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Abstract

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A method for achieving prolonged nutrient limited growth of Neurospora mycelium.

It is difficult to achieve sustained growth under supplement limited conditions of filamentous fungi such as Neurospora which cannot easily be grown in a chemostat. Reducing the initial concentration of a nutrient provided to batch cultures usually results in a rapidly declining supplement concentration and succeeds only in advancing the time at which growth ceases. This seldom produces experimentally amenable variation in growth rate. In order to study the effects of prolonged growth of amino acid auxotrophic mutants at low rates of provision of the required amino acid we have adapted the approach of continuously supplying fresh medium, supplemented with the desired amino acid concentration to batch cultures. The method described should prove applicable to other nutrient limitation conditions such as nitrogen or carbon starvation.

Replicate 1 L round, flat bottomed flasks were used, having three equally spaced 2 cm identations to improve agitation and aeration. These contained an initial volume of 200 ml of medium, inoculated with around 10^7 conidia. The flasks were fitted with silicone rubber stoppers carrying a foil capped vent and a glass tube in contact with the medium through which a combined stream of sterile air (0.42 ml/min) and fresh medium (0.16 ml/min) was pumped by means of a multichannel proportioning pump. Pump tubing was presterilized by passing a 2% solution of hypochlorite, followed by sterile distilled water. The flasks were shaken at 200 rpm on a New Brunswick gyrorotary shaker in 29°C constant temperature room for periods not exceeding 45 h, or a final culture volume of around 600 ml. In the experiments shown in Fig. 1 conidia from an arg-5 strain were inoculated into replicate flasks containing Vogel's medium supplemented with 2.25% (w/v) glucose and 0.12 mM arginine, and supplied at a constant rate with fresh Vogel's/glucose medium containing various concentrations of arginine. Once the initial supply of arginine had been exhausted, growth occurred at a linear rate proportional to the concentration of arginine in the feeding solution. Mycelium growing at a linear rate cannot represent a true metabolic steady state, as is established under conditions of exponential growth. Nevertheless such experiments can yield valuable information on changes in enzyme synthesis (in this case the derepression of amino acid synthetic enzymes - Flint et al., in preparation) and in macromolecular svnthesis during prolonqed periods of nutrient limited growth. A somewhat different 'fed batch' culture technique, suitable for growth and sampling of germinating conidia, has been described by Limon-Lason et al ((1977) BBRC, 78: 1234-1241) for the study of nitrogen and carbon limited conditions

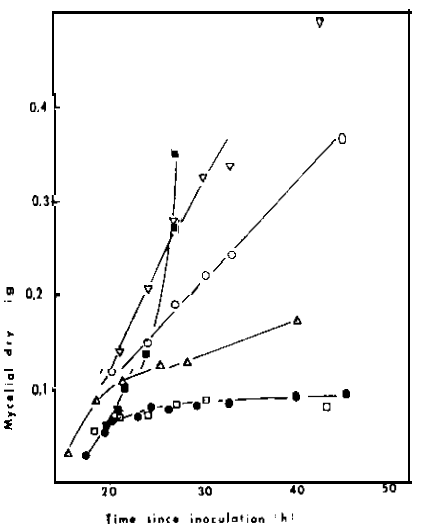

Figure 1. -- Growth of an arg-5 inoculated into-200 ml of medium containing 0.12 mM arginine and supplied continuously with medium supplemented with arginine at the following concentrations: 0.20 mM ( )0.12 mM (○)0.04 (△) 0.0 mM (□). Cultures that received no supply of fresh medium, but were initially supplemented with 0.12 mM (●) or 2.0 mM (■) arginine are shown for comparison. Full details are described in the text.

Using the conditions described above the growth of a wild strain in minimal medium was shown to be exponential and the rate independent of culture volume over the maximum duration of each experiment. Thus the combination of vigorous agitation and forced air ensures adequate aeration throughout, despite the change in culture volume.

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