Characterization of Plant Growth promoting Rhizobacteria isolated from Chickpea (*Cicer arietinum* L.) producing areas in Central and South Gondar zones, Ethiopia

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Abstract

The main objective of this work was to isolate and characterize the plant growth promoting abilities of chickpea rhizobacteria from highly chickpea growing areas of central and south Gondar zones. Soil samples were collected from 89 farmer fields and serial dilutions were carried out to isolate rhizobacteria. A total of 149 chickpea rhizobacteria isolates were isolated. From the total isolates, 48(32%) were phosphate solubilizers with solubilization indices ranging from 1.49 to 3.49. Most of the isolates (87.5%) produced indole acetic acid ranging from 11.56 µg ml^{-1} to 54.53 µg ml^{-1}. Screening for in vitro antifungal activities against F.oxysporum f.sp.ciceris showed that 18 (38%) isolates inhibited growth. Fourteen isolates (29%) produced siderophore, 21 (44%) and 47 (98%) isolates produced HCN and ammonia respectively.

31 (65%), 29 (60%) and 30 (63%) isolates were positive for protease, cellulase and chitinase production respectively. Four isolates GUCRB 4, 21, 76 and 124 were selected for 16S rRNA gene sequence and identified as Alcaligenes sp., Enterobacter mori, Serratia marcescens and Brevibacillus brevis respectively. The potential isolates are recommended for field trials and then they can be used as inoculants to improve the productivity of chickpea.

Keywords: Chickpea, Phosphate solubilization, 16S rRNA gene sequencing.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major food legume crops grown in the tropics, subtropics and temperate regions of the world. In Africa, Ethiopia is the first in chickpea production and production area coverage[14]. Chickpea seeds contain 5% fat, 23% protein, 47% starch, 64% carbohydrates, 7mg/100g iron, 3mg/100g zinc and 140mg/g calcium[40]. Also, chickpea maintains soil fertility through biological nitrogen fixation[10]. In Ethiopia, chickpea has an important role in the diet of small-scale farmers. It serves as a source of income; its straw is used for animal feed[49]. However, the average productivity of chickpea is 2 tons per ha^{-1} (CSA)[11] which is below its potential. Estimates suggest chickpea could generate up to 6 tons per ha^{-1} [13]. The major factors that are responsible for the low productivity of chickpea in Ethiopia include: limited availability and weak adaption of high yielding improved cultivars, damage by pests, failure to follow the best agronomic management practices, nutrient imbalance and insufficient availability of effective indigenous bacterial strains of chickpea[4].

The most important PGPR belongs to the genera *Azospirillum*, *Azotobacter*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia*[45]. In Ethiopia, PGPR has been isolated from crops like lentil[27], faba bean[21,26], soybean[5,48], lupin[43], teff[51] and coffee[31]. These studies showed that diverse rhizobacteria with multiple PGP properties colonize the rhizosphere and significantly improve plant health and productivity under greenhouse conditions. However, there is no sufficient information on chickpea PGPR isolated from Ethiopian soils except a research conducted on isolation and characterization of phosphate solubilizing bacteria by Midekssa et al[28]. In addition to PSB characterization, there is a need to isolate and characterize PGPR as having multiple plant growth promoting traits from chickpea rhizosphere in different parts of the country. Hence, this study aimed to identify and characterize PGPR from the chickpea rhizosphere to boost the growth and productivity of chickpea on a sustainable basis.

Material and Methods

Study Sites and Soil Sample Collection: Soil samples were collected from the rhizosphere of chickpea grown farmer fields in 8 districts of central and south Gondar zones. From central Gondar zone, 5 districts (East and West Dembya, West Belesa, Wogera and Gondar Zuria) and 3 districts from south Gondar zone (Ebnat, Fogera and East Estie) (Figure 1) were included for study. About 1kg of soil samples were excavated and collected in sterile polythene plastic bags and brought to the Injibara University Microbiology Laboratory for further work.

Rhizobacteria isolation: Ten grams of soil were suspended in 90 ml sterile normal saline to create a 10-fold serial dilution. The rhizobacteria were isolated by spreading 0.1 mL of each dilution on Nutrient and King's B agar plates, subsequently purified and preserved at 4^{o}C on nutrient agar slants[36].

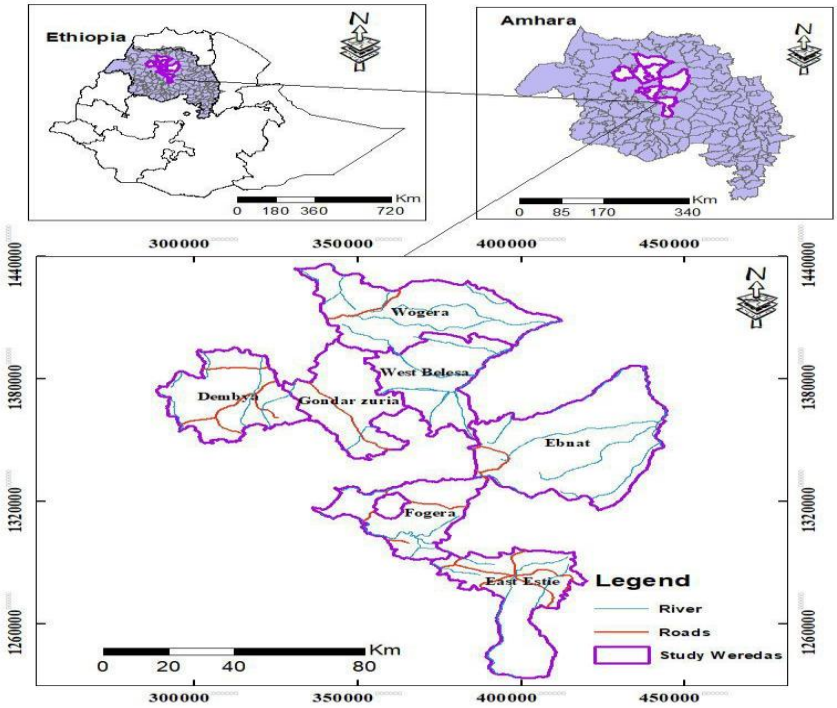

Figure 1: Map of the study area

Gram reaction: The isolates were tested for Gram reaction using the KOH method[7].

Phosphate solubilization: The qualitative phosphate solubilizing efficiency of the isolates was determined on the Pikovskaya agar media[12]. Twenty µl of each isolate was spot inoculated on PK agar plates and incubated at 30°C for 3-9 days. Their solubilization index (SI) was computed by using the formula:

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

IAA production: The isolates were tested for the production of indole acetic acid (IAA) according to Acuña et al[1]. They were inoculated on a nutrient broth medium supplemented with 2mg/mL of L-tryptophan and incubated at 30°C for three days. Each culture was centrifuged at 3,000 rpm for 30 min from which 1mL of the supernatant was mixed with 2 mL of Salkowski reagent. IAA was quantified by comparing the absorbance at 530 nm with a standard curve made from known concentrations of 5, 10, 20, 50, 80 and 100 µg ml^{-1} of IAA using a spectrophotometer.

Siderophore production: The production of siderophore by the bacterial isolates was assayed on the Chrome Azurol S (CAS) agar medium as described by Schwyn and Neilands[41]. The development of an orange-yellow halo around the growth was considered a positive siderophore producing organism.

Production of hydrogen cyanide: The isolates were streaked on a nutrient agar plate amended with 4.4 g/l glycine. Pieces of Whatmann number 1 filter paper were soaked with 0.5% picric acid and 2% sodium carbonate and placed on the undersides of the lid of each plate. The plates were wrapped with parafilm and incubated at 30°C for 4 days. The change from the orange to red color of the filter paper was considered a positive result for the production of HCN[9].

Cellulase production: Cellulase production was tested on carboxymethyl cellulose (CMC) agar plates as described by Kasana et al[19].

Chitinase production: Chitinase production was determined by growing the isolates on colloidal chitin agar (CCA) plates using the method described by Renwick et al[37]. Bacterial cultures were spot inoculated and incubated at 30°C for 3–5 days. The appearance of a clear zone around the colony indicated the production of chitinase enzyme.

Ammonia production: Each isolate was tested for ammonia production in peptone water and incubated for 72h at 30°C. After incubation, 0.5 ml of Nessler's reagent was added. The development of brown to yellow color was considered positive for ammonia production[8].

Protease production: The isolates were tested for protease production on skim milk agar according to Smibert and Krieg[44]. Clear zones around the colonies indicated protease activity.

***In vitro* Antagonistic activity:** The antagonistic properties of the rhizobacteria against the mycelial growth of *Fusarium oxysporum f.sp.ciceri* of chickpea pathogen (obtained from Debrezeit Center, Ethiopian Institute of Agricultural Research, EIAR) were tested by using the dual plate

technique as described by Landa et al[25]. Ten µl of each isolate inoculum were equidistantly streaked on the margins of nutrient agar plates amended with sucrose (0.5%) and incubated at 30°C for 24 h. A 4 to 5 mm diameter agar disc from PDA cultures of the fungal pathogens was placed at the center of the nutrient agar plate for each bacterial isolate and incubated at 30°C for 5-7 days. Percentage inhibition of radial growth (PIRG) was calculated as:

$[(C-T)/C] \times 100$

where C is the radial growth of fungus on control plates and T is the radial growth of the fungus in the dual culture.

Molecular identification of selected isolates: Genomic DNA was extracted from the isolates that showed the best performance in all plant growth promoting properties. Each isolate was grown in a nutrient broth for 24 hours and cells were harvested by centrifugation. Genomic DNA was isolated from the bacterial cultures using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. The quantity of genomic DNA extracted was determined by measuring the absorbance at 260 nm using Thermo Scientific NanoDrop (NanoDrop 2000). The quality and suitability of the DNA PCR reaction were verified on a 1% agarose gel. Universal primers 27F (5'-AGAGTTTGATCCTGGCT CAG-3') as a forward and 1492R (5'-GGTTACCTTGTT GTTACGACTT-3') as a reverse were used for the amplification of 16S rRNA gene[29].

The PCR reaction was as follows: Denaturation at 94 ° C for 4 min, primer annealing at 56°C for 1 min, primer extension at 72°C for 1 min and final extension at 8 min. Finally, the PCR product was visualized by electrophoresis on 1% agarose gel. Then, the 16S partial rRNA amplicons were sent to Macrogen, Netherlands, for sequencing. The quality of the sequence was checked and edited using BioEdit 2013. The partial 16S rRNA gene sequence of isolates was subjected to BLASTn search against the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification. The phylogenetic tree was constructed by the Neighbour-Joining method using molecular evolutionary genetics analysis (MEGA 11) after multiple sequence alignments[47].

Data analysis: The result of phosphate solubilization and IAA production was analyzed using One-way ANOVA and compared and contrasted following Duncan's multiple range test using SPSS version 25. Values were considered significant at $p \leq 0.05$.

Results and Discussion

In this study, a total of 149 chickpea rhizobacteria isolates were isolated and they were dominated by Gram-negative rhizobacteria which accounted for 62% of the isolates, indicating that they represent a majority rather than a minority among PGPR in the study rhizosphere. The rhizospheres of many agriculturally important crops favor more Gram-negative rhizobacteria than Gram-positive[28,30,32].

Phosphate solubilization: Among the rhizobacterial isolates, 48 (32%) were phosphate solubilizers with solubilization indices ranging from 1.49 to 3.49 (Table 1). Rhizobacteria isolates were screened from the rhizosphere of lentil, chickpea and grass pea and produced solubilization indices of 1.34–2.25[27], 1.44–3.06[28], 2–4.81[32] respectively. This research finding revealed the presence of rhizobacteria with the potential of solubilizing inorganic phosphate which can be applied as low-cost microbial inputs to enhance the growth and productivity of chickpea.

IAA production: Most isolates (87.5%) produced IAA (Table 1). Routray and Khanna[38] reported that 26.8% and 25% of the isolates isolated from the rhizospheric soils of lentil and mung bean produced IAA. The isolates in this study produced IAA from 11.56 to 54.53 µg ml^{-1}. Different researchers reported that PGPR isolated from chickpea rhizosphere produced IAA in the range of 15-42 µg ml^{-1} [3], 14-61 µg ml^{-1} [28] and 20-35 µg ml^{-1} [34]. IAA production by PGPR could vary among different species and strains of rhizobacteria but also depends on culture medium conditions[23]. Like phosphate solubilization, bacteria that produce auxins, are likely to have a strong influence on plant growth. Indole-3-acetic acid, one of the most physiologically active auxins, promotes several growth and developmental events, such as cell division, elongation and differentiation[2].

Siderophore, HCN and ammonia production: Fourteen (29%) isolates produced siderophore. Likewise, 21 (44%) and 47 (98%) isolates produced HCN and ammonia respectively (Table 2). Out of the tested isolates, 21 isolates (44%) were HCN producers which were relatively higher than Kumari et al[24] and Temesgen[48] who reported that 23% and 21% of the isolates from chickpea and soybean produced HCN respectively. These HCN producing isolates can be used as biocontrol agents. Ammonia is important in controlling soil-borne pathogens[35], promotes plant growth by making nitrogen available to the plant[18], promotes plant growth by suppressing fungal pathogens in the rhizosphere[6] and enhances plant growth as a result of their ability to fix nitrogen (N_2) to ammonia (NH_3) making more NH_3 available for plant growth[16]. In this study, almost all of the isolates were ammonia producers (98%) which were relatively similar to Kumari et al[24] and Mussa et al[32] who reported the production of ammonia by all rhizobacteria isolated from chickpea and grass pea respectively.

In similar studies, Samuel and Muthukkaruppan[39] reported ammonia production in 95% of strains isolated from the rhizosphere of rice, mangroves and soils contaminated by effluent. Regarding siderophore production, 14 (29%) isolates produced siderophore. Kotasthane et al[22] reported the production of siderophores by the rhizospheric microflora enhances the iron uptake of plants.

Table 1
Plant growth promoting properties of selected chickpea rhizobacterial isolates

Isolates	Gram reaction	Phosphate solubilization (SI)	IAA (µg/ml)
GUCRB2	-ve	1.49 ± 0.03 ^{k}	22.26 ± 0.24 $^{m-r}$
GUCRB4	-ve	3.49 ± 0.25^{a}	51.80 ± 2.90 $^{a-c}$
GUCRB5	-ve	1.94 ± 0.05 $^{g-k}$	29.50 ± 0.30 ^{j}
GUCRB7	-ve	3.21 ± 0.05 $^{a-d}$	47.50 ± 0.17 ^{e}
GUCRB8	-ve	2.47 ± 0.39 $^{e-g}$	37.13 ± 0.14 ^{hi}
GUCRB9	+ve	1.91 ± 0.20 $^{g-k}$	21.66 ± 0.34 $^{n-q}$
GUCRB10	+ve	1.90 ± 0.05 $^{g-k}$	13.23 ± 0.34 ^{vw}
GUCRB12	-ve	1.78 ± 0.07 $^{i-k}$	-
GUCRB13	-ve	2.91 ± 0.17 $^{b-e}$	44.26 ± 0.29 ^{f}
GUCRB14	+ve	1.84 ± 0.18 $^{h-k}$	23.76 ± 0.48 $^{l-n}$
GUCRB18	+ve	1.88 ± 0.05 $^{g-k}$	18.16 ± 0.23 $^{r-t}$
GUCRB20	-ve	3.33 ± 0.19 ^{ab}	50.76 ± 0.23 $^{b-d}$
GUCRB21	-ve	3.45 ± 0.33^{ab}	54.53 ± 0.28 ^{a}
GUCRB22	-ve	1.90 ± 0.05 $^{g-k}$	15.20 ± 0.32 $^{t-v}$
GUCRB23	+ve	2.65 ± 0.10 $^{d-f}$	34.73 ± 0.31 ^{i}
GUCRB26	-ve	1.65 ± 0.10 $^{j-k}$	-
GUCRB27	-ve	2.17 ± 0.09^{f-j}	25.40 ± 0.26 $^{k-m}$
GUCRB28	-ve	1.52 ± 0.09^{k}	-
GUCRB29	-ve	1.77 ± 0.11 $^{i-k}$	27.70 ± 0.11 ^{jk}
GUCRB33	-ve	1.59 ± 0.06 $^{j-k}$	23.63 ± 1.39 $^{l-n}$
GUCRB35	-ve	1.83 ± 0.09 $^{h-k}$	29.46 ± 0.40 ^{j}
GUCRB36	+ve	1.77 ± 0.05 $^{i-k}$	20.00 ± 0.23 $^{p-r}$
GUCRB39	-ve	1.95 ± 0.05 $^{g-k}$	22.63 ± 0.43 $^{m-p}$
GUCRB41	-ve	3.49 ± 0.25^{a}	53.13 ± 0.26 ^{ab}
GUCRB42	-ve	2.95 ± 0.10 $^{a-e}$	44.00 ± 0.56 ^{f}
GUCRB43	+ve	1.89 ± 0.15 $^{g-k}$	11.56 ± 0.08 ^{w}
GUCRB44	-ve	2.41 ± 0.09 $^{e-h}$	26.40 ± 0.81 ^{kl}
GUCRB45	-ve	3.22 ± 0.11 $^{a-d}$	41.06 ± 2.56 ^{g}
GUCRB46	-ve	1.83 ± 0.09 $^{h-k}$	19.26 ± 0.12 $^{q-s}$
GUCRB54	-ve	2.72 ± 0.27 $^{c-f}$	27.63 ± 0.08 ^{jk}
GUCRB55	-ve	2.65 ± 0.08 $^{d-f}$	30.60 ± 0.17 ^{j}
GUCRB57	-ve	1.88 ± 0.11 $^{g-k}$	-
GUCRB58	+ve	1.95 ± 0.05^{g-k}	24.46 ± 0.14 $^{l-n}$
GUCRB60	-ve	2.72 ± 0.27^{c-f}	24.10 ± 0.17 $^{l-n}$
GUCRB69	-ve	1.70 ± 0.07 $^{i-k}$	-
GUCRB70	-ve	1.97 ± 0.17 $^{g-k}$	18.30 ± 0.15 $^{r-t}$
GUCRB71	-ve	2.65 ± 0.08 $^{d-f}$	38.33 ± 1.33 ^{gh}
GUCRB75	+ve	2.41 ± 0.09 $^{e-h}$	20.50 ± 0.15 $^{o-r}$
GUCRB76	-ve	3.25 ± 0.17 $^{a-c}$	50.10 ± 3.06 $^{c-e}$
GUCRB78	+ve	1.70 ± 0.07 $^{i-k}$	23.26 ± 0.76 $^{l-o}$
GUCRB79	-ve	1.88 ± 0.11 $^{g-k}$	30.43 ± 1.30 ^{j}
GUCRB84	+ve	3.32 ± 0.38 ^{ab}	48.16 ± 0.14 ^{de}
GUCRB86	-ve	1.54 ± 0.04 ^{k}	-
GUCRB99	+ve	2.94 ± 0.05 $^{a-e}$	40.93 ± 0.35 ^{g}
GUCRB122	+ve	2.30 ± 0.41^{f-i}	36.16 ± 1.45 ^{hi}
GUCRB124	+ve	3.11 ± 0.29 $^{a-d}$	48.20 ± 2.61 ^{de}
GUCRB126	-ve	1.78 ± 0.07 $^{i-k}$	25.10 ± 1.28 $^{k-m}$
GUCRB135	+ve	1.87 ± 0.12 $^{h-k}$	19.46 ± 1.28 $^{q-s}$

Means with the same letter down the column is not significantly different at P≤ 0.05. Mean ± SD of three replicates using Duncan's multiple range test (P<0.05). **–ve=** Gram-negative +ve = Gram-positive. GUCRB= Gondar University Chickpea rhizobacteria. ' – 'do not produce IAA.

Table 2
Plant growth promoting properties of rhizobacterial isolates

Isolates	Ammonia Production	Cellulase Activity	Chitinase Production	Protease Activity	Siderophore Production	HCN Production	Antagonistic Activity	%
GUCRB2	+	-	+	+	+	-	-	57
GUCRB4	+	+	+	+	+	+	+	100
GUCRB5	+	+	+	+	-	-	-	57
GUCRB7	+	+	+	+	-	+	+	86
GUCRB8	+	+	+	+	-	+	+	86
GUCRB9	+	+	-	+	-	-	-	43
GUCRB10	+	+	-	+	-	-	-	43
GUCRB12	+	+	-	-	-	-	-	29
GUCRB13	+	-	+	+	-	+	+	71
GUCRB14	+	-	-	+	-	-	-	29
GUCRB18	+	+	+	-	-	-	-	43
GUCRB20	+	+	+	+	-	+	+	86
GUCRB21	+	+	+	+	+	+	+	100
GUCRB22	+	+	-	+	+	-	-	57
GUCRB23	+	+	+	+	-	+	+	86
GUCRB26	+	-	-	+	-	-	-	29
GUCRB27	+	+	+	-	-	-	-	43
GUCRB28	+	+	-	-	-	-	-	29
GUCRB29	+	-	-	+	+	-	-	43
GUCRB33	+	-	+	-	-	-	-	29
GUCRB35	+	-	+	-	-	-	-	29
GUCRB36	+	-	-	+	-	-	-	29
GUCRB39	+	+	+	+	+	+	-	86
GUCRB41	+	+	+	+	+	+	-	86
GUCRB42	+	-	+	+	+	+	+	86
GUCRB43	+	+	-	-	-	-	-	29
GUCRB44	+	-	+	-	-	+	+	57
GUCRB45	+	+	-	-	-	+	-	43
GUCRB46	+	+	-	+	-	-	-	43
GUCRB54	+	+	-	+	-	-	-	43
GUCRB55	+	-	+	+	+	+	+	86
GUCRB57	-	-	-	+	-	-	-	14
GUCRB58	+	-	+	+	-	-	-	43
GUCRB60	+	+	+	+	+	-	-	71
GUCRB69	+	-	+	+	-	-	-	43
GUCRB70	+	-	+	-	+	+	+	71
GUCRB71	+	+	+	+	-	+	+	86
GUCRB75	+	-	+	-	-	+	+	43
GUCRB76	+	+	+	+	+	+	+	100
GUCRB78	+	-	-	-	-	-	-	14
GUCRB79	+	-	-	+	-	-	-	29
GUCRB84	+	+	+	-	+	+	+	86
GUCRB86	+	-	+	-	-	-	-	29
GUCRB99	+	+	+	+	-	+	+	86
GUCRB122	+	+	-	-	-	-	-	14
GUCRB124	+	+	+	+	+	+	+	100
GUCRB126	+	+	+	-	-	+	+	71
GUCRB135	+	+	-	-	-	-	-	29
Total/%	**47/98%**	**29/60%**	**30/63%**	**31/65%**	**14/29%**	**21/44%**	**18/38%**	

Eighteen isolates (38%) showed antagonistic activity against the fungal pathogen *Fusarium oxysporum* which was relatively better than Midekssa et al[28] and Temesgen[48] who reported that 25% and 30% isolates from chickpea and soybean showed antagonistic activity respectively.

Enzymes production: 31 (65%), 29 (60%) and 30(63%) isolates produced protease, cellulase and chitinase respectively. Fourteen isolates (29%) produced all the three enzymes (Table 2). The production of protease and chitinase is important in inhibiting fungal spore germination, germ tube elongation and lysis of hyphal tips[33].

In this study, 65%, 60% and 63% of the isolates were positive for protease, cellulase and chitinase production respectively which was relatively better than rhizobacteria positive for chitinase (43%), protease (50%) and cellulase (45.5%) activity reported by Temesgen[48], Kavitha et al[20] and Mussa et al[32] respectively. The production of protease and chitinase by rhizobacteria involved in fungal cell wall degradation during antagonism was reported for various genera of endophytic bacteria including *Enterobacter* and *Serratia*[50].

Antifungal activity of the isolates: Among the rhizobacteria isolates, 18 (38%) inhibited the fungal pathogen (*Fusarium oxysporum f.sp.ciceri*) growth (Table 2).

16S rRNA gene sequence analysis: Based on their overall performance from plant growth promoting and biocontrol activities four isolates GUCRB-4, 21, 76 and 124 were selected for molecular characterization. Based on the analysis of 16S-rRNA gene sequencing, the selected isolates GUCRB-4, 21, 76 and 124 were identified as *Alcaligenes sp.*, *Enterobacter mori*, *Serratia marcescens* and *Brevibacillus brevis* respectively (Table 4). Plant growth-promoting rhizobacteria from the genera *Azotobacter*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Rhizobium*, *Enterobacter* and *Serratia* have been isolated from chickpea and their role in augmenting plant growth has been widely demonstrated[15,17, 42, 46, 52].

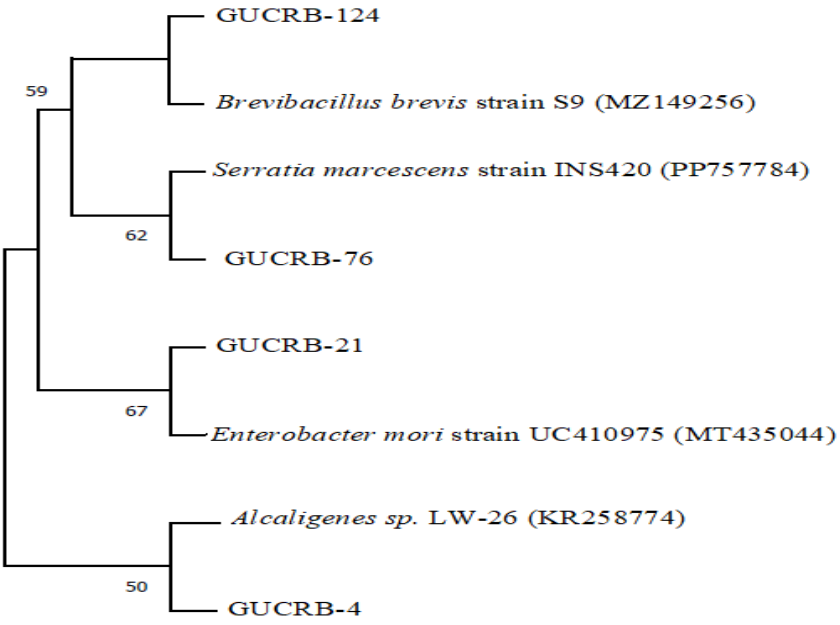

Figure 2: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence shows the relationships between rhizobacteria isolates and some related representative reference species retrieved from NCBI. The numbers on the tree indicate the percentage of bootstrap based on 1000 replications. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

Table 3
16S rRNA gene sequence characterization of selected rhizobacteria isolates

Isolates	The closest gene bank similarity	Best match Id (NCBI)	Query Cover %	E-value	Similarity (%)	NCBI Accession no
GUCRB-4	*Alcaligenes sp.* LW-26	KR258774	99	0.0	92	PP499249
GUCRB-21	*Enterobacter mori* strain UC410975	MT435044	99	0.0	99.2	PP499248
GUCRB-76	*Serratia marcescens* strain INS420	PP757784	100	0.0	100	PP508218
GUCRB-124	*Brevibacillus brevis* strain S9	MZ149256	99	0.0	98.4	PP499250

Conclusion

The four isolates GUCRB-4, 21, 76 and 124 were selected as top performer based on their overall performance in plant growth promoting and biocontrol activities. The four isolates performed top are recommended for additional inoculation experiments under field conditions to confirm and upgrade these stains as microbial inoculants.

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References

1. Acuña J.J., Jorquera M.A., Martínez O.A., Menezes-Blackburn D., Fernández M.T., Marschner P., Greiner R. and Mora M.L., Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals, *Journal of Soil Science and Plant Nutrition*, **11(3)**, 1-12 **(2011)**

2. Ahmed B., Zaidi A., Khan M.S., Rizvi A., Saif S. and Shahid M., Perspectives of Plant Growth Promoting Rhizobacteria in Growth Enhancement and Sustainable Production of Tomato, In Zaidi A. and Khan M., eds., Microbial Strategies for Vegetable Production, Springer International Publishing, 125-149 **(2017)**

3. Ali A., Khalid R., Ali S., Akram Z. and Hayat R., Characterization of plant growth promoting rhizobacteria isolated from chickpea (*Cicer arietinum* L.), *British Microbiology Research Journal*, **6(1)**, 32-40 **(2015)**

4. Altaye A., Tena W.T. and Melese A., Effects of *Mesorhizobium* inoculation, phosphorus and sulfur application on nodulation, growth and yield of chickpea (*Cicer arietinum* L.), *Ethiopian Journal of Applied Science and Technology*, **13(2)**, 11 – 23 **(2022)**

5. Argaw A., Evaluation of co-inoculation of *Bradyrhizobium japonicum* and Phosphate solubilizing Pseudomonas spp. effect on soybean (*Glycine max* L. Merr.) in Assossa Area, *Journal of Agricultural Science and Technology*, **14(1)**, 213-224 **(2012)**

6. Bhattacharyya P.N. and Jha D.K., Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture, *World Journal of Microbiology and Biotechnology*, **28**, 1327-1350 **(2012)**

7. Buck J.D., Nonstaining (KOH) method for determination of Gram reactions of marine bacteria, *Applied and Environmental Microbiology*, **44(4)**, 992-993 **(1982)**

8. Cappuccino J.G. and Sherman N., Microbiology: A laboratory manual, 10th Edition, Pearson Education Limited, London **(2013)**

9. Chandra S. et al, Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*), *Brazilian Journal of Microbiology*, **38**, 124-130 **(2007)**

10. Chang Y.W., Alli I., Konishi Y. and Ziomek E., Characterization of protein fractions from chickpea (*Cicer arietinum* L.) and oat (*Avena sativa* L.) seeds using proteomic techniques, *Food Research International*, **44(9)**, 3094-3104 **(2011)**

11. CSA, Agricultural sample survey, area and production of major crops (Private Peasant Holdings, Meher Season), Central Statistical Agency, Addis Ababa, Ethiopia **(2022)**

12. Edi Premono M., Moawad A.M. and Vlek P.L.G., Effect of phosphate-solublizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere, *Indonesian Journal of Crop Science*, **11,** 13-23 **(1996)**

13. FAO, F., United Nation's Food and Agricultural Organization, Statistical Division, Rome **(2019)**

14. FAO, F., United Nation's Food and Agricultural Organization, Statistical Division, Rome **(2021)**

15. Fierro-Coronado R.A., Quiroz-Figueroa F.R., García-Pérez L.M., Ramírez-Chávez E., Molina-Torres J. and Maldonado-Mendoza I.E., IAA-producing rhizobacteria from chickpea (*Cicer arietinum* L.) induce changes in root architecture and increase root biomass, *Canadian Journal of Microbiology*, **60(10)**, 639-648 **(2014)**

16. Hayat R., Ali S., Amara U., Khalid R. and Ahmed I., Soil beneficial bacteria and their role in plant growth promotion, a review, *Annals of Microbiology*, **60**, 579-598 **(2010)**

17. Joseph B., Patra R.K. and Lawrence R., Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.), *International Journal of Plant Production*, **1(2)**, 141-152 **(2007)**

18. Kandjimi O.S., Uzabakiriho J. and Chimwamurombe P.M., Isolation and characterization of culturable bacteria from bulk soil samples and the rhizosphere of arid-adapted Tylosema esculentum (Burchell). A. Schreiber (Marama bean) in Namibia, *African Journal of Biotechnology*, **14(11)**, 944-952 **(2015)**

19. Kasana R.C., Salwan R., Dhar H., Dutt S. and Gulati A., A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine, *Current Microbiology*, **57**, 503-507 **(2008)**

20. Kavitha T., Nelson R. and Jesi S.J., Screening of rhizobacteria for plant growth promoting traits and antifungal activity against charcoal rot pathogen *Macrophomina phaseolina*, *International Journal of Pharma and Bio Sciences*, **4**, 177–186 **(2013)**

21. Keneni A., Assefa F. and Prabu P.C., Isolation of phosphate solubilizing bacteria from the rhizosphere of faba bean of Ethiopia and their abilities on solubilizing insoluble phosphates, *Journal of Agricultural Science and Technology*, **12(1)**, 79-89 **(2010)**

22. Kotasthane A.S., Agrawal T., Zaidi N.W. and Singh U.S., Identification of siderophore producing and cynogenic fluorescent Pseudomonas and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea, *3 Biotech*, **7(2)**, 1-8 **(2017)**

23. Kumar P., Dubey R.C. and Maheshwari D.K., Bacillus strains isolated from rhizosphere showed plant growth promoting and

antagonistic activity against phytopathogens, *Microbiological Research*, **167(8)**, 493-499 **(2012)**

24. Kumari P., Khanna V. and Kumar P., Multifaceted rhizobacteria-mediated growth augmentation in chickpea, *Agricultural Research*, **6**, 368-377 **(2017)**

25. Landa B.B., Hervás A., Bettiol W. and Jiménez-Díaz R.M., Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium oxysporum f. sp. ciceris*, *Phytoparasitica*, **25**, 305-318 **(1997)**

26. Mengstie G.Y., Awlachew Z.T. and Degefa A.M., Screening of rhizobacteria for multi-trait plant growth-promoting ability and antagonism against *B. fabae*, the causative agent of chocolate spot disease of faba bean, *Heliyon*, **10(3)**, 1-9 **(2024)**

27. Midekssa M.J., Loscher C., Schmitz R. and Assefa F., Characterization of phosphate solubilizing rhizobacteria isolated from lentil growing areas of Ethiopia, *African Journal of Microbiology Research*, **9(25)**, 1637-1648 **(2015)**

28. Midekssa M.J., Löscher C.R., Schmitz R.A. and Assefa F., Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea (*Cicer aeritinum* L.) producing areas of Ethiopia, *African Journal of Biotechnology*, **15(35)**, 1899-1912 **(2016)**

29. Miller C.S., Handley K.M., Wrighton K.C., Frischkorn K.R., Thomas B.C. and Banfield J.F., Short-read assembly of full-length 16S amplicons reveals bacterial diversity in subsurface sediments, *PLOS ONE*, **8(2)**, 1-11 **(2013)**

30. Muleta D., Assefa F. and Granhall U., *In vitro* antagonism of rhizobacteria isolated from *Coffea arabica* L. against emerging fungal coffee pathogens, *Engineering in Life Sciences*, **7(6)**, 577-586 **(2007)**

31. Muleta D., Assefa F., Börjesson E. and Granhall U., Phosphate-solubilising rhizobacteria associated with *Coffea arabica* L. in natural coffee forests of southwestern Ethiopia, *Journal of the Saudi Society of Agricultural Sciences*, **12(1)**, 73-84 **(2013)**

32. Mussa A., Million T. and Assefa F., Rhizospheric bacterial isolates of grass pea (*Lathyrus sativus* L.) endowed with multiple plant growth promoting traits, *Journal of Applied Microbiology*, **125(6)**, 1786-1801 **(2018)**

33. Ordentlich A., Elad Y. and Chet I., The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*, *Phytopathology*, **78(1)**, 84-88 **(1988)**

34. Pandey S., Gupta S. and Ramawat N., Unravelling the potential of microbes isolated from rhizospheric soil of chickpea (*Cicer arietinum*) as plant growth promoter, *3 Biotech*, **9(7)**, 1-9 **(2019)**

35. Paulitz T., Nowak-Thompson B., Gamard P., Tsang E. and Loper J., A novel antifungal furanone from *Pseudomonas aureofaciens*, a biocontrol agent of fungal plant pathogens, *Journal of Chemical Ecology*, **26**, 1515-1524 **(2000)**

36. Pingping Sun, Jianchao Cui, Xiaohui Jia and Wenhui Wang, Isolation and Characterization of *Bacillus Amyloliquefaciens* L-1 for Biocontrol of Pear Ring Rot, *Horticultural Plant Journal*, **3(5)**, 183-189 **(2017)**

37. Renwick A., Campbell R. and Coe S., Assessment of in vivo screening systems for potential biocontrol agents of *Gaeumannomyces graminis*, *Plant Pathology*, **40(4)**, 524-532 **(1991)**

38. Routray S. and Khanna V., Characterization of rhizobacteria for multiple plant growth promoting traits from mung bean rhizosphere, *International Journal of Current Microbiology and Applied Sciences*, **7(1)**, 2264-2269 **(2018)**

39. Samuel S. and Muthukkaruppan S.M., Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil, *Current Botany*, **2(3)**, 22-25 **(2011)**

40. Sanjeewa W.G.T., Wanasundara J.P.D, Pietrasik Z. and Shand P.J., Characterization of chickpea (*Cicer arietinum* L.) flours and application in low-fat pork bologna as a model system, *Food Research International*, **43(2)**, 617-626 **(2010)**

41. Schwyn B. and Neilands J.B., Universal chemical assay for the detection and determination of siderophores, *Analytical Biochemistry*, **160(1)**, 47-56 **(1987)**

42. Singh O., Gupta M., Mittal V., Kiran S., Nayyar H., Gulati A. and Tewari R., Novel phosphate solubilizing bacteria *'Pantoea cypripedii* PS1'along with *Enterobacter aerogenes* PS16 and *Rhizobium ciceri* enhance the growth of chickpea (*Cicer arietinum* L.), *Plant Growth Regulation*, **73**, 79-89 **(2014)**

43. Sintie E., Diversity, Symbiotic Effectiveness and Plant Growth Promoting Characteristics of Rhizobia and Rhizospheric Bacteria on Growth and Production of White lupin (*Lupinus albus* L.) under Greenhouse and Field Conditions in North Western Ethiopia, Ph.D. dissertation, Addis Ababa University **(2018)**

44 Smibert R.M. and Krieg N.R., Phenotypic characterization, In Methods for General and Molecular Bacteriology, Eds., Gerhardt P., Murray R.G.E., Wood W.A. and Krieg N.R., Washington DC, American Society of Microbiology, 607–654 **(1994)**

45. Swarnalakshmi K., Yadav V., Tyagi D., Dhar D.W., Kannepalli A. and Kumar S., Significance of plant growth promoting rhizobacteria in grain legumes: Growth promotion and crop production, *Plants*, **9(11)**, 1-25 **(2020)**

46. Taghavi S., van der Lelie D., Hoffman A., Zhang Y.B., Walla M.D., Vangronsveld J., Newman L. and Monchy S., Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638, *PLOS Genetics*, **6(5)**, 1-15 **(2010)**

47. Tamura K., Stecher G. and Kumar S., MEGA11: molecular evolutionary genetics analysis version 11, *Molecular Biology and Evolution*, **38(7)**, 3022-3027 **(2021)**

48. Temesgen D., Genetic diversity of rhizobia and rhizobacteria from soybean (*Glycine max* (L) Merr.): Implication for the commercial production and application to enhance soybean production under low input agriculture in Ethiopia, Ph.D. dissertation, Addis Ababa University **(2017)**

49. Tena W., Wolde-Meskel E., Degefu T. and Walley F., Genetic and phenotypic diversity of rhizobia nodulating chickpea (*Cicer arietinum* L.) in soils from southern and central

Ethiopia, *Canadian Journal of Microbiology*, **63(8)**, 690-707 **(2017)**

50. Wang S., Wang W., Jin Z., Du B., Ding Y., Ni T. and Jiao F., Screening and diversity of plant growth promoting endophytic bacteria from peanut, *African Journal of Microbiology Research*, **7(10)**, 875-884 **(2013)**

51. Woyessa D. and Assefa F., Effects of Plant Growth Promoting Rhizobaceria on Growth and Yield of Tef *Eragrostis tef* Zucc. Trotter) under Greenhouse Condition, *Research Journal of Microbiology*, **6(4)**, 343-355 **(2011)**

52. Yadav J., Verma J.P. and Tiwari K.N., Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinum* L.) under in vitro conditions, *Biological Forum — An International Journal*, **2(2)**, 15-18 **(2010)**.

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