Use of plant growth-promoting rhizobacteria isolates as a potential biofertiliser for wheat

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Abstract: Plant growth-promoting rhizobacteria (PGPR) isolated from the rhizosphere soil of eight field crops at different locations in Egypt were identified. Rhizobacteria strains were identified as *Bacillus endophyticus* AW1 5, *B. filamentosus* EM9, ET3, *Micrococcus luteus* KT2, FW9, FC13, SaW4, *Enterobacter cloacae* SK18, *Pseudomonas azotoformans* TPo10, *Citrobacter braakii* TC3. All isolates solubilised insoluble phosphate and produced *IAA*, while only six were able to produce siderophores *in vitro*. Vegetative growth and yield of wheat cv. 'Sakha 94' were enhanced after the application of single inoculation of each isolate compared to the control. Grain yield was increased by 20.7–96.5% over the control according to bacterial isolates. Available phosphorus (P) and counts of total bacteria in soil were observed to be significantly increased in treatments than in control. After the wheat harvest, soil pH was observed to be decreased, and a highly significant negative correlation was observed between soil pH and the levels of available phosphorus. Significant increases in grain and straw yields, as well as uptake of nitrogen (N) and P by plants, were observed due to inoculation with PGPR isolates. Levels of photosynthetic pigments, free amino acids, free phenolics, and reducing sugars in flag leaf and spikes were significantly enhanced by the application of all PGPR isolates compared to the control. Thus this study identifies the PGPR isolates for the improvement of the growth, yield, and quality of wheat. The study may be also useful for field evaluation under different soils and environmental conditions before generalising PGPR isolates as biofertilisers.

Keywords: nitrogen, phosphorous, plant growth-promoting rhizobacteria, siderophores

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are specific strains of soil bacteria inhabiting around or on the root surface and could stimulate plant growth [BACKER *et al.* 2018; DASGUPTA *et al.* 2015]. It can directly promote plant growth via nitrogen fixation, production of phytohormones such as auxins and gibberellins, solubilisation of phosphate, and improve the availability of iron, or indirectly, as biological control agents. DIAS *et al.* [2013], KUNDAN *et al.* [2015] and DOS SANTOS [2020] obtained 78 isolates

of fluorescent rhizobacteria from rhizospheric soil samples of kale, lettuce, parsley, and rudbeckia plants and found that all isolates were able to produce indole-3-acetic acid (*IAA*). It was known that *IAA* promoted plant growth and development through the extension and differentiation of plant cells and stimulated the formation of the lateral root [GAMALERO, GLICK 2011; GROVER *et al.* 2021].

Different PGPR, such as *Bacillus* and *Pseudomonas*, could dissolve insoluble phosphate (P) and increase the availability of P to plants via secreting organic acids, which decrease soil pH or

chelate calcium and iron, resulting in the effective releasing of phosphates [BECHTAOUI et al. 2019]. Phosphorus is an essential microelement, required for the growth and development of plant organs, especially roots, and necessary for photosynthesis and respiration.

Siderophores are produced by numerous members of the genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Rhodococcus*, and other rhizobacteria [RAZA, SHEN 2010]. Iron is an essential, immobile micronutrient in plants. It is a cofactor for different enzymes, such as superoxide dismutase, cytochrome oxidase, and chlorophyll synthase, which are involved in important physiological processes, such as respiration.

PGPR inoculants improved the plant growth and yield of various crops under both: greenhouse and field conditions. In this regard, inoculation with *Pseudomonas fluorescens*, *P. putida*, and *P. cepacia* enhanced the grain yield of wheat by 46–75% compared to uninoculated ones [DE FREITAS, GERMIDA 1992]. In addition, both root and shoot weight of barley increased by 16.7 and 34.7%, respectively, compared to untreated seedlings after the application of *Bacillus* spp. [CANBOLAT et al. 2006]. Length of wheat roots and shoots increased after inoculation with *Paenibacillus brasiliensis* (2025-11), *Acinetobacter* (2077-5), *Serratia proteamaculans* (2025-1), *Bacillus cereus* (2026-2), *Bacillus cereus* (2027-2), and *Bacillus* sp. (2015-1) compared to the uninoculated control [MOUSTAINE et al. 2016].

Enhancement of plant growth and its yield after the application of different types of PGPR is mainly due to the vital role of these bacteria in improving the physiological process of plants through an increase in their contents of photosynthetic pigments and macroelements, such as reducing sugar and amino acids. In this respect, SHARMA et al. [2003] found that the application of the siderophore-producing *Pseudomonas* strain GRP3 to mungbean increased the chlorophyll a and b contents compared to the control. Also, WANI and KHAN [2010] found that the application of *Bacillus* species strain PSB10 to potted chickpea significantly improved growth, chlorophyll, seed yield, and protein content.

The protein quantity and quality of different crops are limiting factors in industrial and nutritional usage, particularly in bread wheat. STEFAN et al. [2007] reported that inoculated soybean with eight strains of PGPR (RS1 to RS8) caused high protein content in seeds compared to the uninoculated control. Electrophoretic analysis of protein using SDS-PAGE techniques showed that PGPR-treated soybean differed in its quantity in some unique bands with molecular masses of 105, 86, 80, 52, 38, and 31.5 kDa. On the contrary, PRATHIBHA and SIDDALINGESHWARA [2013] observed that the pattern of protein bands significantly differed in seeds of two varieties of sorghum treated with the PGPR *Pseudomonas fluorescence*, *Bacillus subtilis,* in comparison to the control. Description of protein electrophoretic gel showed that bands with 35 and 50 kDa were present in all three treatments. A 40 kDa protein band was a unique one, present only in seeds, both treated with *P. fluorescence* and *B. subtilis*. A 14 kDa protein band was present in control and seeds treated with *P. fluorescence*, with greater intensity in seeds treated with *P. fluorescence*, and was absent in *B. subtilis*-treated lane. It is obvious that the presence or absence of unique band (s) of protein of plant seed indicates the types of PGPRs inhabiting the rhizosphere.

Therefore, this research was planned to isolate and identify the PGPR from different field crops cultivated at different sites, and evaluate them for their capacity for phosphate solubilisation, production of siderophores, and indole acetic acid. The study will also help to identify the PGPR isolates based on their impact on P, pH, total bacterial counts in soil, and ultimately their influence on the growth and yield of wheat.

MATERIALS AND METHODS

SOIL SAMPLE COLLECTION AND PGPR ISOLATION

Rhizospheric soil samples were collected from the rhizosphere of eight field crops grown at various sites in Ismailia, Egypt during the 2015–2016 seasons. The samples were collected from roots by gently shaking to remove the adhering soil [ABD EL-AZEEM 2006]. The rhizobacterial isolates were denominated based on the location of samples: Abu Sawyer (A), Experimental Farm of the Faculty of Agriculture, Suez Canal University (E), Fayed (F), El-Kassasin (K), El-Salhia (Sa), Sarabium (S), El-Tal El-Kabeer (T), and the host plants, clover (C); kidney bean (K); maize (M); potato (Po), tomato (T) and wheat (W).

Bacterial strains were isolated by dilution plate method using two media. First was tryptic soy agar, which comprised in g·dm^{-3}: Tryptone 15, soybean peptone (Soytone) 5, NaCl 5, agar 15. The second was King's B agar medium (pH 7.20) comprising in g·dm^{-3}: protease peptone 20, glycerol, 10 cm^3, MgSO$_4$ 7, H$_2$O 6, K$_2$HPO$_4$ 2.5, agar 15 [STARR et al. 1981]. The plates were incubated at 28 ±2°C for 3–5 days. Morphologically different colonies appearing on the media were isolated and streaked on plates with the same medium for purification. The bacterial isolates were kept in 20% glycerol and stored in a refrigerator. Ten isolates showing prolific growth and having a different morphological appearance on agar medium were selected and stored for completing this study.

Phenotypic characterisation of isolates

Cultural, morphological and biochemical properties of the bacterial isolates were examined based on the scheme of bacterial identification of MAC FADDIN [1976], and the methods described in Bergey's manual of determinative bacteriology [HOLT et al. 1994] and Bergey's manual of systematic bacteriology 2nd ed. [VOS et al. 2009]. All isolates were characterised using Gram stain, endospore, and light microscope as well as some biochemical tests.

Identification of isolates using the sequencing of 16S rRNA gene

Ten bacterial isolates were identified according to 16S rRNA gene partial sequences. Bacterial genomic DNA was extracted from isolates according to STANLEY [1990]. After the extraction of bacterial DNA, the polymerase chain reaction (PCR) was done to amplify the 16S rRNA gene using a universal primer (Invitrogen, USA). The right primer was 5'-ATTCCGGTTGATCCTGCCGG-3' and the left one was 5' AGGAGGTGATCCAGCCGCAG-3' [VENTOSA, NETO 1995]. The PCR program was, 95°C predenaturation 5 min, 94°C denaturation 1 min, 60°C annealing 1 min, 72°C extension 90 s, 72°C final extension 10 min, 4°C hold. The PCR product was electrophoretic on agarose (1.3%) gel using ethidium bromide. 50 ng·mm^{-3} of each PCR product was used to

prepare the sample, which was delivered to MacroGen company in Korea following their specifications. The sequences were analysed using BLAST [undated] to get a preliminary identification of the PGPR strains.

PLANT GROWTH-PROMOTING TRAITS OF SELECTED BACTERIAL ISOLATES

Phosphate solubilisation

The efficiency of the isolates to dissolve insoluble mineral phosphate (tricalcium phosphate-$Ca_3(PO_4)_2$) was tested in liquid cultures *in vitro*. Solubilisation of tricalcium phosphate was estimated using Erlenmeyer flasks (100 cm^3) containing 40 cm^3 of National Botanical Research Institute's phosphate (NBRIP) medium, which comprised in $g \cdot dm^{-3}$: glucose 10, $Ca_3(PO_4)_2$ 5, $MgCl_2 \cdot 6H_2O$ 5, $MgSO_4 \cdot 7H_2O$ 0.25, KCl 0.20, $(NH_4)_2SO_4$ 0.10, agar 15. The pH value of the medium was initially kept at 7.10 to ensure a minimal concentration of soluble phosphate. Insoluble tricalcium phosphate was sterilised individually by autoclaving and added to the flasks at a rate of 5 $g \cdot dm^{-3}$. All flasks were inoculated with the rhizobacterial isolates (0.5 cm^3 inoculum with approximately 10^7–10^8 $CFU \cdot cm^{-3}$). The uninoculated medium served as a control. The flasks were incubated at 30°C for 10 days. The culture's suspensions were centrifuged at 3000 rpm for 30 min. Soluble phosphate and pH in supernatants were determined [JACKSON 1973].

Indole acetic acid (IAA) equivalents production

Production of *IAA* by bacterial isolates was determined using the colorimetric method [SARWAR *et al.* 1992]. Bacterial cultures, 0.5 cm^3 (4-days old and 10^7–10^8 $CFU \cdot cm^{-3}$), were inoculated in 25 cm^3 of nutrient broth and incubated at 30°C for 48 h. Uninoculated flasks were taken as a control. The bacterial cultures were centrifuged at 3000 rpm for 30 min. The 3 cm^3 of the collected supernatant were pipetted into test tubes and 2 cm^3 of Salkowski reagent were added as a colouring agent. The tubes containing the mixture were left for 30 min in the dark for colour development. The intensity of the colour was measured using a spectrophotometer (detection limits 0.2–45.0 $\mu g \cdot cm^{-3}$) at 535 nm. The concentration of *IAA* was estimated with a standard curve of *IAA*.

Siderophore production

The bacterial isolates were qualitatively screened for siderophores production using Chrome Azurol S (CAS) agar medium [ZUBERER, ALEXANDER 1991] containing the ternary complex CAS/Fe^{3+}/hexadecyltrimethyl-ammonium bromide as an indicator. 10 cm^3 of sterilised CAS agar medium were poured into a 9 cm Petri dish. The bacterial inoculum was placed as far as possible in the centre medium. The plates were incubated in the dark at 30°C for 7 days. The CAS agar colour changed from blue to orange, purple, or dark purplish-red surrounding a bacterial colony and was considered positive for siderophore production.

GREENHOUSE EXPERIMENT

Preparation of bacterial inoculum

To prepare bacterial inoculum, a sterile wire loop was utilised to inoculate some bacterial cells from stock culture, suspended in tryptic soy broth into 100 cm^3 flasks containing 40 cm^3 of nutrient broth medium and incubated at 30°C for 4 days. After this period, the counts of all bacterial cultures in the cell suspensions ranged from 10^7 to 10^8 $CFU \cdot cm^{-3}$. The 10 g of wheat seeds were soaked in 40 cm^3 of the cell suspension for 1 h before planting.

Experimental design and treatments

A pot experiment was conducted in the greenhouse of the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt to evaluate the effects of single inoculation with the 10 selected isolates of the rhizobacteria on some soil properties, growth, and yield parameters of wheat. During the experimental period (December 2015–April 2016), the temperature, relative humidity, and light density fluctuated between 12 and 25°C, 58 and 65%, and 15 and 20 klx, respectively. The sandy soil (0–30 cm depth) and cattle manure (*CM*) used in this study were collected from the above-mentioned farm. The soil and *CM* were air-dried, crushed, and sieved through a 2 mm sieve. Some properties of the soil and *CM* were determined according to GEE and BAUDER [1986], and SWIFT [1996] and shown in Table 1.

The soil was uniformly packed in plastic pots with a height of 18 cm and a mean diameter of 17 cm at a rate of 5 kg of soil per pot. A drainage hole of about 1 cm in diameter was made at the bottom of each pot. The soil in each pot was completely mixed with 50 g of *CM* (1% w/w) as an organic fertiliser. The experimental design was a completely randomised block design with five replicates for each treatment. All pots received the same P fertilisation at a rate of 1.0 g superphosphate (15.5% P_2O_5) per pot (equivalent to 31.0 kg P_2O_5 per fed) mixed with the soil before cultivation. Thereafter, 10 inoculated wheat seeds (*Triticum aestivum* cv. 'Sakha 94') were sown in each pot and irrigated to soil field capacity using Ismailia Canal water (0.36 $dS \cdot m^{-1}$). The seedlings were thinned to 5 plants per pot two weeks after cultivation. Ammonium sulphate and potassium sulphate at rates of 0.60 g N and 0.25 g K_2O per pot (equivalent to 120 kg N and

Table 1. Some characteristics of the experimental soil and cattle manure (*CM*)

Medium	Sand	Silt	Clay	Textural grade	pH	EC_e (dS·m⁻¹)	Organic C	Total N	Available N	Available P
							g·kg⁻¹		mg·kg⁻¹	
Soil	94.6%	3.50%	1.90%	sand	8.02[1)]	1.41	1.55	0.17	4.68	4.10
CM	–	–	–	–	7.45[2)]	12.8	139.5	12.2	127	116

[1)] pH in soil-water suspension (1:2.5). [2)] pH in *CM* water suspension (1:5).
Explanations: EC_e = electrical conductivity in soil and *CM* saturated extract.
Source: own elaboration based on GEE, BAUDER [1986], SWIFT [1996].

50 kg K_2O per fed, respectively) were applied to all pots in three split dressings (20, 30, and 50% of the total amounts) 20, 45 and 65 days after sowing date. Wheat plants were harvested 90 and 120 days after the planting date. Plant height (cm) and shoot dry weight (g·pot⁻¹) were estimated 90 days after sowing. After 120 days from sowing, plant height, grains, straw, and biological yield were also investigated. Total N and P were determined in straw and grains. Rhizosphere soil samples were collected from each treatment and analysed for total bacterial counts after 90 and 120 days, as well as for pH and available P after 120 days.

Soil and plant analyses

Rhizosphere soil analyses were conducted as described by Jackson [1973] and Swift [1996]. Briefly, rhizosphere soil pH was determined in soil-water suspensions (1:2.5 w/v) after 1 h of shaking at 25°C. Available P was extracted by 0.5 M NaHCO$_3$ and the P concentration was determined colourimetrically [Kuo 1996]. The total bacterial count was determined using serial dilutions and tryptic soy agar medium [Starr et al. 1981] with an incubation period of 5 days at 28 ±2°C.

The concentrations of N and P in straw and grains were determined and expressed as mg of total P and N per pot. Total N was determined by the Kjeldahl method [Bremner 1996]. Phosphorus was determined after wet digestion of plant samples using a nitric (HNO$_3$)-sulfuric (H$_2$SO$_4$)-perchloric (HClO$_4$) acids mixture (4:1:8, equal volume). P was measured spectrophotometrically using the molybdenum-blue method [Jackson 1973].

Photosynthetic pigments (chlorophyll a, b and carotenoids and expressed in mg·(100 g)⁻¹ of fresh weight) were determined in wheat flag leaf 90 days after sowing. To determine chlorophyll a, b and carotenoids, 0.5 g of leaf ground with 10 cm³ 85% acetone and the optical density was measured at 662, 644, and 441 nm, respectively [Arnon 1949].

Determination of some organic compounds

Free phenolic compounds, reducing sugars and free amino acids were also determined. Briefly, 1 g of flag leaf lamina and the main spike were blended for 1 minute and extracted with 96% ethanol for 72 h at 5°C as described by Abdel-Rahman et al. [1975]. Free phenolics were determined by a modified Folin-Ciocalteu method and measured at 650 nm [William et al. 1965]. Reducing sugars were determined by Nelson's method with alkaline copper and arsenomolybdate reagents and measured at 540 nm [Moore 1974]. Concentrations of total free amino acids were estimated using the method of Rosen [1957] with ninhydrin reagent. The blue-coloured samples were measured against a blank sample at 570 nm.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of soluble storage proteins in wheat grains

One-dimensional SDS-PAGE gel electrophoresis, according to the method of Laemmli [1970], was used to fractionate the soluble storage proteins in grains for inoculated and non-inoculated wheat plants after harvesting. Wheat grains at the amount of 20 mg were dispersed in 1 cm³ SDS 10% with 100 mm³ β-mercaptoethanol for 15 min, then centrifuged at 11,000 g for 10 min; 20 mm³ of extraction were mixed with 20 mm³ of SDS-loading sample buffer (SDS 4%, β-mercaptoethanol 3%, glycerol 20%, tris(hydroxymethyl)aminomethane (TRIS) HCl 50 mM, pH 6.8 and bromophenol blue traces), heated at 96°C for 3 min

and 10 mm³ aliquot was electrophoresed (10 mm³ of protein/lane). The resolving and stacking gels were prepared according to the standard procedure of Davis [1964]. The electrode buffer contained TRIS 50 mM, glycine 0.384 M, and SDS 0.1%. The protein bands were developed with Coomassie brilliant blue R-250 dye (0.2% solution, freshly prepared in 45% methanol, 10% glacial acetic acid, and 45% distilled water) at room temperature overnight. The gel was photographed and made by scan apparatus as densitometry (optical density) analysis at 600 nm using standard protein maker (Pharmacia).

STATISTICAL ANALYSIS

All growth and yield parameters of wheat were analysed statistically by analysis of variance (ANOVA) using Costat statistical software. Correlation between levels of solubilised inorganic phosphate, produced indole acetic acid in vitro or counts of total bacteria in the rhizosphere soil and each of the growth and yield parameters was also calculated by using the SPSS program version 22 [Leech et al. 2005]. The least significant difference test (LSD) was applied to make a comparison between the means ($P < 0.05$).

RESULTS

ISOLATION AND PHENOTYPIC CHARACTERISATION OF BACTERIAL ISOLATES

Approximately 200 isolates were obtained from the rhizosphere of cereals, legumes, and vegetable crops cultivated at different sites in Ismailia and El-Sharkia Governorates, Egypt. Only 10 isolates were selected based on their colony morphology, shape, gram stain, and biochemical properties (Tab. 2) as well as due to their plant growth-promoting activities in vitro (Tab. 3). The isolates were found to belong to five genera viz. Bacillus (3), Micrococcus (4), Pseudomonas (1), Enterobacter (1) and Citrobacter (1).

Most isolates are gram-positive (70%); the cell is straight rod-like or spherical in shape. Specifically, Bacillus has white or creamish white colonies, is irregular or circular with a diameter of 5–8 mm, raised or flat, opaque or translucent, and has lobate or entire edge (Tab. 2). They were straight rod-like, spore-forming and motile except for B. endophyticus. It was variable for oxidase, gelatin liquefaction, nitrate reduction to nitrite, starch hydrolysis, Voges-Prosqauer test, urease production, and utilisation of citrate, as well as produce catalase. Micrococcus has yellow or cream-white colonies, irregular with a diameter of 5–8 mm, convex or flat, opaque or translucent, smooth, viscid, and undulate or erose edge (Tab. 2). It was non-spore-forming, non-motile, and gram-positive cocci. It was positive for the production of oxidase, catalase and gelatin liquefaction. However, it was negative for nitrate reduction to nitrite, Voges-Prosqauer test, and utilisation of citrate. The gram-negative (30%) include three isolates (Enterobacter, Pseudomonas, and Citrobacter) as follows: Enterobacter had cream-white colonies, circular with a diameter of 5–8 mm, raised, opaque, rough, and viscid with an entire edge (Tab. 2). These were non-spore-forming, motile, and gram-negative straight rods. It was positive for the production of oxidase, catalase and Voges-Prosqauer tests, and negative for gelatin liquefaction and methyl red tests. Pseudomonas had

Table 2. Selected physiological and biochemical characteristics of PGPR strains isolated from the rhizosphere of different plants at Suez Canal region

Isolate	Host plant	Colony morpho-logy	Shape	Gram stain	Endos	Cata lase	Oxid ase	NR	Gelatin lique faction	Starch hydro-lysis	MR	VP	Citrate	Urease	O.F glucose
AW15	wheat	IR, CW	straight rods	+	+	+	+	–	–	–	+	–	+	–	O
EM9	maize	IR, W	straight rods	+	+	+	+	–	–	–	+	–	+	–	O
ET3	tomato	IR, W	straight rods	+	+	+	+	–	–	–	+	–	+	–	O
KT2	tomato	IR, Y	spherical	+	–	+	+	–	–	–	–	–	–	–	O
FW9	wheat	IR, Y	spherical	+	–	+	+	–	–	–	–	–	–	–	O
FC13	clover	IR, Y	spherical	+	–	+	+	–	–	–	–	–	–	–	O
SaW4	wheat	IR, Y	spherical	+	–	+	+	–	–	–	–	–	–	–	O
SK18	kidney bean	CI, CW	straight rods	–	–	+	+	–	–	–	–	+	+	–	O/F
TPo10	potato	IR, CW	straight rods	–	–	+	+	+	+	–	–	+	+	–	O
TC3	clover	IR, YW	straight rods	–	–	+	–	–	–	+	+	–	+	–	O/F

Explanations: + = positive, – = negative, *IR* = irregular, *CI* = circular, *CW* = cream-white, *W* = white, *Y* = yellow, *YW* = yellow-white, endos = endospore, *NR* = nitrate reduction, *MR* = methyl red, *VP* = Voges-Proskauer, *O* = oxidative, *F* = fermentative.
Source: own study.

Table 3. *In vitro* characterisation of plant growth promoting activities

Isolate	P-solubilisation		IAA[1] production (mg·dm^{-3})	Siderophore production
	mg P·dm^{-3}	pH		
AW15	111.7 ±3.15	4.91 ±0.140	14.40 ±1.51	–
EM9	149.8 ±25.0	4.32 ±0.105	6.34 ±0.311	–
ET3	79.4 ±0.653	4.80 ±0.051	4.05 ±0.178	+
FC13	159.1 ±3.71	4.66 ±0.197	17.14 ±0.278	+
FW9	136.2 ±4.65	4.80 ±0.075	15.04 ±1.14	–
KT2	81.1 ±3.43	4.90 ±0.068	11.32 ±0.411	+
SaW4	211.5 ±12.9	4.41 ±0.121	2.41 ±0.062	+
SK18	117.0 ±6.43	4.94 ±0.152	4.36 ±0.998	+
TC3	154.1 ±8.16	4.12 ±0.070	2.18 ±0.155	–
TPo10	84.4 ±5.23	4.76 ±0.072	1.54 ±0.247	+
$LSD_{0.05}$	31.72	0.138	1.280	
r		–0.5364		

[1] Indole acetic acid (*IAA*) production in the absence of tryptophan addition.
Explanations: $LSD_{0.05}$ = least significant difference at 0.05 significance level, values represent average (n = 3), r = correlation coefficient between solubilised P and pH in liquid medium.
Source: own study.

cream-white or orange colonies, irregular with a diameter of 6–8 mm, raised, opaque or translucent, smooth and viscid with erose or undulate edge. These were non-spore-forming, motile, and gram-negative straight rods. It was positive for nitrate reduction, production of oxidase and catalase, liquefaction of gelatin, and negative for methyl red and Voges-Prosqauer tests. *Citrobacter* had yellow-white colonies, was irregular with a diameter of 7–8 mm, raised, opaque, smooth, and viscid with a lobate edge. These were non-spore-forming, motile, and gram-negative straight rods. It was positive for the production of catalase, utilisation of citrate, and reduction of nitrate, but it is negative for the production of oxidase and the Voges-Prosqauer test.

IDENTIFICATION OF ISOLATES USING THE SEQUENCING OF 16S RRNA GENE

Based on genetic marker analysis using the 16S rRNA technique (Fig. 1, Tab. 4), the isolates had the 15 kilobase pair (15 kbp) of nucleotides characterised by the gene-encoded 16S rRNA as shown in Figure 1. It was grouped into two genera of gram-positive bacteria (*Bacillus* and *Micrococcus*) and three genera of gram-negative bacteria (*Enterobacter, Pseudomonas,* and *Citro-bacter*) with similarities of 86–98% to known species using the gene bank database ABDEL-RAHMAN et al. [2017]. Three strains were identified as *Bacillus* sp. Strain AW15 was identified as *Bacillus endophyticus* and strains EM9 and ET3 were identified as *B. filamentosus*, which are like gene sequencing of 16S rRNA of *Bacillus endophyticus* 2DT and *Bacillus filamentosus* SGD-14 by 94 and 95%, respectively (Tab. 4). Strains FC13, FW9, KT2, SaW4
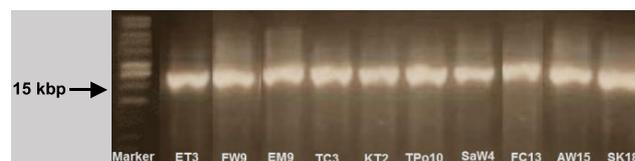

15 kbp ➤

Marker ET3 FW9 EM9 TC3 KT2 TPo10 SaW4 FC13 AW15 SK18

Fig. 1. Electrophoretic agarose-gel of the polymerase chain reaction (PCR) product after amplification of gene encoded the 16SrRNA (15 kbp of nucleotides) of rhizobacterial isolates; source: own study

Table 4. Identification of bacterial isolates based on 16S rRNA partial sequence analysis

Isolate	Identified organism	GenBank accession number	Closet type strain in *RDP* database	16S rRNA identity (%)
AW15	*Bacillus endophyticus*	NR_025122.1	*Bacillus endophyticus* 2DT	95
EM9	*Bacillus filamentosus*	NR_134701.1	*Bacillus filamentosus* SGD-14	96
ET3	*Bacillus filamentosus*	NR_134701.1	*Bacillus filamentosus* SGD-14	98
FC13	*Micrococcus luteus*	NR_075062.2	*Micrococcus luteus* NCTC 2665	88
FW9	*Micrococcus luteus*	NR_075062.2	*Micrococcus luteus* NCTC 2665	88
KT2	*Micrococcus luteus*	NR_075062.2	*Micrococcus luteus* NCTC 2665	87
SaW4	*Micrococcus luteus*	NR_075062.2	*Micrococcus luteus* NCTC 2665	87
SK18	*Enterobacter cloacae*	NR_117679.1	*Enterobacter cloacae* DSM 30054	97
TC3	*Citrobacter braakii*	NR_028687.1	*Citrobacter braakii* 167	99
TPo10	*Pseudomonas azotoformans*	NR_113600.1	*Pseudomonas azotoformans* NBRC 12693	93

Explanations: *RDP* = Ribosomal Database Project.
Source: own study.

were identified as *Micrococcus luteus* and the gene sequence of 16S rRNA was similar to *Micrococcus luteus* NCTC 2665 by 87–98% (Tab. 4). Strain SK18 was identified as *Enterobacter cloacae* and had 16S rRNA gene sequencing like *Enterobacter cloacae* DSM 30054 by 97% (Tab. 4). Strain TC3 was identified as *Citrobacter braakii* and had the same sequence of 16SrRNA gene as *Citrobacter braakii* 167 (Tab. 4). Strain TPo10 was identified as *Pseudomonas azotoformans* which is genetically similar to *P. azotoformans* NBRC 12693 by 93% (Tab. 4).

SCREENING OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) FOR PLANT GROWTH-PROMOTING (PGP) ACTIVITIES

The PGPR activities involved solubilisation of inorganic phosphate, production of indole acetic acid (*IAA*) and siderophores.

Phosphate solubilisation

All the tested bacteria were able to dissolve $Ca_3(PO_4)_2$ and exhibited various levels of solubilised phosphate (Tab. 3). After 10 days of incubation, the amounts of P dissolved from $Ca_3(PO_4)_2$ by the tested rhizobacteria varied from 79.4 mg P·dm^{-3} with the isolate *B. filamentosus* ET3, which was isolated from tomato-rhizosphere soil, to 211.5 mg P·dm^{-3} with the *M. luteus* SaW4 (isolated from wheat-rhizosphere soil). The significant reductions in pH values were observed in bacterial cultures after 10 days of incubation as compared to the uninoculated control (pH = 6.97), the values varied from 4.12 to 4.94.

Indole acetic acid (IAA) equivalents production

All the tested isolates were able to produce *IAA* equivalents in the absence of tryptophan as a precursor with significant differences between them in most cases (Tab. 3). The amounts of produced *IAA* ranged from 1.54 to 17.14 mg·dm^{-3}. The isolate *Micrococcus luteus* FC13 was the most active in producing *IAA*, while *P. azotoformans* TPo10 produced the lowest amount. These two isolates were separated from clover and potato-rhizosphere soil, respectively. Similarly, the amounts of *IAA* produced by bacteria

isolated from the rhizosphere of wheat, at various locations significantly differed from 2.41 (with *Micrococcus lylae* SaW4) to 15.04 mg·dm^{-3} (with *Micrococcus luteus* FW9). However, the amounts of *IAA* produced by bacteria from the rhizospheres of clover and wheat grown at the same location were not significantly different.

Siderophore production

As shown in Table 3, the six tested isolates possess the ability to produce siderophores *in vitro*. However, *M. luteus* FC13 isolates have the capacity to produce both siderophores and *IAA* as well as to solubilise inorganic phosphate.

GREENHOUSE POT EXPERIMENT

Total bacteria in soil

Generally, the counts of the total bacteria in all inoculated rhizosphere soil samples increased relative to the uninoculated control and were always higher at those after 90 days than those after 120 days (Fig. 2). Concerning the rhizosphere soil samples taken after 90 days from the sowing date, the total bacterial counts varied between 1.8·10^6 g^{-1}, dry soil with the uninoculated control, and 11.8·10^6 g^{-1}, dry soil with the strains *Micrococcus luteus* FC13 and *Pseudomonas azotoformans* TPo10. Likewise, at those after 120 days, the counts ranged from 1.01·10^6 g^{-1}, dry soil with the control, to 8.51·10^6 g^{-1}, dry soil with the strain *Pseudomonas azotoformans* TPo10.

Soil pH and availability of P in soil

After wheat harvest (after 120 days from the sowing date) soil samples were taken from each pot to determine the impact of the bacterial inoculants on pH and available P. Figure 3 shows that all the bacterial inoculants significantly decreased soil pH values as compared to the uninoculated control. The obtained pH reductions reached their maximum of 0.27 pH unit with the strains *Pseudomonas azotoformans* TPo10 and *Micrococcus luteus* SaW4 relative to the control. Data in Figure 3 indicate that the seed inoculation with the tested strains caused increases in soil
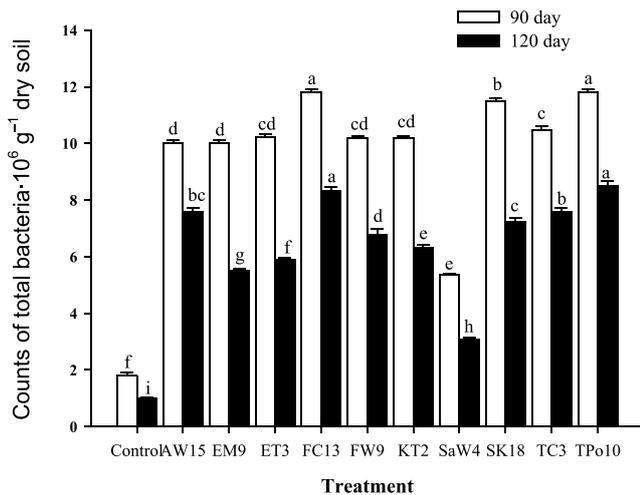
Fig. 2. Counts of total bacteria in the rhizosphere of wheat plants as affected by inoculation with plant growth-promoting rhizobacteria after 90 and 120 days from sowing date; error bars = mean standard error (n = 3); the same letters in the Figure indicate no substantial difference at 0.05 significance level, determined by least significant difference (*LSD*) test (n = 3); source: own study

available P, without significant differences among most of them. The increases over the uninoculated control ranged from 67.57%, with the strains *B. filamentosus* ET3, to 87.39%, with *E. cloacae* SK18.
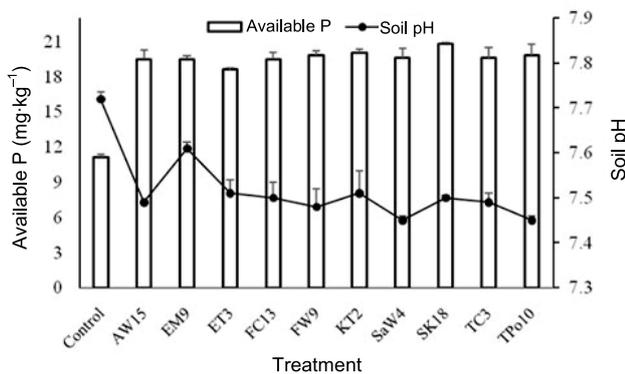

Fig. 3. Effect of plant growth promoting rhizobacteria on pH and availability of P in the soil after 120 days from sowing date; error bars = mean standard error (n = 3); source: own study

Wheat growth and yield

Regarding the 90-day-old plants, results presented in Table 5 show that all isolates significantly increased plant height by 25.5–46.8% and shoot dry weight by 32.8–141.9% as compared to the uninoculated control. The highest plant height and shoot dry weight were obtained with the strains *Bacillus filamentosus* ET3 and *M. luteus* KT2, respectively. However, the differences between the bacterial inoculants were not always significant. Concerning the 120-day-old plants, it also indicates that the seed inoculation with all the tested isolates significantly increased plant height by 26.3–32.2%, grain yield by 80.2–96.5%, straw yield by 66.9–90.9%, and the biological yield (grains plus straw) by 76.2–91.4% relative to the control. The highest plant height, grain, straw, and biological yields were recorded with the strains *M. luteus* KT2, *Enterobacter cloacae* SK18, *Pseudomonas azotofor-*

mans TPo10, and *Enterobacter cloacae* SK18, respectively. Table 5 also shows that no significant differences in the grain/straw ratio were observed between all the inoculated treatments and the uninoculated control, but the difference between some isolates was significant. The ratio of grain and straw for all the bacterial inoculants varied from 0.79, with the strain *M. luteus* KT2, to 0.91, with *B. filamentosus* EM9. Table 6 shows significant or highly significant positive linear correlations between the levels of *IAA* or P, solubilised by the tested rhizobacteria *in vitro* and all the above-mentioned growth and yield parameters.

Nitrogen and phosphorus contents in plant

Nitrogen and phosphorus contents in the plant were only determined in 120 day-old-plants. As shown in Table 5 the concentrations of the two nutrients in grains and straw varied between the bacterial inoculants, but the differences between the tested isolates were not always significant. Though, the nutrient uptake of the two nutrients by plants was significantly increased due to seed inoculation by all tested bacterial inoculants as compared to the uninoculated control. Significant increases over the uninoculated control in the uptake of N ranged from 16 to 100% for grains, from 15 to 127% for straw, and from 16 to 105% for the total (grains plus straw). The highest N uptake by grains and total (grains plus straw) was observed with the isolate *Enterobacter cloacae* SK18, while that of straw was obtained with *P. azotoformans* TP₀10. Similarly, the increases in the uptake of P over the uninoculated control were significant and varied between 31 and 153% for grains, between 31 and 136% for straw, and between 35 and 144% for the total (grains plus straw). The highest uptake of P by grains, straw, and plant (grains plus straw) was obtained with the isolates *B. endophyticus* AW15, *Pseudomonas azotoformans* TP010 and *Enterobacter cloacae* SK18, respectively.

Photosynthetic pigment contents

The PGPR inoculated-wheat plants showed higher contents of all photosynthetic pigments (chlorophyll a, b and carotenoids) in the flag leaf than the uninoculated plants (Tab. 7). In this respect, the isolates *M. luteus* FC13, *M. luteus* SaW4, *M. luteus* FW9, *B. filamentosus* EM9, and *P. azotoformans* TPo10 gave the highest values of chlorophyll-a (46.3 to 52.0 mg·(100 g)$^{-1}$ FW) compared to the other isolates. Application of the strain *P. azotoformans* TP₀10 to wheat led to an increase in the amount of chlorophyll by about 18.5% compared to the uninoculated plants. Inoculation of wheat by the isolate *M. luteus* KT2 gave a higher amount of chlorophyll b (142.9 mg·(100 g)$^{-1}$ FW) than other isolates with an increment of about 8.8 times compared to the control. Also, the same isolate donated the highest value of the total chlorophyll a + b (189.2 mg·(100 g)$^{-1}$ FW) compared to other treatments with an increment of about 3.2 times compared to the control. On the contrary, plants inoculated with the same isolate (KT2) recorded the lowest value of chlorophyll a/b (0.32). Application of the strain *M. luteus* SaW4 gave the maximum value of carotenoids (28.2 mg·(100 g)$^{-1}$ FW) with about a 42.5% increment compared to untreated plants.

Phytochemical constituents

As shown in Table 7 the contents of free amino acids, free phenolic compounds, and reducing sugars were significantly enhanced in flag leaf and spikes by application of all PGPR

Table 5. Effect of plant growth promoting rhizobacteria strains on growth parameter, yield and uptake of N and P in wheat plants after 120 days from sowing date

Plant growth-promoting rhizobacteria strain	90-day-old plant		120-day-old plant			Nitrogen uptake			Phosphorus uptake		
	plant height (cm)	shoot *DW* (g·pot^{-1})	grains	straw	total yield	grains	straw	total	grains	straw	total
			g·pot^{-1}			mg·pot^{-1}					
Control	37.6	4.15	6.77	8.33	15.1	165	44	209	25.5	11.4	36.9
B. endophyticus AW15	53.2	8.08	12.8	14.9	27.7	323	96	419	64.4	24.9	89.3
B. filamentosus EM9	46.0	6.05	12.7	13.9	26.6	311	82	393	57.5	21.0	78.5
B. filamentosus ET3	55.2	10.04	12.8	14.8	27.6	321	95	416	62.8	24.4	87.2
M. luteus FC13	49.6	6.90	12.3	14.6	26.9	303	92	395	60.6	23.2	83.8
M. luteus FW9	49.2	7.11	12.7	15.1	27.8	315	95	410	62.6	24.8	87.4
M. luteus KT2	53.4	8.81	12.1	15.4	27.5	301	95	396	58.6	25.6	84.2
M. luteus SaW4	48.4	7.01	12.3	15.0	27.3	288	78	366	54.7	21.8	76.5
E.cloacae SK18	48.4	7.29	13.3	15.6	28.9	330	98	428	64.0	25.9	89.9
C. braakii TC3	50.6	8.15	12.2	14.8	27.0	300	91	391	60.6	24.4	85.0
P. azotoformans TP$_0$10	47.2	5.51	12.9	15.9	28.8	316	100	416	62.7	26.9	89.6
LSD$_{0.05}$	3.23	0.505	0.821	0.844	1.132	26.0	16.7	33.2	8.14	4.45	9.08

Explanations: *DW* = dry weight, *LSD* = least significant difference.
Source: own study.

Table 6. Correlation coefficient between P-solubilisation, indole acetic acid (*IAA*) production by the tested bacteria *in vitro* or counts of total bacteria in the rhizosphere and some growth and yield parameters of wheat plants harvested after 90 and 120 days from sowing date

Growth and yield parameter	90-day-old plant			120-day-old plant		
	P	*IAA*	total bacterial count	P	*IAA*	total bacterial count
Plant height	0.6038**	0.5441**	0.6121**	–	–	–
Shoot dry weight	0.6217**	0.4083*	0.4748**	–	–	–
Straw yield	–	–	–	0.5862**	0.4113*	0.6395**
Grain yield	–	–	–	0.6242**	0.5305**	0.6487**
Biological yield	–	–	–	0.6246**	0.4762*	0.6579**

Explanations: * = significant at 0.05, ** = significant at 0.01.
Source: own study.

Table 7. Effect of plant growth promoting rhizobacteria on photosynthetic pigment and phytochemical constituents of wheat flag leaf and spikes after 90 days from sowing date

Plant growth-promoting rhizobacteria strain	Chl a	Chl b	Carotenoids	Amino acids		Free phenols		Reducing sugars	
				mg·100 g^{-1} fresh weight					
	flag leaf			flag leaf	spikes	flag leaf	spikes	flag leaf	spikes
Control	43.9	16.1	19.5	0.31	0.75	2.88	1.50	1.02	1.99
B. endophyticus AW15	49.5	61.3	26.0	1.09	1.36	5.17	2.47	1.90	6.74
B. filamentosus EM9	51.4	42.0	21.9	1.73	2.21	5.39	3.10	4.60	7.69
B. filamentosus ET3	48.4	88.0	23.2	1.62	1.61	3.00	3.45	4.52	8.45
M. luteus FC13	50.9	83.5	26.4	0.62	2.72	6.47	2.60	5.39	7.47
M. luteus FW9	51.0	39.7	23.7	1.21	1.89	5.55	3.36	4.28	6.63
M. luteus KT2	46.3	142.9	22.6	0.91	3.14	6.66	3.50	3.78	7.62

cont. Tab. 7

Plant growth-promoting rhizobacteria strain	Chl a	Chl b	Carotenoids	Amino acids		Free phenols		Reducing sugars	
	mg·100 g^{-1} fresh weight								
	flag leaf			flag leaf	spikes	flag leaf	spikes	flag leaf	spikes
M. luteus SaW4	50.6	67.5	25.5	1.34	1.84	6.11	3.32	5.35	7.48
E. cloacae SK18	49.6	78.0	25.7	1.72	1.44	6.39	3.26	3.02	6.19
C. braakii TC3	50.3	63.9	25.3	0.83	1.64	3.16	1.68	4.42	10.52
P. azotoformans TP$_0$10	52.0	51.1	24.5	1.31	1.66	6.52	3.44	3.45	6.07
$LSD_{0.05}$	5.42	3.94	6.88	0.242	1.002	1.729	1.410	1.377	1.523

Explanations: Chl a = chlorophyll a, Chl b = chlorophyll b.
Source: own study.

isolates compared to the untreated control. Inoculation by the strain *B. filamentosus* EM9 gave the highest value of free amino acids in the flag leaf (1.73 mg·g^{-1} FW) with a 4.6 times increment compared to the control. The highest contents of free amino acids and reducing sugars in spikes (3.14 and 12.4 mg·g^{-1} FW, respectively) were obtained in wheat plants inoculated with the strains *M. luteus* KT2. Likewise, the maximum contents of free phenols in flag leaf and spikes (6.66 and 3.5 mg·g^{-1} FW, respectively) were observed in wheat plants inoculated with the strain *M. luteus* KT2. The isolates *M. luteus* FC13 and *C. braakii* TC3 recorded the maximum values of reducing sugars in both flag leaf and spikes (5.39 and 10.52 mg·g^{-1} FW, respectively) with a 4.3 times increment relative to the control.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of wheat grains protein profiles

Grain protein of wheat cv. 'Sakha 94' was analysed to identify protein patterns involved in response to inoculation with different types of PGPR isolates. Soluble proteins extracted from grains of 10 different treatments were separated by electrophoresis on one-dimensional SDS-PAGE gel (Fig. 4). Results revealed that soluble storage protein of treated and non-treated grains was fractioned into eight varied bands with the molecular weights (MW): 109.7, 105.0, 101.8, 95.8, 91.5, 84.9, 66.8, and 45.4 kDa. All eight bands had the highest values of optical densities (OD) as a final product of gene expression of specific proteins in all inoculated grains compared to the control as shown in Figure 4. The highest OD of the first band with a high MW of 109.7 kDa was recorded in grains inoculated with *B. endophyticus* AW15 and *M. luteus* FC13 isolates. The grains inoculated with isolate *M. luteus* SaW4 gave the highest values of OD of the second band with MW of 105.0 kDa. Inoculation with EM9 isolate led to enhancement of the OD of the third protein band with MW 101.8 kDa. The isolate *B. endophyticus* AW15 gave the maximum values of OD of the fourth protein band with MW 95.8 kDa. The grains treated with *M. luteus* FW9 and *P. azotoformans* TPo10 isolates gave the highest OD of the fifth protein band with MW 91.5 kDa (HMW glutenins). The sixth protein band with 84.9 kDa (Gliadins) was more expressed after the application of *E. cloacae* SK18 isolate. Isolates kT2 gave the maximum values of OD of the seventh protein band with 66.8 kDa. The eighteenth protein band with 45.4 kDa (LMW glutenins) was more expressed after inoculation with *M. luteus* FC13 isolate.
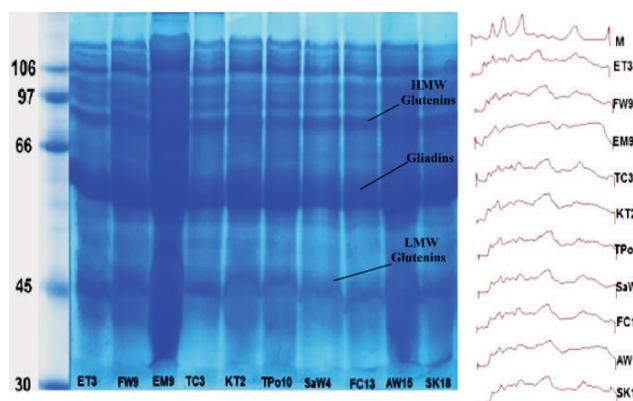

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins and its optical densities of bands at 660 nm extracted from grains of wheat cv. 'Sakha 94' as affected by rhizobacterial isolates; HMW = high molecular weight, LMW = low molecular weight; source: own study

DISCUSSION

Results showed that the rhizosphere of clover, faba bean, kidney bean, maize, pepper, potato, tomato, and wheat had five genera of plant growth promoting rhizobacteria (PGPR) from different sites in Egypt. PGPR isolates were identified morphologically, biochemically, and genetically as *Bacillus, Micrococcus, Enterobacter, Citrobacter,* and *Pseudomonas* (Tab. 2, 4; Fig. 1). The same genera were found by different researchers such as Ahemad and Khan [2010], Ahemad and Kibret [2014], Goswami et al. [2016], Vinayarani and Prakash [2018], which induced the plant growth traits through phosphate solubilisation, siderophore, and phytohormone production. Similar sequencing (86–98%) of 16S rRNA gene among isolates and known species in gene bank database reclassified isolates as cited in Table 4.

Tested PGPR isolates had different capacities for solubilising the inorganic phosphate *in vitro* (Tab. 3). A high correlation between the amounts of dissolved P and pH values was observed in this study (Fig. 3), this finding may be attributed to the production of organic acids by tested isolates. Similar results were reported by many investigators [Maldonado et al. 2020; Maliha et al. 2004; Song et al. 2008]. For instance, Abdul Wahid and Mehana [2000], and Mehana and Farag [2000] reported that PGPR had different potentials and types of organic acids which

are responsible for releasing phosphorous due to reducing soil pH or chelate calcium. PGPR may have different mechanisms to encourage the growth and yield of plants, *M. luteus* SaW4 isolates had high P availability *in vitro* as well as siderophores producers, which save available iron. All tested isolates from different rhizospheric environments produced *IAA in vitro* in different quantities as shown in Table 3. These results indicated that the location of host plants, host plant species, and the inherent characteristics of each bacterial isolate were major factors in the amounts of *IAA* produced by the isolated bacteria. Previous reports cited that 80% of microorganisms isolated from the rhizosphere of different crops were able to produce *IAA* as secondary metabolites [Patten, Glick 1996].

Only six out of ten isolates (60%) produced siderophores (Tab. 3). Therefore, this activity besides other traits was responsible for promoting wheat growth and yield. Many researchers proved the efficiency of siderophores as a plant growth-promoting trait in increasing the growth and yield of crops by increasing the availability of iron for plants [Chen et al. 2021; Sharma et al. 2003]. In addition, Abd El-Azeem et al. [2008] found that 41 out of the tested 46 strains had the capacity to produce siderophores *in vitro*, which enhanced the growth and yield of wheat.

Increasing the bacterial counts in all inoculated treatments compared to the control can be attributed to the application and growth of the tested microorganisms in the rhizosphere of wheat plants (Fig. 2). This result indicates that all the studied isolates were able to proliferate in soil without antagonism from the indigenous microorganisms. It was reported by Kloepper [1994] that the PGPR must be able to colonise the root surface, survive, multiply and compete with other microorganisms at least for the time required to achieve their plant growth-promoting/protection activities. The decrease in the number of bacteria at 120 days compared to 90 days may be attributed to the unfavourable soil moisture condition due to soil drying before wheat harvest and also to the exhaustion of available organic materials which are necessary for the growth of heterotrophic bacteria. Similar results were also reported by Mehana [1994] and Ali [1999].

The increment of bacterial count (Fig. 2) led to reducing the soil pH and an increase in the P availability (Fig. 3) as a result of the isolates application. Decreasing values of pH in soil cultivated with wheat due to the inoculation with *M. luteus* SaW4 was also reported by Al-Attar [2013] who found that the maximum decrease in soil pH reached 0.38 pH unit with *B. globisporus* EF3 as compared to the uninoculated control. Such increases in soil available P may be attributed to applying rhizobacteria, such as *Pseudomonas* spp. and *Bacillus* spp., which were the most efficient phosphate dissolvers by producing organic acids such as acetic and succinic acids [Khanafari et al. 2012].

The significant differences respecting the C:N ratio between some tested isolates may be attributed to the ability of some bacteria to fix nitrogen in soil non-symbiotically, which encourages vegetative growth and delays flowering of the crop, and consequently decreases the grain/straw ratio. It was reported that the soil rich in nitrogen over the satisfactory range will tend to keep down the C:N ratio which delays flowering in nitro-negative crops such as wheat [Martin et al. 1976]. Similar results and conclusions were reported by Abd El-Azeem et al. [2008] and Ahmed [2008].

High correlations between the levels of *IAA* or P solubilised by the tested rhizobacteria *in vitro* and all the growth and yield parameters (Tab. 6) proved the importance of both plant growth traits as essential mechanisms for promoting the growth and yield of wheat plants. This result also demonstrated that the screening method based on determining the ability of bacteria to produce *IAA* and to solubilise insoluble mineral phosphate *in vitro* may be considered a good approach to selecting PGPR. A highly significant positive linear correlation between *in vitro* auxin production by the tested PGPR and each of the growth and yield parameters of wheat was also reported by Khalid et al. [2004] and Abd El-Azeem et al. [2008].

Total rhizobacteria and all growth and yield parameters were highly correlated as shown in Table 5, confirming the importance of seed inoculation by these bacteria for improving the growth and yield of the wheat plant.

Increment in the uptake of N and P by wheat plants inoculated with PGPR, which was also reported by many researchers [Ghanem et al. 2013; Hakim et al. 2021; Naveed et al. 2008; Turan et al. 2010], led to an increase in the photosynthetic pigments as shown in Table 7. Moustaine et al. [2016] found that the strain of *Pantoea agglomerans* enhanced chlorophyll content and the fresh weight of wheat plants. Similarly, significant increases in chlorophyll a, chlorophyll b, and total chlorophyll contents with the application of PGPR in different plant species were reported by other investigators [Abulfaraj, Jalal 2021; Ma et al. 2011; Sharma et al. 2003; Wani, Khan 2010]. Reducing sugars as a final product of photosynthesis was also increased in rhizobacterial-inoculated wheat. The positive effects of PGPR inoculants on the carbohydrate content in the plant were reported by Prathibha and Siddalingeshwara [2013], who found that the carbohydrate content significantly increased during grain germination in two varieties of sorghum after inoculation with the PGPR *Pseudomonas fluorescence* and *Bacillus subtilis*. The increment of the carbonic skeleton and nitrogen resources by PGPR isolates led to the enhancement of the amino acid concentration in leaves and spike (Tab. 7). Ekinci et al. [2014] reported that the content of the amino acid in cauliflower seedlings was affected by inoculation with different types of PGPR. Inoculation with *B. megaterium* strain TV-3D increased the amounts of serine, glycine, arginine, tyrosine, valine, lysine, and sarcosine. *B. megaterium* TV-91C enhanced the content of histidine, alanine, cystine, and phenylalanine. The addition of *B. subtilis* TV-17C increased the content of methionine and hydroxyproline. The application of *B. megaterium* TV-87A led to an increase in the amount of methionine and tryptophan. The application of *B. megaterium* KBA-10 increased the amount of proline.

Availability of nitrogen and phosphorus as a result of PGPR inoculation has a significant influence on protein bands as shown after electrophoresis (Fig. 4). Dupont and Altenbach [2003] demonstrated that the flour quality of wheat grains was influenced by specific proteins such as gluten, which play a crucial role in forming the strong and cohesive dough. It consisted of two groups of glutenins (*HMW* subunits, 65–90 kDa, and *LMW* subunits, 30–45 kDa) and gliadins (30–80 kDa) as reported in the work of D'Ovidio and Masci [2004].

The overexpression of specific proteins in grains can correlate with the specific genera of the PGPR habitat in the rhizosphere region. As previously mentioned, *M. luteus* FC 13

isolate was the most efficient *IAA* and siderophores producer *in vitro*, therefore, inducing the expression of specific protein bands (109.7 kDa). *P. azotoformans* TPo10 isolates gave the highest OD of the fifth protein band with MW 91.5 kDa or *HMW* glutenins, which may be due to maximising the bacterial count around wheat rhizosphere after 90 and 120 days of sowing, as shown in Table 5, which subsequently increased the enzyme activity and released macro and micro nutrients for plants, as well as siderophore producers. Undetectable unique protein band(s) after application of all PGPR isolates under study, as shown in Figure 4, indicated the biosafety effect of using PGPR as biofertilisers without allergic effects from the new protein bands for humans [Battais *et al.* 2008].

The results reported herein were in line with Stefan *et al.* [2007], who found that protein bands in soybean seeds differed only in their quantity in some unique bands with the molecular masses 105, 86, 80, 52, 38, and 31.5 kDa after inoculation with PGPR strains. On the contrary, Prathibha and Siddalingeshwara [2013] found that protein bands significantly differed in seeds of sorghum treated with PGPR (*Pseudomonas fluorescence, Bacillus subtilis*, and control).

CONCLUSIONS

We conclude that significant increases in grain and straw yields, as well as uptake of nitrogen and phosphorus by plants, were observed due to inoculation with plant growth-promoting rhizobacteria (PGPR) isolates. Thus, the results of this study showed beneficial impacts of the tested PGPR on wheat growth and yield, some soil properties, contents of all photosynthetic pigments (chlorophyll a, b and carotenoids) in flag leaf, levels of free amino acids, free phenols, and reducing sugars in flag leaf and spikes. The tested PGPR is recommended for field evaluation under different soil and environmental conditions before generalising it as a biofertiliser.

REFERENCES

Abd El-Azeem S.A.M. 2006. Studies on plant growth promoting rhizobacteria microorganisms. PhD Thesis. Ismailia, Egypt. Suez Canal University pp. 159.

Abd El-Azeem S.A.M., Mehana T.A., Shabayek A.A. 2008. Effect of seed inoculation with plant growth-promoting rhizobacteria on the growth and yield of wheat (*Triticum aestivum* L) plants cultivated in a sandy soil. Catrina. Vol. 3(2) p. 69–74.

Abdel-Rahman H.M., Salem A.A., Moustafa M.M.A., El-Garhy H.A.S. 2017. A novice *Achromobacter* sp. EMCC1936 strain acts as a plant growth-promoting agent. Acta Physiologiae Plantarum. Vol. 39(61) p. 1–15. DOI 10.1007/s11738-017-2360-6.

Abdel-Rahman T., Thomas H., Doss G.J., Howell L. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiologia Plantarum. Vol. 34(1) p. 39–43. DOI 10.1111/j.1399-3054.1975.tb01852.x.

Abdul Wahid O.A., Mehana T.A. 2000. Impact of phosphate solubilizing fungi on the yield and phosphorus-uptake by wheat and faba bean plants. Microbiological Research. Vol. 155(3) p. 221–227. DOI 10.1016/S0944-5013(00)80036-1.

Abulfaraj A.A., Jalal R.S. 2021. Use of plant growth-promoting bacteria to enhance salinity stress in soybean (*Glycine max* L.) plants. Saudi Journal of Biological Sciences. Vol. 28(7) p. 3823–3834. DOI 10.1016/j.sjbs.2021.03.053.

Ahemad M., Khan M.S. 2010. Influence of selective herbicides on plant growth promoting traits of phosphate solubilizing *Enterobacter asburiae* strain PS2. Research Journal of Microbiology. Vol. 5(9) p. 849–857. DOI 10.3923/jm.2010.849.857.

Ahemad M., Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University – Science. Vol. 26(1) p. 1–20. DOI 10.1016/j.jksus.2013.05.001.

Ahmed K.G.E. 2008. Use of nitrification inhibitors as a means for improving nitrogen fertilizer efficiency in soils. MSc Thesis. Ismailia, Egypt. Suez Canal University pp. 193.

Al-Attar O.M.G. 2013. Influence of seed inoculation with plant growth promoting rhizobacteria on mineral nutrition of plant and soil fertility. MSc Thesis. Ismailia, Egypt. Suez Canal University pp. 205.

Ali M.S.M. 1999. Use of sewage sludge and chicken manure for improving productivity of a sandy soil. MSc Thesis. Ismailia, Egypt. Suez Canal University pp. 176.

Arnon D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology. Vol. 24(1) p. 1–15. DOI 10.1104/pp.24.1.1.

Backer R., Rokem J.S., Ilangumaran G., Lamont J., Praslickova D., Ricci E., Subramanian S., Smith D.L. 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science. Vol. 9, 1473 p. 1–17. DOI 10.3389/fpls.2018.01473.

Battais F., Richard C., Jacquenet S., Denery-Papini S., Moneret-Vautrin D.A. 2008. Wheat grain allergies: An update on wheat allergens. European Annals of Allergy and Clinical Immunology. Vol. 40(3) p. 67–76.

Bechtaoui N., Raklami A., Tahiri A.I., Benidire L., El Alaoui A., Meddich A., Göttfert M., Oufdou K. 2019. Characterization of plant growth promoting *rhizobacteria* and their benefits on growth and phosphate nutrition of faba bean and wheat. Biology Open. Vol. 8(7) p. 1–8. DOI 10.1242/bio.043968.

BLAST undated. Basic local alignment search tool [online]. National Library of Medicine, National Center for Biotechnology Information. [Access 07.03.2022]. Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi

Bremner J.M. 1996. Nitrogen-Total. Ch. 37. In: Methods of soil analysis. Part III. Eds. D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner. SSSA Book Series 5. Madison, WI, USA. ASA, SSSA p. 1085–1121. DOI 10.2136/sssabookser5.3.c37.

Canbolat M.Y., Bilen S., Cakmakci R., Sahin F., Aydin A. 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biology and Fertility of Soils. Vol. 42(4) p. 350–357. DOI 10.1007/s00374-005-0034-9.

Chen J., Zhao G., Wei Y., Dong Y., Hou L., Jiao R. 2021. Isolation and screening of multifunctional phosphate solubilizing bacteria and its growth-promoting effect on Chinese fir seedlings. Scientific Reports. Vol. 11(1), 9081 p. 1–13. DOI 10.1038/s41598-021-88635-4.

Dasgupta D., Ghati A., Sarkar A., Sengupta C., Paul G. 2015. Application of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Sesbania bispinosa* on the growth of chickpea (*Cicer arietinum* L.). International Journal of Current Microbiology and Applied Sciences. Vol. 4(5) p. 1033–1042.

DAVIS B.J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Annals of the New York Academy of Sciences. Vol. 121(2) p. 404–427. DOI 10.1111/j.1749-6632.1964.tb14213.x.

DE FREITAS J.R., GERMIDA J.J. 1992. Growth promotion of winter wheat by fluorescent pseudomonads under field conditions. Soil Biology and Biochemistry. Vol. 24(11) p. 1137–1146. DOI 10.1016/0038-0717(92)90064-5.

DIAS A., DOS SANTOS S.G., VASCONCELOS V.G.S., RADL V., XAVIER G.R., RUMJANEK N.G., RIBEIRO R.L.D. 2013. Screening of plant growth promoting rhizobacteria for the development of vegetable crops inoculant. African Journal of Microbiology Research. Vol. 7(19) p. 2087–2092. DOI 10.5897/AJMR12.2258.

DOS SANTOS R.M., DIAZ P.A.E., LOBO L.L.B., RIGOBELO E.C. 2020. Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. Frontiers in Sustainable Food Systems. Vol. 4, 136 p. 1–15. DOI 10.3389/fsufs.2020.00136.

D'OVIDIO R., MASCI S. 2004. The low-molecular-weight glutenin subunits of wheat gluten. Journal of Cereal Science. Vol. 39(3) p. 321–339. DOI 10.1016/j.jcs.2003.12.002.

DUPONT F.M., ALTENBACH S.B. 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of Cereal Science. Vol. 38(2) p. 133–146. DOI 10.1016/S0733-5210(03)00030-4.

EKINCI M., TURAN M., YILDIRIM E., GUNEŞ A., KOTAN R., DURSUN A. 2014. Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (Brassica oleracea L. var. botrytis) transplants. Acta Scientiarum Polonorum – Hortorum Cultus. Vol. 13(6) p. 71–85.

GAMALERO E., GLICK B.R. 2011. Mechanisms used by plant growth-promoting bacteria. In: Bacteria in Agrobiology: Plant Nutrient Management. Ed. D. Maheshwari. Heidelberg, Berlin. Springer p. 17–46. DOI 10.1007/978-3-642-21061-7_2.

GEE G.W., BAUDER J.W. 1986. Particle-size analysis. Ch. 15. In: Methods of soil analysis. Part 1: Physical and mineralogical methods. Ed. A. Klute. 2nd ed. Madison, Wisconsin, USA. ASA, SSSA p. 383–409. DOI 10.2136/sssabookser5.1.2ed.c15.

GHANEM O.M., RABIE R.K., ABD EL-AZEEM S.A.M., MEHANA T.A. 2013. Response of wheat to inoculation with plant-growth promoting rhizobacteria at different P fertilization levels. Journal of Soil Sciences and Agricultural Engineering. Vol. 4(6) p. 563–575. DOI 10.21608/JSSAE.2013.51950.

GOSWAMI D., THAKKER J.N., DHANDHUKIA P.C. 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food and Agriculture. Vol. 2(1), 1127500 p. 1–19. DOI 10.1080/23311932.2015.1127500.

GROVER M., BODHANKAR S., SHARMA A., SHARMA P., SINGH J., NAIN L. 2021. PGPR mediated alterations in root traits: way toward sustainable crop production. Frontiers in Sustainable Food Systems. Vol. 4, 618230 p. 1–28. DOI 10.3389/fsufs.2020.618230.

HAKIM S., NAQQASH T., NAWAZ M.S., LARAIB I., SIDDIQUE M.J., ZIA R., MIRZA M.S., IMRAN A. 2021. Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. Frontiers in Sustainable Food Systems. Vol. 5, 617157 p. 1–23. DOI 10.3389/fsufs.2021.617157.

HOLT J.G., KRIEG N.R., SNEATH P.H.A., STALEY J.T., WILLIAM S.T. (eds.) 1994. Bergey's manual of determinative bacteriology. 9th ed. Baltimore, MD, USA. Williams and Wilikins. ISBN 9780683006032 pp. 787.

JACKSON M.L. 1973. Soil chemical analysis. New Delhi, India. Prentice Hall pp. 498.

KHALID A., ARSHAD M., ZAHIR Z.A. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology. Vol. 96(3) p. 473–480. DOI 10.1046/j.1365-2672.2003.02161.x.

KHANAFARI A., BELADI M., SHARIFNIA F. 2012. An investigation of rhizobacteria as biofertilizer on Mentha L. compounds change. Annals of Biological Research. Vol. 3(9) p. 4293–4302.

KLOEPPER J.W. 1994. Plant growth-promoting rhizobacteria (other systems). In: Azospirillum/Plant associations. Ed. Y. Okon. Boca Raton, FL, USA. CRC Press p. 137–166.

KUNDAN R., PANT G., JADON N., AGRAWAL P.K. 2015. Plant growth promoting rhizobacteria: Mechanism and current prospective. Journal of Fertilizers & Pesticides. Vol. 6(2) p. 1–9. DOI 10.4172/2471-2728.1000155.

KUO S. 1996. Phosphorus. Ch. 32. In: Methods of soil analysis. Part III. Chemical methods. Eds. D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner. SSSA Book Series 5. Madison, WI, USA. American Society of Agronomy and Soil Science Society of America p. 869–919. DOI 10.2136/sssabookser5.3.c32.

LAEMMLI U.K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature. Vol. 227(5259) p. 680–685. DOI 10.1038/227680a0.

LEECH N.L., BARRETT K.C., MORGAN G.A. 2005. SPSS for intermediate statistics: Use and interpretation. 2nd ed. Mahwah, New Jersey, USA. Lawrence Erlbaum Associates, Inc. publishers pp. 255.

MA Y., RAJKUMAR M., VICENTE J.A., FREITAS H. 2011. Inoculation of Ni-resistant plant growth promoting bacterium Psychrobacter sp. strain SRS8 for the improvement of nickel phytoextraction by energy crops. International Journal of Phytoremediation. Vol. 13(2) p. 126–139. DOI 10.1080/15226511003671403.

MAC FADDIN J.F. 1976. Biochemical tests for identification of medical bacteria. Baltimore, USA. Waverly Press pp. 527.

MALDONADO S., RODRÍGUEZ A., ÁVILA B., MORALES P., GONZÁLEZ M.P., ARAYA ANGEL J.P.A., ..., STOLL A. 2020. Enhanced crop productivity and sustainability by using native phosphate solubilizing rhizobacteria in the agriculture of arid zones. Frontiers in Sustainable Food Systems. Vol. 4, 607355 p. 1–14. DOI 10.3389/fsufs.2020.607355.

MALIHA R., SAMINA K., NAJMA A., SADIA A., FAROOQ L. 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pakistan Journal of Biological Sciences. Vol. 7(2) p. 187–196. DOI 10.3923/pjbs.2004.187.196.

MARTIN J.H., LEONARD W.H., STAMP D.L. 1976. Principles of field crop production. 3rd ed. New York. MacMillan. ISBN 0023767200 pp. 1030.

MEHANA T.A. 1994. Microbiological properties and nutrient availability in a salt–affected calcareous soil as influenced by certain amendments under leaching conditions. Bulletin of Suez Canal University. Vol. 3 p. 384–402.

MEHANA T.A., FARAG F.M. 2000. Influence of phosphate dissolving microorganisms and elemental sulfur on phosphorus and micronutrient availability in a calcareous soil treated with rock phosphate. Journal of Agricultural Science Mansoura University. Vol. 25(5) p. 2983–2993.

MOORE T.C. 1974. Research experiences in plant physiology. A laboratory manual. Berlin, Heidelberg. Springer pp. 462.

MOUSTAINE M., KAHKAHI E.R., BENBOUAZZA A.E., BENKIRANE R., ACHBANI E.H. 2016. The role of plant growth-promoting rhizobacteria (PGPR) in stimulating the growth of wheat (Triticum aestivum L.) in Meknes region, Morocco. Plant Cell Biotechnology and Molecular Biology. Vol. 17(7–8) p. 363–373.

NAVEED M., ZAHIR Z.A., KHALID M., ASGHAR H.N., AKHTAR M.J., ARSHAD M. 2008. Rhizobacteria containing ACC-deaminase for improv-

ing growth and yield of wheat under fertilized conditions. Pakistan Journal of Botany. Vol. 40(3) p. 1231–1241.

Patten C.L., Glick B.R. 1996. Bacterial biosynthesis of indole-3-acetic acid. Canadian Journal of Microbiology. Vol. 42(3) p. 207–220. DOI 10.1139/m96-032.

Raza W., Shen Q. 2010. Growth, Fe^{3+} reductase activity and siderophore production by *Paenibacillus polymyxa* SOR-21 under differential iron conditions. Current Microbiology. Vol. 61(5) p. 390–395. DOI 10.1007/s00284-010-9624-3.

Prathibha K.S., Siddalingeshwara K.G. 2013. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as rhizobacteria on seed quality of sorghum. International Journal of Current. Microbiology and Applied Science. Vol. 2(3) p. 11–18.

Rosen H. 1957. A modified ninhydrin colourimetric analysis for amino acids. Archives of Biochemistry and Biophysics. Vol. 67(1) p. 10–15.

Sarwar M., Arshad M., Martens D.A., Frankenberger J.W.T. 1992. Tryptophan – dependent biosynthesis of auxins in soil. Plant and Soil. Vol. 147(2) p. 207–215.

Sharma A., Johri B.N., Sharma A.K., Glick B.R. 2003. Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in Mung bean (*Vigna radiata* L. Wilzeck). Soil Biology and Biochemistry. Vol. 35(7) p. 887–894. DOI 10.1016/S0038-0717(03)00119-6.

Song O.R., Lee S.J., Lee Y.S., Lee S.C., Kim K.K., Choi Y.L. 2008. Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. Brazilian Journal of Microbiology. Vol. 39(1) p. 151–156. DOI 10.1590/S1517-838220080001000030.

Stanley R.M. 1990. Experimental techniques in bacterial genetics. Boston. Jones and Bartlett Publishers pp. 180.

Starr M.P., Stolp H., Trüper H.G., Balows A., Schlegel H.G. 1981. The prokaryotes. A handbook on habitats, isolation and identification of bacteria. Berlin, Germany. Springer-Verlag pp. xlviii + 2596.

Stefan M., Ungureanu E., Oprica L., Dunca S. 2007. The effect of some rhizobacterian strains on soluble proteins content in soybeans (*Glycine max* L. MERR.). Analele Ştiinţifice ale Universităţii

"Alexandru Ioan Cuza", Secţiunea Genetică şi Biologie Moleculară. T. 7 p. 23–26.

Swift R.S. 1996. Organic matter characterization. Ch. 35. In: Methods of soil analysis. Part III. Chemical Methods. Eds. D.L. Sparks, A. L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner. SSSA. Book Series 5. Madison, WI, USA. American Society of Agronomy and Soil Science Society of America p. 1011–1020. DOI 10.2136/sssabookser5.3.c35.

Turan M., Gulluce M., Cakmakci R., Oztas T., Sahin F. 2010. The effect of PGPR strain on wheat yield and quality parameters. In: 19th World Congress of Soil Science: Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August 2010. [DVD]. Brisbane, Australia.

Ventosa A., Neto J.J. 1995. Biotechnological applications and potentialities of halophilic microorganisms. World Journal of Microbiology and Biotechnology. Vol. 11(1) p. 85–94. DOI 10.1007/BF00339138.

Vinayarani G., Prakash H.S.2018. Growth promoting rhizospheric and endophytic bacteria from *Curcuma longa* L. as biocontrol agents against rhizome rot and leaf blight diseases. The Plant Pathology Journal. Vol. 34(3) p. 218–235. DOI 10.5423/PPJ.OA.11.2017.0225.

Vos P., Garrity G., Jones D., Krieg N.R., Ludwig W., Rainey F.A., Schleifer K.H., Whitman W.B. 2009. Bergey's manual of systematic bacteriology. 2nd ed. New York. Springer-Verlag. Vol. 3. ISBN 987-0-387-95041-9 pp. 1422.

Wani P.A., Khan M.S. 2010. *Bacillus* species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. Food and Chemical Toxicology. Vol. 48(11) p. 3262–3267. DOI 10.1016/j.fct.2010.08.035.

William H.P., Chichilo A.C., Reynolds H. 1965. Official methods of analysis of the association of agricultural chemists. 10th ed. Washington, DC. Association of Agricultural Chemicals pp. 158.

Zuberer D.A., Alexander D.B. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of Soils. Vol. 12 p. 39–45. DOI 10.1007/BF00369386.