Enrichment of urban waste compost samples allows the isolation of highly efficient phosphate solubilizing bacteria

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Declaration of Competing Interest

The authors reported no declarations of interest.

Author contribution

Sebastian Espinoza: Conceptualization, Methodology, Formal analysis and investigation, Writing original draft preparation. **Cintya Gordon-Falconí:** Conceptualization, Methodology, Writing – review and editing, Funding acquisition, Supervision. **María Daniela Groppa:** Conceptualization, Methodology, Writing – review and editing, Supervision. **Erika Llumiquinga-Marcillo:** Methodology, Formal analysis and investigation.

Abstract

Phosphorus plays a fundamental role in plant growth, development, and defense against pathogens and abiotic stress. However, most soil phosphorus is presented in forms unavailable to plants, thereby reducing crop yield and threatening global food security. Phosphate-solubilizing bacteria (PSB) have emerged as a promising strategy to improve the productivity and sustainability of agroecosystems, although many reported isolates show limited solubilization efficiency, highlighting the need for new isolation approaches. In this study, five isolates identified as *Pantoea cypripedii* and one isolate assigned to the genus *Klebsiella* were obtained for waste compost samples through an enrichment process and evaluated for phosphate-solubilization efficiency. All the isolates showed high solubilization index (SI) values, ranging from 4.06 to 5.38 in 12 days, and high soluble phosphorus production, ranging from 584.15 to 732.12 mg/L after 5 days of incubation. This approach enabled the rapid isolation of highly efficient PSB with potential for biofertilizer development. However, the strong dominance of a single genus raises the possibility that the enrichment process may have caused the underrepresentation of other functional taxa. In addition to meeting the quality standards for their application in crop soils, the use of urban waste compost used in this study underscores the importance of these waste management strategies in urban settings for minimizing environmental impact.

Keywords: phosphorus, urban waste, compost, phosphate solubilizing bacteria, biofertilizer, waste management.

1. Introduction

Phosphorus plays a fundamental role in molecular and cellular processes in plants such as DNA synthesis, cell division, phospholipid biosynthesis [1]. It is also a component of energy-carrying molecules such as ATP, ADP and other metabolic intermediates that support plant growth and development [2] as well as protection against abiotic stresses [3] and defense against pathogens [4]. Therefore, it is not surprising that phosphorus deficiency causes alterations on vegetative growth, root development, flowering, and fruit and tuber formation, thereby reducing the quality and yield of major commercial crops and threatening global food security [1, 5].

In soils, plants can take phosphorus as orthophosphate anions (PO_4^{-3}). However, most soil phosphorus (63-72% of the total phosphorus present in soil) is presented in forms that are not available to plants [6]. This non-labile phosphorus can be slowly released into the available pool and may sustain plant growth in natural ecosystems, but not in cropping systems, which require high levels of available phosphorus and therefore depend on the continuous input of this macroelement in the form of synthetic, mineral or organic fertilizer [7]. However, these phosphorus inputs are also rapidly fixed in the soil, and producers must apply this macroelement in quantities greater than those required by the crop [8]. This overapplication not only causes economical losses to the farmers [9] but also affects the environment through the eutrophication of water bodies, a process that leads to oxygen depletion, disruption of the food chain and restriction on drinking water use [8, 10].

To counteract these negative impacts in environment and human health, phosphate solubilizing bacteria (PSB) have emerged as a promising strategy for improving productivity and sustainability of agroecosystems [11]. These microorganisms contribute to phosphorus mobilization in soils through two principal mechanisms, solubilization and mineralization [12]. Solubilization usually occurs from inorganic phosphorus present in secondary and primary minerals [13] through the production of siderophores, hydroxyl ions, and organic acids. The latter release phosphorus by lowering pH or by acting as chelators of $Ca^{+2,}$ Fe^{+3}, and Al^{+3} cations that usually sequester phosphorus in soils [11]. Mineralization involves dephosphorylation of organic phosphorus forms that are present in plant, animal debris and microbial cell membranes [13]. This process involves the action of three principal enzymes: phosphatases, phytases, and C-P cleavage enzymes [14]. Additionally, PSB are capable of stimulate the activation of phosphate transporters and phosphatase activity in plants root systems [15] , alter positively the microbial indigenous

80 community in crop soils [16], produce phytohormones that promote plant growth [17] and protect plants
81 against pathogens [18].
82 Phosphate-solubilizing bacteria have been isolated from a broad range of sources, including rhizosphere of
83 crops and wild plants, different plant hosts and plant tissues, different types of soils, climate conditions and
84 ecosystems, as well as freshwater, seawater and sediments [19, 20]. In recent years, composting has
85 emerged as a promising technology for managing solid waste in developing countries [21] , and it also
86 represents a valuable organic fertilizer that provides nutrients, improves soil structure and fertility, and
87 enhances the structure and diversity of microbial communities in agricultural soils [22]. In compost
88 systems, it is possible to find a great abundance of microorganisms that secrete hydrolytic enzymes, such
89 as proteases, cellulases, and phosphatases, which decompose complex organic matter into simpler nutrients
90 that are readily available for plant nutrition [23].
91 Previous studies have established the high abundance and adaptability of PSB in composting systems
92 through direct bacteria culture and the use of gene markers [24, 25] especially during the initial and cooling
93 phases of composting [26]. However, PSB in compost piles derived from urban waste remains poorly
94 studied and characterized [27] and the few isolates described in literature have shown limited solubilization
95 rates [28]. This characterization is relevant in a context in which composting is no longer restricted to
96 agricultural and rural settings, but it has also expanded into urban areas, in order to reduce the proportion
97 of organic residues send to landfills and to prevent adverse environmental impacts, principally the
98 uncontrolled release of greenhouse gases [29]. This is specifically noteworthy in urban centers in Ecuador,
99 such as Quito, where organic residues account for 53.18% of total solid wastes, according to official data
100 [30].
101 The isolation of potential biofertilizers candidates from urban compost piles could provide an alternative to
102 synthetic fertilization while also adding value to these systems and supporting their wider implementation
103 in cities, thereby ameliorating waste management issues [21]. The aim of the present study was to isolate
104 and *in vitro* characterize highly efficient PSB from composting systems containing different kinds of urban
105 residues from Quito, Ecuador, with biofertilizer potential to promote more sustainable agriculture practices.
106 **2. Material and methods**
107 **2.1. Chemicals and reagents**
108 **Compost piles locations:** Urban Compost samples were collected from three organic waste management
109 facilities in the Metropolitan District of Quito: (1) "*Agrovivas*" (0°14'57.26" S, 78°29'53.68" W), (2)

"*Anuna*" (0°11'10.43" S, 78°29'08.12" W), and (3) the Environmental Education and Waste Management Center "*ECO-Centro CEGAM Quitumbe*" (0°17'22.58" S, 78°33'43.45" W).

Microbiology Analysis: D-Glucose ($C_6H_{12}O_6$, 99.0%), Nutrient Broth, Luria-Bertani Miller Broth and Agar powder were purchased from TM Media. Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, ≥99.5%) and sodium chloride (NaCl, ≥99.5%) were purchased from Isolabs Chemicals. Ammonium sulphate ($(NH_4)_2SO_4$, 20.98 to 21.38%), potassium chloride (KCl, 99.0%), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 99.0-102.0%), and ascorbic acid ($C_6H_8O_6$, 99.8 %) were purchased from Fischer Scientific. Calcium phosphate (Ca_2PO_4, ≥96.0%) was purchased from Sigma-Aldrich. Antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, 100.6%), was purchased from Mallinckrodt. Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot H_2O$, 99.0%) and sulfuric acid (H_2SO_4, 98.0%) were purchased from Sisco Research Laboratories (SRL).

Chemical analysis: All reagents were of analytical grade or higher and used without further purification. Deionized water (resistivity ≥ 18.2 MΩ·cm) was used throughout. Sulfuric acid (H_2SO_4, 95–97%) was obtained from Scharlau. Nitric acid (HNO_3, 68%, trace metal grade) and sodium bicarbonate ($NaHCO_3$, ≥95%) were purchased from Thermo Fisher Scientific. Hydrochloric acid (HCl, 37%, trace metal grade) was acquired from Supelco. Sodium hydroxide (NaOH, ≥98%) and boric acid (H_3BO_3, ≥99.5%) were supplied by Sigma-Aldrich. Kjeldahl catalyst titanium tablets ($K_2SO_4/CuSO_4/TiO_2$) were obtained from BÜCHI.Multi-element standard solutions for ICP-OES calibration were obtained from Inorganic Ventures.

2.2. Sample process of urban compost piles

Three compost piles at maturity phase, one from each facility, were selected on the basis that they were primarily composed of organic waste from urban households, while also incorporating other types of urban waste. In *Agrovivas*, discarded food from medium-and large-scale food processing and retail companies such as restaurants, hotels and supermarkets is also incorporated. *Anuna* used waste from urban tree pruning and green maintenance, whereas *Eco-CEGAM Quitumbe* incorporated ruminal residues from the municipal slaughterhouse.

Composite sampling was performed following the methodology described by López-Gonzales [31] with some modifications. Subsamples of 250 g were collected in nine different points in each compost pile: three subsamples from the surface (1-10 cm depth), three from the middle (45 cm depth), and three from the bottom (90 cm depth). The subsamples were thoroughly mixed and reduced via the quartile method. Composite samples were collected in sterile resealable plastic bags and stored at 4 ºC for the

physicochemical and microbiology analyses. For identification purposes, *Agrovivas* samples were coded as "PAG", *Anuna* samples as "PAN" and *ECO-CEGAM Quitumbe* as "PQT".

2.3. Chemical characterization of compost samples

For all chemical analyses, compost samples were oven-dried at 60 °C for 24 h and subsequently sieved to <500 µm using a No. 35 ASTM mesh. Compost pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-to-deionized water suspension. Measurements were performed using a multiparameter meter equipped with an Orion ROSS Ultra pH electrode and an Orion COND 4-electrode conductivity probe, Thermo Scientific [32].

Total nitrogen was quantified using the Kjeldahl digestion method (BUCHI Labortechnik AG). Briefly, 0.5 g of compost was digested with concentrated sulfuric acid in the presence of a catalyst, followed by distillation with sodium hydroxide and boric acid, and titration [33]. Urea was used as a quality control standard, yielding a recovery of 103.6%. Total carbon was determined by catalytic combustion oxidation according to Standard Method 5310B, using a TOC-L analyzer coupled with an SSM-5000A module, Shimadzu [34]. Dextrose ($C_6H_{12}O_6$, ≥99.5%) was used as a control standard, with a recovery of 104.65%. Total phosphorus was determined after acid digestion. Approximately 0.5 g of compost was mineralized with 3 mL of concentrated nitric acid (HNO_3, trace metal grade) at 90 °C for 2 h, followed by the addition of 6 mL of concentrated hydrochloric acid (HCl, trace metal grade) and further digestion at 90 °C for 2 h, simulating aqua regia conditions. The digested samples were diluted to 50 mL with deionized water, filtered through a 0.45 µm membrane, and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES; iCAP P 7400, Thermo Scientific) [35]. Calibration curves were prepared using Inorganic Ventures standards. The certified reference material San Joaquin Soil (NIST 2709) was used for quality control, with an average recovery of 75%. Available phosphorus was extracted using the Olsen method. Briefly, 1 g of compost was shaken with 0.5 M $NaHCO_3$ solution for 24 h [36, 37]. Calibration standards, blanks, and quality controls were prepared in the same Olsen extractant matrix and analyzed by ICP-OES under the same conditions described above.

2.4. Enrichment of samples and isolation of potential PSB

For the isolation and characterization of phosphate-solubilizing bacteria (PSB), National Botanic Research Institute phosphate (NBRIP) medium was used [38]. The composition of the medium was as follows: glucose, 10 g; $MgCl_2 \cdot H_2O$, 5 g; NH_4SO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KCl, 0.2 g; Ca_2PO_4, 5 g as the

insoluble phosphorus source, and agar powder, 12 g per liter. Cycloheximide (75 ug/mL) was added to agarized media to inhibit fungal growth.

A prior enrichment step in NBRIP broth was performed for 21 days following the methodology described by Alemneh et al [39] for isolation of more efficient PSB. Briefly, 500 mg of compost was inoculated in 100 mL Erlenmeyer flasks containing 50 mL of fresh sterile NBRIP broth and incubated at 30 ºC and 180 rpm. The enrichment process lasted three weeks, and every 7 days, 100 µL of inoculum was transferred into 50 mL of fresh sterile NBRIP broth and incubated under the same conditions. After 21 days, 100 µL of the enriched inoculum was used for PSB detection according to the methodology of Janati et al. [40] with some modifications. Serial dilution up to 10^{-7} was prepared in 0.9% p/v NaCl, and aliquots from the 10^{-5}, 10^{-6} and 10^{-7} dilutions were inoculated onto NBRIP agar using the spread plate method and incubated for 7 days at 30 ºC. Colonies showing halo formation were selected and purified on NBRIP agar by repeated streaking until uniform colony morphology was observed and assuring that the halo-forming solubilization phenotype was maintained [40, 41]. Finally, pure colonies were transferred to Nutrient Agar and stored at 4 ºC [42] and then transferred to Nutrient Broth, growth for 18 h, and preserved in 15% glycerol at –80 ºC for further analysis [43].

2.5. Estimation of phosphate solubilization efficiency of bacterial isolates

2.5.1. Qualitative estimation of phosphate solubilizing activity

The purified isolates were grown in Nutrient Broth until they reached ~10^9 CFU/mL. A 7 µL aliquot of each bacterial isolate was then spot inoculated on NBRIP agar. Colonies and halo diameters were measured on days 4, 8, and 12 after inoculation, using a vernier caliper. The phosphate solubilization index (SI) was calculated according to the formula proposed by Ibañez et. al [44]:

$$SI = \frac{colony\ diameter\ (mm)\ +\ halozone\ diameter\ (mm)}{colony\ diameter\ (mm)}$$

2.5.2. Quantitative estimation of phosphate solubilizing activity

A 1 mL aliquot of active liquid inoculum (~10^9 CFU/mL) from each bacterial isolate was dispensed in 250 mL Erlenmeyer flasks containing 100 mL of fresh sterile NBRIP broth. One milliliter of sterile fresh Nutrient Broth was used as a negative control. The flasks were incubated at 30 ºC and 180 rpm for 5 days. On days 1, 3 and 5 after inoculation, 2 mL aliquots were taken and centrifuged at 10 000 rpm for 10 minutes according to methodology proposed by Cumpa-Velásquez et al. [45] with slight modifications. An aliquot of 168 uL was diluted with distilled water 1:1000 (v/v) and used for soluble phosphate determination using the Ascorbic Acid Method with slight modifications [46] and determined by UV-Vis spectrophotometry (at

880 nm) (Optizen Pop Spectrophotometer, K LAB Co., Ltd., Korea). Phosphate concentration was calculated using a calibration curve with KH_2PO_4 ranging from 0.075 to 1.5 mg/L. The rest of the volume was used to measure supernatant pH values (Orion Lab Star PH111 pH/mV Bench Meter, ThermoFisher Scientific).

2.7. Molecular identification of bacterial isolates

Genomic DNA extraction of each bacterial isolate was performed using the E.Z.N.A.® Bacterial DNA Kit (Omega Bio Tek) according to manufacturer's protocol with some modifications. To increase the lysis efficiency, incubation with lysozyme was performed for 45 min. DNA samples were quantified before PCR amplification and Sanger sequencing (*Biosequence* Services). The16S rRNA gene region was amplified using primers 27F and 1492R according to [12] with slight modifications. Forward and reverse sequences were assembled to generate a consensus sequence using *BioEdit* software, which was subsequently analyzed using BLAST for genus and species assignment.

2.8. Statistical analysis

A two-way ANOVA was performed to evaluate the solubilization index (SI) of the isolates in the qualitative phosphate solubilization assay, as well as soluble phosphorus concentrations obtained in the quantitative assay. These assays were carried out in triplicate. Data obtained was analyzed using R Studio. Post hoc comparisons were conducted using Tukey's test, and differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical characterization of compost samples

Compost piles evaluated in the present study showed different chemical compositions (Table 1). pH values were strongly alkaline, ranging from 8.95 to 9.70 across the piles evaluated. Similarly, Oviedo-Ocaña et al (2019) reported alkaline pH values ranging from 8.00 to 9.00 during the co-composting of green waste and household organic waste. According to Gaspar et al. [47], increased pH values may result from the mineralization and degradation of organic acids produced by the microbial activity during the initial phases of food waste composting, and due to the complete degradation of amino acids and release of ammonia. Alkaline pH values are generally indicative of compost stability and maturity. However, the very high pH values reported in the present studio could also be attributed to ammonium accumulation in the piles and may lead to ammonia volatilization into the atmosphere [48], which could reduce the quality of the final product [49].

Electrical conductivity values ranged from 1.13 to 1.62 mS/cm in the compost piles evaluated, all below the maximum recommended threshold of 3.5 mS/cm [50]. These values indicate a low salt concentration and suggest a low risk of phytotoxicity effects. Likewise, C/N ratios ranged from 9.86 and 11.83, which, according to Ji et al. [51], are consistent with compost maturity and suitability for application to crop soils. Finally, phosphorus fractions in the compost piles ranged from 3.37 to 4.22 g/kg for total phosphorus (TP) and from 0.81 to 1.36 g/kg for plant-available phosphorus. These values were consistent with those reported by Wei et al. [52], who found similar concentrations of total and available phosphorus in composts derived from kitchen waste, fruit and vegetable waste, and green waste, with total P ranging from 3.29 to 4.62 g/kg and Olsen P ranging from 0.78 to 1.86 g/Kg. In the PQT pile, available P values were considerably higher than those observed in the other compost piles. This may be attributed to the addition of ruminal residue as a raw material during pile preparation. Previous studies have reported that co-composting fruit and vegetables waste with animal manure can increase the concentration of available phosphorus in compost to levels as high as 3.70 g/kg [53].

Table 1. Physicochemical characterization of the urban compost piles analyzed.

Compost Pile	pH	EC (mS/cm)	Total C (%)	Total N (%)	C/N ratio	Total P (g/kg)	Available P (g/kg)
PAG	9.23	1.62	11.94	1.21	9.86	4.22	0.86
PAN	8.95	1.13	13.92	1.34	10.35	3.37	0.81
PQT	9.70	1.51	14.10	1.19	11.83	3.46	1.36

PAG, Agrovivas; PAN, Anuna; PQT, ECO-CEGAM Quitumbe. EC, electrical conductivity; C/N, carbon-to-nitrogen ratio; Total P, total phosphorus; Available P, plant-available phosphorus.

3.2. Estimation of phosphate solubilization efficiency of bacterial isolates

From the three compost piles sampled, the enrichment process allowed the isolation of two representative strains per pile. Selection was based on colony morphology and halo formation. The isolates obtained were P1A and P1C from PAG (Fig. 1A-B), P2A4 and P2B from PAN (Fig. 1C-D), and P3B and P3E from PQT (Fig. 1E-F). All isolates produced a visible halo on NBRIP agar and showed high solubilization index (SI) values (Table 2). In the qualitative phosphate-solubilization assay, SI tended to increase over time, reaching

its highest values on day 12 (4.06 to 5.38). On that day, no statistically significant differences were detected among the isolates, except for P1A, which showed the lowest SI in this study (4.06±0.07).

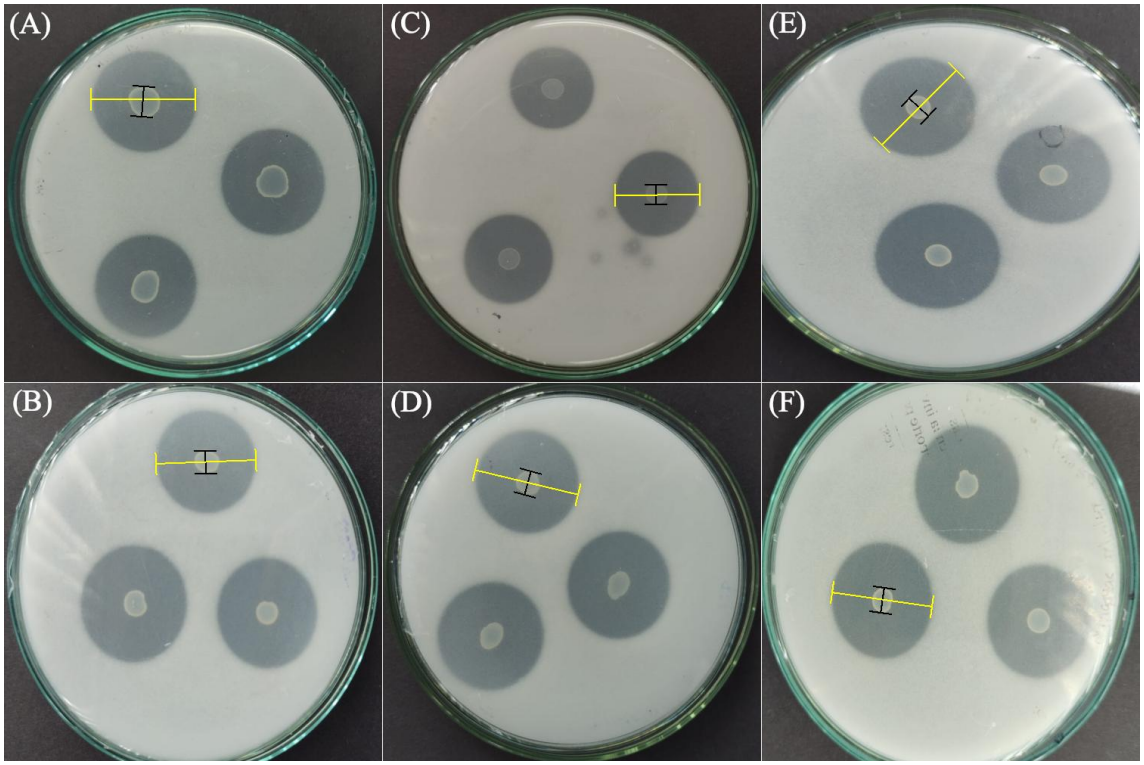

Figure 1. Representative phosphate-solubilizing bacterial isolates from the three urban compost piles on NBRIP agar: A) P1A, B) PIC, C) P24A, P2B, D), P3B, and E) P3E. The yellow bar indicates the solubilization halo diameter, and the black bar indicates the colony diameter.

In the quantitative assay, all isolates showed high concentration of soluble phosphate in the supernatant, ranging from 584.15 to 732.12 mg/L after 5 days of incubation. These values were considerably higher than those reported for isolates obtained from the co-composting of food waste and sawdust [28], which ranged from 82.8 to 126.8 mg/L after 9 days of incubation. The elevated soluble P levels observed in the present study may be attributed to the enrichment process applied prior to the isolation of PSB. Previous studies have indicated that the most efficient PSB may be less abundant in plant rhizosphere [39]; therefore, an enrichment step may favor the proliferation of these less abundant but more effective strains. For example, isolates belonging to the genus *Burkholderia* showed a SI value of 3.29 when obtained without enrichment process, whereas strains from the same genus showed an average SI of 4.08 after enrichment. Similar results were observed in NBRIP broth, where *Burkholderia spp* showed a 1.2-to-4.2-fold greater P solubilization than isolates obtained without enrichment.

In the present study, no statistically significant differences were detected among the isolates in the quantitative assay, except for isolate P1C, which showed a differed pattern that observed in the qualitative test. This discrepancy highlights that halo formation and SI values do not always reflect the actual solubilization potential of PSB. Previous studies have also shown that some isolates that did not produce visible solubilization halos on solid media were still able to release soluble P in liquid culture and even in the soil. Therefore, the qualitative assay alone may not be sufficient for PSB screening [54]. Regarding pH dynamics, all isolates showed a marked decrease during the first 24 hours of incubation, from initial values of approximately 6.8 before inoculation to ranging from 4.09 to 4.20 by day 5 (Table 2). This pattern may be attributed to the production of organics acids by PSB, such as oxalic, formic, and citric acid, which have been reported in previous studies [12, 55]. Additionally, no statically significant differences in soluble phosphorus concentrations were detected among days 1, 3 and 5, indicating a rapid onset of solubilization followed by a stable release of phosphorus in liquid medium.

284 **Table 2.** Solubilization index (SI) and soluble phosphorous concentration of bacterial strains isolated from three compost samples.

Compost Pile	Isolate	Solubilization Index (SI)			Soluble P concentration (mg/L)			
		Day 4	Day 8	Day 12	Day 1	Day 3	Day 5	pH final values
PAG	P1A	3.29±0.03 f	3.75±0.02^{ef}	4.06±0.07^{de}	690.92±80.28 ^{a}	651.28±40.67^{ab}	732.12±108.28^{a}	4.11±0.01
	P1C	3.78±0.16ef	4.50±0.1^{bcd}	4.78±0.11^{abc}	420.21±48.98 ^{b}	579.29±43.31^{ab}	593.96±72.34^{ab}	4.20±0.04
PAN	P2A4	4.14±0.14c^{de}	4.55±0.2^{bcd}	4.82±0.29^{abc}	644.52±91.77^{ab}	590.06±48.57 ^{ab}	584.15±51.06^{ab}	4.15±0.01
	P2B	4.08±0.28b^{cde}	4.36±0.17^{ab}	4.98±0.53^{ab}	617.77±42.22^{ab}	697.12±150.55 ^{a}	631.82±9.16^{ab}	4.13±0.02
PQT	P3B	4.23±0.11c^{de}	4.82±0.24 ^{abc}	5.38±0.11^{a}	646.66±48.41^{ab}	711.37±178.66^{a}	651.53±45.25^{ab}	4.10±0.01
	P3E	4.22±0.20^{cde}	4.78±0.26 ^{abc}	4.37±0.33^{abc}	661.96±27.51^{a}	650.39±19.83 ^{ab}	629.21±46.56^{ab}	4.09±0.00

285 Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. Statistically significant differences are indicated by different lowercase letters

3.3. Molecular identification of bacterial isolates

The six phosphate-solubilizing bacterial isolates obtained from the compost enrichment process were identified by 16S rRNA gene sequencing and BLAST analysis (Table 3). All sequences showed high similarity values (98.33–98.94%) and E-values of 0.0, supporting robust taxonomic assignment at the genus level.

Table 3. Bacterial strains isolated from compost samples and their identity percentages (≥ 98%) based on GenBank accession sequences obtained by BLAST analysis.

Isolate	Microorganism Name	Gen Bank Accession Code	Identity percentage	E value
P1A	*Pantoea cypripedii* strain DFJ-4	PQ836518.1	98.88%	0.0
P1C	*Klebsiella sp.* Strain H7	MH588303.1	98.54%	0.0
P2A4	*Pantoea cypripedii* strain Ghg22-3	MH001548.1	98.73%	0.0
P2B	*Pantoea cypripedii* strain P11	OP810910.1	98.33%	0.0
P3B	*Pantoea cypripedii* ATCC 29267	NR_118857.1	98.66%	0.0
P3E	*Pantoea cypripedii* ATCC 29267	NR_118857.1	98.94%	0.0

Five out of six isolates were assigned to the genus *Pantoea*, showing highest similarity to *Pantoea cypripedii*, while one isolate (P1C) was affiliated with *Klebsiella sp.* This marked predominance of *Pantoea* suggests a strong selection for this genus under the composting conditions and/or the isolation strategy employed. Although compost is typically considered a microbiologically diverse environment [56] the low taxonomic diversity observed here may reflect enrichment of specific functional groups with phosphate-solubilizing capacity.

Members of the genus *Pantoea* have been increasingly reported as plant-associated bacteria with multiple plant growth-promoting traits, including inorganic phosphate solubilization, organic acid production, phytohormone synthesis as wells as biocontrol agents of fungal and bacterial diseases in commercially relevant crops [54, 57, 58]. Their dominance in the present study is therefore consistent with their ecological versatility, adaptability to different environments, robust competition mechanisms and biodegradative capabilities [57, 59] that allows them to thrive in nutrient-rich but competitive environments such as compost. In contrast, the detection of a single *Klebsiella* sp. isolate indicates the presence of less abundant but potentially relevant taxa contributing to phosphorus mobilization, as it has been previously reported for

Klebsiella strains [60]. Despite the high sequence identity values (>98%), species-level assignment based solely on 16S rRNA gene sequences should be interpreted with caution, particularly within genera such as *Pantoea*, where closely related species often exhibit limited resolution using this marker [61]. Therefore, the identification of isolates as *Pantoea cypripedii* should be considered provisional, pending further phenotypic or genotypic characterization with other housekeep genes as described in other studies [62].

4. Conclusions

The results obtained in this study indicate that urban waste compost can serve as an effective source of phosphate-solubilizing bacteria, specifically when an enrichment methodology is applied prior to isolation. The isolates described in this study can decrease pH and release soluble phosphorus rapidly after inoculation in NBRIP medium with Ca_2PO_4 and maintaining this activity over time. Future studies should focus on characterization of acid organic profiles produced by these isolates as well as other plant growth promoting traits such as IAA and siderophore production, nitrogen fixation, and pathogen antagonism.

Most of the isolates were assigned to the genus *Pantoea*, suggesting that members of this group are well adapted to compost environments and may play a relevant role in phosphorus mobilization. The detection of a *Klebsiella* isolate, although less frequent, also points to the contribution of other taxa that might be overlooked under the applied isolation conditions. In this sense, it is likely that the diversity of phosphate-solubilizing bacteria present in the original material was underestimated however it allowed the isolation of highly efficient PSB that represent promising candidates for further evaluation as bioinoculants. Additional studies addressing their functional performance, stability, and interaction with plants under controlled and field conditions are required to validate their biotechnological potential. Additionally, it was demonstrated that compost samples from three different urban organic waste management facilities not only met quality standards for their application in crop soils as an organic fertilizer but were shown to be a great source of plant growth promoting bacteria, specifically PSB with a biofertilizer potential. These findings also highlight the importance of implementing and expanding composting as an organic waste management strategy in urban settings.

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