Axenic Growth and Development of the Cellular Slime Mould, *Dictyostelium discoideum*

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Dictyostelium discoideum has been used in numerous studies of development; it has been grown on living or dead bacteria (Gezeliuss, 1962) but Sussman & Sussman (1967) reported that it could be adapted to grow in a medium without bacteria or bacterial fractions. The use of *D. discoideum* in molecular studies of development has increased the need for an axenic medium for its growth. The present paper concerns simplification of the previous axenic medium, observations on growth kinetics and the kinetics of appearance and disappearance of a developmentally-controlled enzyme in axenically grown organisms.

METHODS

Axenic cultivation. Organisms were grown in 7 ml. of medium in 125 ml. Erlenmeyer flasks; when larger amounts were required, 20 ml. of medium in 125 ml. flasks were used. The medium previously reported (Sussman & Sussman, 1967) was adjusted to the following composition: 10 g. Proteose peptone (Difco) and 5 g. yeast extract (Difco) were dissolved in 650 ml. distilled water. Sixty-five ml. samples of this solution were autoclaved separately (121° for 20 min.). To each of the above samples was added 4 ml. of sterile glucose solution (27 g./100 ml.) and 1 ml. of sterile phosphate buffer (6.7 g. Na₂HPO₄·7H₂O + 3.4 g. KH₂PO₄ in 100 ml. distilled water). Flasks were inoculated with organisms from a previous culture to 1 to 5 × 10⁵/ml. and incubated on a reciprocating shaker (110 cycles/min.) at 22°.

Organisms were counted with a haemocytometer or a Coulter electronic counter. Dilutions for counting were made with a salt solution (NaCl 0.6 g., KCl 0.75 g., CaCl₂ 0.3 g./1000 ml. water; Bonner, 1947).

Cloning procedure. Axenically-grown organisms were cloned monthly (seven to eight transfers), by diluting organisms in axenic medium to an appropriate concentration, and plating them on SM/5 agar medium (Sussman & Lovgren, 1965) in association with *Klebsiella (Aerobacter) aerogenes*. This method was used since the organisms grew poorly on semisolid axenic medium and did not fruit on any medium which they could not deplete of nutrients. Spores from a clone which produced typical fruiting bodies were placed in a culture tube containing 5 ml. axenic medium + 500 µg. streptomycin sulphate (sterilized by filtration)/ml. These tubes were incubated without shaking at 22° for 2 to 4 days until microscopic examination revealed abundant growth of the amoebae. These were then transferred to 125 ml. flasks with 7 ml.

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axenic medium and shaken. Growth rate was determined on the second passage. The cloning procedure prevented the accumulation of poorly-fruited organisms which often become a significant proportion of the population after many passages without cloning.

Fruiting. Axenically-grown organisms were made to fruit synchronously by a modification of a technique already reported for cells grown with bacteria (Sussman & Lovgren, 1965). Late log phase organisms were collected by centrifugation, then washed once in axenic medium without glucose. The organisms were diluted in this solution to 5×10^7 /ml. One-half ml. of this suspension was distributed on a membrane filter (Millipore) or on a 4.25 cm. filter paper disc (Whatman no. 50) supported by two filter pads containing 3.2 ml. pad diluting fluid in 0.04 M-phosphate buffer (pH 6.4) (Sussman, 1966). After 15 min. the filter with organisms was transferred to fresh pads and diluting fluid; this transfer removed most of the axenic medium. The filters were then incubated at 22° in a moist chamber.

Assays. UDP-galactose polysaccharide transferase was assayed by the method of Sussman & Osborn (1964) as modified by Yanagisawa, Loomis & Sussman, (1967). Organisms were broken by ultrasonic treatment. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Composition of the medium. It was soon evident that the foetal calf serum and liver concentrate present in the previously reported medium (Sussman & Sussman, 1967) were not required for maximum growth rate or yield. Except for the increase in glucose concentration noted in METHODS, no short-term improvements were observed after adjusting the relative concentration of the constituents or by using other peptones or sugars. Addition of powdered milk or lecithin, which were useful in the axenic cultivation of *Polysphondelium pallidum* (Sussman, 1963), did not aid *Dictyostelium discoideum*. The high concentration of buffer used previously was not necessary. Dialysis of the yeast extract and proteose peptone and reconstitution in differing proportions, adjustment of pH over the range 4.5 to 7.0, and addition of trace elements did not result in any improvement in growth.

Kinetics of growth. Fig. 1 shows the kinetics of growth of *Dictyostelium discoideum* in the present axenic medium. No changes in growth rate or yield have been observed over 1 year. The mean doubling time after 12 hr of growth was about 12 hr. During the first 12 hr after a transfer from late log phase, the doubling time was 8 hr and the protein doubling time 12 hr. It appears that the protein content/organism of an inoculum of late log. organisms decreases during the first 12 hr of growth on fresh medium (e.g. from 0.12 to 0.06 m μ g. protein/organism).

When early log. phase organisms (about 12 hr) were used as an inoculum, there was no initial growth phase, and the initial generation time was 12 hr. An examination of the size distribution of axenically-grown organisms with the Coulter counter showed an increase in cell size during late log. phase growth which was lost soon after transfer to fresh medium. Analysis of the ploidal composition of the spores from axenic organisms (Sussman & Sussman, 1962) revealed a low grade, metastable, haploid condition.

Development of axenically grown organisms. Organisms grown axenically and placed on a filter support at a density of 2.5×10^7 had the same developmental pattern both

morphogenetically and temporally over 24 hr as organisms grown with bacteria (Sussman & Lovgren, 1965) developing from a higher density (10^8 organisms/filter). Increasing the concentration of axenic developmental organisms to approach the optimal concentration for bacteria-fed organisms caused morphological abnormalities and loss of synchrony.

Fig. 1. Kinetics of growth of *Dictyostelium discoideum* amoebae in axenic medium. Inoculum from late log. phase organisms.

Several enzymes have been shown to be under developmental regulation in *Dictyostelium discoideum* (Sussman & Osborn, 1964; Ashworth & Sussman, 1967; Roth & Sussman, 1968). We examined the activity of UDP-galactose polysaccharide transferase during the development of axenically-grown organisms; the transferase activity was quantitatively and temporally identical to that for organisms grown with bacteria (Sussman, 1966).

DISCUSSION

Although the 12 hr doubling time of axenically grown organisms is significantly slower than the 3 to 4 hr found for growth with bacteria (Sussman, 1961; Sussman & Sussman, 1963), it is still fast enough to be of practical use. The total yield of organisms is the same for growth with bacteria in liquid medium. During late log. phase the axenically grown organisms accumulate an excess of protein, perhaps because of the slowing in rate of cell division at this time. When transferred to fresh medium they divide rapidly, presumably until they reach the proper protein content for balanced growth. The over-all rate and sequence of morphogenetic events and the accumulation and loss of a developmentally-regulated enzyme of axenically grown organisms were the same as those of cells grown with bacteria. These results indicate that many of the basic aspects of vegetative growth in *Dictyostelium discoideum*, including growth rate, type of growth and type of medium, do not affect the subsequent events associated with starvation-induced development.

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