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# EVALUATION OF DIVERSITY OF FREE LIVING PLANT GROWTH PROMOTING RHIZOBACTERIA OF WHEAT GROWN IN SALINE SOIL

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**ABSTRACT**
A study was conducted to find out the microbial diversity of the rhizosphere of wheat and screening of the effective PGPR isolate with multiple traits under saline soil conditions. Total 59 rhizobacteria were isolated from rhizosphere using different media *viz*., NA, King's B medium, and Jensen's medium while predominant genera found were *Pseudomonas, Bacillus* and *Azotobacter*. All the isolates were screened in vitro for their plant growth promoting traits. Ammonia production was most common trait of *Pseudomonas* (37.03%) and *Azotobacter* (100.00%) and *Bacillus* (100.00%). Phosphorus solubilization was detected in the isolates of *Azotobacter* (66.23%), *Pseudomonas* (45.35%), and *Bacillus* (23.80%). Thirty seven (63%) isolates restricted the growth of the test fungus *Alternaria alternate*. Twelve isolates (four isolates from each medium) were selected on the basis of qualitative screening for plant growth promoting traits. The amount of IAA produced by selected isolates was in the range of 63.60$\mu$g/ml to 306.60$\mu$g/ml. The selected isolates were also tested for salt (NaCl) tolerance at 3% to 10% concentration and found *Pseudomonas* (75%) *Bacillus* (75%) and *Azotobacter* (100%) isolates tolerant at 10% NaCl concentration . With this work it can be concluded that the rhizobacteria isolated from the rhizospheric soils of wheat would be useful as inoculants for saline soil conditions.

## INTRODUCTION

Rhizosphere is a rich niche of microbes and should be explored for obtaining potential plant growth promoting rhizobacteria (PGPR), which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants. Wheat is one of the major cereal crops in India. Wheat is grown in temperate climate and it is staple food for 35% of world population. Plant productivity in saline soils is considerably reduced due to improper nutrition of plant along with osmotic and draught stress (Benlloch-Gonalez *et al.,* 2005). About 65% yield loss occurs in moderately saline area. The use of PGPR may prove to be useful in developing strategies to facilitate wheat growth in saline area. Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that actively colonize plant root and increase plant growth by production of various plant growth hormones, phosphate solubilizing activity, $N_2$ fixation HCN, siderophore , ammonia production and other biological activities (Deshwal *et al.,* 2011,Deshwal& Kumar 2013, Kushwaha *et al.,* 2013, Mohite, 2013).Few strains from genera such as *Pseudomonas*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are well known PGPRs (Kloepper, 1993, Joshi and Bhatt, 2011). Reports suggest that amount of salt that affected land worldwide is estimated to be 900 million ha; it's 6% of the global total land mass. The saline area under agriculture is increasing every year across the globe (Paul and Nair, 2008). Salinity reported as one of the major anthropogenic as well as environmental stresses that reduced plant growth (Tank and Saraf, 2010). Previously, it has been observed that increasing salinity in the soil decreased plant growth, photosynthesis, stomatal conductance, chlorophyll content and mineral uptake compared to soil without salinity (Hans and Lee 2005). Salinity also adversely affects plant growth and development of the plants (Shukla *et al.,* 2012). Salt tolerant plant growth promoting rhizobacteria reduced the impact of salinity on plant growth and improved productivity. Salt-tolerant PGPR can play an important role in alleviating soil salinity stress during plant growth and bacterial exopolysaccharide (EPS) can also help to mitigate salinity stress by reducing the content of Na+ available for plant uptake (Upadhyay *et al*., 2009). Any microbial utilization in agriculture requires on evaluation of the environmental risks associated with the introduction of indigenous and un-indigenous microorganisms into the plant rhizosphere as well as assessment of most desirable condition for the effective and successful establishment of plant growth promoting rhizobacteria (PGPR) as inoculants in the rhizosphere of host plant (Rangrajan *et al.,* 2002). The isolated PGPR strains from region may not perform in the same way in other soil and climate conditions (Johnson *et al.,* 1998). Isolation of native strains adapted to the environmental then study may contribute to the formulation of inoculants to be used in region crops. The different stages of life cycle of wheat consist of elongation, flowering stage, fruiting stage and ripening. It is found that rate of roots exudates released by the

root of the wheat at flowering stage is higher as compared to other stages (Huddedar *et al.,* 2000).

The present study has the view to isolate the salt tolerant bacteria from rhizosphere of wheat grown in various salt affected regions of Uttar Pradesh and screening for their ability to enhance the growth and yield of wheat under the saline condition. As the cultures are isolated from the saline soil samples that show the bacterial cultures have the salt tolerance power. We can treat the problem of salinity stress on wheat plants with the help of these isolates, as they are naturally occurring in the rhizospheric saline soil where wheat plants grow. The future aspect of our work is to make the formulation of these cultures so that the problem of salinity stress on wheat plants can be treated. These formulations cannot only provide tolerance against the salt but also have antifungal activity, catalytic activity and can work as plant growth promoting rhizobacteria.The work was designed to evaluate the diversity of free living plant growth promoting rhizobacteria of wheat grown in saline soils.

## MATERIALS AND METHODS

### Sample collection

The root adhering soil (RAS) samples were collected from wheat crop. The soil was sampled from different locations in the districts viz., Faizabad (FZD), Sultanpur(SUT), Gonda(GND), Barabanki(BBK), Lucknow(LKO), Unnao(UNO), Kanpur Dehat (KNP) and Mathura (MTH) of Uttar Pradesh Uttar Pradesh, India.

### Soil analysis

Physiochemical parameters of soil were analyzed viz., pH, electrical conductivity (EC) andESP (%) presented in table-1.

### Isolation of rhizobacteria

Serial dilution of each soil sample was made upto $10^{-4}$, $10^{-3}$ and $10^{-4}$ dilution were plated on to three media, Jensen's medium, King's B (KB) medium and nutrient agar (NA) medium. Plates were incubated at 28ºC for 72 hours.

### Morphological and biochemical characterization by Bergey's manual

On the basis of cultural, morphological (Form, Elevation, Margin, Color, Appearance, Texture and Surface) , cell morphology (Shape, Gram reaction and Arrangement) and biochemical characteristics a total of 59 bacterial isolates were identified as *Pseudomonas Bacillus* and *Azotobacter*as described in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994).

### Screening of isolates for salt tolerance

Salt tolerance in PGPRs was determined by procedure given by Yildirim *et al.,* 2008. The tolerance of the isolates against salinity was evaluated by observing the growth on Nutrient Agar (NA) medium amended with different concentration of NaCl (i.e., 3% to 10%). Control plate was also maintained with 0.05% NaCl. Plates were incubated for 72 hours at 28ºC and the growth on NaCl amended medium was compared with control plates.

### Screening for plant growth promoting attributes

### IAA production

The production of indole acetic acid (IAA) was determined as per the method of Gordon and Weber (1951) with some modifications using IAA as standard. Cultures were incubated at 28ºC for 24 hrs in Luria Bertani (LB) broth. Cells were removed from the media by centrifugation at 10,000 rpm for 15 minutes. The Ortho-phosphoric acid (2-3 drops) was added to 2ml of supernatant and was mixed vigorously with 4ml of Salkouski's reagent (0.5M solution prepared in 50ml of 35% $HClO_4$) and incubated at room temperature for 25 minutes and observed for the color formation (Fisher *et al.,* 2007). Concentration of IAA in supernatant was calculated by spectrophotometer at 530nm.

### Ammonia production

The ammonia production was determined by the method as described by Cappuccino and Sherman (1992) and Shifraw *et al*. (2004). Isolates were incubated for 4 days in peptone water (Peptone 10 g, NaCl 5g in litre, pH 7.0). Nessler's reagent (1ml) was added in each tube and observed for color formation.

### Phosphate solubilization

Test for phosphate solubilisation was done as per the method of Goldstein (1986) and Frioni(1990)with some modifications. The plates were prepared with Pikovskaya's medium. All the bacterial isolates streaked on the surface of Pikovskaya's agar plate and phosphate solubilizing activity was estimated after 4 days of incubation at 28ºC. Phosphate solubilization activity was determined by the development of the clear zone around the bacterial colonies.

### Antifungal activity test

The antagonistic activity of each selected bacterial isolate against *Alternaria alternate* was studied by using a dual culture plate assay (Sharma *et al.,* 2003). A loopful of 48 hrs old culture was spotted in the centre of the potato dextrose agar plate and 6 mm disc of pre grown phyto-pathogenic fungi inoculated on both sides of the plate. The plates with only fungal disc without bacterial streaks served as control. All in vitroantagonism assays were done in triplicate. The percent inhibition was determined after incubating for 3-5 days at 28ºC. The percentage growth inhibition was calculated using the following calculation:

$$I = \frac{C-T}{C} X\ 100$$

Where, I= Per cent inhibition, C= Growth in control, T= Growth in treatment

### Catalase test

Isolates were tested for catalase activity by adding 3-4 drops of hydrogen peroxide ($H_2O_2$) on bacterial culture and observed for exclusion of air bubbles.

## RESULTS

A total of 59 bacteria were isolated from the rhizosphere soil of wheat growing in salt affected soils of Faizabad, Sultanpur, Gonda, Barabanki, Lucknow, Unnao, Kanpur Dehat and Mathura districts of Uttar Pradesh, India. The number of isolates obtained on different media viz., King's B, nutrient agar and Jensen's medium were 16, 27and 16 respectively.

**Table 1: Chemical analysis of soil samples**

| SN | Name of Districts | pH | EC (ds/m) | ESP (%) | Available nutrients (kg/acre) | | |
|---|---|---|---|---|---|---|---|
| | | | | | N | P | K |
| 1 | Faizabad (FZD) | 9.51 | 5.0 | 55 | 227 | 10 | 211 |
| 2 | Sultanpur (SUT) | 8.96 | 3.5 | 52 | 225 | 15 | 209 |
| 3 | Gonda (GND) | 893 | 3.6 | 47 | 225 | 13 | 210 |
| 4 | Barabanki (BBK) | 8.61 | 3.5 | 49 | 230 | 18 | 205 |
| 5 | Lucknow (LKO) | 8.85 | 3.4 | 48 | 227 | 15 | 211 |
| 6 | Unnao (UNO) | 8.60 | 3.3 | 49 | 225 | 16 | 209 |
| 7 | Kanpur Dehat (KNP) | 8.82 | 3.5 | 48 | 225 | 13 | 210 |
| 8 | Mathur (MTH) | 8.73 | 5.8 | 44 | 227 | 14 | 210 |

**Table 2: Morphological and cultural characteristic of rhizobacterial test isolates**

| Biochemical characters | *Pseudomonas spp.* | *Bacillus spp.* | *Azotobacter spp.* |
|---|---|---|---|
| Number of isolates | 16 | 27 | 16 |
| Grams reaction | -ve | +ve | -ve |
| Shape | Rods | rod | rods |
| Pigment | Cream , light to green | Cream | Transparent to light milky most isolates become light brown to black after 10 days of incubation |
| Colony morphology | Smooth margin, flat to raised | Circular, lobate to serrated margin | Watery mucilaginous with smooth margins |
| Sucrose | + | + | + |
| Dextrose | + | + | + |
| Mannitol | - | + | + |
| $H_2S$ production | - | - | - |
| Indole | - | - | - |
| Methyl red | - | - | - |
| Vogues Prokauer | - | - | - |
| Citrate Utilization | + | + | + |
| Catalase test | + | + | + |
| Nitrate reduction | + | - | - |
| Lipid hydrolysis | + | + | + |
| Casein hydrolysis | + | + | + |
| Starch | + | + | + |
| Gelatin hydrolysis | + | - | - |

### Morphological and biochemical characterization

On the basis of cultural, morphological (Form, Elevation, Margin, Color, Appearance, Texture and Surface), cell morphology (Shape, Gram reaction and Arrangement) and biochemical characteristics a total of 59 bacterial isolates were identified as *Pseudomonas Bacillus* and *Azotobacter* as described in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994). The general characteristics of the isolates were illustrated (Table 2).

### Screening for plant growth promoting attributes

Each isolate was screened for the production of ammonia, phosphate solubilization and antifungal activity. Plant growth promoting activities compared in Fig. 3. The percentage of distribution of isolates for PGP activity was found as P-solubilization (16.94%), IAA production (20.33%), ammonia production (30.50%) and antifungal activity (63.00%) against *Alternaria alternate*. (Fig. 1)

### Quantitative IAA production

On the basis of multiple plant growth promoting traits, twelve isolates (four from each medium) selected and screened for quantitative production of IAA. The selected isolates produced IAA in range of 63.60$\mu$g/mL to 304.6$\mu$g/mL. (Fig. 2)

### Salt tolerance

The twelve selected isolates screened for salt tolerance at graded concentrations (3% to 10%) of NaCl. All the selected isolates with multiple PGP activity could tolerate NaCl (9%) concentration. Out of 12 isolates, *Pseudomonas* (75%) and *Azotobacter* (100.00% and *Bacillus* (75.00%) were able to tolerate NaCl stress up to 10% as shown in Table 3.

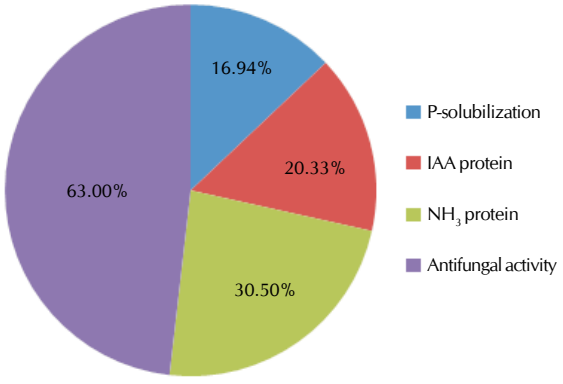
## DISCUSSION

The salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity (Borneman *et al.,* 1996). Wheat is considered to be moderately tolerant to salinity (Mass 1986). IAA production by PGPR isolates is important attribute for improvement of plant growth (Deshwal and Kumar 2013, Kivil *et al.,* 2014). Till date, 80% rhizobacteria have been reported to produce IAA (Loper and Schroth,1986). However, in the present study, out of 59 isolates only 12 isolates (20.33%) shown production of IAA. IAA production was seen maximum by MTH-KB1 strain that is 304.6$\mu$g/ml but the lowest by UNO-JM2 that is 63.6$\mu$g/ml however, Zahid *et al.,* 2015 showed that isolates had the potential to produce IAA in the range of

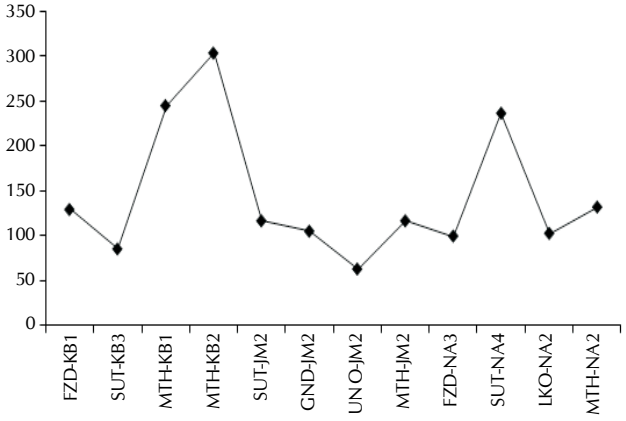
**Table 3: Determination of salt tolerance of Rhizobacterialisolates**

| SN | Isolate Designation | Salt concentration 3% NaCl | 4% NaCl | 5% NaCl | 6% NaCl | 7% NaCl | 8% NaCl | 9% NaCl | 10% |
|---|---|---|---|---|---|---|---|---|---|
| 1. | FZD-KB1 | + + + + | + + + + | + + + + | + + + + | + + + + | + + | + + | + |
| 2. | SUT-KB3 | + + + + | + + + + | + + + + | + + + + | + + | + | + _ | |
| 3. | MTH-KB1 | + + + + | + + + + | + + + + | + + + + | + + + + | + + | + + | + |
| 4. | MTH-KB2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + | + + | + |
| 5. | SUT-JM2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + | + + | + |
| 6 | GND-JM2 | + + + + | + + + + | + + + + | + + + + | + + | + | + + | |
| 7. | UNO-JM2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + | + + + | + + |
| 8. | MTH-JM2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + | + + | + + |
| 9. | FZD-NA3 | + + + + | + + + + | + + + + | + + + + | + | + | + + | |
| 10. | SUT-NA4 | + + + + | + + + + | + + + + | + + + + | + + + + | + | + + | |
| 11. | LKO-NA2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + + | + + | |
| 12. | MTH-NA2 | + + + + | + + + + | + + + + | + + + + | + + + + | + + | + + | |

- = No growth, + = Poor growth , + + = Medium growth, + + + = High growth, + + + + = Very high growth




**Fgiure 1: Percentage distribution of isolated rhizobacterial strains for PGP activities**




**Fgiure 2: Screening of selected isolates for IAA production after 48 hours of incubation**

0.9–5.39$\mu$g mL$^{-1}$ and promote plant growth. Ammonia production is also an important PGP trait, out of 59 isolates only 18 isolates (30.5%) shown production of ammonia. The establishment and performance of phosphate solubilizing microorganisms are severely affected by environmental factors, especially under stressful condition (Beneduzi *et al.,* 2008) making it essential to isolate microorganisms from these condition (such as saline-alkali soils) with high efficiency. In the present study, 10 isolates were found to solubilise phosphate. Similar pattern was reported by (Yasmin *et al.,* 2009) who analyzed 15 PGPR isolates for phosphate solubilisation out of which only six were able to solubilise insoluble phosphate. The population and activity of these PGPRs are greatly influenced by the soil conditions. All the selected isolates with multiple PGP activity could tolerate NaCl stress up to 9% and ten isolates (*Azotobacter* 4, *Pseudomonas* 3 and *Bacillus* 3) found tolerant even at 10% NaCl concentration. The screening of 133rhizospheric bacterial strains was done for salt tolerance, out of only 24 strains could grow at 8 % NaCl concentration and no strains was able to grow at 9 % NaCl (Upadhyay *et al.,* 2009) and l *Pseudomonas aeruginosa* and *P. fluorescens* strains showed growth in medium containing 1.5% NaCl and above 1.75% NaCl concentration in medium, the survival number of *Pseudomonas* gradually reduced (Deshwal and Kumar, 2013).

Fifteen strains from wheat rhizosphere screened for in-vitro antifungal activity against multiple plant pathogenic fungi and found effective to antagonism(Sachdev *et al.,* 2009) while in the present study, all 59 isolates were in-vitro screened for antifungal activity against plant pathogenic fungi *Alternaria alternate* and 63% isolates shown antagonistic activity. The inoculation by *R. solani* (without PGPR) showed that all the roots were fully infected (100%) and application of PGPR strains with *R. solani* showed that the PGPR controlled the infection at various degrees in wheat (Fatima *et al.,* 2009).

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**Discussion** must not recapitulate results but should relate the author's experiments to other work on the subject and give their conclusions.

All tables and figures must be cited sequentially in the text. Figures should be abbreviated to Fig., except in the beginning of a sentence when the word Figure should be written out in full.

The figures should be drawn on a good quality tracing/ white paper with black ink with the legends provided on a separate sheet. Photographs should be black and white on a glossy sheet with sufficient contrast.

References should be kept to a minimum and listed in alphabetical order. Personal communication and unpublished data should not be included in the reference list. Unpublished papers accepted for publication may be included in the list by designating the journal followed by "in press" in parentheses in the reference list. The list of reference at the end of the text should be in the following format.

1. **Witkamp, M. and Olson, J. S. 1963.** Breakdown of confined and non-confined Oak Litter.*Oikos*. **14**:138-147.
2. **Odum, E.P. 1971.** *Fundamentals of Ecology*. W. B. Sauder Co. Publ. Philadelphia.p.28.
3. **Macfadyen, A.1963.** The contribution of microfauna to total soil metabolism. In:*Soil organism*, J. Doeksen and J. Van Der Drift (Eds). North Holland Publ. Comp., pp 3-16.

References in the text should be quoted by the **author's name and year** in parenthesis and presented in year order. When there are more than two authors the reference should be quoted as: first author followed by *et al.,* throughout the text. Where more than one paper with the same senior author has appeared in on year the references should

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