Can soil Chytridiomycota survive and grow in different osmotic potentials?

Frank H. GLEASON*, David J. MIDGLEY, Peter M. LETCHER, Peter A. MCGEE

aSchool of Biological Sciences A12, University of Sydney, Sydney 2006, Australia
bDepartment of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA

ARTICLE INFO
Article history:
Received 12 December 2005
Received in revised form
11 February 2006
Accepted 1 March 2006
Corresponding Editor:
Nicholas P. Money

Keywords:
Chytridiomycota
Growth
Hypersaline conditions
Osmotic potential
Salinity
Soil chytrids
Survival

ABSTRACT
Twenty isolates from soil in the orders Spizellomycetales, Blastocladiales and Chytridiales (Chytridiomycota) grew on complex solid media supplemented with 10 g l⁻¹ sodium chloride. In a synthetic liquid medium, 4.4 g l⁻¹ sodium chloride strongly inhibited growth in three of the five isolates, possibly because of the effect of the ions or osmolarity of the solution. The maximum concentration for growth in synthetic liquid medium with different osmotic potentials using polyethylene glycol (PEG) varied considerably amongst the isolates. Three patterns of growth with increasing concentrations of PEG were evident among isolates within the genus Rhizophydium. Up to the concentration where growth ceased, the dry weight of each isolate either decreased, remained constant, or in one case, increased. Most of the fungi survived when incubated at room temperature for 7 d in complex liquid media supplemented with 35 g l⁻¹ sodium chloride or 300 g l⁻¹ PEG. These data indicate that soil Chytridiomycota can survive various osmotic potentials that may occur during the wetting and drying phases in soils.

© 2006 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction
Fungi and other microbes have been isolated from terrestrial, freshwater and marine environments where salt content (salinity) varies from hyposaline to hypersaline. Examples of environmental extremes include hyposaline mountain lakes and streams (Barr 2001; Sparrow 1960), hypersaline salt lakes such as the Dead Sea (Kis-Papo et al. 2001; Kis-Papo et al. 2003) and hypersaline salterns (Gunde-Cimerman et al. 2000). Hypersaline environments range in concentration of sodium chloride from slightly greater than sea water (approximately 35 g l⁻¹, 600 mM) up to almost complete saturation. Hypersaline water contains a variety of other monovalent and divalent cations in addition to the sodium cation. In any one habitat, salinity will fluctuate with rainfall and other factors (Kis-Papo et al. 2001).

The effect of salinity on growth and reproduction (Tresner & Hayes 1971; Byrne & Jones 1975; Juniper & Abbott 1993; Chen et al. 2003) and metabolic activities (Malik et al. 1979) of fungi varies enormously. Fungi adjust to an increase in sodium chloride concentration in the external medium by excluding the ions from the cytoplasm and by formation of compatible solutes within the cytoplasm (Blomberg & Adler 1993; Davis et al. 2000; Hocking 1993; Jennings & Burke 1990). Increased salinity resulted in the endogenous accumulation of polyols in Aspergillus and Penicillium (Adler et al. 1982) and Fusarium (Ramirez et al. 2004). Osmotic adjustment has not been documented in Chytridiomycota.

Most species of chytrids have been isolated from soil or freshwater and some only from estuarine or marine habitats (Barr 1987; Barr 2001; Sparrow 1960). The growth response of 57 isolates of soil chytrids in complex liquid media (MHU
medium) at 0, 5 and 15% salinity was correlated by Booth (1971) with the habitat of each isolate, and ecotypes with different tolerances to salinity were suggested. Nielsen (1982) tested nine isolates of Allomyces for growth on complex solid media (YpSs agar) supplemented with seven concentrations of sodium chloride ranging from 150–300 mM. Overall, chytrids appear to be less tolerant of salinity than other groups of fungi (Tresner & Hayes 1971; Byrne & Jones 1975; Chen et al. 2003; Kis-Papo et al. 2003). The growth of an individual organism is the result of interaction of all aspects of the growing environment. Response to salinity may involve a direct response to the ions in addition to the osmotic potential of the solution. Testing the growth of chytrids in a complex medium may mask the impact of salinity because essential nutrients are readily accessed (Blomberg & Adler 1993), and the concentration of individual ions in complex media is unknown.

Lack of moisture, high temperatures and high osmotic potential are all sources of stress, which when combined may limit the growth and survival of fungi in soil (Magan 1997). In a normal growth cycle, soil organisms are subjected to changes in osmotic potential due to wetting and drying. The cycle of wetting and drying creates microcosms within the soil. A sporangial fungus may be subject to hypersaline conditions when the soil dries and to hyposaline conditions after substantial rainfall. Mechanisms that enable growth and survival in changing osmotic potential are unknown for chytrids but are presumably important in soils from arid and periodically dry habitats.

Three basic patterns of growth response might be predicted. First, as osmotic potential increases the fungus may be unaffected until the response mechanisms are overwhelmed and growth ceases. Second, growth of the fungus may slow in response to increasing osmotic potential. Slowed growth may be seen in fungi from moist temperate climates such as where soil chytrids are commonly found. Third, the fungus may be adapted to relatively high osmotic potential and so growth will increase with increasing osmotic potential until the response mechanisms are overwhelmed. The latter case may be exemplified by fungi that exist in arid environments. The life cycle will only be completed if growth continues as the soil dries. The purpose of this study was to clarify the effects of osmotic potential, such as is found with increasing salt concentration, on growth and survival of soil chytrids.

Materials and methods

Twenty chytrids, isolated into pure culture from vegetated and cropping soils in eastern New South Wales, Australia, were used in this investigation (Table 1). Most of these habitats experience drought. The identity and origin of the 20 isolates of fungi and some of the procedures used in the present study have been described previously (Commandeur et al. 2005; Gleason et al. 2004; Gleason et al. 2005; Letcher et al. 2004a; Letcher et al. 2004b). The isolates include nine genera in three orders, three clades in the Chytridiales and several subclades of the genus Rhizophydia (Letcher et al. 2004c).

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Survival after immersion in complex media</th>
<th>Growth in synthetic media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl (g l⁻¹)</td>
<td>PEG (g l⁻¹)</td>
</tr>
<tr>
<td>Allomyces arbuscula Allo Mar CW16a</td>
<td>70</td>
<td>300</td>
</tr>
<tr>
<td>Catenaria sp. Poly Ad 2-0a</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Catenophlyctis sp. Dec CC 4-10Za</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Spizellomyces sp. Mar Ad 2b</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Spizellomyces sp. Mar C/C2b</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Spizellomyces sp. Dec CC 4-10Fb</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophlyctis rosea AUS 13b</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophlyctis sp. AUS 16b</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Powellomyces sp. AUS 17 b</td>
<td>105</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 2c</td>
<td>70</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 3c</td>
<td>35</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 6c</td>
<td>35</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 7c</td>
<td>17.5</td>
<td>150</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 8c</td>
<td>17.5</td>
<td>0</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 9c</td>
<td>35</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 11c</td>
<td>17.5</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. AUS 12c</td>
<td>17.5</td>
<td>300</td>
</tr>
<tr>
<td>Chytridiomyces hyalinus AUS 14c</td>
<td>17.5</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. Mar Ad 14c</td>
<td>35</td>
<td>300</td>
</tr>
<tr>
<td>Rhizophydia sp. Mar R2c</td>
<td>35</td>
<td>300</td>
</tr>
</tbody>
</table>

—, Not tested.

a Blastocladiaceae.
b Spizellomycetales.
c Chytridiales.
Stock cultures of all of the fungi were maintained for up to three months on half strength YpsSs in 2% agar except for Rhizophlyctis rosea A13 which was grown on PYG in 2% agar (Gleason et al. 2005). Before the experimental work the fungi were subcultured onto fresh media. The inoculum consisted of thalli or plugs of agar containing the fungus on solid media or zoospores in liquid media, except where indicated. Zoospores were obtained by flooding cultures on solid medium in Petri dishes with sterile de-ionized water for 2 h. In all experiments the cultures used for obtaining zoospores of the monocentric isolates was less than 7 d old. Some of the other fungi grow slowly, and inoculum may have been up to 14 d old.

Because complex media may influence the capacity of a fungus to tolerate increased osmotic potential and because chemically defined media were needed, growth response to osmotic potential was tested in a synthetic medium. Cladochytrium sp. AUS 11 was the only isolate grown on chytrid synthetic medium (CSM). The composition of CSM is as follows: K2HPO4 600 mg l−1, (NH4)6Mo7O24 2H2O 200 mg l−1, Mg(NO3)2 2H2O 200 mg l−1, CaCl2 2H2O 50 mg l−1, FeEDTA 500 μg l−1, MnCl2 4H2O 10 μM, ZnCl2 10 μM, H3BO3 33 μM, CuSO4 5H2O 1 μM, (NH4)2MnO4 2H2O 0.2 μM, thiamin 133 μg l−1 and glucose 5 g l−1. All other fungi required inclusion of amino acids in the medium to attain measurable growth. Chytridiomycetes hyalinus AUS 14, Rhizophlyctis sp. AUS 16, Powellomyces sp. AUS 17, Spizellomyces sp. Mar Ad 2-0 and Mar CC2, and Rhizophyllum sp. AUS 3, AUS 6, AUS 7, AUS 9, AUS 12 and Mar Ad 14 were grown on CSM to which two amino acids were added (CSMAA), l-alanine 0.9 g l−1 and l-methionine 0.1 g l−1, and the glucose concentration was reduced to 4 g l−1.

Growth on solid complex media supplemented with sodium chloride

All of the isolates were inoculated onto solid half strength YpsSs or PYG in 2% agar supplemented with 0, 10, 20 or 35 g l−1 sodium chloride. The cultures were incubated at room temperature for 7 d. Growth on solid media for monocentric isolates was determined by visual observation as an increase in size, diameter or number of colonies and for polycentric and hyphal was determined by visual observation as an increase in size, thalli or plugs of agar containing the fungus on solid media were subcultured onto fresh media. The inoculum consisted

Growth in liquid synthetic media supplemented with sodium chloride or polyethylene glycol (PEG)

The use of sodium chloride as an osmoticum in media may indicate the general response of the fungus to changes in osmotic potential. However, sodium chloride also has a direct and immediate impact on physiological activity of fungi (Blomberg & Adler 1993) and need not indicate the specific effect of altered osmotic potential of the surrounding soil solution. Thus the fungal response to sodium chloride was compared with a less readily absorbed osmoticum, polyethylene glycol (PEG, Fluka), to indicate the underlying response mechanism.

Chytridiomycetes hyalinus AUS 14, Rhizophlycits sp. AUS 16, Rhizophyllum sp. AUS 6, AUS 7, and Mar Ad 14 were grown in liquid CSMAA supplemented with NaCl. Chytridiomycetes hyalinus AUS 14, Rhizophlyctic sp. AUS 16, Powellomyces sp. AUS 17, Spizellomyces sp. Mar Ad 2-0 and Mar CC2, and Rhizophyllum sp. AUS 3, AUS 6, AUS 7, AUS 9, AUS 12 and Mar Ad 14 were grown in liquid CSM supplemented with PEG. Cladochytrium sp. AUS 11 were grown in liquid CSM supplemented with PEG.

Sodium chloride was added at concentrations of 0, 4.375 (75 mM), 8.75 (150 mM), 13.125 (225 mM), 17.5 (300 mM), or 26.25 (450 mM) g l−1. PEG 6000 was added at concentrations of 0, 37.5 (6.25 MM), 75 (12.5 mM), 112.5 (18.75 mM), 150 (25 mM), or 225 (37.5 mM) g l−1. The osmotic potential of all synthetic liquid media was measured in three replicates samples (size 20 l) using a micro-osmometer (Model 210, Fiske Associates, Norwood, MA). The standards used for calibration were 0, 50, 850 mOsm kg−1. Any readings above 1000 mOsm kg−1 were questionable because of calibration range. Estimated concentration of molecules other than sodium chloride or PEG in CSMAA was 55 mM (Table 2).

Twenty-five millilitres of sterile medium was dispensed into each sterile 50 ml polypropylene screw cap centrifuge tube (Cellstar, Greiner Bio-One). Five replicates of each tube were inoculated twice with 0.2 ml of zoospore suspension, except AUS 11, which was inoculated with one plug of culture. Tubes were incubated on a rotary shaker (Ratek, model EOMS orbital mixer) for 14 d. The thalli and plugs were spun down on a Centrins bench top centrifuge (at 2000 rev min−1), for 10 m), washed with 25 ml de-ionized water for 8 h while stationary, and centrifuged again. The liquid was poured off, and the thalli and plugs washed from the tube into aluminium foil dishes, dried at 70 °C overnight, and then weighed. Any weights less than 1 mg were considered to be no growth and were excluded from statistical analysis.

Data on growth were analysed using analysis of variance (ANOVA). Where significant differences were found, and a trend was visually apparent, a linear regression line was fitted.

Survival on solid complex media supplemented with sodium chloride

Seven monocentric chytrids from the orders Spizellomycetales and Blastocladiales were tested for survival on the surface of complex solid media. Members of the Chytridiales were not included because the surface of agar supplemented with

| Table 2 – Mean (± variance) osmolarity of solutions of sodium chloride (NaCl) and polyethylene glycol (PEG) in chytrid synthetic medium to which amino acids were added (CSMAA) |
|----------------|----------------|----------------|----------------|
| PEG g l−1 [mM] | PEG mOsm kg−1 | NaCl g l−1 [mM] | NaCl mOsm kg−1 |
| 0 | 49 ± 0 | 54 ± 0.5 |
| 37.5 [6.25] | 75 ± 2 | 4.375 [37.5] | 195 ± 1.4 |
| 75 [12.5] | 131 ± 3 | 8.75 [150] | 340 ± 1.3 |
| 112.5 [18.75] | 221 ± 5 | 13.125 [225] | 479 ± 1.5 |
| 150 [25] | 357 ± 8 | 17.5 [300] | 622 ± 1.3 |
| 225 [37.5] | 965 ± 14 a | 26.25 [450] | 898 ± 0.5 a |

a Beyond the upper standard used for calibration of the solutions.
NaCl dries rapidly, and members of the Chytridiales do not survive dry conditions (Gleason et al. 2004). Thalli of Spizellomyces sp. Mar C/C2, Spizellomyces sp. Mar Ad 2, Spizellomyces sp. Dec CC 4-10F, Catenophlyctis sp. Dec CC 4-10Z, Rhizophlyctis rosea AUS 13, Rhizophlyctis sp. AUS 16 and Powellomyces sp. AUS 17 were subcultured to the surface of solid PYG growth media supplemented with 35 g l\(^{-1}\) sodium chloride and incubated at room temperature. After 7 d, at least 30 groups of thalli of each species were transferred to fresh solid PYG growth media lacking NaCl. After a further 7 d on fresh media, the cultures were examined visually for growth (Gleason et al. 2005).

**Survival in liquid complex media supplemented with sodium chloride or PEG**

Thalli on the surface of solid media are subjected to changing osmotic potential as the media dries. Survival after a period of total immersion in liquid media was examined in all fungi. Thalli of the monocentric species or small pieces of agar with the polycentric species were placed into 2 ml liquid PYG media supplemented with either 17.5, 35, 70 or 105 g l\(^{-1}\) sodium chloride, or 150 or 300 g l\(^{-1}\) PEG in 25 ml glass screw cap test tubes. After 7 d incubation at room temperature, the fungi were removed from the complex liquid medium and spread onto fresh solid PYG medium lacking sodium chloride and PEG, and then incubated at room temperature for 7 d. In monocentric species at least ten blocks of agar with the fungi, were placed on the surface of the solid media. Survival was estimated by visual observation of growth (Gleason et al. 2005). All survival experiments with sodium chloride were repeated three times. Limited solubility of PEG prevented the testing of higher concentrations.

**Results**

**Growth on solid complex growth media supplemented with sodium chloride**

All of the fungi grew rapidly on solid complex growth media supplemented with zero and 10 g l\(^{-1}\) (170 mM) sodium chloride. None of these fungi appeared to grow on solid media containing either 20 or 35 g l\(^{-1}\) sodium chloride. All fungi on supplemented media secreted a viscous colourless liquid that visually resembled glycerol.

**Growth in liquid synthetic media supplemented with sodium chloride or PEG**

In one replicate for one treatment where the value for dry weight was zero the data were not included in the statistical analyses. The growth of Rhizophyllum sp. AUS 6 and AUS 7 and Rhizophlyctis sp. AUS 16 was totally inhibited by 4.375 g l\(^{-1}\) sodium chloride in the medium, significantly reduced in Chytridiomycetes hyalinus AUS 14 (9.6 ± 0.7 to 5.3 ± 0.3 mg, P < 0.05) and similar in Rhizophyllum sp. Mar Ad 14 (5.2 ± 0.1 to 4.3 ± 1.1 mg, P > 0.05). No isolate grew in media supplemented with 8.75 g l\(^{-1}\) sodium chloride or higher (Table 1).

The fungi can be placed in five groups according to the maximum concentration of PEG at which significant dry weight was recorded. AUS 2 and AUS 12 grew at 37.5 but not 75 g l\(^{-1}\) PEG. AUS 6 and AUS 7 grew at 75 but not 112.5 g l\(^{-1}\) PEG. AUS 9 grew at 112.5 but not 150 g l\(^{-1}\) PEG. AUS 14, AUS 16, Mar Ad 2 and Mar Ad 14 grew at 150 but not 225 g l\(^{-1}\) PEG. The remainder grew to just over 1 mg at 225 g l\(^{-1}\) PEG (Table 1).

Three patterns of response to increasing concentrations of PEG were evident (Table 3). First, AUS 3, AUS 6, AUS 12, AUS 16, and Mar Ad 2 had statistically similar dry weights at all concentrations of PEG (P > 0.1 in all cases) until the maximum above which growth ceased. AUS 9 had the same pattern if the result for growth at zero PEG is ignored (P > 0.8). Dry weights were similar in AUS 17 and Mar CC 2 if the data for growth at 225 g l\(^{-1}\) PEG are ignored (P > 0.8). Second, a statistically significant decline in growth with increasing concentration of PEG was observed in AUS 7 (ANOVA, P < 0.001; y = 9.1 – 0.3x, R\(^2\) = 0.79, P < 0.001), AUS 11 (ANOVA, P < 0.001; y = 4.3 – 0.08x, R\(^2\) = 0.85, P < 0.001), and AUS 14 (ANOVA, P < 0.001; y = 8.3 – 0.3x, R\(^2\) = 0.63, P < 0.001). Third, a statistically significant increase in growth with increasing concentration of PEG until the maximum above which growth ceased was observed in AUS 1 (ANOVA, P < 0.001; y = 21.3 + 0.2x, R\(^2\) = 0.86, P < 0.001).

### Table 3 – Mean (± SD) dry weight (mg) of chytrids in increasing concentrations of polyethylene glycol (PEG)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>0</th>
<th>37.5</th>
<th>75</th>
<th>112.5</th>
<th>150</th>
<th>225</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS 3</td>
<td>5.7 ± 0.9</td>
<td>5.5 ± 1.0</td>
<td>0.3 ± 0.2*</td>
<td>0.3 ± 0.1*</td>
<td>0.3 ± 0.1*</td>
<td></td>
</tr>
<tr>
<td>AUS 6</td>
<td>5.1 ± 2.9</td>
<td>5.5 ± 0.4</td>
<td>6.7 ± 1.0</td>
<td>0.3 ± 0.1*</td>
<td>0.3 ± 0.1*</td>
<td></td>
</tr>
<tr>
<td>AUS 7</td>
<td>9.5 ± 0.5</td>
<td>6.4 ± 0.7</td>
<td>5.8 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>AUS 9</td>
<td>15.2 ± 0.3</td>
<td>13.1 ± 0.4</td>
<td>11.3 ± 0.4</td>
<td>11.9 ± 0.5</td>
<td>0.3 ± 0.1*</td>
<td>0.3 ± 0.1*</td>
</tr>
<tr>
<td>AUS 11</td>
<td>4.5 ± 0.7</td>
<td>3.5 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>2.9 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>AUS 12</td>
<td>6.9 ± 1.4</td>
<td>6.1 ± 0.9</td>
<td>0.3 ± 0.4*</td>
<td>2.5 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>AUS 14</td>
<td>8.5 ± 0.9</td>
<td>6.4 ± 0.7</td>
<td>7.0 ± 1.0</td>
<td>6.0 ± 1.3</td>
<td>3.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>AUS 16</td>
<td>6.3 ± 0.6</td>
<td>6.0 ± 0.2</td>
<td>6.2 ± 0.7</td>
<td>5.9 ± 0.8</td>
<td>4.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>AUS 17</td>
<td>6.0 ± 0.1</td>
<td>5.2 ± 0.5</td>
<td>5.0 ± 0.6</td>
<td>5.5 ± 0.5</td>
<td>5.1 ± 1.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Mar Ad 2</td>
<td>7.9 ± 0.6</td>
<td>7.6 ± 0.6</td>
<td>7.7 ± 1.1</td>
<td>9.0 ± 1.5</td>
<td>4.5 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Mar C/C2</td>
<td>13.3 ± 0.4</td>
<td>9.9 ± 0.6</td>
<td>9.6 ± 1.5</td>
<td>10.0 ± 0.9</td>
<td>10.1 ± 0.4</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Mar Ad 14</td>
<td>4.5 ± 0.2</td>
<td>8.2 ± 1.1</td>
<td>8.8 ± 0.6</td>
<td>9.0 ± 1.3</td>
<td>11.5 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

\* Excluded from statistical analysis.
observed in Mar Ad 14 (ANOVA, P < 0.001; y = 5.5 + 0.23x, R² = 0.75, P < 0.001).

Survival on solid complex growth media supplemented with sodium chloride

All seven fungi resumed growth after transfer to fresh media lacking sodium chloride.

Survival in liquid complex growth media supplemented with sodium chloride or PEG

All fungi resumed active growth after immersion in at least one of the media supplemented with sodium chloride. The tolerance of fungi to sodium chloride concentration differed. Fungi could be placed in one of four groups according to the maximum concentration of sodium chloride in which they survived (Table 1).

First, five members of the Chytridiales (Rhizophydium sp. AUS 7, AUS 8 and AUS 12, Chytridiomycetes hyalinus AUS 14 and Cladochytrium AUS 11) resumed growth after immersion in medium supplemented with 17.5 but not 35 g l⁻¹ sodium chloride. Second, five members of the Chytridiales (Rhizophydium sp. AUS 3, AUS 6, AUS 9, Mar Ad 14 and Mar R2) resumed growth after immersion in medium supplemented with 35 but not 70 g l⁻¹ sodium chloride. Third, one member of the Chytridiales (Rhizophydium sp. AUS 2) and one Blastocladiales (Allomyces arbuscula Mar CW 16) resumed growth after immersion in medium supplemented with 70 but not 105 g l⁻¹ sodium chloride. Fourth, eight of nine isolates from the Spizellomyctes and Blastocladiales (Catenaria sp. Poly Ad 2-0, Spizellomyctes sp. Mar C/C2, Spizellomyctes sp. Mar Ad 2, Spizellomyctes sp. Dec CC 4-10F, Catenophyctis sp. Dec CC 4-10Z, Rhizophyctis rosea AUS 13, Rhizophyctis sp. AUS 16 and Powellomyces sp. AUS 17) resumed growth after immersion in the medium supplemented with sodium chloride at 105 g l⁻¹.

All fungi except Rhizophydium sp. AUS 8 resumed active growth after immersion in media supplemented with PEG (Table 1). Rhizophydium sp. AUS 7 returned to growth after immersion in 150 g l⁻¹PEG, and the remaining fungi after immersion in the medium supplemented with 300 g l⁻¹PEG.

Discussion

This research is part of a programme designed to clarify the response of soil chytrids to common environmental stress to better understand which species will survive in particular habitats, and what environmental factors influence their survival and growth. Australian soils are commonly subject to drought. Osmotic potential increases as the soil dries. The purpose of the present research was to clarify the responses of soil chytrids to increasing osmotic potential.

All 20 fungi in the present study grew on complex solid media supplemented with sodium chloride at a concentration up to 10 g l⁻¹ (170 mM or 10 %w) but not 20 g l⁻¹ (340 mM or 25 %w). Similarly, Nielsen (1982) observed growth of nine isolates of Allomyces on complex media with sodium chloride at a concentration of up to 175 mM but not above 275 mM. Booth (1971) observed growth in complex liquid media made with diluted sea water of a number of soil chytrids at either 5 and/or 15 %w. Some of the latter chytrids were isolated from environments with salinities higher than normally found in soil (Booth 1971). No isolate in the three studies grew at 35 g l⁻¹ (600 mM or 35 %w) NaCl, the approximate salinity of undiluted sea water. Therefore, it might be inferred that soil chytrids cease growth before salinity reaches that of sea water.

The data from experiments on growth on complex solid media supplemented with sodium chloride are problematic for several reasons. Growth on solid media is difficult to interpret because the elevated parts of the colony are drier than the lower parts attached to agar. Growth on complex media may mask the underlying response to osmotic potential. In this study the growth of five isolates in synthetic liquid media was inhibited by concentrations less than 8.75 g l⁻¹ sodium chloride. The response to sodium chloride as the osmoticum may indicate a complex reaction as fungi may be intolerant of either Na⁺ or Cl⁻ ions, or both. Finally, use of established colonies for the inoculum to study the response may mask vulnerability of one part of the life cycle to stress. Total immersion of fungi in liquid media provides a more homogeneous environment to study growth. Use of zoospores as inoculum requires the fungus to pass through the entire life cycle for growth to be detected. The nutrients in the growth media are also important. Many building blocks for the synthesis of complex macromolecules are provided in complex media. Synthesis of the building blocks from inorganic compounds may add additional stress to the osmotic stress caused by the presence of sodium chloride.

In general, fungi in Spizellomyctes grew at higher concentrations of PEG (maxima of 150 and 225 g l⁻¹) than in Chytridiales (37.5–150 g l⁻¹) indicating greater tolerance of high osmolarity. The growth response of members of the genus Rhizophydium varied. AUS 3, AUS 6, AUS 7 and AUS 12, all from moist temperate regions near Sydney, appear to be less tolerant of high osmotic stress than Mar Ad 14, the sole isolate in present study from an arid hot environment at Narrabri. In addition, the Sydney fungi had similar growth patterns under increasing osmotic potential, which ceased presumably as the cells were overwhelmed by comparatively low concentrations of PEG. In contrast, growth increased with increasing osmotic potential in Rhizophydium Mar Ad 14. It might be argued that the growth response to zero PEG by Rhizophydium Mar Ad 14 might be a statistical anomaly. However, if the data for zero PEG are removed, a significant regression can be fitted to the data, indicating that growth of the fungus increased with increasing PEG until the fungus was overwhelmed. These differences might indicate different ecotypes among isolates of Rhizophydium (Booth 1971).

The growth responses to sodium chloride and PEG in liquid synthetic growth media can be compared in only five fungi. The maximum osmolarity for growth in a solution of sodium chloride was 195 mOsm kg⁻¹ for two fungi (AUS 14 and Mar Ad14), and in PEG it was 357 mOsm kg⁻¹ for three fungi (AUS 14 and Mar Ad14, plus AUS16: Table 2), indicating that the effect of the concentration Na⁺ and Cl⁻ ions may be in addition to the effect of total osmolarity of the solution. Indeed, AUS 16 appears comparatively sensitive to NaCl. The mechanisms of tolerance to solutes such as sodium chloride and PEG in the growth media were not examined in this study.
The stages of the life cycle sensitive to increasing osmotic potential remain to be determined. However, the data on growth in increasing concentrations of PEG indicate interesting possibilities. If sporangia are sensitive to increasing osmotic potential, then the growth response might decline, as observed in AUS 7, AUS 11 and AUS 14 (all Chytridiales). Response might be seen as a sudden change in growth if zoospores are the sensitive stage (AUS 9, Mar AD 14 and Mar CC 2). Clarification of the physiological processes involved in sensitivity of different stages requires a clearer understanding of the mechanism of response by chytrids to increasing osmotic potential, which is currently under investigation.

All of the chytrids survived brief immersion (for 7 d) in concentrated salt solutions. Four general patterns were observed. All Blastocladiales and Spizellomycetales survived exposure to 70–105 g l\(^{-1}\) sodium chloride, and all Chytridiales 17.5 to 70 g l\(^{-1}\). The isolates in the Chytridiales that failed to survive exposure to 35 g l\(^{-1}\) all came from habitats in moist temperate environments. However, tolerance of sodium chloride and geographic region of the isolates were unrelated, evidence against the ecotype hypothesis. All of the fungi except for Rhizophydatum sp. AUS 7 and AUS 8 survived exposure to 300 g l\(^{-1}\) PEG. Both fungi also failed to survive exposure to 35 g l\(^{-1}\) sodium chloride, indicating greater sensitivity to osmotic stress. Unfortunately, it was not possible to test higher concentrations of PEG because of the limits of solubility. Not surprisingly, all fungi ceased growth at lower concentrations of sodium chloride and PEG than the concentration from which they recovered growth. Thus the response of soil chytrids to dry periods is likely to involve a period of quiescence when osmotic potential is high, and recovery of growth when soil moisture returns.

Most of the isolates in the present study can be classified as halotolerant fungi, because they survive immersion in liquid complex growth media with salt at a concentration of 35 g l\(^{-1}\) (approximating sea water) for 7 d. Furthermore eight fungi survived 105 g l\(^{-1}\) sodium chloride, hypersaline conditions three times the concentration of sea water. Hypersaline conditions of this magnitude are rarely found in nature and concentrations of NaCl. Physiologia Plantarum 56: 139–142.


Acknowledgements

The authors thank David Porter and Naresh Magan for their helpful suggestions. This research was supported in part by NSF-PEET Grant no. DEB-9978094.

References


Can soil Chytridiomycota survive and grow in different osmotic potentials?