

## ISOLATION, CHARACTERIZATION AND EFFECT OF AUXIN PRODUCING BACTERIA ON GROWTH OF *TRITICUM AESTIVUM*

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### ABSTRACT

Auxin, one of the most important phytohormones enhances the root architecture, nutrient acquisition and plant growth. Present study provides the screening, characterization and optimization of auxin producing bacteria from wheat rhizosphere. Effects of auxin producing isolates on seed germination and growth of *T. aestivum* were also evaluated. The *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus marinus* (AUX36, AUX53, AUX137 and AUX142 respectively) were selected by salkowski reagent as auxin producers. Colorimetric analysis showed that all selected isolates produced maximum auxin in the presence of tryptophan at 37 °C and pH 7 after 72 hours of incubation. AUX36 and AUX137 produced maximum auxin at 0.98% (w/v) NaCl concentration, while AUX53 and AUX142 at 2% NaCl. Glucose supplementation enhanced the auxin in the culture filtrate of AUX53 and AUX137, while addition of sucrose in the growth medium enhanced the auxin production for all isolates except AUX142. The AUX36, AUX53 and AUX137, produced highest concentration of auxin at 0.1% peptone. Seed germination experiments using surface sterilized seeds of *T. aestivum* revealed that all isolates could increase root length of seedlings as compared to control. Comparison of various growth parameters after 15 days of growth showed that plant growth was significantly improved by bacterial treatments. All isolates had significant effect on root and shoot length of *T. aestivum*. It is concluded that *Bacillus megaterium* AUX36, *Escherichia coli* AUX53, *Klebsiella pneumonia* AUX137 and *Bacillus marinus* AUX142 may be used to develop indigenous biofertilizers in future after further investigations.

**Key words:** Auxin, Phytohormones, Optimization, *T. aestivum*, *B. megaterium*

### INTRODUCTION

Auxin, a phytohormones, plays an important role in growth and development of plant even in very discrete amounts (Ahmed and Hasnain, 2010; Hussain *et al.*, 2013). Phytohormones are defined as organic substances produced by plants in specific organs and then transported to other parts of plant to enhance and control the certain physical, biochemical, and morphological characteristics and responses. Other than plants many bacteria and fungi residing in soil especially in close proximity of plant roots also produce different kinds of phytohormones such as auxin, cytokinins, gibberellins, abscisic acid and ethylene (Spaepen and Vanderleyden, 2011).

Auxin is quantitatively the most abundant phytohormones secreted by plant associated rhizobacteria (Lin and Xu, 2013; Spaepen and Vanderleyden, 2011). Indole-3-acetic acid (IAA) is the principle and first auxin isolated from plants (Woodward and Bartel, 2005). It is a secondary metabolite synthesized from tryptophan by different metabolic pathways (Pedraza *et al.*, 2004). Auxin is critical for virtually all aspects of plant growth and orchestrates many developmental processes (Woodward and Bartel, 2005). Auxin is also involved in true leaf formation, senescence, apical dominance and

flowering (Pedraza *et al.*, 2004). Auxin plays important role in formation and elongation of roots and root hairs, and initiation and emergence of lateral roots. Increase in root surface area promote nutrient and water uptake which causes overall development of the plant (Mishra *et al.*, 2009). It is not yet clear how bacteria benefit from auxin production (Spaepen *et al.*, 2007). Some authors have suggested that auxin is a signaling molecule in microorganisms (Remans *et al.*, 2006; Spaepen and Vanderleyden, 2011).

With increasing world population and ever increasing demand for food, chemical fertilizers are used excessively in agricultural set ups to increase productivity and yield (Chaiham and Lumyong, 2011). However, problems such as higher costs, pollution of natural resources and safety risks caused by chemical fertilizers have compelled the scientist to explore the alternative methods for safe and increased crop productivity. One such alternative is use of plant growth promoting rhizobacteria as biofertilizers (Akbari *et al.*, 2007; Kende and Zeevaart, 1997). Although the science of biofertilizers and auxin producing bacteria is not new to the world, work is still needed to be done in developing countries such as Pakistan to develop indigenous biofertilizers. Keeping in view its potential, present study was designed for the isolation and characterization of auxin producing bacteria from the rhizosphere of plants

and to determine their beneficial effects on the growth of *T. aestivum*.

## MATERIALS AND METHODS

### Isolation and Purification of bacterial isolates:

Rhizospheric soil samples serially diluted, plated on nutrient agar (peptone 5.0g/l, beef extract 3.0g/l, sodium chloride 8.0g/l and agar 12.0g/l, pH 7.3±0.2) plates and incubated for 24 hours at 37 °C. Morphologically distinct colonies from agar plates were selected, purified and stored in nutrient broth containing glycerol (15%).

**Screening for auxin production:** Isolates were screened for auxin production by using salkowski reagent. Broth cultures were centrifuged and supernatants were mixed with salkowski reagent (1: 2). Salkowski reagent was prepared by mixing 150 ml of concentrated H<sub>2</sub>SO<sub>4</sub>, 250 ml of distilled H<sub>2</sub>O, and 7.5 ml of 0.5 M FeCl<sub>3</sub>·6H<sub>2</sub>O as described previously (Patten and Glick, 2002). The mixtures were allowed to stand for 30 minutes at room temperature in dark for color production. Isolates showing pink to red color were selected as auxin producers and used in further experiments.

**Identification of bacteria:** Selected bacterial isolates were identified by colony morphology, microscopic examination (gram staining, spore staining, and acid fast staining) and biochemical characteristics following Bergey's Manual of Determinative Bacteriology (Bergey, 2009) by performing tests such as catalase, oxidase, indole, methyl red, voges proskeur, citrate utilization, starch hydrolysis, carbohydrate fermentation i.e. glucose, sucrose, mannitol and lactose etc., and urea utilization.

### Optimization of the selected bacteria for auxin production:

Optimization of auxin production was done by providing different physical conditions (temperature, pH, time and osmotic pressure) and chemical factors (glucose, sucrose, peptone and tryptophan). Bacterial cultures were incubated at different pH (5.0, 7.0, and 9.0), osmotic pressure (0.98%, 2% and 4%), and temperature (24°C, 37°C and 42°C) for different time (24, 48 and 72 hours) for physical optimization. Affect of different chemicals and their concentration on auxin production were studied by supplementing the nutrient broth with various concentrations (0, 0.1 and 1%) of glucose, sucrose, peptone and tryptophan. After incubation, supernatants were obtained, mixed with salkowski reagent for color development. Mixture was incubated for 30 minutes in dark and color intensity was measured by spectrophotometer (UV-1700 Pharma Spec, UV-VIS Spectrophotometer, Shimadzu) at 535 nm. Absorbance values were compared with a standard curve to quantify the auxin. Different concentrations of commercially available synthetic IAA (Merck Pvt.

Limited, Pakistan) were used to prepare the standard curve.

**Seed germination test:** The surface sterilized seeds of *T. aestivum* were treated with bacterial cultures (Approximately 10<sup>6</sup>-10<sup>7</sup> cells per ml) for 10 minutes, dried and sown on soft agar plates (0.8%). Four seeds were sown in each plate at equal distance and incubated at room temperature. The number of roots, shoot, root length and root mass were recorded after 3 days of germination as these were considered the main parameter in determining the effect of auxin. The sterile seeds soaked in non-inoculated media for 10 minutes were served as control. Two replicate plates were used for each treatment.

**Plant pot experiment:** For pot experiment, soil was sieved through 2-10mm mesh and sterilized by autoclaving. Seeds previously treated with bacterial cultures were transferred to pots containing 100 grams of autoclaved soil to a depth of 5 mm. Six seed were sown in each pot and the experiment was performed in duplicate. Sterile seeds treated with non-inoculated media were used as control. The pots were kept at 25±2°C, 60% relative humidity and 12 hours photoperiod for 15 days. After 15 days, plants were harvested and their growth parameters including root number, root length, root hairs, shoot length and leaf number were analyzed.

**Statistical Analysis:** Different treatments were compared by one way ANOVA at P<0.05 followed by Tukey's multiple comparison tests by Graph Prism 5 software.

## RESULTS AND DISCUSSION

Auxins play an important role in coordination of various growth and physiological processes in the plant's life cycle. Use of auxin producing bacterial strains as biofertilizers may solve the problems caused by chemical fertilizers such as heavy cost and environmental pollution (Akbari *et al.*, 2007).

In the present study, 16 auxin producing isolates were screened but only four bacterial isolates (AUX36, AUX53, AUX137 and AUX142) were selected for further studies on the basis of their ability to immediately change the color of salkowski reagent. The AUX36, AUX53, AUX137 and AUX142 were identified as *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus marinus*, respectively. Auxin production is well-known and common feature of root associated bacteria (Ahmad *et al.*, 2004). Different bacterial genera such as *Bacillus*, *Burkholderia*, *Enterobacter*, *Vibrio*, *Klebsiella*, *Pseudomonas*,

Optimization of physical conditions for auxin production revealed that all isolates were able to produce auxin over a temperature range from 28 to 42 °C with a

significantly higher ( $P < 0.05$ ) amount of auxin at 37 °C (Fig 1).

Similar finding has also been reported in previous studies (Ahmed and Hasnain, 2010; Sachdev *et al.*, 2009; Sudha *et al.*, 2012). All isolates produced maximum amount of auxin after 72 hours of incubation. Concentration of auxin increased in the culture supernatant with incubation time, as it is a secondary metabolite (Sachdev *et al.*, 2009). The pH of the medium considerably affected the bacterial auxin biosynthesis, producing significantly highest ( $P < 0.05$ ) amount of auxin at neutral pH. In a previous study, Sudha *et al.* (2012) also reported that *Bacillus spp.* produce maximum amount of auxin at neutral pH. The varying levels of NaCl also effected the auxin production. AUX36 and AUX137 produced optimum level of auxin at 0.98% NaCl concentration. However, AUX53 and AUX142 produced highest level of auxin in medium containing 2% NaCl. Glucose supplementation also significantly enhanced the concentration of auxin in the culture filtrate of AUX53 and AUX137 (Fig 2).

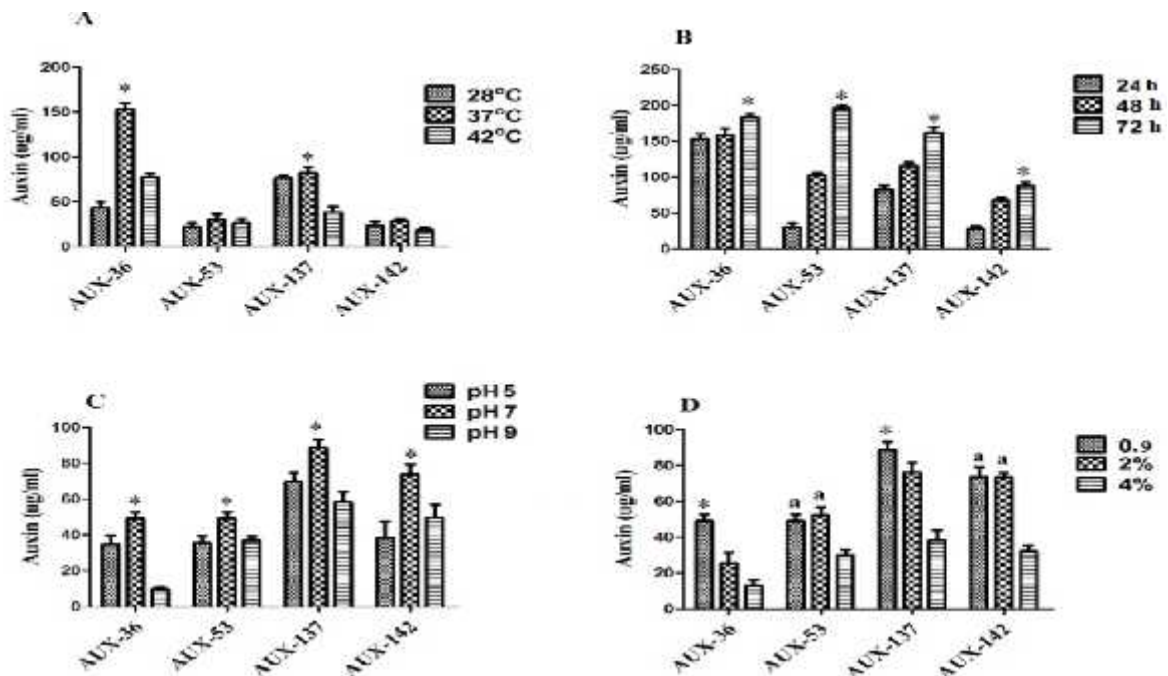
Addition of sucrose (1%) and peptone (0.1%) in the growth medium significantly ( $P < 0.05$ ) enhanced the auxin biosynthesis in the culture filtrate of AUX36, AUX53 and AUX137, but not for AUX142. Addition of tryptophan (1-1.5%) in the growth medium significantly enhanced ( $P < 0.05$ ) the auxin production in the culture filtrate of all the isolates. Microorganisms use tryptophan

as a precursor for auxin biosynthesis so tryptophan presence stimulates the auxin production (Akbari *et al.*, 2007; Tsavkelova *et al.*, 2007).

Data obtained from seed germination experiments demonstrated isolate specific positive effect on seed germination (Fig 3).

All isolates increased the root length, root number and root mass of the germinated seeds as compared to control group ( $P < 0.05$ ). Effect of *B. megaterium* AUX36 and *E. coli* AUX53 had significantly higher effect on seed germination as compared to AUX137 and AUX142 ( $P < 0.05$ ). Increased seed germination of *T. aestivum* has also been reported previously (Selvakumar *et al.*, 2008). Bacterial isolates also increased the growth parameters of *T. aestivum* in plant pot experiment (Fig 4). The *B. megaterium* AUX36 had significantly higher effect on growth parameters. Our results are in accordance with the previous studies, where *Bacillus spp.* showed positive effect on plant growth (Acuña *et al.*, 2011; Ali *et al.*, 2009; Qureshi *et al.*, 2011; Selvakumar *et al.*, 2008). Effects of other bacteria such as *Azospirillum spp.* on the growth of *T. aestivum* has also been studied previously (Akbari *et al.*, 2007; Tsavkelova *et al.*, 2007).

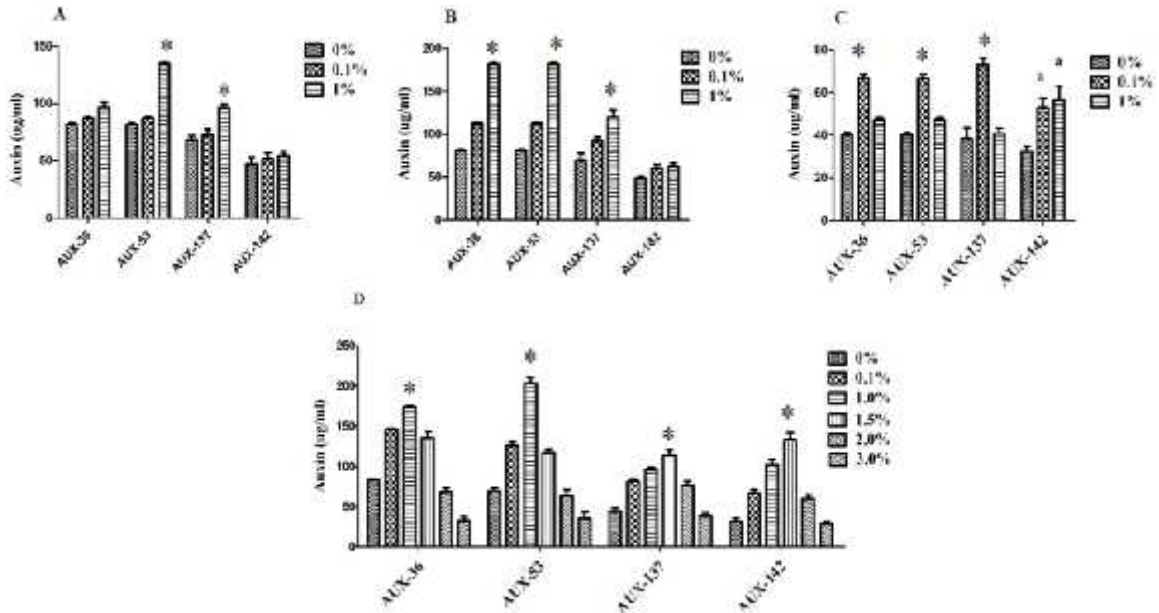
It is concluded that bacterial isolates enhance the germination and growth parameters of *T. aestivum* and may become good candidates for the development of indigenous biofertilizers after further investigations.



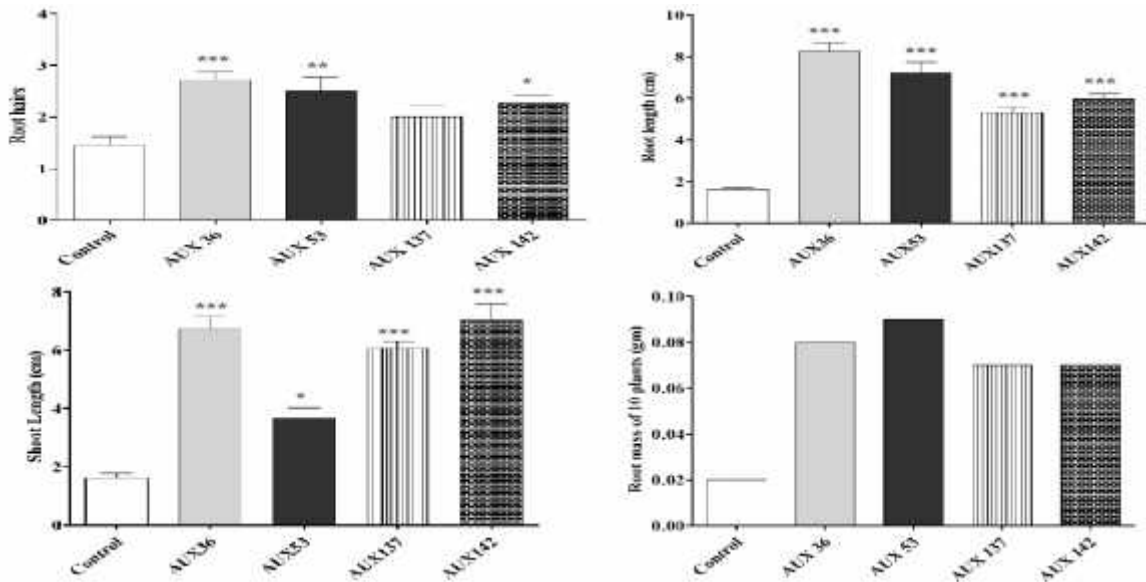
**Fig 1: Effect of physical conditions on the auxin ( $\mu\text{g/ml}$ ) production.** (A) Effect of temperature (B) Effect of incubation time (C) Effect of pH (D) Effect of NaCl

\*indicates statistically significant difference as compared to other treatments in the same group ( $P < 0.05$ )

<sup>a</sup> represents statistically non-significant difference as compare to each other but significant difference when compared with the third treatment in the same group at  $P < 0.05$



**Fig 2: Effect of chemical parameters on the auxin (µg/ml) production** (A) Effect of glucose (B) Effect of sucrose (C) Effect of peptone (D) Effect of tryptophan  
 \*indicates statistically significant difference in growth of isolate as compared to other two treatments at P<0.05  
<sup>a</sup> represents statistically non-significant difference as compare to each other and statistically significant difference when compared with the third treatment in the same group at P < 0.05



**Fig 3: Effect of bacterial inoculation on different parameters of germination of seeds.** (A) Effect on root hairs (B) Effect in root length (C) Effect on shoot length (D) Effect on root mass  
 \*indicates statistically significant difference in growth of isolate as compared to other two treatments at P<0.05  
<sup>a</sup> represents statistically non-significant difference as compare to each other and statistically significant difference when compared with the third treatment in the same group at P < 0.05.

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