ORIGINAL ARTICLE

Diversity and Plant Growth Promoting Properties of Rhizobacteria Isolated from tef (Eragrostis tef)

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Abstract

The purpose of this study was to evaluate and assess the plant growth promoting characteristics and diversity of major tef rhizosphere isolates from central Ethiopia. A total of 162 bacteria were isolated from rhizosphere of tef [Eragrostis tef (Zucc.) Trotter] and characterized. While screening using some plant growth promoting characteristics, only about sixty six of the isolates were found to show some characteristics of plant growth promoting rhizobacteria (PGPR) and hence selected for further study. The sixty six isolates were further characterized by several morphological, physiological and biochemical tests and identified to species level using API kit method. Numerical analysis of phenotypic data by a computer cluster analysis using UPGMA (NTSYSpc software version 2.1) was done based on carbohydrate fermentation profile. The isolates displayed diverse morphological, physiological, biochemical and PGPR characteristics [(Phosphate solubilization (52%), antmicrobial property (41%) and HCN production (17%)] in addition to tolerance to a wide range of pH by most of the isolates. The 66 isolates were found to belong to 7 genera and 15 species; two species with each of two sub-species. Analysis of phenotypic data revealed that at boundary levels of 50% average similarity, two major clusters were formed by both Gram-negative and positive isolates while the respective groups form six and seven clusters at about 75% and 78.4% average similarity. The fact that the tef rhizosphere bacteria to be positive for various PGPR characteristics suggests that the isolates have better potential for greenhouse and field testing and application in improving yield of tef.

Key words/phrases: API kit, Bacteria, Ethiopia, P solubilization, Screening of PGPR

INTRODUCTION

Ethiopia is both the origin and major diversity center of tef [*Eragrostis tef* [Zucc.) Trotter]. Tef is a fine stemmed tufted annual grass and produces small seeds (Seyfu Ketema, 1997). The plant is the most popular crop has high demand by consumers.

However, the yield of this crop is still low regardless of several breeding attempts and release of varieties such as DZ-Cross-37 and DZ-01-974 (Solomon Zewdie et al., 2000). Furthermore, the ever-increasing cost of such agricultural inputs is also becoming neither available nor affordable to most Ethiopian poor farmers that are struggling to improve food security and their living standard (Woyessa and Assefa, 2011). In conventional agricultural practices, the increased use of agrochemicals including pesticides has also led to the frequent contamination of cultivated soils. These chemicals may also adversely affect the rhizospheric organisms including PGPR and associated biotic processes, which are governed by the rate of application, the activity spectrum of the pesticides and the persistence and availability of chemicals (Moorman 1989, Srinivas et al. 2008). Such excessive use of chemicals and change in traditional cultivation practices has resulted in the deterioration of physical, chemical and biological health of the cultivable soil. Hence, microbial diversity in soil is considered important for maintaining for the sustainability of production systems in agriculture (Joshi and Bhatt, 2011). Though the links between microbial diversity and ecosystem processes is not well understood (Stark, 2007), microbial diversity in soil is considered important for maintaining for the sustainability of agriculture production systems.

Therefore, these problems necessitate study of microbial diversity as well as looking for additional, environmentally friendly potential options such as phyto-beneficial rhizosphere microorganisms as well.

The use of rhizosphere microorganisms particularly, plant growth promoting microorganisms as inoculum to boost production of the crop could be one of the potential alternatives. It is also evident that the use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system (Sturz *et al*., 2000; Shoebitz *et al*., 2009).

Several attempts are currently undertaken to manipulate soil microorganisms to improve cereal production. These microorganisms are capable of adding more nitrogen (Çakmakçi *et al*., 2006), providing plant growth promoting metabolites to enhance growth (Gutierrez *et al*., 1996). Some of them produce iron scavenging compounds to improve iron nutrition to the roots of plants, and release secondary antagonistic organic compounds to protect plants from root disease (Lucy *et al*., 2004). These microorganisms are generally known as plant growth promoting rhizosphere rhizobacteria (PGPR). Plant growth promoting rhizobacteria (PGPR) accounts for about 2-5% of total the rhizobacteria involved in plant growth promotion (Antoun and Kloepper, 2001). Such PGPR use one or more direct or indirect mechanisms to improve the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth. Among these, P solubilization, biological nitrogen fixation, improvement of other plant nutrients uptake, and phytohormone production like, indole-3-acetic acid are some of the regulators that profoundly influence plant growth (Zaidi *et al*., 2009).

Isolation and identification of rhizosphere microorganisms is important for proper utilization of their beneficial effects to increase plant growth in general and tef in particular. In Ethiopia, only few studies on tef root-associated microorganisms have been undertaken. Accordingly, effects of PGPR on growth and yield of tef was evaluated by

Woyessa and Assefa (2011). Tekalign Mamo (1984) investigated the effect of vesicular arbuscular mycorrhizal (VAM) fungi (*Glomus fasiculatum*) on mineral content of tef. The effect of phosphate solubilizing fungus on growth and yield of tef was studied by Asfaw Hailemariam (1993) and effect of *Azosprillum* bacterial isolates on the growth and nitrogen content of tef was undertaken by Solomon Zewdie *et al*., (2000). However, information on the diversity and PGPR properties of rhizosphere microorganisms is scanty. Therefore, this study was initiated to assess PGPR characteristics and diversity of isolates from tef roots.

MATERIALS AND METHODS

Description of the study area, sample collection and isolation of rhizobacteria

Soil samples were collected from four sites agricultural fields of: Alemgena (8° 55' 0" North, 38° 39' 0" East) which is located about 15 km south west of Addis Ababa, Sebeta (8° 55' 0" North, 38° 37' 0" East), that is found about 25 km south west of Addis Ababa, Addis Ababa (Gergi and CMC areas) and Debre Zeit (9° 6' 0" North, 37° 15' 0" East) about 40 km south east of Addis Ababa). The soil samples were immediately transported to the Addis Ababa University, Applied Microbiology laboratory for further study.

Soils from the rhizosphere of tef were carefully removed and bulked together. The larger particles were removed using 2mm sieve. The roots were then thoroughly washed with sterile distilled water to remove as much PGPR as possible. Ten gram of each soil sample was mixed separately in 90 ml of root-washed water in150 ml flasks. Then, a series of dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were made by transferring 1 ml from the flasks into 9 ml sterile distilled water after vortexing the diluents in each test tube. From dilutions of 10^{-4} and 10^{-5}, 0.1 ml suspensions were spread on to pre solidified nutrient agar plates and incubated at 30 ± 2°C for 48 hr.

Representative colonies of all morphological types were separately picked at random and subcultured on nutrient agar media for purification. The purified cultures were maintained on nutrient agar slant at 4°C and preserved in 80% glycerol at –22°C for subsequent experiments. All the cultures were checked for purity for each experiment.

Screening of the isolates *Screening phosphate solubilizing isolates*

All the isolates were tested *in vitro* for their phosphate solubilization activity following the method described by Donate-Correa *et al*. (2004) on Pikovskaya agar medium containing the ingredients (g^{-1}): Glucose (5), $Ca_3(PO_4)_2$ (5), $(NH_4)_2SO_4$ (0.5), Yeast Extract (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.1), $MnSO_4$ (0.002), $FeSO_4$ (0.002) and Agar (15).

The medium was autoclaved at 121°C for 15 minutes. About 20 ml of the molten agar medium was poured into each petridish and allowed to solidify before inoculating the isolates. A 24 hr broth culture was spot inoculated on the petridishes in triplicate using sterile loop and incubated at 30 ± 2°C for 5-7 days. Bacterial colonies that formed clear zones (haloes) were considered as phosphate solubilizers and clear zone diameters were measured in cm.

Screening for cyanide production

Hydrogen cyanide (HCN) production from glycine was tested following the procedure of Lorck (1948). Tryptic soya agar (TSA) supplemented with glycine (4.4 g^{-1}) was prepared and autoclaved. A loop full of 24 hr old broth culture of the isolates was inoculated to the plates. Sterile filter paper strips soaked with 0.5% (w/v) picric acid were fixed to the underside of the petridish lids. The plates were then sealed with parafilm and

incubated for 5-7 days at 30°C. An uninoculated plate and an inoculated plate without picric acid impregnated papers were used as controls. A change in the color of the filter strips from yellow to brown or reddish-brown was regarded as indication of cyanogenic potential.

Screening for antimicrobial property

This test was done using a modified method of Brinkhoff *et al*. (2004). A reference test organism, E. *coli* ATCC 25922 was obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI). A nutrient broth 8.0 (g^{-1}) and NaCl, 5.0 (g^{-1}) was prepared in two 250 ml flasks and autoclaved for 15 minutes at 121°C. One of the flasks was used for control to adjust optical density (OD) reading and the other was used for inoculation of the test organism. A loopful (about 10^6 cfu) of the test organism was inoculated to the flask and incubated at 37°C in a Gallenkamp orbital shaker at 120 rpm until the OD_{600} nm reached 1.3 (the control flask was kept at 4°C and used for calibration of the spectrophotometer.

All isolates to be tested for antibiotic production were separately incubated up to their stationary phases (36-48 hrs) at 30°C in a sterile 5 ml nutrient broth. Plates with sterile medium containing (g^{-1}) nutrient broth (8.0), NaCl (5.0) and agar (15.0) were prepared and overlaid with 0.7% top agar containing 1% cell suspension of the test organism (*E.coli*). Sterile blank antibiotic discs of 6 mm (Aldrich Chem. Co., USA) were soaked with stationary phase culture broth of the soil isolates to be tested for antibiotic production and put on the plates at four corners, the un-soaked being placed at the center for control purpose. The plates were then incubated at 37°C for 3-5 days to check for inhibition zones.

Characterization and identification of isolates

Morphological and cultural features of colonies were determined using King's B agar medium (King *et al*., 1954) containing (g^{-1}) Proteose peptone No. 3 (20), K_2HPO_4 (1.5), $MgSO_4.7H_2O$ (1.5), Agar (15), Cyclohexamide (0.1), Ampicillin (0.05), CAF (0.0125) and Glycerol (10 ml). Gram reaction of the isolates was determined by using 3% potassium hydroxide test (Suslow *et al*.,1982) and spore staining was done for Gram-positive isolates using Wirtz-Conklin method indicated by Tiwari *et al*. (2004). Catalase (Collins and Lyne, 1970) and Cytochrome oxidase (Kovacs, 1956) tests were done.

Growth of the isolates at different pH values (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 8.5, 9.0, 9.5 and 10.0) on nutrient agar medium was assessed. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH before autoclaving. Each of the sterile nutrient agar plate was streaked with a loop full of 24 hr old culture grown in nutrient and incubated at 30 ± 2°C. Duplicate plates were used for each isolate. For control plates, the pH was adjusted at 7.0. Growth was finally recorded qualitatively as (+) for growth or (-) for no growth.

Biochemical identification

Isolates were identified to species and sub species level according to Analytical Profiles Index, API 20 NE and API 50 CHB kits (bio-Mérieux, Marcy, I'tole, France) for Gram-negatives and Gram-positives, respectively following the manufacturer's instructions. The results were analyzed using API software package (APIWeb, Version-1.1.0).

Numerical analysis

Using data from carbohydrate fermentation profile of isolates, phenotypic dendrogram was constructed based on unweighted-pair group method

with average, UPGA method of clustering using NTSYSpc software (version 2.1). For cluster analysis, positive results of carbohydrate fermentation profiles of isolates were converted to '1' and negative results into '0'.

RESULTS

Isolation, screening, characterization and identification of PGPR from tef rhizosphere

Morphological and cultural characteristics

The characterized tef rhizosphere isolates (a total of 162 bacterial isolates) showed diverse morphological and cultural characteristics as indicated from variations in colony size and shape. On the basis of their Gram reaction, 94 (58%) of the isolates were found to be Gram positive and about 68 (42%) were Gram negative bacteria. All the Gram negative bacteria were rod-shaped, non-spore forming; where as the Gram positive bacteria were rod-shaped, except isolate AURB54 (*Bacillus megaterium* 1) that displayed a coccoid shape. Like wise 67 (71%) of the Gram-positive isolates were found to be spore formers with oval, cylindrical or ellipsoidal spores, where as 27 (29%) did not show spore-forming ability (Table1).

Table 1. Some cellular and morphological characteristics of tef rhzosphere isolates

Isolate code	Gram reaction	Shape	Spore
AURB1- AURB15	Gm -	Rod	-
AURB16- AURB40	Gm-	Rod	-
AURB67- AURB94	Gm-	Rod	-
AURB41- AURB50	Gm+	Rod	+
AURB50	Gm+	Rod	-
AURB51- AURB53	Gm+	Rod	+
AURB54	Gm+	Cocci	+
AURB55- AURB66	Gm+	Rod	+
AURB122- AURB162)	Gm+	Rod	+
AURB95- AURB121	Gm+	Rod	-

(+) = positive for the test
(-) = negative for the test
(Gm+) = Gram positive, (Gm-) = Gram negative

Screening of isolates based on PGPR and biochemical properties

The isolates were reduced to 66 (40 Gram negative isolates and 26 Gram positive isolates) based on screening for three PGPR properties: P-solublization, antibiotic production and HCN production (Table 2). Some of the clear zone formation by P-solubilizers on Pikovskaya agar medium, inhibition zone by antimicrobial activities against the test organism (*E. coli* ATCC 25922) and HCN production from glycine were

indicated. Consequently, about 52% of the 66 isolates were found to be phosphate solubilizers. Furthermore, about 41% of them showed antimicrobial property against the test organism and about 17% were found to produce HCN.

All tef rhizosphere isolates were found to be catalase positive where as only 31 (47%) were oxidase positive, the majority of which (29%) being Gram negative isolates (Table 2).

Table 2. Some PGPR and biochemical properties of tef rhizosphere isolates

Isolate	PGPR property of the isolates							Biochemical property			
	P-solubilization			Antibiotic production		HCN production		Catalase +		Oxidase +	
	No.	%	Clear zone (cm)	No.	%	No.	%	No.	%	No.	%
AURB1- AURB40	20	50%	0.1-0.5	13	33%	9	23%	40	100%	19	48%
AURB41- AURB49	5	56%	0.1-0.4	4	44%	0	0	9	100%	3	33%
AURB50	1	100%	0.1	0	0	0	0	1	100%	0	0
AURB51- AURB52	0	0	0	2	100%	0	0	2	100%	1	50%
AURB53- AURB55	1	33%	0.4	2	67%	1	33%	3	100%	1	33%
AURB56-AURB66	7	64%	0.1-0.4	6	55%	1	9%	11	100%	7	64%
AURB67-AURB162	0	0	0	0	0	0	0	-	-	-	-
Total positive (out of the 66 isolates)	34	52%	-	27	41%	11	17%	-	100%	31	47%

(-) = Isolates with no PGPR property were not tested for catalase and oxidase

Characterization and Identification of the isolates

Based on biochemical test and API (20NE and 50 CHB) kit method, identity of the 66 isolates was as presented in Table 3. The most diverse bacterial groups were represented by the genera: *Bacillus* with 8 species (each of *B. megaterium* and *B. cereus* with two sub species), *Pseudomonas* with two species and each of the remaining five genera were represented only by one species. As far as the species frequency is concerned, *Pseudomonas fluorscens* represented 26% of all the isolates followed by *Chryseomonas luteola* (17%), *Bacillus licheniformis* (8%) and *Pseudomonas putida* (7%). Like wise, each of the remaining four species (*Aeromonas hydrophila*, *Bacillus cereus* 2, *Bacillus subtilis* and *Bacillus sterothermophilus*) was represented by only 2% of the isolates.

Table 3. Taxonomic diversity of the isolates based on API kits

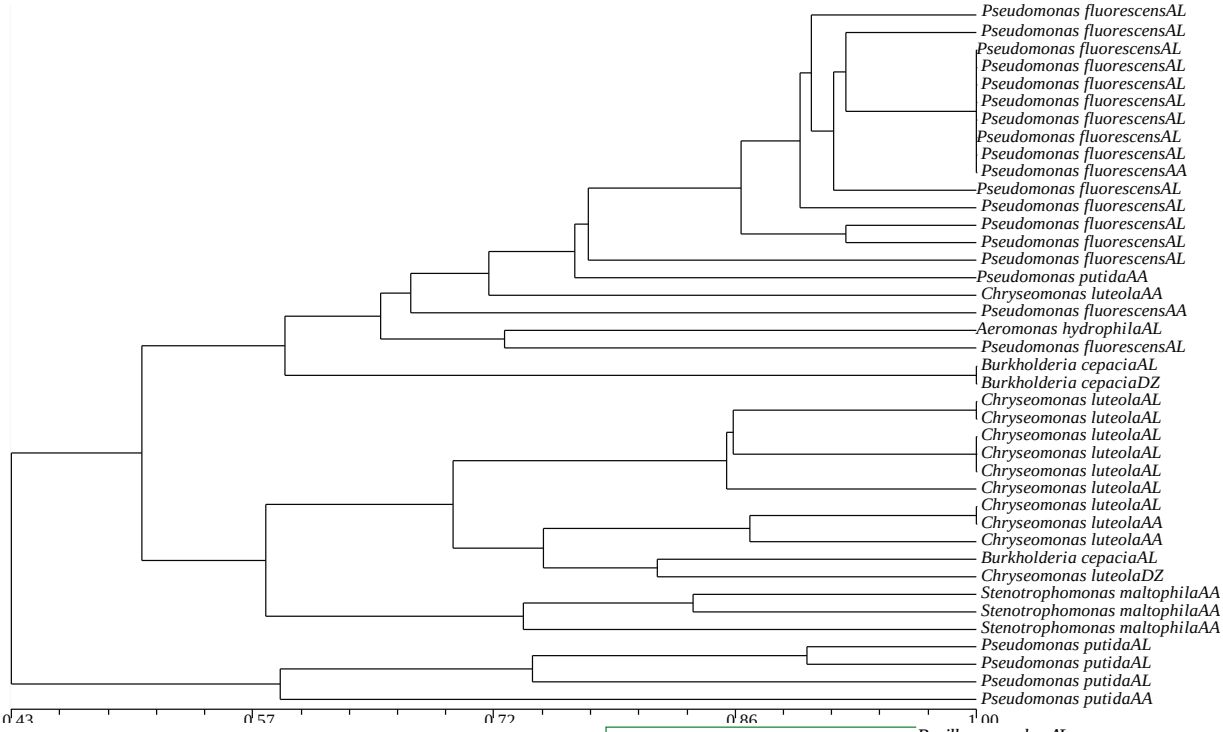
Isolates	Number of isolates	Genus	Species/subspecies	Density (%)
AURB1- AURB17	17	*Pseudomonas*	*Pseudomonas fluorscens*	26
AURB18- AURB28	11	*Chryseomonas*	*Chryseomonas luteola*	17
AURB29- AURB33	5	*Pseudomonas*	*Pseudomonas putida*	7
AURB34- AURB36	3	*Burkholderia*	*Burkholderia cepacia*	4
AURB37- AURB39	3	*Stenotrophomonas*	*Stenotrophomonas maltophila*	4
AURB40	1	*Aeromonas*	*Aeromonas hydrophila*	2
AURB41- AURB45	5	*Bacillus*	*Bacillus licheniformis*	8
AURB46- AURB49	4	*Bacillus*	*Bacillus firmus*	6
AURB50- AURB52	3	*Brevibacillus*	*Brevibacillus brevis*	4
AURB53- AURB55	3	*Bacillus*	*Bacillus megaterium* 1	4
AURB56- AURB57	2	*Bacillus*	*Bacillus coagulans*	3
AURB58- AURB59	2	*Bacillus*	*Bacillus cereus* 1	3
AURB60- AURB61	2	*Bacillus*	*Bacillus pumilus*	3
AURB62- AURB63	2	*Bacillus*	*Bacillus megaterium* 2	3
AURB64	1	*Bacillus*	*Bacillus cereus* 2	2
AURB65	1	*Bacillus*	*Bacillus subtilis*	2
AURB66	1	*Bacillus*	*Bacillus sterothermophilus*	2
Total	**66**	**7**	**17**	**100%**

Numerical *analysis*

The degree of similarity of PGPR isolated from tef rhizosphere was analyzed based on results from API 20 NE kit (for Gram negatives) and API 50 CHB kit (for Gram positives) (Fig. 1, a and b). At 50% average similarity, about two major clusters and one minor cluster were recognized (Fig. 1a). One of the major clusters contained more than 50% of the isolates analyzed by API 20 NE and the second major cluster included 14 of the 40 Gram negative isolates. The minor cluster contained only 4 isolates, all of which are *P. putida*. At a boundary level of about 75% similarity, six clusters of Gram negatives were recognized and three isolates (one *P. putida*AA, *B. cepacia*AL and *B. cepacia*DZ) did not form any cluster, the latter two showing 100% similarity.

Two major clusters were recognized at a boundary level of 50% average similarity based on API 50 CHB analysis for Gram positive tef rhizosphere isolates (Fig. 1b). One of the major clusters contained 14 of the 26 Gram positive isolates and the second cluster contained the remaining 12 isolates, which did not include any isolate from Addis Ababa (AA) while the former included some isolates from all the collection areas, Addis Ababa (AA), Alemgena (AL), Sebeta (SB) and Debrezeit (DZ). *Bacillus megaterium*2 DZ did not form cluster beyond 64% level of average similarity and became distinct.

At about 78.4% similarity, all of the Gram positive isolates were grouped into seven clusters except *Bacillus megaterium*2 DZ and *Bacillus pumilus* DZ, which were not clustered with any other isolates. An average similarity of 100% was observed only between two *Bacillus firmus* SB isolates and between *Bacillus megaterium*1 AL and *Bacillus stearothermophillus* AA at a boundry level of 97% average similarity.


Pseudomonas fluorescensAL
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Pseudomonas fluorescensAA
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Pseudomonas fluorescensAL
Pseudomonas fluorescensAL
Pseudomonas fluorescensAL
Pseudomonas putidaAA
Chryseomonas luteolaAA
Pseudomonas fluorescensAA
Aeromonas hydrophilaAL
Pseudomonas fluorescensAL
Burkholderia cepaciaAL
Burkholderia cepaciaDZ
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAL
Chryseomonas luteolaAA
Chryseomonas luteolaAA
Burkholderia cepaciaAL
Chryseomonas luteolaDZ
Stenotrophomonas maltophilaAA
Stenotrophomonas maltophilaAA
Stenotrophomonas maltophilaAA
Pseudomonas putidaAL
Pseudomonas putidaAL
Pseudomonas putidaAL
Pseudomonas putidaAA
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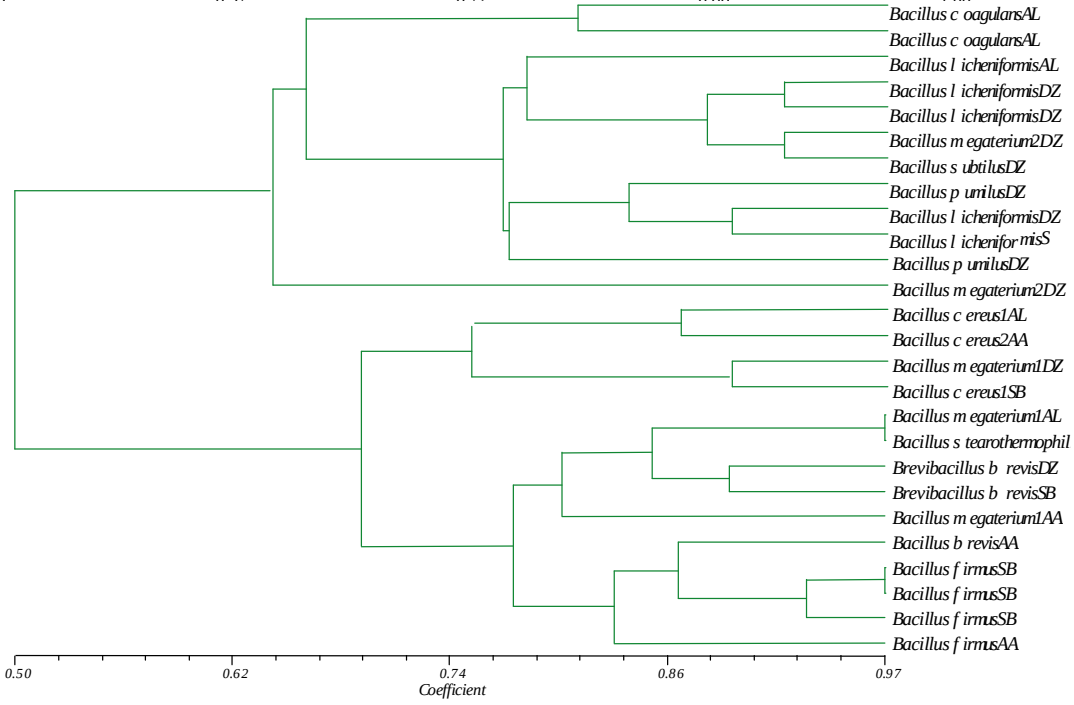
0.57
0.72
0.86
1.00


Bacillus coagulansAL
Bacillus coagulansAL
Bacillus licheniformisAL
Bacillus licheniformisDZ
Bacillus licheniformisDZ
Bacillus megaterium2DZ
Bacillus subtilusDZ
Bacillus pumilusDZ
Bacillus licheniformisDZ
Bacillus licheniformisS
Bacillus pumilusDZ
Bacillus megaterium2DZ
Bacillus cereus1AL
Bacillus cereus2AA
Bacillus megaterium1DZ
Bacillus cereus1SB
Bacillus megaterium1AL
Bacillus stearothermophil
Brevibacillus brevisDZ
Brevibacillus brevisSB
Bacillus megaterium1AA
Bacillus brevisAA
Bacillus firmusSB
Bacillus firmusSB
Bacillus firmusSB
Bacillus firmusAA
0.50
0.62
0.74
0.86
0.97
Coefficient

a

b

Figure 1. Dendrogram derived from carbohydrate fermentation study

a) Gram negative tef rhizosphere isolates using API 20 NE kit b) Gram positive tef rhizosphere isolates using API 50 CHB kit. AA= Isolate from Addis Ababa, AL= Isolate from Alemgena, SB= Isolate from Sebeta, DZ= Isolate from Debre Zeit

All isolates grew in the pH ranges of 6.0-8.5 and most of the isolates managed to grow at alkali pH (9) (91%) and acidic pH (5.5) (95%). About 37 (56%) of the isolates were found to tolerate and grow at the lowest (4.5) pH level and 55 (83%) of them tolerated the highest (10.0) pH levels tested in this experiment (Table 4). All isolates of the genus *Pseudomonas* were found to grow in the pH

range of 4.5 to 10.0, except two isolates that failed at pH value of 4.5. All isolates of 4 species (*Bacillus megaterium* 1, *Bacillus coagulans*, *Bacillus cereus* 1 & *Bacillus cereus* 2 and *Bacillus pumilus*) tolerated and grew from the pH range of 4.5 to 10.0.

Isolates of *Stenotrophomonas maltophila* and *Aeromonas hydrophila* were found to grow only at pH values of 5.5-8.0 and 6.0-9.0, respectively. Whereas all isolates of *Chryseomonas luteola* and *Bacillus sterothermophilus* tolerated 5.5 to 10.0 pH values.

Table 4. Growth of tef rhizosphere isolates at different pH levels.

Species of isolates	No. of isolates	Number of positive isolates at specific pH level						
		pH level						
		4.5	**5.0**	**5.5**	**6.0-8.5**	**9.0**	**9..5**	**10**
Pseudomonas fluorscens	17	+ (14)	+(17)	+ (17)	+ (17)	+ (17)	+ (17)	+ (17)
Chryseomonas luteola	11	-	-	+ (11)	+ (11)	+ (11)	+ (11)	+ (11)
Pseudomonas putida	5	+ (4)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)
Bacillus licheniformis	5	+ (5)	+ (5)	+ (5)	+ (5)	+ (3)	+ (3)	+ (1)
Bacillus firmus	4	+ (2)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
Burkholderia cepacia	3	-	-	+ (1)	+ (3)	+ (3)	+ (3)	+ (3)
Stenotrophomonas maltophila	3	-	-	+ (3)	+ (3)	-	-	-
Brevibacillus brevis	3	-	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)	+ (2)
Bacillus megaterium 1	3	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)	+ (3)
Bacillus coagulans	2	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+(2)
Bacillus cereus 1	2	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
Bacillus pumilus	2	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
Bacillus megaterium 2	2	+ (1)	+ (1)	+ (2)	+ (2)	+ (2)	+ (1)	+ (1)
Aeromonas hydrophila	1	-	-	-	+ (1)	+ (1)	-	-
Bacillus cereus 2	1	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)
Bacillus subtilis	1	+ (1)	+ (1)	+ (1)	+ (1)	-	-	-
Bacillus sterothermophilus	1	-	-	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)
Total No.	**66**	**37**	**46**	**63**	**66**	**60**	**58**	**55**
% at specific pH level		56%	69%	95%	100%	91%	88%	83%

(+) = growth and (-) = no growth

Numbers in parenthesis show number of positive isolates at a given pH level

DISCUSSION

Most of the isolates were rod-shaped and dominated by Gram-positive bacteria.

Based on PGPR screening, about 52%, our isolates were P solubilizers. Whereas, 41% and 17% of our isolates showed antimicrobial activity and cyanide production, respectively. However, there was a notable

difference in the degree of phosphate solubilization as indicated by the diameters of clear zone formed by the various isolates. Phosphate solubilization, antibiotic and cyanide production have been reported to be among the mechanisms by which microorganisms promote plant growth (Lucy *et al*., 2004; Tilak *et al*., 2005), supporting the potential of our isolates to be used for further evaluation on plants.

The identification of our isolates using API kit based on carbohydrate utilization showed that tef rhizosphere contains a diverse flora of microorganisms. Consequently, they were classified into 7 genera and 15 species. The genera were: *Pseudomonas*, *Chryseomonas*, *Burkholderia*, *Bacillus*, *Brevibacillus*, *Stenotrophomonas* and *Aeromonas.* All the Gram-negative bacteria were slightly lower in terms of diversity, though they represented about 60% proportion of the total identified rhizosphere bacterial isolates. However, the diversity study in this study is only based on API kit method and other few morphological and biochemical tests and hence may not necessary reflect the true picture of tef rhizosphere bacteria.

All species of *P. fluorescens* in this experiment were included in the largest cluster irrespective of where they were collected (AA or AL) as in the case of the minor cluster, though one *P. putida* was

not included suggesting that they are very much related in their carbohydrate fermentation profile. Such result was explained by Kumar *et al*. (2002) as organisms having similar properties may share some genetic relationship among themselves irrespective of geographical location. Complete (100%) similarity was observed within 8 *P. fluorescens*; 2 *Burkholderia cepacia*; 2 *Chryseomonas luteola* species at each of two different clusters and 3 *Chryseomonas luteola* species at another cluster. The Gram-positive isolates of tef rhizosphere were more diverse in carbohydrate fermentation as compared to the Gram-negative isolates.

Based on extrapolation of the 66 identified isolates to the PGPR properties, it was revealed that the majority (71%) of phosphate solubilizers were Pseudomonas and Bacillus and about 63% of the total HCN producers were Pseudomonas fluorscens. Tilak *et al*. (2005) reported Pseudomonas and Bacillus to be the main phosphate solubilizer bacteria. Similarly, the genus Pseudomonas has been described for its production of a wide spectrum of antibiotics, siderophores and other secondary metabolites (Raaijmakers *et al*., 1997; Smirnov *et al*., 1997; Shtark *et al*., 2003). Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of a chemical fertilizer, a large portion of soluble inorganic

phosphate applied to the soil is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Microorganisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle. Hence, the finding of the present study demonstrated that a diverse group of potential phosphate solubilizing bacteria are associated with tef rhizosphere and could serve as efficient biofertilizer candidates for improving the P-nutrition of crop plants

Soil pH is among the obvious influencing factors of microbial activity and populations as revealed by several studies and hence can ultimately influence plant growth significantly. The growth of most of our tef rhzosphere isolates over a wide range of pH including acidic pH values suggests that there is a potential to inoculate them under different soil pH conditions if other conditions could not significantly affect their performance. It is evident that there is high rainfall in tropics including Ethiopia. Previous studies indicate that such high rainfall results in an increase of acidity-related soil toxicity factors in many tropical soils (Woomer, *et al*., 1988). Such increase in soil moisture ultimately results in a less favorable, low-pH environment which is disadvantageous to crops such as legumes as reported by (Hosaka and Ripperton, 1944) and similarly, Keyser and Munns (1979) reported that the low pH also decreases rhizobial persistence. Curl and Truelove (1986) have reported that the growth of large number isolates at acidic pH values could probably be because of their adaptation to pH of soil near roots that is usually assumed to be acidic due to CO2 and organic acid. As pH is one of the major environmental stresses that limits crop production by affecting plant function (Rhoades and Loveday, 1990), isolation and characterization of PGPR adapted to diverse soil pH conditions is of paramount importance. Currently, crop tolerance of low soil pH has become extremely important in the agricultural development of the humid tropics because so many of those soils have low pH (Kamprath and Foy, 1985). Hence, the fact that most of our isolates to be tolerant to diverse pH indicates significant positive implication regarding their adaptation particularly to acidic tropical soils including that of Ethiopia to increase crop tolerance and performance. Hence, it will be important to test the performance of our isolates at greenhouse condition, which will to ultimately enable as further evaluation of the most promising candidates at field conditions.

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