

## Further Studies on the Water Relations of Xerophilic Fungi, Including Some Halophiles

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Germination and growth of six fungi, *Basipetospora halophila*, *Polypaecilum pisce*, *Aspergillus penicilloides*, *A. wentii*, *Eurotium repens* and *Exophiala werneckii*, isolated from salt fish, and two new undescribed species from other sources, a *Geomyces* sp. and a *Eurotium* sp., were examined on media in which water activity ( $a_w$ ) was controlled over a wide range by glucose/fructose, glycerol or NaCl. Three species, *B. halophila*, *P. pisce* and *Ex. werneckii*, grew more rapidly on media containing NaCl in comparison with glucose/fructose, and hence can be considered to be halophilic. Two other species, *A. penicilloides* and *A. wentii*, were also unusually tolerant of NaCl: *A. penicilloides* could grow on media saturated with NaCl, while *A. wentii* grew more rapidly on NaCl-based media down to 0.82  $a_w$  than any other xerophile examined so far. In contrast, the *Geomyces* sp. was intolerant of salt. The *Eurotium* sp. was obligately xerophilic, with growth confined to the range 0.935–0.68  $a_w$ .

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### INTRODUCTION

Xerophilic fungi were defined by Pitt (1975) as those capable of growth under at least one set of environmental conditions at a water activity ( $a_w$ ) below 0.85. Among xerophiles for which solute tolerances have been defined (Pitt, 1975; Pitt & Hocking, 1977, 1982; Hocking & Pitt, 1979; Luard & Griffin, 1981) germination and growth were either not influenced by solutes or growth was more vigorous on media containing high concentrations of sugars rather than polyols or NaCl. This paper describes the physiological characterization of some further xerophiles, particularly species isolated from salt fish, which might be expected to possess quite different solute tolerances. Two newly isolated and undescribed putative xerophiles from other sources were also included.

### METHODS

*Fungi.* Eight xerophilic fungi were studied: *Basipetospora halophila* FRR 2608, from Japanese dried fish, 1984, FRR 2187, from imported Asian dried fish, 1979; *Exophiala werneckii* FRR 2561, from salted fish, 1980; *Polypaecilum pisce* FRR 2606, *Aspergillus penicilloides* FRR 2612, *Aspergillus wentii* FRR 2624, *Eurotium repens* FRR 2625, all from Indonesian dried fish, 1983; *Geomyces* sp. FRR 2375, from layer pellets, 1983; *Eurotium* sp. FRR 2471, from cardamom seeds, 1982. FRR denotes the culture collection of the CSIRO Division of Food Research, North Ryde, NSW, Australia.

*Media.* Media were prepared with water activities ranging from 0.997 to 0.747  $a_w$  (NaCl) and 0.645  $a_w$  (glucose/fructose, glycerol), in increments of approximately 0.02  $a_w$ , by adding NaCl, glycerol or a mixture of equal weights of glucose and fructose to a basal medium as described by Pitt & Hocking (1977). Media with water activities above 0.92 were sterilized by 15 min in steam at 100 °C on three successive days, the remainder by a single steam treatment. Actual  $a_w$  values of media were checked at the time of pouring into Petri slides with a Sina-Scope instrument (Sina, Zürich, Switzerland) calibrated using saturated salt solutions, ranging from  $a_w$  0.643 to  $a_w$  0.980 (Robinson & Stokes, 1955).

*Growth and observation of fungi.* Inoculation and examination of fungal germination and growth were as described by Pitt & Hocking (1977). Incubation was at 25 °C for a maximum of 100 d.

## RESULTS

### Germination

Representative data for germination times of the fungi studied are shown in Fig. 1, in which log reciprocal germination times are plotted against water activity. Data for three fungi, *B. halophila* FRR 2608, *P. pisce* and *Eurotium* sp. FRR 2471 are shown in detail, as germination times on the three solutes were obviously different. For the other fungi, data are shown for only one solute, as solute type did not markedly affect germination time over most of the  $a_w$  range studied.

The minimum  $a_w$  values for germination of all the fungi on media containing the three different solutes are summarized in Table 1. All of the fungi germinated at the highest  $a_w$  tested (0.997) except *Eurotium* sp. FRR 2471, which failed to germinate on any solute above 0.935  $a_w$ . Germination times for most of the fungi were shortest on the high  $a_w$  media (0.997–0.970); however, germination of *B. halophila* occurred most rapidly between 0.95 and 0.90  $a_w$ , with an optimum at 0.91  $a_w$  on NaCl media. The optimum  $a_w$  for germination of *Eurotium* sp. FRR 2471 was very low, approximately 0.88. Five of the fungi germinated on the medium containing saturated NaCl ( $a_w$  0.747). *B. halophila* germinated within 6 d at 25 °C, but germination of *Geomyces* sp. took 90 d (Table 1). Spores of *E. repens* and *P. pisce* were the least tolerant of high NaCl concentrations and failed to germinate on salt media below 0.83  $a_w$ .

### Radial growth rates

Radial growth rates of seven of the fungi are shown in Fig. 2. Data for *E. repens* are not shown as they were qualitatively similar to those of *E. chevalieri* as reported by Pitt & Hocking (1977).

The effects of solute on growth were generally similar to those on germination, although more pronounced. The effect of solute on *A. wentii* and *A. penicilloides* was minimal (Fig. 2*a*). *P. pisce* grew more rapidly on NaCl than on glycerol at high  $a_w$ , and more rapidly on NaCl than on glucose/fructose over the entire  $a_w$  range studied, especially below 0.90  $a_w$  (Fig. 2*b*). *Ex. werneckii* showed faster growth on salt media, especially below 0.90  $a_w$ .

The most pronounced solute effects were shown by the remaining fungi. *Geomyces* sp. FRR 2375 was intolerant of NaCl (Fig. 2*c*), while *B. halophila* exhibited much more rapid growth on NaCl media than in the presence of the other solutes (Fig. 2*d*). Germination of *Eurotium* sp. FRR 2471 was restricted to below 0.936  $a_w$ ; growth from inocula other than spores, at higher values, has not been tested. This fungus was the most xerophilic studied, growing

Table 1. Minimum  $a_w$  for germination and corresponding germination times for eight xerophilic fungi

Solute . . .	NaCl		Glycerol		Glucose/fructose	
	Min. $a_w$	Germination time (d)	Min. $a_w$	Germination time (d)	Min. $a_w$	Germination time (d)
<i>B. halophila</i>	0.747	5.8	0.780	8.1	0.770	14
<i>P. pisce</i>	0.831	7.5	0.749*	7.1	0.770*	3.9
<i>Ex. werneckii</i>	0.747	25	0.780	25	0.770	15
<i>E. repens</i>	0.831	1.8	0.721	18	0.685*	85
<i>A. wentii</i>	0.791	6.7	0.749*	28	0.734	21
<i>A. penicilloides</i>	0.747	8.3	0.734*	14	0.770	5.8
<i>Geomyces</i> sp.	0.747*	90	0.749*	38	0.734*	90
<i>Eurotium</i> sp.	0.747	32	0.721	44	0.675	38

\* Although germination occurred at this  $a_w$ , the formation of at least microcolonies did not follow.

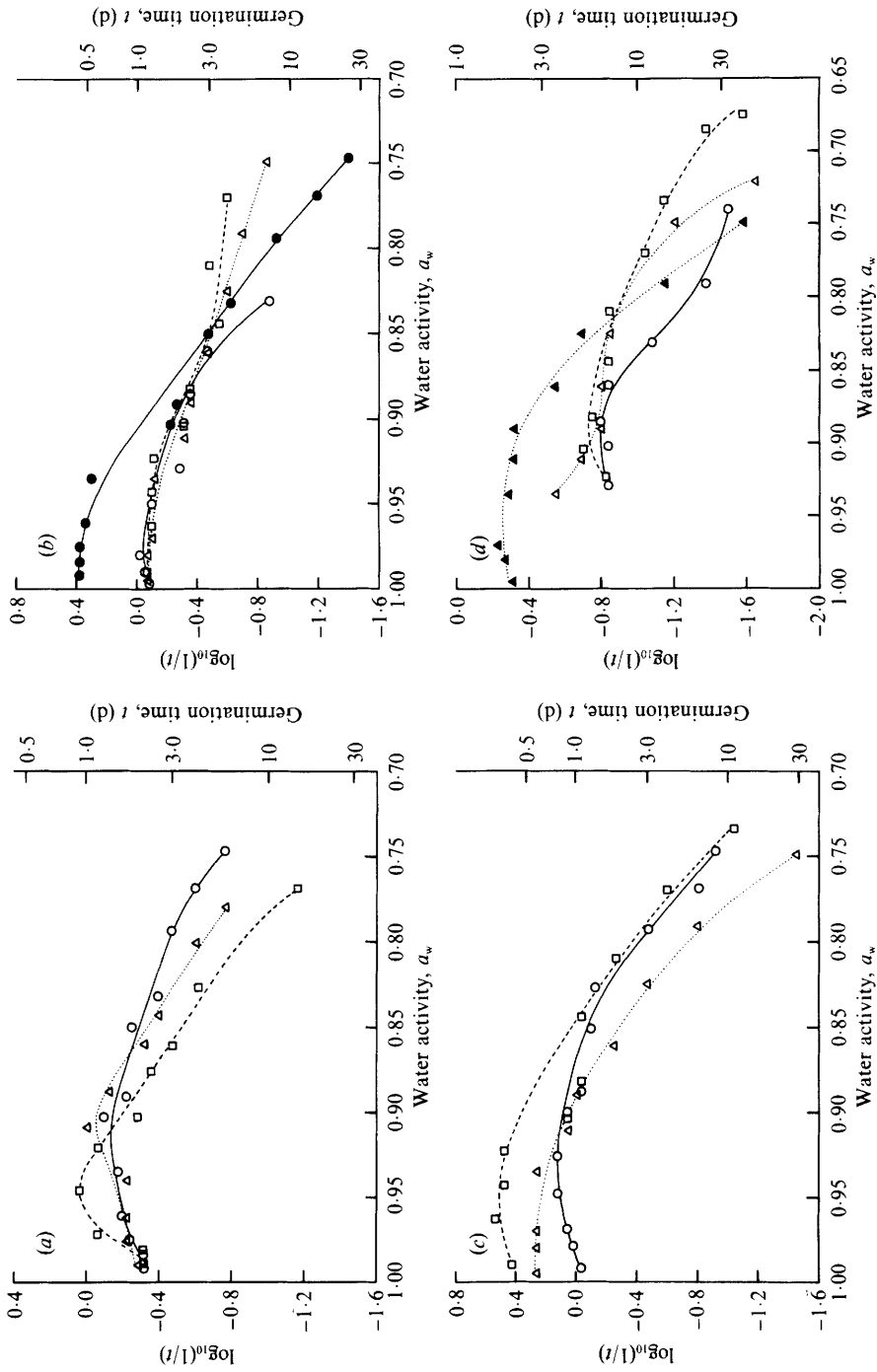


Fig. 1. Effect of  $a_w$  and solute on the time in days ( $t$ ) for germination of eight xerophilic fungi: (a) *B. halophila* FRR 2608 on NaCl (O), glucose/fructose (□), glycerol (Δ); (b) *Ex. werneckii* on NaCl (●), glucose/fructose (□), glycerol (Δ); (c) *E. repens* on glucose/fructose (□); *A. wentii* on glycerol (Δ); *A. penicilloides* on NaCl (O); (d) *Geomyces* sp. FRR 2375 on glycerol (▲); *Eurotium* sp. FRR 2471 on NaCl (O), glucose/fructose (□), glycerol (Δ). Where data from only one solute are shown, results from the other solutes were very similar.



optimally at 0.88–0.84  $a_w$ , and as low as 0.68  $a_w$ . Growth was markedly more rapid on glucose/fructose than on the other media. The other fungi studied grew at or near 0.997  $a_w$ , although growth of *A. penicilloides* and *B. halophila* was very slow at the higher water activities.

In general the minimum  $a_w$  for growth on the three solutes was the same as the minimum  $a_w$  for germination. In a few cases, where germination times were long, growth did not occur within the time limits of the experiment. Such cases are indicated in Table 1. The minimum recorded  $a_w$  for growth of both *P. pisce* and *Geomyces* sp. FRR 2375 was 0.79.

The optimal  $a_w$  values for growth of the fungi examined were as follows: *A. wentii*, 0.96–0.94; *P. pisce*, 0.96–0.94; *Ex. werneckii*, 0.96–0.93; *Geomyces* sp. FRR 2375, 0.95–0.92; *E. repens*, 0.95–0.91; *A. penicilloides* 0.93–0.90; *B. halophila*, 0.89–0.86; and *Eurotium* sp. FRR 2471, 0.88–0.84.

#### DISCUSSION

Pitt (1975) discussed the status of fungi which had been claimed to exhibit enhanced growth in NaCl solutions, i.e. were considered to be halophilic, and concluded that there was no evidence for halophilism in fungi. However, the experiments reported here demonstrate both the superior growth of *B. halophila* in media with NaCl as the major solute (Fig. 2*d*), and the ability to grow in media saturated with NaCl. Clearly, *B. halophila* is a halophilic fungus. In contrast with obligately halophilic bacteria, but in common with facultatively halophilic bacteria and the alga *Dunaliella* (Brown, 1976), growth of *B. halophila* is stimulated by NaCl but there is no obligate requirement. Two isolates of *B. halophila* from dried fish showed similar water relations, including enhanced growth in NaCl. A third strain isolated from indigo solution has also been examined, and shared these properties (unpublished observations), indicating that this is a stable physiological characteristic of this species.

Our results also indicate that *Ex. werneckii* and *P. pisce* can be considered to be halophilic because of their superior growth on media containing NaCl compared with glucose/fructose below 0.90  $a_w$ . *Ex. werneckii* was able to grow in the presence of saturated NaCl, although slowly. *A. penicilloides* also grew on media saturated with salt, but grew equally well on other solutes.

*Aspergillus* and *Eurotium* species are generally less well adapted to growth in the presence of high NaCl concentrations, as previously reported for *A. flavus*, *A. ochraceus* and *E. chevalieri* (Pitt & Hocking, 1977) and observed here for *E. repens*. The results reported here for *A. wentii* are noteworthy on three accounts: (1) the high growth rates on both NaCl- and glucose/fructose-based media near 0.95  $a_w$ ; (2) its strongly xerophilic nature, which exceeds that of most of the other frequently studied species of *Aspergillus*; and (3) its salt tolerance, as it exhibits the fastest growth of any fungus thus far studied on NaCl-based media down to 0.82  $a_w$  at 25 °C. The latter two observations are unexpected, as Pitt & Christian (1986) reported the minimum  $a_w$  for growth of *A. wentii* to be 0.84, and considered this species to be a xerophile of little consequence.

Pitt & Hocking (1977) suggested that the less specialised xerophilic fungi such as *Aspergillus* species were little affected by solute type, but that more highly adapted fungi, such as *Xeromyces bisporus* and *Chrysosporium fastidium*, were more sensitive, particularly to NaCl. The results of these studies confirm this. *B. halophila*, *Eurotium* sp. FRR 2471 and *Geomyces* sp. FRR 2375 can all be considered to be highly adapted xerophilic fungi. Like *X. bisporus* and *C. fastidium*, they have specialized growth requirements, and appear to have evolved to occupy specific ecological niches, *B. halophila* being successful in salt environments, while the others are adapted to high-sugar environments.

*Ex. werneckii* is particularly interesting, because it is usually isolated as a human pathogen, i.e. from environments of very high  $a_w$ . Adaptation to environments of low  $a_w$ , as reported by Mok *et al.* (1981) and confirmed here, is surprising.

Previous studies with xerophiles indicated that glycerol was a satisfactory solute in media of reduced  $a_w$  for the isolation and enumeration of xerophiles (Pitt & Hocking, 1977) and it was used as the basis of Dichloran 18% glycerol agar (DG18) (Hocking & Pitt, 1980). This medium has been effective for the isolation and enumeration of most xerophiles from dried and concentrated foods. However, the results shown above indicate that the halophilic fungi studied here grow poorly on glycerol. In examining foods high in NaCl an alternative isolation medium,

such as malt extract/yeast extract/5% salt/12% glucose agar (Pitt & Hocking, 1985a), is recommended for the isolation of halophilic fungi (Pitt & Hocking, 1985b).

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