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Original Article

Alkaliphilic and alkalitolerant fungi in mud and salt crusts collected from Wadi El-Natrun lakes

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A B S T R A C T

A total number of alkaliphilic and alkalitolerant fungi (37 species related to 16 genera) were collected from mud and salt crusts of Wadi El- Natrun lakes on Czapek Dox agar adjusted at pH10 and pH13 during the seasons of study. The most dominant genera at both pH levels were *Aspergillus* (9 species) followed by *Penicillium* (9), *Acremonium* (3) and *Fusarium* (3), Of these genera, *Aspergillus terreus* and *Pencillium chrysogenum* were the most commonly encountered species.

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Introduction

Microorganisms have an optimum pH for growth, and only few species can grow at pH values of less than 2.0 (acidophiles) or greater than 10.0 (alkaliphiles), for examples, *Aspergillus nidulellus* (*Emericella nidulans*) is able to grow over a wide range of pH values as acid as 2.5 or as alkaline as 10.5 (Rossi and Arst, 1990).

Alkaliphiles include two main physiological groups of microorganisms, alkaliphiles and haloalkaliphiles. Alkaliphiles require an alkaline pH of 9 or more for their growth and have an optimal growth pH of around 10, whereas haloalkaliphiles require both an alkaline pH (pH 9) and high salinity (up to 33% [w/v] NaCl) (Horikoshi, 2004). Nagai *et al.* (1998) classified fungi based on the pH growth patterns into 3 subgroups: (i) alkaliphobic: which could not grow at pH 10, (ii) alkalitolerant: which grew at pH 10 and (iii) alkaliphilic species which could grow at pH 10 but not at all at pH 5.

The bioenergetics that alkaliphiles face in maintaining pH homeostasis in a highly alkaline environment have been increasingly studied in the last two decades. Alkaliphilic microorganisms exhibit a remarkable ability to maintain cytoplasmic pH much lower than external pH values of 10 to 11. Measurements of the optimum pH of intracellular enzymes and several indirect methods such as DMO (5, 5-dimethyl-2, 4-oxazlidinedione), BCECF (2, 7-Biscarboxyethyl) carboxy fluorescein), and methylamine have been applied to measure the internal pH of microorganisms. Studies have shown that alkaliphiles had a lower internal pH than external pH (8-11) Horikoshi (1999). The internal pH was maintained at around 8, despite high external pH. Also, organisms growing at neutral pH usually maintain their external pH is more alkaline than the internal pH. At very high environmental pH levels, however, alkaliphilic bacteria reverse their pH gradient (inside more acidic than the exterior) in order to maintain their external or internal pH near neutral (Ivey *et al.*, 1998b and Ni and Boone, 1998).

Fungi are often dominant members of soil microbiota, especially in acidic environments, and may operate over a wider pH range than that of many heterotrophic bacteria (Gadd, 2004). While there are numerous reports on fungi isolated from acidic to neutral soils, there are only a few reports about fungi from alkaline soils. Alkaline soils were examined for their fungal biota by Rai *et al*. (1971) and Nagai *et al.* (1995, 1998). Soils with large amounts of calcium carbonate were studied by Vardavakis (1990) in Greece, whereas in Argentina, Cabello and Arambarri (2002) and Elíades *et al.*, (2004, 2006a) have reported fungi from alkaline soils. Moreover, haloalkaliphiles have been mainly found in extremely alkaline saline environments, such as the Rift Valley lakes of East Africa and the western soda lakes of the United States (Horikoshi, 2004).

Nagai *et al.* (1995) found that the use from acidic and alkaline soils of alkaline medium facilitates the isolation of alkaliphilic fungi with the number of species of *Acremonium* and *Fusarium* increased on alkaline medium (pH 9.7) than on acidic medium. Many species Cabello and Arambarri (2002) studied the rhizospheric soil microfungi from native forests (undisturbed and disturbed) using slightly acid and alkaline media and isolated 54 taxa from undisturbed forest soil (49) and disturbed forest soil (37). *Acremonium* sp., *Aspergillus ustus, Coemansi apectinata*,*Doratomyces stemonitis, Fusarium solani, F. oxysporum, Gliocladium roseum, Humicola fusco-atra, Mortierella* sp., *Penicillium lilacinum, Trichoderma harzianum,* and *T. koningii* showed the highest frequency, in both undisturbed and disturbed forests.

They also noted that *Fusarium solani, Aspergillus ustus, Penicillium lilacinum* and *Gliocladium roseum* were more frequent on alkaline medium (pH9.7) and *Acremonium* sp. and *Doratomyces stemonitis* were only isolated on the alkaline medium.

Emericellopsis minima, *Neosartory astramenia* and *Melanospora zamiae* were isolated at pH 10 to11 (alkaliphilic) (Elíades *et al*., 2006b).

Materials and Method

Collection of samples

Samples (salt crusts and mud) were collected during January 2006 – May 2007, from eight lakes (Fasida, Umm-Risha, Rosetta, Hamra, El ElZugm, Al Beida, Khadra, Al Gaar) of Wadi El-Natrun (depression) region, Egypt.

Mud samples (nearly a depth 0.5 m) were collected by hands at random from different sites inside and along shore of lakes.

Salt crust samples were collected by hands at random from mineral formation present along shores of Lake (Figure1).

Figure 1. Showing the salt crusts (top) and salt crusts of El Al Gaar Lake (bottom).

Isolation of fungi

From mud and salt crusts

The dilution plate method was used for enumeration of different fungal species as described by Johnson *et al.*, (1972) and employed in this laboratory by Moubasher *et al.* (1977).

Media used for isolation of alkaliphilic and alkalitolerant fungi

Modified CzapekDox agar in which pH was adjusted at 10, 13 using NaOH were used for isolation of alkaliphilic and alkalitolerant fungi.

Identification of fungi

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium.

Results and discussion

Alkaliphilic and alkalitolerant fungi in mud collected from Wadi El- Natrun lakes.

Twenty eight species related to 12 genera of alkaliphilic and alkalitolerant fungi were recovered on CzapekDox agar adjusted at pH10 (25 species and 9 genera) and pH13 (17 species and 9 genera).

Aspergillus followed by *Penicillium, Acremonium* and *Fusarium* were the most commonly encountered fungi at both pH levels (Table 1).

Aspergillus (9 species) was the most commonly encountered fungus in mud of the 8 lakes on both alkaline media and also on the control medium. It comprised 85.6% and 84.6% of the total propagules on both alkaline media, respectively. *Aspergillus* showed its peak from Al Gaar during spring 2007 at both pH levels. These numbers were higher on medium adjusted at pH10 than on that one adjusted at pH13 and on control medium. Of *Aspergillus*, *A. terreus* (42.6% and 64.4%), *A. niger* (15.4% and 2.2%) and *A. flavus* (13.2% and 4.5%) were recorded from all lakes during almost all seasons of the study. *A.fumigatus* (19.9% and 21.2%) was recorded from all lakes exceptRosetta during almost all seasons. *A. ochraceus* was recovered from 5 lakes during 4 seasons of the study. The remaining *Aspergillus* species were recorded from either 2 lakes. *A. aculeatus, A. phoenicis* (Al Gaar and Khadra each during spring and summer 2006) and *A. carbonarius* (Umm Risha and Al Gaar during spring 2006) or from one lake: *A. sydowii* (El Zugm during only spring 2006). In this respect, El-Wahid *et al.* (1982) found that the genera *Aspergillus* (17 species) and *Pencillium* (14) showed the widest spectrum of species among all genera isolated from the tidal mud-flats of Khuwait (pH value fluctuated between 8.1. and 8.6). However only seven species of *Aspergillus* (*A. ochraceus, A. niger, A. fumigatus, A. sydowii, A. flavus, A. terreus* and *A. oryzae*) were frequent. Also, Mehdi & Saifullah (1992a,b) recorded *Aspergillus* to be the most diverse fungus isolated from mud sample from Clifton and Korangi Creek in Pakistan with *A. flavus* being the most abundant.

The genus *Aspergillus* is considered one of the most characteristic taxa of saline soils (Moubasher *et al.*, 1990; Abdel-Hafez *et al.*, 2009; Ismail *et al.* 2017).

Rai *et al*. 1966) found that *A. flavus* and *A. niger* were quite common from mangrove mud ((pH 7.3-7.5) collected from West Bengal, India. *A. flavus, A. fumigatus* and *A. niger* were

common from the benthic sediment samples isolated in the EEZ of Indian coast (Gupta and Prabhakaran, 1990).

Penicillium (6 species) was recorded from all lakes except Hamra during almost seasons of the study. Its peak was recorded from Rosetta during summer 2006 on medium adjusted at pH10 while at pH13 from Khadra during spring 2006. It comprised 3.2% and 2.1% of the total propagules on both media respectively. Its dominant species *P. chrysogenum* (1.1% and 1.2%) and *P. puberulum* (0.4%and 0.8%) were recorded from 5 lakes during some seasons of the study. The remaining *Penicillium* species were recorded from 2 lakes (*P. purpurgenum* during only summer 2006 season), 1 lake: (*P. brevicompactum* from Al Beida during winter 2007, *P. griseofulvum* from Rosetta during summer 2006 and *P. oxalicum* from Rosetta during summer and autumn 2006). In this respect, El-Wahid *et al.* (1982) isolated 14 *Pencillium* species from the tidal mud-flats of Khuwait (pH 8.1- 8.6). Of which *P. frequentans, P. cyclopium*, and *P.notatum* (= *P. chrysogenum*) were common.

Three species of *PeniciIlium* (*P. nigricans, P. roseopureum* and *P. stipitatum*) were isolated from mangrove mud in India (Rai *etal*., 1966), and *P. chrysogenum* and unidentified *Penicillium* species from the benthic sediment samples in India (Gupta and Prabhakaran, 1990). *Acremonium* (3 species) and its dominant species *A. hyalinulum* and *A. strictum* were recorded each from muds of 3 lakes (at pH 10 and 13) (Hamra, El Zugm and Umm Risha) during 3 seasons of the study (spring and autumn 2006 and winter 2007). *A. furcatum* was recorded from only Hamra Lake during two seasons (autumn 2006 and winter 2007). In this respect, Nagai *et al.* (1995 &1998), Cabello and Arambarri (2002) and Elíades *et al*. (2004) found that species of the genus *Acremonium* could grow well under alkaline conditions.

Fusarium (3 species, 1.4% and 0.9% of the total propagules on both alkaline media respectively) and its dominant species *F.solani* (0.8%, 0.6%) were recorded from 3 lakes (El Zugm, Rosetta and Fasida) during only one season of the study (spring 2006 and 2007). Its peak was recorded from El Zugm during spring 2007 at both pH levels. *F. subglutinans* was recorded from 2 lakes (Rosetta and Fasida) while *F. semitectum* and some unidentified *Fusarium* species were recorded from Fasida during the same season (spring). In agreement with our results, *F. solani* was most commonly isolated on alkaline medium (Elíades *et al.* 2004, 2006a) and *Fusarium solani, F. lateritium* and *F. subglutinans* (Nagai *et al.* 1995), *Fusarium solani* and *F. oxysporum* (Cabello and Arambarri, 2002) showed high tolerance of alkaline conditions. However, El-Wahid *et al.* (1982) isolated six species of the genus *Fusarium* from the tidal mud-flats of Kuwait of which only *F. oxysporum* was common and Rai *et al*. (1966) isolated two species of *Fusarium* (*F. oxysporum* and *F. aquaeductum*) from mangrove mud. However this species was isolated in frequently in the current work.

The remaining genera were recorded from either muds collected from 2 lakes: *Trichoderma* spp. (El Zugm and Khadra during only spring 2006 and 2007), or from one lake: *Acrophialophora fusispora* (Al Beida, winter 2007), *Alternaria alternata* (El Zugm, winter 2007), *Cladosporium cladosporioides* (Al Beida, winter 2007), *C. sphaerospermum* (Al Beida, winter 2007), *Emericella nidulans* (Khadra, winter 2006), *Fennellia flavipes* (Hamra, summer 2006), *Myrothecium verrucoria* (El Zugm, spring 2006) and *Phoma herbarum* (Fasida, summer 2006). In this respect, El-Wahid *et al.* (1982) isolated *Alternaria* (5 species), *Chaetomium*, *Pithornyces* and *Ulocladium* (4 species each) of which *A. alternata, A. chlamydospora, C. olivaceum, C. gracile, P. atro-olivaceus, U. consortiale* and *U. atrum* were the most frequent from the tidal mud-flats of Kuwait. Also, species of *Cladosporium*, *Fusarium* and *Alternaria* were reported from the benthic sediment samples collected from East coast of India (Gupta and Prabhakaman, 1990).

Working on sediment samples collected from hypersaline environments of solar salterns, Cantrell *et al.* (2006) also isolated *Aspergillus* sp., *A. japonicus, Chaetomium globosum, Cladosporium cladosporioides, Penicillium* sp., and *P. variabile*, in addition to many isolates of dark and white sterile mycelia.

Some species could be isolated on medium adjusted at pH10 (*Myrothecium verrucaria, F. semitectum, Penicillium brevicompactum, P. griseofulvum, P. purpurgenum*) or pH13 (*Acrophialophora fusispora*) or on both pH levels (*Acremonium hyalinulum* and *Fusarium subglutinans*), but were absent on bothacidic media.

Table 1. Summarized of alkaliphilic and alkalitolerant fungi from mud in different lakes of Wadi El –Natrun.

Fungal taxa	Hamra	AL Beida	El Zugum	Rosetta	Umm Reisha	Al Gaar	Khadra	Fasida
Acremonium	5		2356	236	236	2346	2346	23456
A. hyalinulum	5					2	3	
A. strictum	5				2	2		
Acremonium spp.	4		235	23	246	234	1246	256
Acrophialophorafusispora		5	3		246	2346	126	6
Alternaria alternata			2	236	2	236	1236	2346
Aspergillus	236	2346				2	3	
A. aculeatus			2356	236	36	236	246	23456
A. carbonarius								
A. flavus	236	236						
A. fumigatus	36	246						
A. niger	23	236					1	
A. ochraceus		4						
A. phoenicis			6	26				26
A. terreus	26	23						2
Cladosporium		5	6	26				26
C. cladosporioides		5		2				26
C. sphaerospermum		5						2
Emericella nidulans			2					
Fennellia flavipes	3		23	2346	46	2	1	36

Fungal taxa	Hamra	AL Beida	El Zugum	Rosetta	Umm Reisha	Al Gaar	Khadra	Fasida
Fusarium								
F. semitectum				23	46	2	1	3
F. solani				3				
F. subglutinans				34				
Fusarium spp.			23	6	4	2		6
Myrothecium verrucoria				3				3
Penicillium		25			4			3
P. brevicompactum		5						3
P. chrysogenum			6				2	
P. griseofulvum			11	11	10	9	8	12
P. oxalicum			7	3	3	2	4	4
P. puberulum								
P. purpurgenum								
Penicillium spp.		2						
Rhizopus oryzae								
Trichoderma spp.								
No. of genera (12)	8	9						
No. of species (28)	3	4						

1 = winter 2006, 2 = spring 2006, 3 = summer 2006, 4 = autumn 2006, 5 = winter 2007 and 6 = spring 2

Alkaliphilic and alkalitolerant fungi in salt crusts collected from Wadi El- Natrun lakes

Thirty two species related to 11 genera of alkaliphilic and alkalitolerant fungi were recovered from salts of different Wadi El-Natrun lakes on CzapekDox agar adjusted at pH10 (27 species and 10 genera) and pH13 (21 species and 8 genera.

Aspergillus (9 species), *Acremonium* (3), *Penicillium* (7) and *Fusarium* were the most common genera on both alkaline media (Table 2).

Aspergillus was the most commonly encountered fungus insalts of the 8 lakes on control medium, and on medium adjusted at pH10 and in 7 lakes on medium adjusted at pH13. Its peak was recorded from Al Beida during spring 2006 on medium adjusted at pH10 and from Khadra during winter 2006 at medium adjusted at pH13. It comprised 81.3% and 54.6% of the total propagules on both media respectively.

The number of CFUs of *Aspergillus* was recorded during the 6 seasons of the study was regularly higher on medium adjusted at pH10 than on medium adjusted at pH13. CFUs on medium adjusted at pH13 were lower than those recorded on control medium. Of *Aspergillus*, *A. terreus* (9.6% and 24.2%), *A. flavus* (28.3% and14.4%), *A. niger* (28.4% and 1.1%) and *A. fumigatus* (3.3% and 2.2%) were the most commonly encountered species. *A. flavus* was recorded from salts of all lakes while *A. terreus* and *A. niger* were recorded from all lakes except Hamra and *A. fumigatus* was isolated from only 5 lakes. The remaining *Aspergillus* species were recorded from 3 lakes: *A. phoenicis* (from Al Beida, El Zugm and Al Gaar during spring 2006 only, *A. sydowii* (El Zugm, Rosetta and Khadra during winter and spring 2006), from 2 lakes (*A. ochraceus* from Rosetta and Umm Risha), or one lake (*A. aculeatus* and *A. carbonarius* both from Al Beida during spring 2006). These results where Aspergilli were the most common are in agreement with those reported by Rai *et al*. (1971), Rele (2004) who found that fungi commonly encountered at alkaline pH were *Aspergillus* species. On the other hand, *A. terreus* was reported to be the most common among aspergilli on alkaline media adjusted at pH8 and pH11 (Elíades *et al.*, 2004).

Acremonium was recorded from 4 lakes (Hamra, El Zugm, Rosetta and Umm Risha). It comprised 7.1% and 38.4% of the total propagules on both media, respectively. *Acremonium* count was higher on alkaline media than on control medium. The peak of *Acremonium* was recorded on medium adjusted at pH10 from Rosetta during autumn 2006 while at pH5 from El Zugm in spring 2006. *A. hyalinulum* and *A. strictum* were recorded each from 3 lakes during 3 seasons of the study while *A. furcatum* from 2 lakes (El Zugm and Rosetta) during 3 seasons of the study. In this respect Nagai *et al.* (1995 &1998), Cabello and Arambarri (2002) and Elíades *et al*. (2004) who found that all isolates of the genus *Acremonium* grew well under alkaline conditions.

Penicillium comprised 6.8% and 3.8% of the total propagules, on both pH levels, respectively. The peak of *Penicillium* was recorded from Al Beida during spring 2006 at both pH levels. *Pencillium* and its dominant *P. chrysogenum* (2.1% and 1.6%) were isolated from all lakes except Umm Risha during almost all seasons. *P. duclauxii* was isolated from 5 lakes during 4 seasons of the study. The remaining *Penicillium* species were isolated from 4 lakes: *P. puberulum* (Hamra, Al Beida, Rosetta and Khadra during winter, spring and summer 2006 and 2007), 3 lakes: *P. viridicatum* (Al Beida, Rosetta and Al Gaar during spring, autumn and winter 2006 and 2007), from 2 lakes: *P.oxalicum* (Al Beida and El Zugm during spring 2006), from 1 lake: *P. funiculosum* (Fasida, spring 2007) and *P. griseofulvum* (Al Gaar during autumn 2006). In this respect, Siegel and Siegel (1979) reported that *Penicillium* and *Aspergillus* could grow in a variety of brines or on moist salt crystals, simulating a range of natural terrestrial habitats such as salt flats. Cabello and Arambarri (2002) found that *Penicillium lilacinum* (*Paecilomyces lilacinus*) was frequently isolated on alkaline medium (pH 9.7) from soils of disturbed and undisturbed forests.

Fusarium (0.8% and 0.6%) was recorded from 3 lakes (Hamra, Rosetta and Umm Risha) during 3 seasons of the study (winter, autumn and spring). It showed its peak in Rosetta during autumn 2006 at both pH levels. *Fusarium solani* (0.5%and 0.4%) was isolated from Rosetta and Umm Risha while *F. sambucinum* was isolated from Hamra and Rosetta. In accordance with the present results, *Fusarium* and *Aspergillus* species were commonly encountered at alkaline pH (Rele, 2004). *F. solani* was most common *Fusarium* species in alkaline medium (Nagai *et al.* 1995; Cabello and Arambarri, 2002; Elíades *et al*. 2004, 2006a).

The remaining fungi were recorded from either 2 lakes: *Alternaria alternata* (Rosetta and Fasida), *Cladosporium cladosporioides* (Al Gaar and Fasida), from 1 lake: *Cladosporium sphaerospermum* and yeasts (Fasida, autumn

2006), *Cochliobolus tuberculatus* (Rosetta, autumn 2006), *Paecilomyces variotii* and *Stachybotrys chartarum* (El Zugm, spring 2007), *Phoma herbarum* (Hamra, winter 2006), *Scopulariopsis brumptii* (Fasida, winter 2007), *S. sphaerospora* (Hamra, winter 2007) and *Talaromyces* spp. (Al Beida, spring 2006).

Domsch *et al*., (1980) showed that a number of fungi have been recorded from salt marshes, mangrove mud, estuaries, and other coastal habitats include *Absidia corymbifera*, *Alternaria* spp., *Aspergillus* spp., *Chaetomium indicum*, *Cladosporium oxyporum*, *Drechslera* spp., *Fusarium* spp., *Nigrospora sphaerica*, *Paecilomyces* spp., *Penicillium* spp., *Rhizoctonia solani*, *Rhizopus stolonifer*, *Syncephalastrum racemosum*, and *Trichoderma viride*.

Some species could be isolated on medium adjusted at pH10 (yeasts) or pH13 (*Cladosporium sphaerospermum*, *Paecilomyces variotii* and *Scopulariopsis spherospora*) or on both pH levels (*Acremonium hyalinulum*), but were not isolated on both acidic media.

Table 2. Summarized of alkaliphilic and alkalitolerant fungi from salt crusts in different lakes of Wadi El –Natrun.

Fungal taxa	Hamra	AL Beida	El Zugum	Rosetta	Umm Reisha	Al Gaar	Khadra	Fasida
Acremonium	3		256	4	2			
A. furcatum			56	4				
A. hyalinulum			25	4	2			
A. strictum	3		5	4				
Acremonium spp.	3							
Alternaria alternata				4				3
Aspergillus	135	23456	256	2346		2346		56
A.aculeatus		2						
A. carbonarius		2						
A. flavus	135	246	2	2346	2456	46	135	6
A. fumigatus		246	26		23	36		6
A. niger		235	26	34	246	2346	35	56
A. ochraceus				24			1	
A. phoenicis		2	2			2		
A. sydowii			2	2			1	
A. terreus		34	256	2346	26	6	3	45
Cladosporium						34		45
C. cladosporioides						34		45
C. sphaerospermum								4
Cochliobolus				4				
C. tuberculatus				4				
Fusarium	1			46	2			
F. sambucinum	1			4				
F. solani				46	2			
Paecilomyces variotii			6					
Penicillium	15	256	2	3		34	35	6
P. chrysogenum	15	26	2	3		34	5	6
P. duclauxii	1	2	2			4	5	
P. funiculosum								6
P. griseofulvum						4		
P. oxalicum		2	2					
P. puberulum	1	2		3			35	
P. viridicatium		5		2		4		
Pencillium spp.	1	2						
Phoma herbarum	1							
Scopulariopsis	5							5
S. brumptii								5
Stachybotrys			6					
S. chartarum			6					
Trichoderma spp.		2						
YEASTS								4
No. of genera (11)	6	3	5	6	3	3	2	6
No. of species (32)	9	12	14	15	6	10	8	11

1 = winter 2006, 2 = spring 2006, 3 = summer 2006, 4 = autumn 2006, 5 = winter 2007 and 6 = spring 2

Conclusion

Twenty eight species related to 12 genera of alkaliphilic and alkalitolerant fungi were isolated from mud samples while thirty two species related to 11 genera were isolated from salt crusts of different Wadi El-Natrunlakes**,** The most commonly encountered fungi at both pH levels were *Aspergillus* (9 species) followed by *Penicillium* (9), *Acremonium* (3) and *Fusarium* (3) from mud and salt crusts, Of these genera, *Aspergillus terreus* and *Pencillium chrysogenum* were the most commonly encountered species.

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