Endophytic and rhizospheric bacteria associated with the phytoremediators crown flower and candle bush, and their roles in phosphate–potassium solubilization

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Abstract. Crown flower and candle bush show strong tolerance to the multi-constraint conditions of the Putri Cempo landfill, including drought and heavy-metal stresses that are worsening with climate change and contributing to soil degradation. This resilience is likely supported by beneficial microorganisms, particularly phosphate- and potassium-solubilizing bacteria that improve nutrient availability under adverse conditions. This study compared the population density, colony morphotypes, and nutrient-solubilization capacity of endophytic and rhizospheric bacteria from both plants to identify promising isolates for biofertilizer and bio-phytoremediation. Bacteria were isolated from rhizosphere soil and surface-sterilized leaf and root tissues using Pikovskaya medium, and their PSI and KSI were assessed on Pikovskaya and Aleksandrov media. Plant growth traits and rhizosphere characteristics were also evaluated. Candle bush growing directly on the dump-site showed lower total bacterial and PSB densities than the crown flower located 200 m away. Despite higher nutrients, elevated heavy-metal levels likely suppressed bacterial abundance. Among 38 PSB isolates, 52.63% exhibited dual phosphate- and potassium-solubilizing activity. The highest PSI (5.90) and KSI (5.11) were produced by isolates KRhizS5 and KRe8 from candle bush, while isolates BRhizS7 and BRhizS3 yielded the highest values in crown flower. These isolates show strong potential as biofertilizers and partner agents in bio-phytoremediation.

1 Introduction

One of the major consequences of rapid growth of urbanization is the increase in waste, from both industrial and domestic sources. Most of this waste is ultimately disposed of in landfills.

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In many countries including Indonesia, many landfills still operate using open-dumping practices [1], which pose substantial environmental risks when not properly managed, such as emission of greenhouse gases produced during waste decomposition, the accumulation of heavy metals, xenobiotic contaminants and micro-plastic [2] that can contaminate surrounding ecosystems.

The Putri Cempo landfill in Surakarta, Indonesia, has been operating since 1986 and still employs an open-dumping disposal system. This landfill spans approximately 17 ha, with 16 ha occupied by accumulated waste and facility buildings, while the remaining 1 ha comprises open space and leachate disposal areas [3]. Multiple studies have consistently demonstrated that the area surrounding the Putri Cempo landfill is contaminated with heavy metals. [4] reported Pb and Cd enrichment in soils from 14 sampling locations at depths of 10, 30, and 60 cm, with concentrations exceeding background levels (Pb: 20.1–91.2 mg/kg; Cd: 0.1–2.8 mg/kg). Notably, Pb concentrations increased with proximity to the waste pile. In addition, [5] detected Cr (1.2279 mg/L) and Ni (0.94842 mg/L) at levels surpassing national quality standards (SNI). The ecological conditions at the site further limit plant establishment. The landfill has leachate drainage system but no irrigation system for the plant growth on the surrounding area, leaving all vegetation entirely dependent on natural rainfall. Over the past five years, data from the Surakarta City Central Bureau of Statistics (2020–2024) indicate that the area has experienced very low precipitation during the dry season, particularly from June to October, with rainfall dropping to zero in August in the last two years. These increasingly severe dry-season conditions reflect broader climate change phenomena, characterized by reduced rainfall, altered seasonal regimes, and longer, more frequent droughts. Such climatic shifts intensify environmental stresses that broadly constrain plant growth [6].

Despite these harsh conditions, several phytoremediator species, most notably crown flower (*Calotropis gigantea*) and candle bush (*Senna alata*) are able to grow successfully in this multi-constraint environment. In addition to their inherent phytoremediation capacity and tolerance to heavy metals, the persistence of *S. alata* and *C. gigantea* in such challenging habitats is likely supported by beneficial microorganisms, such as phosphate- and potassium-solubilizing bacteria, which can improve nutrient availability and enhance plant resilience to multiple environmental stresses [7,8].

Phosphorus is an essential macronutrient that supports plant growth under stress by promoting ATP and membrane synthesis, stimulating root development and soil exploration, improving stomatal regulation and photosynthetic efficiency, and increasing osmolyte and antioxidant accumulation [9]. It also reduces heavy-metal availability through immobilization and promotes phytochelatin synthesis, thereby lowering metal toxicity [9]. Similarly, potassium plays a central role in metabolism and stress adaptation by activating enzymes, regulating protein synthesis, photosynthesis, osmoregulation, and ionic balance, as well as controlling stomatal aperture to limit water loss during drought [10]; it also mitigates heavy-metal stress by supporting enzymatic activity and reducing oxidative damage [11].

In addition to enhancing soil nutrient availability and improving overall soil health, phosphate and potassium solubilizing bacteria also function as soil microorganisms capable of contributing to carbon sequestration [12]. These bacteria, like other soil microbial communities, can capture atmospheric carbon dioxide (CO_2) through carbon fixation processes and promote its stabilization within soil organic matter. By increasing the amount of carbon stored in the soil, these microorganisms help reduce atmospheric CO_2 concentrations and thereby support climate change mitigation [12].

In landfill environments characterized by heavy metal contamination and seasonal drought, the availability of phosphorus and potassium becomes critical for sustaining plant growth and stress tolerance. Within this context, the ability of crown flower and candle bush to persist under such constraints positions them as promising hosts for investigating

beneficial microbial communities. In particular, isolating multifunctional phosphate-solubilizing and potassium-solubilizing bacteria from both their rhizosphere and endophytic tissues provides an opportunity to identify superior microbial isolates that enhance nutrient acquisition, improve stress tolerance, and potentially contribute to metal immobilization. Accordingly, this study aimed to assess and compare the population density, colony morphotype diversity, and phosphate- and potassium-solubilization capacities of endophytic and rhizospheric bacteria associated with these two plant species. Such superior isolates hold considerable potential for development as biofertilizers, bioremediation agents, and plant bioresistance inducers, thereby supporting the growth of these species and potentially other crops under similarly adverse environmental conditions.

2 Material and Methods

2.1 Survey and sampling

Field surveys were carried out around the Putri Cempo landfill in Surakarta, Central Java, Indonesia. Sampling was carried out within the coordinate range 7°32'15"S, 110°51'14"E to 7°32'18"S, 110°51'17"E within the landfill vicinity to capture zones most directly affected by heavy-metal accumulation and seasonal water scarcity. Two plant species, candle bush (*Senna alata*; Indonesia: ketepeng cina) which grown on garbage dump area, and crown flower (*Calotropis gigantea;* Indonesia: biduri) grown on open area 200 m distance from candle bush, were chosen due to their high abundance in the Putri Cempo landfill area and their documented tolerance to drought stress and heavy metal toxicity. For each selected plant species, three individual plants were sampled as replicates. From each plant, three sample types were collected: leaves, roots, and their rhizosphere soil. Rhizosphere soil samples were used both for bacterial isolation and for soil chemical analyses. Leaves and roots were used for endophyte bacterial isolation.

2.2 Measurement of the several plant growth variables and rhizosphere soil chemical properties

The crown flower and candle bush plants selected for sampling were measured for plant height, stem diameter, and leaf dimensions (length and width). Plant height was measured from the soil surface to the highest point of growth, with stem diameter taken at a height of about 10 cm from the soil surface. Leaf length and width were measured from the third fully expanded leaf. Rhizospheric soil collected from these plants was also subjected to soil chemical analysis, including measurements of soil pH (H_2O), organic C (Walkey and Black methode), total N (Kjeldahl), available P (Olsen), and available K (1N ammonium acetate extraction).

2.3 Isolation of endophytic and rhizospheric soil bacteria

Endophytic bacteria were isolated from leaf and root tissues following surface sterilization. For each plant part (leaf and root) of crown flower and candle bush, 10 g were weighed and subjected to a modified surface-sterilization method [13]. The samples were surface-sterilized by immersion in 70% alcohol (1 min), 3.25% NaOCl (3 min), and again in 70% alcohol (1 min), followed by 4–5 rinses with sterile distilled water. Following surface sterilization, the leaf and root tissues were aseptically ground and subjected to serial dilutions in sterile 0.85% NaCl solution (10^{-1}–10^{-5}). Rhizospheric soil samples underwent the same dilution procedure but were extended to 10^{-7}. Subsequently, 0.1 mL from each dilution was

inoculated onto a medium specific for phosphate-solubilizing microorganisms (PSM), Pikovskaya's agar, using the spread plate method. All cultures were incubated at room temperature for a 72-hour period, after which population density and colony morphotype diversity were assessed. Population density was calculated using the following formula:

$$\text{Population density} = \frac{Number\ of\ colonies \times Dilution\ factor}{volume\ plated\ (mL)} \quad (1)$$

2.4 Purification and assessment of phosphate and potassium solubilization indices (PSI and KSI)

Isolates that exhibited clear halo zones were subsequently purified to the same medium of Pikovskaya medium agar. Then, the purified isolates were streaked onto two types of medium: the first to Pikovskaya medium agar to determine the phosphate solubilization index (PSI), and the second to Aleksandrov medium agar to measure the potassium solubilization index (KSI). The PSI or KSI value was calculated using the following formula:

$$PSI\ or\ KSI = \frac{Total\ diameter\ (colony + halo\ zone)\ (mm)}{Colony\ diameter\ (mm)} \quad (2)$$

2.5 Statistical analysis

A t-test was applied to evaluate differences in plant sampling measurements and rhizosphere soil chemical properties. Population density, along with PSI and KSI were subjected to one-way ANOVA, and significant differences among means were determined using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

3 Results and discussion

3.1 Plant Growth Parameters and Soil Chemical Properties

The plants used as sampling sources for bacterial isolation exhibited plant growth parameters as presented in Table 1. Crown flower showed larger size characteristics, including plant height, stem diameter, and leaf dimensions compared to candle bush. These size differences were not attributed by soil nutritional status, as the rhizosphere of candle bush had higher levels of organic C, total N, available P, and available K than that of crown flower (Table 2). Instead, the variation in plant size is principally due to species-specific differences and the fact that these plants were collected from natural habitats, where their exact ages were unknown.

Table 1. Plant growth parameters

Plant types	Plant height (cm)	Stem diameter (cm)	Length of the third leaf (cm)	width of the third leaf (cm)
Crown flower (*Calotropis gigantea*)	205 ± 11.36	34.5 ± 1.51 a	16 ± 2.23 a	9.5 ± 0.72
Candle bush (*Senna alata*)	184 ± 9.64	22.1 ± 2.90 b	10.6 ± 2.65 b	6.1 ± 2.50
T-test	NS	**	*	NS

Note: **: $p<0.01$; *: $0.01 \leq p \leq 0.05$; ns: >0.05 (non-significant)

Table 2. Soil chemical characteristics

Rhizosphere soil of plant types	pH H_2O	Organic C (%)	Total N (%)	Available P	Available K
Crown flower (*Calotropis gigantea*)	6.1 ± 0.05	1.40 ± 0.01 b	0.42 ± 0.02	7.99 ± 0.16 b	0.59 ± 0.004 b
Candle bush (*Senna alata*)	6.0 ± 0.02	2.92 ± 0.03 a	0.42 ± 0.02	10.68 ± 1.11 a	1.85 ± 0.005 a
T-test	NS	**	NS	**	**

Note: **: $p<0.01$; *: $0.01 \leq p \leq 0.05$; ns: >0.05 (non-significant)

The differences in soil nutritional status between the two plant species are likely attributed to their distinct sampling locations. The candle bush samples were collected closer to the waste piles than the crown flower samples. Increased proximity to waste piles generally elevates soil nutrient levels, largely due to the higher organic matter input from decomposing waste, which subsequently enhances soil fertility [14].

3.2 Population density and diversity of the colony morphotypes of bacterial isolates

Total bacterial colonies grown on the isolation media (Pikovskaya medium agar) were calculated as total bacteria, while the colonies capable of halo zone formation were identified as phosphate-solubilizing bacteria (PSB). In the present study, all these data are presented in Table 3. Diversity of colony morphotypes observed from the total colonies grown on the isolation media were 22 from crown flower and 27 from candle bush, in which 20 and 18 distinct colonies from these two plants were identified as the phosphate-solubilizing bacteria (PSB).

Table 3. Population density and colony morphotype diversity of total Total bacteria and Phosphate solubilizing bacteria

Source of bacterial isolate		Population density total bacteria (10^5 CFU/g)	Diversity of colony morphotypes of total bacteria	Population density halozone-forming bacteria (10^5 CFU/g)	Diversity of colony morphotypes of halozone-forming bacteria
Crown flower (*Calotropis gigantea*)	Leaf endophyte	0.033 ± 0.02 c	6	0.017 ± 0.01 d	5
	Root endophyte	1.693 ± 4.32 c	5	0.243 ± 2.51 cd	5
	Rhizosphere soil	13.375 ± 4.80 a	11	1.3 ± 0.52 a	10
Candle bush (*Senna alata*)	Leaf endophyte	0.029 ± 0.01 c	7	0.007 ± 0.00 bc	4
	Root endophyte	0.064 ± 0.01 c	9	0.016 ± 0.01 d	8
	Rhizosphere soil	7.4 ± 2.94 b	11	1 ± 0.55 ab	6
ANOVA		**		**	

Note: **: $p<0.01$; *: $0.01 \leq p \leq 0.05$; ns: >0.05 (non-significant)

Population density for total bacteria and PSB exhibiting halo zones showed significant differences between plant species and among endophytic and rhizosphere bacterial groups based on ANOVA (Table 3). The rhizosphere soil of crown flower exhibited the highest population densities for both total bacteria and PSB (Fig. 1), reaching 13.375 × 10^5 CFU g^{-1} and 1.3 × 10^5 CFU g^{-1}, respectively. Similarly, endophytic bacterial population density of total bacteria and PSB, both root and leaf endophytes, were also higher in crown flower compared with the candle bush. In crown flower, the root endophytes reached 1.693 × 10^5 CFU g^{-1} for total bacteria and 0.243 × 10^5 CFU g^{-1} for PSB, while the leaf endophytes reached 0.033 × 10^5 CFU g^{-1} and 0.017 × 10^5 CFU g^{-1}, respectively.

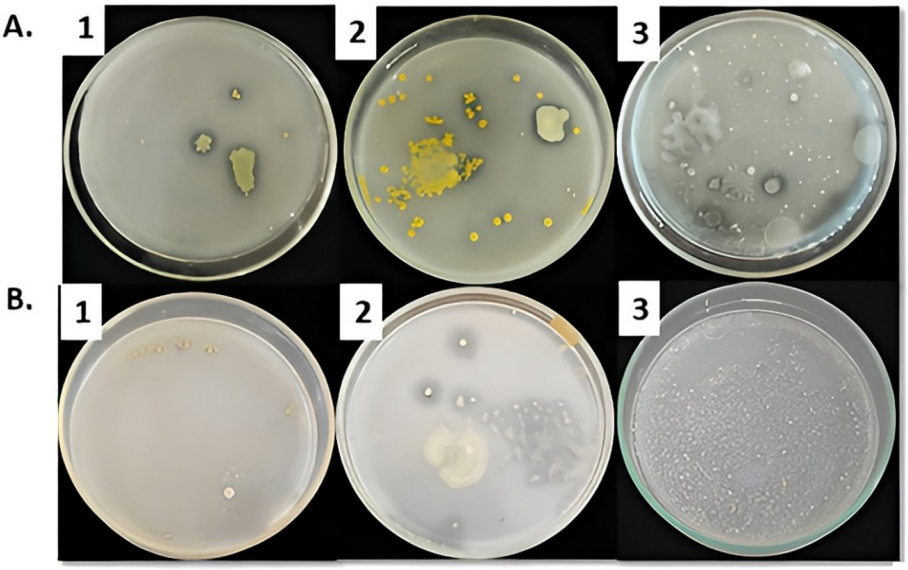

Fig. 1. Isolated phosphate-solubilizing bacteria (PSB) on Pikovskaya medium from rhizospheric soil and endophytic tissues (leaf and root) of crown flower and candle bush. A: crown flower; B: candle bush; 1: leaf bacterial endophytes; 2: root bacterial endophytes; 3: rhizospheric soil bacteria.

Despite the larger total number of isolates obtained from candle bush, crown flower exhibited a greater abundance of phosphate-solubilizing bacteria (PSB) and higher population densities for both total bacteria and PSB. These differences are likely driven by the contrasting environmental conditions at the sampling sites. Crown flower was collected from an area located farther from the waste pile, whereas candle bush grew in closer proximity to the waste pile. Previous studies at the Putri Cempo landfill showed that heavy-metal concentrations increase as the distance from the waste pile decreases [4]. This pattern is further supported by findings from [14], who reported that bacterial diversity in landfill soils decreases in response to elevated heavy-metal concentrations.

Across both host plants, a consistent trend was observed in bacterial population density, with rhizosphere soils exhibiting the highest values, followed by root endophytes, and the lowest densities detected in leaf endophytes. This pattern is consistent with the findings of [8], who reported that in endophytic and rhizospheric bacterial communities from five plant species in the Alas Bromo agroforestry system, rhizosphere bacterial populations exceeded those of endophytes, and root endophytes exhibited higher densities than leaf endophytes. Consistent results were also documented by [13] in organic wetland rice, where rhizospheric bacteria were more abundant than root endophytes.

3.3 Phosphate and Potassium solubilization indices of bacterial isolates

Of the 38 phosphate-solubilizing bacterial isolates obtained from the rhizosphere and endophytic sources of crown flower and candle bush, all selected for further screening (Table 4) showed significant variation in their phosphate and potassium solubilization indices.

Table 4. Phosphate and potassium solubilization indices (PSI and KSI) of bacterial isolates

Source of bacterial isolate		Isolate code	PSI	KSI
Crown flower (*Calotropis gigantea*)	Leaf endophyte	BLe1	2.31 ± 0.09 defgh	2.10 ± 0.13 def
		BLe2	2.68 ± 0.32 cdef	1± 0 k
		BLe3	2.25 ± 0.31 efgh	1.87 ± 0.39 efgh
		BLe4	2.37 ± 0.22 cdefg	1.59 ± 0.04 ghi
		BLe5	2.59 ± 0.23 cdef	1.72 ± 0.07 fgh
	Root endophyte	BRe1	1.36 ± 0.10 jkl	2.27 ± 0.35 de
		BRe2	1.81 ± 0.29 ghij	2.25 ± 0.48 de
		BRe3	1.40 ± 0.07 ijkl	1 ± 0 k
		BRe4	1.64 ± 0.10 hijkl	1 ± 0 k
		BRe5	3.00 ± 0.40 c	0 ± 0 l
	Rhizosphere soil	BRhizS1	2.68 ± 0.21 cdef	1 ± 0 k
		BRhizS2	1.89 ± 0.19 ghij	0 ± 0 l
		BRhizS3	2.94 ± 0.19 cd	2.73 ± 0.24 c
		BRhizS4	1.38 ± 0.16 jkl	1.47 ± 0.26 hij
		BRhizS5	1.11 ± 0.11 kl	1.63 ± 0.11 ghi
		BRhizS6	2.38 ± 0.15 cdefg	0 ± 0 l
		BRhizS7	4.38 ± 0.31 b	1.65 ± 0.24 ghi
		BRhizS8	1.38 ± 0.18 jkl	2.24 ± 0.76 de
		BRhizS9	2.85 ± 0.07 cde	0 ± 0 l
		BRhizS10	2.60 ± 0.23 cdef	0 ± 0 l
Candle bush (*Senna alata*)	Leaf endophyte	KLe1	1.29 ± 0.08 jkl	0 ± 0 l
		KLe2	2.24 ± 0.02 efgh	0 ± 0 l
		KLe3	2.09 ± 0.16 fghi	0 ± 0 l
		KLe4	1.55 ± 0.13 ijkl	1 ± 0 k
	Root endophyte	KRe1	2.40 ± 0.46 cdefg	1.90 ± 0.02 efg
		KRe2	1.79 ± 0.12 gkijk	2.45 ± 0.45 d
		KRe3	1.46 ± 0.04 ijkl	3.36 ± 0.08 b
		KRe4	1.80 ± 0.06 gkijk	1.25 ± 0.20 ijk
		KRe5	1.44 ± 0.08 ijkl	1 ± 0 k
		KRe6	1.87 ± 0.43 ghij	3.29 ± 0.26 b
		KRe7	1.38 ± 0.10 jkl	1.52 ± 0.1 ghij
		KRe8	3.01 ± 0.68 c	5.11 ± 0.30 a
	Rhizosphere soil	KRhizS1	0 ± 0 m	0 ±0 l
		KRhizS2	1.57 ± 0.06 ijkl	1.16 ± 0.16 jk
		KRhizS3	1.45 ± 0.37 ijkl	1 ± 0k
		KRhizS4	1.54 ± 0.12 ijkl	1 ± 0 k
		KRhizS5	5.90 ± 1.60 a	3.63 ± 0.42 b
		KRhizS6	1 ± 0 l	1 ± 0 k
ANOVA			**	**

Note: **: $p<0.01$; *: $0.01 \leq p \leq 0.05$; ns: >0.05 (non-significant)

A total of 20 isolates were able to solubilize potassium, as indicated by halo formation on Aleksandrov medium. The highest phosphate solubilization index (PSI) and potassium solubilization index (KSI) were recorded in isolates from candle bush, specifically the rhizospheric isolate KRhizS5 with a PSI of 5.90 and the root endophytic isolate Kre8 with a KSI of 5.11.

In crown flower, five leaf endophytic isolates exhibited PSI values of 2.25–2.68, of which four demonstrated potassium-solubilizing activity with KSI values of 1.00–2.10. Among the five root endophytic isolates, PSI values ranged from 1.36 to 3.00, and two were capable of solubilizing potassium, producing KSI values of 0–2.27. In the rhizosphere, ten isolates showed PSI values of 1.11–4.38, and five exhibited potassium-solubilizing ability with KSI values of 0–2.73.

In candle bush, four leaf endophytic isolates had PSI values of 1.29–2.24, but none showed potassium-solubilizing activity. Among the eight root endophytic isolates, PSI values ranged from 1.38 to 3.10, and seven were able to solubilize potassium, with KSI values of 1.00–5.11. In the rhizosphere soil, six isolates displayed PSI values of 0–5.90, and two exhibited potassium-solubilizing activity with KSI values of 0–3.63.

The PSI values recorded in this study were lower than those reported by [8] for five plant species in the Alas Bromo agroforestry system. However, the KSI values obtained in this study were higher, whereas the KSI values reported for the Alas Bromo isolates were only in the range of 1.00 to 3.84. These differences are likely attributable to variations in environmental conditions and soil chemical properties across study sites, both of which substantially influence microbial phosphate- and potassium-solubilization performance [15].

The high PSI and KSI values of the isolates obtained from candle bush consistent with the higher available P and K detected in its rhizosphere compared to that of crown flower. This pattern is further supported by previous findings showing that isolates with a phosphate-solubilization index above 3 can increase soil available P by more than 50–90% relative to basal treatment [8].

4 Conclusion

The population densities of total bacteria and phosphate-solubilizing bacteria (PSB) isolated using Pikovskaya medium were lower in candle bush growing directly on the garbage-dump area than in crown flower growing 200 m from the dump site. This pattern indicates that elevated heavy-metal concentrations at the dump site exerted a stronger suppressive effect on bacterial abundance than the benefits of higher soil nutrient levels. Although candle bush displayed greater morphotype diversity among total bacterial colonies, crown flower exhibited higher morphotype diversity among PSB capable of halo-zone formation. Across plant types, PSB population density was consistently higher in rhizosphere soil than in plant tissues. Of the 38 PSB isolates obtained, 20 (52.63%) exhibited dual phosphate- and potassium-solubilizing activity. Two isolates from candle bush, KRhizS5 and KRe8, showed the highest PSI (5.90) and KSI (5.11), respectively, indicating higher rhizosphere phosphorus and potassium availability at this site. Future studies should investigate how these bacterial partners support phytoremediation under multiple environmental stresses, especially as climate change intensifies drought, temperature extremes, and soil degradation in contaminated areas.

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