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Isolation and Characterization of *Acinetobacter baumannii* from Soils of Three Major Onion-Producing Provinces in the Philippines

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Abstract

One of the key strategies for sustainable agriculture is the utilization of indigenous soil microorganisms as inoculant. In this report, three major onion-growing provinces in the Philippines were identified and soils were collected for the isolation of beneficial microorganisms which were characterized for potential to be used as inoculant, particularly as phosphate solubilizer. A laboratory experiment was carried out to isolate potential phosphate solubilizing bacteria (PSB) employing Pikovskaya (PK) medium with tricalcium phosphate and 16S rRNA gene sequencing. From a total of 157 isolates that showed a halo zone on PK plates, three representative isolates (one from each location) were selected based on their population dominance and halo zone size for genetic identification using 16SrRNA sequencing. Interestingly, the isolated PSBs from the three sites showed highly similar nucleotide sequences with *Acinetobacter baumannii*, which were reported in the Philippines as multi-drug-resistant disease-causing microorganisms. However, in this present report, the *A. baumannii* isolates were characterized to have the ability to solubilize Phosphorus; with the Mindoro Occidental isolate (MOc PSB-RGL-2023) possessing the highest solubilizing index of 3.42 mm, followed by Ilocos Sur (IS PSB-RGL-2023) with 3.2 mm, and Nueva Ecija (NE PSB-RGL-2023) with 2.7 mm. These findings suggest the potential of *A. baumanii* as bioinoculant for improving P availability in the soil thus, provides a valuable insight as the pioneer report that isolated and characterized *A. baumannii*-similar isolates from the three provinces in the Philippines as a prospective PSB bioinoculant.

Keywords: 16S rRNA Gene*, A. baumannii, Allium cepa*, PSB.

Introduction

To attain sustainable food production, it is necessary that nutrient depletion and soil degradation must be prevented by replacing the nutrients removed after crops were harvested. One of the different nutrients important for crops is Phosphorus (P), which is required in the largest quantities (1). In all living cells, P is important and its functions cannot be replaced by other elements; life on Earth is not possible without it (2). Plants absorb large quantities of P from the soil solute in the form of phosphate, primarily dihydrogen phosphate (H_2PO_4) (3). But the amount of soluble P in the soil is usually [400–1260 mg kg^{-1}] which is very low (4). P activators encompass a variety of techniques designed to enhance and expedite the conversion of soil P into forms that are soluble in plants within the soil solution. One method involves is utilizing the bacteria that solubilize phosphate (5). To transform the insoluble phosphate to soluble phosphates in the soil, the PSB produce enzymes and organic acids and also excrete siderophores that can form complexes and chelate the metal ions (6, 7). There are various common genera of PSB being isolated in the soil such including *Acinetobacter, Pseudomonas, Erwinia, Bacillus, Enterobacter, Burkholderia, Flavobacterium, Agrobacterium, Arthrobacter, and Micrococcus* (8). In addition, it was reported that *Acinetobacter* spp. with *Enterobacter cloacae* have been recognized as active phosphate-solubilizing strains (9). In another study, it was revealed that the most effective solubilizers of tricalcium phosphate-containing agar were the *Acinetobacter* strains. After 5 days of incubation, phosphorus in liquid cultures was dissolved by *Acinetobacter* strains, with the mean ranging from 167 to 888

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µg/ml of P or 167 and 1022 ppm (10). There were many studies that phosphate solubilizing bacteria were utilized as biofertilizer in root crops production. In an experiment using the mixture of PSB in garlic production, the observations include an improved size of bulbs, dry mass, concentration of P in the leaf, and the crop's yield (11). Similarly, the germination, diameter of bulb, and growth of onion crops were positively affected by PSB inoculation (12). Meanwhile, another report revealed that using biofertilizer containing phosphate solubilizing bacteria as foliar spray, the bulb weight, bulb diameter, yield, and total soluble solids of onion increased by 6.7%, 4.10%, 19.14%, and 91.50%, respectively (13). The onion, scientifically known as *Allium cepa*, is a globally important part of daily cuisine, especially in the Philippines. The total area dedicated for onion production is 19,824.02 hectares in the country and typically, a 1-ha onion production requires at least 45kg of available phosphate. From the total area devoted to onion, Nueva Ecija has the largest coverage with 9,495.16 hectares, followed by Mindoro Occidental with 4,023.15 hectares, and Ilocos Sur with 1,689.84 hectares (14). Onion farmers in the country particularly in Bongabon, Nueva Ecija experience the problem in the expensive cost of chemical fertilizers (15) resulting to high cost of production. To reduce dependence on synthetic P sources, harnessing the ability of PSB as potential bioinoculant for onion production may provide cheaper cost of production, environment-friendly, and sustainable P management approach. In addition, exploring the potentials of locally-isolated soil microorganisms for the development of bioinoculants and/or biofertilizers that are adapted to local agro-environment gradients is a more ecological manner to protect the local biodiversity than using introduced microorganisms. In particular, the goal that this report was able to achieve is the isolation and characterization of PSB from onion-producing provinces with prospect of biofertilizer development specific to increase phosphate availability for onion.

Methodology

Soil Collection and Analysis

A total of three [3] soil samples were collected from top three onion producing regions in the Philippines namely: I, III, and IVB. The Region I soil sample was collected at Brgy. Tay-ak, Bantay, Ilocos Sur [17°35'55"N 120°28'7"E]; Region III soil sample was collected at Brgy. Vega, Bongabon, Nueva Ecija [15°39'12"N 121°8'8"E]; while Region IVB soil sample was collected at Brgy. La Curva, San Jose, Mindoro Occidental [12° 24' 17" N · 121° 2' 45" E]. Soil sampling methods followed the standard protocol according to the procedure stated at the leaflet of the Department of Agriculture (DA) - Bureau of Soils and Water Management (16). In summary, a total of 10 subsamples [15 – 25 cm depth] per location were obtained and were mixed thoroughly until a 1-kg of composite sample was taken. The 100 grams was put in an ice box and used for microbial analysis; the 900 grams of each sample were brought to the DA-Regional Field Office Number III-Regional Soils Laboratory in City of San Fernando, Pampanga, Philippines, for chemical and physical properties analyses. Different methods were used to determine the parameters in Cation Exchange Capacity (CEC), pH, Electrical Conductivity (EC). The measurement of pH and EC were done thru Potentiometric; CEC was determined thru Cation Displacement Kjeldahl Distillation; organic matter was measured thru Walkley - Black-Colorimetric; Total Nitrogen was measured thru Kjeldahl; Phosphorus and Sodium were measure thru Olsen; Potassium was determined thru Leaching Flame Atomic Emission Spectroscopy (AES); Calcium and Magnesium were measured thru Leaching-Flame Atomic Absorption Spectroscopy (AAS); Iron, Zinc, Manganese were tested thru DTPA Extraction Flame AAS; Sulfate was tested thru Turbidimetric; and textural class was tested thru Bouyoucos Hydrometer.

Isolation of Phosphate Solubilizing Bacteria from the Soil Samples

The soil samples were mixed in sterilized distilled water with a ratio of 99 ml sterile distilled water: 1 gram of soil and conducted the serial dilution to 10^{-1} up to 10^{-9} in the 9ml conical plastic tube, thereafter. The 10^{-1} up to 10^{-9} soil samples that were diluted serially were plated (0.1 ml) on the PVK agar having TCP as the source of phosphate. PVK agar medium contained the following ingredients for 1liter capacity: Yeast extract 0.5g, Dextrose 10g, Tricalcium phosphate ($Ca_3(PO_4)_2$) 5g, Ammonium sulfate $(NH_4)^2SO_4$) 0.5g, Potassium chloride (KCl) 0.2g, Magnesium sulfate ($MgSO_4$) 0.1g, Manganese sulfate ($MNSO_4$) 0.0001g, Ferrous

sulfate ($FeSO_4$) 0.0001g, Bacteriological Agar/Agar 15g, purchased at (RTC Laboratory Services and Supply House, Quezon City, Phil.) and Distilled water 1 liter. The solution was mixed in Erlenmeyer Flask (EF) thoroughly until all the chemicals were dissolved then, autoclaved (TRIUP, Model TRS-50L) at 15 lbs pressure, 121^{0}C for 20 minutes. In an inverted position, all petri dishes were incubated at 27-30 ^{0}C for 7 days. The colony forming units (CFUs) of *Acinetobacter baumannii* isolated from soil samples across the three study sites were 42 CFUs in Ilocos Sur, 55 CFUs in Mindoro Occidental, and 60 CFUs in Nueva Ecija. These values show minor differences in abundance but indicate that *A. baumannii* was present in all locations at comparable levels. After incubation, the colonies having clear halo zone were screened and selected. They were streaked on the freshly made PVK agar plates to get the pure colonies. Repeated streaking was done out of the same medium to validate the result. Isolated bacteria on petri plates were kept at 4^{o}C for further study.

Screening and Measurement of Solubilizing Index (SI) of Isolated PSB

On the PVK agar plates, the isolated bacteria were screened for their capability of solubilizing tricalcium phosphate. Aseptically, the isolated bacteria were spot inoculated on the middle of petri plates with PVK agar. At 28^{o}C ±2 ^{0}C, all petri plates were incubated for the duration of 7 days. A clear halo zone which surrounds the growing colony is an indicator of the microorganisms in solubilizing phosphate. Measuring the solubilizing index (SI) was done and computed as (colony diameter + halo zone diameter) divided to the colony diameter (17).

$$PSI = \frac{colonydiameter + halozonediameter}{colonydiameter}$$

Ilocos Sur (IS PSB-RGL-2023) PSI = $\frac{[10.2\ mm] + [22.3\ mm]}{[10.2\ mm]}$ = [3.20 mm]

Mindoro Occidental (MOc PSB-RGL-2023) PSI = $\frac{[5.5\ mm] + [13.3mm]}{[5.5\ mm]}$ = [3.42 mm]

Nueva Ecija (NE PSB-RGL-2023) PSI = $\frac{[7.2\ mm] + [12.3\ mm]}{[7.2\ mm]}$ = [2.70 mm]

Selection of Representative Isolate for Sequence Analysis

The isolated bacteria were spot inoculated quarterly on the petri plate containing PVK agar. The colony with biggest halo zone around was selected, sliced from the agar plate with the use of aseptic surgical blade and put in the micro centrifuge tube then, covered with parafilm tightly. The samples were sent to MACROGEN Laboratory, Seoul, South Korea thru Kinovett Scientific Solutions Corporation, Quezon City, Phil. The study used a universal primer EU49f [5'-TTAACACATGCAAGTCGAACGG-3'] and EU1070r [5'-GGACTTAACCCAACATCTCACGA-3'] and run in the Polymerase Chain Reaction (PCR) condition of: initial denaturation for 5 minutes at 94^{o}C, followed by 30 cycles [94^{o}C for 60 seconds, 55 ^{0}C for 60 seconds and 74^{o}C for 60 seconds] and final extension at 74^{o}C for 5 minutes (18).

Construction of Phylogenetic Tree

The Basic Local Alignment Search Tool (BLAST) tool was used to align the sequences to the bacterial lineages that deposited in the GenBank of the National Center for Biotechnology Information (NCBI). The alignment was done using ClustalW and Neighbor-Joining method was utilized to build the phylogenetic trees. The distances of the genetic were computed using Molecular Evolutionary Genetic Analysis (MEGA v11) software. Consequently, the phylogenetic trees were bootstrapped with 1000 replications.

Results and Discussion

Physical and Chemical Properties of the Soils

A summary of soil's selected physical and chemical properties is presented in Table 1. The study collected 3 soil samples from three sites intended for onion production. The microbial community structure was strongly influenced by rainfall, location, temperature, soil pH, and soil type, and showed some correlation with microbial activity (19). The phylum *Proteobacteria* was the most dominant bacterial group, aligning with findings from several studies on agricultural ecosystems (20-22), *Acinetobacter baumannii* belong to

Proteobacteria (23). Members belonging to *Proteobacteria* showed the highest richness in soil, common in many soil ecosystems, including rhizospheres, saline soils, and semi-arid soils (24). The soil pH in Ilocos Sur, Mindoro Occidental, and Nueva Ecija were 6.95, 6.97, and 6.92 respectively which are considered neutral. The pH of soil is directly linked to the populations of soil microbial communities and is commonly regarded as a general indicator of the structure characteristics of the communities of bacteria (25). Soil pH was the primary factor determining bacterial diversity, richness, and community composition at broad spatial scales (26, 27). Soil pH also had significant effects on soil enzyme activities, respiration and soil metabolic quotient (qCO_2) (28, 29). A principal mechanism by which pH can influence microbial dynamics is through the alteration of nutrient availability (30).

The estimated bacterial population of the higher soil pH of 6.8 is 60% more than that of the soil having the pH 5.1. The estimated bacterial abundance in soil with pH of 5.5 was 26% which was in larger quantity compare to more acidic soil of having a 4.1 pH. Bacterial population in soil pH 2.5-2.7 is relatively in low level (26). In acidic soil also, the availability of P may be a strong factor affecting microbial community composition. Rising soil acidity can greatly reduce the bioavailability of inorganic P (Pi) because mobilized Aluminum (Al) will geochemically bind with Pi (31). The CEC is an essential gauge in the determination of soil quality and it indicates the capacity of the soil to retain the positively charge ions (32). The CECs in soils from Ilocos Sur and Nueva Ecija were found to be 35.19cmol/kg and 15.44cmol/kg, respectively, both falling within the normal range while Mindoro Occidental which is clay loam in texture and has a CEC of 13.42cmol/kg, is considered low. Soils with high amount of negative charge are more fertile because they can retain more cations influencing microbial population (33). Total Phosphorus and CEC of the soils also influenced the distribution of the microbial community (34) affecting both its composition and spatial variability across different soil types. The values of EC in three sites are considered non-saline. The three study sites possess a very low organic matter. Ilocos Sur has 1.40%; 1.43% belonged to Mindoro Occidental, and 0.87% to Nueva Ecija. The soils generally had low organic matter, which could be attributed to the rapid mineralization (35). The relative bacterial abundance, which increases with high organic matter availability in soils, is consistent with finding from previous studies (36). The accumulation of soil organic matter enhances various aspects of soil fertility, including bacterial diversity (37). Farm management techniques, such as the massive and rampant application of chemical fertilizers and pesticides in crops, have direct correlation with the low concentration of organic matter in the soils. This statement is supported by a previous report indicating that the reduction of quality of soil used in agricultural production and the decline of the organic matter in the soil is caused by the continuous application of chemical fertilizers (38). In Ilocos Sur, 17 to 21 bags/hectare of chemical fertilizers were used. A total of 24 to 32 bags of fertilizer were used in one hectare of onion field in Mindoro Occidental. And in Nueva Ecija, a total of 21 to 31 bags/hectare of chemical fertilizer were used. This was based on the interview with the onion farmers in the three areas. Agricultural practices can affect the bacterial population richness and diversity of soils through changes in soil physical and chemical properties (39, 40). In Ilocos Sur, total Nitrogen was 0.07%, 0.04% in Mindoro Occidental, and 0.05% in Nueva Ecija. In case of Potassium, Ilocos Sur has 0.61cmol/kg, Mindoro Occidental has 0.28cmol/kg, and Nueva Ecija has 0.24cmol/kg. The macronutrients such as Nitrogen and Potassium are very low in three sites. However, the Phosphorus was in normal ranged at 30.62ppm to 49.44ppm. P content in the soil was mainly because of the application of fertilizer containing P over a long period of time. The residual P fertilizer not recovered by the crops is believed to be permanently bound or fixed in the soil in forms unavailable to plant (3). Accordingly, the P retention is dominated by precipitation reactions in neutral-to-calcareous soils (41). Exchangeable bases such as Calcium, Magnesium, and Sodium are high in three study sites. Furthermore, the textural class for Ilocos Sur was Sandy Clay Loam, for Mindoro Occidental was Clay Loam, and for Nueva Ecija was Sandy Loam. The clay fraction contains a more diverse bacterial community compared to the silt or sand fraction (42).

Table 1: Soil Chemical and Physical Properties of the Three Study Sites: Ilocos Sur, Mindoro Occidental, and Nueva Ecija

	Location		
Parameters	**Ilocos Sur**	**Nueva Ecija**	**Mindoro Occidental**
pH	6.95	6.92	6.97
CEC, cmol/kg	35.19	15.44	13.42
EC, mS/cm	0.52	0.09	0.45
OM (%)	1.40	0.87	1.43
Total Nitrogen (N), %	0.07	0.05	0.04
Phosphorous (P), ppm	30.62	49.44	49.24
Potassium (K), cmol/kg	0.61	0.24	0.28
Calcium (Ca), cmol/kg	25.93	14.07	14.18
Magnesium (Mg), cmol/kg	7.33	4.71	1.96
Sodium (Na), cmol/kg	0.97	0.26	0.22
Manganese (Mn), ppm	7.33	18.30	28.71
Zinc (Zn), ppm	1.18	1.46	0.79
Sulfate (SO_4), ppm	1.19	1.11	1.35
Iron (Fe), ppm	41.75	43.12	146.42
Soil Texture			
Sand, %	46.52	63.85	42.82
Silt, %	20.93	19.28	28.59
Clay, %	32.55	16.87	28.59
Textural Classes	Sandy Clay Loam	Sandy Loam	Clay Loam

Morphological Characterization and P-Solubilization Ability of the Isolated Bacteria

All bacterial isolates were assessed for their morphological characteristics. The bacterial colonies exhibited circular shapes with raised elevations, smooth, convex, entire, and whitish pigmentation on the agar plates, which is the same to the results of the study of (6, 43). As earlier described, *Acinetobacter* isolates were recognized in different characteristics such as negatively oxidase, positively catalase, gram-negative, non-motile, and short rods or coccobacilli (44, 45). The capacity of the isolated bacteria to solubilize phosphate using tricalcium phosphate as the P source was assessed. The ability of the bacterial isolates in the three research sites to solubilize tricalcium phosphate was demonstrated by the distinct halo zones surrounding the colonies. Meanwhile, effective PSB in soil has been reported from bacterial strains, including *Acinetobacter* (46).For clarity, the representative bacterial isolates were assigned with codes as follows: isolate from Nueva Ecija – NE PSB-RGL-2023; from Ilocos Sur – IS PSB-RGL-2023; from Mindoro Occidental – MOc PSB-RGL-2023.The solubilization index was used to quantify the capacity of isolated PSB to solubilize TCP on the PVK agar media (47). The results of solubilizing indices were measured on the basis of clear halo zone and the SI was calculated in millimeter (mm). The clear halo zone formed around the colony of bacteria could be the polysaccharide, organic acid, enzyme phosphatase, and phytase productions (48). It was discovered that the phosphate solubilizing capability of the isolates obtained in the soils from the three study sites are varied. The three bacterial isolates showed varying P solubilizing activity, as evidenced by the size differences between their colonies and P solubilizing halos. The isolates' halo zone to colony zone ratios varied from 2.70 mm to 3.42 mm (Table 2). The highest proficient bacterial isolates solubilizing tricalcium phosphate was Mindoro Occidental (MOc PSB-RGL-2023) isolate of 3.42 mm, Figure 2. Like the previous isolates, it displayed notable colony morphology and produced measurable halo zone on selective media, further supporting its potential as a PSB. Ilocos Sur isolate (IS PSB-RGL-2023) has an SI of 3.20 mm in Figure 1. Upon culturing, the colony

exhibited distinct morphological characteristics, including shape, texture, and pigmentation. Measurements indicated the colony size and the presence of clear halo zone surrounding the colony on Pikovskaya's agar, suggesting its phosphate-solubilizing ability. The last is NuevaEcija (NE PSB-RGL-2023) isolate with an SI of 2.70 mm in Figure 3. This isolate also demonstrated phosphate-solubilizing potential, as evidenced by the halo zone formation. Based on the result, the three locally-isolated bacteria showed efficient phosphate solubilization that can be utilized to increase the availability of P for plant's uptake. Another study found that *Acinetobacter baumannii*, after the 7- and 14-days incubation period, has a mean P dissolved in liquid cultures of 10.8 mg l^{-1} and 39.3 mg l^{-1}, respectively and with the solubilization index of 1.83 mm; which are smaller SI than the isolates in this present report (46). After 7 days of incubation, a SI of 4.0cm was garnered and 387 µg/ml of soluble phosphate was produced by *Acinetobacter*SuKlC24 in PVK media through the process of phosphomolybdate method, and the result demonstrates the strong capability of phosphate solubilization (49). It was reported that the highest solubilization index from 12 PSB isolates obtained from mangrove soil of East Java was only 2.82, and that a highest solubilization index of 2.36 was recorded from 19 isolates (50). Meanwhile, researchers from Egypt evaluated 40 isolates for phosphate solubilizing property and obtained the highest SI of 2.3 wherein a combination of some of these isolates [with SI of 1.3, 1.8, and 2.0] significantly increased the P content of wheat plants by 76% and 12% over the full fertilized plants, suggesting that these SI values are highly efficient in solubilizing high amount of P from the soil for plant's uptake.

Table 2: Solubilization Index of Isolates from the Three Study Sites: Ilocos Sur, Mindoro Occidental, and Nueva Ecija

Representative Isolates	Size in mm
IS PSB-RGL-2023	3.20
MOc PSB-RGL-2023	3.42
NE PSB-RGL-2023	2.70

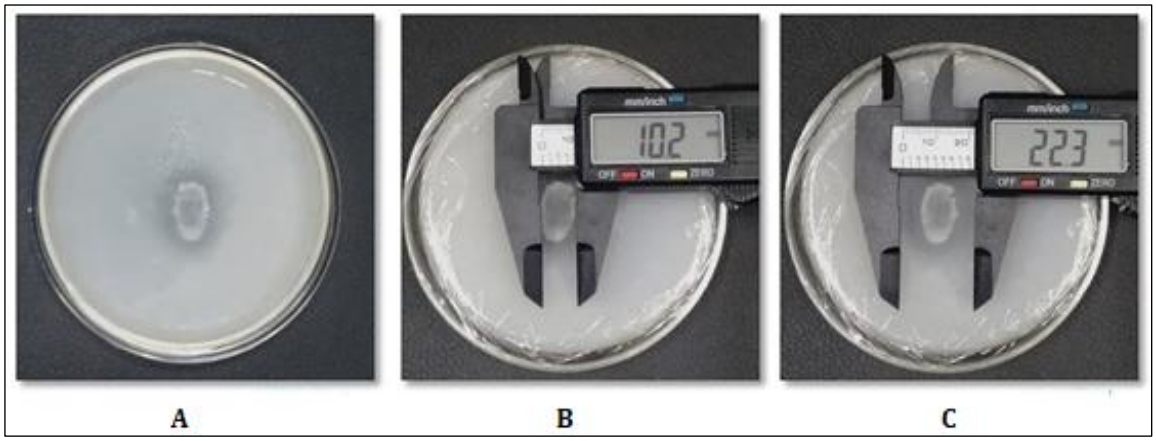

Figure 1: Isolate IS PSB-RGL-2023 from Bantay, Ilocos Sur, Philippines, showing (A) Colony Morphology, (B) Colony Size, and (C) Halo Zone Size. The16SrRNAGene Sequence of the Isolate Showed High Similarity to *Acinetobacter baumannii*

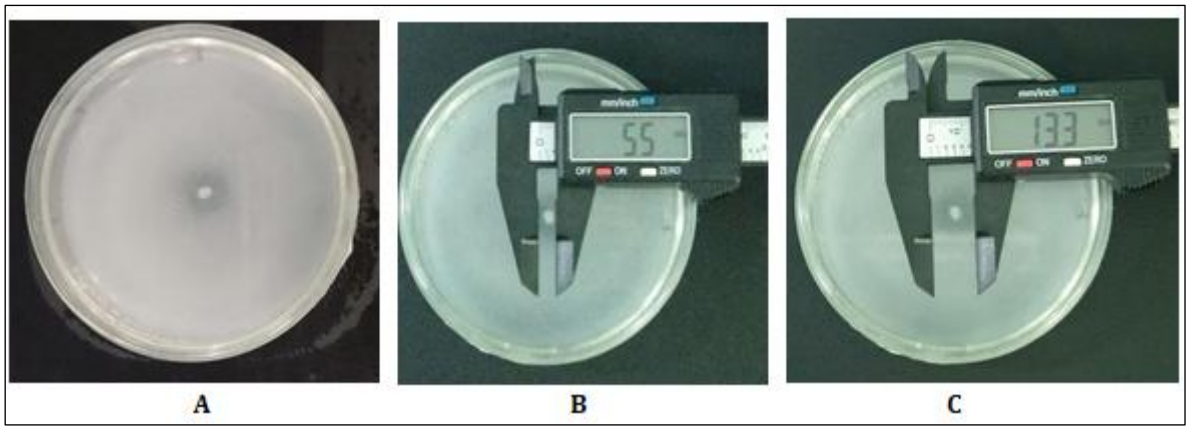

Figure 2: Isolate MOc PSB-RGL-2023 from San Jose, Occidental Mindoro, Philippines, showing (A) Colony Morphology, (B) Colony Size, and (C) Halo Zone Size. The16SrRNAGene Sequence of the Isolate Showed High Similarity to *Acinetobacter baumannii*

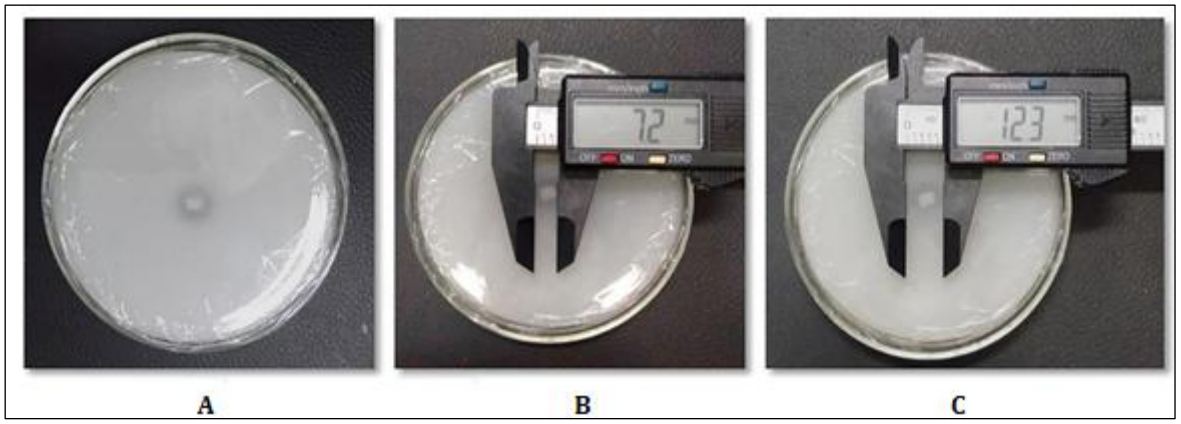

Figure 3: Isolate NE PSB-RGL-2023 from Bongabon, Nueva Ecija San Jose, Philippines, showing (A) Colony Morphology, (B) Colony Size, and (C) Halo Zone Size. The16SrRNAGene Sequence of the Isolate Showed High Similarity to *Acinetobacter baumannii.*

Genetic Characterizations of Isolated PSB from Soil Samples

Utilizing 16S rRNA sequences and matching them to the database stored in NCBI GenBank, *A. baumannii* were isolated from the rhizospheric soils in onion fields at Bantay, Ilocos Sur, Bongabon, Nueva Ecija, and San Jose, Mindoro Occidental. *Acinetobacter* is a genus of gram-negative, oxidase-negative, strictly aerobic bacteria, belong to *γ-Proteobacteria*, and order *Pseudomonadales* (23). *Acinetobacter* species exist in natural ecosystems such as soils, aquatic, marine, sediments, the polar region, and even in site with hydrocarbon contamination (51, 52). *Acinetobacter*are abundant bacteria comprehensively distributed in water and soil ecosystems and contains 17 validly well-explained species and 14 unidentified genomic species (53). *Acinetobacter* cells population estimate in soil and water was 10^5/mg (54). There were existed 924 genomes of *Acinetobacter*in the Integrated Microbial Genome database as of September 2014, of which 728 genomes or equivalent to 81% belong to *A. baumannii* (55). *Acinetobacter* are chemoheterotrophs and nutritionally diverse, the different substrates they utilized as derivatives of lone carbon and energy are the same of the aerobic *Pseudomonad* (56). Due to soil complexity, *A. baumannii* has been found to coexist with closely related *Acinetobacter* species such as *A. bohemicus* (57). *Acinetobacter*is an important Plant Growth Promoting Bacteria (PGPR) because it is known to solubilize phosphate, potassium, and zinc, produce antibiotics, siderophores, gibberellin, and Indole Acetic Acid (IAA). *Acinetobacter* has a wide range of uses in the removal of phosphate from wastewater environments due to its ability to sequester high amounts of inorganic phosphate (58). Meanwhile, it was reported that *A. baumannii* solubilized 10.8 mgl^{-1} and 39.3 mgl^{-1} of P after the incubation of 7 and 14 days, respectively (46). In

addition, a study reported that *A. baumannii* PUCM1029 strain solubilized phosphate of 64 mg/ml, produced IAA of 10 µg/ml, and produced siderophore of 74.20SU (44). Moreover, *A. baumannii* was discovered to produce siderophores quantitatively, at a rate of 65.54SU (59). By making iron available to plants and creating a shortage of iron for pathogenic fungi, siderophores promote plant growth (60). A certain *Acinetobacter* sp. SK2 solubilized 682 µg ml^{-1} of TCP and 86 µg ml^{-1} of Rock Phosphate (RP), resulting in a pH drop of up to 4 owing to gluconate formation. The produced gluconate was mediated by enzymes membrane-bound bound GDH (*mGDH*) and soluble GDH (*sGDH*) and this is the biochemical basis of the P solubilization (61). The *Gluconobacter, Pseudomonas,* and *Acinetobacter* species which are gram-negative have the membrane-bound *mGDH,* however the *sGDH* is less particular (62). *Acinetobacter baumannii* LRFN53 found to produce ethylene, thus, has the capability in the nitrogen fixation (63). In the case of iron-limiting situations, and after 48 hours of incubation, *A. baumannii* HIRFP40 secreted siderophore of 94.77SU, and *A. baumannii* LRFP52 strain solubilized Zinc most efficiently. *Acinetobacter* spp. is capable of accumulating amount of phosphate larger than what is needed for cell synthesis (64). The process is called luxury phosphate absorption. This supports the claim that *Acinetobacter* spp. is the primary microorganism responsible in improved absorption of Phosphorus. The isolates of *Acinetobacter* spp. grow best in neutral media and are less resistant to extremely acidic environments (65). Therefore, aside from its ability as plant growth promoter and P-solubilizer, the species of *Acinetobacter* can be explored to be inoculated on acidic soil conditions to increase P availability that are being rendered unavailability by low soil pH. Although in this report, the isolated bacteria were only sequenced based on 16S rRNA gene and its genetic identity has to be further verified through sequencing of other chromosomal, metabolic, and functional genes. Yet, this is the first report in the Philippines about *A. baumanii* isolated from agricultural soils. Although the first report about *Acinetobacter* in the Philippines was about the 117 *A. baumanii* isolates collected from the Philippines' hospitals which were carbapenem-resistant was on December 2021, the isolates were not related to crop production. However, an earlier study reported the isolation of *Acinetobacter baumannii* from non-clinical environments, including agricultural soils, emphasizing its existence outside clinical settings (66). The present study confirms the occurrence of *A. baumannii* in agricultural soil, that support its potential role as an environmental reservoir. The result raises essential deliberations regarding the ecological behavior of *A. baumannii* and its potential interaction with soil microbiota. In another study investigating the environmental distribution of *Acinetobacter*, 45 agricultural rhizosphere soil samples were collected from Baghdad City, Iraq, of which *Acinetobacter baumannii* was isolated in 18% of the samples (67). The result showed the highest activity on inulin agar plates. These results underscore the ecological presence and functional potential of *Acinetobacter baumannii* in soil environments, particularly in the aerobic mineralization of organic matter. Also, current findings have demonstrated the presence of *Acinetobacter baumannii* in fresh produce such as vegetables and fruits, with some isolates exhibiting extensive drug resistance and strong biofilm-forming abilities. Genetic analysis even revealed novel sequence types, suggesting diverse origins and adaptive potential (68). While *Acinetobacter baumannii* isolate identified in this study demonstrated potential phosphate-solubilizing activity, it is important to consider its biosafety implications. A *baumannii* is recognized as an opportunistic human pathogen with known multi-drug-resistant strains in clinical settings. The identification of this species in agricultural soil underscores the need to further investigate its genomic profile, including presence of antibiotic resistance genes and potential virulence factors. Such studies are crucial to ensure that the use of environmental strains as biofertilizer components doesn't pose a risk to human health or contribute to the spread of antibiotic resistance. The presence of *Acinetobacter baumannii* in onion-producing soils, as observed in this study, warrants cautious interpretation. While this species is known in clinical contexts, environmental strains may pose beneficial agricultural properties. In fact, *Acinetobacter baumannii* AP1 has been commercialized as a multifunctional biofertilizer in Malaysia as GoGrow BioNPK, with proven nitrogen fixation, phosphate and potassium

solubilization, and antagonistic activity against bacterial wilt in crops. The product was shown to maintain acceptable viability over six months under various storage conditions at 10^8 CFU mL^{-1} as set by Department of Agriculture Malaysia (69). Shown on Figures 4, 5, and 6 are the phylogenetic trees constructed to indicate the 16S rRNA gene nucleotide similarity obtained from the DNA database through BLAST between the known strains and the locally-isolated bacterial isolates of this study. The phylogenetic trees were generated using the Neighbor-Joining method with 1,000 bootstrap replications in MEGA 11 software to ensure the reliability of the branching patterns. The isolates in this study are using letter and number combinations, such as IS PSB-RGL-2023, which refers to phosphate-solubilizing bacterial isolate obtained in Ilocos Sur, while MOc PSB-RGL-2023 and NE PSB-RGL-2023 are isolates from Mindoro Occidental and Nueva Ecija respectively. It is evident that the phosphate solubilizing bacteria in the three study sites are categorized under the genus of *A. baumannii* with IS PSB-RGL-2023 obtaining a 98% similarity to *A. baumannii* NY13623 strain (Figure 4); NE PSB-RGL-2023 with 98% similarity to three strains such as *A. baumannii* APP 173, *A. baumannii* AE7, *A. baumannii* ABF9692 (Figure 5); and MOc-PSB RGL-2023 with 99% similarity to *A. baumannii* 1326924-1 (Figure 6). The high degrees of similarities (98% and 99%) confirm that the isolates belong to the *Acinetobacter baumannii*. Meanwhile, *Acinetobacter baumannii* is well-documented as a nosocomial pathogen in clinical environments due to its capacity for multi-drug resistance on abiotic surfaces (70). However, its occurrence in natural ecosystems, including agricultural soils, has gained increasing attention. In these environments, *Acinetobacter baumannii* has been isolated as part of the rhizospheric and bulk soil microbial communities. There was a laboratory study that combination of *Pseudomonas azotoformans*, *Acinetobacter baumannii*, and *Bacillus paramycoides* solubilize the largest amount of inorganic phosphate ($Ca_3(PO_4)_2$. These three bacteria combinations resulted in the highest dissolved P with .29% potential dissolution contained in the liquid medium (71, 72). The isolates identified in this study exhibited phosphate-solubilizing activity, suggesting a potential ecological role in phosphorus cycling. However, given its dual identify as both a potential biofertilizer agent and a clinical concern, careful ecological assessment is needed. Its introduction in agricultural systems must be evaluated for biosafety, population stability, and interaction with indigenous microbiota.

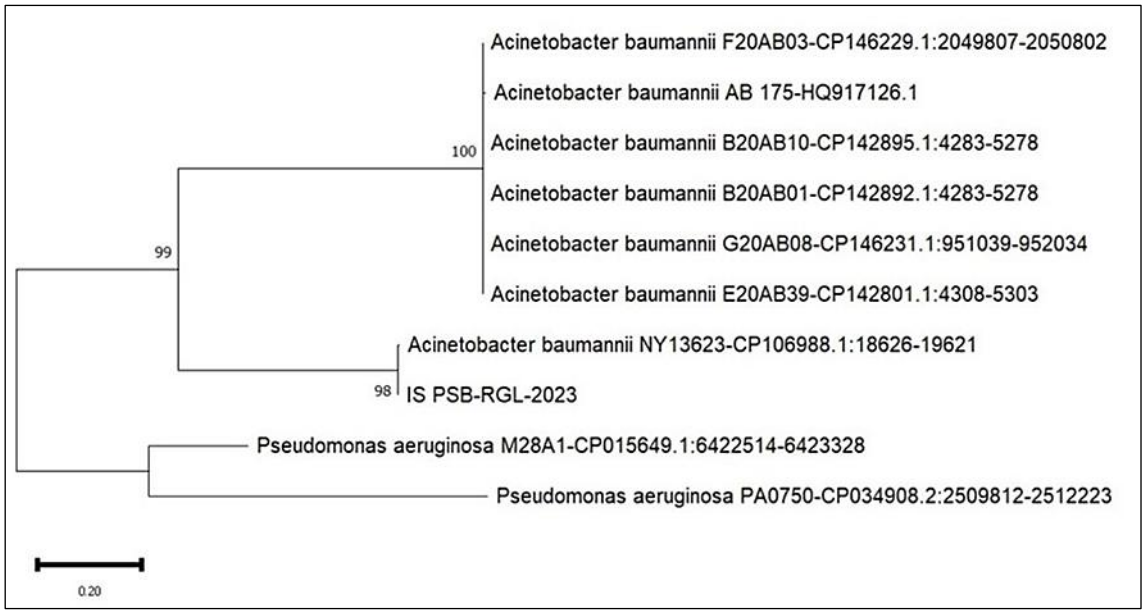

Figure 4: Phylogenetic Tree based on the 16S rRNAGene Sequences, Constructed using the Neighbor-Joining Method in MEGA 11 with 1,000 Bootstrap Replications. The Isolates from this Study are Labeled with Letter-Number Codes; IS PSB-RGL-2023 Refers to the Isolate Ilocos Sur, Philippines

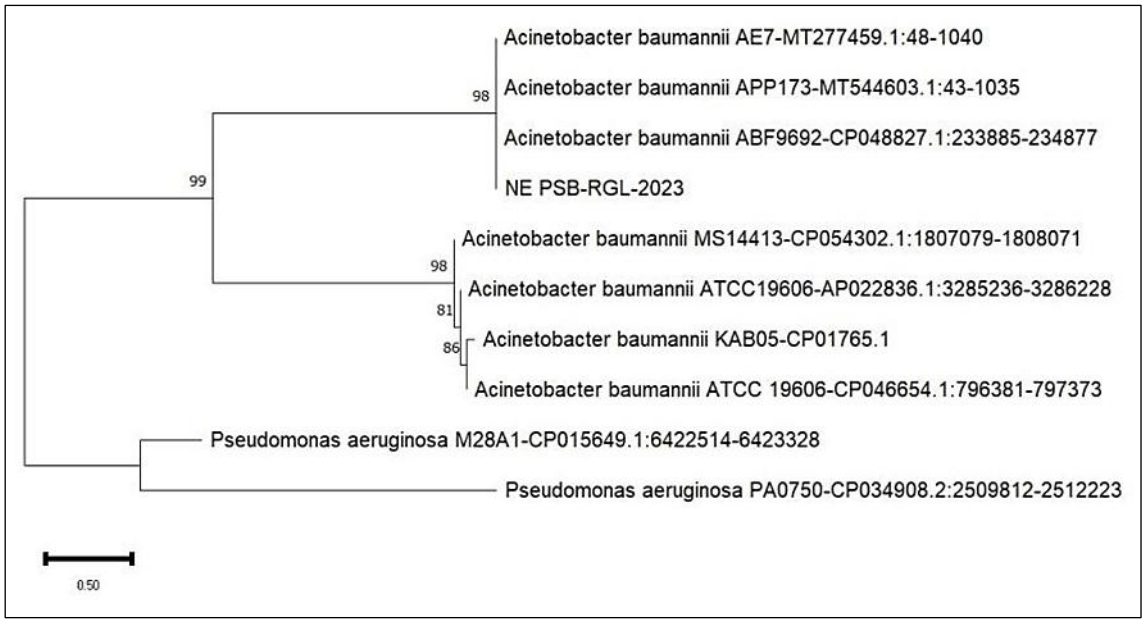

Figure 5: Phylogenetic Tree based on the 16S rRNAGene Sequences, Constructed using the Neighbor-Joining Method in MEGA 11 with 1,000 Bootstrap Replications. The Isolates from this Study Are Labeled with Letter-Number Codes; NE PSB-RGL-2023 Refers to the Isolate from Nueva Ecija, Philippines

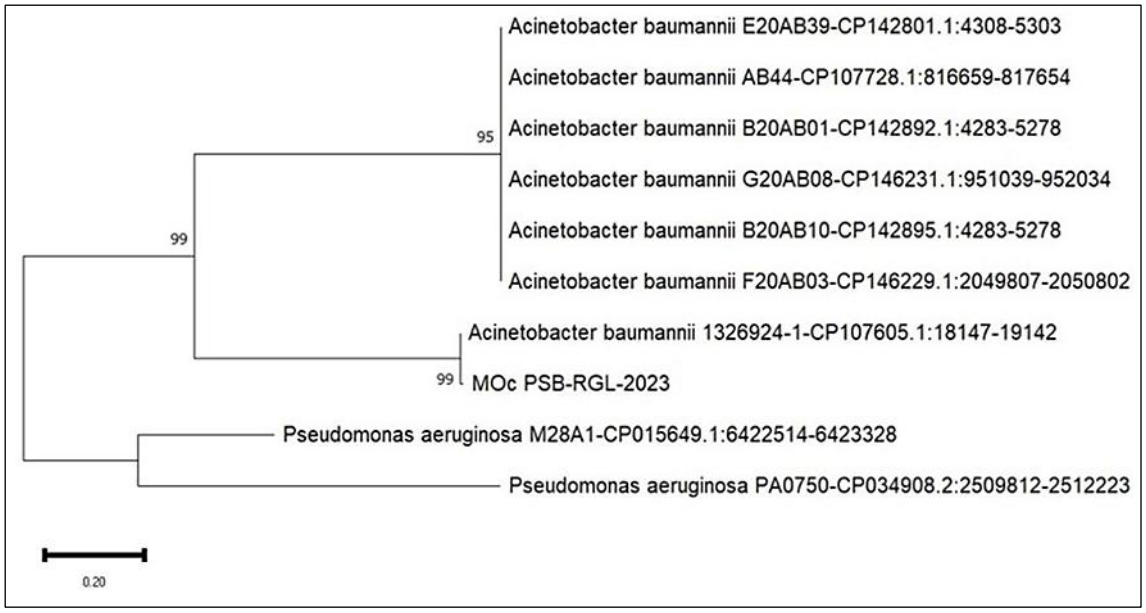

Figure 6: Phylogenetic Tree based on the 16S rRNAGene Sequences, Constructed using the Neighbor-Joining Method in MEGA 11 with 1,000 Bootstrap Replications. The Isolates from this Study are Labeled with Letter-Number Codes; MOc PSB-RGL-2023 Refers to the Isolate from Mindoro Occidental, Philippines

Conclusion

PSB help to the increased availability of soluble phosphates in the soils for easy absorption of plants, thus reducing the usage of chemical fertilizers that contribute to lessen the environmental degradation. This study reports the presence of the PSB isolated from the soils of onion fields in Ilocos Sur, Nueva Ecija, and Mindoro Occidental, Philippines which has genetic identity similar to genus *Acinetobacter baumannii*via 16S rRNA gene sequencing. The three representative isolates showed the ability to solubilize phosphate indicated by clear halo zones on PVK agar plates.

To date, it is the first report from the Philippines identifying *A. baumannii* isolates as a potential phosphate solubilizer that can be utilized for onion production. This study serves as an initiative in the formulation of biofertilizer using *A. baumannii* for increasing P availability for crops that require a high amount of P application, such as onion. However, the study utilized only the 16S rRNA gene, so further research should focus on the identification of specific functional genes of phosphate solubilization and/or quantification of siderophore and gluconic acid, phytase enzyme productions, and other plant growth promoting hormones that are potentially released by these beneficial microorganisms.

In response to biosafety concerns, it is acknowledged that *A. baumannii* is associated with multi-drug resistance in clinical settings. Therefore, future research must include comprehensive biosafety evaluations, including whole genome sequencing or molecular screening to confirm the absence of antibiotic resistance and virulence genes. Thede assessments are essential to ensure the safe use of environmental *A. baumannii* strains in sustainable agricultural practices.

Abbreviations

AAS: Atomic Absorption Spectroscopy, AES: Atomic Emission Spectroscopy, BLAST: Basic Local Alignment Search Tool, CEC: Cation Exchange Capacity, DA: Department of Agriculture, EC: Electrical Conductivity, EF: Erlenmeyer Flask, IAA: Indole Acetic Acid, IS: Ilocos Sur, MEGA: Molecular Evolutionary Genetic Analysis, mGDH: membrane-bound GDH, Moc: Mindoro Occidental, NCBI: National Center for Biotechnology Information, NE: Nueva Ecija, PGPR: Plant Growth-Promoting Rhizobacteria, PK: Pikovskaya, PSB: Phosphate solubilizing-bacteria, RGL: RosaleeGrajo Leander, sGDH: soluble GDH, SI: Solubilizing Index, TCP: Tri-calcium Phosphate.

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Author Contributions

Rosalee G. Leander: performed the experiments, analyzed and interpreted the data, wrote and edited the paper, Maria Luisa T. Mason: conceived and designed the experiments, performed the experiments, contributed reagents, analyzed and interpreted the data, wrote and edited the paper, Ariel G. Mactal: conceived and designed the experiments, provided the materials for the research, edited the paper, Fernan T. Fiegalan: conceived and designed the experiments, edited and suggested for the improvement of the paper, Marilou M. Sarong: conceived and designed the experiments, edited the paper, Elaida R. Fiegalan: conceived and designed the experiments, edited the paper.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

There are no human subjects in this article and informed consent is not applicable

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