

Rock-phosphate solubilising bacteria and their effect on soybean (*Glycine max*) growth under pot grown conditions

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Abstract: The modern agriculture is dependent on phosphorus (P) derived from phosphate rock. However, the direct application of low-grade rock phosphate as a P source in soils need an addition of inoculums of phosphate solubilising microorganisms to improve the rock phosphate efficiency as a phosphorus source. Phosphate solubilising bacteria (PSB) were screened for their phosphate solubilising ability on plates and in liquid cultures supplemented with Malian, Moroccan or Mexican rock phosphates. They were subsequently tested on soybean grown in pots filled with non sterile soil amended with Moroccan rock phosphate for their aptitude in promoting soybean growth. The activity of the different strains on plates indicates *Pantheoa* sp. and *Bacillus* sp. as the most efficient strains able to show halo zone on plates supplemented all different rock phosphates, with a solubilisation index (SI) of 3.65, 4.10 and 5.42 (*Pantheoa* sp.) and 2.93, 3.13 and 2.13 (*Bacillus* sp.) respectively for Malian, Moroccan and Mexican rock phosphates. *Pantheoa* sp. remains the strains showing the highest concentration of the solved P with all rock phosphates: 1038.25, 996.67 and 1207.87 µgP/g for Malian, Moroccan and Mexican rock phosphates respectively. It is followed by *Klebsiella* sp. (862.57, 615.19 and 426.29 µgP/g respectively) and *Bacillus* sp. (810.86, 270.92 and 180.95 µgP/g). In general, *Pantheoa* sp. and *Bacillus* sp. better contribute to the soybean growth with the effect of 35% and 34% respectively compare to non inoculated control supplied with non soluble Moroccan rock phosphate. The activity of *Klebsiella* sp. (13%) that is low in general seems to be stimulated when associated with the two other strains (33%). This suggests that the use of rock phosphate combined with the co-inoculation with those strains would ensure soybean production in economically profitable and environmentally friendly conditions.

Keywords: Phosphate Solubilising Bacteria, Rock Phosphate, Rock Phosphate Solubilisation, Soybean Growth

1. Introduction

After nitrogen, phosphorus (P) is an essential plant nutrient whose deficiency restricts crop yields severely [1]. It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration [2]. Tropical and sub tropical soils are predominantly acidic, and often extremely phosphorus deficient [3] with high phosphorus fixation capacities. Phosphorus is added to soil in the form of phosphatic fertilizers, part of which is utilized by plants and the

remainder converted into insoluble fixed forms. It is well documented that major fractions of soil phosphorus are usually present in the forms which are unavailable to plant [4]. This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and environmentally undesirable [5]. Therefore, the necessity to develop economical and eco-friendly technologies is steadily increasing [6, 7, 8]. Natural rock phosphates have been recognised as a valuable alternative for P fertilisers, the modern agriculture being dependent on phosphorus derived from phosphate rock [9]. However, the direct application of low-grade rock

phosphate as a P source in neutral and alkaline soils was of little importance [10, 11], but addition of an inoculum of phosphate solubilising microorganisms to soil has also been found to improve the rock phosphate efficiency as a phosphorus source [12]. Many soil microorganisms, including bacteria and fungi, are able to mobilize phosphorus from sparingly soluble rock phosphates, and they have an enormous potential in providing soil phosphates for plant growth [13, 14]. These organisms are ubiquitous but vary in density and mineral/rock phosphate solubilising ability from soil to soil or from one production system to another. They are generally isolated from rhizosphere and nonrhizosphere soils, rhizoplane, phyllosphere, and rock P deposit area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique [4]. Once a potential isolate is identified, it must be further tested for direct contribution to P plant nutrition and not necessarily to general growth promotion, as commonly done because promotion of growth, even by phosphate solubilising bacteria (PSB), can be the outcome of other mechanisms [15] and ability to solubilise P is not necessarily correlated with the ability to promote plant growth [16]. Therefore, this study is conducted to characterize three bacterial strains in solubilising rock phosphates as well as their impact in promoting soybean growth under pot grown conditions.

2. Materials and Methods

2.1. Microorganisms

All the strains used in this study are from the strain bank of the Laboratory of Biotechnology, Faculty of Science, University of Douala. Strains were isolated from soil collected in three agro ecological zones of Cameroon; *Klebsiella* sp. in zone I, *Pantthoea* sp. in zone II, and *Bacillus* sp. in zone IV. They have been previously screened for their ability in solubilising sparingly soluble phosphates including $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , FePO_4 and sodium-phytate, and would be recognized as inorganic/organic phosphate solubilisers.

2.2. Rock Phosphates

Rock phosphates of different origins and analyzed for their chemical contents (Table 1) were used: the Tilemsi rock phosphate from Mali, Gafsa rock phosphate from Mexico and Moroccan rock phosphate.

To get rid of their soluble fractions, the different rock phosphates were washed 4 times with warm water following the cycle: 1 hour - 24 hours - 1 hour - 24 hours. They were then dried at 60 °C until complete evaporation of water and homogenized before use.

Table 1. Mineralogical composition of the different rock phosphates used (Magallón-Servín, unpublished).

Origin rock P	Mineral elements (%)								mg/kg		
	Total P_2O_5	Available P	K	Ca	Mg	Na	Fe	Al	Mn	Zn	Cu
Mali	30	12.98	0.056	28.19	0.131	0.232	3.844	0.80	8360	87	51
Mexico	28	8.87	0.219	25.94	0.222	0.358	0.442	0.58	788	103	18
Morocco	13	9.33	0.093	28.83	1.93	0.552	0.267	0.42	96	219	38

2.3. Preparation and Evaluation of the Concentration of the Inoculums

To prepare inoculums from each bacterial strain, pure bacterial colony was individually suspended into 50 ml Nutrient Broth (NB) (5 g Peptone, 1 g Beef extract, 2 g Yeast extract, 5 g Sodium chloride, 1000 ml Distilled water, pH 7.0) and incubated at 28 °C, 150 rpm, for 3 days. Cultures were then spin at 10,000g, 10 minutes at 4 °C, followed by three times washing with 0.85% sterile NaCl at the same conditions. Bacterial cells were resuspended into 0.85% sterile NaCl and the OD adjusted to 0.2 at 620 nm. To assess the number of bacterial cells per milliliter, 1 mL of bacterial suspension, OD 0.2 was serially diluted until 10^{-7} . 200 μL of dilutions 10^{-7} , 10^{-6} , 10^{-5} were used to inoculate Nutrient Agar (NA) (5 g Peptone, 1 g Beef extract, 2 g Yeast extract, 5 g Sodium chloride, 15 g Agar, 1000 ml Distilled water, pH 7.0) plates in duplicate. After incubation at 28 °C, for 4 days, bacterial colonies were counted and the number of Colony Forming Unit (CFU) per mL recorded. Counting colonies allowed the determination of the concentration of the inoculum evaluated to $1-1.7 \times 10^9$ CFU / mL. Consortia of strains were freshly prepared by

mixing equal volumes of the different strain suspensions.

2.4. Bacterial Rock Phosphate Solubilising Capacity on Agar Plate

The characterisation of strains for their rock phosphate solubilising ability was assessed on plates filled with the National Botanical Research Institute's Phosphate growth medium (NBRIP) [17] with some modifications and containing per litre of distilled water: 20 g glucose, 5 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g KCl, 0.1 g $(\text{NH}_4)_2\text{SO}_4$ and one rock phosphate type at 5 g.L⁻¹ (Malian RP, Moroccan RP or Mexican RP), plus 0.5% bromocresol green [18], pH 7.5. A stock solution of 0.5% dye was prepared by dissolving a corresponding weight of bromocresol green into 70% ethanol and the final pH adjusted to 6.5 with 1M KOH. Five microliters of each bacterial suspension obtained as described above were transferred onto a single point of compartmented Petri dish. The plates were sealed and incubated at 28 °C for 5 days and the phosphate solubilisation recorded through the halo/yellow zone surrounding the bacterial colony. The index of solubilisation (IS) as defined by Qureshi et al. [19]

was used as an indicator for the isolate efficiency: $IS = (\text{Colony diameter} + \text{diameter of halo zone}) / \text{Colony diameter}$.

2.5. Quantitative Estimation of Phosphate Solubilisation by Bacteria in Liquid Media

Bacteria were tested in liquid media to assess their capability in releasing phosphorus from insoluble rock phosphate sources. 50 ml NBRIP medium were distributed into 250 mL Erlenmeyer flasks, individual rock phosphate types (Malian RP, Moroccan RP or Mexican RP) were added to the medium at the concentration of 5 g.L^{-1} and the pH adjusted to 7.5. After sterilization and cooling, 200 μL bacterial suspensions of $1\text{--}1.7 \times 10^9 \text{ CFU / mL}$ were used to inoculate flasks containing the different rock phosphates. Each treatment was performed in triplicate and non-inoculated flasks supplemented with the different rock phosphates supplied with 200 μL 0.85% sterile NaCl served as controls. Incubation was made at 28°C , 150 rpm for 7 days. At end of the incubation time and in all cases, the cultures were transferred into sterile falcon tubes, centrifuged at $10,000g$ for 10 minutes at 4°C , a part of the supernatant taken for pH measurement and another part for P determination following the method described by Murphy & Riley [20].

2.6. Pot Experiment

The pot experiment was conducted to assess the effect of a single strain or consortia of strains on soybean (*Glycine max*) growth. The experiment was conducted in 3 liters pots containing homogenized non-sterile soil with the following characteristics: pH H_2O , 5.5; pH KCl, 4.4; nitrogen, 0.06%; available phosphorus, 7.54 ppm; organic matter, 0.59%; organic carbon, 0.34%; iron, 1.75 ppm; aluminium, 0.29 $\text{m}\text{eq/g}$; calcium, 1.04 $\text{m}\text{eq/g}$; magnesium, 1.60 $\text{m}\text{eq/g}$; potassium, 0.15 $\text{m}\text{eq/g}$; sodium, 0.07 $\text{m}\text{eq/g}$. The experiment consisted of 12 treatments including 10 microbial treatments and two controls. The microbial treatments consisted of three single inoculations respectively labeled A (*Panthoea* sp.), B (*Klebsiella* sp.), C (*Bacillus* sp.) and two consortia AC (*Panthoea* sp. + *Bacillus* sp.) and ABC (*Panthoea* sp. + *Klebsiella* sp. + *Bacillus* sp.). All the pots, except the positive control were amended with Moroccan rock phosphate at the rate of 80 kg.ha^{-1} (0.3625 g for 0.8836 dm^3) to increase the amount of phosphorus in soil. All the pots were equally supplied with potassium at the rate of $80 \text{ kg K}_2\text{O.ha}^{-1}$. In all inoculated pots, one pre-germinated seed was soaked with 1 mL of bacterial suspension at sowing. The control treatments consisted of a positive control (C^+) supplemented with soluble KH_2PO_4 at the concentration of 350 mgP/g soil, and a negative control (C^-) supplemented with Moroccan rock phosphate, both without bacterial inoculation.

The experimental design is a completely randomized block system with 12 treatments, 1 host plant and 4 replications, resulting in a total of 48 experimental units.

Plant growth was followed during 6 weeks within which, each pot received 500 ml water three times per week. Growth parameters (number of leaves, plant high, stem base diameter) were taken the third and the sixth week. At the end of the growth, plants were harvested, the aerial part separated from the root part, and then dried at 60°C until the dry mass of materials became stable to determine shoot, root and total dry weight.

2.7. Statistical Analysis

Statistical analyses were performed with Sigma plot 12.0. The analysis of variance (ANOVA) was run to find difference between factors and the HSD Turkey test to compare the different treatments.

3. Results

3.1. Strains Characterization on Plates Supplemented with Rock Phosphates of Different Origins

3.1.1. Solubilisation Index of Strains

The efficacy of the different strains in solubilising sparingly soluble rock phosphates was first assessed on plates using solubilisation index (SI) an indicator for the strain efficiency, higher the SI, greater the solubilisation ability. *Panthoea* sp. and *Bacillus* sp. were able to solubilise the three rock phosphate types ($SI > 1$), while *Klebsiella* sp. could only solubilised the Malian rock phosphates (Figure 1). *Panthoea* sp. with the solubilisation index of 3.65, 4.10 and 5.42 respectively for Malian, Moroccan and Mexican rock phosphates is the strain showing the greatest solubilisation index regarding the different rock phosphates. Significant differences can be clearly observed within *Panthoea* sp. and *Bacillus* sp. (2.93, 3.13 and 2.13 respectively) and within *Bacillus* sp. and *Klebsiella* sp. (1.81, 1 and 1 respectively). Regarding the activity of the different strains on plates, *Panthoea* sp. can be considered as the most efficient strains, followed by *Bacillus* sp. and *Klebsiella* sp. (Figure 2).

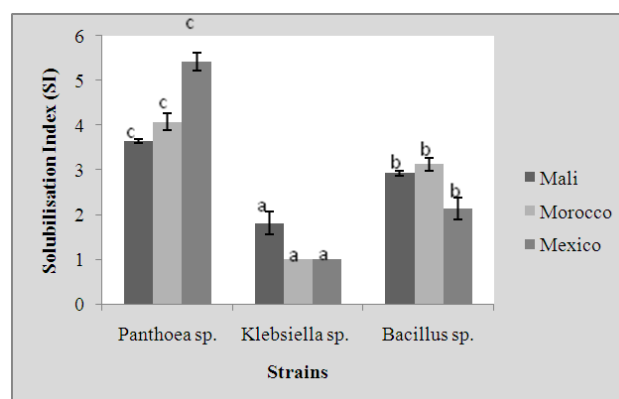


Figure 1. Solubilisation of different types of phosphate by each strain on plates. The different letters within the same phosphate type are significantly different ($p < 0.05$).

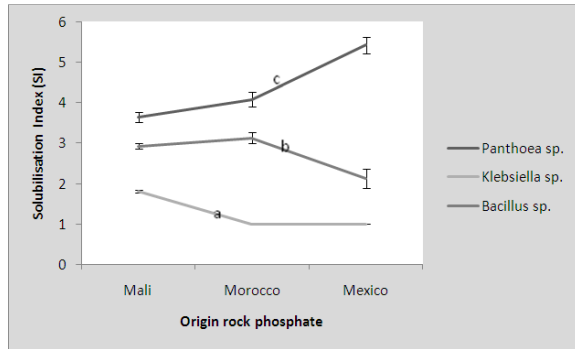


Figure 2. Relative efficiency of strains in solubilising rock phosphates of different origins on plates. The different letters indicate a significant difference ($p < 0.05$) between strains regardless the phosphate origin.

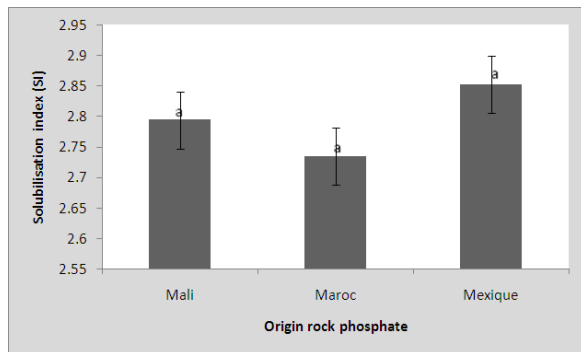


Figure 3. Fluency of the different rock phosphates to be solved by the strains on plates. The different letters indicate a significant difference between rock phosphates ($p < 0.05$).

3.1.2. Fluency of the Different Rock Phosphate to be Solved on Plates

There is no significant difference between the different rock phosphate solubilisation on plates (Figure 3). The different rock phosphates with an average solubilisation index of 2.794 (Mali), 2.734 (Morocco) and 2.852 (Mexico) show the same capacity to be solved.

3.2. Strains Characterization in Liquid Cultures Supplemented with Rock Phosphates of Different Origins

3.2.1. Concentration of the Solved-P in Liquid Cultures and pH of the Corresponding Media

In general, all the strains were able to mobilise phosphorus from insoluble sources, with significant difference within strains and control (Figure 4). Same like on plates, *Panthoea* sp. is the strain showing the highest efficiency in mobilising phosphorus from insoluble rock phosphates sources with an average amount of 1038.25, 996.67 and 1207.87 $\mu\text{gP/g}$ for Malian, Moroccan and Mexican rock phosphates respectively. Contrary to the result obtained on plates, *Klebsiella* sp. appears much more efficient in liquid culture with average solved P of 862.57, 615.19 and 426.29 $\mu\text{gP/g}$ respectively, compared to *Bacillus* sp. with average solved P of 810.86, 270.92 and 180.95 $\mu\text{gP/g}$ respectively. Furthermore, *Klebsiella* sp. that was not able to show any visible activity on plates amended

with Moroccan and Mexican rock phosphates could conversely mobilised significant amount in broth containing the same rock phosphate types. In general, the rock phosphate solubilisation is accompanied with drop of pH of the media, but no strong correlation could be established between the amount of the solved P and the pH of the corresponding media.

3.2.2. Efficiency of Strains According to the Concentration of the Solved P

There is a significant difference ($p < 0.05$) between ability of strains in solubilising the different rock phosphates (Figure 5). *Panthoea* sp. presents the highest efficiency in solubilising all rock phosphates in liquid cultures followed by *Klebsiella* sp. and finally by *Bacillus* sp. Great differences can be easily observed within *Panthoea* sp. and *Klebsiella* sp. and within *Klebsiella* sp. and *Bacillus* sp.

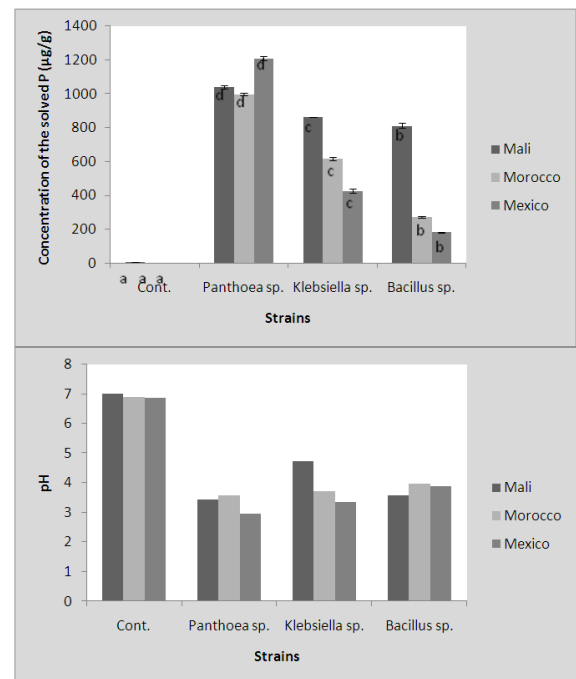


Figure 4. Concentration of the solved P in liquid cultures amended with rock phosphates of different origins and the pH of the corresponding media. The different letters within the same phosphate type are significantly different ($p < 0.05$).

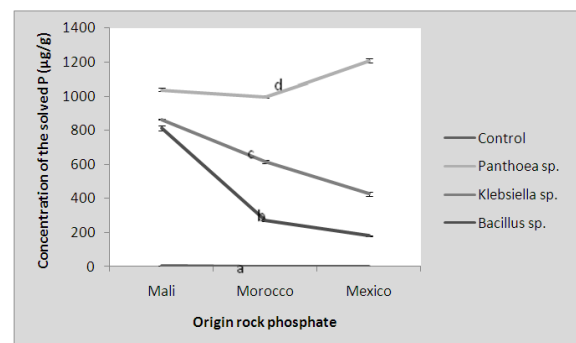


Figure 5. Aptitude of strains in solubilising rock phosphates of different origins in liquid media. The different letters indicate a significant difference between strains regardless the phosphate type ($p < 0.05$).

3.2.3. Fluency of the Different Rock Phosphate to be Solved in Liquid Media

Contrary to the result obtained on plates with no significant difference on the ability of the different rock phosphates to be solved, there is a significant difference between the different rock phosphates in liquid cultures (Figure 6). The Malian rock phosphate (679.59 $\mu\text{gP/g}$) is the easiest phosphates to be solved by the different strains, followed by Moroccan (470.69 $\mu\text{gP/g}$) and Mexican rock phosphate (453.79 $\mu\text{gP/g}$).

3.3. Effect of Inoculation by Strains on the Soybean Growth

3.3.1. Growth Parameters Three Weeks After Planting

The number of leaves varies between 5 and 6 while the stem base diameter varies between 2.48 and 3.40 cm with no significant difference between treatments in both cases at three weeks after planting (Table 2). However, there is a significant difference ($p < 0.05$) between treatments regarding the plant high. While the negative control (C-)

shows the lowest score (12.40 cm), the best one is obtained with *Bacillus* sp. (15.68 cm) followed by *Panthoea* sp. (15.00 cm) and the consortium of the three strains ABC (14.58 cm). This shows the real aptitude of strains, either in single or in consortia to promote the plant growth regarding the plant high parameter.

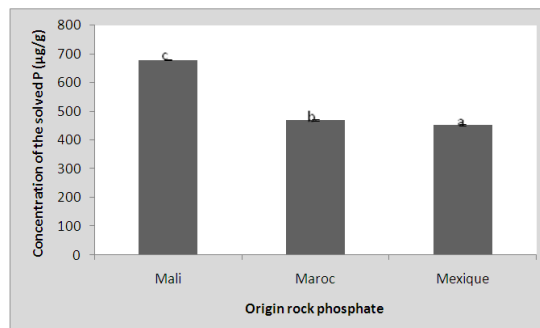


Figure 6. Facility of the different rock phosphates to be solved by the strains in liquid media. The different letters indicate a significant difference between rock phosphates ($p < 0.05$).

Table 2. Number of leaves, plant high and stem base diameter of soybean plants the third week after planting.

Treatment	(a) Number of leaves	Standard deviation	(b) Plant high (cm)	Standard deviation	(c) Stem base diameter (cm)	Standard deviation
C-	5 a	0.50	12.40 a	0.43	2.70 a	0.48
C+	6 a	0.58	13.78 bc	0.33	3.40 a	0.43
A	5 a	0.00	15.00 cd	0.71	2.78 a	0.39
B	5 a	0.00	13.08 ab	0.82	2.48 a	0.36
C	5 a	0.50	15.68 d	0.40	3.27 a	0.21
AC	5 a	0.50	14.08 bc	0.15	2.75 a	0.35
ABC	5 a	0.50	14.58 cd	0.66	2.63 a	0.21
(a) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	1.857	0.310	1.625	0.190	
Residual	21	4.000	0.190			
Total	27	5.857				
(b) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	30.244	5.041	16.970	<0.001	
Residual	21	6.238	0.297			
Total	27	36.481				
(c) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	2.810	0.468	3.645	0.012	
Residual	21	2.698	0.128			
Total	27	5.508				

The different letters within the same column indicate a significant difference ($p < 0.05$) between treatments. A (*Panthoea* sp.), B (*Klebsiella* sp.), C (*Bacillus* sp.), AC (*Panthoea* sp. + *Bacillus* sp.), ABC (*Panthoea* sp. + *Klebsiella* sp. + *Bacillus* sp.).

3.3.2. Growth Parameters Six Weeks After Planting

Six weeks after planting, no real differences can be observed between treatments regarding the number of leaves. However, significant differences are noted between

treatments as far as concern the plant high and the stem base diameter. Same like three weeks after planting, the best record is obtained with *Bacillus* sp. in both plant high (40.50 cm) and stem base diameter (4.93 cm) among the inoculation treatments. In general, the stem base diameter varies with treatments, the highest performance obtained by the positive control (5.20 cm), followed by *Bacillus* sp., *Panthoea* sp. (4.37 cm) and the consortium (AC) of both *Bacillus* sp. and *Panthoea* sp. (4.58 cm). This shows the real aptitude of strains, either in single or in consortia to promote the plant

growth regarding the different parameters.

Table 3. Number of leaves, plant high and stem base diameter of soybean plants six weeks after planting.

Treatment	(a) Number of leaves	Standard deviation	(b) Plant high (cm)	Standard deviation	(c) Stem base diameter (cm)	Standard deviation
C-	9 ab	0.50	32.33 a	3.09	3.69 ab	0.21
C+	10 ab	0.50	39.75 bc	2.75	5.20 e	0.28
A	10 ab	0.58	35.33 a	0.94	4.37 cd	0.45
B	9 ab	0.50	34.93 a	0.90	3.30 a	0.22
C	10 ab	1.00	40.50 c	0.35	4.93 d	0.25
AC	9 ab	0.50	36.21 ab	0.85	4.58 cd	0.19
ABC	8 a	0.50	35.61 ab	1.76	4.22 bc	0.14
(a) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	7.214	1.202	3.258	0.020	
Residual	21	7.750	0.369			
Total	27	14.964				
(b) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	194.355	32.392	9.960	<0.001	
Residual	21	68.299	3.252			
Total	27	262.653				
(c) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	10.658	1.776	25.403	<0.001	
Residual	21	1.469	0.0699			
Total	27	12.127				

The different letters within the same column indicate a significant difference ($p < 0.05$) between treatments. A (*Panthoea* sp.), B (*Klebsiella* sp.), C (*Bacillus* sp.), AC (*Panthoea* sp. + *Bacillus* sp.), ABC (*Panthoea* sp. + *Klebsiella* sp. + *Bacillus* sp.)

3.3.3. Root, Shoot and Total Plant Dry Weight Six Weeks After Planting

Significant differences ($p < 0.05$) are observed within strains regarding the root, shoot and total plant dry matter. Concerning the root dry mass, only one inoculated treatment *Panthoea* sp. (2.89 g) is significantly different from the negative control C- (1.95 g), but the best score is performed by the positive control C+ (3.92 g) supplied with soluble phosphate (Table 4). Regarding the root dry matter,

all inoculated treatments are significantly different from the negative control. The best record is obtained by the consortium of the three strains ABC (2.75 g) followed by *Bacillus* sp. (2.30 g), *Panthoea* sp. (2.24 g) and then *Klebsiella* sp. (1.79 g). As far as concern the total plant dry matter, the best record is still obtained with the positive control C+ (6.21 g). Among the inoculated treatments, *Panthoea* sp. (5.14 g) and the consortium of the three strains ABC (4.97 g) show the best performance, followed by *Bacillus* sp. (4.60 g) and *Klebsiella* sp. (4.00 g). This shows the real aptitude of strains, either in single or in consortia to promote the plant growth regarding the shoot, root and total plant dry masses.

Table 4. Shoot dry weight, root dry weight and total dry weight of soybean plants six weeks after planting.

Treatment	(a) Shoot dry weight (g)	Standard deviation	(b) Root dry weight (g)	Standard deviation	(c) Total plant dry weight (g)	Standard deviation
C-	1.95 a	0.04	1.32 a	0.24	3.27 a	0.27
C+	3.92 c	0.17	2.29 cd	0.11	6.21 e	0.19
A	2.89 b	0.11	2.24 cd	0.09	5.14 d	0.04
B	2.21 a	0.16	1.79 b	0.12	4.00 bc	0.22
C	2.30 a	0.18	2.30 d	0.11	4.60 cd	0.16
AC	2.25 a	0.28	1.43 ab	0.25	3.69 ab	0.52
ABC	2.23 a	0.19	2.75 e	0.21	4.97 d	0.30
(a) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	10.884	1.814	60.890	<0.001	
Residual	21	0.626	0.0298			
Total	27	11.509				
(b) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	6.471	1.079	36.136	<0.001	
Residual	21	0.627	0.0298			
Total	27	7.098				
(c) Source of Variation	DF	SS	MS	F	P	
Between Groups	6	23.857	3.976	51.568	<0.001	
Residual	21	1.619	0.0771			
Total	27	25.476				

The different letters within the same column indicate a significant difference ($p < 0.05$) between treatments. A (*Panthoea* sp.), B (*Klebsiella* sp.), C (*Bacillus* sp.), AC (*Panthoea* sp. + *Bacillus* sp.), ABC (*Panthoea* sp. + *Klebsiella* sp. + *Bacillus* sp.)

3.3.4. Effect (%) of the Different Treatments on Growth, Root, Shoot and Total Dry Weight of Soybean Six Weeks After Planting

Based on the effect of inoculation on the different measured plant parameters compared to the negative

treatment without inoculation, the different treatments can be classified as follow:

$C+ > A = C = ABC > B = AC$. In general, *Panthoea* sp. (35%) and *Bacillus* sp. (34%) better contribute to the soybean growth compare to non inoculated control supplied with non soluble Moroccan rock phosphate. The activity of *Klebsiella* sp. (13%) that is low in general seems to be stimulated when associated with the two other strains (33%).

Table 5. Effect (%) of the treatments on growth, root, shoot and total dry weight of soybean plants six weeks after planting under pot grown conditions.

Treatment	Number of leaves	Plant height	Stem base diameter	Root dry weight	Shoot dry weight	Total dry weight	Average
C+	11	23	41	101	73	90	57
A	9	9	18	48	70	57	35
B	0	8	0	13	35	22	13
C	9	25	34	18	74	41	34
AC	0	12	24	15	9	13	12
ABC	0	10	14	14	108	52	33

The effect (%) is calculated according to the formula: $Effect = \frac{(T-C-)}{C-} \times 100$. Where, T is a given treatment and C- the negative control. A (*Panthoea* sp.), B (*Klebsiella* sp.), C (*Bacillus* sp.), AC (*Panthoea* sp. + *Bacillus* sp.), ABC (*Panthoea* sp. + *Klebsiella* sp. + *Bacillus* sp.)

4. Discussion

Several strains of bacterial and fungal species have been described and investigated in detail for their rock phosphate solubilising capabilities [21, 22]. Bacterial strains exhibiting P solubilising activity were detected on plates by the formation of halo/yellow zone around their colonies. This reaction is used to assess the P solubilisation activity of strains with the SI value as an indicator for the strain efficiency [23]. Although *Klebsiella* sp. did not showed any visible halo zone on plates amended with Moroccan and Mexican rock phosphates, it could conversely mobilized important amounts of phosphorus from these rock phosphate sources and could be considered as rock phosphate solubilisers. These contradictory results between plate halo detection and phosphate solubilisation in liquid cultures found earlier by Deubel and Merbach [24] and Fankem *et al.* [25] indicates that liquid culture should be associated to halo zone for rock phosphate solubilisers characterization. However, the plate method is still a feasible way to pre-screen the isolates that possess phosphate solubilising ability. While clear correlation was found between solubilisation ability on plate and in liquid culture with *Panthoea* sp., no correlation was observed with *Klebsiella* sp. and *Bacillus* sp. between solubilisation ability on plate and in liquid culture as mentioned in the previous investigations by Azziz *et al.* [26] and Fernández *et al.* [27]. Although all the strains showed good

solubilisation in liquid cultures amended with the different rock phosphates, the amount of the solved P varied with the strain as well as the rock phosphate origin. Solubilisation of phosphate is commonly accompanied by a remarkable drop in pH [22, 28, 29], though no strong correlation was observed within the drop of pH and the amount of the solved P. Regarding the efficiency of strains on plates and in liquid cultures, *Panthoea* sp. shows the highest performance, followed by *Bacillus* sp. This performance was maintained on soybean growth regarding the different parameters measured, with a significant increase of 35% compared to the negative control. Although the activity of *Klebsiella* sp. (13%) was not strong enough in this study, that strain altogether with *Pseudomonas*, *Enterobacter* and *Microbacterium* have been reported in several papers [30] as plant promoting strains. This value is therefore in agreement with Tandon [31] who reported that inoculation with phosphate solubilising microorganisms generally induces a growth increase of approximately 10-15%. However, values greater than that range have been reported by Abou-el-Seoud and Abdel-Megeed [32] and Fankem *et al.* [33] who respectively found an increase of 26% and 36%, with corn, and in this case 35% with soybean. Increasing the bioavailability of P in the soils with combined inoculation and rock materials has been reported by many researchers [34, 35, 36], which may lead to increased P uptake and plant growth [37, 38, 39]. In general, while *Bacillus* and *Pseudomonas* [40], *Aspergillus* and *Penicillium* [41] are considered as important genera of mineral phosphate solubilising bacteria and fungi respectively, only few reports indicated both *Panthoea* sp. and *Klebsiella* sp. as mineral phosphate solubilising bacteria and rare reports indicating their rock phosphate solubilising ability. The present study highlights the

aptitude of *Panthoea* sp., *Klebsiella* sp. and *Bacillus* sp. in single or in consortia in solubilising rock phosphates of different origins as well their aptitude in promoting the soybean growth. This came to confirm the aptitude of those strains in solubilising sparingly soluble inorganic/organic phosphates including $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , FePO_4 and sodium-phytate, and would be recognized as inorganic/organic/rock phosphate solubilisers. Growth enhancement by bacteria may relate to their ability to produce extensive root length [42] and can also improve root development and increase the rate of water and mineral uptake [43, 44]. This increase in growth may be attributed to auxin production [45], ACC-deaminase activity [46, 47] and production of organic acids [33] or phosphatases [48] to solubilise/mineralize P, thereby increasing phosphate nutrition of inoculated plants. Therefore, the positive effect on growth with non-soluble P may result from the synergic combination of both bacterial capacities for IAA production and P mobilisation. The use of phosphate-solubilising bacteria as inoculants simultaneously increase phosphorus uptake by the plant and crop yield [49]. Moreover, according to Amellal et al. [50] *Panthoea agglomerans* NAS206 can play an important role in the regulation of the water content (excess or deficit) of the rhizosphere of wheat by improving soil aggregation. This may hence justified its efficiency on the performed tests.

5. Conclusion

Natural rock phosphates have been recognised as a valuable alternative for P fertilisers, the modern agriculture being dependent on phosphorus derived from phosphate rock. However, the direct application of low-grade rock phosphate as a P source in soils is of little importance and need an addition of inoculums of phosphate solubilising microorganisms to improve the rock phosphate efficiency as a phosphorus source. The organisms possessing phosphate solubilising ability can convert the insoluble phosphatic compounds into soluble forms into soil and make it available to the crops. While *Bacillus* and *Pseudomonas* are generally considered as important genera of mineral phosphate solubilising bacteria, only few reports indicated both *Panthoea* sp. and *Klebsiella* sp. as mineral phosphate solubilising bacteria, and rare reports their rock phosphate solubilising ability. The present study highlights the aptitude of *Panthoea* sp., *Klebsiella* sp. and *Bacillus* sp. in solubilising rock phosphates of different origins as well their aptitude in promoting the soybean growth. These bacterial strains can be therefore recognized as inorganic/organic/rock phosphate solubilisers.

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