced in these three isolates when exposed to Fe-citrate of the concentration 100 µg/ml or above (Fig. 2-D, Table 2).

Discussion

It was found that the growth of *Rhizobium* was maximum obtained at 28°C and with little increase or decrease of temperature had a significant effect on their growth of rhizobia. Soil temperature in the tropics usually exceeds 45°C at 5 cm and 50°C in 1 cm depth [16,18], and which may limit nodulation in relation to rhizobial growth. The upper limit ranges between 32°C and 47°C, although tolerance varies among species and strains because high temperature decreases rhizobial survival and

Table 2. Modification of protein synthesis in three different *Rhizobium* sp. UU-23, UU-25 and UU-27 at different pH, temperature, salinity (NaCl) and Fe (Fe-citrate)

***Rhizobium* strain**	**pH**			**Temperature (^{0}C)**			**NaCl (M)**			**Fe (Fe-citrate)**		
	6	**7**	**9**	**25**	**30**	**35**	**0.0**	**0.025**	**0.25**	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**
UU-23												
Induction of protein (kDa)												
Repression of protein (kDa)	37,50	37,50									45	45
Overproduction of protein (kDa)	28		37,55			16,45,	130				62,105	62,105
UU-25						62,130						
Induction of protein (kDa)												
Repression of protein (kDa)				62							45	45
Overproduction of protein (kDa)						62,130	67,130				62	62
UU-27												
Induction of protein (kDa)	50	-50										
Repression of protein (kDa)	-			45							45	45
Overproduction of protein (kDa)	45		45,55,130			16,45,62, 67,130	67,130				62	62

For pH: The control cultures were grown at pH-7.8
for temperature: The control cultures were grown at 28^{o}C
for salinity: The control cultures were grown at 0.0025M
for iron: The control cultures were grown without Fe source

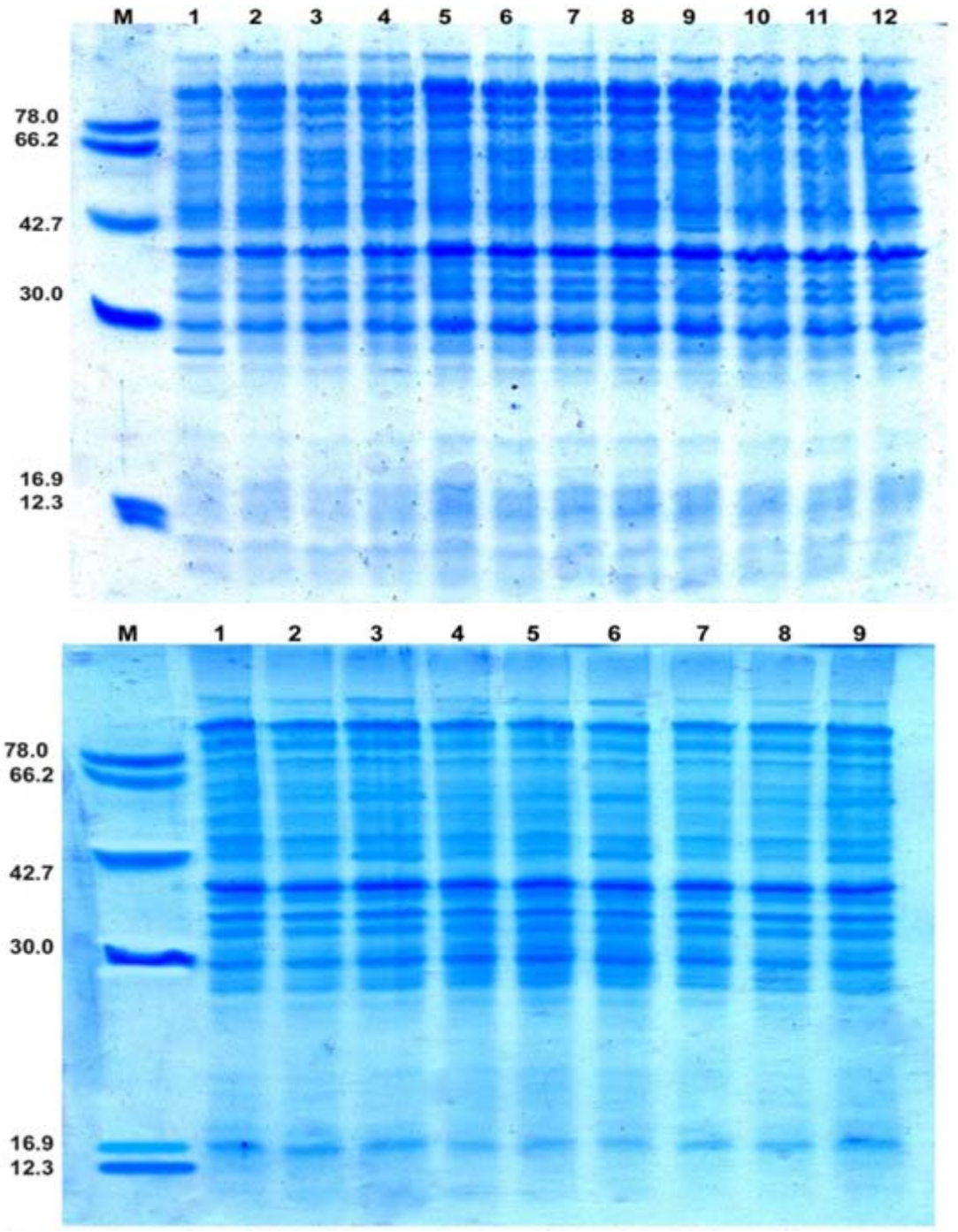

Fig. 2. A -B. Protein profile of *Rhizobium* sp. isolated from *Vigna mungo* collected from various locations of Odisha state, India at different pH and temperature.

Fig-2-A pH: Lane 1-4: (*Rhizobium* strain UU-23) 1. pH-6, 2. pH-7, 3. pH-7.8 (control), 4. pH-9
Lane-5-8: (*Rhizobium* strain UU-25): 5. pH-6, 6. pH-7, 7. pH-7.8 (control), 8. pH-9
Lane-9-12: (*Rhizobium* strain UU-27): 9. pH-6, 10. pH-7, 11. pH-7.8 (control), 12. PH-9
Fig-2-B Temperature: Lane-1-3: (*Rhizobium* strain UU-23) 1. 25^{o}C 2. 30^{o}C 3.35^{o}C
Lane-4-6: (*Rhizobium* strain UU-25) 4. 25^{o}C 5. 30^{o}C 6.35^{o}C
Lane-7-9: (*Rhizobium* strain UU-27) 7. 25^{o}C 8. 30^{o}C 9.35^{o}C

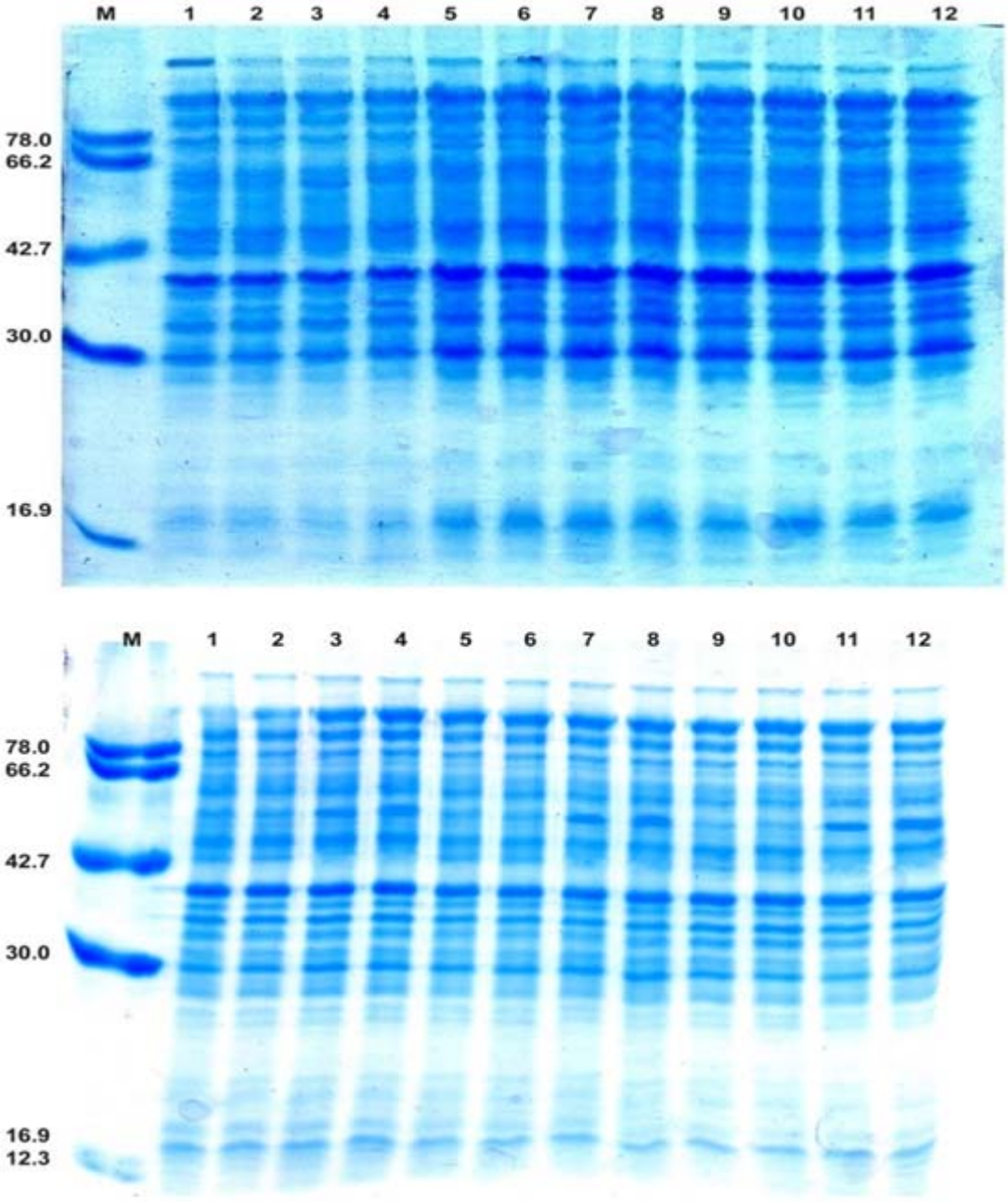

Fig. 2. C-D. Protein profile of *Rhizobium* sp. isolated from *Vigna mungo* collected from various locations of Odisha state, India at different salinity and iron (Fe-citrate)

Fig-2-C, Salinity

Lane-1-4: (*Rhizobium* strain UU-23) 1. Zero 2. 0.0025M (control) 3. 0.025M 4. 0.25M
Lane-5-8: (*Rhizobium* strain UU-25): 5. Zero 6. 0.0025M (control) 7. 0.025M 8. 0.25M
Lane-9-12: (*Rhizobium* strain UU-27): 9. Zero 10. 0.0025M (control) 11. 0.025M 12. 0.25M

Fig-2-D, Fe (Ferric citrate)

Lane-1-4: (*Rhizobium* strain UU-23) 1. Zero 2.50 µg/ml 3.100 µg/ml 4.200 µg/ml
Lane-5-8: (*Rhizobium* strain UU-25): 5. Zero 6. 50 µg/ml 7.100 µg/ml 8.200 µg/ml
Lane-9-12: (*Rhizobium* strain UU-27): 9. Zero 10. 50 µg/ml 11.100 µg/ml 12.200 µg/ml

establishment in tropical soils. Hence, repeated and higher rates of inoculation may frequently be needed. The alternative is inoculated strains capable of surviving at the higher temperature of tropics so as to make the inoculation successful. There have been number of investigations on the effect of temperature on infection process of temperate species of *Rhizobium*. The results showed that at 4°C the growth of *Rhizobium* was suppressed where as at 24°C and above the growth was enhanced, the growth was retarded when the *Rhizobium* exposed at higher temperature. However, these results were dependent on variation between *Rhizobium* strains and host cultivars [2,27]. *Rhizobium* also grew well at near neutral pH and with variation of the pH to acidic or alkaline side affected their growth though there were minor deviations among the strains to tolerate higher and lower pH levels. The optimum pH for rhizobial growth has also been found between pH-6 to pH-7 [15] and relatively few rhizobia grew in acidic pH [10]. Intrinsic tolerance cannot be predicted from the pH at the site of isolation because when fast growing rhizobial strains were isolated from nodules that have been inoculated with soil from certain sites where the pH ranges from 3 to 5. Only 37 % were able to grow in buffered medium at pH 4 and 60 % grew at pH 9.5 [12]. The microsymbiont is usually the more pH sensitive partner. Some rhizobial species can tolerate acidity better than others, however, similar results that the tolerance may vary among strains within a species has been reported earlier [6,13].

Odisha has a long coast line of nearly 460 km and the nearby fields are often saline due to seawater influx. In coastal areas of Odisha where the experiments were principally based, the farmers also withdraw ground water for irrigation resulting an increase in concentration of salt in the fields. Hence introduction of salt resistant *Rhizobium* was considered important to make the biofertilizer programme successful in this region. There are three interrelated factors involve in studying the gross effect of salinity in legume establishments: (i) Effect of the salt on the growth of *Rhizobium,* (ii)limits of salt tolerance of the legume, and (iii) limits of salt tolerance of the host as well as the bacterium for successful nodulation and symbiosis. Different species of rhizobia withstand different levels of NaCl which was invariably higher than the host plant [5,26]. Further, degree of salinity/alkalinity conducive for good nodulation was different from the limits of tolerance of *Rhizobium* and the host to the salt [5]. In the present study on the growth responses of several strains of *Rhizobium* from *Vigna mungo* to different concentrations of NaCl ranging from 0.002 to 1M showed wide variation in the capabilities of these strains to tolerate the salt. There are reports that salt tolerant strains significantly enhance their capacity to oxidize carbon sources by increasing growth rate and EPS production that involve in adhesion resulting in a greater adapting capacity to colonize on favourable saline environment [3].

All the *Rhizobium* species isolated from *V. mungo* grew well in presence of Iron. Although iron is abundant in soil (1 to 6 %) and it ranks 4th among all elements on surface of earth it is often unavailable to the microbes and plants because of its solubility which is dependent on pH. Under aerobic soil conditions most iron exists in the insoluble ferric form [8]. It is a component of the cell and its deficiency causes growth inhibition and can also change the cell morphology. To meet the requirement of iron the organisms evolve a specific high affinity mechanism and when the medium and the soil is low in soluble iron this mechanism becomes operative, and this happens with involvement of siderophores which are low molecular weight iron chelators [8]. Iron plays special role in root nodules for the symbiotic nitrogen fixation as this is required for leghaemoglobin, nitrogenase and cytochrome synthesis within the bacteroids in the nodules. Research have shown that presence of active nodules indicate iron deficient stress response in soybean [8]. Odisha soils are rich with iron which varies from 8 ppm to 376 ppm [22]. The locations where the region specific strains from *Vigna mungo* were isolated where rich with iron exceeding 100µg/g soils. Hence growth response of these strains to various iron concentrations is a critical factor for their establishment after inoculation to make the biofertilizer programme successful.

The results also showed that in response to a

particular stress a suite of proteins were either induced or over-produced conferring protections to their survival under the stress. Also when any protein is repressed when subjected to a stress it was possibly that the organism is sensitive to that condition resulting in retardation of growth. There are reports that when microorganisms are subjected to stresses, irrespective of the environmental variables they are exposed to, the expression of stress proteins are almost similar with minor deviation.

However, the results of the present experiments showed that when the *Rhizobium* strains, three from the same host *V. mungo* were subjected to low and high pH, temperature, salinity and low and high concentrations of iron, the induction, repression and over production of proteins upon exposure to different stresses were not identical, and also that the modification of protein expression of all the *Rhizobium* strains in response to the same stress was quite different. This shows that there exists a genetic variability among the rhizobial strains from the same host even at different locations of one region and respond to cope with the stress factors differently. The result also showed that the three different strains *V. mungo* UU-24, UU-26 and UU-27 have good response to these stresses than other strains. So these strains could be considered as best strains for *Rhizobium* biofertilizer programme.

Acknowledgement

The authors gratefully acknowledge the University Grants Commission (UGC), New Delhi, India for financial assistance through a Research Fellowship grant to Santosh Kumar Sethi.

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