PHENOTYPIC CHARACTERIZATION OF MODERATE HALOPHILIC FUNGI FROM ATHALASSOHALINE LAKE IN SAUDI ARABIA

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Abstract

The isolation of moderate halophilic fungi from the Dowmatal Jandal Lake reported here for the first time. In 2000, Fungi were first reported to thrive in solar salterns . The mycobiota of the Dowma lake in this study, was dominated by *Aspergillus*, *Penicillium* and *Rhizomucor*, where *Mucor* was found in lesser numbers. *Aspergillus* and *Penicillium* genera were obtained from the water sample, while *Rhizopus* and *Mucor* were obtained from the sediment samples. There was a significant difference in the growth of each isolate at different salt concentrations. The isolates are *P.Chrysogenum , A. flavus, A. fumigatus, Mucor sp., Mucor circinelloides and Rhizomucor sp.* In the present study, we assessed their growth at a wide range of salinity (0 to 25% NaCl). Furthermore, isolates were found in greater numbers in the sediment sample as compared to that in the water sample. The obligate moderate halophiles isolated, belonged to the genus *Aspergillus* and were identified as *A. flavus* and *A fumigatus.* However, we believe that all the six isolates are suitable model organisms to study halotolerance in eukaryotes.

Keywords: Athalassohaline, Ecosystem, Halophilic Fungi, Hyper saline environments

Introduction

The degree of variation of life form in an entire planet or within a given ecosystem and biome is defined as biodiversity. It is an ideal measure of the health of an ecosystem in the planet [Sharon A et al., 2006]. This biodiversity varies from region to region. For example, tropical regions and

terrestrial habitats are typically rich in species, whereas Polar Regions habitat has fewer species [Litchfield *et.al.* 2009]. Hypersaline environments or athalassohaline environments are formed by sea water evaporation [Oren 2002]. These hypersaline environments are formed by the water evaporation that leads to the precipitation of gypsum and minerals, whereas the salinity increases due to sodium chloride (NaCl) precipitation above 300 psu [Gunde-Cimerman et al. 2000]. These extreme hypersaline environments provide a wide diversity of salinity, temperature, pH, light intensity and nutrient concentrations [Pedro´ s-Alio´ 2004]. Recently, most of the microbial diversity studies have focused on hypersaline salterns mainly on halophilic Archaea bacteria (Halobacteriales) that are the major microbial content of this extreme environmental life [Oren 2002]. Apart from these Archaea bacteria, other microbes such as eubacteria, algae, protozoa, and fungi also inhabit these environmental conditions, but still, it is unclear if these hypersaline conditions are suitable for their survival.[Gunde-Cimerman et al. 2004]

In most of the ecosystems, fungi are ubiquitous and usually colonize a diverse range of substrates. The tropical regions are the major area for the diversity of fungi [Nieves-Rivera 2005, Hawksworth 1991]. However, previous studies showed that most of the fungi can survive in extreme environmental conditions [Griffith 1994]. Earlier studies reported that fungi found in such extreme environment belongs to the Ascomycota and inhabit mainly saline soils, sea water, mangroves and salt marshes. [Kohlmeyer & Volkmann-Kohlmeyer 1991, Newell 1996, Domsch et al. 1993, Moubasher et al. 1990]. Still, the fungal hypersaline environmental adaptation mechanism is unclear [Gunde-Cimerman et al. 2004]. A large number of studies have been done in extreme salt conditions of northern latitudes such as the Dead Sea [Kis-Papo et al. 2003], and few studies have also been carried out in the natural and artificial thalassohaline environments such as Slovenia (Adriatic Sea) Dominican Republic, Czech Republic (Soos National Natural Reserve) and France (Mediterranean Sea) [Tina Kogej et al., 2005, Gunde-Cimerman N et al., 2000, Butinar L et al., 2005, Hujslova M et al., 2010].

The present study describe the isolation of halophilic fungi from Dumat al- Jandal athalossohaline lake which is one of several man made salt lakes in the arid region of the northern region of Saudi Arabia. This is the first of its kind study on this athalossohaline lake to identify the presence of halophilic fungal diversity and that the obtained selective isolates may have potential applications in various fields of environmental settings in this region.

Materials and Methods

Site Description

The study area is located in the Dowma al Jandal, Al Jouf North-Western part of Saudi Arabia. (29° 48′ 41.1″ N, 39° 52′ 5.9″ E). This area is an arid region and is characterized by high solar radiation, low precipitation (51 mm) and high salinity (up to 500 psu). A short rainy season takes place from December to February in which life flourishes. Sampling in this study took place during the dry period (from September to October 2013) that was characterized by higher-than-usual precipitation. Consequently, salinity levels in the lake usually fluctuate.

Physicochemical Analysis

Physicochemical conditions during sampling were determined using portable instrumentation as previously described by MM. enazi et,al (2013) . The pH and temperature of the hypersaline lake were measured using a combined pH and temperature meter (Hanna Instruments). The salinity readings were taken with KL-1385 (Kelilong Electron Co.Ltd,china).

Isolation and Characterization of Fungi

Three soil sediment samples at a depth of (10 cm) and two water samples (500ml each) were collected from random weather of the lake during September and October 2013 from Dowmat al Jandal athalossaline lake. Quantity about 2 gm of the soil sample was suspended in 10 ml of sterile water, shaken and further diluted (10^{-1} to 10^{-3}) in sterile water. The 30 µl of each soil sediment dilution and 10 ml of each water sample was spread onto agar-based isolation media Malt Extract Agar (MEA) and and Czapek Dox Agar (CzA), Thus, the components of MEA are malt extract, (30 g/L) , mycological peptone, (5 g/L), Dextrin (2.75 g/L), Agar (15g/l), 15% NaCl and pH 7.1 ± 0.1 , Czapek Dox Agar (CzA) ingredients are Sucrose (30g/l), Sodium nitrate (2.0 g/l), Magnesium glycerophosphate (0.5 g/l), Potassium chloride (0.5g/l), Dipotassium sulphate (0.350 g/l), Ferrous sulphate (0.01g/l) and Agar (12g/l) , 15% NaCl and pH 7.1 ± 0.1 . All components were added to distilled water and brought volume brought up to 1.0 L. The medium was mixed thoroughly and gently heated until it was dissolved and then autoclaved. Each selected dilution of each soil sediment and water sample was analyzed in triplicate. The plates were incubated at 30 ^{o}C for two weeks. Pure cultures were isolated in MEA and CzA agar . Purified isolates were maintained at 10% CzA. Fungal identification was done on the basis of colony and micro morphology characteristics, and were identified using standard taxonomic references. (Domsch et al., 1980, Ellis, 1971; 1976 and Raper and Fennell, 1965).

Halotolerance Test

The halotolerance test was performed using the standard procedure described by Moubasher et al. (1990). Salt tolerance of the isolates was checked by inoculating the cultures in triplicate on CzA amended with salt up to concentrations: 5,10, and 15% [Nazareth et al., 2012]. Isolates were inoculated in the centre of the plates and incubated at 30 ^{o}C for two weeks . After this period, the diameter of the colony was measured. Plates that did not show growth in 7 days were further incubated till 15 days to check for delayed growth, and then sub-cultured onto Czapek Dox Agar and MEA media to confirm the salt tolerance level(Data not shown).

Results

A total of 06 moderate halophilic fungi were isolated. The isolates obtained from this study belong to the genera *Penicillium, Aspergillus , Mucor and Rhizomucor*. Out of the 6 isolates, three of them which were isolated from water samples are *P.chrysogenum , A. flavus, A. fumigatus* (Figure 1,-3). Furthermore, the other three isolates were isolated from the soil sediment and they are *Mucor sp., Mucorcircinelloides and Rhizomucor sp.* (Figure 4-6).

Discussion

The Dowmat al Jandal athalossaline hypersaline lake is renowned as a habitat for halophiles [MM enazi et al 2013], Therefore, an understanding of the microbial communities in such hypersaline environments is highly desirable due to their potential applications [Mohamed SH, et al, 2005, Gurielidze M ,et al. 2010]. Due to its high salinity, the lake provides an inhospitable environment for most organisms, and conditions have become even harsher in recent decades. Occurrences of microbial blooms are rare events in the lake and are limited to short periods. The water of the athalossaline lake is not only hyper saline but oligotrophic as well. The sampling areas near the outlet of the lake had a diverse fungal community, supported by the lower salinity of the lake water. In this lake, the water interface represents a unique microbial ecosystem mainly determined by a varying salt gradient (1.5 to 19% NaCl) within only a few meters [MM.enazi et al., 2013]. The study conducted by us has shown that the mycobiota present in an extreme environment is believed to be too harsh for fungi to thrive. Isolates in this study, including *Aspergillus flavus, Mucor circinelloides, Penicillium chrysogenum,* have been reported in other hypersaline waters from temperate regions [Kis-Papo, et al. 2003]. The relationship between the concentration of nitrogen, the number of fungal isolates and salinity was established by Butinar et al. (2005b). The low nutrient medium slowed down the growth of fast growing Gram negative

bacteria. The work demonstrates the presence of moderate halophilic fungi belonging to the genera *Penicillium, Aspergillus, Mucor and Rhizomucor*. Higher concentration of salt used in the media for isolation, has helped in the selective isolation of halophilic fungi over that of the non-halophilic fungi. This is the first documented study which shows the presence of moderate halophilic fungi in this lake and hence provides new information on the distribution and occurrence of moderate halophilic fungi. Furthermore in future, we may find the possible role of fungi in the biogeochemical cycling of nutrients in water and sediments in lake. Isolates obtained in this study can tolerate up to 19 % of salt concentration and a few species can grow up to 25 %. Genomic sequencing of fungal moderate halophiles has lagged behind for investigation into economically important halophilic fungi. However, the advances in 16SrDNA sequencing technology are quickly reducing the costs and efforts for the identification of diverse halophilic fungi [Pareek et al., 2011].

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Figure 1: *Penicillium chrysogenum* isolated from water sample and showed growth on MEA media. The P*enicillum chrysogenum* contains penultimate branches which bear a whorl of phialides. Conidiophores are hyaline and are smooth walled. Phialides are flask-shaped, consisting of a cylindrical basal part. Conidia are globose and cylindrical.

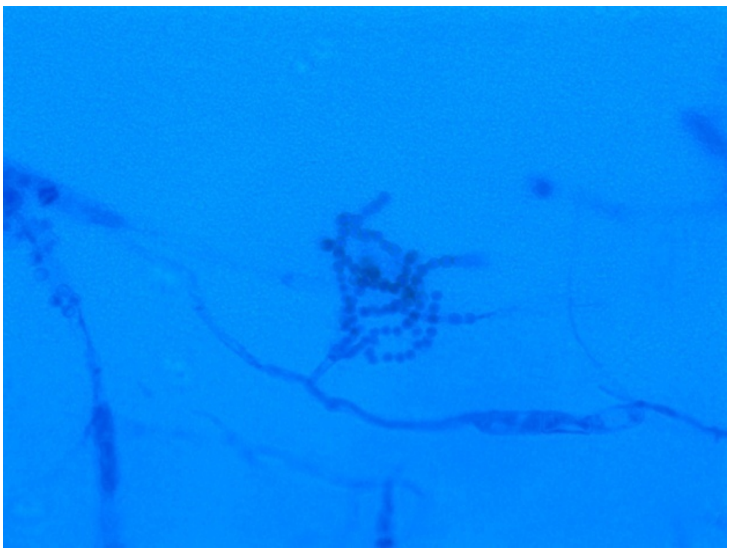

Figure 2: *Aspergillus flavus* isolated from water sample and showed growth on MEA media. Conidial heads are typically radiate, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and coarsely roughened, the roughness often being more noticeable near the vesicle.

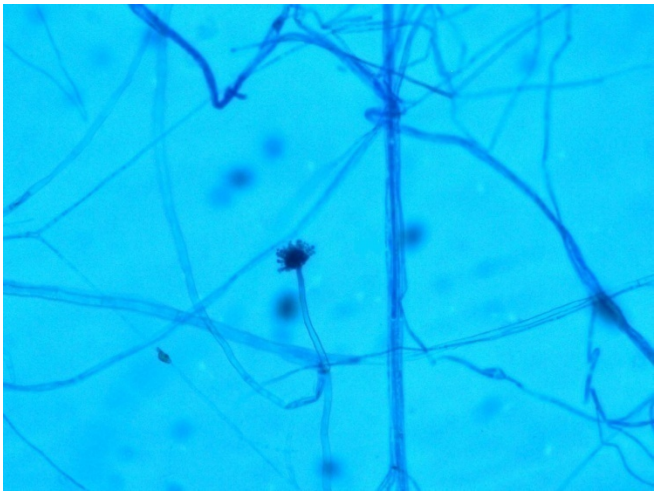

Figure 3: *Aspergillus fumigates* isolated from water sample and showed growth on MEA media. Conidial heads are typically columnar and uniseriate. Conidiophores are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains .

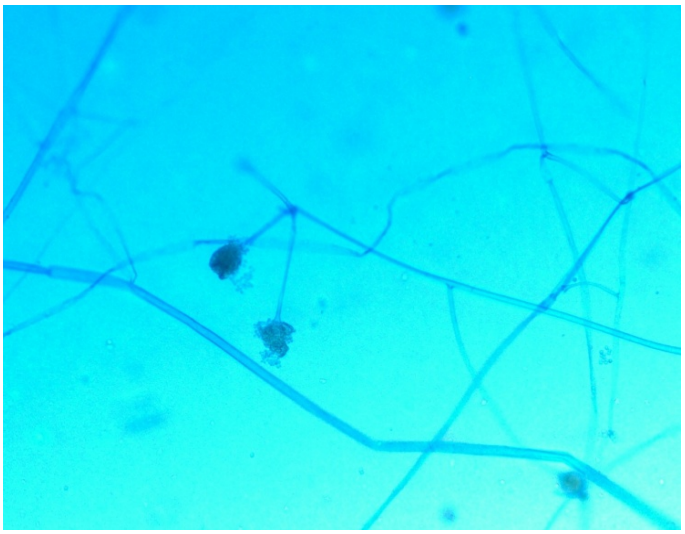

Figure 4: *Mucor* sp. isolated from soil sediment and showed growth on Czapek Dox Agar (CzA). Sporangiophores are erect, simple or branched, multispored sporangia, without apophyses and with well-developed subtending columellae. A conspicuous collarette (remnants of the sporangial wall) is usually visible at the base of the columella after sporangiospore dispersal.

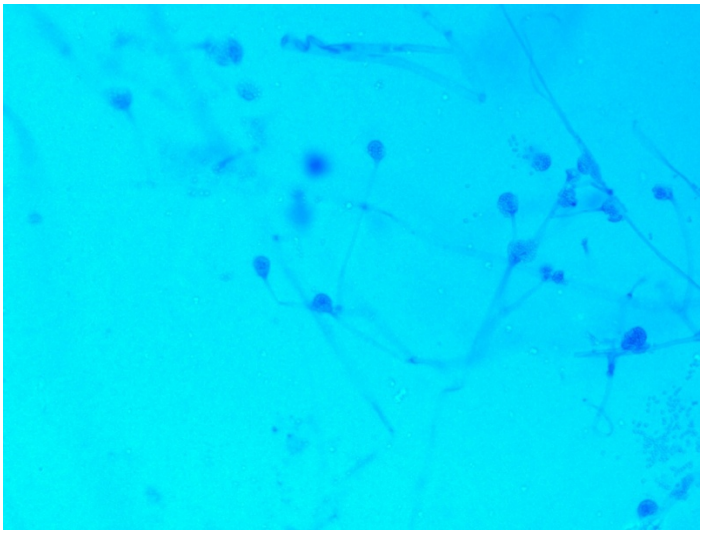

Figure 5: *Mucor circinelloides* isolated from soil sediment and showed growth on Czapek Dox Agar (CzA). Figure 5a showed sporangiophores are hyaline and shorter branches becoming circinate . Sporangia are spherical, columellae are spherical to ellipsoidal . Sporangiospores are hyaline, smooth-walled. Figure 5b showed zygospores are only produced in crosses of compatible mating types and are reddish-brown to dark-brown, spherical with stellate spines.

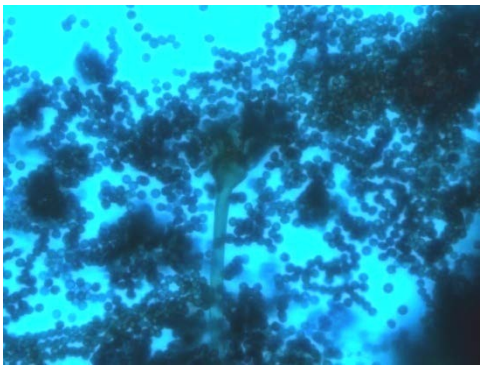

Figure 5a

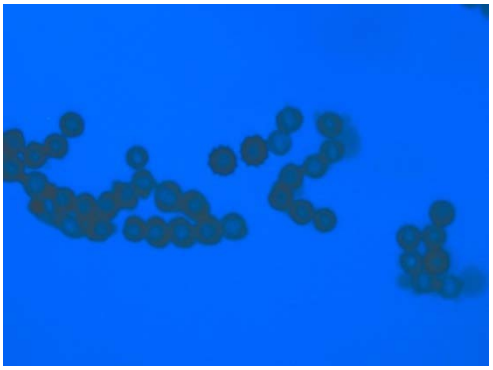

Figure 5b

Figure 6: *Rhizomucor sp.* isolated from soil sediment and showed growth on Czapek Dox Agar (CzA) . Greyish brown colored mycelium and by the development of typical sympodially branched, hyaline to yellow-brown sporangiophores always with a septum below the sporangium. Sporangia are globose, each possessing an oval or pear-shaped columella . Sporangiospores are hyaline, smooth-walled and globose . Chlamydoconidia are absent. Zygospores are rough-walled, reddish brown to black.