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Phosphate Solubilizing Fungi Isolated and Characterized from Teff Rhizosphere Soil Collected from North Showa and Gojam, Ethiopia

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Abstract

Phosphorus (P) is one of the major bio elements limiting agricultural production. Phosphate solubilizing fungi play a noteworthy role in increasing the bioavailability of soil phosphates for plants. The present study was aimed at isolating and characterizing phosphate solubilizing fungi from teff rhizosphere soil. Fungi were identified using lactophenol cotton blue staining confirmation and Biolog micro station. 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 µ-L transferred from each suspension into 96 wells of the biolog yeast micro Plate and filamentous fungi microplate tagged with different carbon source and incubated at 26°C for 24 to 72 h and read by the micro station reader at a single wavelength of 590 nm, results were recorded and processed for identification by micro log3 software ver. 4.20.05. Biolog micro station produce 24 fungi read results. 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 evaluated for phosphate solubilization by the pikovskaya's agar (PVK) selective media. Seven species were positive in phosphate solubilizing ability. *Trichosporon beigelii B, Phichia norvegensis, Cryptococcus albidus var aerius, Candida etchellsii, Cryptococcus albidus var albidus, Rhodotrula aurantiaca A, Rhodotorula aurantiaca B Cryptococcus luteolus, Cryptococcus albidus var diffluens, Neosartorya fisheri var. Fischeri, Cryptococcus terreus A, Candida montana, Penicillium purpurogenum var. Rubrisclerotium, Yeast isolate GTRWS18, GTS9B, GTS7C*. At 15 days' incubation, the solubilizing index ranges 1.2-5.3. The *Trichosporon beigelii B, Phichia norvegensis, Cryptococcus albidus var aerius* were superior in phosphate solubilization with 5.3, 3.35, 3.2 solubilizing index respectively. Therefore, these species can be candidated and exploited after further evaluation as bio fertilizers for teff productivity.

Keywords: Biolog microorganisms; Microstation; Phosphorus; Rhizosphere; Soil solubilization; Teff

Introduction

Phosphorus (P) is the second important nutrient after nitrogen that affects plant growth and metabolism processes making up about 0.2% of plant dry weight [1]. Phosphorus contributes remarkably to photosynthesis, energy and sugar production, nucleic acid synthesis, and promotes N_2 fixation in legumes [2]. In plants, phosphorous increases the strength of cereal straw, promotes flower formation and fruit production, stimulates root development and essential for seed formation [3]. It also plays a role in root development, stalk and stem strength, flower and seed formation, maturity and production, crop quality and resistance to plant diseases [4]. Mobility of phosphate ions in the soil is very low due to their high retention in soil. In 1986 Stevenson and Holford reported that the recovery rate of P fertilizer by plants is only about 10-30% [5]. The remaining 70-90% 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 [6,7]. 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 of fertilizer phosphorus and bound phosphorus in the soil that is environmentally friendly and sustainable [8]. Several groups of microorganisms including fungi, bacteria and actinomycetes are known as efficient fixed P solubilizes [9]. 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 [10]. A wide range of soil fungi are reported to solubilize insoluble phosphorous such as *Aspergillus niger* and *Penicillium sp.* which are the most common fungi capable of phosphate solubilization [11]. Exploration of phosphate solubilizing microorganisms has been conducted by many researchers from soils, mangrove and rhizosphere [1,12-17] respectively. 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, Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium* and *Serratia* have reported to enhance plant growth [18]. Many fungal species can solubilize rock phosphate, aluminum 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 sp., Sclerotium rolfsii, Pythium sp., Aerothecium sp., Phoma sp., Cladosporium sp, Rhizoctonia sp., Rhizoctonia solani, Cunninghamella spp., Rhodotorula sp., Candida sp., Schwanniomyces occidentalis, Oideodendron sp., Pseudonymnoascus sp.* [11,19-22]. The soil yeasts *Candida tropicalis, Geotrichum candidum, Geotrichum capitatum, Rhodotorula minuta* and *Rhodotorula rubra* solubilized insoluble phosphate reported by Al falih.

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Yeast belonging to genus *Saccharomyces, Hansenula, Klockera, Rhodotorula* and *Debaryomyces spp*. were phosphate solubilizing yeast [23]. The principal mechanism for many soil fungi and bacteria can solubilize inorganic phosphate into soluble forms through the process of acidification, chelation, exchange reactions and production of organic acids by Han. 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 [12,24]. Phosphorus deficiency is the most important problem of Ethiopian soil and more than 70-75% of highland soils are characterized by phosphorus deficiency. The deficiency is very severe in the acidic soils of the southern, southwestern and western regions. Areas Al^{3+} and Fe^{3+} are totally incriminated with phosphorus fixation [25]. 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 Al^{3+} and Fe^{3+} [26]. 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) [27]. Teff [*Eragrostistef* (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 [28]. 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. Teff straw, besides being the most appreciated feed for cattle [29]. Teff is the only cultivated of all 300 *Eragrostis spp*. Its agro ecological adaptability has resulted in its cultivation as an important crop in 10 of 18 agro ecological zones of the country. Teff adapted to a wide range of environments and cultivated under diverse agro-climatic conditions [29]. It can be grown in altitudes ranging from near sea level to 3000 ms, but the best performance occurs between 1100 and 2950 m.a.s.l [30]. Annual rainfall of 750-850 millimeter (mm), growing season rainfall of 450-550 mm and a temperature range of 10°C- 27°C. A very good result can also be obtained at an altitude range of 1700-2200 m and growing-season rainfall of 300 mm [31]. 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 [32]. Teff production and productivity have been far below the potential. Currently the average national productivity is estimated to be less than 0.5 ton per ha. This is very low 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 [31]. Declining soil fertility because 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. However chemical fertilizers are neither easily available nor affordable for many poor Ethiopian farmers and not environmentally friendly. 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 fungus on growth and yield of teff was studied, inoculation of teff by vascular arbuscular mycorrhizal (VAM) and other phosphate solubilizing fungi that gives great results on teff yield improvement. So, these previous research works that tell us bio fertilizers made by plant growth promoting rhizobacteria (PGPR) are better indicative to improve teff production and productivity to the significant level. This study was aimed to isolate, identify and evaluating of phosphate solubilizing fungi from teff rhizosphere soil collected from North Showa and Gojam 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 and Gojam in selected districts, particularly in Bichena, Bahirdar zuria, Huletejunaesae, Debrwork, Dejen. Tarmaber, kewot, Sidebirna wayu, Moretna giru, North showa the elevation ranges 1100-3009 meters above sea level. Geographic coordinates Latitude: 9°46'8.4", Longitude: 39°40'4.8". The zone is located approximately average 200 km far from Addis Ababa. Gojam Zone is bordered on the south by the Oromia Region, on the west by west Gojjam, on the north by south Gondar, and on the east by south Wollo; the bend of the Abay Riverdefines the Zone's northern, eastern and southern boundaries.10°31'44.7"N and 37°51'10.2"E. West Gojjam (Mirab Gojjam) is a Zone in the Amhara Region of Ethiopia. West Gojjam isborded by North Gondar, on the north by Lake Tana, and the Abay River which separates it from the South Gondar, and on the east by east Gojam. Coordinates: Latitude: 10.97379 North, Longitude: 37.46814 East. Gojam at Average altitude, 1788 m.a.s.l. (Figure 1).

Sample collection

One hundred fifty teff farmland site were selected based on five teff varieties, two soil types and 200 m difference within 1200-2200 m.a.s.l altitude from the districts of North Showa and Gojam. One hundred fifty rhizosphere soil and 150 teff root 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 farmland were stored in sterile sample tube and icebox during April 08-28/2016 and transported microbial directorate laboratory in Ethiopian biodiversity institute to Addis Ababa and kept in +4°C until processed (Figure 2).

Screening and isolation of fungi from teff rhizospher soil

Soil samples were clustered according to altitude, soil type and teff varieties and merged into 50 composite samples. From each soil samples 1 g was taken and diluted in distilled water serially up to 10^{-6} mL. About 0.1 mL inoculum sample was transferred by swab through and streaked by nichrom loop on extract peptone dextrose agar media (YPDA), Rose Bengal agar, potato dextrose agar. Primary cultures were incubated for 26°C in digital incubator for 48 h. Isolates were subculture twice until pure colony 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 48 h at 26°C for yeast and filamentous fungi micro plate (YT/FF Microplate) inoculum preparation. The yeast and filamentous fungi were identified

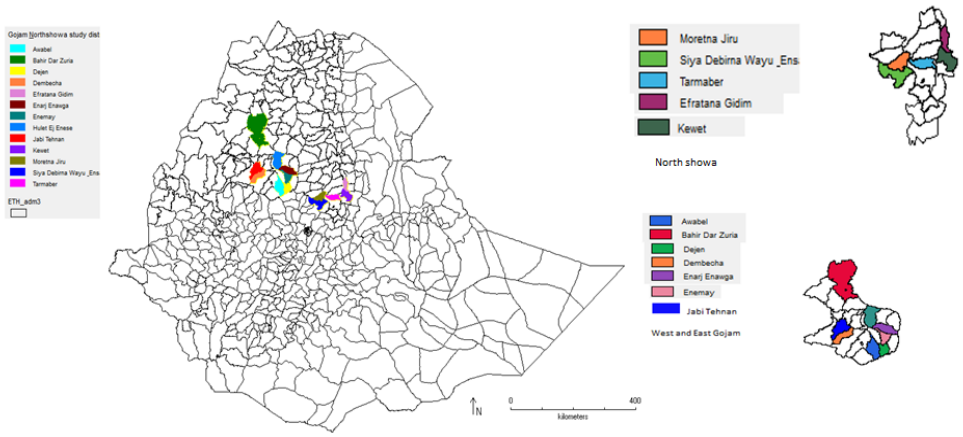

Figure 1: Map of study area.

Figure 2: Activities during teff rhizospher soil collection from Gojam and Northshowa.

according to the Biolog micro station reading and procedure.

Screening and isolation of fungi from teff rhizoplan soil

Fifty composite Teff root sample were washed with sterile distilled water and the root wash solution was kept in test tube. About 0.1 mL inoculum sample was transferred by swab and streaked by nichrom loop on yeast extract peptone dextrose agar media (YPDA), Rose Bengal agar, potato dextrose agar. Primary cultures were incubated for 26°C in digital incubator for 48 h. Isolates were subculture twice until pure colony 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 48 h at 26°C for yeast and filamentous fungi micro plate (YT/FF Microplate) inoculum preparation.

Teff root endophyte identification

Surface sterilization on teff root was carried out using 97% ethanol alcohol and 5% hypochlorate. Teff root were first washed by distilled water until all soil particle release and soaked in 97% ethanol alcohol for 10 minutes. Disinfected teff root again soaked in 5% solution Hypochlorite solution for 10 minutes then again washed by distilled water for six times. 1 cm root is cut and macerated by mortar and pestle until mix together. Each of the samples is transferred into 50 mL distilled water and mixed, shacked by vortex. About 0.1 mL inoculum sample was transferred by swab and streaked by nichrom loop on yeast extract peptone dextrose agar media (YPDA), Rose Bengal agar, potato dextrose agar. Primary cultures were incubated for 26°C in digital incubator for 48 h. Isolates were subculture twice until pure colony 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 48 h at 26°C for yeast and filamentous fungi micro plate (YT/FF Microplate) inoculum preparation.

Colony morphology identification for phosphate solubilizing fungi

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

Identification of yeast from teff rhizosphere, rhizoplane soil and root endophyte using biolog micro station

Pure yeast isolates after grown on yeast extract potato dextrose agar were transferred to biolog universal growth agar and incubated at 26°C for 48 h. Pure colony of yeast suspension were prepared in 9 mL sterile distilled water and adjusted to 47 ± 2T using biolog turbidiameter.100 µL of inoculum was dispensed using digital pipettor to each of 96 wells of yeast microplate(YT) tagged with different carbon source and incubated at 26°C 24-72 h. The YT micro plate measures both metabolic reactions as well as turbidity growth to produce identifications. YT micro Plate is configured with both oxidation tests and assimilation tests. The first 3 rows of the panel (rows A - C) contain carbon source oxidation tests using tetrazolium violet as a colorimetric indicator of oxidation. The next five rows of the panel (rows D - H) contain carbon source assimilation tests. Results from these tests are scored turbid metrically. The last row of the panel (row H) has wells that contain 2 carbon sources. These wells test for the co-utilization of various carbon sources with D-xylose. YT micro plate was read by the micro station reader at 24 h, 48 h, 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 (Biolog) (Figure 3).

Identification of filamentous fungi from teff rhizosphere soil using biolog micro station

Filamentous fungi isolated and screened 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 ± 2T using Biolog turbidiameter. 100 µ-L of inoculum was dispensed using digital pipettor to each of 96 wells of filamentous fungi microplate (FF) tagged with different carbon source and incubated at 26°C, 24-240 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 h, 48 h, 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 (Figure 3).

Identification of phosphate solubilizing microorganisms from teff rhizoshere soil

Fungal isolate identified by Biolog micro station were tested for their phosphate solubilizing ability. Pure fungi colonies were collected using a needle nose and spot at 4 quadrants on sterile solid Pikovskaya media having composition of 2.5 g $Ca_3(PO_4)$, 0.5 g $(NH_4)_2SO_4$, 0.2 NaCl, 0.1 g $MgSO_4.7H_2O$, 0.2 g KCl, 10 g glucose, 0.5 g of yeast extract, 20 g agar, small amounts of $MnSO_4$ and $FeSO_4$, and 1000 mL distilled water [33]. $Ca_3(PO_4)_2$ 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 following formula. Fungi that formed the fastest clear areas with the greatest

Figure 3: Biolog micro station identification steps.

diameter indicate the most superior phosphate solubilizing fungi.

SI=Colony diameter + Halozone diameter/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 290 fungal colonies were grown and counted on different growth media and identified pure colonies having similar morphology were clustered in order to detect percentage frequencies of the microorganisms. Sixty one percent were filamentous fungi and 39% were non-filamentous fungi. From filamentous fungi, *Aspergillus* species were dominant (32%), *Penicillium* species (30%), *Fusarium* species (15%). *Trichoderma* (14%), *Colletotrichum* (9%). From yeast species *Cryptococcus* and *Rhodotrula* were dominant. The phosphate solubilizer fungi isolates were also identified based on their colony morphology that is pigmentation, shape, size, texture, elevation and margin, the following table will summarize (Table 1).

Identification of filamentous fungi species using lactophenol 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 and read by biolog micro station where 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 piluiliferum*, *Fusarium avenaceum*, *Penicillium vulpinum, Neosartorya fischeri var. Fischeri, Fusarium udum, Hypocrea pseudokoningii, Trichoderma cittrinoviride, Trichoderma aureoviride, Penicillium purpurogenum var. rubrisclerotium, Penicillium pinophilum* (Table 2).

Identification of yeast species using biolog micro station

Biolog microstion read at 24, 48 and 72 YT microplate incubation result revealed that yeast ≥ 0.5 similarity index value identify *Rhodotorula aurantiaca A, Candida etchellsii, Kluyveromyces delphensis, Cryptococcus luteolus, Cryptococcus albidus var aerius, Cryptococcus terreus A, Rhodotorula aurantiaca, B, Phichia norvegensis, Zygoascus hellenicus, Candida Montana, Cryptococcus albidus var diffluens* (Table 3).

Phosphate solubilization test

A total of 36 funguses were read by biolog micro station from teff rhizospher soil and tentatively evaluated for their phosphate solubilization efficiency on Pikovskaya's agar selective media. Among all 19 isolates were positive for phosphate solubilization (Table 4), they produced the largest halos around their colony approximately 1.2-5.3 cm within 15 days of incubation. *Trichosporon beigelii B* showed superior solubilization index (PSI) 5.3, followed by 3.35, 3.2 *Phichia norvegensis, Cryptococcus albidus var aerius* respectively the smaller solubilization index recorded 1.2 by yeast isolate GTS7C (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 solubilizes of bound phosphates in soil thus playing an important role making it available to plants in the soluble form [34-37]. Solubilization of insoluble phosphorus by microorganisms was reported by Pikovskaya [38]. During the last two decades' knowledge on phosphate solubilizing microorganisms increased significantly [39]. In this study, a total of 290 yeast isolates were screened from teff rhizosphere soil collected from North showa and Gojam, Ethiopia and 19 yeasts were read by Microstation. Sixteen yeast have got full species identification (ID) and 3 with no species ID (Table 2). All yeast species evaluated for their phosphate solubilization ability on Pikovskaya (PVK) selective media. Among all 16 yeast species and 3 isolates were positive for phosphate solubilization. *Phichia norvegensis*, *Cryptococcus albidus var albidus*, *Cryptococcus luteollus*, *Rhodotrula aurantiaca B*, *Cryptococcus albidus var diffluens*, *Candida etchellsii*, *Cryptococus terrus A, Cryptococcus albidus* var aerius, *Rhodotrula aurantiaca A, Trichosporon beigelii B, Cryptococcus luteolus, Rhodotrula aurantiaca A*, *Penicillium purpurogenum var. rubrisclerotium, Neosartorya fisheri var. fischeri*, *Candida montana, Zygo ascus hellenicus.* Yeast isolate, GTRWS1, GTS9B reported yeast belonging to genus *Saccharomyces, Hansenula, Klockera, Rhodotorula* and *Debaryomyces spp* were phosphate solubilizing yeast [23]. The soil yeasts *Candida tropicalis,*

P-solubilizing fungi	Shape	Elevation	Size	Marigin	Surface texture	Color
Trichosporon beigelii B	Irregular	Flat	Large	Lobate	Concentric	White yellow
Rhodotrula aurantiaca A	Rhound	Flat	Large	Undulate	Radiate	White
Penicillium purpurogenum Var.rubrisclerotium	Circular	Umbonate	Large	Filamentous	Radiate	Gray
Neosartoryafisheri var. fischeri	Circular	Umbonate	Large	Filamentous	Rugose	Olive green
Cryptococcus luteolus	Irregular	Flat	Lobate	Obate	Radiate	Yellow
Zygoascus hellenicus	Irregular	Flat	Lobate	Obate	Radiate	Yellow
Candid montana	Round	Flat	Large	Smooth	Radiate	White pink
Cryptococcus albidus var aerius	Irregular	Flat	Large	Lobate	Concentric	Yellow
Cryptococcus terreus A	Irregular	Flat	Large	Undulate	Smooth	White
Cryptococcus albidus var albidus	Entire	Pulvinate	Large	Entire	Radiate	White yellow
Rhodotorula aurantiaca B	Irregular	Raised	Large	Undulate	Concentric	White
Phichia norvegensis	Circular	Flat	Large	Entire	Concentric	White yellow
Candida etchellsii	Circular	Flat	Medium	Entire	Concentric	Yellow white
Cryptococcus albidus var diffluens	Entire	Flat	Large	Erose	Concentric	Yellow white

Table 1: Colony morphology for phosphate solubilizer fungi.

	Fungus species	LPCB Staining result	Probability	Similarity	Distance	Teff farm land districts
1	*Colletotrichum lindemuthianum (Saccardo & Mangus) Briosi*	+	-	0.001	32.96	Ejersa Qubete
2	*Emericella quadrilineata (Thom & Raper) C.R. Benjamin*	+	-	0.001	29.89	Kewot (Worentele)
3	*Fusarium melanochlorum (Caspary) Sacc.*	+	-	0.001	32.37	Efratana Gidm (Karalgoma)
4	*Aspergillus brevipes G. Sm.*	+	-	0.002	28.24	Tarma Ber (Asfachew)
5	*Fusarium juruanum*	+	-	0.000	48.72	Tarma Ber (Armania)
6	*Trichoderma piluiliferum Webster & Rifai*	+	-	0.000	48.48	Efratana Gidm (Karalgoma)
7	*Fusarium avenaceum s.sp.nurragi Summerell & L.W. Burgess*	+	-	0.002	27.20	Kewot (Korebta)
8	*Penicillium vulpinum (Cooke & Massee) Seifert & Samson*	+	-	0.003	25.98	Tarma Ber (Chira Meda)
9	*Neosartorya fischeri var. Fischeri (Wehmer) Malloch & Cain*	+	-	0.001	32.18	Ejersa Qubete
10	*Fusarium udum E.Butler*	+	-	0.000	39.36	Tarma Ber (Chira Meda)
11	*Hypocrea pseudokoningii*	+	-	0.000	33.5	Tarma Ber(Chira Meda)
12	*Trichoderma cittrinoviride Bissett BGA*	+	-	0.000	46.16	Efratana Gidm(Karalgoma)
13	*Trichoderma aureoviride Rifai*	+	-	0.000	39.81	Efratana Gidm(Karalgoma)
14	*Penicillium purpurogenum var. Rubrisclerotium Thom*	+	-	0.004	24.46	Ejersa Qubete
15	*Penicillium pinophilum Hedge. BGB*	+	-	0.002	26.74	Mendida (Moyesilasie)

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

	Fungus species	Probability	Similarity	Distance	Fungi isolated from North showa	Fungi isolated from Gojam
1	*Rhodotorula aurantiaca A*	100/100/100	0.604/0.533/0.584	6.1/7.77/6.48	Kewot (Abay Atir)/ Kewot (Worentele)	Bichena Gotera Kebele
2	*Candida etchellsii*	74/78	0.5450.658/	4.01/2.34	Deneba (Dacho)	Awabel, Enebi chifri
3	*Kluyveromyces delphensis*	77	0.533	4.75	Kewot (Worentele)	-
4	*Cryptococcus luteolus*	-/-	0.728/0.659	4.13/3.19	Tarmaber (ChiraMeda)	Dejen Zemetin Kebele
5	*Cryptococcus albidus var aerius*	-/100	0.226/0.6	7.22/14.34	Mendida (Moyeselase)	Jabitehnan
6	*Trichosporon beigelii B*	99	0.523	7.51	Mendida (Moyeselase)	-
7	*Cryptococcus albidus var aerius*	100	0.542	7.22	-	Hulet eju enese,Debre Gubae Kebele
8	*Cryptococcus terreus A*	99	0.605	6.03	-	Hulet eju enese,Debre Gubae Kebele
9	*Cryptococcus albidus var albidus*	93	0.598	5.49	-	Jehabitenan,Jiga Yelimdar
10	*Rhodotorula aurantiaca B*	86	0.588	4.86	-	Hulet eju enese,Debre Gubae Kebele
11	*Phichia norvegensis*	82	0.520	5.61	-	Hulet eju enese, Debre Gubae Kebele
12	*Zygoascus hellenicus*	-	0.216	14.1	Tarmaber (ChiraMeda)	
13	*Candida montana*	-	0.47	3	Kewot (Abay Atir)	
14	*Cryptococcus albidus var diffluens*	-	0.358	7.81		Hulet eju enese, Debre Gubae Kebele

Table 3: Biolog micro station yeast identification result read.

Geotrichum candidum, Geotrichum capitatum, Rhodotorula minuta and *Rhodotorula rubra* solubilized insoluble phosphate reported by Al falith. Woyessa and Assefa reported bacteria isolated from teff rhizosphere soil from agricultural fields of Alemgena and Bushoftu Ethiopia, isolates teff rhizosphere contains a diverse flora of microorganisms. The genera were *Pseudomonas, Chryseomonas, Burkholderia, Bacillus, Brevibacillus, Stenotrophomonas and Aeromonas*. These 4 species *Bacillus subtilis, Burkholderia cepacia, Pseudomonas fluorescens, 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 rhizophere yeast and potential phosphate solubilizer yeast. This study will confirm that there are a diverse teff rhizosphere yeast and superior phosphate solubilizer isolated from North showa and Gojam teff farm land (Tables 2 and 3). The yeast species *Rhodotrula aurantiaca A* are phosphate solubilizer fungi species discovered in this study are also similar with the work of [19,40]. In this study phosphate solubilization index (PSI) were measured within 5 days' intervals for 15 days and they showed 1.2 -3.35 PSI clear zone diameter over colony diameter ratio (Table 3). Narsian reported yeast belonging to genus Saccharomyces *Hansenula, Klockera, Rhodotorula* and *Debaryomyces* exhibited highest SI (1.33-1.50). The study by Yasser et al. phosphate solubilization index recorded 1.05- 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), *Penicillium purpuragenum*(SI=1.30). In this study the largest solubilization index recorded by *Phichia norvegensis* (SI. 3.35), *Cryptococcus albidus var aerius* (SI. 3.2), *Candida etchellsii* (SI 2.9). The smallest solubilization index recorded by *Cryptococcus terrus A* (PSI, 1.72) (Figure 3 and Table 3). According to De freitas et al., 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 highest solubilizing index [10]. Phosphate solubilization index (PSI) values up to 2.4 have been recorded for *Aspergillus niger*, with values of 3.1 for *Penicillium italicum* and 3.0 for *Paecilomyces lilacinus* [40-42]. Fungal strains isolated from sugarcane and sugar beet rhizosphere showed SI in range of 1.13 to 1.59 reported PSI of the fungal strains

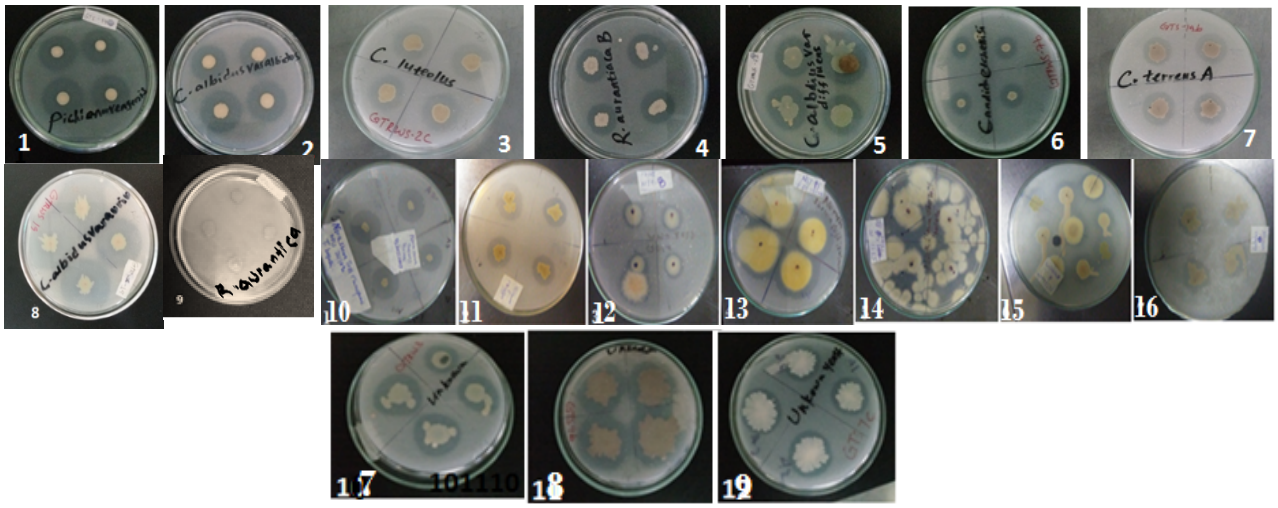

Figure 4: Phosphate solubilizing yeast on Pikovskaya's agar media. (1) *Phichia norvegensis* (2) *Cryptococcus albidus var albidus* (3) *Cryptococcus luteollus* (4) *Rhodotrula aurantiaca B* (5) *Cryptococcus albidus var diffluens* (6) *Candida etchellsii* (7) *Cryptococus terrus A* (8) *Cryptococcus albidus* var aerius (9) *Rhodotrula aurantiaca A* (10) *Trichosporon beigelii B,* (11) *Cryptococcus luteolus,* (12) *Rhodotrula aurantiaca A* (13) *Penicillium purpurogenum var.rubrisclerotium,* (14) *Neosartorya fisheri var. fischeri*, (15) *Candida montana*, (16) *Zygo ascus hellenicus* (17) Yeast isolate GTRWS18 (18) Yeast isolate GTS9B (19) Yeast isolate GTS7C.

	Fungus species isolated from teff rhizosphere soil	Phosphate solubilization index (PSI)		
		5th day	10th day	15th day
	Trichosporon beigelii B	3.8	4.3	5.3
1	*Phichia norvegensis*	2.51	3	3.35
2	*Cryptococcus albidus var aerius*	1.32	2.51	3.2
3	*Candida etchellsii*	1.76	2.54	2.90
4	*Cryptococcus albidus var albidus*	2.49	2.57	2.9
5	*Rhodotrula aurantiacaA*	1.16	1.8	2.6
6	*Rhodotorula aurantiaca B*	1.55	2	2.24
7	*Cryptococcus luteolus*	1.80	1.82	2.22
8	*Cryptococcus albidus var diffluens*	1.54	1.67	1.9
9	*Neosartorya fisheri var. fischeri*	1.53		1.88
10	*Cryptococcus terreus A*	1.34	1.44	1.72
11	*Candida montana*	1.2	1.3	1.6
12	*Penicillium purpurogenum var.rubrisclerotium thom*	1.318	1.4	1.5
13	*Yeast isolatGTRWS18*	1.4	1.5	1.54
14	*Yeast isolatGTS9B*	1.2	1.33	1.49
15	*Yeast isolatGTS7C*	0.9	1.1	1.2

Table 4: Phosphate solubilization index (PSI).

isolated from maize rhizosphere that ranged from 1.53 to 1.80. In this study new phosphate solubilizer yeast *Trichosporon beigelii B, Phichia norvegensis, Cryptococcus albidus var aerius* identified from teff rizhosphere soil with superior solubilization index (PSI) 5.3, 3.35 and 3.2 respectively in 15 days incubation. 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 nine fungi isolated from teff rhizosphere soil using lactophenol cotton blue staining and biolog microstation identification system where equivalent to molecular techniques and the dominant species were filamentous fungi. Seven fungi species *Trichosporon beigelii B, Phichia norvegensis, Cryptococcus albidus var aerius, Candida etchellsii, Cryptococcus albidus var albidus, Rhodotrula aurantiacaA, Rhodotorula aurantiaca B, Cryptococcus luteolus, Cryptococcus albidus var diffluens, Neosartorya fisheri var. Fischeri, Cryptococcus terreusA, Candida montana, Penicillium purpurogenum var. Rubrisclerotium, yeast isolate GTRWS18, GTS9B, GTS7C* were positive for phosphate solubilization efficiency. *Trichosporon beigelii B* was the superior among the isolated fungi in solubilizing index 5.3 followed by *Rhodotrula aurantiaca A* 2.9 and good candidate after further evaluation on *invitro* 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 timely important concern using alternative ecofriendly bio fertilizer to increase yield and productivity of teff crop.

Recommendation

The beneficial effects of plant growth promoting microorganisms(PGPM) have not been exloited well. In the past, some microbial inoculants prepared from *Rhizobium* for leguminous crops, *Azotobacter* and *Azospirillium* 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, 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 prductivity. 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.

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