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Short contribution

Effect of phosphate on antibiotic and extracellular protein production by *Myxococcus coralloides* D

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Summary. The effect of inorganic phosphate concentrations on antibiotic and extracellular protein production by *Myxococcus coralloides* D have been examined. Antibiotic production by growing cells of this myxobacterium was maximal at phosphate concentrations of 10–20 m*M*, but was inhibited by concentrations higher than 20 m*M*. The total extracellular protein and the extracellular protein per cell ratio were independent of phosphate levels in the culture broth.

Introduction

Myxobacteria produce a wide variety of exoenzymes and antibiotics (Shimkets 1984; Rosenberg and Varon 1984). The antibiotic produced by *Myxococcus coralloides* D in particular has been isolated and purified (Arias et al. 1979). This myxobacterium strain shows significant autolytic activity when grown in a liquid medium. The autolysis can be delayed by increasing the phosphate levels in the medium (Fernandez-Vivas et al. 1983). High phosphate concentrations also inhibit the fructification of *M. coralloides* D on a solid medium (Gonzalez et al. 1987).

Phosphate regulates the synthesis of several antibiotics produced by Gram-positive bacteria, including macrolide antibiotics, peptide antibiotics and tetracyclines, and biosynthetically complex antibiotics (Martin 1977; Martin and Demain 1980). Phosphate also diminishes the quantity of protein secreted by an efficient protein-producing bacterium such as *Bacillus brevis* (Tsukagoshi et al. 1981). In this report we note the effects of orthophosphate on antibiotic and extracellular protein production by *M. coralloides* D.

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Materials and methods

Organisms. Myxococcus coralloides strain D, isolated in our laboratory (Arias and Montoya 1978), was employed in this work. A strain of *Staphylococcus aureus* from our departmental stocks was used as test organism.

Growth media and conditions. For antibiotic and extracellular protein production the following protein hydrolysates were used, each at a concentration of 0.5%: trypticase peptone (BBL, Cockeysville, MD, USA), polypeptone peptone (BBL), bacto casitone (Difco, Detroit, Mich), peptone from soymeal or phytone (Merck, Darmstadt, FRG), bioprotein L (Hoechst, Hoechst, FRG), biopeptone (Hoechst) and peptone from meat (Adsa Micro, Barcelona, Spain). Trypticase peptone (BBL) was employed at concentrations of 0.1%, 1%, 1.5% and 2%. All media contained in addition 0.1% $MgSO_4 \cdot 7H_2O$ and 1% glucose in 10 m*M* potassium phosphate buffer, pH 6.5. The cultures were incubated at 28° C with gyratory shaking (200 rpm). Casitone (CT) liquid medium (Fernandez-Vivas et al. 1983) was used for conservation and preparation of inocula. For the quantitative assay of antibiotic activity, bacto antibiotic medium 1 and medium 2 (Difco) were used. The total number of cells was determined using a Petroff-Hausser counting chamber.

Antibiotic production and assay. One-litre erlenmeyer flasks, containing 500 ml of the appropriate medium were inoculated with 5 ml of a dense suspension of a 48-h culture of *M. coralloides* D in CT medium. Samples of the cultures were centrifuged (12 000 *g* for 20 min at 4° C) and the antibiotic was extracted from the supernatant with chloroform, according to Arias et al. (1979). Antibiotic activity against *S. aureus* was determined by the paper disc assay method. Plates of 100 mm diameter were used; each plate contained 5 ml bacto antibiotic medium 2, overlayed with bacto antibiotic medium 1 (2.5 ml) containing 2.7×10^8 *S. aureus* cells. Units of antibiotic were determined from a standard of inhibition-zone curve as a function of the quantity of antibiotic absorbed into a disc of 5.5 mm diameter. One unit of the antibiotic is defined as the concentration that causes an inhibition zone of 9 mm. For the paper disc assay method, the chloroform solution was used directly. All values presented are the mean of at least three different assays from separate cultures. For other experiments, the antibiotic was transferred into phosphate buffer (K_2HPO_4-KH_2PO_4, 10 mM, pH 6.5), by adding the buffer directly to a concentrated solution of the antibiotic and removing the chloroform under vacuum.

Enzyme assay and protein determination. Extracellular phosphatase activities were assayed according to Gonzalez et al. (1987).