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Research Article

Diversity of the Phosphate Solubilizing Fungi (PSFs) Population from the Soybeans Rhizosphere in the Noun Valley, Western Highlands of Cameroon

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Abstract

Soil beneficial microorganisms like bacteria and fungi play a key role on soil properties, nutrients availability and fertility. Phosphate solubilizing fungi (PSFs) in plants rhizosphere have the ability to convert insoluble organic and inorganic phosphate into soluble form and make the phosphorus available to the plants. Many soils in West and North-West of Cameroon lack soluble phosphate for plants growth, development and productivity. The main objective of this study was to evaluate the diversity of phosphate solubilizing fungi under the soybean's rhizosphere in the Noun Valley, Western Highlands of Cameroon. Fifteen soybeans rhizosphere samples were randomly collected in five localities of the Noun Valley, and analyzed. Fungi isolation was done by serial dilution plate method on nutrient agar plates (PDA) amended with antibiotic, and PSF selection were done on Pikovskaya's agar (PVK) media. The phosphate (P) solubilization efficiency was done on PVK broth and modified Pikovskaya's broth amended with $Al(PO_4)$. Functional activities such as metabolites and enzymes production ability were assessing. Fungi diversity was done through macroscopic and microscopic features observation, (colony colors, mycelia shape, growth and sporulation rate, mycelia branching pattern and spores' organization). From 178 isolates obtained from the fifteen sampling sites, 148 strains grouped in four genera (*Rhizopus spp.*, *Aspergillus spp.*, *Penicillium spp.* and *Trichoderma spp.*) and 15 fungi species, showed phosphate solubilization ability. The solubilization efficiency (SE) and P-released were done at temperature range of 25 ℃- 30 ℃ and at pH range of 6-7. The SE ranged between 96. 59% and 15.7% on agar media while the amount of P released in broths ranged from 15.45 to 2.64 mg/L depending on isolates, the broth and the incubation time. Among these, *Rhizopus sp.* (15.45mg/L) showed the highest solubilization efficiency, followed by *Aspergillus sp., Trichoderma sp. and Penicillium sp*. With 13.46 mg/L, 12.03 mg/L and 9.19 mg/L respectively. The entire PSF were able to produce organic acid and showed cellulose hydrolysis activity. Therefore, these fungi species from soybeans rhizosphere are potential PSF for the solid and liquid formulation of PSF based-biofertilizers.

Keywords

Diversity, Phosphate Solubilizing Fungi, Soybeans Rhizosphere, Noun Valley

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1. Introduction

Phosphate and nitrogen are macronutrients needed by plants, especially for various protein formations. Soil phosphates are present in organic and inorganic forms. Phosphorus influence various key metabolic processes such as macromolecular biosynthesis and photosynthesis [22], stimulates roots and shoots development, early fruiting [27], and tuberization [19]. Despite the diverse forms and abundance in the soil, phosphorus is the least accessible and soluble macronutrients and hence most frequently deficient in most agricultural soils because of its low availability and its poor recovery from the applied fertilizers [49, 46]. The soil pH changes are critical for soil nutrients availability, decomposition rate of organic matter, plant nutrients uptake, microorganisms' survival and plants' health. For instance, in acidic soils, phosphorus is associated with aluminum and iron compounds whereas calcium phosphate is the predominant form of inorganic phosphate in calcareous soils. Many of the agricultural soils in subtropical areas, particularly in Cameroon, are predominantly acidic, P-deficient and have an unfavorable condition for P availability [49, 8]. In the Western Highland of Cameroun, phosphorus deficiency (47%) probably due to abundant Ferralsols (55.2%) and Nitosols (15.5%), acidity (pH < 5.5), aluminum (30%) and ferrous fixation or toxicity, leading to low plants phosphorus availability is the most pronounced soil fertility constrains in this area [18, 34].

Phosphate-solubilizing microorganisms (PSM) promote biological nutrients fixation, decomposition of organic matter and enhance nutrient uptake by converting nutritionally important elements (nitrogen, phosphorus and potassium) from unavailable to available form. PSM in the rhizosphere can increase P availability by solubilization (both lowering the pH and solubilizing iron - bound and aluminum - bound P or probably by complexing or chelating the iron and aluminum) and mineralization [42]. Several bacterial, fungal and actinomycetal strains have been identified as PSM. Microbes from genera like *Pseudomonas, Bacillus, Acinetobacter, Rhizobium, Alcaligenes, Arthrobacter, Azospirillum, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Serratia, Penicillium, Aspergillus* [42, 35] and *Trichoderma* [9] have been proved as P-solubilizing bacteria or fungi. P-solubilizing fungi (PSF) is a group of beneficial fungi with the ability to transform unavailable phosphate to availability forms. The most important genera of PSF are *Aspergillus, Penicillium, actinomycetes* and *Arbuscular mycorrhizae* [21, 41, 1]. Many fungi live in symbiotic association with plant species in which they fulfill the requirement of the host plant. Generally associated fungi are present in the three different plants regions; phyllosphere, endophytic region and rhizosphere [51, 43]. The main fungi from plant rhizosphere region are *Penicillium sp*., *Trichoderma sp*. [30], *Aspergillus spp*., *Eupeniccilium sp*., *Isaria sp*., *Leptosphaerulina sp*. [31], *A. terreus*, and *A. Luchuensis* [23].

Many of these fungi are known to be abundant in rhizospheric soil and leguminous plants such as soybeans. Soybeans (*Glycine max*) is a member of the legume plant family (*Leguminosae*); a second largest crops species and it is the most important legumes [12]. Soybeans has been used in Human and animals direct consumption or in food processing. Soybeans plants have been proved to be a good host plant with very rich rhizospheres and has been used in the intercropping system to improve soil fertility [47, 29]. They have a special characteristic that they can absorb nitrogen from the air and use it for their own growth. Soybeans plant growing in intercropping system, improved nutrients uptake by enhancing active biological processes, rhizosphere soil acid phosphatase activity, and P accessibility [53]. Phosphate solubilizing microorganisms have a great impact on symbiotic nitrogen fixation and plant growth [52]. Bacterial communities of soybeans rhizosphere changed significantly during growth, with a higher abundance of potential plant growth promoting rhizobacteria (PGPR), including *Bacillus*, *Bradyrhizobium*, and *Rhizobium* [44]. Soybeans phosphate-solubilizing fungi are fungi living in association with soybeans plant roots that can solubilized or transform phosphate from unavailable form to available form. Fungi can grow in a wide range of soil pH [14] and perform better in acidic soil conditions [3]. Fungi immobilized phosphorus better than bacteria [26]. They produce more acids and consequently exhibit greater phosphate-solubilizing activities than bacteria in both liquid and solid media [50, 40], and do not lose their solubilizing activities by repeating sub - culturing like bacteria [22]. Moreover, Soil fungi can grow in a wide range of soil improving the physio-chemical and biological properties of the soil [7]. Phosphate solubilizing fungi from soybean rhizosphere from different locations of Northern Karnataka (Belagavi, Haveri, Dharwad districts; India) were identified as *Aspergillus spp* and *Penicillium spp*. [38]. *Rhizopus ssp* from soybeans rhizosphere have shown ability to increase tomato plant protection and growth [2]. To our knowledge, none has been done so far in PSF diversity and their biological role in the Noun valley. It is important to evaluate the diversity of PSF in the Noun Valley to identify those with potential in PSF based-biofertilizer formulation.

2. Methodology

2.1. Collection of Soybean Rhizosphere Samples

This study was carried out in the Biology Laboratory of Teachers Training College Bambili and Biochemistry laboratory of the University of Bamenda in 2022-2023. Soybeans rhizosphere samples were collected the Noun Valley, Western Highlands of Cameroon. The soil in this area is acid (pH = 5.3). Systematically selected healthy soybeans plants were

collected in the randomly chosen of cultivated farms in the five localities (Menfoung, Bambili, Ndop, Bangourain and Njingoumbe) of Noun Valley (Figure 1). Six soybean rhizospheres were collected in three sites each of the five localities using a sterile cutlass. Rhizospheric soil around the plants were dug from 0 to 15 cm depth and immediately tied in a sterile black plastic bag and transported to the Laboratory for fungi isolation.

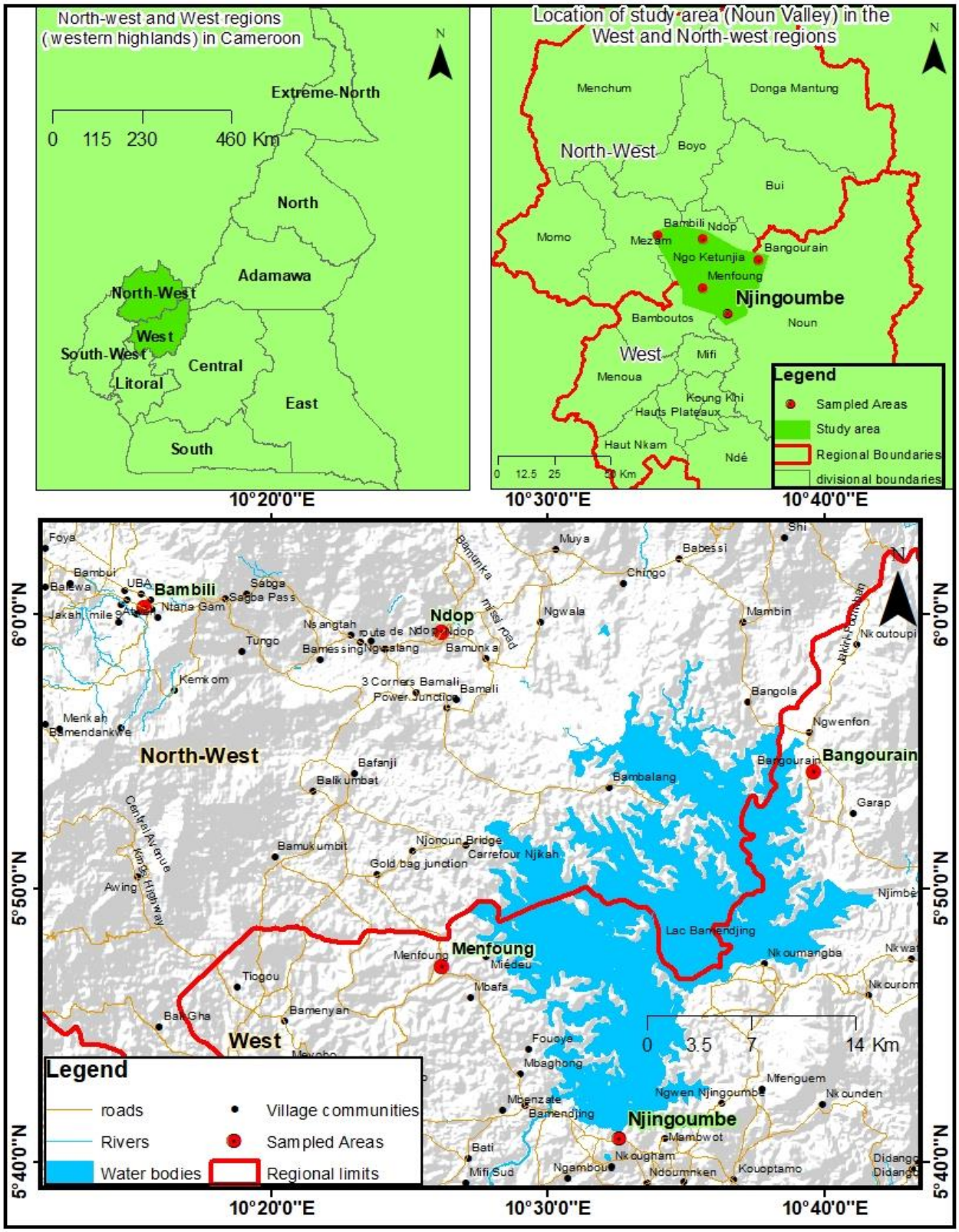

Figure 1. *Noun Valley and samples collection areas.*

2.2. Isolation of Rhizospheric Fungi

Isolation was done by serial diluted plate method on Potato Dextrose Agar (PDA) media amended with antibiotic. 1g of rhizospheric soil samples was dissolved in 9 ml (10^{-1} dilution) of autoclaved distilled water and was thoroughly being shaken. Subsequent dilution was made following the technique of Alexander [4] for serial dilution plate technique. Respectively 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of the solution was added to 9 ml, 8 ml, 7 ml, 6 ml and 5 ml of distilled water to prepare 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} dilution. The process was performed under Bunsen-burner flame to maintain sterile air conditions. A prepared and sterilized media was poured into Petri-dishes (20 ml per Petri-dish). After solidification of the medium, 0.1 ml of each sample was used to inoculate the agar medium plate. The sample was evenly spread over the agar surface in the form of line and then incubated at 25°C (ambient temperature) in darkness conditions. After 48 hours of incubation, colonies that were growing up were picked out and purified by subculturing 6 times on culture media. The Fungi were later grown in a liquid medium at 25°C and stored as a microorganism's bank. The Petri-dishes with a given fungi strain were then silt, protected and stored in microorganisms 'bank.

2.3. Screening of Phosphate Solubilizing Fungi Potentials Isolate on PVK Agar Medium

PSF selection was done on Pikovskaya's (PVK) media agar. A fungus from PDA culture media was further grown on sterilized Pikovskaya (PVK) media agar plates [37]. The sterilized PVK was prepared and poured into Petri-dishes. After 15 minutes of solidification of the medium, 0.1 ml of each sample was used to inoculate the agar medium plate by using L-Rod and then incubated at 25° (dark conditions) for 48 hours. The fungi with phosphate solubilizing ability or selected PSF from each sample was collected, recorded, and conserved in 10% glycerol for identification or diversity.

2.4. Morphological Characterization of Phosphate Solubilizing Fungi

The identification process was done using macroscopic observation, microscopic observation and microorganisms' identification key [32]. Macroscopic observation (macro-features such as colonies size and colors, shape, size elevation, surface margins, surface texture, colony growth rates, and degree of sporulation), were observed daily for their characterization according to [25], and the microscopic observation (Using a light microscope, micro-features such as vesicles shapes, conidiophores and conidia, columnar or radiate conidial heads, Hülle cells, ascomata, ascospores, branching pattern and shape phialide of isolated microorganisms were observed and draw down for their characterization [32].

2.5. Biochemical Characterization of Phosphate Solubilizing Fungi

The selectively isolated PSF strains were tested; enzymes (phosphatase, peroxidase, cellulase, lipase and amylase) and secondary metabolites (organic acid) production ability were done.

2.5.1. Phosphatase Production Ability

A slid of five days old culture of each PSF culture was used to inoculate 20 ml of PVK containing insoluble tri calcium phosphate ($CaPO_4$) and incubate for seven days at 25 ℃. The formation of clear zones around the growth of the microorganism was noted as a positive test. The strains that developed clear zones around the colonies of microorganisms growing on the plate were easily identified as phosphatase producer [36].

2.5.2. Phosphate Solubilization Efficiency

The solubilizing efficiency (SE) was determined on PVK solid media after inoculation in comparison with un-inoculated control.

SE = (Solubilization zone / colony diameter) x 100 [20].

The solubilizing efficiency was further determined on PVK broth in comparison with un-inoculated control and standard curve to better quantify the rates of P solubilization. 0.2 ml of a four-day old culture were inoculated (in three replicate) into 9.8 ml of PVK medium with 0.5% $Ca_3(PO_4)_2$ (w/v) and incubated in a shaker at room temperature. Analogous culture which contained 0.5% of $AlPO_4$ instead of $Ca_3(PO_4)_2$ was done in 250 ml conical flasks each. Un-inoculated flasks were kept for each set of treatment. Water soluble P in the culture filtrates was estimated by the chlorostannous reduced molybdophosphoric acid blue method described by Jackson [17]. Two ml of 5, 10, 15 and 30 day's old culture were centrifuge at 10,000 rpm for 10 min and the supernatant was used to estimate the P release. One ml of this supernatant was mixed with 10 ml of chloromolybdic acid and the volume adjusted to 40 ml with distilled water (mixed thoroughly). To this, 1 ml of chlorostannous acid was added and the volume was made up to 50 ml with distilled water (mixed thoroughly). Potassium dihydrogen phosphate (KH_2PO_4) was used as standard (blank reagent). The P released in the supernatant was measured at 610 nm wavelength with UV-ViS spectrophotometer. The pH of each medium was measured at the various time by pH-meter. Chloromolybdic acid reagent: dissolving 7.5 g of ammonium molybdate in 150 ml distilled water + 162 ml of con. HCl and volume adjusted using distilled water up to 1000 ml. Standard curves for metabolites analysis was done according to Lamenew [24].

2.5.3. Cellulase Production Test or Cellulose Hydrolysis

Cellulose hydrolysis was estimated by the technique proposed by Akpomie [5]. The identified cultures were individually streaked on Carboxy Methyl Cellulose (CMC) agar plates and incubated at 28°C. After 7 days of growth, the plates were observed for clear zones around the culture by treating the plates with Congo red and sodium hydroxide. Clear zones around the growth of the microorganism were regarded as a positive test, and the strain were noted as cellulase producers.

2.5.4. Catalase Production Test or Hydrogen Peroxide Reduction

This test aimed to evaluate the PSF ability to produce catalase or peroxidase that reduces hydrogen peroxide to water and oxygen. On a clean and sterilized glass slide, a smear of the organism was made in a drop of normal saline with a flame sterilized wire loop and left for 30 seconds. Thereafter, a drop of hydrogen peroxide was placed on the smeared organisms and effervescence, or bubbles appearance was observed [39]. After 30 seconds bubble formation was observed because of catalase production.

2.5.5. Amylase Production Test

The ability of the isolates to hydrolyze starch was assessed by the procedure of Aneja [6]. Petri-plates containing starch agar were inoculated with test cultures and incubated at 37°C for seven days. After the seven days of incubation at 37°, the plates were flooded with Lugol's iodine solution and allowed to stand for 15-20 minutes and observed the appearance of clear zone.

2.5.6. Lipase Production Test

Fungi were grown on peptone agar (PA) supplemented with 1% sterile tween 20 to test the production of lipase by the isolate. Four days after the incubation at 27°C, visible precipitate formation was observed around the colonies [10]. The precipitation was due to the formation of the calcium salts of lauric acid liberated by the lipase activity.

2.5.7. Organic Acid Production

Organic acid formation was evaluated on bromothymol blue media. Autolaved medium was poured in sterile Petri-plates (20 ml / plate) under sterilized conditions and allowed to solidify. Fungal colonies were inoculated on petri-plates containing medium for plate assay and the plates were incubated in inverted positions in an incubator for 72 hours at 28°C as has been done by Collins [10]. After seven days of incubation at 37°C, Positive (+) cultures were screened by observing clear halo zones on bromothymol blue medium.

2.6. Statistical Analysis

Statistical analyses were perfumed using SPSS version 23. Similarities index among fungi strains were calculated and dendogram with fungi strains relationship was generated.

3. Results

3.1. Morphological Description of the Isolate

From the five localities (I to V), 148 phosphate solubilizing fungi strains were isolated and the Ndop area showed the highest percentage (30%) followed by Menfoung (20%), Bangourain (17%), Njingoumbe (17) and lastly Bambili (16%). The fungi colony colour varied from white (59%), black (20%), green (12%), yellowish (6%) and brown (3%). Based on macro- features (growth and sporulation rate, colours, mycelia shape) and micro-features (branching pattern and spores' organization), the PSF species were grouped in nine (A, B, C, D, E, F, G, H and I) groups. Group A with five subgroups (A_1 to A_5) are white mould with different growth rates and mycelia branching pattern; and spores found within the sporangia. The group A_1 and A_2 present stolons in addition to rhizoids. Comparatively to the rest A_5 showed a very high number of branches, very high growth and sporulation rate. They belong to zygomycetes group of fungi and were respectively identified as *Rhizomucor* (A_1), *Rhizopus Oryzae* (A_2), *Rhizopus stolonifer* (A_3 and A_4), and *Rhizopus sp* (Figure 2). Group B presented some very long mycelia with two branching hyphae with spore within the sporangia and it is very closer to *Rhizopus Oryzae* (Figure 3). Group C, white at the young age and brownish at the older age, presented long and septate mycelia with three branching hyphae carrying spores within the sporangia (Figure 4). Group D presented short and aseptate yellowish mycelia with monoverticillate *Penicillium* conidia head (Figure 5). Group E showed very long and white multiple mycelium and branching pattern carrying spore along (Figure 6). Group F presented black colonies very height growth and sporulation rate, carrying biseriate *Aspergillus* conidia head with single spores (C and D) and uniseriate *Aspergillus* conidia (H and I) with spores in chains. They were identified as *Aspergillus Niger* (Figure 7). The group G, green-white and white-green in colonies carrying conidiophore and conidia, was identified respectively as *Aspergillus sp* and *Trichoderma sp.* G_1 with uniseriate *aspergillus* head conidia, white at the early stage then green was identified as *Aspergillus fumigatus* (A-E). G_2 with branching conidiophore was closer to *Trichoderma viridea* (Figure 8). Group H with brown and very short mycelia carried *Aspergillus* conidia heads was identified as to *Aspergillus flavus* (Figure 9). The final, group I yellowish or white-yellow in colony and very poor in spore with undergrowth at the old age belong to *Penicillium spp.* (Figure 10).

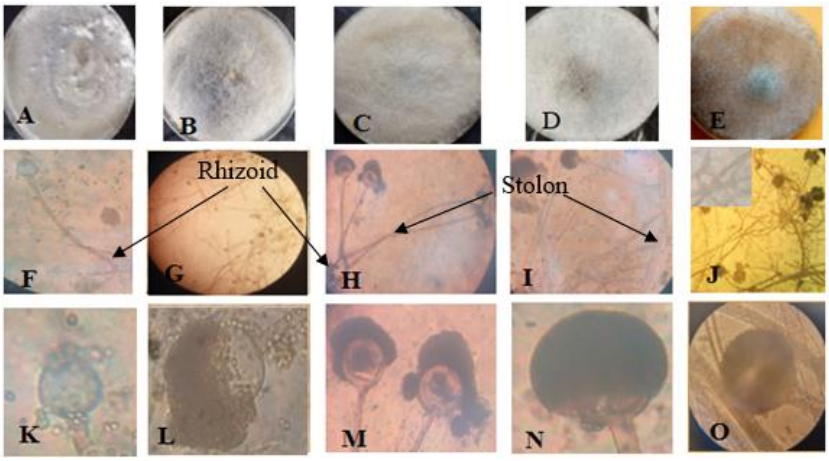

Figure 2. *Colony structure, mycelia branching pattern and spores organization of subgroup A_1 (A, F and K), A_2 (B, C and L), A_3 (C, H and M), A_4 (D, I and N) and A_5 (E, J and O).*

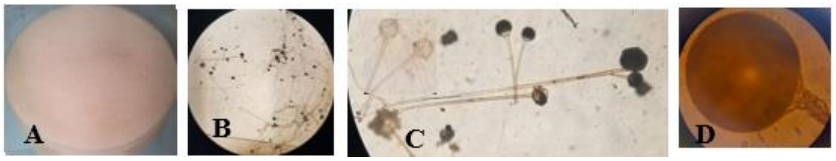

Figure 3. *Colony structure (A), mycelia branching pattern (B and C) and spores organization (D) of group B.*

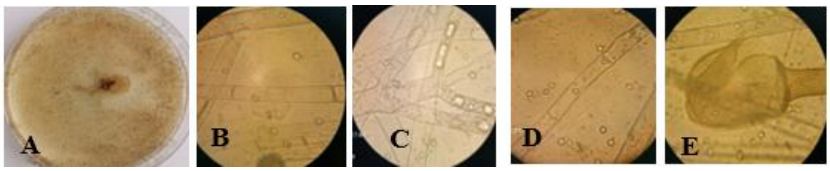

Figure 4. *Colony structure (A), mycelia branching pattern (B and C), and spores organization (E) of group C.*

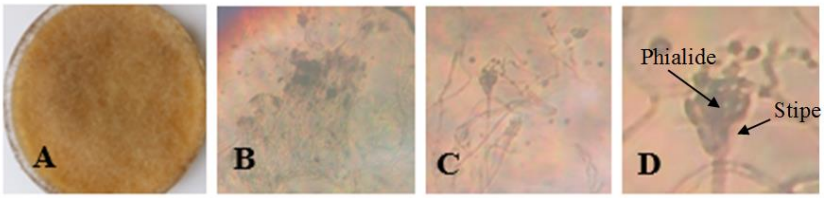

Figure 5. *Colony structure (A), conidia shape (B and C) and spores' organization (D) of group D.*

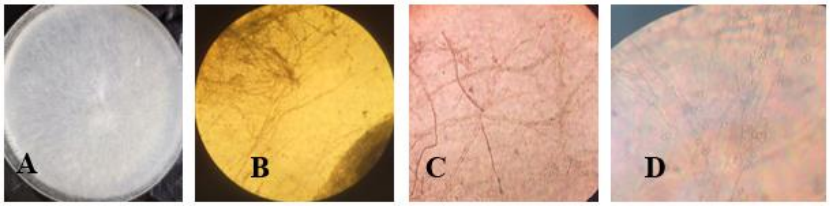

Figure 6. *Colony structure (A), mycelia branching pattern (B and C) and spores organization (D), of group E.*

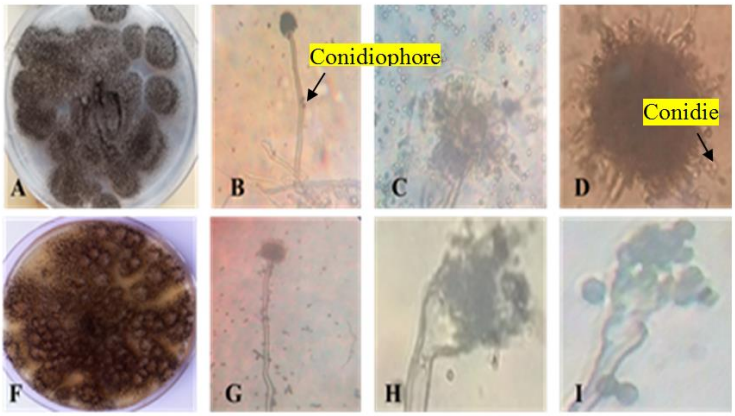

Figure 7. *Colony structure (A and F), mycelia branching pattern (B and G) and spores organization (D and I) of group F.*

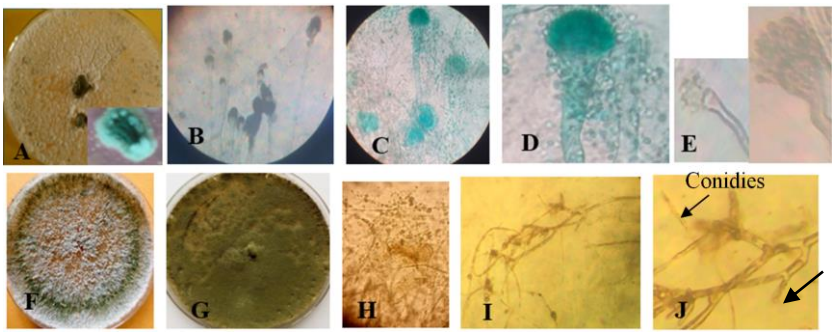

Figure 8. *Colony structure (A and F), mycelia branching pattern (C and H) and spores organization (D, E and J) of group G.*

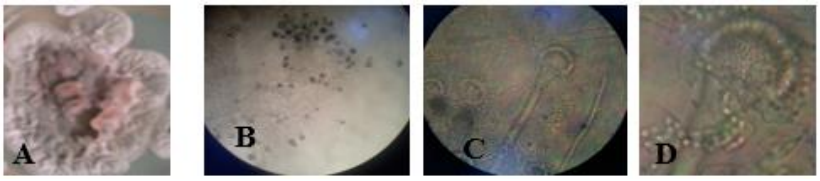

Figure 9. *Colony structure (A), mycelia shape and branching pattern (B and C), spores organization (D), of group H.*

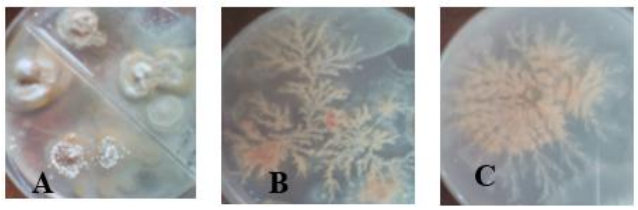

Figure 10. *Young mycelia (A) and old colony structure (B and C) of group I.*

3.2. Morphological Relationships or Diversity of the Phosphate Solubilizing Fungi Strains

Relationship among PSF strains using macro-features and micro-features showed a range of similarities and dissimilarities among isolates. Similarities index varying from 5 to 25% and isolates were distributed into 13 to 2 groups depending on related traits or characters. The distribution showed no relationship with sample origin nor localities. PSF isolates from the collected samples were distributed into three main groups (similarity < 15%) numbered I, II and III. Group I appears to be the highest diversify group follow by group III and group II with 6, 4 and 3 subgroups respectively. The isolate within the same subgroup are closer each to others than in the different subgroups (Figure 11).

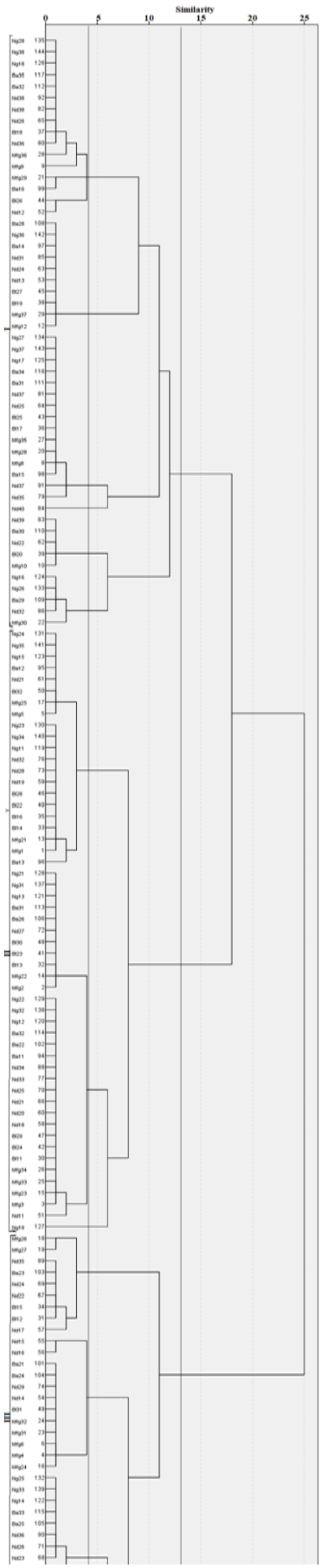

Figure 11. *Dendogram showing morphological relationships among Phosphate solubilizing fungi strains.*

3.3. Biochemical Characteristics or Diversity of the Phosphate Solubilizing Fungi

The selected 148 PSF strains were diversified, and they were identified based on morphological characteristics as *Rhizopus spp.* (44%), *Aspergillus spp*. (31%), *Penicillium spp*. (7%), *Trichoderma spp.* (4%), and *Unidentified spp*. (14%) (Figure 12). All the selected PSF strains showed ability in phosphatase, cellulase and organic acid production. *Rhizopus sp.* and *Aspergillus sp.* exhibited positive test over the entire test carried, and can hydrolyse phosphate complexes, cellulose, starch, lipids, reduce hydrogen peroxide, complexing calcium, aluminium, and iron ions (Table 1). The various fungi species showed phosphate solubilizing ability in both solid and broth media. The phosphate solubilizing efficiency (PSE) on PVK agar media varied from 96.59% to 15.71% and the important ones are *Rhizopus sp* (96.59%), *Aspergillus sp*. (82.26%). On PVK liquid media or broth amended with aluminum phosphate ($AlPO_4$) or tricalcium phosphate ($Ca_3(PO_4)$), the inorganic phosphate released increases with time. Generally, the released amount of P was relatively height on calcium broth than aluminum broth with the optimum values at 30th day of incubation. Some strains showed constant values as from 15th day of inoculation while others delay in solubilization. The overall among of inorganic P- released with incubation time varied from 2.64 to 15.45 mg/L (Table 2).

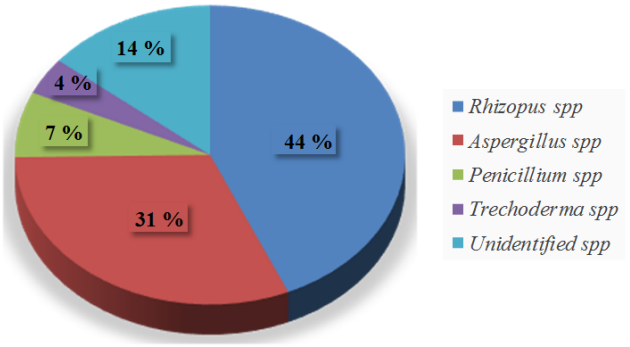

Figure 12. *Isolated phosphate solubilizing fungi genera and percentages.*

Table 1. Biochemical characterization of the selected phosphate solubilizing fungi species.

s/n	Codes	Fungi species	Phosphatase test	Cellulase test	Amylase test	Lipase test	Peroxidase test	Organic acids test
1	A_1	*Rhizopus spp*	+	+	+	-	-	+
2	A_2	*Rhizopus spp*	+	+	+	-	-	+
3	A_3	*Rhizopus spp*	+	+	+	+	-	+
4	A_4	*Rhizopus spp*	+	+	+	-	-	+
5	A_5	*Rhizopus spp*	+	+	+	+	+	+
6	B	*Unidentified spp*	+	+	+	-	-	+
7	C	*Unidentified spp*	±	-	-	-	-	-
8	D	*Penicillium spp*	+	+	-	-	+	+
9	E	*Unidentified spp*	+	-	-	-	+	-
10	F_1	*Aspergillus spp*	+	+	+	+	+	+
11	F_2	*Aspergillus spp*	+	+	-	-	-	+
12	G_1	*Aspergillus spp*	+	+	+	+	-	+
13	G_2	*Trichoderma spp*	+	+	-	-	-	+
14	H	*Aspergillus spp*	+	+	+	+	+	+
15	I	*Penicillium spp*	±	+	-	-	+	+

+ = positive test, - = negative test

Table 2. Phosphate solubilization ability of selected fungi species on PVK solid media and PVK broth.

s/n	Codes	Fungi genera	Percentage / abundance (%)	Insoluble phosphate	Halo zone diameter (mm) at 15th days	P-solubilisation efficience (PSE)	P_i-Release (mg/L)			
							5th day	10th day	15th day	30th day
1	A_5	*Rhizopus sp*	8	$Ca_3(PO_4)_2$	39.7	96.59	11.32	12.26	13.20	15.45
				$AlPO_4$			9.76	12.49	12.50	12.50
2	F_1	*Aspergillus sp*	10	$Ca_3(PO_4)_2$	25.5	82.26	9.91	11.56	11.79	13.46
				$AlPO_4$			3.33	4.75	2.94	2.94
3	B	*Unidentified spp*	6	$Ca_3(PO_4)_2$	22	68.75	4.05	4.52	6.86	8.05
				$AlPO_4$			5.16	5.45	5.45	5.45
4	A_2	*Rhizopus sp*	13	$Ca_3(PO_4)_2$	16	66.66	10.85	10.40	11.09	12.89
				$AlPO_4$			2.64	7.80	7.80	8.037
5	A_3	*Rhizopus sp*	8	$Ca_3(PO_4)_2$	15	65.79	7.80	10.62	10.85	12.60
				$AlPO_4$			7.23	4.99	6.63	6.63
6	F_2	*Aspergillus sp*	10	$Ca_3(PO_4)_2$	19.7	61.56	4.75	7.80	8.27	10.04
				$AlPO_4$			6.54	6.86	6.86	6.86
7	D	*Penicillium sp*	1	$Ca_3(PO_4)_2$	15	60	3.34	7.80	8.037	9.19

s/n	Codes	Fungi genera	Percentage / abundance (%)	Insoluble phosphate	Halo zone diameter (mm) at 15th days	P-solubilisation efficience (PSE)	P_i-Release (mg/L)			
							5th day	10th day	15th day	30th day
				$AlPO_4$			2.64	2.64	3.11	3.11
8	A_4	*Rhizopus sp*	6	$Ca_3(PO_4)_2$	13	56.77	8.04	8.27	10.62	12.60
				$AlPO_4$			5.39	6.16	6.63	6.63
9	A_1	*Rhizopus sp*	8	$Ca_3(PO_4)_2$	12	53.33	3.81	4.28	4.75	6.05
				$AlPO_4$			2.87	2.87	2.92	2.92
10	G_1	*Aspergillus sp*	8	$Ca_3(PO_4)_2$	12.6	48.46	5.45	8.27	8.27	9.47
				$AlPO_4$			2.64	6.16	6.39	6.39
11	C	*Unidentified spp*	1	$Ca_3(PO_4)_2$	12	35.29	4.28	5.45	5.69	6.34
				$AlPO_4$			2.64	8.04	8.04	8.04
12	E	*Unidentified sp*	7	$Ca_3(PO_4)_2$	7	31.81	3.11	5.45	5.92	6.91
				$AlPO_4$			3.33	4.75	2.94	2.94
13	G_2	*Trichoderma sp*	4	$Ca_3(PO_4)_2$	5.1	23.18	7.80	10.15	10.38	12.03
				$AlPO_4$			6.54	6.86	6.86	6.86
14	I	*Penicillium sp*	6	$Ca_3(PO_4)_2$	2.4	20	5.69	5.92	5.92	6.62
				$AlPO_4$			3.33	3.34	3.34	3.34
15	H	*Aspergillus sp*	3	$Ca_3(PO_4)_2$	3.3	15.71	6.63	6.86	7.80	9.19

$Ca_3(PO_4)_2$ (Calcium phosphate) and $AlPO_4$ (Aluminum Phosphate)

4. Discussion

The 15 soil samples of soybeans rhizosphere collected in five different localities of the Noun valley were used for PSF isolation. Rhizospheric soil samples were strongly acid (pH < 5.5) and soil available phosphorus was generally averagely low (11-31 ppm) [13]; characteristic of Ferralsols and Nitosols soil of the Western Highland of Cameroon [11, 33]. Out of 178 fungi isolate, 148 fungi strains showed phosphate solubilization ability. These PSF were unequal distributed in the sampled areas. Ndop area showed the highest percentage (30%) followed by Menfoung (20%), Bangourain (17%), Njingoumbe (17%) and lastly Bambili (16%). This distribution may depend on the type of the crops growing with sampled soybeans rhizosphere, the fertilizers application, the altitude in the valley, the soil colours, type and pH. It's known that land use influences the soil microbial biomass and the PSF distribution in ecosystem was affected by land uses, soil type and soil pH [48, 55].

The morphological description and diversity of PSF strains highlighted some similarities and dissimilarities between the different isolates. For instance, colony colours varied from white (59%), black (20%), green (12%), yellowish (6%) and brown (3%). Macro- features (growth and sporulation rate, colours, mycelia shape) and micro-features (branching pattern and spore organization), were much diversified. The morphological relationship among PSF showed that they are very diversified and all over the Noun Valley, they are distributed into 3 main groups with group I been the highest diversified. The PSF strains were identified as fungi species belonging to the genera *Rhizopus spp.* (44%), *Aspergillus spp*. (31%), *Penicillium spp.* (7%), and *Trichoderma spp.* (4%). These results are in line with Premkumar *et al*. [38] who also realized that PSF strains from soybeans rhizosphere were *Aspergillus spp* and *Penicillium spp.*

The evaluation of solubilization ability showed a very wide range of solubilization efficiency on PVK agar media ranging between 15.71% and 96.59% on the 15th day. The amount of P_i -released in the broth, increased with inoculation time (2.64 - 15. 45 mg/L) and it maximum was at the 30th day. Among the isolate, *Rhizopus sp*. released the maximum amount of P_i (15.45 mg/L) followed by *Aspergillus sp*. with 13.46 mg/L, *Trichoderma sp*. (12, 03 mg/L) and *Penicillium sp.* (9.19 mg/L). The P_i -released on the 15th day ranged from 2.64 to 13.20 mg/L relatively high compared to results (1.70 ppm to 10.78 ppm) obtained by Premkumar *et al*. [38]). The differential solubilization efficiency of PSF isolates for insoluble phosphate can be due to phosphatase

production ability, the nature and quantity of organic acids secreted in the broth [15, 28, 45] and even the type, the source of phosphate complex [54, 16].

5. Conclusion

Soybeans rhizosphere in the Noun Valley presented a very diversified PSF species in terms of macroscopic, microscopic and biochemical characteristic or features. The colour of the colonies varied from white, black, and green, white-green, brown to yellowish. Growth and sporulation rate were very slow, slow, high and very high depending to the strain or species. *Rhizopus sp.* and *Aspergillus Niger* showed a relative high growth rate and degree of sporulation. Mycelia shape and branching pattern varied from unbranched, single branched, monoverticillate to multiple branches, short, relatively long mycelia carrying sporangiophores and conidia. Spores were carried within the sporangia, on *Penicillium* conidia head, *Aspergillus* conidia head or along mycelia. Similarities and dissimilarities studies showed that these PSF strains belong to *Rhizopus spp.*, *Aspergillus spp.*, *Penicillium spp.*, and *Trichoderma spp.* These PSF showed differential ability in enzyme (phosphatase, cellulase) and metabolite (organic acid) production. They showed differential ability in insoluble phosphate complex ($CaPO_4$ and $AlPO_4$) solubilization both in solid media and in broth; therefore, they are potential fungi that can be used in solid or liquid PSF based-biofertilizers formulation.

Abbreviations

CMC	Carboxyl Methyl Cellulose agar Plates
PA	Peptone Agar plates
PDA	Potato Dextrose Agar plates
PSE	Phosphate-solubilizing Efficiency
PSFs	Phosphate Solubilizing Fungi
PVK	Pikovskaya's Media Agar Plates
SE	Solubilization Efficiency

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Conflicts of Interest

The authors declare no conflicts of interest.

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