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# Isolation and biochemical characterization of rhizobium from pea crop at Swabi

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**Key words:** Rhizobium, Nodules, Nitrogen fixation, Phosphate solubilization, HCN.

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**Abstract**

Microorganisms like Rhizobium are symbiotic bacteria forming nitrogen fixing nodules on legumes. Nodule inducing bacteria are capable of colonizing the roots of legumes to fix nitrogen and to produce phosphorus, phytohormones, siderophores and HCN. To study the important characters of Rhizobium was the main objective of this study which will help for growth and improvement of Pea. Nitrogen (N) and Phosphorus (P) are the foremost limiting nutrients in agricultural soils of Pakistan. Through the enhancement of these microorganisms the deficiency of these major plant nutrients can be control. From diverse areas of Swabi pea samples were collected. Rhizobium strains were isolated from nodules on Yeast-Manitol Agar medium (YMA) and were characterized bio-chemically for Nitrogenase activity, phytohormone production, phosphate solubalization, siderophores and HCN production. Nitrogenase was determined through calorimetrically. The production of ethylene by the Rhizobium strains were ranges from 20.7 $\mu$ moles $C_2H_2$/hr to 38.4 $\mu$ moles $C_2H_2$/hr. Phytohormone (IAA) was measured through Electro-Spectrophotometer. The qualitative assessment of phosphorus solubalization was made by size of halo zones around the colonies on Pikovskaya agar medium. Rhizobium strains produced IAA (ranging from 14.25-13.65 $\mu$g/ml), Phosphorus solubilized (18-24$\mu$g $ml^{-1}$) and four Rhizobium strains were siderophores positive. Out of seven Rhizobium strains only five strains were able to produce hydrogen cyanide (HCN). Furthermore, biozotes (Biofertilizer) were prepared from inoculation of these Rhizobium strains and were applied in pot experiment to check their effects on pea. All the inoculants showed positive effect on growth and development of peas and significantly increased root shoot length, dry and fresh weight of pea.

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## Introduction

Pea (*Pisum sativum* L.) is believed to have been domesticated in the Middle East (Zohary, D., and M. Hopf. 1973) and possibly Afghanistan (Govorov *et al.,* 1928; Young *et al.,* 1982). From these centers it is presumed to have radiated, giving rise to various present-day pea cultivars. The' roots of pea are normally nodulated by the nitrogen-fixing bacterial strain *Rhizobium leguminosarum* biovar viciae. In this bacterial genus, the designation of species is on the basis of plant nodulation specificity, and as a rule, *Rhizobium* species nodulate one or a few species of plants; the host range of *R. leguminosarum* bv. *viciae* is known to include species of *Pisum*, *Viciae*, *Lens*, and *Lathyrus* (Jordan, D. C. 1984). Pea (*Pisum sativum* L.) is an important source of food in Pakistan and is an important component of our daily. The total area under pea cultivation in Pakistan was 11.689 thousand hectares with total production of 83.603 thousand tons (Javaid *et al.*, 2002). Khyber Pakhtunkhwa shares an area of 1.781 thousand hectare with production of 12.239 thousand tons with an average yield of 6.7 tones $ha^{-1}$ (Minfa, 2009). Non availability of promising varieties, poor production package and lack of sensitive market are major bottlenecks in the production of pea (Ullah *et al.,* 2008).

*Rhizobium* earlier well recognized as a symbiotic $N_2$ fixer and reported as symbiotic microorganisms. In calculation, most inoculation studies have paying attention on free living diazotrophs; even though few information indicate rhizobia can perform as plant growth promoting rhizobacteria (PGPR) (Hoflich *et al.*, 1995; Noel *et al.*, 1996; Yanni *et al.*, 1997). Rhizobacteria live in plant roots and apply a positive achievement range from direct pressure mechanisms to an indirect achievement. Therefore, the bacteria inhabit the rhizosphere and advantageous to plants are known as PGPR (Kloepper, 1980).

The communication of plants with *Rhizobium* has expected significant concentration due to the high requirement for $N_2$ fixation. This was recognized to the information that beneath preventive conditions of N and P, AM fungi improves Phosphorus uptake thus increasing the plants nitrogenase action, which in circle promotes root and mycorrhizal expansion (Abd Alla *et al.,* 2000; Sylvia *et al.,* 1998; Fitter and Garbaye, 1994). Although *Rhizobium* is fit known inoculants for legumes, they are also been used as inoculants for non-leguminous plants (Chabot *et al.*, 1996). Bacteria that inhabit the rhizosphere were classified on the basis of their effects on plants and the method they interrelate with roots, a number of being pathogens while other triggers valuable effects. *Rhizobia* persuade the structure of root nodules on suitable leguminous host plants. Communications between plants and microbes in the rhizosphere (rhizobacteria) can obviously affect crop yield. Rhizobacteria that advantage plant growth and development are called Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper, 1993). Studies of antagonism between *Rhizobium* strains in nodule formation have usually recommended that nodules contain only a single strain (Ullah *et al.,* 2008 & Abd *et al.,* 2000), Even though dissimilar nodules on the same plant might contain different strains (Koo 2009 & Maliha 2004).

## Materials and methods

### *Isolation and biochemical characterization of rhizobium*

Strains of *Rhizobium* sp. were obtained from root nodules of field grown pea plants collected from Swabi. Root nodules of pea were located on the roots with a pink color. Root nodules was sterilize in 0.1% (w/v) sodium hypochloride for 5 min immersed in 95% (v/v) ethanol for 10 s, and then washed six times with distilled water and streaked on yeast extract mannitol agar (YMA) medium containing 0.0025% (w/v) congo red (Vincent, 1970). After 3 days of inoculation at 30°C, single colony was selected and re-streaked on YMA medium for purification.

### *Colony morphology*

Initial inoculums of $10^8$ cell $ml^{-1}$ were prepared in YMA with a pH initially adjusted at 6.8. Colony morphology (colour, mucoidly, transparency, borders, from) was evaluated by streaking a loop of the initial

inoculums on YMA medium and allowing the bacteria to grow in the dark at 30°C for 3, 5 and 7 days (Vincent, 1970).

*Indole acetic acid (IAA)*

The production of indole acetic acid was evaluated according to Asghar *et al.,* (2002). Briefly, rhizobial strains were grown in yeast-mannitol broth YMB (Afzal 2008) supplemented with 50 mg L-1 tryptophan. After 72hr, bacterial cultures were centrifuged at 10.000rpm for 5 min and 60µL of the supernatants generated were placed in micro plates to react with 40µL Salkowski reagent (2mL 0.5 mol $L^{-1}$ $FeCl_3$ + 98mL 35 % $HClO_4$). The mixture was left in the dark for 30 min at room temperature. Samples that turned red were considered positive for IAA production ability.

*Siderophores production*

For the siderophores production assay, the isolates were grown in iron-deficient King's B medium for 72hrs (Ahmad *et al.,* 2008). At the end of this period, bacterial cultures were centrifuge as described previously. An aliquot of 50µL was collect from each supernatant and pipette into a micro plate along with 50µL of chrome azurol-S (CAS) reagent (Schwyn & Neilands, 1987). After 15 min, isolates that was change the color of the reaction mixture from blue to orange was considered positive for siderophores production.

*Determination of nitrogenase activity by colorimetric method*

The nitrogenase activity was measured by using the acetylene reduction assay (Tejera *et al.*, 2005). Single colony of each Rhizobium strain was inoculated in semi solid vial of N-Free Media (NFM) and incubated for 48 hours at 30°C. The cotton plugs on vials were replaced with suba seals and 10% v/v acetylene. One ml of acetylene per vial was injected. The vials were incubated for 24 hours at 30°C. Production of Ethylene was measured by Spectrophotometer.

*Effect of rhizobium on growth and germination of pea*

To check the effect of Rhizobium on growth and germination of peas study was conducted. It was done in control environment. In the current experiment 7 Rhizobium strains were applied on germination of pea (9001) seeds. Eight treatments with 3 replicates and one control with no inoculation. This experiment was conducted in (CRD) complete randomized design. Treatments were as follow:

*Detail of Treatments*

| | | | |
|---|---|---|---|
| Control (uninoculated) | | $T_3$ | Rhizobium-P3 |
| | | $T_6$ | Rhizobium-P6 |
| $T_1$ | Rhizobium-P1 | $T_4$ | Rhizobium-P4 |
| | | $T_7$ | Rhizobium-P7 |
| $T_2$ | Rhizobium-P2 | $T_5$ | Rhizobium-P5 |

Seeds of peas were surface sterilized with $H_2SO_4$ for 1 min, and after that seeds were washed 2 to 3 times with DDO, and were carefully covered with water in culture of all bacterial isolates individually. Seeds, which were taken as control, were only covered with sterilized distilled water. Three seeds were sown in each pot having 0.7kg autoclaved soil for 1 month in growth chamber. Seed germination was observed on 6 day. Autoclaved distilled water was given to each pot daily during this period. Plants were harvested after 1 month and the following growth parameters were recorded

*Shoot/ Root length (cm)*

Plant Shoot / Root Length was calculated in cm with the help of measuring device
1 month of sowing.

*Shoot/ Root fresh weights (mg)*

Shoot / Root fresh weight was recorded in mg after 1 month of sowing.

*Root/ Shoot dry weights (mg)*

Root/Shoot dry weight were recorded by oven drying them at 65°C.

*Number of leaves/ plant*

Number of leaves/Plant was counted after 1 month of growing.

*Statistical analysis*

All of the experiments were done in three replicates. The data obtained were subjected to analysis of variance (ANOVA) using software STATISTIX 8.1 and means obtained were compared by Least Significance Difference (LSD) test at 5 % level of significance (Steel *et al.*, 1997).

**Results**

*Isolation and Morphological Characterization of Rhizobium*

*Isolation of rhizobium from nodules*

Total ten samples were collected from different areas of Swabi district out of which only in seven samples nodules were present. First of all roots were washed thoroughly to remove soil and other particles. About 10 nodules were taken from each plant/sample. Harsh the nodules from the root by cutting the root about 0.5cm on each side of the nodules. The root appendages were used to control the nodule with forceps and reduce the risk of damaging the nodule.

For surface sterilization the intact, pure nodules were Immerse for 5-10 seconds in concentrated hydrogen peroxide ($H_2SO_4$). Then the nodules were dip for 15 seconds in dilute hydrogen peroxide. After sterilizing in hydrogen peroxide 3-4 wash were given with sterilized water. Then the sterilized nodules were crushed with a pair of blunt tipped forceps in a large drop of sterilized water in a Petri plate. After crushing one loop full of the nodule suspension was streaked on yeast-mannitol agar (YMA) plates. The plates were kept in incubator for 24 hours to observe growth. After 24 hours the following growth was observed.

**Table 1.** Morphological characterization of rhizobium.

| Isolates | Grime Staining | Cell Morphology | Opacity |
|---|---|---|---|
| Rhizobium-P1 | - | Bacillus | Translucent |
| Rhizobium-P2 | - | Bacillus | Opaque |
| Rhizobium-P3 | - | Bacillus | Translucent |
| Rhizobium-P4 | - | Coccus | Opaque |
| Rhizobium-P5 | - | Bacillus | Translucent |
| Rhizobium-P6 | - | Coccus | Translucent |
| Rhizobium-P7 | - | Bacillus | Opaque |

**Note:** P=Pea.

*Morphological characterization of rhizobium*

All the Rhizobium strains were characterized for colony and cell morphology, gram staining, and opacity. All of the 6 Rhizobium strains were gram negative and were observed as bacillus except Rhizobium-P5 and Rhizobium-P7 in cell morphology (Table 1).

**3.2.1** *Quantification of phytohormone (IAA)*

All of the seven Rhizobium strains were subjected to test IAA activity. All the strains produce high concentration of IAA ranging from 14.25µg/ml to 10.50µg/ml (Table 2). Amongst these isolate Rhizobium-P7 and Rhizobium-P1 produced maximum amount of IAA 14.25-13.65µg/ml. The smallest amount was detected by Rhizobium-P1 which was 10.50µg/ml (Figure A).

*Siderophores production*

Rhizobium strains were experienced for production of Siderophores. Siderophores was detected using blue agar assay. Yeast Manitol Agar (YMA) media was prepared and mixed with chrome azurol-S (CAS) as indicator. Rhizobium was sported in them. (Ahmad *et al.,* 2008). After incubation of 72hrs, isolates that alter the color of the plates from blue to orange was precise as positive for siderophores production (Figure B). Out of seven Rhizobium strains only 4 were Siderophores producers (Table 2).

*Phosphate solubalization of isolates in broth cultures*

Rhizobium strains were also subjected to test for

Phosphate solubalization. These strains were inoculated Yeast Manitol Broth (YMB). The culture inoculated flasks were placed on rotatory shaker for 5 days. The available P was quantified after 5 days of incubation. The P solubilized by Rhizobium was expressed as available P (%) in broth culture. Maximum % P was solubilized by Rhizobium-P7 (24$\mu$g ml$^{-1}$) followed by Rhizobium-P6 20$\mu$g ml$^{-1}$ (Table 2).

**Table 2.** Biochemical characterization of rhizobium strains.

| Isolates | Indole Acetic Acid Concentration ($\mu$g/ml) | Siderophores Production | Phosphate solubalization (µg ml$^{-1}$) | µ moles $C_2H_2$/Hr | Hydrogen Cyanide Production |
|---|---|---|---|---|---|
| Rhizobium-P1 | 13.65 | + | 19 | 20.7 | + |
| Rhizobium-P2 | 11.02 | - | - | 38.4 | + |
| Rhizobium-P3 | 10.50 | + | - | 29.1 | - |
| Rhizobium-P4 | 12.25 | - | 18 | 35.4 | - |
| Rhizobium-P5 | 11.30 | - | 20 | 30.3 | + |
| Rhizobium-P6 | 12.10 | + | - | 37.5 | + |
| Rhizobium-P7 | 14.25 | + | 24 | 33.8 | + |

**Note:** P=Pea.

*Determination of nitrogenase activity*

Nitrogenase activity is generally determined by Gas chromatograph but in case of absence there is another protocol of nitrogenase determination by colorimetric method. So in the present study the nitrogenase was determined calorimetrically by acetylene reduction assay (Figure C). The production of ethylene by the Rhizobium isolates were ranges from 20.7$\mu$moles $C_2H_2$/hr to 38.4$\mu$moles $C_2H_2$/hr. Maximum ethylene was produced by Rhizobium-P2; 38.4$\mu$moles $C_2H_2$/hr following by Rhizobium-P7; 37.5$\mu$moles $C_2H_2$/hr (Table 2).

*Effect of rhizobium on growth of peas in pot experiment*

Pot experiment was conducted to test the Rhizobium strains for growth development of pea. It was find out that each isolate is capable positive effect on growth of pea (Figure C)

*Root/Shoot Length (cm)*

Plants Root and Shoot length were recorded after 1 month of sowing and it was observed that all of the bacterial strains increased Root/Shoot length considerably greater than control (un-inoculated) (Figure C).

Shoot length ranges from 10.5-23.3cm. Maximum shoot length was observed in T6 (23.3 cm) inoculated with Rhizobium-P6 following by T7 (Rhizobium-P7) in increasing shoot length; 21.0cm. Minimum shoot length was recorded in control (uninoculated) 10.5 cm (Table 3). All of the treatments inoculated with bacterial strains were statistically highly significant over control. Among the treatments shoot length of T6 inoculated with (Rhizobium-P6) was highly significant over T2 (Rhizobium-P2). T1 (Rhizobium-P1), T3 (Rhizobium-P3), T4 (Rhizobium-P4), T5 (Rhizobium-P5) and T7 (Rhizobium-P7) were significantly same (Figure D).

Root length of the pea was ranges from 5.6-16.5cm. Highest root length was observed in the T5 (Rhizobium-P5) following by T3 (Rhizobium-P3); 11.5cm. Root length of all the treated plants were highly significant than control. Among the treatments

T5 (Rhizobium-P5) was highly significant over T2 (Rhizobium-P2), T4 (Rhizobium-P4) and T7 (Rhizobium-P7). T1 (Rhizobium-P1), T3 (Rhizobium-P3) and T6 (Rhizobium-P6) were same to each other but significant over control (Table 3).

**Table. 3.** Root/Shoot length and No of leaves/Plant.

| Treatment | Root length (cm) | Shoot length (cm) | No of leaves per Plant |
|---|---|---|---|
| Control | 5.6667 C | 10.5000 C | 9.0000 C |
| T1 (Rhizobium-P1) | 15.667 AB | 21.333 AB | 17.667 AB |
| T2 (Rhizobium-P2) | 14.500 B | 19.000 B | 19.000 AB |
| T3 (Rhizobium-P3) | 16.500 AB | 20.667 AB | 16.000 B |
| T4 (Rhizobium-P4) | 14.333 B | 20.667 AB | 19.000 AB |
| T5 (Rhizobium-P5) | 13.833 A | 20.667 AB | 20.000 A |
| T6 (Rhizobium-P6) | 12.167 AB | 23.333 A | 18.667 AB |
| T7 (Rhizobium-P7) | 14.333 B | 21.000 AB | 18.333 AB |
| LSD value (5%) | 3.2692 | 3.9145 | 3.9660 |
| Standard Error | 0.7569 | 1.3320 | 1.3123 |

**Note:** T=Treatment, P=Pea.

*Number of leaves/plant*

Numbers of leaves were counted after 30 days. Number of leaves ranges from 9-20/Plant (Table 3). Highest no of leaves was found in T5 inoculated with Rhizobium-P5 (20 leaves/Plant) following by T4 (Rhizobium-P4) 19 leaves/Plant (Figure 3). All the treated plants produced significantly more number of leaves than control. T5 (Rhizobium-P5) was highly significant than T3 (Rhizobium-P3). Among the treatments T1 (Rhizobium-P1), T2 (Rhizobium-P2), T4 (Rhizobium-P4), T6 (Rhizobium-P6) and T7 (Rhizobium-P7) were statistically same (Table 3). Minimum number of leaves was produced by uninoculated control (9 leaves/Plant).

**Table. 4.** Root/Shoot fresh and dry weights.

| Treatment | Root fresh weight (g) | Shoot fresh weight (g) | Root dry weight (g) | Shoot dry weight (g) |
|---|---|---|---|---|
| Control | 0.5667 B | 1.1333 B | 0.1333 B | 0.2667 C |
| T1 (Rhizobium-P1) | 1.0000 AB | 1.7333 A | 0.4333 A | 0.6333 AB |
| T2 (Rhizobium-P2) | 1.0000 AB | 1.5333 AB | 0.4000 AB | 0.7000 AB |
| T3 (Rhizobium-P3) | 1.1000 A | 1.5667 AB | 0.4333 A | 0.5000 BC |
| T4 (Rhizobium-P4) | 0.9333 AB | 1.7667 A | 0.3667 AB | 0.6333 AB |
| T5 (Rhizobium-P5) | 0.9000 AB | 1.7333 A | 0.3667 AB | 0.7667 AB |
| T6 (Rhizobium-P6) | 1.1667 A | 1.8333 A | 0.5000 A | 0.7333 AB |
| T7 (Rhizobium-P7) | 1.1667 A | 1.9667 A | 0.5000 A | 0.8333 A |
| LSD value (5%) | 0.5136 | 0.4550 | 0.2946 | 0.3165 |
| Standard Error | 0.1047 | 0.0928 | 0.0601 | 0.0645 |

**Note:** T=Treatment, P=Pea.

*Root/shoot fresh weight (mg/plant)*

Root/Shoot fresh weight was much improved by inoculation with Rhizobium and PGPR strains. Shoot fresh weight of one month old plants were measured, ranges from 1.1-1.9g/plant (Table 4). Highest shoot fresh weight was recorded in T7 due to inoculation with Rhizobium-P7 1.9g/plant, which is statistically at the bar with T1 (Rhizobium-P1), T3 (Rhizobium-P3), T4 (Rhizobium-P4) and T5 (RhP5).

Root fresh weight of treatments was significantly high than control. Root fresh weight of pea plants were ranges from 0.56-1.16g/plant. The maximum root fresh weight was increased by the isolate T5

(Rhizobium-P5) and T6 (Rhizobium-P5) 1.16g/Plant. Fresh root weight of uninoculated (control) was observed as 0.56g/plant, which was statistically smaller than all of the treatments, inoculated with Rhizobium and PGPR strains (Table 4). All the treatments were significantly same but high than uninoculated control.

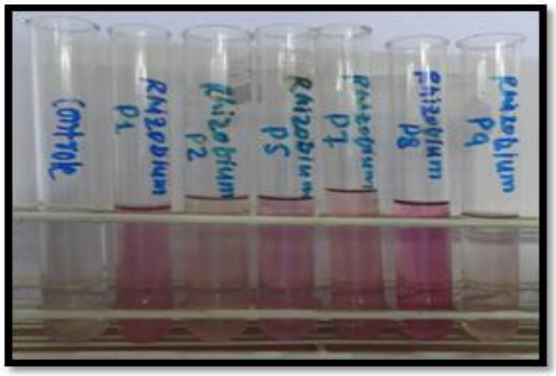


**Fig. 1.** Quantification of IAA by Rhizobium strains.

*Root/shoot dry weight (mg/plant)*

The Root/Shoot was oven dried on 65°C and weighed after 30 days of growing. Root/Shoot dry weight was better appreciably, observed in all of the treatments inoculated with PGPR and Rhizobium strains (Table 4).

Shoot dry weight ranges from 0.26-0.83g/plant. Maximum shoot dry weight was measured in the T7 (Rhizobium-P7) 0.83g/Plant which was highly significant statistically over T3 (Rhizobium-P3) among the treatments. Among the treatments T1 (Rhizobium-P1), T2 (Rhizobium-P2), T4 (Rhizobium-P4), T5 (Rhizobium-P5) and T6 (Rhizobium-P6) were statistically same but highly significant over control (Table 4).


**Fig. 2.**Siderophores Producing Rhizobium Strains.

Root dry weight ranges from 0.13-0.50g/plant. Highest root dry weight was recorded by which is statistically at bar with T6 (Rhizobium-P6) and T7 (Rhizobium-P7) 0.50g/plant which is statistically at bar with T3 (Rhizobium-P3). Among the treatments T2 (Rhizobium-P2), T4 (Rhizobium-P4) and T5 (Rhizobium-P6) were statistically similar. Minimum root dry weight was observed in the uninoculated (control) 0.13g/plant (Table 4).

**Discussion**

The objective of the current study was to study the important characters of Rhizobia which will be accommodating the growth development of Pea in Swabi. Pea (*Pisum sativum* L.) is a major resource of food in Pakistan and is a vital component of our daily dishes. The total area in pea cultivation in Pakistan is 11.689 thousand hectares (Javaid *et al.*, 2002). Khyber Pakhtunkhwa comprise an area of 1.781 thousand hectare with production of 12.239 thousand tons with an average yield of 6.7 tones $ha^{-1}$ (Minfa, 2009).

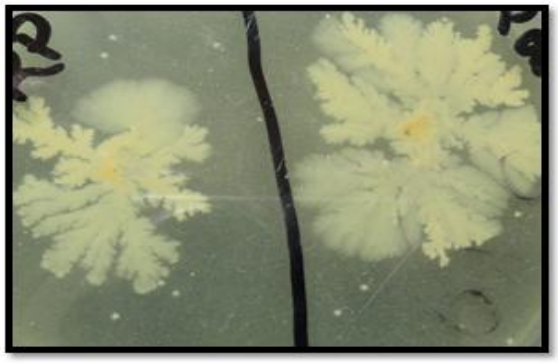

**Fig. 3.** Nitrogenase Activity of Rhizobium Strains.

Rhizobium occupies a vigorous position in soil through their natural capability to supply vital nutrients to the plants. Among the plant nutrients, P and N are the 2 major plant nutrients supplied by these bacteria naturally. In this condition, the inoculation properties of Rhizobium including symbiotic $N_2$ fixation, phosphate solubalization (Whipps 2001) and phytohormone production, siderophores and HCN production (Ahmad *et al.,* 2008) are getting enhanced meditation for their use to extend microbial inoculants in order to increase production.

Ten samples of pea were collected from diverse sites in district Swabi out of which only 7 samples having nodules from which Rhizobium strains were isolated and characterized for nitrogenase activity, phosphate solubalization IAA production, HCN and siderophores production.


**Fig. 4.** Comparison among inoculants & Control.

Nitrogenase was determined through calorimetrically. The production of ethylene by the Rhizobium strains were ranges from 20.7$\mu$moles $C_2H_2$/hr to 38.4$\mu$moles $C_2H_2$/hr (Table 2) which are in the line of (Maliha *et al.*, 2004 and Sahin *et al.,* 2004).

IAA was measured in the presence of L-tryptophan (Asghar *et al.,* 2002). In culture solution the production of IAA was 10.50-14.25$\mu$gml$^{-1}$ (Table 2). The current results are according to Ahmad *et al.*, (2008). Who isolated 72 bacterial strains of rhizobacteria from rhizosphere. IAA was produced at a significant amount by most of the isolates.

Among the seven strains of Rhizobium only four strains were competent to solubilized phosphate. The P-solubalization of the Rhizobium strains reduce in the series specified by Biswas *et al.,*(1980) and reported by Illmer and Schinner (1992) which is from 60 to 99$\mu$gml$^{-1}$, while the P-solubilized in the current study was measured through spectrophotomer which were recorded in range of 18-24$\mu$gml$^{-1}$ by Rhizobium (Table 2).

HCN a secondary metabolite produced by numerous Rhizobium strains is provide protection to plants against weeds and various pathogens therefore serve as bio-control agent (Sahin *et al.,* 2004). Out of seven Rhizobium strains only five strains were able to produce hydrogen cyanide (Table 2).

Siderophores is a bio-molecule of low molecular weight, which helps to plant in Iron acquisition and also have antagonist effect against plant pathogenic microbes (Datta M. 2011). Among seven rhizobium isolates only four Rhizobium strains were siderophores positive producers (Table 2). These investigations are in line with (Bashan *et al.,* 2004) studied that some rhizospheric bacteria can produce siderophores.

Furthermore these Rhizobium strains were evaluated in pot experiment for their effect on growth and yield of pea. For this purpose inoculums were prepared with most excellent proficiency with crop and synergistic or neutral interface amongst the microbes and applied in different pots. Diverse growth and yield parameters were taken in this study. Development of growth of Plant by Rhizobium in various crops is obviously confirmed (Cheung *et al.*, 2005).

The current observations of the pot experiment of pea was close to Fischer *et al.* (2007), who find out the ability of Rhizobium on wheat by development of shoot/ root fresh and dry weights. All the Rhizobium strains improved the root and shoot dry biomass by 100% and 70% respectively. The current conclusion goes to the results of (Hilali *et al.*, 2001; Ahmad *et al.*, 2008 and Khalid *et al.*, 2004), who obtained 70% increase in pea root/shoot dry biomass by PGPRs inoculation as contrast to control (uninoculated).

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