Effects of Water Activity on Growth and Sporulation of *Paecilomyces farinosus* in Liquid and Solid Media

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The effects of water activity on colony radial growth rate, specific growth rate, growth yield and blastospore production of *Paecilomyces farinosus* were studied. The compound (PEG 200) used to adjust water activity was not used for growth either when it was the sole carbon source in the medium or when the medium also contained glucose. Daily additions of water were made to cultures ('compensated' cultures) to compensate for evaporation; when this was not done the water activity of the medium decreased during incubation and a water activity at which growth ceased was eventually attained. A correlation was observed between the inhibitory effects of water activity on specific growth rate (μ) in 'compensated' shake flask cultures and on colony radial growth rate (K_r) on solid medium. Thus, K_r can be used to assess the effects of water activity on mould growth. Growth yield decreased linearly with decrease in water activity, and a decrease in water activity from 0.985 to 0.958 caused an approximately sixfold increase in the yield of blastospores per unit biomass dry weight.

INTRODUCTION

Trinci (1971) showed that the radial growth rate of a fungal colony (K_r) is a function of the width of its peripheral growth zone (w) and the organism's specific growth rate (μ). Thus,

$$K_r = w\mu \quad (1)$$

It follows from equation (1) that colony radial growth rate can only be used as a reliable indicator of specific growth rate when the width of the colony's peripheral growth zone is not altered by the experimental variable being considered. For example, temperature does not have an appreciable effect on peripheral growth zone width, and therefore colony radial growth rate can be used to determine the optimum temperature for mould growth (Trinci, 1971). By contrast, although L-sorbose has no appreciable effect on the maximum specific growth rate of *Neurospora crassa*, it causes a considerable decrease in peripheral growth zone width and hence in colony radial growth rate on solid medium (Trinci & Collinge, 1973). Similarly, although the colony radial growth rate of *Aspergillus nidulans* varies with glucose concentration over the range 0.01 to 80 g glucose l⁻¹ (Trinci, 1969), glucose concentration would only be expected to influence the mould's maximum specific growth at the lowest extreme of this range (Monod, 1942; Fiddy & Trinci, 1975). Thus, colony radial growth does not always provide a reliable way of assessing the effect of an environmental variable on mould growth.

Although colony radial growth rate is often used to determine the effect of water activity on fungal growth (Luard & Griffin, 1981; Luard, 1985; Kuthubutheen & Webster, 1986; Magan & Lynch, 1986; Eamus & Jennings, 1986), no study has been made to assess the validity of this practice. In this paper we show that when the growth rate of *Paecilomyces farinosus* is altered by changing the water activity of the medium, there is a direct relationship between specific growth rate in liquid media and colony radial growth rate on solid medium.

METHODS

Organism and media. *Paecilomyces farinosus* (Holm ex S. F. Gray) Brown & Smith was obtained from the Glasshouse Crops Research Institute, Littlehampton, UK. The defined medium (DM) used has been described previously (Trinci, 1971); it was buffered at pH 6.8 with 0.05 M (final concentration) phosphate buffer and was prepared and sterilized as described by Trinci (1971). When necessary, medium was solidified with agar (final concentration 15 g l⁻¹; Taiyo, Davis Gelatine). Polyethylene glycol of M_r 200 (PEG 200) was obtained from Sigma and was sterilized (121 °C for 15 min) in water (liquid medium) or water agar (solid medium); the final concentration of PEG 200 in media ranged from 0.5 to 1.25 M, giving water activities (a_w) of 0.985–0.947 for liquid media and 0.994–0.946 for solid media.

Measurement of water activity. The water potentials of liquid and solid media were determined using an HR 33T dew point microvoltmeter (Wescor) and water activities were calculated from these measurements (Griffin, 1981).

Inoculation, cultural conditions and growth measurements. Shake flask cultures were prepared by inoculating 20 ml volumes of media in 250 ml nephlos flasks (Trinci, 1972) with 1 ml volumes of a blastospore suspension. The blastospore inoculum was obtained by filtering stationary phase (65 h) cultures of *P. farinosus* through two layers of sterile lens tissue (Whatman). All shake flask cultures were incubated at 25 °C and 200 r.p.m. on a rotary shaker with a 2.5 cm stroke; incubations lasted up to 600 h (initial a_w 0.947). Under these experimental conditions each flask lost about 0.5 ml of water per day by evaporation. Therefore, in some experiments (lasting 350 h) daily additions of sterile distilled water (at 25 °C) were made to compensate for evaporation.

Petri dishes (9 cm diameter) containing 20 ml volumes of solid media were put in perspex boxes (28 × 16 × 10 cm) and left to equilibrate at 25 °C for 72 h. Five plates in each box were then inoculated with plugs (5 mm diameter) taken from a spread plate inoculated with 1.0 ml of a conidial suspension, and incubated at 25 °C for 24 h; the remaining five plates in each box were left uninoculated and helped to maintain the relative humidity in the box at an appropriate level.

Colony diameter was measured using a Shadowmaster (Baty & Co., Burgess Hill, Sussex, UK) with a ×10 objective. Culture optical density was measured using an EEL colorimeter (Evans Electroselenium) with a green filter (540–560 nm). Each measurement was the mean of three replicate flasks. Dry weights were determined after filtering cultures through previously washed, dried and weighed Whatman no. 5 filter papers in Buchner flasks. The biomass on the filter was washed with 100 ml distilled water and then dried at 70 °C to constant weight. Counts of blastospores were made in early stationary phase using an improved Neubauer haemocytometer. Each result was the mean of nine replicates and the standard error was determined. Analysis of variance (95% confidence level) was calculated after transformation of the data to log₁₀.

Glucose determinations. Glucose was measured using the Reflocheck system (Boehringer Mannheim).

RESULTS AND DISCUSSION

P. farinosus had a specific growth rate of 0.116 h⁻¹ on DM (a_w 0.985) and a slightly lower specific growth rate of 0.110 h⁻¹ on DM containing 0.5 M-PEG 200 (a_w 0.977). This result suggests that PEG 200 is inhibitory to growth because of its effect on water activity rather than because it is toxic. Fig. 1 shows growth of 'compensated' (daily additions of water to replace that lost by evaporation) and 'non-compensated' batch cultures of *P. farinosus*. Cessation of growth of 'compensated' cultures (initial a_w 0.949) was associated with glucose exhaustion. By contrast the deceleration in the rate of growth of the 'non-compensated' cultures (initial a_w 0.954) which occurred after 120 h incubation was associated with a decrease in water activity to about 0.940. After 325 h incubation, the 'non-compensated' cultures had stopped growing although the medium still contained 18.4 mM glucose; presumably evaporation had resulted in the establishment of a water activity (0.92) which was inhibitory to further growth. This method (allowing evaporation to increase water activity) could be used to determine the water activity at which mould growth ceases.

Figs 2 and 3 and Table 1 show the effects of water activity on growth and sporulation of 'compensated' shake flask cultures of *P. farinosus*. At water activities of 0.977 (DM with 0.5 M-PEG 200) and below, there was a reduction in specific growth rate with decrease in water activity of the medium; over the range of water activities from 0.947 to 0.977 there was a direct relationship between the logarithm of specific growth rate and the water activity of the medium (Fig. 3). The basis of this relationship is not known. Watson (1970) also found that the specific growth rate of batch cultures of *Saccharomyces cerevisiae* decreased with decrease in water activity of the medium.

PEG 200 was not used for growth of *P. farinosus* when it was the sole carbon source supplied in

Fig. 1. Growth of shake flask cultures of *P. farinosus* at 25 °C in DM containing 1.1 M-PEG 200. Daily additions of water were made to some cultures (●) to maintain the water activity approximately constant; no additions were made to other cultures (○). The water activities of 'compensated' (water added) (■) and 'non-compensated' (□) cultures, and the glucose concentrations of 'compensated' (▲) and 'non-compensated' (△) cultures were also measured.

Table 1. Effects of water activity on specific growth, colony radial growth rate, growth yield and sporulation of *P. farinosus* at 25 °C on DM containing various concentrations of PEG 200

Sterile distilled water was added to the shake flask cultures at daily intervals to compensate for evaporation.

Growth of 'compensated' shake flask cultures					Growth on solid medium	
a_w of medium	Specific growth rate, μ (h^{-1}) $\pm$ SEM*	Growth yield [g dry biomass (g glucose utilized) $^{-1}$]	Production of blastospores in early stationary phase*		a_w of medium	Colony radial growth rate, K_r ($\mu\text{m h}^{-1}$) $\pm$ SEM†
			Spores (ml culture) $^{-1}$ $\pm$ SEM‡	Spores (mg biomass) $^{-1}$		
0.985§	0.116 $\pm$ 0.002	0.506	(1.7 $\pm$ 0.12) $\times 10^7$ a	3.4 $\times 10^6$	0.994§	67.5 $\pm$ 1.7
0.977	0.110 $\pm$ 0.003	0.425	(5.3 $\pm$ 0.30) $\times 10^7$ b	1.2 $\times 10^7$	0.981	65.2 $\pm$ 0.5
0.966	0.055 $\pm$ 0.003	0.323	(5.7 $\pm$ 0.52) $\times 10^7$ b	1.7 $\times 10^7$	0.972	48.5 $\pm$ 0.7
0.958	0.028 $\pm$ 0.001	0.215	(4.1 $\pm$ 0.25) $\times 10^7$ b	1.9 $\times 10^7$	0.963	34.2 $\pm$ 0.5
0.951	0.017 $\pm$ 0.001	0.137	(2.0 $\pm$ 0.17) $\times 10^7$ a	1.5 $\times 10^7$	0.954	13.5 $\pm$ 0.4
0.947	0.010 $\pm$ 0.001	0.112	(6.4 $\pm$ 0.51) $\times 10^6$ c	5.7 $\times 10^6$	0.946	7.5 $\pm$ 0.3

* Each result is the mean of three cultures.

† Each result is the mean of five cultures.

‡ Each result is the mean of nine replicates. Results with different letters (a, b or c) are significantly different at the 95% confidence level (calculated by analysis of variance using $\log_{10}$ transformed data).

§ DM lacking PEG.

|| DM containing 0.5 M-PEG.

the medium (result not shown), and 0.5 M-PEG (final concentration in the medium) did not have an appreciable effect on specific growth rate or cause an increase in the final biomass of cultures grown on glucose (Table 1). However, bacteria which can utilize PEG 200 as a carbon source have been isolated and characterized (Kawai *et al.*, 1984).

The growth yield [g biomass produced (g glucose utilized) $^{-1}$] of *P. farinosus* was reduced from

Fig. 2

Fig. 2. Growth and glucose concentrations of 'compensated' (daily additions of water) shake flask cultures of *P. farinosus* at 25 °C in DM (●) and in DM containing various concentrations of PEG 200; the water activities of the media were 0.985 (●), 0.977 (○), 0.966 (■), 0.958 (□), 0.957 (▲) and 0.947 (△).

Fig. 3

Fig. 3. Effect of water activity on the specific growth rate of 'compensated' (daily additions of water) shake flask cultures of *P. farinosus* at 25 °C on DM (○) and DM containing various concentrations of PEG 200 (●).

0.506 at a_w 0.985 (DM) to 0.112 at a_w 0.947 (DM with 1.25 M-PEG 200) (Table 1) and a linear relationship was observed between decrease in the water activity of the medium and decrease in growth yield (Fig. 4). This relationship reflects the increased requirement of *P. farinosus* for maintenance energy (Pirt, 1965) at low water activities and this can be largely attributed to the increased requirement of the organism for energy for 'osmotic work' and the reduction in its specific growth rate. Watson (1970) also found that the growth yield of *S. cerevisiae* in batch culture was reduced when the water activity of the medium was lowered by addition of NaCl.

The effects of water activity on the specific growth rate of *P. farinosus* in submerged culture and on colony radial growth rate on solid medium are compared in Fig. 5. The results suggest that specific growth rate and colony radial growth rate are affected by water activity to a similar extent and that therefore colony radial growth rate is a valid way of assessing the effect of water activity on mould growth. The result also suggests that, over the PEG 200 range tested, water activity has no appreciable effect on colony peripheral growth zone width (equation 1).

Finally, Table 1 shows the effect of water activity on the number of blastospores produced by *P. farinosus* in submerged culture; at low water activity there was a significant increase in

Fig. 4

Fig. 5

Fig. 4. Effect of water activity on the growth rate of 'compensated' (daily additions of water) shake flask cultures of *P. farinosus* at 25 °C on DM (○) and DM containing various concentrations of PEG 200 (●).

Fig. 5. Comparison of the effects of water activity on the specific growth rate (□, ○) and colony radial growth rate (■, ●) of cultures of *P. farinosus* at 25 °C on DM (□, ■) and DM containing various concentrations of PEG 200 (○, ●). Growth rates are expressed as percentages of the growth rate obtained on DM alone.

number of blastospores formed per unit biomass. *P. farinosus* is a pathogen of brown planthopper (*Nilaparvata lugens*) and the present results suggest that blastospore yield of this fungus in submerged culture may be increased by adjusting the water activity of the culture medium.

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