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Biodiversity of the phosphate solubilizing microorganisms (PSMs) population from the rice rhizosphere soils of the two agro-ecological zones of Cameroon

Henri FANKEM[1*], Hélène Laure MAFOKOUA[1], Laurette NGO NKOT[1], Claude SIMO[1], Donald TCHOUOMO DONDJOU[1], Gylaine Vanissa TCHUISSEU TCHAKOUNTE[1], Dieudonné NWAGA[2] and François-Xavier ETOA[2]

[1]Department of Plant Biology, Faculty of Science, University of Douala, Douala, Cameroon.
[2]Department of Microbiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon.
[*]Corresponding author; E-mail: fankemhenri@yahoo.fr

ABSTRACT

The plant rhizosphere microorganisms having the phosphate solubilizing capacity can convert the insoluble soil organic and inorganic phosphates into a soluble form and make the phosphorus (P) available to the plant. With the objective of evaluating the phosphate solubilizing microorganism populations under the rice rhizosphere, soils samples were collected in three locations of two agro-ecological zones of Cameroon and analyzed for their PSMs diversity. Isolation of microorganisms was made on non selective nutrient agar plates and the phosphate solubilizing activity of isolates was tested on National Botanical Research Institute's Phosphate growth medium (NBRIP) amended with sparingly soluble rock phosphates of different origins. The morphological description of isolates allowed evaluating the phosphate solubilizing microorganism's diversity under the rice rhizospheric soil. The most probable number of PSMs ranged between 22 and 53% with an average of 48%, 52.80% and 22.44% for Nkolbisson, Nyokon and Santchou respectively. The 65 isolates obtained from all locations were distributed in four diverse groups. The index of solubilization ranged between 2.70 and 7.24 depending on isolate. From a total of 65 isolates obtained from the three sampling sites, 46 were phosphate solubilizing isolates among which: 20 were of low solubilization, 16 of medium and 10 of high solubilization. This is the first work reporting phosphate solubilizing microorganisms on rice rhizosphere in Cameroon. However, the selection of phosphate solubilizing microorganisms as possible inoculation tools for phosphate-deficient soils should focus on the integral interpretation of laboratory assays, greenhouse experiments as well as field trials.

Keywords: Diversity, index of solubilization, isolates, rice, rock phosphates.

INTRODUCTION

Phosphorus (P) is an essential mineral nutrient required for plant growth because it is a major component of important molecules (Collavino et al., 2010). It is intimately involved in a wide range of physiological and biochemical processes that include; cellular energy systems, structure and function of nucleic acids (Antoun, 2012), membrane integrity and sugar metabolism (Richardson, 2009). Orthophosphate anions (mainly $H_2PO_4^-$ and HPO_4^{2-}) are the form of P taken up by plants from soil solution; however their concentration is very low because they are chemically very active and they react rapidly with cations like calcium in alkaline soils or aluminum and iron in acid soils to form sparingly soluble precipitates, not available to plants (Antoun, 2012; Oteini et al., 2015). The overuse of chemicals and chemical fertilizers as an alternative to overcome the P deficiency has led to the lethal consequences to useful

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arthropods and other beneficial microbes as well as led to soil pollution (Popavath Ravindra Naik et al., 2008). Furthermore, the efficiency of applied phosphorus rarely exceeds 30% due to fixation in soil (Sharma et al., 2013).

Nowadays, the new approach to farming, often referred to as sustainable agriculture, requires agricultural practices that are friendlier to the environment and that maintain the long-term ecological balance of the soil ecosystem (Khan et al., 2009). In this context, the use of microbial inoculants (biofertilizers) including PSM in agriculture represents an environment friendly alternative to further applications of mineral fertilizers. The association between PSM and plant roots plays a key role in P nutrition in many agro ecosystems, particularly in P-deficient soils (Goldstein 2007; Jorquera et al., 2008). Although several phosphate solubilizing bacteria (PSB) occur in soil and in plant rhizosphere, the amount of P released by these micoorganisms is generally not sufficient to fulfill the requirements of growing plants (Rodríguez et al., 2006). The inoculation of plants with selected PSB to increase native population can mobilize P from poorly available sources and therefore improve plant nutrition (Richardson 2001; Guiñazu et al., 2010). Phosphate Solubilizing Microbes (PSM) has the potential to reduce application rates of phosphate fertilizer by 50% without significantly reducing crop yield (Yazdani et al., 2009). Phosphate Solubilizing Bacteria (PSB) may also be useful in the phyto-remediation of heavy metal impacted soil (Ahemad, 2015; Monica and Harshada, 2015) or for bioleaching of rare Earth elements for mined ores (Shin et al., 2015). Among the alternative P sources, the most important are locally available rock phosphate (RP) resources. 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 of the RP resources are readily plant available and agronomically reactive when applied directly to the soils.

It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (Kim et al., 1997; Fankem et al., 2008), which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994). Continued exploring the natural biodiversity of soil microorganisms and the optimization/manipulation of microbial interactions in the rhizosphere represents a prerequisite step to develop more efficient microbial inoculants with phosphate solubilizing ability.

Therefore, knowledge on biodiversity of phosphate solubilizing microorganisms is essential to understand their ecological role and their utilization in sustainable agriculture. The aim of this research is to evaluate the phosphate solubilizing microorganism populations in the rice rhizosphere from two agro-ecological zones of Cameroon.

MATERIALS AND METHODS

Sampling sites

The soil samples were collected in three locations located in two agro-ecological zones of Cameroon (Figure 1). The Santchou locality in the agro-ecological zone III, characterized by an equatorial mountain climate and high rainfall (2000 to 3000 mm). Temperatures are fairly fresh for several months and the dry period is very short and lasts more than 2 months (Martin and Segalen, 1966). Located between 9°50' and 10°06' North latitude and between 5°20' and 5°12' longitude East, Santchou is an area estimated to approximately 900 km^2, with reddish soils, ferralitiques melanized, existing on the volcanic hills of the Eastern part of West Cameroon (Claisse and Laplante, 1953). Here, sampling was made on an area of 500 m^2, intended mainly to the rice cultivation within the five past years.

The agro-ecological zone V, composed of Nkolbisson and Nyokon localities, is a moist forest zone with bimodal rainfall of about 1545 mm/year, with maxima in May and October. The annual average temperatures range between 23 °C (July and August) and 26 °C in February (Fankem, 2007). It is situated below 3°52' North latitude and under 11°32' longitude (Bachelier, 1959). That zone is characterized by ferralitique, reddish or yellowish soils according to locations (Ambassa-Kiki and Babalola, 2000). Soil samples were collected on IRAD

Nkolbisson's experimental field of 500 m^2 of 2 years rice cultivation, and in Nyokon farm field of 1ha where rice is grown for the first time (Figure 1).

Sampling

The sampling technique consisted of zigzag sampling because it takes into account the intrinsic variability of soil related to the land use and cultural practices. A composite soil sample was obtained by mixing 30 sub-samples collected at the rhizosphere of rice plants from 0 to 30 cm depth. Samples were taken into sterile containers and took to the laboratory where they were air dried, crushed and a part sieved with a 2 mm sieve mesh to remove pebbles and large organic debris while the other part remained unsieved for soil physico-chemical analysis. The samples were kept in sterile plastics bags and stored in the refrigerator at 4 °C for further biological analysis.

Evaluation of the most probable number of phosphate solubilizing microorganisms

The most probable number of microorganisms solubilizing phosphate was evaluated on NBRIP medium (Nautiyal, 1999) containing per liter of distilled water: glucose 20 g; $MgCl_2 \cdot 6H2$ 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.25 g; KCl, 0,2 g; $(NH_4)_2SO_4$ 0,1 g; $Ca_3\ (PO_4)_2$ 5.02 g, Agar 15 g; pH 7.2. For this evaluation, 5 g of soil sample were suspended in 45 ml of sterile distilled water. After stirring and homogenization, decimal dilutions were made 10^{-1} to 10^{-7} dilution. One (1) ml of 10^{-5}, 10^{-6} and 10^{-7} dilution was introduced into sterile Petri dishes in triplicate, which subsequently received approximately 12 ml of NBRIP medium. After 5 days incubation at 28 °C, the total number of microorganisms and the number of microorganisms showing halo zone around their colonies were assessed by visual and direct counting.

Isolation of microorganisms

The isolation of microorganisms was made on non-selective medium to allow the greatest diversity of microorganisms to grow. To do this, 5 g of soil were suspended in 45 ml of sterile distilled water. After stirring and homogenization, decimal dilutions were made as described above. One (1) ml of 10^{-5}, 10^{-6}, 10^{-7} dilution was introduced in sterile Petri dishes that further received about 12 ml nutrient agar containing per liter of distilled water: NaCl, 3 g; Yeast extract, 3 g; Peptone, 5 g; Agar, 15 g; pH 7. The Petri dishes were then incubated at 28 °C for 5 days. The different bacterial colonies were transferred into new Petri dishes containing the same medium. The operation was repeated several times until obtaining pure bacterial colonies. The obtained isolates were kept in tubes containing the same medium and stored in at 4 °C. The tubes were renewed every two months to maintain the isolates viable.

Assessment of the diversity of isolates

The diversity of the isolates was made from the macroscopic description of the bacterial colonies. It was based on the shape (round, irregular, star and invasive), relief (convex, flat concentric wave), the contour (regular, irregular), size (very small colony, ϕ<1 mm; small colony, 1<ϕ<2 mm; average colony, 3<ϕ<5 mm; big colony, ϕ>5 mm), surface (smooth, rough) and color. This description allowed highlighting the similarities and the differences between isolates. The construction of the phylogenetic dendrograms was made using SLSTAT Software version 2014.

Assessment of the ability of isolates in solubilizing rock phosphate of different origins in solid medium

Rock phosphates

Four rock phosphates from different origins have been used, including the Malian (total P, 30%; available P, 12.98%) Mexican (total P, 28%; available P, 8.87%), Moroccan (total P, 13%; available P, 9.33%) and Algerian (total P, 29%; available P, nd) rock phosphates (Fankem et al., 2014). In order to get rid of their soluble factions, they have been washed 4 times with warm water following the cycle: 1 hour - 24 hours - 1 hour - 24 hours. They were then oven dried at 60 °C until complete evaporation of the water and then homogenized before use.

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 centrifuged at 10.000 g for 10 minutes 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 the concentration of the inoculums of $4.5 – 5\times10^{8}$ CFU / ml.

Activity of isolates on plates

Evaluation of the ability of all isolates in solubilizing the different types of phosphate was made in Petri dishes containing NBRIP medium (Nautiyal, 1999) as previously described. Sterile Petri dishes were subdivided into four compartments and the centre of each compartment received 3 µl of bacterial suspension obtained as previously described. The operation was performed in triplicate to allow the statistical analyses. Incubation was made at 28 ^{o}C for five days within which the index of solubilization was recorded according to Qureshi et al. (2012): "IS = (diameter of the colony + diameter of halo zone)/(diameter of the colony)".

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 at $p<0.05$.

RESULTS

Characteristics of soils of the different sites

The physico-chemical analyses of soils samples from different sites have shown different characteristics from each other (Table 1). In general, soils are all acidic soils with a pH of 4.87 for Nkolbisson, 5.52 for Nyokon and 4.76 for Santchou. The amounts of available phosphorus in general are low with respectively 15.42, 10.61 and 6.54 mg/kg for Nkolbisson, Santchou and Nyokon. Organic matter in Nyokon soil (50.53 mg/kg), is much more higher than those of Nkolbisson (35.77 mg/kg) and Santchou (17.15 mg/kg). The bases exchangeable (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) are more represented in soil of Nyokon, followed by that of Nkolbisson and Santchou. The Cation Exchange Capacity (CEC) decreases from Nyokon soil (12.52 Cmol/kg) Santchou (9.73 Cmol/kg) and Nkolbisson (8.99 Cmol/kg) (Table 1).

The most probable number of phosphate solubilizing microorganisms

The phosphate solubilizing microorganisms was viewed over the non solubilizers microorganisms through the halo zone (translucent) around their colonies. In general the values of the most probable number of PSM ranged between 22 and 53%. The soil from Nkolbisson showed a total microorganism number of $25\text{x}10^{7}$ out of which $12\text{x}10^{7}$ (48%) were PSM. At Nyokon, a total microorganism number was $6.34\text{x}10^{7}$ with $3.35.10^{7}$ PSM (52.80%), while Santchou sample showed a total microorganism number of $49.43.10^{7}$ with $10.8\text{x}10^{7}$ PSM, accounting for 22.44% (Table 2).

Isolation of microorganisms

The different isolates were obtained on the basis of their morphological and physical characteristics. A total of 65 isolates were obtained from the three soil samples. Rhizospheric soils of Nkolbisson and Nyokon allowed obtaining 23 isolates that have been labeled NK and NY respectively, while 19 isolates were obtained from Santchou and labeled SA.

Diversity of isolates from the rice rhizosphere of the different sites

Diversity of isolates from Nkolbisson soil sample

The results shows some similarities and dissimilarities between isolates obtained from soil sample collected at Nkolbisson. Three broad groups are represented in this case with similarity index ranging between 200 and 300% (Figure 2a). Isolates within the same group are closer each to others than in the different groups.

The first group (I) is represented by seven isolates among which: NK44, NK41, NK43, NK42, NK32, NK31 and NK21. Among the seven isolates, five (NK44, NK41, NK43, NK32 and NK31) was characterized as rock phosphate solubilizers. The second group (II) is represented only by NK10 that is a rock phosphate solubilizer, while the third group (III) is constituted of fifteen isolates (NK16, NK14, NK24, NK22, NK17, NK23, NK19, NK11, NK2, NK9, NK6, NK8, NK13, NK3, NK1) among which only three (NK22, NK23, NK19) did not show the rock phosphates solubilizing capacity.

Diversity of isolates from Nyokon soil sample

The results show some similarities and dissimilarities between isolates obtained from this soil sample collected in Nyokon rice field. Three broad groups are also represented here with a dissimilarity index below 100 (Figure 2b).

Like in Nkolbisson rice field, 23 isolates were obtained from the soil sample collected in this area, among which a total of 18 have displayed the aptitude for rock phosphate solubilization. The first group is made of two isolates (NY10 and NY22), both the rock phosphate solubilizers. The second group is constituted of 7 isolates (NY14, NY15, NY16, NY17, NY18, NK20 and NY21) among which only NY16 could not displayed the aptitude in solubilizing the different rock phosphates. The third group is the most diverse and consists of 14 isolates (NY1 NY2 NY3 NY4, NY7, NY8, NY9, NY11, NY12, and NY13) among which ten solubilizers, the rock phosphates being recalcitrant to NY19, NY7, NY23 and NY1.

Diversity of isolates from Santchou soil sample

The results show some similarities and dissimilarities between the isolates obtained from that soil sample. Four major groups are represented at a less than 50% similarity index (Figure 2c). A total of 19 isolates distributed into four main groups were obtained from soil sample collected in Santchou rice field.

The first group is constituted of four isolates (SA12, SA5, SA1 and SA15) with 50% of rock phosphate solubilizers (SA5 and SA1). The second group is the most diverse and consisted of 8 isolates (SA3, SA2, SA11, SA18, SA13, SA9, SA8 and SA6) among which only two (SA3 and SA8) did not showed ability in solubilizing rock phosphates. The third group consisted of 6 isolates (SA22, SA19, SA16, SA10, SA14 and SA23) among which only one (SA10) was not able to solubilize rock phosphates. The last group, the fourth is made of only one isolate (SA21) which is also a rock phosphate solubilizer

Diversity of combined isolates from the different sites

While grouping all the isolates (65) obtained from the different locations, the results allow seeing the closer relationships between all isolates, with the total number of groups not far from those obtained in a single location. Four major groups are represented at a level of 250% similarity index (Figure 3). The first group is the wider group constituted of 33 isolates, representing 51% of the total isolates. Among these, there are 12 (36.4%), 7 (21.2%) and 14 (42.4%) for Santchou, Nkolbisson and Nyokon respectively.

The second group is made of 19 isolates representing 29% of the total isolates among which there are 6 (31.6%), 6 (31.6%) and 7 (36.8%) for Santchou, Nkolbisson and Nyokon respectively. The third group consisted of 12 (18%) isolates of the total. Among these, there are 1 (8.3%), 9 (75.0%) and 2 (16.7%) for Santchou, Nkolbisson and Nyokon respectively. The last and the smallest group is represented only by one isolate (2% of the total isolates) of Nkolbisson.

Ability of isolates in solubilizing rock phosphate of different origins in solid media

The screening of isolates in Petri dishes amended with different rock phosphates allowed the determination of their ability in solubilizing these rock phosphates.

Isolates from Nkolbisson soil sample

The results indicate that 18 isolates over 23 have solubilized at least one rock phosphate type. The Algerian and Malian rock phosphates were solubilized by most of isolates (15). The Mexican RP showed five solubilizations over 18, while the Moroccan rock phosphate was the most recalcitrant with only one solubilization (Table 3).

The rock phosphate solubilization varies from one isolate to another. Significant differences in the solubilization ability of the different isolates are noted. Therefore, NK43 isolate was the only one able to solubilize all the four rock phosphate types, while three isolates (NK44, NK40 and NK31) showed positive activity on three RP (Algeria, Mali and Mexico) over the four tested. Regarding the Algerian RP, NK10 is the isolate that showed the greatest solubilization ability with an index of solubilization of 5.8 while NK2, NK11, NK16 and NK24 were not able to solubilize the Algerian RP. As far as concern the Malian RP, NK16 and NK13 are the most efficient isolates with an index of 5.3 and 5 respectively. On the other hand, NK6 is the only isolate unable to dissolve this type of phosphate. NK43 is the most efficient isolate with an index of solubilization of 3.5 and the only one that was able to solubilize the Moroccan rock phosphate. For Mexican RP, NK43 remains the most efficient isolate with an index of 5.24; it is followed by NK31, NK16, NK44 and NK40 with the index of 4.7, 4.5, 3.7 and 2.9 respectively.

It appears from this result that the most efficient isolate is the NK43 with 4.3 as index of solubilization, followed by NK31 and NK44 with respectively 3.80 and 3.46 as index of solubilization (Table 3). The less efficient isolates are NK4 and NK2 with an index of solubilization of 1.71 and 1.75 respectively. The speed of solubilization also varied from one isolate to another. The fastest isolates (NK31 and NK17) showed the solubilization ability the first day, while isolates NK44, NK13, NK11, NK9, NK6, NK3, NK2 and NK1 could show the activity the second day. The slowest ones are: NK43, NK41, NK40, NK32, NK16, NK14, NK10 and NK8 could show the activity only the third day.

Isolates from Nyokon soil sample

Nineteen isolates over the 23 obtained from Nyokon soil sample showed an ability to solubilize at least one rock phosphate type. Significant differences in the solubilization ability of different isolates as well as the rock phosphate type are observed. One isolate (NY5) was able to solubilize all the four rock phosphate types (Table 4). Two isolates, NY10 and NY11 solubilized three rock phosphate types (Algerian, Malian and Mexican RP). Three isolates (NY6, NY12 and NY21) showed the activity only on plated supplemented with Malian RP. The Malian RP is the easiest RP that is solubilized by the 19 solubilizers. The Algerian RP is the second rock phosphate to be solubilized with 7 isolates over the 19, while the Mexican and Moroccan RP seem to be the most recalcitrant.

NY5 is the most efficient isolate on Malian RP with an index of solubilization of 6, followed by NY17 (IS=5.18). For Algerian RP, NY14 is the most efficient isolate with an index of 4.63, followed by NY22 (IS=4.37), while for Moroccan RP, NY5 is the most efficient isolate with an index of 3.5. In addition, NY5 is the only one among the 19 isolates that is able to solubilize the Moroccan RP. NY11 is the most efficient isolate on Mexican RP with an index of 5.09, followed by NY5 (IS=3.51) and NY10 (IS=3.33).

The results indicate that NY11 is the isolate with the greatest ability in RP solubilization with an average index of 3.94 (Table 4); It is followed by NY10 with an index of solubilization of 3.65. The less efficient isolates are NY12, NY6 and NY21 with index of solubilization equal of 1.33, 1.39 and 1.49 respectively.

The speed of solubilization of the different rock phosphates varied from one isolate to other. Twelve isolates (NY2, NY3 NY4, NY5, NY9, NY11, NY12, NY13, NY15, NY18, NY19 and NY20) over the 19 solubilizers showed the activity from the first day after incubation, 3 isolates (NY14, NY17, NY21) on the second day and finally, 4 isolates (NY6, NY8, NY10 and NY22) the third day (Table 4).

Isolates from Santchou soil sample

The same trend was seen as in other locations. Here, the solubilization depends on the isolate and according to the rock phosphate type. Significant differences are observed within isolates and within RP types (Table 5). The Malian rock phosphate is the easiest RP that was solubilized by 8 isolates over the 9; it is followed by the Algerian RP which is solubilized by 5 isolates. The Moroccan and the Mexican RP are the least solubilized with only 3 solubilizers. Two isolates, SA18 and SA19 were able to solubilize all the four rock phosphate types, while one isolate (SA23), solubilized 3 rock phosphates (Algerian, Malian and Moroccan RP) over the four RP.

For Algerian RP, isolates SA18 and SA23 were the most efficient with respective index of solubilization of 5.77 and 5.59. While considering the Malian RP, SA23 is the most efficient isolate with an index of 4.94, followed by SA13 and SA1, which have respective index of solubilization of 4.42 and 4.43. Regarding the Moroccan RP, A23 is the most efficient isolate with an index of 4.57; it is followed by SA19 and SA18 with solubilization indexes respectively equal to 4.5 and 3.92. Mexican RP, SA9 is the most efficient isolate with an index of 7.24, the highest index obtained from all isolates. It is followed by SA18 and SA19 with index of solubilization respectively equal to 3.69 and 2.7 (Table 5).

Regarding the solubilization of the different RP, the isolate SA23 is the most efficient of all with an index of solubilization of 4.8, followed by SA18 isolate (4.17), while the least efficient isolate is SA22 with an index of solubilization of 1.4 (Table 5).

According to the facility of the different RP to be solubilized, the Malian RP was the easiest with an average index of 2.70, followed by Algerian RP (IS=2.20), the Moroccan and Mexican RP being the most recalcitrant RP with respectively 1.71 and 1.76 as index of solubilization.

While considering the speed of solubilization, two isolates (SA9 and SA23) solubilized faster than isolates SA5, SA13, SA18 and SA19 which solubilized the second day, and finally than SA1, SA2 and SA11 which showed activity only the third day.

Solubilization according to the phosphate origin

The solubilization of the different rock phosphates varied according to the phosphate type. Significant differences were observed within the solubilization rate of the different RP (Table 6). In general, the Malian rock phosphate appeared the most solubilized in all locations with index of solubilization ranging between 2.70 and 3.63, with an average of 3.28. The Algerian RP comes second with an average index of solubilization of 2.56, followed by the Mexican RP with an average of 1.69. In all locations and in all cases, the Moroccan RP appeared the most recalcitrant RP for all isolates.

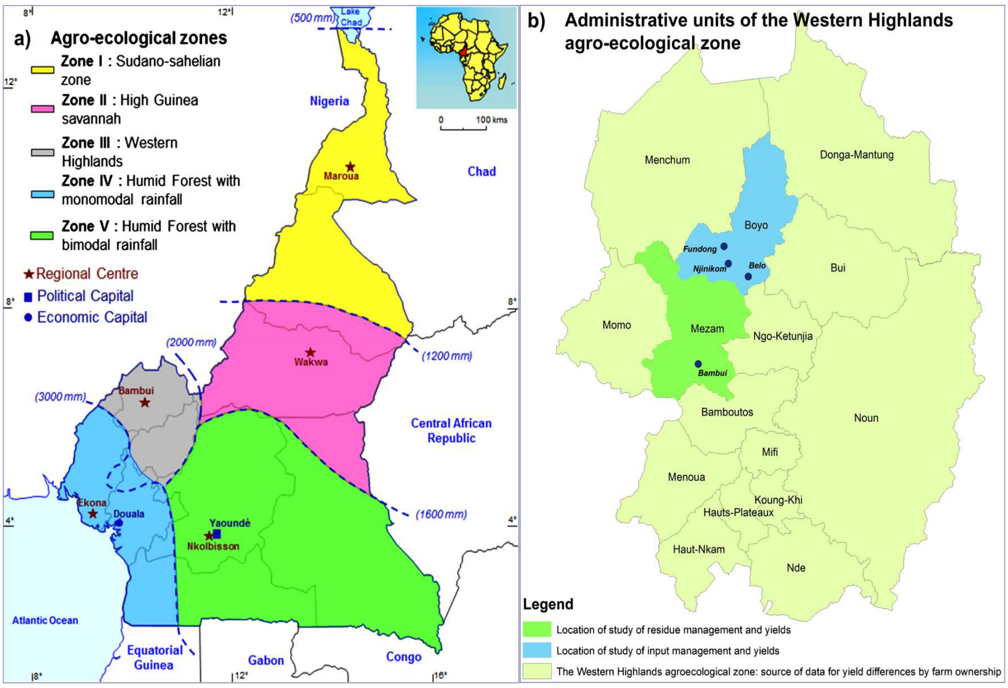
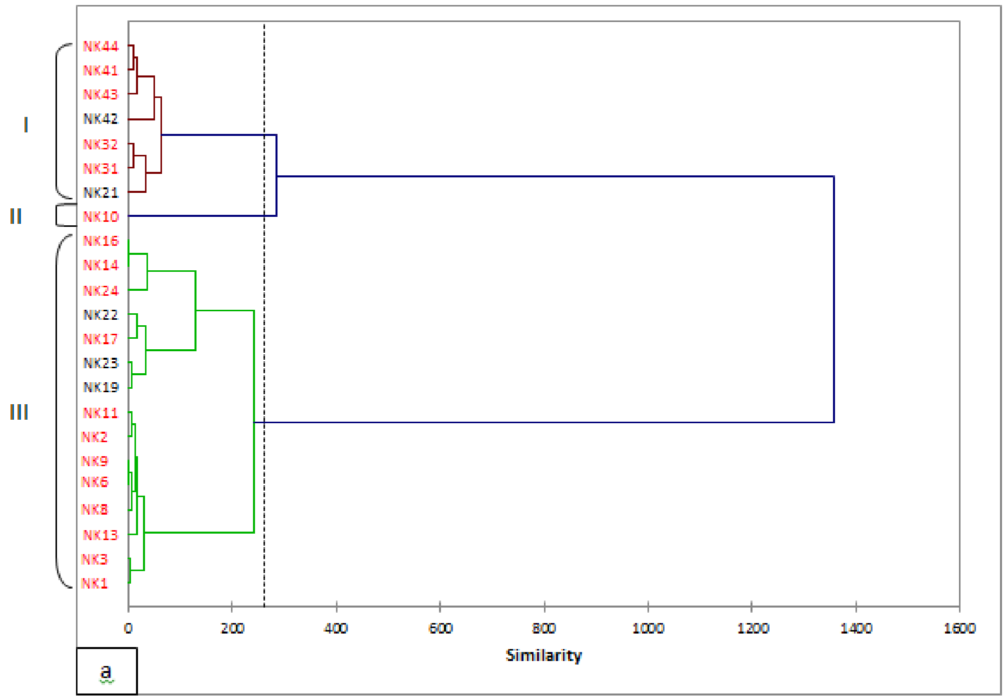


Figure 1: Sampling sites (yellow stars) in two out of the five agro-ecological zones of Cameroon: Nkolbisson and Nyokon in zone V (green color), Santchou in zone III (grey color).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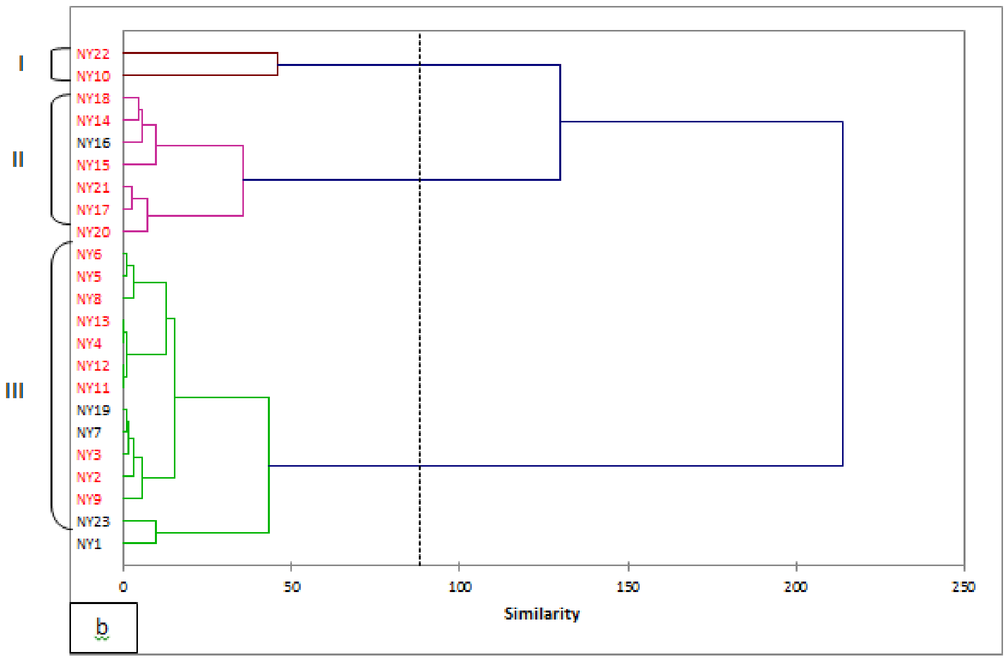
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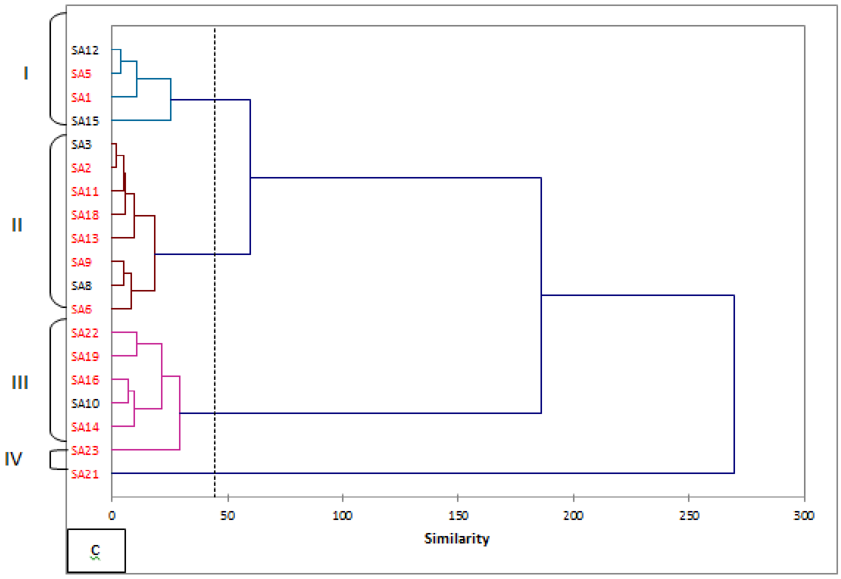

Figure 2: Dendrogram showing diversity within rock phosphate solubilizing isolates (in red color) obtained from soil samples collected at Nkobisson (a, similarity < 300%), Nyokon (b, similarity < 100%) and Santchou (c, similarity < 50%). Isolates are distributes into four main groups numbered I, II, III, IV.

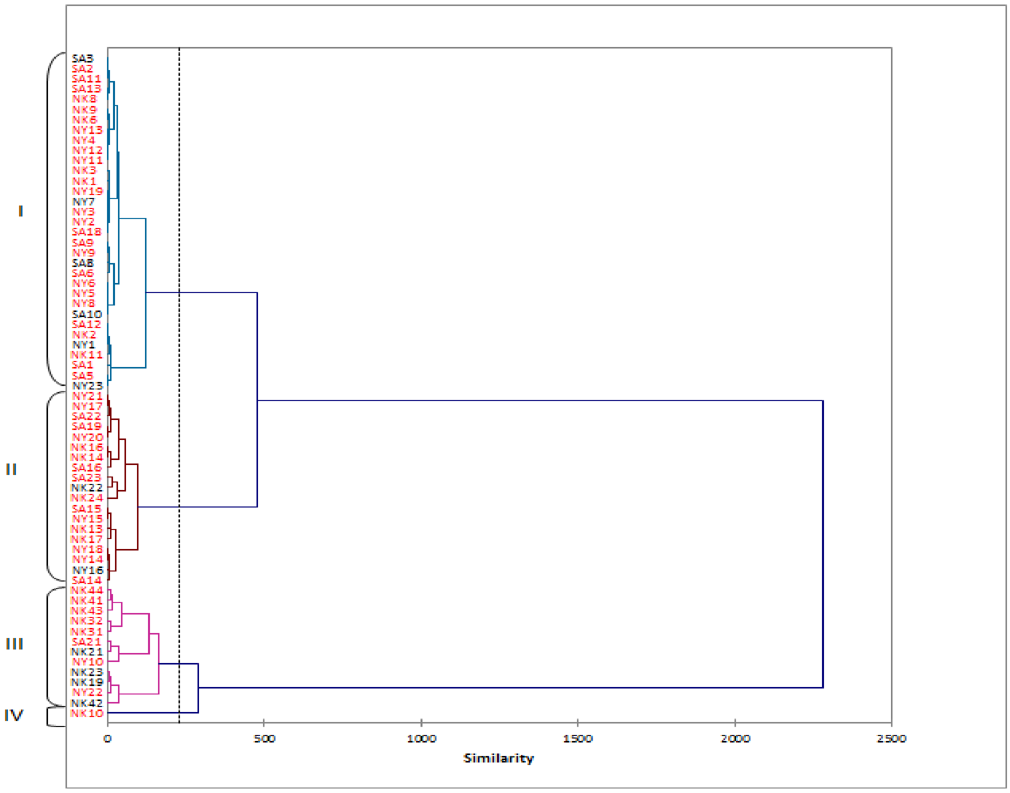

Figure 3: Dendrogram showing cumulative diversity within rock phosphate solubilizing isolates (in red color) obtained from soil samples collected at the three sampling sites (similarity < 250%). Isolates are distributes into four main groups numbered I, II, III, IV.

Table 1: Physico-chemical characteristics of the soil from the three sampling sites.

Origin of the soil		Element (mg/kg)					Element (cmol/kg)				
	pH	**Available P**	**Org mat.**	**Org C**	**Total N**	**C/N**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**CEC**
Nkolbisson	4.87	15.42	35.77	20.75	2.48	8.36	5.21	0.49	0.25	0.09	9.00
Nyokon	5.52	6.54	50.53	29.31	2.86	10.26	7.20	1.17	0.39	0.13	12.52
Santchou	4.76	10.61	17.15	9.95	1.76	5.64	1.91	0.28	0.22	0.07	9.73

Org mat (Organic matter), Org C (Organic carbon), P (Phosphorus), CEC (Cation Exchange Capacity).

Table 2: The most probable number (MPN) of phosphate solubilizing microorganisms from soils collected in the three sampling sites.

Number of microorganisms (10^7 CFU)	**Origin of the soil**			**Total number**
	Nkolbisson	**Nyokon**	**Santchou**	
Total number	25	6 .34	49.43	80.77
Number of PSM	12	3.35	10.80	26.15
Pourcentage of PSM (%)	48	52.8	22.44	32.37

CFU (Colony forming unit), PSM (Phosphate solubilizing microorganism).

Table 3: Solubilization of the different rock phosphates (RP) by isolates from Nkolbisson soil sample.

	Index of solubilization (IS)					Speed of solubilization (day)
Isolates	**Algerian** RP	**Malian** RP	**Moroccan RP**	**Mexican RP**	**Average**	
NK1	3.85 cde	3.83 cde	1.00 a	1.00 a	2.42 bc	2nd
NK2	1.00 a	2.83 b	1.00 a	1.00 a	1.46 a	2nd
NK3	3.95 cde	3.67 cd	1.00 a	1.00 a	2.41 bc	2nd
NK6	4.77 fgh	1.00 a	1.00 a	1.00 a	1.94 b	2nd
NK8	3.05 b	4.15 cdef	1.00 a	1.00 a	2.39 bc	3rd
NK9	4.93 gh	1.00 a	1.00 a	1.00 a	1.98 b	2nd
NK10	5.80 i	1.00 a	1.00 a	1.00 a	2.20 bc	3rd
NK11	1.00 a	5.00 hi	1.00 a	1.00 a	2.00 b	2nd
NK13	3.88 cd	5.00 hi	1.00 a	1.00 a	2.72 c	2nd
NK14	4.98 h	4.77 fghi	1.00 a	1.00 a	2.94 cd	3rd
NK16	1.00 a	5.30 i	1.00 a	4.54 d	2.96 cd	3rd
NK17	4.28 efg	4.47 efgh	1.00 a	1.00 a	2.69 c	1st
NK31	4.22 def	4.90 ghi	1.00 a	4.71 de	3.71 d	3rd
NK32	3.60 bcd	3.70 cd	1.00 a	1.00 a	2.33 bc	1st
NK40	3.33 bc	3.61 c	1.00 a	2.94 b	2.72 c	3rd
NK41	3.00 b	2.81 b	1.00 a	1.00 a	1.95 b	3rd
NK43	4.43 efgh	4.30 defg	3.55 b	5.24 e	4.38 e	3rd
NK44	3.00 b	4.52 fgh	1.00 a	3.70 c	3.06 cd	3rd

Different letters within a column indicate significant differences between treatments at *P<0.05*.

Table 4: Solubilization of the different rock phosphates (RP) by isolates from Nyokon soil sample.

	Index of solubilization (IS)					Speed of solubilization (day)
Isolates	**Algerian RP**	**Malian RP**	**Moroccan RP**	**Mexican RP**	**Average**	
NY2	1.00 a	2.83 ab	1.00 a	1.00 a	1.46 a	1st
NY3	1.00 a	3.8 3 cd	1.00 a	1.00 a	1.71 ab	1st
NY4	1.00 a	3.74 cd	1.00 a	1.00 a	1.69 ab	1st
NY5	4.10 de	6.00 g	3.50 b	3.51 b	4.28 d	1st
NY6	1.00 a	2.92 ab	1.00 a	1.00 a	1.48 a	3rd
NY8	1.00 a	4.04 de	1.00 a	1.00 a	1.76 ab	3rd
NY9	1.00 a	3.37 bc	1.00 a	1.00 a	1.59 ab	1st
NY10	3.40 bc	4.51 e	1.00 a	3.33 b	3.06 c	3rd
NY11	3.77 cd	3.62 cd	1.00 a	5.09 c	3.37 c	1st
NY12	1.00 a	2.64 a	1.00 a	1.00 a	1.41 a	1st
NY13	3.70 cd	2.53 a	1.00 a	1.00 a	2.06 b	1st
NY14	4.63 e	3.43 c	1.00 a	1.00 a	2.52 bc	2nd
NY15	1.00 a	3.51 cd	1.00 a	1.00 a	1.63 ab	1st
NY17	2.92 g	5.18 f	1.00 a	1.00 a	2.53 bc	2nd
NY18	1.00 a	2.77 a	1.00 a	1.00 a	1.44 a	1st
NY19	1.00 a	4.03 de	1.00 a	1.00 a	1.76 ab	1st
NY20	1.00 a	2.99 ab	1.00 a	1.00 a	1.50 a	1st
NY21	1.00 a	3.45 c	1.00 a	1.00 a	1.61 ab	2nd
NY22	4.37 e	3.65 cd	1.00 a	1.00 a	2.51 bc	3rd

Different letters within a column indicate significant differences between treatments at *P<0.05*.

Table 5: Solubilization of the different rock phosphates (RP) by isolates from Santchou soil sample.

Isolates	Index of solubilization (IS)					Speed of solubilization (day)
	Algerian RP	Malian RP	Moroccan RP	Mexican RP	Average	
SA1	3.23 **b**	4.34 **cd**	1.00 **a**	1.00 **a**	2.39 **c**	3^{rd}
SA2	1.00 a	4.13 c	1.00 a	1.00 a	1.78 ab	3^{rd}
SA5	1.00 a	3.32 b	1.00 a	1.00 a	1.58 a	2^{nd}
SA9	3.33 b	1.00 a	1.00 a	7.24 d	3.14 d	1st
SA11	1.00 a	4.00c	1.00 a	1.00 a	1.75 ab	3^{rd}
SA13	1.00 a	4.43 cd	1.00 a	1.00 a	1.86 b	2^{nd}
SA18	5.77 c	3.33 b	3.92 b	3.69 c	4.18 e	2^{nd}
SA19	3.83 b	3.33b	4.50 bc	2.70 b	3.59 de	2^{nd}
SA23	5.59 c	4.94 d	4.57 c	1.00 a	4.03 e	1^{st}

Different letters within a column indicate significant differences between treatments at *P<0.05*.

Table 6: Ability of rock phosphates to be solubilized by the different isolates from the three sampling sites.

Type PR	Index of solubilization (IS)			
		Nyokon	Santchou	Average
Algérie	3,43 **c**	2,05 **c**	2,20 **b**	2.56 **c**
Mali	3,52 **c**	3,63 **d**	2,70 **c**	3.28 **d**
Maroc	1,13 **a**	1,13 **a**	1,71 **a**	1.32 **a**
Mexique	1,85 **b**	1,47 **b**	1,76 **a**	1.69 **b**

Different letters within a column indicate significant differences between the treatments at *P<0.05*.

DISCUSSION

The soil samples collected from the three different locations are all acidic with pH below 6. Acidity generally characterized the soils from the southern part of Cameroon.

The soils available phosphorus are 6.54 ppm, 10.61 ppm, and 15.42 ppm respectively for Nyokon, Santchou and Nkolbisson and can be qualified as soils of low available phosphorus (Giroux and Tran, 1996). However, according to the rice minimum requirement of soil available phosphorus which is about 10 ppm (IRRI, 1970; Boudoudou et al., 2009), these soils can be considered as deficient for Nyokon, with moderate amounts for Santchou and Nkolbisson. The last two cases may be due to the amendment applied in those rice fields respectively belonging to the Centre of Agricultural Research for Development in Nkolbisson, and for SODERIM, an old rice Cameroonian company in recovery for Santchou site. Thus, in order to improve their production, considerable amendments are made on these farm fields. Furthermore, the amounts of available phosphorus obtained from those soil samples highlight the limits of the use of soluble phosphorus amendments in southern Cameroonian soils (Fankem et al., 2006), because of their acidity and their high iron and aluminum concentration, leading most (75-90%) of the soluble phosphate into insoluble complexes such as iron and aluminum phosphates (Supanjani et al., 2006). The Nyokon site, which is cultivated for the first time, received no amendment.

The proportions of isolates solubilizing rock phosphates obtained from the different locations allowed considering the Nyokon soil as the densest with 52.80%. It is followed by Nkolbisson with 48% and finally by Santchou soil which is less populated in PSM with

22.44%. Those proportions are not far from the findings of Kucey et al. (1989), according to whom the proportions of PSM in arable soils range between 10 and 50%, with an average of 40%. In addition, the proportion of PSM in Santchou soil is almost the half of those of Nkolbisson and Nyokon soils. This can be explained by the soil composition in small quantities of organic matter and would be explained also by a low value of the C: N ratio, against strong values of organic matter encountered in Nyokon soil. This relationship been highlighted earlier by Garcia et al. (1974) in a study on microbial activities in soils of paddy fields in Senegal. More important is the amount of available phosphorus in soil, less is the proportion of MSP.

The microorganisms were isolated and purified on nutrient agar which is a non-selective medium, to promote the growth of the greater diversity of microorganisms in the soil samples. Several researchers have also used this medium to isolate microorganisms; for instance, Islam et al. (2006) in isolation and assessment of the potential of bacteria in solubilizing the rice rhizoplane phosphate in Bangladesh.

The morphological description of pure colonies is an attempt for preliminary identification of isolates. This allowed assessing the microbial diversity of the different sites and highlighting the similarities and differences between the different isolates. This technique was earlier used by Atekan et al. (2014) who, on the same basis determined 8 isolates solubilizing phosphate from sugar cane waste.

The evaluation of the solubilization ability of isolates shows a wide range index of solubilization ranging between 2.70 and 7.24, depending on isolate. That range is close to the one (2.16 and 6.23) obtained by Maliha et al. (2004). With an average index of solubilization of 4.38, the NK43 isolate from Nkolbisson is the strongest, it is followed by isolate NY5 (IS=4.28) from Nyokon and then by isolate SA18 (IS=4.18). Based on the solubilization potential, the different isolates can be grouped according to the IS range established by Berraquero et al. (1976) and Silva Filho and Vidor (2000): low ($SI < 2$), medium ($2 < SI < 3$) or high solubilization capacity ($SI > 3$). In general, of a total of 46 phosphate solubilizing isolates recorded in the three sampling sites, 20 are of low solubilization, 16 of medium solubilization and 10 of high solubilization. Obvious correlation can be observed while comparing the MPN of phosphate solubilizing microorganisms from the different soil samples (52.8% for Nyokon, 48% for Nkolbisson and 22.44 for Santchou) with those obtained during the assessment of the ability of those isolates in solubilizing phosphates on plates, (82.6% for Nyokon, 78.26% for Nkolbisson and 47.36 for Santchou). However, the high percentages obtained on plates might be based on physical and chemical properties of phosphates used during the two processes.

Conclusion

The isolates able to solubilize the rock phosphates of different origins were found in rice rhizospheric soils collected in all the sampling sites. This ability was found through halo detection method on plates supplemented with different sparingly soluble rock phosphates. The morphological description of the isolates allowed highlighting the relationships between the different isolates from the different sites. These results support the findings of several authors concerning the phosphate solubilizing ability of some main genera of bacteria and fungi. This is the first work reporting phosphate solubilizing microorganisms on rice rhizosphere in Cameroon. Studies are in progress for identifying and evaluating the effect of inoculation with those isolates on the growth and phosphorus uptake of rice plants under nursery and field conditions.

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