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Characterization and Identification of Tef (*Eragrostis tef*) Seed Endophytic Bacterial Species and Evaluate their Effect on Plant Growth Promotion

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Abstract

In the present day there is no report on characterization and identification of bacterial endophytes isolated from tef (*Eragrostis tef*) seeds. This study was conducted to screen, identify, and characterize bacterial endophytes isolated from tef seeds germplasm repository and determine if the bacterial provided plant growth promotion. Nine endophytic bacterial species were identified from 83 tef seed accessions using a Biolog microbial identification system, which utilize 95 different carbon sources. Eight of the identified bacterial species could produce amylase, seven of the species could solubilize phosphate and six of the bacteria could degrade cellulose. All the bacteria were shown to enhance growth of wheat (*Triticum aestivum*) under laboratory condition. *Pseudomonas stutzeri, Rhizobium radiobacter, Bacillus butanolivorans*, *Pseudomonas putida biotype B, Enterobacter cowanii*, *Pantoea dispersa, Enterobacter cloacae ss dissolvens*, *Serratia ficaria* and *Pantoea agglomerans* significantly increased mean root dry mass of inoculated *Triticum aestivum* up to 9.8%, 9.3%, 8.1%, 7.9%, 7.7%, 7.5%, 7%, 6.9% and 5.5% respectively and increase the mean shoot dry mass of *Triticum aestivum* up to 29%, 25%, 23%, 26%, 23%, 20%, 22% and 19% respectively. In addition, several seed endophytic bacterial species exhibited tolerance to salinity up to 6% and only *Bacillus butanolivorans* tolerated salinity up to 15% and temperature up to 60°C, this suggested the potential for possible application under stressed environmental conditions and possible utility as bioinoculant for maintaining sustainable agricultural production and productivity without affecting human health.

Keywords: Abiotic; Biolog; Endophytes; Seed; Stress and Tef

Introduction, Background and Justification

Tef (*Eragrostis tef*) is an indigenous tropical cereal crop of Ethiopia and it has been cultivated for thousands of years in Ethiopian high lands [1]. It is a daily staple and co-staple food for about fifty millions of Ethiopian people. Tef nutritionally contains 72.1%-75.2% carbohydrate, 14%-15% proteins, 3% fiber, 2% fat and its mineral contents i.e., 11 mg -33 mg iron, 100 mg -150 mg calcium, and also rich in potassium and phosphorous. Shahidur [2] indicated that tef has strong inseparable cultural and traditional ties for more than ninety millions of Ethiopian people. Its grain is free from gluten and is an increasingly important dietary component for individuals who suffer from celiac disease [3]. Tef seeds, like other plant organs such as root, stem, leaf and flower, harbor endophytic microorganisms [4], which have significant role on plant growth promotion and plant health.

Endophytes are microorganisms that survive and colonize internal tissues of host plants and do not cause visible harm [5]. They may be transferred directly from parent to progeny through seeds or plant to plant by entering the plant tissue through root zone or aerial portions such as flowers, stems and cotyledons [6]. Seeds acquire their microbiome by three major pathways:

(i) Internal transmission through the vascular system,

(ii) Floral transmission by the stigma and

(iii) External transmission via contact of the seed with microorganisms present on fruits, flowers [7].

Seed endophytes offer a wide range of benefits to plants such as growth promotion [8], production of anti-biotic compounds [9], nutrition acquisition [10], induction of plant defense system and tolerance to biotic and a biotic stress [11]. Besides, they produce excess number of secondary metabolites of potential application in medicine, agriculture, and industry [12]. And also play a role in the preservation and germination of the seed [13].

Seed endophytes have ability to produce lytic enzymes such as amylase in order to utilize starch and resume growth after long-term survival inside the seeds [14]. Some seed endophytes were also reported to be able to use phytate, which is the main storage form of phosphorus in seeds, as a source of phosphate [15]. Johnston-Monje and Raizada [16] reported that most of the bacterial isolates from seeds of different maize varieties were able to solubilize phosphorus and fix nitrogen. In addition, Zawoznik [17] reported that eight endophytic bacteria designated to *Pseudomonas*, *Azospirillum*, and *Bacillus* genera were isolated from barley seeds under selective pressure for nitrogen-fixing bacteria.

Now-a-days there is no report on characterization and identification of bacterial endophytes isolated from tef seeds. Thus, the present study was conducted to isolate, characterize, and identify tef seed endophytic bacterial species have a significant role on plant growth promotion and plant health used for producing endophytic bacterial bio inoculum to maintain sustainable agricultural production and productivity without affecting environment and human health. In addition, we also performed brief physiological tests to have better understanding of seed endophytic bacterial species on adapting a biotic stress condition.

Material and Methods

Sample collection

Eight three tef seeds accessions were collected from Ethiopian biodiversity institute germplasm repository in 2017 G.C.

Isolation of tef seed endophytic bacteria

Collected seed samples were washed in sterile water and immersed in 70% ethanol for 3 min and followed by fresh sodium hypochlorite solution (2% NaClO) for 5 min; and then transferred to 70% ethanol (v/v) for 30 secs to remove the remaining NaClO. Finally, the seeds were

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rinsed five to seven times with sterilized distilled water for 30 secs. The water samples of the last washes were inoculated onto nutrient agar plates to check for sterility. Simultaneously, the surface sterilized seeds were pressed onto nutrient agar plate to test the sterilization efficiency. The seeds that were detected as free from contamination by cultivable microorganisms were used for the isolation of endophytic bacteria [18]. The surface sterilized seeds were crashed using sterile pestle and mortal and homogenized with 2 mL of 0.9% NaCl. The homogenates samples were diluted up to 10^{-6} and transferred on Nutrient agar and King's B-agar medium. Single colonies were subculture several times on the same agar media to obtain pure endophytic bacterial cultures (Figure 1).

Identification of tef seed endophytic bacteria

Morphological identification: Morphological identifications of endophytic bacteria were carried out by observing bacterial cultural or colonial morphology such as color, colony shape, elevation, edge, shape, and surface texture on agar medium, by following Bergey's manual of determinative bacteriology [19]. And also, cellular morphology of the endophytic bacteria was performed using gram staining techniques [20].

Biochemical identification: Biochemical identification of tef seed endophytic bacteria were conducted by using Biolog microbial identification system. Gene III Biolog micro plates were originally developed for the rapid identification of bacterial isolates by sole-carbon source utilization, through the inoculation of 95 individual carbon sources plus a water control on a 96 well plate [21]. For Endophytic bacterial identification, bacterial suspension was prepared using inoculated fluid protocol (A) and adjusted to 90% to 98%T of the isolates using Biolog standard turbidimeter. Prepared endophytic bacterial suspensions were transferred (100 µl) into each well of the micro Plate filled with different carbon sources and also incubated at 30°C. The plates are read between 18 h and 48 h following inoculation with a pre-grown isolate. Metabolism of the substrate in particular wells results in formazan production, producing color change in the tetrazolium dye. Individual endophytic bacterial species may be identified by the specific pattern of color change on the plate, providing an identifiable metabolic fingerprint.

Screening of bacterial endophytes for a biotic stress tolerance: The isolated tef seed endophytic bacteria were characterized for optimal growth temperature, salt tolerance, lytic enzymes secretion capacity, seeds germination and plant growth properties.

Figure 1: Endophytic bacterial isolates growth on agar medium containing different salt concentration.

Optimal growth temperature test: Tef seed endophytic bacterial isolates were transferred on prepared nutrient agar medium and incubated at different temperature ranges such as 27°C, 32°C, 37°C, 42°C, 50°C and 60°C for 5 to 7 days and bacterial growth was observed at every 24 h to confirm tolerance of the different ranges of temperature.

Salt tolerance test: Tef seed endophytic bacterial isolates were inoculated in to nutrient agar medium supplemented with different concentrations of salt (NaCl) 2%, 4%, 6%, 8%, 10%, 12%, 15% and 20%). All plates were incubated at 30°C for 5 to 7 days and bacterial growth was observed at every 24 h to confirm its tolerance of different concentration of NaCl.

Screening of bacterial endophytes for lytic enzyme production activity: Endophytic bacterial isolates were screened for production of lytic enzymes such as, amylase and cellulase. For amylase production all the isolates were spot inoculated on nutrient agar medium amended with 1% starch of (pH 6.06) and incubated at 30°C for 5 days. Inoculated plates were flooded with Logoi's iodine. Formation of clear colorless zone around the growing bacterial colonies was confirmed as positive result for amylase production. For cellulase synthesis, all endophytic bacterial isolates were spot inoculated on cellulose congo-red agar medium with the following composition: KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, cellulose 2 g, agar 15 g, congo-red 0.2 g, and gelatin 2 g; distilled water 1 L and at pH 6.8 to 7.2. Bacterial colonies exhibiting discoloration of congo-red was confirmed as positive result for cellulase synthesis.

Test for phosphate solubilization activity: Phosphate solubilization activity was performed according to Sgroy (22) protocol. Phosphate solubilisation test was carried out by spot inoculated tef seed endophytic bacterial isolates on Pikovaskaya's agar medium (tricalcium phosphate agar medium). Clear zone formation around bacterial colonies after 7 days of incubation at 30°C was confirmed as positive result for bacterial phosphate solubilization ability.

Seed germination and bacterial plant growth test: To study effects of the seeds associated endophytic bacteria on seed germination and plant growth performance was conducted under laboratory condition using *Triticum aestivum* seeds. Healthy seeds of *Triticum aestivum* were collected from Ethiopian biodiversity institute germplasm repository. Collected seeds were surface sterilized with 2% sodium hypochlorite and 70% ethanol, followed by successive washing with sterile distilled water and then the water was decanted. Sterilized seeds were kept for 30 minutes in 48hr old cultures with optical density (OP) = 10. The inoculated seeds were kept on sterilized plate containing wet blotting paper and incubated at room temperature and also plates were sprayed with sterile water for about 2-15 days. Seeds only treated with distilled water were used as negative control. The root and shoot lengths were recorded regularly from 2-15 days.

Methods of data analysis: Data analysis was carried out using table, frequency, and percentiles to evaluate enzymatic activities, a biotic stress tolerance capacity, *Triticum aestivum* seed germination and growth performance.

Results

Isolation of the endophytic bacterial strains

Successful seed surface sterilization was confirmed by observing absence of microbial growth on the nutrient agar plates inoculated with final wash water after overnight incubation. Nutrient and king's B agar media has been used for the isolation of endophytic bacteria and different colonies have been recovered after 24 h to 48 h of incubation at 30°C. A total of sixty putative endophytic bacteria isolates were isolated from eighty-three tef seed accessions.

Identification of endophytic bacterial strains

Cultural and cellular morphology: Cultural morphological identification was done by observing colony of tef seeds endophytic bacteria such as color, size, form, margin, texture, elevation (Table 1). The highest percentage occurrence of the tef seed endophytic bacterial isolates on culture medium were recorded as 35% *Pseudomonas*, 25% *Pantoea,* 15% *Enterobacter*, 10% *Bacillus,* 8% *Serratia* and 7% *Rhizobactera*. Cellular morphological identification of the tef seed endophytic bacterial isolates were carried out by using gram staining techniques. Results of the gram staining under light microscope observation were as Gram-negative road (60%), Gram-negative Cocci (30%), and Gram-positive road (10%) (Table 2).

Screening of the endophytic bacterial isolates for a biotic stress tolerance

Optimal growth temperature test: The ability of the tef seed endophytic bacterial isolates to tolerate extreme temperatures was verified by plating onto nutrient agar medium and incubated at different ranges of temperature i.e., 27°C, 32°C, 37°C, 42°C, 50°C and 60°C for 48 h [22,23]. All bacterial isolates belonged to tef seed endophytic bacterial species such as *Pseudomonas stutzeri*, *Pantoea dispersa*, *Enterobacter cowanii*, *Pseudomonas putida biotype B*, *Serratia ficaria, Pantoae agglomerans* and *Rhizobium radiobacte* were grow well at temperature 37°C, *Enterobacter clocaea ss dissolvens* were grow well at temperature 42°C and only endophytic isolate belonged to bacterial species such as *Bacillus butanolivorans* was grow well at temperature 60°C (Table 3).

Salt tolerance test: Tef seed endophytic bacterial strains (6W1 (1), 6W1 (2), 8M, 9LR, 11, 13M, 16M, 16M2, and 25RH) were screened for tolerance of different ranges of salt concentrations. Endophytic bacterial isolates belonged to species of *Pseudomonas stutzeri*, *Pantoea dispersa*, *Enterobacter cowanii*, *Enterobacter clocaea ss dissolvens, Pseudomonas putida biotype B*, *Serratia ficaria, Pantoae agglomerans* and *Rhizobium radiobacte* could tolerate salinity levels up to 2%, 4%

Cods	Colony morphology						
	Color	Size	texture	Shape	Margin	Opacity	Elevation
25RH	Yellow	Small	Smooth	Circular	Entire	Transparent	Raised
13M	Yellow	Medium	Smooth	Circular	Entire	Transparent	Flat
1M	White	Small	Smooth	Circular	Entire	Transparent	Flat
11	White	Large	Smooth	Rod	Erose	Transparent	Raised
9LR	Golden	Large	Smooth	Irregular	undulate	Transparent	Raised
16M	White	Small	Smooth	Circular	Entire	Transparent	Raised
16M2	Orange	Large	Concentric	Irregular	Lobate	Transparent	Convex
8M	White	Large	Flamentous	Rugose	Lobate	Dell	Flat
6W1st	Brown	Large	Concentric	Irregular	Erose	Dell	Flat
6W1st2	White	Large	Concentric	Circular	Entore	Transparent	Pulvinate

Table 1: Colonial morphology of tef seeds endophytic bacterial isolates on agar medium.

Cods	Gram staining result			
	Color	Shape	Gram positive	Gram negative
25RH	Pink	Road	-	√
13M	Red	Road	-	√
1M	Purple	Raod	√	-
11	Pink	Road	-	√
9LR	Pink	Cocci	-	√
16M	Pink	Cocci	-	√
16M2	Pink	Road	-	√
8M	Pink	Road	-	√
6W1st	Red	Road	-	√
6W1st2	Pink	Cocci	-	√

Table 2: Cellular morphology of bacterial endophytes growth on agar medium.

Code	Endophytic bacterial species	Temperature (T°C)					
		27	32	37	42	50	60
8M	*Pseudomonas stutzeri*	+	+	+	-	-	-
6W1st	*Pantoea dispersa*	+	+	+	-	-	-
9LR	*Enterobacter cowanii*	+	+	+	-	-	-
25RH	*Enterobacter clocaea ss dissolvens*	+	+	+	+	-	-
1M	*Bacillus butanolivorans*	+	+	+	+	+	+
11	*Pseudomonas putida biotype B*	+	+	+	-	-	-
6W1st (2)	*Serratia ficaria*	+	+	+	-	-	-
16M2 and 13m	*Pantoae agglomerans*	+	+	+	-	-	-
16M	*Rhizobium radiobacte*	+	+	+	-	-	-

Table 3: Bacterial endophytes optimal growth temperature test.

and 6%, and only 1M isolate belonged to endophytic bacterial species of *Bacillus butanolivorans* could tolerate salinity levels up to 8%, 10%, 12% and 15% (w/v) (Table 4).

Screening for lytic enzyme production and phosphate solubilization test

Tef seed endophytic bacterial strains were characterized for lytic enzyme production such as amylase and cellulase and phytase using suitable substrates (Table 5). Majority of the tef seeds endophytes belonged to species of *Pseudomonas stutzeri, Pantoea dispersa, Enterobacter clocaea ss dissolvens, Bacillus butanolivorans, Pseudomonas putida biotype B, Serratia ficaria*, *Pantoae agglomerans* and *Rhizobium radiobacte* could produce amylase and some of the bacterial isolates belonged to species of *Enterobacter cowanii, Enterobacter clocaea ss dissolvens, Bacillus butanolivorans, Pseudomonas putida biotype B, Serratia ficaria*, and *Rhizobium radiobacte)* could produce cellulose (Figure 2) and *Pseudomonas stutzeri, Pantoea dispersa, Enterobacter clocaea ss dissolvens, Bacillus butanolivorans, Pseudomonas putida biotype B, Serratia ficaria,* and *Rhizobium radiobacte* could solubilize insoluble phosphate (Table 5).

Biochemical identification: Biochemical identification of the tef seed endophytic bacterial isolates was done using Biolog microbial identification system according the manufacturer's instructions on the basis of their ability to utilize 95 different carbon sources. Bacterial isolates were grown on Biolog Universal Growth (BUG) medium and incubated at 30°C for 24 h. Fresh tef endophytic bacterial cultures were suspended in to inoculums fluid protocol A and adjusted turbidity to required optical density up to 90-98 T using standard Biolog turbidimeter. Prepared endophytic bacterial suspensions were transferred (100 µl) into each well of the micro Plate filled with different carbon sources and chemicals and incubated at 30°C. The plates are read between 18 h and 48 h following inoculation with a pre-grown bacterial isolate. Metabolism of the different Carbone sources in particular wells results in formazan production, producing color change in the tetrazolium dye. Eight individual tef seed endophytic bacterial species were accurately identified and one is partially identified by the specific pattern of color change on the plate (Table 6).

Seed germination and plant growth test: Seed germination test were performed under laboratory condition to evaluate seed germination status, plant root, and shoot growth. Seeds of the *Triticum aestivum* inoculated with *Pseudomonas stutzeri* were 100% germinated after 72 h of incubation and significantly increase *T. aestivum* root dry mass up to 9.8% and increase shoot dry mass up to 29% comparing to the non-inoculated seeds, seeds of the *T. aestivum* inoculated with *Rhizobium radiobacte* were 100% germinated after 72 h incubation and increase mean root dry mass up to 9.3% and increase mean shoot dry mass up to 25%, seeds inoculated with *Bacillus butanolivorans* were 100% germinated after 72 h incubation and increase mean root dry mass up to 8.1% and increase shoot dry mass up to 23%, seeds inoculated with *Pseudomonas putida biotype B* were 100% germinated after 72 h incubation and increase mean root dry mass up to 7.9% and increase mean shoot dry mass up to 26%, seeds inoculated with *Enterobacter cowanii* were 90% germinated after 72 h incubation and increase mean root dry mass up to 7.7% and increase mean shoot dry mass up to 23%, seeds inoculated by *Pantoea dispersa* were 90% germination after 72 h incubation and increase mean root dry mass up to 7.5% and mean shoot dry mass up to 24%, seeds inoculated with *Enterobacter clocaea ss dissolvens* were 90% germinated after 72 h incubation and increase mean root dry mass up to 7% and increase mean shoot dry mass up to 20%, seeds inoculated with *Serratia ficaria* were 90% germinated

Code	Endophytic bacterial species	Salt concentration (w/v) % of Nacl							
		2%	4%	6%	8%	10%	12%	15%	20%
8M	*Pseudomonas stutzeri*	+	+	+	_	_	_	_	_
6W1st	*Pantoea dispersa*	+	+	+	_	_	_	_	_
9LR	*Enterobacter cowanii*	+	+	+	_	_	_	_	_
25RH	*Enterobacter clocaea ss dissolvens*	+	+	+	_	_	_	_	_
1M	*Bacillus butanolivorans*	+	+	+	+	+	+	+	_
11	*Pseudomonas putida biotype B*	+	+	+	_	_	_	_	_
6W1st (2)	*Serratia ficaria*	+	+	+	_	_	_	_	_
16M2 & 13m	*Pantoae agglomerans*	+	+	+	_	_	_	_	_
16M	*Rhizobium radiobacte*	+	+	+	_	_	_	_	_

Table 4: Tef seed endophytic bacterial NaCl tolerance test result.

Code	Endophytic bacterial species	Enzymatic test				Phosphate solubilization	
		Amylase		Cellulase			
		+	-	+	-	+	-
8M	*Pseudomonas stutzeri*	√	-		-	√	-
6W1st	*Pantoea dispersa*	√	-		-	√	-
9LR	*Enterobacter cowanii*		√	√	-		-
25RH	*Enterobacter clocaea ss dissolvens*	√	-	√	-	√	-
1M	*Bacillus butanolivorans*	√	-	√		√	
11	*Pseudomonas putida biotype B*	√	-	√		√	-
6W1st (2)	*Serratia ficaria*	√	-	√	-	√	-
16M2 & 13m	*Pantoae agglomerans*	√	-		-		-
16M	*Rhizobium radiobacte*	√	-	√		√	

Table 5: Endophytic bacterial lytic enzymes production and phosphate degradation test results.

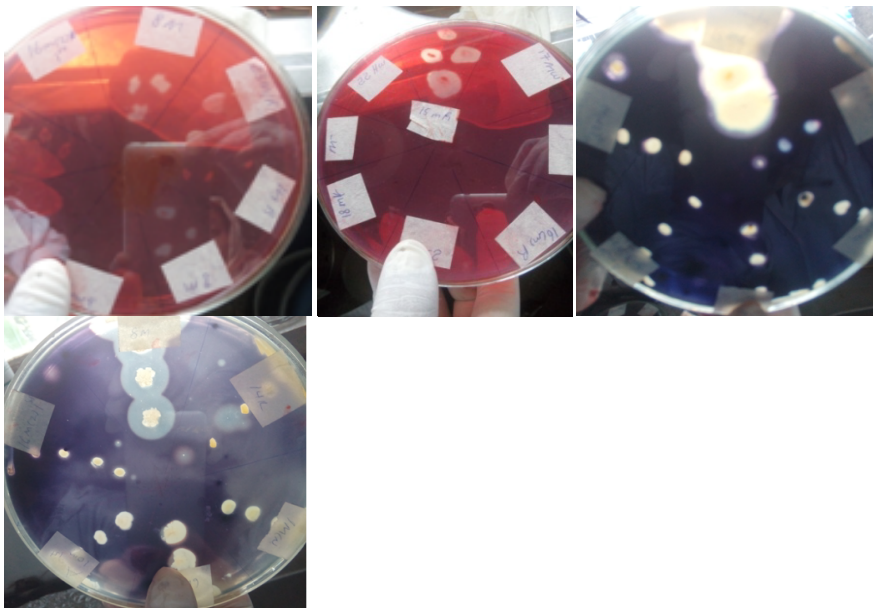

Figure 2: Test of endophytic bacterial isolates for enzyme secretion capability.

Tef endophytic bacterial species	Probability	Similarity	Distance	Types of Organism	Status
Pseudomonas stutzeri	0.694	0.589	5.968	GN-Nent	Fully identified
Pantoea dispersa	0.616	0.529	6.949	GN-Nent	Fully identified
Enterobacter cowanii	0.523	0.660	4.919	GN-Nent	Fully identified
Enterobacter clocaea ss dissolvens	0.673	0.549	6.650	GN-Nent	Fully identified
Bacillus butanolivorans	0.637	0.653	4.985	GP-Rod-SB	Fully identified
Pseudomonas putida biotype B	0.584	0.582	6.127	GN-Nent	Fully identified
Serratia ficaria	0.523	0.555	6.547	GN-Ent	Fully identified
Pantoae agglomerans	0.9994	0.790	2.852	GN-Ent	Fully identified
Rhizobium radiobacte	0.0	0.234	7.854	GN-Nen	Partially identified

Table 6: Biolog identification results.

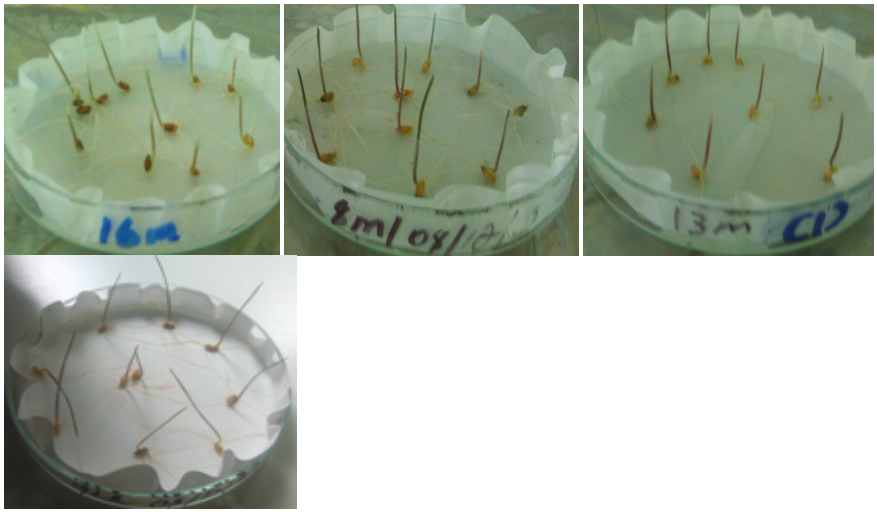

Figure 3: Bacterial endophyte inoculated *T. aestivum* seed germination and growth status.

Endophytic bacteria inoculated in to *T. aestivum*	Growth parameter							
	Root length (mm)			Root dry mass (%)	Shoot length (mm)			Shoot dry mass (%)
	3 day	5 day	7 day	7 days	3 days	5 days	7 days	7 days
Pseudomonas stutzeri	6.5	8.7	9.4	9.8	4.6	6.3	7.5	29
Pantoea dispersa	4.5	6.7	7.0	7.5	3.3	4.6	6.7	24
Enterobacter cowanii	6	7.2	8.7	7.7	4.2	5.3	5.9	23
Enterobacter clocaea ss dissolvens	3.7	4.9	6.5	7	2.1	3.5	4.8	20
Bacillus butanolivorans	4.5	5.3	7.8	8.1	4.5	5.6	7.6	23
Pseudomonas putida biotype B	4.0	4.9	7.6	7.9	4.8	5.3	7.8	26
Serratia ficaria	3.8	4.5	6.6	6.9	3.8	6.2	7.7	22
Pantoae agglomerans	2.8	4.1	4.8	5.5	2.0	3.9	4.1	19
Rhizobium radiobacte	6.1	7.2	8.5	9.3	4.5	5.6	6.2	25
Control	1.1	1.4	2	0.1	0.3	0.5	0.8	0.17

Table 7: Growth status of *T. aestivum* inculated with Tef seed endophytic bacterial species.

after 72 h incubation and increase mean root dry mass up to 6.9% and increase mean shoot dry mass up to 22% and seeds inoculated with *Pantoae agglomerans* were 80% germinated after 72 h incubation and increase mean root dry mass up to 5.5% and increase mean shoot dry mass up to 19% (Figure 3 and Table 7).

Discussion

Tef is one of the endogenous tropical cereal crops of Ethiopian and adapted to a wide range of environments and also cultivated under diverse agro climatic conditions such as high land, middle land and low land. The crop performs well in both water logged vertisoils as well as salt, water and extreme temperature-stressed in the semi-arid regions throughout the country. Endophytes are microorganisms that colonize internal plant tissues without causing apparent harm to the host [24]. The association between plants and bacterial endophytes developed very early in evolution and it is likely that this association occurs in all plant species. Plant endophytic microbial interactions have a significant role in plant growth and development in their lifetimes by direct and indirect mechanisms [25]. Host plants provides diverse protective niches for endophytic organisms, and endophytes can produce useful metabolites and signals which can increase nutrient uptake [25], modify plant growth activity [5] and increase resistance to osmotic stress condition, increase resistance to heavy metal, produce antibiotics and lytic enzymes, induce resistance to plant pathogens [26].

The most predominant seed endophytic bacteria belong to the Proteobacteria and mainly the γ-Proteobacteria. In general, common bacterial genera reported in seeds of very different plant species are Bacillus and *Pseudomonas*. Also, *Micrococcus, Staphylococcus, Pantoea* and *Acinetobacter* are often found colonizing the seed. In this study sixty endophytic bacterial strains were isolated from EBI germplasm repository tef seed accessions, from these isolates majority of them are gram negative and few of them are gram positive. From all identified tef seed endophytic bacterial strains, majority of them are belonged to genus *Pseudomonas*, some of the endophytes belonged to genus *Pantoae* and *Enterobacter*, whereas few of them belonged to genus *Bacillus, Serratia* and *Razobium*. The present study result was correlated with the previous work of Zawoznik [17], he reported that eight endophytic bacteria isolates assigned as *Pseudomonas*, *Azospirillum*, and *Bacillus* genera were isolated from barley seeds under selective pressure for nitrogen-fixing bacteria. For a biotic environmental stress condition, salinity is one of the major factors that adversely affect crops production and productivity in the world. It affects crops productivity in two ways: high concentrations of salts in the soil make it harder for roots to extract water and high concentrations of salts within the plant can be toxic that easily affect crop production and productivity.

Nine identified tef seed endophytic bacterial species were *in vitro evaluated* for tolerance to salinity problem under laboratory condition. Almost all of the identified endophytes such as *Pseudomonas stutzeri*, *Pantoea dispersa*, *Enterobacter cowanii*, *Enterobacter clocaea ss dissolvens, Pseudomonas putida biotype B*, *Serratia ficaria, Pantoae agglomerans* and *Rhizobium radiobacter* were grow well at 2%, 4% and 6% w/v of salt concentration and only *Bacillus butanolivorans* was grow well at 8%, 10%, 12% and 15% w/v of salt concentration. It may have ability to produce environmental stress tolerant enzymes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase that reduce production of ethylene and thus bacterial species were identified from tef seed is one of the best alternative bacterial bio-inoculum, which have a significant role in maintaining sustainable agricultural production and productivity under high soil salinity conditions. According to Siddikee [27] report canola seeds were inoculated with ACC deaminase producing halotolerant endophytic bacterial species under high salinity conditions increase fresh and dry mass of the inoculated plants up to 47%. In addition, Gamalero [28] also reported that inoculation of plant growth-promoting bacterial consortium such as *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 shows a significant change on the growth of cucumber under salt stress conditions.

Based on temperature tolerance test, majority of the identified endophytic bacterial species such as *Pseudomonas stutzeri*, *Pantoea dispersa*, *Enterobacter cowanii*, *Pseudomonas putida biotype B*, *Serratia ficaria, Pantoae agglomerans* and *Rhizobium radiobacte* were grow well at temperature 37°C, and only *butanolivorans* was grow well at a temperature up to 60°C. This finding is correlated with the work of Shrey [29]; he reported that maize seed endophytic bacteria exhibited tolerance to salinity (10%), and temperature up to 60°C. Thus, plant growth promoting traits of the endophytic bacterial species identified from tef seed is very important in climate change scenario with projections of increased intensities of a biotic stresses condition in near future posing problem to agricultural production and productivity. Other evaluation was carried out to assess tef seed endophytic bacterial plant growth promoting characteristics such as lytic enzyme production and phosphate solubilization. Result shows that almost

all of bacterial species (90%) were produce amylase and few of them (10%) secrete cellulase. This result is correlated with the work of Mano [14], he reported that seed endophytes often seem to possess amylase activity in order to utilize starch and resume growth after long-term survival inside the seeds. In addition to this, 70% of the newly identified tef seed endophytic bacterial species were degraded phosphate. This is supported by the work of López-López [15], he reported that some seed endophytic bacterial species were used phytate, which is the main storage form of phosphorus in seeds, as a source of phosphate during dormancy period. In addition, Johnston-Monje and Raizada [16] also reported that most of the bacterial species were isolated from seeds of different maize genotypes were able to degrade phosphorus, which increase phosphorus availability to the plants.

During seed germination and plant growth test, seed of *T. aestivium* inoculated with identified endophytic bacterial species such as *Pseudomonas stutzeri, Rhizobium radiobacte, Bacillus butanolivorans*, *Pseudomonas putida biotype B, Enterobacter cowanii*, *Pantoea dispersa Enterobacter clocaea ss dissolvens* and *Serratia ficaria*. majority of the tef seed endophytic bacterial inoculums were germinate *T. aestivium seeds* up to 90% to 100% after 72 h incubation and significantly increase mean root dry mass of *T. aestivium* up to 9.8%, 9.3%, 8.1%, 7.9%, 7.7%, 7.5%, 7%, 6.9% and 5.5% respectively and also increase mean shoot dry mass up to 29%, 25%, 23%, 26%, 23%, 20%, 22% and 19% respectively and only *Pantoae agglomerans* was germinate inoculated seed up to 80% after 72 h incubation. This result is correlated with the work of Pradhan [30], he reported that seeds inoculated with *Bacillus* sp. was significantly increased the germination percentage, root and shoot length of the crops as compare to the untreated one. In addition, Woyessa and Assefa [31] reported that inoculation of tef crops with *Pseudomonas fluorescent* increases mean root dry weight (39%), root shoot ratio (42%), and grain yield (28%) and also inoculation of *Bacillus subtilis* increase mean root dry weight (28%), root shoot ratio (19%) and grain yield (44%).

Conclusion and Recommendations

To our knowledge, this study is the first report of endophytic bacteria identified from tef seed conserved in EBI germplasm repository. All bacterial genera isolated from tef seeds have previously been described as seed endophytes conferring some beneficial effects to their host plants. The presence of these bacterial species in tef seeds likely reflects selection facilitating the vertical transmission of these microbes, transferring valuable properties to the seedlings, flowering, and fruiting. Interestingly, majority of tef seed identified endophytic bacterial species exhibiting excellent traits of plant growth promoting activities such as phosphate solubilization, high salinity tolerance, extreme temperature tolerance and production of lytic enzymes and suggested the potential for possible application under stressed environmental conditions and possible utility as bioinoculant for maintaining sustainable agricultural production and productivity without affecting human health.

Based on the result of this study, the following recommendation is given: (i) further study must be carried out using a combination of the phenotypic and genotypic identification system.

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