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Phosphate solubilizing fungi isolated and characterized from Teff rhizosphere soil collected from North Showa zone, Ethiopia

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Phosphorus is one of the major bio elements limiting agricultural production. About 95 to 99% phosphorus in agricultural soil is unavailable form for plant growth. Phosphate solubilizing microorganisms can increase soil phosphate availability. This study was aimed to identify and evaluate phosphate solubilizing fungi from Teff rhizosphere soil. Fungi were identified using lactophenol cotton blue staining confirmation and Biolog Microstation identification system. Fungi isolates were screened and transferred to biolog universal yeast agar media. Pure yeast cells and filamentous fungi were suspended in sterile water and filamentous fungi (FF) inoculum fluid at 49 ± 2 and 75 ± 2 turbidity measured by biolog turbidimeter, respectively. 100 μL transferred from each suspension into 96 wells of the biolog yeast microplate and filamentous fungi microplate tagged with different carbon source and incubated at 26°C for 24 to 72 h and read by micro station at a single wavelength of 590 nm, results were recorded and processed for identification by micro log3 software ver. 4.20.05. Biolog microstation read 24 fungi species. Filamentous fungi ≤ 0.5 similarity index (62.5%), yeast ≥ 0.5 similarity index (25%), yeast ≤ 0.5 similarity index (12.5%). The identified fungi were tested for phosphate solubilization by the Pikovskaya's agar (PVK) selective media. Seven species were positive in phosphate solubilizing ability: *Trichosporon beigellii* B, *Rhodotrula aurantiaca* A, *Cryptococcus luteolus*, *Zygoascus hellenicus*, *Penicillium purpurogenum* var. *rubrisclerotium*, *Neosartorya fisheri* var. *fischeri*, and *Candida montana*. At 15 days incubation, *T. beigellii* B and *R. aurantiaca* A was able to solubilize phosphate with solubilizing index of 5.3 and 2.6, respectively. *T. beigellii* B, were superior in phosphate solubilization. Therefore, these species can be candidated and exploited after further evaluation as biofertilizers for agriculture productivity.

Key words: Biolog Microorganisms, micro station, phosphorus, rhizospher, soil, solubilization, Teff.

INTRODUCTION

Phosphorus (P) is the second essential macronutrient for plant growth and development. It accounts 0.2% of plant dry weight, limits the growth of plants and crop yield (Sharma et al., 2013). Phosphorus contributes remarkably to photosynthesis, energy and sugar production, nucleic

acid synthesis, and promotes N_2 fixation in legumes (Saber et al., 2005).

The mineral nutrition of plants mainly depends on soil P content that can be assimilated as a soluble phosphate (Ehteshami, 2011). Phosphorous increases the strength

of cereal straw, promotes flower formation and fruit production, stimulates root development and also essential for seed formation (Sharma et al., 2011). It also plays a role in stalk and stem strength, maturity and production crop quality and resistance to plant diseases (Richardson, 2007). Mobility of phosphate ions in the soil is very low due to their high retention in soil. Stevenson (1986) and Holford (1997) reported that the recovery rate of P fertilizer by plants is only about 10 to 30%. The remaining 70 to 90% is accumulated in soil or in the form of immobile that is bound by Al or Fe in acid soils, or Ca and Mg in alkaline soils (Prochnow et al., 2006; Yang et al., 2010). Phosphorus is highly insoluble and unavailable to plants. It must be converted into soluble form. Phosphate solubilizing microorganisms can play an important role in dissolving both fertilizer phosphorus and bound phosphorus in the soil that is environmentally friendly and sustainable (Khan et al., 2007). Several groups of microorganism including fungi, bacteria and actinomycetes are known as efficient fixed P solubilizers (Sundara et al., 2002). Fungi are the important components of soil microbes typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996). A wide range of soil fungi are reported to solubilize insoluble phosphorous such as *Aspergillus niger* and *Penicillium* species, which are the most common fungi capable of phosphate solubilization (Whitelaw et al., 1999). Exploration of phosphate solubilizing microorganisms has been conducted by many researchers from soils (Chen et al., 2006; Widawati et al., 2008; Gupta et al., 2012), mangrove (Vazquez et al., 2000; Holguin et al., 2001), and rhizosphere (Chung et al., 2005; Poonguzhali et al., 2008; Oliveira et al., 2009). From such explorations various types of phosphate solubilizing microorganisms have been successfully identified. In last few decades, a large array of rhizosphere bacteria and fungi including species of *Penicillium*, *Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Arthrobacter ilicis*, *Escherichia coli*, *Pantoea agglomerans*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Microbacterium laevaniformans*, and *Micrococcus luteus* have been identified as P-fertilizers (Kumar et al., 2014). The principal mechanism for many soil fungi and bacteria can solubilize inorganic phosphate into soluble form through the process of acidification, chelation, exchange reactions and production of organic acids (Han, 2006). Acid phosphatases play a major role in the mineralization of organic phosphorus in soil phosphate solubilization effect is mainly through the reaction between organic acids excreted from organic

matters with phosphate binders such as Al, Fe, and Ca, or Mg to form stable organic chelates to free the bound phosphate ion (Arcand and Schneider, 2006; Gupta et al., 2012). Phosphorus deficiency is the most important problem of Ethiopian soil and more than 70 to 75% of highland soils are characterized by phosphorus deficiency (Beyene, 1982). The deficiency is very severe in the acidic soils of the southern, southwestern and western regions. Areas Al³⁺ and Fe³⁺ high are totally incriminated with phosphorus fixation (Sertsu and Ali, 1983). The fixed forms of P in acidic soils are aluminum and iron phosphates, while in alkaline soils they are calcium phosphates (Rfaki et al., 2014). Around 70% of Ethiopian vertisol have available phosphorus below 5 ppm, which is very low for supporting good plant growth and fixation in vertisols is related more to calcium, which is the predominant cation in all profiles than Al³⁺ and Fe (Mamo et al., 1988). Vertisols are dark, montmorillonite-rich clay soils with characteristic shrinking and swelling properties. They have high clay content (>30% to at least 50 cm depth from the surface) and when dry they show cracks of at least 1 cm wide and 50 cm deep. They have high calcium and magnesium contents (FAO, 2000). Teff [*Eragrostis tef* (Zucc.) Trotter] is the major indigenous cereal crop of Ethiopia, where it was originated and diversified. It is a highly demanded and a staple food grain for majority of the Ethiopian people. In a country of over 80 million people, teff accounts for about 15% of all calories consumed in Ethiopia (Bekabil et al., 2011). The teff grain is ground to flour which is mainly used for making popular pancake-like local bread called injera and sometimes for making porridge. The grain is also used to make local alcoholic drinks, called tela and katikala. Tef straw, besides being the most appreciated feed for cattle (Ketema, 1997). Teff is the only cultivated of all 300 *Eragrostis* species. Its agro ecological adaptability has resulted in its cultivation as an important crop in 10 of 18 agro ecological zones of the country. It can be grown in altitudes ranging from near sea level to 3000 ms, but the best performance occurs between 1100 and 2950 masl (Hailu and Seyfu, 2000). Annual rainfall of 750 to 850 mm, growing season rainfall of 450 to 550 mm and a temperature range of 10 to 27°C. A very good result can also be obtained at an altitude range of 1700 to 2200 m and growing-season rainfall of 300 mm (Seyfu, 1993). The crop performs well in both water logged vertisol in the highlands as well as water-stressed areas in the semi-arid regions throughout the country and consequently it is preferred over other grain crops such as maize or barley (Zelege, 2010). Teff production and productivity have been far below the potential (Demeke, 2013). Currently, the average national productivity is estimated to be less than 0.5 ton per ha. This is very low

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compared to other cereals such as wheat and sorghum grown in the region. Lower grain yield is mainly attributed to low soil fertility, especially nitrogen (N) and phosphorus (P) deficiencies and weed control practices (Seifu, 1993). Declining soil fertility as a result of continuous cropping without replenishing soil nutrients, continues application of phosphate fertilizer and soil erosions is the major factors that reducing production and productivity of the crop in Ethiopia. Higher grain yield of teff was recorded by applying inorganic fertilizers (Abate, 1993). However, chemical fertilizers are neither easily available nor affordable for the majority of poor Ethiopian farmers and not environmentally friendly (FAO, 1987). Such economic considerations necessitate for an alternative less expensive and environmentally friendly agricultural technologies to improve yield and quality of grain. Screening and characterization of phosphate solubilizing microorganisms are important for proper utilization of their beneficial effects to increase crop production and sustain agricultural productivity of the country without contaminating environments. In Ethiopia, only few studies on teff root-associated microorganisms have been undertaken. The effect of phosphate solubilizing some fungus on growth and yield of teff was studied by Asfaw (1993). Inoculation of teff by vascular arbuscular mycorrhizal (VAM) and plant growth promoting rhizobacteria (PGPR) give good result on teff productivity. So previous research works tell us using biofertilizer is better indicative to improve teff productivity to a significant level. However, there are some trials on rhizobacteria and vascular arbuscular mycorrhizal using as biofertilizer, phosphate solubilizing fungi were not studied well. This study was aimed to isolate, identify and evaluating of phosphate solubilizing fungi from teff rhizosphere soil collected from North Showa farm land and selecting superior solubilizing fungi that will be candidated for bio fertilizer after further evaluation for agricultural productivity.

MATERIALS AND METHODS

Study area

The study was conducted in North Showa zone in five selected districts, particularly in Kewot, Tarmaber, Efratana gidim, and Siadeberna wayu. North showa zone is one of the 10 zones of Amhara regional state. The elevation ranges from 1100 to 3009 m above sea level. Geographic coordinate latitude: 9°46'8.4" and longitude: 39°40'4.8". The zone is located in approximately average 200 km far from Addis Ababa (Figure 1).

Sample collection

Twenty five (25) teff farmland site were selected based on three teff varieties, two soil types and 200 m difference within 1200 to 2200 m.a.s.l altitude in the study area. Seventy five rhizosphere soil samples were collected through drillings at 5, 10, and 15 cm depth (Figure 2). Approximately, 15 g of soil were taken from each depth of sampling point and a total of 45 g composite soil per sampling

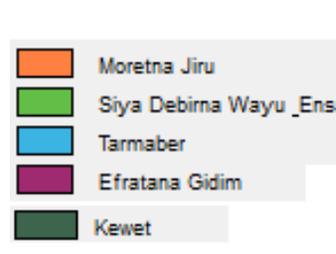


Figure 1. Map of study area.

farmland were stored in sterile sample tube and icebox during April 08 to 28/2016 and transported to microbial directorate laboratory in Ethiopian Biodiversity Institute to Addis Ababa and kept in +4°C until processed.

Screening and isolation of fungi from teff rhizosphere soil

One gram of soil from each sample was serially diluted up to 10^{-6} mL in distilled water. About 0.1 mL inoculum sample was transferred to yeast extract peptone dextrose agar media (YPDA), rose bengal agar, potato dextrose agar by cotton swab and streaked using nichrom loop. Primary cultures were incubated for 26°C in digital incubator for 48 h. Isolates were subculture twice until pure colony was obtained for morphological identification. A single yeast colony and pure filamentous fungi was streaked to Biolog universal yeast agar (BUY agar plate (60 g/1 L) and incubated for 48h at 26°C for yeast and filamentous fungi micro plate (YT/FFMicroplate) inoculum preparation). The yeast and filamentous fungi were identified according to the Biolog micro station reading and procedure.

Colony morphology identification

The colony morphology of the isolated fungi were examined after grown on yeast extract peptone dextrose agar media and biolug universal yeast agar media at 26°C for 48 h and its colony morphology, form, size, elevation, margin/edge, and colony color were observed using hand lens as well as its percentage frequency were recorded.

Identification of yeast from teff rhizosphere soil

Pure yeast isolates after being grown on yeast extract, potato dextrose agar were transferred to biolug universal growth agar and incubated at 26°C for 48 h. Pure colony of yeast suspensions were prepared in 9 mL sterile distilled water and adjusted to $47 \pm 2T$ using biolug turbidimeter. 100 μ L of inoculum was dispensed using digital pipettor to each of 96 wells of yeast micropate (YT) and incubated at 26°C 24 to 72 h.

The YT micro plate is tagged with 96 carbon source. An isolate ability to metabolize each carbon source is measured in the presence or absence of purple hue in the wells. Tetrazolium violet a redox dye forms a purple color when oxidized by cellular respiration of microorganisms. The YT micro plate measures both metabolic reactions as well as turbidity growth to produce identifications. YT micro plate was read by the micro station reader at 24, 48, and 72 h at a single wavelength of 590 nm. The biolug software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score produces similarity index value and



Figure 2. Activities during teff rhizosphere soil collection.

probability for species identification (Biolog1993).

Identification of filamentous fungi from teff rhizosphere soil

Filamentous fungi screened and isolated on rose bengal agar and potato dextrose agar were stained by lactophenol cotton blue in order to confirm to which genera the fungi is belonged to then pure filamentous fungi were transferred into biolog universal growth agar media and incubated at 26°C for 48 h. Pure sporulate filamentous fungi suspension were prepared using 15 mL filamentous fungi inoculum fluid and adjusted to $75 \pm 2T$ using biolog turbidimeter. 100 μ L of inoculum was dispensed using digital pipettor to each of the 96 wells of filamentous fungi micropate (FF) tagged with different carbon source and incubated at 26°C and 24 to 240 h. After incubation, the FF micro plate measures both metabolic reactions as well as turbidity growth to produce identifications. Filamentous fungi micro plate (FF) was read by the micro station reader at 24, 48, and 72 h at a single wavelength of 590 nm. The biolog software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score produces similarity index value and probability for species identification (Biolog1993).

Identification of phosphate solubilizing microorganisms

Fungal isolate identified by biolog microstation were tested for their phosphate solubilizing ability. Pure fungi colonies were collected using a needle nose and spotted at 4 quadrants on sterile solid Pikovskaya media (2.5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 NaCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 10 g glucose, 0.5 g of yeast extract, 20 g agar, 0.0001 g $MnSO_4$, 0.0001 g $FeSO_4$, and 1000 mL distilled water) (Rao, 1982). $Ca_3(PO_4)_2$ was used as a source of phosphate. Observations were made until the formation of a clear zone around the colonies of fungi that indicated the occurrence of phosphate dissolution. At 5 days intervals, solubilization index (SI) was measured using the following formula (Premono et al., 1996). Fungi that formed the fastest clear areas with the greatest diameter indicate the most superior phosphate solubilizing fungi.

$$SI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Statistical analysis

The data analysis involved various descriptive statistics such as means and percentages frequency. STATA ver.13 was used for phosphate solubilization index data analysis.

RESULTS

Percentage frequency of fungal species isolated from teff rhizosphere soil

A total of 450 fungal colonies were grown and counted on different growth media and identified pure colonies having similar morphology were clustered in order to detect the incidence frequencies of the microorganisms encountered. Sixty five percent were filamentous fungi and 35% were non filamentous fungi. From filamentous fungi, *Aspergillus* species were dominant (33%), *Penicillium* species (29%), *Fusarium* species (16%), *Trichoderma* (13%), and *Colletotrichum* (9%). The phosphate solubilizer fungi isolates were also identified based on their colony morphology that is pigmentation, shape, size, texture, elevation and margin) (Table 1).

Identification of filamentous fungi species using lacto phenol cotton blue staining (LPCB) and biolog micro station

Representative filamentous fungal isolates from clustered group were stained using lacto phenol cotton blue to confirm to which genera filamentous fungi belonged to

Table 1. Colony morphology for phosphate solubilizer fungi.

P-solubilizing fungi	Shape	Elevation	Size	Marigin	Surface texture	Color
<i>Trichosporon beigellii</i> B	Irregular	Flat	Large	Lobate	Concentric	White yellow
<i>Rhodotrula aurantiaca</i> A	Rhound	Flat	Large	Undulate	Radiate	White
<i>Penicillium purpurogenum</i> Var. <i>rubrisclerotium</i>	Circular	Umbonate	Large	Filamentous	Radiate	Gray
<i>Neosartoryafischeri</i> var. <i>fischeri</i>	Circular	Umbonate	Large	Filamentous	Rugose	Olive green
<i>Cryptococcus luteolus</i>	Irregular	Flat	Lobate	Obate	Radiate	Yellow
<i>Zygoascus hellenicus</i>	Irregular	Flat	Lobate	Obate	Radiate	Yellow
<i>Candida montana</i>	Round	Flat	Large	Smooth	Radiate	White pink

Table 2. Biolog micro station filamentous fungi identification result read.

Index value	Fungus species	LPCB Staining result	Probability	Similarity	Distance	Teff farm land districts
	<i>Colletotrichum lindemuthianum</i> (Saccardo & Mangus) Briosi	+	-	0.001	32.96	Ejersa Qubete
	<i>Emericella quadrilineata</i> (Thom & Raper) C.R. Benjamin	+	-	0.001	29.89	Kewot (Worentele)
	<i>Fusarium melanochlorum</i> (Caspary) Sacc.	+	-	0.001	32.37	Efratana Gidm (Karalgoma)
	<i>Aspergillus brevipes</i> G. Sm.	+	-	0.002	28.24	Tarma Ber (Asfachew)
	<i>Fusarium juruanum</i>	+	-	0.000	48.72	Tarma Ber (Armania)
	<i>Trichoderma piluiferum</i> Webster & Rifai	+	-	0.000	48.48	Efratana Gidm (Karalgoma)
Filamentous fungi ≤0.5 Similarity index (62.5%)	<i>Fusarium avenaceum</i> s.sp.nurragi Summerell & L.W. Burgess	+	-	0.002	27.20	Kewot (Korebta)
	<i>Penicillium vulpinum</i> (Cooke & Massee) Seifert & Samson	+	-	0.003	25.98	Tarma Ber (Chira Meda)
	<i>Neosartorya fischeri</i> var. <i>Fischeri</i> (Wehmer) Malloch & Cain	+	-	0.001	32.18	Ejersa Qubete
	<i>Fusarium udum</i> E.Butler	+	-	0.000	39.36	Tarma Ber (Chira Meda)
	<i>Hypocrea pseudokoningii</i>	+	-	0.000	33.5	Tarma Ber (Chira Meda)
	<i>Trichoderma citrinoviride</i> Bissett BGA	+	-	0.000	46.16	Efratana Gidm (Karalgoma)
	<i>Trichoderma aureoviride</i> Rifai	+	-	0.000	39.81	Efratana Gidm (Karalgoma)
	<i>Penicillium purpurogenum</i> var. <i>Rubrisclerotium</i> Thom	+	-	0.004	24.46	Ejersa Qubete
	<i>Penicillium pinophilum</i> Hedge. BGB	+	-	0.002	26.74	Mendida (Moyesilasie)

and read by biolog micro station equivalent to molecular method. The result revealed that 15 filamentous fungi species associated teff rhizosphere soil. Both lacto phenol cotton blue staining result and biolog microstation read showed that a filamentous fungi ≤0.5 similarity index (62.5%) *Colletotrichum lindemuthianum*, *Emericella quadrilineata*, *Fusarium melanochlorum*,

Aspergillus brevipes, *Fusarium juruanum*, *Trichoderma piluiferum*, *Fusarium avenaceum*, *Penicillium vulpinum*, *Neosartorya fischeri* var. *Fischeri*, *Fusarium udum*, *Hypocrea pseudokoningii*, *Trichoderma citrinoviride*, *Trichoderma aureoviride*, *Penicillium purpurogenum* var. *rubrisclerotium*, and *Penicillium pinophilum* (Table 2).

Identification of yeast species using biolog micro station

Biolog microstation read at 24, 48 and 72 YT microplate incubation result revealed that yeast ≥0.5 similarity index (25%) *Rhodotrula aurantiaca* A, *Candida etchellsii*, *Kluyveromyces delphensis*, *R. aurantiaca* A, *Cryptococcus luteolus*, and

Table 3. Biolog micro station yeast identification result read.

Index value	Fungus species	Probability	Similarity	Distance	Teff farm land districts
Yeast \geq Similarity index 25 (%)	<i>Rhodotorula aurantiaca A</i>	100	0.604	6.1	Kewot (Abay Atir)
	<i>Candida etchellsii</i>	74	0.545	4.01	Deneba (Dacho)
	<i>Kluyveromyces delphensis</i>	77	0.533	4.75	Kewot (Worentele)
	<i>Rhodotorula aurantiaca A</i>	100	0.512	7.77	Kewot (Tere)
	<i>Cryptococcus luteolus</i>	-	0.728	4.13	Tarmaber (ChiraMeda)
	<i>Trichosporon beigellii B</i>	99	0.523	7.51	Mendida (Moyeselase)
Yeast \leq 0.5 Similarity index (12.5%)	<i>Cryptococcus albidus var aerius</i>	-	0.226	14.34	Mendida (Moyeselase)
	<i>Zygoascus hellenicus</i>	-	0.216	14.1	Tarmaber (ChiraMeda)
	<i>Candida montana</i>	-	0.47	3	Kewot (Abay Atir)

Table 4. Phosphate solubilization index (PSI).

Fungus species isolated from teff rhizosphere soil	Phosphate solubilization index (PSI)		
	5th days	10th days	15th days
<i>Trichosporon beigellii B</i>	3.8	4.3	5.3
<i>Rhodotrula aurantiacaA</i>	1.425	2.2	2.6
<i>Cryptococcus luteolus</i>	1.33	1.88	2.01
<i>Penicillium purpurogenum var.rubrisclerotium thom</i>	1.318	1.4	1.5
<i>Zygo ascus hellenicus</i>	1.47	1.56	1.7
<i>Neosartorya fisheri var. fisheri</i>	1.53		1.88
<i>Candida montana</i>	1.2	1.3	1.6

Trichosporon beigellii B. and yeast \leq 0.5 similarity index (12.5%), *Cryptococcus albidus var aerius*, *Zygoascus hellenicus*, and *Candida Montana* (Table 3).

Phosphate solubilization test

A total of 24 fungus species were evaluated for their phosphate solubilization efficiency on Pikovskaya's agar selective media. Among all 7 isolates were positive for phosphate solubilization (Table 4). From 1.0 to 3.4 cm clear zone diameter were recorded within 15 days of incubation (Figure 4). *T. beigellii B* showed superior solubilization index (PSI) of 5.3, followed by *R. aurantiaca A* which is 2.6, the smaller solubilization index recorded 1.5 by *P. purpurogenum var. rubrisclerotium* (Table 4 and Figure 4).

DISCUSSION

Phosphorus deficiencies are wide spread on soil throughout the world and one of the limiting factors for crop productivity. Phosphorus fertilizers represent major cost for agricultural production. Many bacteria, fungi and a few actinomycetes are potential solubilizers of bound phosphates in soil thus playing an important role making it available to plants in the soluble form (Halder et al.,

1991; Abd-Alla, 1994; Whitelaw, 2000; Goldstein, 1986). Solubilization of insoluble phosphorus by microorganisms was reported by Pikovskaya (1948). During the last two decades knowledge on phosphate solubilizing microorganisms increased significantly (Richardson, 2001; Rodriguez and Fraga, 1999). The 3 main phosphate solubilization mechanisms employed by soil microorganisms are (1) release of organic acid anions, siderophores, protons, hydroxyl ions, CO₂, that release of complexing or mineral dissolving compounds, (2) extracellular enzymes, and (3) the release of phosphatase enzyme (McGill and Cole, 1981). Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996). Many fungal species can solubilize rock phosphate, aluminium phosphate and tricalcium phosphate, such as *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus awamor*, *Penicillium italicum*, *Penicillium radicum*, *Penicillium rugulosum*, *Fusarium oxysporum*, *Curvularia lunata*, *Humicola* spp., *Sclerotium rolfsii*, *Pythium* spp., *Aerothecium* spp., *Phoma* spp., *Cladosporium* spp., *Rhizoctonia* spp., *Rhizoctonia solani*, *Cunninghamella* spp., *Rhodotorula* spp., *Candida* spp., *Schwanniomyces occidentalis*, *Oideodendron* spp., and *Pseudonymnoascus* spp. (Isbelia et al., 1999; Sparks, 1999; Whitelaw et al., 1999; Didiek and Sugiarto, 2000; Helen et al., 2002). Mäder et al. (2002) also reported *Penicillium albidum*, *Penicillium*

thomii, *Penicillium restrictum*, *Penicillium frequentans*, *Gliocladium roseum*, *Myrothecium roridum*, *Penicillium jensenii* and *Eupenicillium javanicum* have been phospho fungi, Varsha et al. (2010) reported that yeast belonging to genus *Saccharomyces*, *Hansenula*, *Klockera*, *Rhodotorula* and *Debaryomyces* spp. were phosphate solubilizing yeast. Fungi commonly reported to effectively solubilizing phosphorus include species of *Aspergillus candidus*, *A. niger*, *Aspergillus parasiticus*, *Aspergillus rugulosus*, *Aspergillus terreus*, *Penicillium*, *Pseudeurotium*, *Trichoderma* spp. and some mycorrhizal fungi (Aseri et al., 2009; Muraleedharan et al., 2010). Firew et al. (2016) reported that from haricot bean, faba bean, cabbage, tomato, and sugarcane phosphate solubilizing fungi *Aspergillus* spp. (55.69%), *Penicillium* spp. (23.35%), and *Fusarium* spp. (9.58%) were isolated from Jimma Ethiopia. Increased growth and P uptake of several crop plants due to PSB inoculation have been reported in a number of studies conducted under both growth chamber and greenhouse conditions (Dey et al., 2004). In this study, a total of 24 fungus were isolated from teff rhizosphere soil collected from North Showa, Ethiopia (Tables 3 and 4) and tentatively evaluated for their phosphate solubilization efficiency on Pikovskaya (PVK) selective media. Among all, 7 isolates were positive for phosphate solubilization: *T. beigelii* B, *R. aurantiaca* A, *C. luteolus*, *P. purpurogenum* var. *rubrisclerotium*, *Z. hellenicus*, *Neosartorya fisheri* var. *fischeri*, and *C. montana* (Table 4). Woyessa and Assefa (2011) reported bacteria isolated from teff rhizosphere soil from agricultural fields of Alemgena and Bushoftu Ethiopia, isolates tef rhizosphere contains a diverse flora of microorganisms. The genera were *Pseudomonas*, *Chryseomonas*, *Burkholderia*, *Bacillus*, *Brevibacillus*, *Stenotrophomonas* and *Aeromonas*. These 4 species *B. subtilis*, *Burkholderia cepacia*, *Pseudomonas fluorescens*, and *Bacillus coagulans* were superior phosphate solubilizer bacteria. However, many rhizospheric bacteria and fungi isolated from different crop rhizosphere soil, there is little information regarding teff rhizosphere fungi and potential phosphate solubilizer. This study will confirm that there are a diverse teff rhizosphere fungi and superior phosphate solubilizer fungi isolated from North Showa teff farm land (Table 3). The soil yeasts *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum*, *Rhodotorula minuta* and *Rhodotorula rubra* solubilized insoluble phosphate reported by Al-Falih (2005). The fungi species *P. purpurogenum* var. *rubrisclerotium* and *R. aurantiaca* A are phosphate solubilizer fungi species discovered in this study are also similar with the work of Yasser et al. (2014) and Isbelia et al. (1999). In this study, phosphate solubilization index were measured within 5 days intervals for 15 days and they showed 1.5 to 5.3 PSI clear zone diameter over colony diameter ratio (Table 4). Narsian et al. (2008) reported yeast belonging to genus *Saccharomyces* *Hansenula*, *Klockera*, *Rhodotorula* and *Debaryomyces* exhibited the highest SI

(1.33 to 1.50). The study by Yasser et al. (2014), phosphate solubilization index recorded 1.05 to 1.45. *A. japonicas* (SI=1.45), *A. niger* (SI=1.12), *Penicillium expansum* (SI=1.20), *Penicillium funiculosum* (SI=1.40), *Penicillium variable* (SI=1.13), and *P. purpurogenum* (SI=1.30). In this study, the largest solubilization index recorded by *T. beigelii* B (PSI, 5.3), *R. aurantiaca* A (PSI, 2.6), the smallest solubilization index recorded by *P. purpurogenum* var. *rubrisclerotium* (PSI, 1.5) (Figure 3 and Table 4). According to De Freitas et al. (1997), good phosphate solubilizers produce halos around their colonies with diameters higher than 1.5 cm. Most efficient phosphate solubilizer on Pikovskaya's agar plates with PSI = 3.29. Whereas among fungi *P. canescens* showed the highest solubilizing index (Chabot et al., 1993; Nahas, 1996). Phosphate solubilization index (PSI) values up to 2.4 have been recorded for *A. niger*, with values of 3.1 for *Penicillium italicum* and 3.0 for *Paecilomyces lilacinus* (El-Azouni, 2008; Hernandez-Leal et al., 2011). Fungal strains isolated from sugarcane and sugar beet rhizosphere showed SI in range of 1.13 to 1.59 (Mahamuni et al., 2012). Alam et al. (2002) reported PSI of the fungal strains isolated from maize rhizosphere that ranged from 1.53 to 1.80. In this study, *P. purpurogenum* recorded the smallest SI value 1.5, but Anju et al. (2015) reported that *P. purpurogenum* SI is 2.25±0.65. In this study, new phosphate solubilizer yeast *T. beigelii* isolated from teff rhizosphere soil with superior solubilization index (PSI) is 5.3 in 15 days incubation. Therefore, demonstration of high phosphatase activity and releasing high amount of phosphorus may be due to the specificity of the phosphatase (Deepa et al., 2010). Therefore, these strains can be candidated and exploited as bio fertilizers through further evaluation and optimization test to increase agricultural productivity of teff crop.

Conclusion

Twenty four (24) fungi isolated from teff rhizosphere soil using lactophenol cotton blue staining and biologic microstation identification system where equivalent to molecular techniques and the dominant species were filamentous fungi. Seven fungi species *T. beigelii* B, *R. aurantiaca* A, *C. luteolus*, *P. purpurogenum* var. *rubrisclerotium*, *Z. hellenicus*, *N. fisheri* var. *fischeri*, and *C. montana* were positive for phosphate solubilization efficiency. *T. beigelii* B was the superior among the isolated fungi in solubilizing index of 5.3 followed by *R. aurantiaca* A with 2.6 and good candidate after further evaluation on *in vitro* test, green house and field trials as bio fertilizer. The rise in the cost of chemical fertilizer, the lack of fertilizer industries in developing countries and the growing environmental issue and biodiversity loss using chemical fertilizer are timely important concern using alternative ecofriendly bio fertilizer to increase yield and productivity of teff crop.

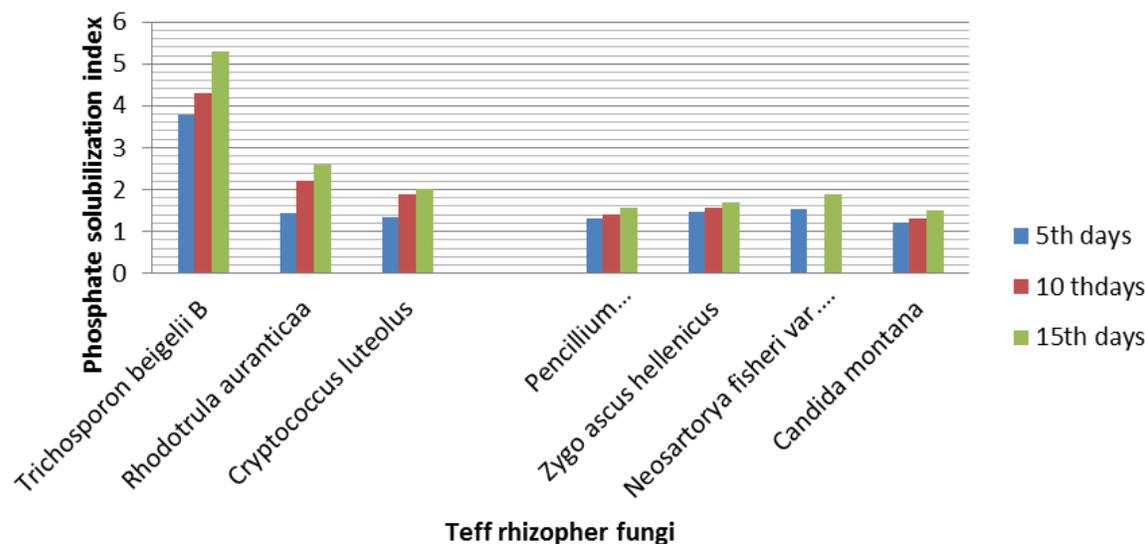


Figure 3. Graph of phosphate solubilization index at 5, 10 and 15 days incubation.

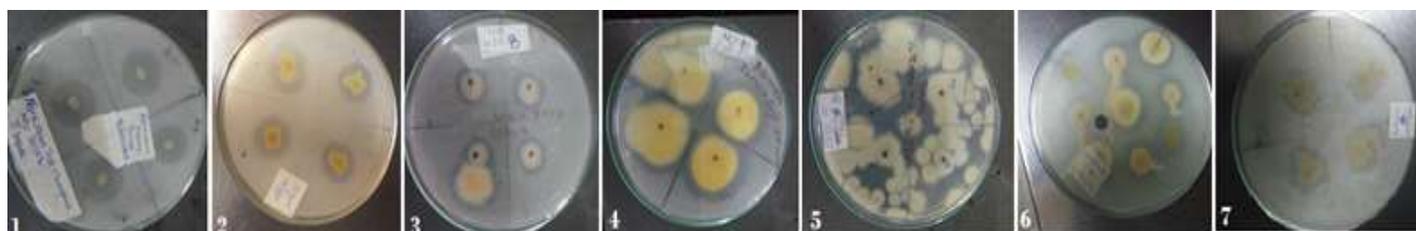


Figure 4. Phosphate solubilizing fungi photo from back side of culture plate: (1) *Trichosporon beigelii B*, (2) *Cryptococcus luteolus*, (3) *Rhodotrula aurantiaca A*, (4) *Pencillium purpurogenum var. rubrisclerotium*, (5) *Neosartorya fisheri var. fisheri*, (6) *Candida montana*, (7) *Zygo ascus hellenicus*.

RECOMMENDATION

The beneficial effects of plant growth promoting microorganisms (PGPM) have not been exploited well. In the past, some microbial inoculants prepared from *Rhizobium* for leguminous crops, *Azotobacter* and *Azospirillum* for cereal crops and *Frankia* for tree crops have been used as nitrogen providers in many developed and developing countries. However, enormous interest increase in research in recent years in PGPM such as nitrogen fixer, phosphate solubilizer, and pathogen suppressor. There is no well-organized microbial inoculant industry for bio fertilizer production especially for phosphate solubilizer and there is no link with researcher working on microbial bio fertilizer in Ethiopia, therefore, Agricultural Research Institute, microbiologist, soil scientist agronomist, and stockholders in general must work together in depth on structural and functional diversity of PGPM and selecting superior biofertilizer, biopesticide, biostimulant to increase crop yield and productivity. Further research should be continued with

selecting efficient phosphate solubilizer microorganism (PSM) isolates. These may be used for inoculum production and their inoculation effect on the plant growth must be studied *in vitro*, green house and field trials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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