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Potential Plant Growth-Promoting Activity of Rhizobacteria *Pseudomonas sp* in *Oryza sativa*

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ABSTRACT

Plant growth – promoting rhizobacteria (PGPR) are free living bacteria, isolated from the rhizosphere, which when applied to seeds or crops, enhanced the growth of the plant or reduce the damage from soil–borne plant pathogens. It had been estimated that more than 100 million tones of nitrogen, potash and phosphate – chemical fertilizers had been used annually in order to increase plant yield. The potential negative effect of chemical fertilizers on the global environment and the cost associated with production had lead to research with the objective of replacing chemical fertilizers with bacterial inoculants. Fluorescent Pseudomonas strains were isolated from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. and these Pseudomonas strains showed fluorescent colour on Kings B medium under U.V light used for further studies, The maximum optical density (0.16) was observed at 30˚C which depicts the maximum growth of the bacteria. At low temperatures and with the increase in the temperature the optical density decreases which indicates that the growth of bacteria decreases.

Keywords: *Pseudomonas, Oryza sativa, Oryzaglaberrima. Rhizobacteria, PGPR*

INTRODUCTION

Rice is a seed of the monocot plant *Oryza sativa* (Asian rice) or *Oryzaglaberrima* (African rice). As a cereal grain, it is the most important staple food for a large part of the world's human population, especially in East Asia, South east, and the West Indies It is the grain with the third highest worldwide production, after maize (corn) and wheat in 2011. India is one of the world's largest white rice producer according for 20% of all world rice production. Rice is India's preeminent crop, and is the staple food of the people of the eastern and southern parts of the country

Indian scenario
India accounted for the bulk of this month's downward revision in global production. India's 2012/13 crop was lowered 2.5 million tons to 100.0 million due to a delayed and weak monsoon that has lowered yield prospects for growers dependent on the annual rains. Despite the downward revision, the 2012/13 crop is India's second largest on record and India is still expected to have near-record supplies of rice. Severe problems with disease, weather, and pests have lowered Ecuador's 2012/13 production forecast 250,000 tons to 600,000 tons, little changed from the year-earlier abnormally small crop and weak yield.

Global scenario

Global rice production for 2012/13 is forecast at a record 465.1million tons (milled basis),down almost 1.4 million tons from last month's forecast, but up 1.0 million tons from a year earlier. The bumper crop is the result of expanded area. At 160.0 million hectares, global rice area is up 1.1 million hectares from a year earlier and the highest on record. Southeast Asia and Sub-Saharan Africa account for most of the projected year-to-year expansion in global rice area in 2012/13. The average yield remains forecast at 4.33 tons per hectare, fractionally below the year-earlier record. Global rice supplies are expected to be plentiful in 2012/13. Record crops are projected for four Asian exporters—Cambodia, China, Thailand, and Vietnam. Near-record crops are projected for two additional Asian exporters—India and Pakistan. Among the non-Asian exporters, Australia, Brazil, Egypt, and the United States are project to harvest larger crops in 2012/13. Several major importers are projected to produce record crops in 2012/13.

Health benefits of rice

1. Cholesterol Free: Eating rice is extremely beneficial for health, just for the factthat it does not contain harmful fats, cholesterol or sodium. It forms an integral part of balanced diet.
2. Rich in Vitamins: Rice is an excellent source of vitamins and minerals like niacin, vitamin D, calcium, fibre, iron, thiamine and riboflavin.
3. Resistant Starch: Rice abounds in resistant starch, which reaches the bowel in undigested form. It aids the growth of useful bacteria for normal bowel movements.
4. High Blood Pressure: As rice is low in sodium, it is considered best food for those suffering from high blood pressure and hypertension.
5. Cancer Prevention: Whole grain rice like brown rice is rich in insoluble fibre that can possibly protect against many types of cancers. Many scientists believe that such insoluble fibres are vital for protecting the body against cancerous cells.
6. Dysentery: The husk part of rice is considered as an effective medicine to treat dysentery. A three month old rice plant's husks is said to contain diuretic properties. Chinese people believe that rice considerably increases appetite, cures stomach ailments and indigestion problems.
7. Skin Care: Medical experts say that powdered rice can be applied to cure some forms of skin ailments. In Indian subcontinent, rice water is duly prescribed by ayurvedic practitioners as an effective ointment to cool off inflamed skin surfaces.
9. Alzheimer's Disease: Brown rice is said to contain high levels of neurotransmitter nutrients that can prevent Alzheimer's disease to a considerable extent.
10. Heart Disease: Rice bran oil is said to have antioxidant properties that promotes cardiovascular strength by reducing cholesterol levels in the body.

Plant Growth – Promoting Rhizobacteria

Plant growth – promoting rhizobacteria (PGPR) are free living bacteria, isolated from the rhizosphere, which when applied to seeds or crops, enhanced the growth of the plant or reduce the damage from soil–borne plant pathogens. It had been estimated that more than 100 million tones of nitrogen, potash and phosphate – chemical fertilizers had been used annually in order to increase plant yield. The potential negative effect of chemical fertilizers on the global environment and the cost associated with production had lead to research with the objective of replacing chemical fertilizers with bacterial inoculants.

Plant growth – promoting bacteria are of two types:
1. Those that form a symbiotic relationship with the plants and
2. Those that are free – living in the soil but are found near or even the roots of plants.

The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial. Beneficial free living bacteria referred to as PGPR are found in the rhizosphere of the roots of many different plants. Different bacteria that had been reported as PGPR belong to the following genera: *Pseudomonas, Bacillus, Azospirillium, Agrobacterium, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Enterobacter, Burkholderia, Klebsiella, Clostridium, Vario – vovax, Xanthomonas* and *Phyllobacterium.*
Breakthrough research in the field of PGPR occurred in the mid 1970s with studies demonstrating the ability of Pseudomonas strains capable of controlling soil–borne pathogens to indirectly enhance the plant growth and increased the yield of potato and radish plants. The effect of PGPR on agricultural crops has been investigated and published by various authors in the last two decades with recent applications on trees. Some strains demonstrated the

ability to reduce acetylene and colonize roots of canola when grown at low temperature. Salamone (2000) reported the growth – promoting effect of P.florescence strain G20-18 on wheat and radish plants by production of cytokinin phyto hormones. As the effect of PGPR on plants was demonstrated, the concept of PGPR began to gain importance and a large number of bacterial strains have been isolated, screened and evaluated for plant growth promotion. The mechanisms by which PGPR can exert a positive effect on plant growth can be of two types:

1. Direct
2. Indirect

Rhizosphere bacteria promote plant growth and yield either directly or indirectly. The direct mechanisms of plant growth promotion may involved the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host. The direct growth promoting mechanisms are as follows:

1. Nitrogen fixation
2. Solubalization of phosphorus
3. Sequestering of iron cytokinins, gibberellins
4. Lowering of ethylene concentration.

The indirect mechanisms of plant growth promotion by PGPR include:
1. Antibiotic production
2. Depletion of iron from the rhizosphere
3. Synthesis of antifungal metabolites
4. Production of fungal cell wall lysing enzymes
5. Competition for sites on roots
6. Induced systemic resistance. Several works suggested that combinations of biocontrol agents could be more effective in controlling soil–borne pathogens than a single agent could be more effective in controlling soil–borne pathogens than a single agent.

Induced systemic resistance (ISR) has been reported as one of the mechanisms by which PGPR reduce plant disease, through the manipulation of host plant's physical and biochemical properties. The exact mechanisms by which combinations provide increased plant growth promotion, biocontrol of disease and ISR are not fully understood, although a number of hypotheses, including the synergistic action of antifungal metabolites such as antibiotics and hydrolytic enzymes, have been proposed. PGPR antagonize soil pathogens by competing for resources such as iron, or by the production of antibiotics or lytic enzymes. In China, PGPR had been in commercial development for over 20 years and were referred to as "yield – increasing bacteria" (YIB) that are applied to over 20 million hactares of crops. A PGPR *Pseudomonas*sp.(*fluorescens*B16) isolated from the roots of graminaceous plants had been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit weight of shoots and roots, yield and plant growth. Under salinity conditions inoculation of plants (particularly soyabean) with beneficial microorganisms promotes the plant growth. Another major benefit of PGPR is to produce antibacterial compounds that were effective against certain plant pathogens and pests. Moreover, PGPR mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases.

MATERIALS AND METHODS

Collection of soil sample
Soil sample was collected from the plant rhizosphere from various plants of Bhojia institute of life sciences, Budh (Baddi), Himachal Pradesh garden. **Isolation of *Pseudomonas* sp. from rhizospheric soil:**

For the isolation of *Pseudomonas*sp, serial dilution agar plate technique was followed. 10g Soil sample was collected from the rhizospheric region of healthy plants and mixed in 100ml of sterile distilled water. Now 6 test tubes were taken and labeled as 1 to 6.Each test tube was filled with the 9ml of sterile distilled water. 1ml of suspension was taken from the mixture and poured into the test tube I and this gave the dilution of 10^{-1}. Now 1ml of suspension from dilution 10^{-1} was transferred to next test tube and was labeled as II. This gave the dilution of 10^{-2}. Procedure was repeated up to 6 dilutions. *King's B* agar medium was used for the plating. 1ml of suspension was

taken from the 10^{-5} and 10^{-6} dilutions and then transferred to *King's B*agar medium plates and incubated at 30°C for 4 to5 days. For the isolation of pure bacterial culture streak plate technique was followed.

Streak plate method
Colonies were isolated on the basis of their shape, size, color, opacity and mucosity. With the help of sterilized inoculation loop individual bacterial isolates were streaked on the plates and incubated at 30°C for 4 to 5days.

Biochemical characterization of bacteria
Gram staining
This is a differential staining method used for differentiating bacterial species as Gram positive or Gram-negative on the basis of physical and chemical composition of their cell walls. The smear was prepared from 1-2 drops of culture on clean slide and heat fixed. 1-2 drops of crystal violet solution A was applied on the fixed smear for 1 min and then washed with sterile distilled water. Gram's iodine solution B was applied for 1 min and then washed with 95%alcohol. Finally stain the smear with counter stain safranin for 30 seconds again washed with sterile distilled water. The smear was air dried and examined under light microscope by using oil immersion. The Gram positive bacterial cells appeared violet while gram negative bacteria turned pink to red (Vincent, 1970).

Endospore staining
Endospore stain is used to visualize bacterial Endospore. The smear was prepared and heat fixed. Flooded the slide with 0.5% malachite green and kept the slide over a water bath for 5 minutes. The slide was washed and counter stained with safranin for 30 seconds. Again washed the slide and blot dried. The slide was observed under the oil immersion objective of microscope.

Sugar hydrolysis
Different sugars were tested as isolated bacterial culture has ability to hydrolyze them such as glucose, sucrose, maltose, and xylose. Different sugars in 6 different test tubes were taken, each containing sufficient amount of beef extract and peptone. For the detection of acid formed, the pH indicator i.e. phenol red was added. 5 test tubes inoculated with the bacterial culture and 6^{th} test tube was kept as control and incubated at 30±2°C for 24 hours.

Production of HCN
Nutrient medium was mixed with 4.4g of glycine and bacteria were streaked on the nutrient agar plate. A whatsman filter paper soaked in the solution of 2% sodium carbonate and 0.5% picric acid solution was placed on streaked plates and sealed with parafilm. Plates were incubated at 30±2°C for 4 days. The change in the color of filter paper from orange to red indicates the HCN production.

Ammonia production
Peptone broth was prepared and bacterial culture was inoculated in it. The broth was incubated for 24 hours in test tubes. 0.5g of soil was added to the different test tube and again incubated the test tubes for the 7 days and Nessler's reagent was added. The production of ammonia is indicated by the color changes from yellow to brown precipitate.

Starch hydrolysis
Starch is an insoluble polymer of glucose act as a carbon source for the microorganism andmicroorganism has ability to degrade starch. Starch agar plates were prepared and streaked with the bacterial culture. The plates were incubated at the 30±2°C for 48 hours. After starch incubation plates were flooded with iodine solution. Due to the formation of starch and iodine complex blue black color appeared. The clear zone around streaked culture indicates the degradation of starch due to production of amylase.

Phosphate solubilization
Pikovskaya medium was prepared for the phosphate solubilization test after bacterial inoculation. Plates were incubated at 30±2°C for 4-5 days. The clear zone of solubilization was formed around the colony that indicate the phosphate solubilization.

Nitrate reduction test
Nitrate broth was inoculated with different bacterial culture and incubated at 30±2°C for 48 hours. 3 drops of reagent and 1 drop of sulphuric acid were added in a china dish plate and one drop of culture was added into it. The

blue color appeared and indicates that the nitrite was produced. Zinc dust was also added, if no blue colour appeared that means bacteria reduced nitrate and nitrite was formed.

Urease production test
Bacteria growing naturally in an environment are exposed to the urine and with the help of enzyme Urease they decompose the urea. The plates were streaked with different bacterial cultures and incubated at 30±2°C 4 days. The purple-pink color in the test tubes indicated the positive result for the test.

Citrate utilization test
The microorganism having ability to utilize the citrate as a carbon and energy source for the growth and ammonium salts as the source of nitrogen. The Simmons citrate medium was prepared and the pH was set at 6.8. The plates were streaked with different bacterial cultures and incubated at 30±2°C for 48 hour. Blue colour and growth indicate positive result while original green colour and no growth indicated the negative result.

Voges- proskauer test
The glucose phosphate peptone water medium was prepared and adjusted the pH to 7.6. The broth was incubated with bacterial culture at 30±2°C for 48 hours. 1ml of 40% KOH and 3ml of a 5% solution of α-naphthol is added in absolute ethanol. Development of pink colour indicated the positive result.

Antibiotic resistance test
Nutrient agar media was prepared and autoclaved at 121°C for 15 min. Then this media was poured into petri plates. On cooling, the bacterial broth was spreaded with the help of spreader and antibiotic discs were applied. The plates were incubated at 30±2°C for 24 hours.

Effect of pH and temperature on the isolates
Effect of pH
Nutrient broth was taken in 5 test tubes and their pH was adjusted to 4, 5, 6, 7, and 8 respectively. Then tubes were autoclaved and bacterial culture was inoculated into the test tubes adjusted at different pH and incubated at 30^{o}C for 24 hrs. Absorbance was recorded at 620nm.

Effect of temperature
Nutrient broth was taken in 5 test tubes. All the test tubes were adjusted at pH-7. These test tubes were autoclaved and inoculated with bacterial isolate and then incubated at different temperatures, viz. 28^{o}C, 30^{o}C, 32^{o}C, 34^{o}C and 36^{o}Crespectively for 24 hrs. Absorbance was recorded at 620 nm.

Plantation of Rice plant
For the plantation, we used gritty pots for the pot experiments. These pots filled with the soil that were sterilized and teeming by the loam soil.

For the surface sterilization, the seeds of rice plant were sterilized in the 1% $Hgcl_2$ solution for 2 minutes and washed with sterilized distilled water for 10 minutes to remove the traces of toxic $Hgcl_2$. After the air drying of rice seeds were sown into sterilized pots (3 seeds per pot) and non sterilized pots. Out of four pots two of pots (sterilized and non sterilized) used as control.

Standard 1: Sterilized soil + sterilized seed
Standard 2: Soil + seed
Treatment 1A: Sterilized soil + sterilized seed (Control)
Treatment 2A: Sterilized soil + sterilized seed (Test) + Pseudomonas
Treatment 1B: Non-Sterilized soil + Sterilized seed (Control)
Treatment 2B: Non-Sterilized soil + Sterilized seed (Test) + Pseudomonas

100µl of log culture (10° cells) of bacterial isolates was transferred as inoculums in the corresponding treatments. Treated and non treated pots were irrigated with sterilized water two times daily. After every 7 days interval 100 µl of microorganism inoculum was inoculated in the corresponding pot as booster dose. Pots were irrigated daily and harvested after 20 days. Different plant growth parameters like root length, shoot length, root dry weight, shoot dry weight and leaf numbers were measured.

RESULTS AND DISCUSSION

Fluorescent *Pseudomonas* strains were isolated from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. And these Pseudomonas strains showed fluorescent colour on Kings B medium under U.V light used for further studies, one strain was selected on the basis of its PGPR activity. All strains were Gram negative and morphologically rod-shaped (Table 1 and Fig 1).PGPR strain was grown on *King's B agar* medium (Fig 1). The biochemical characteristic of PGPR strain was studied as described in Bergey's Manual of determinative Bacteriology (Holt *et al.)*.

Fig. 1: Isolated *Pseudomonas sp.*

Table 1: Colony characteristics of isolated *Pseudomonas sp.*:

COLONY SIZE	**LARGE**
SURFACE	**IRREGULAR**
OPACITY	**OPAQUE**
COLOR	**GREENISH YELLOW**

Sugar fermentation

Cultures were transferred in King's B medium broth, which had different carbon sources (Table 2).The isolated bacterial isolates fermented glucose, maltose, sucrose and xylose (figure 2 (a),(b),(c) and (d) as a result of which colour change occurred.

Table 2: Sugar Hydrolysis

S.no.	Carbohydrate source	Observation	Result
1.	**Glucose**	Colour changes from red to yellow.	**Positive**
2.	**Maltose**	Colour changes from red to yellow.	Positive
3.	**Xylose**	Colour changes from red to yellow.	Positive
4.	**Fructose**	Colour changes from red to yellow.	Positive

Table 3: Biochemical test results for the isolated bacterial sp.

S.No.	Biochemical Test	Result
1.	**Catalase Test**	**Positive**
2.	**Citric Acid Test**	**Positive**
3.	**Triple Sugar Iron Test (TSI)**	**Positive**
4.	**Starch Hydrolysis**	Negative
5.	**Indole Production**	Negative
6.	**Nitrate Reduction**	Positive
7.	**Urease Production**	Negative
8.	**Gelatin Hydrolysis**	Negative
9.	**Methyl-Red**	Negative
10.	**Voges- Proskauer**	Negative
11.	**Hydrogen Sulphite**	Negative
12.	**Ammonia Production**	Negative

Biochemical tests

All the strains were found to be Catalase positive (figure 3 (a)), Citric acid positive (figure 3 (b)), phosphate solublization positive (figure 3 (c)), Nitrate positive, TSI positive (figure 3 (d)).After studying all the biochemical characteristics, it has been noted that isolated strains show biochemical characteristics related to *Pseudomonas* genus. These isolated bacterial isolates were further tested for their PGPR activity with rice plant.

On the basis of biochemical tests performed and the software, "Advanced Bacterial Identification software" (www.tgw1916.net/bacteria_logare.html) used for identification, we found that the isolated bacterium is *Pseudomonas* sp.

Antibiotic sensitivity test

The isolated *Pseudomonas*sp. showed resistance against Ciprofloxacin, Erythromycin, Ampicillin and Vanamycin (Table 5.4 and Figure 5.4).The zone of inhibition was observed around all the antibiotics used. The largest zone was observed in Vanamycin which is 15 mm. The Ciprofloxacin and Ampicillin showed 13.3 mm zone of inhibition. The smallest zone of inhibition was observed in Erythromycin and is 11.6 mm

Table 4: Antibiotic sensitivity Test

S.no	Antibiotic	Observation	Result	Zone of inhibition (mm)
1.	**Ciprofloxacin (CX)**	Formation of zone of inhibition around the colony was observed	Sensitive	**13.3**
2.	**Erythromycin (E)**	Formation of zone of inhibition around the colony was observed	Sensitive	**11.6**
3.	**Vanamycin (VA)**	Formation of zone of inhibition around the colony was observed	Sensitive	**15**
4.	**Ampicillin (AP)**	Formation of zone of inhibition around the colony was observed	Sensitive	**13.3**

(a)

(b)

(c)

(d)

Fig. 2: Utilization ability of isolated bacterial strainwithdifferent carbohydrate sources (a): Glucose; (b): Maltose; (c): Xylose; (d) Sucrose

(a)

(b)

(c)

(d)

Figure 3: Biochemical activity results for isolated bacterium (a): Catalase test; (b) Citrate utilization; (c): phosphate solubilization; (d): TSI

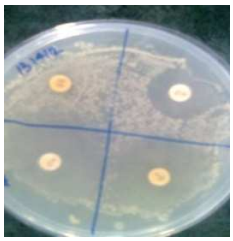


Figure 4: Antibiotic sensitivity Test

The isolated *Pseudomonas* sp. showed maximum growth at pH 6(figure 5) and at temperature 30˚C (figure 6). The maximum optical density (0.13) was observed at pH 6 which indicates that the maximum growth of the bacteria is at neutral pH. It also shows growth at slightly alkaline pH but with increase in pH the growth of bacteria decreases. At very low pH, the optical density (0.1) is very low which indicates that the bacteria have minimum growth in acidic conditions. The maximum optical density (0.16) was observed at 30˚C which depicts the maximum growth of the bacteria. At low temperatures and with the increase in the temperature the optical density decreases which indicates that the growth of bacteria decreases

Table 5: Effect of pHon growth of bacterial isolate *Pseudomonas sp.*

S.No.	pH	Absorbance (620 nm)
1	5	.10
2	6	.13
3	7	.11
4	8	.06
5	9	.04

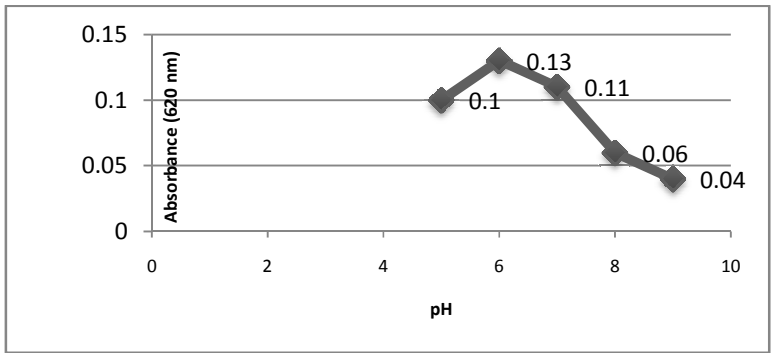


Fig. 5: Effect of pH on growth of bacterial isolate *Pseudomonassp.*

Table 6: Effect of Temperature on growth of bacterial isolate *Pseudomonassp*

S.No.	Temperature (˚C)	Absorbance (620 nm)
1	26	0.8
2	30	0.11
3	35	0.13
4	37	0.16

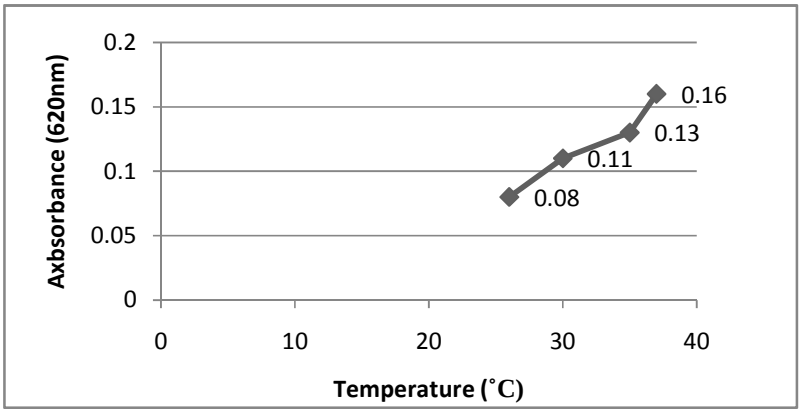


Fig.6: Effect of Temperature on growth of bacterial isolate *Pseudomonassp.*

Result observed after 32 days

Rice plants which were inoculated with the culture of *Pseudomonas* sp. (Table 7 and Fig 7).

Table 7: Effect of *Pseudomonas* sp. (PGPR strain) on shoot length

Days of Plantation	Shoot length (cm)			
	Treatment 1A	Treatment 2A	Treatment 1B	Treatment 2B
Day7	5.2	9.7	0.03	1.1
Day12	5.9	10.4	1.4	1.7
Day17	6.0	11.2	2.0	3.3
Day22	6.5	13.0	3.2	5.33
Day27	7.4	13.7	5.7	8.4
Day32	8.1	15	5.9	10.2

Isolated PGPR's showed positive effect by increase in their shoot length (Table 7 and figure 7), root length and root weight (Table 4.8 and figure 5.9 and 5.10) as compared to un-inoculated plants.

Table 8: Effect of *Pseudomonas* sp. (PGPR strain) on root length and root weight.

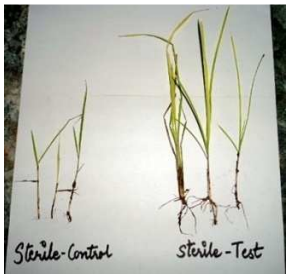
	Treatment 1A	Treatment 2B	Treatment 1B	Treatment 2B
Root length (cm)	3.1	5	2.9	3.3
Wet root weight (mg)	0.014	0.041	0.006	0.029
Dry root weight (mg)	0.004	0.017	0.003	0.017

Also, the growth in the pots containing sterile soil is high as compared to the pots containing non-sterile soil (Figure 6 and 7)

(a) Growth in sterile soil

(b) Growth in non- sterile soil

Figure 5: Growth of Rice in sterile and non-sterile soil



(a) Shoot height and root length in-sterile soil

(b) Shoot height and root length in non-sterile soil

Figure 6: Shoot height and root length of Rice plant

Results revealed that shoot length was increased in the PGPR treated plants over un-inoculated control as shown in Figure. 7. The length of the shoot was recorded on 7thday after plantationand was found that, in treatment 2A and treatment 2B the shoot length is 9.7 cm and 1.1cm respectively which is more as compared to treatment 1A (5.2 cm) and treatment 1B (0.03 cm). On day 12, in treatment 2A the shoot length is 10.4 cm which is more as compare to treatment 1A (5.9 cm). In treatment 1B and treatment 2B the shoot length is 1.4 cm and 1.7cm. On 17th day, in treatment 2A and treatment 2B the shoot length is 11.2 cm and 3.3 cm respectively which is more as compared to treatment 1A (6.0 cm) and treatment 1B (2.0 cm). On 22nd day the shoot length in treatment 2A and treatment 2B is 13.0 cm and 5.33 cm respectively which is more as compared to treatment 1A (6.5 cm) and treatment 1B (3.2 cm). On 27th day the shoot length in treatment 2A and treatment 2B is 13.7 cm and 8.4 cm respectively which is more as compared to treatment 1A (7.4 cm) and treatment 1B (5.7 cm). On day 32, the shoot length in treatment 2A and treatment 2B is 15 cm and 10.2 cm respectively which is more as compared to treatment 1A (8.1 cm) and treatment 1B (5.9 cm). The largest shoot length was recorded in treatment 2A (15 cm) and after that in treatment 2B (10.2 cm). The plants grown in sterile soil (PGPR treated) have large shoot length as compared to the plants grown in non-sterile soil (non-PGPR treated). The PGPR treated plants have increased root length as compared to the un-inoculated control. The root length of treatment 2B is 5 cm which is largest among all the treatments. Treatment 1B has the least root length i.e. 2.9 cm. The wet root weight and dry root weight has shown positive results (Table 8, figure 9 and figure 10). The wet root weight of treatment 2B is 0.041 mg which is highest among all the treatments. Treatment 1B has the lowest wet root weight i.e. 0.006 mg.Treatment1A and treatment 2B have 0.014 mg and 0.041 mg wet root weight.The dry root weight of treatment 2B is 0.017 mg which is highest among all the treatments. Treatment 1A has the lowest dry root weight i.e. 0.001 mg. Treatment 1B and treatment 2B have 0.006 mg and 0.029 mg wet root weight respectively. In treatment 2B the dry root weight has shown positive result as compared to treatment 1B as the weight increases.

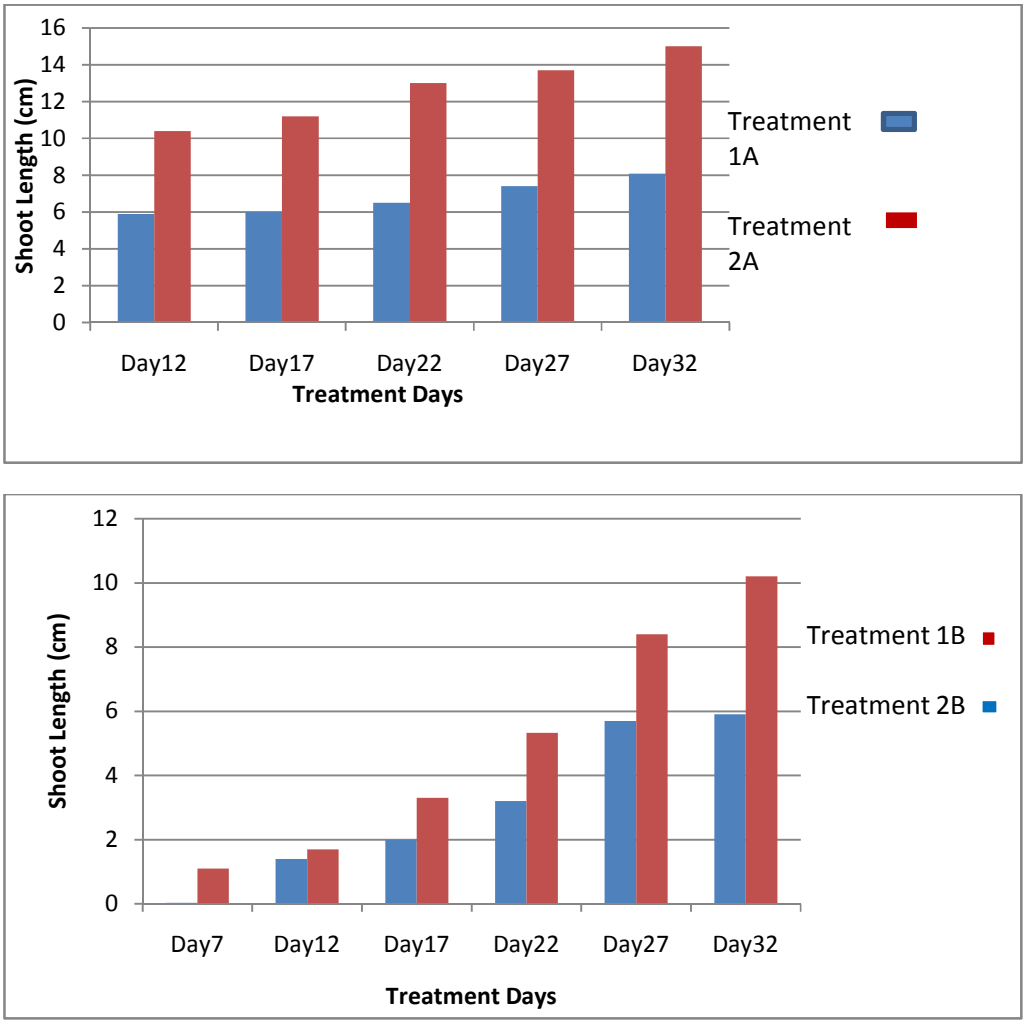


Figure 7: Effect of *Pseudomonas* sp.(PGPR) on shoot length of the treatments.

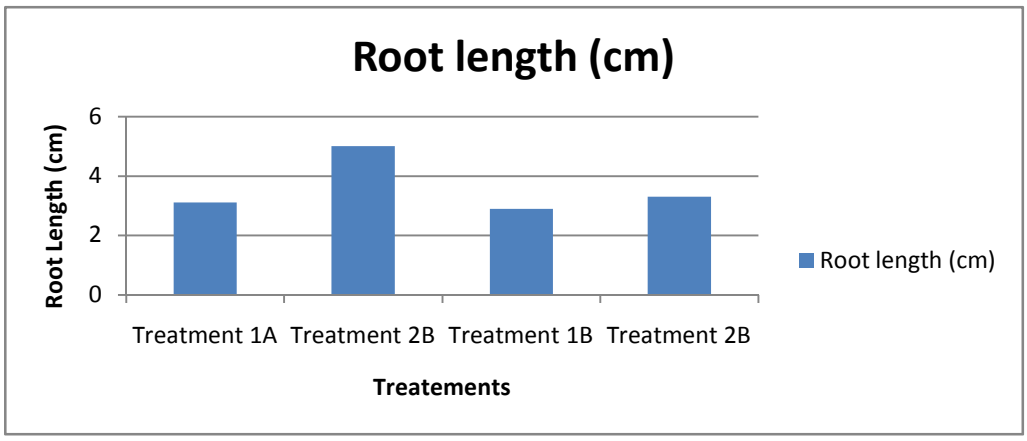


Figure 8: Effect of *Pseudomonas* sp.(PGPR strain) on root length of the treatments

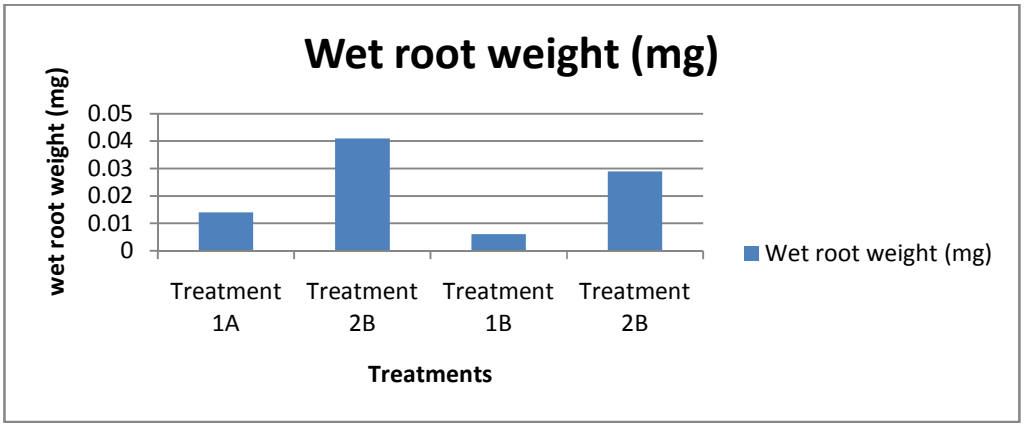


Figure 9: Effect of *Pseudomonas* sp.(PGPR strain) on root wet.

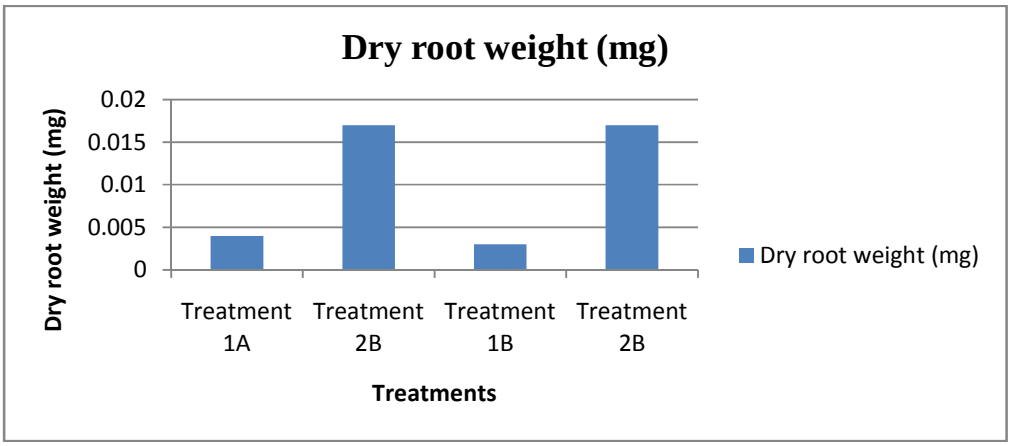


Figure 10: Effect of *Pseudomonas sp.* (PGPR strain) on root dry weight of the treatments.

CONCLUSION

Pseudomonas strain was isolated from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. All strains were Gram negative and morphologically rod shaped (Table 5.1). All *Pseudomonas* strains were grown on Kings B medium (KBM). The biochemical characteristics of all isolated Pseudomonas strains were studied as described in Bergey's Manual of Determinative Bacteriology (Holt *et. al*., 1994).

Sugar fermentation

Cultures were transferred in King's B medium broth, which had different carbon sources (Table 4.2).The isolated bacterial isolates fermented glucose, maltose, sucrose and xylose (figure 4.2 (a),(b),(c) and (d) as a result of which colour change occurred.

Biochemical test

All the strains were found to be Catalase positive (figure 5.3 (a)), Citric acid positive (figure 5.3 (b)), phosphate solublization positive (figure 5.3 (c)), Nitrate positive, TSI positive (figure 5.3 (d)).After studying all the biochemical characteristics, it has been noted that isolated strains show biochemical characteristics related to *Pseudomonas* genus. These isolated bacterial isolates were further tested for their PGPR activity with rice plant.

Results of plantation of rice plant

Result observed after 32 days growth of Rice plant

The *pseudomonas* sp. strain was show positive effect by increasing both roots and shoots length of Rice plant as compared with control (Figure 6). Both dry shoots and dry root weight of plants inoculated by the *pseudomonas* sp.

strain was show positive result as compared with control. Similarly, both wet shoots and dry shoot weight of plants inoculated by pseudomonas sp. strain was also show the positive results as compared with control.

On the basis of microscopic examination and Gram's staining it was confirmed that the isolated bacterial cultures have rod shaped structures and Gram negative. On the basis of biochemical analysis it was recorded that isolated bacterial colony is of *Pseudomonas* sp. Isolated bacteria were tested for their PGPR activity. Isolated rhizobacteria showed positive results same was recorded by De-Freitas *et al*.(1997) and Gaur in (1990). Isolated bacteria show positive results by increasing shoot length, root length and root weight as compared with control. As discussed above same result has been reported by Ashra fuzzaman *et al.,* (2009) that plant growth-promoting rhizobacteria (PGPR) enhanced the rice growth. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable rice cultivation in Bangladesh. Further investigations, including efficiency test under greenhouse and field conditions, are needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on plant growth and development

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