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Full Length Article

High Fungal Diversity and Dominance by Ascomycota in Dam Reservoir Soils of Arid Climates

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Abstract

The study analyzed fungal diversity in soil trapped behind dams in three arid regions of Oman, in the south-eastern part of the Arabian Peninsula. Physicochemical analysis indicated that soils are of the loamy sand or sandy loam types and they are deficit in some macro and micro elements. Culture-based techniques revealed variation between dam soils in the number and type of phyla, classes, orders, genera and species. *Ascomycota* was the most dominant phyla in the three samples, contributing to 89% of the total species. *Eurotiomycetes*, *Sordariomycetes* and *Dothideomycetes* were the most common classes in dam soils. *Zygomycetes*, *Pezizomycetes* and *Oomycetes* were also detected, but at lower levels. Fifty fungal taxa were recovered from the three dam soils, with *Aspergillus*, *Penicillium* and *Trichoderma* being the most common genera. Most of the detected species were saprophytes; however some were either plant pathogens or mycotoxin producers, affecting humans directly or indirectly. This study appears to report for the first time 20 fungal species in Oman. It is also the first study from arid regions to characterize high fungal diversity in soil trapped behind dams. The sources of these fungi in dam soils and the implications of fungal diversity are discussed. © 2017 Friends Science Publishers

Keywords: Fungal diversity; *Oomycota*; Direct plating; Arid climate; Pathogens

Introduction

Water shortage is the most serious challenge facing people living in arid areas of the world (Pereira *et al.*, 2002; Chaves and Davies, 2010; Cummings, 2012; El-Ghonemy, 2012; Erol and Randhir, 2012; Levy *et al.*, 2013). The short periods of rain, coupled with high infiltration rates and extremely high temperatures in desert areas, make the benefits from water in dry areas very limited. However, people living in these areas have developed means by which they can save or extract water for drinking and irrigation purposes. The construction of dams is one of the methods adopted (Petts and Gurnell, 2005; de Araújo and Medeiros, 2013). These dams trap the water flowing in streams "usually referred to as *Wadis*". Consequently, groundwater recharge increases and the trapped water is sometimes used for irrigating crops, watering livestock or drinking purposes, before it evaporates because of the usual high temperatures in these regions.

The trapped water behind dams usually carries sediments in the form of soil particles and plant debris. These sediments sink to the bottom of the reservoir. Although it is prohibited in some areas of the world to move these sediments, some people move soil trapped behind dams to be used instead of the overused soil in their farms (Al-Sa'di *et al.*, 2008).

Fungi play important roles in soils as decomposers and plant symbionts (Větrovský and Baldrian, 2013). Several fungal species are known to work as symbionts with plants, improving their growth (Cavagnaro, 2014; Van Geel *et al.*, 2015). Fungi also help improve soil stability and functions (Abed *et al.*, 2013). Previous studies have observed differences between microbial communities in fields with different histories of soil amendment, tillage, irrigation and plant community structure (Liebig *et al.*, 2004). In addition, other studies addressed fungal diversity in decaying plant material from dams and other sources (Colas *et al*., 2016). However, no information is available concerning fungal diversity in dam soil from arid regions of the world. This establishes a barrier towards understanding the population structure of fungal communities in these systems, their sources and their potential roles.

Different techniques have been used for characterizing fungal diversity in soil samples, including the use of culture-based techniques and molecular approaches (Johnsen *et al.*, 2001; Nannipieri *et al.*, 2003;

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Langarica-Fuentes *et al.*, 2014; Al-Mazroui and Al-Sadi, 2015; Kazeeroni and Al-Sadi, 2016). Among several culture-based techniques, serial dilution and direct plating proved to be efficient in analyzing fungal diversity from different soils and substrates (Al-Mazroui and Al-Sadi, 2015, 2016; Al-Sadi *et al.*, 2015b).

Oman is located in the south eastern part of the Arabian Peninsula. It is characterized by hot and dry weather most of the year. Temperatures in deserts can reach as high as 50°C in the summer. Water for agricultural purposes usually comes from underground water pumped from wells, as well as in some cases from water flowing in tunnels called *Aflaj*. Construction of dams is common in this part of the world to help increase the water table.

The overall objective of this study is to characterize fungal diversity in dam soils from three arid regions in Oman using culture-based technique. Knowledge into this area will help understand the microbial make-up of these soils and its potential contribution to soil health and function.

Materials and Methods

Site Description and Sample Collection

Soil samples were collected from three dams in Oman, namely Al Khoudh (KH), Wadi Tanuf (WT) and Madha (MA) during 2011 (Table 1). Approximately 500 g of soil was collected from dry soil at the bottom of the reservoir (water already evaporated/drained at least 3 months before the collection of samples). Soil samples were collected from the top 10 cm of surface soil from three randomly selected areas within the dam. The soil samples were subjected to analysis within 48 h.

Physicochemical Analysis

The proportion of sand, silt and clay in the soil samples was determined (Klute, 1986). Determination of electrical conductivity (EC) and pH were as explained by Zhang *et al.* (2005). Total nitrogen was analyzed using Kjeltec Analyser (FOSS TECATOR, Sweden) (Kazeeroni and Al-Sadi, 2016). Potassium (K), sodium (Na) and calcium (Ca) contents were analyzed with flame photometric method (Sheerwood 450 flame photometer). Phosphorus (P) and magnesium (Mg) were determined by using Inductively Coupled Plasma (Perkin Elmer, USA) (Al-Ghaithi *et al.*, 2016).

Isolation and Identification of Fungi

Detection of fungi in the soil samples was achieved using serial dilution and soil plating techniques (Al-Sa'di *et al*., 2008) in 2.5% potato dextrose agar amended with 50 mg L^{-1} rose Bengal and 10 mg L^{-1} rifampicin. For direct plating technique, 100 mg of each soil sample was spread onto the surface of PDA plates. After three to seven days, fungal colonies appearing on the PDA plates were transferred to fresh PDA plates amended with 200 mg L^{-1} ampicillin and 10 mg L^{-1} rifampicin. The obtained isolates were purified for further use.

The identification was based on sequence analysis of the internal transcribed spacer region of the ribosomal RNA (ITS rRNA). DNA extraction was according to Lee and Taylor (1990) and Al-Sadi *et al*. (2011a) from freeze dried mycelia. The ITS region was amplified using the universal primers ITS1 and ITS4 (White *et al.*, 1990). Thermocycling was run with the following parameters: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 90 s and a final extension at 72°C for 10 min. Sequencing was carried out at Macrogen Inc. Seoul, Korea. Phylogenetic analysis was carried out using the parsimony optimality criterion in PAUP* 4.0b10 (Swofford, 1998) using 1000 bootstrap replicates.

Statistical Analysis

Tukey's studentized range test (SAS, v8, SAS Institute Inc., Cary, NC) was used to assess the differences in the soil chemical properties between the four samples of dams used in this study.

Results

Physicochemical Characteristics

The results of physical properties analysis revealed that soils from the three dams differ in their textures (Tables 1 and 2). Soils from MA and KH were loamy sand, while the soil from WT was sandy loam (Table 1). Soils differed significantly in some of their chemical properties. The pH ranged from 7 to 8, while the EC ranged from 0.69 to 2.29 dS m^{-1}. Soil from WT had significantly higher EC and N compared to the other soils. Soil from KH had significantly more Na and the lowest values of K and Mg. Soil from MA had the lowest levels of Ca (Table 2).

The Major Fungal Groups in Dam Soils

Evaluation of fungal diversity in KH, WT and MA soils revealed variation between the samples in the number and type of detected phyla, classes, orders, genera and species (Table 1; Figs. 1 and 2).

Ascomycota was the most dominant phyla of fungi in all samples, accounting for 89% of the total isolates. This was followed by *Zygomycota* (9.6%) and *Oomycota* (Heterokontophyta) (1.4%). *Eurotiomycetes* was the most dominant class in all samples (39.7%), followed by *Sordariomycetes* (27.4%), *Dothideomycetes* (20.5%), *Zygomycetes* (9.6%), *Pezizomycetes* (1.4%) and *Oomycetes* (1.4%) (Fig. 1).

Table 1: Characteristics of locations from which dam soils were collected

Sample	District	GPS Location	Year of establishment	Texture (% clay : silt : sand)
KH	AlKoud	23°37'37"N 58°9'43"E	1985	Loamy sand (7 : 8 : 86)
WT	Nizwa	23°3'29"N 57°28'8"E	1989	Sandy loam (7 : 24 : 69)
MA	Madha	25°15'50"N 56°17'50"E	2004	Loamy sand (7 : 11 : 82)

Table 2: Chemical characteristics of soils

Sample	pH	EC (dS m^{-1})	N	P	K	Na	Mg	Ca
KH	6.97 b	0.69 b	458 b	0.13 a	6.09 b	61.24 a	30.67 b	521.52 a
WT	7.90 a	2.29 a	1941 a	0.25 a	12.01 a	36.59 b	71.19 a	382.93 a
MA	8.00 a	0.99 b	687 b	0.11 a	11.71 a	32.31 b	70.28 a	84.91 b

Values with the same letter in the same column are not significantly different from each other at $P < 0.05$ (Tukey's Studentized range test, SAS, v8). The values of nutrients are expressed in ppm

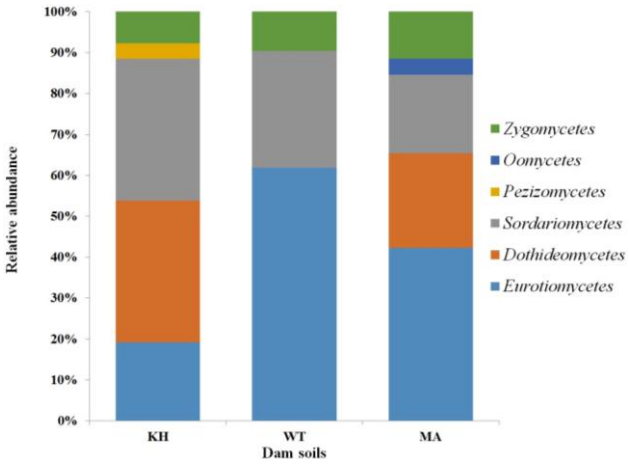

Fig. 1: Relative abundance of fungal classes in the three dam soils

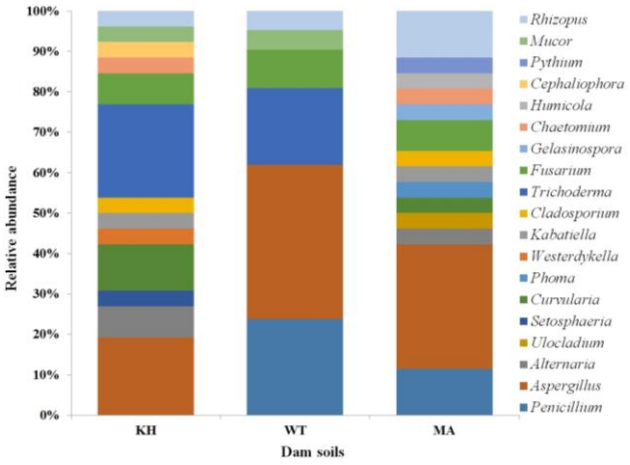

Fig. 2: Relative abundance of the 19 fungal genera in the three dam soils

A total of 19 different genera and 50 different species were recovered from the different dam soils (Table 3; Fig. 2). *Aspergillus*, *Trichoderma* and *Penicillium* were the most dominant genera in most of the dam soils. More than 53% of the total species recovered from all soils belong to one of the three genera. The ITS sequences of 73 isolates representing 50 different fungal species were deposited in GenBank (Table 3; Fig. 3). Twenty of the recovered species are new reports in Oman after checking the list of fungi in Oman (Maharachchikumbura *et al*., 2016) (Table 3).

Fungal Diversity across Dams

Three classes, *Eurotiomycetes*, *Sordariomycetes* and *Zygomycetes*, were detected in the three dam soils. *Oomycetes* was only detected in MA, *Pezizomycetes* was detected in KH, while *Dothideomycetes* was detected in KH and MA. *Aspergillus* sp., *A. tubingensis*, *Fusarium falciforme*, *F. brachygibbosum* and *Rhizopus delemar* were detected in soils from the three dams (Fig. 4). The other classes, genera and species were detected in one or two of the dam soils.

Discussion

The presence of 50 fungal species belonging to 19 genera and six classes in dam soils in arid regions indicates the presence of relatively high fungal diversity in these areas. The main source of fungal propagules in dam reservoir soils could be sediments carried out from under vegetation and trees by the flowing water streams. *Wadi* streams in Oman and in many dry areas usually extend tens and hundreds of kilometers before they reach dams. Various grasses, shrubs and trees are common in the *Wadi* streams. A previous study in Oman indicated the presence of relatively high fungal diversity under vegetation from a *Wadi* stream in Oman (Abed *et al.*, 2013). It is therefore likely that the flowing water carries soil rich with such fungi from under vegetation and accumulates fungal propagules in the reservoir of dams.

Variation was observed in the type of fungal species among different dams. This could be related to several reasons, including the length of the *Wadi* stream, the type of plants growing in such streams, soil texture and the type of fungi associated with plants growing in *Wadi* streams (Ullmann and Büdel, 2001).

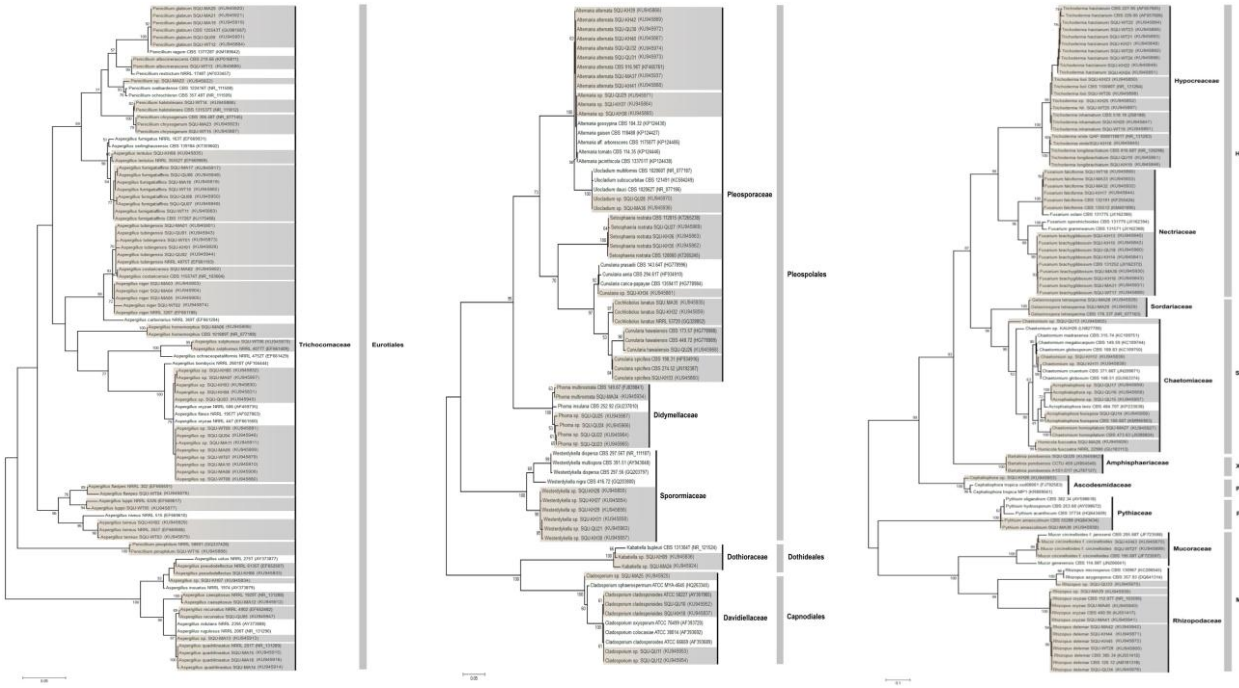

Fig. 3: Phylogenetic trees generated from maximum parsimony analysis of the ITS rRNA gene sequences of fungal isolates from this study and fungal type strains obtained from GenBank (1000 replicates; values above 50% are indicated). The bar indicates nucleotide substitutions per site

Although higher fungal diversity was observed in samples from two dams having loamy sand soils, no conclusion can be made on the relationship between soil texture and fungal diversity because only three dam soils were studied.

Our study demonstrated dominance by the phylum Ascomycota in the three dam soil samples. Previous studies have indicated the dominance of *Ascomycota* in truffle grounds (Mello *et al*., 2011) and in the rhizosphere of *Xinjiang Jujube* (Liu *et al*., 2015). *Zygomycota* was the second dominant phylum, which has also been reported as sub-dominant species in the Negev desert (Bates *et al.*, 2010). Oomycota was present at a very low percentage in dam soils, which agrees with previous studies that reported that such water molds are not very common in dry soils compared to species of Ascomycota (Abed *et al*., 2013).

The study indicated that *Aspergillus*, *Trichoderma* and *Penicillium* were the most dominant fungi in most samples tested in this study. These fungi grow very fast and have high sporulation capacity and are tolerant to heat. Dominance of *Trichoderma* in dam soils may provide benefits to growers using dam soils in their farms as several species of *Trichoderma* have been reported as potential biological control agents (Watanabe *et al.*, 2005; John *et al.*, 2010; Al-Sadi *et al.*, 2015c; Diánez Martínez *et al.*, 2015).

Detection of fungi in dam soil samples indicated that some of the recovered fungal species belong to species known to cause diseases of crops. These include *Alternaria alternata*, *Curvularia spicifera*, *C. lunatus* and *Cladosporium cladosporioides*, that have been reported as potential foliar pathogens (Al-Sadi *et al.*, 2011b, 2014 and 2015a), as well as *Fusarium falciforme*, *F. brachygibbosum*, and *Phoma multirostrata* that have been reported as potential root pathogens (Al-Sadi *et al.*, 2012). In addition, some of the detected fungi, e.g. *Aspergillus niger*, have been reported as producers of mycotoxins that can affect humans (Elshafie *et al.*, 2007; Nielsen *et al.*, 2009).

Several farmers in Oman practice the transfer of soil from dam reservoirs to their farms. The texture of soils can influence crop yields (Jalota *et al.*, 2010). The results of physicochemical analysis indicated that KH and MA soils are loamy sand, whereas WT soil is sandy loam. Loamy sand soils are commonly used for growing several crops, including tomatoes and cucumbers (Kazeeroni and Al-Sadi, 2016). Soil pH is an important consideration for farmers. If the soil pH level is higher than the standard pH requirement for a certain crop, then this could result in micronutrient deficiencies and nutrient imbalances (Silva and Uchida, 2000). The EC values of soils, ranging from 0.69–2.29, are still acceptable for growing several crops (Senthil Kumar *et al.*, 2001; Al-Sadi *et al.*, 2010).

Physicochemical analysis indicated that dam soils are not rich in macro and micro minerals, and are below the critical levels for agricultural purposes. Fertilization may be required to provide some of the necessary

Table 3: A list of fungal species recovered from the three dam soils

Order	Fungal species*	Location		
		KH	WT	MA
Capnodiales	*Cladosporium cladosporioides*	KU945837	-	-
	Cladosporium sp.	-	-	KU945925
Dothideales	*Kabatiella* sp.	KU945836	-	KU945924
Eurotiales	*Aspergillus caespitosus*	-	-	KU945912
	Aspergillus costaricensis	-	-	KU945902
	Aspergillus flavipes	-	KU945876	-
	Aspergillus fumigatiaffinis	-	KU945882	KU945917
	Aspergillus homomorphus	-	-	KU945906
	Aspergillus lentulus	KU945835	-	-
	Aspergillus luppii	-	KU945877	-
	Aspergillus niger	-	KU945874	KU945903
	Aspergillus pseudodeflectus	KU945833	-	-
	Aspergillus quadrilineatus	-	-	KU945914
	Aspergillus sp.	KU945830	KU945879	KU945907
	Aspergillus sulphureus	-	KU945878	-
	Aspergillus terreus	KU945829	KU945875	-
	Aspergillus tubingensis	KU945828	KU945873	KU945901
	Penicillium albocinerascens	-	KU945885	-
	Penicillium chrysogenum	-	KU945887	KU945923
	Penicillium glabrum	-	KU945884	KU945919
	Penicillium halotolerans	-	KU945886	-
	Penicillium pinophilum	-	KU945888	-
	Penicillium sp.	-	-	KU945922
Hypocreales	*Fusarium brachygibbosum*	KU945840	KU945889	KU945930
	Neocosmospora falciformis (*Fusarium falciforme*)	KU945844	KU945890	KU945932
	Trichoderma harzianum	KU945848	KU945892	-
	Trichoderma inhamatum	KU945847	KU945891	-
	Trichoderma lixii	KU945850	KU945898	-
	Trichoderma longibrachiatum	KU945846	-	-
	Trichoderma sp.	KU945852	KU945897	-
	Trichoderma viride	KU945845	-	-
Mucorales	*Mucor circinelloides* f. *circinelloides*	KU945870	KU945899	-
	Rhizopus arrhizus (*Rhizopus delemar*)	KU945871	KU945900	KU945942
	Rhizopus oryzae	-	-	KU945940
	Rhizopus sp.	-	-	KU945939
Pezizales	*Cephaliophora* sp.	KU945853	-	-
Pleosporales	*Alternaria alternata*	KU945866	-	KU945937
	Alternaria sp.	KU945864	-	-
	Curvularia lunata	KU945859	-	KU945935
	Curvularia sp.	KU945861	-	-
	Curvularia spicifera	KU945860	-	-
	Phoma multirostrata	-	-	KU945934
	Setosphaeria rostrata	KU945862	-	-
	Ulocladium sp.	-	-	KU945936
	Westerdykella sp.	KU945854	-	-
Pythiales	*Pythium amasculinum*	-	-	KU945938
Sordariales	*Chaetomium homopilatum*	-	-	KU945927
	Chaetomium sp.	KU945838	-	-
	Gelasinospora tetrasperma	-	-	KU945928
	Humicola fuscoatra	-	-	KU945926

*Species in bold are reported for the first time in Oman (Maharachchikumbura *et al*., 2016)

elements for plant growth, which usually varies from one crop to the other (Al-Azizi *et al.*, 2013; Hernández *et al.*, 2014; Lehrsch *et al.*, 2015).

Conclusion

Our study demonstrates that soils from dam reservoirs in arid regions may have high fungal diversity. Fungal taxa are dominated by saprophytes from Ascomycota, with some potential plant pathogens or species affecting human health through mycotoxin production. This study appears to be the first to investigate fungal diversity in dam soils from arid regions in the Arabian Peninsula. It also appears to report 20 fungal species for the first time in Oman. Future studies should focus on the sources of fungi in these soils and also on the roles such fungi may play in the ecosystem.

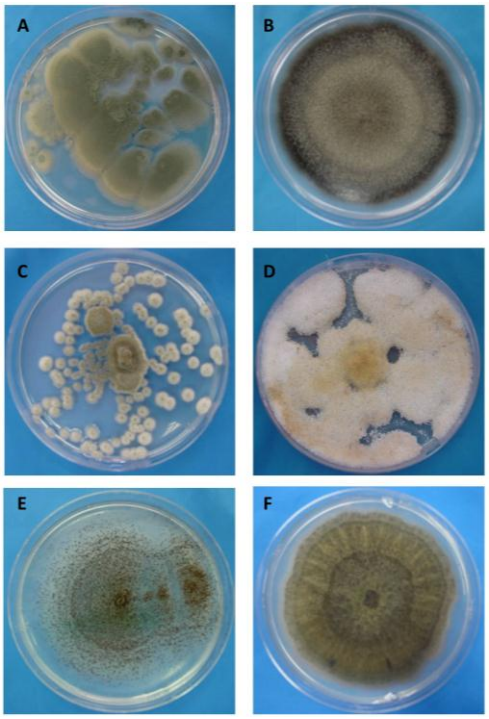

Fig. 4: Some of the common fungal species in dam soils (A: *Penicillium* sp., B: *Alternaria* sp., C: *Cladosporium cladosporioides*, D: *Fusarium* sp., E: *Aspergillus* sp., F: *Curvularia lunata*)

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