**World Research Journal of Geoinformatics**
ISSN: 2278-7003 & E-ISSN: 2278-7011, Volume 1, Issue 2, 2012, pp-21-26.
Available online at http://www.bioinfo.in/contents.php?id=177

# ISOLATION OF ROOT ASSOCIATED BACTERIA FROM THE LOCAL VARIETY OF RICE GJ-17

**YACHANA JHA[1] AND SUBRAMANIAN R.B.[2]**

N.V. Patel College of Pure & Applied Sciences, S.P. University, V.V. Nagar, Anand, Gujarat, India.
B.R.D. School of Biosciences, Sardar Patel University, Post Box no. -39, VV Nagar- 388120, Gujarat, India.
*Corresponding Author: Email- yachanajha@ymail.com

Received: April 05, 2012; Accepted: April 30, 2012

**Abstract-** Root associated bacteria were isolated from the rice root from the rice field of Gujarat by using nitrogen free selective medium. Various tests were preformed for the identification of isolates, but for identification upto genus level the 16S rDNA analysis was performed. Then 16S amplicons sequences obtained were analyzed by using bioinformatics software and the cultures were identified as *P. pseudoalcaligenes*, *B. pumilus* and *Stenotrophomonas maltophilia* respectively. Then these organisms were evaluated for their plant growth promoting activities by *in-vitro* inoculation to paddy plants. Results obtained during characterization and greenhouse studies indicated its good candidates as PGPR and also showed good response in paddy for growth promotion.
**Keywords-** rDNA analysis, amplicons sequences, rice field, identification of isolates, growth promotion

**Citation:** Yachana Jha and Subramanian R.B. (2012) Isolation of Root Associated Bacteria from the Local Variety of Rice GJ-17. World Research Journal of Geoinformatics, ISSN: 2278-7003 & E-ISSN: 2278-7011, Volume 1, Issue 2, pp-21-26.



## Introduction

The complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, which is modulated by the environment that is prevailing in different conditions [5]. The variety of genetic and functional activities performed by the vast microbial population, have a critical impact on the soil functions. This is because microorganisms are the driving forces for the fundamental metabolic processes [21]. Many microbial interactions are responsible for key environmental processes, such as the biogeochemical cycling of nutrients and maintenance of plant health and soil quality [4]. PGPR can be of two different types when associated with host tissue that is endophytes or epiphytes, otherwise they can be rhizospheric bacteria, those that are present in the root adhering soil.

With increasing emphasis on green technologies that are environmental friendly, researchers are looking for viable alternatives to conventional methods of crop production. Though a number of physical factor are directly or indirectly involved in plant growth and development, soil play a major role in the process. The rhizosphere is a volume of soil around plant where plant growth is stimulated. It is a place where many biologically important function and interaction takes place. The rhizosphere is populated by a diverse range of microorganism and the bacteria colonized this habitat are called rhizobacteria.

An intensive farming practice that warrants high yield and quality requires the extensive use of chemical fertilizers, which are costly and may create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices [12]. In this context, the use of biofertilizers containing plant growth-promoting rhizobacteria (PGPR) strains instead of synthetic chemicals may serve as an effective alternative and environmental friendly practice to improve plant growth, through the supply of plant nutrients and soil productivity [22]. Moreover, it has been found that exploiting these PGPR strains for the growth promotion could reduce the need for chemical fertilizers as well as the cost of cultivation.

Among different groups of biofertilizers, nitrogen fixing and phosphorus solubilizing bacteria may be considered to be important since they improve plant nutrition by increasing N and P uptake by plants and they play a significant role as plant growth promoter of crops [16]. It has been a well-known fact that these PGPR strains

may promote growth either by fixation of atmospheric nitrogen or by solubilization of minerals such as phosphorus [17] and they can also promote growth through production of plant growth regulators [15]. A diverse group of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Xanthzomonas* and *Serratia* have been shown to promote plant growth. During the last couple of decades, the use of PGPR for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation by PGPR have been reported [3]. One possible approach is to explore soil microbial diversity of PGPR, well adapted to particular soil environment. So keeping in view the above constrains, the present study was designed to screen certain rhizospheric bacterial isolates for their multiple plant growth promoting activities on rice.

## Materials and Methods

### Isolation and Identification of Bacteria

Bacterial strains were isolated from rhizosphere soil and root of paddy variety GJ-17 were obtained from Main Rice Research Center, Navagam, Anand, Gujarat. Both the rhizosphere soil and root sample were taken from a depth of 0-15cm, kept in plastic bags in an icebox and carried to the laboratory. Samples were kept in refrigerator at 4 ºC. For rhizosphere soil, clump of soil loosely adhering to the roots were removed and with firmly adhering 10gm of each soil sample were suspended in 90ml of sterile saline (0.85% NaCl/100ml) and agitated at 150rpm for 30min. The soil suspension was diluted with sterile saline to $10^{-1}$ to $10^{-5}$. Then, roots were removed from these suspensions, washed thoroughly under a running tap water for 5min to remove adhering soil particles and then cut into 2-3cm pieces. The root surface of 10gm of each root sample was surface sterilized with 1% chloramines T for 15 min [23]. The root was rinsed with sterilized distilled water and then macerated with mortar and pestle in sterile 0.85g NaCl in 100ml (endorhizosphere). The crushed root was put into 90ml of sterile saline and agitated at 150 rpm for 30min to disperse the bacterial cells from the root. Both samples were diluted and inoculated into vials containing 5ml of semi-solid nitrogen-free medium (NFb) [11] with 0.05g yeast extract/100ml and N-free semi-solid medium with bromthymol blue. After 48hrs. in incubation at 30 ºC, one loop of pellicle forming culture was transferred into fresh semi-solid NFb medium. Cultures with subsurface pellicle were streaked into solid NFb (supplemented with $NH_4Cl$) and congo red medium [6]. Distinct colonies were maintained on nutritive agar plates at 4 ºC.

### Phenotypic Characterization of the Bacterial Isolates

Morphology and Gram staining were determined using a light microscope (1,000X) (Zeiss, Argentina S.A). Pellicle-forming ability and microaerobic $N_2$-dependent growth were assessed in semi-solid NFb medium with different carbon sources. For this purpose, malate was replaced by fructose, glucose, glycerol or sucrose [13].

### Extraction of Genomic DNA and PCR Amplification of nifH gene

For DNA extraction, colonies from bacterial isolates were cultured in 3 ml of liquid 1/2 DYGS medium overnight at 30 ° C. The cells were centrifuged and further used for DNA extraction. Genomic DNA was extracted and purified by use of the Fast DNA spin kit (Qbiogene Inc., CA, USA) according to the manufacturer's protocol. Amplification of the nifH gene from the extracted DNA was performed using the primers Pol F (50 TGCGAYCCSAARGCBGA CTC-30) and Pol R (50-ATSGCCATCATYTCRCCGGA-30). Amplification was performed in 50 ml final volume containing 1 ml genomic DNA (50 ng), 20 pmol each of forward and reverse primer, PolF and PolR, a 200 mM concentration of each of dNTPs (Sigma, USA), 10XTaq polymerase buffer and 2.5 U of Taq polymerase (Sigma, USA). PCR conditions consisted of initial denaturation step at 94 °C for 4 min, 30 amplification cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and primer extension at 72 °C for 2 min; followed by a final extension at 72 ° C for 5 min with MyCycler™ PCR System (BioRad, USA). Aliquots of the PCR products were analyzed in 1.5% (wt/vol) agarose gels (Sigma, USA) by horizontal gel electrophoresis. DNAs were visualized by UV excitation after staining with ethidium bromide (0.5 mg $l^{-1}$). PCR products were eluted from agarose gels, purified and sequenced.

### Evaluation of PGPR for Their Plant Growth Promoting Activities

#### Assay for $NH_3$ Production

Isolates were tested for the production of ammonia in peptone water by Cappucino method [7]. Freshly grown cultures were inoculated into 10 ml peptone water in each tube and incubated for 48 hrs. at 30 °C. 0.5 ml Nessler's reagent was added to each tube. Development to brown to yellow colour was a positive test for ammonia production.

### Exopolysaccharide (EPS) Production

For the estimation of EPS production, the isolates were inoculated into conical flasks containing 100 ml YEM broth supplemented with 1% of carbon source. The inoculated flasks were incubated at 30 °C on a gyro-rottatory shaker at 200 rpm for 72 hrs. After incubation, the culture broth was centrifuged at 3500x g and the supernatant was mixed with two volumes of chilled acetone (Qualigen, India). The crude polysaccharide developed was collected by centrifugation at 3500x g for 30 min. The EPS was washed with distilled water and weighed after overnight drying at 105 °C [10].

### Production of HCN and Catalase

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck [18]. Briefly, Nutrient broth and RBA broth was amended with 4.4g glycine/l bacteria were streaked on modified agar plate. A Whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated for HCN production. Bacterial cultures were grown in a Nutrient agar and RBA medium for 24-72 hrs. at 37 °C and 28 °C. The cultures were mixed with appropriate amount of $H_2O_2$ on a glass slide to observe the evolution of oxygen.

### Acetylene reduction Assay

Bacteria were pre-grown at 37 °C in minimal NFb-medium supplemented with 10 mM $NH_4Cl$, harvested at midlog phase, and washed twice with N-free minimal medium [2]. Equal amounts of

cells of each strain were resuspended in 10 ml of semisolid N-free NFb-medium in 40 ml bottles, which were made airtight with subaseal caps and incubated at 30 °C for the formation of pellicle. After 48 hrs. of incubation, air (10%, v/v) was replaced with acetylene gas, and the bottles were incubated for 24 hrs. These cultures were then used to assay acetylene-reducing activity by measuring the amount of ethylene produced from acetylene using a TRACOR 540 gas chromatograph equipped with a flame ionization detector and a Porapak R column.

**Rice Cultivation and Inoculation**

Seeds of rice variety GJ-17 were washed thoroughly with distilled water followed by surface sterilization with 0.1% $HgCl_2$ solution for 4 min and 70% ethanol for 10 min. The seeds were washed thoroughly with sterile distilled water and kept in a shaker for 5 - 6 hrs. in autoclaved distilled water on a rotary shaker. Later the seeds were transferred to Petri dishes containing tryptone glucose yeast extract agar medium and incubated in dark at 30 °C to test for possible contamination. The germinated seedlings devoid of any contamination were used for inoculation experiments.

To study the effect of the isolated bacteria on the biochemical parameters selected, rice seedlings were transferred to culture tubes containing 400 ml Hoagland's nutrient medium, 400 ml micronutrients and 1% agar in 40 ml distilled water. Before the transfer, bacterial inoculum of the isolated bacteria was added with the medium at a concentration of 6 x $10^8$ cfu $ml^{-1}$. To obtain a mixture of both bacterial cultures, an equal volume of both the cultures were mixed in the medium to give a concentration of 6 x $10^8$ cfu $ml^{-1}$. All experiments were carried in 5 replicates. The tubes were incubated at 27 °C in a 12 hrs. light - dark cycle in a growth chamber.

**Visualization of Association of Bacteria in the Paddy Root**

Association of PGPR within the root was confirmed by TTC staining (2, 3, 5-triphenyl-2H-tetrazolium chloride) which consisted of maleic acid and 1.5 gm of TTC in sterile potassium phosphate buffer (pH 7). Paddy roots inoculated with isolates were surface sterilized with sodium hypochlorite and were incubated overnight in the TTC stain and cross sections of root were examined under image analyzer microscope (Carl Zeiss).

**Effect of PGPR under Greenhouse Conditions**

Plant were transferred to pots containing sterilized sand-perlite (1:1) and kept in a greenhouse. The plants were irrigated with water and with Hoagland nutrient solution once a week.

**Statistical Analysis**

Data were analyzed by one way ANOVA (analysis of variance). All treatments were replicated 3 times, with 7-15 plants per experiment. Differences were considered to be significant at the $P < 0.05$ level. Means were compared by Fisher's protected LSD.

**Results and Discussion**

**Isolation and identification of Root Associated Bacteria**

A variety of beneficial bacteria colonize the roots and aerial parts of rice [19]. Interest in beneficial rhizobacteria associated with rice has increased recently due to their potential use as biofertilizers [20]. The beneficial effects of plant growth promoting rhizobacteria (PGPR) have been attributed to biological N2 fixation [20] and production of phytohormones that promote root development and proliferation resulting in more efficient uptake of water and nutrients [14].

Thirty five bacterial isolates were obtained from the soil samples of paddy field at botanical garden, S.P university, Gujarat using semisolid nitrogen-free medium (Nfb). Isolates obtained from rhizosphere and endorhizosphere of rice plants of variety GJ-17 were mostly Gram-negative and non motile, only few were Gram-positive and motile. Isolates having different colony character and morphology were selected for further identification. The plant growth promoting potentials were determined on three functions. First, N2-fixing efficiency was evaluated and isolates showed $NH_3$ and acetylene reduction activity (ARA). Secondly presence of NifH gene and ability to grow on $N_2$ free medium. Thirdly, isolates showed P-solubilizing efficiencies. On the basis of phosphate solubilizing ability for 12 continuous days, 8 different isolates were selected for further evaluation (table-1). Isolate 1A showed continuous increase in phosphate solubilization activity and it increased to 8.8 times on 12th day, while 1B and 2D showed increase in phosphate solubilization activity upto to 9th day and decreased on 12th day. The isolate 1B had 11 times higher phosphate solubilization activity on 9th day and it decreased to 1.2 times on 12th day. At the same time duration isolate 2D showed 10 times higher phosphate solubilization activity on 9th day and it decreased to 1.4 times on 12th day, while in all other isolates phosphate solubilization activity increased only by 3 times. Out of these 8 isolates, 3 isolates (1A, 1B and 2D) showing the highest phosphate solubilization activity compared to all others, were selected for further identification as after nitrogen, phosphorus is next important parameter for PGPR. Chaiharn and Lumyong, [8] reported that phosphate solubilizing microbes have been routinely isolated from rhizospheric soil of various plants such as rice.

*Table 1- Phosphate released at 3 days interval for a period of 12 days by the isolates*

| | Mean±S.D | | | |
|---|---|---|---|---|
| Isolates | Phosphate released (µg P $ml^{-1}$) on 3rd day | Phosphate released (µgP $ml^{-1}$) on 6th day | Phosphate released (µgP $ml^{-1}$) on 9th day | Phosphate released (µgP$ml^{-1}$) on 12th day |
| 1A | 26.72±1.29 | 97.80±0.10 | 152.5±6.24 | 230.00± 5.63 |
| 1B | 31.20±1.76 | 183.3±0.15 | 337.5±09.04 | 272.5±10.10 |
| 1C | 16.72±1.41 | 77.0±0.15 | 112.5±16.26 | 137.5±09.04 |
| 1D | 24.48±1.25 | 87±0.3 | 80.0±04.26 | 132.5±13.2 |
| 2A | 21.72±0.03 | 67.8±0.11 | 107.0±12.50 | 145.0±9.16 |
| 2B | 21.720±0.04 | 76.8±0.1 | 126.5±08.50 | 157.5±1.25 |
| 2C | 19.48±0.02 | 94.40±0.11 | 135.0±09.71 | 182.5±1.00 |
| 2D | 24.41±0.090 | 96.23±0.25 | 240.5±08.52 | 178.16±1.04 |

*Values indicate mean (±S.D) of three replicates*

**Molecular Identification of Bacterial Isolates**

PCR amplicons of 16S rDNA was sequenced and analyzed using bioinformatics softwares. Distance Matrix based on Nucleotide Sequence Homology (Using Kimura-2 Parameter) and alignment using combination of NCBI GenBank and RDP database were carried out, which are shown in table- 2, 3 and 4, respectively. The sequences were submitted to NCBI data Bank having accession nos. EU921258, EU921259 and FJ602871. The cultures were identified as *P. pseudoalcaligenes*, *B. pumilus* and *Stenotrophomonas maltophilia* respectively. Based on nucleotides homology and phylogenetic analysis the microbe (Sample: YJ1) was detect-

ed to be *Pseudomonas pseudoalcaligenes* (GenBank Accession Number: AB276371). Nearest homolog species was found to be *Pseudomonas mendocina* (Accession No. D84016). Information about other close homologs for the microbe can be found from the Alignment View Table 2.

*Table 2- Alignment View for YJ1 using combination of NCBI GenBank and RDP database*

| Alignment View | ID | Alignment Results | Sequence description |
|---|---|---|---|
| | YJ1 | 0.94 | Studied sample |
| | CP000680 | 0.95 | Pseudomonas mendocina |
| | AB276371 | 0.93 | Pseudomonas pseudoalcaligenes strain :14 |
| | AB276372 | 0.93 | Pseudomonas pseudoalcaligenes strain :15 |
| | D84016 | 0.99 | Pseudomonas mendocina |
| | D84022 | 1.00 | Pseudomonas nitroreducens |
| | Z76666 | 1.00 | Pseudomonas pseudoalcaligenes st.LMG1225T |
| | AF494091 | 0.97 | Pseudomonas nitroreducens strain 0802 |
| | DQ178223 | 0.98 | Pseudomonas mendocina strain PC7 |
| | DQ674859 | 0.98 | Rhizobium sp. CCNWYC 119 |
| | AJ551094 | 0.95 | Hyphomicrobium sp. Isolates wp13 |

Based on nucleotides homology and phylogenetic analysis the microbe (Sample: YJ2) was detected to be *Bacillus pumilus* (GenBank Accession Number: EF32674). Nearest homolog species was found to be *Bacillus subtilis* (Accession No. EF563825). Information about other close homologs for the microbe can be found from the Alignment View Table 3.

*Table 3- Alignment View for YJ2 using combination of NCBI GenBank and RDP database*

| Alignment View | ID | Alignment Results | Sequence description |
|---|---|---|---|
| | YJ2 | 0.95 | Studied sample |
| | EF488975 | 0.98 | Bacillus pumilus Strain BSH-4 |
| | EF491624 | 0.98 | Bacillus pumilus isolates ZB13 |
| | EF032674 | 0.97 | Bacillus pumilus Strain AU17 |
| | AY548949 | 0.97 | Bacillus pumilus Strain 8N-4 |
| | EU620418 | 0.98 | Bacillus pumilus Strain S8-11 |
| | EU624442 | 0.98 | Bacillus pumilus Strain SS-02 |
| | EU231625 | 0.98 | Bacillus pumilus Strain TCCC 11012 |
| | EF197942 | 0.96 | Bacillus pumilus Strain J |
| | EU660365 | 0.96 | Bacillus pumilus Strain CT13 |
| | AY149473 | 0.96 | Bacillus pumilus Strain Bobby 2007 |

Based on nucleotides homology and phylogenetic analysis the microbe (Sample: YJ3) was detected to be *Stenotrophomonas* sp. (GenBank Accession Number: EU816585). Nearest homolog species was found to be *Stenotrophomonas maltophilia* (Accession No. EU430096). Nearest homolog genus was found to be *Pseudomonas geniculata* (Accession No. EU239476). Information about other close homologs for the microbe can be found from the Alignment View Table 4.

*Table 4- Alignment View for YJ3 using combination of NCBI GenBank and RDP database*

| Alignment View | ID | Alignment Results | Sequence description |
|---|---|---|---|
| | YJ3 | 0.88 | Studied sample |
| | EU816585 | 0.98 | Stenotrophomonas sp. MB-1-6-5 |
| | AB294557 | 0.98 | Stenotrophomonas maltophilia St.NCB0306-284 |
| | AY162068 | 0.97 | Gamma proteobacterium |
| | DQ146981 | 0.97 | Vibrio sp. V205 |
| | EU430096 | 0.97 | Stenotrophomonas maltophilia St.776 |
| | EU239476 | 0.96 | Pseudomonas geniculata St.XJUHX-18 |
| | AB021406 | 0.98 | Pseudomonas beteli St.ATCC 19861T |
| | DQ490311 | 0.96 | Xanthomonadaceae bacterium KVD |
| | AY841369 | 0.98 | Xanthomonas retroflexus |
| | FM178869 | 0.99 | Acetobacter pasteurianus St.CCM 3606 |

**Evaluation of PGPR for their Plant Growth Promoting Activities**

All three isolates were positive for acetylene reduction and *P. pseudoalcaligenes* reduces 1.2 times more than *S. maltophilia* and 1.3 times from *B. pumilus*. All three isolates were also positive for EPS production and *P. pseudoalcaligenes* produced 2.3 times more than *B. pumilus* and 1.4 times from *S. maltophilia*. The ESP production is an important trait of bacteria as it helps bacteria to protect against desiccation, phagocytosis and phage attack besides supporting $N_2$ fixation by preventing high oxygen tension [26]. Production of ammonia is an important attribute of PGPR that influences plant growth indirectly [28].All three isolates were positive for ammonia, catalase as well as HCN production as represented in table-5. The ammonia released by the rhizobacteria play a signaling role in the interaction between PGPR [9]. Thus the ability to produce HCN is a desired quality of plant growth promoting rhizobacteria. By synthesizing HCN some rhizobacteria inhibit plant disease development thus strengthening the host's disease resistance mechanism [25]. The presence of HCN in the soil can also act as an efficient biological weed control.

*Table 5- Acetylene reduction activities, EPS, ammonia, catalase, and HCN production potentials of the isolates*

| Isolates | Acetylene Reduction Activity (nmol/h) | EPS (µg/ml) | Ammonia Production | Catalase Production | HCN Production |
|---|---|---|---|---|---|
| B. pumilus | 113$^{bc}$ | 12$^{c}$ | +ve | +ve | +ve |
| P.pseudoalcaligenes | 156$^{a}$ | 27$^{a}$ | +ve | +ve | +ve |
| S. maltophilia | 132$^{b}$ | 20$^{ab}$ | +ve | +ve | +ve |

*For each Parameter, values in columns followed by the same letter are not significantly different at (P≤0.05).*

**Amplification of nifH Gene**

Amplification of nifH gene to confirm the potential for nitrogen

fixation, the presence of the structural gene for nitrogenase reductase (nifH) was determined by nifH gene amplification with genomic DNA extracted from all three representative isolates (shown in fig. 1). Amplification with the gene specific primers yielded the expected 420bp size product on agarose gel. The DNA sequence data matched with the predicted nifH sequence (data not shown) and study was supported by Rösch [24]. Identification of the isolates on the basis of their 16SrRNA gene sequences and the results of ARA and nifH gene amplification show that the majority of the strains isolated from the enrichments in the N-free semisolid medium were PGPR [27].

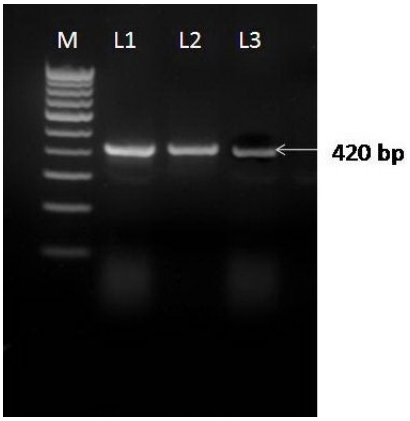


**Fig. 1-** Agarose gel showing the amplified nifH gene of 3 isolates. M is 100bp marker, L-1, L-2 and L-3 lane are of nifH gene *P. pseudoalcaligenes*, *Bacillus pumilus* and *Stenotrophomonas maltophilia* having molecular weight of about 420 bp each respectively

### *In vivo* localization of PGPR in the Root

Bacteria within the plant root could be observed as red coloured cells under the microscope after TTC staining. The presence of bacterial colony in the root cortex region could be clearly visualized as red colored spots because TCC stains living cells (respiring) only, while dead cell of root cortex remains colorless (Fig. 2).

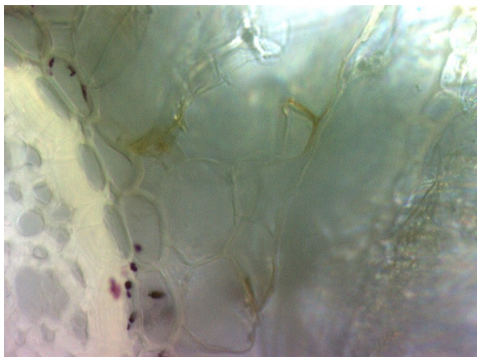

**Fig. 2-** The Photomicrograph of a section of paddy root showing the association of bacteria in root cortex as red spots due to TTC staining.

### Effect of PGPR on Growth of Paddy under Greenhouse

The bacterial isolates *B. pumilus, P. pseudoalcaligenes* and *S. maltophilia* were evaluated under greenhouse condition in pots having soil with following physio-chemical properties; pH: 7.79, electrical conductivity 1063 µS/cm, CEC:3 cmol, organic carbon: 5500 mg per kg , available nitrogen 200 mg per square decimeter, available Ca: 12.1cmol, available P 205 : 9.5 mg per square decimeter, available K 20 : 265 mg per kg, Fe: 3.1 mg per kg , Zn: 285 mg per kg, Mn: 3.7 mg per kg, Cu : 2.2 mg per kg . All the individual isolates and their mixtures significantly enhance plant growth as root length, shoot length and dry weight and among all the combination *P. pseudoalcaligenes* is most effective (table- 6). Plants inoculated with PGPR showed 3-20% enhanced shoot length, 10-60% enhanced root length, 3-25% enhanced plant height and 8-55% enhanced dry weight. In a similar study Adesemoye [1] also confirm growth promotion by one representative each from both species of bacteria *Pseudomonas* and *Bacillus*), but little variations were observed in bacterial effectiveness among parameters and crop types.

*Table 6- Effect of B.pumilus, P.pseudoalcaligenes and S. maltophilia in growth parameter under greenhouse study (n-=5)*

| Bacterial Strain Co-inoculated | Shoot Length (cm) | Root Length (cm) | Plant Height (cm) | Dry Weight (g plant $^{-1}$) |
|---|---|---|---|---|
| Control | $68.8^{h}$ | $11.2^{h}$ | $81.4^{h}$ | $2.3^{h}$ |
| Control + B. pumilus | $70.6^{g}$ | $13.5^{f}$ | $84.2^{g}$ | $2.74^{d}$ |
| Control + P. pseudoalcaligenes | $77.4^{b}$ | $17.1^{b}$ | $95.7^{b}$ | $3.85^{a}$ |
| Control + S. maltophilia | $72.3^{ef}$ | $15.6^{d}$ | $88.3^{ef}$ | $3.63^{b}$ |
| Control + B. pumilus+ P. pseudoalcaligenes | $74.6^{de}$ | $16.3^{c}$ | $91.6^{de}$ | $2.63^{de}$ |
| Control + S. maltophilia + P. pseudoalcaligenes | $76.7^{bc}$ | $14.1^{e}$ | $92.1^{cd}$ | $2.41^{f}$ |
| Control + S. maltophilia+ B. pumilus | $75.7^{cd}$ | $12.2^{g}$ | $89.7^{bc}$ | $2.32^{fg}$ |
| Control + S. maltophilia + B. pumilus + P. pseudoalcaligenes | $82.1^{a}$ | $18.3^{a}$ | $102.1^{a}$ | $2.94^{c}$ |

*For each Parameter, values in columns followed by the same letter are not significantly different at (P≤0.05).*

### Conclusion

An important factor to be considered when screening new isolates is their activity in the range of environments in which they would be expected to be used; in particular different soil types. Further studies are in progress to evaluate the interaction of these PGPR with rice plant under biotic and abiotic stress. Based on these results for plant growth promotion activities and disease resistant ability *P. pseudoalcaligenes, B. pumilus* and S. *maltophilia* in this study is a better PGPR. Rhizosphere bacteria that promote plant growth are considered an alternative to the use of chemicals in agriculture. So, certainly bacteria represent a potentially valuable crop protection tool in high-value cropping systems like cereals and vegetables, where high use of chemicals leads to contamination. In addition to direct control of soil pathogens, the study demonstrated that bacteria represented an attractive alternative to chemical pesticides for systemic protection against pathogens. A major advantage of bacteria is that once systemic resistance is induced, the natural defense mechanism of the plant is operative for prolonged periods even if population of inducing bacteria decline across time.

### References

[1] Adesemoye A.O., Torbert H.A. and Kloepper J.W. (2008) *Canadian Journal of Microbiology* 54, 876-886.
[2] Albrecht S.L. and Okon Y. (1980) *Culture of Azospirillum.*

*Methods in Enzymology*, 69, Academic, New York, 740-749.
[3] Asghar H.N., Zahir Z.A., Arshad M. and Khaliq A. (2002) *Biology and Fertility of Soils* 35, 231-237.
[4] Barea J.M., Toro M., Orozco M.O., Campos E. and Azco´n R. (2004) *Nutrient Cycling in Agro-ecosystems* 63, 35-42.
[5] Buscot F. (2005) *Microorganisms in soils, roles in genesis and functions*, 3-18.
[6] Ca´ceres E. (1982) *Applied Environmental Microbiology* 44, 990-991.
[7] Cappuccino J.C. and Sherman N. (1992) *Microbiology, A Laboratory Manual*, Wesley Pub. Co., New York.
[8] Chaiharn M. and Lumyong S. (2009) *World Journal of Microbiology and biotechnology* 25, 305-314.
[9] Chitra R.S., Sumitra V.C. and Yash D.S. (2002) *Bulgarian Journal of Plant Physiology* 28, 46-56.
[10] Damery J.T., Alexander M. (1969) *Soil Sciences* 103, 209-215.
[11] Do bereiner J. (1995) *Methods in applied soil microbiology and biochemistry*.
[12] Esitken A., ErcilI S., Sevik I. and Sahin F. (2005) *Turkish Journal of Agriculture and Forestry* 27, 37-42.
[13] Hartmann A., Zimmer W. (1994) *Azospirillum/plant associaction*. 15-39.
[14] *Jacoud C.,* Job D., Wadoux P. and Bally R. **(**1999) *Canadian Journal of Microbiology* 45, 339-342.
[15] Jaleel C.A., Manivannan P., Sankar B., Kishorekumar A., Gopi R., Somasundaram R. and Panneerselvam R.(2007) *Colloids and Surfaces B, Biointerfaces,* 60, 7-11.
[16] Karlidag H., Esitken A., Turan M., Sahin F. (2007) *Scientia Horticulturae* 114, 16-20.
[17] Karthikeyan B., Muthuselvam K., Parthasarathi R. Deiveekasundaram M. (2008) *Crop Research* 33, 244-247.
[18] Lorck H. (2004) *Plant Physiology* 1, 142-146.
[19] *Mehnaz S*., Mirza M.S., Haurat J., Bally R., Normand P., Bano A. and Malik K.A. **(**2001) *Canadian Journal of Microbiology and biotechnology* 47, 110-117.
[20] *Meunchang S.,* Panichsakpatana *S*.A., Yokoyama T. **(**2004) *Soil Science and Plant Nutrition* 50, 413-421.
[21] Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pietramellara G. and Renella G. (2003) *European Journal of Soil Science* 54, 655-670.
[22] O'Connell P.F. (1992) *Outlook Agriculture* 21, 5-12.
[23] Patriquin D.G., Dandoring J. Bereiner (1987) *Canadian Journal of Microbiology* 24, 734-742.
[24] R¨osch C., Mergel A., Bothe H. (2002) *Applied Environmental Microbiology* 68, 3818-3829.
[25] Schippers B., Bakker A.W., Bakker R., van Peer R. (1990) *Plant and Soil* 129,75- 83.
[26] Tank N., Saraf M. (2003) *Indian Journal of Microbiology* 43, 37 -40.
[27] Tripathi A.K., Verma S.C., Ron E.Z. (2002) *Research in Microbiology* 153, 579-584.
[28] Wani P.A., Khan M.S., Zaidi A. (2007) *Chemosphere* 70, 36-45.