Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques

Sharon A. CANTRELLa,*, Lilliam CASILLAS-MARTÍNEZb, Marirosa MOLINAc

aDepartment of Biology, School of Science and Technology, Universidad del Turabo, P. O. Box 3030, Gurabo, PR 00778, USA
bBiology Department, University of Puerto Rico, CUH Station, Humacao, PR 00791, USA
cEnvironmental Protection Agency, ORD, Athens, GA 30602 USA

ABSTRACT

The Cabo Rojo Solar Salterns located on the southwest coast of Puerto Rico are composed of two main ecosystems (i.e., salt ponds and microbial mats). Even though these locations are characterized by high solar radiation (mean light intensity of 39 mol photons m\(^{-2}\) d\(^{-1}\)) they harbour a diverse microscopic life. We used morphological and molecular techniques to identify a series of halotolerant fungi. A total of 183 isolates and 36 species were cultured in this study. From the water from the salt ponds, 86 isolates of 26 species were cultured. The halotolerant fungi isolated from water were: Cladosporium cladosporioides, nine Aspergillus sp., five Penicillium sp. and the black yeast Hortaea werneckii. A distinctive isolate with a blue mycelium was cultured from the salt ponds, representing a new species of Periconia based on morphology and rDNA analysis. Forty-four isolates from eight species were cultured from the sediments around the salt ponds. Most of the sediment isolates formed only sterile mycelium, while several were Chaetomium globosum. A total of 53 isolates from 16 species were isolated from the three layers of the microbial mats, of which Aspergillus niger was the most frequent isolate. Phospholipid fatty acid profiles generated from the different layers of the microbial mats indicated that the uppermost layers of the mats contained fungal biomarker, 18:2w6. This fatty acid decreased with depth, the highest concentration was observed in the green upper layer and it disappeared in the black bottom anoxic layer. This correlates with the isolation of fungi using the serial dilution technique. This is the first study that documents the presence of fungi in microbial mats.

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INTRODUCTION

Living organisms can be found over a wide range of extreme conditions. Most of the organisms living in extreme environments (i.e., extremophiles) belong to the prokaryotes, specifically to the domain Archaea. Extremophiles from the Eukarya domain can also be found living in extreme conditions, as long as there are enough energy sources available to sustain their metabolism. Most eukaryotic extremophiles belong to the algal and fungal categories; some examples are Cyanidium caldarium, a red thermophilic alga, Chlamydomonas nivalis, a psychrophilic green alga, and Trichosporum cerebriae, an
acidophilic fungus. These groups also include Dunaliella salina, a halophilic green alga known to dominate in hypersaline environments (Schleper et al. 1995).

Hypersaline environments originate by the evaporation of sea water and are also called thalassohaline environments (Oren 2002). As water evaporates, gypsum and other minerals precipitate, eventually, sodium chloride (NaCl) precipitates and salinity increases above 300 psu (Oren 2002; Gunde-Cimerman et al. 2000). An example of an extreme hypersaline environment is the crystallization ponds or solar salterns. These ponds provide a diversity of environments where different conditions of salinity, pH, temperature, light intensity, oxygen and nutrient concentrations are found, allowing the study of different microbial communities (Pedrós-Alió 2004; Pedrós-Alió et al. 2000; Gunde-Cimerman et al. 2000). Most microbial diversity studies in salterns have focused on halophilic Archaea bacteria of the order Halobacterales, which comprise the main microbial component in these environments (Oren 2002). Other organisms such as algae, protozoa, eubacteria and even fungi are also found in the salterns, even though it was thought that they could not survive under extreme salt conditions (Gunde-Cimerman et al. 2004).

Fungi are ubiquitous in most ecosystems where they usually colonize a diverse range of substrates. The highest diversity of fungi is found in tropical regions, mainly in tropical forests (Hawksworth 1991). In Puerto Rico, fungal studies have concentrated on forest and coastal ecosystems (Acevedo 1987, 2001; Cantrell et al. 2004; Lodge et al. 2002, Nieves-Rivera 2005). However, many fungi can adapt to extreme environmental conditions of water, temperature, pH and salinity (Griffith 1994). Most of the fungi that can be found in extreme environments belong to the imperfect stage of the Ascomycota, which have been reported in mangroves, saline soils, marine sediments, sea water, salt marshes, and sand dunes (Domisch et al. 1993, Kohlmeyer & Volkmann-Kohlmeyer 1991, Hyde and Pointing et al. 2000, Newell 1996, Guiraud et al. 1995, Moubasher et al. 1990). Because fungi were only recently isolated from hypersaline environments, their function in these extreme conditions is still unclear (Gunde-Cimerman et al. 2004). Most of the studies of fungi in extreme salt conditions have been performed in the northern latitudes, in the region of Russia and the Dead Sea (Gunde-Cimerman et al. 2004, Kis-Papo et al. 2001, 2003, Buchalo et al. 1998). Butinar et al. (2005a,b) described fungi isolated from several natural and man-made hypersaline environments including those found in France, Namibia, Portugal, Slovenia, Spain, and Dominican Republic.

Montalvo-Rodríguez et al. (1998) described Halogemeticum boricuense, a new genus of halophilic Archaea isolated from the solar salterns in Puerto Rico. Díaz-Muñoz & Montalvo-Rodríguez (2005) reported for the first time Hortaea werneckii from Puerto Rico. Casillas-Martínez et al. (2005) describe the microbial mat associated with the surrounding environment of the Cabo Rojo Solar Salterns. The microbial mats located in the Cabo Rojo salterns are approximately 50–70 mm in depth with three distinct layers (green, pink and black). As observed by transmission electron micrographs the top green layer is dominated by cyanobacteria, diatoms and benthic dinoflagellates, which are abundant during the rainy periods. The pink zone underneath this top layer harbours an abundance of unidentified filamentous anoxygenphotrophs with their characteristic lamellae and other bacteria especially spore formers. The most abundant organisms present in anoxic black layers are spirochetes with their characteristic axial filaments. These different communities of the mats are subjected to the typical changes in pluvial precipitation of Caribbean ecosystems (rainfall decreases from 177 mm in the rainy season to 51 mm in the dry period). Increases in the salinity of the locations causes specific changes in the community structure of the different mat layers as reported after terminal RFLP (T-RFLP) analysis. We reported a decrease in the number of peaks present in the electropherograms generated from mats samples collected during the dry season. This result indicates the relationship between an increase in salinity and a decrease in the diversity of the community structure.

The Cabo Rojo salterns are composed of two ecosystems, the salt ponds and the microbial mats. Physicochemical conditions at this location were monitored during sampling to optimize our growth media for the enrichment of a series of halophilic and halotolerant fungi. Specific layers of the microbial mats were analysed for their content of the 18:2ω6 fungal biomarker. Fungal isolates were characterized based on morphological and molecular traits.

Materials and methods

Site description

The study area is located in the Southwest coast of Puerto Rico in Punta Los Morrillos in the municipality of Cabo Rojo (lat. 17°56′25″ N, long. 67°11′W). The area is known as the Cabo Rojo Solar Salterns (in Spanish Las Salinas de Cabo Rojo; Fig 1) and is characterized by a high solar radiation (46 mol photons m⁻² d⁻¹), low precipitation (51 mm) and salinity (up to 600 psu) (Casillas-Martínez et al. 2005). The salterns are composed of an estuary surrounded by mangroves. Natural mats are fed from an inlet that also feeds a series of artificial salt ponds with seawater.

Pluvial precipitation in the salterns varies depending on the time of the year, a dry period with a mean precipitation of 51 mm runs from April to November. A short rainy season takes place from December to March in which life flourishes again at the salterns as pluvial precipitation rises to 177 mm. Sampling in this study took place during a transient period within the dry period (from May through July, 2003–2004) that was characterized by higher-than-usual precipitation. Consequently, salinity levels in the ponds fluctuated and the mats were always submerged with the incoming water from the inlet (in contrast to the usual dry period in which the mats are completely dry).

Physicochemical analysis

Physicochemical conditions during sampling were determined using portable instrumentation as previously described by Casillas-Martínez et al. 2005. Briefly, the pH and temperature of salt pond water and overlying water of the mats was measured using a combined pH and temperature meter (Hanna Instruments, Woonsuckel, RI, HI 9024C). The salinity readings were taken with a handheld refractometer
Isolation and characterization of fungi

A sediment core (10 × 2.5 cm) and water samples (500 ml) were collected during June 2003 and 2004. Microbial mat samples were collected during June 2004. Four aliquots of 10 ml of each water sample were filtered through a 0.45 μm membrane. Filters were placed onto two different media: marine agar (MA) prepared using water from the same sample at two concentrations (10 %, 90 %) and malt extract agar (MEA). Isolation of fungi from sediments and microbial mats was performed using the serial dilution technique in combination with two selective media prepared in the laboratory: MA prepared with sea water and MEA. Four 2 × 2 inch squares of microbial mats were collected and divided into three distinctive layers: green (first layer), pink (second layer), and black (third layer; Fig 2). Isolations were made from a composite of each layer. In addition, fungi were isolated from dry microbial mats. The plates were incubated at 30°C for one month. Pure cultures were isolated in MEA and MA. Fungi were identified using standard taxonomic references.

Some isolates were characterized using a Biolog MicroStation Plate Reader and MicroLog 3 (Hayward, CA) to determine the different carbohydrates metabolized. Fungi were grown in MEA and a spore suspension was made in the inoculating fluid FF-IF. The spore suspension was inoculated in FF microplates that are specific for fungi and incubated at 25°C for 96 h.

Halotolerance test

The halotolerance test was performed following a modified procedure described in Moubasher et al. (1990). The culture medium used in this study was MEA amended with the following NaCl concentrations: 10, 15, 20 and 25 %. Isolates were inoculated in the centre of the plates and incubated at 27°C for 10 d. After this period, the diameter of the colony was measured.

Fig 1 – Detail map showing Los Morrillos point in the southwest coast of Puerto Rico where the Cabo Rojo Solar Salterns are located. Two main ecosystems comprise the salterns salt ponds and microbial mats. Top right photo shows the constructed salt ponds (SP) and the natural salt flat (NSF) within which microbial mats will develop (January 2006). Bottom right photo shows the inlet within which microbial mats have developed (June 2004).

Fig 2 – Layers in a microbial mat collected in June 2004 at the Cabo Rojo Solar Salterns in Cabo Rojo, Puerto Rico.
DNA extraction and phylogenetic analysis

Genomic DNA of selected pure cultures was extracted using CTAB following the protocol of Graham et al. (1994). DNA was stored in a –20 °C freezer. The ITS1–5.8 S ITS region was amplified using a combination of primers ITS1 and ITS4 (White et al. 1990) with the Qiagen PCR Core Kit (Qiagen, Valencia, CA). PCR amplifications were conducted using the standard protocol suggested by the manufacturer using 2–4 μl of DNA template. Thermal cycling parameters for amplification consisted of one initial cycle of denaturing at 95 °C for 2 min, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. This cycle was followed by 38 cycles with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. A final cycle was added consisting of an extension time of 10 min.

Sequencing in both directions was performed on an ABI 310 automated sequencer (ABI, Foster city, CA) using primers ITS1 and ITS4. A few amplicons were cloned with the Topo TA cloning system (Invitron, Carlsbad, CA). Sequences were compiled using Auto Assembler (ABI) software. Sequence similarities were obtained using the BLAST tool from NCBI. Alignments and phylogenetic trees were reconstructed in Clustal X version 1.81 and the NJ distance method with Jukes-Cantor correction (Thompson et al., 1997). BS confidence levels were defined from 1K iterations of tree reconstruction.

Phospholipid fatty acid (PLFA) extraction and analysis

Mat and sediment samples collected for lipid analysis were frozen within a few hours of collection and stored at –20 °C until freeze-dried. Phospholipid fatty acids were extracted, methylated and purified from 2 g of the freeze-dried samples following the procedure described by Burke et al. (2003). The extracted fatty acid methyl esters (FAMEs) were analysed with an Agilent 6890 Series gas chromatography (GC) system (Agilent; Palo Alto, CA) equipped with a flame ionization detector (FID) and a 50-m DB-5 capillary column (film thickness 0.33 μm, internal diameter 0.2 mm; Agilent; Palo Alto, CA). Individual compounds were quantified by FID response relative to an internal standard (20:0 ethyl ester) added prior to GC analysis. Identifications were assigned based on relative retention times compared to a prepared standard mixture (Sigma, St Louis, MO; and Matreya, Pleasant Gap, PA) that was run with each batch of samples. Subsequent analysis by GC–mass spectrometry (MS) was used to verify compound identification and to identify FAMES not in the standard mixture. The same temperature program and similar 30-m DB-5 (film thickness 0.25 μm, internal diameter 0.25 mm) as used in the GC–FID instrument were used in an HP 5890 Series II GC interfaced to an HP 5972 mass selective detector. The oven temperature program was programmed to hold at 70 °C for 2 min, ramp from 70 to 160 °C at 40 °C min⁻¹, then ramp from 160 to 280 °C at 5 °C min⁻¹, with a final isothermal period of 20 min.

Results

Physicochemical parameters

We determined the physicochemical parameters that prevail in four different locations within the Cabo Rojo salterns that include: the inlet that feeds the ponds, the water column on top of the microbial mats and three salt ponds (A, E and New Pond). Even though all locations were subjected to the typical seasonal changes of the Caribbean, the summer months are considered a transient period as the dry period is interrupted with occasional rainy events. Mean rainfall during sampling was 68 mm and the daylight intensity was 39 mol photons m⁻² d⁻¹. The mean salinity in the locations sampled ranged 250–600 psu in the salt ponds and 50–80 psu in the water column of the mats and the inlet (Fig 3). As all locations exceeded salinity of 35 psu they were considered hypersaline. Other parameters such as temperature values (ranging from 26–32 °C) and pH (7.3 to 8.2) also differ depending on the sample location.

Salt pond water samples

The total number of isolates was calculated by the addition of the number of isolates in each aliquot. A total of 86 isolates from 26 species were obtained, most of them are halotolerant (Table 1, Fig 4). These isolates were obtained primarily from MEA plates. Only four specimens were isolated from MA prepared with 10 % pond water; no isolates grew on MA + 90 % seawater. In 2004, it was not possible to obtain water samples from pond E and New Pond because they were in the process of salt extraction. The most frequent species in water samples was Cladosporium cladosporioides, which accounted for 27 % of the isolates. Several species of Aspergillus and Penicillium were identified, such as A. candidus, A. caespitosus, A. flavus, A. flavipes, A. melleus, A. nidulans, A. ochraceus, A. penicilloides, A. unguis, P. citrinum, P. chrysogenum, P. oxalicum and P. variabile. The black yeast, Hortaea werneckii was also isolated from one of the water samples. This yeast has been isolated from soil and hypersaline environments in temperate regions, where it seems to be more abundant (Butinar et al. 2005a, Gunde-Cimerman, personal communication). In addition, Díaz-Muñoz & Montalvo-Rodríguez (2005) have isolated H. werneckii from the Cabo Rojo salt ponds. Other dematiaeous fungi isolated in this study were Curvularia lunata, and Nigrospora sphaerica.

The number of total isolates and the number of isolates that belong to the dematiaeous fungi from water samples taken in 2003 were compared with the salinity of the water. The results indicate that there is a high correlation between

![Fig 3 – Average salinity measurements in the different water samples.](image-url)
the total number of fungal isolates present in the ponds and salinity. Pond A produced both the highest number of isolates and the highest salinity, while the inlet and mat channel had the least number of isolates and the lowest salinity (Figs 3–5).

A distinctive isolate was obtained from one water sample and based on cultural, morphological and phylogenetic characteristics; it belongs to a new species of *Periconia* (Figs 6–7).

This new species is characterized by producing a blue pigmented mycelium on MEA and FDA, and spherical brown ornamented spores produced in a brown conidiophore. The new species was characterized using Biolog and can metabolize several carbohydrates such as arabinose, fucose, maltitol, mannitol, methyl-D-Glucoside, palatinose, d-psicose, raffinose, rhamnose, salicin, threhalose and quinic acid. In liquid media, this species grew differently depending on the medium and the percentage of dextrose. In malt extract (MEB) and Sabouraud dextrose broth (SDB), the species produced a blue-pigmented mycelium (Fig 6). Plates containing MEA and Sabourand Dextrose Agar (SDA) also turned blue. In contrast, in marine and Sabouraud broths, the mycelium is white and there is no pigment produced in the medium. The pigment was extracted in acetone and acetonitrile and was analysed using hplc. The results show three peaks between 600–630 nm, suggesting that the blue colour is produced by three isomers of the same pigment (data not shown).

**Sediment samples**

A total of 44 isolates from eight species were obtained from sediment samples (Table 1, Fig 8). All the species were isolated on MEA; none on MA. Most of the sediment isolates...
were Sterile mycelium belonging to the "Moniliaceae" (23 %) and “Dematiaceae” (36 %) but several isolates belonged to Aspergillus sp., A. japonicus, Chaetomium globosum, Cladosporium cladosporioides, Penicillium sp., and P. variabile.

Microbial mat samples
A total of 53 isolates from 16 species were obtained from the different layers of the microbial mats (Table 1, Fig 9). All the species were isolated on MEA; none on MA. In contrast to the water samples, 83 % of the isolates belong to the moniliaceous fungi. Aspergillus niger was the most common species, accounting for 38 % of the isolates. Chaetomium globosum, as well as Cladosporium cladosporioides, C. sphaerospermum and Nigrospora sphaerica were isolated from the dry microbial mat. Most fungal isolates obtained from the live and active microbial mat belonged to Aspergillus and Penicillium. The number of isolates decreased from the first (green) to the third (black) layer, which agrees with the results obtained by PLFA analysis.

Halotolerance test
The majority of the species isolated in this study can grow in 0–25 % NaCl, however, none of them can be classified as haloophilic because they all can grow in NaCl-free media. Based on their growth rate in the presence of 25 % NaCl, species were classified as (see Table 1): (1) highly halotolerant (species with a growth rate > 3 cm over 10 d in 25 % NaCl): Penicillium sp. (SACCR #10 and #50AA), P. chrysogenum, Aspergillus caespitosus, A. candidus, A. carneus, A. nidulans, Nigrospora sphaerica and moniliaceous Sterile mycelium (SACCR #62AA). In MEB Hortaea...
werneckii can grow up to 25 % NaCl. (2) Moderately halotolerant (species with a growth rate between 2–3 cm over 10 d in 25 % NaCl): Aspergillus sp. (SACCR #48AA), A. flavus, A. melleus, A. ochraceus, A. penicillioides, Cladosporium cladosporioides, Humicola sp., moniliaceous mycelia sterilia (SACCR #61AA), Myrothecium roridum and P. citrinum. (3) Weakly halotolerant species can be divided into two categories, those that present a growth rate < 2 cm over 10 d in 25 % NaCl and those that can grow in up to 15 % NaCl. The following species are in the first category: A. flavipes, A. niger, C. oxysporum, C. sphaerospermum, Chaetomium globosum, Curvularia lunata, Dreschlera sp. (SACCR #121), and P. oxalicum. Periconia sp., and Graphium-like fall in the second category.

Phylogenetic relationships

Several isolates were hard to identify and for their identification nucleotide sequences from the ITS1–5.8 S ITS2 region of the ribosomal DNA were used. We selected representatives of all the different genera obtained in this study. In Fig 7, the phylogenetic relationship of these isolates is presented. Isolates that were confirmed using nucleotide sequences are Aspergillus ochraceus (SACCR #97), Chaetomium globosum (SACCR #13M1), Cladosporium oxysporum (SACCR #103), and Hortaea werneckii (SACCR #16). Also, phylogenetic analysis confirmed that the isolate thought to belong to the genus Periconia (SACCR #64) was indeed a new species. Isolates that were identified to the species level and supported by morphology are A. penicillioides (SACCR #43B), C. sphaerospermum (SACCR #25) and Penicillium chrysogenum (SACCR #2). The above halotolerant isolates from the Cabo Rojo Solar Saltern relate phylogenetically with non-halophilic or non-halotolerant species. More isolates and species obtained in this study will be sequenced.

PLFA analysis

PLFA analysis was used to assess the structure of the microbial communities inhabiting the different mat layers. Although the types of fatty acids identified in each layer were similar, the relative composition of individual PLFAs suggests drastic changes in the structure of the microbial community across layers. The top layer (green) contained the highest percent composition of PLFAs commonly found in G- bacteria (16:1ω7), diatoms (20:5ω3) and fungi (18:2ω6), while the bottom layer (black) had a higher composition of the markers for anaerobic bacteria (cy17:0, cy19:0; Fig 10). Both diatoms and fungi biomarkers were absent in this bottom layer. Seasonal differences (dry versus wet periods) were mostly noticed in the composition of the intermediate, pink layer. Fungi, G- and G+ bacteria (i15:0, i16:0) were all found at a higher percent composition during the dry season in this layer, while anaerobic bacteria were more abundant during the wet season. In contrast, the fungi biomarker was more abundant during the wet season in the top layer and anaerobic bacteria were more abundant during the dry season in both the top and bottom layers. Overall, the fungi biomarker accounted for less than 5 % of the total PLFA composition.

Discussion

In this study, we identified species of fungi present in an extreme environment believed to be too harsh for fungi to thrive. Our results support recent reports presented by others.
isolates are dominant and Dead Sea and vegetative mycelia, and spores stored in for 11 % of the isolates.

Mycelia Sterilia and third layer of the mat can be related to the ability of this characterized by brown spores and its presence in the second ing protection. Conversely, the presence of photosynthetic organism that might be provid-

tively). The dominance in the first layer of moniliaceous fungi lium were the dominant species. In addition, the presence

of the isolates belong to moniliaceous fungi. The second and third layer was dominated by A. niger (90 % and 75 %, respec-

tively). The dominance in the first layer of moniliaceous fungi that produce hyaline mycelium and spores might be related to the presence of photosynthetic organism that might be provid-

ing protection. Conversely, A. niger is a moniliaceous species characterized by brown spores and its presence in the second and third layer of the mat can be related to the ability of this fungus to grow in the absence of oxygen and the production of pigmented spores that are more resistant than hyaline spores to extreme conditions (Domsch et al. 1993).

It is particularly interesting that 60 % of the sediment isolates belong to Mycelia Sterilia, of which dematiaceous isolates are dominant and Chaetomium globosum accounted for 11 % of the isolates. C. globosum was reported from the Dead Sea and vegetative mycelia, and spores stored in undiluted Dead Sea water were viable after 12 weeks (Kis-Papo et al. 2003). The C. globosum isolates obtained in this study were from the sediments and dry mat, and can grow up to 2 cm in diameter in 10 d in MEA amended with 25 % NaCl. Interestingly, this species was not found in live microbial mat.

The exact number of total species that can thrive in hyper-

saline environments is unknown, and it is possible that the number varies as a function of latitude and surrounding habi-
tat (Gunde-Cimmerman et al. 2004; Pedrós-Aliós 2004). In the present study, 36 species, 15 genera and several isolates of mycelia sterilia were obtained, representing less than 1 % of the total number of species described in the world and tropical regions. From these species, 75 % are halotolerant fungi and might be growing and reproducing in this extreme environment.

The relationship between the concentration of nitrogen, the number of fungal isolates and salinity was established by Butinar et al. (2005b). They observed two peaks of nitrogen, one at salinities of 8–10 (0.3–1.7 mg l\(^{-1}\) nitrogen) and the other at salinities of 21–25 (4–13.5 mg l\(^{-1}\) nitrogen). These two peaks correspond to the highest number of colony forming units. Some of the nutrients in the solar saltern come from bacteria, algae and the brine shrimp that bloom in the ponds right before salt starts to precipitate above a salinity of 300 (Pedrós-Aliós 2004). As the salinity increases above 450 psu many organisms start to die and the ecosystem might be devoid of life. Fungi will live on the excreted nutrients, and the dead organic matter that is produced from the die-off of the algae and brine shrimp. Fungal colonies then develop on the surface of the dead algae and shrimp, forming aggregates. The outer cells of the aggregate are exposed to high salt concentra-

tions, and help protect the centre of the colony (Gunde-Cimmerman et al. 2004). In our study, results indicate that the number of isolates increases with salinity. This peak in numbers of fungal isolates coincides with the highest measured concentration of nutrients and organic matter in the saltern, suggesting that a similar sequence of events as de-

scribed by Gunde-Cimmerman et al. (2004) could be taking place at the Cabo Rojo salterns. Also, the number of dematiaceous fungi increases with salinity which is an expected shift of the microbial community due to its adaptation advantage. These fungal species with dark cell walls in the spores and mycelium can tolerate dehydration, and solar radiation better than the moniliaceous fungi whose cells are devoid of pig-

ments. The dark pigment is melanin, and at high salt concentra-

tions melanin granules will be densely packed protecting the fungal cell (Gunde-Cimmerman et al. 2004). Another adapta-
tion to this hypersaline environment consists of an alteration in the composition of phospholipid fatty acids in the fungal cell membrane. At high salt concentrations the membrane needs to be more fluid to overcome the higher osmotic pres-

sure. This fluidity is achieved by increasing the unsaturated PHFA:sterol ratio (Gunde-Cimmerman et al. 2004).

In general, the results obtained in the present study agree with similar studies on fungi from hypersaline environments. A total of 36 species were identified including a new species of Periconia. Most of the isolated species are fungi that are com-

mon in other non-saline environments. None of the identified species are truly halophilic because they present extensive growth on non-salt media. Most of the species obtained in this study (75 %) can tolerate up to 25 % salt concentration and a few species can grow up to 15 %. This is the first study that documents the presence of fungi in microbial mats pro-

viding new information on the occurrence and distribution of fungi in these habitats. Future work will concentrate on the possible role of fungi in the biogeochemical cycling of nutrients in water, sediments and microbial mats in these hypersaline environments. In addition, we will implement modern molecular techniques such as T-RFLP and large-scale
sequencing to augment acknowledge on the diversity and function of fungal species in hypersaline environments particularly microbial mats.

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