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Isolation and Characterization of Halotolerant Soil Fungi from the Great Salt Plains of Oklahoma (USA)

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Abstract – The Great Salt Plains (GSP) of Oklahoma is an inland terrestrial hypersaline environment where saturated brines leave evaporite crusts of NaCl. The current report examines the fungal community, complementing earlier reports on the bacterial and archaeal communities. Twenty-five fungal isolates from GSP soils were obtained on medium containing 10% NaCl and characterized. Based on 18S rRNA gene sequence analysis, all of the isolates fall within the ascomycetes, with a predominance of Trichocomaceae, represented by *Aspergillus*, *Eurotium*, and *Penicillium* species. Representatives of *Anthrinium*, *Cladosporium*, *Debaryomyces*, *Fusarium*, and *Ulocladium* also were isolated. Overall the isolates were widely halotolerant, with best growth observed at lower salinities and no halophilism. The fungal genera observed were all cosmopolitan, without strong specialization. Taken together, these results support the conclusion that hypersaline environments do not have a characteristic community, in contrast to what was observed at the GSP for bacteria and archaea.

Biodiversity / extreme environment / fungi, halophilic / halotolerant / hyperhaline, hypersaline / 18S rRNA / salt flats / terrestrial

Résumé – [Isolement et caractérisation des champignons du sol halotolérant des Great Salt Plains de l'Oklahoma, USA]. Les Great Salt Plains (GSP) de l'Oklahoma est un environnement hypersalin sur voie terrestre où les saumures saturées laissent des croûtes évaporites de NaCl. Le présent rapport examine la communauté fongique, en complément des rapports antérieurs sur les communautés bactériennes et archées. Vingt-cinq isolats fongiques du GSP sols ont été obtenus sur un milieu contenant 10 % de NaCl et caractérisé. Basé sur l'analyse de la séquence du gène rRNA 18S, tous les isolats relèvent des ascomycètes, avec une prédominance de Trichocomaceae, représentée par les espèces de *Aspergillus*, *Eurotium*, et *Penicillium*. Les représentants de *Anthrinium*, *Cladosporium*, *Debaryomyces*, *Fusarium*, et *Ulocladium* ont également été isolés. Globalement les isolats étaient largement halotolérants, avec la meilleure croissance observée à des salinités inférieures et aucun halophilisme. Les genres fongiques observés étaient tous cosmopolites, sans une forte spécialisation. Pris ensemble, ces résultats appuient la conclusion que les zones hypersalines n'ont pas une communauté caractéristique, contrairement à ce qui a été observé à la GSP pour les bactéries et les archées.

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INTRODUCTION

Evaporite salt crusts form on soil surfaces at the Great Salt Plains (GSP) of Oklahoma, a 65-km^2 tract of barren salt flats fed by capillary flow of saturated brines from subterranean NaCl deposits (Johnson, 1980; Reed, 1982; Major *et al.*, 2005). Our earlier studies of the GSP soil microbial community included the isolation and characterization of > 60 bacteria that clustered into 46 phylotypes based on 16S rRNA sequences (Caton *et al.*, 2004; Wilson *et al.*, 2004; Litzner *et al.*, 2006). While bacteria were dominant at the GSP, abundant archaea were isolated and characterized (Caton *et al.*, 2009). Culture-independent diversity analysis demonstrated an abundant collection of anaerobes, many of which are from groups associated with sulfur metabolism. However, overall diversity was lower than typically seen for oligohaline soils (Caton *et al.*, 2009; Caton & Schneegurt, 2012). Most microbiological studies of hypersaline environments have been done on aquatic systems (lakes and salterns), often in marine locales. In contrast, the GSP presents a terrestrial environment far inland. We extend our analysis of the GSP microbial community to fungi in the current report.

Studies on fungal diversity in hypersaline systems have predominantly focused on aquatic environments, particularly solar salterns where ocean water is concentrated during salt mining operations (Anastasiou, 1961; Gunde-Cimmerman *et al.*, 1997, 2000; Buchalo *et al.*, 1998; Kis-Papo *et al.*, 2001, 2003; Butinar *et al.*, 2005b,c, 2011; Cantrell *et al.*, 2006; Mbata, 2008; Nayak *et al.*, 2012). Fungal diversity studies in terrestrial environments have been limited to arid desert soils in the Middle East (Borut, 1960; Ranzoni, 1968; Moubasher *et al.*, 1985, 1990; Abdel-hafez, 1989a,b; Guiraud *et al.*, 1995; Steiman *et al.*, 1995; Mahdy *et al.*, 1996; Mulder & El-Hendawy, 1999; Grishkan *et al.*, 2003; Mandeel, 2006; Al-Musallam *et al.*, 2011), with a few exceptions (Khodair *et al.*, 1991; Hujslová *et al.*, 2010). In general, hypersaline desert soils tend to have a low abundance of fungi, often 10^2 or 10^3 per gram soil (Borut, 1960; Ranzoni, 1968; Salama *et al.*, 1971; Abdel-hafez, 1989a,b; Khodair *et al.*, 1991; Gunde-Cimmerman *et al.*, 2000; Grishkan *et al.*, 2003).

The suggestion by Ranzoni (1968) that there is no characteristic arid or hypersaline fungal community has been echoed by Guiraud *et al.* (1995) and Grishkan *et al.* (2003). In nearly all cases, hypersaline fungal communities are dominated by *Aspergillus* and *Penicillium* species, with melanized dematiaceous forms commonly observed (Borut, 1960; Salama *et al.*, 1971; Moubasher *et al.*, 1985, 1990; Abdel-hafez, 1989a,b; Guiraud *et al.*, 1995; Steiman *et al.*, 1995; Grishkin *et al.*, 2003; Hujslová *et al.*, 2010; Al-Musallam *et al.*, 2011), similar to the communities observed in marine salterns (Gunde-Cimmerman, *et al.*, 1997, 2000; Butinar *et al.*, 2005b,c, 2011; Cantrell *et al.*, 2006) and the Dead Sea (Buchalo *et al.*, 1998; Kis-Papo *et al.*, 2001,2003; Mbata, 2008). *Alternaria*, *Chaetomium*, *Cladosporium*, *Eurotium*, *Fusarium*, and *Hortaea* are commonly observed. Also seen in moderate abundance are *Mucor*, *Rhizopus*, and *Ulocladium,* along with dozens of other genera detected at lower abundance or as rare members of a community.

Salinity can directly affect sporulation and growth of fungi. For instance, at higher salinities (> 5%) there tends to be increased sporulation with more chlamydospores observed, an inhibition of conidiogenesis, and fewer hyphae (Mulder *et al.*, 1989; Mahdy *et al.*, 1996; Mulder & El-Hendawy, 1999; Mandeel, 2006). Most fungal isolates from hypersaline natural environments are halotolerant rather than halophilic, with many growing best at lower salinities (Hujslová *et al.*, 2010; Nayak *et al.*, 2012). Xerophilic fungi (*Debaryomyces, Hortaea,*

Xeromyces, Zygosaccharomyces) that require high osmotica and grow at the lowest water activities known to date are often associated with high-sugar foods (Kroemer & Krumbholz, 1931; Onishi 1980, Andrew & Pitt, 1987; Jermini & Schmidt-Lorenz, 1987; Gock *et al.*, 2003; Plemenitaš *et al.*, 2008; Leong *et al.*, 2011). Broad tolerances to salinity without halophilicity and inhibition of growth and development at higher salinities, supports the suggestion that fungal communities in hypersaline environments are not highly specialized. The dominance of cosmopolitan fungal genera, such as *Aspergillus* and *Penicillium*, suggests that the hypersaline fungal community is a halotolerant subset of common soil fungi in these areas.

The culturable fungal community at the GSP appears to follow these trends, being composed mainly of *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* species. The isolates tended to be euryhaline and not halophilic, with most growing best at lower salinities. We further investigated their physiology by determining pH and temperature tolerances and their ability to grow on different carbon substrates. This study of fungal diversity at the GSP is the first to examine an inland non-arid hypersaline soil. Preliminary accounts of this work have been presented previously (Evans *et al.*, 2005, 2009).

MATERIALS AND METHODS

Soil sample collection: Surface (top 4 cm) grab samples were cleanly taken using sterile tools along a salinity gradient near the edge of the salt flats near the crystal dig area (N 36° 42.856' W 93° 15.725'). Each bulk sample was mixed by hand in sterile Whirl-Pak bags, transported at 25°C, using a cold-pack when needed, and processed within 1 to 2 h of collection. For salinity measurements, soils were dried to constant weight at 110°C, diluted 1:10 with distilled water, agitated for 1 h, and settled. The liquid phase was filtered (0.22 µm; Millipore) and examined with a handheld salinity refractometer with automatic temperature compensation (Fisher).

Fungal isolation and characterization: Soil samples were collected and either plated directly or enriched for 24 h in liquid culture before serial dilution plating. Enrichment and maintenance was on Sabouraud dextrose broth supplemented with 10% salt and 100 µg ml^{-1} ampicillin. Colony morphology was determined by direct plating of isolates onto Sabouraud dextrose agar. The medium was supplemented with 5% salt and 100 µg ml^{-1} ampicillin. Plates were grown at 25°C for 21 d.

Salt tolerances were determined by plating of isolates onto Sabouraud dextrose agar plates supplemented with various salt concentrations and 100 µg ml^{-1} ampicillin. Plates were grown at 25°C for 21 d. Temperature tolerances were determined by inoculation of Sabouraud dextrose broth tubes supplemented with 5% salt and 100 µg ml^{-1} ampicillin and incubation at 4, 25, 37, or 50°C for 21 d. Gelatinase activity was measured in Czapek broth with 5% NaCl and 12% gelatin after incubation for 2 wk at 25°C. Substrate utilization was determined in Czapek broth without sucrose at 5% NaCl supplemented with carbon sources at 30 g L^{-1}.

Phlylogenetic Analyses: Crude DNA extracts from each isolate were prepared using a freeze-thaw technique as described in Caton *et al.* (2004). Genomic DNA in the supernatant was the target of PCR amplification of 18S rRNA gene fragments using fungal primers (combinations of either EF4: 5'GGAAGGGRTGTATT TATTAG-3' and FUNG5: 5'-GTAAAAGTCCTGGTTCCCC-3' [Smits *et al.*,

1999] or Fun817F 5'-TTAGCATGGAATAATRRAATAGGA-3' and Fun1536R: 5'-ATTGCAATGCYCTATCCCCA-3' [Borneman & Hartin, 2000]). PCR was performed in a thermal cycler (Eppendorf Mastercycler) as 25-μL reactions containing 0.2 μM of each primer, 1 U of ExTaq DNA polymerase and associated master mix (Takara), and 5 μL of cell extract. DNA was denatured at 95°C for 2 min, followed by 40 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final 5-min extension at 72°C. PCR amplicons were single-pass sequenced using the appropriate forward primer at the University of Kansas Biodiversity Institute or at University of Tulsa.

Sequences were automatically aligned using Clustal-W (Thompson *et al.*, 1994) and then manually examined and trimmed in MacClade v4.08 (Sinauer Associates). Contextual 18S rRNA gene sequences were identified in GenBank using BLAST (Altschul *et al.*, 1990) or from comparison to relevant literature. PAUP 4.0 b10 (Swofford 1998) generated phylogenetic trees using distance analysis with Jukes-Cantor rules and the neighbor-joining algorithm. Sequences were trimmed to equal lengths and positions with gaps and ambiguous bases ignored, giving ~300 positions for analysis, including a variable region. Jackknife analysis was used to assess the relative support for each branch with a total of 100 replicates conducted heuristically using the distance-based neighbor-joining algorithm in PAUP. The trees were rooted using a basidiomycete, *Gymnosporangium* sp., as the functional outgroup. No chimeras were identified using Pintail within Sequin (GenBank). The sequences appear in GenBank with the accession numbers KF562819 to KF562843.

RESULTS

Isolation, description, and identification

Fungal isolates were obtained from GSP soils by direct plating and enrichment in medium containing 10% NaCl. Axenic strains of 25 isolates were established and characterized phenetically and phylogenetically. By 18S rRNA gene sequence analysis, all of the isolates were determined to be within the saccharomyceta of the Ascomycetes (Fig. 2). Representatives of six fungal families were recovered from the GSP. Most isolates were closely related to the Trichocomaceae, represented by *Aspergillus, Eurotium,* and *Penicillium* species. Three other isolates clustered with *Cladosporium* in the Davidiellaceae (F5, F7, and F15). Three isolates (F4, F9, and F14) were related to the Pleosporaceae genus *Ulocladium* and two others (F11 and F16) clustered with *Fusarium* in the Nectriaceae. One isolate (F2) was related to *Arthrinium* in the Apriosporaceae. Two isolates (F6 and F17) were most closely related to the halotolerant yeasts of *Debaryomyces*. Morphological descriptions and conidia measurements for each GSP isolate are given in Table 1.

Environmental tolerances

Halotolerances of GSP fungal isolates were determined by measuring growth in media with different salt concentrations (Fig. 3). All of the isolates grew at the lowest salinity tested (0.1%) with most isolates (76%) growing best at this

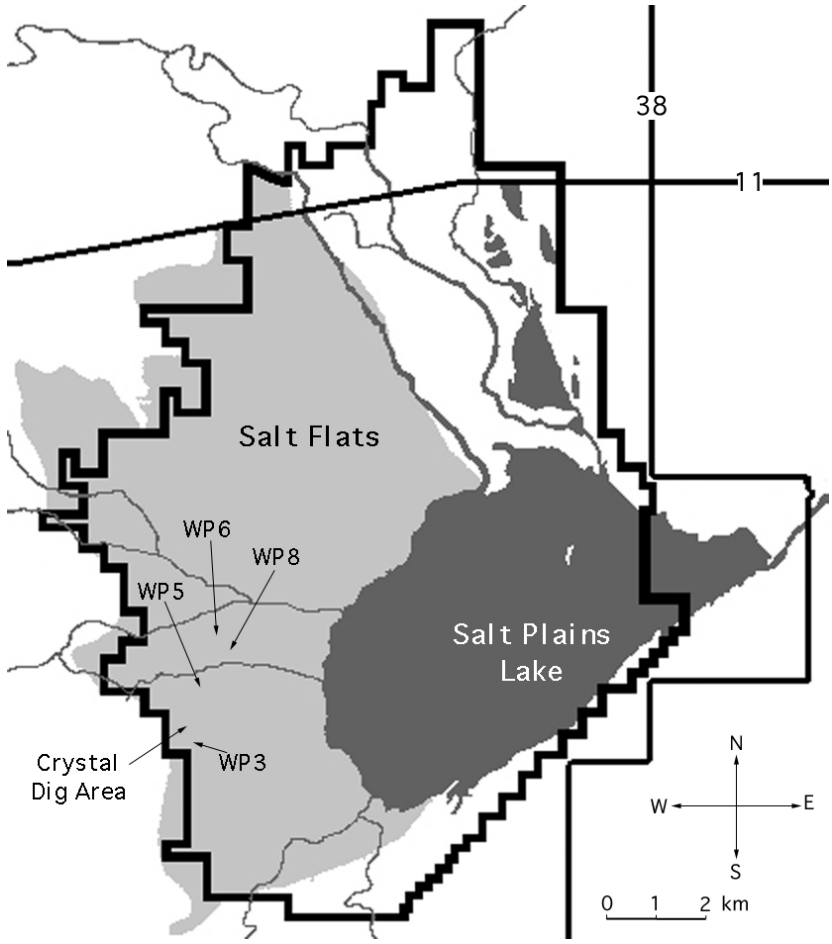

Fig. 1. Map of the Salt Plains National Wildlife Refuge showing the salt flats and sampling sites used for the current study.

salinity. The six remaining isolates, including the two yeasts, grew best at 5% NaCl. While all of the isolates were halotolerant, even euryhaline, none of the isolates were halophilic. Five euryhaline isolates grew across the entire range of salinities tested, including three Trichocomaceae (F10, F12, and F13) and the two *Debaryomyces* (F6 and F17). Only isolate F9, in the *Ulocladium* cluster, did not grow above 10% NaCl, the salinity used for isolation.

Growth temperature tolerances of the GSP fungal isolates are shown in Figure 4. The majority of the isolates (80%) were restricted to growth in the mesophilic range of 25 to 37°C, approximately evenly split between growing best at each temperature. Five isolates grew at 45°C, including three Trichocomaceae (F19, F22, and F23), a *Cladosporium* (F15), and a *Debaryomyces* (F6). It is interesting to note that the *Debaryomyces* isolate F6 grew across the entire temperature range, from 4 to 45°C, and grew best at 4°C. Remarkably, the closely related *Debaryomyces* isolate F17 was restricted to the mesophilic range.

Table 1. Morphological descriptions of GSP fungal isolates. Colonies were grown on Sabouraud dextrose agar supplemented with 5% NaCl for 21 d before examination

Isolate	*Colony Morphology*	*Reverse*	*Dimensions*
F1	Loose mycelia, cream to yellow; rugose; brown conidiophores and conidia; radial conidial heads split into columns with age; spherical conidia	Ridged; cream to olive-brown	Conidia 12-15 µ; Conidial head 350-390 µ; Vesicle 132-160 µ
F2	Fast growing, high, loose mycelia; blastic conidiogenesis; elliptical conidia	Smooth; white to cream	Conidia 19-23 µ
F3	Fast growing; yellow-green pigmented spores darken with age; radial conidial heads; spherical conidia	Smooth; cream to green-brown	Conidia 27-30 µ; Conidial head 662-680 µ; Vesicle 290-295 µ
F4	Intermediate compact mycelia; cream to olive-brown with a thick cream margin; blastic conidiogenesis with elliptical conidia	Smooth to wrinkled; cream to olive-brown	Conidia 27-30 by 32-36 µ
F5	Compact mycelia; velvety texture; olive green; brown in center with age and cream margin; ubonate topography; elliptical conidia	Rugose blue-green	Conidia 16-19 by 27-36 µ
F6	Yeast; dull cream color with a smooth edge	Smooth; cream	Cells 12-18 µ
F7, F15	Loose white mycelia; yellow-green conidial heads; white margin around colony; radial conidial heads; spherical conidia	Smooth; cream to green-brown	Conidia 19-23 µ; Conidial head 145-160 µ; Vesicle 78-85 µ
F8	Fast growing; loose white mycelia with large black radial conidial heads; spherical conidia	Rugose; white to dull yellow	Conidia 34-36 µ; Conidial head 554-600 µ; Vesicle 290-318 µ
F9	Compact mycelia; velvety texture; dark green with cream margin; dictyospores	Smooth; orange-brown to dark brown	Dictyospores 78-90 by 195-265 µ
F10	Fast growing; low colony; powdery texture; blue-green to olive with white margin; conidial heads branch into asymmetrical, undefined columns; spherical conidia	Smooth; grey-green	Conidia 27-32 µ
F11	Loose mycelia; light pinkish-orange; blastal acropetal conidia; elliptical conidia	Smooth; white to dark salmon	Conidia 10-14 by 24-32 µ
F12	Light yellow myelia with dark yellow, wet conidial heads; uneven edge; secretes dark yellow pigment; spherical conidia	Rugose; white to dull yellow	Conidia 38-46 µ; Conidial head 260-490 µ
F13	Intermediate compact mycelia; rugose ; dull yellow to yellow-brown center; white to cream margin; clear to white hypha; dull yellow to brown conidia; radial conidial head; spherical conidia	Rugose; white to dull yellow	Conidia 8-12 µ; Conidial head 155-205 µ; Vesicle 60-72 µ
F14	Intermediate compact mycelia; velvety; yellow-green to dark green with a cream colored margin; dictyospores	Rugose; cream to green-brown	Dictyospores 82-175 by 28-56 µ
F15	Intermediate compact mycelia; slight blue-green spore growth in the center; cream to peach hypha; white margin; rugose; spherical conidia	Smooth; green-brown	Conidia 12-18 u
F16	Loose mycelia; light pinkish-orange; blastal acropetal conidia; elliptical conidia	Smooth; white to dark salmon	Conidia 10-14 by 24-32 µ
F17	Yeast; dull cream color with a smooth edge	Smooth; cream	Cells 12-18 µ

Table 1. Morphological descriptions of GSP fungal isolates. Colonies were grown on Sabouraud dextrose agar supplemented with 5% NaCl for 21 d before examination *(continued)*

Isolate	*Colony Morphology*	*Reverse*	*Dimensions*
F18	Compact mycelia; velvety texture; white to peach in center; cream margin; umbonate; conidial heads branch into asymmetrical columns; spherical conidia	Rugose; cream to dark salmon	Conidia 13-15 µ
F19	Intermediate compact mycelia; cream to yellow center, white margin; umbonate; radial conidial heads; spherical conidia	Rugose; white to dull yellow	Conidia 11-14 µ; Conidial head 374-402 µ; Vesicle 100-145 µ
F20	Intermediate compact mycelia; blue-green center with cream to peach hypha; white margin; spherical conidia	Rugose; dark brown	Conidia 12-16 µ
F21	Intermediate compact mycelia; cream to peach in center; white margin; umbonate; spherical conidia	Rugose; orange-brown	Conidia 14-18 µ
F22	Compact mycelia; green center; yellow-green spores that darken with age; cream margin; elliptical conidia	Smooth; blue-green	Conidia 14-22 by 24-28 µ; Conidial head 410-485 µ
F23	Intermediate compact mycelia; slight blue-green spore growth in the center; cream to peach hypha; white margin; rugose; spherical conidia	Smooth; green-brown	Conidia 12-18 µ
F24	compact mycelia; velvety texture; white to blue-green in center; umbonate; conidial heads branch into asymmetrical columns; spherical conidia	Rugose; salmon to dark brown	Conidia 9-12 µ
F25	Intermediate compact mycelia; blue-green in center; white margin; yellow spore growth; elliptical conidia	Rugose; dark yellow	Conidia 11-20 by 24-43 µ

Tolerances to pH among the GSP fungal isolates were broad (Fig. 5). Nearly a third grew across the entire range from pH 4 to 11. Half of the isolates grew best at pH 7. Only one isolate (F16), a *Fusarium*, grew best above pH 7 and did not grow below pH 6. The closely related isolate F11 grew best at pH 6 and grew across the entire pH range. One *Aspergillus* isolate (F13) grew best at pH 5, while two closely-related others (F3 and F24), grew best at pH 4. Isolates F13 (*Aspergillus*) and F25 (*Penicillium*) did not grow above pH 7 and growth of isolate F1 (*Aspergillus*) was restricted to pH 5 to 8.

Substrate utilization

Defined Czapek medium was used to test for growth of 20 GSP fungal isolates on seven individual carbon substrates. Five of these were sugars and all of the isolates grew to some extent on all five sugars. Glucose was preferred by three isolates (F11, F19, and F20), including a *Fusarium* and two *Penicillium*. While most isolates (75%) grew on lactose, none grew strongly on this carbon substrate. Only half of the isolates could grow on citrate and these did so weakly. The two *Debaryomyces* isolates showed weak growth on Czapek medium without an added carbon source, apparently being capable of utilizing the agar.

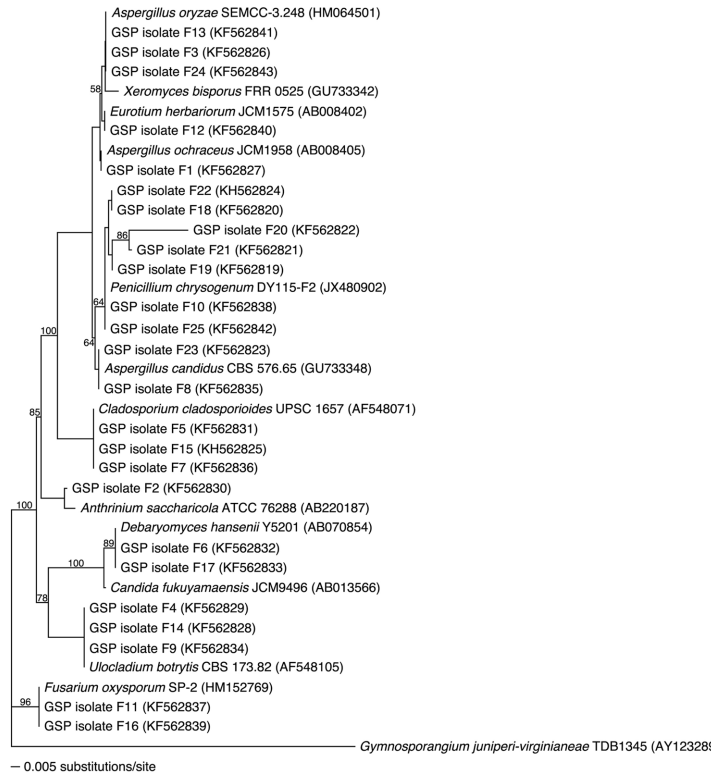

Fig. 2. Phylogenetic tree for GSP fungi based on 18S rRNA gene sequences. Bootstrap values greater than 50% are shown.

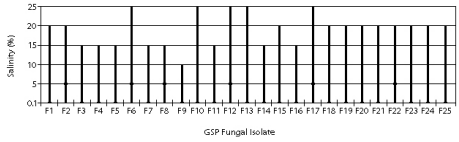

Fig. 3. Salinity tolerance of GSP fungal isolates. The ranges permissible for growth are indicated by bars and the optimal salinities for growth are indicated by closed squares.

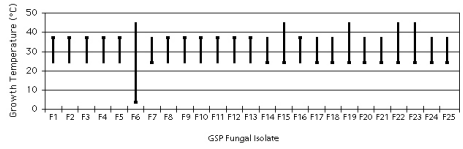

Fig. 4. Temperature tolerance of GSP fungal isolates. Bars indicate the ranges permissible for growth and the optimal temperatures for growth are indicated by closed squares.

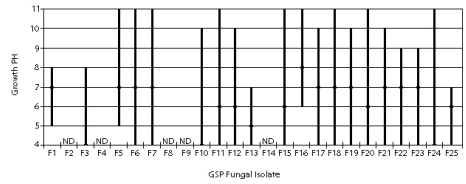

Fig. 5. pH tolerance of GSP fungal isolates. Bars indicate the ranges permissible for growth and the optimal pH for growth are indi- cated by closed squares.

Table 2. Carbon substrate utilization by GSP fungi. Growth was observed in Czapek broth without sucrose at 5% NaCl supplemented with carbon sources at 30 g L^{-1}

Isolate	*None*	*Glucose*	*Galactose*	*Lactose*	*Sucrose*	*Mannose*	*Xylose*	*Citric Acid*
F1	–	+++	+++	+	+	++	++	–
F3	–	++	++	–	+	++	++	–
F5	–	+++	+++	+	++	++	++	+
F6	+	+	+	+	++	++	++	+
F7	–	+++	+++	++	++	++	++	–
F10	–	+++	+++	++	+++	+++	++	–
F11	–	+++	++	+	++	++	++	+
F12	–	++	+	–	++	++	++	–
F13	–	+++	+++	++	++	++	++	–
F15	–	+++	++	+	+++	+++	+++	+
F16	–	+	+	–	+++	++	+++	+
F17	+	+	+	+	+++	+++	+++	+
F18	–	+++	+++	++	+++	++	++	+
F19	–	+++	+	–	++	++	++	+
F20	–	+++	++	–	+	++	++	–
F21	–	+++	+++	+	+++	++	++	–
F22	–	+++	+++	++	++	+++	++	+
F23	–	++	++	+	++	++	++	+
F24	–	+++	+++	+	+++	++	++	–
F25	–	+++	+++	++	+++	+++	+++	–

DISCUSSION

Hypersaline conditions at the GSP require successful microbes to develop coping mechanisms to tolerate high salt concentrations. Most of the bacteria isolated previously from GSP soils exhibited broad salinity tolerances (euryhaline) and only a few were halophilic (requiring high salinities for growth) (Caton *et al.*, 2004; Litzner *et al.*, 2006). The same is true of the GSP fungal isolates, broad halotolerance but no halophilicity. Broad tolerances demonstrate that GSP microbes are ecological generalists with respect to salinity. One suggestion is that generalists may be enriched by the rapidly changing salinity accompanying rain events. This proposition is supported by genetic evidence that there is a great deal of subspecies-level genetic variation driven by high rates of lateral gene transfer and recombination among bacteria at the GSP (Wilson *et al.*, 2004; Schneegurt, 2013). While no bacterial or fungal halophiles were found at the GSP, the archaea at the site are halophilic (Caton *et al.*, 2009).

The findings that there is limited fungal diversity at the GSP and that these are mainly cosmopolitan genera supports the conclusion that terrestrial hypersaline environments do not harbor a characteristic fungal community of specialized taxa (Ranzoni, 1968; Guiraud *et al.*, 1995; Grishkan *et al.*, 2003).

Instead it seems that these populations are halotolerant relatives of fungi that are widespread in oligohaline environments. Certainly any isolation methods and media chosen limit the diversity observed in culture collections. However, when similar methods were applied to the prokaryotic community at the GSP, representatives of many specialized taxa were isolated, including halophiles (Caton *et al.*, 2004, 2009).

All of the GSP isolates were within the Ascomycota and saccharomyceta. The GSP collection was predominately common Trichocomaceae (*Aspergillus, Eurotium,* and *Penicillium*) as observed in several previous studies of fungi in hypersaline soils (Borut, 1960; Salama *et al.*, 1971; Moubasher *et al.*, 1985, 1990; Abdel-hafez, 1989a,b; Guiraud *et al.*, 1995; Steiman *et al.*, 1995; Grishkin *et al.*, 2003; Hujslová *et al.*, 2010; Al-Musallam *et al.*, 2011). *Eurotium* is noted for its halotolerance and xerophilicity, being found in salterns and around the Dead Sea (Andrews & Pitt, 1987; Abdel-hafez *et al.*, 1989a; Kis-Papo *et al.*, 2003; Butinar *et al.*, 2005a). *Debaryomyces* also are well known for their halotolerance (Jermini & Schmidt-Lorenz, 1987; Butinar *et al.*, 2005b). The Cabo Rojo saltern fungal community included *Aspergillus candidus* and *Cladosporium cladosporioides* (Cantrell *et al.*, 2006). *Cladosporium* are very widespread in the environment and have been found in sabkhas and salterns (Gunde-Cimmerman *et al.*, 1997; Butinar *et al.*, 2005b; Cantrell *et al.*, 2006; Al-Musallam *et al.*, 2011). *Fusarium* and *Ulocladium* also are widespread and have been found in Dead Sea coastal soils and arid hypersaline soils (Moubasher *et al.*, 1990; Khodair *et al.*, 1991; Guiraud *et al.*, 1995). *Arthrinium* is a cosmopolitan soil fungus that has been reported in salterns (Gunde-Cimmerman *et al.*, 1997). *Hortaea* have been repeatedly observed in hypersaline soils, but none were isolated from the GSP (Gunde-Cimmerman *et al.*, 2000; Cantrell *et al.*, 2006; Plemenitaš *et al.*, 2008; Fettich *et al.*, 2011; Nayak *et al.*, 2012). The GSP collection did not include representatives of *Chaetomium* or *Pichia* (Steiman *et al.*, 1995; Mulder & El-Hendawy, 1999; Butinar *et al.*, 2005b). Xerophilic fungi typically associated with foods, such as *Xeromyces* and *Zygosaccharomyces*, were not expected (Kroemer & Krumbholz, 1931; Jermini & Schmidt-Lorenz, 1987).

Given the occurrence of cosmopolitan fungi at the GSP, it is not surprising that the vast majority of the isolates grew best in oligohaline medium. The GSP is typically hot in the summer with median June-September high temperatures > 45°C. Most of the isolates grew at 37°C, but not much above that. The GSP soils tend to be alkaline (mean of pH 8.75), so it is surprising that a few isolates did not grow above pH 8 and in particular that F13 and F25 did not grow above pH 7. GSP fungi apparently use different strategies to persist at the GSP. Environmental tolerances differ even among isolates closely related phylogenetically. Examples include the pH tolerances of *Fusarium* isolates (F11 and F16) and the salinity tolerances of *Aspergillus* isolates (F3 and F13). This was observed for some groups of GSP bacteria as well (Litzner *et al.*, 2006). The *Debaryomyces* isolates were particularly tolerant of salinity and pH, with isolate F6 being tolerant across the entire range of all three stressors.

Carbon substrate utilization patterns among the GSP fungi can be compared to the patterns reported for GSP bacteria (Litzner *et al.*, 2006). Here only moderate or strong growth (++ or +++ in Table 2) is considered. The use of glucose, galactose, and sucrose were high and lactose use low in both GSP fungi and bacteria. The GSP fungi are distinguished by greater use of mannose and xylose and less use of citrate than GSP bacteria. This pattern is most similar to the GSP bacterial Phenon D, particularly in its high use of mannose and xylose.

Galactose and xylose are typically associated with hemicellulose in decaying plant debris. It is interesting to note that Phenon D also utilized cellobiose.

Halotolerance in fungi from oligohaline environments may be more widespread than might be expected. Initial abundance measurements by most probable number analysis of turf and prairie soils using SP media with 10 or 20% salinity (A_ws of 0.92 and 0.85, respectively) found that culturable microbes were greatly reduced with the addition of a fungicide cocktail (Porazka *et al.*, 2011). When sucrose is used as the osmoticum, fungi account for 70 and 90% of MPN counts of osmotolerant microbes at 50 and 70% sucrose (A_ws of 0.93 and 0.90, respectively), respectively (Moore *et al.*, 2013). Despite the widespread occurrence of halotolerant bacteria in oligosaline soils, those at the GSP were mainly from taxa associated with hypersaline environments. In contrast, the GSP fungi do not seem to be from specialized groups and there is no apparent characteristic fungal community in hypersaline soils.

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