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Isolation of Peanut (*Arachis hypogaea* L.) Nodulating Rhizobia and Assessment of Their Phosphate Solubilizing Activity

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Abstract: Phosphate-solubilizing bacteria can enhance the dissolution of insoluble phosphorus in the soil, promoting the availability of soluble phosphorus. Thus, their application can reduce the use of chemical fertilizers and ease sustainable agriculture. The study aimed at isolating and assessing the phosphate solubilizing activity of peanut rhizobia isolated from root nodules of peanut in three Cameroonian soils (Douala, Bafoussam and Ebolowa). Rhizobia were trapped by seeding peanut in plastic pots containing different soils. 45 days later, at the flowering time, root nodules were harvested. From these nodules, rhizobia were isolated, purified on Yeast Extract Mannitol Agar (YEMA) medium and authenticated by inoculating them on sterile sand containing peanut plants and watered with a nutrient solution without nitrogen. The phosphate solubilizing activity of the rhizobia isolates was then assessed in Modified Mineral Salt Medium (MMSM) containing bromocresol green and where the soluble phosphate was replaced by insoluble inorganic phosphates from Cameroon, Algeria, Senegal and tricalcium phosphate in both solid and broth media. The results were analysed statistically by one-way ANOVA using SPSS. A collection of twenty-five isolates was constituted among which 04 from Douala, 12 from Bafoussam and 09 from Ebolowa. All isolates were distinct morphologically. There was significant solubilization of inorganic phosphate in both solid and broth media ($p<0.05$). Isolated bacteria were characterized as being phosphate solubilizers with values ranging from 1.75 (AhBf1 on Algeria rock phosphate) to 18.9 mg/L (AhDa3 on Algeria rock phosphate). The Algeria rock phosphate was the most solubilized by peanut nodulating bacteria, followed by the tricalcium phosphate, the Senegal rock phosphate and the Cameroon rock phosphate. The solubilizing activity of inorganic phosphates in the broth medium was associated with a global decrease in the pH of the culture medium. The peanut nodulating rhizobia isolates that show the best solubilizing capacity could alleviate the problem of phosphorus availability in agricultural soils.

Keywords: Peanut, *Rhizobium*, Phosphate Solubilizing Rhizobia, Cameroon

1. Introduction

After nitrogen, phosphorus is one of the most essential nutrients for plant growth and development. It is also a growth-limiting nutrient in the soil due to its liability to fixation [1]. Phosphorus deficiency in soil can severely limit

plant growth productivity, particularly in legumes, where both the plants and their symbiotic bacteria are affected, and this may have a deleterious effect on nodule formation, development and function [2]. In order to alleviate the problem of phosphate deficiency, a large amount of phosphate fertilizers is applied to the soil in modern agriculture [3], which will quickly enhance phosphate availability. However, the utilization rate of that phosphate is very low in the long run because it is easy to be complexed again by metal ions such as Fe, Al and Ca in soil [4]. Long periods and repeated applications of phosphate fertilizers not only raise environmental issues but also compromise the soil micro-ecosystem balance, resulting in the loss of soil activities. Thus, environmentally friendly substitutes for phosphate fertilizers must urgently be found to avoid the adverse effects of agricultural production [5]. Alternative and sustainable ways to alleviate soil phosphate deficiency are emerging with the discovery and research of phosphate-solubilizing microorganisms. Besides, symbiotic nitrogen fixation, also some species of Rhizobium are involved in phosphate solubilization. But studies on the phosphate solubilizing ability of Rhizobium strains are very limited. The main advantage of using rhizobia as a phosphate-solubilizing microorganism will be their beneficial nutritional effect resulting both from phosphate mobilization and nitrogen fixation. The aim of the study is to isolate and assess the phosphate solubilizing activity of peanut rhizobia isolated from root nodules in three Cameroonian soils (Douala, Bafoussam and Ebolowa).

2. Materials and Methods

2.1. Soil Sampling and Analysis

The sampling was carried out in 3 sites belonging to three agro-ecological zones: the Highland zone in Bafoussam, with 1500 to 2000 mm of rainfall per year, the humid forest zone with bimodal rainfall in Ebolowa, with 1500 to 2000 mm of rain per year and the humid forest zone with monomodal rainfall in Douala, with 2500 to 4000 mm of rain per year.

2.2. Trapping of Peanut Nodulating Bacteria

Vincent's trapping technique (1970) was used with peanut as a trap plant, on a device consisting of three batches of perforated plastic bags of about 2.5 kg of soil. The three batches representing the different sampling sites were separated to avoid any transfer of microorganisms from one site to another. Peanut seeds of the local variety acquired on the Douala market were disinfected with 3% (w/v) sodium hypochlorite for 3 minutes, then rinsed abundantly with sterile distilled water. The seeds were then soaked in alcohol at 90° for 2 minutes and rinsed abundantly with sterile distilled water to rid them of residual alcohol [6], then germinated for 48 hours in darkness in a sanitized enclosure, then sown in plastic bags, at a rate of 5 seeds per bag. The plants were watered regularly with tap water so that the only factors that could limit nodulation were the absence of rhizobia in the soil and the characteristics of the soil.

2.3. Isolation and Authentication of Peanut Nodulating Bacteria

The isolation of peanut-nodulating bacteria was carried out according to Vincent's method (1970). The nodules preserved by desiccation are rehydrated by immersion in sterile distilled water for 2 hours. For each site, 30 nodules are used. The nodules are then disinfected by soaking in 95°ethanol for 10 s, then soaking in an acidified solution of mercuric chloride ($HgCl_2$) at 1 % for 30 s. The nodules are rinsed 4 to 5 times in sterile distilled water to remove traces of mercuric chloride. The isolation of peanut-nodulating bacteria was carried out on Yeast Extract Mannitol Agar (YEMA) medium supplemented with Congo Red. The appearance of colonies was observed after 48 h and 72 h. Colonies that did not absorb Congo Red were picked and subcultured regularly in Petri dishes containing YEMA medium and incubated in an inverted position at 28°C until pure and homogeneous colonies were obtained. The authentication test ensures that isolates obtained from peanut nodules are indeed rhizobia isolates, through their ability to form nodules on the roots of a host legume [6]. For this test, peanut has been used as a host plant. The seedlings inoculated with the purified isolates were watered twice a week with Jensen's nutrient solution, the composition of which per litre of sterile distilled water is as follows: K_2HPO_4: 0.2 g; $MgSO_4$ ($7H_2O$): 0.2 g; NaCl: 0.2 g; $CaHPO_4$: 1 g; $FeCl_2$: 0.14 g; HBO_3: 2.86 mg; $MnSO_4$ ($6H_2O$): 2.03 mg; $ZnSO_4$: 0.22 mg; $CuSO_4$: 0.08 mg; $NaMbO_4$: 0.09 mg. The pH of the medium was adjusted to 6.5 and the medium was then autoclaved at 120°C for 20 min.

2.4. Morphological Characterization of Peanut Nodulating Bacteria

Morphological characterization of peanut nodulating rhizobia isolates consisted of separating bacteria according to growth rate, diameter, shape, opacity, viscosity, outline, presence of mucus, fluorescence, relief, colour of the bacterial colonies [7]. The microscopic characterization consisted of observing the morphology and the nature of their cell wall after Gram staining of the bacteria under the optical microscope at 40X.

2.5. Assessment of Phosphate Solubilizing Activity

The rock phosphates to be solubilized are rock phosphate from Algeria, rock phosphate from Senegal, rock phosphate from Cameroon and tricalcium phosphate. The rock phosphate solubilization test by rhizobia isolates was carried out in solid medium and in liquid medium.

2.5.1. Assessment of Phosphate Solubilizing Activity in Solid Medium

The test on solid medium, the purpose of which is to evaluate the solubilizing capacity of isolates of rhizobia nodulating peanut, was carried out on Modified Mineral Salt (MMS) medium [8] supplemented with Bromocresol green

(5 mL/L). The composition per litre of Bromocresol green solution is as follows: NH_4Cl: 0.4 g; KNO_3: 0.78 g; NaCl: 0.1 g; $CaCl_2$ $2H_2O$: 0.25 g; $FeSO_4$: 0.27 mg; $MnSO_4$: 1.56 mg; $ZnSO_4$, $7H_2O$: 1.40 mg; glucose: 10 g; Agar: 20 g. A stock solution of bromocresol green at 0.5 % was previously prepared according to the method of Gadagi [9]. The source of soluble phosphate (0.5 g of K_2HPO_4) was replaced by 0.5 g of a given insoluble rock phosphate. The different phosphates were washed 4 times in lukewarm water every 24 hours and then dried in the oven at 60°C until the water has completely evaporated. For 50 ml of solution, 35 ml of ethanol is diluted in 1 ml of distilled water, and then 0.25 g of Bromocresol green is added. After stirring, the pH is adjusted to 6.5 with 1M NaOH. The culture media containing various rock phosphates and supplemented with 0.5 % Bromocresol green are autoclaved and poured into sterile Petri dishes. After cooling, each Petri dish is divided into 4 compartments using a marker to inoculate the centre of three-quarters of each Petri dish with 10 µL of each isolate, the 4th quarter of the uninoculated dish serving as a control. A preliminary test was carried out to determine the solubilizing isolates of each type of phosphate. Three repetitions were carried out for each isolate showing an ability to solubilize phosphate. The solubilization test involved all the rhizobia isolates. The concentration of rhizobial inocula was evaluated using a 0.5 Mc Farland solution, which corresponds to a bacterial concentration equal to 108 bacteria/ml. The solubilization of phosphate by the isolates was evaluated over a period of 5 days. The solubilizing activity was evaluated by measuring the diameter (N) of the colony and the diameter (Z) of the solubilization halo using graph paper. The solubilization halo perceived as a translucent area around the colony was observed daily. From these measurements, the z/n ratio or solubilization index (SI) of each isolate was given by the formula [10]:

$$SI = Z\ (mm) / N\ (mm)$$

2.5.2. Assessment of Phosphate Solubilizing Activity in Broth Medium

The basic YEM medium was prepared without K_2HPO_4 which was replaced by rock phosphate from Algeria, Senegal, Cameroon or tricalcium phosphate. The inorganic phosphate is introduced into a 250 mL Erlenmeyer flask containing 50 mL of basic YEM. The pH is adjusted to 6.5 and the medium is autoclaved at 120° C. for 20 min. After cooling, each Erlenmeyer flask is inoculated with 200 µL of a 2-days culture of each isolate (0.5 Mc Farland). For each inorganic phosphate, three replicates were used per isolate. The control Erlenmeyer flasks were not inoculated. The culture media were incubated at 28°C under the shaker at 150 rpm. On the 5th day of incubation, 5 mL of each culture medium is withdrawn aseptically and introduced into Eppendorf tubes. Of this 5 mL, 3 mL are used for measuring the pH and 2 mL are centrifuged and used for the determination of the solubilized phosphorus according to the method of Murphy and Riley [11]. After centrifugation of the bacterial culture obtained previously, 500 µL of supernatant from each Eppendorf is introduced into a 50 mL test tube, then supplemented to 8 mL with 7.5 mL of distilled water. Subsequently, 2 mL of the reagent solution is introduced into the test tubes. The test tubes are placed in the dark for one hour until the colour of the medium becomes stable. The O. D. is read with a spectrophotometer (BIOBASE) at 665 nm.

2.6. Statistical Analysis

Statistical analysis was performed using the SPSS 16.0 software package. and the results were expressed as the means ± standard deviation of three replicates. Data were examined by ANOVA, and mean comparison was performed by Duncan's multiple range test at p = 0. 05.

3. Results and Discussion

3.1. Soil and Rock Phosphates Physicochemical Analysis

Table 1 indicates the physicochemical properties of sites from where peanut nodulating bacteria were sampled. According to the pH analysis, all soils have an acid pH. In addition, assimilable phosphorus analysis showed that soil sampled from Douala soil displayed the highest level (12.95 µg/g), whereas the lowest levels of phosphorus were noted on Ebolowa soil (4.47 µg/g). The cation exchange capacity tested on soils is very low at Ebolowa (6.65 cmol /Kg) and high at Bafoussam (20.27 cmol/Kg).

Table 1. *Soil properties of sampling sites.*

Parameters	Bafoussam	Douala	Ebolowa
Sand (%)	65.10	56	49.03
Clay (%)	9.33	28	35.40
Loam (%)	25.57	16	15.57
pH (H_2O)	5.53	6.1	4.38
EC (µS/cm)	287	0.06	100.30
Organic carbon (%)	3.81	1.28	1.31
total N (%)	0.30	0.10	0.10
Assimilable phosphorus / Bray II (µg/g)	7.13	12.95	4.47
Total phosphorus (µg/g)	1003.37	26.16	58.71
C/N	12.53	13	12.80
CEC (cmol (+)/Kg)	20.27	10	6.65

The rock phosphates' phosphorus content is displayed in table 2. It appears from this table that the assimilable phosphorus (P_2O_5) is higher in the Algeria rock phosphate (2.9 %) than in the other sites (0.113 %). The total phosphorus is lower for the Algeria rock phosphate and higher for the Senegal rock phosphate.

Table 2. *Rock phosphates phosphorus content analysis.*

Parameters		Rock phosphates origin		
		Cameroon	Senegal	Algeria
Assimilable P	Bray II (mg /Kg)	489.95	493.11	496.03
	$\%P_2O_5$	0.113	0.113	2.9
Total P (mg /Kg)		698.5	776.56	680.4

3.2. Isolation of Peanut Nodulating Bacteria

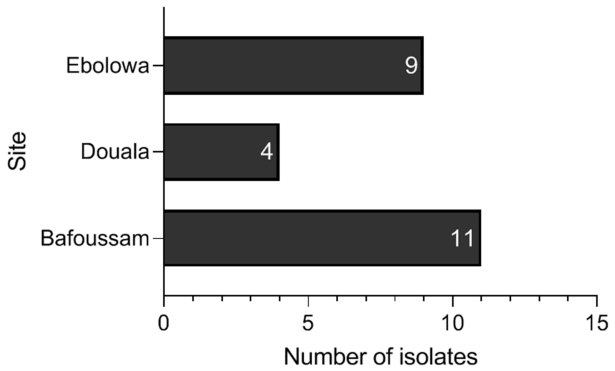


Figure 1. *Number of rhizobia isolates nodulating peanut in the different sites.*

After isolation, purification and authentication, a collection of 25 isolates of peanut nodulating bacteria was established (Figure 1). From this collection, 9 rhizobia were isolated from Ebolowa, 4 from Douala and 12 from Bafoussam after authentication test on peanut as a host plant in the greenhouse.

The morphological characters of the isolates are displayed in table 3 which shows that all the colonies are rounded in shape, fast-growing, viscous and Gram-negatives (Figure 2). The highest colony diameter (0.42 cm) is registered on AhDa4 and the lowest on AhEb6 (0.01 cm). These characteristics are in agreement with those of tropical leguminous [12]. The growth speed of the isolates could be a survival strategy, as fast-growing isolates are more tolerant than slower one [12].

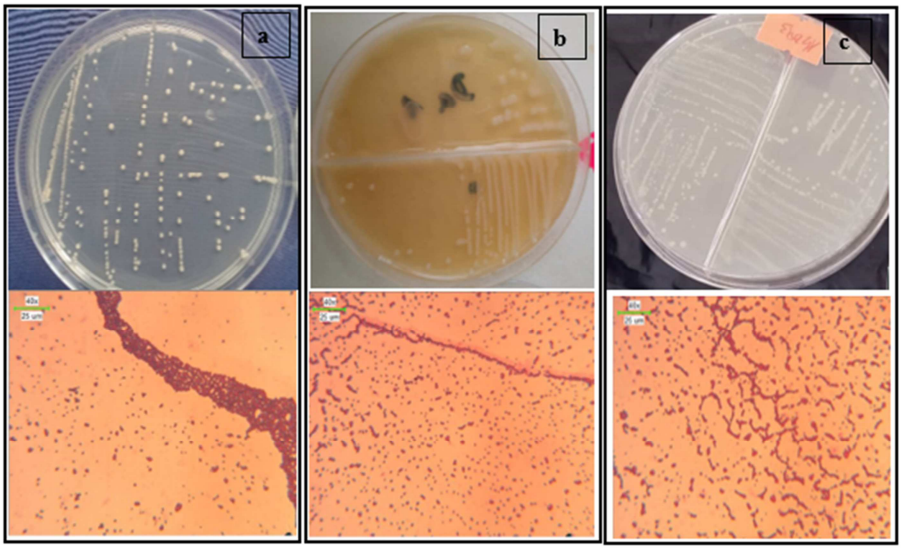


Figure 2. *Macroscopic (on top) and microscopic (down) aspect of AhEb3 (a), AhBf5 (b) and AhDa3 (c) after Gram staining.*

Table 3. *Morphological characters of peanut nodulating bacteria.*

Sites	Colonies	Diameter (cm)	Form	Colour	Growth	Outline	Mucus
Bafoussam	AhBf1	0.1	Rounded	Whitish	Fast	Regular	Absent
	AhBf2	0.2	Rounded	Milky white	Fast	Regular	Present
	AhBf3	0.2	Rounded	Milky white	Fast	Regular	Present
	AhBf4	0.1	Rounded	Yellow	Fast	Regular	Present
	AhBf5	0.3	Rounded	Milky white	Fast	Regular	Present
	AhBf6	0.18	Rounded	Milky white	Fast	Regular	Present
	AhBf7	0.1	Rounded	Milky white	Fast	Regular	Absent
	AhBf8	0.19	Rounded	Milky white	Fast	Regular	Present
	AhBf9	0.11	Rounded	Milky white	Fast	Regular	Present
	AhBf10	0.12	Rounded	Milky white	Fast	Regular	Present
	AhBf11	0.13	Rounded	Whitish	Fast	Regular	Present
	AhBf12	0.19	Rounded	Milky white	Fast	Regular	Present
Ebolowa	AhEb1	0.05	Rounded	Milky white	Fast	Regular	Absent
	AhEb2	0.04	Rounded	Milky white	Fast	Regular	Absent
	AhEb3	0.2	Rounded	Milky white	Fast	Regular	Present
	AhEb4	0.1	Rounded	Whitish	Fast	Regular	Present
	AhEb5	0.1	Rounded	Milky white	Fast	Regular	Absent
	AhEb6	0.01	Rounded	Milky white	Fast	Regular	Absent
	AhEb7	0.1	Rounded	Milky white	Fast	Regular	Present
	AhEb8	0.1	Rounded	Milky white	Fast	Regular	Absent
	AhEb9	0.2	Rounded	Milky white	Fast	Regular	Absent
Douala	AhDa1	0.15	Rounded	Milky white	Fast	Regular	Absent
	AhDa3	0.3	Rounded	Whitish	Fast	Regular	Present
	AhDa4	0.42	Rounded	Whitish	Fast	Regular	Present
	AhDa5	0.33	Rounded	Milky white	Fast	Regular	Present

Table 3. *Continued.*

Sites	Colonies	Diameter (cm)	Viscosity	Opacity	Fluorescence	Relief	Gram staining
Bafoussam	AhBf1	0.1	Viscous	Opaque	No	Bulging	-
	AhBf2	0.2	Viscous	Opaque	No	Bulging	-
	AhBf3	0.2	Viscous	Opaque	No	Bulging	-
	AhBf4	0.1	Viscous	Opaque	Yes	Bulging	-
	AhBf5	0.3	Viscous	Opaque	No	Bulging	-
	AhBf6	0.18	Viscous	Opaque	No	Bulging	-
	AhBf7	0.1	Viscous	Opaque	No	Bulging	-
	AhBf8	0.19	Viscous	Opaque	No	Bulging	-
	AhBf9	0.11	Viscous	Opaque	No	Bulging	-
	AhBf10	0.12	Viscous	Opaque	No	Bulging	-
	AhBf11	0.13	Viscous	Opaque	Yes	Bulging	-
	AhBf12	0.19	Viscous	Opaque	No	Bulging	-
Ebolowa	AhEb1	0.05	Viscous	Opaque	Yes	Flat	-
	AhEb2	0.04	Viscous	Opaque	Yes	Bulging	-
	AhEb3	0.2	Viscous	Opaque	Yes	Bulging	-
	AhEb4	0.1	Viscous	Opaque	Yes	Bulging	-
	AhEb5	0.1	Viscous	Opaque	Yes	Bulging	-
	AhEb6	0.01	Viscous	Opaque	Yes	Flat	-
	AhEb7	0.1	Viscous	Opaque	Yes	Bulging	-
	AhEb8	0.1	Viscous	Opaque	Yes	Bulging	-
	AhEb9	0.2	Viscous	Opaque	Yes	Bulging	-
Douala	AhDa1	0.15	Viscous	Opaque	Yes	Bulging	-
	AhDa3	0.3	Viscous	Opaque	Yes	Bulging	-
	AhDa4	0.42	Viscous	Opaque	No	Bulging	-
	AhDa5	0.33	Viscous	Opaque	No	Bulging	-

3.3. Assessment of Phosphate Solubilizing Activity

3.3.1. Assessment of Phosphate Solubilizing Activity in Solid Medium

The solubilization test in the solid medium is displayed by the production of a yellow halo surrounding the colony (Figure 3). This halo is a function of the quantity of solubilized phosphate.

Solubilization indexes (SI) as a function of isolates and phosphate types are determined in table 4. From the results, it appears that all the Bafoussam and Douala isolates have solubilized Algeria rock phosphate, while 77.77 % of the Ebolowa isolates were able to do so. The isolates of Douala were the only ones showing a solubilization halo of the Senegal rock phosphate, with a significantly highest value of 5.4 ± 0.40 for AhDa3. Except for the isolates of that site, only 3 isolates (AhEb3, AhEb4 and AhEb7) of Ebolowa induced a solubilization halo for the Cameroon rock phosphate. Overall, 52 % of isolates were able to solubilize tricalcium phosphate. The highest solubilization index (5.5 ± 0.71) was observed on AhDa3 with is significantly different from those of other isolates.

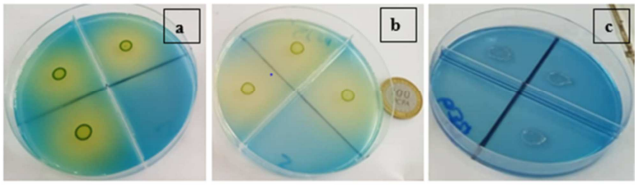


Figure 3. *Solubilization of Algeria rock phosphate by AhDa3 (a) and AhEb4 (b) and no solubilization of Cameroon rock phosphate by AhBf1 (c).*

Table 4. *Solubilization of phosphate in solid medium.*

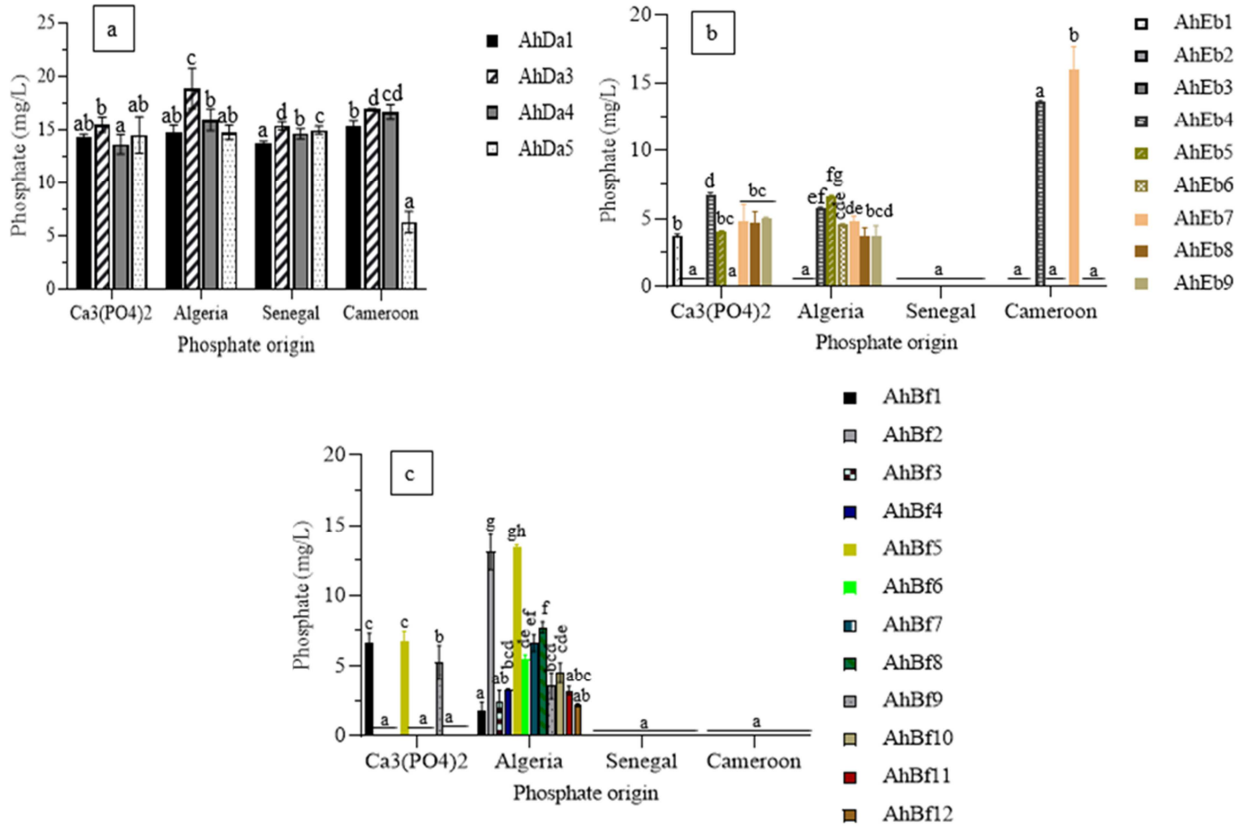
Sites	Isolates	Phosphate origin			
		Algeria	Senegal	Cameroon	$Ca_3(PO_4)_2$
Bafoussam	AhBf1	1.66 ± 0.23bcd	-	-	2.125 ± 1.41b
	AhBf2	3.215 ± 0.47g	-	-	-
	AhBf3	1.745 ± 0.12bcd	-	-	-
	AhBf4	1.575 ± 0.11bcd	-	-	-
	AhBf5	3.425 ± 0.18g	-	-	2.045 ± 0.25b
	AhBf6	2.6 ± 0.14ef	-	-	-
	AhBf7	2.37 ± 0.10cde	-	-	-
	AhBf8	1.535 ± 0.09bc	-	-	-
	AhBf9	1.38 ± 0.03b	-	-	1.80 ± 0.28b
	AhBf10	2.38 ± 0.10de	-	-	-
	AhBf11	3.21 ± 0.30ef	-	-	-
	AhBf12	1.18 ± 0.10b	-	-	-

Sites	Isolates	Phosphate origin			
		Algeria	Senegal	Cameroon	$Ca_3(PO_4)_2$
Douala	AhDa1	4.625 ± 0.88h	4.28 ± 0.54b	5.36 ± 0.50c	4.375 ± 0.25cd
	AhDa3	5.250 ± 0.35hi	5.4 ± 0.40d	6.95 ± 0.07d	5.5 ± 0.71d
	AhDa4	5.955 ± 1.00j	4.65 ± 0.49bc	6.68 ± 0.68d	4.9 ± 0.85d
	AhDa5	4.775 ± 0.67h	4.975 ± 0.39c	6.3 ± 0.99d	4.505 ± 1.70cd
Ebolowa	AhEb1	1.4 ± 0.14b	-	-	1.785 ± 0.12b
	AhEb2	-	-	-	-
	AhEb3	-	-	3 ± 0b	-
	AhEb4	5.775 ± 0.09j	-	3.635 ± 0.09b	2 ± 0.00b
	AhEb5	1.69 ± 0.04bcd	-	-	2.06 ± 0.08b
	AhEb6	1.6 ± 0.06bcd	-	-	-
	AhEb7	4.86 ± 0.37h	-	8 ± 1.13e	4.855 ± 1.21d
	AhEb8	1.21 ± 0.06b	-	-	3.5 ± 0.00c
	AhEb9	2.61 ± 0.44ef	-	-	2.25 ± 0.35b

Means with the same letter are not significantly different at p= 5% by Duncan test

3.3.2. Assessment of Phosphate Solubilizing Activity in Broth Medium

The results of the phosphate solubilization in broth showed that the amount of solubilized phosphate varies from one isolate to another and depends on the phosphate types (Figure 4). The analysis of variance showed a significant difference ($P<0.05$) among isolates for all phosphate types and in all study sites. In Douala (Figure 4a), AhDa3 is significantly the highest of all the isolates for all the phosphate types with respectively 15.5 mg/L for $Ca_2(PO_4)_2$, 18.88 mg/L for Algeria rock phosphate, 15.4 mg/L for Senegal rock phosphate and 16.95 mg/L for Cameroon rock phosphate. All the isolates of this site showed an ability to solubilize phosphate.



Means with the same letter are not significantly different at p=5%

Figure 4. *Solubilization of phosphate in broth medium. a: Douala; b: Ebolowa; c: Bafoussam.*

3.3.3. Effect of the Phosphate Solubilizing Activity of Rhizobia on the pH of the Culture Medium

The solubilizing activity of inorganic phosphates in the broth medium was associated with a global decrease in the pH of the culture medium (Table 5).

Table 5. *Effect of the solubilization of phosphate on the pH of the culture medium.*

Sites	Isolates	Phosphate origin			
		Algeria	Senegal	Cameroon	$Ca_3(PO_4)_2$
Bafoussam	AhBf1	6.55 ± 0.6klm	6.4± 0.05de	6.5 ± 0h	5.15 ± 0.1d
	AhBf2	5± 0b	6.5± 0.02de	6.5 ± 0h	6.5 ± 0k
	AhBf3	6.39 ± 0.03jkl	6.85± 0.4f	6.5 ± 0gh	6.5 ± 0k
	AhBf4	6.42 ± 0.1lmn	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhBf5	4.93 ± 0.7b	6.5 ± 0de	6.5 ± 0h	5.2 ± 0.3e
	AhBf6	5.41 ± 0.3d	6.65 ± 0.6ef	6.5 ± 0g	6.5 ± 0k
	AhBf7	5.02 ± 0.61b	6.5± 0de	6.5 ± 0h	6.5 ± 0k
	AhBf8	5.35 ± 0.4d	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhBf9	6.12 ± 0.17fg	6.5 ± 0d	6.5 ± 0h	5.4 ± 0.2f
	AhBf10	6.03 ± 0.05ef	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhBf11	6.25 ± 0.26hi	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhBf12	6.31 ± 0.28ijk	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
Douala	AhDa1	6.2 ± 0.4gh	5.7 ± 0.06c	4.65 ± 0.08d	4.87 ± 0.15b
	AhDa3	4.68 ± 0.3a	4.7 ± 0.03a	5.1 ± 0.45e	4.75 ± 0.2a
	AhDa4	6.09 ± 0.21ef	4.7 ± 0.1a	4.2 ± 0.56b	5.02 ± 0.05c
	AhDa5	4.78 ± 0.05a	5 ± 0.15b	6.3 ± 0.06f	5 ± 0.6c
Ebolowa	AhEb1	6.5 ± 0mno	6.5 ± 0de	6.5 ± 0h	6.45 ± 0.17j
	AhEb2	6.5 ± 0no	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhEb3	6.5 ± 0no	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhEb4	5.23 ± 0.02c	6.5 ± 0de	4.1 ± 0.1a	5.8 ± 0.1g
	AhEb5	5.15 ± 0.062c	6.5 ± 0de	6.5 ± 0h	6.6 ± 0.08l
	AhEb6	6.23 ± 0.07hi	6.5 ± 0de	6.5 ± 0h	6.5 ± 0k
	AhEb7	6 ± 0.01e	6.5 ± 0de	4.28 ± 0.12c	5.9 ± 0.12h
	AhEb8	6.54 ± 0.28o	6.5 ± 0.02de	6.5 ± 0h	6.05 ± 0.03i
	AhEb9	6.7 ± 0p	6.5 ± 0.01de	6.5 ± 0h	5.8 ± 0.25g

Means with the same letter are not significantly different at p=5 % by Duncan test

For Algeria and the Senegal rock phosphates, the highest significant ($p < 0.05$) decrease in the pH was due to AhDa3 (4.68 ±0.3 and 4.7 ± 0.03 respectively). With a pH of 4.1 ± 0.1, AhEb4 induced a significantly major decrease in the pH of the medium containing Cameroon rock phosphate. For the tricalcium phosphate, the lowest value of pH was obtained for AhDa3 (4.75 ± 0.2). These results are similar to those obtained by Youagang et al. [13] and Marra et al. [14] who showed that bacteria nodulating respectively common bean and cowpea can solubilize inorganic phosphates. This capacity to solubilize phosphate by peanut nodulating rhizobia could be explained by their adaptation to assimilable phosphorus-lacking soils. The inorganic phosphate solubilization by rhizobia is linked to an acidification of the culture medium. According to Otieno et al. [15] and Lin et al. [16], this drop in the pH is due to the release of organic acids in the culture medium. This implies explicitly that the mechanism of solubilization is mainly based on the chelation of oxo acids from sugar to produce organic acids that ease the uptake of inorganic phosphates [17-19]. Maliha et al. [20] reported that during the solubilization process, gluconic acids are usually produced by phosphate-solubilizing bacteria in the rhizosphere.

4. Conclusion

Phosphorus is an important limiting factor in agriculture production, and microbial phosphorus solubilization is an effective process to release the precipitated phosphorus in soil. The present work was carried out in order to isolate and assess the phosphate solubilizing capacity of peanut nodulating rhizobia. A total of 25 distinct isolates were obtained from three different sites in Cameroon, with 12 at Bafoussam, 04 at Douala and 09 at Ebolowa. Their screening for the inorganic phosphate solubilizing ability led to see that the solubilization of phosphate varied from one isolate to another and depends on the phosphate origin. Isolated bacteria were characterized as being phosphate solubilizers with values ranging from 1.75 (AhBf1 on Algeria rock phosphate) to 18.9 mg/L (AhDa3 on Algeria rock phosphate). Not surprisingly, the pH of all bacterial cultures dropped significantly from 6.5 to 4.1 (AhEb4 on Cameroon rock phosphate), likely due to organic acid production. Overall, the highest solubilizers were AhDa3, AhBf5 and AhEb4. These isolates will be used in further study as inoculants for the peanut culture in different Cameroonian soils, where assimilable phosphorus absence is a significant hindrance.

Conflict of Interest

The authors declare that they have no competing interests.

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References

[1] Schachtman D. P., Reid R. J. and Ayling S. M. 1998. Phosphorus uptake by plants: From soil to cell. Plant Physiol. 116 (2): 447-453.

[2] Alikhani HA, Saleh-Rastin N and Antoun H. 2006. Phosphate solubilization activity of rhizobia native to Iranian soils. Plant Soil 287: 35-41.

[3] Elser JJ. Phosphorus: a limiting nutrient for humanity? 2012. Curr Opin Biotechnol. 23 (6): 833-838.

[4] Goldstein AH. 1986. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Alternative Agr.; 1 (2): 51-57.

[5] Singh, H., and Reddy, M. S. 2011. Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. Eur. J. Soil Biol. 47, 30–34. doi: 10.1016/j.ejsobi.2010.10.005.

[6] Vincent, J. M. 1970. A manual for the practical study of the root nodule bacteria. IBP. Handbook n°15 – Blackwell scientific publishers, Oxford. Edingbourgh. p. 104.

[7] Jordan, D. C. 1984. Family III. Rhizobiaceae Conn. 1938. In: Bergey's manual of systematic bacteriology. Krieg, N. R. and Holt, J. G. (eds) 1 pp. 234-244.

[8] Reyes, F. et Rodriguez, H. 1999. phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 17: 319-339.

[9] Gadagi, R. S., Sa, T. 2002. New isolation method for microorganisms solubilizing iron and aluminium phosphates using dyes. Soil Science and Plant Nutrition. 48: 615-618.

[10] Berraquerro, F. R., Baya, A. M. and Cormenzana, A. R. 1976. Establishment of indices for the study of phosphate solubilzing soil bacteria Ars pharmaceutica. 17: 399-406.

[11] Murphy J, and Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31-36. ws in Plant Sciences 22, 107-149.

[12] Zhang, W. T., Yang, J. K., Yuan, T. Y., Zhou, J. C. 2007. Genetic diversity and phylogeny of indigenous rhizobia from cowpea (*Vigna unguiculata* (L.) Walp.). Biology and Fertility of Soil, 44 (1): 201-210.

[13] Youagang G. H. S., Ngo Nkot L., Asseng C. C., Nyaka Ngolisa A. I. C., Ngakou A. et Nwaga D. 2020. Isolation and characterization of Legume Nodulating Bacteria Isolated from Common bean (*Phaseolus vulgaris* L.) nodules. Global Scientific Journals. 8 (5): 1777-1792. Online: ISSN 2320-9186.

[14] Marra, L. M., Oliveira, S. L., Soares, C. R. F. S., Moreira, F. M. S. 2011. Solubilization of inorganic phosphates by strains from tropical legumes. Sci. Agric. 65: 603-609.

[15] Otieno N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN. 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Frontiers in Microbiology 6: 745.

[16] Lin, T. F., Huang, F. T., Youg, C. C., 2006. The proton of gluconic acid are major factor responsible for the dissolution of tricalcium phosphate by *Burkolderia cepacia* CC- A174. Bioresource Technology 97: 957-960.

[17] Adnane B, Elhaissoufi W, Said K, Benmrid B, Borden K, Rchiad Z. 2021. Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus. Microbiol Res. 252: 126842.

[18] Antoun H., and Kloepper, J. W. 2001. Plant Growth promoting rhizobacteria. Encyclopedia of Genetics. Brenner, S. and Miller, J. F. Academic Press. 1477-1480.

[19] Peix, A. Rivas-Boyero., A. A. Mateos., P. F. Rodriguez-Barrueco., C. Martínez-Molina., E. Velazquez, E. 2001. Soil Biology & Biochemistry. 33: 103-110.

[20] Maliha R., S. Khalil, N. Ayub, S. Alam and F. Latif. 2004. Pak. J. Biol. Sciences. 7 (2) 187-196.