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Biological Studies on the Effect of Plant Growth Promoting Rhizobacteria on Tomato (*Solanum lycopersicum*) Plants

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Abstract: One of the recent trends to improve plant productivity under abiotic stress and biotic stress is the use of rhizobacteria. Accordingly, this study is based on the isolation of rhizobacteria from several different locations from Mecca region and to identify the ability of these bacterial isolates to indole acetic acid (IAA) production and nitrogen fixation. As the results indicated that all isolates (16 isolates) have the ability to produce IAA, while the ability of nitrogen fixation varied. The bacterial isolates were classified into three groups based on the strength of their ability to nitrogen fixation. The results also revealed that the increase in tomato germination increasing with PGPR treated seeds over control. These results were a good indicator for using the activity of *Bacillus simplex* ET1, *B. megaterium* NT1 and *B. subtilis*in a future experiment to improve the growth and yield of tomatoes under field conditions.
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1. Introduction:

Plants are exposed to a wide scope of environmental stresses which decreases and confines the productivity of agricultural crops. Two types of environmental stresses are encountered to plants which can be categorized as (1) Abiotic stress and (2) Biotic stress . The abiotic stresses that affect crop growth vary, including water stress, salinity, cold stress, higher temperature and heavy metals(Agarwal and Grover, 2006; Hsu and Kao, 2003; Lu et al., 2008; Rodríguez-Serrano et al., 2009; Wang et al., 2003). For example, salinity pressure affects the development of reproductive structures or translocation of nutrient reserves and impairs plant growth by causing osmotic imbalance, reduce photosynthesis, ion toxicity, disrupt nucleic acid metabolism and protein synthesis(Juan et al., 2005; Karakas et al., 2016). These stresses affect plant growth and productivity, including rice, wheat and tomato crops, resulting in annual economic losses (Banerjee et al., 2017; Shriram et al., 2016). Furthermore it, attacks by various pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores are included in biotic stresses.

To overcome the negative effect of environmental stresses on the plant, modern biological methods, such as Plant growth Promoting Rhizobacteria (PGPR) will be used in this study. The hypotheses of the action of this type of bacteria is summarized in stimulating the growth of plants lies in the production of many compounds that belong to growth regulator in the vicinity of rhizosphere, while indirectly effect based on decreasing the inhibitory affects of pathogens(Ahemad and Kibret, 2014; Singh et al., 2018). PGPR also acts as a biological control, helping to maintain soil fertility by removing heavy metals and other soil pollutants, thus serving as a promising alternative to hazardous chemical fertilizers used indiscriminately in sustainable agriculture. The application of PGPR to mitigate the effects of environmental stresses is a cost-effective and environmentally friendly biologic approach to physical-chemical methodologies(Singh et al., 2018).

This study was conducted to isolation and identification some of PGPR from plant rhizosphere and studied their morphological characteristics, nitrogen fixation and IAA production to improve the productivity of plant under abiotic stress and biotic stress.

2. Materials and Methods:

1. Isolation PGPR from soil samples:

1.1 Sample collection

Bacteria were isolated from the rhizosphere soil from different plants growing in Mecca region in the summer season 2018 according to (Wollum, 1982) **Fig. (1).**

In each sampling point, Multiple samples were collected consisted of rhizosphere soil (soil around the root zone) plants. Soil samples have been taken at a depth of 15-20 cm of the soil surface. Soil samples were collected in perforated plastic bags using a shovel.

1.2 Isolation of rhizobacteria

Rhizospheric bacteria were isolated from 10 g of dried soil samples by serial dilution method. The soil samples suspension was spread on Glucose Peptone Agar Medium (GPAM) plates (Wollum, 1982). The plates were incubated at 28 ± 2°C till the appearance of bacterial colonies.

1.3 Purification:

Individual colonies of bacteria, which varied in morphology (shape, size and color) were picked up using a sterile inoculating wire loop and subcultured to purify by repetitive streaking on (GPAM) and Nutrient agar) NA (plates. The purity of each bacterial isolate was checked under the microscope using standard staining. The purified isolates were maintained on NA media and kept at 4 °C and subcultured at an interval of every 4 weeks and preserved in glycerol 20% added to nutrient broth at -80˚C for long period of time.

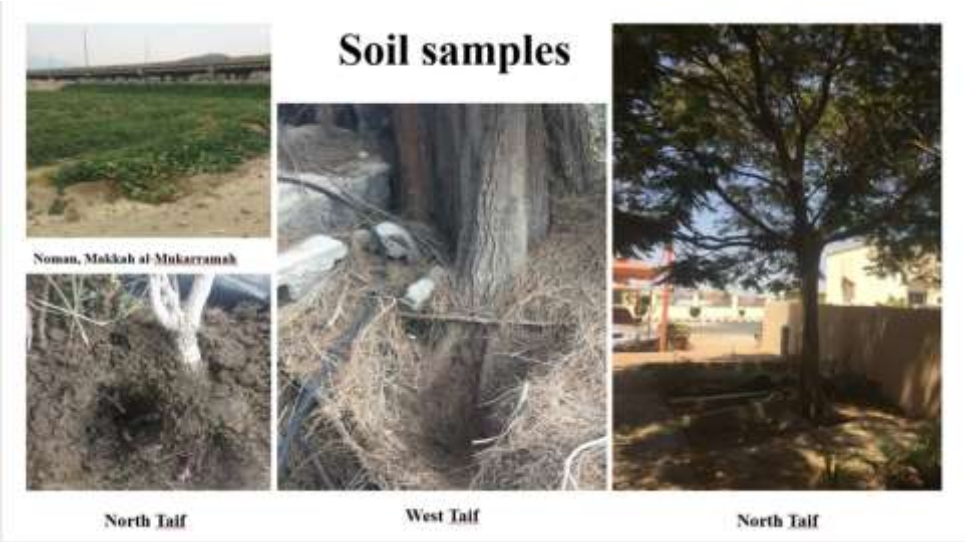

Fig. (1): Some pictures of the sites from which soil samples were collected

1.4 Identification:

Bacterial isolates were defined by the Bruker MALDI Biotyper system. To measure a unique molecular fingerprint of an organism the MALDI Biotyper CA System identifies microorganisms using MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight) Mass Spectrometry, Bruker Corporation, USA was used. Specifically, the MALDI Biotyper CA System measures highly abundant proteins that are found in all microorganisms. The characteristic patterns of these highly abundant proteins are used to reliably and accurately identify a particular microorganism by matching the respective pattern with an extensive FDA-cleared database to determine the identity of the microorganism (Ferreira et al., 2011)

1. Indole acetic acids (IAA) production:

Auxin production by the PGPR isolates was tested in the presence and absence of L-tryptophan (L-TRP) and determined by colorimetry. For this purpose, 20 ml of glucose peptone medium (GPM) broth will added in 100 ml Erlenmeyer flasks, autoclaved and cooled. Five milliliters of filter sterilized (0.2 µm membrane filter, Whatmann) L-TRP solution (5%) was added to the liquid medium GPM to achieve a final concentration of 1.0 g l^{-1}. The flask content was inoculated by adding 1.0 ml of 4-day-old bacterial broth. The flasks were plugged and incubated at 28 ± 1°C for 48 h at100 rpm shaking. Non-inoculated/untreated control was kept for comparison. After incubation, the contents were filtered through Whatmann filter paper no. 2, then transferred this filter paper to another Petri dish filed with Salkowski's reagent (4.5 gm of $FeCl_3$ per liter in 10.8 M H_2SO_4) let paper saturated, and incubated at room temperature under dark conditions for 30 min. Organisms which producing IAA gave pink to red color after incubation period as described by (Sarwar et al., 1992).

2. Nitrogen fixation:

Jensen's Mediumis formulated according to Jensen and is recommended for detection and cultivation of nitrogen fixing bacteria(Jensen, 1942;

Kapoor et al., 2017). Cultural characteristics observed after incubation at 30°C for 8 days.

3. Effect of PGPR on tomato germination rate and seedling growth (in-vitro)

To study the effect of PGPR on plant germination rate, Lab experiment was carried out at fully control conditions in one growth room chambers at Microbiology lab, Faculty of Sciences, University of Jeddah, Saudi Arabia. 51 seeds from cultivar of tomato were sterilized by soaking in 2% sodium hypochlorite for 5 min and then washed by sterile distilled water for 5 times. sixteen treatments were replicated three times as following: (1) Seeds were incubated in 100 ml of 1% glucose solution as a suspension of the PGPR isolates separately at room temperature for 4 h. (2) Seeds were incubated with 1% glucose solution free from bacterial cells as control. After incubation period, the soaked seeds were placed in sterilized pot containing wet cotton (3 seeds/cup and 1 cup/each treatment). Pots were incubated in growth room at 28±2°C for 3 days to calculate the final germination percent (FGP)(Association, 1993, 1999) based on the following equation:

$$FGP = \frac{\text{number of germinating seeds}}{\text{Total number of seeds}} \times 100$$

Mean germination time (MGT) was calculated according to the following equation (Moradi et al., 2008):

$$MGT = \sum Dn / \sum n$$

Where, "*n*" is the number of seeds germinated on day "*D*", and "*D*" is the number of days counted from the beginning of germination.

3. Results and discussion:

The expanding significance of beneficial bacteria in agriculture has brought about numerous endeavors to isolate and identify bacteria associated with the rhizosphere of plants so as to follow their jobs in plant development advancement.

The number of bacterial isolates obtained was 45 pure isolates from soil samples from rhizosphere from Mecca region. Morphological characteristics of bacterial colonies were used to distinguish between different isolates and purify them on plate agar. The results showed that, bacterial isolates defined in two genera, with sixteen species **(Table 1).** The benefit isolates of PGPR, which were isolated and identified in this study are *Bacillus simplex ET1, B.cereus ET1, B.megaterium NT1, B.licheniformis, B.simplex ET2, B.megaterium NT2, B.subtilis, Bacillus sp, B.cereus WT1, B.cereus WT2, B.cereus WT3, B.cereus WT4, B.cereus NT1, B.cereus WT5, Enterobacter cloacae, B.cereus ET1*. Using Gram stain, bacterial isolates were identified (Gram-positive and Gram-negative) by the chemical and physical properties of their cell walls, **Fig. (2).**

Table 1: Morphological and biochemical characteristic of bacterial isolates

Bacteria	Shape	Gram stain		Spore test		Motility		Colony character
		Gram +ve	Gram -ve	Spore forming	Non-Spore forming	Motile	Non-Motile	
Bacillus simplex ET1	rod	✓			✓	✓		White, circular, smooth
B.cereus ET1	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
B.megaterium NT1	rod	✓		✓		✓		White, circular, smooth
B.licheniformis	rod	✓		✓		✓		Wrinkled, dull, opaque, adherent colonies
B.simplex ET2	rod	✓			✓	✓		White, circular, smooth
B.megaterium NT2	rod	✓		✓		✓		White, circular, smooth
B.subtilis	rod	✓		✓			✓	circular, white, adherent, colonies with membranous growth
Bacillus sp	rod	✓		✓		✓		Gray-white round, opaque, drying, medium-size colony
B.cereus WT1	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency

B.cereus WT2	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
B.cereus WT3	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
B.cereus WT4	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
B.cereus NT1	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
B.cereus WT5	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency
Enterobacter cloacae	rod		✓		✓	✓		White, shiny, circular colony-entire edge
B.cereus ET1	rod	✓		✓		✓		large, grey-white, granular colonies, less wavy edge and less membranous consistency

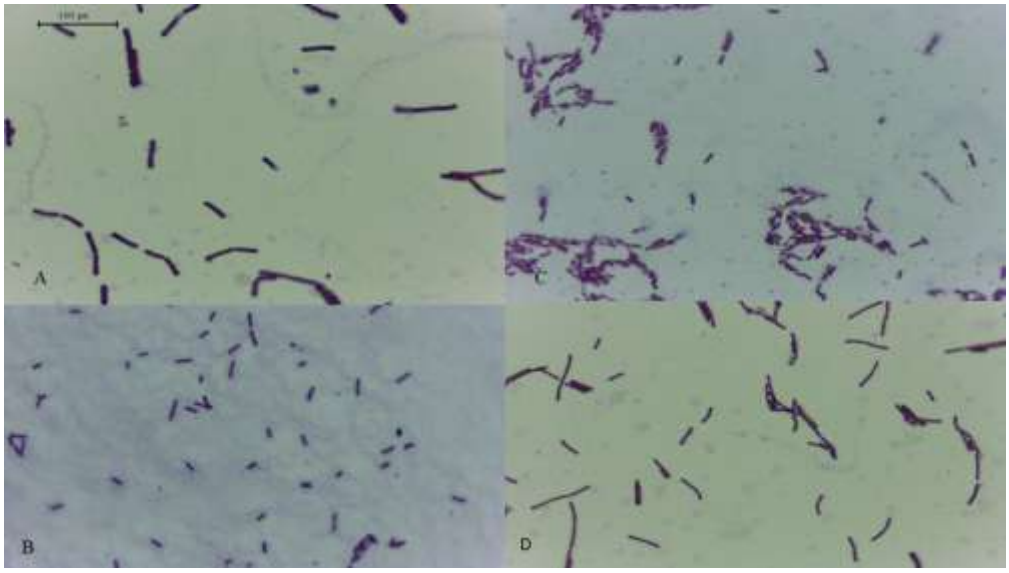

Fig. (2): Microscopic images of some PGPR in Gram stain, A= *B.megaterium NT1,* B=*B.subtilis,* C= *B.cereus WT4,* D= *Bacillus simplex ET1*

Bacterial isolates were screened for plant growth promoting traits such as production of IAA and nitrogen fixation. Sixteen isolates form fifty-four had the ability to produce of IAA **(Table 3)**. Auxin production by the PGPR isolates was tested in the presence and absence of L-tryptophan (L-TRP) and determined by colorimetry **Fig. (3).** This result was agreement in some isolates of PGPR which recorded by Kashyap et al. (2019), Dolkar et al. (2018), Xu et al. (2015), Rahman et al. (2018) and Esitken et al. (2010). While Hidri et al. (2019) found that the *B. subtilis* reduced the effect of the abiotic stress on the plant. The reason is due to the efficiency of the strain of *B.subtilis* that produce high IAA under stress conditions.

As for ratios of nitrogen fixation, bacteria were classified into 3 groups (good producer) *B. simplex ET1, B. cereus ET1, B. megaterium NT1, B. licheniformis, B. simplex ET2, B. megaterium NT2, B. subtilis, Bacillus sp*, followed by (medium producer*) B. cereus WT1, B. cereus WT2, B. cereus WT3, B. cereus WT4, B. cereus NT1,* and the last group (weak producer) *B. cereus WT5, Enterobacter cloacae, B. cereus ET1* (Table 3). This classification was made based on the growth of isolates in a nitrogen-free medium (Jensen's Medium) Fig. (4). This

classification of these results has been found in several researches of the world (Liu et al., 2017)(Orhan, 2016).(Kim et al., 2011)) mentioned that *B. cereus* EU104735 do not fix nitrogen, this is in agreement with the results in *B. cereus* ET1 and *B.cereus WT5*, in addition to that it was found that *B. simplex*, *B. megaterium* does not fix nitrogen, and this is in contrast to the results of *B. simplex*, *B. megaterium* in this study. The difference is due to two reasons: the host plant and the location.

Table 1: Detection for production of IAA and nitrogen fixation by bacterial isolates

Bacteria	IAA production	Nitrogen fixation
Bacillus simplex ET1	++	++
B.cereus ET1	++	++
B.megaterium NT1	++	++
B.licheniformis	++	++
B.simplex ET2	++	++
B.megaterium NT2	++	++
B.subtilis	++	++
Bacillus sp	++	++
B.cereus WT1	++	+-
B.cereus WT2	++	+-
B.cereus WT3	++	+-
B.cereus WT4	++	+-
B.cereus NT1	++	+-
B.cereus WT5	++	--
Enterobacter cloacae	++	--
B.cereus ET1	++	--

-- = Weak producer; -+ = medium producer and ++ =good producer

PGPR isolates significantly affect tomato seed germination**.** The results reveal that the increase in tomato germination increased in PGPR treated seeds over control. Data presented in **Fig. (5-6)** showed the effects of seed treatments by seed soaking with the sixteen isolates of PGPR on final germination percentage (FGP), mean germination time (MGT) after 3 day from planting.

The best PGPR bacteria for FGP and MGT in seed soaking treatment were *Bacillus simplex ET1, B.megaterium NT1, B.licheniformis, B.simplex ET2, B.megaterium NT2, B. subtilis, B.cereus WT3, B.cereus NT1*.

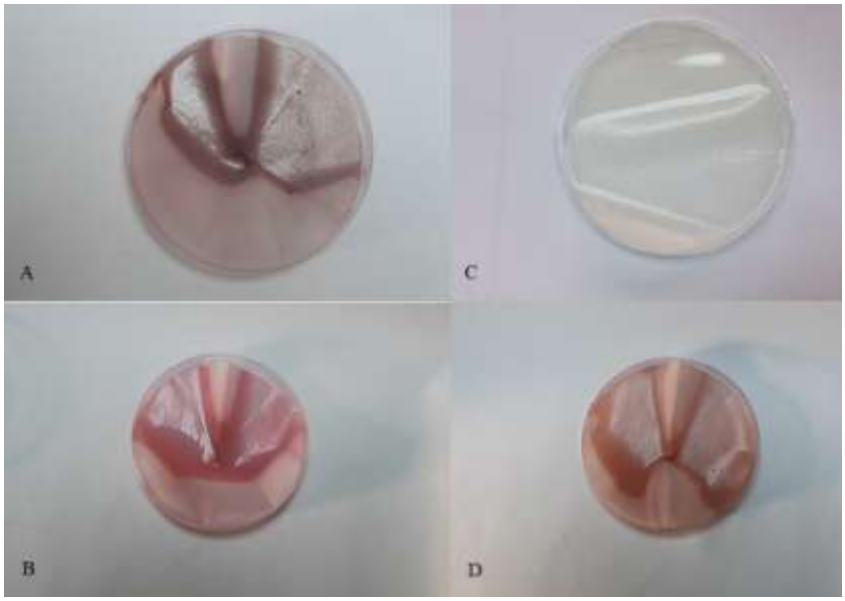

Fig (3): The auxin production by some PGPR isolates was detected by Salkowiski's reagent *A=*B.subtilis,* B=*Enterobacter cloacae*, C= Control, D= *B.simplex ET1*.

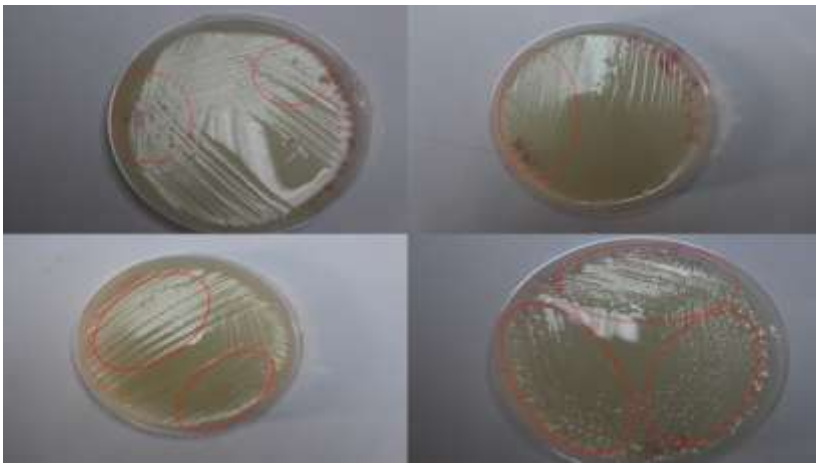

Fig. (4): The red range shows the extent of growth of some bacterial isolates (*B.megaterium NT1, B.subtilis, B.cereus WT4, Bacillus simplex ET1*) in a nitrogen-free medium (Jensen's Medium)

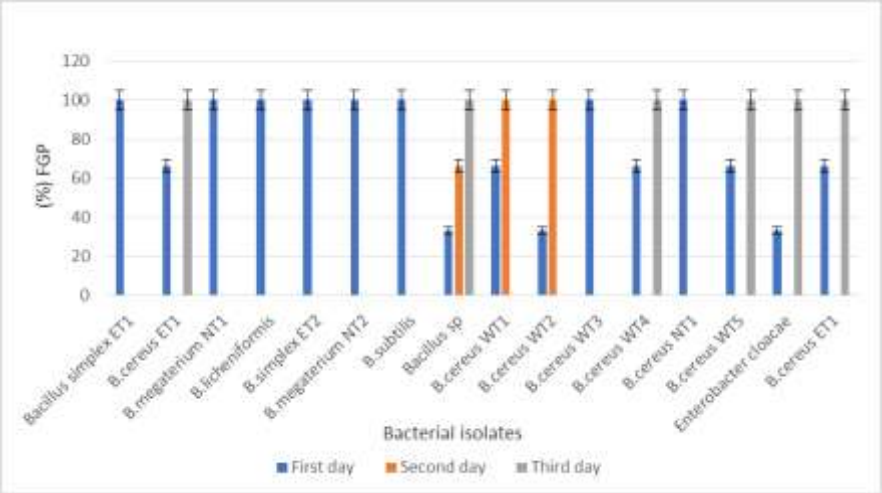

Fig. (5): Percentage distribution of PGPR on final germination percentage after 3 day

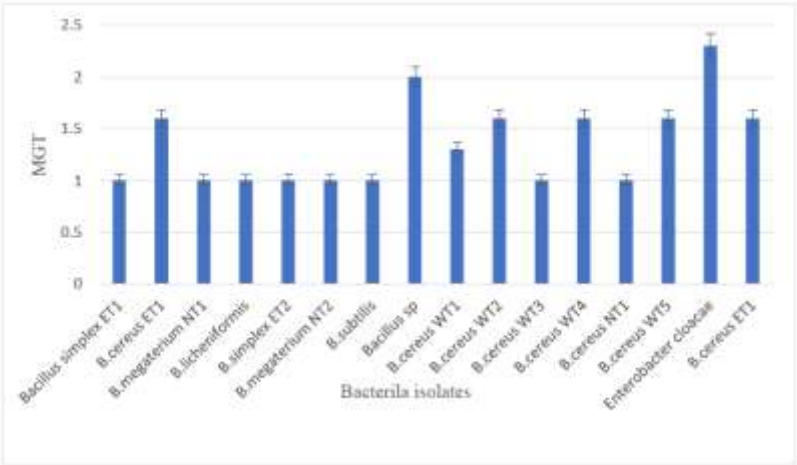

Fig. (6): Percentage distribution of PGPR on mean germination time after 3 day

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