*Available online at: www.awkum.edu.pk/PJLS*

# Plant growth promotion by locally isolated IAA producing rhizobacteria

**Saima Noor and Anwar Hussain***

Department of Botany, Shankar Campus Abdul Wali Khan University Mardan, Pakistan

*Corresponding author: anwarhpu@yahoo.com

## ABSTRACT

Fifteen strains of rhizobacteria were isolated from the rhizosphere of wheat grown in district Sawabi. Nine strains were able to produce IAA in the culture media. Production of IAA was modulated by time of incubation, temperature and tryptophan concentration in the culture media. Optimum conditions for IAA production were incubation of 24 hours at 28 C in culture media containing 1000 µg $mL^{-1}$ of tryptophan. Maximum IAA (268 µg $mL^{-1}$) was recorded in the culture of WC-5 when grown for 24 hours in the presence of 1000 µg $mL^{-1}$ of tryptophan. Most of the strains were able to mobilize phosphate on agar plate. Growth of okra was promoted under natural condition when its seeds were primed with the selected rhizobacteria. Root growth was significantly enhanced (74%) in seedlings inoculated with WC-9 as compared to that of control seedlings. Other growth parameters such as fresh and dry weight and chlorophyll contents were also significantly enhanced by IAA producing strains. It may be concluded that wheat rhizosphere host friendly rhizobacteria which produce IAA, solubilize phosphate and enhance plant growth.

Key words: Rhizobacteria, IAA, Phosphate solubilisation, Plant growth promotion

## Introduction

The portion of soil beside and inclined by roots is called rhizospheres [1], or the rhizospheres is defined as the soil directly influenced by root secretions with intensive microbial activities [2]. A gradual rhizospheres effect is created by the particular growth conditions found in proximity to roots [3] and leads to the selection of the microbial populations modifying the diversity of bacteria [4]. Its biotic composition performs an absolutely vital function for root metabolism and development, the microbial community actively participates in defining the composition of the rhizospheres by degrading and secreting complex organic compounds, and by lysing plant cells. These types of molecules are part of mucilage's and lysates, respectively [5]. Bacteria which are isolated from terrestrial rhizospheres have more capacity to produce and release auxins, e.g. IAA, as secondary metabolites than bacteria which are isolated from bulky soil because of more and rich supply of substrates is available in the rhizospheres environment [6,7].

Bacteria isolated from rhizospheres increase the growth of plants when applied to the seeds of crops are called as plant growth promoting rhizobacteria (PGPR). They have the ability to reduce the harmful effect of soil borne plant pathogens. Plant growth promoting rhizobacteria may enhance the plant growth directly or indirectly. It has also been reported that such bacteria increase the root length and root surface area [8].

Rhizospheric bacteria produce plant growth regulators *in vitro*, such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene [9,8]. About 80% rhizospheric bacteria can synthesize IAA [10]. Rhizospheric bacteria which produce IAA can affect the levels of endogenous plant auxin . Most of the PGPR are accepted to produce IAA, IAA-producing PGPR are accepted to increase root growth, development and producing larger surface area to allow the plants to get more nutrient from the soil [8].

Mobilization of insoluble phosphate is another important tool of rhizobacteria employed for promoting plant growth by making this element available to plant root for absorption.

Objective of the current study was to investigate the potential of local rhizobacteria (the unexplored microbial flora) for IAA production and plant growth promotion.

## 1. Material and Method

Isolation of Rhizobacteria

Two different plant species were selected for the isolation of Rhizospheric bacteria and studying their effect on plant growth and development. The selected plants were Wheat (*Triticum Aestivum*) and Garlic (*Allium Sativum*). Healthy plants of *Allium Sativum* and *Triticum aestivum* were collected from the local regions of district Swabi, Khyber Pukhtonkhwa, Pakistan. The selected plants were first identified, then digged out as whole, sealed in paper bags, labelled and carried to the Plant physiology laboratory, Department of Botany, Shankar campus of Science, Abdul Wali Khan University Mardan. Collected Plants were carefully stored at 4˚C till further processing.

Rhizobacteria were isolated from roots of the selected plants as described previously [8]. Purity of the cultures was established by examining the smears of rhizobacteria after subjecting them to Gram's straining.

Screening of isolates for IAA production

Production of IAA by axenic cultures of our isolates was done as described by Hussain and Hasnain [8]. All the test strains of bacteria were screened for IAA production. Bacterial cultures were inoculated in the sterilized L-Broth medium and incubated in the shaking incubator at 28˚ C for 24 hours. Bacterial cell were harvested by centrifuging the overnight cultures at 12000 rpm for 2 minutes. 1 mL of the supernatant was mixed with 2 mL of salkowski reagent. Development of pink colour after 30 minutes of incubation at room temperature in dark indicated the presence of IAA in the cultures.

Optimizing condition for IAA production

The bacterial strains containing IAA were then selected for the optimization. To check the concentration of IAA production in the bacterial strains already checked for IAA were grown at the following conditions. Rhizobacteria were inoculated in L-Broth medium containing different amount of tryptophan (0 µg $mL^{-1}$, 500 µg $mL^{-1}$, 1000 µg $mL^{-1}$) and incubated in shaking incubator for 24 hours, 48 hours and 72 hours. Incubation temperature was 28 and 37 ˚C. Bacterial cultures were centrifuged at 12000 rpm for 2 minutes. IAA was quantified in culture supernatant by colorimetric method. Bacterial growth was checked by noting optical density at the wave length of 600.

Phosphate solubilisation

Phosphate solubilisation ability was assayed on plates containing Pikovskaya medium [11] supplemented with 1.2% agar. Pure Bacterial colony was picked with sterile inoculation loop and stabbed on agar plate. Three plates were inoculated per Bacterial strain. After incubationof 3 days at 30 ˚C the

plates were observed for clear zone around Bacterial growth.

Plant Growth Experiment

Experiment was designed to investigate the influence of 13 isolated bacterial strains on the two crop species okra (*Abelmoschus Esculentus*) plant. Each treatment was replicated five times. Effect of the rhizobacteria was then determined by comparing the plants treated with the strains with control plants. Inoculumn dessity of bacterial cultures was set to $10^6$ cfu. Selected seeds of okra and chilli were soaked for 1 hour in bacterial suspension separately. All the experiments were performed in the laminar flow.

The pots were prepared by filling sterilized soil in the pots. Five replicates of isolated strain were germinated in the prepared pots. Two seeds were sown in each pot and allowed to grow for two weeks. Different growth parameters such as root length, shoot length, root number, chlorophyll content, fresh weight and dry weight were checked out.

Statistical Analysis

Data was analysed for significance by ANOVA and Duncan multiple range test (p=0.05) by using SPSS (16.0) for window.

## 2. Results

Strain Isolation and screenin for IAA

Bacterial isolates from the rhizospheres of *Triticum Aestivum* were labelled as WC-1, WC-2, WC-3..... WC-15. Isolated colonies from the rhizospheres of *Allium Sativum* plates were labelled as Com-1, Com-2, Com-3 ...Com-15 and were also further purified.

Nine of the isolates from the rhizosphere of whear and 3 from that of garlic were able to produce IAA. Cell morphology is shown in Table-1.

Effect of culture conditions of growth of bacteria

All the strains were grown in L-Broth medium with 0, 500, 1000 µg $mL^{-1}$ concentration of tryptophan at 28 or 37 ˚C. Growth of rhizobacteria was significantly modulated by time, temperature and tryptophan concentration in the culture media. Strain WC-5 showed time and tryptophan dependent growth. Optimum temperature for its growth was 28 ˚C (Figure 1). Strain WC-7 was time dependent and also tryptophan dependent and the more suitable incubation temperature was 28 ˚C. The strain grew maximally in media having 1000 µg $mL^{-1}$ tryptophan at day 3 when incubated at 28 ˚C (Figure 2).

Table: 1 Morphological cell characteristics of 13 selected Bacterial isolates

| S. | Strains | Gram's | Shape |
|---|---|---|---|
| 1 | WC-1 | Positive | Cocci |
| 2 | WC-2 | Positive | Cocci |
| 3 | WC-3 | Positive | Small |
| 4 | WC-4 | Positive | Big rod |
| 5 | WC-5 | Positive | Big rod |
| 6 | WC-6 | Positive | Big rod |
| 7 | WC-7 | Positive | Big rod |
| 8 | WC-8 | Positive | Small |
| 9 | WC-9 | Positive | Small |

Optimization of IAA production by the isolates

Optimum conditions for IAA production by strain WC-5 were day 1, 1000 µg $mL^{-1}$ tryptophan and 28 ˚C (Figure 3). Maximum amount of this hormone (268.04 µg $mL^{-1}$) was detected in cultures on day 1 with 1000 µg $mL^{-1}$ tryptophan at 28 ˚C. Decrease in the production of IAA was obvious on the following days with all concentrations of

tryptophan. On the other hand, a steady increase in the amount of IAA was recorded till day 2 when cultures were incubated at 37 ˚C. The amount of this hormone was below detection limit in cultures containing 0 or 500 µg $mL^{-1}$ kept at 37 ˚C. Maximum production was 31.9 µg $mL^{-1}$ recorded on day 2 in cultures containing 1000 µg $mL^{-1}$ tryptophan at this temperature. On day 3, sharp decrease was obvious in the amount of IAA with all concentrations of tryptophan.

Strain WC-7 also shows a trend of increase in IAA concentration by increasing the amount of tryptophan in the culture media of bacteria. The suitable temperature for this strain was 28 ˚C. By increasing the incubation time it show the decrease in amount of IAA. The highest amount of IAA (189.52 µg $mL^{-1}$) was recorded after 24 hours at 1000 µg $mL^{-1}$ tryptophan (Figure 4).

Screening of Rhizospheres Bacteria for Phosphate solubilization

Phosphate Solubilization were followed by plate assay in the selected bacterial strains The isolates exhibited different sorts of phosphate solublizing index (PSI) ranging from 1.59 to 6.27. Nine out of 13 bacterial isolates were able to solubilize phosphates. The isolates WC-6 showed high PSI which was more than four. WC-7 showed 4 PSI and the other bacterial isolates (WC-3, WC-4 and WC-8) showed less than 4 PSI (Auxin producing).

Plant growth experiment

Okra seedlings were inoculated with the isolated bacterial strains and their effect was observed by comparing different growth parameters with control seedlings. The parameters observed and recorded were shoot length, root length, number of roots, chlorophyll content, fresh weight and dry weight. Only few of the strain were able to induce significant difference in shoot length of the seedlings as compared to control (Table 2). Bacterial strains WC-3, WC-5, WC-7 and Com-10 induced significant increase in shoot length of Okra seedling as compared to control. Maximum increase (6%) in shoot length was observed with strain WC-6. However, all the strains were able to enhance root length in the inoculated seedlings. Root growth was significantly enhanced (74%) in seedlings inoculated with WC-9 as compared to that of control seedlings. 70% increase in root length was recorded when seedlings were inoculated with WC-8. Only few strains could cause significant increase in chlorophyll content of the inoculated seedlings. These strains were WC-1, WC-2, WC-4, WC-5, WC-7 and WC-9 (Table 2). Maximum increase in this parameter was up to 11% in WC-4 inoculated seedlings. Number of lateral roots was significantly greater in bacterially inoculated seedlings than that of control seedlings. More than 2-fold increase was observed in okra seedlings inoculated with WC-1, WC-5 and WC-6. The rest of the strains also enhanced the number of lateral root significantly. Increase of 93% in number of roots was observed with strains WC-2, WC-4 and WC-9. WC-3 enhances number of roots by 87% as compared to control. Except two strains (WC-1 and WC-6) all the strains induced significant increase in fresh weight of the okra seedling. Maximum increase of 28% was observed with strain WC-7. However, none of the strain was able to improve dry weight except WC-3 which caused more than 2-fold increase in dry weight of the seedlings.

## 3. Discussion

Plant growth promoting rhizobacteria (PGPR) are a natural source for improving the efficiency and vitality of crop plants. PGPR encompass all the bacteria that inhibit plant roots and exert a positive effect by mechanisms, ranging from direct influence e.g., increased solubilization and uptake of

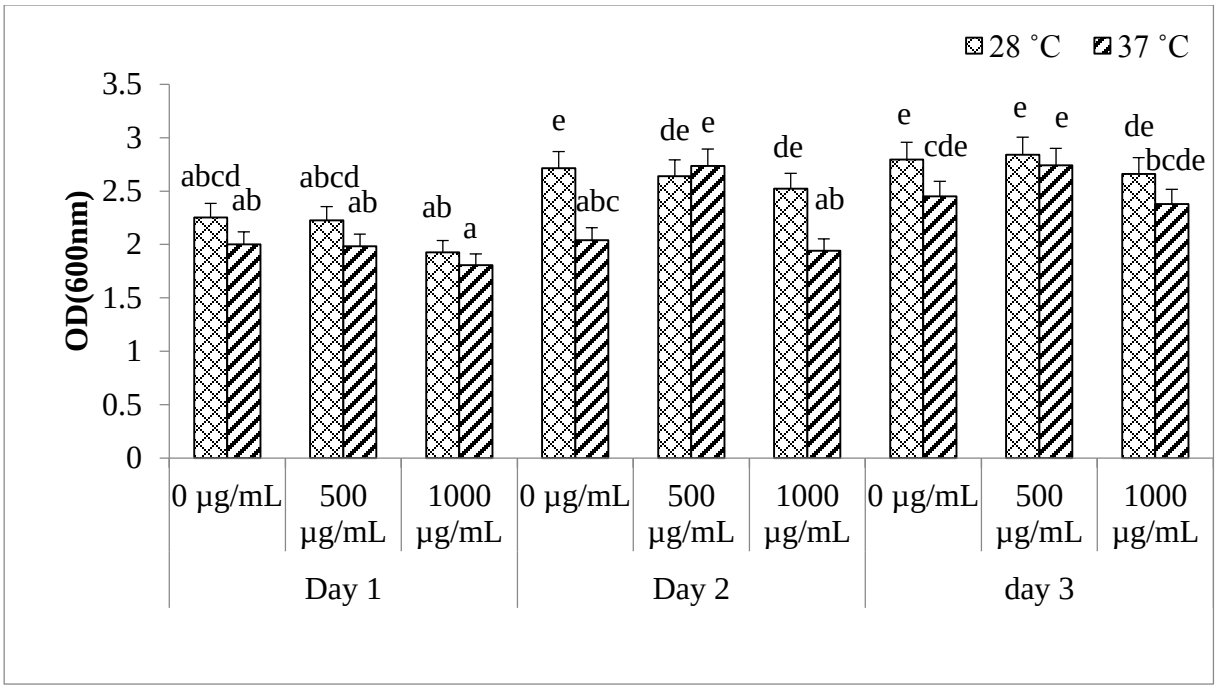


**Figure-1:** Effect of incubation period, tryptophan concentration and different temperature on growth of WC-5. Growth was determined by recording OD at 600 nm. Mean bars labelled with different letters are significantly different ($p < 0.05$)

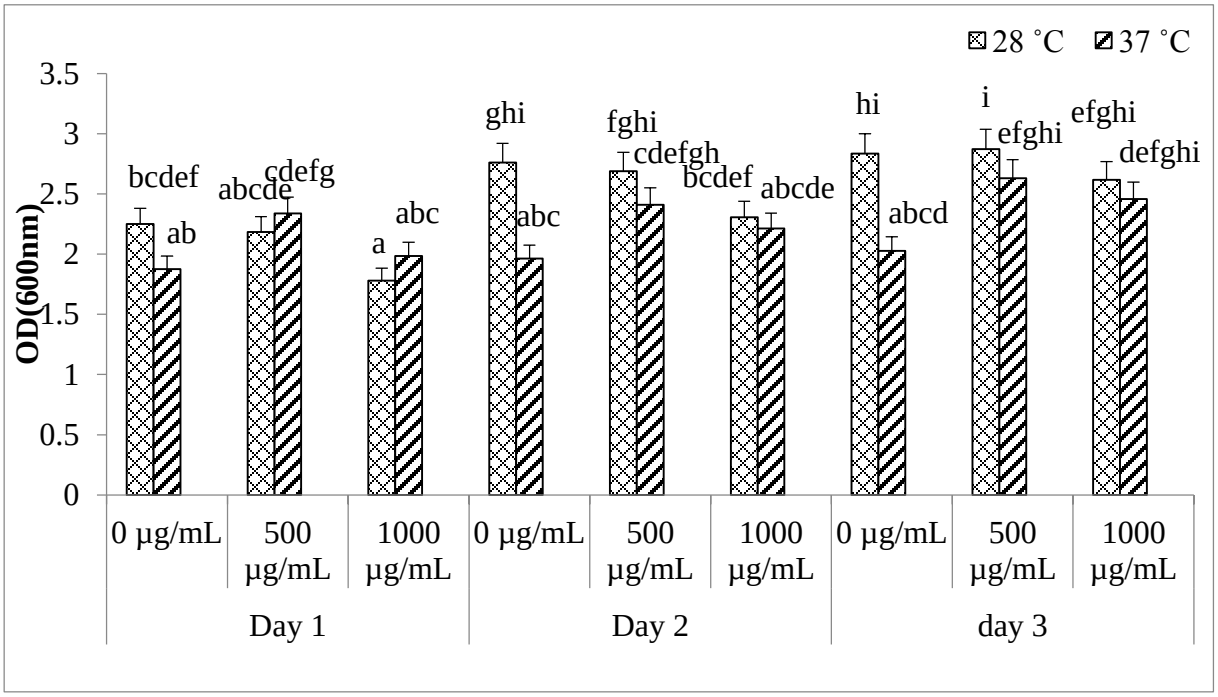


**Figure 2:** Effect of incubation period, tryptophan concentration and different temperature on growth of WC-7. Growth was determined by recording OD at 600 nm. Mean bars labelled with different letters are significantly different ($p < 0.05$)

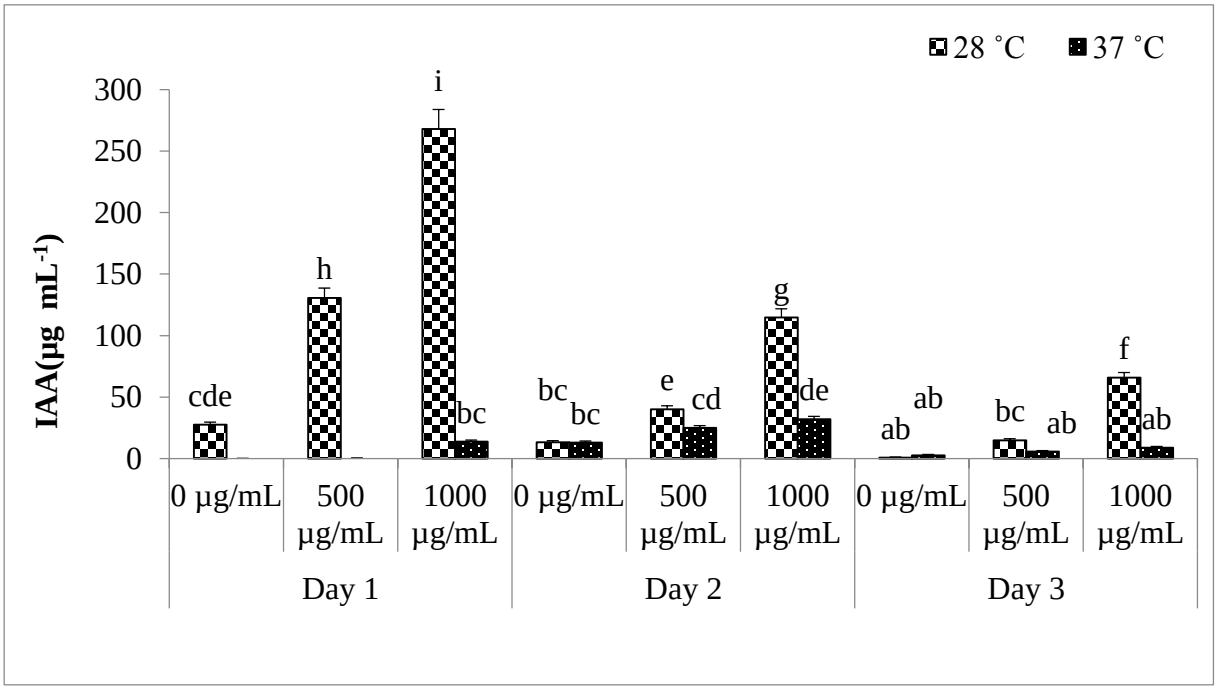


**Figure 3:** Auxin production by bacterial strain WC-5 under the influence of different concentration of tryptophan, temperature and incubation period. Mean bars labelled with different letters are significantly different ($p < 0.05$)

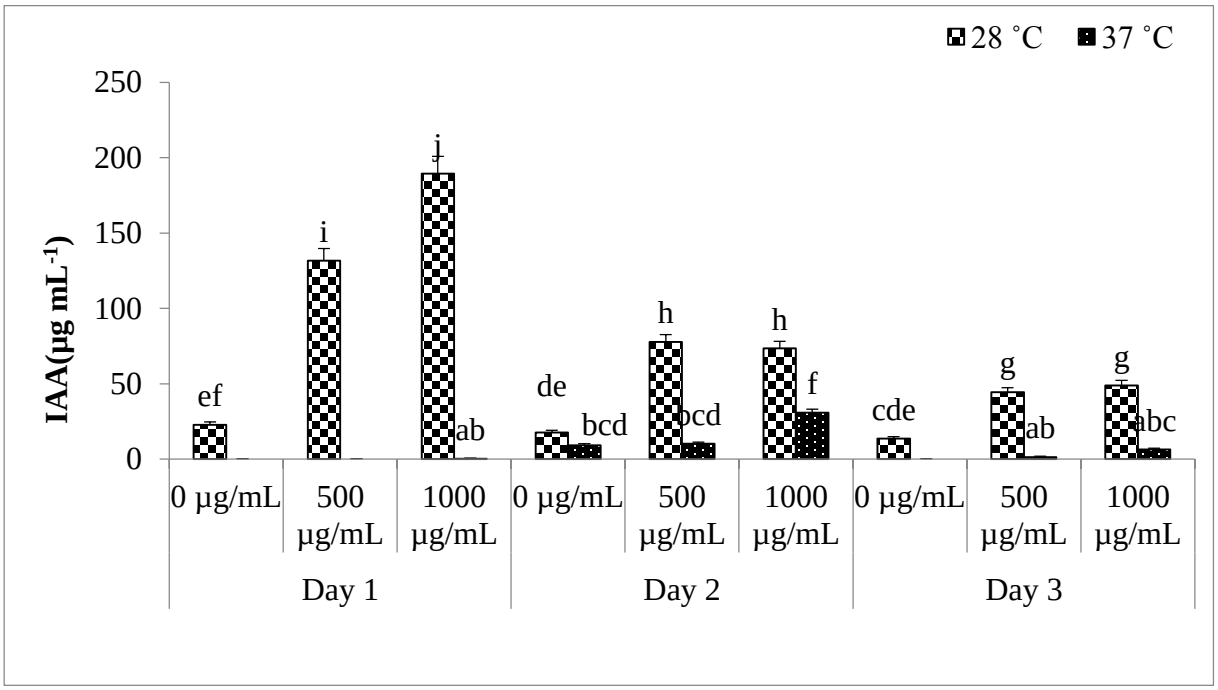


**Figure 4:** Auxin production by bacterial strain WC-7 under the influence of different concentration of tryptophan, temperature and incubation period. Mean bars labelled with different letters are significantly different ($p < 0.05$)

nutrients or production of plant growth regulators, to an indirect effect e.g., pathogen suppression such as biocontrol, production of siderophores or antibiotics [12].`Isolation of bacteria from intrinsic of rhizospheres of the target crop is essential for successful identification of potential biocontrol and biofertlizing agents Rhizobacteria associated with wheat were the most efficient producers of IAA during current study. Difference in the ability of rhizobacteria to produce auxins could be attributed to difference in their genetic makeup, growth kinetics and enzymatic activities involved in auxin synthesis under given cultural conditions. Results are conformity to the findings of many workers [10,13]. Among total 15 isolates auxin production was recorded in 86% of strain isolated from wheat rhizosphere and garlic rhizospheres. Berea *et al*., [13], reported that 58 of the isolated rhizobacteria were able to produce auxin. In the absence of L-Tryptophan the auxin excretion was between 3.316 mg/l to 76.697 mg/l, meanwhile it was between 3.997 mg/l to 67.987 mg/l in the presence of 50 mg L- Tryptophan [14]. Biosynthesis of IAA by rhizobacteria occurs in two ways; tryptophan dependent and tryptophan independent pathways [15]. During current study, our isolates seemed to use both the pathways to produce IAA. Addition of tryptophan to the culture media enhanced IAA production universally in all bacterial strains. IAA production was time and tryptophan dependent. It has been reported previously that IAA production enhance when bacteria are fed by tryptophan [15]. Optimum concentration of tryptophan in bacterial culture media was 1000 µg $mL^{-1}$ for IAA production. Increasing its concentration beyond this point may cause decrease in IAA production due to its inhibitory effect on bacterial growth [16]. Production of IAA was also enhanced with increase in incubation time. Contrary to provious findings, maximum IAA production was recorded in cultures incubated for 24 hours, when bacterial strains were in early stationary phase. Stationary phase of growth has been known as the most efficient phase of bacterial growth for the production of secondary metabolites including IAA [17].

Table: 2 Impact of IAA producing bacterial strains on different growth parameters of okra determined by pot experiment

| S# | Shoot length (cm) | Root length (cm) | Chlorophyll (SPAD) | No of roots | Fresh weight (g) | Dry weight per 10 plants (g) |
|---|---|---|---|---|---|---|
| **Cont** | 14.6±0.5bc | 2.7±0.4a | 39.58±3.5abc | 3.2±0.5a | 0.42±0.02a | 0.4±0.03a |
| **WC-1** | 13.8±0.6ab | 2.9±0.3a | 43.86±2.1bc | 7±0.9c | 0.45±0.02abc | 0.4±0.02a |
| **WC-2** | 14.2±0.6bc | 3.06±0.3ab | 41.04±1.4abc | 6.2±0.9bc | 0.43±0.02ab | 0.4±0.02a |
| **WC-3** | 14.74±0.6b | 3.1±0.4ab | 35.36±2.1a | 6±1bc | 0.48±0.02abcd | 2.6±0.93b |
| **WC-4** | 12.62±0.1a | 3.1±0.2ab | 43.98±1.1c | 6.2±1.2bc | 0.48±0.00abcd | 0.4±0.01a |
| **WC-5** | 15.6±0.5d | 3.4±0.5abc | 41.78±2.8abc | 6.8±0.9c | 0.42±0.02ab | 0.4±0.01a |
| **WC-6** | 14.1±0.8bc | 3.7±0.2abc | 39.44±2.7abc | 7.4±0.9c | 0.54±0.04d | 0.4±0.04a |
| **WC-7** | 15.06±0.3c | 4.4±0.6bcd | 42.36±0.5abc | 3.8±0.5ab | 0.48±0.01abcd | 0.4±0.02a |
| **WC-8** | 13.6±1.2ab | 4.6±0.3cd | 35.98±2.7a | 5.4±0.2abc | 0.45±0.03abc | 0.4±0.04a |
| **WC-9** | 12.3±0.9ab | 4.7±0.6cd | 40.78±2.3abc | 6.2±1.1bc | 0.5±0.0bcd | 0.4±0.02a |

Values are mean of five replicates followed by standard error of mean. Mean labelled with different letters are significantly different (Duncan Multiple Range test; $p < 0.05$)

In line to previous findings [18], suitable temperature for IAA production was 28 ˚C decreases in temperature decrease IAA production. In present research the best and suitable temperature for IAA production was 37 ˚C. Phosphate-dissolving bacteria with the ability to produce growth regulators are frequently found in the rhizosphere. The bacteria belong to several genera and growth regulator production is not specific to any genus. It seems reasonable to suggest that the effects on plant growth that result from inoculation with these bacteria are caused primarily by growth regulators. Qualitative Phosphate Solubilization potential estimated by observing the large clear/halo zones on agar media revealed that out of thirteen bacterial isolates tested, nine isolates had Phosphate solublizing ability. The isolates exhibited different sorts of phosphate solublizing index (PSI) ranging from 1.59 to 6.27. This variation in utilization of substrate by these strains could be due to difference in their organic acids production [19].

Most of the plants get benefit from the plant growth promoting rhizospheric bacteria due to the result of multifarious mechanisms. In the present study the effect of rhizobacteria, significant increase in chlorophyll content, fresh weight, dry weight, shoot length and root length was observed in seedlings of Okra primed with our isolates. IAA producing bacteria has been previously implicated in plant growth promotion [20,21]

## 5. References

1. Hartmann A, Rothballer M and M Schmid (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and soil 312 (1-2):7-14

2. Lynch JM and F Leij (1990) Rhizosphere. Wiley Online Library,

3. Hinsinger P, Gobran GR, Gregory PJ and WW Wenzel (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytologist 168 (2):293-303

4. De Boer W, Kowalchuk GA and JA Van Veen (2006) Root, food and the rhizosphere microbial community composition. New Phytologist 170 (1):3-6

5. Bertin C, Yang X and LA Weston (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant and soil 256 (1):67-83

6. Frankenberger Jr WT and M Arshad (1995) Phytohormones in soils: microbial production and function. Marcel Dekker Inc.,

7. Lebuhn M, Heulin T and A Hartmann (1997) Production of auxin and other indolic and phenolic compounds by Paenibacillus polymyxa strains isolated from different proximity to plant roots. FEMS Microbiology Ecology 22 (4):325-334

8. Hussain A and S Hasnain (2011) Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. World Journal of Microbiology and Biotechnology 27 (11):2645-2654

9. Zahir ZA, Abbas SA, Khalid M and M Arshad (2000) Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. Pak J Biol Sci 3:289-291

10. Khalid A, Arshad M, Zahir ZA and M Khalid (2001) Relative efficiency of rhizobacteria for auxin biosynthesis. Online Journal of Biological Sciences 1:750-754

11. Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362-370

12. Kloepper JW, Lifshitz R and RM Zablotowicz (1989) Free-living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7 (2):39-44

13. Barea JM, Navarro E and Montoya (1976) Production of Plant Growth Regulators by Rhizosphere Phosphate-solubilizing Bacteria. Journal of Applied Bacteriology 40 (2):129-134

14. Khakipour N, Khavazi K, Mojallali H, Pazira E and H Asadirahmani (2008) Production of auxin hormone by fluorescent pseudomonads. Am Eurasian J Agric Environ Sci 4 (6):687-692

15. Spaepen S, Vanderleyden J and R Remans (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS microbiology reviews 31 (4):425-448

16. Harari A, Kigel J and Okon (1989) Involvement of IAA in the interaction between Azospirillum brasilense and Panicum miliaceum roots. In: Nitrogen Fixation with Non-Legumes. Springer, pp 227-234

17. Akbari GA, Arab SM, Alikhani HA, Allakdadi I and MH Arzanesh (2007) Isolation and selection of indigenous Azospirillum spp. and the IAA of superior strains effects on wheat roots. World Journal of Agricultural Sciences 3 (4):523-529

18. Gray WM, Ã–stin A, Sandberg Gr, Romano CP and M Estelle (1998) High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proceedings of the National Academy of Sciences 95 (12):7197-7202

19. Rashid M, Khalil S, Ayub N, Alam S and F Latif (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak J Biol Sci 7 (2):187-196

20. Ambardar S and J Vakhlu (2013) Plant growth promoting bacteria from Crocus sativus rhizosphere. World Journal of Microbiology and Biotechnology 29 (12):2271-2279

21. Chang P, Gerhardt KE, Huang X-D, Yu X-M, Glick BR, Gerwing PD and BM Greenberg (2014) Plant Growth-Promoting Bacteria Facilitate the Growth of Barley and Oats in Salt-Impacted Soil: Implications for Phytoremediation of Saline Soils. International Journal of Phytoremediation 16 (11):1133-1147