

Micromycetes in Sand and Water along the Algerian Western Coastal Areas

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Abstract

The littoral represents the final compartment of the entropic rejections generating great quantities of organic matters. These areas represent an ideal place for the development of saprotrophic fungal communities. The distribution and diversity of microfungi in the littoral region of the Algerian Western Coast was investigated for the first time. Sixty four samplings of sand (surface and 5 cm deep) and water (surface and 1 m deep) were carried out during the dry and rainy seasons. From the study, more than 250 strains belonging to 15 different genera had been isolated from water and sandy beaches. Data indicated a clear abundance (85%) of *Penicillium*, *Aspergillus*, Mucorales, *Cladosporium*, *Trichoderma* and *Fusarium* in the littoral region of the Algerian Western Coast.

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1. Introduction

In the seas and oceans, the fungus occupied all the habitats of surface water, and some fungal biotypes are adapted to living deeply in the ocean. There are many environmental problems, like marine erosion, pluvial water and domestic waste disposal on the beach, causing the water to be inappropriate for bathing and contact sports in some areas.

Fungi are important components of ecosystems as they are cosmopolitan and usually isolated from tropical, subtropical and temperate countries (Smith and Berry, 1975). They are considered the most active microorganisms in the decomposition of organic compounds both in sand and water (Moore-Landecker, 1996). When compared to thousands of fungal species known from terrestrial environments, only 500 have been described for oceans and estuaries although they comprehend the largest part of the Earth's surface (Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer and Volkmann-Kohlmeyer, 1991). The described species are mainly the anamorphous of Ascomycota and Basidiomycota, including some marine yeast. Some of these marine filamentous fungi are parasites of marine algae or marine angiosperms, or they grow symbiotically with brown algae (Kingham and Evans, 1986). Most of the publications referring to filamentous fungi in marine

environments are centered on Europe and North America (Dabrowa *et al.*, 1964, Kishimoto and Baker, 1969; Kirk, 1983; Tan, 1985). However, in Brazil, studies such as Faraco and Faraco (1974), Mattede *et al.* (1986), Purchio *et al.* (1988), Pinto *et al.* (1992), and Sarquis and Oliveira (1996) may be highlighted. Very recently, a special interest, by certain scientists in the world, was established to investigate marine fungi because of their important utility in agroalimentary and health and their great harmful effects through producing toxic substances that cause digestive dysfunctions to humans.

By following up the causes behind many food poisoning cases recorded in Canada and France, researchers identified marine fungi as the only reason behind these pathological signs that usually appear in individuals who consume seafood containing shells (Brewer *et al.*, 1993). Many authors emphasized that fungal species in marine environment are known to produce mycotoxins. Among those authors, Landreau (2001) studied the metabolites of *Trichoderma koningii* Oudemans; Grovel (2002) and Kerzaon *et al.* (2008) characterized the gliotoxin secreted by *Aspergillus fumigatus*; Petit *et al.* (2004) investigated the griseofulvin of *Penicillium waksmanii*, and Mohamed-Benkada (2006) evaluated the fungal risk in conchylaceous zone of *Trichoderma* genus.

Oran city has approximately two million inhabitants and about 70% of the population is served by a sewage collection system. The sewage is discharged in the sea without treatment. Ain Eturk, Eden, Andalouses, and Madagh represent the main beaches in Oran. However,

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they present serious environmental problems as they are intensively visited by tourists and local visitors (Boutiba *et al.*, 2003). The mycota of these beaches is practically unknown, especially with respect to human pathogens. Therefore, considering the lack of studies on the diversity of filamentous fungi in Oran beaches, the aim of this work was to isolate and identify filamentous fungi from four sandy beaches during the dry and rainy seasons.

2. Material and Methods

2.1. Study area

Oran city is approximately 2121 km² located at 35°43'N 0°37'W. The beaches of Ain Eturk (S1), Eden (S2), Andalouses (S3), and Madagh (S4) are the main beaches in Oran (Fig. 1). As urban beaches, they are intensively visited by tourists and locals, and present serious environmental conflicts (Boutiba *et al.*, 2003).



Figure 1. A map showing the study area, Oran city and its main beaches.

The sampling points were chosen in the regions that have the greatest access of bathers and the highest release of domestic sewage.

2.2. Sand and water samplings

Sand and water samplings were concomitantly collected in Ain Eturk, Eden, Andalouses and Madagh beaches, during rainy season (December/2008 and January/2009) and during dry season (June and July/2009). Sixty four sand and water samples were concomitantly collected. The sand sampling in each site was carried out using a little garden shovel, in mid-seashore at one meter from the tideline. Samples were taken from surface soil and below at 5 cm deep. The samples were placed in labeled plastic bags.

In each sampling site, water samples were collected from surface water and 1 m deep using sterile labeled glass tubes. The sand and water samples were kept at room temperature and transferred to the laboratory for later experimental work.

2.3. Isolation, purification and identification of filamentous fungi

Each sand sample (50 g) was diluted in 90 ml of sterilized distilled water, with addition of some drops of Tween 80 making it possible to suspend the spores which could adhere to the grains of sand. The solution was centrifuged at 2500 r/min for 15 min, and then 0.5 ml of the supernatant was spread, in triplicate, onto Petri dishes containing Sabouraud's dextrose Agar (SDA) with chloramphenicol (500 mg/l). Each water sample (0.5 ml, undiluted) was also spread onto Petri dishes as above. The cultured plates were incubated at 27 °C (±2 °C). As soon as the first colonies were developed, they were transferred to test tubes containing SDA. After the purity of the colonies was confirmed, they were subcultured onto Potato Dextrose Agar, or Czapeck's Agar, in glass tubes. Fungal identification was carried out by macroscopic and microscopic observations of colonies and when needed,

mainly for identification of species level, by microculture on a microscopic glass slides (Riddell, 1950; and Pitt, 1985)

2.4. Sand and water abiotic analysis

Sand and water pH and temperature were measured in the morning with a digital pH-Meter and digital thermometer (Hanna), respectively. Salinity was obtained using a handheld salinity refractometer.

3. Results and Discussion

3.1. Sand and water abiotic data for Ain Eturk, Eden, Andalouses and Madagh beaches

Factors such as water salinity, temperature and pH may influence the activity, abundance and distribution of marine fungi (Dix and Webster, 1995; Sridhar, 2009). Moulds can grow between 0 and 35°C. Certain species are able to grow with extreme temperatures; *Cladosporium herbarum* can develop with temperatures lower than 0°C, and *Aspergillus flavus* or *Aspergillus fumigatus* can tolerate temperature until 60°C (Bourgeois *et al.*, 1996).

The water temperature of Ain Eturk, Eden, Andalouses and Madagh beaches ranges from 20.9 °C to 23.6 °C in the dry season (June and July). In the rainy season (December and January), the minimum water temperature was 13.3 °C while the maximum temperature was 18.2°C. The sand temperature in the dry season reached its minimum at 21.1 °C and maximum at 23.6 °C, and in the rainy season, it was between 13.1 and 19.4 °C. Salinity and the temperature are the most important factors affecting the marine fungi diversity (Booth and Kenkel, 1986).

In Ain Eturk, the water and sand salinity was of 24 and 38.1‰ in the rainy and dry seasons, respectively. Water and sand salinity in Eden was of 20 and 37.8‰ in the dry and rainy seasons, respectively. Water and sand salinity in Andalouses was of 24 and 37‰ for Madagh, water and sand salinity was between 15 to 37.4‰ (Table 1). All water

and sand samples had a slightly alkaline pH, varying from 7.78 to 9.21.

In general, marine fungi need high temperatures (usually between 25 and 30 °C) to reproduce (Griffin, 1981). According to Gambale *et al.* (1977), salinity has a great influence in the microbiota of the estuaries. Different studies showed that salinity seemed to affect the metabolism of fungi, as unusual physiological or morphological effects were observed on terrestrial fungal strains when grown on media containing high sodium chloride concentrations (Tresner and Hayes, 1971).

Sodium chloride also induced inhibition of cellulolytic ability of fungi belonging to the genus *Aspergillus* (Malik *et al.*, 1980), and Frisvad and Samson (2004) proposed the use of a culture medium with 5% of sodium chloride for the study of fungal secondary metabolites to enhance their production. The tolerance of fungal growth toward salt in the medium seemed to change from species to species and it appeared to be dependent on the culture temperature (Rai and Agarwal, 1973; Mert and Dizbay, 1977; Mert and Ekmekci, 1987).

Table 1. Temperature, pH and salinity of the water and sand from Ain Turk, Eden Andalouses and Madagh beaches.

Sampling date	Ain Turk						Eden						
	water			Sand			water			Sand			
	pH	T (°C)	S (%)	pH	T (°C)	S (%)	pH	T (°C)	S (%)	pH	T (°C)	S (%)	
Rainy saison	12/04/08	7.84	13.3	30	8.15	15.2	25.0	7.78	14.3	29	8.36	13.1	20
	12/20/08	7.87	13.3	31	8.60	16.5	26.0	7.88	14.5	28	8.70	15.2	20.5
	01/03/09	7.88	13.4	29.5	8.61	18.9	24.0	7.94	13.6	27.5	8.85	13.9	30
Dry saison	01/29/09	7.94	17.0	32	8.70	19.4	28.0	7.94	18.2	30	8.80	14.0	36
	06/04/09	8.12	21.0	37	8.82	21.1	26.0	7.98	20.9	36	8.85	22.0	37
	06/23/09	8.17	21.3	37	8.80	21.9	32.0	7.97	22.0	36.2	8.83	22.3	37
	07/01/09	8.25	21.5	37.5	8.85	22.5	36.5	8.12	22.2	36.5	8.85	23.4	37.5
	07/18/09	8.56	22.2	38	9.18	22.9	38.1	8.22	22.3	37.4	9.21	23.6	37.8

Sampling date	Andalouses						Madagh						
	Water			Sand			Water			Sand			
	pH	T (°C)	S (%)	pH	T (°C)	S (%)	pH	T (°C)	S (%)	pH	T (°C)	S (%)	
Rainy saison	12/04/08	7.87	14.1	32	8.34	16.5	24.0	7.88	14.3	34	8.72	16.5	15
	12/20/08	7.84	14.3	31.5	8.60	16.4	26.0	7.98	14.0	33	8.75	16.7	20
	01/03/09	7.94	17.4	33	8.60	18.0	29.5	8.12	18.0	34	8.80	17.3	30
Dry saison	01/29/09	8.07	16.0	34	8.65	18.4	32.0	8.22	18.2	35	8.82	17.0	33
	06/04/09	8.12	21.5	35	8.72	23.0	35.0	8.94	21.9	36	8.88	22.3	36
	06/23/09	8.15	22.0	35	8.83	25.9	36.0	8.94	22.0	36.5	8.73	22.6	36.4
	07/01/09	8.46	22.3	36	8.95	23.4	37.6	8.97	22.3	37	8.95	23.4	37
	07/18/09	8.88	22.3	36.5	9.17	23.5	37.0	9.18	22.4	37.4	9.16	23.5	37.1

Furthermore, those strains from saline environment also showed a reduction in growth rate related to the increase of salt concentration in solid medium (Tresner and Hayes, 1971; Atapattu and Samarajeewa, 1990; Rojas *et al.*, 1991; Tepsic *et al.*, 1997).

Cantrell *et al.* (2006) found 86 isolates from hypersaline environments: *Cladosporium*, *Aspergillus* and *Penicillium*. In Portugal, Barata (2006) reported that the water temperature was (12 to 24°C), the pH (6.6 to 8), and the salinity (2.57 to 31.81). High salt concentrations also affected intracellular growth activities of a strain of *A. foetidus* grown successively on culture media with increasing salt concentrations. It was assumed that this

phenomenon would result from the requirement of the organism to synthesize osmo-protective compounds for cell-wall integrity and vacuolar morphology (Thangavelu *et al.*, 2006).

According to Kerazon *et al.* (2008), while fungal development is limited to *A. fumigatus* strains occurring in marine environment, their excretion of gliotoxin is stimulated by a salinity of 33 g/L which would lead to an amplification of toxin release in marine surroundings. In Brazil, Gomes *et al.* (2008) found that the temperature range of water and sand in both rainy and dry seasons was (24.3 to 28.8°C); however the range of pH was 7.70 to 8.22 and the range of salinity was 15 to 40%.

Table 2. Number of strains isolated from each sample collected from the Algerian western coastal areas. S1: Ain Eurk, S2: Eden, S3: Andalouses, S4: Madagh.

	Total	%	Water								Sand							
			Rainy season				Dry season				Rainy season				Dry season			
			S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
<i>Penicillium</i>	105	41.66	3	1	2	1	3	2	2	1	19	10	13	6	15	11	7	9
<i>Aspergillus</i>	49	19.4	2	1	1	-	1	1	1	2	9	7	6	4	5	4	2	3
Mucorales	29	11.50	2	-	-	-	1	-	-	1	4	3	2	3	5	3	1	4
<i>Cladosporium</i>	16	6.34	2	1	-	-	2	-	2	1	2	2	1	0	2	0	1	0
<i>Trichoderma</i>	8	3.17	-	-	-	-	-	-	1	-	3	2	1	0	0	0	0	1
<i>Fusarium</i>	7	2.77	-	-	-	-	-	-	-	-	2	3	0	0	1	1	0	0
<i>Alternaria</i>	6	2.38	-	-	-	-	-	-	-	-	2	1	1	0	1	1	0	0
<i>Pullularia</i>	4	1.58	1	-	-	-	-	-	-	-	2	0	0	0	0	0	0	1
<i>Verticillium</i>	3	1.19	-	-	-	-	-	-	-	-	1	1	0	0	0	0	0	1
<i>Geotrichum</i>	3	1.19	-	-	-	-	-	-	-	-	2	1	0	0	0	0	0	0
<i>Scopulariopsis</i>	3	1.19	-	-	-	-	-	-	-	-	1	2	0	0	0	0	0	0
<i>Gliocladium</i>	2	0.79	-	-	-	-	-	-	-	-	1	0	0	0	1	0	0	0
<i>Phialophora</i>	2	0.79	-	-	-	-	-	-	-	-	1	0	0	0	1	0	0	0
<i>Acremonium</i>	1	0.39	-	-	-	-	-	-	-	-	1	0	0	0	0	0	0	0
<i>Chrysosporium</i>	1	0.39	-	-	-	-	-	-	-	-	1	0	0	0	0	0	0	0
Unknowns	13	5.15	2	1	1	-	2	-	1	3	0	0	0	0	1	0	1	1
Total	252		12	4	4	1	9	3	7	8	51	32	24	13	32	20	12	20

About 252 fungal strains were isolated (Table 2, Figure 2) and identified from Algerian Western coastal areas for all types of samples combined, belonging to 15 genera of Ascomycetes subphylum. Fungal communities were represented predominantly by strains of *Penicillium* (41.66%), *Aspergillus* (19.44%), Mucorales (11.50%), *Cladosporium* (6.34%), *Trichoderma* (3.17%), *Fusarium* (2.77%), *Alternaria* (2.38%), *Pullularia* (1.58%), *Verticillium* (1.19%), *Geotrichum* (1.19%), *Scopulariopsis* (1.19%), *Gliocladium* (0.79%), *Phialophora* (0.79%), *Acremonium* (0.39%), *Chrysosporium* (0.39%), and "unknowns" (5.15%). These results are in agreement with Oliveira (1996) who particularly studied the diversity of filamentous fungi on Ipanema beach, Sarquis and isolated 34 genera and 170 species. The genera with the most frequent species were: *Aspergillus* (30.4%) and *Penicillium* (16.2%). In Brazil, fifty seven species were isolated with *Aspergillus*, and *Penicillium* were the most frequent genera in both sand and water, with a total of 11 and 19 species, respectively (Gomes *et al.*, 2008). Similar to the present study, Tauk-Tornisielo (2005) isolated soil filamentous fungi from the Ecological Park of Juréia-Itatins, finding that *Aspergillus* and *Penicillium* were the genera with the highest diversity of species. Studying the incidence of anemophilous fungi isolated from Praia do Laranjal, Pelotas, RS, Bernardi and Nascimento (2005) identified 18 genera. The authors found that *Cladosporium* (18.22%), *Alternaria* (13.84%), *Penicillium* (10.20%), *Curvularia* (7.47%) and *Aspergillus* (3.28%) were the

genera with the species most frequently found. Moreover, Migahed (2003) studied the fungal genera of highest incidence and their respective numbers of species, concluding that *Penicillium* and *Aspergillus* were the most. Thirty-two manglicolous marine fungi belonging to 23 ascomycetes, 1 basidiomycete and 8 mitosporic fungi were observed from South India coast (Gayatri *et al.*, 2008). It should be noted that a large number of strains isolated from seawater samples could not be identified because their mycelium remained sterile (Gomes *et al.*, 2008).

During the dry and rainy seasons, from sea water and sand, 104 isolate of filamentous fungi, mostly anamorphous, were isolated and identified from S1, 59 from S2, 47 from S3 and 42 from S4 (Figure 2). Some species were common for all the beaches, like *Penicillium*, *Aspergillus*, Mucorales and *Cladosporium*.

The number of genera in S1 was higher than that in other beaches; this may be due to the fact that this beach is more frequently visited by tourists and local bathers than the other beaches. It may be also due to the proximity of this site to sources of waste water rejections, which certainly contributes to increasing pollution. Similar facts were observed in a study carried out by Purchio *et al.* (1988) and Gomes *et al.* (2008). Conversely, the opposite was found by Mattede *et al.* (1986). The authors evaluated dry and wet sand samples from polluted and non-polluted beaches of the city of Vitória, Espírito Santo and found that the incidence of fungal genera was greater in non-polluted beaches (55%) than in polluted ones (45%).

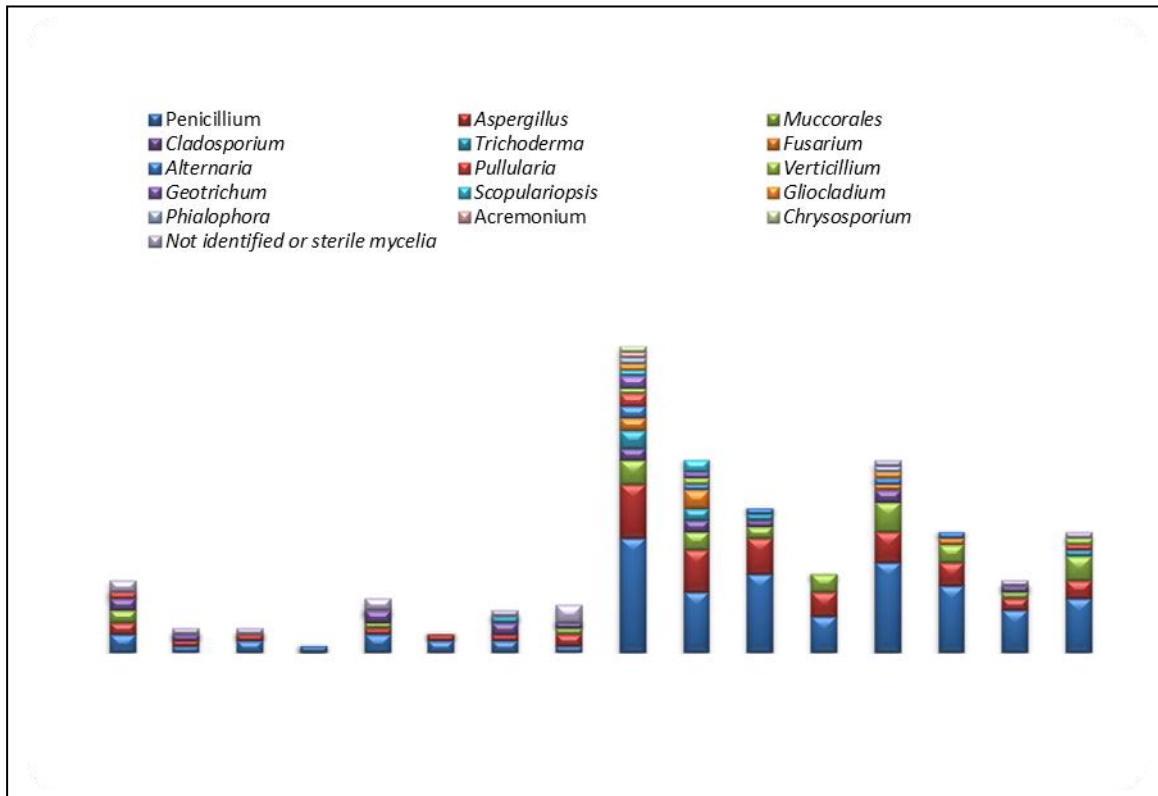


Figure 2. Micromycetes isolated from the four studied beaches of Algerian Western coast.

S1: Ain Eturk, S2: Eden, S3: Andalouses and S4: Madagh

The comparison of the total number of strains isolated by sample type (Table 2) shows that the strain diversity of individual samples was less in seawater (20%) than that of sand (80%). A comparison of the strains found in sand with those isolated from water sand samples had a greater number of strains in which *Penicillium* spp, *Aspergillus* spp, *Cladosporium* spp, Mucorales and *Trichoderma* spp were presented in all types of samples including both sand and water. The similarity in species composition and abundance suggests that these genera are adapted to the marine environment. Genera like *Fusarium*, *Alternaria*, *Pullularia*, *Verticillium*, *Geotrichum*, *Scopulariopsis*, *Gliocladium*, *Phialophora*, *Acremonium* and *Chrysosporium* were isolated only from sand samples (Table 2). Thus, the strains found in sand would appear to be more representative of those really present and well-established in the marine environment (Sallenave, 2000). The number of isolates from samples collected during the rainy season (56%) is greater than that collected during the dry season (44%). The results obtained in the present study agreed with similar studies (Gomes *et al.*, 2008). Harrison and Jones (1975) clearly showed that many mould saprolegniales from fresh water cannot reproduce with salinity higher than 30%. During the dry seasons, when the salinity is raised, marine fungi prevail, contrary to that is the case of the rainy season when the salinity is low and the terrestrial fungi are dominant (Sadaba, 1996).

The investigation carried out by Jessica (2004) on three beaches in Bay Mayagüez to Puerto Rico revealed elevated

levels of fungi species during the rainy season. The author correlated this distribution with salinity. Salinity variations had a significant effect upon abundance of fungi, especially during the months of May through August, when the temperatures were higher, causing an elevation of salinity levels in sand due to seawater evaporation.

The main pathogenic fungi to humans and animals can be found within the anamorphic fungi. These fungi are saprophytic and occasionally pathogenic, and can be isolated from water, soil, animals and humans. Species of *Aspergillus*, *Cladosporium* and *Penicillium*, are found in these beaches, and can be a source of infection for superficial and deep mycosis (Sidrim and Moreira, 1999).

In conclusion, this study shows that Algerian Western coastal areas could represent a wide source of fungal communities. Thus, it would appear that littoral coast represents a large fungal reservoir whose role is little understood but is possibly important for animals, plants and the marine and terrestrial ecosystems. Ain Eturk had the highest number of filamentous fungi contamination (41.2%), followed by Eden (20.6%), Andalouses (17.5%), and Madagh (16.6%). The presence of some toxinogenic moulds in marine environment, such as *Penicillium* and *Trichoderma* strains, could represent a potential risk of toxicities for shellfish, involving contaminations and possibly intoxications of shellfish consumers (Sallenave *et al.*, 2000). It would be interesting to evaluate the toxicity of some micromycetes isolated from these coastal areas by realising a bioassay screening.

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