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Original Article

Acidophilic and acidotolerant fungi in mud and salt crusts collected from Wadi El-Natrun lakes

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A B S T R A C T

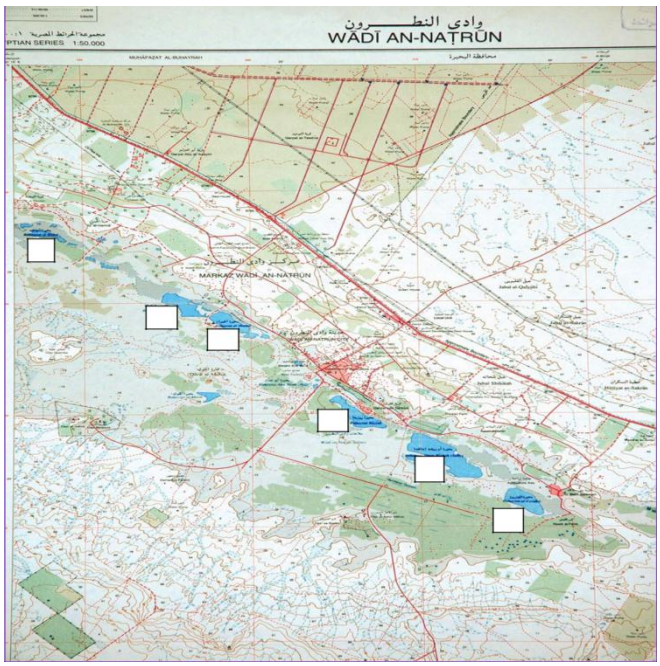
Isolation some fungi from extreme source under extreme media and these were 42 species related to 14 genera of acidiphilic or/ aciditolerant fungi were collected from mud and salt crusts of Wadi El- Natrun lakes on Czapek Dox agar adjusted at pH 4 and pH 5 during the seasons of study. *Aspergillus* and *Penicillium* were the most common genera of the total propogules on the two pH levels.

Introduction

The Wadi El-Natrun Depression is situated at the western side of the Nile Delta of Egypt and includes some water bodies characterized by high salinity. Wadi El-Natrun area is a part of the Western (Libyan) desert adjacent to the Nile Delta. It belongs to the Behira Governorate. It is a narrow depression located approximately 90 km south of Alexandria and 110 km North West of Cairo.

Wadi El-Natrun depression includes two principal ecosystems, the salt marsh ecosystem of the depression, and the gravel desert ecosystem of the surrounding highlands (Zahran and Girgis, 1970).

Most fungi living in acidic habitats should be regarded as acid-tolerant rather than strictly acidiphilic because they are also able to grow under neutral or even alkaline pH. Acidic natural environments having pH ranges from 3 to 4 are relatively common and include soils, lakes, swamps and peat bogs (Middelhoven *et al*., 1992).



Extreme acidic environments, having pH values < 3, are found in many parts of the world and are of both natural and anthropogenic origin. Natural acidic habitats with pH ranges from 1 to 3 are mainly solfatara soils and have been reported in U.S.A., Japan, Russia, Italy, Iceland and New Zealand. The mechanisms with which hyperacidophiles cope with their environment by pumping protons out of the cell and by establishing a low proton membrane permeability to maintain a relatively neutral pH (Nicolay *et al*., 1987). Fungi are probably common in the acidic environment because of this internal pH regulation (Gross and Robbins, 2000). Raven (1990) provided that pH is an all-pervasive variable in the environment of phototrophs. Phototrophs as a whole can grow over essentially the whole range of pH values found in nature.

The fungal community in acidic soils has been studied extensively (Gross and Robbins, 2000). Nagai *et al*., (1995) isolated thirty fungal species from acidic soils samples in Japan on slightly acidic medium and the comprised most commonly species were *Mortierella, Mucor*, *Trichoderma, Gliocladium* and *Fusarium* Also Nagai *et al*., (1998) isolated sixteen on slightly acidic medium (cornmeal agar) from alkaline soils in Japan of which species were recorded: *Chaetomium*, *Cladosporium*, *Cylindrocarpon*, *Epicoccum*, *Geomyces*, *Mortierella*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Trichoderma*.

A detailed listing of acidiphilic and acid-tolerant fungi growing in pH < 4 environments, primarily soils has been compiled by Gross and Robbins, (2000) including 81 fungal species and yeasts and Ascomycetes (1 species), Zygomycetes (16 sp), yeasts (8 sp) and Mitosporic (56 sp).

All the isolated Ascomycota, *Talaromyces* species were the most sensitive to high pH, although they were isolated at pH 6 to pH 11. Their anamor pH levels belonging to *Penicillium* preferred acid pH (Gams, 1992).

Humicola fusco-atra and *Trichoderma koningii*, and all species of Zygomycetes (*Absidia spinosa, Coemansia pectinata*, *Mortierella alpine*, and *Mortierella* sp., *Mucor hiemalis*, and *Rhizopus stolonifer*) were exclusively isolated on slightly acidic cornmeal medium, pH 6 collected from disturbed and undisturbed forests in Argentina (Cabello and Arambarri, 2002).

Most species of terrestrial fungi are considered to germinate and grow well in weakly acidic to neutral pH range (Park, 1968). Several poly-extremophiles thrive in various ranges of pH, from milder solutions to very acidic media (Seckbach, 2005). The *Acontium cylatium, Cephalosporium* spp., and *Trichosporon ceribriae* were growing at near pH 0 (Schleper *et al*., 1995).

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 El Zugm, Al Beida, Khadra, Al Gaar) of Wadi El-Natrun (depression) region, Egypt.

Mud samples were collected at random from different sites inside and along shore of lakes.

Salt crust samples were collected at random from mineral formation present along shores of Lake.

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 acidiphilic and aciditolerant fungi

Modified Czapek Dox agar media in which pH was adjusted at 4 or 5 using diluted HCl for isolation of acidiphilic and aciditolerant fungi

Identification of fungi

The following references were used for the identification of fungal genera and species (purely morphologically, based on macroscopic and microscopic features).

Results and discussion

Acidophilic and acidotolerant fungi in mud collected from Wadi El- Natrun lakes.

Twenty nine species related to 11 genera of acidiphilic or/ aciditolerant fungi were recovered on Czapek Dox agar adjusted at pH 4 (19 species and 9 genera) and pH 5 (20 and 9). *Aspergillus* and *Penicillium* were the most common genera constituting 88% and 85.6% of the total propogules on the two acidic (pH 4 and pH 5) media compared to 76% on the control medium. The results were in agreement with Ismail *et al.* 2017 who found *Penicillium* was the second most dominant behind *Aspergillus* in the mud from Wadi El- Natrun lakes. Contrary to our results, Lee and Baker (1971) found *Trichoderma* (2 species) to be the most common followed by *Penicillium* (14), *Fusarium* (7), and *Aspergillus* (6) from the Hawaiian mangrove swamp soil. *Aspergillus* (88.0%, 85.6% of the total propagules, respectively) was the most commonly encountered fungus in mud of the 8 lakes on control as well as on both acidic media. The peak of *Aspergillus* was recorded from Al Gaar during spring 2007 at both pH levels. The number of Colony Forming Units (CFUs) of *Aspergillus* recorded from the 8 lakes during the 6 seasons of study was regularly higher on medium adjusted at pH5 than at pH4 and both were higher than those recorded on the control medium. *A. terreus* (55.8% and 55.2%), *A. fumigatus* (23.5% and 18.7%), *A. niger* (16.5%, 20.1%) and *A. flavus* (7.7% and 5.7%) were recorded from all lakes during all seasons of study. The remaining *Aspergillus* species were recorded either from 4 lakes: *A. carbonarius* (Hamra, El Zugm, Rosetta and Al Gaar during spring and summer 2006), 3 lakes: *A. ochraceus* (El Zugm, Khadra and Fasida during autumn 2006 and winter 2007), 2 lakes: *A. phoenicis* (Al Gaar and Khadra during spring and summer 2006) or one lake: *A. aculeatus* (from Khadra during summer 2006) and *A. sydowii* (from Rosetta during spring 2006). In previous reports, the genus *Aspergillus* was abundant and is considered one of the most characteristic taxa of arid mycobiotas (Grishkan *et al.* 2003a,b; Klich, 2002) and mycobiotas of saline soils (Moubasher *et al*., 1993; Abdel-Hafez *et al.*, 2009).

Gupta and Prabhakaman, (1990) isolated also several species of yeasts and filamentous fungi from the benthic sediment samples collected from the east coast of India belonging to the genera *Aspergillus, Penicillium, Fusarium, Cladosporium* and *Alternaria.* Of *Aspergillus, A. flavus, A. fumigatus* and *A. niger* were reported.

Penicillium (8 species) came behind *Aspergillus* in its count comprising 5.8% and 5.7% on both acidic media compared to 12.8% of the total fungi on the control medium. *Pencillium* was recorded from all lakes except El Zugm during almost all seasons of study. The peak of *Penicillium* was recorded from Rosetta during summer 2006 at both pH levels. *P. chrysogenum* (1.8% and 1.2%) and *P. puberulum* (0.8% and 1.2%) were both recorded during 3 seasons of study

(summer, autumn and spring), however the former was recorded from all lakes except Al Beida and El Zugm lakes and the latter was recorded from 4 lakes (El Zugm, Rosetta, Umm Risha and Khadra). The remaining *Penicillium* species were recorded either from 3 lakes: *P. duclauxii* (Hamra, Rosetta and Al Gaar) during 3 seasons (summer, autumn and spring), 2 lakes: *P. funiculosum* (Al Beida and Fasida during summer 2006 and spring 2007), *P. purpurgenum* (Hamra and Umm Risha during autumn 2006), or from Hamra Lake only: *P. echinulatum* (autumn 2006), *P. expansum* and *P. oxalicum* (summer 2006), *P. janczewskii* (spring 2006).These results are in agreement with those reported by Gams,(1992) who found that *Penicillium* species prefer acid pH. Also, Elíades *et al* (2004) found P. *purpurogenum* as most common from a native forest in Argentina followed by *P. chrysogenum* and *Penicillium* spp. on acidic medium adjusted at pH5. (Gupta and Prabhakaman, 1990) isolated *P. chrysogenum* and some unidentified *Penicillium* species from the benthic sediment samples in the east coast of India. On the other hand, (Lee and Baker,1972) isolated 14 species of *Penicillium* from the mangrove swamp soil (pH ranged from 5.5-6.0) and these were *P. corylophilum, P. decumbens, P. diversum, P. funiculosum, P. frequentans, P. janthinellum, P. lilacinum, P. melinii, P. ochrochloron, P. oxalicum P. purpurogenum, P. simplicissimum, P. variabile, P. vermiculatum* and *Penicillium* sp.

Fusarium (0.5% of the total fungi on both acidic media) and its dominant species *F. solani* were recorded from 5 lakes (El Zugm, Rosetta, Umm Risha, Al Gaar and Fasida). The peak of *Fusarium* was recorded from El Zugm during spring 2007 at both pH levels while *Emericella* (0.5% and 0.2%) and its dominant species *E. nidulans*(0.3% and 0.1%) was recorded from 4 lakes (El Zugm, Rosetta, Umm Risha and Al Gaar) with the highest number of CFUs being recorded from Umm Risha during spring 2006 at both pH levels. Both genera were recorded during 2 seasons of study (spring and summer). *E. quadrilineata* was recorded from Umm Risha Lake during spring 2006. In this respect, Lee and Baker (1972) isolated seven species of *Fusarium* from the mangrove swamp soil (pH ranged from 5.5-6.0) and these were *Fusarium bostrycoides, F. dimerum F. lateritium F. roseum F. solani, F. sporotrichioides* and *Fusarium* sp. Elíades *et al*. (2006a) isolated also *F. oxysporum, F. solani* in addition to some other unidentified *Fusarium* species from xeric forest soils in Argentina on acid media with pH 5 and 6. The remaining fungal species were recorded from 3 lakes: *Trichoderma* spp. (El Zugm, Al Gaar and Fasida during spring and summer), 2 lakes: *Cladosporium sphaerospermum* (Umm Risha and Fasida during spring and autumn 2006) or one lake: *Acremonium furcatum, A. strictum* (both Hamra during autumn 2006), *Cladosporium cladosporioides* (Rosetta, spring 2007), *Cochliobolus tuberculatus* (Hamra, spring 2007), *Eurotium chevalieri* (El Zugm, spring 2006), *Fennellia flavipes* (Umm Risha, spring 2007), *Humicola grisea* (Rosetta, spring 2006) and *H. insolens* (Fasida, spring 2006) (Table 1). In earlier reports, *Trichoderma* (represented by *T. viride* and *T. glaucum*) was the most common genus in the mangrove swamp soil in Hawaiian (pH ranged from 5.5-6.0) (Lee and Baker, 1972), while it (as *T. hamatum* and *T. koningii*) was moderately isolated from soil on acid media of pH 5 or 6 (Elíades *et al*., 2004). Also, (Gupta and Prabhakaman, 1990) isolated species of *Cladosporium*, *Fusarium* and *Alternaria* from the benthic sediment samples in the east coast of India. Cantrell *et al.,* (2006) recorded also *Aspergillus japonicus, Aspergillus* sp., *Chaetomium globosum, Cladosporium cladosporioides, Penicillium variabile* and *Penicillium* sp. in addition to many isolates of dark and white sterile mycelia from sediment samples collected from hypersaline environments of solar salterns.

The current results show that some species could be isolated on medium adjusted at pH5 (*Humicola insolens, Emericella quadrilineata, Penicillium echinulatum, P. expansum, P. janczewskii* and *P. funiculosum*) not on alkaline media.

Table 1. Summarized of acidiphilic and aciditolerant 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	4							
A. furcatum	4							
A. strictum	4							
Aspergillus	23456	23456	2356	2346	2346	2346	12346	2356
A. aculeatus							3	
A. carbonarius	3		2	2	2			
A. flavus	23	235	26	26	2	2346	246	23
A. fumigatus	6	236	356	2	26	236	26	36
A. niger	35	234	26	2346	246	236	2346	23
A. ochraceus			5				4	5
A. phoenicis						2	3	
A. terreus	6	3	256	236	2346	236	246	256
Cladosporium				6	2			4
C. cladosporioides				6				
C. sphaerospermum					2			4
Cochliobolus tuberculatus	6							
Emericella			2	2	2	3		
E. nidulans			2	2	2	3		
E. quadrilineata					2			
Eurotium chevalieri			2					
Fennellia flavipes					6			
Fusarium solani			6	6	2	3		6
Humicola				2				2
H. grisea				2				
H. insolens								2

Fungal taxa	Hamra	Al Beida	El Zugum	Rosetta	Umm Reisha	Al Gaar	Khadra	Fasida
Penicillium	46	23		2346	2134	2	2	236
P. chrysogenum	46			36	23	2	2	2
P. duclauxii	4			36		2		
P. echinulatum				4				
P. expansum				3				
P. funiculosum		3						36
P. janczewskii				2				
P. oxalicum				3				
P. puberulum			3	23	24		2	
P. purpurgenum	4				4			
Pencillium spp.		2	3		4			
Trichoderma spp			36			2		6
No. of genera (11)	4	2	6	6	5	5	2	6
No. of species (29)	11	5	11	16	13	10	9	10

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

Acidiphilic and aciditolerant fungi in salt crusts collected from Wadi El- Natrun lakes.

Thirty four species related to 11 genera of acidiphilic and aciditolerant fungi were recovered on Czapek Dox agar adjusted at pH 4 (27 species and 10 genera) and pH 5 (31 species and 10 genera).

Aspergillus (10 species), *Emericella* followed by *Penicillium* (9) and *Fusarium* were the most common genera at both pH levels. Studies of (Siegel and Siegel, 1979) have shown 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, or special water-bodies. On other hand, (Mabyalwa and Mathabatha, 2006) found *Scopulariopsis* to be more dominant and could be isolated from the solar salterns, South Africa consecutive evaporation ponds, whereas *Verticillium* was infrequent.

Aspergillus was the most commonly encountered fungus in salts of the 8 lakes on acidic media as well as the control medium. It comprised 86.1% and 80.1% of the total propagules on both media respectively. Its highest CFUs was recorded from Al Beida during spring 2006. The total CFUs of *Aspergillus* recorded from the 8 lakes during the 6 seasons of study was higher on medium adjusted at pH4 than on medium adjusted at pH5 and both were higher than that on the control medium. Of *Aspergillus*, *A. terreus* (13.5 % and 19.8%) was the most commonly encountered fungus, recovered from all lakes except Khadra during almost all seasons while *A. flavus* (24.1% and 16.1%), *A. niger* (26.8% and 26.5%) and *A. fumigatus* (3.9% and 12.6%) were recovered from all lakes during almost all seasons of study. *A. carbonarius* was isolated in salts of 5 lakes during spring, summer and autumn 2006. The remaining *Aspergillus* species were recorded from either 3 lakes: *A. phoenicis* from Umm Risha, Al Gaar and Khadra during spring, summer and autumn 2006) or from 1 lake during spring 2006: *A. aculeatus*, *A. puniceus* (both from Al Beida), *A. sydowii* (Fasida) and *A. ustus* (El Zugm).

Emericella total counts were higher at pH5 than those on pH4. *Emericella* (represented by 2 species) was recorded from 2 lakes during spring and summer 2006. *E. nidulans* (0.2% and 3.8%) was isolated from (El Zugm during spring 2006 and Fasida during summer 2006) while *Emericella quadrilineata* (0% and 1.3%) was isolated from (El Zugm during spring 2006). Shearer, (1972) showed that the ratio of Ascomycetes to Fungi Imperfecti increased with increasing salinity, while the number of species generally decreased.

Penicillium was higher at both pH levels (3.6% and 4.6%). It was recovered from all lakes during almost all seasons. The peak of *Penicillium* was recorded from Rosetta on medium adjusted at pH4 while at pH 5 from Khadra during winter 2007. *P. chrysogenum* (1.7% and 2.0%) was recorded from all lakes except Hamra during 4 seasons of study. *P. puberulum* was isolated in 5 lakes during 3 seasons (spring, summer and autumn) while *P. viridicatium* was recorded from 4 lakes (Hamra, Al Beida, Rosetta and Al Gaar during summer and autumn 2006 and winter 2007). The remaining *Penicillium* species were recorded from 3 lakes: *P. duclauxii* (El Zugm, Rosetta and Al Gaar during summer and spring), *P. oxalicum* (Rosetta, Al Gaar and Khadra during 4 seasons of study) or from 1 lake: *P. funiculosum* (Rosetta, summer 2006), *P. griseofulvum* (Al Gaar, autumn 2006), *P. purpurgenum* (Rosetta, summer 2006). These results are in agreement with those reported by Ismail *et al*., (2017). Also, Gams, (1992) who found that *Penicillium* species prefer acid pH. Elíades *et al*. (2004) reported also *P. purpurogenum* followed by *P. chrysogenum* to be isolated on acidic media adjusted at pH5.

Fusarium CFUs were much less at both pH levels (0.5% and 0.6%, respectively) than those on the control medium. *Fusarium* (represented by 4 species) and its dominant species *F. solani* (0.5% and 0.3%) were recorded from salts of only two lakes (Rosetta during autumn and spring 2006 and 2007 and Umm Risha during spring 2006 and 2007). The remaining *Fusarium* species were recorded from Rosetta lake during only one season: *F. oxysporum* and *F. subglutinans* (autumn 2006), *F. semitectum* (spring 2006). In agreement with current results, (Elíades *et al*., 2004, 2006a) found that *F. oxysporum* and *F. solani* were the most common on acidic media.

The remaining fungi were recorded from salts of either 2 lakes: *Acremonium strictum* (Hamra and El Zugm during summer and autumn 2006), *Stachybotrys chartarum* (El Zugm and Rosetta during autumn and spring 2006) or one lake: *Acremonium hyalinulum*, *Alternaria alternata*, *A. tenuissima* (each from El Zugm during autumn 2006), *Cladosporium cladosporioides* (Fasida, autumn 2006), *Cochliobolus australiensis* (Rosetta, autumn 2006), *Humicola grisea* (Fasida during spring and summer 2006), *Nigrospora sphaerica* (Rosetta, spring 2007) and *Paecilomyces lilacinus* (El Zugm, spring 2006) (Table 2). In this respect, Bhatnagar and Bhatnagar (2005) found that the dominant fungal genera in desert soil crusts were *Alternaria*, *Fusarium* and *Phialomyces* whereas in non-crusted soils were *Alternaria* and *Penicillium* followed by *Fusarium*. Also, Cantrell *et al.*, (2006) isolated *A. japonicus*, *Aspergillus* sp., *Chaetomium globosum*, *Cladosporium cladosporioides*, *P. variabile* and *Penicillium* sp. and many isolates of dark and white sterile mycelia from sediment samples collected from hypersaline environments of solar salterns (salterns salt ponds).

The current results show that some species could be isolated on medium adjusted to pH4 (*Aspergillus puniceus*) or pH5 (*Cochliobolus australiensis, Paecilomyces lilacinus* and *Penicillium crustosum*) but not on alkaline media.

Table 2. Summarized of acidiphilic and acidtolerant 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
Aspergillus	236	23456	2356	2346	23456	2346	135	256
A. aculeatus		2						
A. carbonarius		2	2	4		2	3	
A. flavus	25	23456	236	236	23456	246	135	26
A. fumigatus	356	34	26	3	2	26	1	6
A. niger	256	236	2	2346	246	236	13	26
A. phoenicis				4		2	3	
A. puniceus		2						
A. sydowii								2
A. terreus	23	3	2356	2346	26	6		25
A. ustus			2					
Cladosporium								4
C. cladosporioides								4
Cochliobolus				4				
C. austaliensis				4				
C. tuberculatus				4				
Emericella			2					3
E. nidulans			2					3
E. quadrilineata			2					
Fusarium				246	2			
F. oxysporum				4				
F. semitectum				2				
F. solani				46	2			
F. subglutinans				4				
Humicola grisea								23
Nigrospora sphaerica				6				
Paecilomyces lilacenus			2					
Penicillium	25	56	26	36	4	24	5	6
P. chrysogenum		6	2	3	4	4	5	6
P. crustosum								6
P. duclauxii			6	3		2		
P. funiculosum				3				
P. griseofulvum						4		
P. oxalicum				36		4	5	
P. puberulum			2		4	4	5	6
P. purpurgenum				3				
P. viridicatium	5	5		3		4		
S. rostrata				4				
Stachybotrys			4	46				
S. chartarum			4	46				
No. of genera	2	2	5	7	3	2	2	5
No. of species	5	13		22	7	12	8	11

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

Conclusion

A total number of acidiphilic and aciditolerant fungi (42 species related to 14 genera) were collected from mud and salt crusts of Wadi El- Natrun lake. *Aspergillus* and *Penicillium* were the most common genera of the total propogules on the two pH levels.

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