Effect of Varying Growth Rate on the Morphology of *Arthrobacter*

By B. M. LUSCOMBE AND T. R. G. GRAY

Hartley Botanical Laboratories, University of Liverpool, Liverpool, L69 3BX

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INTRODUCTION

The genus *Arthrobacter* was erected by Conn & Dimmick (1947) to contain pleomorphic bacteria with nonsporing, Gram-positive rods. In batch culture with appropriate media, *Arthrobacter* spp. have a life cycle in which coccoid organisms change to rods and back to cocci (Mulder *et al.* 1966). The rod stage is only present for a relatively short period of a few hours during which the environment and physiology of the organism is in a dynamic state, making studies on the rods difficult.

Ensign & Wolfe (1964) produced rod and coccus forms of *Arthrobacter crystallopoietes* by varying the composition of the growth medium. Whereas cocci were produced on a glucose salts medium, rods were formed by enriching the medium with additional carbon sources, e.g. succinate. The effect of using an enriched medium on morphology suggests that the transformation from coccus to rod is either induced by specific chemicals or is related to changes in growth rate. The aim of this experiment was to determine whether morphology was related to growth rate, and this was achieved by growing a strain of *Arthrobacter* in a chemostat under carbon-limited conditions and observing changes in morphology that occurred with changes in dilution rate.

METHODS

A strain of *Arthrobacter* (Cluster 3 NCIB 10683) described by Lowe & Gray (1972) and isolated from an acid forest soil was selected. It was grown in a chemostat with a working volume of 150 ml. and vortex aeration, using the basal simple salts medium of Owens & Keddie (1969), with biotin (1 µg/l.), and glucose (0.2 g./l.) as the limiting carbon source. Carbon-limited populations were grown at 10°, 15°, 20° and 25° at dilution rates between 0.01 h.⁻¹ and 0.4 h.⁻¹. Samples were taken and observed under phase contrast (× 800) when a steady state had been obtained at any temperature and dilution rate. Bacterial density was determined by direct counts using a Helber slide and biomass was measured by weighing washed bacteria, dried to constant weight at 110°.

RESULTS

By varying the dilution rate, the morphology of the organisms in the chemostat could be changed. At 25° rods were produced at dilution rates above 0.25 h.⁻¹, at 20° at rates above 0.11 h.⁻¹, at 15° at rates above 0.05 h.⁻¹ and at 10° at rates above 0.02 h.⁻¹. At lower dilution rates, the bacteria were always coccoid. Both types could be maintained indefinitely by holding the dilution rate at an appropriate level.

The transformation from cocci to rods at 25° was accompanied by a reduction in numbers from 6.19 to 4.47 × 10⁸ per ml. However, the biomass remained constant at all

dilution rates (0.099 g./l.), the reduction in numbers being compensated for by the increase in size, implying that both forms have similar efficiencies in the conversion of substrate to bacteria.

DISCUSSION

The results suggest that morphology is related to specific growth rate and that the change from cocci to rods does not require a specific inducer. In batch cultures where an inducer has been added to produce rods, the resulting enrichment probably causes faster growth. The rod form appears during the log phase of growth when the specific growth rate is at a maximum but in the lag and stationary phases, when the growth rate is lower, the cells are coccoid. The effect of growth rate on length has been noted with other bacteria (Herbert, 1959) although it had no effect on the diameter of true cocci. This suggests that in the present experiments, the cocci are not true cocci but rods with greatly reduced lengths.

In the natural environment (a forest soil), temperatures rarely exceed 15° so one might expect the change from coccus to rod to occur at a specific growth rate of about 0.05, corresponding to a generation time of 13.8 h. Calculations based on energy flow in soil (Gray & Williams, 1971) have suggested that bacterial generation times are likely to be greater than this value so one would expect *Arthrobacter* strains to be present in soil in the coccoid form. Direct observation of *Arthrobacter* strains growing in forest soil (Lowe, 1969) has shown this to be the case.

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