Estimation of Substrate Affinities (K_s Values) of Filamentous Fungi from Colony Growth Rates

By S. J. PIRT

Microbiology Department, Queen Elizabeth College (University of London),
Campden Hill, London, W.8

(Received 6 November 1972)

The analysis described below indicates that the relation between growth-limiting substrate concentration and the rate of spread of a fungal colony on agar represents the actual relation between the biomass specific growth rate and substrate concentration, whereas this is not so for bacterial colonies.

Colonies of filamentous fungi on a solid substrate such as nutrient agar increase in radius at a constant rate represented by the equation

$$r = K_r t + r_0 \quad (1)$$

where r is the radius at time t and r_0 is the radius at time zero. It has been found that $K_r = \mu w$ where μ is the specific growth rate (t^{-1}) of the mycelium and w is the width of the growing zone at the periphery of the colony (Trinci, 1971). Thus K_r is a direct measure of the specific growth rate of a mould provided that w is constant, a condition which appears to be generally met (Trinci, 1971). Relation (1) also holds for bacterial colonies for a limited period (Pirt, 1967), but for the bacterial colony $K_r \propto \mu^{\frac{1}{2}}$.

Also for the bacterial colony it is found that $K_r \propto s_0^{\frac{1}{2}}$ where s_0 is the initial concentration of growth-limiting substrate. This relation between K_r and s_0 for bacterial colonies probably reflects the development of a concentration gradient of nutrient around a bacterial colony. That this concentration gradient must extend far from the boundary of a bacterial colony is shown by the calculations and experiments of Cooper, Dean & Hinshelwood (1968).

The linear relation between K_r and the square root of the initial concentration of growth-limiting substrate does not hold for fungal colonies. Gillie (1968) and Trinci (1969) suggested that $K_r \propto \log s_0$ but this holds over only a small range of s_0 values. The colony growth rates reach their maximum values at extremely low concentrations of growth-limiting substrate, for example, < 0.1 g glucose/l for *Mucor hiemalis*. In contrast, in bacterial colonies the maximum K_r value is not attained until the carbon source concentration is 2.0 g/l or greater. Furthermore, this upper limiting s_0 value for glucose in a bacterial colony can be raised several-fold by increasing the oxygen partial pressure; however, the limiting glucose concentration for a mould cannot be so increased. The absence of any apparent limitation of mould colony growth rate by substrate concentration except at extremely low values suggests that the relation between K_r and s_0 for a mould colony is similar to that expected between specific growth rate and growth-limiting substrate concentration (s) formulated by Monod (1942), i.e.,

$$\mu = \mu_m s / (s + K_s) \quad (2)$$

in which μ_m is the theoretical maximum specific growth rate and K_s , known as the 'saturation constant', is an inverse measure of the organism's affinity for the substrate. Conformation of data to relation (2) can be tested for by making a Lineweaver-Burk plot of $1/\mu$ against $1/s$, when a straight line should be obtained. For a fungal colony K_r can be substituted for μ ; data from the work of Gillie (1968) and of Trinci (1969) plotted in this way (Fig. 1) give

Fig. 1. Plots of the reciprocal of radial colony growth rate (K_r) against the reciprocal of growth-limiting substrate concentration in nutrient agar for: *Mucor hiemalis* (glucose limited), $\triangle$, and *Aspergillus nidulans* (strain BWB 480) (glucose limited), $\circ$, based on data of Trinci (1969); *Neurospora crassa* (arginine limited), $\times$, based on data of Gillie (1968). Ordinates (h.mm^{-1}): A, *M. hiemalis*; B, *A. nidulans*; C, *N. crassa*. Abscissae: D (l.g^{-1}), *M. hiemalis*, *A. nidulans*; E (l.mg^{-1}), *N. crassa*.

good straight lines until K_r is near the theoretical maximum. With glucose as growth-limiting substrate, near the maximum growth rate, the graph deviates in the direction of lower growth rates. This effect is accounted for by a decrease in the width of the peripheral growth zone (Trinci, 1971). From the intercepts on the abscissa the K_s values obtained are: 5.0 mg glucose/l for *Aspergillus nidulans*; 4.24 mg glucose/l for *Mucor hiemalis* and 0.5 mg arginine/l for the *Neurospora crassa* auxotrophic mutant. The K_s values for glucose are about the same as those obtained for bacteria (Monod, 1942) and lower than those found for a yeast by chemostat culture (Pirt & Kurowski, 1970). There do not appear to be any previous estimates of K_s values for filamentous moulds. The K_s values of substrates for different micro-organisms have received little systematic study because of the technical difficulty involved in making the measurements with liquid cultures. In contrast, colony growth rate measurements present a relatively simple method for determining K_s values of organisms which grow in mycelial form. The K_s values are of fundamental importance for predicting microbial growth rates and for quantitative determination of the effects of growth inhibitors and stimulators.

These results indicate that there is no substantial difference between the resistance to nutrient diffusion between hypha and medium in the peripheral zone of a fungal colony on agar and the resistance in an agitated liquid culture. This similarity of the nutrient concentration gradients may be attributed to the rapid advance of the leading hyphae on solid medium and to the restriction of the hyphal density at low substrate concentrations.

Further tests are needed of the validity of the assumption that the width of the peripheral growth zone (w) is constant, particularly when the substrate is growth-limiting. It should be noted that impurities in the agar used in experiments may lead to erroneously high growth rates, especially at the lower growth-limiting substrate concentrations; therefore the agar should be selected with care and purified, and glassware thoroughly cleaned.

I thank my colleague Dr A. P. J. Trinci for allowing me to use his raw data for Fig. 1.

REFERENCES

- COOPER, A. L., DEAN, A. C. R. & HINSHELWOOD, C. (1968). Factors affecting the growth of bacterial colonies on agar plates. *Proceedings of Royal Society B* **171**, 175-199.
- GILLIE, O. J. (1968). Observations on the tube rate of measuring growth rate in *Neurospora crassa*. *Journal of General Microbiology* **51**, 185-194.
- MONOD, J. (1942). *Recherches sur la croissance des cultures bactériennes*. Paris: Hermann.
- PIRT, S. J. (1967). A kinetic study of the mode of growth of surface colonies of bacteria and fungi. *Journal of General Microbiology* **47**, 181-197.
- PIRT, S. J. & KUROWSKI, W. M. (1970). An extension of the theory of the chemostat with feedback of organisms. Its experimental realization with a yeast culture. *Journal of General Microbiology* **63**, 357-366.
- TRINCI, A. P. J. (1969). A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *Journal of General Microbiology* **57**, 11-24.
- TRINCI, A. P. J. (1971). Influence of the width of the peripheral growth zone on the radial growth rate of fungal colonies on solid media. *Journal of General Microbiology* **67**, 325-344.