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Research Article

Potential of Locally-Isolated *Acinetobacter Baumannii* Strains as Phosphate Solubilizer from three Major Onion-Producing Provinces in the Philippines

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Abstract

The fixed insoluble Phosphorus in the soil limits its utilization by crops and thus, the role of Phosphate Solubilizing Bacteria (PSB) is beneficial to increase the Phosphorus availability in the soil for crops. In order to obtain native isolates that may be used for onion production, three major onion-producing provinces in the Philippines were selected to collect soil samples. A laboratory experiment was carried out to isolate probable PSB employing Pikovs Kaya (PK) medium with tricalcium phosphate. 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 16S rRNA sequencing. The isolated PSB strains from the three sites showed highly similar nucleotide sequences with *Acinetobacter baumannii* terms of ability to solubilize Phosphorus, the Mindoro Occidental isolate (MOc PSB-RGL-2023) showed 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 *Acinetobacter baumanii* as bioinoculant for improving P availability. This study provides a valuable insight as the pioneer report that isolated and characterized possible *A. baumannii* Strains from the three provinces in the Philippines as a prospective PSB bioinoculant.

Keywords: *A. baumannii* • Allium cepa • PSB • 16S rRNA gene

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, Phosphorus 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 Phosphorus from the soil solute in the form of phosphate, primarily dihydrogen phosphate (H_2PO_4) (Roberts & Johnston, 2015) [3]. But the amount of soluble Phosphorus in the soil is usually (400 mg kg^{-1}–1260 mg kg^{-1}) which is very low [4]. Phosphorus activators encompass a variety of techniques designed to enhance and expedite the conversion of soil Phosphorus 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]. 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, Acinetobacter spp.* with Enterobacter cloacae have been recognized as active phosphate-solubilizing strains [7]. In the research, result revealed that the most effective solubilizers of tricalcium phosphate containing agar were the Acinetobacter strains. In the 5 days incubation, Acinetobacter strains dissolved Phosphorus in liquid cultures with the mean ranged from 167 μg/ml to 888 μg/ml Phosphorus or 167 ppm and 1022 ppm.There were many studies that phosphate solubilizing bacteria were utilized as biofertilizer in root crops production. In the experiment of using the mixture of PSB in garlic production, improved the size of bulbs, dry mass, concentration of Phosphorus in the leaf, and the crop's yield. The germination, diameter of bulb, and growth of onion crops were positively affected by PSB inoculation [8,9]. 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 [9,10].

The onion, scientifically known as *Allium Cepa L.,* 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 according to the Onion Production Guide (https://www.da.gov.ph/wp-content/uploads/2021/04/), a 1-ha onion production requires at least 45 kg 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 (https://psa.gov.ph, 2022).Onion farmers in the country particularly in Bongabon, Nueva Ecija experience the problem in the expensive cost of chemical fertilizers (Domingo, 2023) resulting to high cost of production.

To reduce dependence on synthetic Phosphorus sources, harnessing the ability of PSB as potential bioinoculant for onion production may provide cheaper cost of production, environment-friendly, and sustainable Phosphorus 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.

Materials and Method

Soil collection and analysis

A total of 3 samples were collected from top three onion producing regions in the Philippines namely: I, III, and IVB. 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 (https://www.bswm.da.gov.ph/download/how-to-collect-soil samples-for-analysis/). In summary, a total of 10 subsamples (15 cm–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 (https://rfo3.da.gov.ph/index.php/regional-soils-laboratory/, n.d.) 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 AES; Calcium and Magnesium were measured thru Leaching-Flame AAS; Iron, Zinc, Manganese were tested thru DTPA Extraction Flame AAS; Lead was measured thru Acid Digestion ICP OES; Sulfate was tested thru Turbidimetric; and textural class was tested thru Bouyoucos Hydrometer (https://rfo3.da.gov.ph/index.php/regional-soils-laboratory/, n.d.).

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 9 ml 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 (RI, P., 1948). PVK agar medium contained the following ingredients for 1liter capacity: Yeast extract 0.5 g, Dextrose 10 g, Tricalcium phosphate($Ca_3(PO_4)_2$) 5 g, Ammonium sulfate ($(NH_4)_2SO_4$) 0.5 g, Potassium chloride (KCl) 0.2 g, Magnesium sulfate ($MgSO_4$) 0.1, Manganese sulfate ($MNSO_4$) 0.0001 g, Ferrous sulfate ($FeSO_4$) 0.0001 g, Bacteriological Agar/Agar 15 g, 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-50 L) at 15 lbs pressure, 121°C for 15 minutes. In an inverted position, all petri dishes were incubated at 27°C-30°C for the duration of 7 days. After incubation, the colonies having clear halo zone were screened and selected. They were streaked in 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°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°C ± 2°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 (Edi Premono, 1997).

$$PSI = \frac{colonydiameter + holozone\ diameterPSI}{colony\ diameter}$$

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, Philippines. 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°C, followed by 30 cycles (94°C for 60 seconds, 55°C for 60 seconds and 74°C for 60 seconds) and final extension at 74°C for 5 minutes.

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) (http://www.ncbi.nlm.nih.gov/, n.d.). 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

The characteristics of physical and chemical properties of the soils are shown in table 1. The study collected 3 soil samples from three sites intended for onion production. 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 [11]. 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 [12]. 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 [13]. The CECs in soils from Ilocos Sur and Nueva Ecija were found to be 35.19 cmol/kg and 15.44 cmol/kg, respectively, both falling within the normal range while Mindoro Occidental which is clay loam in texture and has a CEC of 13.42 cmol/kg, is considered low. 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. 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, that the reduction of quality of soil used in agricultural production and the 150 decline of the organic matter in the soil is caused by the continuous application of chemical fertilizers [14].

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 0.61 cmol/kg, Mindoro Occidental has 0.28 cmol/kg, and Nueva Ecija has 0.24 cmol/kg. The macronutrients such as Nitrogen and Potassium are very low in three sites. However, the Phosphorus was in normal ranged at 30.62 ppm to 49.44 ppm. Phosphorus content in the soil was mainly because of the application of fertilizer containing Phosphorus over a long period of time. According to, the Phosphorus retention is dominated by precipitation reactions in neutral-to-calcareous soils. 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 [15].

Table 1. Soil chemical-physical properties of the three (3) locations in 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 (%)	1.4	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	18.3	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 phosphorus 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 as described by Acinetobacter isolates were recognized in different characteristics such as negatively oxidase, positively catalase, gram-negative, non-motile, and short rods or coccobacilli. The capacity of the isolated bacteria to solubilize phosphate using tricalcium phosphate as the Phosphorus 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. Effective PSB in soil has been reported from bacterial strains, including Acinetobacter [16,17].

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 [18]. 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 [19]. 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 Phosphorus solubilizing activity, as evidenced by the size differences between their colonies and Phosphorus 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, followed by Ilocos Sur isolate (IS PSB-RGL-2023) of 3.20 mm and the last, Nueva Ecija (NE PSB-RGL-2023) isolate of 2.70 mm (figures 1-3). Based on the result, the three locally-isolated bacteria showed efficient phosphate solubilization that can be utilized to increase the availability of Phosphorus for plant's uptake.

In a report by Adi Nughoro *et al.* (2020), it was found that *Acinetobacter baumannii*, after the 7 days and 14 days incubation period, has mean Phosphorus dissolved in liquid cultures of 10.8 mgl^{-1} and 39.3 mgl^{-1}, respectively and with the solubilization index of 1.83 mm; which is smaller SI than the isolates in this present report. Another study by indicated that after 7 days of incubation, Acinetobacter strain SuKIC24 garnered a SI of 4.0 cm and produced 387 μg/ml of soluble phosphate in PVK media thru the process of phosphomolybdate method and the result demonstrates the strong capability of phosphate solubilization. Fatimah *et al.* (2023) reported that the highest solubilization index from 12 isolates from mangrove soil of East Java, Indonesia was only 2.82 and the author's earlier report (Fatimah *et al.*, 2021) from 19 isolates had a highest solubilization index of 2.36. 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 Phosphorus 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 Phosphorus from the soil for plant's uptake. In this present report, the lowest SI is 2.70 while the highest is 3.42 which is a positive indication that these local isolates have the ability to solubilize a high amount of Phosphorus as proven by several studies [20].

Table 2. Solubilizing index of isolates from the three (3) locations in Ilocos Sur, Mindoro Occidental, and Nueva Ecija.

Representative Isolates	Size in mm
IS PSB-RGL-2023	3.2
MOc PSB-RGL-2023	3.42
NE PSB-RGL-2023	2.7

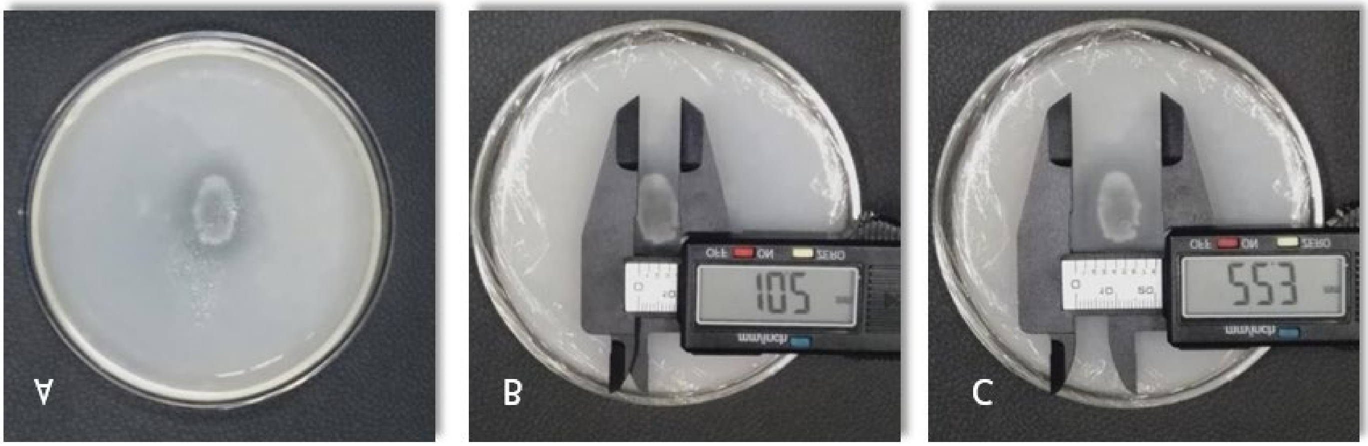

Figure 1. The isolate (IS PSB-RGL-2023) from Bantay, Ilocos Sur, Philippines indicating the 1A) colony morphology; 1B) colony size; and 1C) halo zone size with 16S rRNA gene similar to *Acinetobacter baumannii.*

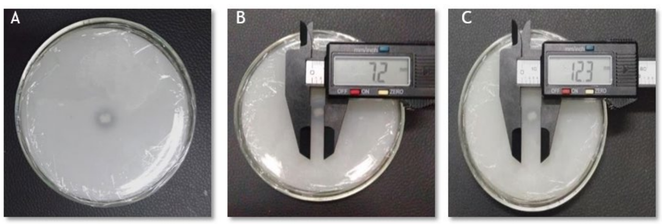

Figure 2. The isolate (NE PSB-RGL-2023) from Bongabon, Nueva Ecija, Philippines indicating the 2A) colony morphology; 2B) colony size; and 2C) halo zone size with 16S rRNA gene similar to *Acinetobacter baumannii.*

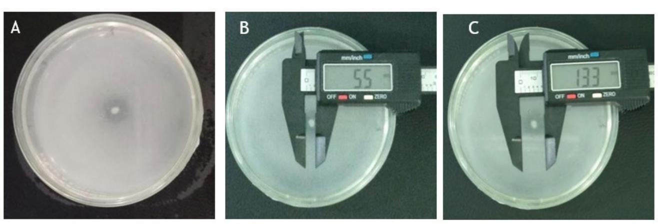

Figure 3. The isolate (MOc PSB-RGL-2023) from San Jose, Occidental Mindoro, Philippines indicating the 3A) colony morphology; 3B) colony size; and 3C) halo zone size with 16S rRNA gene similar to *Acinetobacter baumannii.*

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. *Acinetobacter* species exist in natural ecosystems such as soils, aquatic, marine, sediments, the polar region, and even in site with hydrocarbon contamination [21, 22].

According to, *Acinetobacter* is abundant bacteria comprehensively distributed in water and soil ecosystems and contains 17 validly well-explained species and 14 unidentified genomic species. *Baumann* (1968) reported that the estimate population of Acinetobacter cells in soil and water was 105/mg. cited that there existed 924 genomes of Acinetobacter in the Integrated Microbial Genome database (IMG; www.img.jgi.doe.gov) as of September 2014, of which 728 genomes or equivalent to 81% belong to *A. baumannii.* Acinetobacter are chemoheterotrophs and nutritionally diverse, the different substrates they utilized as derivatives of lone carbon and energy are the same of the aerobic Pseudomon. Due to soil complexity, *A. baumannii* has been found to coexist with closely related Acinetobacter species such as *A. bohemicus* [23-26].

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 [27, 28]. Meanwhile, it was reported that *A. baumannii* solubilized 10.8 mgl^{-1} and 39.3 mgl^{-1} of Phosphorus after the incubation of 7 and 14 days, respectively (ADI [1]. 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 in a research conducted by Farokh *et al.* (2011). Moreover, *A. baumannii* was discovered to produce siderophores quantitatively, at a rate of 65. 54SU [29]. By making iron available to plants and creating a shortage of iron for pathogenic fungi, siderophores promote plant growth. A certain Acinetobacter sp. SK2 solubilized 682 μgml^{-1} of TCP and 86 $\mu g\ ml^{-1}$ 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 Phosphorus solubilization (Bharwad and Rajkumar, 2020). According to the Gluconobacter, Pseudomonas, and *Acinetobacter species* which are gram-negative have the Membrane-Bound mGDH, however the sGDH is less particular [9]. In a research of Sachdev, it was found that *A. baumannii* LRFN53 produced ethylene, thus, has the capability in the nitrogen fixation. 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. According to Sidat (1999) *Acinetobacter spp.* is capable of accumulating amount of phosphate larger than what is needed for cell synthesis. The process is called luxury phosphate absorption. This supports the claim that *Acinetobacter spp.* Is the primary microorganism responsible in improved absorption of Phosphorus. According to isolates of *Acinetobacter spp*. grow best in neutral media and are less resistant to extremely acidic environments. Therefore, aside from its ability as plant growth promoter and Phosphorus-solubilizer, the species of Acinetobacter can be explored to be inoculated on acidic soil conditions to increase Phosphorus availability that are being rendered unavailability by low soil pH [30-40].

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* strains that can be explored as a microbial inoculant or biofertilizer for crop production in the country. 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. However, this report was not related to crop production. Shown on 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. 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; NE PSB-RGL- 2023 with 98% similarity to three strains such as *A. baumannii* APP 173, *A. baumannii* AE7, *A. baumannii* ABF9692; and MOc-PSB RGL-2023 with 99% similarity to *A. baumannii* 1326924-1(Figures 4-6).

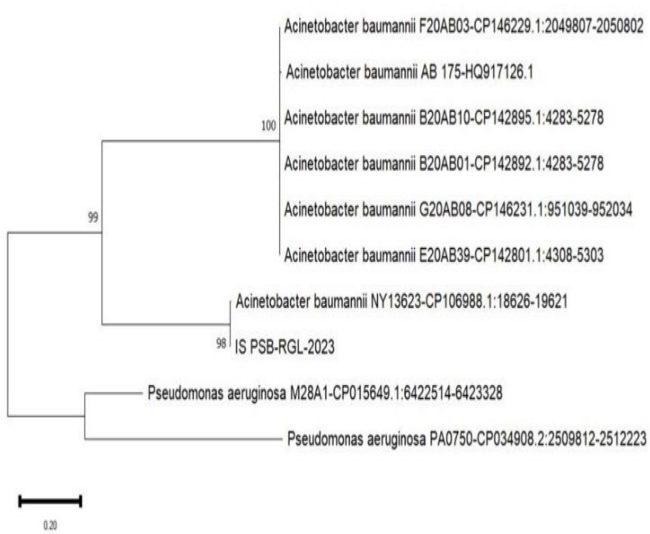

Figure 4. A phylogenetic tree based on the 16S rRNA gene sequences.

The tree was created in MEGA 11 software using the Neighbor-Joining method and 1000 bootstrap replications. The isolates in this investigation are identified by letter and number combinations: IS PSB-RGL-2023 isolate from Ilocos Sur, Philippines.

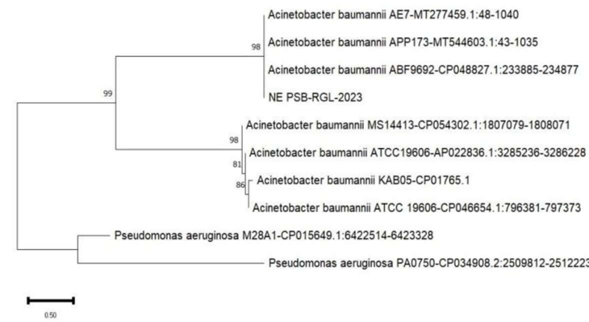

Figure 5. A phylogenetic tree based on the 16S rRNA gene sequences.

The tree was created in MEGA 11 software using the Neighbor-Joining method and 1000 bootstrap replications. The isolate in this investigation is identified by letter and number combinations: NE PSB-RGL-2023 isolate from Nueva Ecija, Philippines.

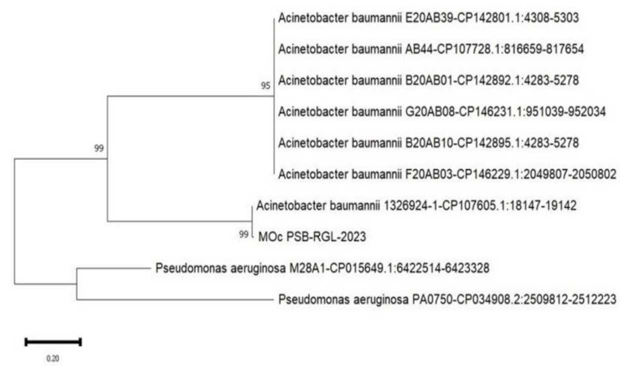

Figure 6. A phylogenetic tree based on the 16S rRNA gene sequences.

The tree was created in MEGA 11 software using the Neighbor-Joining method and 1000 bootstrap replications. The isolate in this investigation is identified by letter and number combinations: MOc PSB-RGL-2023 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 discovered the PSB present in the soils of onion fields in Ilocos Sur, Nueva Ecija, and Mindoro Occidental, Philippines which is dominated by genus Acinetobacter. This genus has the effective capacity of solubilizing phosphate, possess different mechanisms such as organic acid, IAA, siderophores productions which contribute to soil fertility and plant growth and development. With these, A. baumanni can be a potential biofertilizer that enhances crop productivity while also protecting or maintaining environmental conditions 286. In the Philippines, this is the first report as of this time on *A. baumannii* strain as a phosphate solubilizer for onion production. This study serves 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 addition, future research will include the molecular analysis of other genes related to its functions, evolution, and ecological niche to infer on its possible endemism.

Declarations

Author contribution statement

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

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Competing interest statement

The authors declare no conflict of interest.

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