Maize (Zea mays) growth promotion by rock-phosphate solubilising bacteria isolated from nutrient deficient soils of Cameroon

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Rock phosphate is an alternative strategy for less expensive natural sources of plant nutrients. However, this can be efficiently used by plants only when associated with phosphate solubilising microorganisms. Phosphate solubilising bacteria (PSB) from soils of two agro ecological zones of Cameroon 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 maize grown in pots filled with unsterile soil amended with Malian rock phosphate for their aptitude in promoting maize growth. Under in vitro condition, Enterobacter sp. showed halo zone on plates supplemented with the different rock phosphates with an index of solubilization (IS) varying between 2.10 and 2.71. This strain also showed the highest concentration of mobilized P with all rock phosphates: 1075.17, 1161.04, 862.57 µg P/g for Malian, Moroccan and Mexican rock phosphates, respectively, followed by Klebsiella sp. with the concentrations of 862.57, 615.19, 426.29 µg P/g, respectively. The Malian (402.5 µg P/g) and Moroccan (403.7 µg P/g) rock phosphates appeared to be the easiest phosphates to be solubilised by the different strains. However, the Mexican rock phosphate (345.3 µg P/g) was less solubilised in broth. In general, all the strains in single and in consortia significantly increased the number of leaves, stem base diameter, total dry mass, shoot dry mass and root dry mass as compared to non-inoculated control. The effect of inoculation with single strain varied between 27.5 and 59.3% growth increase, while the effect of inoculation with consortia varied between 54.1 and 109.3% as compared to non-inoculated control. The findings of the current study suggest the potential use of rock phosphate and PSB that would enhance maize productivity in economically profitable and environmentally friendly ways.

Key words: Maize growth, phosphate solubilising bacteria, plant nutrient, rock phosphate.

INTRODUCTION

Phosphorus (P) is the second nutrient element after nitrogen mostly required by plant. It is the main component of nucleic acids, phospholipids and adenosine triphosphate (ATP). To overcome the specific P nutrient deficiency, various forms of P, varying from processed rock phosphates (P-fertilizers) to ground in the form of
phosphatic fertilizers, part of which is utilized by plants and the remainder converted into insoluble fixed forms. The effectiveness of using phosphate fertilizer is very low; only about 10 - 25% (Isherword, 1998; Hosseini et al., 2010). Among the alternative P sources, the most important are locally available rock phosphate (RP) resources (Rajan et al., 1996). While the use of commercial P-fertilizers is not cost effective, rock phosphate as a source of P is not expensive but the availability of P is low (Jayadi et al., 2013). Not all the RP resources are readily plant available and agronomically reactive when applied directly to the soils. Reactivity is defined as the combination of RP properties that determine the rate of dissolution of RP in a given soil under field conditions. Solubility of RP can be increased by phosphate solubilising microorganisms (PSMs). Increasing RP solubility by microorganisms is due to the lowering of pH and or organic acid excretion (Vessey et al., 2004; Fankem et al., 2008). Microbial solubilisation of rock phosphate, especially low grade and its use in agriculture is receiving great attention. This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (Narsian and Patel, 2000). 

PSMs include different groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but they also cause a large portion of soluble fractions to be released in quantities in excess of their requirements. Among the bacterial genera identified to have phosphate solubilising capabilities are Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium and Erwinia (Rodriguez et al., 2006). Seed or soil inoculation with PSMs is known to improve solubilisation of fixed soil phosphates in order to improve crop yields (Jones and Darrah, 1994). PSMs are low cost solutions that enrich the soil giving a thrust to economic development without disturbing ecological balance. The present study aims at characterizing bacterial strains in solubilising rock phosphate of different origins on plate and liquid culture media as well as assessing their impact on maize growth under pots grown conditions.

### MATERIALS AND METHODS

#### Microorganisms

All the strains used in this study are from the culture collection of the Laboratory of Biotechnology, Faculty of Science, University of Douala. Strains were isolated from soils of two agro ecological zones in Cameroon; Pseudomonas sp. and Klebsiella sp. in zone I (The Sudano-Sahelian lowland region with savanna scrub and grass, an arid region with sparse rainfall and high median temperatures), Burkholderia sp., Enterobacter sp. in zone IV (The Monomodal Humid Forest, exceedingly hot and humid with a short dry season, densely forested and includes some of the wettest places on earth). They have been previously screened for their ability in solubilising sparingly soluble phosphates including calcium-phosphate (Ca$_3$(PO$_4$)$_2$), aluminium-phosphate (AlPO$_4$), iron-phosphate (FePO$_4$) and sodium-phytate, and could be recognized as inorganic/organic phosphate solubilisers.

#### Rock phosphates

Rock phosphates of different origins were used: the Tilemsi rock phosphate from Mali, rock phosphate from Gafsa in Mexico and rock phosphate from Morocco. They were analyzed for their chemical contents (Table 1). To get rid of their soluble fractions, the different rock phosphates were washed four times with warm water following the cycle: 1 - 24 h - 1 - 24 h. They were then dried in an oven at 60°C until complete evaporation of water and homogenized before use.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Total P$_2$O$_5$</th>
<th>Available P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>Fe</th>
<th>Al</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mali</td>
<td>30</td>
<td>12.98</td>
<td>0.056</td>
<td>28.19</td>
<td>0.131</td>
<td>0.232</td>
<td>3.844</td>
<td>0.80</td>
<td>8360</td>
<td>87</td>
<td>51</td>
</tr>
<tr>
<td>Mexico</td>
<td>28</td>
<td>8.87</td>
<td>0.219</td>
<td>25.94</td>
<td>0.222</td>
<td>0.358</td>
<td>0.442</td>
<td>0.58</td>
<td>788</td>
<td>103</td>
<td>18</td>
</tr>
<tr>
<td>Morocco</td>
<td>13</td>
<td>9.33</td>
<td>0.093</td>
<td>28.83</td>
<td>1.93</td>
<td>0.552</td>
<td>0.267</td>
<td>0.42</td>
<td>96</td>
<td>219</td>
<td>38</td>
</tr>
</tbody>
</table>

#### Preparation and evaluation of the concentration of the inoculum for P solubilisation and greenhouse experiments

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 three days. Cultures were then centrifugated at 10,000 g for 10 min at 4°C, followed by three washing with 0.85% sterile NaCl at the same conditions. Bacterial cells were re-suspended in 0.85% sterile NaCl and the optical density (OD) of the suspension adjusted to 0.2 at 620 nm wavelength. To assess the number of bacterial cells per milliliter, one ml of the bacterial suspension with OD 0.2 was serial diluted until 10$^{-7}$. A 200 µl of dilutions 10$^{-7}$ was 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 evaluated. Counting colonies allowed the determination of

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the concentration of the inoculum of 1.28 - 2.08×10^8 CFU/ml. Consortia were prepared by mixing equal volumes of the different strain suspensions.

**Bacterial rock phosphate solubilising capacity on agar plate**

The characterization of strains for their rock phosphate solubilising ability was assessed on plates filled with the National Botanical Research Institute’s Phosphate growth medium (NBRIP) (Nautiyal, 1999) with some modifications that contains (g/l): 20 g glucose, 5 g MgCl₂6H₂O, 0.25 g MgSO₄7H₂O, 0.2 g KCl, 0.1 g (NH₄)₂SO₄ and each rock phosphate type at a rate of 5 g l⁻¹ (Malian RP, Moroccan RP or Mexican RP) plus 0.5% BCG dye (Gadagi and Sa, 2002) for better observation, pH 7.5. Five microliters of each bacterial suspension OD 0.2 obtained as described above were transferred onto a single point of compartmented Petri dish. The plates were sealed with parafilm and incubated at 28°C for 5 days. The halo/yellow zone surrounding the bacterial colony indicates extension of phosphate solubilisation. The index of solubilisation (IS) as defined by Qureshi et al. (2012) was used as an indicator for the strain efficiency: IS = (Colony diameter + diameter of halo zone) / colony diameter.

**Quantitative estimation of phosphate solubilisation by bacteria in liquid media**

Bacteria were tested in liquid media to assess their capability in releasing phosphates from insoluble rock phosphate sources. A 50 ml NBRIP medium was 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 bacteria suspensions of 1.28 - 2.08×10^8 CFU/ml were used to inoculate flasks containing the different rock phosphates. Each treatment was replicated three times and non-inoculated flasks supplemented with different rock phosphates supplied with 200 µl of 0.85% sterile NaCl served as control. Incubation was made at 28°C, 150 rpm for 7 days. At end of the incubation time (7th day) tubes, centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was taken for P determination following the method described by Murphy and Riley (1962).

**Pot experiment**

The pot experiment was conducted to assess the effect of a single strain or consortia of strains on maize (Zea mays) growth. The maize seeds consisted of the variety CMS8704 with a life cycle of 115 days and cultivated in the Nord, Centre, Littoral, South-East and Nord-West regions of Cameroon. The experiment was conducted in 3 L pots containing homogenized non-stereile soil with the following characteristics: pH H₂O: 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 měq/g; calcium, 1.04 měq/g; magnesium, 1.60 měq/g; potassium, 0.15 měq/g; sodium, 0.07 měq/g. The soil used was therefore very low in available phosphorus (7.54 ppm).

The experiment consisted of 12 treatments including 10 microbial treatments and two controls. The microbial treatments consisted of four single inoculations labeled A (Klebsiella sp.), B (Pseudomonas sp.), C (Burkholderia sp.) and D (Enterobacter sp.), and six consortia (AB, CD, ABC, ACD, BCD and ABCD). Consortia were obtained by mixing equivalent volumes of the bacterial suspension OD 0.2 of each strain. All the pots, except the positive control were amended with Malian rock phosphate at the rate of 80 kg ha⁻¹ (0.3625 g for 0.8836 dm³) to increase the amount of phosphate in soil. In all inoculated pots, one pre-germinated seed was placed in a pit, soaked with 1 ml of bacterial suspension and finely covered with soil. The control treatments consisted of a positive control (Cont+) supplemented with soluble K₂HPO₄ at the concentration of 350 mg P/g soil, and a negative control (Cont-) supplemented with Malian rock phosphate, both without bacterial inoculation.

The experimental design was 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/week and 200 ml of a solution of 200 mg l⁻¹ of N (1.4285 g l⁻¹ of 14:0:14 NK fertilizer) was added to each pot once a week. Growth parameters (number of leaves, plant height, stem base diameter) were taken every two weeks. At the end of the growth (six weeks after planting), the 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 mass.

**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 means.

**RESULTS**

**Strains characterization on plates supplemented with rock phosphates of different origins**

In general, all the strains were able to solubilise at least one rock phosphate type but the index of solubilisation were different within strains and the different rock phosphates were not equally solubilised. *Enterobacter* sp. was the only strain able to solubilise the three rock phosphate types (IS>1), with the IS of 2.45, 2.10 and 2.71 for Malian, Moroccan and Mexican rock phosphates, respectively. The Malian rock phosphate was solubilised by all the strains (Table 2). However, Moroccan and Mexican rock phosphates were recalcitrant to *Pseudomonas* sp., *Burkholderia* sp. and *Klebsiella* sp. (IS=1). In Malian rock phosphate plate, *Enterobacter* sp. showed the highest value (IS=2.45) followed by *Klebsiella* sp. (IS=1.81), *Burkholderia* sp. (IS=1.65) and *Pseudomonas* sp. (IS=1.33). Additionally, *Enterobacter* sp. often showed the solubilising activity on the first day of incubation regardless of the rock phosphate type, while the rest strains showed the activity only on the second day. There is a significant difference between the different rock phosphate solubilisation on plates. The Malian rock phosphate is the easiest phosphate (IS=1.8) to be solubilised by the different strains, followed by Mexican rock phosphate (IS=1.43) and the Moroccan rock phosphate (IS=1.28) being the most recalcitrant phosphate on plates.

**Quantification of mobilized phosphate in liquid medium**

In general, all the strains were able to mobilise phosphate
Table 2. Rock phosphate solubilisation on plates and concentration of the solved P in liquid cultures amended with rock phosphates of different origins.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Malian RP</th>
<th>Moroccan RP</th>
<th>Mexican RP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Index of Solubilisation (IS)</td>
<td>Concentration of the solved P (µg/g)</td>
<td>Index of Solubilisation</td>
</tr>
<tr>
<td>Control</td>
<td>6.67±0.0a</td>
<td>0±0.0a</td>
<td>0±0.0a</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>1.33±0.00a</td>
<td>17.24±1.2a</td>
<td>156.24±14.7c</td>
</tr>
<tr>
<td>Burkholderia sp.</td>
<td>1.65±0.11b</td>
<td>50.85±0.8b</td>
<td>86.03±5.7b</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>2.45±0.05d</td>
<td>1075.17±9.4d</td>
<td>1161.04±10.2e</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>1.81±0.04c</td>
<td>862.57±1.7c</td>
<td>615.19±9.1d</td>
</tr>
</tbody>
</table>

The different letters within the same column are significantly different (p < 0.05).

from insoluble sources, although no significant (p<0.05) difference was observed within Pseudomonas sp. (17.24 µg P/g) and control (6.67 µg P/g) regarding the Malian rock phosphate (Table 2). Enterobacter sp. showed the highest concentration of P in all rock phosphate types: 1075.17, 1161.04, 862.57 µg P/g for Malian, Moroccan and Mexican rock phosphates, respectively followed by Klebsiella sp. with the concentrations of 862.57, 615.19, 426.29 µg P/g for Malian, Moroccan and Mexican rock phosphates, respectively (Table 2). Pseudomonas sp. and Burkholderia sp. obtained the lowest concentration of P from the sparingly soluble rock phosphate sources. However, the strains that were not able to show any visible activity on plates amended with Moroccan and Mexican rock phosphates mobilised important amount of phosphate in liquid cultures containing the same rock phosphate types. For instance, Pseudomonas sp. solubilized 156.24 and 95.4 µg P/g; Burkholderia sp. 86.03 and 112.32 µg P/g and Klebsiella sp. 615.19 and 426.29 µg P/g for Moroccan and Mexican rock phosphates, respectively (Table 2).

There was a significant (p<0.05) difference between ability of strains in solubilising the different rock phosphate types. Enterobacter sp. showed the highest efficiency to solubilise the three types of phosphate in liquid cultures followed by Klebsiella sp., Burkholderia sp. and Pseudomonas sp. (Figure 1).

Regarding the facility of the different rock phosphates to be mobilised, there was a significant (p<0.05) difference between different rock phosphates solubilisation in liquid cultures (Figure 2). The Malian (402.5 µg P/g) and Moroccan (403.7 µg P/g) rock phosphates were the easiest phosphates to be mobilised by the different strains with no significant difference
between them, while the Mexican rock phosphate (345.3 µg P/g) was the most recalcitrant phosphate in liquid cultures.

**Effect of inoculation by strains on the maize growth**

**Number of leaves**

In general, the number of leaves varied within the treatments for each week (Table 3). Two weeks after planting, the number of leaves varied between 3 and 5/plant (Table 3), while the highest record (5 leaves/plant) was obtained from the positive control supplied with soluble phosphate and most of consortia (AB, ABC, ACD and BCD). Most of the treatments with single strain had 4 leaves/plant like the negative control, except strain A with 3 leaves/plant (Table 3). The highest number of leaves (7) was still obtained from the Cont + treatment (7) at the fourth week but the smallest observed in Cont. (5 leaves/
Table 4. Stem base diameter at second, fourth and sixth week after planting and root shoot and total plant dry mass at sixth week.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2nd Week Stem base diameter (cm)</th>
<th>4th Week</th>
<th>6th Week</th>
<th>Shoot (g)</th>
<th>Root (g)</th>
<th>Total plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont-</td>
<td>0.95±0.1ab 1.88±0.3a 5.08±0.4a 2.33±0.1a 2.41±0.3a 4.74±0.3a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont+</td>
<td>1.55±0.5b 5.88±0.3d 10.58±0.5f 7.10±0.3f 5.88±0.2d 12.98±0.4g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.98±0.1ab 3.25±0.5abc 7.00±0.0bcd 3.40±0.3b 3.50±0.1b 6.90±0.3b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.43±0.4a 2.75±0.5ab 6.58±0.7b 3.34±0.2b 3.54±0.3b 6.87±0.3b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.78±0.5ab 4.03±0.2bc 8.08±1.2cd 4.25±0.2c 4.04±0.3bc 8.28±0.3c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.00±0.0ab 3.25±0.5abc 6.88±0.5bc 3.14±0.4b 3.76±0.1b 6.90±0.5b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1.13±0.3ab 4.50±0.7cd 7.46±0.3bcde 5.22±0.3d 5.55±0.3d 10.77±0.6de</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CD</td>
<td>1.48±0.4b 4.38±0.8bcd 7.63±0.5bcde 5.22±0.3d 4.45±0.3c 9.66±0.6d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABC</td>
<td>1.40±0.7ab 4.20±1.1bc 8.71±0.7e 4.14±0.3c 3.95±0.3bc 8.08±0.6c</td>
<td></td>
<td></td>
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<tr>
<td>ACD</td>
<td>1.18±0.6ab 3.73±1.0bc 6.87±0.5bc 5.45±0.1de 5.66±0.4d 11.11±0.4e</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BCD</td>
<td>1.25±0.5a 3.75±1.0bc 7.70±0.7bcd 6.10±0.3e 5.52±0.4d 11.63±0.7ef</td>
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<td></td>
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</tr>
<tr>
<td>ABCD</td>
<td>1.13±0.3ab 3.25±0.5abc 8.43±0.3de 5.72±0.3d 6.62±0.1e 12.34±0.2fg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cont- = negative control with rock phosphate without inoculation; Cont+ = positive control with soluble phosphate (KH2PO4); A (Klebsiella sp.); B (Pseudomonas sp.); C (Burkholderia sp.); D (Enterobacter sp.); AB (Klebsiella sp. + Pseudomonas sp.); CD (Burkholderia sp. + Enterobacter sp.); ABC (Klebsiella sp. + Burkholderia sp.+ Enterobacter sp.); ACD (Pseudomonas sp. + Burkholderia sp.+ Enterobacter sp.); BCD (Pseudomonas sp. + Burkholderia sp.+ Enterobacter sp.); ABCD (Klebsiella sp. + Burkholderia sp. + Pseudomonas sp. + Enterobacter sp.). The different letters within the same column indicate a significant difference (p<0.05) between treatments.

Plant height

In general, the plant height varied with treatments over time with significant (p<0.05) differences between treatments starting from two weeks after planting (Table 3). At the second week, the greatest height (8.33 cm) was observed in positive control but the smallest in negative control (5.18 cm). Treatment BCD was among the consortia that gave the highest (7.68 cm) value (Table 3). Similarly, treatment C was among inoculation with single strain that showed the highest height (6.75 cm). Four weeks after planting, the situation was identical with positive control (19.78 cm) (Table 3).

The greatest (16.63 cm) value for consortia was observed from CD and in A (13.73 cm) for treatments with single inoculation. Six weeks after planting, the negative control showed a height of 18.7 cm, while the positive control showed 24.28 cm. However, the highest score was obtained mainly by some consortia ABCD (27.75 cm) and BCD (26.91 cm). Treatment C had the highest (24.60 cm) height among those inoculated with single bacterium, while inoculation with single or with consortia gave an increase of 29.8% for Cont., 31.6% for C, 43.9% for BCD and 48.4% for ABCD. In general, except the B treatment, inoculation with bacterial strain either in single or in consortia had positive effect on plant with regard to height parameter.

Stem base diameter

In general, the stem base diameter varied with treatments over time, with significant differences between treatments starting from two weeks after planting (Table 4). In the second week, positive control had the biggest (1.55 mm) stem diameter, while the smallest (0.43 mm) value was recorded from treatment B. Treatment D (0.70 mm) had the greatest value among the single inoculation treatments but CD had the highest diameter (1.48 mm) among the consortia.

Four weeks after planting, the greatest (5.88 mm) diameter was recorded from positive control but the smallest (1.88 mm) was from negative control. The AB consortium showed the largest (4.50 mm) diameter among the consortia but C (4.03 mm) among the single
Table 5. Effect (%) of the treatments on growth, root, shoot and total dry weight of maize six weeks after planting under pot grown conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of leaves</th>
<th>Plant height</th>
<th>Stem base diameter</th>
<th>Root dry weight</th>
<th>Shoot dry weight</th>
<th>Total dry weight</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont+</td>
<td>40</td>
<td>29.8</td>
<td>108.5</td>
<td>144.0</td>
<td>205.2</td>
<td>174.0</td>
<td>116.9</td>
</tr>
<tr>
<td>A</td>
<td>40</td>
<td>18.3</td>
<td>37.9</td>
<td>45.2</td>
<td>46.2</td>
<td>45.7</td>
<td>38.9</td>
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<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>29.7</td>
<td>46.7</td>
<td>43.4</td>
<td>45.1</td>
<td>27.5</td>
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<tr>
<td>C</td>
<td>40</td>
<td>31.6</td>
<td>59.1</td>
<td>67.5</td>
<td>82.6</td>
<td>74.9</td>
<td>59.3</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>18.6</td>
<td>35.5</td>
<td>56.1</td>
<td>34.8</td>
<td>45.7</td>
<td>38.5</td>
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<tr>
<td>AB</td>
<td>60</td>
<td>33.6</td>
<td>46.9</td>
<td>130.3</td>
<td>124.4</td>
<td>127.4</td>
<td>87.1</td>
</tr>
<tr>
<td>CD</td>
<td>40</td>
<td>21.2</td>
<td>50.2</td>
<td>84.4</td>
<td>124.3</td>
<td>104.0</td>
<td>70.7</td>
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<tr>
<td>ABC</td>
<td>20</td>
<td>20.6</td>
<td>71.6</td>
<td>63.7</td>
<td>134.4</td>
<td>134.6</td>
<td>95.5</td>
</tr>
<tr>
<td>ACD</td>
<td>60</td>
<td>24.1</td>
<td>53.3</td>
<td>134.9</td>
<td>126.5</td>
<td>145.5</td>
<td>87.2</td>
</tr>
<tr>
<td>BCD</td>
<td>40</td>
<td>43.9</td>
<td>134.9</td>
<td>134.6</td>
<td>134.6</td>
<td>134.6</td>
<td>95.5</td>
</tr>
<tr>
<td>ABCD</td>
<td>60</td>
<td>48.4</td>
<td>66.2</td>
<td>174.8</td>
<td>145.9</td>
<td>160.6</td>
<td>109.3</td>
</tr>
</tbody>
</table>

The effect (%) is calculated according to the formula: \( \text{Effect} = \frac{(T-\text{Cont})}{\text{Cont}} \times 100 \). Where, T is a given treatment and Cont- the negative control. A (Klebsiella sp.), B (Pseudomonas sp.), C (Burkholderia sp.), D (Enterobacter sp.), AB (Klebsiella sp. + Pseudomonas sp.), CD (Burkholderia sp. + Enterobacter sp.), ABC (Klebsiella sp. + Pseudomonas sp. + Burkholderia sp.), ACD (Klebsiella sp. + Burkholderia sp. + Enterobacter sp.), BCD (Pseudomonas sp. + Burkholderia sp. + Enterobacter sp.), ABCD (Klebsiella sp. + Pseudomonas sp. + Burkholderia sp. + Enterobacter sp.).

inoculation. The highest (10.59 mm) diameter was observed at the sixth week on the positive control but the smallest (5.08 mm) value from the negative control (Table 4). After sixth week, ABC treatment showed the highest diameter (8.71 mm) among consortia but C (8.08 mm) among single inoculation treatments. Positive control inoculation in single or in consortia showed an increase of 108.5% for Cont+, 71.6% for ABC, 59.1% for C, and 66.2% for ABCD as compared to the negative control (Table 5). In general, except the B treatment, inoculation with strain either in single or in consortia had positive effect on plant stem base diameter.

**Root, shoot and total plant dry weight**

ABCD consortium had the highest (6.62 g/plant) value of root dry weight followed by the positive control with the mass of 5.88 g/plant (Table 4) six weeks after planting. The negative control had the smallest root dry weight (2.41 g/plant). Treatment C had the highest (4.03 g/plant) weight among single inoculation treatments. Concerning the shoot dry weight, the greatest value was obtained from positive control (7.10 g/plant), while the smallest (2.32 g/plant) recorded from negative control. Treatment BCD (6.10 g/plant) was the best among consortia, but C (4.24 g/plant) was the best among single inoculation treatments. Regarding the variation of the total dry weight, the greatest and the smallest values are, respectively obtained from positive control (12.98 g/plant) and C (4.74 g/plant). Treatment ABCD with a weight of 12.34 g/plant was the best treatment among consortia, but treatment C had the greatest weight of 8.24 g/plant among single inoculation treatments. There was an increase of 174% for positive control, 74.9% for C, 145.5% for BCD and 160.6% for ABCD as compared to the negative control (Table 5). In general, all the inoculation treatments, either in single or in consortia had positive effect on plant dry weight.

Based on the effect of inoculation on the different plant parameters measured as compared to the negative treatment without inoculation, the different treatments can be classified as follows: Cont+ > ABCD > BCD > ACD = AB > CD > C > ABC > A = D > B six weeks after planting (Table 5). In general, consortia of strains had better effect than inoculation with single strain. Among the consortia, ABCD and BCD were the best in promoting maize growth, while C was the best among the single inoculations. Pseudomonas sp. (B) showed better performance when associated with any other bacteria.

**DISCUSSION**

The role of phosphate solubilising microorganisms capable of solubilising various forms of insoluble phosphates as well as rock phosphates is well known (Babana et al., 2013; Fankem et al., 2014). The activity on plates was assessed at least three times to confirm the real ability of strains in solubilising rock phosphates, since some bacteria can lose their solubilisation property after several successive subcultures (Kucey et al., 1989; Shankarrao, 2012). A halo zone on the plate is used to assess the P solubilisation activity of strains with the IS value as an indicator for the strain efficiency (Fernandez et al., 2007). Many bacteria strains isolated from Malian soils have been reported to solubilise the Malian (Tilemsi) rock phosphate and could be considered as rock phosphate solubilisers (Babana et al., 2013). Except Enterobacter sp., the three other bacterial strains did not
show any visible halo zone on plates amended with Moroccan and Mexican rock phosphates. This might be due to the low diffusion of the organic acids produced by these bacteria during their growth (Babu-Khan et al., 1995; Kloeppe et al., 1999; Azziz et al., 2012; Fernández et al., 2012). Regarding the efficiency of strains on rock phosphate solubilisers characterization. However, the plate method is still a feasible way to pre-screen the isolates that possess phosphate solubilising ability. In line with the previous investigations (Azziz et al., 2012; Fernández et al., 2012), there was no clear correlation between solubilisation ability on plate and in liquid culture. Although all the strains showed good solubilisation in liquid cultures amended with the different rock phosphates, the amount of the solubilized P varied with the strain involved as well as the rock phosphate origin. Solubilisation of phosphate is commonly accompanied by a remarkable drop in pH (Kloeppe et al., 1999; Azziz et al., 2012; Fernández et al., 2012) as was the case for all of our strains. Regarding the efficiency of strains on plates and in liquid cultures, Enterobacter sp. showed the highest performance followed by Klebsiella sp. In agreement with this study, Park et al. (2011) found that Enterobacter cloacae could solubilise 17.5% P in the growth medium and can be associated with RP as an alternative technique to soluble P compounds. Moreover, the ability of phosphate solubilisation by plant-associated Pseudomonas, Klebsiella, Enterobacter and Microbacterium species has been reported by Rodríguez and Fraga (1999). Increasing the bioavailability of P in the soils with combined inoculation and rock materials has been reported by many researchers (Schilling et al., 1998; Lin et al., 2002; Han and Lee, 2005; Han et al., 2006; Marschner, 2009), which may lead to increased P uptake and plant growth (Han et al., 2006; Chen et al., 2006; Eweda et al., 2007; Jorquera et al., 2008; Sabannavar and Lakshman, 2009). The present study highlights the ease of the four strains in single or in consortia in promoting the maize growth. In general, all the strains in single and in consortia have significantly increased the number of leaves, stem base diameter, total dry weight, shoot dry weight and root dry weight as compared to non-inoculated control. The effect of inoculation with single strain varied between 27.5 and 59.3%, while the effect of inoculation with consortia is between 54.1 and 109.3% showing that bacteria are capable of promoting the plant growth when combined inoculation is done. While studying the response to the pots inoculation of corn seeds by bacteria, Hussain et al. (2013) found significant increases in plant height, shoot dry weight and root dry weight of 16, 42 and 29%, respectively. In the present study, the average values obtained for the same parameters are 26.03, 97.63 and 93.28%, respectively, with the average total plant dry matter of about 95.41%. However, the average total plant dry matter obtained with single strain is about 52.85%. This value remains greater than that of Abou-el-Seoud and Abdel-Megeed (2012) who reported the increase of dry weight of maize plants inoculated with single bacterium of 26% as compared to non-inoculated treatment. In addition, there are some similar reports on enhanced dry matter content of maize and groundnut due to inoculation of phosphate solubilising bacteria (Hameeda et al., 2008; Pandey et al., 2006; Walpola and Yoon, 2013).

Growth enhancement by bacteria may relate to its ability to produce extensive root length (Sheng and Huang, 2001) and can also improve root development and increase the rate of water and mineral uptake (Alexander, 1997; Saghir et al., 2007). Many other researchers have found that phosphate solubilising microorganisms could increase maize growth and increase yield (Chabot et al., 1996; Yazdani et al., 2009; Hussain et al., 2013). This increase in growth may be attributed to auxin production (Gyaneshwar et al., 2002), ACC-deaminase activity (Naik et al., 2008; Jayadi et al., 2013), production of organic acids (Fankem et al., 2008) or phosphatases (Chabot et al., 1996) to solubilise/mineralize P, thereby increasing phosphate nutrition of inoculated plants. Collavino et al. (2010) testing E. aerogenes R4M-A and Burkholderia spp. R4M-F strains for IAA production in culture medium found that both produced and released free IAA. So, IAA produced by several bacteria can stimulate the development and proliferation of roots, with increases in uptake of water and nutrients (Bashan and De-Bashan, 2005). 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 phosphates uptake by the plant and crop yield (Mehta and Nautiyal, 2001). Moreover, bacteria belonging to genera Bacillus, Pseudomonas, Serratia and Enterobacter are reported to solubilise the insoluble phosphate compounds and aid in plant growth (Frey-Klett et al., 2005; Hameeda et al., 2008).

Burkholderia sp. is the best of the four used strains because of its efficiency in greatly increasing all the growth parameters. This is in agreement with the results of Linu et al. (2009) who found that Burkholderia sp. gave better results in improving growth of cowpea, and this strain had been previously evaluated by Pandey et al. (2005) to have phosphate solubilisation, auxin production, ACC deaminase activity and also nitrogen fixing ability. In addition to phosphate solubilisation, Burkholderia spp. and especially Burkholderia cepacia have potential for biological control and promoting plant growth (Babu-Khan et al., 1995; Kloeppe et al., 1999;
Rodríguez et al., 2000; Bhattacharyya and Jha, 2012). The growth and yield of maize increased when inoculated with *Burkholderia* sp. J62 in metal-polluted soil (Jiang et al., 2008).

**Conclusion**

The results obtained in the present study indicate that *Pseudomonas* sp., *Burkholderia* sp., *Enterobacter* sp. and *Klebsiella* sp. can effectively improve the solubility of rock phosphate applied as fertilizer, to increase the amount of available phosphate in the soil and increase the productivity of maize. This suggests that the use of rock phosphate combined with the co-inoculation of phosphate solubilising bacterial strains in soil with low fertility provides a sustainable alternative to the use of industrial fertilisers for maize production. This approach would ensure maize production in economically profitable and environmentally friendly manner.

**Conflict of Interest**

The author(s) have not declared any conflict of interests.

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