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Exploring the Potential of Groundnut Rhizobia in Ethiopia: A Comprehensive Screening for Multiple Plant Growth Promoting Activities

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ABSTRACT

Rhizobia, known for their nitrogen-fixing abilities, have been explored for their potential plant growth-promoting (PGP) mechanisms beyond nitrogen fixation. This study aimed to identify efficient rhizobia isolates exhibiting multiple PGP traits. A total of 72 groundnut nodulating rhizobia isolates were screened in vitro for various PGP traits, including phosphate solubilization, production of indoleacetic acid (IAA), ammonia (NH3), hydrogen cyanide (HCN), different hydrolytic enzyme production, and antifungal activity. Results revealed that 72% of the isolates produced IAA, ranging from 7.4 to 78.8 µg.ml−1, while 23.6% of the isolates demonstrated tri-calcium phosphate solubilization capabilities. Although all isolates produced ammonia, two isolates exhibited particularly strong production. Only two isolates demonstrated the ability to produce hydrogen cyanide. Enzymatic production analysis indicated that 50% and 48% of the tested isolates displayed protease and cellulase activities, respectively, while chitin hydrolysis was not observed. Furthermore, only 13.8% of the isolates exhibited inhibitory effects against the test pathogen F. oxysporum, with GNR-07 displaying the highest inhibition potential (42.3%), followed by GNR-03 (28%) and GNR-28 (25.8%). Remarkably, all isolates (100%) exhibited multiple PGP properties, ranging from 3 to 7 out of 8 traits (37.5% - 87.5%). Isolates GNR-37 and GNR-28 demonstrated superior performance, exhibiting the highest number of PGP properties (87.5%), including antifungal activity. Additionally, isolates GNR-43, GNR-19, GNR-03, and GNR-07 displayed 75% of the tested PGP characters. These findings suggest that these isolates hold promising potential as plant growth-promoting rhizobial (PGPR) inoculants. However, further evaluation of their performance under greenhouse and field conditions is necessary.

Keywords: Rhizobia, plant growth-promoting traits, groundnut nodulating rhizobia, PGP properties, PGPR inoculant.

1. Introduction

Plant growth promoting rhizobacteria, constitute a diverse group of bacteria that inhabit the rhizosphere, the root surfaces, and establish associations with roots. These bacteria have the capability to directly and/or indirectly enhance plant growth by improving its extent and quality(MEENA et al., 2012; Moustaine et al., 2017). The genera of bacteria encompassed within PGPR include Acetobacter, Acinetobacter, Alcaligenes, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Derxia, Enterobacter, Gluconacetobacter, Herbaspirillum, Klebsiella, Ochrobactrum, Pantoae, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas, and Zoogloea(Jha & Saraf, 2015).

PGPR exhibit their ability to promote plant growth through both direct and indirect mechanisms. Direct mechanisms involve the utilization of specific bacterial traits that directly contribute to the enhancement of plant growth. These mechanisms encompass the production of ACC deaminase, auxin, gibberellin, cytokinin, phosphorous solubilization, nitrogen fixation, and iron sequestration through siderophore production. Additionally, certain PGPR possess the characteristic of inhibiting the functioning of plant pathogenic microorganisms, including both fungi and bacteria.

On the other hand, indirect mechanisms rely on the production of various substances and activities that indirectly influence plant growth. These mechanisms include the production of cell wall degrading enzymes, antibiotics, competition, induced systemic resistance, hydrogen cyanide, quorum quenching, and siderophores(Olanrewaju et al., 2017). It is worth mentioning that various studies conducted by researchers such asEl-Saadony et al. (2022) andNaz et al. (2022) have further demonstrated the effectiveness of PGPR in antagonizing phytopathogens through mechanisms like competition, antibiotic production, lytic enzyme production, and hydrogen cyanide release.. PGPR employ these mechanisms to antagonize phytopathogens. They compete for nutrients and space, produce antibiotics and lytic enzymes, such as chitinases, cellulases, proteases, and lipases, which cause the lysis and destruction of cell walls in pathogenic bacteria and fungi. This ability to synthesize these enzymes equips PGPR with biocontrol activity against a wide range of pathogenic fungi(Sathya et al., 2017).

Rhizobia, which establish symbiotic relationships with leguminous plants and form root nodules, are among the PGPR that exhibit numerous distinctive traits promoting plant growth in both leguminous and non-leguminous crops(Jaiswal et al., 2021; Knežević et al., 2022).These rhizobia

have the capacity to directly enhance plant growth through the production of various metabolites that possess plant growth-promoting properties. Furthermore, they contribute to biocontrol by producing different lytic enzymes(Gopalakrishnan et al., 2015).

According to reports, rhizobia that form nodules on groundnut plants possess plant growth-promoting (PGP) properties. These properties include the ability to solubilize inorganic phosphate(Afzal & Asad, 2019)produce phytohormones such as Indole Acetic Acid (IAA)(Kumar et al., 2014; Panigrahi et al., 2020)and produce siderophores(Vargas et al., 2017).

In a broader context, rhizobia found in root nodules that possess multiple plant growth-promoting (PGP) traits offer added advantages as inoculants for both legume and non-legume crops grown in rotation or simultaneously. While numerous studies have been conducted on the phenotypic and symbiotic characteristics of groundnut rhizobia under greenhouse and field conditions in Ethiopia, there has been limited exploration of their additional PGP properties(Degefu et al., 2018). Therefore, the purpose of this research is to isolate rhizobia strains that exhibit various PGP traits beyond nitrogen fixation. This investigation aims to identify local groundnut rhizobia and fully unlock their potential to enhance groundnut production within the country.

2. Material and Methods

2.1. *Source of rhizobial isolates*

For the PGP tests, a collection of 72 groundnut rhizobia isolates was utilized. These isolates were obtained from soil samples collected from the primary groundnut cultivation regions in Ethiopia, employing the groundnut variety known as Babile 1. To ensure accuracy and reliability, the isolates were authenticated on the Babile 1 variety (Asnake*et al*, 2024). It was observed that the majority of these isolates corresponded to slow-growing rhizobia, specifically identified as belonging to the Bradyrhizobium spp.

2.2. *Screening for plant growth promoting (PGP) characteristics of groundnut rhizobia*

2.2.1. Quantitative estimation of indole acetic acid (IAA) production

The ability of rhizobial isolates to produce indole acetic acid (IAA) was determined colorimetrically accordingly. The rhizobial cultures were grown at 28°C in YEM broth supplemented with filter sterilized L-tryptophan (2 g l-1) for 72 hrs on a shaker at 150 rpm, and

centrifuged at 6,000 rpm for 15 minutes at 4ºC. 2 ml of the supernatant was mixed with 4 ml of Salkowski reagent (1 ml of 0.5M FeCl3 solution in 50 ml of 35% perchloric acid) and the mixture was reserved at room temperature for 25 minutes in darkness. IAA production was confirmed due to the development of pink color. The intensity of pink color was read at 530 nm spectrophotometrically and the amount of IAA produced was extrapolated from the standard curve constructed from pure IAA (LobaChemie) in the range of 5 to 100 µg/ml. Non-inoculated L-tryptophan supplemented YEM broth medium was used as control(Md HoirulAzri, 2018).

2.2.2. Solubilization of inorganic phosphates

A loopful of fresh rhizobial culture (10µL; 10^8 cells mL^{-1}) was spot inoculated on Pikovskay's medium containing (in g l^{-1} of distilled water): glucose 10.0, yeast extract 0.5, KCl0.2, MgSO4.7H2O 0.1, $Ca_3(PO_4)_2$ 2 and agar 15.0 and incubated at 28-30°C for one week. Clear zone formation around the colonies was recorded as positive for inorganic phosphate solubilization. Phosphate solubilization index (the extent of phosphate solubilizing ability of bacterial isolates) was also determined (Paul & Sinha, 2017).

Phosphate Solubilization Index (SI) = B/A

Where; A = Colony diameter B = Total diameter (colony + halo zone)

2.2.3. Ammonia (NH3) production

The isolates were tested for ammonia production by inoculating a loopful of freshly grown cells in to 10 ml of pre-sterilized peptone water tubes and incubated at 28^0C for 3 days. The tubes were treated with 0.5 ml of Nessler's reagent (potassium iodide -50 g, saturated mercuric chloride -35 mL, distilled water -25 mL, potassium hydroxide (40%) -400 mL) to detect development of brown to yellow color as a positive test for ammonia production (Manasa*et al*., 2017).

2.2.4. In vitro antagonistic activity against test pathogenic fungus (Fusarium oxysporum)

The inhibitory effect of the rhizobia isolates against the pathogen, *Fusarium oxysporum*, was evaluated *in vitro* on YEMA plates using the dual culture technique as described by Dinesh *et al*. (2015). A loopful (10µL; 10^8cells mL-1) of each rhizobial culture was spot-inoculated on YEMA

plates amended with 0.5% sucrose at a distance of 2.0 cm from the center at four equidistant points and incubated at 28ºC for 5 days. Then, five days old mycelial discs (4 mm diameter) of *Fusarium oxyporum*was positioned at the center of the Petri dishes and incubated at the same temperature until the test pathogen in the control plates (plates without rhizobia isolate) reached the edges of the plates. The radial growth of fungal mycelium and the inhibition percentage was compared with control using the formula, I = [C − T/C] × 100, where I is the percent inhibition and C and T are the pathogen radial growth in control and treatment, respectively.

2.2.5. Test for the production of different lytic enzymes

Cellulase production activity:

Isolates were tested for their ability to produce cellulase according to the method. A loopful of broth culture (10µL; 10^8 cells mL-1) of each isolate was streaked on a cellulose agar media containing composition of KH_2PO4 0.5 g, $MgSO_4$ 0.25 g, cellulose 2 g, agar 15 g, and gelatin 2 g; distilled water one liter and at pH 6.8–7.2. The plates were incubated at 28^0C for 72 hours and flooded with Gram's iodine for 5 minutes to detect clear zone formation around colonies(Dar et al., 2015).

Protease production activity:

Protease activity was assayed with YEM agar containing 5% skimmed milk. (Mohamad et al., 2018). After incubation at 28^0C for 5–7 days, formation of clear halo zone around the bacterial colonies was positive reaction for milk casein hydrolysis.

Production of chitinase:

Chitinase activity of the isolates was tested on chitin agar plates constituting (g l-1) colloidal chitin (4), MgSO4.7H2O (0.5), K2HPO4 (0.7), KH2PO4 (0.3), FeSO4.7H2O (0.01), MnCl2 (0.001), NaCl (0.3), yeast extract (0.2) and agar (20).After adding iodine the development of clear zone around the colony wasreflected as positive for the enzyme chitinase production (Madison et al., 2017).

2.2.6. Production of hydrogen cyanide (HCN)

The rhizobial isolates (100 µL; 10^8 cells mL-1) were inoculated into YEMA plates supplemented with 4.4 g L-1 of glycine to detect HCN production.Stripes of filter paper (Whatman filter paper No.1) were soaked in the yellow picric acid solution (2.5 g of picric acid and 12.5 g of Na_2CO_3

dissolved in 1L of distilled water) and fixed to the underside of the upper lids and sealed with parafilm (to avoid the escaping of volatiles like HCN) and incubated at 28^{o}C for 5 days. A color change of the yellow filter paper to brown was recorded as positive for HCN production(Mir et al., 2021)..

3. Results

3.1. Indole acetic acid (IAA) production

The groundnut rhizobia isolates exhibited significant variations in their ability to produce Indole Acetic Acid (IAA) when cultured in tryptophan-supplemented YEM broth media. Out of the 72 isolates tested, 52 isolates (72%) demonstrated detectable levels of IAA production, ranging from 7.4 to 78.8 µg.ml−1. However, the remaining 20 isolates had IAA levels below the detection limit. Notably, isolate GNR-43 displayed the highest IAA production at 78.8 µg.ml-1, followed by isolates GNR-37 (77.2 µg.ml-1), GNR-28 (67.8 µg.ml-1), GNR-34 (66.8 µg.ml-1), GNR-03 (65.8 µg.ml-1), GNR-07 (58.8 µg.ml-1), and GNR-54 (56.8 µg.ml-1) (Table 1). These findings highlight the considerable variability in IAA production among the groundnut rhizobia isolates.

Fig.1. IAA production of tested isolates

Table .1. IAA production of groundnut rhizobia collected from different parts of Ethiopia

Isolates	Sampling site	IAA produced (µg.ml−1)	Isolates	Sampling site	IAA produced (µg.ml−1)
GNR04	Babile(fite)	23.5	GNR38	Babile(ARC)	11.6
GNR01	Babile(tula)	11.8	GNR40	Babile (Tula)	7.4
GNR03	Babile(ARC)	65.8	GNR41	Berhet 01	17.5
GNR09	Pawe(M6)	25.6	GNR42	Babile(ARC)	42.4
GNR05	Babile(tula)	8	GNR43	Berhet 01	78.8
GNR08	Pawe(PARC)	32.5	GNR44	Babile(ARC)	33.4
GNR06	Pawe(ARC)	15.2	GNR45	Berhet	19
GNR02	Pawe(M6)	36	GNR46	Babile (Tula)	7
GNR07	Pawe(M6)	58.8	GNR47	Pawe(ARC)	33.7
GNR15	Pawe(ARC)	23.4	GNR49	Babile(ARC)	18.5
GNR16	Babile(tula)	14.6	GNR51	Berhet	29.8
GNR18	Pawe(M6)	21.4	GNR52	Babile(ARC)	30.1
GNR19	Pawe(ARC)	76	GNR53	Babile(ARC)	22
GNR20	Babile	11.2	GNR54	Babile (Tula)	56.8
GNR21	Babile(fite)	9.5	GNR57	Babile (Tula)	13.4
GNR22	Babile(ARC)	15	GNR58	Babile (Tula)	34
GNR24	Babile(fite)	32.2	GNR59	Babile(ARC)	27.5
GNR25	Pawe(ARC)	24.4	GNR61	Pawe(ARC)	39.2
GNR28	Berhet	67.8	GNR63	Pawe(M6)	21.5
GNR29	Pawe(M6)	32.4	GNR64	Pawe(ARC)	20.8
GNR30	Berhet 01	23	GNR65	Pawe(ARC)	9.6
GNR31	Babile(fite)	16.5	GNR67	Berhet 01	41.2
GNR32	Babile(fite)	26.8	GNR68	Pawe(ARC)	7.4
GNR34	Berhet 01	66.8	GNR69	Pawe(M6)	8.1
GNR36	Babile(Tula)	28	GNR71	Pawe(ARC)	34.8
GNR37	Babile(Tula)	77.2	GNR72	Berhet 01	20.6

3.2. *Phosphate solubilizing ability of isolates*

Phosphate-solubilizing bacteria were identified based on their ability to form a clear zone around the colonies after 48 hours of incubation on Pikovskaya's agar plates following spot inoculation. These clear zones indicated positive phosphate solubilization tests. Out of the 72-rhizobial isolates tested, seventeen isolates (23.6%) demonstrated the capability to solubilize tri-calcium phosphate. The diameter of the halo-zones surrounding each colony varied among these seventeen phosphate-solubilizing bacteria, indicating differences in their phosphate solubilization capacity (Table 2). The clear zone diameters ranged from 1 mm to 3 mm, with isolates GNR-67 and GNR-28 displaying the largest and smallest clear zones, respectively. Additionally, the phosphate solubilization indices formed by these isolates varied between 1.7 and 2.45, indicating variations in their effectiveness in solubilizing phosphate.

Fig.2. Clear zones of phosphate solublization on Pikovskaya’s agar plate

3.3. *Ammonia (NH3) production*

Screening ofthe rhizobial isolates for ammonia production were, an important trait of PGPR that indirectly influences the plant growth and performance. Of all the tested Rhizobium isolates, two isolates were (GNR-28, GNR37) exhibited as strong (+++) ammonia producer, six isolates (GNR-3.GNR-7, GNR-19, GNR-34, GNR-43 and GNR-54) were produce moderately (++) .Whereas the rest isolates were scored as weak (+) for Ammonia production.

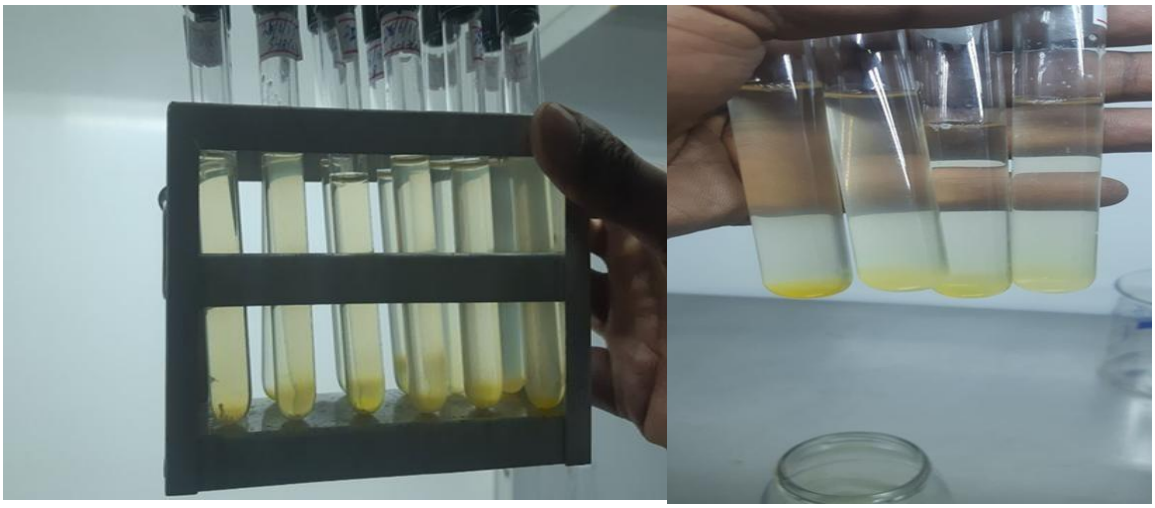

Fig 3.Test for ammonia production of rhizobial isolates

3.4. Antagonistic activity of *Rhizobium* against fungal pathogen

In vitro antagonistic potential of the *Rhizobium* isolates were tested against *Fusarium oxysporum*in dual culture under *in vitro* conditions and the percentage of inhibition was recorded. After five days of incubation, inhibition zone was clearly visible. Hence, ten isolates were found to be inhibitory against fungal strain; yet, maximum inhibition potential was exhibited by the rhizobial isolates GNR-07 followed by GNR-03 and GNR-28 with growth inhibition of 42.3%, 28%and 25.8% respectively against *Fusarium oxysporum*(Table 2.).

3.5. *Production of different lytic enzymes*

Screening of bacterial isolates for lytic enzymes production was done. Lytic enzymes includes Protease, cellulase and chitinase positive isolates showed distinct, clear, and prominent zones of clearance around the colonies showing lytic enzymes production.In this study, of the tested isolates, 35 and 36 isolates showed positive for cellulase andprotease activity, respectively. The

majority (90%) of the isolates that produce cellulase enzyme also produce protease enzyme. However, all the tested isolates were negative for chitinase activity (data not shown).

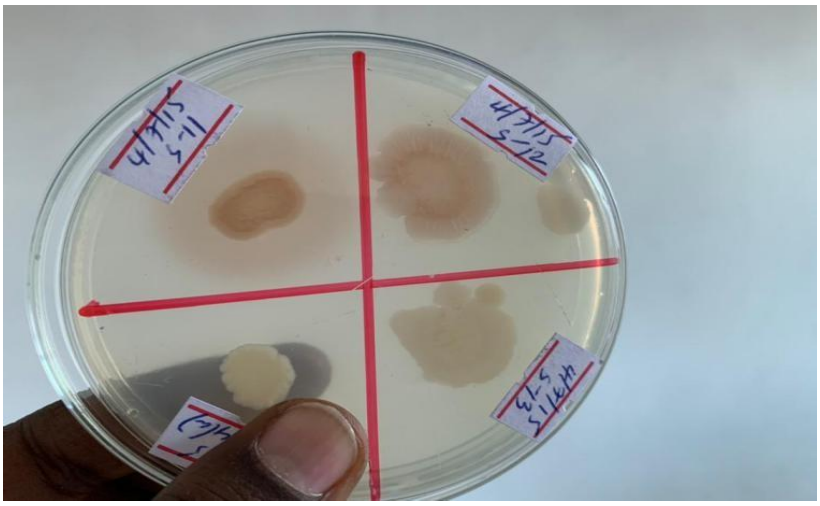

Fig 4. Protease enzyme production by rhizobial bacteria on SMA media

3.6. *Production of Hydrogen Cyanide (HCN)*

HCN production is ascribed as one of the mechanisms of biocontrol activity of rhizobia, the ability of the 72 isolates to produce HCN was determined by the picric acid assay. Only two *Rhizobium* cultures, were produced HCN scored as moderate (++) for GNR-37 and weak for GNR-28 whereas the remaining70 isolates not changed the yellow color of the picric acid solution treated filter paper, implying majority of the groundnut isolate were failed to produce HCN (Table 2).

Fig 5. Plate based qualitative assays showing HCN production

Table 2. Summary of Multiple Plant Growth Promoting traits of groundnut rhizobial isolates

Isolate	IAA produced	Phosphate solublization index	Ammonia Production	Protease	Cellulase	Chitinase	Antifungal activity	HCN	%PGP
GNR03	65.8	2.1	++	+	+	−	28	−	75
GNR05	8	NS	+	+	+	−	0	−	50
GNR08	32.5	2.21	+	−	+	−	12.3	−	40
GNR02	36	NS	+	+	−	−	0	−	37.5
GNR12	0	NS	+	+	+	−	0	−	37.5
GNR07	58.8	2.34	++	+	+	−	42.3	−	75
GNR15	23.4	NS	+	+	−	−	0	−	37.5
GNR17	0	NS	+	+	+	−	0	−	37.5
GNR19	76	2.42	++	+	+	−	22.5	−	75
GNR20	11.2	2.1	+	+	+	−	0	−	62.5
GNR24	32.2	2.24	+	+	+	−	0	−	62.5
GNR25	24.4	1.8	+	−	−	−	0	−	37.5
GNR26	0	NS	+	+	+	−	0	−	37.5
GNR27	0	NS	+	−	+	−	0	−	37.5
GNR28	67.8	2.45	+++	+	+	−	25.8	+	87.5
GNR29	32.4	2.33	+	+	−	−	0	−	50
GNR31	16.5	NS	+	−	+	−	0	−	37.5
GNR35	0	NS	+	+	+	−	12.3	−	50
GNR34	66.8	2.41	++	+	+	−	0	−	62.5
GNR36	28	NS	+	+	−	−	0	−	37.5
GNR37	77.2	2.4	+++	+	+	−	25	++	87.5
GNR38	11.6	NS	+	−	+	−	0	−	37.5
GNR42	42.4	1.8	+	+	−	−	0	−	50
GNR43	78.8	2	++	+	+	−	12.5	−	75
GNR44	33.4	NS	+	+	+	−	0	−	50
GNR46	7	NS	+	−	−	−	15.5	−	37.5
GNR47	33.7	2.22	+	−	+	−	0	−	50
GNR49	18.5	NS	+	+	+	−	0	−	50
GNR50	0	NS	+	+	+	−	0	−	37.5
GNR51	29.8	NS	+	−	+	−	0	−	37.5
GNR53	22	NS	+	+	−	−	0	−	37.5
GNR54	56.8	1.8	++	+	+	−	26.7	−	75
GNR55	0	NS	+	+	+	−	0	−	37.5
GNR58	34	2.31	+	−	+	−	0	−	50
GNR60	0	NS	+	+	+	−	0	−	37.5
GNR61	39.2	NS	+	+	−	−	0	−	37.5
GNR64	20.8	NS	+	+	+	−	0	−	50
GNR65	9.6	NS	+	−	+	−	0	−	37.5
GNR67	41.2	1.7	+	+	−	−	0	−	50
GNR68	7.4	NS	+	+	+	−	0	−	50
GNR71	34.8	1.74	+	+	+	−	0	−	62.5

4. Discusion

Out of the tested rhizobial isolates, 17 isolates (23.6%) were found to produce a halo zone around their colonies after 24 hours of incubation on Pikovskaya's agar medium. The size of the halo zone gradually increased up to 72 hours. The solubilization efficiency (SE) of the Rhizobium strains on solid media ranged from 71% to 150%. Among the isolates, GNR-28 exhibited the highest solubilization efficiency, followed by GNR-19 and GNR-34.In a study conducted by Muhammad Adnan (2016), it was observed that 21% of the tested rhizobia were phosphate-solubilizing bacteria, with a solubilization efficiency ranging from 38% to 270%. Similarly, the phosphate-solubilizing microorganisms Bacillus subtilis and Bacillus cereus, which were isolated by Maheshwar and Sathiyavani (2012) from the groundnut rhizosphere soil, demonstrated a significant phosphate-solubilizing zone.

The isolates in the current study varied in their intrinsic ability to produce IAA as the production varied under the same condition. Hence, 72% of the tested isolates produced a detectable amount of IAA, which ranged from 7.4 to 78.8 $\mu g.ml^{-1}$. The biosynthesis of IAA is widespread among plant-associated bacteria (Ulrich et al., 2021).Several studies also showed that many soybean rhizobia produced IAA irrespective of the type of rhizobia nodulating groundnut host varieties (Dlamini et al., 2021; Ibny et al., 2019). Ibny et al. (2019) working with 89 rhizobial strains and found that 39 % of the strains produce IAA. The IAA produced by some of the isolates in the present study was higher than previously reported for both fast and slow growing groundnut rhizobia in pakistan (0.02 to 69.71 μg.ml−1) Ibny et al.(2019) and lower than which has been reported from Ghana (56 to 290 $\mu g\ ml^{-1}$) (Khalid et al., 2020). The roduction of IAA by PGPR can be differ with in species and strains, and influenced by culture conditions, substrate availability and growth stage (Ashrafuzzaman et al., 2009; Kumar et al., 2012; Ngoma et al., 2013).

Another important trait of rhizobia is the production of ammonia that indirectly influences the plant growth. The plants can take up ammonia produced by the rhizobia as a source of nitrogen for their growth. The ammonia production results of the test isolates showed varied outcome (table 2). All isolates (100%) were able to produce ammonia. Among these, two isolates were exhibited as strong ammonia producer, six isolates were produce moderately the remaining isolates were scored as weak ammonia producer. SimillarlyAjilogba et al. (2022) works Shows that, even if all the tested isolates were positive for NH3 production, two of them were recorded

as moderate and the rest as weak ammonia producers. In general, having such rhizobia characteristics suggests that it is vital to select a nitrogen fixer with ammonia production in a bio-fertilizer consortium for agriculture practices.

To check the efficacy of antagonism of selected rhizobial isolates against soil borne fungal isolates (*Fusarium oxysporum*) infecting groundnut plant, dual culture method was adapted and the percentage of inhibition of growth was recorded (Table 2). As a result of the plate assay, some rhizobial isolates curtailed the growth of pathogenic fungi tested and were found to be highly inhibitory to *Fusarium oxysporum*, whereas other strains showed only nominal antifungal activity. Rhizobial Strains GNR-07, GNR-03 and GNR-28 suppressed the growth of tested fungi at higher percentage when compared to other strains tested. Similar results were observed by Antoun et al. (1998), who found that 49 of his rhizobial strains inhibited the growth of *Fusarium oxysporum*.,.A significant reduction in damping -off or wilt disease of groundnut plant could be achieved by inoculating the strains that have antifungal ability and capable to reduce the percentage of crop loss. Such biocontrol agents have also been reported to produce toxic metabolites, enzymes or volatile compounds that have inhibitory effects on soil-borne pathogens.

Hydrolytic enzymes act as agents for prevention of plant diseases by causing lysis of pathogenic microbes in the close vicinity of the plant as they secrete increased level of cell wall lytic enzymes (Protease,cellulose and chitinase) (Neeraja et al., 2010). In this study, 50 % isolates were positive for protease production and 48 % isolates were positive for cellulose enzyme production. Even if there is no work on groundnut rhizobia in production of different lytic enzymes in Ethiopia, similar result reported by Diriba (2017) that indicate 33% and 38% of soybean rhizobia isolated from Ethiopian soils showed protease and cellulase activity, respectively whereas, none of them utilized chitin. PGPR that able to produce these lytic enzymes are expected to have biocontrolproperty against a wide range of fungi and bacteria that are potentially pathogenic for the plant and led the crop toenhance crop yield.

Bacterial isolates for their production ability of hydrogen cyanide (HCN) was also screened. Of all the tested *Rhizobium* isolates (72) for Hydrogen Cyanide, only two isolates showed moderate and weak production (Table 2). Irrespective of the types of host plants, out of 22-tested soybean isolates which isolated from Ethiopia soil only one isolate produced HCN (Diriba et al., 2017). Earlier, Antoun*et al*. (1998) had also reported that only 3% of rhizobial strains from different genera and species were found to produce HCN, implying the rare occurrence of HCN

production among rhizobia species, indicating that PGP rhizobia are relatively inefficient in theproduction HCN (Datta *et al*., 2015). In general, presence or absence and intensity of Hydrogen Cyanide production can play a significant role in antagonistic potential of bacteria against phytopathogens.

5. Conclusion

The current study paves the way for the selection of ideal Rhizobium strains that possess multiple plant growth-promoting properties (PGPR) and confirmed antagonistic activity, thereby contributing to consistent growth and yield improvements in groundnut. The selection of appropriate rhizobial inoculants plays a crucial role in enhancing nitrogen fixation and food production. The focus of this study was to screen and identify rhizobial isolates with multiple PGPR traits, isolated from the major groundnut growing areas in the country, and utilize them as biofertilizers to stimulate groundnut growth.The tested Rhizobium isolates were evaluated for various PGPR traits, including indole-3-acetic acid (IAA) production, phosphate-solubilizing ability (PSB), ammonia production, hydrogen cyanide (HCN) production, lytic enzyme production (protease, cellulose, and chitinase), as well as antagonistic activity under in vitro conditions. Among the tested isolates, GNR-37 and GNR-28 demonstrated an efficiency of 85%, followed by GNR-43, GNR-19, and GNR-XX, which exhibited a 75% efficiency across all the tested traits. Consequently, isolates displaying multiple desirable traits hold a selective advantage for practical applications, such as formulating effective inoculants for groundnut cultivation.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
BA conducted the main experiments, designed the work, and wrote themanuscript.
AF analyzed the data and contributed to the part ofexperimental designs.
MD participated in some experiments analyzing the data.
LG participated in some experiments.
All authors read and approved the final manuscript

6. References

Afzal, A., & Asad, S. A. (2019). Microbial Applications for Sustainable Agriculture. *Innovations in Sustainable Agriculture*, 43-77.

Ajilogba, C. F., Babalola, O. O., Adebola, P., & Adeleke, R. (2022). Bambara Groundnut Rhizobacteria Antimicrobial and Biofertilization Potential. *Frontiers in Plant Science*, *13*.

Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R., & Lalande, R. (1998). Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (Raphanus sativus L.). *Molecular microbial ecology of the soil: results from an FAO/IAEA co-ordinated research programme, 1992–1996*, 57-67.

Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hoque, A., Islam, M. Z., Shahidullah, S., & Meon, S. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, *8*(7).

Dar, M. A., Pawar, K. D., Jadhav, J. P., & Pandit, R. S. (2015). Isolation of cellulolytic bacteria from the gastro-intestinal tract of Achatina fulica (Gastropoda: Pulmonata) and their evaluation for cellulose biodegradation. *International Biodeterioration & Biodegradation*, *98*, 73-80.

Degefu, T., Wolde-meskel, E., Ataro, Z., Fikre, A., Amede, T., & Ojiewo, C. (2018). Groundnut (Arachis hypogaea L.) and cowpea (Vigna unguiculata L. Walp) growing in Ethiopia are nodulated by diverse rhizobia. *African Journal of Microbiology Research*, *12*(9), 200-

217.

Dlamini, S. T., Jaiswal, S. K., Mohammed, M., & Dakora, F. D. (2021). Studies of phylogeny, symbiotic functioning and ecological traits of indigenous microsymbionts nodulating Bambara groundnut (Vigna subterranea L. Verdc) in Eswatini. *Microbial ecology*, 1-16.

El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., El-Tahan, A. M., Ebrahim, A. A., Abd El-Mageed, T. A., & Negm, S. H. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Frontiers in plant science*, *13*, 923880.

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L., & Krishnamurthy, L. (2015). Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech*, *5*, 355-377.

Ibny, F. Y., Jaiswal, S. K., Mohammed, M., & Dakora, F. D. (2019). Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (Vigna subterranea L. Verdc.) in Africa and their relationship with phylogeny. *Scientific reports*, *9*(1), 1- 17.

Jaiswal, S. K., Mohammed, M., Ibny, F. Y., & Dakora, F. D. (2021). Rhizobia as a source of plant growth- promoting molecules: Potential applications and possible operational mechanisms. *Frontiers in Sustainable Food Systems*, *4*, 619676.

Jha, C. K., & Saraf, M. (2015). Plant growth promoting rhizobacteria (PGPR): a review. *Journal of Agricultural Research and Development*, *5*(2), 108-119.

Khalid, R., Zhang, X. X., Hayat, R., & Ahmed, M. (2020). Molecular characteristics of rhizobia isolated from Arachis hypogaea grown under stress environment. *Sustainability*, *12*(15), 6259.

Knežević, M., Buntić, A., Delić, D., & Stajković-Srbinović, O. (2022). Root Nodule Bacteria-Rhizobia: Exploring the Beneficial Effects on Non-legume Plant Growth. In *Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes* (pp. 129-168). Springer.

Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., & Negi, S. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent research in science and technology*, *4*(1), 1-5.

Kumar, V., Kayasth, M., Chaudhary, V., & Gera, R. (2014). Diversity of diazotrophs in arid and semi-arid regions of Haryana and evaluation of their plant growth promoting potential on Bt-cotton and pearl millet. *Annals of microbiology*, *64*, 1301-1313.

Madison, J. D., Berg, E. A., Abarca, J. G., Whitfield, S. M., Gorbatenko, O., Pinto, A., & Kerby, J. L. (2017). Characterization of Batrachochytrium dendrobatidis inhibiting bacteria from amphibian populations in Costa Rica. *Frontiers in microbiology*, *8*, 290.

MEENA, B., ALTAF, M. M., & AHMAD, I. (2012). Functional diversity of indigenous plant growth promoting rhizobacteria (PGPR) isolated from Aligarh soils. *Advances in Life Sciences*, *1*, 58-61.

Mir, M. I., Kumar, B. K., Gopalakrishnan, S., Vadlamudi, S., & Hameeda, B. (2021). Characterization of rhizobia isolated from leguminous plants and their impact on the growth of ICCV 2 variety of chickpea (Cicer arietinum L.). *Heliyon*, *7*(11), e08321.

Mohamad, O. A., Li, L., Ma, J.-B., Hatab, S., Xu, L., Guo, J.-W., Rasulov, B. A., Liu, Y.-H., Hedlund, B. P., & Li, W.-J. (2018). Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by Bacillus atrophaeus against Verticillium dahliae. *Frontiers in microbiology*, *9*, 924.

Moustaine, M., Elkahkahi, R., Benbouazza, A., Benkirane, R., & Achbani, E. (2017). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (Solanum Lycopersicum L.) and characterization for direct PGP abilities in Morocco. *International Journal of Environment, Agriculture and Biotechnology*, *2*(2), 238708.

Naz, R., Khushhal, S., Asif, T., Mubeen, S., Saranraj, P., & Sayyed, R. (2022). Inhibition of Bacterial and Fungal Phytopathogens Through Volatile Organic Compounds Produced by Pseudomonas sp. In *Secondary Metabolites and Volatiles of PGPR in Plant-Growth Promotion* (pp. 95-118). Springer.

Neeraja, C., Anil, K., Purushotham, P., Suma, K., Sarma, P., Moerschbacher, B. M., & Podile, A. R. (2010). Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. *Critical reviews in biotechnology*, *30*(3), 231-241.

Ngoma, L., Esau, B., & Babalola, O. O. (2013). Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *African Journal of Biotechnology*, *12*(26).

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, *33*, 1-16.

Panigrahi, S., Mohanty, S., & Rath, C. (2020). Characterization of endophytic bacteria Enterobacter cloacae MG00145 isolated from Ocimum sanctum with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. *South African Journal of Botany*, *134*, 17-26.

Paul, D., & Sinha, S. N. (2017). Isolation and characterization of phosphate solubilizing bacterium Pseudomonas aeruginosa KUPSB12 with antibacterial potential from river Ganga, India. *Annals of Agrarian Science*, *15*(1), 130-136.

Sathya, A., Vijayabharathi, R., & Gopalakrishnan, S. (2017). Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech*, *7*, 1-10.

Ulrich, K., Kube, M., Becker, R., Schneck, V., & Ulrich, A. (2021). Genomic analysis of the endophytic Stenotrophomonas strain 169 reveals features related to plant-growth promotion and stress tolerance. *Frontiers in microbiology*, *12*, 687463.

Vargas, L. K., Volpiano, C. G., Lisboa, B. B., Giongo, A., Beneduzi, A., & Passaglia, L. M. P. (2017). *Potential of rhizobia as plant growth-promoting rhizobacteria*. Springer.