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Multifunctional plant growth promoting potential of *Burkholderia vietnamiensis*-OP984178 and *B. ambifaria*-OP984173 isolated from rhizosphere soils, Tanzania

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ABSTRACT

This study aimed to isolate and identify phosphate solubilizing microorganisms from rhizosphere soils and evaluate their plant growth-promoting traits *in vitro*. Fifty-six potential phosphate-solubilizing bacteria were isolated, and ten isolates were selected for further characterization based on their performance. *Burkholderia* species were found to be the predominant solubilizers of insoluble phosphorus sources. Two strains, *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984173, showed the ability to release soluble phosphorus from different sources, including tricalcium phosphate, ferric phosphate, and hard Minjingu rock phosphate powder. Both isolates also demonstrated production of indole acetic acid, with *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984173 producing up to 23.45 µg/ml and 24.24 µg/ml, respectively. Furthermore, the strains showed antifungal efficiencies of 31.48% and 24.81%, respectively, when co-inoculated with *Fusarium proliferatum*-MZ497514 on potato dextrose agar. The study also identified the nitrogen fixation potential of the two strains. Overall, the findings highlight the potential of the selected *Burkholderia* strains as biofertilizers and biocontrol agents, making them valuable candidates for sustainable agriculture practices. Further research is recommended to explore the potential of these strains using test crops.

Keywords: phosphate solubilization, isolation, *Burkholderia* spp, antifungal efficiency, Minjingu phosphate rock, ferric phosphate

INTRODUCTION

The use of expensive chemical fertilizers and pesticides in agricultural production is proving unsustainable as it adversely affects human health and negatively affects the environment. Hence, the safe use of microorganisms that improve soil fertility, enhance plant growth, and limit the growth of phytopathogenic fungi has been receiving immense attention from researchers (Adesemoye et al., 2009).

Burkholderia is a genus of about 100 species characterized by great diversity in function and distribution over a wide range of ecosystems (Madhaiyan et al., 2021). While there are some species with detrimental influence on humans and animals or plants, other species are of beneficial value. Members of the genus have received little attention in agricultural production application technologies due to the early discovery that a large segment of the members, particularly the *B. cepacia* complex (Bcc), comprising 20 species and the *B. pseudomallei* group are known to cause opportunistic infections in cystic fibrosis patients and mellidiosis, respectively.

There are, however, members of the genus *Burkholderia* that perform beneficial interactions with plants, functioning as active rhizosphere components (Castanheira et al., 2016). Some of these members may exhibit one or a few beneficial traits, while others may

have multifunctional plant-growth-promoting properties. Members of the Genus *Burkholderia* have been reported to be capable of phosphate solubilization, production of IAA and siderophores, nitrogen fixation and antipathogenic traits (An et al., 2022; Brito et al., 2018).

The main objective of this study was to isolate and identify phosphate solubilizing microorganisms with potential multifarious plant growth-promoting traits.

MATERIALS AND METHODS

Description of the study area

The study area encompassed two agroecological zones of Tanzania; the Southern Highlands and the Eastern Zone, from where soil sampling was done. The Southern Highlands zone has a tropical climate with seasonal and altitudinal temperature variations and distinct dry and rainy seasons. The rainfall ranges from 823 to 2,850 mm, and the annual temperatures from 13 to 19 °C. Sampling was done across four districts in this zone: Madaba and Namtumbo in the Ruvuma region, and Njombe TC and Njombe district council in the Njombe region. Madaba has a bimodal rainfall pattern, with an annual rainfall of about 1,200 mm, and a mean annual temperature of about 20 °C. Namtumbo has a unimodal rainfall pattern, with annual rainfall from 800 to 1,600 mm, and mean annual temperature from 18 to 24 °C. Njombe TC and Njombe district council have a bimodal rainfall pattern, with annual rainfall of about 1,000 mm, and a mean annual temperature of about 16 °C. The Eastern Zone has a hot and humid climate throughout the year, especially from November to April. Sampling was done across two districts in this zone: Mvomero and Morogoro DC in the Morogoro region. Mvomero has a bimodal rainfall pattern, with annual rainfall from 600 to 1,200 mm, and mean annual temperature from 22 to 28 °C. Morogoro DC has a bimodal rainfall pattern, with annual rainfall from 700 to 1,000 mm, and mean annual temperature from 23 to 27 °C.

Soil sampling and screening for plant growth promotion traits

Soil samples were collected from the rhizosphere (crop plant roots with their adhering soil to a depth of 20 cm). Ten-used rhizobacteria in this study were isolated from *Ipomea batatas* (Mzm spo, Ksptk, Ksptn, Ksptz and Mzm spk), *Zea mays* (SUA Mz4, K3MZ AND MK34), *Oryza sativa* (SUA R1) and *Cajanus cajan* (Mzw Pgp) crops. To prevent contamination and moisture loss, the soil samples were packed in sterile plastic zipper bags, sealed tightly, and transported to the Soil Science Laboratory, Sokoine University of Agriculture, Morogoro, where the samples were processed immediately.

Phosphate solubilizing bacteria (PSB) from soil samples were isolated according to Khan et al. (2014). One gram of soil sample was dispersed in 9 ml of autoclaved distilled water and serially diluted. An aliquot of 100 µl from each dilution was then spread on Pikovskaya's agar (PVK) medium (Pikovskaya, 1948). The pH of the medium was adjusted to 7.0 before being autoclaved. The plates were incubated on a stationary incubator (BBC Goerz Metrawatt, GTR 0214) at 28 ± 1 °C for 7 days. Colonies that exhibited clear zones around them were identified as PSB and were subsequently transferred to liquid broth and agar slants for further analysis.

The resultant isolates were subjected to multiple screening tests to detect some of the most important plant-growth promotion traits namely, phosphate solubilization, IAA and siderophore production, nitrogen fixation as well as antifungal activity against pathogenic fungus *Fusarium proliferatum*-MZ497514.

Assays for phosphate solubilization by various isolates

Tests for phosphate solubilization were performed using three insoluble phosphate resources, tricalcium phosphate $Ca_3(PO_4)_2$, ferric phosphate ($FePO_4$), and hard Minjingu rock phosphate, as the only sources of phosphorus in the growth medium.

In the first setup, $Ca_3(PO_4)_2$ was used as a component of the PVK agar medium to assess phosphate solubilizing potentials of isolates. In the second assay, PVK was modified by replacing the $Ca_3(PO_4)_2$ with $FePO_4$ and in the third assay, the hard Minjingu rock phosphate powder was used as a source P in the growth medium (Simfukwe and Tindwa, 2018; Kwaslema et al., 2022).

Quantitative estimation of individual isolate's potential for phosphate solubilization was done by inoculating them on broth cultures (Paul and Sinha, 2016) and incubation on an orbital incubator (Stuart SI500/120/60 Incubator, Orbital Shaker, 120 VAC) at 28 °C and 150 rpm for 192 hours, followed by centrifugation of broth solution at 10,000 rpm for 10 min using a multiSPIN Centrifuge (Clever Scientific ltd, CR2000R). The amount of soluble P was determined at 24, 72, 144, and 192 hours of incubation. The colorimetric molybdate blue method was used for the determination of available soluble phosphate in the culture supernatant (Olsen et al., 1982). Qualitative estimation of phosphate solubilization was performed after seven days of incubation of individual isolates at 28 ± 2 °C on three different media: PVK agar media modified PVK agar media with either $Ca_3(PO_4)_2$ or the powdered hard Minjingu rock phosphate.

Assay Indole-3-acetic acid (IAA) production and quantification

Strains with proven phosphate solubilization abilities were further subjected to additional tests for their potential to produce Indole-3-acetic acid (IAA) using a spectrophotometric-Salkowski method, as described by Gordon and Weber (1951). A minimal medium (MM) broth with or without 0.5 % DL-tryptophan was used for the IAA test. Briefly, each isolate was inoculated in the MM broth and incubated on a shaking incubator set at 150 rpm 28 °C for 48 hours. Uninoculated broth was used as a negative control. After 48 hours of incubation, the culture was centrifuged at 8,000 rpm for 10 min and 3 ml of the supernatant was mixed with 6 ml of Salkowski's reagent with vigorous shaking. The mixture was incubated for 30 min at 25 °C in the dark before absorbance reading on a UV/VIS Spectrometer (Wagtech, UNICAM 5625) set at 536 nm wavelength.

Siderophore production assay

The universal Chrome Azurol S (CAS) agar plate assay was used to qualitatively test for the siderophore-producing potential of the isolates. CAS agar was prepared according to the protocol described by Louden et al. (2011). Each isolate was inoculated on a CAS agar plate and incubated at 28 °C for 24 to 36 hours. The development of a halo zone around the colony with a distinct colour change of the medium from deep blue to purplish-red or orange was taken to signify the production of siderophore by the isolate.

Assay for nitrogen-fixation test

Ashby nitrogen-free agar plate assay was used to detect the nitrogen-fixing-ability of the isolates (Sun et al., 2018). The strains that grew on this medium, after incubation at 28 °C for 7 days, were considered to possess the ability to fix nitrogen.

Detection of antifungal activity of isolates

For the selected isolates, the amended agar disk diffusion method (Balouiri et al., 2016) was used to qualitatively screen the antifungal activity against *F. proliferatum*. Briefly, each PSB strain was inoculated on one side of the petri dish onto a potato dextrose agar (PDA) plate and incubated at 28 °C. Forty-eight hours later, a 5 mm diameter disk of fungal mycelium from a 7-day-old culture of *F. proliferatum* was inoculated on the other side of the same petri dish about 2 cm away from the margin. All treatments were performed in triplicate. After incubation at 28 °C, fungal colony diameter was measured on both control and treatment plates at the 6^{th}, 9^{th} and 11^{th} days post-fungal inoculation and the antifungal efficiency was determined as described by Li et al. (2012) and Pham et al. (2019).

$$AE = \frac{DC - DT}{DC} * 100 \qquad \text{Equation 2.2}$$

whereby

DC - Fungal colony diameter on the control plate

DT - Fungal colony diameter on the treatment plate

AE - Antifungal efficiency.

Morphological and Molecular identification of isolates

The morphological characterization of isolates was done by studying bacterial colony morphology such as colony form, elevation, margin, and surfaces. Bacterial cell suspension using fresh culture was used for microscopic examination of isolates with gram staining.

Molecular identification was done by partial sequencing of 16S rRNA gene fragments. For each of the bacterial isolates, total DNA was prepared by using a commercial DNA extraction kit (Quick-DNA™ Fungal/ Bacterial Miniprep Kit-Inqaba Biotec East Africa Ltd) by following manufacturer's instructions. The 16S rRNA gene fragments were amplified using universal primers (27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-TACGGYTACCTTGTTACGACTT-3') (Frank et al., 2008). The sq-PCR reaction mixture comprised of 25 µl OneTaq® Quick-Load® 2X Master Mix with Standard Buffer,1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer and the 2 µl genomic DNA and 50 µl nuclease-free water. The mixture was incubated at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, elongation 72 °C for 30 s and a final extension at 72 °C for 10 minutes using a GTS96 Thermal Cycler (Clever Scientific ltd, TC9610 /TC9610-230). The amplified products were purified from 1.5% agarose gels by gel electrophoresis, and sequences were determined by BrilliantDye™ Terminator v3.1 and ABI 3500XL Genetic Analyzer, POP7™ (Inqaba Biotechnical Industries Ltd, SA). The gene sequences were aligned using CLUSTALW software version 2.1 and the homology trees were constructed using Mega11 software (Tamura et al., 2021; Yadav et al., 2022).

Statistical analysis

Statistical analysis and data visualization were carried out using Genstat (ver. 15) and Microsoft Excel 2016, respectively. Quantitative data including the amount of phosphorus solubilized, quantities of indole acetic acid, and antifungal activities of PSB isolates over the incubation period were subjected to Two-way Analysis of Variance (ANOVA) with explanatory factors bacterial isolates and incubation duration. One-way ANOVA was deployed to assess the solubilization index of isolates. The mean separation was done by Duncan's multiple range test at a 5% level of significance.

RESULTS

Characterization and identification of isolated strains

In this study, a total of fifty-six bacterial isolates were qualitatively screened as potential phosphate solubilizers. Only ten isolates showed the highest phosphate solubilizing abilities and other plant-growth promotion traits (Table 2) were characterized. The results of morphological and molecular identification of studied isolates are presented in Table 1. Two of the selected isolates (K3MZ and MK 34) were from the soil collected at the southern highlands zone while eight isolates (Ksptk, Ksptn, Ksptz, Mzm spk, Mzm spo, Mzw-pgp, SUA MZ4, and SUAR1) were from the eastern zone. All ten isolates that performed best the properties belonged to the genus *Bulkholderia*, the major dominant species were – *B. vietnamiensis* (only one strain), *B. ambifaria* (six stains) and *B. puraquae* (three stains). Colonies of the selected isolates differed in form, elevation, margin, color and Molecular characteristics (Table 1). Most of the colonies of the isolated bacteria were whitish in color, irregular and circular in shape, undulate, entire, raised, flat, opaque, rod in shape and gram-negative.

B. vietnamiensis-OP984178 and B. ambifaria-OP984173 outperformed other isolates on phosphate solubilization

Results on the comparative performance of isolates on phosphate solubilization on $Ca_3(PO_4)_2$-based PVK over selected time intervals are presented on Table 2. Accordingly, the highest recorded quantitative phosphate solubilization was by *B. vietnamiensis*-OP984178 at 510 mg/L after 96 hours of incubation in PVK broth. This was closely followed by *B. ambifaria*-OP984173 and *B. ambifaria*-OP984180 which were able to solubilize 398.7 mg/L and 363.9 mg/L, respectively under same conditions.

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Table 1. Morphological and molecular characterization of an isolate of plant growth-promoting rhizobacteria isolated from agricultural soils of Tanzania

Isolate Lab ID	Molecular characterization			Macro-morphological characteristics					Micro-morphological characteristics			Agro-ecological zones
	Species name	Accession No.	% Identity	Colony color	Colony shape	Colony margin	Elevation	Opacity	Gram stain	Cell shape	Cell arrangement	
K3MZ	*Burkholderia ambifaria*	OP984175	96.1	Whitish	Irregular	Undulate	Raised	Opaque	Negative	Rod	Single	Southern highlands
Ksptk	*Burkholderia ambifaria*	OP984182	99.8	Whitish	Irregular	Undulate	Raised	Opaque	Negative	Rod	Cluster	Eastern
Ksptn	*Burkholderia ambifaria*	OP984173	95.9	Whitish	Irregular	Undulate	Flat	Opaque	Negative	Rod	-	Eastern
Ksptz	*Burkholderia ambifaria*	OP984179	96.9	Whitish	Irregular	Undulate	Raised	Opaque	Negative	Rod	Single	Eastern
MK 34	*Burkholderia ambifaria*	OP984174	97.1	Whitish	Circular	Entire	Raised	Opaque	Negative	Rod	Chain	Southern highlands
Mzm spk	*Burkholderia vietnamiensis*	OP984178	98.4	Whitish	Circular	Entire	Raised	Opaque	Negative	Rod	Single	Eastern
Mzm spo	*Burkholderia ambifaria*	OP984180	98.9	Whitish	Irregular	Undulate	Flat	Transparent	Negative	Rod	Single	Eastern
Mzw-pgp	*Burkholderia puraquae*	OP984176	97.4	Whitish	Irregular	Undulate	Flat	Transparent	Negative	Rod	Single	Eastern
SUA MZ4	*Burkholderia puraquae*	OP984181	95.3	Whitish	Irregular	Undulate	Raised	Opaque	Negative	Rod	Cluster	Eastern
SUAR1	*Burkholderia puraquae*	OP984177	99.3	Whitish	Irregular	Undulate	Flat	Opaque	Negative	Rod	Single	Eastern

Table 2. Performances of *Burkholderia* species from agricultural soils of Tanzania on solubilizing tricalcium phosphate ($Ca_3(PO_4)_2$) at varying incubation periods

Isolate	Soluble P (mg/ L) over time			
	24 hours	72 hours	144 hours	192 hours
Control	26.5 ± 2.60^{a}	25.8 ± 1.35^{a}	25.8 ± 3.93^{a}	21.2 ± 3.62^{a}
B. ambifaria-OP984175	71.2 ± 24.37^{b}	148.4 ± 6.40^{fg}	213.2 ± 10.20^{klmm}	289.8 ± 10.35^{rs}
B. ambifaria-OP984182	127.9 ± 3.24^{cdef}	210.1 ± 12.26^{jklm}	261.2 ± 1.13^{pqr}	280.5 ± 5.26^{qr}
B. ambifaria-OP984173	107.8 ± 1.10^{cd}	184.8 ± 7.62^{hijk}	247.1 ± 17.69^{op}	398.7 ± 22.51^{v}
B. ambifaria-OP984179	125.1 ± 7.84^{cdef}	137.1 ± 6.00^{defg}	130.5 ± 2.76^{cdef}	126 ± 0.09^{cdef}
B. ambifaria-OP984174	116.5 ± 22.05^{cdef}	178.5 ± 8.57^{hi}	218.2 ± 5.93^{lmno}	244.6 ± 4.22^{nop}
B. vietnamiensis-OP984178	114.8 ± 22.77^{cdef}	167.8 ± 7.72^{gh}	241.6 ± 1.74^{mnop}	510.1 ± 2.83^{w}
B. ambifaria-OP984180	119.5 ± 27.96^{cdef}	143.8 ± 7.02^{efg}	203.8 ± 1.39^{ijkl}	363.9 ± 10.13^{u}
B. puraquae-OP984176	134.3 ± 46.39^{def}	180.3 ± 8.61^{hij}	253.3 ± 12.56^{pq}	316.1 ± 12.34st
B. puraquae-OP984181	113 ± 22.77^{cde}	235.8 ± 8.12^{mnop}	290.1 ± 0.23^{rs}	336.3 ± 8.33^{tu}
B. puraquae-OP984177	100.1 ± 0.76^{c}	137.4 ± 18.23^{defg}	246.9 ± 29.56^{op}	349.3 ± 11.28^{u}

Data represents the means ± standard errors of three independent replicates.
The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).

On PVK agar plates, *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984180 showed impressive mean phosphate solubilization indices (PSI) of 2.8 and 2.6 respectively, but *B. puraquae*-OP984176 had the highest solubilization index of 3.4. Data represent the means ±SE of eight independent replicates. The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$) (Figure 1).

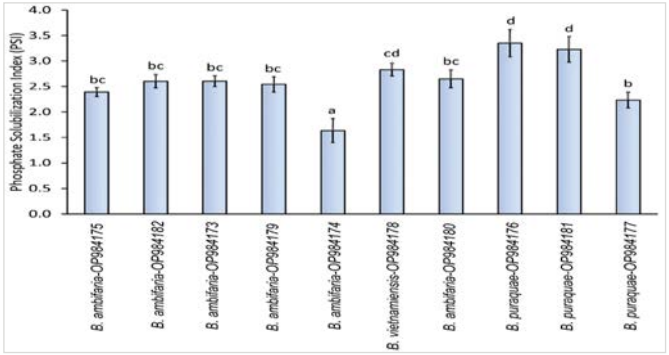


Figure 1. Phosphate solubilization indices (PSI) of *Burkholderia* species grown on Pikovskaya agar (PVK) media for seven days

When the PVK's tricalcium phosphate was replaced with an equal amount of ferric phosphate as the sole source of phosphorus in the growth medium (Table 3), *B. vietnamiensis*-OP984178 demonstrated the greatest solubilization ability, releasing 82.76 mg/L. *B. ambifaria*-OP984179 followed closely behind with 74.94 mg/L, while *B. ambifaria*-OP984180 produced a slightly lower amount of solubilized phosphorus at 61.65 mg/L but still maintained comparable phosphate solubilizing abilities.

On a modified PVK broth with Minjingu rock phosphate powder (Table 4), *B. vietnamiensis*-OP984178 released the highest amount of soluble phosphorus (491.2 mg/L) in 144 hours of incubation, followed by *B. puraquae*-OP984176 and *B. ambifaria*-OP984173 which solubilized an average amount of soluble P of 378.1 mg/L and 304.5 mg/L, respectively after 192 hours of incubation. In general, *B. vietnamiensis*-OP984178 exhibited a superior phosphate solubilization ability across all the insoluble P substrates used. Moreover, *B. vietnamiensis*-OP984178 demonstrated a higher solubilization index on PVK media amended with the rock phosphate (Figure 3).

Table 3. Performances of *Burkholderia* species from agricultural soils of Tanzania on solubilizing ferric phosphate ($FePO_4$) at varying incubation periods (24, 72, 144, and 192 hours)

	Soluble Phosphorus (mg/L)			
	24 hours	72 hours	144 hours	192 hours
Control	29.58 ± 1.06^{abcde}	26.96 ± 5.38^{abc}	37.09 ± 8.27^{abcdefgh}	23.9 ± 15.30^{a}
B. ambifaria-OP984175	34.34 ± 1.71^{abcdefg}	41.62 ± 3.93^{abcdefgh}	62.54 ± 0.19^{efghijkl}	61.42 ± 6.43^{defghijkl}
B. ambifaria-OP984182	26.92 ± 1.75^{abc}	48.86 ± 14.43^{abcdefghijk}	61.77 ± 17.23^{defghijkl}	53.52 ± 1.46^{abcdefghijkl}
B. ambifaria-OP984173	22.62 ± 0.86^{a}	47.27 ± 8.56^{abcdefghij}	64.89 ± 13.02^{ghijkl}	65.14 ± 3.20^{ghijkl}
B. ambifaria-OP984179	43.91 ± 12.27^{abcdefghi}	60.87 ± 14.65^{defghijkl}	79.28 ± 17.63^{jkl}	74.94 ± 12.67^{ijkl}
B. ambifaria-OP984174	41.82 ± 17.50^{abcdefghi}	47.58 ± 11.44^{abcdefghij}	65.91 ± 9.84^{ghijkl}	61.57 ± 5.56^{defghijkl}
B. vietnamiensis-OP984178	26.66 ± 6.88^{ab}	44.49 ± 4.93^{abcdefghi}	69.61 ± 8.31^{hijkl}	82.76 ± 10.77^{l}
B. ambifaria-OP984180	28.79 ± 3.08^{abcd}	45.47 ± 3.51^{abcdefghi}	65.76 ± 1.31^{ghijkl}	61.65 ± 4.23^{defghijkl}
B. puraquae-OP984176	24.53 ± 10.69^{a}	51.54 ± 8.55^{abcdefghijkl}	68.04 ± 9.88^{hijkl}	64.26 ± 5.29^{fghijkl}
B. puraquae-OP984181	31.53 ± 2.85^{abcdef}	43.74 ± 11.02^{abcdefghi}	53.14 ± 11.75^{abcdefghijkl}	45.17 ± 4.96^{abcdefghi}
B. puraquae-OP984177	27.19 ± 0.48^{abc}	46.31 ± 4.18^{abcdefghij}	59.84 ± 1.45^{cdefghijkl}	57.9 ± 8.97^{bcdefghijkl}

Data represents the means ± standard errors of three independent replicates.

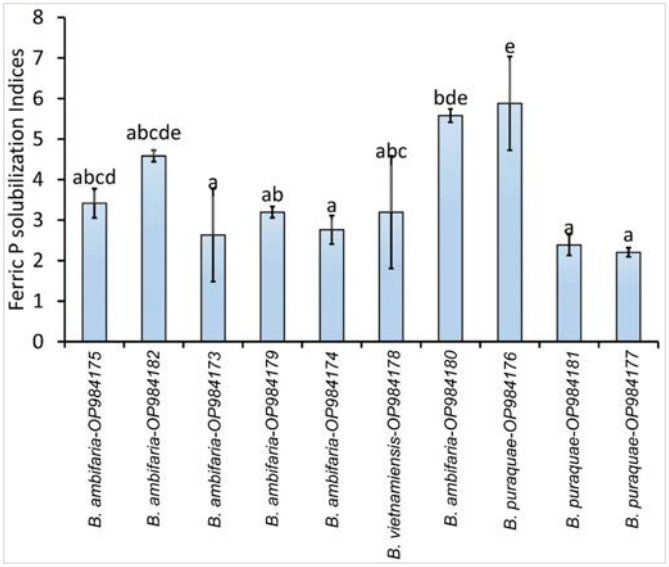
The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).

Table 4. Performances of *Burkholderia* species from agricultural soils of Tanzania on solubilizing the powdered hard Minjingu rock phosphate at varying incubation periods (24, 72, 144, and 192 hours)

PSB Strain	Soluble Phosphorus (mg/L) over the Incubation Time			
	24 hours	72 hours	144 hours	192 hours
Control	48.7 ± 7.81^{a}	50.3 ± 3.21^{a}	61.1 ± 0.4^{a}	50.4 ± 3.64^{a}
B. ambifaria-OP984173	55.8 ± 11.88^{a}	260.7 ± 5.7^{efghij}	296.9 ± 2.67^{ij}	304.5 ± 29.36^{j}
B. ambifaria-OP984174	45.2 ± 6.24^{a}	141.1 ± 12.67^{b}	170.6 ± 4.87^{bc}	163.4 ± 11.86^{bc}
B. ambifaria-OP984175	52.2 ± 2.76^{a}	176.7 ± 26.9^{bcd}	255.6 ± 24.49^{efghij}	309.2 ± 9.89^{j}
B. ambifaria-OP984179	58.3 ± 4.6^{a}	210.4 ± 25.62^{bcdefg}	244.6 ± 18.06^{defghij}	305.2 ± 32.16^{j}
B. ambifaria-OP984180	58.9 ± 5.53^{a}	214.2 ± 5.74^{cdefgh}	259.9 ± 16.83^{efghij}	271.5 ± 5.94^{fghij}
B. vietnamiensis-OP984178	140.1 ± 50.76^{b}	269.4 ± 21.61^{fghij}	491.2 ± 19.91^{l}	415.3 ± 32.67^{k}
B. puraquae-OP984176	58.7 ± 8.29^{a}	309.1 ± 50.94^{j}	377.6 ± 23.79^{k}	378.1 ± 58.57^{k}
B. puraquae-OP984177	60.2 ± 2.04^{a}	192 ± 7.01^{bcde}	223.9 ± 20.85^{cdefghi}	205.7 ± 28.97^{bcdef}
B. puraquae-OP984181	58.2 ± 7.49^{a}	244.3 ± 6.01^{defghij}	274.1 ± 4.61^{fghij}	279.4 ± 25.29^{ghij}
B. ambifaria-OP984182	63.2 ± 3.79^{a}	284.2 ± 14.42^{hij}	304.7 ± 20.62^{j}	306 ± 40.08^{j}

Data represents the means ± standard errors of three independent replicates.

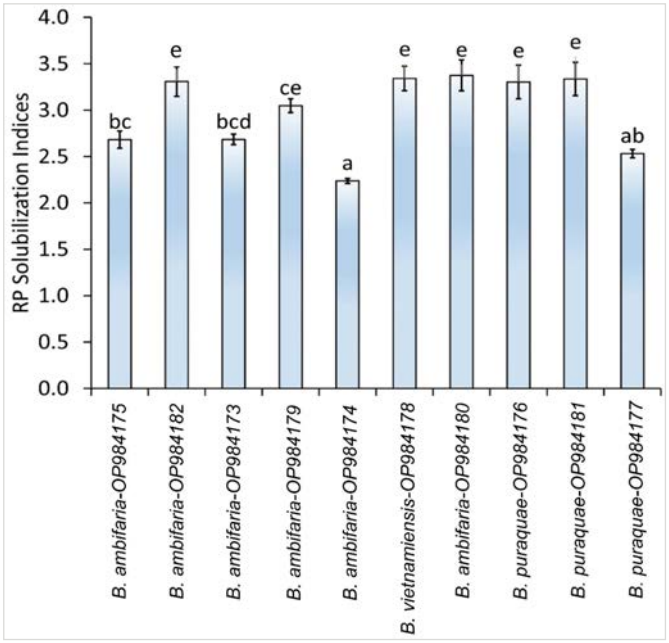
The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).



Data represent the means ±SE of three independent replicates.

The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).

Figure 2. Solubilization indices (SI) of *Burkholderia* species grown on modified Pikovskaya Agar (PVK) media having ferric phosphate as a sole phosphorus source for seven days

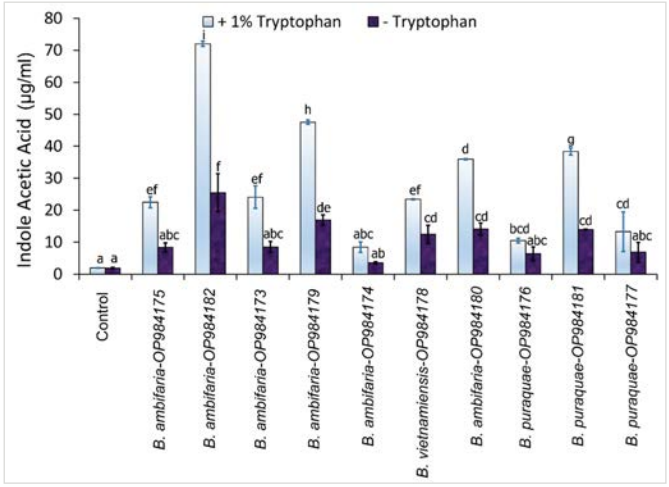


The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).

Figure 3. Solubilization indices of *Burkholderia* species after 7 days of incubation on modified PVK Agar amended with Minjingu rock phosphate as a sole P source

Effectiveness of B. vietnamiensis-OP984178 and B. ambifaria-OP984173 in production of other plant-growth-promoting substances

Indole acetic acid production tests showed that all ten isolates could produce IAA in the presence or absence of tryptophan (Figure 3). Despite that the both strains - *B. vietnamiensis*-OP984173 and *B. ambifaria*-OP984180 did not produce the highest quantity of IAA in comparison to the other tested isolates, they still exhibited significant production capacities. *B. vietnamiensis*-OP984178 produced 23.45 and 12.61 µg/ml while *B. ambifaria*-OP984173 produced 24.24 and 8.473 µg/ml of IAA with and without L-Tryptophan substrate, respectively. The highest amount of IAA (71.92 and 25.62 µg/ml, respectively, with and without L-Tryptophan) was produced by *Burkholderia ambifaria*-OP984182. The addition of L-tryptophan roughly doubled the IAA production capacity of all studied isolates within the same incubation time (Figure 4).



The significance differences according to Duncan's test ($P < 0.05$) were indicated by letters.

Key: + 1% Tryptophan stands for the media with tryptophan precursor added at 1% of the media while – Tryptophan stands for the media with no tryptophan added.

Figure 4. Indole acetic acid production by *Burkholderia* species grown on media in the presence or absence of tryptophan precursor

The siderophore production and nitrogen fixation abilities of the isolated strains are shown in Table 6. Eight isolates exhibited siderophore production on CAS agar medium, as indicated by the formation of orange halos around the colonies, suggesting different levels of siderophore secretion among the strains. No siderophore activity was detected in *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984173. However, all ten isolates, including the non-siderophore producers, demonstrated nitrogen fixation potential on Ashby nitrogen-free agar medium, as manifested by their growth and colony formation. These results reveal that the isolated strains possess diverse plant growth-promoting traits that could enhance plant growth under different environmental conditions.

Antifungal properties of B. vietnamiensis-OP984178 and B. ambifaria-OP984173 against Fusarium proliferatum-MZ497514

Tested isolates exhibited a differential ability to inhibit the growth of a phytopathogenic fungus - *Fusarium proliferatum*-MZ497514. Among the tested isolates, *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984173 displayed mean antifungal efficiencies of 31.34% and 12.96%, respectively, against *F. proliferatum*. However, these values were not the highest among the tested isolates. The best-performing isolate (*B. ambifaria*-OP984179) demonstrated an antifungal efficiency of 40% (Table 5).

Table 5. Antifungal efficiencies of *Burkholderia* species against *Fusarium proliferatum*-MZ497514 co-cultivated on potato dextrose agar (PDA)

PSB Isolate	Antifungal Efficiency (%)		
	Day 3	Day 6	Day 9
B. ambifaria-OP984175	31.93 ± 1.67^{def}	29.68 ± 1.35^{cde}	30 ± 1.11^{cde}
B. ambifaria-OP984182	35.45 ± 2.12^{efg}	33.75 ± 1.31^{efg}	31.85 ± 1.85^{def}
B. ambifaria-OP984173	8.69 ± 2.45^{a}	8.88 ± 1.73^{a}	12.96 ± 0.74^{a}
B. ambifaria-OP984179	42.52 ± 0.90^{h}	36.86 ± 3.09^{fgh}	40 ± 3.33^{gh}
B. ambifaria-OP984174	26 ± 1.22^{bcd}	24.04 ± 2.17^{bc}	24.07 ± 0.37^{bc}
B. vietnamiensis-OP984178	30.41 ± 2.25^{cdef}	29.73 ± 0.58^{cde}	31.48 ± 0.74^{def}
B. ambifaria-OP984180	24.84 ± 3.62^{bc}	22.93 ± 2.29^{b}	24.81 ± 0.37^{bc}
B. puraquae-OP984176	35.35 ± 1.23^{efg}	32.87 ± 2.37^{ef}	35.56 ± 1.11^{efg}
B. puraquae-OP984181	33.28 ± 2.28^{ef}	33.78 ± 2.39^{efg}	30 ± 0.64^{cde}
B. puraquae-OP984177	9.76 ± 3.87^{a}	8.87 ± 2.89^{a}	12.22 ± 0.64^{a}

Data represents the means ± standard errors of three independent replicates.
The significance differences, indicated by letters, were generated according to Duncan's test ($P < 0.05$).

Table 6. Qualitative tests for siderophore production and nitrogen fixation abilities of the strains of *Burkholderia* species studied

Isolate	Siderophore Production			N fixation test
	Test	Color	Pattern	
Control	-	Blue	Clear	-
B. ambifaria-OP984175	+	Orange	Diffused	+
B. ambifaria-OP984182	+	Orange	Diffused	+
B. ambifaria-OP984173	-	Blue	Clear	+
B. ambifaria-OP984179	+	Orange	Diffused	+
B. ambifaria-OP984174	+	Orange	Diffused	+
B. vietnamiensis-OP984178	-	Blue	Clear	+
B. ambifaria-OP984180	+	Orange	Diffused	+
B. puraquae-OP984176	+	Orange	Diffused	+
B. puraquae-OP984181	+	Orange	Diffused	+
B. puraquae-OP984177	+	Orange	Diffused	+

DISCUSSION

The current study reports that two species of the genus *Burkholderia* namely *B. vietnamiensis*-OP984178 and *B. ambifaria*-OP984173 have multifunctional plant growth-promoting potential. These findings have important implications for the development of biofertilizers based on phosphate-solubilizing bacteria (PSB).

The molecular identification of the PSB isolates obtained in this study revealed their affiliation with the *Burkholderia* genus known for its diverse and versatile bacteria with various ecological roles and biotechnological applications (Coenye and Vandamme, 2003). *Burkholderia* genus has a wide range of habitats, from the environment to clinical settings (Coenye and Vandamme, 2003; Martina et al., 2018). This diversity underscores the importance of distinguishing between strains that can provide agricultural benefits and those that may pose risks. The species identified in this study include *B. ambifaria*, *B. vietnamiensis*, and *B. puraquae*.

The results of our study regarding the use of various types of phosphate substrate and the incubation time are in line with the findings of previous research that documented *Burkholderia* species' strong phosphate solubilization capabilities for various phosphate substrates (Ghosh et al., 2016). A large number of other studies (Battini et al., 2016; Ghosh et al., 2016; Tagele et al., 2018) indicate that the solubilization process is influenced by several factors, such as the production of organic acids, pH changes, enzyme activities, and bacterial growth Further studies are needed to elucidate the molecular mechanisms and biochemical pathways involved in the solubilization of insoluble phosphorus sources by *Burkholderia* species.

Several studies have reported the plant growth-promoting potential of *Burkholderia* spp. The bacteria can colonize the roots of several different plant species and form symbiotic relationships with the plants. For example, *Burkholderia vietnamiensis* can promote plant growth by improving soil nutrient availability and suppressing plant pathogens (Liu et al., 2022).

Burkholderia ambifaria has been shown to promote plant growth by improving soil fertility and plant nutrient uptake, while *Burkholderia puraquae* has been reported to enhance plant growth and tolerance to various abiotic stress factors such as drought, salinity, and heavy metal toxicity (An et al., 2022; Brito et al., 2018; Parra-Cota et al., 2014).

In addition to the solubilization of insoluble phosphorus sources, PSB can also promote plant growth by producing other beneficial substances or activities, such as indole acetic acid (IAA), siderophores, antifungal compounds, and nitrogen fixation (Battini et al., 2016). In this study, all 10 tested strains of *Burkholderia* species exhibited positive plant growth-promoting traits, indicating their multifunctional potential for biofertilizer production. IAA is a phytohormone that can stimulate root growth and development, thereby increasing the root surface area and enhancing nutrient uptake by plants (Adeleke and Babalola, 2021). All the PSB isolates under this study were able to produce IAA in the presence of tryptophan, with *Burkholderia ambifaria*-OP984182 producing the highest amount (41.2 - 71.92 µg/ml). These quantities are among the highest to be produced by *Burkholderia* strains compared to most of the reported values in the literature. Previous studies have shown that *Burkholderia* strains generally produce lower amounts of IAA than non-*Burkholderia* strains. For instance, Kong and Hong (2020) reported that *Burkholderia* sp. SSG produced only 2.9 to 4.5 µg/ml of IAA, while other bacterial strains reached up to 43 µg/ml. However, the addition of high concentrations of tryptophan, the precursor of IAA biosynthesis, can significantly increase the IAA production by some *Burkholderia* strains. Santos et al. (2022), for example, demonstrated that *B. gladioli* could produce up to 226 µg/ml of IAA when supplemented with 5 mM of tryptophan in broth culture.

CONCLUSIONS

The results of this study demonstrate that the two isolated strains, *Burkholderia vietnamiensis*-OP984178 and *B. ambifaria*-OP984173, exhibit promising multifunctional plant growth-promoting properties, including high phosphate solubilization, IAA production, and antifungal properties. These results highlight the potential for these strains to be used as biofertilizers. However, further research is necessary to validate their effectiveness under field conditions.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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