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Isolation and identification of a phosphate-solubilizing *Paenibacillus polymyxa* strain GOL 0202 from durum wheat (*Triticum durum* Desf.) rhizosphere and its effect on some seedlings morphophysiological parameters

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ABSTRACT

Paenibacillus polymyxa is a common bacterium, belonging to Plant Growth Promoting Rhizobacteria (PGPR). It is found in soils, roots and rhizosphere of various crop plants especially wheat. This bacterium has a great biotechnological potential in sustainable agriculture. Indeed, in the rhizosphere, *P. polymyxa* is involved in nitrogen fixation, production of phytohormones, exoplysaccharides, hydrolytic enzymes and antibiotics, in the enhancement of soil porosity and in soil phosphorus solubilization. In this study, we have identified GOL 0202 strain as *Paenibacillus polymyxa,* isolated from durum wheat (*Triticum durum* Desf.) rhizosphere collected from arid region soil of Southern. This genomic identification was performed by 16S rRNA gene sequencing. It was shown that this strain possesses an interesting phosphate solubilization activity on NBRIP (National Botanical Research Institute Phosphorus) medium containing tricalcium phosphate (Ca_3 $(PO_4)_2$). This activity, was recorded over five days, it was negatively correlated with pH medium whose decline was due probably to the release of organic acids. In addition to other activities associated with *P. polymyxa*, this availability of soluble phosphate has allowed the improvement of durum wheat seedlings growth estimated through dry weight, chlorophylls and proteins contents. Hence, *P. polymyxa* GOL 0202 could be used for their targeted application as bioinoculant.

1. Introduction

Phosphorus (P) is one of the most essential plant nutrients limiting plant growth (Wang et al., 2009). However, and although it is abundant in soils, P concentration in soil solution is very low and ranges from 1 $\mu g\,mL^{-1}$ to 1 $mg\,mL^{-1}$ (Brady and Weil, 2002). Indeed, the greater part of P remains in insoluble form in soil and, hence, unavailable for the plant. Many of the phosphate present in soil become incorporated into organic or mineral compounds, and most plants are unable to use these P sources (Richardson, 2001). Chemical phosphorus fertilizers are therefore used to supplement soluble phosphorus on most agricultural soils, but these are costly and adversely impact the environment in term of eutrophication, soil fertility depletion and carbon footprint (Sharma et al., 2013). More than that, the efficiency of the applied P fertilizers in chemical form rarely achieved to 30% due to its fixation, in the form of iron/aluminum phosphate in acidic soils (Norrish and Rosser, 1983) or in the form of calcium phosphate in neutral to alkaline soils (Lindsay et al., 1989). In order to solve this problem, for a long time scientists have been interested in the ability of some bacteria to dissolve soluble mineral phosphates such as tricalcium phosphate (Goldstein and Liu, 1987; Goldstein et al., 1993). Therefore, many researchers have tried to develop biofertilizer for P-supply by means of Phosphate-Solubilizing Bacteria (PSB). The PSB allow transforming insoluble phosphate into soluble form through the process of acidification, chelation and exchange reaction in soils (Banik, 1983; Berthelin et al., 1991; Rodriguez et al., 2000; Barroso et al., 2006; Son et al., 2006). Some *Paenibacillus polymyxa* strains have been studied as PSB (Singh and Singh, 1993; Wang et al., 2012a; Weselowski et al., 2016). *Paenibacillus polymyxa* is found in different niches such as soils, roots, rhizosphere of various crop plants including wheat, maize, sorghum, sugarcane and barley (Guemouri-Athmani et al., 2000; Von der Vonder Weid et al., 2000). In addition to its capacity to dissolve soil phosphate, *Paenibacillus polymyxa* (formerly known as *Bacillus polymyxa*) has many other properties as nitrogen fixation (Lindberg et al., 1985; Heulin et al., 1994),

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production of antibiotics (Rosado and Seldin, 1993; Kajimura and Kaneda, 1996, 1997; Choi et al., 2007), exopolysaccharides (Haggag, 2007), auxin (Lebuhn et al., 1997), cytokinin (Timmusk et al., 1999), chitinase (Mavingui and Heulin, 1994), and hydrolytic enzymes (Nielsen and Sørensen, 1997) as well as promotion of increased soil porosity (Gouzou et al., 1993). All these activities might be of importance for plant growth promotion.

The strain GOL 0202 is part of a bacterial collection provided by the Soil Biology Team which studies exopolysaccharides potentiel production of telluric bacteria. The choice of this bacterium is based on its phosphate solubilization ability, compared to the other bacteria. The screening strategy employed during this research enabled the identification of PSB colonies on NBRIP medium containing $Ca_3(PO_4)_2$ as a sole P source. Bacterial isolates showing clear halos of solubilization were selected among thousands of colonies. Based on these results and for this study, we selected this isolate exhibiting PS activities and a high production of exopolysaccharides. Therefore, the aims of the present study were:

- The isolation and identification of a phosphate-solubilizing strain from durum wheat rhizosphere taken from South-Algerian soil.
- The assessment of its phosphate-solubilizing mechanisms in relation with the pH medium variation.
- Some biometric and biochemical analyses on seedlings wheat inoculated with this bacteria.

2. Materials and methods

2.1. Sample collection, characterization and identification

The bacterial strain GOL 0202, used in this study, was obtained from the laboratory of Biological Soil, FSB, USTHB-Algeria. This bacterium was isolated from the soil of durum wheat plants growing in fields at El Golea (30° 34′ N, 2° 53 ′E) in arid region of Southern Algeria. The strain was cultured on Trypticase Soya Agar (TSA) at 30 °C for 48 h.

Tests for the biochemical and physiological properties of bacteria were performed. The Gram reaction, endospore forming and the biochemical analyses including catalase, oxidase and urease reactions; nitrate reduction, gelatine liquefaction and starch hydrolysis; indole production, methyl red and Voges-Proskauer reactions; and H_2S formation were investigated as described by Barrow and Feltham (1993). The carbohydrate fermentation was tested according to Leifson (1963) by using microtiter plate (including Agar, carbohydrate and Bromothymol blue as pH indicator). Inoculated plate was incubated at 30 °C for 48 h, and the results were checked by medium color changing.

Genomic identification of bacterial strain GOL 0202 was performed through 16S rRNA gene sequencing by the laboratory of Microbial Ecology of the Rhizosphere and Extreme Environment (CEA Cadarache, Saint Paul-lez-Durance, France). Briefly, total DNA of bacteria was extracted as described by Ranjard et al. (2003). Amplification of the 16S rRNA was performed by PCR (Achouak et al., 1999) using universal bacterial primers Fd1 (5′-AGAGTTTGATCCTGGCTCAG-3′, position 8–27 of the *E. coli* rrs gene) Tm 60 °C and S17 (5′-GTTACCTTGTTACG ACTT-3′, position 1492–1509 of the *E. coli* rrs gene) Tm 50 °C (Haichar et al., 2007). Those primers enabled to obtain the nearly complete 16S rRNA gene sequences. The 16S rRNA sequence has been deposited in the GenBank data library. The phylogenetic tree was constructed using the Maximum-likelihood method in MEGA7 software (Kumar et al., 2016). The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987) with the Tamura 3-parameter model (Tamura, 1992). The topology of the tree was evaluated by bootstrap analysis based on 1000 replicates (Felsenstein, 1985).

Table 1
Biochemical and physiological characters of *Paenibacillus polymyxa* strain GOL 0202.

Characters of GOL 0202			
Gram	+	Arginine dihydrolase	+
Motility	+	Indole	-
Endospore forming	+	Urea	-
Catalase	+	EPS production on YESA	+
Oxidase	-	H_2S	-
Nitrate reductase	+	Citrate	-
Denitrification	-	Glucose	A
Gelatinase	+	Lactose	A
Amylase	+	Sucrose	A
Ornithine decarboxylase	+	Xylose	+
Lysine decarboxylase	+	Arabinose	A
Methyl red	-	Voges-Proskauer	+

(+) positive reaction, (-) negative reaction, (A) medium acidification

2.2. Phosphate solubilization efficiency and pH medium variation

2.2.1. P solubilization activity on agar medium

The bacterial solubilization activity of phosphate was performed on NBRIP (National Botanical Research Institute Phosphorus) agar media containing tricalcium phosphate $Ca_3(PO_4)_2$ (Nautiyal, 1999). The qualitative study was carried out by spot-inoculation of the bacteria on medium. After 10 days of incubation at 30 °C, the formation of a halo zone around the colony indicated the presence of a solubilization activity of the phosphate by the bacterium. The solubility potential of P was calculated on the basis of the Phosphate solubilization index (PSI) in triplicate, according to the following equation (Morales et al., 2011):

$$PSI = \frac{\text{Total diameter of halo zone}}{\text{Colony diameter}}$$

2.2.2. Quantitative analysis of phosphate solubilization and variation in pH of the liquid medium

The estimation of this activity and the variation of the pH were carried out from bacterial culture in 100 mL of liquid NBRIP medium containing 1 g glucose, 0.5 g $MgCl_2 \cdot 6H_2O$, 0.025 g $MgSO_4 \cdot 7H_2O$, 0.02 g KCl, 0.01 g $(NH_4)_2SO_4$, 2.0 g NaCl, 0.5 g of tricalcium phosphate as a sole source of phosphate. The bacteria was pre-inoculated in 20 mL of the sterilized NBRIP broth (pH = 7) and then incubated at 30 °C for 2 days with rotary shaking at 150 rpm. Three 250 mL Erlenmeyer flasks containing 100 mL of the sterilized NBRIP broth (pH = 7) were inoculated with 1 mL each of the pre-culture and then incubated at 30 °C with orbital shaking at 150 rpm. Aliquots of 10 mL were taken every 24 h for 5 days and centrifuged at 8000 rpm for 10 min. The supernatant was used to determine the phosphate concentration using ascorbic acid method as described by Murphy and Riley (1962) at 882 nm. The values corresponding to the amount of solubilized phosphate were calculated from a standard curve established from a stock solution of KH_2PO_4 ($r^2 = 0.99$). The solubilization of phosphate is mainly associated with the production of organic acids which lower the pH of the medium, it was measured during the 5 days (Vyas and Gulati, 2009). Control flasks without bacterial inoculation were used for samples and their values were subtracted from the experimental values.

2.3. Effect on growth, chlorophylls and proteins content of durum wheat Triticum durum Desf. var. Vitron

2.3.1. Inoculum preparation

From a 48 h bacterial culture in a Petri dish, the inoculum was prepared in 250 mL Erlenmeyer flasks containing 50 mL TSB (Trypticase Soja Broth, 1/10th dilution) medium. The incubation was carried at 28 °C on an orbital shaker at 100 rpm for 48 h. The bacterial culture was concentrated by centrifugation at 5000 rpm for 15 min. The

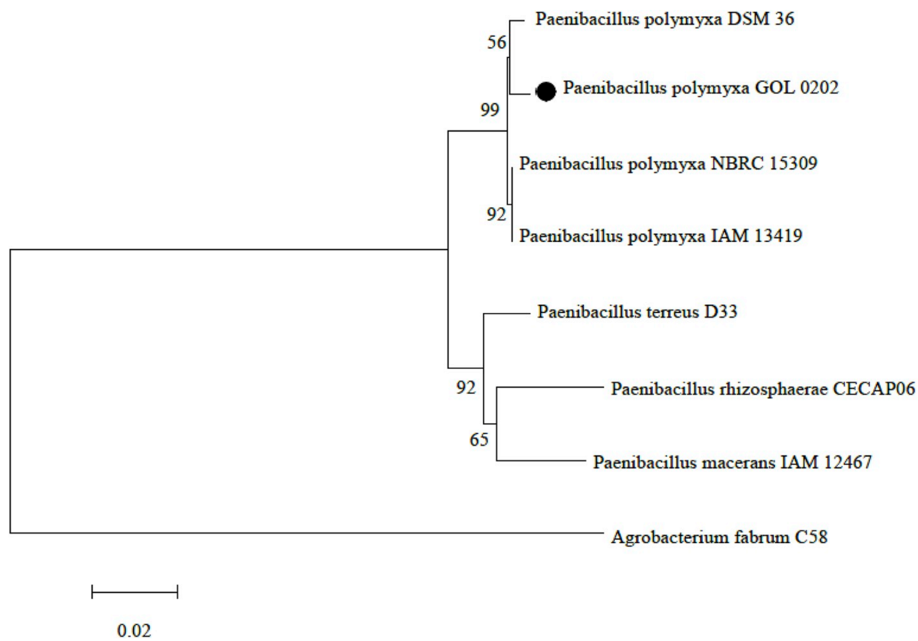

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequence of *Paenibacillus polymyxa* GOL 0202 and its closest 16S rRNA gene matches in the NCBI GeneBank database. Strain isolated in this study is indicated by black circle.

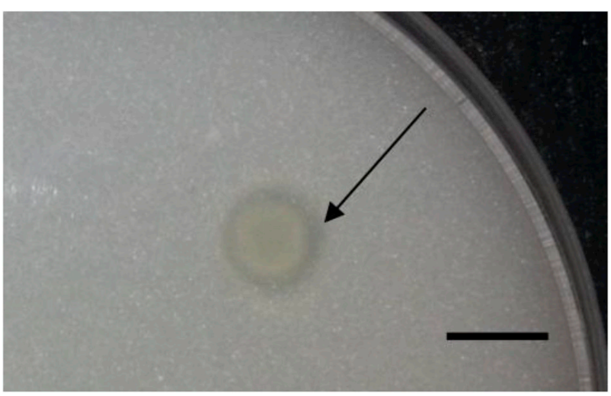

Fig. 2. Halo showing tricalcium phosphate solubilization by *P. polymyxa* GOL 0202 on solid NBRIP medium plates. Arrow indicates the solubilization halo.

pellet was washed 3 times in a sterile potassium chloride solution (0.9%, w/v) and then re-suspended in this saline solution. The density of the bacterial suspension to be inoculated was estimated to 10^6 CFU mL^{-1} corresponding to optical density at 600 nm of 0.6–0.7.

2.3.2. Wheat seeds germination

The experiment was carried out on durum wheat seeds *Triticum durum* Desf. var. Vitron. In order to obtain uniform results for germination as well as for the morpho-physiological characteristics of wheat seedlings, we have selected the grains to keep only the healthiest and most homogeneous ones. Wheat seeds belonging to the modal weight class between 55 and 65 mg, were surface sterilized by dipping them in calcium hypochlorite (5%, w/v) supplemented with 1 mL of ethanol under partial vacuum for 10 min, followed by five to six washings with distilled sterile water. The grains thus treated are soaked for 30 min in sterile distilled water in dark condition and allowed to germinate on 1.5% (w/v) water agar plates. The germination took place in the oven at 25 °C for 48 h. These manipulations were realized in aseptic conditions under a horizontal laminar-flow hood.

2.3.3. Wheat seedlings growth

A triplicate experimental protocol was divided into 2 batches: one consisted of non-inoculated seeds (T) and the other consisted of inoculated seeds (Ti). The durum wheat seedlings were grown, in the presence or absence of bacteria, in 1-L pots containing a sterilized soil mixture (1/3 sand, 1/3 soil, and 1/3 of universal potting soil KB at pH = 6.4). Ten sterile durum wheat seedling, germinated on the agar, were transplanted in the pots. The plants were then inoculated by the bacteria, with a volume of 5 mL of the bacterial suspension, while the non-inoculated controls received 5 mL of sterile distilled water. The pots were placed under 16/8 h light/dark cycle and 50–60% RH at 25 ± 2 °C in an Environment Control Chamber. The plants were removed carefully under a gentle flow of tap water after 21 days of sowing. Data on root length, plant height (aerial parts), root dry weight and shoot dry weight were recorded. The samples were oven-dried at 70 °C for 48 h to a constant weight for determining the dry weight.

2.4. Biochemical assays

2.4.1. Chlorophylls content

Fifty mg of fresh leaves are ground in 2 mL of 80% acetone. After a centrifugation for 10 min at 3000 rpm, the supernatant was recuperated. The results were expressed as chlorophyll concentration (μg g^{-1} of fresh weight) and were obtained from the McKinney equations (1941).

2.4.2. Total water-soluble proteins content

Fresh leaves from 21 day-old plants were ground in 0.1 M Tris-HCl grinding buffer and centrifuged at 13000 rpm for 20 min at 4 °C. The supernatant was collected and the total soluble protein concentration was determined by the Bradford method (Bradford, 1976) with 1 mg mL^{-1} bovine serum albumin (BSA) solution as a standard.

2.4.3. Total phosphorus content

The extraction of foliar phosphorus was conducted according to Gautheyrou and Gautheyrou (1962). An equal weight of 250 mg of foliar dry matter was carbonized at 350 °C until obtaining ashes. The ashes were ground and some drops of distilled water were added. Then, 1 mL of HCl 5.5N was also added. The solution was boiled on gentle heat for 3 min, filtered with Whatman membrane and adjusted to 25 mL with distilled water. The solution was used to determine the

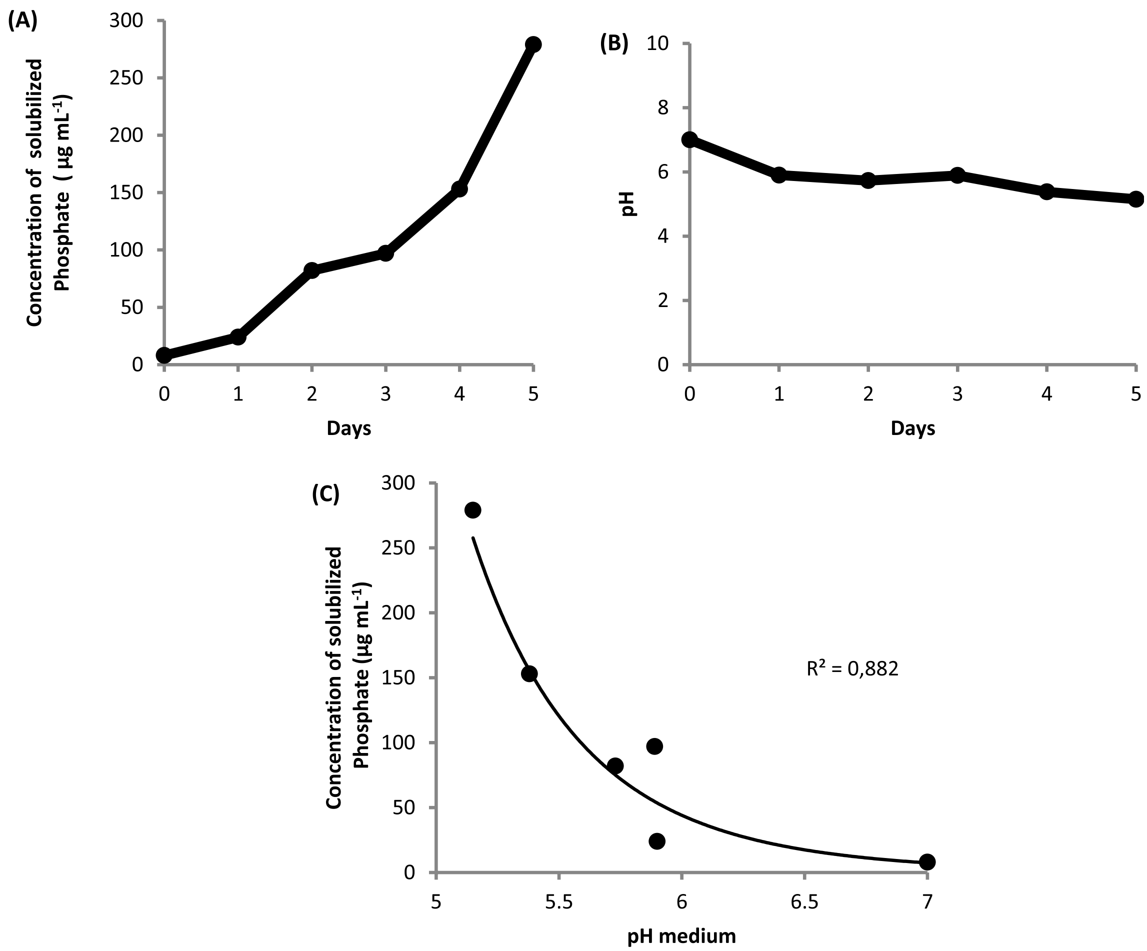

Fig. 3. A: Solubilization activity of the tricalcium phosphate by *P. polymyxa* GOL 0202 on the liquid NBRIP culture medium after 5 days of incubation. B: pH variation of the liquid NBRIP culture medium after 5 days of incubation. C: correlation between phosphate solubilization and pH value.

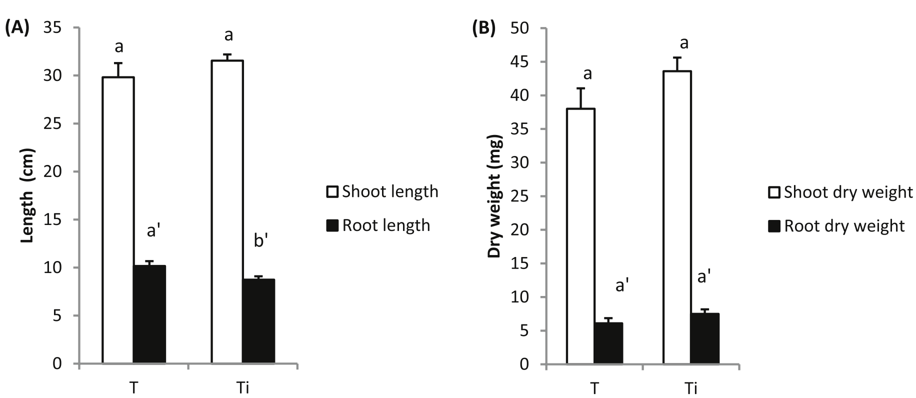

Fig. 4. A: Effect of *P. polymyxa* GOL 0202 on shoot and root length of durum wheat (*Triticum durum*) seedlings. T, control and Ti, inoculated. Different letters indicate statistically significant differences ($p < 0.05$). B: Effect of *P. polymyxa* GOL 0202 on shoot and root dry weight of durum wheat (*Triticum durum*) seedlings. T, control and Ti, inoculated. Different letters indicate statistically significant differences ($p < 0.05$).

phosphorus concentration using ascorbic acid method as described by Murphy and Riley (1962) at 882 nm. The values, corresponding to the amount of phosphorus, were calculated from a standard curve, established from a stock solution of KH_2PO_4 ($r^2 = 0.781$).

2.5. *Statistical analysis*

The results were expressed as mean ± standard error, all experiments were conducted in five replicates. Averages were compared by a one-way ANOVA variance analysis, at significance levels of 5%. A Student test was applied between the means of the results expressing significant differences following the ANOVA test.

3. Results

3.1. *Species identification*

The isolate GOL 0202 was Gram-positive, cells were rod shaped, motile bacteria which produced endospores. The bacteria was positive for: gelatine liquefaction and starch hydrolyzation; catalase, Voges-Proskauer test and nitrate reduction; glucose, lactose, sucrose and arabinose fermentation. Negative for: oxidase, urease and indole production; methyl red reaction and H_2S formation (Table 1). The sequence of the 16S rRNA gene of the isolate GOL 0202 (GenBank Accession no. MF406149) compared with known bacterial sequences in NCBI GenBank using the Basic Local Alignment Search Tool (BLAST,

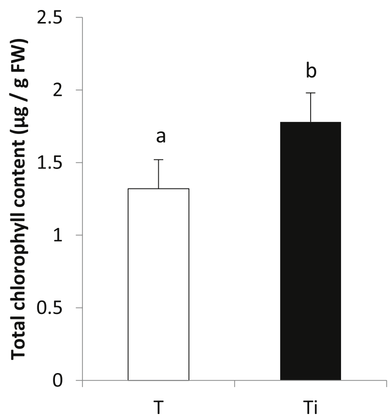

Fig. 5. Effect of *P. polymyxa* GOL 0202 on total chlorophylls content of durum wheat (*Triticum durum*) seedlings. T, control and Ti, inoculated. Different letters indicate statistically significant differences ($p < 0.05$).

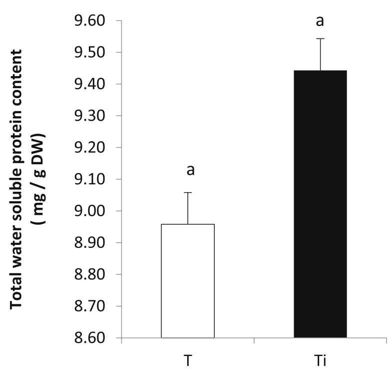

Fig. 6. Effect of *P. polymyxa* on the total water-soluble protein content in durum wheat (*Triticum durum*) seedlings. T, control and Ti, inoculated. Different letters indicate statistically significant differences ($p < 0.05$).

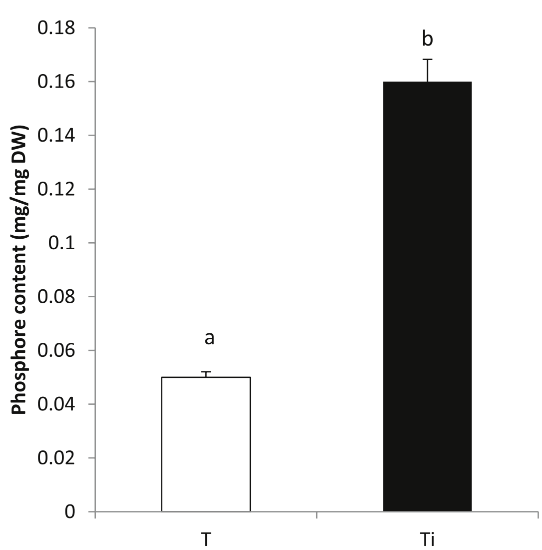

Fig. 7. Effect of *P. polymyxa* on the phosphore content in durum wheat seedlings *Triticum durum*. T, control and Ti, inoculated by *P. polymyxa*. Different letters indicate statistically significant differences ($p < 0.05$).

http://www.ncbi.nih.gov/BLAST), showed the belonging of bacterial strain GOL 0202 to *Paenibacillus polymyxa* DMS36 with an identity value of > 99% (Fig. 1). A phylogenetic tree of the strain GOL 0202 and its relatively proximate species was generated in MEGA7, on the basis of the 16S rRNA gene sequence alignment using the neighbor-joining method.

3.2. Qualitative and quantitative analysis of phosphate solubilization and pH medium variation

Culture on solid NBRIP medium showed a phosphate solubilization halo around the colonies with a PSI of 1.32 ± 0.04 (Fig. 2). On liquid medium, solubilization of the inorganic phosphate measured at 24-h intervals was enhanced with increasing incubation time up to 5 days (Fig. 3A). Conversely, there was a decreasing trend in pH with increasing incubation time. The maximum amount of soluble phosphate in the culture supernatant after five days for *P. polymyxa* GOL 0202 was estimated to be 279 µg mL^{-1}, with a 5.15 pH value (Fig. 3A and B). The solubilization of $Ca_3(PO_4)_2$ was accompanied with a decrease of the pH of the NBRIP medium, correlation analysis indicated that the values soluble P released and pH have a high negative relationship (r = -0.939) (Fig. 3C).

3.3. Growth parameters of durum wheat (Triticum durum)

3.3.1. Length and dry weight of shoot and root

Inoculated *P. polymyxa* had no significant effect on shoot and on root dry weight of durum wheat seedling, but the rate of increase in shoot dry weight reached 14.91%, while the root dry weight increased by 24.01%. (Fig. 4B). However, the ANOVA test showed that the bacteria factor revealed a significant variation in the root lengths. Indeed, inoculated durum wheat seedlings had higher root lengths values than non-inoculated seedlings with 16.24% of increase and 5.76% for shoot lengths (Fig. 4A). We observed that the number of roots in inoculated plants (Ti) was higher than uninoculated plants (T).

3.3.2. Foliar chlorophylls content

Following the inoculation of the *P. polymyxa* strain, a significant increase in total chlorophylls content was recorded. It ranged from 1.32 ± 2.7 µg g^{-1} of Fresh Weight (FW) in the non inoculated seedlings (T) to 1.78 ± 1.85 µg g^{-1} of FW in the inoculated seedlings (Ti) (Fig. 5) corresponding to an increase of 34.84%.

3.3.3. Total water-soluble protein content

The Plant Growth Promoting Rhizobacteria (PGPR) effect of *P. polymyxa* was no significant but showed an increased level of water-soluble proteins. Inoculated wheat seedlings (Ti) had a protein content of 109 ± 7.5 mg g^{-1} of Dry Weight (DW), whereas only 105 ± 12.16 mg g^{-1} of DW on the control seedlings (T) (Fig. 6) corresponding to an increase of 3.67%.

3.3.4. Total phosphorus content

The amount of phosphorus in the inoculated durum plants (Ti) was significantly increased by *P. polymyxa*. The concentration of phosphorus achieved a rate of 0.160 ± 0.008 mg mg^{-1} of Dry Weight (DW). While, the control seedlings (T) had 0.050 ± 0.002 mg mg^{-1} of DW (Fig. 7). This defined an increase of 220% in the total phosphorus content of durum wheat.

4. Discussion

16S rRNA gene amplification and sequencing was used to identify isolate GOL 0202, as this fragment is considered to be the most authentic taxonomic marker for bacterial identification at the genus and species level. Bacterial isolate from rhizosphere durum wheat, was assigned the GenBank accession no. MF406149. The sequence obtained was compared with NCBI databases and the blast revealed a sequence homology of 99% with *P. polymyxa* strain DSM36 (GenBank accession No. NR 117726.2) identified as *Paenibacillus polymyxa* strain GOL 0202. Fig. 1 also showed the relationship of the isolate with their closest relatives retrieved from NCBI GenBank. Various strains of the species *P. polymyxa* have been reported in the rhizosphere of various crops as maize, barley and cucumber (Nielsen and Sørensen, 1997; Vonder Weid et al. 2000; Wang et al., 2012b) and have been isolated from wheat roots (Guemouri-Athmani et al., 2000) and studies for their plant growth-promoting characteristics as nitrogen fixation, production of phytohormones, exoplysaccharides, hydrolytic enzymes and antibiotics (Lebuhn et al., 1997; Haggag, 2007; Weselowski et al., 2016).

On NBRIP medium and at the fifth day of incubation, the P-soluble concentration reached 279 µg mL^{-1} ± 0.01 with a PSI equal to 1.32. Interestingly, Teymouri et al. (2016) showed that the production of soluble P by *Pseudomonas* sp and *Bacillus* sp reached its maximum (357 µg mL^{-1} and 221 µg mL^{-1} respectively) at the fifth day with a PSI equal to 2.6 and 3.5 these strains exhibit high P solubilization on broth media while giving a small halo zone on agar media. In this regard, Qian et al. (2010) explained that P solubilization activity is mainly dependent on bacterial activity, since the mineralization of these compounds is carried out by means of several phosphatases and organic acids which are secreted by bacteria, and may be influenced by media composition and culture conditions. In the same way, Son et al. (2006) found that longer incubation periods decreased soluble P concentration, because a part of the liberated orthophosphate was assimilated by PSB to grow and to form microbial biomass (Mardad et al., 2013). The value of soluble P recorded after five days was relatively high and with agreement with previous results, obtained after five days incubation, which ranged from 111.7 µg mL^{-1} for *Enterobacter* Hy-402 (Yi et al., 2008) to 821.4 µg mL^{-1} for *Pseudomonas trivialis* BIHB 769 (Vyas and Gulati, 2009). So *P. polymyxa* probably excrete organic acids into the medium, such as gluconic acid, citric acid, or others, decreasing the pH of medium and solubilize mineral phosphates (Vyas and Gulati, 2009; Xie et al., 2016; Li et al., 2017). The inorganic acids so released, convert $Ca_3(PO_4)_2$ to di- and monobasic phosphates with the net result of an enhanced availability of the element to plants (Khan et al., 2014), which was clearly demonstrated by the high values of foliar phosphorus content obtained after the inoculation of durum wheat by *Paenibacillus. polymyxa* GOL 0202.

Thus, we observed a significant improvement in seedlings growth. This is in perfect agreement with several works, in particular undertaken by Puri et al. (2015a) who reported an increase of up to 35% in length and up to 30% in biomass after 30 days of inoculated corn seed with *P. polymyxa* strain P2*b*-2R. Also, Puri et al. (2015b) reported that the same strain, *P. polymyxa* P2*b*-2R inoculation, enhanced canola seedling height and biomass.

In addition to the availability of soluble phosphate, this growth stimulation may be due to nitrogen fixing ability by *P. polymyxa* which was demonstrated by Guemouri-Athmani et al. (2000). However, it hasn't been demonstrated that plant growth promotion by *P. polymyxa* is primarily correlated with its nitrogen-fixing ability (Lindberg et al., 1985; Lindberg and Granhall, 1984). Also, this bacteria releases regulators like Indole-3-butyric acid (IBA), tryptophan and tryptophol or indole-3-ethanol that can indirectly contribute to plant growth (Lebuhn et al., 1997). If the production of phytohormones as auxins and ethylene by phosphate-solubilizing microorganisms are considered a common microbiological trait (Khan et al., 2014), the synthesis of cytokinins by some PSM such *Paenibacillus polymyxa* is less common (Timmusk et al., 1999).

The results of chlorophylls content are in perfect agreement with the work of Akhtar and Siddiqui (2007) who reported an enhanced growth parameters, chlorophyll content and consequently plant biomass of chickpea. This chlorophylls increase may be linked to enhanced nitrogen fixation as nitrogen is part of the enzymes associated with chlorophyll synthesis (Chapman and Barreto, 1995) and the chlorophyll concentration represent a relationship of crop nitrogen content and yield level (Blackmer and Schepers, 1995). The positive effect of *P. polymyxa* on recorded protein content could be explained by improved plant nitrate uptake by bacteria (Näsholm et al., 2009).

5. Conclusion

The solubilization activity of inorganic phosphate by *Paenibacillus polymyxa* is one of the aspects of plant growth promotion that these bacteria possess. Indeed, the experimental tests showed that the solubilization activity of the phosphate was proportional to the time and that this activity required acidification of the medium. This property confers to the plant a high level of total phosphorus content which is an important element. Thus, the quantities of phosphate made available to the seedlings enabled an improvement in the growth parameters. It was confirmed by an increase in foliar photosynthetic pigments which act directly on photosynthetic yield. The PGPR effect of this bacterial strain would also imply improved absorption of other mineral elements such as nitrogen which resulted in protein accumulation in inoculated plants. Due to its beneficial effects, this strain could be developed and formulated commercially for possible application in order to improve the plant productivity of certain crops and, in particular, durum wheat. However, additional studies are needed to establish optimal growth parameters for *Paenibacillus polymyxa*, to determine the factors influencing its soil compatibility and to confirm its benefits in field trials.

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References

Achouak, W., Christen, R., Barakat, M., Marte, M.H., Heulin, T., 1999. Burkholderia caribensis sp. now, an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique. Int. J. Syst. Bacteriol. 49, 787–794.
Akhtar, M.S., Siddiqui, Z.A., 2007. Biocontrol of a chickpea root-rot disease complex with Glomus intraradices, *Pseudomonas putida* and *Paenibacillus polymyxa*. Australas. Plant Pathol. 36 (2), 175–180.
Banik, S., 1983. Variation in potentiality of phosphate solubilizing soil microorganisms with phosphate and energy source. Zentralbl. Mikrobiol. 138, 209–216.
Barroso, C.V., Pereira, G.T., Nahas, E., 2006. Solubilization of $CaHPO_4$ and $AlPO_4$ by *Aspergillus niger* in culture media with different carbon and nitrogen sources. Braz. J. Microbiol. 37, 434–438.
Barrow, G.I., Feltham, R.K.A., 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria, third ed. Cambridge University Press, Cambridge.
Berthelin, F., Leyval, C., Laheurte, F., DeGiudici, P., 1991. Involvements of roots and Rhizospere microflora in the chemical weathering of soil minerals. In: Plant Root Growth an Ecological Perspective. Special Publication series of the British Ecological Society, N° 10, Black-well Scientific, Oxford.
Blackmer, T.M., Schepers, S., 1995. Use of a chlorophyll meter to monitor N status and schedule fertigation of corn. J. Prod. Agric. 8, 56–60.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. Anal. Biochem. 72, 248–254.
Brady, N.C., Weil, R.R., 2002. The Nature and Properties of Soils. Pearson Education, NJ, USA, pp. 976.
Chapman, S.C., Barreto, H.J., 1995. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. Agron. J. 89, 557–562.
Choi, S.K., Park, S.Y., Kim, R., Lee, C.H., Kim, J.F., Park, S.H., 2007. Identification and functional analysis of the fusaricidin biosynthetic gene of *Paenibacillus polymyxa* E681. Biochem. Biophys. Res. Commun. 365, 89–95.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Gautheyrou, J., Gautheyrou, M., 1962. Méthode rapide de dosage des éléments minéraux -N-P-K-Na-Ca- Mg dans les feuilles de cannes à sucre, deuxième édition. Notes de laboratoire, Providence, Abymes.
Goldstein, A.H., Liu, S.T., 1987. Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwivia herbicola*. Bioresour. Technol. 15, 72–74.
Goldstein, A.H., Rogers, R.D., Mead, G., 1993. Separating phosphte from ores via bioprocessing bioresour. Technol. 11, 1250–1254.
Gouzou, L., Burtin, G., Philippy, R., Bartoli, F., Heulin, T., 1993. Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination. Geoderma 56, 479–491.
Guemouri-Athmani, S., Berge, O., Bourrain, M., Mavingui, P., Thiéry, J.M., Bhatnagar, T., Heulin, T., 2000. Diversity of *Paenibacillus polymyxa* in the rhizosphere of wheat (*Triticum durum*) in Algerian soils. Eur. J. Soil Biol. 36, 149–159.
Haggag, W.M., 2007. Colonization of exopolysaccharide-producing *Paenibacillus*

polymyxa on peanut roots for enhancing resistance against crown rot disease. Afr. J. Biotechnol. 6, 1568–1577.

Haichar, F.Z., Achouak, W., Christen, R., Heulin, T., Marol, C., Marais, M.F., Mougel, C., Ranjard, L., Balesdent, J., Berge, O., 2007. Identification of cellulolytic bacteria in soil by stable isotope probing. Environ. Microbiol. 9, 625–634.

Heulin, T., Berge, O., Mavingui, P., Gouzou, L., Hebbar, K.P., Balandreau, J., 1994. *Bacillus polymyxa* and *Rahnella aquatilis*, the dominant N_2-fixing bacteria associated with wheat rhizosphere in French soils. Eur. J. Soil Biol. 30, 35–42.

Kajimura, Y., Kaneda, M., 1996. Fusaricidin A, a new depsipeptide antibiotic produced by *Bacillus polymyxa* KT-8. Taxonomy, fermentation, isolation, structure elucidation and biological activity. J. Antibiot. 49, 129–135.

Kajimura, Y., Kaneda, M., 1997. Fusaricidins B, C and D, new depsipeptide antibiotics produced by *Bacillus polymyxa* KT-8: isolation, structure elucidation and biological activity. J. Antibiot. 50, 220–228.

Khan, M.S., Zaidi, A., Ahmad, E., 2014. Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. In: Khan, M.S., Zaidi, A., Aamil, M. (Eds.), Phosphate Solubilizing Microorganisms. Springer International Publishing Switzerland, pp. 31–62.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lebuhn, M., Heulin, T., Hartmann, A., 1997. Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiol. Ecol. 22, 325–334.

Leifson, E., 1963. Determination of carbohydrate metabolism of marine bacteria. J. Bacteriol. 85, 1183–1184.

Li, Y., Liu, X., Hao, T., Chen, S., 2017. Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates. Int. J. Mol. Sci. 18 (7), 1253.

Lindberg, T., Granhall, U., 1984. Isolation and characterization of dinitrogen-fixing bacteria from the rhizosphere of temperate cereals and forage grasses. Appl. Environ. Microbiol. 48, 683–689.

Lindberg, T., Granhall, U., Tomenius, K., 1985. Infectivity and acetylene reduction of diazotrophic rhizosphere bacteria in wheat (Triticum aestivum) seedlings under gnotobiotic conditions. Biol. Fertil. Soils 1, 123–129.

Lindsay, W.L., Vlek, P.L.G., Chien, S.H., 1989. Phosphate minerals. In: Dixon, J.B., Weed, S.B. (Eds.), Minerals in Soil Environment. Soil Science Society of America, Madison, pp. 1089–1130.

Mavingui, P., Heulin, T., 1994. In vitro chitinase and antifungal activity of a soil, rhizosphere and rhizoplane population of *Bacillus polymyxa*. Soil Biol. Biochem. 26, 801–803.

Mardad, I., Serrano, A., Soukri, A., 2013. Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. Afr. J. Microbiol. Res. 7 (8), 626–635.

McKinney, 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140, 315–332.

Morales, A., Alvear, M., Valenzuela, E., Castillo, C.E., Borie, F., 2011. Screening, evaluation and selection of phosphate-solubilising fungi as potential biofertiliser. J. Soil Sci. Plant Nutr. 11, 89–103.

Murphy, Y., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytol. 182, 31–48.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 17, 265–270.

Nielsen, P., Sørensen, J., 1997. Multi-target and medium independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. FEMS Microbiol. Ecol. 22, 183–192.

Norrish, K., Rosser, H.S., 1983. Mineral Phosphate an Australian Viewpoint. CSIRO/ London, UK, Australia Academic Press, Melbourne, pp. 335–361.

Puri, A., Padda, K.P., Chanway, C.P., 2015a. Can a diazotrophic endophyte originally isolated from lodgepole pine colonize an agricultural crop (corn) and promote its growth? Soil Biol. Biochem. 89, 210–216.

Puri, A., Padda, K.P., Chanway, C.P., 2015b. Evidence of nitrogen fixation and growth promotion in canola (*Brassica napus*L.) by an endophytic diazotroph *Paenibacillus polymyxa*P2b-2R. Biol. Fertil. Soils 52 (1), 119–125.

Qian, Y., Shi, J., Chen, Y., Lou, L., Cui, X., Cao, R., Li, P., Tang, J., 2010. Characterization of phosphate solubilizing bacteria in sediments from a shallow eutrophic lake and a wetland: isolation, molecular identification and phosphorusrelease ability determination. Molecules 15, 8518–8533.

Ranjard, L., Lejon, D.P.H., Mougel, C., Schehrer, L., Merdinoglu, D., Chaussod, R., 2003. Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environ. Microbiol. 11, 1111–1120.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol. 28, 897–906.

Rodriguez, H., Gonzalez, T., Selman, G., 2000. Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J. Biotechnol. 84, 155–161.

Rosado, A.S., Seldin, L., 1993. Production of a potentially novel anti-microbial substance by *Bacillus polymyxa*. World J. Microbiol. Biotechnol. 9, 521–528.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2, 587.

Singh, H.P., Singh, T.A., 1993. The interaction of rockphosphate, *Bradyrhizobium*, vesicular-arbuscular mycorrhizae and phosphate solubilizing microbes on soybean grown in a sub-Himalayan mollisol. Mycorrhiza 4, 37–43.

Son, H.J., Park, G.T., Cha, M.S., Heo, M.S., 2006. Solubilization of insoluble inorganic phosphate by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Bioresour. Technol. 97, 204–210.

Tamura, K., 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Mol. Biol. Evol. 9, 678–687.

Teymouri, M., Akhtari, J., Karkhane, M., Marzban, A., 2016. Assessment of phosphate solubilization activity of Rhizobacteria in mangrove forest. Biocatalysis and Agricultural Biotechnology 5, 168–172.

Timmusk, S., Nicander, B., Granhall, U., Tillberg, E., 1999. Cytokinin production by *Paenibacillus polymyxa*. Soil Biol. Biochem. 31, 1847–1852.

Vonder Weid, I.A., Paiva, E., Nóbrega, A., Van Elsas, J.D., Seldin, L., 2000. Diversity of *Paenibacillus polymyxa* strains isolated from the rhizosphere of maize planted in Cerrado soil. Res. Microbiol. 151, 369–381.

Vyas, P., Gulati, A., 2009. Organic acid production in vitro and plant growth promotion in maize undercontrolled environment by phosphate-solubilizing fluorescent Pseudomonas. BMC Microbiol. 9, 174.

Wang, L., Baek, S.H., Cui, Y., Lee, H.G., Lee, S.T., 2012a. *Paenibacillus sediminis sp. nov*., a xylanolytic bacterium isolated from a tidal flat. Int. J. Syst. Evol. Microbiol. 62, 1284–1288.

Wang, Y., Shi, Y., Li, B., Shan, C., Ibrahim, M., Jabeen, A., Xie, G., Sun, G., 2012b. Phosphate solubilization of *Paenibacillus polymyxa* and *Paenibacillus macerans* from mycorrhizal and non-mycorrhizal cucumber plants. Afr. J. Microbiol. Res. 6, 4567–4573.

Wang, X., Wang, Y., Tian, J., Lim, B.L., Yan, X., Liao, H., 2009. Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. Plant Physiol. 151, 233–240.

Weselowski, B., Nathoo, N., Eastman, A.W., MacDonald, J., Yuan, Z.C., 2016. Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. BMC Microbiol. 16, 244.

Xie, J., Shi, H., Du, Z., Wang, T., Liu, X., Chen, S., 2016. Comparative genomic and functional analysis reveal conservation of plant growth promoting traits in *Paenibacillus polymyxa* and its closely related species. Sci. Rep. 6, 21–329.

Yi, Y., Huang, W., Ge, Y., 2008. Exo-polysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J. Microbiol. Biotechnol. 24, 1059–1065.