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OBSERVATIONS ON THE GROWTH OF *DICTYOSTELIUM DISCOIDEUM* IN AXENIC MEDIUM: EVIDENCE FOR AN EXTRACELLULAR GROWTH INHIBITOR SYNTHESIZED BY STATIONARY PHASE CELLS

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SUMMARY

By analysing the growth kinetics of *Dictyostelium discoideum* amoebae in varying combinations of the components of the nutrient medium HL-5 (Cocucci & Sussman, 1970), we have demonstrated that *Dictyostelium* amoebae enter stationary phase when they deplete the medium of one or more nutrient components. However, if the medium of a stationary phase culture is supplemented with any of the initial components of HL-5 medium and even when supplemented with all components of HL-5 medium, it does not support the growth of freshly inoculated cells, indicating that stationary phase cultures produce a potent growth inhibitor. The possible function of this inhibitor is discussed.

INTRODUCTION

Amoebae of an axenic strain of *Dictyostelium discoideum* grow and divide in suspension in the nutrient medium HL-5 (Cocucci & Sussman, 1970). When these amoebae are washed free of this medium and dispersed on filter pads saturated with a non-nutrient buffered salt solution, they stop growing and dividing, and enter a developmental programme which leads to the construction of a multicellular fruiting body structure (Sussman, 1966). This structure is composed of a base and stalk of highly vacuolated, cellulose-sheathed cells, and a cap containing ellipsoidal spores.

Although the removal of all nutrients leads to both the cessation of cell multiplication and entrance into the developmental programme, cells which have stopped multiplying do not enter development in all cases. When cells enter stationary phase in nutrient medium, they also stop multiplying, but they do not appear to enter the developmental programme. Studies of these stationary phase cells have demonstrated that they exhibit a phenotype distinct from either log phase cells or early developing cells (Cocucci & Sussman, 1970; Malkinson & Ashworth, 1973; M. Mirick, J. Yarger, & D. R. Soll, in preparation). In addition, stationary phase cells are not terminally differentiated, since they resume growth when washed and added back to fresh growth medium (Mirick, Yarger & Soll, in preparation), and enter development when washed and deposited on a filter pad saturated with a non-nutrient solution. Therefore, log phase cells either enter the developmental programme leading to a fruiting body when

all nutrients are removed, or, alternatively, express the stationary phase phenotype when they cease to multiply after reaching a maximum cell concentration in nutrient medium.

It has been assumed that *Dictyostelium* amoebae enter stationary phase because they deplete the medium of one or more nutrient components. In this report, we will present evidence which demonstrates that this is probably the case. However, if stationary phase medium is supplemented with the depleted nutrient components, it does not support renewed cell multiplication after inoculation with fresh cells, indicating that a stationary phase culture produces a potent growth inhibitor in the medium.

METHODS

Maintenance of stocks

Stocks of the *Dictyostelium discoideum* axenic strain Ax-3 were maintained on lawns of the bacterium *Aerobacter aerogenes* on nutrient agar according to the methods of Sussman (1966). Single fruiting bodies from these plates were picked at 1-month intervals and inoculated into 2 ml of the nutrient medium HL-5 (see next section for formula) containing 500 μg/ml of streptomycin sulphate. After 1 week, these starter cultures were inoculated into fresh HL-5 and grown as described below. These larger cultures were tested for normal morphogenesis according to methods outlined by Cocucci & Sussman (1970) and were then used as experimental cultures. Stock experimental cultures were maintained for no longer than 1 month by serial inoculation into fresh HL-5 every 4 days.

Growth of Ax-3 cells in HL-5

2×10^7 exponentially growing cells were inoculated into 140 ml of sterile HL-5 medium (2 g proteose peptone, 1 g yeast extract, 2 g dextrose, 2 ml of 0·5 M phosphate buffer, pH 6·5, per 140 ml) in a 1-l. Erlenmyer flask and agitated at 20 °C on a reciprocal shaker at 60 strokes per min. Cell concentration was monitored by removing 1 ml of cell suspension and counting in a haemocytometer. Any variation in this method is noted either in the text or figure legend.

Obtaining stationary phase medium

Cell cultures at stationary phase for 48 h were centrifuged at 5000 *g* for 5 min; the supernatant was removed and recentrifuged at 9000 *g* for 5 min. The final supernatant was sterilized by passing it through a Millipore filter (0·45 μm pore diameter).

Materials

Both proteose peptone and yeast extract were purchased from Difco Laboratories, Detroit, Michigan.

RESULTS

Standard growth kinetics in the axenic medium HL-5

Log phase cells inoculated into fresh HL-5 medium continued to divide without interruption with a generation time of between 10 and 12·5 h (Fig. 1). This rate remained constant up to a titre of $1{\cdot}2 \times 10^7$ cells per ml and then decreased to zero in stationary phase at a final concentration of approximately 2×10^7 cells per ml, regard-

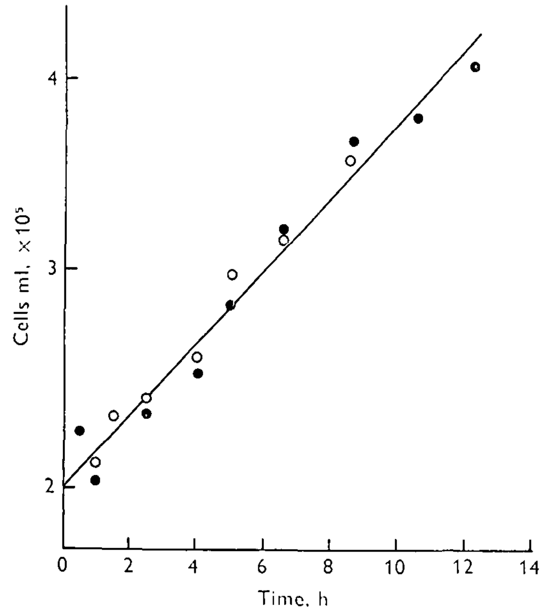

Fig. 1. Growth kinetics during a single generation of logarithmically dividing cells inoculated at time zero into fresh HL-5 medium. Symbols (●, ○) represent parallel cultures.

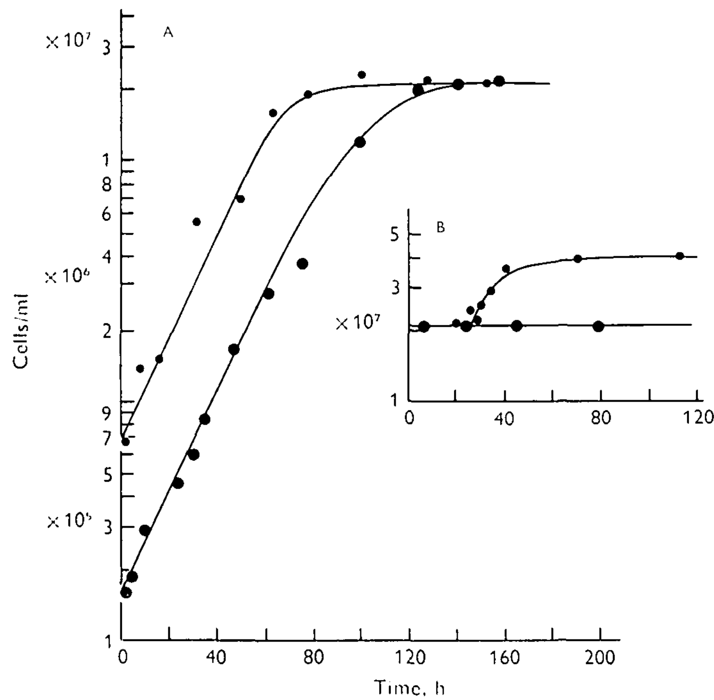

Fig. 2. Growth kinetics through several generations in HL-5 medium. A, fresh log phase cells were inoculated in HL-5 medium to initial concentrations of $1 \cdot 7 \times 10^5$ per ml and 7×10^5 per ml (Large and small circles, respectively). B, cells in stationary phase (2×10^7 cells per ml, ●) for 24 h were washed free of medium and reinoculated into fresh HL-5 medium at an initial concentration of 2×10^7 cells per ml (•).