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Phosphate Solubilization by *Enterobacter cloacae* and its Impact on Growth and Yield of Wheat Plants

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THE MOST efficient phosphate solubilizing bacterial strain was isolated from rhizosphere of healthy rice plants and identified as *Enterobacter cloacae* (B1) based on morphological and biochemical characteristics and 16S r DNA. The optimum phosphate solubilization conditions for pH, temperature and incubation period were obtained at 8, 30 °C and 3 days, respectively. Accordingly, *Enterobacter cloacae* (B1) was evaluated under field conditions through its impact on growth and yield characteristics of wheat (*Triticumaestivum* L.) cultivar Masr 1 in comparison with chemical fertilizers (phosphoric acid,calcium super phosphate and NPK). Results of this study showed that, *Enterobacter cloacae* (B1) effectively increased growth characteristics including plant height (cm) (9.96 %), fresh weight (g/plant) (86.78 %), dry weight (g/plant) (58.36 %) and flag leaf area (cm^2) (53.68 %) and physiological characteristics including chlorophyll pigments content, chl. a, b and total ($\mu g/cm^2$) (31.91 %, 35.81 % and 33.33 %, respectively)as well as yield characteristics such as spike length(cm) (13.14 %), spikelets number (13.58 %), grains number/spike (23.16 %), 1000 kernels weight (g) (6.49 %), spike weight(g) (29.37 %), biological weight(g/m^2) (55.92 %) and grains weight(g/m^2) (41.18 %) in comparison with control treatment.

Keywords: Phosphate solubilizing bacteria, *Enterobacter cloacae*, Wheat, Chemical fertilizers, Phosphoric acid.

Introduction

Phosphorus is one of the highly important macro nutrient required by plants. It is a key nutrient for morphological, physiological and biochemical development. Also, it contributes to photosynthesis, energy and sugar production and nucleic acid synthesis (Saber *et al*., 2005).

Phosphorous is added to cultivated soil in different forms as mineral phosphate fertilizers or organic manure, it is rapidly converted into insoluble complexes such as iron and aluminium phosphate in the acidic soil and calcium phosphate in alkaline or normal soil (Gyaneshwar *et al*., 2002). This problem is well known in Egyptian soils specially those rich in calcium carbonate (El-Gamal, 1996).

To overcome phosphorus deficient problems in soils by safe ways, less expensive costs and friendly environment strategies, Phosphorus Solubilizing Microorganisms (PSMs) have been used to solubilize the precipitated phosphates through converting them into soluble forms, H_2PO^{4-} and HPO_4^{2-} that are available to plant (Coutinho *et al*., 2012). This occurs through principal mechanisms such as acidification of the medium, chelation, ion-exchange reactions and production of various acids (Chung *et al*., 2005 and Gulati *et al*., 2010).

The most powerful PSMs that belong to bacteria as *Pseudomonas*, *Enterobacter* and *Bacillus* in addition to fungus such as *Penicillium* and *Aspergillus* (Whitelaw, 2000, Wakelin *et al*., 2004, Yadav *et al*., 2010 and Xiao *et al*., 2011). *Rhizobium leguminosarum*bv. *Viciae*have been demonstrated to solubilize inorganic phosphorus by Belal *et al*. (2013).

Efficiency of PSMs differs significantly with cultural conditions such as pH, temperature and incubation period. Inoculation with PSMs have an important contribution to overall plant P nutrition and growth, and have increased yields of many crops (Whitelaw, 2000 and Leggett *et al*., 2001), In particular, under glasshouse conditions (Zaidi *et al*., 2009 and Khan *et al*., 2010). More

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importantly, investigations conducted under field level using wheat and maize plants have revealed that PSMs could drastically reduce the usage of chemical or organic fertilizers (Singh and Reddy, 2011).

The present study was designed to isolate, characterize the efficient phosphate solubilizing bacteria and application on wheat plant to improve its growth and yield.

Materials and Methods

Collection of soil samples

Soil samples were collected from rhizosphere of healthy plants (rice, maize, cotton, pepper and cucumber) in Kafr Elsheikh Governorate, Egypt stored in polyethylene bags and brought to the laboratory for further studies.

Isolation and screening of Phosphorus Solubilizing Microorganisms

Ten grams from each soil sample was suspended in a 90-ml sterilized saline solution and serially diluted. The dilutions were plated on Pikovskaya's Agar medium and the plates were incubated at 28 ºC for 3-6 days. The bacterial colonies surrounded with a halo zone were purified then maintained on PVK slants at 4 ºC. All bacterial isolates were screened according to the formed halo zone around the colonies. The highest phosphorus solubilizing activity isolate (wider halo zone) were selected for morphological, biochemical and molecular characterization (included DNA extraction and polymerase chain reaction (PCR) at Sigma Scientific Services Co., Giza, Egypt and 16S rDNA gene sequencing technique was conducted using ABI 3730xl DNA sequencer at GATC Company, Germany).

Effect of pH and temperature on phosphate solubilization efficiency

Pikovskaya's agar medium (PVK) was adjusted at different pH values 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 to determine the optimum pH. The selected bacterial strain was spot inoculated at the center Pikovskaya's plate and incubated at 30 ºC for 7 days. Diameter of phosphate solubilization zone was recorded. To determine the optimum temperature, Pikovskaya's agar medium's plates were inoculated with the bacteria then the plates were incubated for 7 days at temperatures 20 ºC, 30 ºC and 40 ºC. Diameter of phosphate solubilisation zone was recorded.

Effect of incubation period on growth and phosphate solubilization efficiency

Pikovskaya's broth medium was used to detect the effect of incubation period on quantative of phosphate solubilization in addition to the growth of the bacterial isolate. 100 ml of the medium was inoculated by 1 ml (10^9cfu/ml) of culture of the bacterial strain. a sterilized uninoculated medium was served as a control. The cultures were incubated on a rotatory shaker at 30 ºC and 150 rpm/min. Every 24 hr,solubilized P was measured spectrophotometrically at 430 nm according to Subba (1993) and Belal*et al*. (2013) and also cells number of the bacteria was determined by plating appropriate dilutions of the liquid medium onto nutrient agar medium

Inoculation effect of Enterobacter cloacae (B1) on wheat growth and yield

Field experiment was carried out to evaluate the impact of the selected isolate on growth and yield of wheat (*Triticumaestivum* L.) cultivar Masr 1. The experiment was carried out in experimental fields of Faculty of Agriculture, Kafrelsheikh University, Egypt during two growing winter successive seasons of 2014/2015 and 2015/2016.

Plots of 1m long and 1m wide were prepared in the field and hand-sown with 17g wheat grains. The wheat grains were inoculated at the time of planting as follows, grains were witted with 10 % sugar syrup, air-dried under shadow and thoroughly mixed with a volume of bacterial suspension previously prepared enough to obtain 10^8 CFU per gram of grains for half an hour. As per control treatments, plots were sown with uninoculated grains.

The experiment included 9 treatments, T1 (C) control, T2 (P) Phosphorus fertilizer, T3 (N+P+K), T4 (A) phosphoric acid, T5 (A+P), T6 (A+NPK), T7 (B1), T8 (B1+P), T9 (B1+NPK).

Chemical fertilizers (NPK) were applied with the respective treatments at the recommended rates as follows: Phosphorus fertilizer dose was applied before sowingas calcium super phosphate (15.5 % P_2O_5), Nitrogen fertilizer was added in three doses in the form of urea (46 % N): the first dose (20%) at the time of sowing, the 2nd dose (40%) before the 1st irrigation and the 3rd dose (40%) before the 2nd irrigation and potassium fertilizer dose was applied before the 2nd irrigation in the form of potassium sulphate (48 % K_2O).

As per phosphoric acid 85%,it was applied after 1st irrigation (35 days from sowing) at the rate of 3cm^3/liter.The cultural practices, irrigation and pest control were carried out as commonly used.

The following data were recorded:

- Growth characteristics, plant height (cm), fresh weight / plant (g), dry weight / plant (g): Plants were dried at 70 ºC till constant weight in electric oven and Flag leaf area / plant (cm^2); measured according to Muller (1991).
- Physiological characteristics, chlorophyll pigments such chlorophyll a, b and total chlorophyll content (µg / cm^2) were determined in the flag leaf lamina using the spectrophotometer method described by Moran and Porath (1980).
- Yield characteristics, spike length (cm) was measured by the length of the main spike, spikeletsnumber / spike was determined by counting number of fertile spikelets per spike, grains number / spike was computed by counting number of grains of the main spike, 1000-kernel weight (g) was determined as the mean weight of 1000 kernel random sample, spike weight (g) was determined as the weight of the main spike, Biological weight (g / m^2) was determined as the weight of harvested plants of 1 sqr meter and grains weight (g / m^2) was determined as the weight of harvested grains of 1 sqr meter.

Statistical analysis

The treatments were distributed in a Randomized Complete Block Design (RCBD). Each treatment was represented by 3 plots as replicates.The collected data were statistically analyzed by CoStatsoftware. Duncan's multiple range tests (DMRT) were used for comparisons among treatments means at 0.05 probability level (Duncan, 1955).

Results and Discussion

Isolation and screening of phosphorus solubilizing bacteria

Forty - four bacterial isolates were obtained from different plants. The strain B1 showed the highest phosphate solubilization activity (wider halo zone) among all other strains.

Identification of Enterobacter cloacae (B1) using 16S rDNA

The selected bacterial strain (B1) was morphologically and biochemically characterized according to Bergey's manual of Systematic Bacteriology (Krieg and Holt, 1984) as well as using analysis of 16S rDNA.

The gene coding of 16S rDNA of the strain was amplified by PCR using universal primer. Results in Fig. 1 showed that, 1500 bp DNA fragment was obtained by PCR amplification of 16S rDNA gene of the bacterial strain. Then the amplified PCR product has been sequenced, and data of sequence obtained was compared with data base in the Nucleotide Database of National Center for Biotechnology Information (NCBI).

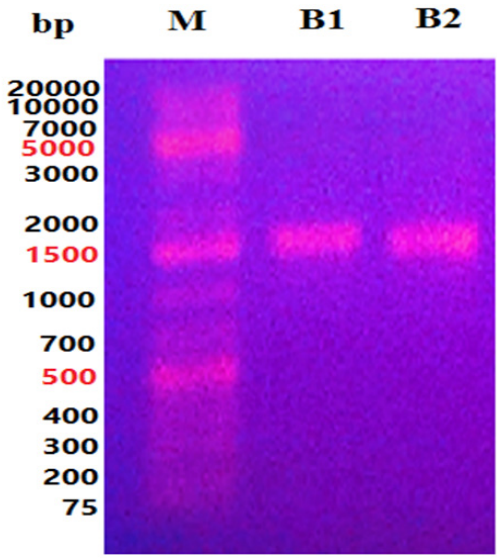

Fig. 1. Agarose gel electrophoresis of purified PCR product B1: *Enterobacter cloacae* and M: DNA marker

Phylogenetic analysis of the bacterial strain and related bacterial species according to 16S rDNA gene sequence was provided in Fig. 2.This revealed that bacterial strain was most closely related to *Enterobacter cloacae*strain ECNH4 (99%). This result is the same as the finding of Kampfer *et al.* (2005) who indicated that plant growth promoting bacterial strain D5/23T most closely related to *Enterobacter cloacae* with 99.0% and *Enterobacter dissolvens* with 98.5% sequence similarity.

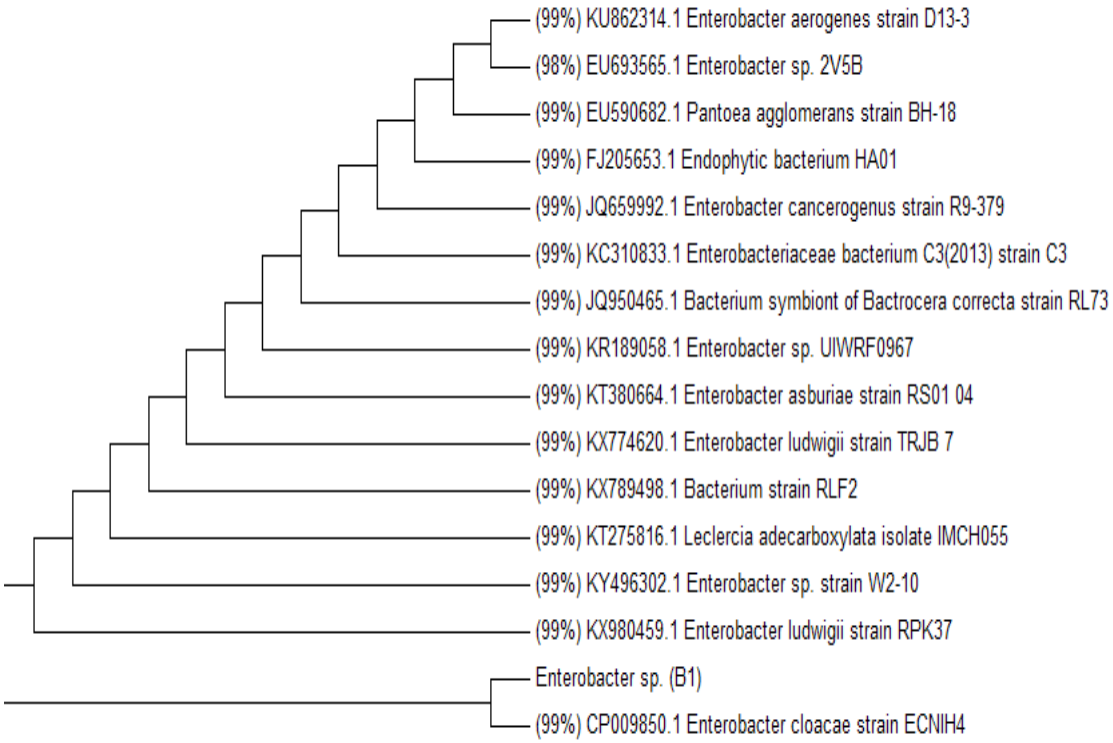

Fig. 2. Phylogenetic tree of 16S rRNA gene sequences of *Enterobacter cloacae* (B1)

Effect of pH and temperatureon phosphate solubilization efficiency of Enterobacter cloacae (B1)

Data presented in Fig. 3 and 4 show the influence of pH and temperature on phosphate solubilization efficiency of *E. cloacae* (B1) in Pikovskaya's agar medium.

Results showed that *E. cloacae* (B1) is able to solubilize P at a wide range of pH from 4.5 to 9. and at different temperatures 20,30 and 40 °C. Whereas the optimum pH and temperature were 8 and 30°C, respectively.These obtained results are in agreement with Nopparat *et al*. (2008) who found that the bacterial strain SD02P3218 recorded the highest tricalcium phosphate solubilization at pH8.84. and temperature 34.7 °C.Previous workers have found that bacteria showed higher phosphate solubilization at pH 7-8 (Seshadri *et al*., 2002). Also, Mardad *et al*. (2014) found that, the highest production of orthophosphateby *Enterobacter hormaechei* was at pH 7 and temperature 30°C.

Effect of incubation Period on growth and phosphate solubilization efficiency of Enterobacter cloacae (B1)

Data presented in Fig. 5 revealed that, phosphate solubilization has been started when the strain grew on the medium. The maximum phosphate solubilization occurred at the end of logarithmic phase (on the third day). These results were in line with Walpola and Arunakumara (2015) who found that the highest phosphate solubilization of *Enterobacter ludwigii* and *Enterobacter hormaechei* was recorded at day 2 and 3 of the incubation, respectively. Also, Jena and Rath (2013) reported that the optimum incubation period of phosphate solubilizing activities was found to be 3 days for five bacterial isolates. phosphate solubilization by *Rhizobium leguminosarum*bv. *Viciae* gradually increased up to 7 days on Pikovskaya's Agar medium (Belal *et al*., 2013).

Inoculation effect of Enterobacter cloacae (B1)on growth characteristicsand yield of wheat plants

Growth characteristics

Data concerning growth characteristics (plant height, fresh and dry weight and flag leaf area) are presented in Table 1. Also, chlorophyll pigments content (chl. a, b and total) is presented in Table 2.

Plant height (cm)

Presented results show that the highest values of plant height were obtained by

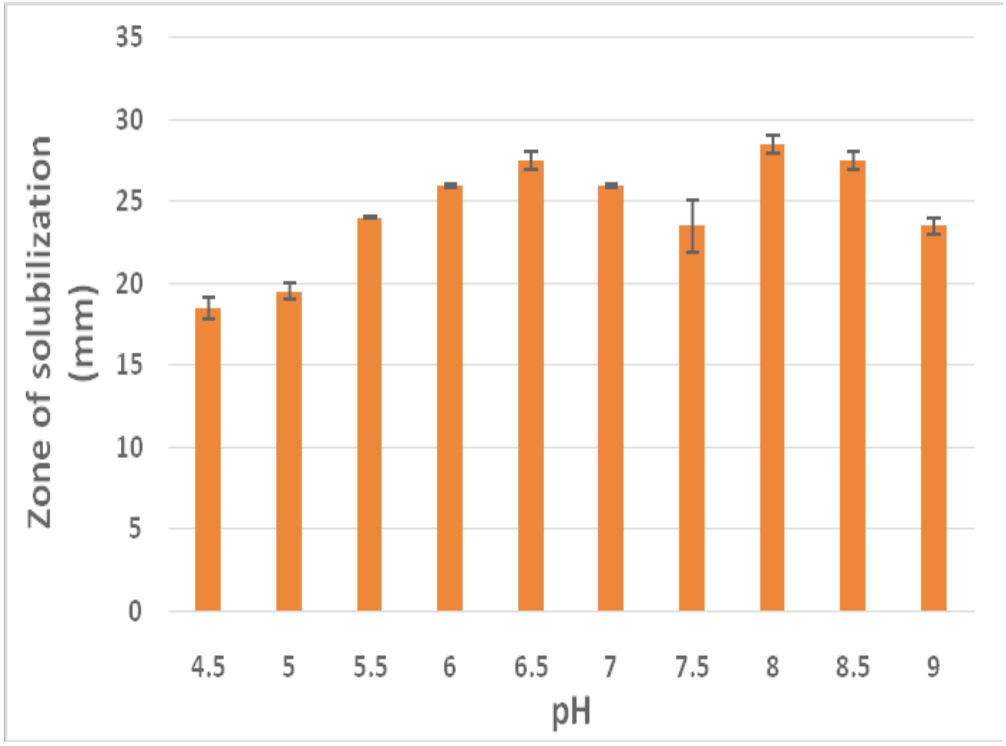

Fig. 3. Effect of pH on phosphate solubilization of *Enterobacter cloacae* (B1)

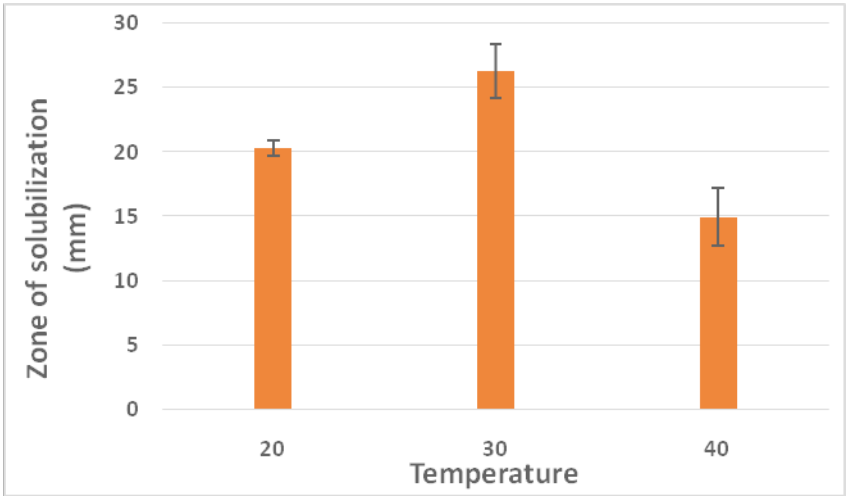

Fig. 4. Effect of temperature on phosphate solubilization *Enterobacter cloacae* (B1)

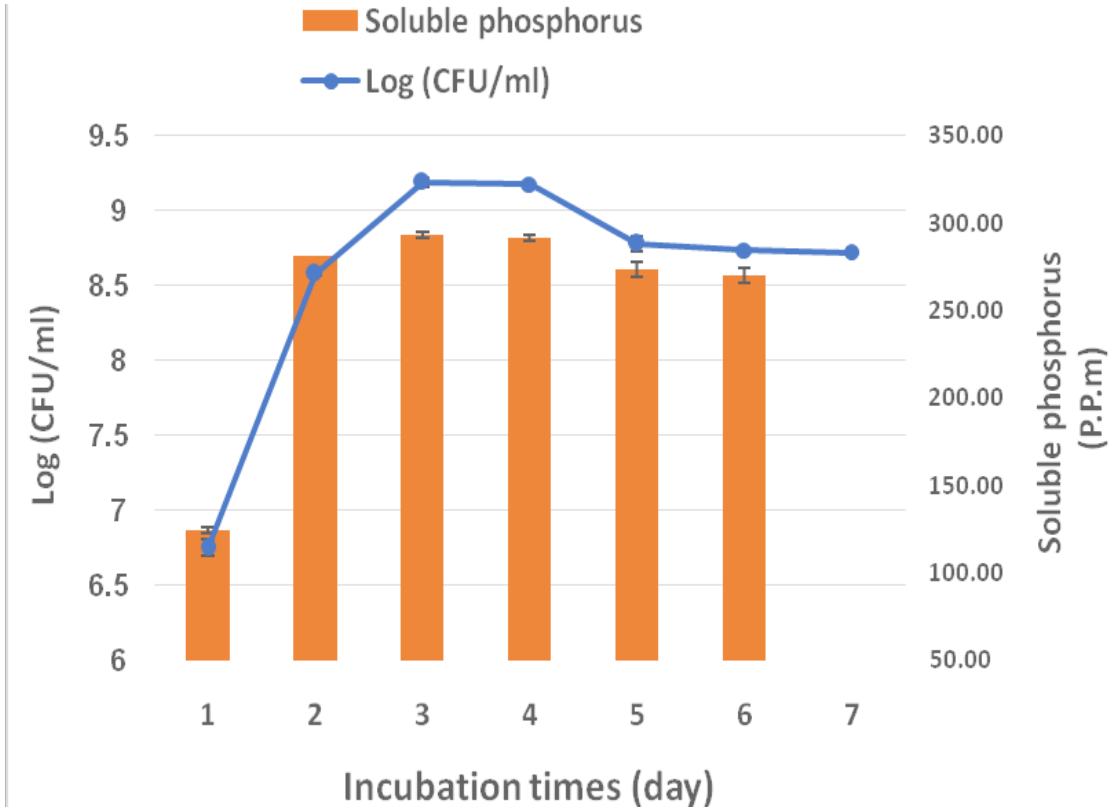

Fig. 5. Effect of incubation period on growth and phosphate solubilization of *Enterobacter cloacae* (B1).

application of bacterial strain and phosphoric acid in combination with NPK (B1+NPK and A+NPK treatments) while the lowest value of plant height was recorded in the control treatment (uninoculated and unfertilized) during the both successive seasons. The treatments (B1+NPK) and (A+NPK) increased plant height insignificantly during the first season and significantly during the second season in comparison with the treatment (NPK) except the second record in the first season of the treatment (A+NPK) which showed a significant increase. It could be observed that, inoculation of phosphate solubilizing bacteria (B1) increased plant height by about 9.96% over un-inoculated plants. Also, application of phosphoric acid led to increase in wheat plant height up to 11.53% over control. The treatment (B1+NPK) increased plant height by 12.97 % over (NPK) treatment and the treatment (A+NPK) increased plant height by 14.92% over (NPK) treatment.These obtained results are in harmony with those finding by Ramesh *et al.* (2014) who reported that, plant height increasing in wheat and soybean plants due to inoculation with *Enterobacter cloacae* subsp. *dissolvens* MDSR9. Similar increase in growth parameters with the inoculation has been previously reported with inoculation of *Enterobacter* (Mehnaz *et al.*, 2010, Montanez *et al.*, 2012 and Shoebitz *et al.*, 2009). Kumar *et al*. (2001) found the combined effect of bacterial inoculants and fertilizer showed maximum increase in plant height.

The increasing in plant growth due to the inoculation with microorganisms having phosphate solubilizing ability may be attributed to auxin production (Gyaneshwar *et al.*, 2002 and Fankem *et al.*, 2008), ACC-deaminase activity (Zafarul- Hye *et al.,* 2007 and Naik *et al.,* 2008), production of organic acids (Fankem *et al.,* 2008) or phosphatases (Abd- Alla, 1994 and Chabot *et al.*, 1996) to solubilize/mineralize P , thereby increasing phosphate nutrition of inoculated plants.

Fresh weight and dry weight (g / plant)

The treatment (B1+NPK) recorded the highest mean values of fresh and dry weight during

TABLE 1. Plant height, fresh weight (g), dry weight (g) and flag leaf area (cm^2) of wheat plant cultivar Masr 1 as affected by PSB (B1), chemical fertilizersand their interactions during 2014 / 2015 and 2015 / 2016 seasons.

Treatments	Plant height (cm)				Mean	* Fresh weight (gm)		Mean	* Dry weight (gm)				Mean	Flag Leaf area (cm^2)		Mean
	2014 / 2015		2015 / 2016			2014 / 2015	2015 / 2016		2014 / 2015		2015 / 2016			* 2014 / 2015	2015 / 2016	
	70 Days	130 days	70 days	130 days		70 days			70 days	130 days	70 days	130 days		70 days		
Control	40.28^{c}	75.33^{d}	41.17^{d}	64.13^{c}	55.23	5.29^{c}	9.98^{e}	7.64	1.69^{c}	7.81^{b}	2.36^{e}	11.58^{d}	5.86	17.87^{bc}	14.47^{e}	16.17
P	49.67^{bc}	82.40^{bcd}	49.57^{bc}	67.13^{c}	62.19	7.38^{bc}	12.08^{de}	9.73	2.16^{bc}	11.95^{b}	4.10^{d}	12.89^{cd}	7.78	18.27^{c}	17.71^{cde}	17.99
NPK	54.78^{ab}	85.20^{bc}	53.67^{bc}	79.07^{ab}	68.18	11.25^{abc}	24.57^{abc}	17.91	4.27^{abc}	15.69^{ab}	10.15^{bc}	23.13^{b}	13.31	27.80^{abc}	27.76^{b}	27.78
A	47.78^{bc}	76.27^{cd}	51.43^{bc}	70.93^{c}	61.60	13.58^{abc}	10.71^{de}	12.15	4.36^{abc}	17.59^{ab}	3.41^{de}	15.53^{cd}	10.22	23.60^{abc}	15.92^{de}	19.76
A + P	56.44^{ab}	73.87^{d}	49.67^{bc}	71.87^{bc}	62.96	11.39^{abc}	16.27^{cde}	13.83	4.15^{abc}	10.85^{b}	5.20^{d}	13.85^{cd}	8.51	27.01^{abc}	17.69^{cde}	22.35
A + NPK	65.67^{a}	96.47^{a}	65.67^{a}	85.60^{a}	78.35	17.75^{ab}	25.54^{ab}	21.65	5.45^{a}	26.77^{a}	13.08^{b}	30.68^{a}	19.00	33.79^{a}	34.31^{a}	34.05
B_1	50.66^{bc}	73.73^{d}	47.10^{cd}	71.43^{bc}	60.73	15.67^{ab}	12.87^{de}	14.27	4.67^{ab}	9.71^{b}	4.81^{d}	17.91^{bc}	9.28	30.21^{ab}	19.48^{cd}	24.85
B_1+ P	53.78^{ab}	79.40^{bcd}	56.33^{b}	67.13^{c}	64.16	19.28^{a}	17.40^{bcd}	18.34	5.31^{a}	14.67^{ab}	7.83^{c}	14.13^{cd}	10.49	29.87^{ab}	20.91^{c}	25.39
B_1+ NPK	64.11^{a}	86.33^{b}	71.33^{a}	86.30^{a}	77.02	17.80^{ab}	30.25^{a}	24.03	4.92^{ab}	29.06^{a}	19.39^{a}	38.12^{a}	22.87	30.90^{a}	31.22^{ab}	31.06

* Data have been transformed by the square root.

Control= (uninoculated and unfertilized) **P**= Phosphate fertilizer **NPK**= N+P+K fertilizers **A**= phosphoric acid**B1**= *Enterobacter coloacea*

Means are value of three replications, Means followed by the same letter in a column are not significantly different but by different letters are significantly different (P= 0.05) using the Duncan multiple range test.

TABLE 2. Chlorophylla, b and total chlorophyllcontent (µg / cm²) of wheat plant cultivar Masr 1 as affected by PSB (B1), chemical fertilizersand their interactions during 2014 / 2015 and 2015 / 2016 seasons.

Treatments	Chlorophyll a (µg / cm²)		Mean	Chlorophyll b (µg / cm²)		Mean	Total Chlorophyll (µg / cm²)		Mean
	2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016	
Control	21.20^{h}	22.30^{h}	21.75	12.83^{f}	14.93^{g}	13.88	34.14^{g}	37.31^{g}	35.73
P	28.07^{g}	31.10^{g}	29.59	19.70^{e}	22.80^{b}	21.25	47.84^{f}	53.98^{f}	50.91
NPK	40.27^{c}	42.17^{c}	41.22	20.77^{c}	21.60^{cd}	21.19	61.14^{c}	63.86^{c}	62.50
A	29.23^{f}	31.90^{f}	30.57	16.60^{d}	17.00^{f}	16.80	45.90^{f}	48.91^{g}	47.41
A + P	30.47^{e}	36.93^{e}	33.70	14.90^{e}	22.13^{bc}	18.52	45.46^{f}	59.19^{d}	52.33
A + NPK	45.30^{b}	45.40^{b}	45.35	22.17^{b}	21.37^{d}	21.77	67.58^{b}	66.83^{b}	67.21
B_1	30.57^{e}	26.80^{h}	28.69	19.70^{c}	18.00^{e}	18.85	50.33^{e}	44.94^{h}	47.64
B_1+ P	36.17^{d}	37.80^{d}	36.99	16.40^{d}	18.60^{e}	17.50	52.67^{d}	56.54^{e}	54.61
B_1+ NPK	54.80^{a}	52.10^{a}	53.45	23.73^{a}	23.70^{a}	23.72	78.59^{a}	75.84^{a}	77.22

Control= (uninoculated and unfertilized) **P**= Phosphate fertilizer **NPK**= N+P+K fertilizers **A**= phosphoric acid**B1**= *Enterobacter coloacea*
Means are value of three replications, Means followed by the same letter in a column are not significantly different but by different letters are significantly different (P= 0.05) using the Duncan multiple range test

the both seasons. While the lowest values were recorded by the control treatment (uninoculated and unfertilized) during the both seasons.The treatment (B1+NPK) increased insignificantly fresh weight during both seasons and increased dry weight insignificantly during the first season and significantly during the second season in comparison with (NPK) treatment. The treatment (B1) significantly increased fresh weight and dry weight (first record) during the first season whereas during the second season increased insignificantly fresh weight and significantly dry weight in comparison with its respective control (uninoculated and unfertilized).

Generally, the treatment (B1) increased fresh weight and dry weight by 86.78 % and 58.36 %, respectively over control and the treatment (B1+NPK) increased fresh weight and dry weight by 34.17 % and 71.83 %, respectively over (NPK) treatment.These findings are almost similar to those of Schoebitz *et al.* (2007) who found a statistically significant increase in shoot fresh weight evident in plants inoculated with *Enterobacter ludwigii*. Ramesh *et al*. (2014) also reported that inoculation with *Enterobacter cloacae* subsp. *dissolvens* MDSR9 led to an increase in dry weight in both soybean and wheat crops over un-inoculated control.All quantitative plant traits showed significant increase in response to chemical fertilizer treatments, this response was further substantiated with bacterial inoculation (Kumar *et al*., 2001).

Flag leaf area (cm²)

Based on data in Table 1, the highest values of flag leaf area (cm²) were obtained by the treatments (A+NPK) and (B1+NPK) while the lowest value was obtained in control treatment (uninoculated and unfertilized) during the both seasons. The treatment (B1+NPK) insignificantly increased flag leaf area (cm²) in comparison with (NPK) treatment during the both seasons. The treatment (B1) increased flag leaf area (cm²) insignificantly during the first season and significantly during the second season in comparison with its respective control. The treatment (B1) increased flag leaf area (cm²) by 53.68% over control and the treatment (B1+NPK) increased flag leaf area (cm²) by 11.81 % over (NPK) treatment.

These obtained results are similar to other investigators (Panhwar *et al.,* 2011 and Al-Shamma and Al-Shahwany, 2014).

Physiological characteristics (chlorophyll pigments (µg / cm²)

Data presented in Table 2 cleared that, the treatment (B1+NPK) recorded the highest values of chlorophyll pigments (chl. a, b and total) content (µg / cm²) during the both seasons except chl. b of the second season. (B1+NPK) treatment significantly increased chlorophyll pigments (chl. a, b and total) content (µg / cm²) in comparison with (NPK and A+NPK) treatmentsduring the both seasons. On the other hand, the treatment (B1) significantly increased chlorophyll pigments (chl. a, b and total) content (µg / cm²) in comparison with untreated control which recorded the lowest value. The treatment (B1) increased chl. a, b and total by 31.91 %, 35.81 % and 33.33 %, respectively over control and the treatment (B1+NPK) increased chl. a, b and total by 29.67 %, 11.94 % and 23.55, respectively % over (NPK) treatment.These findings are similar to those of Panhwar *et al.* (2011) who found that PSB inoculated treatments on aerobic rice demonstrated a significant increase in chlorophyll content and leaf photosynthesis compared to non-inoculated treatments. Mehrvarz *et al.* (2008) also reported similar results with mycorrhiza along with PSB (*Pseudomonas putida*) which showed an increase in leaf chlorophyll content in barley. Al-Shamma and Al-Shahwany (2014) reported that the combination of biofertilizer (*A. chroococcum, A. brasilense* and *P. fluorescens*) and 100% NP significantly affected flag leaf chlorophyll content (55.05 SPAD). Application of *Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2) strains individually increased chlorophyll pigment contents(µg / cm²) for two wheat cultivars compared with untreated plant (El-Afry *et al.*, 2012).

Yield and its components

Data concerning yield characteristics (spike length, spikelets number, grains number, 1000 kernels weight and spike weight) are presented in Table 3. As well , biological and grains yield are presented in Table 4.

Spike length (cm) and spikelets number

Data in Table 3 revealed that, the treatment (A+NPK) gavet he highest value of spike length (cm) and spikelets number during the first season and increased insignificantly spike length and spikelets number compared to (B1+NPK) and (NPK) treatments during the first season. On the contrary,during the second season the treatment (B1+NPK) was the highest and increased significantly spike length and spikelets number compared to (A+NPK) and (NPK) treatments. The treatment (B1) increased significantly spike length and spikelets number during the both seasons in comparison with uninoculated and unfertilized treatment which recorded the lowest value.

The treatment (B1) increased spike length and spikelets number by 13.14 % and 13.58 %, respectively over control and the treatment (B1+NPK) increased spike length and spikelets number by 6.31 % and 5.69 %, respectively over (NPK) treatment.

These obtained results are in line with similar previous findings (Khalid *et al*., 1997, Hilali*et al*., 2000 and Afzal & Bano, 2008), who reported an increase in spikelets per spike and spike length of various crop plants by microbial inoculation. Also, Kumar *et al*. (2001) reported the combined effect of bacterial inoculants and fertilizer showed maximum increase in spike length (11.3%) and spikelet spike^{-1} (11.1%) in wheat crop. The agronomic traits and yield have been increased when plants inoculated with bio-fertilizers combined with mineral fertilizers, and that is because of the application of bio-fertilizers which may be attributed to their role by enhancing plant growth due to the availability of different nutrients including N, P and K in addition to several micronutrients (Al-Shamma and Al-Shahwany, 2014).

Grains number / spike

Concerning grains number, the obtained results exhibited that, the highest value during the first season was recorded by the treatment (A+NPK) and increased insignificantly grains number per spike in comparison with (B1+NPK) and (NPK) treatments. Whereas, during the second season the treatment (B1+NPK) was the highest value and increased significantly grains number in comparison with (A+NPK and NPK) treatments.The treatment (B1) increased grains number insignificantly during the first season and significantly during the second season in comparison with control which recorded the lowest value in the both seasons. The treatment (B1) increased grains number by 23.16 % over control and the treatment (B1+NPK) increased grains number by 7.92 % over (NPK) treatment.

These obtained results are in agreement with many other investigators (Kumar *et al.*, 2001, Minaxi *et al.*, 2013 and Al-Shamma &

Al-Shahwany, 2014). In contrast, Bulut (2013) reported that the highest number of kernels per spike was obtained from N and N+P treatments while the lowest values were obtained in treatments of bacteria and control. Fertilization improves nutrition and increases the number of fertile spikelets and flowers, and consequently increases the number of kernels per spike (Singh and Prasad, 2011).

1000 kernels weight (g)

Based on data exhibited during the first season, none of the treatments had any influence on 1000 kernels weight of wheat as compared to control. These findings are almost similar to those of Afzal *et al.* (2005) who found that, there is no influence of treatments on spike length, total and fertile spikelets per spike and grains per spike of wheat as compared to control.

Whereas during the second season (A+NPK) treatment gave the highest one followed by the treatment (B1+NPK) which increased insignificantly 1000 kernels weight in comparison with (NPK) treatment. The treatment (B1) increased significantly 1000 kernels weight in comparison with control which recorded the lowest value. The treatment (B1) increased 1000 kernels weight by 6.49 % over control. The obtained results are similar to the findings of Kumar *et al*. (2001) and Minaxi (2013) who reported 1000 grain weight increasing over control due to the inoculation of phosphate solubilizing bacteria. Also, Al-Shamma and Al-Shahwany (2014) showed that, the combination of biofertilizer and 100% NP significantly affected average weight of 1000 grains. similarly, the highest values of 1000-kernel weight were observed in single N and bacteria treatments (Bulut, 2013).

Spike weight (g)

Data regarding spike weight presented in Table 3 showed that, the treatment (B1+NPK) recorded the highest value and increased significantly spike weight in comparison with (NPK) treatment. Also, the treatment (B1) increased significantly spike weight (g) in comparison with control treatment which recorded the lowest value.The treatment (B1) increased spike weight by 29.37 % over control and the treatment (B1+NPK) increased spike weight by 12.22 % over (NPK) treatment. These obtained results are similar to the findings of Minaxi *et al*. (2013) who reported maximum increase in spike weight evident in the treatment having combination of 2 rhizobacterial strains along with arbuscular mycorrhizal and tricalcium phosphate at the time of harvesting.

Biological and grains weight (g/m^2)

Biological and grains weight (g) of wheat cultivar Masr 1 were presented in Table 4. The obtained results showed that, the treatment (A+NPK) gave the highest values of biological and grains weight (g) and the lowest value was recorded by control treatment during the both seasons. the treatment (B1+NPK) increased insignificantly biological and grains weight in comparison with (NPK) treatment during the both seasons. Also, the treatments (B1+P) and (B1) increased biological and grains weight (g) insignificantly during the first season and significantly during the second season in comparison with their respective controls (P and control).The treatment (B1) increased biological and grains weight by 55.92 % and 41.18 %, respectively over control and the treatment (B1+NPK) increased biological and grains weight by 7.57 % and 4.46 %, respectively over (NPK) treatment.These results confirmed with the findings of Afzal and Bano (2008) whose reported that inoculation of phosphorus (P) solubilizing bacteria with fertilizer (P_2O_5) was better than only P fertilizer for improving grain yield of wheat. Similarity, Vahed *et al*. (2012) indicated that PSB and Phosphate chemical fertilizer had a significant influence on grain yield, biological yield and grain phosphorus uptake. The yield components (biological and grains yield) have been increased when plants inoculated with bio-fertilizers combined with mineral fertilizers, and that because the application of bio-fertilizers which may be attributed to their role by enhancing plant growth due to the availability of different nutrients including N, P and K in addition to several micronutrients (Al-Shamma and Al-Shahwany, 2014).

Concerning phosphoric acid, application of P as phosphoric acid (fluid P source) produced significantly higher wheat grain yield and grain weight over commercial phosphate fertilisers, i.e. diammonium phosphate (DAP) and triple superphosphate (TSP) (Akhtar et al., 2016).

Some Australian scientists confirmed the superiority of fluid P fertilizers over the granular P-fertilizer and produced 31% more wheat yield

TABLE 3. Spike length (cm), spikelets number, grains number, 1000 kernels weight and spike weight (g) of wheat plant cultivar Masr 1 as affected by PSB (B1), chemical fertilizersand their interactions during 2014 / 2015and 2015 / 2016 seasons.

Treatment	Spike length (cm)		Mean	Spikelet number		Mean	Grains number		Mean	1000 kernels weight(g)		Mean	Spike weight (g)	
	2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016		2014 / 2015	2015 / 2016
Control	9.19^{d}	9.52^{d}	9.36	16.67^{c}	16.60^{e}	16.64	47.53^{c}	51.53^{d}	49.53	40.16^{a}	42.15^{d}	41.16	3.03^{e}	
P	10.30^{bcd}	9.71^{d}	10.01	18.27^{abc}	17.2^{de}	17.74	60.40^{bc}	53.3^{cd}	56.85	43.12^{a}	47.07^{bc}	45.10	3.27^{e}	
NPK	11.08^{abc}	11.09^{b}	11.09	18.67^{ab}	18.60^{bc}	18.64	66.67^{ab}	63.13^{b}	64.90	45.88^{a}	49.08^{ab}	47.48	4.09^{bc}	
A	10.07^{cd}	10.42^{c}	10.25	17.67^{bc}	18.63^{bc}	18.15	56.93^{bc}	59.30^{bc}	58.12	44.54^{a}	47.83^{ab}	46.19	3.74^{cd}	
A + P	10.45^{a-d}	9.83^{d}	10.14	18.40^{abc}	17.53^{de}	17.97	57.80^{bc}	53.67^{cd}	55.74	42.11^{a}	44.45^{cd}	43.28	3.22^{e}	
A + NPK	11.61^{a}	11.21^{b}	11.41	19.80^{a}	19.07^{b}	19.44	75.47^{a}	62.80^{b}	69.14	45.93^{a}	50.93^{a}	48.43	4.20^{ab}	
B_1	10.50^{abc}	10.67^{c}	10.59	18.67^{ab}	19.13^{ab}	18.90	59.93^{bc}	62.07^{b}	61.00	41.10^{a}	46.56^{bc}	43.83	3.92^{bc}	
B_1 + P	10.87^{abc}	9.85^{d}	10.36	19.40^{ab}	17.93^{cd}	18.67	61.47^{b}	57.13^{bcd}	59.30	43.74^{a}	44.36^{cd}	44.05	3.36^{de}	
B_1 + NPK	11.49^{ab}	12.09^{a}	11.79	19.27^{ab}	20.13^{a}	19.70	68.40^{ab}	71.67^{a}	70.04	43.94^{a}	49.46^{ab}	46.70	4.59^{a}	

Control= (uninoculated and unfertilized) **P**= Phosphate fertilizer **NPK**= N+P+K fertilizers**A**= phosphoric acid**B1**= *Enterobacter coloacea*
Means are value of three replications, Means followed by the same letter in a column are not significantly different but by different letters are significantly different (P= 0.05) using the Duncan multiple range test.

TABLE 4. Biological weight and grains weight of wheat cultivar Masr 1 as affected by PSB (B1), chemical fertilizersand their interactions during 2014 / 2015and 2015 / 2016 seasons.

Treatments	Biological weight (g / m²)		Mean	Grains weight (g / m²)		Mean
	* 2014 / 2015	2015 / 2016		* 2014 / 2015	2015 / 2016	
Control	884.44^{b}	704.2^{c}	793.32	389.12^{c}	334.26^{d}	361.69
P	982.22^{b}	891.36^{c}	4959.29	406.48^{c}	403.18^{d}	404.83
NPK	2004.44^{a}	1581.09^{a}	1756.77	896.15^{ab}	728.83^{b}	812.49
A	1388.89^{b}	1265.68^{b}	1328.79	589.99^{bc}	595.38^{c}	592.69
A + P	1191.11^{b}	1264.37^{b}	1214.24	469.54^{c}	575.48^{c}	522.51
A + NPK	2443.70^{a}	1751.52^{a}	2098.11	1092.50^{a}	815.20^{a}	953.85
B_1	1080.00^{b}	1335.93^{b}	1236.97	428.52^{c}	592.76^{c}	510.64
B_1 + P	1193.33^{b}	1162.64^{b}	1178.99	526.88^{c}	534.40^{c}	530.64
B_1 + NPK	2153.33^{a}	1669.26^{a}	1889.80	944.14^{a}	753.35^{ab}	848.75

* Data have been transformed by the square root.
Control= (uninoculated and unfertilized) **P**= Phosphate fertilizer **NPK**= N+P+K fertilizers**A**= phosphoric acid**B1**= *Enterobacter coloace*
Means are value of three replications, Means followed by the same letter in a column are not significantly different but by different letters are significantly different (P= 0.05) using the Duncan multiple range test.

using fluid P-fertilizers as compared to granular ones (Holloway *et al.*, 2001).

Holloway *et al.* (2001) also found superiority of phosphoric acid over commercial P fertilizers while evaluating different solid versus liquid P fertilizers. The superiority of PA over DAP and TSP for producing grain yield may be attributed to its ability to maintain higher solution P in soil as found by Naeem and Akhter (2013). Moreover, PA induced reduction in nitrogen loss as ammonia volatilization (Akhtar and Naeem, 2012) could be another factor making it more efficient than the solid fertilizers.

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