Influence of Culture Conditions on the Physiology and Composition of *Trichoderma aureoviride*

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The biomass and residual limiting substrate profiles of chemostat grown *Trichoderma aureoviride* were typical of carbon- and of nitrogen-limited micro-organisms. Maintenance energy requirements partly accounted for the discrepancy between observed and predicted values of biomass concentration at low dilution rates under glucose limitation. At such low dilution rates the fungal mycelium became differentiated and sporulation occurred. The steady state glucose concentrations in glucose-limited cultures were higher than those predicted by chemostat theory; much closer agreement between observed and predicted values was achieved when a corrected $\mu_{\max}$ term (Pitt & Bull, 1982*a*) was used to calculate the residual glucose concentration. The measured growth parameters ($\mu_{\max}$, $\mu_{\min}$, K_s , Y and m) for *T. aureoviride* had values similar to those reported for other species of filamentous fungi. The biomass concentration of fed-batch cultures increased linearly with time but glucose supplied at a rate three times higher than the maintenance requirement was insufficient to prevent sporulation. Mycelial RNA and protein concentrations increased with increasing dilution rate and were 30 and 40% lower under nitrogen limitation than under carbon limitation. DNA concentration was not influenced by dilution rate. The molar ratio of RNA:Mg²⁺:K⁺ was 8:1:8 and was dilution rate independent. Only two polyamines, spermine and spermidine, were detected in *T. aureoviride*; they increased in concentration and proportion of the biomass as the dilution rate was raised but there was no evidence of their functional interchange ability with Mg²⁺ ions as reported for other fungi.

INTRODUCTION

Descriptions of the mycelial growth of fungi have been derived, with only very few exceptions, from studies of batch cultures either in liquid or on solid media. The use of continuous flow culture techniques to explore fungal physiology has been slight (Bull & Trinci, 1977) and even experimental tests of chemostat theory with these organisms are incomplete. Furthermore, understanding of the phenotypic variability of fungal chemistry and metabolism remains in a primitive state in comparison to our knowledge of bacteria.

This paper reports on studies of fungal behaviour in chemostat culture and attempts to refine the mathematical description of mycelial growth by taking into account a true specific growth rate (μ_h) based on the proportion of actively growing hyphae in the population (Pitt & Bull, 1982*a*). These analyses have been made with a wild-type strain of *Trichoderma aureoviride* and details of its macromolecular and cationic composition are also included.

METHODS

Organism and culture conditions. A strain of *Trichoderma aureoviride* Rifai agg. (Rifai, 1969; isolated by Chesters & Bull, 1963) was used throughout this study. Continuous culture of the fungus was carried out in a 3.0 l

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magnetically stirred fermenter (LH Engineering, Stoke Poges, Bucks) (Pitt & Bull, 1982*a*). The working volume, temperature, pH, agitation and aeration rates were maintained at 2.8 l, 30 °C, 4.5, 1000 rev. min⁻¹ and 2.8 l min⁻¹, respectively. Fed-batch cultures were established by reducing the volume of a steady state (dilution rate, $D = 0.10 \text{ h}^{-1}$) culture to 1.5 l, maintaining the operating conditions as stated above and feeding the remaining culture at a slow rate (about 21 ml h⁻¹) with complete medium containing 30 g glucose l⁻¹ in order to provide the desired input rate of glucose. The feed rate was calculated such that the initial supply of glucose to the culture was three times the maintenance ration [i.e. $0.060 \text{ g glucose (g biomass)}^{-1} \text{ h}^{-1}$]. This procedure was similar to that used by Bainbridge *et al.* (1971) to establish non-growing maintained and autolysing cultures of *Aspergillus nidulans*.

The chemostat was inoculated with a suspension of filamentous mycelia (about 150 ml) grown from a spore inoculum in a magnetically stirred flask culture. The growth media contained (g l⁻¹): (a) for glucose limitation – glucose, 10; (NH₄)₂SO₄, 5.0; K₂HPO₄, 0.75; Na₂HPO₄, 0.75; trace elements (Carter & Bull, 1969), 60 ml l⁻¹ of a $\times 20$ strength stock solution; (b) for ammonia limitation – as above except glucose, 25 and (NH₄)₂SO₄, 2.0. The trace element stock solution contained: Na₂EDTA.2H₂O, 12 g; MgSO₄.7H₂O, 5 g; CaCl₂ 1.0 g; ZnSO₄.7H₂O, 0.4 g; MnSO₄.4H₂O, 0.4 g; CuSO₄.5H₂O, 0.1 g; FeSO₄.7H₂O, 2.0 g (plus 0.5 ml concentrated H₂SO₄); Na₂SO₄, 8.5 g; Na₂MoO₄.2H₂O, 0.1 g; distilled water, 1 litre. The pH was controlled by the automatic addition of either 2 M-KOH or 0.75 M-H₂SO₄.

Mycelia were harvested from the fermenter via a spring-loaded, rapid sample device (Pitt & Bull, 1982*b*) which enabled approximately 20 ml of culture to be collected in a plastic vial containing 2 ml perchloric acid (30% v/v) in less than 1 s.

Analytical procedures. Biomass concentrations were determined in duplicate on 10 ml samples of culture passed through 0.45 μm pore filters, washed with isotonic saline and dried to constant weight overnight at 105 °C. Glucose was assayed with a commercial glucose oxidase kit (Boehringer Corporation) and, during ammonia-limited cultivation, the residual ammonia concentration was determined by an indophenol blue method (Allen *et al.*, 1974).

Mycelial samples for DNA (10 ml) and RNA (5 ml) determinations were extracted with HClO₄ (30%, v/v) and assayed by the diphenylamine and orcinol procedures, respectively (Herbert *et al.*, 1971). All samples were analysed soon after harvesting from the chemostat in order to avoid degradation during storage.

Protein was determined in fresh samples (0.5 ml and 1.0 ml) of the culture using the biuret method and bovine serum albumin as a standard (Herbert *et al.*, 1971). Mycelial magnesium was extracted with ice-cold 20% (v/v) HClO₄ according to the procedure of Herbert *et al.* (1971). A further extraction was made at 70 °C in order to ensure maximum release of structurally bound magnesium. Quantitative extraction of potassium from micro-organisms is difficult because of losses resulting from leakage during harvesting. Consequently, the intracellular potassium concentration was determined as the difference between that in the biomass plus supernatant (i.e. whole culture) and that in the supernatant alone, as described by Herbert *et al.* (1971). The final extracts for potassium and magnesium assays were diluted with 0.2% (w/v) aqueous lanthanum chloride in order to minimize chemical interference during subsequent analysis in an SP190 atomic absorption spectrophotometer (Pye Unicam).

Polyamines were extracted from the mycelium by the procedures described previously (Bushell & Bull, 1974) and separated on silica gel plates (Kieselgel G type 60; Merck). The development of chromatograms in the ethyl acetate/cyclohexane (2:3, v/v) of Dion & Herbst (1970) failed to resolve cadavarine and putrescine; the best separation of reference polyamines was achieved with ethyl acetate/petroleum ether (b.p. 40–60 °C) (3:5, v/v). Eluted polyamines were assayed spectrofluorimetrically (SFR100 ratio recording fluorimeter, Baird Atomic, Braintree, Essex) using an excitation wavelength of 350 nm (slit 5 nm) and emission wavelength of 490 nm (slit 10 nm). This method gave linear calibrations of cadavarine, putrescine, spermine and spermidine over the range 1 to 30 nmol ml⁻¹.

Calculations. The maximum specific growth rates of glucose-limited and ammonia-limited *T. aureoviride* were determined by a washout method (Jannasch, 1969). A plot of $\ln x$ against time (t) was analysed by linear regression to derive the gradient, which is equal to $(\mu_{\text{max}} - D)$.

The maintenance energy coefficient for glucose (m) and the specific maintenance rate (a) were calculated according to the method of Pirt (1975) using the specific rate of glucose consumption (q) in glucose-limited chemostat cultures. A plot of q against D gives m as the intercept and the inverse of the gradient is equivalent to the true growth yield, Y_G ; a is the product of $m \cdot Y_G$.

Theoretical steady state values for biomass ($\bar{x}$) and limiting substrate ($\bar{s}$) concentrations were calculated using the equations of Pirt (1975, p. 42) and took account of the maintenance energy requirement.

RESULTS

Kinetics of carbon-limited and ammonia-limited growth

The observed and calculated values for steady state biomass and residual substrate concentrations in glucose-limited cultures are presented in Fig. 1. The biomass concentration

Fig. 1. Observed and calculated values of biomass and growth-limiting substrate concentrations during glucose-limited culture of *Trichoderma aureoviride*: observed biomass (●); calculated biomass (—); observed glucose (■); calculated glucose (---); calculated glucose concentration using a corrected value for $\mu_{\max}$ (see Discussion) (Δ --- Δ).

Fig. 2. Observed concentrations of biomass (●) and ammonia (■) during ammonia-limited culture of *Trichoderma aureoviride*.

Fig. 3. Experimental growth yields of *Trichoderma aureoviride*: Y_{gluc} , glucose-limited growth (Δ); Y_{NH_3} , ammonia-limited growth ($\blacktriangle$).

Table 1. *Growth parameters for glucose- and ammonia-limited growth of Trichoderma aureoviride*

The following nomenclature is used in this paper: $\mu_{\max}$, maximum specific growth rate; $\mu_{\min}$, minimum specific growth rate; D_{crit} , critical dilution rate; Y , growth yield; Y_G , true or maximum growth yield; K_S , limiting substrate saturation constant; m , maintenance energy coefficient; a , specific maintenance rate.

Parameter	Nutrient limitation	
	Glucose	Ammonia
$\mu_{\max}$	0.22 h ⁻¹	0.20 h ⁻¹
$\mu_{\min}$ *	0.02–0.04 h ⁻¹	0.02–0.04 h ⁻¹
D_{crit} †	0.218 h ⁻¹	0.195 h ⁻¹
Y_{gluc} ‡	0.54 g biomass g ⁻¹	—
Y_G	0.64 g biomass g ⁻¹	—
Y_{NH_3} ‡	—	13.75 g biomass g ⁻¹
K_S (gluc)	0.102 g l ⁻¹	—
K_S (NH ₃)	—	0.048 g l ⁻¹
m	0.02 g biomass g ⁻¹ h ⁻¹	—
a	0.038 h ⁻¹	—

* Based on morphological differentiation (see Text).

† Critical dilution rate was calculated from the formula $D_{\text{crit}} = \mu_{\max} \cdot S_R / (S_R + K_S)$, where S_R is the concentration of limiting substrate in the inflowing medium; the observed value for $\mu_{\max}$ (glucose) was used in this calculation.

‡ Maximum observed values.

Fig. 4. Proportion (m/q_{glucose}) of the energy source used by *Trichoderma aureoviride* for maintenance purposes as a function of dilution rate in a glucose-limited culture.

was reasonably constant over most of the dilution rate range, a situation which was not observed when the fungus was grown under ammonia limitation (Fig. 2). The biomass yield from glucose obtained during glucose-limited growth was constant at about 0.54 g biomass (g glucose)⁻¹ over most of the dilution rate range which was investigated (0.07 to 0.18 h⁻¹) (Fig. 3). During ammonia-limited cultivation, the yield of biomass from ammonia showed a linear increase as the dilution rate was lowered from 0.16 h⁻¹ to 0.05 h⁻¹ [$Y_{\max} = 13.75$ g biomass (g NH₃)⁻¹]; the yield fell at the most extreme dilution rates investigated.

The substrate affinity coefficients for glucose and ammonia and the maintenance energy coefficient are given in Table 1 together with other growth parameters. The minimum specific growth rate ($\mu_{\min}$) for *T. aureoviride* was of the order 0.02 to 0.04 h⁻¹, as judged by the criterion of morphological differentiation of the mycelium. At dilution rates below 0.04 h⁻¹ the frequency of mycelial branching, hyphal swelling and vacuolation, increased progressively

Fig. 5. Fed-batch culture of *Trichoderma aureoviride*. Biomass (●), yield from glucose (△) and glucose feed rate expressed as a function of the maintenance requirement rate (□). A proportion of the culture was drawn off after 48 h to enable a further period of fed-batch operation.

and chlamydospore-like structures were constant features of the culture. The proportion of catabolized glucose which was used for maintenance varied as a function of the dilution rate (Fig. 4).

As D approached $\mu_{\max}$ only about 6% of the glucose consumed was used for maintenance but at the lowest dilution rates examined (0.024 h^{-1}) the maintenance energy requirement was equivalent to 36% of the catabolized glucose. The proportion of glucose consumed for maintenance purposes rose at a greater rate below a dilution rate of 0.06 h^{-1} and this coincided with the onset of morphological differentiation.

The behaviour of *T. aureoviride* at low specific growth rates also was studied in fed-batch culture. The addition of glucose to a culture previously growing at 0.10 h^{-1} (see Methods) was calculated to provide the total biomass with, initially, three times its maintenance energy requirement. This rate of addition was sufficient to allow a net increase in the culture biomass (Fig. 5). However, marked morphological differentiation occurred after about 30 h of fed-batch operation and, although poorly branched, non-autolysed hyphae were present throughout the 96 h of operation, the culture became progressively more differentiated with time. The presence of thick-walled, birefringent spores was clearly evident after about 48 h. These spores originated as terminal hyphal swellings and from fragmented hyphae and germinated without requiring a prior period of dormancy. Indeed, such spores were often seen to have associated germ tubes during the fed-batch cultivation period and still others were observed to have a short germ tube from which a terminal spore developed. During the course of fed-batch cultivation the biomass yield from glucose decreased linearly with time from about 0.70 to $0.44 \text{ g biomass (g glucose)}^{-1}$, whereas total biomass showed a linear increase with feed time (Fig. 5).

The rate of supply of glucose corresponded to an initial dilution rate in the fed-batch culture of 0.014 h^{-1} . The quasi-steady state specific growth rate in fed-batch culture can be calculated from the changes in biomass concentration with time (Pirt, 1975) and during the first 48 h of culture a value of 0.027 h^{-1} was calculated for *T. aureoviride*; this is equivalent to 12% $\mu_{\max}$. The biomass yields at this specific growth rate (i.e. 0.027 h^{-1}) were very similar for steady state chemostat [$0.45 \text{ g biomass (g glucose)}^{-1}$; Fig. 3] and fed-batch [$0.44 \text{ g biomass (g glucose)}^{-1}$; Fig. 5] culture. As shown in Fig. 1, steady state growth was established at a dilution rate as low as 0.004 h^{-1} . However, at this dilution rate the predominant morphology was unicellular, with many of the spores possessing germ tubes and the biomass yield was reduced to $0.34 \text{ g biomass (g glucose)}^{-1}$ (Fig. 3).

Fig. 6. Macromolecular composition of *Trichoderma aureoviride* (a) during glucose-limited growth and (b) during ammonia-limited growth. RNA (●), protein (○) and DNA (□). All values are expressed as a percentage of mycelial dry weight.

Macromolecular composition

The mycelial concentrations of RNA, DNA and protein for glucose-limited growth and of RNA and protein for ammonia-limited growth were measured over the range of dilution rates between $\mu_{\min}$ and $\mu_{\max}$ (Fig. 6*a, b*). The RNA concentration during glucose-limited growth was always higher than equivalent estimates made during ammonia limitation but under both limitations the maximum RNA contents were observed at about $0.8 \mu_{\max}$. Mycelial protein concentrations increased gradually as the dilution rate of glucose- and ammonia-limited chemostats was raised, although a sharp 20% increase was noted as D approached $\mu_{\max}$ under ammonia limitation. Growth under glucose limitation always resulted in protein contents of about 5 to 15% greater than those observed at equivalent dilution rates during nitrogen-limited growth. Maximum protein concentrations (50 to 55%) in both cases occurred as the dilution rate approached $\mu_{\max}$. In contrast to RNA and protein, the DNA concentration of glucose-limited mycelia was comparatively stable at approximately 0.4 to 0.5% dry weight over the dilution range studied. Calculations of the RNA efficiency in protein synthesis as a function of the dilution rate (Alroy & Tannenbaum, 1973; McGetrick & Bull, 1979) were made from the above data (Fig. 7).

Cation composition

Only two polyamines, spermidine and spermine, could be determined quantitatively during glucose-limited cultivation of *T. aureoviride* (Fig. 8*b*). However, a compound was detected that co-chromatographed with cadaverine, but its concentration was too low to permit its quantification. Spermidine was the major polyamine present at all growth rates and its concentration was markedly dependent upon the dilution rate. Spermine was present in much lower concentrations which did not show any obvious relationship to dilution rate. Extracellular polyamines were not detected under any conditions of carbon-limited growth.

The intracellular potassium concentration increased from about 250 to 550 $\mu\text{mol (g dry wt)}^{-1}$ over the dilution rate range investigated (Fig. 8*a*). Magnesium concentrations also increased with dilution rate (Fig. 8*a*) from approximately 30 to 90 $\mu\text{mol (g dry wt)}^{-1}$ with most of this increase [about 50 to 90 $\mu\text{mol (g dry wt)}^{-1}$] occurring above 0.14 h^{-1} .

Fig. 7. The efficiency of RNA in protein synthesis (E) during glucose-limited ($\square$) and ammonia-limited ($\blacksquare$) growth of *Trichoderma aureoviride*. A linear regression analysis was used to fit the glucose-limited plot (regression coefficient, 0.90).

Fig. 8. Cation composition of *Trichoderma aureoviride* during glucose-limited growth: (a) magnesium ($\square$), potassium ($\blacksquare$); (b) spermidine ($\bullet$), spermine ($\circ$).

Table 2. Molar ratios* of RNA, polyamines, Mg^{2+} and K^+ as functions of dilution rate in glucose-limited mycelia of *Trichoderma aureoviride*

Dilution rate (h^{-1})	RNA	Mg^{2+}	K^+	Mg^{2+}/K^+	Polyamines†	Polyamines + Mg^{2+} / RNA
0.02	1	0.15	1.25	0.12	0.07	0.22
0.06	1	0.20	1.04	0.19	0.07	0.27
0.10	1	0.13	1.06	0.12	0.15	0.28
0.14	1	0.12	0.90	0.13	0.14	0.26
0.17	1	0.14	0.78	0.18	0.23	0.37
0.20	1	0.18	1.15	0.16	0.29	0.47

* Values for polyamines, Mg^{2+} and K^+ are expressed as molar ratios of the concentration of RNA which is standardized.

† Spermidine plus spermine.

The molar ratios of Mg^{2+} , K^+ , polyamine and RNA in glucose-limited mycelia are presented in Table 2. Although the molar concentrations of all of these constituents increased with increasing dilution rate, the molar ratio, $RNA:Mg^{2+}:K^+$ appeared reasonably constant at approximately 8:1:8. The polyamine concentration, on the other hand, increased relative to these other constituents as the dilution rate was increased and, relative to the RNA concentration, showed a maximum increase of approximately fourfold.

DISCUSSION

The behaviour of *T. aureoviride* in chemostats is generally in agreement with that reported for the few other species of filamentous fungi studied in continuous-flow cultures (Bull & Trinci, 1977). The biomass concentration profiles as a function of dilution rate are typical of carbon- and of nitrogen-limited micro-organisms (see, Herbert, 1961). It is reasonable to assume that the increasing biomass concentration with decreasing dilution rate under ammonia limitation is a reflection of carbon storage synthesis, probably glycogen and/or lipid, but we have not investigated the nature of such compounds. Of interest is the degree of morphological differentiation which occurred at low specific growth rates, a feature common among fungi. Such dilution rate-dependent heterogeneity may be responsible, in part, for the divergence of the experimental data and the calculated values for steady state biomass and limiting glucose concentrations (Fig. 1). This divergence is not fully accounted for by maintenance energy expenditure, particularly when *T. aureoviride* was growing at dilution rates less than 0.02 h^{-1} despite the fact that 38% of the glucose consumed is utilized for maintenance purposes at this dilution rate (Fig. 4). The equation for steady state limiting substrate concentration suggests three possible explanations for the disagreement between observed and calculated glucose concentrations: (1) catabolism of glucose continued during sample collection and manipulation, thereby producing falsely low values; (2) the value determined for the glucose saturation constant (K_s) was falsely high; or (3) the value determined for the maximum specific growth rate ($\mu_{\max}$) was falsely low.

The first explanation is extremely unlikely; samples were collected and inactivated in perchloric acid within approximately 1 s and it can be calculated from the steady state q_{glucose} values that less than 0.1% of the glucose in the sample would be catabolized during this period. This also argues against an overestimate of the K_s being made. The $\mu_{\max}$ of glucose-limited *T. aureoviride* was determined from washout kinetics to be 0.22 h^{-1} . Recently we have shown, via autoradiographic analysis, that the true specific growth rate of growing hyphae in chemostat culture (μ_h) can be significantly higher than the apparent specific growth rate which, according to chemostat theory, is equivalent to the imposed dilution rate at steady state (Pitt & Bull, 1982a). Consequently, the true maximum specific growth rate of *T. aureoviride* is underestimated by about 25% and the corrected value becomes 0.29 h^{-1} . As shown in Fig. 1, use of this revised value of $\mu_{\max}$ produced calculated concentrations of the limiting substrate that are in very much closer accordance with the observed concentrations.

The minimum specific growth rate of *T. aureoviride* was judged to be approximately 0.03 h^{-1} under carbon- and nitrogen-limited conditions; this value is equivalent to $0.10\mu_{\max}$. Similar values of $\mu_{\min}$ have been estimated for *Penicillium chrysogenum*, $0.13\mu_{\max}$ (Righelato, 1975) and *A. nidulans*, $0.10\mu_{\max}$ (McGetrick & Bull, 1979). The significance of a minimum specific growth rate concept is strengthened when metabolic parameters complement morphogenetic changes. Thus, conidiation in chemostat populations of *P. chrysogenum* was accompanied with an increased rate of nucleic acid turnover and a decay in the specific rate of penicillin synthesis (Righelato *et al.*, 1968). In *A. nidulans* the morphological definition of $\mu_{\min}$ has been correlated with a minimum efficiency of RNA in protein synthesis (Bull & Trinci, 1977); in the present study $\mu_{\min}$ and RNA efficiency were not so correlated.

The maintenance energy coefficient for carbon-limited *T. aureoviride* was $0.02\text{ g glucose (g biomass)}^{-1}\text{ h}^{-1}$, a value which is similar to that found for other filamentous fungi (P.

chrysogenum, $0.022 \text{ g g}^{-1} \text{ h}^{-1}$, Righelato *et al.*, 1968; *A. nidulans*, $0.029 \text{ g g}^{-1} \text{ h}^{-1}$, Carter *et al.*, 1971; *A. terreus* $0.010 \text{ g g}^{-1} \text{ h}^{-1}$, *Cylindrocarpon lichenicola*, $0.038 \text{ g g}^{-1} \text{ h}^{-1}$, J. A. Rodriguez & A. T. Bull, unpublished data). No systematic study has been made of the maintenance energy requirement and factors which control it in fungi. However, the available data suggest that, under optimal growth conditions, the maintenance requirement for filamentous fungi is remarkably constant (average about 25 mg energy source per g biomass per h). Maintenance energy frequently constitutes a substantial proportion of the total energy consumed, the proportion clearly varying as a function of dilution rate in energy-limited systems (e.g. glucose-limited chemostat culture of *T. aureoviride*, Fig. 4). At dilution rates of about 0.02 h^{-1} , 30 to 40% of the energy source may be diverted to mycelial maintenance (Bull & Trinci, 1977; this paper) but when the dilution rate is 0.005 h^{-1} , as much as 70% of the energy source is required for maintenance functions (Righelato *et al.*, 1968). A supply of 1.7 to 3 times the maintenance ration of glucose to a fed-batch culture of *T. aureoviride* (Fig. 5) was sufficient to ensure continued biomass synthesis at a quasi-steady state rate of 0.027 h^{-1} . Nevertheless, these rates of energy source supply were insufficient to maintain the biomass in an undifferentiated mycelial form. Bainbridge *et al.* (1971) found that supplying the energy source at 1.5 times the maintenance ration to fed-batch cultures of *A. nidulans* did not allow net biomass synthesis but maintained the biomass concentration at 80% of its initial value; under these conditions hyphal vacuolation and partial autolysis occurred together with some conidia production.

The environmental control of chemical composition of fungi, other than yeasts, has received scant attention and in particular very few chemostat data have been reported. Over most of the growth rate range examined the RNA and protein patterns of glucose- and ammonia-limited *T. aureoviride* were very similar although the respective maximum concentrations were 30 and 40% lower under nitrogen limitation. Such dilution rate dependent change in RNA content is similar to that originally reported by Herbert (1961) for bacteria. The decline in RNA content at the highest dilution rate analysed is not understood. The RNA content of *T. aureoviride* mycelium is higher than most values quoted for filamentous fungi (e.g. Griffin *et al.*, 1974; McGetrick & Bull, 1979) and, in part, is probably due to the rapid quenching and analysis of culture samples; RNA degradation in stored material is known to occur at significant rates (Herbert *et al.*, 1971). The lower RNA content under nitrogen-limited cultivation contrasts with Herbert's (1961) data for bacterial composition and the difference between our carbon- and nitrogen-limited data is only partly accounted for in terms of an increased proportion of carbon storage materials under the latter condition at low D (i.e. $< 0.125 \text{ h}^{-1}$); cf. Figs 1 and 2.

The DNA content of *T. aureoviride* was independent of dilution rate, a response which has been noted in other fungi such as *Gibberella fujikuroi* (Bu'Lock *et al.*, 1974) and *A. nidulans* (McGetrick & Bull, 1979). The ratio of RNA to DNA maintained a linear relationship to dilution rate at all except very low values ($D < 0.05 \text{ h}^{-1}$), an observation which is consistent with an increasing rate of transcription per stable DNA cistron (Smith, 1979).

Previous determinations of the efficiency of RNA in fungal protein synthesis (g protein synthesized per g RNA per h) (Alroy & Tannenbaum, 1973) have been few but available data suggest that maximum efficiencies are attained at dilution rates equivalent to 65 to 80% $\mu_{\max}$ under energy limitation (Bull & Trinci, 1977). Such findings have been interpreted as implying that all ribosomes and ribosomal subunits are associated in functional polysomal complexes even when growth is substantially below the maximum specific rate. The exact relationship between efficiency and dilution rate shows variation: Alroy & Tannenbaum's (1973) early studies with yeast revealed a linear relationship which extrapolated to the origin whereas in highly sporulating populations of *A. nidulans* zero efficiency was extrapolated to a dilution rate equivalent to $\mu_{\min}$ (Bushell & Bull, 1976). In the current work with *T. aureoviride* the dilution rate dependence of efficiency was different under glucose and ammonia limitation. Under glucose limitation the relationship was reasonably linear ($r = 0.90$) over the whole

range of D . Efficiency appeared to reach a plateau at $D = 0.17 \text{ h}^{-1}$ ($77\% \mu_{\max}$) under ammonia limitation then rose sharply at $D = 0.20 \text{ h}^{-1}$ under which condition ammonia limitation was largely relieved (Fig. 2). These observations suggest that maximum translational capacity in *T. aureoviride* was controlled by the nature, as well as the availability, of the growth-limiting substrate.

The mycelial content of magnesium increased with dilution rate but the molar ratio of Mg^{2+} to RNA remained fairly constant at 1:8 (Table 2). These findings point to a role for Mg^{2+} in ribosome stabilization and are consistent with data obtained from bacteria (Tempest, 1969) and *Candida utilis* (Aiking & Tempest, 1976). In contrast to *T. aureoviride*, the Mg^{2+} :RNA molar ratio of carbon-limited mycelia of *A. nidulans* varied with dilution rate. The potassium content of *Trichoderma* mycelia was much higher than that of magnesium and it occurred in a 1:1 molar ratio with RNA. Although K^+ is also associated with ribosomes in growing micro-organisms its concentration in *T. aureoviride* seems to be in far excess of this requirement and its primary role may be related to glucose utilization. Previous studies with *Neurospora crassa* (Viotti *et al.*, 1971) and *A. nidulans* (Bushell & Bull, 1974) have led to the postulate that a degree of functional interchangeability between Mg^{2+} and polyamines may occur in the regulation of RNA stability and synthesis. Glucose-limited cultures of the latter fungus produced mycelia in which the molar ratio of Mg^{2+} plus total polyamines:RNA remained constant irrespective of the imposed dilution rate. This relationship was not a feature of *T. aureoviride* growth (Table 2) where, instead, the molar ratio of Mg^{2+} plus polyamines:RNA rose progressively as D was increased.

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