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Original Research Article

Microbial Fungal Community Fingerprint of Medicinal Plant Rhizosphere Collected Form Sinai, Egypt

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Abstract

The rhizosphere is a narrow region of soil that is directly affected by roots and associated soil microorganisms. Research on rhizosphere microbes of various medicinal plants is essential for microbial ecology, applied microbiology and industrial biotechnology with regard to the sustainable utilization of egyption medicinal resources. However, the inability of culturing most rhizosphere microorganisms (around 99%) in the laboratory obviates the research progress. In recent years, there are enormous advances in applying non-culturing techniques based on molecular biology and omics to the study of rhizosphere microbial diversity and plant-microbe interactions terminal restriction fragment length polymorphism (T-RFLP). Dry Rhizosphere of medicinal plants collected form Sinai, Egypt and water treated represents a common physiological stress for the microbial communities residing in surface of these medicinal plants. A dry and wetting by water induce lysis in a significant proportion of the microbial biomass and, for a number of reasons, Effect directly or indirectly on microbial community composition. In this study Dry sand and water treated as common stress in the laboratory by exposing three different rhizosphere immersed in water to 10 day and 20 day period. The three rhizospheric medicinal plants were collected from Saint Katherine Mountain, Sinai, Egypt. Fungal community of dry and immersed with distilled water was evaluated every 10 day of incubation. Total DNA was extracted from sand samples and characterized its fungal communities using the T-RFLP method. This work revealed that water changed fungal community in all samples compared with dry sand due to water may induced shifts in bacterial community.

Key words: Microbial fingerprint, bacterial community, TRFLP, medicinal plants, soil DNA extraction, 16S ribosomal RNA

INTRODUCTION

Microbes are fundamental to the maintenance of life on Earth, yet we understand little about the majority of microbes in environments such as soils, oceans, the atmosphere and even those living on and in our own bodies. Culture-based techniques have allowed isolated microbes to be studied in detail, and molecular techniques such as metagenomics are increasingly allowing the identification of microbes in situ. The microbial communities, or microbiomes, of diverse environments have been studied in this way, with the goal of understanding their ecological function (Gilbert et al., 2010)

The plant microbiome is a key determinant of plant health and productivity (Berendsen et al., 2012) and has received substantial attention in recent years (Bulgarelli et al., 2013)a testament to the importance of plant-microbe interactions are the mycorrhizal fungi. Molecular evidence suggests that their associations with green

algae were fundamental to the evolution of land plants about 700 million years ago (Heckman et al., 2001).Classic microbiology involves isolating and culturing microbes from an environment using different nutrient media and growth conditions depending on the target organisms. Although obtaining a pure culture of an organism is required for detailed studies of its genetics and physiology, culture-dependent techniques miss the vast majority of microbial diversity in an environment. Numerous culture-independent, molecular techniques are used in microbial ecology. For studying prokaryotes, PCR amplification of the ubiquitous 16S ribosomal RNA (rRNA) gene is commonly used. Sequencing the variable regions of this gene allows precise (species- and strain level) taxonomic identification. The use of high-throughput sequencing technologies has been widely adopted as they allow identification of thousands to millions of sequences in a sample, revealing the abundances of even rare microbial species. For studying eukaryotic microbes such as fungi, the equivalent rRNA gene (18S) may not provide sufficient taxonomic discrimination so the hypervariable internally transcribed spacer is often used (Bentley et al., 2008).

The rhizosphere is the region of soil influenced by plant roots through rhizodeposition of exudates, mucilage and sloughed cells. Root exudates contain a variety of compounds, predominately organic acids and sugars, but also amino acids, fatty acids, vitamins, growth factors, hormones and antimicrobial compounds (Bertin et al., 2003). Root exudates are key determinants of rhizosphere microbiome structure (Badri et., 2013).

El –Badry, 2016 evaluate Bacterial community community and variation of some medicinal plant rhizosphere collected form Sinai for three medicinal plant collected from The Saint Katherine Mountain.

In this Study the fungal metagenomics indicate number species type of microorganisms were present, also find the relationship between activities of all fungal community with ecosystem functions. In this study deals also with study effect of water addition in fungal metagenomics and their interactions with each other from three sample of rhizospher of medicinal plants collected from Sinai Egypt.

MATERIAL AND METHODS

Site and soil sampling

Saint Katherine Mountain is the capital city in the South Sinai Governorate in the Sinai Peninsula, Egypt. an elevation of about 1600 meters from sea level It is located at the outskirts of El-Tur Mountains at an elevation of 1,586 m (5,203 ft), 120 km (75 mi) away from Nuweiba, at the foot of Mount Sinai and the Saint Catherine's Monastery. Saint Catherine Town lies at the foot of the Sinai high mountain region, the "Roof of Egypt", where Egypt's highest mountains are found. Sampling was performed from the rhizosphere by collecting the soil near the roots (a maximum of 5 cm from the root) and 10 cm from the soil surface. Rhizospheric soil samples were randomly taken from beneath three medicinal plants species common in Saint Cathren mountain Capparis spinosa, Chiliadenus iphionoides and Artemisia herba-alba. All the samples were sealed in plastic bags and transported on ice. In the laboratory, samples were stored at - 80 °C until use.

Microcosms design and total DNA isolation from three medicinal plants rhizospheres (El –Badry, 2016).

Polymerase Chain Reaction (PCR)

The quality of the DNA obtained was tested by its ability to produce polymerase chain reactions with primers ITS1 5- TCCGTAGGTGAACCTGCGG-3 and ITS4 5- TCCTCCGCTTATTGATATGC-3 (White et al. 1990). The following standard conditions were used for bacterial 16S rRNA gene amplification: initial denaturation at 95°C for 5 min; 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 55°C), and extension (1.5 min at 72°C); and a final extension at 72°C for 5 min. The PCR products were purified with a The UltraClean® PCR Clean-Up Kit (MO BIO laboratories, Inc) according to the manufacturers suggested protocol.

DNA digestion from PCR product with Restriction Enzymes for TRFLP profiles (El–Badry, (2016). Hae III restriction Enzyme was used in this work for DNA digestion

RESULTS

Fungal metagenomics of *Capparis spinosa* rhizosphere

Total fungal community in *C. spinosa* rhizosphere in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature was 136 DNA fragments only. Five fungal fragments were present in all condition with percentage of 3.7**%** of total fungal community. All experiment was done duplicate and DNA isolated by Mo Bio kit Method and PCR primers for Fungal ITS gene to characterize fungal community then digest with Hae III restriction Enzyme and analysis fingerprint of fungal community by TRFLP which is popular fingerprinting technique used to monitor changes in microbial community. (Table 1) *C. spinosa* dry and microcosms DNA Fragments shared and recurrent by its DNA length was varied as presented in Microcosms 20 day of *C.spinosa* which was richness community with different 41fungal species duo to the presence of water for 20 day and fungal metabolism began in decomposition in rhizosphere whereas Dry sand for 20

Table 1. Richness microbial community and Percentage of share fungal DNA fragment with its community

Samples	Total Fungal community	Percentage to each other %	Percentage of shared DNA fragments %	Percentage of difference DNA fragments %
*C.spinosa*Cont. 10	36	26.47	13.88	86.11
*C.spinosa*Cont. 20	32	23.52	15.62	84.37
C.spinosa M 10	27	19.85	18.51	81.48
C.spinosa M 20	41	30.14	12.19	87.80

Table 2. Richness microbial community of TRFLP for *C. iphionoides*microcosms experiment sand rhizosphere of Saint Catherine Mountain

Sample	Total Fungal community	Percentage to each other %	Percentage of shared DNA fragments %	Percentage of difference DNA fragments %
C. iphionoides Cont. 10	36	25.17	36.11111	63.88889
C. iphionoides cont. 20	36	25.17	36.11111	63.88889
C. iphionoides M10	34	23.77	38.23529	61.76471
C. iphionoides M 20	37	25.87	35.13514	64.86486
Sum of total community	143	100	9	

Table 3. Richness microbial community of TRFLP for *A. herba-alba* microcosms experiment sand rhizosphere of Saint Catherine Mountain

Sample	Total Fungal community	Percentage to each other %	Percentage of shared DNA fragments %	Percentage of difference DNA fragments %
A. herba-alba cont. 10	32	26.01626	15.625	84.375
A. herba-alba cont 20	42	34.14634	11.90476	88.09524
A. herba-alba M10	23	18.69919	21.73913	78.26087
A. herba-alba M20	26	21.13821	19.23077	80.76923
Sum of total community	123	100	4	

day was the less fungal community. In the other hand Percentage of shared DNA fragments in *C.spinosa* M 20 was less no of fungal species near to *C.spinosa* dry. 10 that mean 12 fungal species was dominant in all rhizospheres.

Fungal metagenomics of *Chiliadenus iphionoides* rhizospher

Total fungal community in *C. iphionoides* rhizosphere in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature was 143 DNA fungal fragment only 13 different fungalDNA fragment were presence in all condition with percentage 9 % of total fungal community. (Table 2) *C. iphionoides* and microcosms Fragments shared and recurrent by its DNA length. As mentioned in this table the presence of water slightly influenced on fungal community with dry rhizosphere and also the Percentage of shared DNA fragments % was from 36 to 35 % higher than *C. spinosa* fungal community but with higher number in fungal species in its rhizoshere.

Fungal metagenomics of *Artemisia herba-alba* rhizospher

Total fungal community in *A. herba-alba* rhizosphere in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature was 123 only 5fungal strains were presence in all condition with percentage 4 % of total fungal community (Table 3) *A. herba-alba* and microcosms Fragments shared and recurrent by its DNA length.

Terminal restriction fragment length polymorphism (TRFLP) results for all control samples:

The results of total fungal community in all dry rhizosphere as control in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature were evaluated in (Figure 1). Percentage of shared DNA fragments % for all was 4 % may due to the antimicrobial activity of these medicinal plants effect on microbial community structure and numbers.

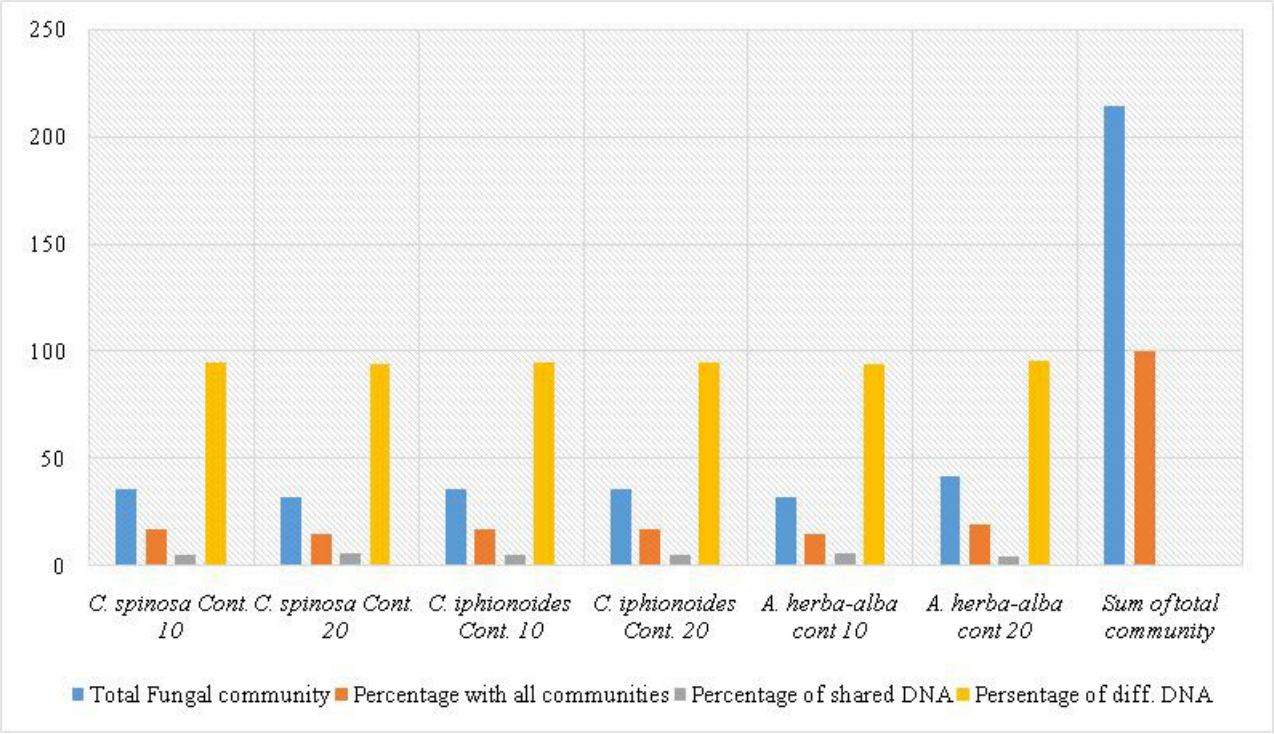

Figure 1. Terminal restriction fragment length polymorphism (TRFLP) results for all control samples

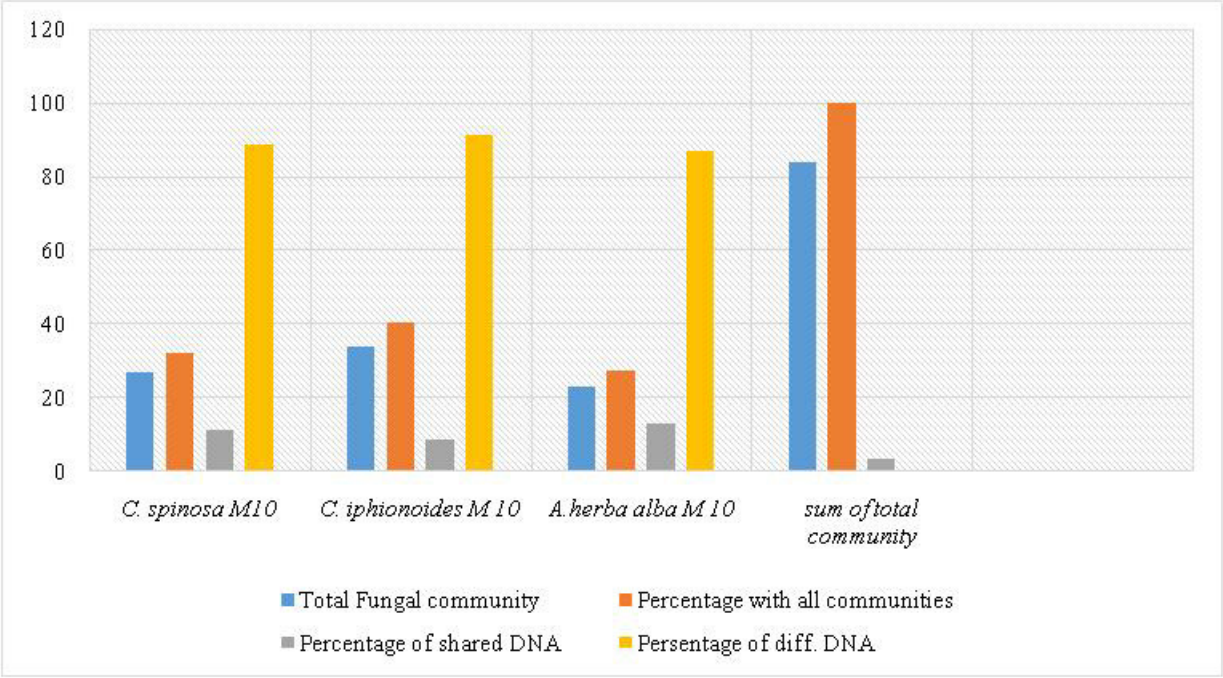

Figure 2. Terminal restriction fragment length polymorphism (TRFLP) results for all microcosms' samples after 10 day

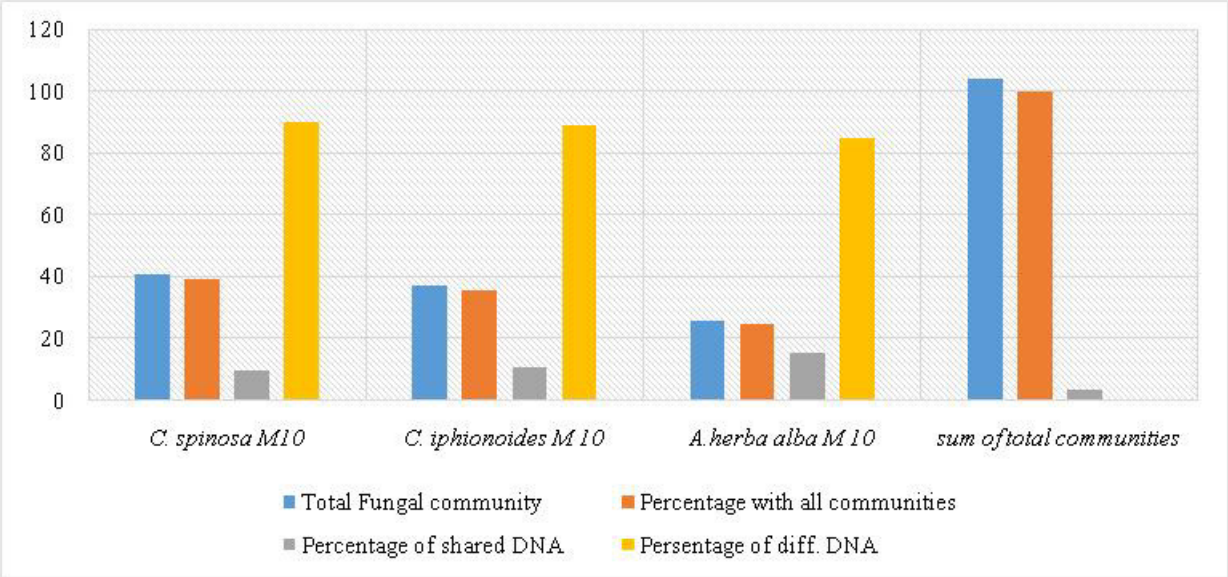

Figure 3. Terminal restriction fragment length polymorphism (TRFLP) results for all microcosms' samples after 20 day

Terminal restriction fragment length polymorphism (TRFLP) results for all microcosms' samples after 10 day

Total fungal community in all microcosms samplesafter 10 days of incubation in room temperature was 84 only 3 fungal species were presence in all condition with percentage 3.5 % of total bacterial community.

Also, microcosms of *A. herba alba* was richness community with different 48 bacterial strains as shown in Figure 2.

Terminal restriction fragment length polymorphism (TRFLP) results for all microcosms' samples after 20 day

Total fungal community in all microcosms samples after 20 days of incubation in room temperature was 104 only 4fungal DNA fragment length were presence in all condition with percentage 3.8 % of total fungal community as shown in Figure 3

DISCUSSION

Desert medicinal plants were used in folkloric medicine for treatment vast disease. Recent studies indicated that these medicinal plants have medicinal characteristics. Current ongoing studies subjected these medicinal plants to detailed genomic studies along with its associated microflora. Therefore, it's very important to evaluate the rhizosphere fungal communities associated with these plants. The rhizosphere fungal communities must be subjected to deep. This may result to constitute valuable resources that could be screened for enzymes of environmental and industrial importance. Identifying novel organisms would be exploited biotechnologically for medicinal uses and biodegradation of this work will make available large genomics data sets to the global research community. This paper is considered the first article dealing with fungal metagenonics from three medicinal plants rhizosphere collected from Sinai Egypt. It is also considered as reference for all researcher to begin serious work in this field of relationship between medicinal plants and its fungal communities

All plant-associated fungi and bacteria are found in high abundances especially in the nutrient-rich rhizosphere region, the soil area influenced by plant roots, hosts of microbes that are of central importance for plant nutrition, health, and quality (Berg, 2009; Mendes et al., 2011, 2013). The rhizosphere can tolerate up to 10^{11} microbial cells per gram root with more than 30,000 different microbial species (Berendsen et al., 2012). These rhizospheric microorganisms from a highly diverse reservoir of soil microbes are attracted by the rhizosphere's plant root secretions and other rhizodeposits (Compant et al., 2010), and driven via the compositional variability of these exudates (Bais et al., 2006; Doornbos et al., 2012). Each medicinal plant species have a specific rhizosphere microbiome dependent of the present soil community (Smalla et al., 2001). Besides plant species, the composition and diversity of microbial rhizosphere communities is shaped by soil type and pedoclimate, plant health and developmental stage, climate and season, pesticide treatments, grazers and animals, and several other biotic and abiotic factors (Singh and Mukerji, 2006; Berg and Smalla, 2009; Barnard et al., 2013). Some genera are ubiquitous and can be found distributed over the entire

Table 4. Similarity index of microbial community of Terminal restriction fragment length polymorphism (TRFLP) results for all control and microcosms' samples after 10 and 20 day

	C10	C20	CM10	CM20	CH10	CH20	CHM10	CHM20	HE10	HE20	HEM10	HEM20
C10	1	0.45	0.34	0.28	0.22	0.30	0.31	0.16	0.31	0.32	0.15	0.21
C20		**1**	0.32	0.34	0.19	0.20	0.21	0.19	0.22	0.25	0.10	0.16
CM10			**1**	0.44	0.26	0.26	0.27	0.21	0.23	0.24	0.12	0.18
CM20				**1**	0.26	0.37	0.36	0.35	0.24	0.27	0.10	0.19
CH10					**1**	0.51	0.56	0.46	0.28	0.35	0.196	0.24
CH20						**1**	0.75	0.43	0.39	0.51	0.14	0.26
CHM10							**1**	0.52	0.45	0.55	0.17	0.22
CHM20								**1**	0.23	0.3	0.21	0.26
HER10									**1**	0.57	0.19	0.23
HER20										**1**	0.15	0.2
HERM10											**1**	0.43
HERM20												**1**

Table 5. Distance matrix based on the similarity matrix

	C10	C20	CM10	CM20	CH10	CH20	CHM10	CHM20	HE10	HE20	HEM10	HEM20
C10	0	0.55	0.66	0.72	0.78	0.7	0.69	0.84	0.69	0.68	0.85	0.79
C20		0	0.68	0.66	0.81	0.8	0.79	0.81	0.78	0.75	0.9	0.84
CM10			0	0.56	0.74	0.74	0.73	0.79	0.77	0.76	0.88	0.82
CM20				0	0.74	0.63	0.64	0.65	0.76	0.73	0.9	0.81
CH10					0	0.49	0.44	0.54	0.72	0.65	0.8	0.76
CH20						0	0.25	0.57	0.61	0.49	0.86	0.74
CHM10							0	0.48	0.55	0.45	0.83	0.78
CHM20								0	0.77	0.7	0.79	0.74
HE10									0	0.43	0.81	0.77
HE20										0	0.85	0.8
HEM10											0	0.57
HEM20												0

Cophenetic Correlation Coefficient (CP) = 0.89

plant, such as the well-known plant-associated genera *Bacillus* and *Pseudomonas* (Berg et al., 2011). However, a high degree of specificity for each microenvironment was also observed via comparison of microbial colonization patterns of different microhabitats (Fürnkranz et al., 2012)

Medicinal plants harbor a distinctive microbiome due to their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microorganisms (Qi et al., 2012).

In comparison to soils of humid areas, the soil microbiome of the Egyptian desert farm Sekem was comprised of a high abundance of Gram-positive, spore-forming bacteria primarily of the Firmicutes branch with 37% of the total bacterial soil community as revealed through a pyrosequencing-based amplicon sequencing approach (Köberl et al., 2011).

Metagenomic approaches have recently been employed for comparing microbial communities from different soils (Luo, 2014): including soils collected from cold deserts, hot deserts, forests, grasslands, and tundra. These studies fulfilled the expected results and led to the characterization of microbiome composition and functional attributes across the various environments studied. More specifically, the study by (Fierer et al., 2014) revealed that biotic stress genes are less abundant in the desert soils than in the other soils, which led them to suggest that abiotic conditions "are more important in shaping the desert microbial communities". They also concluded that metagenomic approaches can be useful in understanding how the microbial diversity and gene functions could vary across terrestrial biomes (Table 4 and 5)

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