

Plant growth-promoting capabilities of microorganisms and PCR amplification of nitrogen fixing (*nif*) gene in bacteria isolated from cassava plant rhizospheric soil from Ota, Ogun State, Nigeria

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Abstract

The application of potent bacteria and fungi as bio-fertilizer can improve the soil nitrogen status leading to enhanced soil well-being and agricultural production. Bacteria and fungi were isolated from soil samples collected around 20 randomly selected cassava plants obtained from Bells University farm, Ota. The isolates were screened for phosphate solubilization activity, indole acetic acid (IAA) production and heavy metal tolerance using standard methods. Isolates were identified based on morphological and sequencing of the amplified 16S rRNA and ITS genes for bacteria and fungi respectively. PCR amplification of the *nif* gene was done using two primers. Results of the molecular identification showed that the bacterial and fungal isolates were *Pseudomonas resinovorans* NOU6 (PP506413), *Klebsiella variicola* NOU5 (PP506412), *Kosakonia radicincitans* NOU1 (PP493938) and *Aspergillus assiutensis* NOU2 (PP493939), *Aspergillus brunneoviolaceus* NOU3 (PP493940) and *Aspergillus tubingensis* NOU4 (PP493941) respectively. Indole acetic acid produced by bacterial and fungal isolates ranged from 121-147 µg/ml and 14-33 µg/ml respectively. The three fungal isolates solubilized phosphate with solubilization index (SI) ranging from 4.25 – 6.25. Results of the heavy metal tolerance test showed that all bacteria isolates were sensitive to mercury while they showed tolerance to tin, cobalt, chromium and iron though at varying degrees. Only *Kosakonia radicincitans* strain NOU1 (C5) harbours the *nif* gene that codes for nitrogenase enzyme. This research offers insights into the possible use of these microorganisms as bio-fertilizers for crop improvement and also underscores the importance of using molecular techniques to target bacteria strains that possess the potentials to fix atmospheric nitrogen.

Keywords: PGPB, phosphate solubilization, fungi, *Nif* gene, heavy metal

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INTRODUCTION

Large-scale food production is becoming necessary as a result of increase in world population, which has led to the development of various production methods, higher input levels, diverse productive varieties, and an acceleration of crop cycles. Notably, using excessive amounts of synthetic chemical pesticides and fertilizers to improve crop yield damages the environment and may pose health concerns to humans (Gómez-Godínez, 2023; Vejan *et al.*, 2016). As an alternative, soil and plant-derived microorganisms, particularly fungi and rhizobacteria, which have coexisted and developed with plants, can be employed as bio-fertilizers. Plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR) are examples of plant growth-promoting microorganisms (PGPMs) found in soil. Through a variety of methods, PGPR can generally be either endosymbionts or free-living in the rhizosphere and aid in the growth and development of plants (Gopalan *et al.*, 2023; Kumar *et al.*, 2022). In nature, biological nitrogen fixation, phosphorus solubilization, siderophore synthesis, and the creation of phytohormones like auxins and gibberellin are just a few of the tactics used by microorganisms that support plant growth in the rhizosphere (Gómez-Godínez, 2023). Auxin synthesis is one of the most commonly described processes for promoting plant growth by about 80 % of rhizobacteria (Canarini *et al.*, 2019; Park *et al.*, 2021). Inoculating plants with PGPRs has been known for decades to shorten the length of plant roots and increase the quantity of lateral roots and root hairs (Park *et al.*, 2021; Ludwig-Müller, 2015; Duca *et al.*, 2014;) and auxin-mediated bacterial effects that promote plant growth are referred to as phytostimulation (Singh *et al.*, 2019). Fungi that promote plant growth (PGPF) include *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and other species. By forming mycorrhizal symbiotic associations with plant roots, these fungi help plants absorb vital nutrients from the soil and give them to the plants which promote plant growth and development (Kumar *et al.*, 2022; Hossain *et al.*, 2017). On the other hand, many previous studies also have reported various types of bacterial species with the ability to promote plant growth such as *Rhizobium* (Sridevi *et al.*, 2008), *Azospirillum* (Chen *et al.*, 2024; Bolete *et al.*, 2020), *Agrobacterium* (Mashiguchi *et al.*, 2019), *Bacillus* (Tsotetsi *et al.*, 2022; Liu *et al.*, 2016; Radhakrishnan *et al.*, 2016), *Ignatzschineria* (Park, 2021), *Bradyrhizobium* (Torres *et al.*, 2018), *Erwinia* (Chen *et al.*, 2023), *Pseudomonas* (Ejeagba *et al.*, 2023;

Iqbal and Hasnain, 2013) etc. Numerous reports demonstrate that bacteria have a favourable impact on crop growth and yield in a variety of soil types, climatic conditions, and biotic and abiotic stress situations (Chen *et al.*, 2023; Ma *et al.*, 2020). One of the most crucial macronutrients for plant growth is nitrogen and approximately 78 % of the nitrogen is present in the atmosphere, and plants are unable to absorb it in such form. However, PGPRs that may either freely or in symbiotic relationships with legumes perform biological nitrogen fixing are numerous in the soil; these includes *Herbaspirillum*, *Burkholderia*, *Azorhizobium*, *Rhizobium*, *Allorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, and *Sinorhizobium* (Chittora *et al.*, 2020). Also, Cyanobacteria (e.g., *Anabaena* and *Nostoc*), *Azotobacter*, *Enterobacter*, *Azoarcus*, *Azospirillum*, *Gluconacetobacter*, *Klebsiella*, *Herbaspirillum*, and *Pseudomonas* can be free-living nitrogen fixers or establish associations with plants as endophytes (Meena, *et al.*, 2021). Inoculating seeds, seedlings, roots, or soil with nitrogen-fixing bacteria promotes plant growth, enhances soil quality, and keeps the soil's nitrogen content stable. Nitrogenase is the fundamental nitrogen fixing enzyme. According to Timofeeva *et al.* (2023), free-living nitrogen-fixing bacteria can be facultative anaerobes, obligate aerobes, or obligatory anaerobes that exist in various settings with varying quantities of molecular oxygen. N₂ fixation can be suppressed and nitrogenase inhibited by oxygen. By employing structures like heterocyst, where the oxygen content is maintained at a low level, nitrogen-fixing bacteria can circumvent the potentially harmful effects of O₂ (Timofeeva *et al.*, 2023; Ladha *et al.*, 2022). Iron (Fe) atoms are necessary for all known forms of nitrogenase, and the majority of them also contain metals like molybdenum. The ability of bacteria to fix atmospheric nitrogen and the distribution of nitrogen-fixing microorganisms in communities like rhizospheric soils is both commonly assessed using the *nifH* gene, which codes for nitrogenase reductase and a number of other genetic markers. In this study, we amplified *nifH* gene from our organisms using two sets of degenerate primers. Phosphorus, a macro element is involved in several metabolic activities like photosynthesis and can be found in molecules like ATP and nucleic acids (Khuna *et al.*, 2023). About 90 % of the phosphorus in the soil is insoluble, immobilized, or precipitated and these make it challenging for plants to absorb. However, phosphate-solubilizing bacteria are able to dissolve inorganic phosphorus in the soil example *Pseudomonas*, *Mesorhizobium*, *Flavobacterium*, *Arthrobacter*,

Bacillus, *Rhizobium*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Rhodococcus*, and *Serratia* (Ejeagba *et al.*, 2023; Alori *et al.*, 2017). They convert insoluble organic and inorganic phosphate into a bioavailable form for plant growth. In addition, many fungal species have been reported to solubilize phosphate; they include members of the genus *Aspergillus* (Khuna *et al.*, 2023; Coutinho *et al.*, 2012), *Penicillium* (Doilom *et al.*, 2020; Coutinho *et al.*, 2012), *Fusarium* (Radhakrishnan *et al.*, 2015), *Trichoderma* (Shaban and El-Bramawy, 2011; Hoyos-Carvajal *et al.*, 2009) etc. Therefore, the objective of this research was to investigate the plant-growth-promoting abilities (production of indole-3-acetic acid (IAA), phosphate solubilisation) of bacterial and fungal species isolated from cassava rhizospheric soils and to analyse the presence of *nif H* gene that encodes a key subunit of the nitrogenase enzyme necessary for atmospheric nitrogen fixation by the isolated rhizobacteria.

MATERIALS AND METHODS

Sample collection and isolation of bacteria and fungi

Twenty (20) samples of soil and root from the rhizospheric region of Cassava cultivars were collected aseptically from various pulse hubs situated in different locations of a cassava farm in Bells University, Ota. The roots (<5 cm length and 2–3 mm diameter) and soil samples (20 g each) were collected for isolation of plant growth promoting microbes (PGPMs) and stored in the refrigerator at 4 °C prior to analysis. For serial dilution, ten (10) grams of each soil sample were placed in a 250 mL Erlenmeyer flask with 90 mL of sterile distilled water. Meanwhile, root samples were aseptically cleaned and surface sterilized with 70 % ethanol for 90 seconds. Using a sterile mortar and pestle, the surface-sterilized samples were pulverized before being seeded on agar substrate devoid of nitrogen to isolate bacteria. Ashby nitrogen-free agar plates were used to cultivate the cultures. The plates contained 0.2 g KH₂PO₄, 5 g CaCO₃, 0.2 g MgSO₄·7H₂O, 10 g mannitol, 0.2 g NaCl, 15 g agar, and 0.1 g CaSO₄·2H₂O per liter. The plates were then incubated at 28 °C for five to seven days. Following the duration of incubation, the cultures were moved to new media devoid of nitrogen and incubated for two days at 28 °C. The transfer procedure was carried out three times to isolate a single strain. Fungi were isolated by spread plating serially diluted soil samples on Potato Dextrose Agar

(PDA), followed by incubation at 28 °C for 3 – 4 days. Isolates in different colonies were selected and subcultured on nutrient agar and PDA to obtain pure cultures of bacteria and fungi respectively for screening (Hamza and Alebejo, 2017). The obtained bacterial and fungal isolates were screened further for various plant growth promoting traits.

Molecular identification of isolates

Total genomic DNA was extracted from bacterial isolates grown in Luria-Bertani (LB) broth for 24 hours using a commercial kit: illustra bacteria genomicPrep Mini Spin Kit (General Electric Company, UK) and quantified using a Nanodrop spectrophotometer (GenovaNano™, Jenway, UK). The 16S rRNA gene region of extracted DNA was amplified by Polymerase Chain Reaction (PCR) using an automated thermocycler (Applied Biosystem Inc., USA) and the universal primers: 27F and 1492R (Inqaba Biotech Ltd, Pretoria, South Africa). PCR thermal cycling programme used was as follows: initial denaturation at 95 °C for 5min; 30 cycles of denaturation, annealing and extension at 94 °C, 52 °C and 72 °C for 30 s, 30 s and 1min 25s followed by a final extension at 72 °C for 10 min and kept at a hold temperature of 4 °C. The size of the amplified fragments was verified by electrophoresing the products on 2 % agarose gel stained with ethidium bromide and viewed using a UV transilluminator. Similarly, Fungal DNA was extracted using the protocol described by Trindade *et al.* (2007) while PCR amplification of the ITS region was done as described by (Wawrik, *et al.*, 2005; Frank *et al.*, 2008). Briefly, universal primer set which flank the ITS1, 5.8S and ITS2 region was used. The cocktail preparation for polymerase chain reaction (PCR) consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM MgCl₂, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5'-TCC GTA GGT GAA CCT GCG G-3' and - ITS 4: 5'-TCC TCC GCT TAT TGA TAT GC-3' primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water and 8 µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition which include a cycle of initial denaturation at 94 °C for 5 min, followed by 35 cycles of each cycle comprised of 30 sec denaturation at 94 °C, 30 secs annealing of primer at 55 °C, 1.5 min extension at 72 °C, a final extension for 7 min at 72 °C and kept at a hold temperature of 4 °C. The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems according to

manufacturer's instruction while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 7.0 were used for all genetic analysis.

Phosphate solubilization screening

Screening was carried out with sterile solidified Pikovskaya's agar medium containing 10 g/L glucose; 5g/L tricalcium phosphate (TCP); 0.5g/L, yeast extract; 0.5 g/L (NH₄) SO₄; 0.2 g/L KCl; 0.1G L MgSO₄.7H₂O; trace quantities of FeSO₄.7H₂O and MnSO₄ as well as 15 g/L agar powder. The medium was sterilized by autoclaving at 121°C for 15 min. The pure bacterial cultures were pre-incubated in nutrient broth for 24 h at 37 °C then a loopful of the broth culture was dropped on the Pikovskaya's agar medium in Petri dish using a sterile inoculating wire loop. This was incubated at 37 °C for 3-5 days. The fungal isolates were inoculated using a sterile inoculating needle to pick up the fungal spores and dropped at the middle of the Pikovskaya's sterile agar plate and incubated at 28 °C for 5-7 days. Appearance of clear zones around the colonies indicated solubilization of phosphate. Solubilization index (SI) was recorded as the ratio of diameter of the zone of clearance of halo zone compared to the diameter of colony (Nosrati *et al.*, 2014).

$$SI = \frac{\text{Diameter of halo zone in mm}}{\text{Colony diameter in mm}}$$

Colony diameter in mm

Quantification of indole acetic acid (IAA) production

Indole acetic acid production assay of the isolates was performed according to the method described by Noha and Shixue (2018). Briefly, bacterial and fungal cultures were cultivated in potato dextrose broth (PDB) and Luria Bertani (LB) broth, respectively, supplemented with 0.1 g/L L-tryptophan, the precursor of IAA, and incubated for seven days at 250 rpm and 30 °C in a shaker. Following the incubation times, cultures were centrifuged for 10 minutes at 13,000 rpm in order to pellet the microbial cells. After thoroughly mixing five milliliters (5 ml) of the supernatant with one milliliter (12 g/l-1 FeCl₃ in 429 ml /L H₂SO₄) of Salkowski's reagent, the mixture was allowed to sit at room temperature in the dark for twenty-four hours (Rahmad *et al.*, 2020). A positive result for IAA generation was shown by the test tubes appearing pink (i.e., the supernatant changing color to pink). Absorbance was measured at 535 nm for the IAA using UV spectrophotometer. Auxin concentration was obtained using a standard curve with a regression equation. The equation was generated from serially diluted commercially available IAA solutions ranging from 0 - 0.12 mg/ml. The absorbance of known concentrations of indole acetic acid solutions was measured spectrophotometrically at 535 nm, and a graph of the concentrations against the absorbance was plotted to obtain the standard equation. Using this equation, the concentration of indole acetic acid produced by each fungal isolate was calculated (Noha and Shixue, 2018).

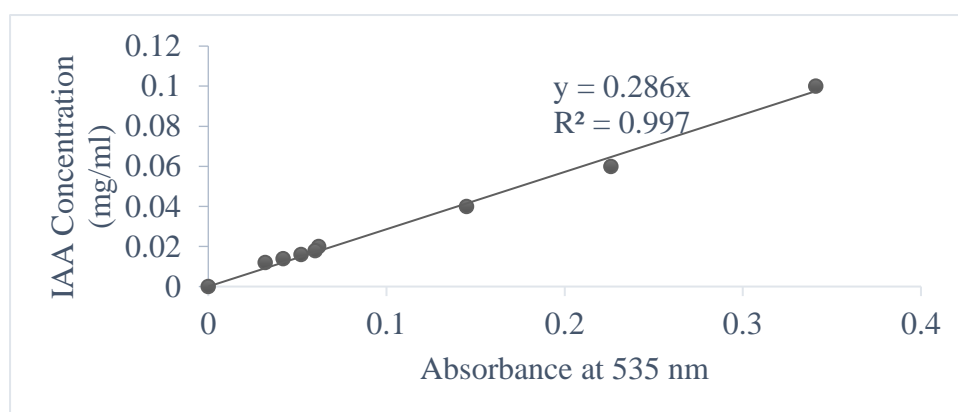


Figure 1: Indole acetic acid standard curve

Where X = Absorbance at 535 nm, Y = Concentration of Indole acetic acid (mg/ml), R² = Regression Coefficient

Heavy metal tolerance test

Heavy metal tolerance of the bacterial isolates was assessed according to the method described by Oyetibo *et al.* (2010). Pure cultures of bacterial isolates were grown on sterile LB broth for 24 h at 30 °C. Different concentrations of the various heavy metals including Chromium (1.0 - 10 mM), Iron (1.0 - 10 mM), Tin (1.0 - 10 mM), Mercury (1.0 - 10 mM), Cobalt (1.0 - 10 mM) were obtained by transferring appropriate amounts of the stock solution into test tubes already containing sterilized LB broth. Controls included uninoculated growth media containing heavy metals and inoculated growth media free of heavy metals. Isolate growth was monitored by visual turbidity. Minimum inhibitory concentrations (MIC) were determined by streaking out tubes where turbidity is low or absent on nutrient agar plates. All experiments were carried out in triplicates.

PCR detection of nitrogen fixation gene (*nif* gene)

The *nifH* gene was amplified using an automated PCR thermocycler (Applied Biosystem Inc., USA). Using two degenerate primer sets, PolF/PolR and IGK3/DVV, the *nifH* gene was amplified from the isolated genomic DNA. For the PCR amplification reaction, a 25 µl reaction mix was prepared, which included 12.5 µl of 2X ready master mix (containing dNTPs, Taq DNA polymerase, MgCl₂, and the reaction buffer), 2 µl of template genomic DNA, 1 µl of forward and reverse primers (5 µM), and 8.5 µl of nuclease-free water. The following PCR heat cycling protocol was used to the PolF/PolR primer pair: initial denaturation for five minutes at 94 °C; forty denaturation, annealing, and extension cycles at 94 °C, 55 °C and 72 °C for 30 s, 1min and 30 s respectively, followed by a final extension at 72 °C for 10 min and kept at a hold temperature of 4 °C. The PCR thermal cycling program for the IGK3/DVV primer pair: 5 minutes of initial denaturation at 95 °C; 30 cycles of denaturation, annealing, and extension at 95 °C, 58 °C, and 72 °C for 45 s, 30 s, and 1 min, respectively; a final extension at 72 °C for 10 min; and a hold temperature of 4 °C. The results were electrophoresed on 2 % Agarose gel stained with ethidium bromide and examined under a UV transilluminator to confirm the size of the amplified fragments (Garby and Buckley, 2012).

RESULTS AND DISCUSSION

Microbial isolation and identification

Three bacterial and fungal spp isolated from cassava roots and rhizospheric soils were investigated for their plant growth-promoting capabilities (the production of IAA and the solubilisation of phosphorus minerals) and their heavy metal tolerant. Gram reaction results showed that bacterial isolates were all Gram negatives. Agarose gel plates of the resolved amplified 16S rRNA gene from the bacterial and the targeted genes within the internally transcribed spacer (ITS) of the fungal isolates are shown in plates 1 and 2 respectively. The plates indicated that the gene fragments of the expected base pair sizes for fungi (550bp – 650bp) and bacterial isolates (1500bp) were amplified. Multiple sequence alignments of the nucleotide sequences obtained from the three identified bacterial (C5, C7 and C8) and fungal isolates (B1F, C2F and CF1) with the nucleotide sequences in the NCBI databases revealed a homology with the members of the genus *Kosakonia*, *Pseudomonas*, *Klebsiella* and *Aspergillus* exhibiting strong relationships with 97-99 % relatedness (Table 1). The characterized sequences were deposited at the National Centre for Biotechnology Information (NCBI) database Maryland US with accession of these numbers PP493938- PP493941 and PP506412- PP506413 (Table 1). Based on the nucleotide sequences obtained, the neighbor joining tree constructed showed that these organisms had strong relationships with other members of the genus *Kosakonia*, *Pseudomonas*, *Klebsiella* and *Aspergillus* (Figure 2 and 3). *A. tubingensis* strain NOU4 had close relationship with other strains of *A. tubingensis*. However, *A. assiutensis* strain NOU2 and *A. brunneoviolaceus* strain NOU3 are more closely related and clustered far away from *A. tubingensis* (Fig 3). Different Species from the *Enterobacteriaceae* family such as *Kosankonia* sp., *Klebsiella* sp. and *Enterobacter* sp. have been described previously to be associated with roots of several plant crops (da Silva *et al.*, 2024; Ali and El-Kholy, 2022; Silva *et al.*, 2022; Girma *et al.*, 2020). Ali and El-Kholy in their work reported the isolation of *Kosankonia radicincitans* that produced indole-3-acetic acid, solubilized phosphate and fixed atmospheric nitrogen (Ali and El-Kholy, 2022). These organisms have been reported previously to promote plant growth through phosphate solubilization and indole acetic acid production (Park *et al.*, 2021). Many plant growths processes, including elongation, division, cell differentiation, and root initiation, depend on IAA, a common form of auxin present in plants (Khuna *et al.*, 2023; Park *et*

al., 2021; Keswani *et al.*, 2020). However, due to numerous factors that influence the successful application of these organisms as biofertilizers, it is necessary to understand how

different strains function in biological control and plant growth promotion, and distinctly define the factors that contribute to their more efficient use in the field.

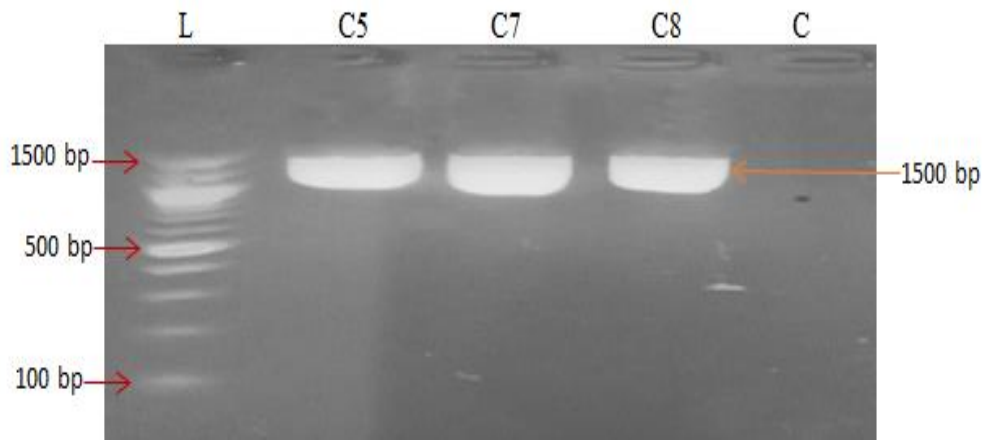


Plate 1: Agarose gel (2.0 %) electrophoresis of 16S rRNA genes amplified from genomic DNA of the bacterial isolates. L: ladder, C: Control

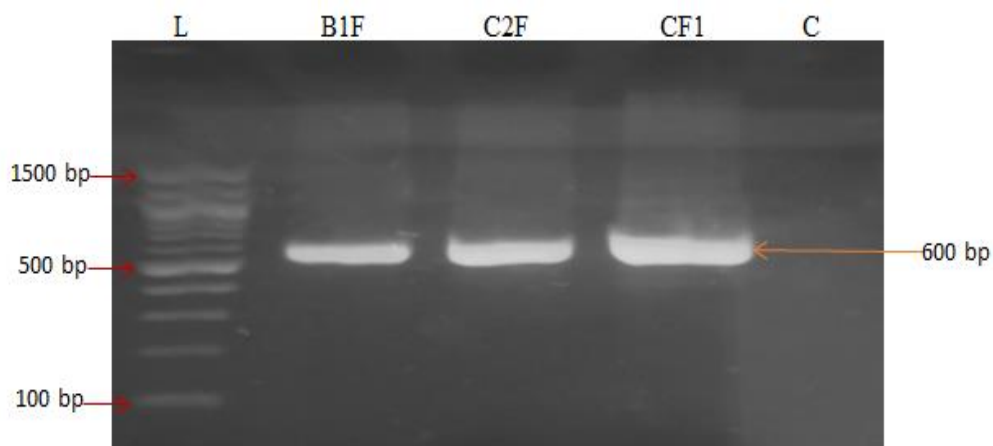


Plate 2: Agarose gel (2.0 %) electrophoresis of PCR product showing amplification of the targeted genes within the internally transcribed spacer (ITS) of the fungal isolates. L: ladder, C: Control

Table 1: Molecular identification of bacterial and fungal isolates based on 16S rRNA gene and ITS region of the 18S rRNA sequencing data

Isolate Code	Identified Bacteria	% Similarity to First Hit on GenBank	Assigned GenBank Accession Number
C5	<i>Kosakonia radicincitans</i> strain NOU1	98.19%	PP493938
C7	<i>Klebsiella variicola</i> strain NOU5	97.42%	PP506412
C8	<i>Pseudomonas resinovorans</i> strain NOU6	99.24%	PP506413
Identified Fungi			
B1F	<i>Aspergillus assiutensis</i> strain NOU2	99.44%	PP493939
C2F	<i>Aspergillus brunneoviolaceus</i> strain NOU3	99.63%	PP493940
CF1	<i>Aspergillus tubingensis</i> strain NOU4	99.46%	PP493941

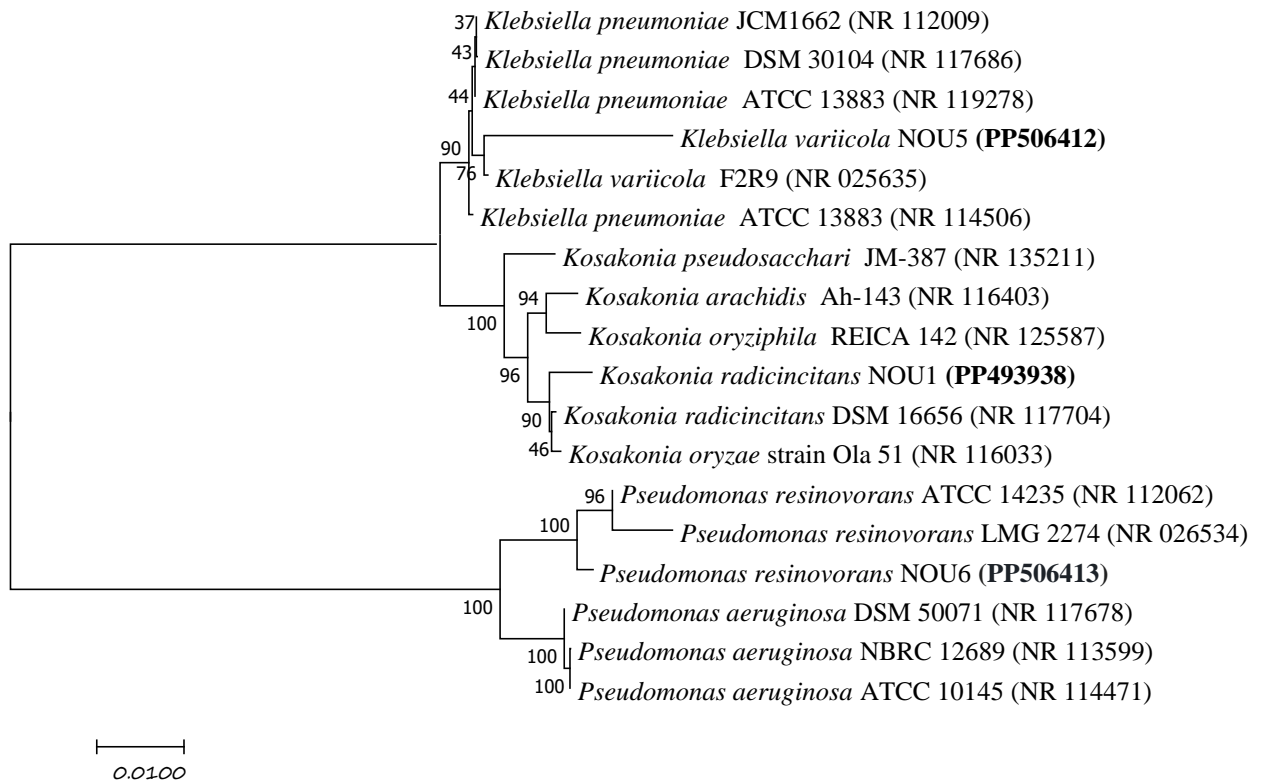


Figure 2: Phylogenetic relationship of the 16S rRNA gene sequences from the bacteria to those in the GenBank database. The accession numbers of the bacteria are in parentheses while the bold accession numbers were those of the bacteria isolated in this study.

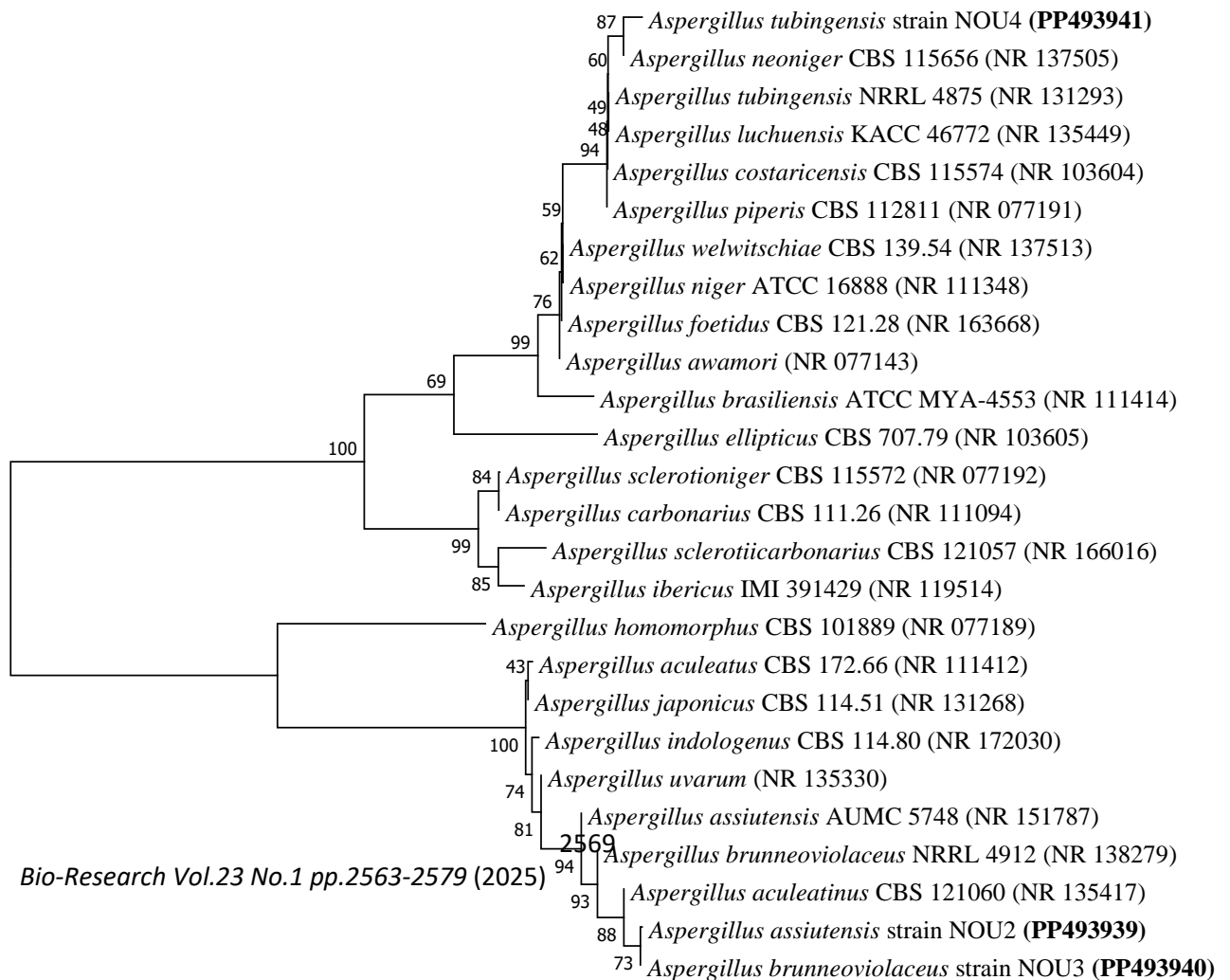


Figure 3: Phylogenetic tree indicating the phylogenetic relationship of rRNA gene sequences within the ITS region of the fungi isolated in this study to those in the GenBank database. The accession numbers of the fungi are in parentheses while the bold accession numbers were those of the fungi isolated in this study.

Assessment of plant-growth promotion characteristics

Production of indole acetic acid:

Bacterial and fungal isolates from this study was examined for their plant growth promoting attributes in terms of indole-3-acetic acid (IAA) production, nitrogen fixation, phosphate (P) solubilization and ability to tolerate heavy metal. Results obtained indicated that these isolates were able to produce indole acetic acid that ranged from 14 µg/ml - 33 µg/ml for fungi and 121 µg/ml to 147 µg/ml for bacteria in the culture filtrate (Table 2). Notably, though all the fungal isolate produced IAA, from our observation bacterial isolates from this study produced higher concentration of IAA. Notably, a number of plant-associated bacterial and fungal species stimulate plant growth by producing hormones that stimulate plant growth (Naziya *et al.*, 2019; Spaepen and Vanderleyden) and fostering greater resilience to plant diseases (Preston, 2003). Growing *Aspergillus* species in a medium enriched with L-tryptophan has been shown to produce indole acetic acid over the years (Ratnam, 2020; Ziaullah *et al.*, 2020; Mehmood *et al.*, 2019). A component of auxin, indole acetic acid (IAA) is the primary precursor of cell division and elongation that affect plant stem growth (Kumar *et al.*, 2022). Fungi's IAA can cause roots and root hairs to develop and form, improving the corresponding plants' ability to absorb nutrients (Ziaullah *et al.*, 2020). Interestingly, the concentrations of IAA produced by other fungal species falls within the range we reported here. For instance, Mehmood and coworker reported that *Aspergillus awamori* isolated from plant leaf produced 24.2 µg/ml IAA which is very close to the amount of IAA produced by our isolates (Mehmood *et al.*, 2020). In contrast, *Aspergillus niger* isolated from Chilli pepper produced 110 µg/ml of IAA (Ratnam *et al.*, 2020) which is far above our values for the three species we reported here. This suggests that the quantity of IAA production varies with

different species of *Aspergillus* and possibly due to cultural conditions. The function of IAA produced by fungi in various plant-fungal interaction systems suggests that fungi can be linked to plants for pathogenesis or a symbiotic approach using IAA and related compounds, which promotes plant growth and modifies the plant's basic defense mechanisms (Ziaullah *et al.*, 2020). *Klebsiella variicola* strain NOU5 isolated in this study produced the highest concentration of IAA (147 µg/ml) compared to *Pseudomonas resinovorans* strain NOU6 and *Kosakonia radicincitans* strain NOU1. Yang and Yang (2020) isolated *Klebsiella variicola* from maize rhizospheric soil that produced about 399.4 ±11.16 mg/L IAA. Furthermore, *Kosakonia radicincitans* has been reported severally to possess the ability to produce IAA (da Silva *et al.*, 2024; Ali and El-kholy, 2022; Kumar *et al.*, 2022; Park *et al.*, 2021). The production of IAA by bacteria isolated from Odisha's acidic soil was documented and measured by Pradhan and Mishra (2015). In their study, they investigated the effectiveness of PGPR isolates on seed germination percentage and growth of rice seedlings to observe the effect of PGPR isolates on rice seedlings. They reported that Seed germination increased by 94% and 80 % when seeds were pretreated with *Bacillus licheniformis* and *Bacillus tequilensis* respectively with significant increase in the root & shoot length of rice seedlings. Additionally, they believed that these isolates improved the growth variable and germination percentage of rice, peanuts, and green gram. *Pseudomonas* species are well known for their capacity to stimulate plant development by producing IAA, according to earlier research conducted by many researchers (Parvin *et al.*, 2020; Meliani *et al.*, 2017; Marathe *et al.*, 2017; Bharucha *et al.*, 2013; Malik *et al.*, 2011). IAA production by *Pseudomonas resinovorans* strain NOU6 was 121 µg/ml, which is marginally greater than the levels reported in the aforementioned papers. Therefore, based on our findings these bacteria species could serve potential candidates for use as plant growth-promoting rhizobacteria.

Table 2: Indole acetic acid (IAA) production

Isolate Code	Name	Absorbance Value (A _{535nm})	IAA Production (µg/ml)
FUNGI			
B1F	<i>Aspergillus assiutensis</i> strain NOU2	0.05	14.00
CF1	<i>Aspergillus brunneoviolaceus</i> strain NOU3	0.115	33.00
C2F	<i>Aspergillus tubingensis</i> strain NOU4	0.101	29.00
BACTERIA			
C5	<i>Kosakonia radicincitans</i> strain NOU1	0.437	125.00
C7	<i>Klebsiella variicola</i> strain NOU5	0.515	147.00
C8	<i>Pseudomonas resinovorans</i> strain NOU6	0.423	121.00

Phosphate solubilization by fungal isolates

Phosphate solubilizing fungi plays important roles in increasing the bioavailability of soil phosphates for plants assimilation. Results obtained from phosphate solubilization screening of the three *Aspergillus* species is presented in Table 3 and plate 3. *Aspergillus tubengensis* strain NOU2 (C2F) had the highest phosphate solubilization index of 2.80 followed by *Aspergillus brunneoviolaceus* strain NOU3 (C1F) with SI of 2.23 and *Aspergillus assiutensis* strain NOU4 (B1F) with SI 1.12. According to Khuna et al. (2023), phosphate-solubilizing fungi (PSF) are extremely important microorganisms that can transform insoluble phosphorus into soluble forms that plants may readily absorb. According to numerous reports over the past few decades, *Aspergillus* species have the capacity to convert insoluble phosphate to soluble form in the rhizosphere of plants as well as in vitro in order to promote plant growth (Khuna et al., 2023; Doilom et al.,

2020; Kaur and Reddy 2017; Rinu and Pandey 2010). This could be attributed to their great efficiency in root colonization (Elias et al., 2016). Interestingly, phosphate solubilization index (SI) recorded by the *Aspergillus* spp from this study is very close to the values reported by previous researchers (Li et al., 2021). In their work, they isolated *A. brunneoviolaceus* and *A. tubingensis* from immature soil without plants in wastelands. However, there exists slight variation with the value reported by Elais and coworker, in their work they obtained SI of 3.05 and 2.87 for the two *Aspergillus* spp isolated (Elias et al., 2016). According to the research, *Aspergillus niger* strains are the most successful in terms of the amount of tricalcium phosphate (TCP) solubilization (Doilom et al., 2020; Ceci et al., 2018). However, the kind of carbon, nitrogen, and metal ions in soils, as well as the growing conditions, all affect solubilization by PSF (Doilom et al., 2020).

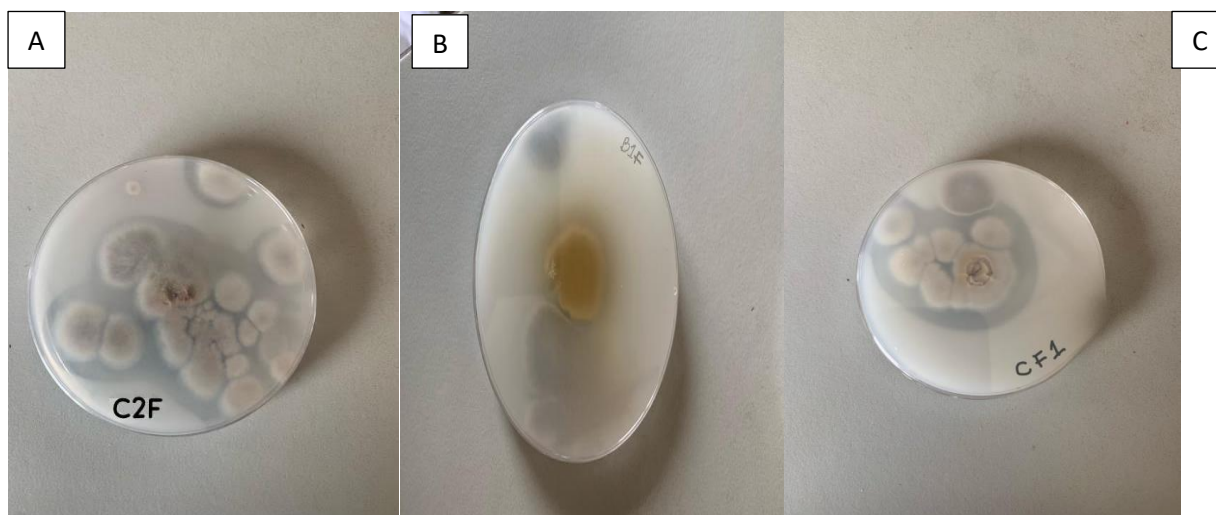


Plate 3: Formation of clear zones by fungal isolates during Phosphate solubilization. A= Fungal isolate C2F; B= Isolate B1F; C= Isolate CF1

Table 3: Qualitative estimation of phosphate solubilization efficiency on fungal isolates

Fungal Isolates	Name	Solubilization index
CF1	<i>Aspergillus brunneoviolaceus</i> strain NOU3	2.23
B1F	<i>Aspergillus assiutensis</i> strain NOU2	1.12
C2F	<i>Aspergillus tubingensis</i> strain NOU4	2.80

Heavy metal tolerance test

Bacterial isolates that had plant growth promoting capabilities were analyzed for heavy metal tolerance at metal concentrations ranging from 1mM to 10mM. Result of the minimum inhibitory concentration (MIC) assay carried out on the bacterial isolates is presented in Table 4. All bacterial isolates were highly sensitive to mercury at the lowest concentration of 1mM after 24 h incubation while *Klebsiella variicola* strain NOU5 and *Pseudomonas resinovorans* strain NOU6 were inhibited by Chromium even at 1 mM concentration. Nevertheless, all the isolates were resistant to Tin, Iron and Cobalt though at different concentrations. According to Enebe and Babalola (2018), a group of metal-resistant PGPB, including *Pseudomonas*, *Klebsiella*, and *Enterobacter* species that are comparable to the bacterial genus isolated from this work, has the potential to greatly enhance the growth of a variety of plants in environments

contaminated with metals. According to Guo and coworker, these metal-resistant PGPB were found to improve plant metal tolerance through soil pH change, plant detoxification rates, and enzymes released by plant roots (Guo *et al.*, 2020). Additionally, some metal-resistant PGPB can affect the mobility and bioavailability of metals, which in turn enhances the rate at which plants use them. This is achieved by the release of chelating agents, acidification, and redox changes (Verma and Kula, 2019). In order to reduce the negative effects of metals and increase plants' ability to accumulate metals, these metal-resistant PGPB strains can be employed as good candidates for metal phytoremediation. According to Manoj *et al.* (2020), some metal-resistant PGPB have been shown to increase plant bioaccumulation/phytoextraction capability by secreting organic acids and siderophores, which lower soil pH and increase metal bioavailability.

Table 4: Heavy Metal Tolerance Assay

Heavy Metal	<i>Kosakonia radicincitans</i> strain NOU1									
	1 mM	2 mM	3 mM	4 mM	5 mM	6 mM	7 mM	8 mM	9 mM	10 mM
Chromium (Cr)	+	+	+	+	-	-	-	-	-	-
Mecury (Hg)	-	-	-	-	-	-	-	-	-	-
Tin (Sn)	+	+	+	+	-	-	-	-	-	-
Iron (Fe)	+	+	+	+	+	+	+	+	+	+
Cobalt (Co)	+	+	+	+	+	+	-	-	-	-
Heavy Metal	<i>Klebsiella variicola</i> strain NOU5									
	1 mM	2 mM	3 mM	4 mM	5 mM	6 mM	7 mM	8 mM	9 mM	10 mM
Chromium (Cr)	-	-	-	-	-	-	-	-	-	-
Mecury (Hg)	-	-	-	-	-	-	-	-	-	-
Tin (Sn)	+	+	+	+	-	-	-	-	-	-
Iron (Fe)	+	+	+	+	+	+	+	+	+	+
Cobalt (Co)	+	+	+	+	-	-	-	-	-	-
Heavy Metal	<i>Pseudomonas resinovorans</i> strain NOU6									
	1 mM	2 mM	3 mM	4 mM	5 mM	6 mM	7 mM	8 mM	9 mM	10 mM
Chromium (Cr)	-	-	-	-	-	-	-	-	-	-
Mecury (Hg)	-	-	-	-	-	-	-	-	-	-
Tin (Sn)	+	+	-	-	-	-	-	-	-	-
Iron (Fe)	+	+	+	+	+	+	+	+	+	+
Cobalt (Co)	+	+	+	+	-	-	-	-	-	-

KEY: - = Negative (Sensitive); + = Positive (Resistant)

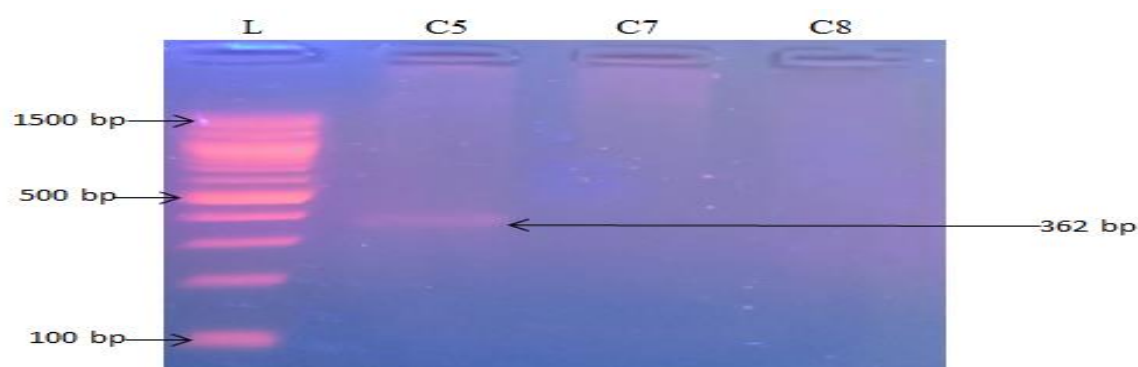


Plate 4: Ethidium bromide-stained agarose gel (2.0 %) electrophoresis of PCR product showing amplification of the targeted nitrogen fixing gene in bacterial isolate C5; L: ladder

PCR amplification of the *nifH* gene from bacterial isolates

Result obtained from PCR amplification of the nitrogen fixation gene (*nifH* gene) from the

three bacterial isolates is shown on plate 4. The Primer set PolF/PolR amplified nitrogen fixing gene from only one bacterial isolate *Kosakonia radicincitans* strain NOU1 (C5) with the expected base size of 362 bp while there was no amplification from *Klebsiella variicola* strain NOU5 (C7) and *Pseudomonas resinovorans* strain NOU6 (C8). For cassava to thrive,

nitrogen is very essential. According to a recent study, cassava might be grown with less fertilizer on low-fertilized soil (Omondi *et al.*, 2018). Orr and coworkers amplified *nifH* gene from *Pseudomonas aeruginosa* from organic and conventionally managed soil using quantitative PCR (qPCR) with primer set PolF/PolR (Orr *et al.*, 2011). In this study, nitrogen fixing gene (*nifH* gene) was amplified from *Kosakonia radicincitans* strain NOU1 (C5) isolated from cassava rhizospheric soil which is a free-living nitrogen fixing bacteria. In both free-living and symbiotic bacteria, the nitrogenase enzyme is predominantly encoded by the *nifH* genes, which are marker genes that can indicate an organism's efficiency in the nitrogen fixation process (Wang *et al.*, 2022). Numerous free-living bacteria are capable of fixing nitrogen in the atmosphere, according to earlier research (Zhang *et al.*, 2022; de Matoso *et al.*, 2021; Luo *et al.*, 2010). Therefore, developing and applying microbial inoculants containing nitrogen fixing bacteria isolated from cassava rhizospheric soil can improve nitrogen availability and soil fertility.

Hence, continuous genetic screening of bacterial isolates using PCR amplification of nitrogen fixing genes is essential for identifying effective biofertilizer strains.

CONCLUSION

In conclusion, the use of microorganisms that promote plant development has potential as a substitute for inorganic phosphate fertilizers. Additionally, by improving the accessibility of phosphorus and other plant growth hormones, the discovery of extremely effective microbes that promote plant development may eventually replace artificial fertilizers in agricultural practices. In addition to supporting food security, this will safeguard the environment as a natural practice and the wellbeing of many living forms. Through elucidating the roles of microorganisms in soil ecosystems and harnessing molecular techniques to identify beneficial bacterial and fungal strains, this research not only offers insights into the possible use of plant growth-promoting microbes as biofertilizers for crop improvement but also underscores the importance of using molecular techniques to target bacteria strains that possess the potentials to fix atmospheric nitrogen. By integrating these findings into agricultural management strategies, we can strive towards more resilient and eco-friendly farming systems, ultimately contributing to

global food security and environmental sustainability.

Conflict of interest

Authors have no conflict of interest to declare

Author contribution

OCC and UG designed the study; performed data analysis and interpretation while NPJ collected and assembled the data. OCC wrote the first draft of the article. APC contributed in reading and reviewing the draft. All authors approved the manuscripts for publication after reading.

REFERENCES

- P., Kopińska, M., Kołodziejek, D., Jastrzębska, E. and Brzózka, Z. (2024). Microfluidic system for generating a three-dimensional (3D) vascularized islet-on-a-chip model. *Sensors and Actuators B: Chemical*, **418**, 2024, 136302.
- Ali, A.A. and El-Kholy, A.S. (2022). Isolation and characterization of endophytic *Kosakonia radicincitans* to stimulate wheat growth in saline soil. *Journal of Advances in Microbiology*. **22**(11): 115-126.
<https://doi.org/10.9734/jamb/2022/v22i11698>.
- Alori, E.T., Glick, B.R. and Babalola, O.O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*. **8**: 971.
<https://doi.org/10.3389/fmicb.2017.00971>.
- Bharucha, U., Patel, K. and Trivedi, U.B. (2013). Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). *Agricultural Research*. **2**: 215-221.
<https://doi.org/10.1007/s40003-013-0065-7>.
- Boleta, E.H.M., Shintate, G.F., Jalal, A., Santini, J. M. K., Rodrigues, W.L., Lima, B.H., Arf, O., Silva, M.R., Buzetti, S. and Teixeira, F.M. (2020). Inoculation with growth-promoting bacteria *Azospirillum brasilense* and its effects on productivity and nutritional accumulation of wheat cultivars. *Frontiers in Sustainable Food Systems*. **4**: 607262.
<https://doi.org/10.3389/fsufs.2020.607262>.

- Canarini, A., Kaiser, C., Merchant, A., Richter, A. and Wanek, W. (2019). Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*. **10**: 157. <https://doi.org/10.3389/fpls.2019.00157>.
- Ceci, A., Pinzari, F., Russo, F., Maggi, O. and Persiani, A.M. (2018). Saprotrophic soil fungi to improve phosphorus solubilisation and release: *in vitro* abilities of several species. *Ambio* **47**: 30–40. <https://doi.org/10.1007/s13280-017-0972-0>.
- Chen, F., Ronen, Z. and Arye, G. (2024). Transport of plant growth promoting bacteria (*Azospirillum brasilense*) in sand under transient water flow: effect of inoculation regime. *Biology and Fertility of Soils* **60**: 883–899. <https://doi.org/10.1007/s00374-024-01839-4>.
- Chen, H., Jin, Z., Huang, R., He, L., Tian, W., Zhao, L. and Zhang, Z. (2023). Promotion of growth of alfalfa by *Erwinia persicina* Cp2 exopolysaccharides under NaCl Stress. *Agronomy*. **13**: 2129. <https://doi.org/10.3390/agronomy13082129>.
- Chittora, D., Meena, M., Barupal, T. and Swapnil, P. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochemistry and Biophysics Report*. **22**: 100737. <https://doi.org/10.1016/j.bbrep.2020.100737>.
- Coutinho, E.P., Wagner P.F. and Yano-Melo, A.M. (2012). Solubilization of phosphates *in vitro* by *Aspergillus* spp. and *Penicillium* spp. *Ecological Engineering*. **42**: 85-89. <https://doi.org/10.1016/j.ecoleng.2012.02.002>.
- da Silva, A.M., da Cruz, P. N., Costa, S.S., Silva, A., Schneider, M.P. C., das Gracas, D. A., da Silva, J. K. and Barauna, R. A. (2024). Assessment of plant-growth promoting potential of bacteria isolated from amazonian black pepper roots. *Journal of Soil Science and Plant Nutrition*. **24**: 2825–2837. <https://doi.org/10.1007/s42729-024-01707-y>.
- Doilom, M., Guo, J. W., Phookamsak, R., Mortimer, P. E., Karunarathna, S. C., Dong, W. and Xu, J. C. (2020). Screening of phosphate-solubilizing fungi from air and soil in Yunnan, China: four novel species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. *Frontiers in microbiology*. **11**: 585215. <https://doi.org/10.3389/fmicb.2020.585215>.
- Duca, D., Lorv, J., Patten, C. L., Rose, D. and Glick, B. R. (2014). Indole-3-acetic acid in plant–microbe interactions. *Antonie Van Leeuwenhoek*. **106**: 85–125. <https://doi.org/10.1007/s10482-013-0095-y>.
- Ejeagba, T.E, Obi, C.C. and Umanu, G. (2023). Isolation and molecular characterization of phosphate-solubilizing bacteria from root nodules of cowpea (*Vigna unguiculata*) seeds planted at Ota, Ogun State, Nigeria. *Journal of Applied Science and Environmental Management*. **27** (11): 2525-2531. <https://doi.org/10.4314/jasem.v27i11.23>.
- Elias, F., Woyessa, D. and Muleta, D. (2016). Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *International Journal of Microbiology*. **2016**(1): 5472601. <https://doi.org/10.1155/2016/5472601>.
- Enebe, M.C. and Babalola, O.O. (2018). The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. *Applied Microbiology and Biotechnology*. **102**: 7821–7835. <https://doi.org/10.1007/s00253-018-9214-z>. Epub 2018 Jul 20.
- Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A. and Olsen, G.J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16SrRNA Genes. *Applied and Environmental Microbiology*. **74**: 2461-2470. <https://doi.org/10.1128/AEM.02272-07>.
- Garby, J.C. and Buckley, D.H. (2012). A comprehensive evaluation of PCR primers to amplify the *nifH* gene of nitrogenase. *PLoS One*. **7**(7): 42149. <https://doi.org/10.1371/journal.pone.0042149>.
- Girma, B., Panda, A.N., Mohanty, S., Ray, L. and Chowdhary, G. (2020). Draft genome sequence of the plant growth-promoting rhizobacterium *Klebsiella* sp. strain kbg6.2, imparting salt tolerance to rice. *Microbiology Resource Announcement*. **9**: 10.1128 <https://doi.org/10.1128/mra.00491-20>.

- Gómez-Godínez, L.J., Aguirre-Noyola, J.L., Martínez-Romero, E., Arteaga-Garibay, R.I., Ireta-Moreno, J. and Ruvalcaba-Gómez, J.M. (2023). A look at plant-growth-promoting bacteria. *Plants*. **12**: 1668. <https://doi.org/10.3390/plants12081668>.
- Gopalan, N.R., Nikhil, P.T., Sharma, R. and Mohapatra, S. (2023). The use of microbes as a combative strategy for alleviation of abiotic and biotic stresses. Unravelling plant-microbe synergy. Academic Press, New York, pp 175–193.
- Guo, J., Muhammad, H., Lv, X., Wei, T., Ren, X., Jia, H., Atif, S. and Hua, L. (2020). Prospects and applications of plant growth promoting rhizobacteria to mitigate soil metal contamination: A review. *Chemosphere*. **246**: 125823. <https://doi.org/10.1016/j.chemosphere.2020.125823>.
- Hamza, T.A. and Alebejo, A.L. (2017). Isolation and characterization of rhizobia from rhizosphere and root nodule of cowpea, elephant and lab lab plants. *International Journal of Novel Research and Interdisciplinary Studies*. **4**: 1-7. <https://www.noveltyjournals.com> (Assessed October 21, 2024).
- Hossain, M.M., Sultana, F. and Islam, S. (2017). Plant growth-promoting fungi (PGPF): phy-tostimulation and induced systemic resistance, in: D. Singh, H. Singh, R. Prabha (Eds.), Plant-microbe interactions in agro-ecological perspectives, Springer, Singapore, pp. 135–191. https://doi.org/10.1007/978-981-10-6593-4_6.
- Hoyos-Carvajal, L., Orduz, S. and Bissett, J. (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biological Control*. **51**: 409–416, <https://doi.org/10.1016/j.biocontrol.2009.07.018>.
- Iqbal, A. and Hasnain, S. (2013) Auxin producing *Pseudomonas* strains: biological candidates to modulate the growth of *Triticum aestivum* Beneficially. *American Journal of Plant Science*. **4**: 1693–1700. <https://doi.org/10.4236/ajps.2013.49206>.
- Kaur, G. and Reddy, M.S. (2017). Improvement of crop yield by phosphate-solubilizing *Aspergillus* species in organic farming. *Archives of Agronomy and Soil Science*. **63**(1): 24-34. <https://doi.org/10.1080/03650340.2016.1182161>.
- Keswani, C., Singh, S.P., Cueto, L., García-Estrada, C. Mezaache-Aichour, S., Glare, T.R., Borriss, R., Singh, S.P., Blázquez, M.A. and Sansinenea, S. (2020). Auxins of microbial origin and their use in agriculture. *Applied Microbiology and Biotechnology*. **104**: 8549–8565. <https://doi.org/10.1007/s00253-020-10890-8>.
- Khuna, S., Kumla, J., Srinuanpan, S., Lumyong, S. and Suwannarach, N. (2023). Multifarious characterization and efficacy of three phosphate-solubilizing *Aspergillus* species as biostimulants in improving root induction of cassava and sugarcane stem cuttings. *Plants*. **12**: 3630. <https://doi.org/10.3390/plants12203630>.
- Kiyoshi, M., Hisano, H., Takeda-Kamiya, N, Takebayashi, Y., Ariizumi, T., Gao, Y., Ezura, H., Sato, K., Zhao, Y., Ken-ichiro, H. and Kasahara, H. (2019). *Agrobacterium tumefaciens* enhances biosynthesis of two distinct auxins in the formation of crown galls, *Plant and Cell Physiology*. **6**(1): 29–37, <https://doi.org/10.1093/pcp/pcy182>.
- Kumar, M., Poonam, Ahmad, S. and Singh, R.P. (2022). Plant growth promoting microbes: Diverse roles for sustainable and ecofriendly agriculture. *Energy Nexus*. **7**: 2022, 100133. <https://doi.org/10.1016/j.nexus.2022.100133>.
- Ladha, J.K., Peoples, M.B., Reddy, P.M., Biswas, J.C., Bennett, A., Jat, M.L. and Krupnik, T.J. (2022). Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Field Crop Resource*. **283**: 108541. <https://doi.org/10.1016/j.fcr.2022.108541>.
- Li, X., Li, D., Yan, J., Zhang, Y., Wang, H., Zhang, J., Ahmed, T. and Li, B. (2021). Effect of plant-growth-promoting fungi on eggplant (*Solanum melongena* L.) in New Reclamation Land. *Agriculture*. **11**: 1036. <https://doi.org/10.3390/agriculture11111036>.
- Liu, Y., Chen, L., Zhang, N., Li, Z., Zhang, G., Xu, Y. and Shen, Q.Z.R. (2016). Plant-microbe communication enhances auxin biosynthesis by a root-associated bacterium, *Bacillus amyloliquefaciens* SQR9. *Molecular Plant-Microbe*

- Interactions*. **29**: 324–330. <https://doi.org/10.1094/mpmi-10-15-0239-r>.
- Ludwig-Müller, J. (2015). Bacteria and fungi controlling plant growth by manipulating auxin: Balance between development and defense. *Journal of Plant Physiology*. **172**: 4–12. <https://doi.org/10.1016/j.jplph.2014.01.002>.
- Ma, Y., Dias, M. C. and Freitas, H. (2020). Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science*. **11**: 591911. <https://doi.org/10.3389/fpls.2020.591911>.
- Malik, D.K. and Sindhu, S.S. (2011). Production of indole acetic acid by *Pseudomonas* sp.: effect of coinoculation with *Mesorhizobium* sp. Cicer on nodulation and plant growth of chickpea (*Cicer arietinum*). *Physiology Molecular Biology of Plants*. **17**(1): 25-32. <https://doi.org/10.1007/s12298-010-0041-7>.
- Manoj, S.R., Karthik, C., Kadirvelu, K., Arulselvi, P.I., Shanmugasundaram, T. and Bruno, B. (2020). Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: a review. *Journal of Environmental Management*. **254**: 109779. <https://doi.org/10.1016/j.jenvman.2019.109779>.
- Mattos, K.A., Padua, V.L.M., Romeiro, A., Hallack, L.F., Neves, B.C., Ulisses, T.M.U., Barros, C.F., Todeschini, A.R., Previato, J.O. and Mendonca-Previato, L. (2008). Endophytic colonization of rice (*Oryza sativa* L.) by the diazotrophic bacterium *Burkholderia kururiensis* and its ability to enhance plant growth. *Anais da Academia Brasileira de Ciências*. **80**: 477-493. <https://doi.org/10.1590/s0001-37652008000300009>.
- Marathe, R.J., Phatake, Y.B., Shaikh, A.C., Shinde, B.P. and Gajbhiye, M.H. (2017). Effect of IAA produced by *Pseudomonas aeruginosa* 6A (BC4) on seed germination and plant growth of glycin max. *Journal of Experimental Biology and Agriculture*. **5**(3): 351-358. [https://doi.org/10.18006/2017.5\(3\).351.358](https://doi.org/10.18006/2017.5(3).351.358).
- Meena, M., Zehra, A., Swapnil, P., Harish, Marwal, A., Yadav, G. and Sonigra, P. (2021). Endophytic nanotechnology: An approach to study scope and potential applications. *Frontiers Chemistry*. **9**: 613343. <https://doi.org/10.3389/fchem.2021.613343>.
- Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A. and Khan, N. (2019). In vitro production of IAA by endophytic fungus *Aspergillus awamori* and its growth promoting activities in *Zea mays*. *Symbiosis*. **77**: 225-235. <https://doi.org/10.1007/s13199-018-0583-y>.
- Meliani, A., Bensoltane, A., Benidire, L. and Oufdou, K. (2017). Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. *Research & Reviews: Journal of Botanical Sciences*. **6**(2): 16-24. Available at: <https://www.rroj.com/open-access/plant-growth-promotion-and-iaa-secretion> (Accessed October 20, 2024).
- Noha, I. O. and Shixue, Y. (2018). Isolation and characterization of pea plant (*Pisum sativum* L.) growth-promoting Rhizobacteria. *African Journal of Microbiology Research*. **12**(34): 820-828. <https://doi.org/10.5897/AJMR2018.8859>.
- Nosrati, R., Owlia, P., Saderi, H., Rasooli, I. and Malboobi, M.A. (2014). A contribution to set a legal framework for biofertilisers phosphate solubilization characteristics of efficient nitrogen fixing soil Azotobacter strains. *Iran Journal of Microbiology*. **6**(4): Available at: 285-95. <https://pubmed.ncbi.nlm.nih.gov/25802714/> (Assessed October 20, 2024).
- Omondi, J. O., Lazarovitch, N., Rachmilevitch, S., Boahen, S., Ntawuruhunga, P., Sokolowski, E. and Yermiyahu, U. (2018). Nutrient use efficiency and harvest index of cassava decline as fertigation solution concentration increases. *Journal of Plant Nutrition and Soil Science*. **181**(5): 664-654. <https://doi.org/10.1002/jpln.201700455>.
- Orr, C. H., James, A., Leifert, C., Cooper, J. M. and Cummings, S.P. (2011). Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. *Applied Environmental Microbiology*. **77**(3): 911-919. <https://doi.org/10.1128/AEM.01250-10>.

- Oyetibo, G.O., Ilori, M.O., Adebuseye, S.A., Obayori, O.S. and Amund, O.O. (2010). Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. *Environmental Monitoring and Assessment*. **168**: 305-314. doi.org/10.1007/s10661-009-1114-3.
- Park, S., Kim, A.L., Hong, Y.K., Shin, J.H. and Joo, S.H. (2021). A highly efficient auxin-producing bacterial strain and its effect on plant growth. *Journal of Genetic Engineering and Biotechnology*. **19**(1): 179. https://doi.org/10.1186/s43141-021-00252-w.
- Parvin, W., Rahman, M., Govender, N.T. and Wong, M.Y. (2020). Identification, determination and quantification of indole-3-acetic acid produced by *Pseudomonas aeruginosa* UPMP3 and its effect on the growth of oil palm (*Elaeis guineensis* Jacq). *World Journal of Agricultural Research*. **8**(3): 75-83. https://doi.org/10.12691/wjar-8-3-2.
- Pradhan, A., and Mishra, B.B. (2015). Effect of plant growth promoting rhizobacteria on germination and growth of rice (*Oryza sativa* L.). *The community and Environmental Assesment* **9**(1&2): 213-216. https://www.researchgate.net/publication/303588764 (Assessed February 21, 2025).
- Radhakrishnan, R. and Lee, I.J. (2016). Gibberellins producing *Bacillus methylotrophicus* KE2 supports plant growth and enhances nutritional metabolites and food values of lettuce. *Plant Physiology and Biochemistry*. **109**: 181–189. https://doi.org/10.1016/j.plaphy.2016.09.018.
- Radhakrishnan, R., Khan, A.L., Kang, S. M. and Lee, I.J. (2015). A comparative study of phosphate solubilization and the host plant growth promotion ability of *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01 under salt stress. *Annals of microbiology*. **65**: 585-593. https://doi.org/10.1007/s13213-014-0894-z.
- Rahmad, A.L., Kuswinanti, T. and Musa, Y. (2020). Isolation of fungi producing hormone Indole Acetic Acid (IAA) on sugarcane bagasse and filter cake. IOP Conf. Series: *Earth and Environmental Science*. **486**: 012131. https://doi.org/10.1088/1755-1315/486/1/012131.
- Raja, N.S., Gopalan, P.T., Nikhil, R., Sharma, R. and Mohapatra, S. (2023). The use of microbes as a combative strategy for alleviation of abiotic and biotic stresses, Editor(s): Dinesh Chandra, Pankaj Bhatt, In *Developments in Applied Microbiology and Biotechnology, Unravelling Plant-Microbe Synergy*, Academic Press, pp, 175-193. https://doi.org/10.1016/B978-0-323-99896-3.00010-2.
- Ratnam, D.P.S.K. (2020). Indole 3-acetic acid production by *Aspergillus* species isolated from chilli rhizospheres. *Bioscience Biotechnology Research Communications*. **13**: 4. http://dx.doi.org/10.21786/bbrc/13.4/80
- Rinu, K. and Pandey, A. (2010). Temperature-dependent phosphate solubilization by cold-and pH-tolerant species of *Aspergillus* isolated from Himalayan soil. *Mycoscience*. **51**(4): 263-271. https://doi.org/10.1007/S10267-010-0036-9.
- Shaban, W. I. and El-Bramawy, M.A. (2011). Impact of dual inoculation with *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions. *International Research and Journal of Agricultural Science*. **1**(3): 098–108.
- Silva, K, Quisen, R.C., Goldbach, J.D., Pepe, K.B., Kalil, F. and Filho, A.N. (2022). Plant growth-promoting endophytic bacteria in peach palm seedlings. *Pesquisa Agropecuária Brasileira*. **57**: e02962. https://doi.org/10.1590/S1678-3921.
- Singh, M., Singh, D., Gupta, A., Pandey, K.D., Singh, P.K. and Kumar, A. (2019). Plant growth promoting rhizobacteria: application in biofertilizers and biocontrol of phytopathogens, in: A.K. Singh, A. Kumar, P.K. Singh (Eds.), *PGPR Amelioration in Sustainable Agriculture: Food Security and Environmental Management*, Elsevier, pp. 41–66, https://doi.org/10.1016/B978-0-12-815879-1.00003-3.
- Spaepen, S. and Vanderleyden, J. (2011). Auxin and Plant-Microbe Interactions. *Cold Spring Harbour Perspectives in Biology*. **3**: 001438. https://doi.org/10.1101/cshperspect.a001438.

- Sridevi, M., Yadav, N. and Mallaiah, K. (2008). Production of indole-acetic-acid by Rhizobium isolates from Crotalaria species. *Research Journal of Microbiology*. **3**(4): 276–281. <https://scialert.net/abstract/?doi=jm.2008.276.281>(Assessed November 12, 2024).
- Timofeeva, A.M., Galyamova, M.R. and Sedykh, S.E. (2023). Plant growth-promoting soil bacteria: Nitrogen fixation, phosphate solubilization, siderophore production, and other biological activities. *Plants*. **12**: 4074. <https://doi.org/10.3390/plants12244074>.
- Torres, D., Benavidez, I., Donadio, F., Mongiardini, E., Rosas, S. and Spaepen, S. (2018). New insights into auxin metabolism in *Bradyrhizobium japonicum*. *Research Microbiology* **169**: 313–323. <https://doi.org/10.1016/j.resmic.2018.04.002>.
- Trindade, L. C., Eder, M., Daniela, B. L. and Marisa A. (2007). Development of a molecular method for detection and identification of *Xanthomonas campestris*. *Summa Phytopathologica*. **33**(1): 16-23. <https://doi.org/10.1590/S0100-54052007000100002>.
- Tsotetsi, T., Nepali, L., Malebe, M. and Tugizimana, F. (2022). *Bacillus* for plant growth promotion and stress resilience: What have we learned? *Plants*. **11**: 2482. <https://doi.org/10.3390/plants11192482>.
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. and Nasrulhaq, B.A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. *Molecules*. **21**: 573. <https://doi.org/10.3390/molecules21050573>.
- Verma, S. and Kuila, A. (2019). Bioremediation of heavy metals by microbial process. *Environmental Technology & Innovation*. **14**: 100369. <https://doi.org/10.1016/j.eti.2019.100369>.
- Wang, Y., Narayanan, M., Shi, X., Chen, X., Li, Z., Natarajan, D. and Ma, Y. (2022). Plant growth-promoting bacteria in metal-contaminated soil: Current perspectives on remediation mechanisms. *Frontiers in Microbiology*. **13**: 966226. <https://doi.org/10.3389/fmicb.2022.966226>.
- Wawrik, B., Kerkhof, L., Zylstra, G.J. and Kukor, J.J. (2005). Identification of unique type II polyketide synthase genes in soil. *Applied Environmental Microbiology*. **71**(5):2232-2238. <https://doi.org/10.1128/AEM.71.5.2232-2238.2005>.
- Yang, L. and Yang, K. (2020). Biological function of *Klebsiella variicola* and its effect on the rhizosphere soil of maize seedlings. *Peer Reviewed Journal*. **8**: e9894. <https://doi.org/10.7717/peerj.9894>.
- Ziaullah, S. M., Asim, S., Nayab, A. and Zahid, A. (2020). IAA production and maize crop growth promoting potential of endophyte *Aspergillus niger* (AO11) under salt stress. *Current Botany*. **11**: 175-181. <https://doi.org/10.25081/cb.2020.v11.5751>.