Rashmi Fotedar*, Anna Kolecka, Teun Boekhout, Jack W. Fell, Ameena Al-Malki, Aisha Zeyara and Masoud Al Marri

Fungal diversity of the hypersaline Inland Sea in Qatar

<https://doi.org/10.1515/bot-2018-0048>

Received 1 May, 2018; accepted 9 October, 2018; online first 17 November, 2018

Abstract: The hypersaline Inland Sea in Qatar constitutes a unique ecosystem characterized by salinities up to saturation, extreme temperature fluctuations, and limited rainfall. To reveal the fungal diversity of this environment, we isolated fungi from water samples collected at the Inland Sea. Taxonomic identification of the isolates was done via DNA barcoding of the ITS1 and ITS2 ribosomal DNA (rDNA) domains and the D1/D2 domains of the nuclear large subunit rDNA. Additional genes, including glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1-alpha (*tef1*), were included for isolates of *Alternaria*, actin (*Act*) for *Cladosporium*, part of the beta-tubulin (*BenA*) and calmodulin (*CaM*) genes for *Aspergillus* and *Penicillium*. In total, 159 fungal isolates, including 85 Ascomycota and 74 Basidiomycota, were obtained from the water samples collected during four samplings in the winter and summer seasons. About 14% (22/159) of the strains, presumably novel species, were preliminarily identified to the genus level. This is the first report highlighting the diversity of fungi from the hypersaline Inland Sea in Qatar.

Keywords: Arabian Gulf; extremophiles; fungal diversity; hypersaline; marine fungi.

Introduction

Fungi are ubiquitous microorganisms that are part of the microbiota occurring in natural ecosystems, including fresh and marine waters ranging from the ocean surface to the deep sea, eutrophic to ultra-oligotrophic lakes, lagoons, rivers, ground waters, melting water and ice of glaciers (Fell et al. 2011, Turchetti et al. 2013, Gunde-Cimerman and Zalar 2014, Grum-Grzhimaylo et al. 2016, Mokhtarnejad et al. 2016). Fungal diversity in so-called extreme environments may be affected by a variety of abiotic and biotic factors, including temperature, pressure, UV radiation (UVR), salinity, presence of fauna, flora and other microorganisms, run-off from soils and spills, and anthropogenic effluents. In these extreme habitats the interactions between abiotic factors, for instance temperature, salinity, and pH, result in the colonization by microorganisms adapted to these environmental conditions (Gunde-Cimerman et al. 2000, Pedros-Alio et al. 2000, Pedros-Alio 2004).

High concentrations of salt (hypersalinity) result in an extreme environment with limited fungal and other microbial growth. Due to this strong selection pressure the diversity of halophilic and halotolerant fungi decreases with increasing salt concentrations (Gunde-Cimerman et al. 2000, Butinar et al. 2005a, Gunde-Cimerman and Plemenitaš 2005, Grum-Grzhimaylo et al. 2016). Many genera of fungi such as *Aspergillus* P. Micheli ex Halle, *Alternaria* Nees, *Aureobasidium* Viala et G. Boyer, *Cladosporium* Link, *Penicillium* Link and *Hortaea* Nishim. et Miyaji, have been reported from hypersaline environments (Kis-Papo et al. 2001, Diaz-Munoz and Montalvo-Rodrigue 2004, Gunde-Cimerman et al. 2004, Butinar et al. 2005a, Coelho et al. 2010, Al-Musallam et al. 2011, Nazareth et al. 2012, Jaouani et al. 2014, Mokhtarnejad et al. 2016).

The Inland Sea is a hypersaline lagoon at the inlet of the Arabian Gulf located in southeast Qatar, bordering Saudi Arabia. This lagoon extends 15 km from north to south and 12 km from east to west and is connected to the Arabian Gulf by a 10-km long channel that allows exchange of water mass and subsequent interaction with bottom substrata (Figure 1). The area surrounding the Inland Sea is characterized by the presence of large mobile

***Corresponding author: Rashmi Fotedar**, Department of Genetic Engineering, Biotechnology Centre, Ministry of Municipality and Environment, P.O Box 20022, Doha, Qatar,
e-mail: rfotedar@mme.gov.qa, rfotedar@hotmail.com

Anna Kolecka: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

Teun Boekhout: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; and Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands

Jack W. Fell: Bahamas Marine EcoCentre, Palmetto Bay, FL, USA

Ameena Al-Malki, Aisha Zeyara and Masoud Al Marri: Department of Genetic Engineering, Biotechnology Centre, Ministry of Municipality and Environment, P.O Box 20022, Doha, Qatar

Figure 1: Map of Inland Sea of Qatar and location of sampling sites. Image Map courtesy: Centre for GIS, Ministry of Municipality and Environment. www.mme.gov.qa.

sand dunes, a tidal embayment system, inland and coastal salt pans, salt hills, stony deserts, elevated areas, and rocky ridges. The Inland Sea is a largely uninhabited area of global importance (Schwarze et al. 2005), which was nominated for the World Natural Heritage List (<http://whc.unesco.org/en/tentativelists/5317/>). In 1993 Inland Sea was declared a water sanctuary by Ministerial Decree No.78 of the Ministry of Municipal Affairs and Agriculture of Qatar that banned all commercial fishing activities. Because of its connectivity to the Arabian Gulf, Inland Sea is characterized by a pronounced salt gradient with salinities ranging from 57 to 75 in the tidal embayment area with saturated salt conditions in the salt pans near the coastal zones. These characteristics offer a unique opportunity to investigate microbial communities along a natural pronounced salinity gradient, with the original microbiota recruited from the Arabian Gulf.

The aim of this investigation was to assess the fungal diversity in the hypersaline ecosystem of Inland Sea. Sequencing of different genes was done via DNA barcoding of the ITS1 and ITS2 ribosomal DNA (rDNA) domains and the D1/D2 domains of the nuclear large subunit rDNA. Additional genes, including glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1- α (*tef1*), were included for isolates of *Alternaria*,

actin (*Act*) for *Cladosporium*, part of the beta-tubulin (*BenA*) and calmodulin (*CaM*) genes for *Aspergillus* and *Penicillium*.

Materials and methods

Inland Sea sampling sites and collection of water samples

The Inland Sea is located at the inlet of the Arabian Gulf in southeast Qatar (N 24.625308; E 051.297259; Figure 1). This area is an arid region characterized by high solar radiation, high salinities ranging from 57 to 75, and limited rainfall (75.2 mm per year). Sampling at eight locations (Figure 1, Table 1) took place during the winter (December 2013) and summer (September 2014) seasons. Seven coastal sites (INS1-INS7) were sampled near the littoral zone in the vicinity of salt pans at a depth of 0.5 m. One site (INS8) was sampled at a depth of 2.5 m in the pelagic zone at approximately 250 m from the coast (Table 1). INS8 was located at the entrance of the 10-km-long channel that allows exchange of water from the Arabian Gulf to the lagoon. Sites INS2, INS6, and INS7 were influenced by

Table 1: Details of sampling sites at the Inland Sea, characteristics of water samples and fungal counts.

Station/depth (m)	Coordinates	Sampling season/month	Salinity	pH	Temp. (°C)	Yeast count (CFU ^a /l)	Filamentous fungi count (CFU ^a /l)
INS1/0.5	N 24.6706; E 051.2884	Winter/ Dec 2013	73	8.06	24–25	70	100
INS2/0.5	N 24.6171; E 051.3573	Winter/ Dec 2013	75	8.06	24–25	140	100
INS3/0.5	N 24.65787; E 51.28368	Summer/ Sept 2014	72	8.07	30–32	90	15
INS4/0.5	N 24.66417; E 51.29282	Summer/ Sept 2014	73	8.2	30–32	0	15
INS5/0.5	N 24.64786; E 51.30041	Summer/ Sept 2014	75	8.1	30–32	0	15
INS6/0.5	N 24.64357; E 51.33078	Summer/ Sept 2014	74	8.2	30–32	710	10
INS7/0.5	N 24.62436; E 51.34179	Summer/ Sept 2014	72	8.1	32	910	10
INS8/2.5	N 24.5522; E 051.3325	Summer/ Sept 2014	57.3	7.99	32	69	8

^aColony forming units (CFU) based on gross morphology of colonies seen on the filter paper. The yeast column included all the yeast isolates and also *Aureobasidium* spp. and *Hortaea werneckii* which grew on the medium as yeasts.

human activity due to the proximity of recreational areas and camps, which are allowed in the area from October to April.

The distances between the sampling sites (INS1-INS7) ranged from approximately 1–3 km, whereas INS8 was further away, approximately 8.2 km from INS2 (Figure 1). Water samples were collected in a 1.7-l Niskin Water Sampler (model 1010) with survey outboard motor boats. The water samples were collected in sterile glass bottles of 1 l that were transferred to the laboratory within 2–3 h after collection, and processed immediately for the isolation of fungi with standard microbiological methods. Marine water parameters, such as temperature, salinity, and pH, were measured in three replicate readings with a 650 MDS Data Logger and 6920 V2 multiparameter, water Quality Sonde (Xylem Analytics, UK) calibrated against standard solutions.

Isolation, identification, and characterization of yeasts and filamentous fungi

Culture methods

For the isolation of fungi, five volumes of 200 ml of water per site were filtered through sterile nitrocellulose membranes (0.45 µm pore size; 47 mm diameter, Merck-Millipore, Ltd., Ireland) with a sterilized filtration device. The process was replicated three times for each sampling site. The filters were placed on modified

glucose-yeast extract-peptone agar (GYPA) medium (2% glucose (Conda laboratories, Spain), 1% peptone (Conda laboratories, Spain), 0.5% yeast extract (Conda lab, Spain), 0.05% chloramphenicol (Sigma Aldrich, USA), 1.7% agar, 50% filtered water collected from Inland Sea). The medium was prepared with Inland Sea water obtained from the same sites where the water samples were collected. Initial experiments with the incubation temperatures of >25°C and 30°C for summer and winter seasons, respectively, showed an overgrowth of filamentous fungi, which obscured the yeast colonies. The plates were consequently incubated at 17°C and 25°C for 8–10 weeks for the winter and summer samples, respectively (Fell et al. 2011).

The plates were inspected for fungal growth at intervals of 48–72 h and cell counts (CFU (colony forming unit) l⁻¹) of yeasts and filamentous fungi were made with a stereoscopic microscope (Olympus SZX 16, Japan). The fungal count was scored after 3, 5, 7, 14, 30 and 60 days of incubation and the averages (per site/per sampling) of the total viable yeast and filamentous fungi were calculated. Microscopic and morphological observations were made with an Olympus SZX 16 stereomicroscope (Olympus, Japan). At least one colony of each macro-morphological colony type was picked and subcultured onto a fresh medium for further purification. For yeasts, purification of isolates was performed on modified GYPA plates, whereas potato dextrose agar (PDA, Conda Laboratories, Spain) plates were used for filamentous fungi. The isolates were purified by repeated sub-culturing at least three to five

Table 2: Species of fungi isolated from the Inland Sea, Qatar.

Group	Species recovered	Site/No. of isolates	Strains sequenced ^b	ITS-GenBank accession numbers	LSU-GenBank accession numbers	Gapdh-GenBank accession numbers	Tef1-GenBank accession numbers	Actin (A)/ β -tubulin (BT) Calmodulin (C) GenBank accession numbers	No. of isolates in Winter	No. of isolates in Summer	Total no. (%)
Ascomycota	<i>Alternaria</i> spp. ^a	INS8 (4)	2M108A	KY387606	KY781812	KY387604	KY387608	–	2	4	6 (3.8)
		INS1 (2)	INM7	KY387605	KY781811	KY387603	KY387607	–			
	<i>Alternaria</i> sect.	INS1 (3)	INM4	KY744118	KY744136	MF084400	MF084404	–	8	2	10 (6.3)
	<i>Alternaria</i>	INS2 (5)	INM10	–	–	MF084401	MF084405	–			
	D.P. Lawrence, P.B. Gannibal, T.L. Peever, B.M. Pryor	INS3 (1)									
		INS8 (1)									
	<i>Alternaria</i> sect.	INS1 (1)	INM5	–	–	MF084402	MF084406	–	1	1	2 (1.2)
	<i>Ulocladioides</i>	INS8 (1)	2M109	–	–	MF084403	–	–			
	Woudenberg et Crous										
	<i>Aspergillus citrinoterreus</i>	INS8 (1)	2M110B	KY781744	KY781745	–	–	BT-MF084376 C-MF084381	0	1	1 (0.6)
	J. Guinea, M. Sandoval-Denis, P. Escribano, E. Bouza et J. Guarro										
	<i>Aureobasidium iranianaum</i>	INS1 (1)	YINS1	KY781746	KY781747	–	–	–	1	0	1 (0.6)
	Arzanlou et Khodaei										
	<i>Aureobasidium melanogenum</i> (Hermanides-Nijhof)	INS8 (2)	2Y211 2Y215A	KY781748 KY781750	KY781749 KY781751	–	–	–	0	2	2 (1.2)
	Zalar, Gostincar et Gunde-Cim.										
	<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud	INS8 (1)	2Y101	–	KY781752	–	–	–	0	1	1 (0.6)
	<i>Zalaria obscura</i> Visagie, Z. Humphries et Seifert	INS3 (2)	INY20	KY781753	KY781754	–	–	–	0	4	4 (2.5)
		INS7 (1)	INY28	KY781755	KY781756	–	–	–			
		INS8 (1)	2Y102	KY781757	KY781758	–	–	–			
	<i>Candida metapsilosis</i> Tavanti, A. Davidson, Gow, M. Maiden et Odds	INS7 (1)	INY21	–	KY695002	–	–	–	0	1	1 (0.6)
	<i>Candida orthopsilosis</i> Tavanti, A. Davidson, Gow, M. Maiden et Odds	INS8 (1)	2Y191A	–	–	–	–	–	0	1	1 (0.6)
	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice	INS8 (1)	2Y213	KY694996	KY695003	–	–	–	0	1	1 (0.6)

Group	Species
-------	---------

Group	Species recovered	Site/No. of isolates	Strains sequenced ^b	ITS-GenBank accession numbers	LSU-GenBank accession numbers	Gapdh-GenBank accession numbers	Tef1-GenBank accession numbers	Actin (A)/β-tubulin (BT) Calmodulin (C) GenBank accession numbers	No. of isolates in Winter	No. of isolates in Summer	Total no (%)		
	<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	INS2 (2)	INM21	KY781759	KY781760	–	–	A-MF084386	2	0	2 (1.3)		
			INM29	–	–	–	–	–	–	–	–		
		INS1 (3)	INM1	KY781786	KY781787	–	–	A-MF084398	4	1	5 (3.2)		
			INS2 (1)	INM14	KY781788	KY781789	–	–	A-MF084399	–	–	–	
	INS5 (1)	Zalar, de Hoog et Gunde-Cim.	INS1 (2)	INM3	KY781780	KY781781	–	–	–	–	–		
				INS2 (1)	INM6	KY781782	KY781783	–	–	–	–	–	
	INS4 (1)	Bensch, Crous et U. Braun	INS5 (1)	2INM1	KY781784	KY781785	–	–	A-MF084397	3	6	9 (5.7)	
				INS6 (1)	–	–	–	–	–	–	–	–	
		<i>Cladosporium spp.^a</i>	INS7 (2)	INS8 (1)	INM18	KY781769	KY781770	–	–	A-MF084391	6	1	7 (4.4)
						INS1 (2)	INM24	KY781774	KY781775	–	–	A-MF084394	–
INS2 (3)			INM23	–	KY781773	–	–	A-MF084393	–	–	–		
			INS3 (2)	INM37	KY781776	KY781777	–	–	A-MF084395	–	–	–	
INS1 (5)		<i>Cladosporium tenuissimum</i> Cooke	INS1 (2)	INM34	KY781771	KY781772	–	–	A-MF084392	–	–	–	
				2INM9	KY781778	KY781779	–	–	A-MF084396	–	–	–	
INS2 (2)		<i>Cladosporium xanthochromaticum</i> Sandoval-Denis, Gené et Cano	INS2 (1)	INM8	KY781761	KY781762	–	–	A-MF084387	5	0	5 (3.2)	
				INM12	KY781763	KY781764	–	–	A-MF084388	–	–	–	
		<i>Debaryomyces hanssenii</i> (Zopf) Lodder et Kreger	INS1 (2)	INS2 (3)	INM11	KY781765	KY781766	–	–	A-MF084389	4	0	4 (2.5)
					INS3 (1)	INM16	KY781767	KY781768	–	–	A-MF084390	–	–
	INS6 (2)		<i>Debaryomyces nepalensis</i> Goto et Sugiy	INS1 (2)	YINM36B	KY694997	KY695004	–	–	–	1	0	1 (0.6)
					INS2 (1)	YINS3	KY694998	KY695005	–	–	–	5	0
	INS8 (5)	<i>Fusarium chlamydosporum</i> Wollenw. et Reinking	INS3 (1)	2INM10B	KY781790	KY781791	–	–	–	0	1	1 (0.6)	
				INS6 (2)	INY32C	KY695000	KY695007	–	–	–	–	–	–
	INS10 (2)	<i>Hyphozyma spp.^a</i>	INS8 (5)	INY36	KY695001	KY695008	–	–	–	0	2	2 (1.3)	
				2Y193B	KY781792	KY781793	–	–	–	0	5	5 (3.2)	
		<i>Hortaea werneckii</i> (Horta) Nishim. et Miyaji	INS11 (1)	2Y210	KY781794	KY781795	–	–	–	–	–	–	
				INS12 (1)	–	–	–	–	–	–	–	–	–

Table 2 (continued)

Group	Species recovered	Site/No. of isolates	Strains sequenced ^b	ITS-GenBank accession numbers	LSU-GenBank accession numbers	Gapdh-GenBank accession numbers	Tef1-GenBank accession numbers	Actin (A)/ β -tubulin (BT) Calmodulin (C) GenBank accession numbers	No. of isolates in Winter	No. of isolates in Summer	Total no. (%)
Basidiomycetes	<i>Trichoderma placentula</i> Jaklitsch	INS6 (1)	INY33,	–	KY781797	–	–	–	0	3	3 (1.9)
	<i>Knufia petricola</i> (Wollenz. et de Hoog)	INS7 (2)	INY10B	KY781796	–	–	–	–	0	1	1 (0.6)
	<i>Gorbushina et Gueidan</i>	INS8 (1)	2Y215B	KY781798	KY781799	–	–	–	0	1	1 (0.6)
	<i>Penicillium oxalicum</i> Currie et Thom	INS5 (1)	2INM6A	KY781804	KY781805	–	–	C-MF084384 BT-MF084379	0	2	2 (1.3)
		INS8 (1)	2M110A	KY781806	KY781807	–	–	C-MF084385 BT-MF084380	0	2	2 (1.3)
	<i>Penicillium chrysogenum</i> Thom	INS3 (1)	2INM8	KY781802	KY781803	–	–	C-MF084383 BT-MF084378	0	2	2 (1.3)
		INS4 (1)	2INM4	KY781800	KY781801	–	–	C-MF084382 BT-MF084377	1	0	1 (0.6)
	<i>Sarocladium bacillisporum</i> (Onions et G.L. Barron)	INS1 (1)	YINS5	KY781808	–	–	–	–	0	4	4 (2.5)
	<i>Kondoa</i> spp. ^a	INS8 (4)	2Y188 2Y189 2Y209 2Y214	KY744105 – KY744106 KY744107	KY744120 KY744121 KY744122 KY744123	–	–	–	0	4	4 (2.5)
	<i>Naganishia albida</i> (Saito) X.Z. Liu, F.Y. Bai, M. Groenew. et Boekhout	INS7 (1)	INY12	KY744108	KY744124	–	–	–	0	1	1 (0.6)
Basidiomycetes	<i>Naganishia albidosimilis</i> (Vishniac et Kurtzman) X.Z. Liu, F.Y. Bai, M. Groenew. et Boekhout	INS3 (1)	INY14	KY744109	KY744125	–	–	–	0	4	4 (2.5)
		INS6 (2)	INY25	KY744110	KY744126	–	–	–	0	4	4 (2.5)
		INS7 (1)				–	–	–	0	4	4 (2.5)
	<i>Naganishia qatariensis</i> ^a	INS6 (1)	INY13	KY744111	KY744127	–	–	–	0	2	2 (1.3)
	R. Fotedar, A. Kolecka, T. Boekhout, J.W. Fell A. Anand, A. Al Malaki, A. Zeyara, M. Al Marri.	INS7 (1)	INY29	MG852088	KY744128	–	–	–	0	2	2 (1.3)
	<i>Papiliotrema laurentii</i> (Kuff.) X.Z. Liu, F.Y. Bai, M. Groenew. et Boekhout	INS6 (1)	INY35	KY744112	KY744129	–	–	–	0	1	1 (0.6)
						–	–	–	0	1	1 (0.6)
						–	–	–	0	1	1 (0.6)
						–	–	–	0	1	1 (0.6)
						–	–	–	0	1	1 (0.6)

Table 2 (continued)

Group	Species recovered	Site/No. of isolates	Strains sequenced ^b	ITS-GenBank accession numbers	LSU-GenBank accession numbers	Gapdh-GenBank accession numbers	Tef1-GenBank accession numbers	Actin (A)/ β -tubulin (BT) Calmodulin (C) GenBank accession numbers	No. of isolates in Winter	No. of isolates in Summer	Total no. (%)
	<i>Rhodotorula diobovata</i> (S.Y. Newell et al. L. Hunter) Q.M. Wang, F.Y. Bai, M. Groenew. et T. Boekhout.	INS6 (1)	INY34	–	KY744130	–	–	–	0	1	1 (0.6)
	<i>Rhodotorula mucilaginosa</i> (A. Jörg.) F.C. Harrison	INS3 (6)	INY2	KY744113	KY744131	–	–	–	0	47	47 (29.6)
	<i>Symmetrospora marina</i> (Phaff, Mrak et O.B. Williams) Q.M. Wang, F.Y. Bai, M. Groenew. et Boekhout	INS6 (18)	INY6	KY744114	KY744132	–	–	–	–	–	–
		INS7 (23)	–	–	–	–	–	–	–	–	–
		INS8 (12)	2Y186	KY744115	KY744133	–	–	–	0	12	12 (7.6)
			2Y190A	KY744116	KY744134	–	–	–	–	–	–
	<i>Symmetrospora</i> spp. ^a	INS8 (1)	2Y207	KY744117	KY744135	–	–	–	0	1	1 (0.6)
	<i>Tremellales</i> sp.	INS2 (1)	INM28	KY781809	KY781810	–	–	–	1	0	1 (0.6)
Total isolates (number)									44	115	159 (100)

^aStrains preliminarily identified at the genus level on the basis of the LSU, ITS, TEF1, Gapdh 1 and 2 sequence analyses.^bStrains sequenced and sequences deposited in GenBank.

times and stored in 50% glycerol (Avonchem, Cheshire, UK) in sterile distilled water at -20°C .

PCR amplification, sequencing, and phylogenetic analyses

For molecular identification, genomic DNA was extracted in 96-well plates following the PrepMan™ Ultra (Applied Biosystems, UK) protocol (Stielow et al. 2015). The internal transcribed spacer 1 and 2 regions (ITS), including the 5.8S rDNA and the D1/D2 domains of the nuclear large subunit (LSU) rDNA, were used for molecular identification (Fell et al. 2000, Scorzetti et al. 2002, Vu et al. 2016). The primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS regions. The LSU region was amplified with the primers LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990). PCR reactions for amplification were performed in volumes of 12.5 μl containing 2.5 μl of the genomic DNA as reported by Stielow et al. (2015). The PCR product was checked by agarose gel electrophoresis, purified with Fast AP, the alkaline phosphatase (Fermentas, Thermo Fisher Scientific, USA) and cycle-sequenced with the ABI Big Dye™ v3.1 technology (Thermo Fisher Scientific, USA). To increase the taxonomic resolution for some groups of filamentous fungi, additional barcodes were used. For *Cladosporium* isolates, part of the actin (*act*) gene was amplified with the primer set ACT-512F and ACT-783R (Carbone and Kohn 1999) as described in Groenewald

et al. (2013). For *Alternaria* species, part of the *gapdh* and *tef1* genes were amplified with the primer sets *gpd1* and *gpd2* (Berbee et al. 1999) and EF1-728F and EF1-986R (Carbone and Kohn 1999), respectively (Woudenberg et al. 2013). For *Aspergillus* and *Penicillium* species, part of the beta-tubulin (*BenA*) and calmodulin (*CaM*) genes amplified with the primer sets Bt2a-F and Bt2b-R (Glass and Donaldson 1995) and Cmd5-F and Cmd6-R (Hong et al. 2005), respectively, were sequenced as described in Samson et al. (2014) and Visagie et al. (2014). Forward and reverse sequences were manually corrected with SeqMan software v 8.0.2 (DNA Star Inc., Madison, WI, USA). The resulting consensus sequences were compared by Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1990) with sequences available in the GenBank database for primary identifications (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). All sequences from the Qatar isolates were grouped by genus and aligned with Clustal W in MEGA v.6 software (Tamura et al. 2011, 2013) with sequences of relevant type strains. Phylogenetic analysis of the LSU and ITS regions followed the procedures of Fell et al. (2000) and Scorzetti et al. (2002). Phylogenetic trees based on the D1/D2 sequence were constructed by using the program provided within the MEGA6 software package. Phylogenetic placement of the novel species was analyzed with the maximum likelihood method (Tamura et al. 2011, 2013). Based on this analysis and the initial BLAST scores, the identity of the isolates at genus, section or species (-complex) level is listed in Table 2.

Figure 2: Maximum likelihood multi-gene tree based on ITS, *gapdh* and *tef1* sequences of *Alternaria* sp. nov. (marked in bold), including all reference isolates from *Alternaria* sect. *Chalastospora* and the closest relatives from *Alternaria* sect. *Infectoriae* with *Alternaria penicillata* CBS 116607 (*Alternaria* sect. *Crivellia*) as outgroup.

The RAX ML bootstrap support values $\geq 75\%$ (BS) and Bayesian posterior probabilities ≥ 0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100% and a PP of 1.0. The GenBank accession numbers of the studied sequences are given in brackets [ITS; *gapdh*; *tef1*] after the species names.

Figure 3: Phylogenetic tree showing the placement of *Kondoia* sp. nov. (marked in bold) and some closely related species based on the analysis of the combined ITS region and D1/D2 domains of LSU rDNA.

Type strains indicated by superscript T. Sequences not generated during this study were obtained from GenBank (accession numbers are shown in parentheses). The tree was constructed by maximum-likelihood method based on the Kimura 2-parameter model using MEGA6 software. Numbers on branches represent bootstrap values from 1000 replicates. Scale bar represents 0.05 substitutions per nucleotide position.

Figure 4: Phylogenetic tree showing the placement of *Naganishia qatarensis* (marked in bold) and some closely related species based on the analysis of the combined ITS region and D1/D2 domains of LSU rDNA.

Type strains indicated by superscript T. Sequences not generated during this study were obtained from GenBank (accession numbers are shown in parentheses). The tree was constructed by maximum-likelihood method based on the Kimura 2-parameter model using MEGA6 software. Numbers on branches represent bootstrap values from 1000 replicates. Scale bar represents 0.02 substitutions per nucleotide position (Fotedar et al. 2018).

Results

The physicochemical characteristics of the water samples are summarized in Table 1. The range of pH and salinity for the coastal sites, i.e. INS1-INS7, was similar, whereas INS8 showed a lower pH and salinity. During the winter and summer seasons the seawater temperatures varied between 24–25°C and 30–32°C, respectively (Table 1).

The total average yeast and filamentous fungus counts for each sampling site are shown in Table 1. The average number of isolates (including both yeasts and filamentous fungi) ranged from 15 to 920 CFU l⁻¹ (Table 1), with the highest values corresponding to the sites INS7, INS6 and INS2 that were coastal zones in the vicinity of salt pans.

One hundred and fifty-nine isolates (yeasts and filamentous fungi) were obtained from the water samples (Table 2). Seventy-two percent of the isolates (115/159) were recovered during the summer season and 28% (44/159) during the winter season. Selected sequences of the fungal isolates were deposited in the GenBank database (www.ncbi.nlm.nih.gov), and their GenBank accession numbers are listed in Table 2. Taxonomic assignments of yeasts were done according to the published literature (Kurtzman et al. 2011, Liu et al. 2015a,b, Wang et al. 2015a,b). Twenty-two strains (22/159; 13.8%) in six genera [*Alternaria* spp. (6 isolates), *Cladosporium* spp. (7 isolates), *Hyphozyma* spp. (2 isolates), *Kondoa* spp. (4 isolates), *Naganishia qatarensis* (2 isolates), *Symmetrospora* sp. (1 isolate)] could not be identified to the species level on the basis of the D1/D2 domains of the LSU and ITS rDNA. These strains may represent novel species (Table 2, Figures 2–4). The novel species of *Naganishia* Goto has been described as *N. qatarensis* by Fotedar et al. (2018). Formal taxonomic descriptions of the remaining novel species are in progress.

Rhodotorula mucilaginosa, a halo- and psychrotolerant red-pigmented yeast species, represented almost 30% (47/159 isolates) of all fungal isolates and was obtained at a sampling depth of 0.5 m from three sites (INS3, INS6, INS7) with a salinity of 72–74 and temperatures ranging from 30 to 32°C.

The majority (40.2%; 64/159 isolates) of the fungal isolates consisted of melanized fungi belonging to the genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Hortaea*, *Knufia* L.J. Hutchison et Unter. and *Zalaria* Visagie, Z. Humphries et Seifert. These melanized fungi were isolated from all sites sampled. Among the melanized fungi, *Cladosporium* species (20%; 32/159 isolates) were most commonly isolated, followed by *Alternaria* species (11.3%; 18/159 isolates).

Discussion

The Inland Sea of Qatar is a unique ecosystem with extreme environmental conditions. During sampling we encountered temperatures of 24–32°C, below average rainfall (winter, 0.1 mm; summer, 0 mm) and high salinities (57–75). For sites INS1-7, no trend was observed between the fungal isolates obtained and the environmental factors measured, such as temperature, pH, salinity, and season of isolation. Sites INS7, INS6 and INS2 (approx. 2–3 km apart from each other) located at the east side had the highest fungal counts, and this could be related to the proximity of these sites to the coastal zone, and the recreational area and camps. Sites INS4, INS5, and INS3 showed low fungal counts that could be due to the pristine nature of these sites with absence of human activity. INS8 (salinity 57, pH 7.9, depth 2.5 m) showed a higher species diversity (Table 2), when compared to the other sites (salinity >70, pH 8.02–8.35 and 0.5 m depth).

The yeast communities in Inland Sea were similar to those reported from other hypersaline environments (Coelho et al. 2010, Yurkov et al. 2011, 2012, França et al. 2016, Mokhtarnejad et al. 2016). Several studies reported a predominance of basidiomycetous fungi in hypersaline aquatic environments (Libkind et al. 2003, Butinar et al. 2005a, de Garcia et al. 2007, Brandao et al. 2011, Mokhtarnejad et al. 2016), but in our study ascomycetous and basidiomycetous yeasts occurred almost with equal abundance (Ascomycota 52.8%; Basidiomycota 47.2%). Some psychrotolerant yeasts, such as species of *Naganishia*, *Rhodotorula* F.C. Harrison and *Debaryomyces* Lodder et Kreger-van Rij ex Kreger-van Rij, were isolated in this study. These yeast species have also been reported from cold ecosystems, e.g. the Calderone glacier, Alpine glaciers and high Arctic glaciers (Butinar et al. 2007, Turchetti et al. 2008, 2013, Branda et al. 2010, Zalar and Gunde-Cimerman 2014).

Rhodotorula mucilaginosa was the most frequently isolated yeast species (29.5% of the total isolates) from the sites adjacent to the salt pans (INS6 and INS7), which were impacted by human activity, due to their proximity to the recreational camps. Our results are in accordance with other reports highlighting *R. mucilaginosa* as the dominant yeast in hypersaline environments with a wide range of salinities and pH (Lahav et al. 2002, Burgard et al. 2010). *Symmetrospora marina* was the second most dominant yeast species with 12 isolates and was isolated from the pelagic site INS8 (salinity 57, 32°C). This species was reported from an aquatic environment in Japan (Urano et al. 2001), from the surface washing of shrimp

(Sampaio 2011), and the Florida Everglades (Fell et al. 2011). This is the first report of *S. marina* from a hypersaline environment.

Naganishia albidosimilis (formerly *Cryptococcus albidosimilis* Vishniac et Kurtzman), *Naganishia albida* and *Naganishia qatarensis* (Fotedar et al. 2018) were isolated from three sites with temperatures ranging from 30 to 32°C. *Naganishia albidosimilis* was reported from hypersaline lakes in Iran (Mokhtarnejad et al. 2016). Strains of *Kondoa* species that belong to the Agaricostilbum clade of Pucciniomycotina (Fonseca et al. 2000, Sampaio 2011, Wang et al. 2015a) were isolated in low frequencies from INS8 only. The Qatari isolates are related to other marine *Kondoa* species, such as *K. aerea* Á. Fonseca, J.P. Samp. et Fell, and *K. malvinella* (Fell et I. L. Hunter) Y. Yamada, Nakagawa et I. Banno, on the basis of the phylogenetic analyses, but may present an undescribed species (Figure 3). *Kondoa aerea* is known from terrestrial habitats (Fonseca et al. 2000), waters of the North Island of New Zealand (Francis et al. 2016), and deep sea sediments of Sagami Bay, Japan (Minegishi et al. 2006). To our knowledge, this is the first report of the isolation of *Kondoa* spp. from a hypersaline lagoon.

Species of the genus *Debaryomyces*, i.e. *D. hansenii* and *D. nepalensis*, were recovered during the winter seasons from two sites with a salinity range of 73–75, a depth of 0.5 m and a temperature of 24–25°C. *Debaryomyces hansenii* has a broad salinity tolerance (Yadav and Loper 1999) and the isolation of this halotolerant species has been documented from oceanic and aquatic environments (Hagler and Ahearn 1987, Nagahama 2006, Coelho et al. 2010), high salinity coastal and oceanic environments (Almeida 2005), and natural hypersaline lakes that are exposed to seasonal low temperatures (Butinar et al. 2005a).

Candida metapsilosis and *C. orthoparapsilosis* were isolated in low numbers (2/159 isolates). A single isolate of *Candida parapsilosis* was cultured from INS8. This species was previously reported from aquatic and hypersaline environments (Soares et al. 1997, Butinar et al. 2005a, Hagler 2006, Nagahama 2006).

The largest group (40.2%; 64/159 isolates) of the filamentous fungal isolates belonged to the melanized fungal genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Hortaea*, *Knufia* and *Zalaria*. *Cladosporium* species (20%; 32/159 isolates) were the most dominant, followed by *Alternaria* species (11.3%; 18/159 isolates). These melanized isolates were recovered from different sampling sites (with varying salinities and temperatures) across the Inland Sea suggesting a wide adaptability and ability

to survive in various environments of high salinities and high temperatures. The majority of these black fungi (75%; 48/64 isolates) were isolated from the coastal sites (INS1-7) near the vicinity of salt pans, indicating that they are able to grow in hypersaline waters and this observation is in agreement with those made elsewhere (Gunde-Cimerman et al. 2000, Nazareth et al. 2012).

Cladosporium was the most species-rich genus and was present in almost all sampling sites with high salinity of 72–75, and this observation is in accordance with reports of *Cladosporium* occurring in salterns in Slovenia and Puerto Rico (Gunde-Cimerman et al. 2000, Cantrell et al. 2006), Great Salt Lake, Utah (Cronin and Post 1977), sabkhas (coastal salt flats) and salterns (Gunde-Cimerman et al. 1997, Butinar et al. 2005b, Cantrell et al. 2006, Al-Musallam et al. 2011), deserts, and salty environments (Kis-Papo et al. 2001, Grishkan et al. 2003, Conley et al. 2006, Smolyanyuk and Bilanenko 2011). Several studies have shown that species of *Alternaria* are predominant in deserts and salty environments (Gunde-Cimerman et al. 2000, Kis-Papo et al. 2001, Conley et al. 2006, Smolyanyuk and Bilanenko 2011). These fungi produce thick-walled and strongly melanized spores, which are important to protect against UVR and increase desiccation tolerance (Sterflinger et al. 2012).

Hortaea werneckii, another melanized fungus, was recovered from the water samples collected from the deep water site INS8 (2.5 m depth; salinity 57.3). This yeast-like fungus was previously reported from drying salty pools (de Hoog and van den Ende 1992), hypersaline waters in Europe (Gunde-Cimerman et al. 2000, Plemenitas et al. 2008), saline water of crystallization ponds (Gunde-Cimerman et al. 2003) and solar salterns in Puerto Rico (Diaz-Munoz and Montalvo-Rodriguez 2004), suggesting that hypersaline water is an ecological habitat of this species (Gunde-Cimerman et al. 2000, Kogej et al. 2005). *Hortaea werneckii* thrives in extreme environments characterized by scarce nutrients, low oxygen tension, high temperature, high UVR, high osmotic stress, as well as a mixture of these conditions (Sterflinger 2006). In our study we did not isolate this species from sites near the salt pans, but in contrast *H. werneckii* was isolated from the deeper water samples of INS8.

Based on the D1/D2 LSU and ITS rDNA sequence analyses, 13.8% (22/159) of the isolates, presumably novel species, were preliminarily identified to the genus level. These species include *Alternaria* spp., *Cladosporium* spp., *Hyphozyma* spp., *Kondoa* spp., *Naganishia qatarensis* (Fotedar et al. 2018) and *Symmetrospora* sp. (Table 2; Figures 2–4). Taxonomically undefined yeast and

filamentous fungi have been isolated in various extreme environments, including glaciers (Butinar et al. 2007, Turchetti et al. 2013), alkaline soils (Grum-Grzhimaylo et al. 2013, 2016), and saline as well as hypersaline environments (Buchalo et al. 1998, Jones and Abdel-Wahab 2005, Zalar et al. 2007, Al-Musallam et al. 2011).

This is the first report highlighting the isolation of halotolerant fungi from hypersaline waters of the Inland Sea in Qatar. Due to the rising sea levels this study will be useful as a reference for future monitoring studies in Qatar.

Acknowledgments: This research was supported by research grant NPRP 6-647-1-127 from the Qatar National Research Fund, a member of Qatar Foundation to R. Fotedar (Ministry of Environment, Qatar), T. Boekhout (Westerdijk Fungal Biodiversity Institute Uppsala, Utrecht, Netherlands) and J.W. Fell (Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Key Biscayne, FL, USA). The authors are solely responsible for the content of the manuscript.

References

- Almeida, J.M.G.C.F. 2005. Yeast community survey in the Tagus estuary. *FEMS Microbiol. Ecol.* 53: 295–303.
- Al-Musallam, A., H. Al-Sammar and N. Al-Sané. 2011. Diversity and dominance of fungi inhabiting the sabkha area in Kuwait. *Bot. Mar.* 54: 83–94.
- Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215: 403–410.
- Berbee, M.L., M. Pirseyed and S. Hubbard. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964–977.
- Branda, E., B. Turchetti, G. Diolaiuti, M. Pecci, C. Smiraglia and P. Buzzini. 2010. Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone glacier, Apennines, Italy). *FEMS Microbiol. Ecol.* 72: 354–369.
- Brandao, R.L., D. Libkind, B.M.A. Vaz, L.C. Espirito Santo, M. Moline, V. de Garcia, M. van Broock and C. Rosa. 2011. Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution, and synthesis of photoprotective compounds and extracellular enzymes. *FEMS Microbiol. Ecol.* 76: 1–13.
- Buchalo, A.S., E. Nevo, S.P. Wasser, A. Oren and H.P. Molitoris. 1998. Fungal life in the extremely hypersaline water of the Dead Sea: first records. *Proc. R. Soc. London.* 265: 1461–1465.
- Burgard, G., D. Arzur, L. Durand, M.A. Cambon-Bonavita and G. Barbier. 2010. Marine culturable yeasts in deep-sea hydrothermal vents: species richness and association with fauna. *FEMS Microbiol. Ecol.* 72: 121–133.
- Butinar, L., S. Santos, I. Spencer-Martins, A. Oren and N. Gunde-Cimerman. 2005a. Yeast diversity in hypersaline habitats. *FEMS Microbiol. Lett.* 244: 229–234.
- Butinar, L., S. Sonjak, P. Zalar, A. Plemenitas and N. Gunde-Cimerman. 2005b. Melanized halophilic fungi are eukaryotic members of the microbial communities' in hypersaline waters of solar salterns. *Bot. Mar.* 48: 73–79.
- Butinar, L., I. Spencer-Martins and N. Gunde-Cimerman. 2007. Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. *Antonie van Leeuwenhoek* 91: 277–289.
- Cantrell, A., L. Casillas-Martinez and M. Molina. 2006. Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques. *Mycol. Res.* 110: 962–970.
- Carbone, I. and L.M. Kohn. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
- Coelho, M.A., J.M. Almeida, I.M. Martins, A.J. de Silva and J.P. Sampaio. 2010. The dynamics of the yeast community of the Tagus river estuary: testing the hypothesis of the multiple origins of estuarine yeasts. *Antonie van Leeuwenhoek* 98: 331–342.
- Conley, C.A., G. Ishkhanova, C.P. McKay and K. Cullings. 2006. A preliminary survey of non-lichenized fungi cultured from the hyper arid Atacama Desert of Chile. *Astrobiol.* 6: 521–526.
- Cronin, A.E. and F.J. Post. 1977. Reports of a dematiaceous hyphomycete from Great Salt Lake, Utah. *Mycologia* 69: 846–847.
- de Garcia, V., S. Brizzio, D. Libkind, P. Buzzini and M. van Broock. 2007. Biodiversity of cold-adapted yeasts from glacial melt-water rivers in Patagonia, Argentina. *FEMS Microbiol. Ecol.* 59: 331–341.
- de Hoog, G.S. and A.H.G. van den Ende. 1992. Nutritional pattern and eco-physiology of *Hortaea werneckii*, agent of human tinea nigra. *Antonie van Leeuwenhoek* 62: 321–329.
- Diaz-Munoz, G. and R. Montalvo-Rodriguez. 2004. Halophilic black yeast *Hortaea werneckii* in the Cabo Rojo solar salterns: its first record for this extreme environment in Puerto Rico. *Caribb. J. Sci.* 41: 360–365.
- Fell, J.W., T. Boekhout, A. Fonseca, G. Scorzetti and A. Stätzell-Tallman. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int. J. Syst. Evol. Microbiol.* 50: 1351–1371.
- Fell, J.W., A. Stätzell-Tallman, G. Scorzetti and M.H. Gutiérrez. 2011. Five new species of yeasts from freshwater and marine habitats in the Florida Everglades. *Antonie van Leeuwenhoek* 9: 533–549.
- Fonseca, I., P. Sampaio, J. Inacio and J.W. Fell. 2000. Emendation of the basidiomycetous yeast genus *Kondoa* and the description of *Kondoa aerea* sp. nov. *Antonie van Leeuwenhoek* 77: 293–302.
- Fotedar, R., A. Kolecka, T. Boekhout, J.W. Fell, A. Anand, A. Al Malaki, A. Zeyara and M. Al Marri. 2018. *Naganishia qatarensis* sp. nov., a novel basidiomycetous yeast species from a hypersaline marine environment in Qatar. *Int. J. Syst. Evol. Microbiol.* 68: 2924–2929.
- França, L., C. Sanninon, B. Turchetti, P. Buzzini and R. Margesin. 2016. Seasonal and altitudinal changes of culturable bacterial and yeast diversity in Alpine forest soils. *Extremophiles* 20: 855–873.

- Francis, M.M., V. Webb and G.C. Zuccarello. 2016. Marine yeast biodiversity on seaweeds in New Zealand waters. *N. Zool. J. Bot.* 54: 30–47.
- Glass, N.L. and G.C. Donaldson. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* 61: 1323–1330.
- Grishkan, I., E. Nevo and S.P. Wasser. 2003. Soil micromycete diversity in the hypersaline Dead Sea coastal area (Israel). *Mycol. Progr.* 2: 19–28.
- Groenewald, J.Z., C. Nakashima, J. Nishikawa, H.D. Shin, J.H. Park, A.N. Jama, M. Groenewald, U. Braun and P. Crous. 2013. Species concepts in *Cercospora*: spotting the weeds among the roses. *Stud. Mycol.* 75: 115–170.
- Grum-Grzhimaylo, A.A., M.L. Georgieva, A.J.M. Debets and E.N. Bilanenko. 2013. Are alkalitolerant fungi of the *Emericellopsis* lineage (Bionectriaceae) of marine origin? *IMA Fungus* 4: 213–228.
- Grum-Grzhimaylo, A.A., M.L. Georgieva, S.A. Bondarenko, A.J.M. Debets and E.N. Bilanenko. 2016. On the diversity of fungi from soda soils. *Fungal Divers.* 76: 27–74.
- Gunde-Cimerman, N. and A. Plemenitaš. (eds). 2005. *Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya*. Springer-Verlag, Heidelberg. pp. 576.
- Gunde-Cimerman, N. and P. Zalar. 2014. Extremely halotolerant and halophilic fungi inhabit brine in solar salterns around the globe. *Food. Technol. Biotechnol.* 52: 170–179.
- Gunde-Cimerman, N., P. Zalar and A. Cimerman. 1997. Diversity of fungal community in high salt marine environments. *Proc. Int. Symp. Environ. Biotech (ISEB)*. Oostende, Belgium. pp. 189–191.
- Gunde-Cimerman, N., P. Zalar, G.S. de Hoog and A. Plemenitaš. 2000. Hypersaline waters in salterns-natural ecological niches for halophilic black yeasts. *FEMS Microbiol. Ecol.* 32: 235–240.
- Gunde-Cimerman, N., S. Sonjak, P. Zalar, J.C. Frisvad, B. Diderichsen and A. Plemenitas. 2003. Extremophilic fungi in arctic ice: the relationship between adaptation to low temperature and water activity. *Phy. Chem. Earth. A/B/C.* 28: 1273–1278.
- Gunde-Cimerman, N., P. Zalar, U. Petrovic, M. Turk, T. Kogej, G.S. de Hoog and A. Plemenitas. 2004. Fungi in the Salterns. In: (A. Ventosa, ed) *Halophilic microorganisms*. Springer-Verlag, Heidelberg. pp. 103–113.
- Gunde-Cimerman, N., J. Ramos and A. Plemenitas. 2009. Halotolerant and halophilic fungi. *Mycol. Res.* 113: 1231–1241.
- Hagler, A.N. 2006. Yeasts as indicators of environmental quality. In: (C.A. Rosa and P. Gábor, eds) *Biodiversity and ecophysiology of yeasts*. Springer-Verlag, Berlin. pp. 519–536.
- Hagler, A.N. and D.G. Ahearn. 1987. Ecology of aquatic yeasts. In: (A.H. Rose and J.S. Harrison, eds) *The yeasts, Vol 2, Yeasts and the environment*. Academic Press, London. pp. 181–205.
- Hong, S.B., S.J. Go, H.D. Shin, J.C. Frisvad and R.A. Samson. 2005. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* 97: 1316–1329.
- Jaouani, A., M. Neifar, V. Prigione, A. Ayari, I. Sbissi, S.B. Amor, S.B. Tekaya, G.C. Varese, A. Cherif and M. Gtari. 2014. Diversity and enzymatic profiling of halotolerant micromycetes from Sebkh El Melah, a Saharan salt flat in southern Tunisia. *Biomed. Res. Internat.* 2014: 1–11.
- Jones, E.B.G. and M.A. Abdel-Wahab. 2005. Marine fungi from the Bahamas Islands. *Bot. Mar.* 48: 356–364.
- Kis-Papo, T., I. Grishkan, A. Oren, S.P. Wasser and E. Nevo. 2001. Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. *Mycol. Res.* 105: 749–756.
- Kogej, T., J. Ramos, A. Plemenita and N. Gunde-Cimerman. 2005. The halophilic fungus *Hortaea werneckii* and the halotolerant fungus *Aureobasidium pullulans* maintain low intracellular cation concentrations in hypersaline environments. *Appl. Environ. Microbiol.* 71: 6600–6605.
- Kurtzman, C.P., T. Boekhout and J.W. Fell. 2011. *The Yeasts: a taxonomic study*, 5th ed. Amsterdam, Elsevier. pp. 2354.
- Lahav, R., P. Fareira, A. Nejdat and A. Abeliovich. 2002. The identification and characterization of osmotolerant yeast isolates from chemical wastewater evaporation ponds. *Microbiol. Ecol.* 43: 388–396.
- Libkind, D., S. Brizzio, A. Ruffini, M. Gadanho, M. van Broock and J.P. Sampaio. 2003. Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. *Antonie van Leeuwenhoek* 84: 313–322.
- Liu, X.Z., Q.M. Wang, B. Theelen, M. Groenewald, F.Y. Bai and T. Boekhout. 2015a. Phylogeny of tremellomycetous yeasts and related dimorphic basidiomycetes reconstructed from multi-gene sequence analyses. *Stud. Mycol.* 81: 1–16.
- Liu, X.Z., Q.M. Wang, M. Göker, M. Groenewald, A.V. Kachalkin, H.T. Lumbsch, A.M. Millanes, M. Wedin, A.M. Yurkov, T. Boekhout and F.Y. Bai. 2015b. Towards an integrated phylogenetic classification of the Tremellomycetes. Phylogeny of tremellomycetous yeasts and related dimorphic basidiomycetes reconstructed from multigene sequence analyses. *Stud. Mycol.* 81: 85–147.
- Minegishi, H., T. Miura, Y. Yoshida, R. Usami and F. Abe. 2006. Phylogenetic analysis of pectin degrading yeasts from deep-sea environments. *J. Japan. Soc. Extremop.* 5: 21–26.
- Mokhtarnejad, L., M. Arzanlou, A. Babai-Ahari, Di Mauro, A. Onofri, P. Buzzini and B. Turchetti. 2016. Characterization of basidiomycetous yeasts in hypersaline soils of the Urmia Lake National Park, Iran. *Extremophiles* 20: 915–928.
- Nagahama, T. 2006. Yeast biodiversity in freshwater, marine and deep-sea environments. In: (C.A. Rosa and P. Gábor, eds) *The yeast handbook biodiversity and ecophysiology of yeasts*. Springer, Berlin. pp. 241–262.
- Nazareth, S., V. Gonsalves and A. Shweta. 2012. First record of obligate halophilic *Aspergilli* from the Dead Sea. *Ind. J. Microbiol.* 52: 22–27.
- Pedros-Alio, C., J.I. Calderon-Paz, M.L. MacLean, G. Medina, C. Marase, J.M. Gasol and N. Guixa-Boixereu. 2000. The microbial food web along salinity gradients. *FEMS Microbiol. Ecol.* 32: 143–155.
- Pedros-Alio, C. 2004. Trophic ecology of solar salterns. In: (A. Ventosa, ed) *Halophilic microorganisms*. Springer-Verlag, Heidelberg. pp. 33–48.
- Plemenitas, A., T. Vaupotic, M. Lenassi, T. Kogej and N. Gunde-Cimerman. 2008. Adaptation of extremely halotolerant black yeast *Hortaea werneckii* to increased osmolarity: a molecular perspective at a glance. *Stud. Mycol.* 61: 67–75.
- Rehner, S.A. and G.J. Samuels. 1994. Taxonomy and phylogeny of *Gliocladium* analyzed from nuclear large subunit ribosomal DNA sequences. *Mycol. Res.* 98: 625–634.
- Sampaio, J.P. 2011. *Rhodotorula*. In: (C.P. Kurtzman, J.W. Fell and T. Boekhout, eds) *The Yeasts, a taxonomic study*. Elsevier, Amsterdam. pp. 1873–1927.

- Samson, R.A., C.M. Visagie, J. Houbaken, S.B. Hong, V. Hubka, C.H. Klaassen, G. Perrone, K.A. Seifert, A. Susa, J.B. Tanney, J. Varga, S. Kocsubé, G. Szigeti, T. Yaguchi and J.C. Frisvad. 2014. Phylogeny, identification, and nomenclature of the genus *Aspergillus*. *Stud. Mycol.* 78: 141–173.
- Schwarze, H.H., K. Barth, W. Al-Malki and I. Minz. 2005. Tourism in the Khor Al-Adaid Area, State of Qatar. Report to Qatar Tourism Authority. pp. 29.
- Scorzetti, G., J.W. Fell, A. Fonseca and A. Statzell-Tallman. 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res.* 2: 495–517.
- Smolyanyuk, E.V. and E.N. Bilanenko. 2011. Communities of halotolerant micromycetes from the areas of natural salinity. *Microbiology* 80: 877–883.
- Soares, C.A.G., M. Maury, F.C. Pagnocca, F.V. Araujo and L.C. Mendoca-Hagler. 1997. Ascomycetous yeast from tropical intertidal dark mud of southeast Brazilian estuaries. *J. Gen. Appl. Microbiol.* 4: 265–272.
- Sterflinger, K. 2006. Black yeasts and meristematic fungi: ecology, diversity and identification. In: (C.A. Rosa and P. Gábor, eds) *The Yeast Handbook: Biodiversity and ecophysiology of yeasts*. Springer, Berlin, Heidelberg. pp. 501–514.
- Sterflinger, K., D. Tesei and K. Zakharova. 2012. Fungi in hot and cold deserts with particular reference to microcolonial fungi. *Fungal Ecol.* 5: 453–462.
- Stielow, J.B., C.A. Lévesque and K.A. Seifert. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* 35: 242–263.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731–2739.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30: 2725–2729.
- Turchetti, B., P. Buzzini, M. Goretti, E. Branda, G. Diolaiuti, C. D'Agata, C. Smiraglia and A. Vaughan-Martini. 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microb. Ecol.* 63: 73–83.
- Turchetti, B., M. Goretti, E. Branda, G. Diolaiuti, C. D'Agata, C. Smiraglia, A. Onofri and P. Buzzini. 2013. Influence of abiotic variables on culturable yeast diversity in two distinct Alpine glaciers. *FEMS Microb. Ecol.* 86: 327–340.
- Urano, N., M. Yamazaki and R. Ueno. 2001. Distribution of halotolerant and/or fermentative yeasts in aquatic environments. *J. Tokyo. Univ. Fish.* 87: 23–29.
- Vilgalys, R. and M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172: 4238–4246.
- Visagie, C.M., J. Varga, J. Houbaken, M. Meijer, S. Kocsubé, N. Yilmaz, R. Fotedar, K.A. Seifert, J.C. Frisvad and R.A. Samson. 2014. Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section Circumdati). *Stud. Mycol.* 78: 1–6.
- Vu, D., M. Groenewald, S. Szöke, G. Cardinali, U. Eberhardt, B. Stielow, M. de Vries, G. Verkleij, P.W. Crous, T. Boekhout and V. Robert. 2016. DNA barcoding analysis of more than 9000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. *Stud. Mycol.* 85: 91–105.
- Wang, Q.M., A.M. Yurkov, M. Göker, H.T. Lumbsch, S.D. Leavitt, M. Groenewald, B. Theelen, X.Z. Liu, T. Boekhout and F.Y. Bai. 2015a. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. *Stud. Mycol.* 81: 149–189.
- Wang, Q.M., M. Groenewald, M. Takashima, B. Theelen, P.J. Han, X.Y. Liu, T. Boekhout and F.Y. Bai. 2015b. Phylogeny of yeasts and related filamentous fungi within Pucciniomycotina determined from multigene gene sequence analyses. *Stud. Mycol.* 81: 27–53.
- White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: (M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, California, USA. pp. 315–322.
- Woudenberg, J.H.C., J.Z. Groenewald, M. Binder and P.W. Crous. 2013. *Alternaria* redefined. *Stud. Mycol.* 75: 171–212.
- Yadav, J.S. and J.C. Loper. 1999. Multiple P450alk (cytochrome P450 alkane hydroxylase) genes from the halotolerant yeast *Debaryomyces hansenii*. *Gene* 226: 139–146.
- Yurkov, A.M., M. Kemler and D. Begerow. 2011. Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. *PLoS One* 6: e23671.
- Yurkov, A.M., M. Kemler and D. Begerow. 2012. Assessment of yeast diversity in soils under different management regimes. *Fungal Ecol.* 5: 24–35.
- Zalar, P. and N. Gunde-Cimerman. 2014. Cold-adapted yeasts in Antarctic habitats. In: (P. Buzzini and R. Margesin, eds) *Cold-adapted yeasts: biodiversity, adaptation strategies and biotechnological significance*. Springer, Berlin, Heidelberg. pp. 49–73.
- Zalar, P., G.S. de Hoog, H.J. Shroers, P.W. Crous, J.W. Groenewald and N. Gunde-Cimerman. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. *Stud. Mycol.* 58: 157–183.

Article note: The publication of this article was funded by the Qatar National Library.

Bionotes

Rashmi Fotedar

Department of Genetic Engineering,
Biotechnology Centre, Ministry of
Municipality and Environment, P.O Box
20022, Doha, Qatar
rfotedar@mme.gov.qa,
rfotedar@hotmail.com

Rashmi Fotedar works as an expert, Department of Genetic Engineering, Ministry of Municipality and Environment, Doha, Qatar. She earned her Master's in molecular biology from University of Technology, Sydney, Australia and her PhD from All India Institute of Medical Sciences New Delhi, India. Her research interests include marine microbiology and molecular characterization of fungi and bacteria from environment and humans.

Anna Kolecka
Westerdijk Fungal Biodiversity Institute,
Utrecht, The Netherlands

Anna Kolecka is a researcher at Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. From 2010 to 2017 she was working as a postdoc in the Yeast Research Group, led by Teun Boekhout. During those years she extensively studied the implementation of MALDI-TOF mass spectrometry for yeast identification and DNA-based molecular approaches for fungal taxonomy. She further explored the application of proteomics for fungal discrimination, recently by using high resolution accurate mass-mass spectrometry (HRAM-MS). She completed her MSc Eng studies in environmental protection at Gdansk University of Technology, Poland in 2005 and later received her PhD in microbiology at Comenius University, Bratislava, Slovakia in 2011.

Teun Boekhout
Westerdijk Fungal Biodiversity Institute,
Utrecht, The Netherlands; and
Institute of Biodiversity and Ecosystem
Dynamics (IBED), University of Amsterdam,
Amsterdam, The Netherlands

Teun Boekhout, works as a principal investigator at Westerdijk Fungal Biodiversity Institute (previously known as CBS Fungal Biodiversity Centre, CBS-KNAW), Utrecht, and Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands. He studied biology in Utrecht where he also graduated. Since 1982 he is working in mycology. In 2018 he was elected Fellow of the American Academy of Microbiology. In 2011 he was editor of *The yeasts, a taxonomic study* and presently he is editor in chief of *The yeasts*, an open access platform on yeast diversity.

Jack W. Fell
Bahamas Marine EcoCentre, Palmetto Bay,
FL, USA

Jack W. Fell, PhD, Professor Emeritus, University of Miami. Chairman, Science Advisory Board, Bahamas Marine EcoCentre. Dr Fell a marine scientist, with his colleagues and students, studied microbial ecology from the tropics to Antarctic, mangroves to deep sea. His lab research included basidiomycetous yeast mating genetics, he was a pioneer in basidiomycete molecular phylogeny and

developed molecular probes for rapid species detection of marine microbes and human pathogens.

Ameena Al-Malki
Department of Genetic Engineering,
Biotechnology Centre, Ministry of
Municipality and Environment, P.O Box
20022, Doha, Qatar

Ameena Al-Malki works as a specialist in Genetic Engineering Department, Biotechnology Centre, Ministry of Environment, Doha, Qatar. She is involved in studies related to isolation and molecular identification of microorganism from marine environment, and detection of GMOs in agricultural and food products. Her fields of interest include the molecular characterization of bacteria and fungi from environmental samples.

Aisha Zeyara
Department of Genetic Engineering,
Biotechnology Centre, Ministry of
Municipality and Environment, P.O Box
20022, Doha, Qatar

Aisha Zeyara works as an expert in Department of Genetic Resources, Ministry of Municipality and Environment, Doha, Qatar. She has a degree in microbiology and molecular biology from Qatar University, Doha, Qatar. Aisha is a molecular microbiologist and has been involved in research studies including isolation, identification and characterization of microbial pathogens from Qatari marine environment.

Masoud Al Marri
Department of Genetic Engineering,
Biotechnology Centre, Ministry of
Municipality and Environment, P.O Box
20022, Doha, Qatar

Masoud Al Marri works as Director, Agricultural Research Department, Ministry of Municipality and Environment, Doha, Qatar. He earned his Master's degree from University of Florida, USA and has been involved in research projects related to marine environment and environmental monitoring of marine, ground water and air in Qatar.