Influence of Temperature on the Water Relations of *Polypaecilum pisce* and *Basipetospora halophila*, Two Halophilic Fungi

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Growth characteristics of *Polypaecilum pisce* and *Basipetospora halophila*, two fungi isolated from dried salt fish, were examined at 20, 30, 34 and 37 °C over a range of water activity (a_w) values (0.99–0.70) on media containing various concentrations of NaCl or glucose/fructose. At high a_w growth of *P. pisce* was similar on media containing either solute, but below 0.90 a_w growth was more rapid on NaCl media, indicating that *P. pisce* can be classified as a halophilic fungus. The minimum a_w for growth on NaCl-based media was 0.746; on glucose/fructose media the minimum was 0.700 a_w . Optimal growth, i.e. growth at 80% or more of the maximum growth, occurred between 0.96 and 0.91 a_w at 30 °C. Over the wide range of temperatures studied, *B. halophila* exhibited more rapid growth on NaCl media than on glucose/fructose media over the entire range of a_w at which growth occurred. The optimal growth rate on NaCl-based media occurred between 0.89 and 0.86 a_w , an exceptionally low value. *B. halophila* can be classified as an extreme halophile, markedly intolerant of high a_w , particularly on glucose/fructose-based media at temperatures above 30 °C.

INTRODUCTION

During a study of fungal spoilage of dried fish from Indonesia, several interesting fungi were encountered. The most prevalent fungus was a previously undescribed species, now named *Polypaecilum pisce* (Pitt & Hocking, 1985), which was isolated from 42% of the fish examined, in extreme cases appearing as conspicuous white growth over large areas of the fish surface (Wheeler *et al.*, 1986). Studies of growth at 25 °C in this laboratory indicated that it was a salt-tolerant xerophile (Andrews & Pitt, 1987). *Basipetospora halophila* (van Beyma) Pitt and Hocking (Pitt & Hocking, 1985) was isolated much less frequently from the fish, but preliminary studies (Andrews & Pitt, 1987) indicated that it grew at very low water activities (a_w) and that growth was stimulated by NaCl, although there was no obligate requirement. This paper describes further studies of the water relations of these fungi over a range of temperatures.

METHODS

Fungi. The isolates studied were *Polypaecilum pisce* FRR 2733 and *Basipetospora halophila* FRR 2787. FRR denotes the culture collection of the CSIRO Division of Food Processing, North Ryde, NSW 2113, Australia. Only one isolate of each species was studied. Andrews & Pitt (1987) have shown that the behaviour of different isolates of *B. halophila* was very similar.

Media. Media of various a_w values were prepared by adding NaCl or a mixture of equal weights of glucose and fructose to a basal medium containing 1% (w/v) malt extract, 1% (w/v) yeast extract, 0.1% dipotassium hydrogen phosphate and 2% (w/v) agar. Media with a_w values above 0.92 were sterilized by steaming for 15 min on three successive days, the remainder by a single 15 min steam treatment (Andrews & Pitt, 1987). Nominal a_w values of 0.99–0.70 were used in preparation of the media. The appropriate concentrations of controlling solutes were calculated using the data of Robinson & Stokes (1955) for media containing NaCl, and of Norrish (1966) for glucose/fructose media. Actual a_w values were determined with the Sina-scope instrument (Sina, Zurich, Switzerland).

Cultivation and examination. Inoculation of the fungi and observation of germination and growth were done as described by Pitt & Hocking (1977). Cultures were grown on Millipore Petri Slides containing approximately 2 ml medium. Inoculation was done by placing a needle point of mature spores at the centre of the slide. Incubation was for a maximum of 100 d at 20, 30, 34 and 37 °C. The Petri slides were examined regularly using a transmitted light microscope.

RESULTS

Germination

The minimum a_w values for germination are given in Table 1. *P. pisce* was least tolerant of low a_w at 20 °C, with germination not occurring below 0.799 a_w . At all temperatures, *P. pisce* germinated at lower a_w values on glucose/fructose media than on NaCl-based media (Table 1). However at 30 °C, i.e. close to its optimum temperature, *P. pisce* germinated on media containing saturated NaCl.

Germination of *B. halophila* occurred on the media containing saturated NaCl (0.750–0.746 a_w) at 20, 25, 30 and 34 °C, with most rapid germination occurring at 30 °C. At 37 °C the lowest a_w for germination was 0.770 on NaCl media (Table 1).

Representative data for germination times of *P. pisce* are shown in Fig. 1, in which log reciprocal germination time is plotted against a_w . Data are shown only for 30 °C, the temperature at which germination was most rapid, and for 20 °C, where germination was prolonged. Germination times at 34 and 37 °C were similar to, but slightly longer than, those at 30 °C. *P. pisce* was most tolerant of high concentrations of NaCl at 30 °C. Above 0.87 a_w germination at all temperatures occurred faster on media containing NaCl than glucose/fructose.

Germination data for *B. halophila* at three temperatures, 20, 30 and 37 °C are shown in Fig. 2. Germination times were shortest on media containing NaCl as the a_w -controlling solute. Temperature did not markedly affect germination except at 37 °C where germination on glucose/fructose medium was restricted to a_w values between 0.928 and 0.876, while germination on NaCl-based media occurred between 0.943 and 0.770 a_w .

At all temperatures examined *P. pisce* germinated at the highest a_w tested (0.998), while *B. halophila* failed to germinate above 0.963 a_w at 34 °C and 0.943 a_w at 37 °C.

Growth rates

Radial growth rates of *P. pisce* and *B. halophila* are shown in Figs 3 and 4, respectively. In general the effects of temperature and solute on growth were similar to those on germination.

As growth temperature was raised from 20 to 37 °C, the optimal a_w for growth of *P. pisce* shifted, from 0.95 or higher at 20 and 25 °C to 0.92–0.94 at 37 °C. Growth of *P. pisce* at 30, 34 and 37 °C was considerably faster than at 20 and 25 °C. *P. pisce* grew most rapidly at 30 °C, where, at optimal a_w , growth was two to three times faster than at 20 °C. Also, at 30 °C *P. pisce* grew at the lowest a_w tested for both media.

At most a_w values growth of *P. pisce* was favoured by the addition of NaCl to the medium. It was notable that at a_w values below the optima, particularly below 0.90 a_w , *P. pisce* grew more rapidly on NaCl than on glucose/fructose media (Fig. 3). For example, at 30 and 34 °C and 0.80 a_w growth rates on NaCl were nearly three times higher than those on the glucose/fructose media. This marked difference was still apparent at a_w values below 0.80. Growth above 0.90 a_w was less affected by solute or temperature, except at 20 °C where growth on glucose/fructose was more rapid than on NaCl media between 0.98 and 0.92 a_w .

B. halophila exhibited more rapid growth on NaCl media over the whole range of a_w at which growth occurred (Fig. 4a). Optimal growth, that is growth at 80% or more of the maximum growth recorded at a particular temperature, occurred at very low a_w values, between 0.93 and 0.86 on NaCl-based media and 0.93 and 0.91 on glucose/fructose media. Water activity for optimal growth of *B. halophila* at 20 °C was little affected by solute. However, at 25, 30 and 37 °C optimal growth was solute dependent. *B. halophila* achieved optimal growth at a considerably lower a_w on NaCl media than on glucose/fructose media at 25, 30 and 37 °C. At 34 °C the effect of solute was not as marked but optimal growth was still achieved at a lower a_w on NaCl-based media.

Table 1. Minimum a_w for germination of *P. pisce* and *B. halophila*

Fungus	Solute	20 °C		25 °C*		30 °C		34 °C		37 °C	
		Minimum a_w	Germination time (d)	Minimum a_w	Germination time (d)	Minimum a_w	Germination time (d)	Minimum a_w	Germination time (d)	Minimum a_w	Germination time (d)
<i>P. pisce</i>	NaCl	0.858	7.4	0.831	7.5	0.746‡	17.3	0.843	1.5	0.800	5.6
	Glucose/fructose	0.799	16.7	0.700	3.9†	0.705	8.2	0.762	6.2	0.788	5.6
<i>B. halophila</i>	NaCl	0.746‡	9.8	0.747‡	5.8	0.746‡	4.7†	0.750‡	8.6	0.770	6.0
	Glucose/fructose	0.766	26.8	0.700	14.0	0.776	12.0†	0.849	6.6	0.876	8.6†

* Data from Andrews & Pitt (1987).

† Although germination occurred at this a_w , the formation of at least microcolonies did not follow.‡ 0.750–0.746 a_w represents media containing saturated NaCl, the lowest a_w achievable with this solute.

Fig. 1. Effect of temperature and solute on the time taken (t) in days for germination of *P. pisce*. ■, □, 20 °C; ●, ○, 30 °C; ■, ●, glucose/fructose; □, ○, NaCl.

Fig. 2. Effect of temperature and solute on the time taken (t) in days for germination of *B. halophila*. ■, □, 20 °C; ●, ○, 30 °C; ▲, △, 37 °C; ■, ●, ▲, glucose/fructose; □, ○, △, NaCl.

Fig. 3. Effect of temperature on the radial growth rate of *P. pisce*. (a) NaCl; (b) glucose/fructose. ■, 20 °C; □, 25 °C; ●, 30 °C; ○, 34 °C; ▲, 37 °C.

Fig. 4. Effect of temperature on the radial growth rate of *B. halophila*. (a) NaCl; (b) glucose/fructose. ■, 20 °C; □, 25 °C; ●, 30 °C; ○, 34 °C; ▲, 37 °C.

At 30 °C on NaCl media optimal growth was achieved between 0.91 and 0.87 a_w (Fig. 4a). At 0.87 a_w growth was 40–50% faster on NaCl than on glucose/fructose. The most notable effect of temperature increase above 30 °C was a rapid decrease in growth rate below optimum a_w . At 0.87 a_w an increase of temperature from 30 to 34 °C resulted in a 30–40% decrease in the rate of growth of *B. halophila*. At 37 °C growth on NaCl at 0.86 a_w was only 28% of that at 30 °C.

B. halophila was markedly intolerant of high a_w , especially on glucose/fructose-based media. Growth was very slow at or above 0.95 a_w . As temperature increased tolerance of high a_w decreased considerably. The maximum a_w for growth on NaCl at 20 °C was 0.994 while at 37 °C it was only 0.858.

In general the minimum a_w required for growth of both species was the same as that required for germination. In only a few cases where germination times were long, growth did not occur within the duration of the experiment. This was not the case for *B. halophila* at high a_w : the maximum a_w at which germination occurred was always greater than that at which growth occurred.

DISCUSSION

In recent years the term halophile has been applied only to specialized bacteria. Pitt (1975) reported that at that time there were no known halophilic fungi: fungi reported to be halophilic, such as *Wallemia sebi*, had been shown to grow at least as well in glucose-based media as in salt-based media. The more rapid growth of *B. halophila* on NaCl media over the range of temperatures reported here indicates that it is a true halophile, in agreement with the recent statement of Andrews & Pitt (1987). Optimal growth occurred only in the presence of NaCl over the entire range of a_w values supporting growth. Under unfavourable conditions, i.e. at high a_w values and low temperatures, *B. halophila* may even have an obligate requirement for NaCl. It was very sensitive to high concentrations of sugars.

P. pisce was reported by Andrews & Pitt (1987) to exhibit superior growth below 0.90 a_w on media containing NaCl. It can also be reasonably described as a halophile. Remarkably, germination and growth of *P. pisce* occurred after 17 d on media containing saturated NaCl (0.746 a_w). The ability of *Polypaecilum* spores to germinate after such a long period on NaCl at a very low a_w indicates a special ability to survive in an environment destructive of the spores of most species.

For *P. pisce* at all temperatures studied, the a_w required for optimal growth was little affected by solute, but at low a_w radial growth rates were very much dependent upon solute; an important point when considering competition for colonization of a high-salt environment such as that encountered during drying of fish. The studies reported here indicate that *P. pisce*

appears to be uniquely suited to colonizing partially dried salt fish, and this offers an explanation for its frequent occurrence on this commodity (Wheeler *et al.*, 1986). *B. halophila*, although extremely salt tolerant, is much less likely to cause spoilage of drying or dried salt fish as its maximum growth rates are very low. The optimum growth temperature for both *B. halophila* and *P. pisce* was near 30 °C.

The establishment of the temperature–water relations of these fungi will aid work currently underway to revise the technology used to produce dried fish in tropical countries.

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