The Relationship between Hyphal Branching, Specific Growth Rate and Colony Radial Growth Rate in *Penicillium chrysogenum*

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INTRODUCTION

The rate at which fungal colonies grow on agar has been related to their growth rate in submerged culture by using the concept of a peripheral annulus in which the mycelium grows exponentially (Trinci, 1971). It was supposed that further inside the colony the mycelium is depleted of substrate and does not contribute to radial extension of the colony. By measuring the width of the peripheral growth zone (w) and the linear colony radial growth rate (K_r), Trinci used eqn 1 (below) to calculate the specific growth rate (μ) for nine species of fungi. He found that the computed value coincided with the specific growth rate measured in submerged culture. We supposed that the colony radial growth rate would also be affected by the hyphal density or branching frequency, which may be a determinant of the width of the peripheral growth zone. The spreading colonial mutants of *Neurospora crassa* which have smaller colony radial growth rates but similar specific growth rates (Trinci, 1973*a*) had a higher branching frequency than the wild type (Trinci, 1973*b*). Bainbridge & Trinci (1970) noted that a mutant of *Aspergillus nidulans*, more branched than its parent, had an almost identical specific growth rate in submerged culture, but a substantially lower colony radial growth rate and a smaller peripheral growth zone when cultured on agar. The present work was undertaken to establish a quantitative relationship between colony growth rate and specific growth rate and hyphal branching in submerged cultures.

The expression used by Pirt (1967) and Trinci (1971) to represent the linear colony radial growth rate was

$$\frac{dr}{dt} = \mu w = K_r. \quad (1)$$

This can be re-written

$$\frac{dr}{dt} = \mu b k, \quad (2)$$

in which b is a measurement of hyphal density and k a constant. Hyphal density can be directly related to branching frequency if the internode length is used or the total hyphal length divided by the number of growing tips. We have used eqn 2 to relate measurements of specific growth rate and hyphal branching in submerged culture to colony radial growth rate on agar.

METHODS

Organisms. *Penicillium chrysogenum* Q176 (CMI No. 37767), and a series of mutants of it with different colony radial growth rates, were used.

Media. The complex medium contained (g/l): glucose, 80; corn steep liquor (Garton and Sons Ltd, London), as nitrogen source, 3.0; CaCO_3 , 10. pH was adjusted to 5.5 with 10 N-

Table 1. *Growth characters of seven strains of Penicillium chrysogenum in submerged and surface culture on complex medium*

Strain	Submerged culture		Agar surface culture	
	Specific growth rate (h ⁻¹)	Hyphal growth unit (μm)	Colony radial growth rate (μm/h)	k†
Q176	0.13	99 ± 8*	172 ± 9*	13.5 ± 1.2*
BG _A	0.21	53 ± 4	94 ± 7	8.5 ± 0.9
C5	0.20	51 ± 3	75 ± 8	7.4 ± 0.9
A	0.30	44 ± 3	83 ± 18	6.3 ± 1.4
T14	0.24	43 ± 10	101 ± 6	9.7 ± 2.3
C2	0.17	38 ± 2	58 ± 3	8.9 ± 0.7
B	0.28	29 ± 2	67 ± 5	8.3 ± 0.8

* $P = 0.05$.

† Calculated using eqn (2).

KOH. The simple medium was that of Righelato, Trinci, Pirt & Peat (1968) containing 30 g glucose/l. For submerged cultures 15 ml portions were dispensed in 100 ml conical flasks. For solid cultures 20 g agar/l were added and, after sterilization, 20 ml portions were dispensed into sterile 85 mm diameter Petri dishes. All media were autoclaved at 121 °C for 20 min.

Measurement of growth rate and branching in shake flasks. We inoculated 10⁷ conidia into flasks containing 15 ml complex growth medium. The flasks were shaken at 25 °C and 220 rev./min on an orbital shaker with a 5 cm throw. Every three hours four flasks were removed from the shaker, their contents bulked in pairs and 20 ml samples taken, which were filtered and washed with 20 ml, then 10 ml, water. Whatman No. 54 filter papers (55 mm) were used, pre-dried and weighed. Mycelial pads were dried on the filter papers at 90 °C for 24 h, placed in a desiccator to cool and then weighed. The natural logarithm of the mycelium dry weight was plotted against time and a straight line fitted through the points between 2 and 12 g mycelium dry wt/l. The slope of the line represented the specific growth rate.

Samples of mycelium were fixed in a mixture of (ml): formalin, 13; glacial acetic acid, 5; ethanol, 100; water, 100. Hyphal growth units were estimated by measuring the total length of hyphae in intact pieces of mycelium at × 150 magnification, by using a micrometer eye-piece, and dividing the length by the number of hyphal tips. At least ten pieces of mycelium were measured for each estimate of hyphal growth unit.

Chemostat cultures were operated by using the medium and conditions described previously (Righelato *et al.* 1968).

Measurement of colony radial growth rate. Conidia were inoculated on to agar plates to give 10 to 20 colonies/plate. Incubation was at 25 °C and each day the radii of ten isolated colonies were measured by vernier callipers or by a micrometer eye-piece, depending on colony size.

RESULTS AND DISCUSSION

A survey of seven strains of *Penicillium chrysogenum* (Table 1) showed a fourfold variation in hyphal branching frequency, measured as hyphal growth units. The maximum specific growth rates of the strains varied independently of the branching frequency over a twofold range. Colony radial growth rate varied over a threefold range. Assuming the model described by eqn 2 to be valid, the dimensionless constant k , calculated by dividing the colony radial

growth rate by the product of the hyphal growth unit and specific growth rate, will be common to all the strains. Values of k for individual strains were between 6.3 and 9.7, with the exception of strain Q176 which had a value of 13.5. Closer inspection showed the hyphae of this strain to be thinner: 4 μm diam compared with 5 to 6 μm for the other strains. As the hyphal extension rate is assumed to be a function of the mass growth rate, thinner hyphae would be expected to extend at a faster rate, the branching frequencies being equal. Thus, the smaller hyphal diameter of strain Q176 could have accounted for the very high radial growth rate of its colonies.

The width of the peripheral growth zone can be calculated by dividing the colony radial growth rate by the specific growth rate. With the exception of strain Q176, the calculated widths ranged from 448 μm for strain BG_A, to 239 μm for strain B. These peripheral growth zones were too small for accurate direct measurement by the method of Trinci (1971).

The comparison of the seven strains of *P. chrysogenum* suggests that hyphal branching frequency is an important determinant of the width of the peripheral growth zone of fungal colonies and hence their radial growth rate. Most of the data fitted the model relating colony growth rate to hyphal branching but the model could probably be made more comprehensive by including a term for hyphal diameter.

In the experiment described above the strains were grown at their maximum specific growth rates on a complex medium, and the observed branching frequencies were reproducible characteristics of the strains under those conditions. However, the branching frequency of any one strain is thought to increase when specific growth rate increases. Katz, Goldstein & Rosenberger (1972) proposed that for an exponentially growing mycelium the rate of extension of a hyphal tip reaches a maximum characteristic of the strain. The mass of the hyphal system would only continue to grow exponentially if a new tip were formed. These workers observed that the branching frequency of *Aspergillus nidulans*, on media supporting three different growth rates, increased with increasing specific growth rate. We observed a similar relationship occurring with *P. chrysogenum*. The hyphal growth unit of strain T14 was $43 \pm 10 \mu\text{m}/\text{tip}$ in the complex medium and $60 \pm 9 \mu\text{m}/\text{tip}$ in the simple medium. The specific growth rates were 0.24 h^{-1} and 0.14 h^{-1} respectively. Another test was made of the relationship between growth rate and hyphal growth unit, using the simple medium and with growth rate controlled by chemostat culture. Increasing the growth rate from 0.05 h^{-1} to 0.12 h^{-1} brought about a decrease in the hyphal growth unit from $52 \pm 15 \mu\text{m}$ to $36 \pm 10 \mu\text{m}$. The hyphal growth unit in the chemostat was smaller than in shake-flask culture at a similar growth rate. This may be related to the increased hyphal diameter in the chemostat, 6.5 μm , compared with 5.5 μm in shake flasks, or possibly to shear damage by the stirrer. The results of these two experiments confirm that hyphal branching decreases when the specific growth rate is decreased by nutritional limitations.

Pirt (1973) used colony radial growth rate (K_r) measurements at different substrate concentrations to estimate the substrate affinity constant (K_s), by a double reciprocal plot of K_r and substrate concentration. This method requires that the peripheral growth zone does not change when the specific growth rate is decreased by using media with substrates at growth-limiting concentrations. In a chemostat the specific growth rate is controlled by the concentration of an essential substrate. Our chemostat experiment showed that the hyphal growth unit increased as the growth rate was decreased by lowering the substrate concentration. If a similar change occurs on agar, the peripheral growth zone will increase with decreasing substrate concentration (Trinci, 1971) which would account for the increased radial growth rate for *Aspergillus nidulans* and *Mucor hiemalis* at low substrate concentrations (Trinci, 1969). We inferred, from our measurements of branching frequency and specific growth rates in submerged culture, that the width of the peripheral growth zone of colonies

growing on agar changes as the specific growth rate changes. Accurate measurement of the hyphal growth unit and the peripheral growth zone on agar was not practicable. The strains of *P. chrysogenum* used in this study had high branching frequencies and hence dense colony edges and narrow peripheral growth zones. A more detailed analysis could be made using moulds with lower branching frequencies.

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