



Research article

Genetic and metabolic diversities of rhizobacteria isolated from degraded soil of Ethiopia

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ABSTRACT

Genetic and metabolic diversities of rhizobacteria are the fundamental sources for their adaptation to cope with abiotic and biotic stresses in order to enhance growth and health of plants in the soil. Thus, this study was initiated to assess the genetic and metabolic diversities of rhizobacteria isolated from plants grown in degraded soil through BOX-PCR and partial sequencing of 16S rRNA genes. A total of eighty isolates were recovered and subjected to phenotypic profiling of carbohydrate and amino acid utilization, BOX PCR and 16S rRNA profiling. The phenotypic profiling showed remarkable metabolic versatility with *Ochrobactrum* spp, *Pseudomonas* spp and *Klebsiella* spp, and BOX-PCR showed greater discriminatory power for fingerprinting of rhizobacterial isolates with high degree of polymorphism. *Bacillus* spp showed the highest Simpson's diversity Index. The 16S rRNA genes sequence assigned the rhizobacteria to phyla Proteobacteria with Gammaproteobacteria and Alphaproteobacteria classes and Firmicutes with Bacilli class. The data also showed that the most dominant species were *Pseudomonas* and *Ochrobactrum*. Genetic and metabolic diversities of the rhizobacterial isolates reveal the potential of these microbes for plant growth improvement under water deficient soil after testing other inoculant traits.

1. Introduction

Soil is considered a rich reservoir of diverse groups of microorganisms that involved in the biogeochemical cycles of bio-elements, and untapped resources for agricultural and industrial applications (Mhete et al., 2020). The rhizosphere of plants is the hot spot of microbial activities dominated by bacteria generally known as rhizobacteria.

The rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed as plant growth promoting rhizobacteria (PGPR; Schroth and Kloepper, 1978). Furthermore, in most cases, a single PGPR has often multiple modes of action including biological control (Vessey, 2003).

Metabolic diversity of rhizobacteria is reduced through intensive land-use, which may have implications for the resistance of the soils to stress or disturbance (Ding et al., 2013). This requires the need for selection and exploitation of rhizobacteria for restoration to improve soil fertility, maintain ecological balance and environmental quality (Zahid, 2015).

The rhizobacteria enhance plant growth by improving nutrient availability, increasing nutrient uptake, enhance plant resistance to biotic and abiotic stresses (Mesa et al., 2015). A diverse array of rhizobacteria are used for maintaining soil fertility that include *Azospirillum*, *Bacillus*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Klebsiella*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Enterococcus* (Solanki et al., 2017; King et al., 2016).

A wide-ranging evaluation of genetic and metabolic diversities can be useful for the introduction of new and useful microorganisms into the environment (Joseph et al., 2012). The metabolic assets of an organism could contribute towards a particular environmental adaptation (Mazur et al., 2013).

A significant number of studies have been focused on the isolation and identification of microbes by employing using physiological and biochemical methods (Liu et al., 2006). Recently, molecular methods have been applied as a smartest means to investigate the species diversity. PCR-based methods such as BOX-PCR and analysis of 16S rRNA genes are appropriate tools to examine microbial diversity in a wider range of environments (Fakruddin et al., 2013; Srinivasan et al., 2015).

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Nowadays, bacterial classification involves techniques to determine both phenotypic and genotypic characteristics (polyphasic approach).

There is a clear incentive to exploit this microbial diversity to develop functional microbes that could be used as targeted bio-tools to boost soil fertility. It is hypothesized that degraded land has metabolically and genetically diverse phyto-beneficial soil bacteria. Thus, the main purpose of this study was to assess the metabolic and genetic diversities of culturable indigenous soil bacteria from degraded soil samples.

2. Transparent methods

2.1. Description of the study area

Soil samples were collected from Fiche areas, Oromia National Regional State, Ethiopia. The site is located at 9° 08' 52" N and 38° 56' 13" E with an altitude of 3100 m above sea level. The study site is highly degraded and almost devoid of vegetation cover with sandy clay loamy in texture (>50% clay) having low inorganic matter, organic carbon, available P, K and total nitrogen. The soil pH is 5.69 with soil salinity of 0.2 dS/m (Getahun et al., 2020). In the study area, heavy rain started in June and ends in September and the dry season occurred from October to January which is followed by small rain (February to May).

2.2. Rhizobacteria isolations and selection

Rhizobacteria were isolated from bulk, rhizosphere soils of acacia and juniperus at different sampling sites of Fiche areas, Oromia National Regional State, Ethiopia and purified using standard methods (Somasegar and Hoben, 2012), and maintained in culture collection at Addis Ababa University. The isolates were screened for phenotypic carbohydrate and amino acid profiling and genetic characterization.

2.3. The phenotypic profiling of carbohydrate and amino acid utilization

The nutritional versatility of the potential rhizobacteria isolates was assessed by their ability to utilize 15 carbohydrate and 7 amino acid sources. Growths of the isolates were checked for each microbe on the basal mineral salt medium (MSM) constructed for the tests of carbohydrates and amino acids utilization (Zajic and Supplisson, 1972). The carbon sources were adjusted to a final concentration of 1 g/L to a basal medium containing (per liter of distilled water: 1.8 g K₂HPO₄, 4.0 g NH₄Cl, 0.2 g MgSO₄·7H₂O, 0.1 g NaCl, 0.01 g FeSO₄·7H₂O, 15 g agar. The amino acids were added at a concentration of 0.5 g/L to the same basal medium from which NH₄Cl was omitted and adjusted to pH 6.9 (Amarger et al., 1997). In amino acid utilization test, mannitol was used as a carbon source. All of the substrates were filter sterilized using membrane (pore size 0.45 μm, Millipore). The test rhizobacteria were grown over night in nutrient broth from which 50 μL of culture was streaked on the MSM agar plates and incubated at 30 °C for 72 h. The results were recorded as (+) for growth or (-) for no growth in comparison with the controls. All the experiments were performed in triplicates.

2.4. Genotypic characterization

The genotypic characterization was done via 16S rRNA and BOX-PCR fingerprinting (Ribeiro and Cardoso, 2012; Xavier et al., 2017).

Table 1. PCR primers used for 16S rRNA and BOX profile.

Target gene	Primer	Sequence (5' → 3')	Product size	References
16S	Forward (fD1)	5'-AGAGTTTGATCCTGGCTCAG-3'	1100–1300	(Weisburg et al., 1991)
	Reverse (rD1)	5'-AAGGAGGTGATC CAGCC-3'		
BOX	BOXA1R	5'CTACGGCAAAGCCGACGCTGACG-3'	50–5000	(Guñazú et al., 2013)

Table 2. PCR conditions for BOX-PCR and 16S rRNA.

PCR steps	BOX-PCR		
	Temperature (°C)	Duration (min/sec)	Cycle
Initial denaturation	95	7'	-
Denaturation	94	1'	
Annealing	53	1'	
Elongation	65	8'	30
Final elongation	65	16'	-
16S rRNA			
Initial denaturation	95	2'	
Denaturation	94	15"	
Annealing	55	45"	30
Elongation	72	2'	
Final elongation	72	5'	

2.5. Genomic DNA extraction

The genomic extraction for genetic diversity was done as described before and the conditions are presented in the tables below. Extracted DNA from pure cultures was used for 16S rRNA genes amplification using a universal primer pair for forward and reverse (Table 1). The PCR condition is presented in Table 2.

2.6. Genetic diversity BOX-PCR fingerprinting

In BOX-PCR genomic fingerprint, BOXA1R primer was used (Table 1). To prepare 25 μL of PCR mixture, 1 μL primers, 2 μL of DNA template, 2.5 μL Taq PCR buffer, 5 μL dNTPs, 1.5 μL MgCl₂, and 0.2 U Taq DNA polymerase (Promega) were mixed together. The PCR reaction was carried out according to the condition in Table 2. The PCR products were separated in 1.5% agarose gel with 1 kb DNA ladder (Invitrogen). Then, the gel was stained with ethidium bromide and viewed under a UV trans-illuminator (Loccus, Brazil).

The DNA band patterns were analyzed and a dendrogram was generated for each isolate by using Bionumerics 7.3 software program (Applied Mathematics, Brazil) by applying the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) algorithm and the Jaccard's coefficient with 3% of tolerance (Sneath and Sokal, 1973). Differences among strains were assessed visually on the basis of the banding patterns of PCR products.

Simpson's Index of Diversity, D, was also calculated. The discriminating power of this typing method was calculated by using Simpson's Index of Diversity, D (Hunter and Gaston, 1988). The higher the discriminatory index, the greater the effectiveness of a particular fingerprinting method to discriminate different strains (Yoke-Kqueen et al., 2013). This index was given by the following equation:

$$D = 1 - \frac{\sum ni(ni - 1)}{N(N - 1)}$$

where N is the total number of strains in the sample population, ni denotes the number of strains belonging to the ith type.

Sequences and accession numbers were deposited in Gene Bank database (NCBI) and received accession numbers MN005961-MN006030 for 16S rRNA sequences. The accession numbers are listed in parentheses

Table 3. Carbons and amino acids utilization patterns of some selected rhizobacterial isolates.

S. No. Strains	Carbon sources															Amino acid sources								
	Amylose	Anditol	Cellibiose	Arabinose	Melibiose	Mannose	Dulcitol	Rhafinose	Sorbitol	Trehalose	Inositol	Maltotriose	Mannitol	Glucose	Sucrose	Total (%)	Asparagine	Arginine	Valine	Isoleucine	Serine	Tryptophan	Glycine	Total (%)
1 <i>Enterococcus</i> PS-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
2 <i>Agrobacterium</i> RS-79	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
3 <i>Ochrobactrum</i> RS-70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
4 <i>Ochrobactrum</i> RS-76	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
5 <i>Ochrobactrum</i> RS-77	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
6 <i>Pseudomonas</i> FB-49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
7 <i>Klebsiella</i> PS-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
8 <i>Serratia</i> RS-73	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	+	+	100
1 <i>Bacillus</i> BS-47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	-	+	71.43
2 <i>Enterococcus</i> PS-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	-	-	-	+	+	57.14
3 <i>Enterococcus</i> PS-9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
4 <i>Paenibacillus</i> FB-50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	-	-	+	+	-	+	+	57.14
5 <i>Ochrobactrum</i> RS-58	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
6 <i>Ochrobactrum</i> RS-59	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	-	+	+	-	-	+	57.14
7 <i>Ochrobactrum</i> RS-68	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
8 <i>Ochrobactrum</i> RS-72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	-	+	85.71
9 <i>Pseudomonas</i> BS-52	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
10 <i>Pseudomonas</i> BS-41	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
11 <i>Klebsiella</i> PS-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	-	+	85.71
12 <i>Klebsiella</i> PS-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71
13 <i>Morganella</i> PS-13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	+	-	+	85.71
14 <i>Serratia</i> PS-54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	+	+	+	+	+	-	+	+	85.71

“+” = show bacterial growth on C and N supplemented sources; “-” = show no bacterial growth on C and N supplemented sources, C- carbon and N-nitrogen source.

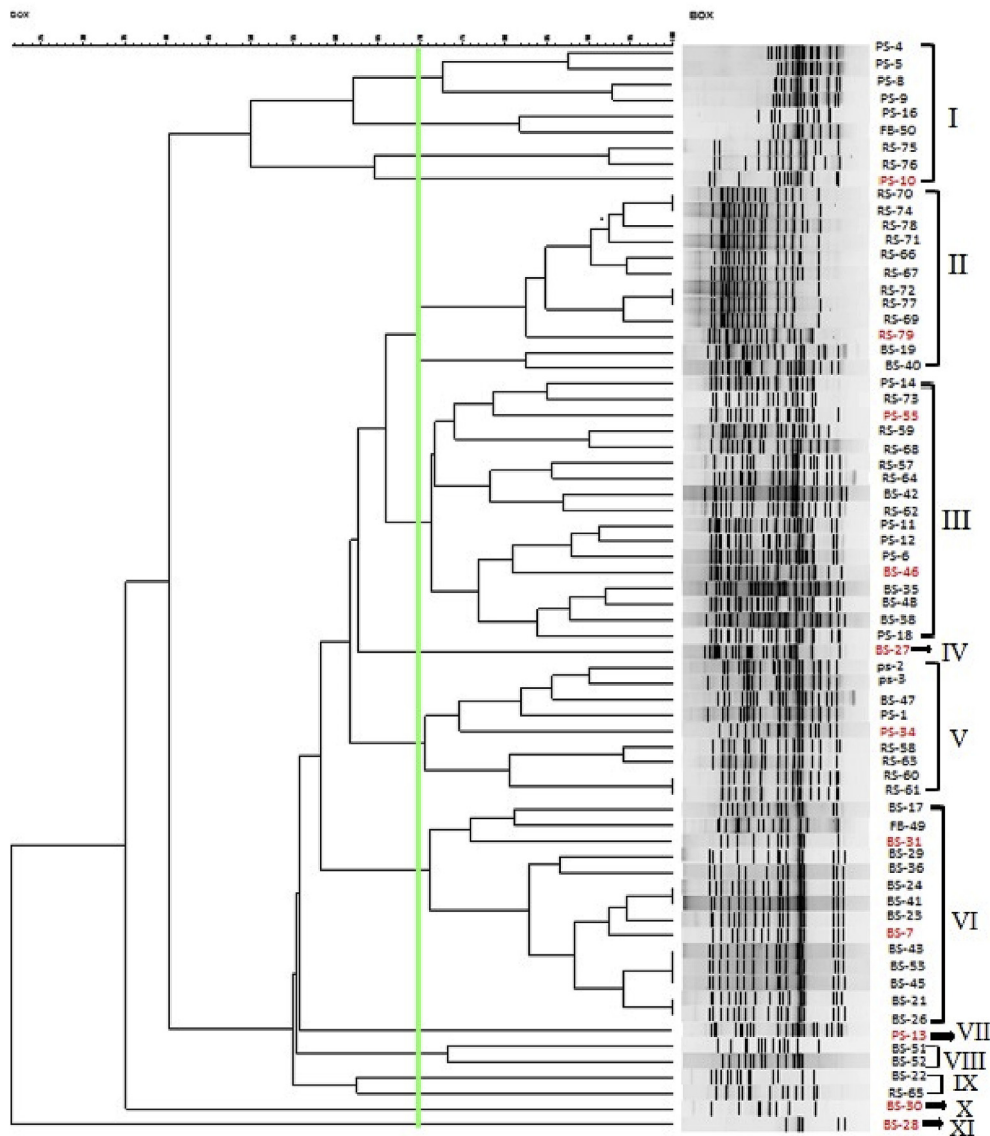


Figure 1. Dendrogram of similarity based on BOX-PCR profiles of rhizobacterial isolates those strains marked in red indicate unique genomic profiles.

Table 4. The Simpson genetic diversity index (D) of rhizobacterial isolates from degraded soil.

Genus	Number (n)	n (n-1)	D	Percent (%)
Bacillus	7	42	0.762	76
Enterococcus	6	30	0	NA
Paenibacillus	3	6	0.667	67
Agrobacterium	1	0	NA	NA
Ochrobactrum	15	210	0.133	13
Acinetobacter	1	0	NA	NA
Pseudomonas	17	272	0.426	43
Klebsiella	3	6	0.667	67
Morganella	9	72	0	NA
Pantoea	3	6	0.667	67
Serratia	8	56	0.607	61
Unidentified	7	42	NA	NA
Total (N)	80	470	0.883	88

NA = not applicable.

in the phylogenetic trees. Four phylogenetic trees were constructed for both Gram positive and Gram negative rhizobacterial strains.

2.7. Statistical data analysis

The carbohydrates and amino acids utilization pattern of rhizobacterial isolates was tabulated using percentage. BOX-PCR dendrogram was established using Bionumerics software (v.7.0.2) (Sneath and Sokal, 1973). All phylogenetic analyses were performed with the software MEGA 7 (Tamura et al., 2013). Pairwise and multiple sequence alignments were generated with Clustal W (Larkin et al., 2007). Tamura 3-parameter model Tamura et al. (2013) with G + I was used to determine the 16S rRNA phylogenies.

3. Results

3.1. Carbohydrates and amino acids utilization

A total of 73 isolates were tested for phenotypic profiling of carbohydrates and amino acids utilization patterns. All the isolates were

Table 5. Summary table of the diversity of the isolates into their respective species and strains.

Isolate	Closely related to	Accession description	%Gene identity	%Query coverage	No isolates
BS 22	<i>Bacillus anthracis</i>	CP033795.1	100	100	1
BS 29	<i>Bacillus cereus</i>	AY853168.1	100	99	1
BS 37	<i>Bacillus cereus</i>	AJ539175.1	99	99	1
BS 45	<i>Bacillus thuringiensis</i>	KX641526.1	99	100	2
PS 4	<i>Enterococcus gallinarum</i>	CP033740.1	99	100	4
PS 11	<i>Enterococcus gallinarum</i>	JF915769.1	92	99	2
BS 51	<i>Paenibacillus polymyxa</i>	CP006872.1	100	100	1
BS 30	<i>Paenibacillus odorifer</i>	CP009281.1	100	100	1
FB 50	<i>Paenibacillus polymyxa</i>	CP025957.1	100	100	1
RS 71	<i>Agrobacterium tumefaciens</i>	CP033032.1	99	100	1
RS 58	<i>Ochrobactrum intermedium</i>	KC146415.1	100	99	4
RS 60	<i>Ochrobactrum intermedium</i>	AJ242582.2	99	99	8
RS 76	<i>Ochrobactrum antropi</i>	KC146415.2	100	100	1
BS 27	<i>Acinetobacter calcoaceticus</i>	KC257031.1	99	99	1
BS 19	<i>Pseudomonas putida</i>	CP025262.1	99	99	1
BS 21	<i>Pseudomonas fulva</i>	CP014025.1	100	99	12
BS 26	<i>Pseudomonas plecoglossicida</i>	MF281997.1	99	100	1
RS 75	<i>Pseudomonas protogens</i>	MK182884.1	99	100	1
FB 49	<i>Pseudomonas fluorescens</i>	KY228953.1	100	100	1
PS 1	<i>Klebsiella michiganensis</i>	CP033824.1	99	99	2
PS 3	<i>Klebsiella oxytoca</i>	CP033824.2	99	99	1
BS 46	<i>Morganella morganii</i>	CP032295.1	99	99	8
PS 13	<i>Morganella morganii</i>	HQ774675.1	99	100	1
BS 35	<i>Pantoea vagans</i>	CP014129.2	99	99	2
BS 20	<i>Serratia grimesii</i>	CP033162.1	99	100	4
BS 42	<i>Serratia grimesii</i>	MG972923.1	100	100	1
PS 54	<i>Serratia fonticola</i>	LR134492.1	99	100	2
RS 65	<i>Serratia marcescens</i>	CP021164.1	99	99	1

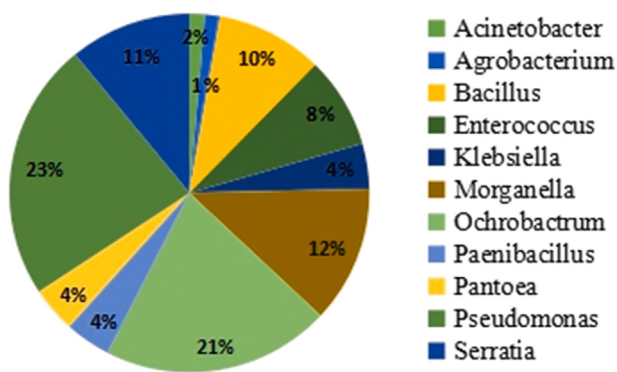


Figure 2. Distribution of rhizobacteria (genus level) identified by 16S rRNA genes sequencing. Values indicate percentages of isolates belonging to each genus amongst the 73 identified isolates.

diversified into nine genera; *Ochrobactrum*, (27% of the isolates) *Enterococcus* (14%), *Klebsiella* (14%), *Pseudomonas* (14%), *Serratia* (10%), *Bacillus* (5%), *Morganella* (5%), *Paenibacillus* (5%), and *Agrobacterium* (5%) (Table 3).

Isolates utilized 15 of the carbohydrates tested (100%), whereas only 37% of the isolates utilized all the nitrogen sources (Table 3), indicating that they were more versatile to utilize carbohydrates than they were to nitrogen sources. Among representative isolates from six genera; *Enterococcus* PS-4, *Ochrobactrum* RS-70, *Ochrobactrum* RS-76, *Ochrobactrum* RS-77, *Agrobacterium* RS-79, *Pseudomonas* FB-49, *Klebsiella* PS-2, and *Serratia* RS-73 utilized all the tested carbohydrate and nitrogen sources indicating the dominance of *Ochrobactrum* in substrate utilization.

3.2. Genotypic diversity

The BOX PCR fingerprint showed a significant genetic diversity of the rhizobacterial isolates (Figure 1). The dominance pattern was different from the phenotypic profiling based on C and N utilization. Thus, *Bacillus* species showed the highest diversity (D = 0.762) followed by *Paenibacillus*, *Klebsiella* and *Pantoea* with D = 0.667 and *Serratia* (D = 0.607) values. The overall Simpson's Index of diversity of the current study indicated a greater bacterial diversity (D = 0.883; Table 4).

3.3. BOX-PCR fingerprinting

The dendrogram displaying the distance relationships between the strains is shown in Figure 1. At a distance of 0.70, eleven clusters were shown (I to XI). The band pattern of BOX-PCR amplification yielded 5–24 bands (Figure 1). BS-35 strain displayed the highest number of bands (n = 24), while BS-28 strain showed the lowest number of bands (n = 5). Strains RS-70/RS-74, RS-72/RS-77, RS-60/RS-61, and BS-24/BS-41 had

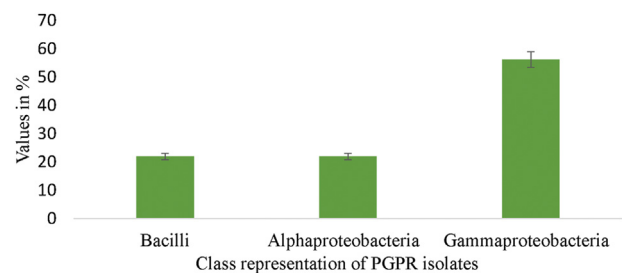


Figure 3. Class representation of each PGPR isolated from degraded land.

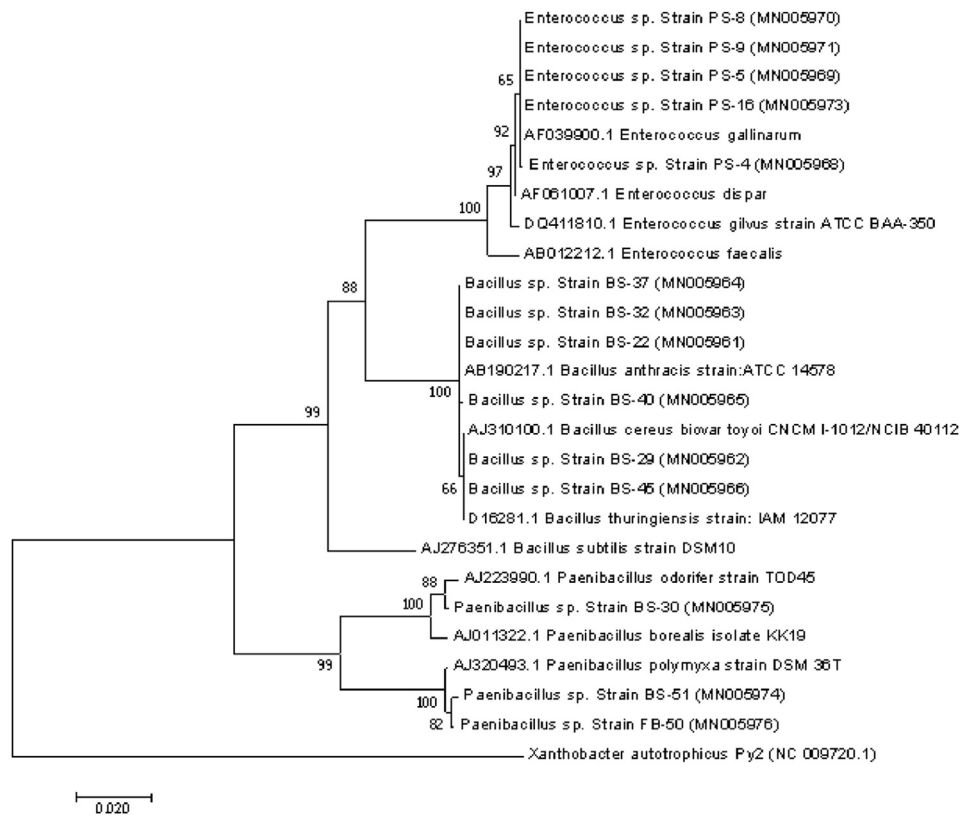


Figure 4. Phylogenetic tree of 16S rRNA gene sequences of Gram positive rhizobacteria from degraded soil and some of their closest phylogenetic relatives using the Neighbor-Joining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Xanthobacter autotrophicus* Py2 (NC-009720.1) species was used for out grouping.

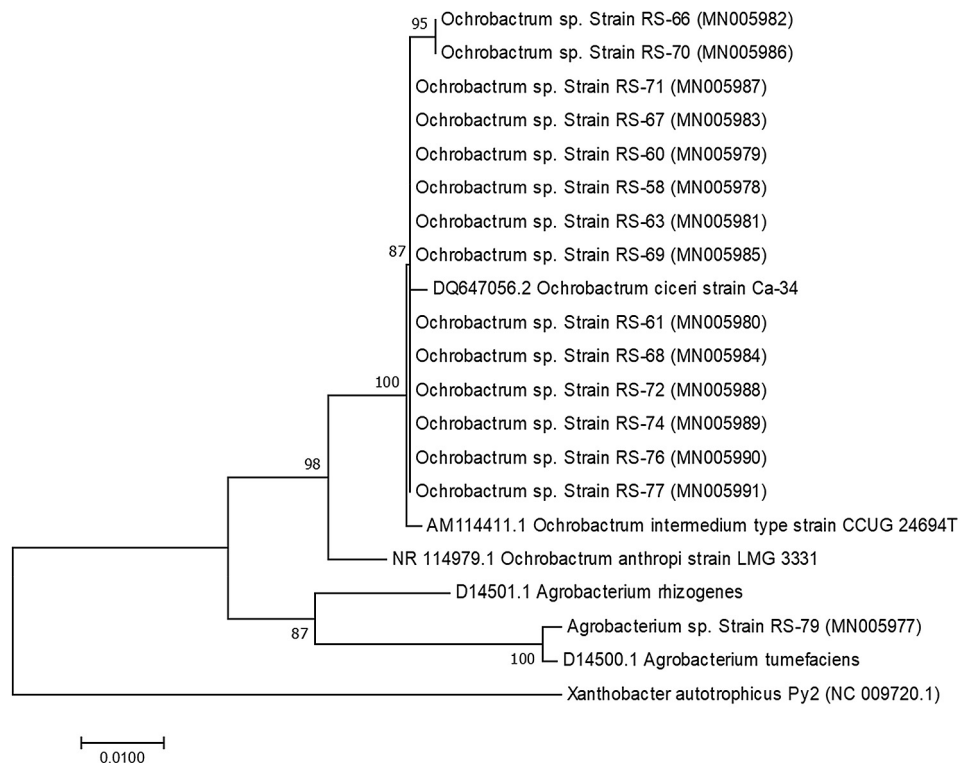


Figure 5. Phylogenetic tree of 16S rRNA gene sequences of Gram negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Xanthobacter autotrophicus* Py2 (NC-009720.1) species was used for out grouping.

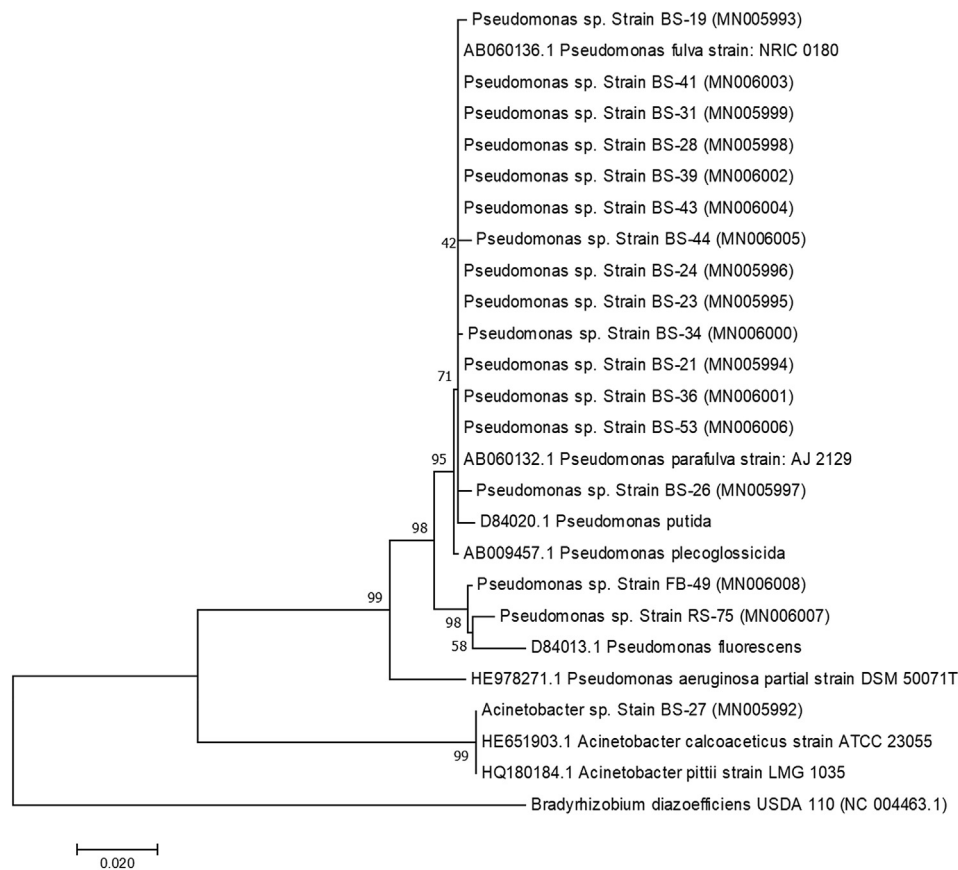


Figure 6. Phylogenetic tree of 16S rRNA gene sequences of Gram negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Bradyrhizobium diazoefficiens* USDA 110 species was used for out grouping.

identical profiles. Moreover, strains BS-43/BS-53/BS-45 showed identical profile. On the other hand, the largest number of strains; PS-10, RS-79, PS-55, BS-46, BS-27, PS-34, BS-31, BS-7, PS-13, BS-30 and BS-28 exhibited unique BOX PCR genomic profiles (Figure 1).

3.4. The 16S rRNA phylogeny of the rhizobacteria

The 16S rRNA gene sequence analysis showed the diversity of the 73 rhizobacteria isolates that were assigned to different genera with 92–100% similarity indices (Table 5). In this study, the 16S rRNA sequence confirmed that Proteobacteria (78.08%) and Firmicutes (21.92%) dominated the bacterial phyla isolated from the study site. The two phyla belonged to three major taxonomic classes, namely, Alphaproteobacteria, Bacilli and Gammaproteobacteria, where the latter was the most dominant (56.16%) of the other groups (21.92% each) (Figure 2).

In general, the rhizobacterial isolates were diversified into 11 genera that were dominated by the genus *Pseudomonas* containing 17 isolates, followed by *Ochrobactrum* (15 isolates; Figure 3). The genus *Morganella*, *Serratia*, *Bacillus* and *Enterococcus* consisted of 9, 8, 7 and 6 isolates, respectively (Figure 3, Table 5). These six genera constituted more than 85% of the population of the rhizobacteria recovered from degraded sampling sites (Table 5). Although the genus *Pseudomonas* was diversified into five species; *P. fulva*, *P. putida*, *P. protogens*, *P. fluorescens*, and *P. plecoglossicida*, the most dominant species was *P. fulva* that contained 75% of the population. The next dominant genus, *Ochrobactrum* was also diversified into *O. intermedium* and *O. anthropi*, where the former constituted more than 90% of the population.

The genus *Morganella* was the third most widely distributed group represented by a single species; *M. morgani* which showed the same

pattern with the genus *Enterococcus* that contained the only species; *Enterococcus gallinarum*. The Gram positive genera; *Bacillus* (*B. cereus*, *B. thuringensis*, and *B. anthracis*) and *Paenibacillus* (*P. odorifer* and *P. polymyxa*) were more diversified than the other dominant Gram negative genera, except *Pseudomonas* and *Serratia*, and the minor group *Klebsiella*.

The percentage distributions of each genera from the study site (Figure 2).

Based on the analysis of 16S-rRNA partial genes sequencing, the phylogenetic trees were constructed (Figures 4, 5, 6, and 7). Analysis of 16S rRNA genes similarity indices ranged from 99%–100%. The identity of Gram positive bacterial genera presented three families that ranged from 96% to 100% similarity indices. The isolate BS-45 had 96% similarity with *B. thuringiensis*, isolate BS-29 had 96% similarity with *B. cereus* and isolates BS-22, BS-32 and BS-37 had 100% similarity with *B. anthracis*. Likewise, *Paenibacillaceae* represented by *P. odorifer* and *P. polymyxa*. However, all the members of the lactobacillales order was represented by *E. gallinarum* with 99% similarity (Figure 4).

The genera *Agrobacterium* with 100% similarity with *A. tumefaciens*, and *Ochrobactrum* with 100% similarity were identified (Figure 5). Sequences of the isolates affiliated to *O. intermedium* ($n = 6$) were more polymorphic with 100% identity, while the isolates belonged to *O. ciceri* ($n = 8$) had 100% similarity. Generally, sequence similarity among *O. anthropi*, *O. ciceri* and *O. intermedium* was 98% identity (Figure 5).

In this study, *Pseudomonas* was the most dominant genus (Figure 6). Strain BS-19 grouped as *Pseudomonas fulva* with 96% similarity, while the majority of the strains were classified under *Pseudomonas para fulva* with 96% identity. Moreover, strain BS-26 fell under *Pseudomonas putida* with 96% similarity, while the strains FB-49 and RS-75 showed 98% similarity with *Pseudomonas fluorescens*. The strain BS-27 was another single genus which had 99% similarity with *Acinetobacter calcoaceticus* (Figure 6).

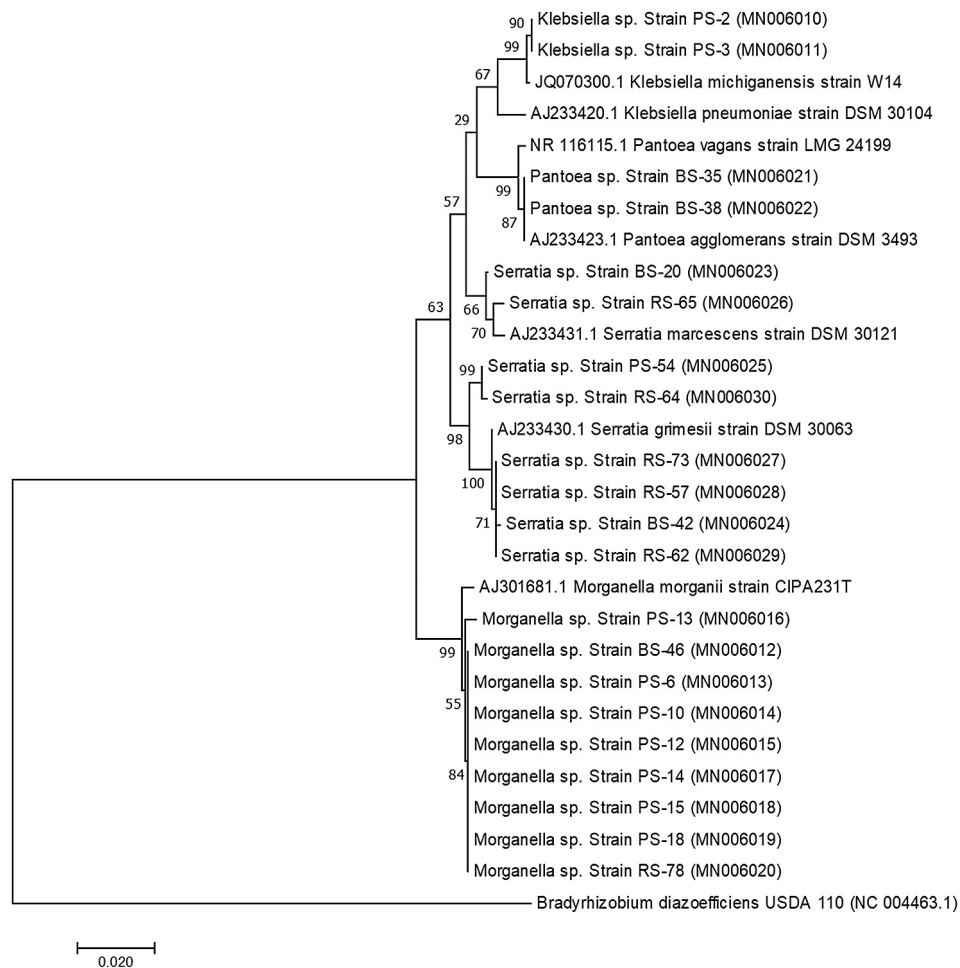


Figure 7. Phylogenetic tree of 16S rRNA gene sequences of Gram negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Bradyrhizobium diazoefficiens* USDA 110 species was used for out grouping.

The genus *Morganella*, *Serratia*, *Klebsiella* and *Pantoea* were also a Gram negative rhizobacterial groups (Figure 7). The genus *Morganella* is the third dominant genus in this study. Accordingly, all of the strains under the genus *Morganella* had 99% similarity with *Morganella morganii* (Figure 7). The isolates BS-20 and RS-65 grouped under *Serratia marcescens*. The remaining strains were classified under *Serratia grimesii* with 99% identity. Similarly, other genera of *Klebsiella* and *Pantoea* had similarity indices with *Klebsiella michiganensis* and *Pantoea agglomerans*, respectively (Figure 7).

4. Discussion

The rhizobacteria strains present high metabolic diversities and can utilize all the carbohydrates (26.67–100%) and fewer amino acids (14.28–100%). This indicates that these rhizobacteria showed a remarkable ecophysiological properties to utilize diverse biomolecules under highly nutrient deficient soil environment. The ability to metabolize various carbon and amino acid sources is an indication that these isolates have numerous enzymes to hydrolyze available biomolecules as energy source to survive under stressful habitat. This may play a significant role in the survival of the rhizobacteria to improve plant growth and yield even in hardy environments (Braga et al., 2018).

The ability of rhizobacteria to utilize diverse organic substrates can be considered as an important trait for rhizosphere competence in order to

make them a good candidate for development of inoculants (Nannipieri et al., 2003). Biomolecules exploitation can permit a greater insight into the ecology and metabolism of microbial species and fundamentally essential in determining the functionality of that particular environment (Deng et al., 2011). Metabolic diversity profiling showed a considerable diversity indices (Chojniak et al., 2015). There is a plethora of information on microbial diversity in a vast range of environments (Escalas et al., 2019).

In this study, the most differentiating DNA patterns for all rhizobacteria were obtained by using BOX - PCR that resulted in complex banding patterns, reflecting high degree of genotypic diversity among them (Menna et al., 2009). The taxonomic data showed that BOX-PCR polymorphism patterns have been effectively used for differentiation of bacterial strains (Louws et al., 1994).

In this study, the highest diversity index was recorded from *Bacillus* species that may indicate their ability to form resistant spores to adapt that particular degraded environment. Simpson's Index gives more weight to the more abundant species in a sample. A similar result of Simpson's Index of Diversity (D) of BOX-PCR (0.888) was reported for *Listeria* spp. and *Listeria monocytogenes* (Maurice Bilung et al., 2018). Moreover, the genotypic diversity in *Bacillus* spp. was reported using BOX PCR patterns (Köberl et al., 2011).

In this study, two major phyla and 11 genera of rhizobacteria were identified with 92–100% similarity indices and confirmed by lower E-

values. The genera *Pseudomonas* and *Ochrobactrum* were the dominant groups in the phylum Proteobacteria where the two genera constituted 44% of the total population of the rhizobacteria. On the other hand, Firmicutes constituted the genera *Bacillus* and *Enterococcus*. Some Gram positive genera of *Bacillus*, *Enterococcus* and *Paenibacillus* were characterized. The dominance of Proteobacteria is of great importance to global carbon, nitrogen and sulfur cycling in order to ensure sustainable biogeochemical cycling processes (Itävaara et al., 2016). The authors reported that Proteobacteria constitutes the largest and phenotypically most diverse and considered a dominant microbial clade.

Similar to this finding, Proteobacteria (25.10%) and Firmicutes (24.8%) reported as the most abundant microbes from the Taklamakan desert, in Asia (China). In another Asian desert, the Gobi desert, the dominance of Firmicutes (69.9 %) and Proteobacteria (12.2%) phyla was also reported (An et al., 2013). In the dry soil, *Ochrobactrum* spp. were the most abundant (79%), while *Bacillus* and *Paenibacillus* consisted 5% of the microbes (Köberl et al., 2011).

In contrast, in pine forest soil, 29.41% and 35.29% Proteobacteria and Firmicutes phyla were distributed (Flores-Núñez et al., 2018). *Pseudomonas* (six species) and *Bacillus* (four species) identified from wild *Coffea arabica*, while *Ochrobactrum* and *Serratia* were also identified as single species (Muleta et al., 2009).

A higher genetic divergence was evident in the *O. intermedium* than that of *O. anthropic*. On the basis of phenotypic characteristics, the genus *Ochrobactrum* could be related to the genera *Alcaligenes*, *Achromobacter*, or to the members of *Pseudomonadaceae*. However, molecular taxonomy places *Ochrobactrum* in the α -subgroup of proteobacteria that closely related to the genus *Brucella* (Velasco et al., 1998). Surprisingly, 16S rDNA-based phylogeny as well as protein profiling (Velasco et al., 1998) and AFLP analysis (Leal-Klevezas et al., 2005) placed *O. intermedium* strains closer to *Brucella* spp. than any other members of the genus *Ochrobactrum*.

Despite the fact that there is no generally accepted cut-off value for the bacterial species delineation, a 97% similarity level in 16S rDNA has been proposed for consideration (Stackebrandt and Geobel, 1994). According to this value, *O. anthropic* and *O. intermedium* were not separated. Although, *Ochrobactrum intermedium* is currently reported as opportunistic pathogen in humans (Teyssier et al., 2005), there are some reports on the presence of *Ochrobactrum* spp. from different environments including soil (Huber et al., 2010) and the rhizosphere and in internal root tissues of different plants (Trujillo et al., 2005). Some nodulating species of *Ochrobactrum* spp. have been described from nodules on *Acacia* (Ngom et al., 2004) and *Lupinus* (Trujillo et al., 2005). *O. intermedium* increased seed germination, root and shoot length, and grain yield in lentil (*Lens esculenta*) (Faisal, 2013). The first plant promoting roles of *O. intermedium* spp. was reported as it increased the peanut shoot and root height as well as dry weight (Paulucci et al., 2015). Moreover, *in vitro* studies confirmed that *Ochrobactrum* spp. and others were the most important isolates to act as potential biofertilizers, biocontrol agents or both (Muleta, 2007).

In this study, some strains of *Morganella* were characterized from the degraded soil. Previous study showed that an endophytic *M. morganii* was reported to be effective when applied to the seeds with significantly higher plant growth promotion than the control (Shiomi, 2007). This may be associated with gene encoding for acid phosphatases. In earlier investigations, several acid phosphatase genes have been isolated and characterized from Gram negative bacteria (Rossolini et al., 1998). For example, the *acpA* gene isolated from *Francisella tularensis* expressed an acid phosphatase with optimum action at pH 6 with a wide range of substrate specificity (Reilly et al., 1996). Similarly, the *napA* phosphatase gene from the soil bacterium *M. morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as a biofertilizer using the broad-host range vector pRK293 (Fraga et al., 2001). Generally, the current study showed degraded soil could harbor metabolically and genetically diverse rhizobacteria. This could help to adapt harsh environments and involve in

plant growth promoting activities with an implication for potential source of inocula development.

5. Conclusion and recommendation

Degraded soil harbored metabolically diverse rhizobacterial genera of *Ochrobactrum* and *Pseudomonas* as the dominant microbes. BOX-PCR showed a better discriminatory power and differentiating DNA patterns for all strains and revealed high genotypic diversity. Based on the genotyping analysis, PGPR isolates were heterogeneous with high index of genetic diversity. Hence, these genetically and metabolically diverse rhizobacteria are potential biotools to be used for rehabilitation of degraded lands upon inoculation at field conditions.

5.1. Limitations of the study

In this study, we only explored the phenotypic diversity with limited carbon and nitrogen sources due to lack of standard kits. Similarly, the genetic assessment of rhizobacteria needs to be supported by whole genome analysis.

Declarations

Author contribution statement

Alemayehu Getahun: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Solomon Kiros, Diriba Muleta, Fassil Assefa: Conceived and designed the experiments.

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Data availability statement

Data associated with this study has been deposited at GenBank under the accession numbers MN005961-MN006030.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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