

Measurement of Growth Rates of Streptomycetes: Comparison of Turbidimetric and Gravimetric Techniques

By T. H. FLOWERS AND S. T. WILLIAMS

Department of Botany, University of Liverpool, Liverpool L69 3BX

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INTRODUCTION

Growth rates of unicellular microbes are usually determined by measurements of culture turbidity or by cell counts. However, growth of filamentous microbes cannot be assessed by counting and the heterogeneous distribution of mycelium generally precludes accurate turbidimetric measurements. Therefore gravimetric determinations, which are laborious and less accurate at low concentration, are required. This partly explains the comparative lack of information on the growth kinetics of such microbes.

The growth kinetics of several fungi grown in submerged culture and on agar media have been studied by Trinci (1969, 1971*a, b*, 1972), who compared specific growth rates, radial extension of colonies and germ-tube growth rates. Most actinomycetes form stable filaments and are generally regarded as slow-growing microbes, but there have been few attempts to determine their growth rates accurately. Haines (1932) recorded exponential germ-tube extension rates of several unidentified isolates, while Marshall & Alexander (1960) claimed that the rate of increase of mass of a *Streptomyces* sp. and a *Nocardia* sp. in submerged culture fitted a cube root relationship. Recently, Schuhmann & Berger (1976) studied the growth kinetics of *Streptomyces hygroscopicus* on solid medium.

Since turbidity measurements can be used to estimate growth rates of fungi which have reasonably dispersed growth in submerged culture (Trinci, 1972; Caldwell & Trinci, 1973), we decided to evaluate the use of turbidimetric methods for determining the specific growth rates of streptomycetes.

METHODS

A preliminary study of the growth form of neutrophilic and acidophilic streptomycetes in several media was made. A medium supporting good, dispersed growth was selected. This was prepared and sterilized in four parts to prevent precipitation: (i) glucose, 1.0 %; (ii) K_2HPO_4 , 0.35 %; (iii) $MgSO_4 \cdot 7H_2O$, 0.05 %; NaCl, 0.05 %; (iv) vitamin-free Casamino acids (Difco), 0.1 %; yeast extract (Difco), 0.1 %; $FeSO_4 \cdot 7H_2O$, 0.001 %; citric acid, 0.001 %; L-proline or L-asparagine, 0.1 %. The concentrations given are the final ones in the medium after mixing the parts. For optimum growth of neutrophilic strains, the medium contained L-asparagine (GCA) and the pH was adjusted to 7.0. Acidophiles were grown with L-proline (GCP) at pH 4.0.

A preliminary survey of the growth form of 22 neutrophilic and 22 acidophilic soil isolates was carried out. These were grown in 40 ml GCA or GCP medium in 250 ml conical flasks at 30 °C in an orbital incubator rotating at 250 rev. min⁻¹. The amount and form of growth was assessed by microscopic examination after 48 h.

Twelve strains grew in a reasonably dispersed form and from these two neutrophiles (F6 and F13) and two acidophiles (MR12 and SW1) were selected for detailed study. Six others

were used solely for turbidimetric determinations of growth rates. Both of the selected neutrophiles were isolated from a pine forest sand (pH 7.5) at Freshfield, Merseyside. The acidophile MR12 was isolated from open cast coal mine spoil (pH 4.5) at Wisconsin, U.S.A., and SW1 from sand under *Calluna vulgaris* (pH 3.5) at Thurstaston, Merseyside.

GCA and GCP media were dispersed in 40 ml volumes into 250 ml conical flasks, each of which had a 20 ml test tube of 15 mm diameter fused to its side. Similar flasks were used by Trinci (1972). Inoculum of the selected strains was prepared by removing spores from plate cultures on oatmeal agar (pH 4.5 or 7.0) and placing them into vials containing 2.5 ml sterile water with a few glass beads. These were shaken vigorously for 5 min and the volume was then made up to 10 ml. Flasks were inoculated with 0.05 ml suspension, giving about 10^5 spores per ml of medium. Use of a more dilute spore suspension or actively growing mycelium led to pellet formation on incubation.

Readings of the turbidity in inoculated flasks were taken hourly. The culture was shaken and the side tube was filled by carefully tipping the flask, avoiding formation of air bubbles. The tube was then placed in an EEL nephelometer head and the reading on the previously standardized galvanometer was noted. Five replicate readings were taken for each flask culture.

Gravimetric determinations were made to calibrate nephelometer readings against dry weight of cells for each strain. They were also used for direct determinations of growth rates for comparison with those obtained from turbidimetric measurements. The mycelium in a flask was harvested by filtration through a tared filter (Sartorius; cellulose nitrate; pore size $0.2\ \mu\text{m}$), and then washed with deionized water and dried at 105°C to constant weight.

Turbidity measurements were converted to dry weight values using the calibration curve and plotted as \log_e dry weight against time. The best fit lines were determined by regression analysis for the linear part of each curve and the specific growth rate and doubling time were calculated. Direct determinations of dry weight were treated similarly.

Samples were taken from cultures at intervals during the exponential and stationary growth phases. Hyphae were sedimented by centrifuging, washed in deionized water and fixed in 10 % (w/v) buffered formalin (pH 7.2) for 24 h. They were then washed twice with deionized water and air dried on coverslips. The coverslips were fixed to metal specimen stubs, coated under vacuum with gold-palladium and examined with a Stereoscan electron microscope (Cambridge Scientific Instruments Ltd, Cambridge) at 20 kV.

RESULTS

A linear relationship between turbidity and dry weight was obtained for all four strains, up to a hyphal density of about $0.4\ \text{mg ml}^{-1}$ (Fig. 1). The relationship for the two neutrophiles (F6 and F13) ceased to be linear above this point due to production of small amounts of soluble brown pigment during the later stages of growth.

Replication of nephelometer readings taken from one flask at a given time was good. There was some variation between flasks due to differences in the length of the lag phase, but growth rates calculated from replicate flask cultures were very similar. Specific growth rates calculated from gravimetric measurements were slightly lower and more variable than those from turbidimetric measurements (Table 1). Rates for neutrophiles were generally a little higher than those for acidophiles.

During exponential growth, all four strains formed small aggregates consisting of a loose network of hyphae (Fig. 2a). Aggregates of SW1 were denser than the others and MR12 also

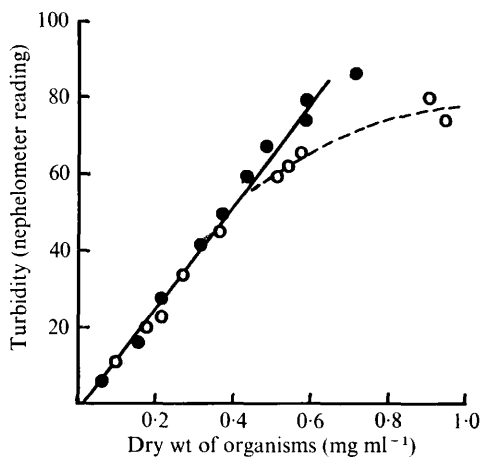


Fig. 1. Relationship between culture turbidity and dry weight of hyphae for neutrophile (○) and acidophile (●) streptomycetes.

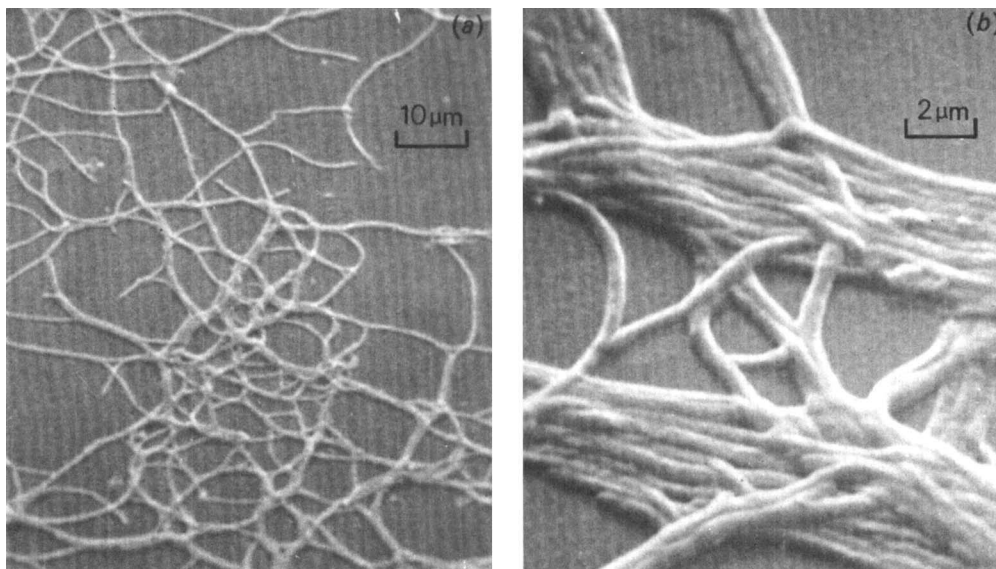


Fig. 2. Growth forms of streptomycete F6 during (a) the exponential phase, (b) the stationary phase. Scanning electron micrographs.

formed many short lengths of hyphae, probably by fragmentation. In the stationary phase, all strains formed hyphal ropes (Fig. 2b).

DISCUSSION

The results show that the growth rates of filamentous actinomycetes can be determined accurately from turbidity measurements, despite theoretical objections (Calam, 1969) to the use of such methods. It is necessary to ensure that reasonably dispersed growth occurs by selecting an appropriate medium, culture conditions and inoculum size. The comparative

Table 1. *Growth rates of streptomycetes determined by turbidimetric and gravimetric measurements*

Strains	Specific growth rate (h ⁻¹)		Doubling time (h)	
	Turbidimetric	Gravimetric	Turbidimetric	Gravimetric
Neutrophiles (pH 7.0, 30 °C)				
F6	0.642 ± 0.011	0.610 ± 0.333	1.08	1.14
F13	0.596 ± 0.008	0.583 ± 0.066	1.16	1.19
F9	0.530 ± 0.005	.	1.31	.
M9	0.620 ± 0.007	.	1.12	.
F15	0.556 ± 0.014	.	1.25	.
Acidophiles (pH 4.0, 30 °C)				
MR12	0.525 ± 0.005	0.494 ± 0.034	1.32	1.40
SW1	0.414 ± 0.009	0.368 ± 0.057	1.68	1.88
H	0.371 ± 0.004	.	1.87	.
SI5	0.249 ± 0.005	.	2.79	.
SW2	0.485 ± 0.009	.	1.43	.

convenience of turbidity measurements facilitates large numbers of determinations of hyphal mass.

Our results are similar to those of Trinci (1972) and Caldwell & Trinci (1973) who showed that turbidity measurements could be successfully used for determining the growth rates of some fungi. The doubling time of *Geotrichum candidum*, determined gravimetrically, was 2.0 h (Trinci 1971*b*), compared with 1.7 h when determined by turbidity measurements (Trinci, 1972). A similar difference between growth rates determined by gravimetric and turbidimetric measurements was noted here.

There is little previous information on the growth rates of actinomycetes with stable hyphae. Haines (1932) obtained values of 0.248 h⁻¹ to 0.576 h⁻¹ by measuring germ-tube extension rates, but the validity of this approach is questionable as the germ-tube specific growth rates of fungi are greater than those calculated from gravimetric measurements of growth in submerged culture (Trinci, 1971*a*). Schuhmann & Berger (1976) noted that germ tubes of *Streptomyces hygroscopicus* had a much higher growth rate than that of mycelium. Specific growth rates of a number of filamentous fungi have been determined by gravimetric methods (Trinci, 1971*b*, 1972). Values ranged from 0.102 h⁻¹ (*Mucor racemosus* at 25 °C) to 0.610 h⁻¹ (*Geotrichum candidum* at 30 °C). The latter is probably the highest fungal growth rate recorded (Trinci, 1972). Values recorded for some of the streptomycetes studied exceeded this and the growth rates were generally higher than those reported for fungi.

Further accurate determinations of the growth rates of other actinomycetes and the effect of culture conditions upon them are now needed.

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REFERENCES

- CALAM, C. T. (1969). The culture of micro-organisms in liquid medium. *Methods in Microbiology* **1**, 255-326.
- CALDWELL, I. Y. & TRINCI, A. P. J. (1973). The growth unit of the mould *Geotrichum candidum*. *Archiv für Mikrobiologie* **88**, 1-10.
- HAINES, R. B. (1932). The influence of temperature on the rate of growth of saprophytic actinomycetes. *Journal of Experimental Biology* **9**, 45-60.
- MARSHALL, K. C. & ALEXANDER, M. (1960). Growth characteristics of fungi and actinomycetes. *Journal of Bacteriology* **80**, 412-416.
- SCHUHMAN, E. & BERGER, F. (1976). Mikroskopische Untersuchungen zur Wachstumskinetik von *Streptomyces hygroscopicus*. *Zeitschrift für allgemeine Mikrobiologie* **16**, 201-215.
- 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 *a*). Experimental growth of the germ tubes of fungal spores. *Journal of General Microbiology* **67**, 345-348.
- TRINCI, A. P. J. (1971 *b*). Influence of the peripheral growth zone on the radial growth rate of fungal colonies. *Journal of General Microbiology* **67**, 325-344.
- TRINCI, A. P. J. (1972). Culture turbidity as a measure of mould growth. *Transactions of the British Mycological Society* **58**, 467-473.