



Rhizosphere Fungi Identified from *Poaceae* and *Cyperaceae* Family Grass in North and East Showa: Ethiopia

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Abstract

The Rhizosphere is a hotspot of plant-microbe interactions with profound influence on plant productivity and ecosystem function. The aim of this research is to isolate and identify Rhizosphere fungi from *Poaceae* and *Cyperaceae* family grass for agricultural application to use as biofertilizer, bio control and bio pesticide. Rhizosphere soil and root sample were collected from 18 *Poaceae* and *Cyperaceae* family grass. Malt and potato dextrose agar were used to primary and secondary fungi culture. Pure fungi isolates were transferred to biolog universal yeast agar media. Pure yeast cells and filamentous fungi were suspended in sterile water and filamentous fungi (FF) inoculums 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 micro plate tagged with different carbon source and incubated at 26°C for 24 to 72 h for yeast and 24-240 h for filamentous fungi and read by micro station 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 results were recorded and processed for identification by micro log³ software ver. 4.20.05. Biolog microstation read 27 fungi species. The result revealed that *Fellomyces fuzhouensis*, *Schizoblastosporion starkeyi-henricii*, *Cryptococcus luteolus*, *Rhodotorula aurantiaca A*, *Cryptococcus Terries A*, *Trichosporon beigelii B*, *Rhodotorula aurantiaca B*, *Cryptococcus albidus var aerius*, were fully identified above 75% probability and 0.5 similarity index. In conclusion these rhizosphere-microbes interaction study useful for agricultural application for bio fertilizer after further evaluation.

Keywords: Cyperaceae; Family; Poaceae; Rhizosphere; *Cryptococcus*; *Luteolus*; Biocontrol

Abbreviations: FF: Filamentous Fungi; PGPR: Plant Growth Promoting Rhizobacteria.

Introduction

Poaceae are the fifth largest family of flowering plants following the *Asteraceae*, *Orchidaceae*, *Fabaceae* and *Rubiaceae*. Globally, about 12,000 grass species in about

771 genera that are classier into 12 subfamilies and the family is economically important because it includes Teff (*Eragrostis tef*), wheat (*Triticum L.*), rice (*Oryza L.*) and corn (*Zea L.*), as well as numerous forage, bamboo and bio fuel grass species [1]. Grasses grow on all continents in tropical, temperate and Arctic zones and are absent only from Antarctica [2]. Grasses have long had significance in human society for feed and fodder for people and

domesticated animals for thousands of years. During seed germination and seedling growth of this grass, there is great interaction with a range of microorganisms present in the surrounding soil. Root exudates from this grass are mainly composed of water soluble sugars, organic acids, and amino acids, hormones, vitamins, amino compounds, phenolics and sugar phosphate esters [3]. Broadly, there are three distinct components recognized in the rhizosphere; the rhizosphere per se (soil), the rhizoplane, and the root itself. The rhizosphere is thus the zone of soil influenced by roots through the release of substrates that affect microbial activity. The rhizoplane is the root surface, including the strongly adhering root particles. The root itself is a part of the system, because certain endophytic microorganisms are able to colonize inner root tissues [4]. Microorganisms present in the rhizosphere play important roles in ecological fitness of their plant host.

The rhizosphere is a hotspot of plant-microbe interactions with profound influence on plant productivity and ecosystem function [5]. Shaped by the release of labile carbon (C) from plant roots and root uptake of nutrients and water (Hinsinger et al., 2005), the physiochemical environment of the rhizosphere supports a microbial community compositionally and metabolically distinct from that found in bulk soil [6]. The resulting rhizosphere micro biome performs critical functions, modulating plant growth and development [7], plant health [6,8], and plant nutrient acquisition [5]. As well as the production of antibiotics, geochemical cycling of minerals and plant colonization [9]. Plant-microbe interactions may thus be considered beneficial, neutral, or harmful to the plant,

depending on the specific microorganisms and plants involved and on the prevailing environmental conditions [10]. Exploring these microorganisms by unraveling their possible relationships with plants has launched a new and fascinating area of investigations in the rhizosphere research. A better understanding of the basic principles of the rhizosphere ecology, including the function and diversity of inhabiting microorganisms is one way to improve agriculture and to reduce improper use of chemical pesticides and fertilizers creating a long list of environmental and health problems. Thus, the exploration of rhizosphere microorganisms from grass family as one of the best options to increase biomass yield of the Teff crops through developing bio fertilizer, bio pesticide, bio control, In general understanding microbial diversity in rhizosphere of grass family useful for compost making, bioremediation and vast agricultural as well as industrial application. There for the aim of this research is isolation and identification of rhizosphere fungi from different grass species at different agro ecology in later for use as agriculture input.

Materials and Methods

Study area

The study was conducted in North and East Showa in 10 selected districts, in North Showa zone particularly in Kewot, Tarmaber, Efratanagidim, Siade bernawayu and Ankober. 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".

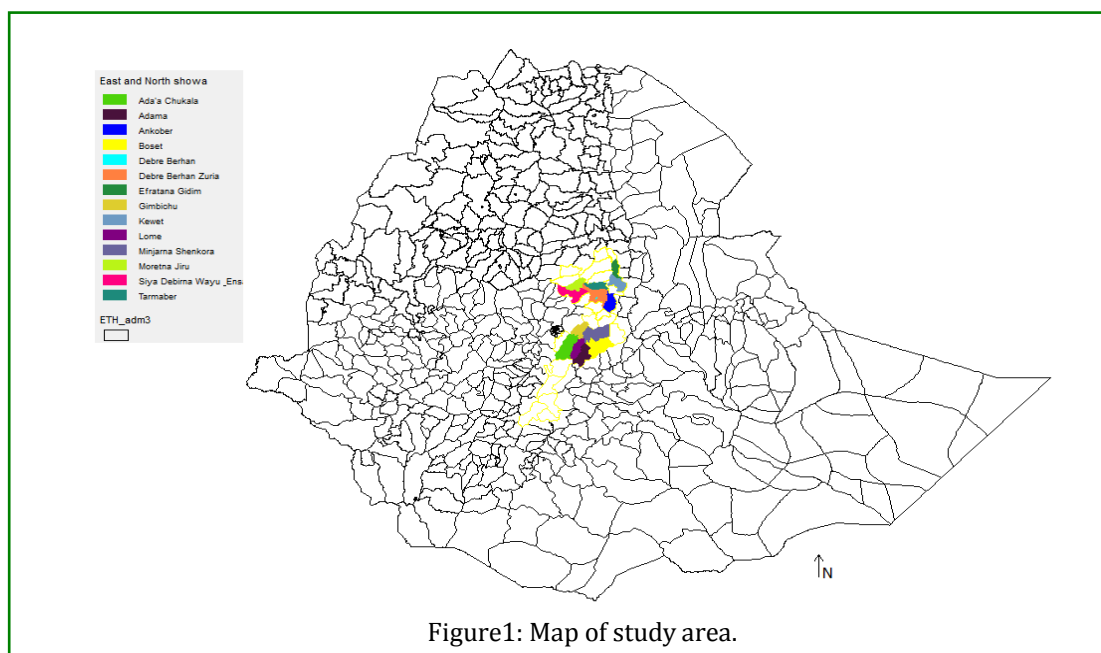


Figure1: Map of study area.

The zone is located in approximately average 200 km far from Addis Ababa. East showa is found in, Oromia regional state, It has 12 districts according to the zonal statistics and information center, study in east showa comprise Adama, Bost, Adaa, Gimbichu and Lomme. East Shewa is found between 38° 57' and 39° 32' E and 7° 12' and 9° 14' N. 93% of the district falls in the lowlands in rift valley. The average altitude is 1600 m, but rises up to 2300 m at the north western and western mountain fringes of the rift on one hand, and it falls to 900-1000m towards northeast. Agro ecology of the zone is divided in to three, 0.2% area of the zone is found in the high land, 61.1% midland and the rest 38.7% is found in the low land (Figure 1).

Sample collection

Six hundred forty rhizosphere soils through drillings at 5, 10, and 15 cm depth and 640 root samples from Poaceae and Cyperaceae family grass in sterile sample tube were collected during August 2017 to March 2018 G.C (Table 1, Figure 2). Approximately 5 g of soil were taken from each depth of sampling point for a total of 15g composite soil stored in sterile sample tube via icebox and transported to micro bial directorate laboratory in Ethiopian biodiversity institute to Addis Ababa and kept in +4°C until processed.

Sample collected area	Poaceae and Cyperaceae family grass species	Sample size
North Showa	<i>Cyperus Eragrostis</i>	20
	<i>Pennisetum Sphacelatum</i>	20
	<i>Beckmannia syzigachne</i>	20
	<i>Eragrostis papposa</i>	20
	<i>Eragrostis variabilis</i>	20
	<i>Eragrostis cilianensis</i>	20
	<i>Eragrostis atropiodes</i>	20
	<i>Milium Yaffle</i>	20
	<i>Hyparrhenia rufa</i>	20
	<i>Eragrostis capillaris</i>	20
	<i>Cynodon Dactylon</i>	20
	<i>Eragrostis spectabilis</i>	20
	<i>Eragrostis capillaris</i>	20
	<i>Eragrostis curvula</i>	20
	<i>Cyperus fuscus</i>	20
<i>Deschampsia cespitosa</i>	20	
	<i>Cyperus Eragrostis/longus</i>	20
East showa	<i>Eragrostis Teff</i>	300
Total rhizosphere soil and roots sample collected each		640

Table 1: Sampling area and Grass family.

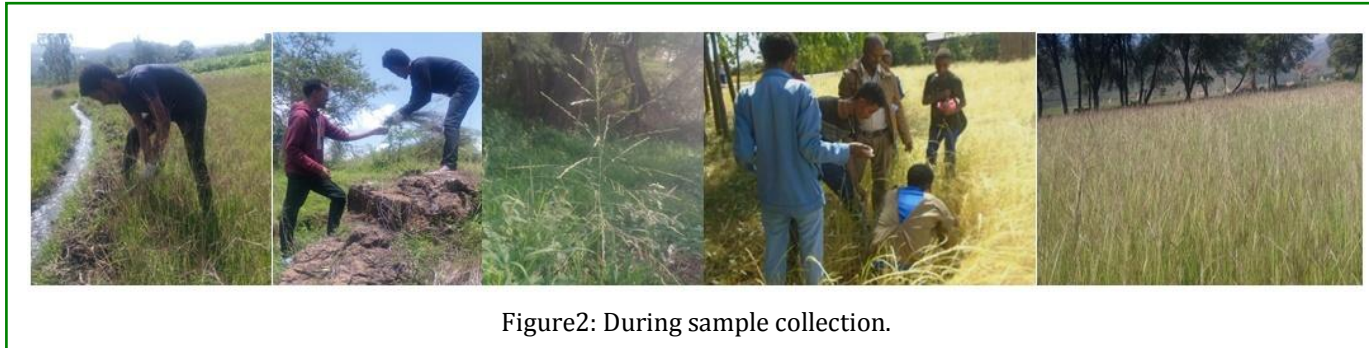


Figure 2: During sample collection.

Screening and isolation of Rhizosphere fungi from Poaceae and Cyperaceae family grass

Six hundred forty soil samples were clustered according to altitude, soil type and grass varieties and merged into 40 composite samples. From each soil samples 1 g was taken and diluted in distilled water serially up to 10⁻⁶ mL. In the same way the root samples were clustered and washed by distilled water. Finally roots were disinfected with 3% Hypochlorate, 70% ethanol alcohol for 3 to 5 minutes and rinse 6 times by distilled water. Finally surface sterilized root were crushed and diluted by distilled water the same way to soil sample. About 0.1 mL inoculums sample was transferred by swab through and streaked by nichrome loop on Malt extract agar and

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 micro plate inoculums preparation.

Identification of Rhizosphere fungi from Poaceae and Cyperaceae family grass

Based on primary and secondary colony morphology fungi isolates were screened and transferred to Biolog universal yeast agar media. Pure fungi cell were

suspended in 9 mL sterile water for yeast and 15mL filamentous fungi (FF) inoculum fluid for filamentous fungi at 49 ± 2 and 75 ± 2 turbidity measured by Biolog turbidimeter, respectively. 100 μ L transferred using digital pipette or from each suspension into 96 wells of the Biolog yeast micro plate (YT) and filamentous fungi micro plate (FF) tagged with different carbon source and incubated at 26°C for 24 to 72 h for yeast and 24-240 h for filamentous fungi and read by micro station reader 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. Acceptable species identification must have similarity index value above or equal to 0.5 or probability above or equal to 75% were chosen only for species identification and characterization [11].

Result

From *Poaceae* and *Cyperaceae* family grass a total of 476 rhizosphere fungal colony were grown on Malt extract agar and Potato dextrose agar. The fungal isolates were

screened and morphologically similar colonies were clustered. Representative clusters were transferred in to YT/FF Micro Plate for Biolog Micro Station reading. Finally 27 fungal species (21 yeast and 6 filamentous fungus) were read by Micro station reader and the result revealed that 8 yeast species >0.5 similarity index and $>75\%$ probability were identified. These are *Fellomyces fuzhouensis*, *Schizoblastosporion starkeyi-henricii*, *Cryptococcus luteolus*, *Rhodotorula aurantiaca* A, *Cryptococcus terreus* A, *Trichosporon beigelii* B, *Rhodotorula aurantiaca*, *Cryptococcus albidus valerius*. Whereas 19 Fungal Species Its Similarity Index Was Below 0.5. There for Eight Rhizosphere, Rhizoplane and Endophyte Yeast were fully identified from five Grass species in the study area (Figure 3, Table 2). Their percentage frequency on MEA media was recorded, the result revealed *Trichosporon beigelii* B (24.5%), *Rhodotorula aurantiaca* A (21.5%), *Cryptococcus toreros* A (20%), *Rhodotorula aurantiaca* B (17.5%), *Cryptococcus luteolus* (9%), *Cryptococcus albidus valerius* (7.5%). The *Cryptococcus* genera (36.5%) yeast was the dominant in this study. Filamentous fungi also confirmed by cellular morphology using Lactophenol cotton blue staining, the result revealed that *Penicillium* genera were dominant.



Figure 3: Five Grass species in the study area (1: *Cyperus Fuscus*, 2: *Eragrostis Cilianensis*, 3: *Pennisetum sphacelatum*, 4: *Beckmannia syzigachne*, 5: *Deschampsia Cespitosa*, 6: *Eragrostis Tef*).

NO	Species	Probability	Similarity	Distance	Lacto phenol blue staining	Isolated from	Species identified from Poaceae Family	Fungi Species Isolation area
1	<i>Fellomyces fuzhouensis</i>	100%	0.622/	5.83		Root wash/Rhizoplane	<i>Eragrostis cilianensis</i>	ChefaDewa (Finchiftu)
2	<i>Fellomyces fuzhouensis</i>	98%	0.51	7.6		Root	<i>Pennisetum sphacelatum</i>	Ankober (Chefa)
3	<i>Schizoblastosporion starkeyi-henricii</i>	100%	0.759	3.61		Root wash	<i>Beckmannia syzigachne</i>	Kewot(Wuruba)
4	<i>Schizoblastosporion starkeyi-henricii</i>	100%	0.711	4.36		Rhizosphere soil	<i>Beckmannia syzigachne</i>	EfratanaGidim (Chiro)
5	<i>Cryptococcus luteolus</i>	99%	0.925	6.62		Root	<i>Deschampsia cespitosa</i>	ChefeDonsa (Woledi)/
6	<i>Rhodotorula aurantiaca</i> A	99%	0.610	5.96		Rhizosphere soil	<i>Eragrostis tef</i>	Bost(Tedecha)
7	<i>Cryptococcus terreus</i> A	100%	0.706	4.43		Rhizosphere soil	<i>Eragrostis tef</i>	Adaa (keteba)
8	<i>Trichosporon beigelii</i> B	100%	0.511	7.8		Rhizosphere soil	<i>Eragrostis tef</i>	Bost(Gere nura)

9	<i>Rhodotorula aurantiaca B</i>	98%	0.734	3.85		Rhizosphere soil	<i>Eragrostis tef</i>	Adda
10	<i>Rhodotorula aurantiaca A</i>	100%	5.00	7.99		Root	<i>Eragrostis tef</i>	Gimbichu
11	<i>Trichosporon beigeli B</i>	100%	0.578	6.59		Root	<i>Eragrostis tef</i>	Adda
12	<i>Cryptococcus terreus A</i>	92%	0.66	4.22		Root	<i>Eragrostis tef</i>	Bost
13	<i>Rhodotorula aurantiaca A</i>	98%	0.548	6.92		Root	<i>Eragrostis tef</i>	Bost
14	<i>Cryptococcus luteolus</i>	100%	0.85	2.18		Root	<i>Eragrostis tef</i>	Lumme
15	<i>Trichosporon beigeli B</i>	100%	0.57	6.64		Rhizosphere soil	<i>Eragrostis tef</i>	Bost
16	<i>Cryptococcus albidus var aereus</i>	100%	0.54	7.10		Rootwash	<i>Eragrostis tef</i>	Adama
17	<i>Rhodotorula aurantiaca B</i>	88%	0.68	3.36		Rootwash	<i>Eragrostis tef</i>	Lumme
18	<i>Candida aaseri A</i>	-	0.361	9.62		Rhizosphere soil	<i>Eragrostis tef</i>	Minjarshenkora (Memeher agar)
19	<i>Pichia guilliermondii A</i>	-	0.315	11.90		Rootwash	<i>Eragrostis tef</i>	Lumme (Dekebora)
20	<i>Rhodotorula acuta</i>	-	0.70	8.24		Rootwash	<i>Eragrostis tef</i>	Adama
21	<i>Pichia norvengensis</i>	-	0.499	4.28		Rhizosphere soil	<i>Eragrostis tef</i>	Bost(Gere nura era)
22	<i>Sterigmatomyces halophilus</i>	-	0.416	9.32		Rhizosphere soil	<i>Eragrostis tef</i>	Gimbichu
23	<i>Trichosporon inkin</i>	-	0.394	1.87		Rhizosphere soil	<i>Eragrostis tef</i>	Lumme
24	<i>Candida incommunis</i>	-	0.415	9.65		Root	<i>Eragrostis tef</i>	Bost(Gere nura)
25	<i>Cryptococcus albidus var diffluens</i>	-	0.444	9.04		Root	<i>Eragrostis tef</i>	Gimbichu
26	<i>Rhodotrula glutinis</i>	-	0.447	8.91		Root	<i>Eragrostis tef</i>	Bost(Gere nura)
27	<i>Cryptococcus luteolus</i>	-	0.54	6.62		Rootwash	<i>Eragrostis cilianensis</i>	EfratanaGidim (Selamaw)
28	<i>Cryptococcus skinneri</i>	-	0.404	4.87		Rootwash	<i>Eragrostis variabilis</i>	EfratanaGidim (Selamaw)
29	<i>Sterigmatomyces halophilus</i>	-	0.499	3.39		Rhizosphere soil	<i>Cyprus fuscus</i>	Ankober (chefa)
30	<i>Trichosporon beigeli B</i>	-	0.452	7.78		Rootwash	<i>Eragrostis cilianensis</i>	EfratanaGidim (Selamaw)
31	<i>Aspergillus restrictus</i>		0.004	24.27	+	Rhizosphere soil	<i>Eragrostis tef</i>	Bost
32	<i>Penicillium italicum Wehmer</i>		0.000	36.00	+	Rootwash	<i>Eragrostis tef</i>	Adaa
33	<i>Penicillium roqueforti Thom BGE</i>		0.000	40.93	+	Rhizosphere soil	<i>Eragrostis tef</i>	Adaa
34	<i>Penicillium argillaceum</i>		0.000	44.03	+	Rootwash	<i>Eragrostis tef</i>	Adaa
35	<i>Penicillium erythromellis Hocking</i>		0.000	41.61	+	Rootwash	<i>Eragrostis tef</i>	Adaa
36	<i>Penicillium digitatum</i>		0.000	48.16	+	Rootwash	<i>Eragrostis tef</i>	Adaa

Table 2: Biolog Micro station fungal identification read result.

Discussion

Rhizodeposition of carbon compounds dramatically increases microbial activity and biomass in soil closely associated with the roots [12]. Integrated interactions between plants and microbes have superior importance

for improving plant growth as well as maintaining proper soil conditions. Rhizosphere interactions that are based on complex exchange are more complicated than those occurring above soil surface or non-rhizosphere soil. Among diverse microbial population, plant growth promoting rhizobacteria (PGPR) gain special attention

owing to their multifarious functional characters like effective root colonization, nitrogen fixation, hormone production, solubilization of nutrients, and production of certain enzymes that are beneficial for sustainable agriculture. An understanding about their ecology, growth-promoting traits, mechanisms of action, and their application for plant growth stimulation has key importance for maximum utilization of this naturally occurring microbial population [13]. Microbial diversity in soil ecosystem exceeds more than eukaryotic organisms. One gram of soil may harbor up to 10 billion microorganisms of possible thousands of different species [14]. Several implications for the management of plant diseases are derived from knowledge of the basis of the specificity of plant-microbial interactions. New biotechnological products are currently being developed based on stimulation of the plant defense response, and on the use of plant-beneficial microbes for biological control of plant diseases (biopesticides) and for plant growth promotion (biofertilizers) as well as bio control. There for the study of this important plant growth promoting micro organisms are possible through extensive exploration from different plant-microbes association ecology. This study aiming to search rhizosphere microbes associated with different grass family at different ecology to know its fungal profile in later to use as biofertilizer, biocontrol, and biopesticide for agricultural and bioremediation application.

The result revealed that *Fellomyces fuzhounsis*, *Schizo blastosporonstarkeyi-henricii*, *Cryptococcus luteolus*, *Rhodotorula aurantiaca A*, *Cryptococcus terreus A*, *Trichosporon beigeli B*, *Rhodotorula aurantiaca B*, *Cryptococcus albidus var aeriis*, yeasts were fully identified using Biolog identification system from *Poaceae* and *Cyperaceae* family grass in North and East Showa, Ethiopia (Table 2). The *Cryptococcus* genera were dominant in this study and *Trichosporon beigeli B* was the dominant species identified from different rhizosphere soil of grass family. This is also reported by [15-18]. The *Basidiomycetes Cryptococcus aeriis*, *Cryptococcus laurentii*, *Cryptococcus terreus*, *Cryptococcus terricola*, *Cryptococcus podzolicus*, *G. (Trichosporon) pullulans* and the *ascomycetous B. (Williopsis) californica*, *B. pratensis*, *Sc. (Debaryomyces) occidentalis*, *L. (Williopsis) saturnus*, *Schizoblastosporion starkeyi-henricii* were yeast identified from most soil. The yeasts, i.e. *Cryptococcus tephrensis*, *Cryptococcus Victoria*, *Rhodotorula glutinis* and *Rhodospidium babjevae*. Although these yeasts are found regularly in soils, they are typically associated with the phylloclade and enter the soil profile with plant material [19]. There are different rhizospheres fungi were identified from different grass family. Mostly PGPR belonging to some important genera include *Serratia*,

Bacillus, *Pseudomona*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Klebsiella pneumoniae*, *Beijerinckia*, *Flavobacterium*, and *Gluconacetobacter* are identified from rhizosphere soil [20,21]. According to Madhugiri et al. 2011 *Acrophialophora fusispora*, *Botryotrichum piluliferum*, *Clonostachys rosea*, *Colletotrichum Dematium*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Khuskia oryzae*, *Verticillium albo-atrum* fungal species were identified from the Rhizosphere and Rhizoplane Subfamily *Panicoideae* Grasses. According to [22].The fungal isolates were exclusively found in the root regions of certain grass species. For example, *Chloris barbata*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Eleusine indica* and *Eragrostis unioides* harboured fungal species. Notable among them were *Graphium penicilloides* from *Chloris barbata*, *Chaetomium indicum*, *Gilmaniella Humicola*, and *Trichoderma pseudokoningii* from *C. dactylon*, *Chaetomium bostrychodes* from *Dactyloctenium aegyptium*, *P. decumbense* and *T. viride* from *Eleusine indica* and, *P. islandicum* and *T. koningii* from *E. unioides*. Rhizobacteria are isolated from teff rhizosphere *Pseudomonas fluorescens*, *Chryseomonas luteola*, *Pseudomonas putida*, *Bacillus licheniformis*, *Bacillus firmus*, *Burkholderia cepaci*, *Stenotrophomonas maltophila*, *Brevibacillus brevis*, *Bacillus megaterium*, *Bacillus coagulans*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus megaterium*, *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis* [23]. 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 eco friendly bio fertilizer from rhizosphere microbes investigation and evaluation for plant growth promoting character to increase yield and productivity of crop.

Conclusion

Understanding the Plant-microbe interactions and rhizosphere microbial diversity has profound role in biotechnological application for plant productivity and ecosystem function. Rhizosphere soil yeast identified is mostly from genera of *Cryptococcus*, *Trichosporon*, *Rhodotrula*, *Fellomyces*, *Schizo blastosporon* in this study. Growth and survival of a particular yeast species in soil may therefore not solely depend on the intrinsic abilities of the yeast, but is the cumulative result of a number of interactions within the soil microbial community. These results are promising in the field of bio-fertilizers. Application of yeasts increased the crop productivity. The mechanisms which could be involved include the bio availability of macro and micronutrients, production of growth hormones, and reduction of the phytopathogens' growth. In addition, they could improve the physical and

chemical properties of soil that increase water holding capacity, prevent nutrient leaching and add more mineral nutrients to the soil. We assume that studying the production of yeasts' secondary metabolites and their bioactivities in the rhizosphere holds exciting promise. We recommend further study dealing with the identification of the secondary metabolites of the yeasts and their bioactivities in the rhizosphere as well as their direct and indirect relationships with the plant growth and productivity.

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