ISOLATION AND CHARACTERIZATION OF HALOPHILIC-ENDOPHYTES FROM EL-HAMRA OASIS, EL-NATRUN VALLEY, EGYPT. I: IMPACT OF ENDOPHYTIC BACTERIAL ISOLATES ON TOMATO (*LYCOPERSICON ESCULENTUM*) GROWTH PROMOTION UNDER GREENHOUSE CONDITIONS.

MANAL F. ABDELALL([1]), M. S. TAWFIK([2*])

1 Microbial Molecular Biology Department, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Street, 12619, Giza, Egypt. E-mail: manalfm@gmail.com; maanlfm@ageri.sci.eg.

2 Oil Crops Biotechnology Lab, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Street, 12619, Giza, Egypt.

E-mail: moh4mon@gmail.com

[*]Correspondent author (moh4mon@gmail.com)

Dehydration-inducing stress conditions are significant problems affecting plant distribution, survival, and productivity worldwide. The severity of dehydration-inducing conditions in a given area is affected by different environmental factors as in high temperatures and increased evaporation/precipitation ratio and affected by the soil drainage system and poor water management in cultivated lands (Egamberdiyeva, 2007). Therefore, finding an efficient, low cost, easily adaptable method for managing dehydration-inducing stress conditions in plants is a major challenge, where minor improvement could have a measurable economic impact (Venkateswarlu and Shanker, 2009).

The majority of cultivated plant species are classified as glycophytes, while halophytic species are primarily found in hypersaline environments, and their growth is severely affected upon removal of NaCl from their environment (Surve *et al*., 2012; Solomon and Viswalingam, 2013). Two distinctive classes of microorganisms are found in a given salinity-affected soil; the first are halophytic microorganisms living in the root-growing zone and requires at least 0.2 M NaCl concentration and cannot grow in the lack of salt. The second is halo-tolerant microorganisms that grow in the absence of salt and the presence of comparably high salt concentrations (Ara *et al*., 2013).

Plant growth-promoting bacteria (PGPB) are soil-borne bacteria that form a symbiotic relationship with plants (Díaz-Zorita and Fernández -Canigia, 2009); they tend to facilitate the growth of plant

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either by colonizing the rhizosphere and endosphere of plants and activate various pathways within plants to facilitate salt-withstanding mechanisms, directly/indirectly (Islam *et al*., 2016). Plant growth-promotion affects plants 1) directly through facilitating nutrient acquisition (phosphorus solubilization, nitrogen fixation), and production of plant growth hormones (Odoh, 2017), or 2) indirectly through siderophore and hydrocyanic (HCN) production and defensive action against biotic pathogens (Goswami *et al*., 2014). Therefore, it could be used in bio-inoculants in agricultural practices to promote sustainable agricultural production (Mei and Flinn, 2010).

The area of El-Natrun Valley, Al-Bahira governorate, is an area known for its hypersaline soda lakes. Salinity reaches up to 5.0 M NaCl, with a pH range of 8.5-11 and temperatures reaching mid-50s during summer, a rich ecosystem for isolation of alkaliphilic, haloalkaliphilic thermo-alkaliphilic microorganisms (Mesbah *et al*., 2007). To our knowledge, most of the available studies on halophilic bacterial isolation and characterization from high-saline lakes in Egypt focused only on phylogenetic analysis of the different classes with limited information on their agricultural potentials (Oren, 2002; Vahed *et al*., 2011).

Keywords: Endophytes, Halophytic bacteria, IAA production, Growth characteristics, Tomato**,** 16S rDNA.

MATERIALS AND METHODS

Sampling site: Hypersaline soda lakes of El-Hamra, located in an extended depression approximately 90 km northwest of Cairo; that area is part of El-Natrun Valley, which is occupies approximately 60 x10 km (Taher, 1999) Fig. (1).

Samples collection: Different plant tissues from four plant species growing in the area (*Phragmites australis*, *Tamarixnilotica*, *Juncus rigidus* and *Halocnemum strobilaceum*) were collected from sites surrounding the lake in triplicates; the samples were placed in sterile containers. All samples were collected in June 2016-17, transported on ice and processed within 2-4 h after collection.

Isolation of halo-endophytic bacteria from plant tissues: All collected plant tissues (roots, stems and leaves) were carefully cleaned with tap water for 30 secs, then rinsing in sterile ddH_2O for 1-2 mins. In laminar flow-hood, surface sterilization was performed by rinsing in 70% ethanol for 1 min, followed by drenched in 20% Clorox solution (1% sodium hypochlorite) for 5 mins. The plant tissues were rinsed 5-6 times with sterile ddH_2O. The samples were allowed to surface dry for 5-10 mins before aseptically dissected into small sections and placed on solid LB medium (Cat # L1704.0500, Duchefa Biochem, Netherland) supplemented with 10% NaCl. The plates were incubated for 48 hr at 28±2 °C. To obtain pure bacterial isolates, bacterial colonies that were morphologically distinct were selected and cultivated repeatedly on fresh solid LB

medium with 10% NaCl. Some uncut disinfected-surface tissues and the last rinsing water were also inoculated onto separate solid LB plates as a control to validate the effectiveness of the surface sterilization procedure. All purified isolates were stored at –80 ºC for further use.

Morphological studies of the isolates: All purified bacterial isolates were characterized morphologically as colony color, form, elevation, margin, etc. Gram staining and morphological studies of cells under a microscope were also performed (data not shown).

Growth characterization of the isolates: Growth of the isolates were determined at different pH (4, 7, and 10), temperature (30, 40, and 55 °C), and NaCl concentrations (5, 10, 15, and 20 %; the cultures were inoculated in LB broth pH 7.0 in a shaker incubator 100 rpm at 30°C for 48 hr.). Bacterial growth was measured at 600 nm wavelength (Table 1).

Molecular analysis of the isolates: All purified isolates were identified using sequences of 16S rDNA. Bacterial-DNA isolation was performed following the procedure by Sambrook *et al.* (2009); amplification of 16S ribosomal-RNA amplification was conducted using 27 F and 1492 R primers (27F **AGAGTTTGATCMTGGCTCAG** and 1492R **CGGTTACCTTGTTACGACTT**). PCR was carried out in 25.0 µL reactions and PCR conditions were as follow: denaturation at 94°C for 5 min, then 35 cycles of 94°C for 1 min, 65°C for 90 sec and 72°C for 1 min, then extending cycle for 7 min at 72°C. Amplified products were gel-separated and visualized using 1.0% agarose gel which stained by ethidium bromide- (0.5 µg/mL). PCR-products were purified using QIAquick Kit (Qiagen, Netherlands), and were sequenced. The sequences were revealed using the basic local alignment search tool (BLAST) and comparisons with the GenBank nucleotide database http://www.ncbi.nlm.nih.gov. Once the sequences were identified, they submitted to the NCBI (accession no. KY608807 - KY608843). Sequences were initially aligned with CLUSTAL Omega method, by MEGA7 software phylogenetic trees were constructed (Kumar *et al.*, 2016) with 1000 bootstrap replications, and evolutionary distances were determined using the p-distance method.

IAA quantification: Following a modified procedure by Patten and Glick, (2002), IAA quantification was conducted using 20 µL aliquots of overnight bacterial growth cultures were used to inoculate 5 ml LB medium fortified with 0.1% tryptophan and incubated in the dark for 24 hr at 30 °C; the cultures were centrifuged (5,500×g, 10 min) and 1.0 ml of supernatant was used for quantification of IAA by mixing with 4.0 ml Salkowski's reagent (150 ml H2SO4, 250 ml ddH_2O and 7.5 ml of 0.5 mol FeCl3•6H_2O; Glickmann and Dessaux, 1994); the reactions were incubated for 30 min at room temperature, then light absorption was measured at 535

nm. **Greenhouse experiment:** Tomato seeds Cv. Castle Rock was germinated in 96-well foam trays filled with soil mixture (1 peat moss:1 washed sand) in the greenhouse. Thirty days post-germination, uniformly growing seedlings were transplanted into 25 cm pots filled with the soil mixture. Five bacterial isolates (HEP3B1, HEP4A2, HEP4A4, HEP1B3, and HEP1B2; Accession no. KY608812, KY608814, KY608816, KY608809, and KY608808, respectively) were inoculated tomato plants separately using different approaches. The plants divided into five groups (1st did not receive any foliage or soil application, 2nd group received IAA spray application at a concentration of 1 mg/L, 3rd group received 50 ml bacterial foliage-spray application, 4th and 5th groups received 10 and 50 ml direct soil application, respectively). A complete randomized block design was used with three replicas (each replica consisted of 3 plants). A week post transplanting into the final pots, the plants were divided into four greenhouse benches (block), and different growth characteristic measurements were recorded (plant height at 37 51 and 65 days post-germination (dpg), as well as above-ground fresh and dry weight at the end of the experiment). The bacterial cultures were prepared as follows, a single colony from each of the chosen bacterial isolates was placed in 5 ml LB liquid medium, fortified with 0.1% tryptophan, and placed in a shaker incubator for 48 hr. The next day, 1 ml of the culture "1 x 107 CFU/ml" was used to inoculate 1-liter LB-tryptone medium, followed by 48 hr incubation in a 30 ºC shaker incubator at 100 rpm. The greenhouse experiment was terminated 65 days post-germination, and the following parameters (plant heights above ground fresh and dry weight) were recorded immediately in the greenhouse and dry weight (60 °C oven for 72 hrs.).

Statistical analysis: Statistical analysis and variance analysis were performed using ASSISTAT software version 7.6 beta by Professor Francisco de Assis Santos e Silva (http://www.assistat.com).

RESULTS AND DISCUSSION

Endophytic bacteria isolation from plants growing in the Hamra Oasis, El-Natrun Valley. The area of El-Natrun Valley is located in the arid region of the northern part of Egypt. It is situated below sea level and consist of several lakes and water feed is provided from underground water from the Nile River, which touches surrounding grass swamp areas (Ali *et al*., 2013), mimicking an estuarine environment (Gutierrez *et al*., 2009a) and making it a rich source for isolating halophytic microorganisms. Different plant species are flourishing under these harsh conditions, including *P. australis, T. nilotica, J. rigidus, and H. strobilaceum*.

A total of 37 isolates were purified from the tissue of *P. australis, T. nilotica, J. rigidus, and H. strobilaceum* (Table (1); Figs. 2A B C and D). The different isolates were then identified using 16S ribosomal DNA sequencing. Then the sequences were defined using a basic alignment search tool (BLAST, http:// www.ncbi.nlm.nih.gov) and comparisons

with the GenBank nucleotide databank, and once the sequences were identified and submitted to the NCBI (accession no. KY608807 to KY608843; Table (1); Fig. (1). Based on the resulted sequences, the different isolates were classified as *Vibrio sp. V. parahaemolyticus*, *V. alginolyticus*, *Bacillus sp.*, *B. pumilus*, *B. sonorensis*, *B. lichenformis*, *B. safensis*, *B. subtilis*, *B. mojavensis*, *B.aerius*, *B. flexus*, *B. axarquiensis*, *B. tequilensis*, *Staphylococcus epidermidis*, and *S. succinus* (Fig. 1).

A phylogenetic tree of endophytic bacterial isolates based on 16S nucleotide sequences was constructed by the neighbor-joining method and evolutionary studies performed in MEGA 7. The sequences' alignment was done with CLUSTALW; bootstrap values were calculated from 1000 re-sampling, with genetic distances shown on scale bars. The tree displayed two major branches, I and II; the 1st is divided into six separate clades (Clade A to F), and while the 2nd consisted only of *Vibrio sp*. *Staphylococcus* specimens we separated into a single clade (A). *Bacillus flexus* did not share enough similarities with the rest of the used samples in this study, so it was separated alone in a clade B. *Bacillus safensis* and *B. pumilus* share genetic similarities to be in the same clade C separated from the rest *Bacillus sp*. Interestingly *B.aerius* had high similarities to *B. pumilus* HEP6C1 and differed from the rest, so resulted in being in clade D. In clade E, there were many similar species of *Bacillus* (*mojavensis*, *axarquiensis*, *subtilis*, and *tequilensis*). Finally, clade F contained all *B. licheniformis* specimens along with *B. sonorensis* HEP3B1.

Morphological and physiological characterization of the different endophytic isolates.

No growth was achieved in all isolated bacteria under acidic conditions, pH 4 (therefore, omitted from Table 1). The numbers present in Table (1) under pH 7 and 10 represent bacterial growth (O.D. ≈ 600) 48 hr post-inoculation. All bacterial isolates grow well at 5% NaCl concentration, equivalent to 0.85 mol NaCl (except for isolates HEP9A2), while no growth was observed under higher NaCl concentrations (10, 15, and 20%; Table 1).

Quantification of IAA production in the different endophytic isolates.

A preliminary screening of all isolates had been performed following the procedure of Bric *et al*., (1991) using a thin nitrocellulose membrane, which indicates that the isolates we had in our hands are indeed IAA-producing bacteria (data not shown). Measurement of IAA isolates-production was conducted a colorimetric method using Salkowski reagent (Gordon and Weber, 1951; Patten and Glick, 1996). The red color was visible within a few mins, yet all samples were measured 30 mins post-incubation in the dark. Bacterial production of IAA is supported in nature *via* the presence of tryptophan from root-exudates, decaying plant cells, or the addition of organic fertilizer (Arkhipchenko *et al*., 2006). Therefore, for IAA quantification, and based upon previous work by

different groups (Patten and Glick, 2002; Swain *et al*., 2007), which indicated that tryptophan added to the bacterial growth medium caused an increase in IAA production (different classes of rhizosphere bacteria), therefore in the present work, LB medium was fortified with 0.1% L-tryptophan was used. Different endophytic bacterial isolates have been found to possess the ability of plant's phytohormone production, thus facilitating plant growth, as in gibberellins (GA3; Khan *et al*., 2014), abscisic acid (ABA; Shahzad *et al*., 2017), and indole acetic acid (IAA; Ali *et al*., 2017). Vendan *et al.,* (2010) confirmed the isolation of more than 50 bacterial endophytes (belonging to *Firmicutes*, *Actinobacteria*, α-*Proteobacteria* γ-*Proteobacteria* families) from ginseng that reported IAA-producing activity. Miliūtė and Buzaitė, (2011) reported that 18 bacterial endophytes were isolated from apple buds, and most of the isolates showed IAA production activity. Sorty *et al.,* (2016) also reported the isolation of different IAA-producing endophytes (belonging to different genera) from *Psoralea corylifolia* L. (a weed). They concluded that the isolates' cell-free extract was able to enhance the germination of wheat seed and seedlings under saline stress *via* IAA production.

In plants, IAA acts as a signaling molecule and affects genes' expression under different conditions (Cassán *et al*., 2014; Egamberdieva *et al.,* 2015). The IAA production and, therefore, plant-growth depends on the IAA gradient in a delicate balance with other growth hormones, resulting in inhibition, stimulation, and differentiation of tissue. Low IAA levels trigger root elongation while a higher-level causes laterals and adventitious root formation (Ghosh *et al.*, 2013). Related-root colonizing phytohormones of rhizobacteria and endophytes effectively colonized and supplied additional IAA for plant growth and development (Sukumar *et al*., 2013). Because of all this, in the present work, we concentrated on identifying isolates based on their ability to produce IAA and found that some of the highest IAA-producing isolates (HEP3B1, HEP4A2, and HEP4A4) belong to *the Bacillus* genus while the other two (HEP1B3, and HEP1B2) belongs to *Vibrio*.

Bacillus sp. is characterized in numerous reports for their IAA production in large quantities (Zhao *et al*., 2011; Bibi *et al*., 2018), yet surprisingly, two isolates are isolated belong to Vibrio sp. (Gutierrez *et al*., (2009b) and later, Kerkar *et al*., (2012) reported the isolation of highly producing IAA Vibrio sp. Gutierrez *et al*. (2009b) reported for the first time, the isolation of 8 Vibrio type strains and five additional species-level clades as IAA-producing from the rhizosphere of Spartina alterniflora and Juncus roemerianus plants. In this study, one of the plants used for the isolation of endophytes belongs to the Juncus genus (J. rigidus) growing in the Hamra Oasis's surrounded environment. At the same time, Kerkar *et al*., (2012) reported the isolation of high IAA-producing Vibrio diazotrophicus from biofilms attached to

the saltern area. Based on the results provided in Table (1), the highest IAA-producing strains were chosen (HEP3B1, HEP4A2, HEP4A4, HEP1B3, and HEP1B2; (Table 1) for further greenhouse studies.

Treatment of tomato plants under greenhouse conditions causes a significant increase in plant height and dry weight. The following isolates (HEP3B1, HEP4A2, HEP4A4, HEP1B3, and HEP1B2) were used separately in a greenhouse experiment to study their effect on promoting the growth of tomato plants under non-stressed terms (Figs. 2 E and F). Different application methods were used (50 ml bacterial foliage-application, 10- and 50 ml direct bacterial application to soil surface), along with two controls (no-application at all, and 50 ml foliage application of 1.0 mg/L IAA solution; Table (2).

The effect of each of the five bacterial isolates for promoting the growth of tomato plants growth (plant height, fresh and dry weight; Figs. 3A, B, and C, respectively) was analyzed. HEP1B3 and HEP4A4 strains cause an increase in plant height significantly compared to other strains and non-inoculated controls (Figure 3A). All tested bacterial strains caused a significant accumulation of above-ground fresh weight, compared to non-inoculated controls (except those inoculated with HEP1B2 strain; Figure 3B). Tomato plants inoculated with the different bacterial strains showed a significant increase in dry weight than non-inoculated controls (Fig. 3C).

The bacterial application method was also tested (foliage spraying vs. direct application to the soil surface at 10 and 50 ml). Results indicate that direct soil application (at 10 and 50 ml) caused a significant increase in tomato plant height (Figure 4A), as well as in fresh and dry weight accumulation (Figs. 4B and C). Although foliage application did not cause a significant increase in plant height compared to non-inoculated controls (Fig. 4A), significant differences were observed at fresh and dry weight levels (Figs. 4B and C, respectively). In general, regardless of the application method used, all the bacterial was separately inoculated-tomato plants resulted in an increment in above-ground fresh and dry weight compared to non-bacterial inoculated controls (Figs. 4B and C, respectively).

Figure (5) summarizes the effect of bacterial treatment on tomato plants growing under greenhouse conditions. It shows that bacterial inoculants were significantly taller (Fig. 5A), accompanied by an accumulation of higher fresh and dry weight non-inoculated controls (Figs. 5B and C, respectively). Our results also indicated that endophytes-inoculated tomato plants showed an increase in fresh (13.2 up to 43.4 %) and dry weight (42.4 up to 52.6%) under non-stressed conditions compared to non-inoculated controls. Previous work using different *Pseudomonas sp.* to examine the effectiveness of different inoculation methods on tomato plant

growth-promotion revealed that the soaking of tomato seedling roots and tomato seed coating had statistically similar results improving plant growth to other methods used (Adesemoye and Ugoji, 2006).

Plant growth promotion due to IAA-production by endophytic bacteria has been reported by different workers (Yasmin *et al*., 2009; Adesemoye and Egamberdeiva, 2013) on potato promotion and tomato plants upon inoculation with endophytic growth-promoting bacteria. Yasmin *et al*. (2009) concluded that improvement in fresh weight and potato yield was connected to IAA production. While Adesemoye and Egamberdeiva, (2013) tested three isolates of *Pseudomonas* (*P. putida*, *P. chlororaphis,* and *P. extremorientalis*) with tomato plant growing under salinated soil, and reported a fresh weight stimulation of 26-28%, coupled with a 22% increase in tomato fruit yield compared to the non-inoculated plants.

Final conclusion. In a world where extensive usage of fertilizers and chemicals is necessary to accommodate the world's growing population, any new non-traditional method is encouraged to maintain highly productive agriculture. In the present study, we investigated the possibility of using halophytic endophytic bacterial strains isolated from salinity tolerant species growing in Egypt's saline area to promote and induce growth characteristic of a commercially important crop in Egypt growing under non-stressed greenhouse conditions. Inoculation of tomato plants with different bacterial strains increased plant height, accompanied by an increase in fresh and dry weight. This approach could provide an environmentally benign technique to increase crop productivity, yet further investigations to evaluate the performance of different endophytic microorganisms-inoculated plant under dehydration-inducing stress conditions is still to follow to identify agronomically-important endophytic microorganisms for commercial crops in Egypt.

SUMMARY

Thirty-seven endophytic bacterial isolates were isolated from tissues of four salt-tolerant plant species (*Phragmites australis*, *Tamarix nilotica*, *Juncus rigidus*, and *Halocnemum strobilaceum*) growing on shores of the El-Hamra Oasis (hypersaline soda lakes, with salinity level, reaches up to 5.0 M NaCl), EL-Natrun Valley, Egypt. Isolates were determined by different morphological, physiological, and molecular characters. Sequencing data of 16S rRNA declare that the 37 isolates belong to 3 genera *Bacillus*, *Staphylococcus*, and *Vibrio* (accessions no. Ky608807 - KY608843). All isolates produced Indol-3-acetic acid (IAA) when allowed to grow in LB media fortified with 0.1% L-tryptophan. The highest five potent isolates "IAA- producer" were selected and used to inoculate tomato (*Lycopersicon esculentum*) plants under greenhouse conditions. Based on plant growth characteristics that were measured at 37, 51, and 65 days post-germination; we found that 10

or 50 ml (1 x 107 CFU/ml) direct addition of bacterial culture to soil surface caused a significant increase in tomato plants' height (above-ground fresh and dry weight) compared to direct foliage spraying. When pooled together, all bacterial treatment caused a significant increase in tomato height (ranging from 4.9 up to 25.8%), fresh weight (13.2 up to 43.4%), and dry weight (42.37 up to 51.58%, with an average of 46.73%), compared to non-bacterial inoculated controls (non-treated, or IAA-sprayed) 65 days post-germination.

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All datasets analyzed in the study are included in the manuscript and presented as tables and figures.

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Table (1): List of the endophytic bacterial strains isolated from tissues of different plant species that grow in the region around the Hamra Oasis, Natrun Valley (*Phragmites australis, Tamarixnilotica, Juncus rigidus and Halocnemumstrobilaceum*). The table also shows their growth characteristic under different pH, temperatures, NaCl concentration, as well as IAA production.

Code	Scientific name	Accession	O.D. at pH 10 30°C 40°C 55°C	O.D. at pH 7 30°C 40°C 55°C	NaCl (%) 5 10 15 20	IAA (ug/100 ml culture)	Plant Species, tissue
HEP1 B1	*V. parahaemolyticus*	KY608807	- - -	3.05 2.84 -	++ - - -	10.77	*T. nilotica*, Root
HEP1 B2*	*Vibrio sp*	KY608808	- - -	2.94 2.67 -	++ - - -	20.00	*T. nilotica*, Root
HEP1 B3*	*V. alginolyticus*	KY608809	1.45 0.46 -	3.00 2.74 -	++ - - -	31.00	*T. nilotica*, Root
HEP3 A1	*B. pumilus*	KY608810	1.79 1.01 -	2.57 1.96 -	++ - - -	9.52	*P. australis*, Root
HEP3 A2	*Bacillus sp*	KY608811	2.43 1.40 -	2.34 1.07 -	++ - - -	9.17	*P. australis*, Root
HEP3 B1*	*B. sonorensis*	KY608812	2.73 2.05 -	2.71 1.87 -	++ - - -	22.60	*P. australis*, Root
HEP3 B2	*B. lichenformis*	KY608813	2.90 2.10 -	2.43 2.07 -	++ - - -	5.52	*P. australis*, Root
HEP4 A2*	*Bacillus sp*	KY608814	2.40 0.99 -	1.81 2.68 -	++ - - -	43.52	*P. australis*, leaves
HEP4 A3	*B. safensis*	KY608815	2.64 0.90 -	1.89 2.91 -	++ - - -	11.05	*P. australis*, leaves
HEP4 A4*	*B. pumilus*	KY608816	2.73 1.27 -	3.00 2.91 -	++ - - -	43.58	*P. australis*, leaves
HEP4 C2	*B. pumilus*	KY608817	2.69 1.81 -	1.53 1.68 -	++ - - -	7.35	*P. australis*, leaves
HEP5 A2	*B. licheniformis*	KY608818	1.57 0.58 -	1.68 1.35 -	++ - - -	7.02	*P. australis*, Root

Table(1): Cont'

HEP5 A3	*Bacillus sp*	KY608 819	1.39	0.58	-	2.09	1.97	-	++	-	-	12.20	*P. australis*, Root
HEP5 A4	*B. licheniformis*	KY608 820	0.85	0.12	-	1.94	2.30	-	++	-	-	9.80	*P. australis*, Root
HEP5 B1	*B. licheniformis*	KY608 821	2.83	1.29	-	2.78	2.74	-	++	-	-	9.30	*P. australis*, Root
HEP5 B2	*B. licheniformis*	KY608 822	1.69	0.33	-	2.09	2.84	-	++	-	-	8.95	*P. australis*, Root
HEP5 C2	*B. licheniformis*	KY608 823	1.28	0.70	-	1.93	2.67	-	++	-	-	5.85	*P. australis*, Root
HEP6 B1	*B. subtilis*	KY608 824	1.09	0.21	-	1.63	0.36	-	++	-	-	5.52	*P. australis*, leaves
HEP6 B11	*B. mojavensis*	KY608 825	0.64	0.11	-	0.85	0.29	-	++	-	-	11.47	*P. australis*, leaves
HEP6 B2	*B. subtilis*	KY608 826	0.47	-	-	1.67	0.48	-	++	-	-	9.05	*P. australis*, leaves
HEP6 C1	*B. pumilus*	KY608 827	2.08	1.77	-	2.05	1.69	-	++	-	-	7.80	*P. australis*, leaves
HEP7 A2	*B. aerius*	KY608 828	2.90	2.53	-	1.79	1.48	-	++	-	-	5.67	*P. australis*, leaves
HEP7 A4	*B. pumilus*	KY608 829	2.30	1.34	-	2.59	2.10	-	++	-	-	5.75	*P. australis*, leaves
HEP8 B1	*B. flexus*	KY608 830	0.67	-	-	1.87	1.79	-	++	-	-	11.47	*J. rigidus*, shoots
HEP8 B2	*S. epidermidis*	KY608 831	0.90	0.43	-	1.98	2.03	-	++	-	-	9.02	*J. rigidus*, shoots
HEP9 A2	*B. safensis*	KY608 832	2.01	1.48	-	2.35	2.94	-	-	-	-	6.02	*J. rigidus*, shoots
HEP9 A4	*B. safensis*	KY608 833	2.40	2.03	-	2.87	3.01	-	++	-	-	12.20	*J. rigidus*, shoots

Table(1): Cont'

HEP9B1	*B. safensis*	KY608834	2.48 2.00 -	1.98 1.60 -	++ - - -	10.10	*J. rigidus*, shoots
HEP10A1	*B. subtilis*	KY608835	0.75 - -	1.99 2.64 -	++ - - -	7.35	*H. strobilaceum*
HEP10A4	*B. axarquiensis*	KY608836	0.68 - -	1.97 1.76 -	++ - - -	6.75	*H. strobilaceum*
HEP11A1	*B. subtilis*	KY608837	0.34 0.13 -	2.68 1.80 -	++ - - -	9.05	*H. strobilaceum*
HEP11A2	*B. subtilis*	KY608838	0.45 0.11 -	2.91 1.96 -	++ - - -	16.00	*H. strobilaceum*
HEP11A3	*B. licheniformis*	KY608839	1.30 0.72 -	2.78 1.68 -	++ - - -	11.47	*H. strobilaceum*
HEP13A2	*B. subtilis*	KY608840	0.74 - -	2.06 1.45 -	++ - - -	7.20	*J. rigidus*, shoots
HEP13A4	*S. succinus*	KY608841	1.08 0.50 -	1.04 0.53 -	++ - - -	5.53	*J. rigidus*, shoots
HEP15A3	*B. tequilensis*	KY608842	1.18 - -	1.10 1.02 -	++ - - -	10.52	*J. rigidus*, Root
HEP15A4	*S. succinus*	KY608843	1.11 0.65 -	2.10 1.99 -	++ - - -	5.85	*J. rigidus*, Root

Table (2): Measurements of plants height (37, 51, and 65 dpg), and above-ground fresh and dry weight (at 65 dpg) of tomato plants treated with 5 endophytic bacterial isolates.

TREATMENT	LENGTH T1	LENGTH T2	LENGTH T3	FRESH WEIGHT	DRY WEIGHT
CONTROL	35.10 ± 2.85	44.17 ± 4.67	55.67 ± 2.42	14.48 ± 0.23	3.98 ± 0.12
IAA SPRAY	33.87 ± 4.58	45.47 ± 2.37	60.67 ± 3.39	17.05 ± 0.26	4.94 ± 0.13
HEP4A2 SPRAY	30.33 ± 4.91	40.33 ± 2.67	53.33 ± 1.26	21.83 ± 1.92	5.72 ± 0.27
HEP4A2 (10ML)	31.33 ± 0.67	44.00 ± 3.05	60.01 ± 2.89	19.37 ± 1.11	5.81 ± 0.03
HEP4A2 (50 ML)	31.67 ± 1.45	49.33 ± 2.60	61.67 ± 1.67	21.11 ± 0.67	5.89 ± 0.14
HEP1B3 SPRAY	42.67 ± 4.70	58.33 ± 4.33	69.51 ± 1.69	16.07 ± 0.46	5.95 ± 0.15
HEP1B3 (10ML)	49.00 ± 1.01	65.51 ± 2.89	78.67 ± 3.21	20.34 ± 1.23	5.82 ± 0.41
HEP1B3 (50 ML)	33.00 ± 2.10	44.67 ± 2.09	62.01 ± 2.11	18.34 ± 0.51	6.05 ± 0.58
HEP4A4 SPRAY	36.00 ± 4.58	41.67 ± 1.67	57.01 ± 2.33	20.3 7± 0.31	5.69 ± 0.17
HEP4A4 (10 ML)	31.67 ± 2.40	51.67 ± 3.67	70.20 ± 1.01	18.99 ± 2.83	5.79 ± 0.25
HEP4A4 (50 ML)	40.33 ± 2.85	53.00 ± 1.00	70.33 ± 2.73	18.51 ± 1.57	5.72 ± 0.11
HEP1B2 SPRAY	31.00 ± 2.08	44.00 ± 3.79	52.67 ± 3.71	19.04 ± 0.75	6.28 ± 0.30
HEP1B2 (10 ML)	30.00 ± 1.15	40.33 ± 2.34	54.00 ± 3.05	15.91 ± 2.02	5.95 ± 0.63
HEP1B2 (50 ML)	26.33 ± 2.18	39.00 ± 2.89	50.67 ± 0.67	14.25 ± 1.55	5.89 ± 0.19
HEP3B1 SPRAY	40.67 ± 2.90	50.67 ± 0.67	58.67 ± 2.67	16.11 ± 1.36	5.78 ± 0.03
HEP3B1 (10 ML)	38.33 ± 1.67	50.00 ± 2.89	64.33 ± 3.48	19.18 ± 1.52	5.68 ± 0.16
HEP3B1 (50 ML)	36.00 ± 2.58	51.33 ± 3.81	65.00 ± 3.64	20.91 ± 1.49	5.54 ± 0.05

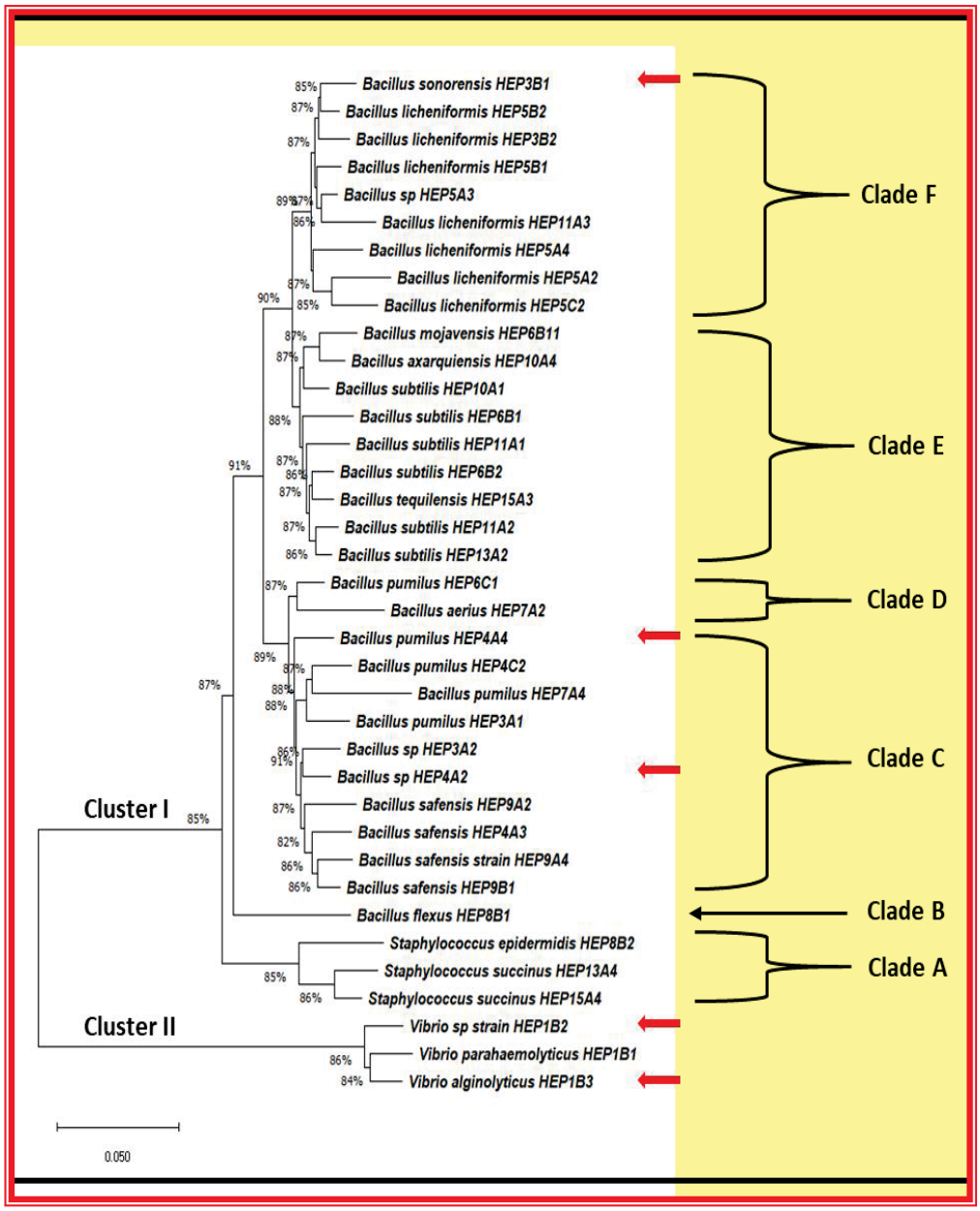

Fig. (1): Phylogenetic of endophytic bacterial isolates distribution based on 16S rDNA gene sequence from wild plants grown in the surroundings El-Hamra Oasis, EL-Natrun Valley. The phylogenetic relationships were inferred using the neighbor-joining approach from the *16 S* rRNA gene, and an evolutionary analysis was performed in MEGA 7. Alignment of the sequences was done with CLUSTALW, bootstrap values were calculated from 1000 re-sampling, with genetic distances shown on scale bars.

Fig. (2): Pictures representing El-Hamra oasis, AL-Natrun valley (A) with the distribution of different halophytic plants (B, C, and D) in the area. E and (F) representing part of the greenhouse experiments 37 days post germination and just before application of the different bacterial strains in the greenhouse.

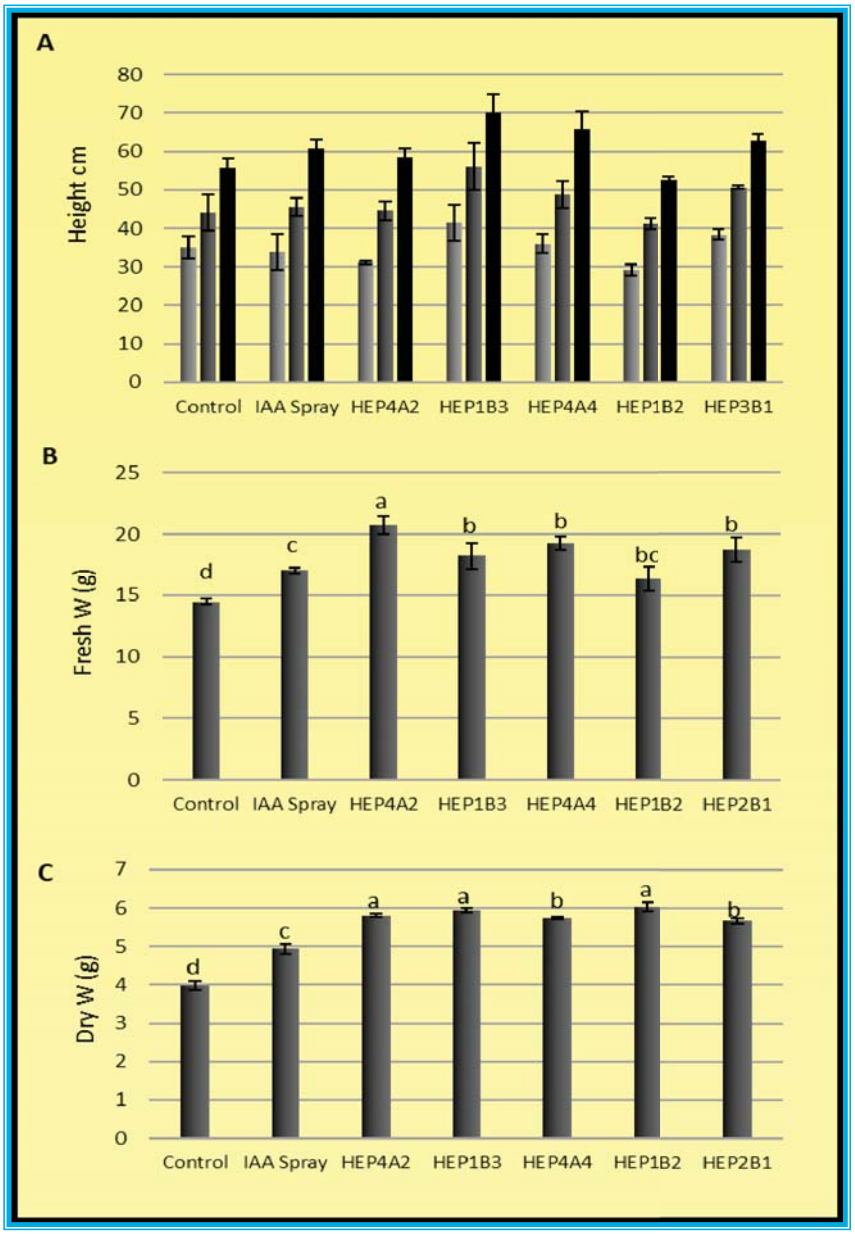

Fig. (3): Impact of endophytic bacterial isolates on tomato plants growing under greenhouse conditions on plant height (A), above ground fresh weight (B) and dry weight (C). Bars are the plant height ± standard error. Bars with same letter are not significantly different at $P \leq 0.05$.

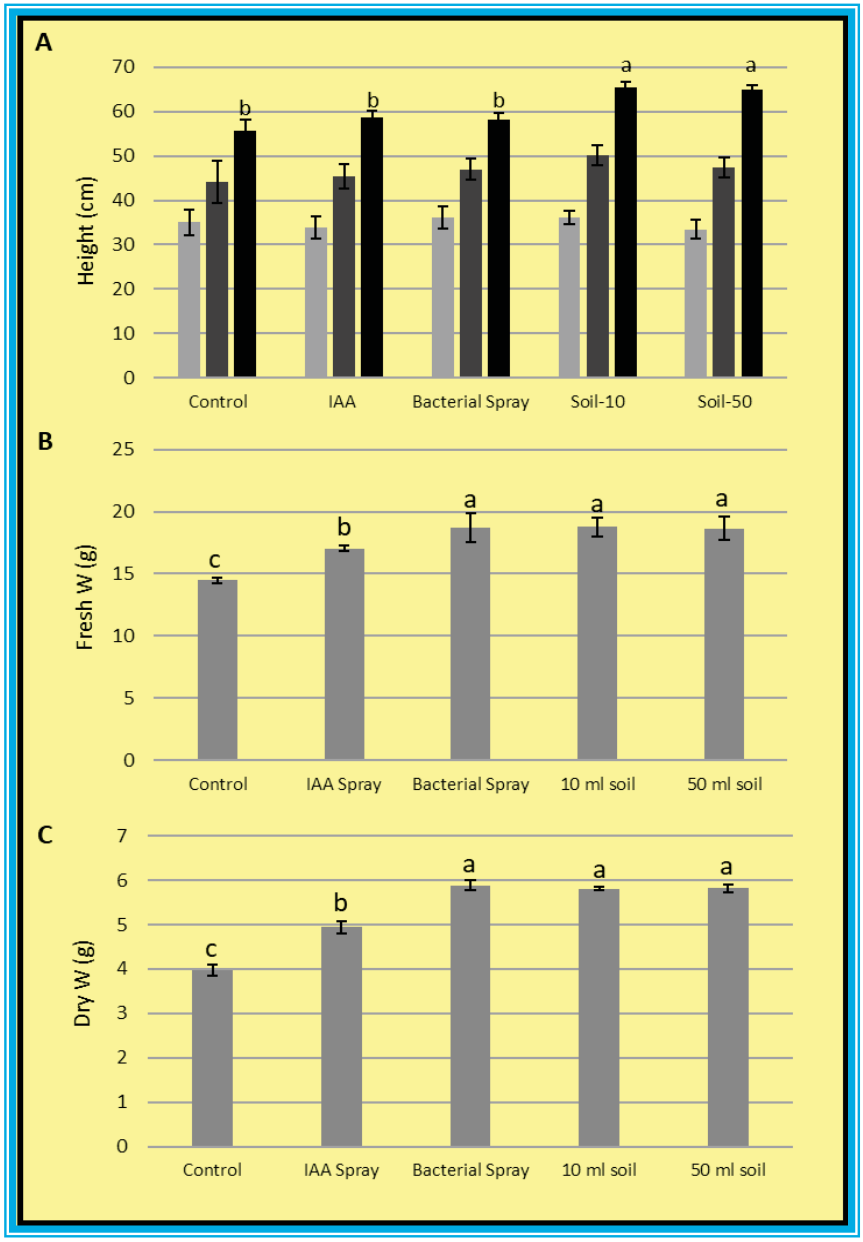

Fig. (4): The effect of different application methods on plant height (A), above ground fresh weight (B) and dry weight (C) of tomato plants growing under greenhouse conditions. Bars represents average plant height ± standard error. Bars with the same letter are not significantly different at $P \leq 0.05$.

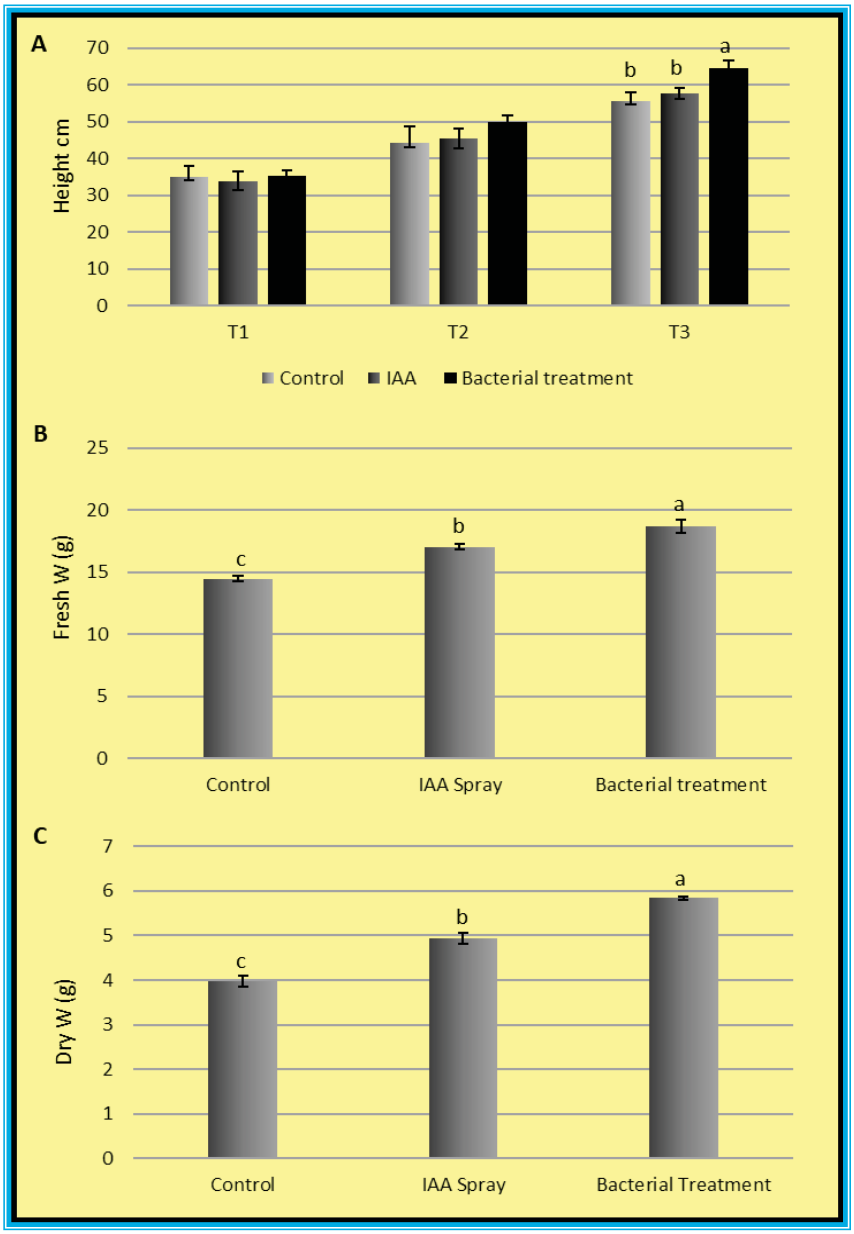

Fig. (5): Endophytic bacterial isolates effect vs. non-inoculated controls (non-treated, and IAA spray) on plants height (A), above ground fresh weight (B) and dry weight (C). (A) bacterial treatment causes a significant rise in plant height compared with IAA and un-treated plants. (B) bacterial treatment had also significant effect on increasing fresh (B) and dry weight (C) of tomato plants compared to non-bacterial treated controls. Bars represent average plant height ± standard error. Bars with same letter are not significantly different at $P \leq 0.05$.