IJIST
P ISSN : 2618-1630
E ISSN : 2909-6130
Recognized Journal

Molecular Characterization of Deciphering Fungal Community Structure in Zea Mays L. and Triticum Aestivum L

Original Article

Kainat Hussain[1], Muhammad Waseem[2], Iqra Mumtaz[3], Samreen Riaz[2]
1. Riphah International University Islamabad(kainatzain739@gmail.com)
2. Institute of Microbiology and Molecular Genetics. University of the Punjab Lahore
3. National University of Modern Languages Islamabad (Iqramumtaz417@gmail.com)
(samreen.mmg@pu.edu.pk)
Correspondence: Muhammad Waseem (waseem.mmg.pu@gmail.com)

Citation | Hussain.K, Waseem.M, Mumtaz.I, Riaz.S "Molecular Characterization of Deciphering Fungal Community Structure in Zea Mays L. and Triticum Aestivum L" International Journal of Innovations in Science & Technology, Vol 4, Issue 3 2022 PP 727-737

Received | April 24, 2022; **Revised |** June 22, 2022 **Accepted |** June 27, 2022; **Published |** June 30, 2022DOI:https://doi.org/10.33411/IJIST/2022040313

Rhizosphere fungi are strongly associated with plant growth and health by providing nutrients and antagonizing pathogens. Commercially, fungus has multipurpose applications in several sectors including beverages, food items and in medicines. Current study aimed to reveal the core fungal community structure of the two leading cereal crops that are *Zea mays* L. and *Triticum aestivum* L. The rhizosphere fungal community was explored via morphology, biochemistry and internal transcribe spacer (ITS) metagenomics. On the basis of morphology, the retrieved fungal strains were imprecisely classified into Ascomycota and Zygomycota. The species including Yeast, *Botyritis californica*, *Rhizopus stolonifer*, *Alternaria tenuissima*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger* and *Microsporum canis* were identified on the basis of macroscopy and microscope. Moreover, the biochemical characterization depicted the role of fungi in promotion of plant growth. Majority of the isolates depicted catalase activity, indole production, phosphate solubilization, ammonia production, nitrogenase activity and urease activity. Metagenomics using amplicon sequencing of ITS region revealed the presence of 805 Operational Taxonomic Units (OTUs) with 647 OTUs in *Zea mays* and 620 OTUs in *Triticum aestivum*. The fungal phyla found in the rhizosphere of *Zea mays* L. and *Triticum aestivum* L. were Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, Incertae sedis fungi. Ascomycota accounted for 93% and 95% of classified fungi in rhizosphere of *Zea mays* L. and *Triticum aestivum* L. respectively. The dominant species found in the rhizosphere soil of *Zea mays* were *Gibberella intricans, Curvularia lunata, Lepidosphaeria nicotiae, Edenia gomezpompae* and *Myrothecium verrucaria*.

Keywords:
Fungi, Metagenomics , Pathogen, Nutrients, Rhizosphere

Conflict of Interest: The author(s) declare that the publication of this article has no conflict of interest.

INTRODUCTION

The food produced by plants is highly dependent upon the nutrients taken for growth and productivity. The microbes are effective for usual plant activities and normal growth. Estimation of complex rhizophere-microbe plant interactions require an insight of rhizosphere and different directions of native microbial communities associated with rhizosphere [1].

Rhizosphere is an area where soil is surrounded by plant's roots. This region or zone is about 1mm wide, but it has no distinct edge. Rhizosphere is densely populated in which roots of one plant compete with lower part (root system) of neighboring plant species and with other soil microorganisms for bacterial activity, fungi role and insects interaction with plants for place, moisture availability, mineral intake, nutrients availability [2]. Root exudates may play vital role in transferring of message from one place to other as messengers; they are helpful in communication and initiation of biological (microorganism and insects) and physical interactions or relationship between soil and underground root microorganisms [3][4]. High number of microorganisms surrounding the plant roots is due to rhizode position, which have 25 percent area below or under the ground with allocated amount of carbon or 10% net fixed carbon [5].

Composition of soil microbial communities is based on soil type and plant roots. Soils have different pH, structure of plants, botanical texture, organic material, micro aggregate and nutrients [6]. These physiochemical properties help the microorganism to create a niche that proves benefits for other types of microbes and also have influence on the root exudates that have effects on the microbial activities. For instance, pH of soil, availability of nutrients has effect on high population rate of crop pathogenic bacteria, fungi, nematodes and other microbes. Plant roots excrete different types of compounds including carbohydrates, amino acids as well as organic acids by diffusion or through ion channels or vesicular system [7]. Plant excretions can change the soil chemistry and provide nutrients activity to microbes in the soil [8].

Zea mays are part of grass family maize with abundance of vitamin A. The maize has special characteristics; it can survive without human activity. It is the next one spring crop (Triticum aestivum L) is third most consumed food product in world. The molecular characterization shows that this plant was originated from weedy grass host. Soil microbes are responsible for major source of nutrients for plants species through the nutrient [9]. Nitrogen cycling system and mineral weathering may have positive or nutritive effect on plant growth [10]. Many beneficial symbiotic link is also found between certain plants for the sake of fixation of nutrients of bacterial species [11]. Soil microorganisms are one of the basic sources of enzymes present in soil. Some fungi cause plant diseases and some can turn off the entire agricultural crop. Other fungi contain antagonistic characters towards plant's pathogens [12]. There is large potential of root microbiome that can be source of solutions and assist in agricultural crops fertilization productivity.

The rhizosphere is a nutritional region of soil which are surrounding plant root. There exists a plethora of the rhizosphere microbiome that stimulates seed germinating physical nutritional health of the seed, progress and up growth of plants, consequently overall production is done by its capacity to hold soil and absorption of nutrients[13][14]. In order to gain insight into these positive interactions, firstly there is need to know the rhizosphere, its structure, complicated and diversity to different directions of native microbial communities' interaction to the rhizosphere. The review focuses on the advances in fungal rhizosphere biology.

Microscopy, macroscopy and biochemical tests give pure identification of yeasts and molds. Fungal microscopy provides information about type, physical appearance, and color scheme of hyphae and fungal concentration. For identification of fungi, main tool is fungal strain. Direct microscopy method is pure and easy to identify fungi by using fungal strains [15]. Scientist identified the biochemical tests are important for identification of fungi. Identification of fungal strains depends on the ability of fungi to grow on substances like carbon and nitrogen sources. Urease test and proteolysis was found important for mold identification [16].

Berendsen *et al*., studied microbiome which appear rhizosphere community and plant health. The study discussed evidence about pathogens or insect attack and effective activity enhances microbial function and to suppress pathogen in rhizosphere [17].

The main objective of this study was to examine the molecular characterization of Deciphering Fungal, Epidemological analysis of Zea mays L. and Triticum aestivum L. and analyzing Structural difference of Zea mays L. and Triticum aestivum L.

Material and Methodology

The current study was done to dissect the rhizosphere fungal community of *Triticum aestivum*L. and *Zea mays*L. Research work was conducted in Microbiology Laboratory at Allama Iqbal Open University Islamabad**.**

Rhizosphere soil samples

Soil was dug on land up-to 10 cm, first plants were grown then wheat of (*Triticum aestivum*) and maize (*Zea mays*) were collected from various locations of same field. These samples were dispatched to AIOU laboratory and stored at -80 ºC.

Isolation and purification of fungi

One gram of rhizosphere soil was diluted in 10 mL sterilized water. Serial dilutions up to 10^{-3} were used for isolation. The isolation was done on Sabouraud Dextrose Agar (SDA). Fungal isolates were obtained after keeping it in incubator at 28 °C for 72 hours. Isolation and purification was done by repeated plating.

Microscopy

For slide preparation, a drop of lacto-phenol cotton blue was added on slide with the help of dropper. The fungal colony was picked with the sterile loop and smear was made on the slide containing drop of water. Slide was allowed to dry and observed under microscope at different magnifications. The observation recorded included septate/aseptate hyphae and conidia shape.

Catalase test

All fungal isolates were tested for catalase activity. The isolates were refreshed on Sabouraud Dextrose Agar (SDA) media. Catalytic activity was determined by mixing fungal culture with a drop of hydrogen peroxide (H_2O_2) on glass slide. Release of bubbles showed the production of oxygen.

Indole test

Tryptophan broth was inoculated with fungal colonies. Inoculated media was incubated for 24-48 hours at 38 ºC. After incubation one drop of Kovac's reagent and two drops of xylene were added in the test tube.

Phosphate solubilization test

All the fungal isolates were tested for phosphate solubilization. Pikovskaya media was prepared and autoclaved at 121 ºC for 20 minutes. Petri dishes were placed in incubator at 32 ºC for three to seven days. Phosphate solubilization by bacterial isolates was indicated by formation of halo zone.

Ammonia test

Peptone broth was taken into test tubes and inoculated with fungal colonies. After inoculation culture plates were kept into incubator for 48-72 hours.

Nitrogenase test

Nitrogenase activity was detected in fungal isolates using nitrogen fixing bacteria (NFB) medium. Spot inoculation was done and petri dishes were placed in incubator at 36-38 ºC for 72hours.

Urease test

Urease test was done under experimental work to detect the capability of bacteria or fungi to break urea into ammonia and carbon dioxide. All the fungal strains were culture in a media containing urea. The emergence of pink color showed urease positive test for urease biochemical test.

Molecular characterization

DNA extraction using pure link TM microbiome DNA purification kit

Good efficient microbial DNA was obtained by Invitrogen, Link™ Microbiome DNA kit. Rhizospherical ground soil was grounded up to 0.2g to clear powder by using pestle and mortar. All process was done by kit method.

Gel electrophoresis

Gel was prepared using 1.5 g of agarose in 100 mL of 1X Tris Borate Ethylene diamine tetra acetic acid (EDTA) buffer (90 mL distilled water and 10 mL 10X TBE buffer). Agarose was dissolved by heating in microwave oven for 1 minute and 30 second. 6 micro liter of ethidium bromide was added before gel was poured into tray dish of casting. The gel was observed cortically and photo was taken by using gel documentation system (Bio Rad).

PCR reaction cocktail and PCR profile

Internal Transcribed Spacer region of fungi was amplified by specific primers (16SV4/16SV3) with barcode. 25 µL of reaction cocktail included: master mix 12.5 µL, PCR water 9.5 µL, forward primer 1 µL, reverse primer 1 µL and subjected DNA template 1 µL. PCR amplification was done according to the reaction conditions as described in Table 1. The product size was found between 400-450 bp.

Table 1. PCR reaction profile for Amplification of ITS region

Steps	Temperature (°C)	Time (Minutes)
Denaturation	95	5
Denaturation	95	1
Annealing	54	1
Extension	72	1
Go to step 2, Cycles	40	1
Final Extension	72	10

Library preparation and sequencing

Work was done on sequencing libraries using (NEB Next Ultra DNA Library PreKit) for Illumine, leading production company's references and index codes with necessary details were added.

Single-end reads

Single ended reads were being assigned to relevant samples which were based upon their unique particular barcode and truncated by dropping off barcode and primer sequence.

OTU production

For all samples the sequences analysis was performed by software (Uparse v7.0.1001) [18]. Sequences with more than 97% similarity were attached to same operational taxonomic units (OTUs).

Species annotation

For all sequences which have to be representative, the Green Gene Database (http://greengenes.lbl.gov/cgi- bin/nph-index.cgi) [19]. was used based on Ribosomal Database Project classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) (Wang et al, 2007) algorithm to over annotate taxonomic information. The Graphlan, Taxonomic trees and Korona figures showed the core bacterial community structure (Lozupone *et al.*, 2005).

RESULTS

Microscopy

The conidia shape observed were club, oval, calumnious globose and columellate. Majority of the isolates revealed club or oval shaped conidia. Few variations were observed between the isolates from rhizosphere soil of *Triticum aestivum* L. and *Zea mays* L. (Table 2, 3).

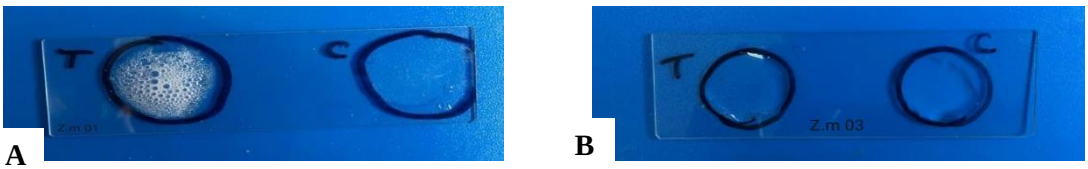

Figure 1 Catalase test; A. Catalase positive test, B. Catalase negative test

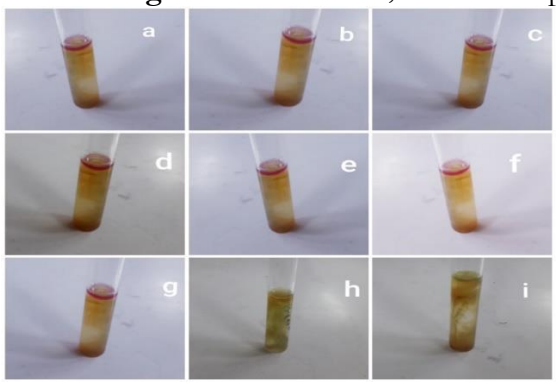

Figure 2. Positive indole test indicated by the formation of pink ring; A. *Aspergillus nidulans*, B. *Rhizopus stolonifer*, C. *Aspergillus niger* and D. *Aspergillus flavus*, E. *Botrytis Californica*, F. *Microsporum canis*, G. Aspergillus niger, H. Yeast and I. *Aspergillus terreus*.

Phosphate solubilization

To detect phosphate solubilization by formation of halo zone in plates and test tubes were used. Approximately, 83% of the fungal strains were found positive for phosphate solubilization test and 53% showed strong phosphate solubilization activity (Figure 3).

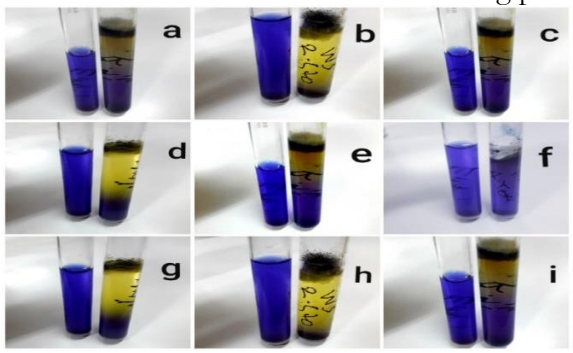

Figure 3. Solubilization activity indicated by; A. *Aspergillus nidulans*, B. *Rhizopus stolonifer*, C. *Aspergillus niger* and D. *Aspergillus flavus*, E. *Botrytis Californica*, F. *Microsporum canis*, G. Aspergillus niger, H. Yeast and I. *Aspergillus terreus*.

Ammonia test

This test was performed to analyze ability of fungi to produce ammonia. Emergence of brown to yellow color by addition of Nesseler's reagent indicted production of ammonia. The results of ammonia were detected in 57% of fungal isolates (Table 1, Figure 4).

Nitrogenase test

Nitrogenase testing experiment was performed to check the capability of fungi to fix nitrogen. The NFB media was used for nitrogenase test. Approximately, 57% of the fungal strains showed nitrogenase activity (Figure 5).

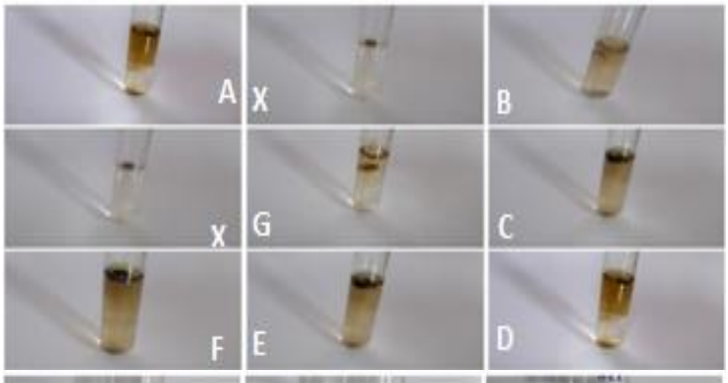

Figure 4. Positive ammonium test was indicated by dark brown precipitates; A. A. nidulans, B. Botrytis californica, C. Yeast, D. Alternaria tenuissima, E. R. While rest of all are negative indicated by "X"

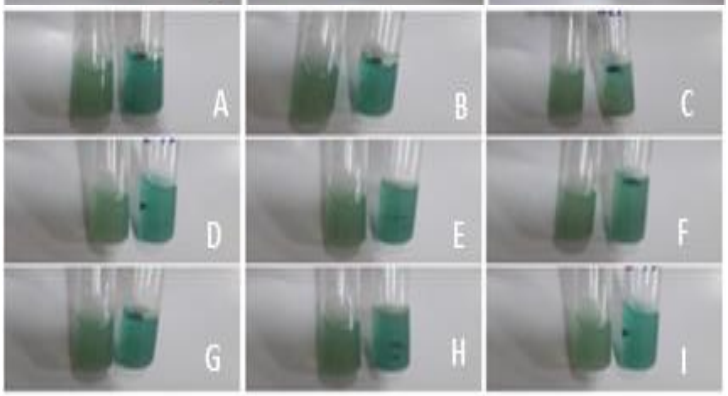

Figure 5. Blue colour produced by fungal isolates depicted nitrogenase positive test; A. A. nidulans, B. R. stolonifer, C A. flavus, D. A. niger, E. Yeast, F. A. terreus, G. Microsporumcanis, H. Botrytis californica and I. Alternaria tenuissima.

Urease test

Urease experiment was also performed to check ability of fungi to hydrolyze the urea rapidly. Approximately, 65% of fungal isolates were positive for urease test (Table 4, Figure 6).

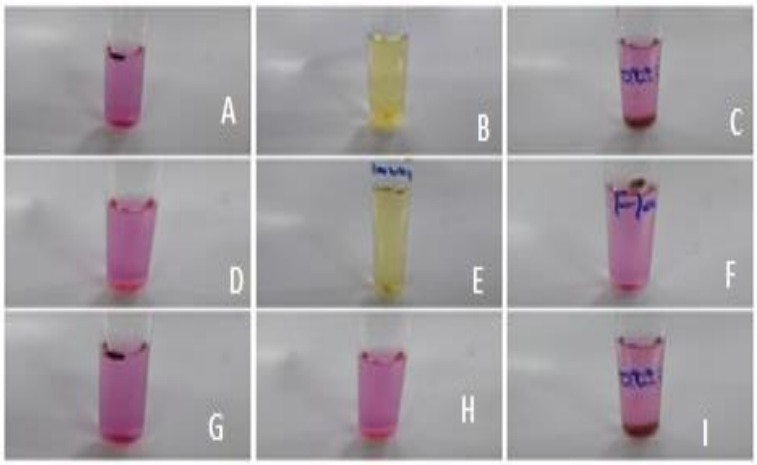

Figure 6 Development of pink colour indicated urease positive test, A. Aspergillus nidulans, B. Yeast, C. Rhizopus stolonifer, D. Alternaria tenuissima, E. Botrytis californica, F. Aspergillus flavus, G. Aspergillus terreus, H. Aspergillus niger and I. Microsporumcanis.

Molecular characterization

Genome DNA isolated from rhizosphere soil of *Zea mays* L. and *Triticum aestivum* L. using Pure Link™ Microbiome DNA Purification Kit was checked for purity on 1.5% agarose gel (Figure 7).

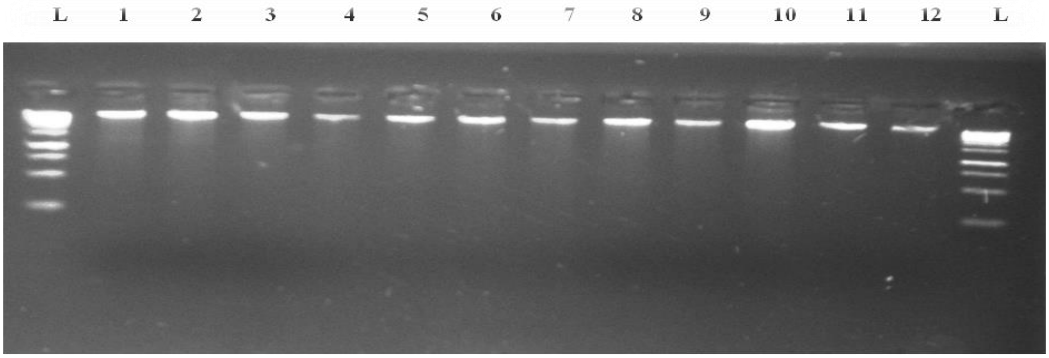

Figure 7. Gel electropherosis showing DNA isolated from rhizosphere soil of *Zea mays* L. and *Triticum aestivum* L.; Lane L: DNA ladder, Lane 1-lane 6: DNA isolated from *Zea mays* L., Lane 7-lane 12: DNA isolated from *Triticum aestivum* L.

Internal transcribed spacer (ITS) metagenomics

The entire fungal community structure of *Zea mays* L. and *Triticum aestivum* L. rhizosphere was explored using ITS metagenomics.

Tags statistics

The Total tags of ITS region gathered from rhizosphere soil of *Triticum aestivum* L. were 70,112 with 55,330 classified tags, 1,501 unclassified tags and 13,281unique tags (Figure 8).

Table 2 Statistical analysis of the sequence reads for ITS region of rhizosphere soil from *Zea mays* L. (ZM.KS) and *Triticum aestivum* L. (TA.KS).

Sample Name	Total tag	Taxon tag	Unclassified tag	Unique tag	OTU
ZM.KS	70195	53717	2359	14119	647
TA.KS	70112	55330	1501	13281	620

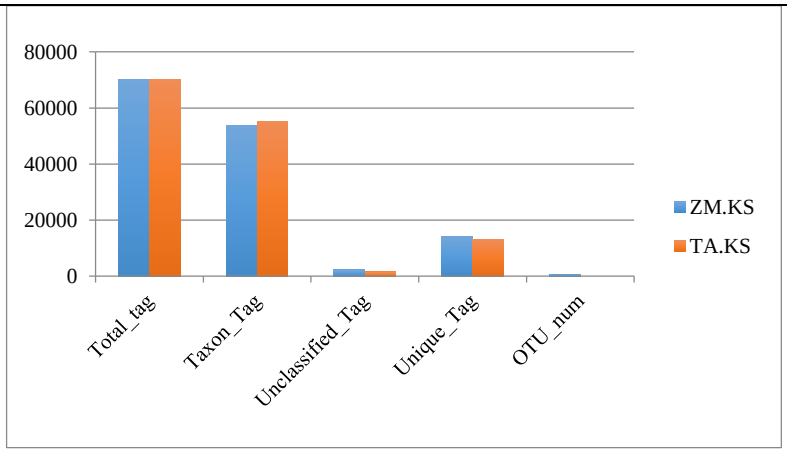

Figure 8. Comparison of the number of total reads, classified reads, unclassified reads and unique reads of ITS Region of rhizosphere soil of *Zea mays* L. (ZM.KS) and *Triticum aestivum* L. (TA.KS)

Table 3 Classified tags for ITS region of rhizosphere soil from *Zea mays* L. (ZM.KS) and *Triticum aestivum* L. (TA.KS)

SamplE Name	Sample Kingdom	Sample Phylum	Sample Class	Sample Order	Sample Family	Sample Genus	Sample Species
ZM.KS	53717	53509	49608	43530	40640	29530	26652
TA.KS	55330	55166	52652	46221	43712	22048	13955

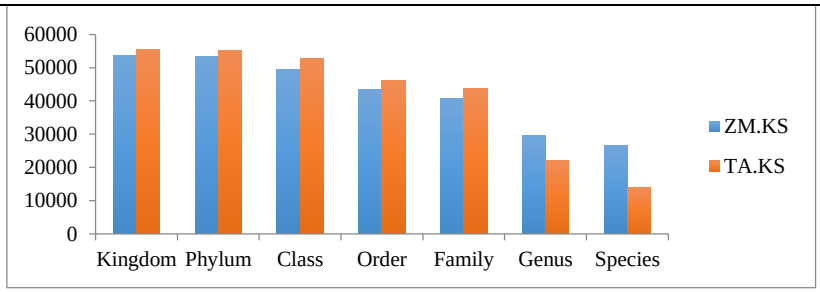

Figure 9. Classified tags for ITS region from rhizosphere soil Samples of *Zea mays* L. (ZM.KS) and *Triticum aestivum* L. (TA.KS)

Operational Taxonomic Units (OTUs)

To analyze the species numerous diversity in each sample, all those effective reads were separately grouped by 97% DNA sequence which have similarity into OTUs. The fungal community of rhizosphere soil of *Zea mays* L. and *Triticum aestivum* L. were clustered into 647 OTUs and 620 OTUs respectively (Table 2).

Beta Diversity

Principle component analysis was performed to expose the variation of fungal species abundance between rhizosphere soils of *Zea mays* L. and *Triticum aestivum* L. Fungal community slightly shows variation between rhizosphere soil from *Zea mays* L. and *Triticum aestivum* L. (Figure 10).

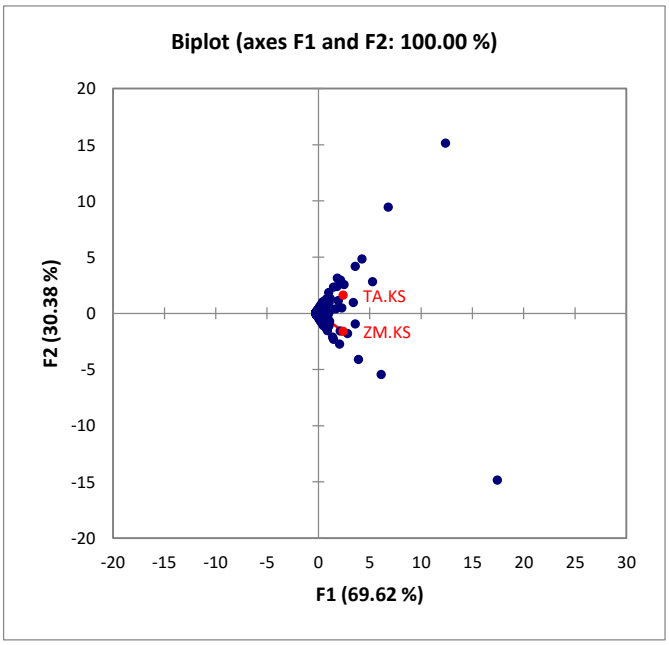

Figure 10. Principle component analyses depicting the variations in rhizosphere soil from *Zea mays* L. (ZM.KS) and *Triticum aestivum* L. (TA.KS)

DISCUSSION

The microorganisms which are associated with plant species are referred to as plant microbiome. Plant microbiome regulates with development of plants. It adds and contributes its characters in nutrient absorption from ground soil. The tolerance ability against soil borne pathogens and modulation of plant immune system depends upon its mineral property. It is quite possible to culture only very limited numbers of microorganisms and analysis of them for beneficial traits by using cultural methodology. However, techniques like metagenomics are very tough in analytical study of whole community interaction and coordination between different components of the community and metabolic pathways or genes activity under particular to relevant environmental condition with own specificity [20][21].

A pervious study by Yadav and coworkers reported phosphate solubilization by *Aspergillus niger*, *Penicillium citrinum* and *Trichoderm harzianum* (Bais *et al*., 2006) [22]. Mittal *et al.* (2008) also documented phosphate solulibizing capacity of Aspergillus and Penicillium sp. after 6 days incubation. *A. niger* and *P. citrinum* caused a marked dropped in logarithm pH of liquid culture media and solubilized expectaated amounts of phosphate (Alam *et al*., 2002). *Trichoderma harzianum* also showed phosphate solubilization in broth culture and solid agar medium of Pikovaskaya (Tarafdar *et al*., 2003). Firew *et al*., [23] reported IAA production in *A. niger*, *T. harzianum* and Penicillium after three days incubation at 30 °C. indole acetic acid (IAA) production in *Aspergillus niger* was experimentally studied for 5-16 days and maximum production up to limit 128 gL to 6.8 gL-1 was observed in Czapek-Dox broth media with 0.1% tryptophan at 6 days of incubation [24]. In similar studies, indole acetic acid (IAA) production and development was found to be maximized at 28 °C [25] isolated and characterized endophytic fungi associated with wheat. A number of the tested endophytes exhibited fairly or poorly good indole acetic acid (IAA) and phosphate solubilizing activities. More than 30% isolates gave positive result for siderophore and ammonia tests, whereas all exhibited catalase activity. The biochemical test results confirmed the effectiveness of fungal strains to be used as bioinoculum [26].

Conclusion:

Current analytical study revealed rhizosphere associated fungal diversity in two major and distinct cereal crops i.e. maize and wheat. Biochemical characterization unveiled the functions of fungi isolated from maize and wheat rhizosphere.

Rhizosphere soil of maize and wheat shared dominant groups of bacteria having similar character. However, differences among various groups were found in relatively more abundance.

Future recommendation

The retrieved fungal strains have been found to involve with plant's growth and developing promoting traits can also be entertained as biofertilizers. Further studies under field conditions are necessary to evaluate the potential ability of plant growth promoting fungal species for commercial applications. The present work can be extended further by using metaproteomics and meta-transcriptomics to gain insight into to elucidate the chemical and microbial markers that stimulate beneficial (micro) organisms. Metagenomics in rhizosphere biology would be very helpful material in understanding the plant and microbe interaction under stresses.

Acknowledgement:

I highly acknowledge to all colleagues, mentor and professors who helped me regarding this article. Their contribution is effective for me to complete this work.

Author's Contribution:

Kainat Hussain helped to find out the research topic and biochemical testing. Muhammad Waseem add his contribution for write-up, managing data and improve the whole article. Iqra Mumtaz improves grammatically mistakes to make it more perfect. Dr. Samreen Riaz work as supervisor and give final approval for publication.

References:

[1] "Proceedings of the National Academy of Sciences, India Section B: Biological Sciences | Home." https://www.springer.com/journal/40011 (accessed Jun. 21, 2022).

[2] P. R. Ryan, E. Delhaize, and D. L. Jones, "FUNCTION AND MECHANISM OF ORGANIC ANION EXUDATION FROM PLANT ROOTS," *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 52, pp. 527–560, 2001, doi: 10.1146/ANNUREV.ARPLANT.52.1.527.

[3] S. Mazhar, R. Yasmeen, A. Chaudhry, and K. Summia, "Role of Microorganisms in Modern Food Industry," *Int. J. Innov. Sci. Technol.*, vol. 4, no. 1, pp. 65–77, 2022, [Online]. Available: https://journal.50sea.com/index.php/IJIST/article/view/82.

[4] S. Bokhari, R. Yasmeen, A. W. Qurashi, S. Habib, and U. Rafi, "Isolation of Keratinolytic from Chicken (Gallus gallus domesticus) Farms and Assessment of their Efficacy in Feathers Degradation," *Int. J. Innov. Sci. Technol.*, vol. 3, no. 4, pp. 142–151, 2021, [Online]. Available: https://journal.50sea.com/index.php/IJIST/article/view/81.

[5] D. L. Jones, C. Nguyen, and R. D. Finlay, "Carbon flow in the rhizosphere: carbon trading at the soil–root interface," *Plant Soil 2009 3211*, vol. 321, no. 1, pp. 5–33, Feb. 2009, doi: 10.1007/S11104-009-9925-0.

[6] H. Höper, C. Steinberg, and C. Alabouvette, "Involvement of clay type and pH in the mechanisms of soil suppressiveness to fusarium wilt of flax," *Soil Biol. Biochem.*, vol. 27, no. 7, pp. 955–967, Jul. 1995, doi: 10.1016/0038-0717(94)00238-V.

[7] M. M. Hossain, F. Sultana, and S. Islam, "Plant growth-promoting fungi (PGPF): Phytostimulation and induced systemic resistance," *Plant-Microbe Interact. Agro-Ecological Perspect.*, vol. 2, pp. 135–191, Dec. 2017, doi: 10.1007/978-981-10-6593-4_6/COVER/.

[8] N. O. Igiehon and O. O. Babalola, "Rhizosphere Microbiome Modulators: Contributions of Nitrogen Fixing Bacteria towards Sustainable Agriculture," *Int. J. Environ. Res. Public Health*, vol. 15, no. 4, Apr. 2018, doi: 10.3390/IJERPH15040574.

[9] D. P. Singh, H. B. Singh, and R. Prabha, "Microbial inoculants in sustainable agricultural productivity: Vol. 1: Research perspectives," *Microb. Inoculants Sustain. Agric. Product. Vol. 1 Res. Perspect.*, pp. 1–343, Jan. 2016, doi: 10.1007/978-81-322-2647-5/COVER.

[10] J. F. Toljander, J. C. Santos-González, A. Tehler, and R. D. Finlay, "Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial," *FEMS Microbiol. Ecol.*, vol. 65, no. 2, pp. 323–338, 2008, doi: 10.1111/j.1574-6941.2008.00512.x.

[11] F. J. Bergersen, "Biochemistry of Symbiotic Nitrogen Fixation in Legumes," *http://dx.doi.org/10.1146/annurev.pp.22.060171.001005*, vol. 22, no. 1, pp. 121–140, Nov. 2003, doi: 10.1146/ANNUREV.PP.22.060171.001005.

[12] A. M. JAROSZ and A. L. DAVELOS, "Effects of disease in wild plant populations and the evolution of pathogen aggressiveness," *New Phytol.*, vol. 129, no. 3, pp. 371–387, Mar. 1995, doi: 10.1111/J.1469-8137.1995.TB04308.X.

[13] P. A. H. M. Bakker, R. L. Berendsen, R. F. Doornbos, P. C. A. Wintermans, and C. M. J. Pieterse, "The rhizosphere revisited: Root microbiomics," *Front. Plant Sci.*, vol. 4, no. MAY, p. 165, May 2013, doi: 10.3389/FPLS.2013.00165/BIBTEX.

[14] I. R. Imran, "Environmental Factors as Diabetogenic Agent in Type 2 Diabetes Mellitus," *Int. J. Innov. Sci. Technol.*, vol. 4, no. April, pp. 288–299, 2022, [Online]. Available:

https://journal.50sea.com/index.php/IJIST/article/view/150.
[15] Z. Hussain, A. Martin, and G. A. Youngberg, "Blastomyces dermatitidis with large yeast forms," *Arch. Pathol. Lab. Med.*, vol. 125, no. 5, pp. 663–664, 2001, doi: 10.5858/2001-125-0663-BDWLYF.
[16] R. Kaine, *Medical microbiology 2003*, vol. 53, no. 9. 2003.
[17] B. R. Glick, "Bacteria with ACC deaminase can promote plant growth and help to feed the world," *Microbiol. Res.*, vol. 169, no. 1, pp. 30–39, Jan. 2014, doi: 10.1016/J.MICRES.2013.09.009.
[18] R. C. Edgar, B. J. Haas, J. C. Clemente, C. Quince, and R. Knight, "UCHIME improves sensitivity and speed of chimera detection," *Bioinformatics*, vol. 27, no. 16, pp. 2194–2200, Aug. 2011, doi: 10.1093/BIOINFORMATICS/BTR381.
[19] D. Rotenberg, L. Cooperband, and A. Stone, "Dynamic relationships between soil properties and foliar disease as affected by annual additions of organic amendment to a sandy-soil vegetable production system," *Soil Biol. Biochem.*, vol. 37, no. 7, pp. 1343–1357, Jul. 2005, doi: 10.1016/J.SOILBIO.2004.12.006.
[20] O. P. Ahlawat, R. Tiwari, and G. P. Singh, "Metagenomics of wheat rhizosphere for abiotic stress management," *Wheat Barley Res.*, vol. 10, no. 2, 2018, doi: 10.25174/2249-4065/2018/79291.
[21] S. N. Abbas and A. Naushad, "COVID-19 pandemic: A remedial measure through convalescent serum," *Int. J. Innov. Sci. Technol.*, vol. 2, no. 2, pp. 46–50, 2020.
[22] H. P. Bais, T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco, "The role of root exudates in rhizosphere interactions with plants and other organisms," *Annu. Rev. Plant Biol.*, vol. 57, no. February 2006, pp. 233–266, 2006, doi: 10.1146/annurev.arplant.57.032905.105159.
[23] F. A. Ripa, W. D. Cao, S. Tong, and J. G. Sun, "Assessment of plant growth promoting and abiotic stress tolerance properties of wheat endophytic fungi," *Biomed Res. Int.*, vol. 2019, 2019, doi: 10.1155/2019/6105865.
[24] F. Elias, D. Woyessa, and D. Muleta, "Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia," *Int. J. Microbiol.*, vol. 2016, 2016, doi: 10.1155/2016/5472601.
[25] C. Hamel, V. Vujanovic, R. Jeannotte, A. Nakano-Hylander, and M. St-Arnaud, "Negative feedback on a perennial crop: Fusarium crown and root rot of asparagus is related to changes in soil microbial community structure," *Plant Soil*, vol. 268, no. 1, pp. 75–87, 2005, doi: 10.1007/s11104-004-0228-1.
[26] S. Basharat, S. Mazhar, R. Yasmeen, and W. Hamid, "Evaluation of Microbial Contamination via Wastewater Collected from Different Oil Industries and its Treatment Using Various Coagulants," *Int. J. Innov. Sci. Technol.*, vol. 4, no. 2, pp. 392–403, 2022, [Online]. Available: https://journal.50sea.com/index.php/IJIST/article/view/224.