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RESEARCH ARTICLE

Isolation and characterization of plant-growth promoting bacteria from Irish potato rhizosphere in Nyandarua county, Kenya

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Abstract

Irish potato, rich in antioxidants and minerals, is an important food source but faces challenges due to yield losses and excessive use of agrochemicals. To address these challenges, biological controls methods are gaining attention as eco-friendly alternatives. The rhizosphere hosts a complex interplay of roots, microbiota and soil, making it a key focus for sustainable agricultural practices. This study aimed to identify and characterize rhizosphere bacteria from Irish potato roots in Nyandarua county, Kenya. Root samples were collected using purposive and zigzag methods. Bacterial isolates were characterized through 16S rRNA gene sequencing and biochemical tests. Twenty-seven bacterial isolates with diverse morphological traits of colonies, including creamy-white, white and pink colonies of varying shapes and sizes were identified. Biochemical tests demonstrated all isolates as catalase and oxidase producers, with 14 isolates producing indole acetic acid (IAA) and 13 isolates producing hydrogen cyanide (HCN). Five isolates KG02 (9), KG02 (1), KG02 (2), KG02 (5) and KLm02 (1) were closely related to *Bacillus aerius*, *Paenibacillus xylanexedens*, *Alcaligenes faecalis* and *Providencia huashanensis* with 100 % sequence identity to known sequences in the NCBI database. This study highlights the importance of microbial diversity in soil ecosystems and their potential applications in sustainable agriculture. Further greenhouse experiments are recommended to evaluate their antagonistic and growth-promoting effects on Irish potatoes.

Keywords: biological control; growth-promoting properties; Irish potato roots; microbial diversity; rhizospheric bacteria

Introduction

Irish potato (*Solanum tuberosum*), a member of the Solanaceae family, is an essential starchy tuberous crop cultivated and consumed globally. Renowned for its nutritional value, the potato is rich in vitamins C and B6, antioxidants and minerals (1). By 2023, 376 million tons of potatoes had been produced globally, with China and India being the main producers (2). Africa's production has increased from 2 million tons in 1960 to over 30 million tons by 2013 (3) and approximately 2.7 million tons in 2022 (4). In Kenya, the Irish potato ranks as the second most important food crop after maize and is cultivated by approximately 800000 small-scale farmers, serving as a vital source of income and food security for these farmers (5). However, potato yields in Kenya are frequently undermined by substantial losses, ranging from 50 % to complete crop failure due to pests, diseases and suboptimal soil health (6, 7). These yield reductions threaten both food security and the economic stability of potato farming communities (6).

The challenges affecting potato production are multifaceted, encompassing both abiotic and biotic factors. Abiotic factors such as soil degradation, nutrient depletion and extreme weather conditions significantly reduce yields. Overreliance on chemical fertilizers and pesticides further exacerbates soil health and contributes to environmental degradation (2). Meanwhile, biotic stresses, including soil-borne pests and diseases like late blight (*Phytophthora infestans*), contribute to the decline in productivity (6). Addressing these challenges requires innovative, sustainable approaches to enhance crop resilience and productivity while minimizing environmental harm. Among these solutions, plant growth-promoting rhizobacteria (PGPR) have gained attention as a viable alternative.

PGPR are a diverse group of beneficial bacteria that colonize plant roots and establish mutually beneficial relationships with their hosts. These bacteria promote plant-growth through direct and indirect mechanisms, offering eco-friendly solutions to modern agricultural challenges (8, 9). Direct mechanisms involve enhancing nutrient availability and plant growth through processes such as nitrogen fixation, phosphorus solubilization and the synthesis of phytohormones like auxins, cytokinins and gibberellins (9, 10).

Additionally, PGPR produce compounds such as hydrogen cyanide (HCN), ammonia and siderophores, which improve nutrient uptake and protect plants from pathogens (11).

Research has been done on the use of PGPR to enhance plant growth, yield and health of Irish potato. For example, the microorganisms like *Azospirillum* sp., *Rhizobium* sp. and lactic acid bacteria such as *Lactobacillus acidophilus* that are found in the rhizosphere of Irish potato showed to have strong potential as biofertilizers by fixing nitrogen and by producing different growth hormones including indole acetic acid (IAA) (12, 13). It has been demonstrated that some PGPR species promote plant growth by acting as biocontrol. For instance, some *Burkholderia cepacia* strains exhibit biocontrol properties against *Fusarium* species. In a similar vein, the latter PGPR showed the ability to use siderophore production to promote maize growth in iron-deficient environments (14) *Pseudomonas* sp., frequently present in the potato rhizosphere, *Enterobacter* sp., *Bacillus subtilis* and *Bacillus megaterium* are known for their plant growth promoting properties, enhancing nutrient availability and biocontrol abilities against soil borne pathogens (13, 15).

The reliance on chemical fertilizers and pesticides in modern agriculture has led to significant environmental and health concerns. Biological control, including the use of rhizobacteria, is an emerging strategy to address these issues. As natural biofertilizers and biocontrol agents, rhizobacteria offer an environmentally friendly and cost-effective alternative to agrochemicals (16, 17). Rhizobacteria contribute to sustainable agriculture by enhancing plant health, improving nutrient cycling and reducing dependency on synthetic chemical inputs. However, despite their potential, the diversity, functionality and mechanisms of PGPR in the rhizosphere of Irish potato, particularly under Kenyan agroecological conditions remain underexplored. Therefore, this study aimed at isolating and characterizing beneficial rhizosphere bacteria associated with Irish potato cultivation in Kenya.

Materials and Methods

Sample collection

Samples were collected from the villages of Githabai and Landmark, Kinangop sub-county, Kenya, lying between latitude 0°8′ to the north and 0°50′ to the south and between 35°13′ East and 36°42′ west (Fig. 1). Soil samples were collected at a depth of 15-20 cm by using purposive techniques in two major fields of Kinangop (Githabai and Landmark), one each in each village according to a zigzag pattern (18, 19). For each field, three different soil samples were taken from potato plants ready to harvest and placed in sterile zip lock bags, transported to the Institute for Biotechnology Research (IBR) laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT) and kept at 4 °C before further analysis.

Isolation and identification of rhizobacteria from potato roots

Soil collected from the potato rhizosphere were used to isolate rhizospheric bacteria using a tenfold serial dilution. From each sample, one gram (1 g) of soil was diluted in 30 mL sterile universal glass bottle containing 9 mL of sterile distilled water. The mixture was vortexed for 1 min at 150 rpm to ensure homogenization. Subsequently, serial dilutions were performed up to a 12-fold dilution. An aliquot (of 0.3 mL) was cultured on ammonium mineral salt (AMS) medium adjusted to a pH 6.8 and supplemented with 0.5 % (v/v) methanol (20). After 5 days of incubation at 27 °C, a 10^{-12}-fold dilution was employed to identify colonies based on morphology. Single colony was obtained by sub-culturing procedures.

Morphological characteristics

Morphological characteristics of the colonies of isolates were recorded considering colony shape, size and colour (10). Biochemical tests such as ability to produce catalase and peroxidase were performed following the method described by Saeed et al. (21).

Catalase production assay

Catalase test was performed to determine the ability of isolated bacteria to break down hydrogen peroxide through the production of enzymes such as catalase or peroxidase. A drop of 3 % hydrogen peroxide was poured to the bacterial colony on a sterile glass slide. The formation of air bubbles was monitored for 10 s. Positive catalase produced air bubbles, whereas negative catalase did not produce any bubbles (21). The assay was done in triplicate.

Oxidase production assay

The oxidase test detects the presence of the oxidase enzyme in bacteria. Oxidase reagents include reducing agents that change colour when oxidized. One loop of bacterial colony was etched onto oxidase discs. The observation of results consisted of colorimetric induced reaction; dark purple on oxidase discs showed that the tested bacteria had a positive oxidase enzyme, whereas no change in colour suggested a negative oxidase result (22). The test was done in triplicate.

Indole acetic acid production assay

Indole acetic acid (IAA) production was assessed using the colorimetric approach (23). Single colonies of each isolate were inoculated in 5 mL of Luria-Bertani Broth supplemented with L-tryptophan (2.0 g/L; w/v) and the resultant supernatant treated with 1 mL of Kovacs reagent (HiMedia, Mumbai, India). The test was done in triplicate and the medium without inoculum was considered as the negative control. Positive IAA production was represented by red colour.

Hydrogen cyanide production assay

Hydrogen cyanide production capacity test was performed as described (24). The isolates were cultured in petri dishes (90 mm in diameter) with nutrient agar supplemented with glycine (4.4 g/L; w/v). Sterilized filter paper discs (Whatman filter paper No 1, GE Healthcare UK Ltd, Amersham Place, Buckinghamshire, UK, CAT No. 1001-125), each measuring 125 mm in diameter, were immersed in a solution containing 2 % sodium carbonate and 0.5 % picric acid. Subsequently, these discs were positioned on the top of each Petri dish inoculated with isolates. Plates were sealed with parafilm and incubated at 28 ± 2 °C for 5 days. One plate containing nutrient agar supplemented with glycine (4.4 g/L; w/v), but without culturing any isolate on it served as a negative control. The colour alteration from yellow to faint reddish-brown on the paper indicated HCN production. The test was done in triplicate to

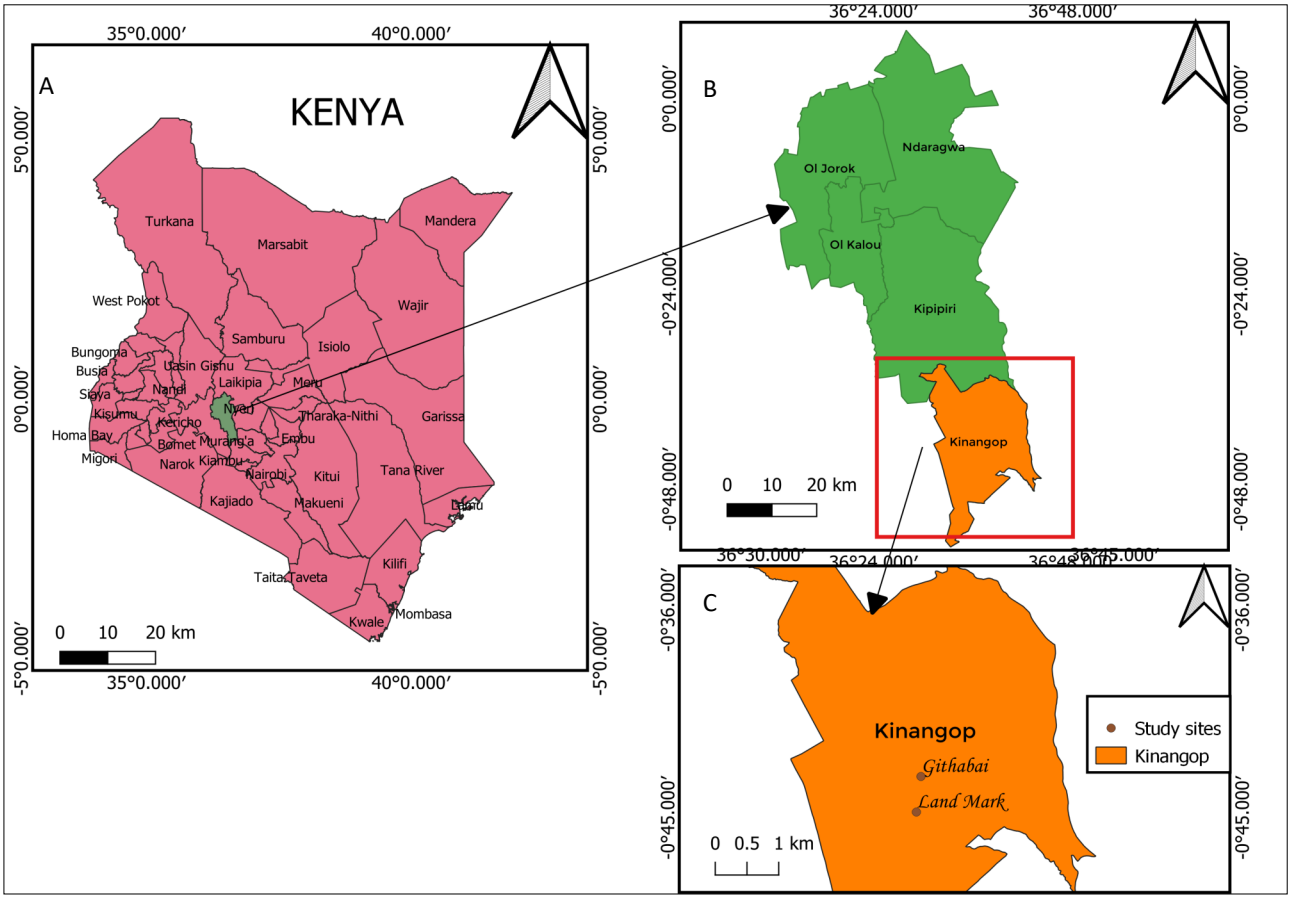

Fig. 1. Description of sample collection sites using QGIS Desktop v3.16.10. The area highlighted in orange indicates the sampling points, at Kinangop sub-county, Githabai and Landmark (brown dots) villages. (A-C) Indicates a map of Kenya, Nyandarua county and Kinangop sub-county, respectively.

ensure the results.

DNA analysis

A single colony of isolate was inoculated in 5 mL of nutrient broth and incubated at 37 °C for 48 hr. To get enough bacteria cells, as much as 1.5 mL of inoculum was centrifuged at 6000×g for 10 min. The genomic DNA was extracted from resuspended bacterial cells with the use of QIAamp DNA Mini Kit (QIAGENGmbH, Hilden, Germany) following the manufacturer's instructions. DNA concentration and purity were measured by using a nanodrop machine (Genova nano, Cole-Parmer Ltd, Stone, Staffs, UK). DNA fragments were separated by electrophoresis on 1 % agarose gel stained with GelRed Nucleic Acid Gel Stain (10000× in water 100 µL) for 45 min. Thereafter, the DNA template was stored at -20 °C for further analysis.

The PCR amplification of 16S rRNA genes was performed in 25 µL of Accuris 2× Taq Red Dye Master Mix and 2 µL of a template of genomic DNA. The PCR amplification was conducted in a total volume of 50 µL reactions Including 21 µL of water, 1 µL of forward primer (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′) and 1 µL of reverse primer (1492R: 5′-CGGTTACCTTGTTACGACTT-3′). The PCR amplification method was as follows: initial denaturation for 5 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 57 °C for 30 s, elongation at 72 °C for 1 min and 30 s and final extension at 72 °C for 5 min (25) in ProFlex PCR System (applied biosystems by life technologies Holdings Pte Ltd, Marsiling industrial Estate, Singapore) Thermoblock thermocyclers. The presence of bands and size of PCR amplicons were confirmed using a 1 % agarose and observed under Gel Doc transilluminator (Uvitec Cambridge, UK). The PCR amplicons was sequenced by Macrogen Asia Pacific Pte Ltd, Singapore. The sequencing result was analysed and trimmed using BioEdit software (26). The nucleotides comparison was performed using Basic local alignment tools (BLASTn) available in the GenBank/NCBI-Database.

The phylogenetic tree was built using the 16S ribosomal RNA (16S rRNA) gene sequences. The acquired nucleotide sequence was compared to sequences of closely related species obtained from the GenBank/NCBI and aligned with MUSCLE software (27). The evolutionary studies were performed using MEGA 11 (28). The evolutionary history was inferred using the Neighbour joining method and bootstrap values of 1000 iterations (29). The evolutionary distances were computed using the p-distance method (30).

The isolates identified from this study including KG01 (2), KG02 (2), KG02 (7), KLm03 (6), KLm02 (1), KLm02 (3), KG01 (3), KLm02 (2), KG02 (5), KG01 (4), KG02 (3), KG02 (1), KG02 (9) and KG01 (6) have been deposited in the NCBI GenBank and assigned the respective accession numbers PV615544, PV615545, PV615543, PV628405, PV618406, PV616974, PV638745, PV638746, PV638770, PV640221, PV640222, PV640223, PV640224 and PV651706.

Results

Morphological characteristics of the culture and biochemical tests

Table 1. Morphological characteristics and biochemical tests (catalase and oxidase) of bacterial isolates obtained from potato fields in Kenya

	Morphological characteristics				Biochemical tests	
No.	Code of isolates	Colony colour	Size	Shape	Catalase	Oxidase
1	KG01 (1)	Creamy	Small	Round	+	+
2	KG01 (2)	Creamy-white	Small	Round	+	+
3	KG01 (3)	Creamy-white	Small	Round	+	+
4	KG01 (4)	Creamy	Small	Round	+	+
5	KG01 (5)	Creamy	Small	Round	+	+
6	KG01 (6)	Creamy	Medium	Round	+	+
7	KG01 (7)	Creamy-white	Small	Round	+	+
8	KG01 (8)	Creamy	Small	Round	+	+
9	KGO1 (9)	Creamy	Small	Round	+	+
10	KGO2 (1)	Creamy	Medium	Round	+	+
11	KG02 (2)	Creamy-white	Small	Round	+	+
12	KG02 (3)	White	Small	Round	+	+
13	KG02 (4)	Creamy	Medium	Round	+	+
14	KG02 (5)	Creamy	Small	Round	+	+
15	KG02 (6)	Creamy	Small	Round	+	+
16	KG02 (6)	Pink	Small	Round	+	+
17	KG02 (8)	White	Small	Round	+	+
18	KG02 (9)	White	Small	Round	+	+
19	KLm02 (1)	Yellow	Small	Round	+	+
20	KLm02 (2)	White	Small	Round	+	+
21	KLm02 (3)	Pink	Small	Round+	+	+
22	KLm03 (1)	White	Small	Round +	+	+
23	KLm03 (2)	Creamy	Small	Irregular +	+	+
24	KLm03 (3)	White	Small	Round +	+	+
25	KLm03 (4)	White	Small	Round +	+	+
26	KLm03 (5)	Yellow	Small	Round +	+	+
27	KLm03 (5)	White	Small	Round +	+	+

Morphology-based identification revealed 27 isolates with pink, white, yellow and creamy colony features, as well as small and medium size and round and irregular shape (Table 1). Githabai location yielded a higher number of isolates, totalling to 18, with 9 isolates recovered from landmark area. All isolates were small in size, except for three medium-sized isolates KG01 (6), KG02 (1) and KG02 (4). Most colonies displayed a round shape, with only KLm03 (2) exhibiting an irregular shape. The predominant colony colour was creamy or creamy-white, while a few isolates exhibited distinct colours, including pink (KG02 (7), KLm02 (3)), yellow (KLm02 (1), KLm03 (5)) and white (KG02 (3), KLm02 (2)). All isolates tested positive for catalase and oxidase activity.

Characterization of rhizobacteria based on growth promoting activities

Thirteen isolates were capable of synthesizing both IAA and HCN. Notably, isolate KG02 (9) exhibited a unique characteristic, demonstrating the ability to produce IAA exclusively (Table 2).

The phylogenetic analysis of the isolates, based on 16S rRNA gene sequences, revealed their taxonomic affiliations.

Table 2. Assessment of IAA and HCN production

Isolate ID	IAA production	HCN production
KG02 (1)	+	+
KG02 (2)	+	+
KLm02 (3)	+	+
KG01 (2)	+	+
KLm02 (2)	+	+
KG02 (7)	+	+
KLm03 (6)	+	+
KG02 (3)	+	+
KG01 (6)	+	+
KG02 (5)	+	+
KG01 (3)	+	+
KG01 (4)	+	+
KLm02 (1)	+	+
KG02 (9)*	+	-

+: positive; -: negative; *: indicates the isolate with a unique characteristic of IAA production.

Most isolates clustered within the genus Serratia, with strong bootstrap support for multiple sub-clusters. Specifically, isolates such as KG01 (4), KG01 (2), KLm02 (2) and KLm03 (6) grouped with *Serratia marcescens* or closely related species, indicating their significant similarity to this genus. Additionally, isolates KG02 (1) and KG02 (9) were closely related to species of the genus *Paenibacillus* and *Bacillus* respectively, while KLm02 (1) clustered with *Providencia* with bootstrap values of 100. Similarly, isolate KG02 (3) and KG02 (2) formed a distinct clade with *Alcaligenes faecalis*, reflecting its unique taxonomic position among the analysed isolates. The tree's robust bootstrap values of 99 or 100 further confirmed the reliability of these phylogenetic relationships. These findings highlight the diversity of bacterial genera within the analysed samples, suggesting varied ecological and functional roles (Fig. 2).

According to the blast analysis results (Table 3), six of the fourteen isolates had similarities to the *Serratia* genus. Five isolates, however, including KG01 (2), KG01 (4), KG02 (7), KLm02 (2) and KLm03 (6), demonstrated a high similarity index of 99-100 % to *Serratia marcescens* strain HA1, *Serratia* sp. (in: enterobacteria), *Serratia marcescens* strain AF816, *Serratia marcescens* strain M86 and *Serratia marcescens* strain Rizhao_64, respectively. Another group of 4 out 14 isolates were closer to *Alcaligenes* genus, including the isolate KG02 (5), which indicated the highest similarity index greater that 99 % to *Alcaligenes faecalis* strain BY-01. Furthermore, the blast analysis result showed that isolates KG02 (9) and KG02 (1) had a 100 % similarity index with *Bacillus* and *Paenibacillus* species, while isolate KLm02 (1) was closer to the genus of *Providencia* with similarity indices of 100 %.

Discussion

The morphological and biochemical characterization of the 27 isolates from potato root soil samples collected in Nyandarua County's Kinangop sub-county revealed notable diversity. Isolates were identified based on colony colour, shape and size, showing variations in characteristics such as round or irregular shape, with creamy or creamy-white as the predominant colour. Other distinct colours included pink, yellow and white, showcasing some degree of heterogeneity

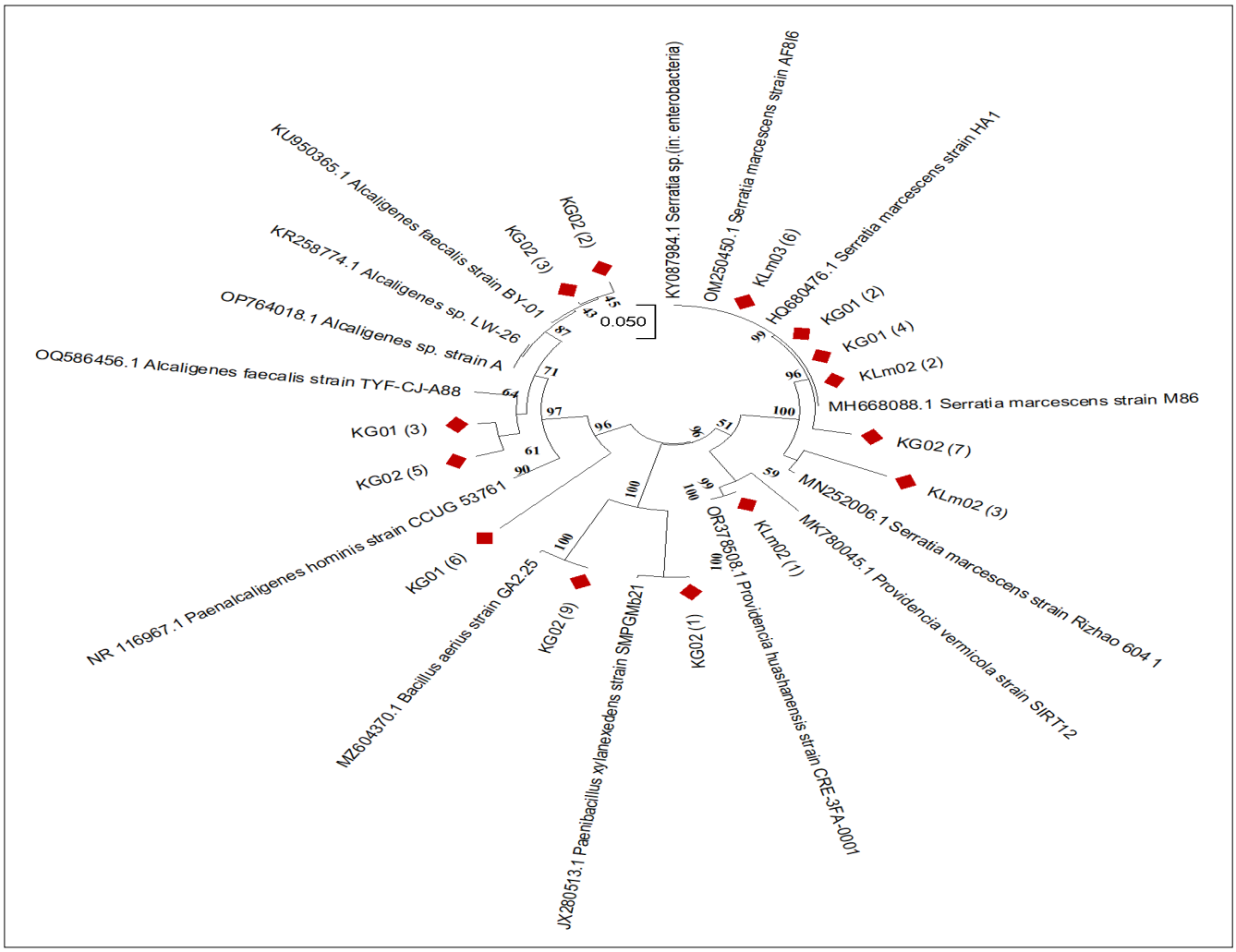

Fig. 2. The phylogenetic tree constructed based on 16S rRNA gene sequences. A taxonomic connection of 14 isolates with the closest hits retrieved from GenBank at NCBI. The *Paenalcaligenes hominis* strain CUG 53761 contained in the tree was utilized to root the tree. The red shaped lozenge on the tree indicates 14 isolates.

Table 3. Results of the blast analysis of rhizobacteria isolated from Irish potato roots

Isolate ID	Max score	Top score	Scientific name of the closest isolate in blast	Query cover	E-value	Percentage of identities	Accession number
KG02 (1)	1214	1214	*Paenibacillus xylanexedens* strain SMPGMb21	100 %	0.0	100 %	JX280513.1
KG02 (2)	383	383	*Alcaligenes faecalis* strain BY-01	100 %	7e-102	93.73 %	KU950365.1
KLm02 (3)	571	571	*Serratia marcescens* strain Rizhao_64_1	100 %	2e-158	90.21 %	MN252006.1
KG01 (2)	1351	1351	*Serratia marcescens* strain HA1	100 %	0.0	100 %	HQ680476.1
KLm02 (2)	1626	1626	*Serratia marcescens* strain M86	100 %	0.0	99.66 %	MH668088.1
KG02 (7)	730	730	*Serratia marcescens* strain AF816	100 %	0.0	97.65 %	OM250450.1
KLm03 (6)	1179	1179	*Serratia* sp. (in: enterobacteria)	100 %	0.0	100 %	KY087984.1
KG02 (3)	588	588	*Alcaligenes* sp. strain A	100 %	2e-163	95.64 %	OP764018.1
KG01 (6)	309	309	*Providencia vermicola* strain SIRT12	99 %	2e-79	83.05 %	MK780045.1
KG02 (5)	374	374	*Alcaligenes faecalis* strain BY-01	100 %	3e-99	99.51 %	KU950365.1
KG01 (3)	436	436	*Alcaligenes faecalis* strain BY-01	99 %	7e-118	89.20 %	KU950365.1
KG01 (4)	1312	1312	*Serratia* sp. (in: enterobacteria)	100 %		100 %	KY087984.1
KLm02 (1)	1543	1543	*Providencia huashanensis* strain CRE-3FA-0001	100 %	0.0	100 %	OR378508.1
KG02 (9)	1668	1668	*Bacillus aerius* strain GA2.25	100 %	0.0	99.46 %	MZ604370.1

(Table 1). Most isolates were small, with three medium-sized isolates (KG01 (6), KGO2 (1) and KG02 (4)). Notably, the Githabai location yielded a higher number of isolates (18), compared to Landmark (9), possibly suggesting environmental or ecological factors influencing microbial diversity at the two sites. Biochemically, all isolates exhibited catalase and oxidase activity, indicating uniform enzymatic functionality across the isolates, although morphological differences suggest that these isolates may perform varied ecological functions. These findings align with previous studies highlighting the importance of morphological and biochemical characterization in understanding microbial diversity and ecological roles (31). Such variations may provide insight into the adaptive capabilities of microorganisms in different soil environments since they can protect themselves and the host plants from oxidative stress by detoxifying harmful compounds and potentially suppressing pathogens, thus promoting healthier and more resilient plants (32).

The biochemical analysis of 27 isolates demonstrated their potential functional diversity, particularly in the production of plant-growth-promoting compounds. Thirteen isolates were found to synthesize both IAA and HCN (Table 2), indicating their potential dual role in promoting plant growth and suppressing soil-borne pathogens, as HCN is known for its antimicrobial activity (33). Interestingly, isolate KG02 (9) exhibited a unique biochemical profile, producing IAA exclusively. This suggests a specialized role for this isolate in enhancing root elongation and overall plant development through auxin-mediated mechanisms (34). The role of PGPR in agricultural sustainability for producing IAA was previously studied (9). PGPR directly promote plant growth by influencing plant hormonal balance and enhancing root system architecture which was supported with this study (35). By producing HCN, PGPR suppress the growth of plant pathogens by inhibiting their respiration, thereby indirectly contributing to improved plant growth and yield in a sustainable way (36). The capacity of these isolates to produce such metabolites highlights their potential as biofertilizers or biocontrol agents, supporting sustainable agricultural practices (9). Future studies should investigate the specific genes and regulatory pathways underlying these traits to better understand their ecological roles and application potential.

The phylogenetic analysis of the 27 isolates based on 16S rRNA gene sequences revealed distinct taxonomic affiliations, providing insights into the microbial diversity of the potato root soil samples. A significant proportion of isolates, including KG01 (4), KG01 (2) and KG02 (7), clustered within the genus Serratia, specifically grouping with *Serratia marcescens* or closely related species, showing that these bacteria are prevalent in Githabai and Landmark villages (Fig. 2). This clustering suggests a strong taxonomic similarity, which is further supported by robust bootstrap values (95-100), confirming the reliability of these phylogenetic relationships. Additionally, isolates KLm02 (1)) was closely related to species from the genus *Providencia*, while KG02 (2) and KG02 (5) were affiliated with *Alcaligenes faecalis*, a bacterium commonly found in diverse environmental niches. Remarkably, isolate KG02 (1) and KG02 (9) formed a distinct clade with *Paenibacillus xylanexedens* and *Bacillus aerius* respectively, indicating its unique taxonomic position. These results emphasize the considerable diversity of bacterial genera present in the soil, suggesting a variety of ecological functions and potential roles in soil health and plant interactions (37). The high sequence identity among the five isolates, with 100 % identity in their phylogenetic positions, underscores their close evolutionary relationship. Overall, these findings align with studies that highlight the complexity and functional diversity of microbial communities in soil environments (38).

Conclusion

The study revealed microbial diversity and functional potential in potato root soil samples from Nyandarua County's Kinangop sub-county. The isolates showed variations in colour, shape and size, with some showing distinct colours. They produced plant-growth-promoting compounds, suggesting potential as biofertilizers and biocontrol agents. The study emphasizes the importance of microbial diversity in soil ecosystems and sustainable agriculture. Future research should explore the genetic basis of these functional traits to further understand their ecological roles and enhance their practical use in agricultural practices.

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Authors' contributions

IP carried out the conceptualization, methodology format analysis, software, investigation, resources, data curation, writing an original draft preparation, visualization, project administration and funding acquisition. PJ carried out the methodology, validation, formal analysis, investigation, review and editing and project administration. AK carried out the conceptualization, methodology, validation, investigation, review and editing, supervision, project administration and funding acquisition. TD carried out the validation, investigation, review and editing, supervision, project administration and funding acquisition. LT carried out the methodology, validation, investigation, review and editing, supervision, project administration and funding acquisition.

Compliance with ethical standards

Conflict of interest: The author declares no conflicts of interest.

Ethical issues: None

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