Properties and significance of an α-amylase produced by *Myxococcus coralloides* D

M. Esther Fárez-Vidal, Antonia Fernández-Vivas, F. González and J.M. Arias
Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, Granada, Spain

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M.E. FÁREZ-VIDAL, A. FERNÁNDEZ-VIVAS, F. GONZÁLEZ AND J.M. ARIAS. 1995. The extracellular amylase activity from *Myxococcus coralloides* D was purified by Sephacryl S-200 gel filtration and by ion-exchange chromatography on DEAE-Sephadex A-25. The molecular weight was estimated by SDS-PAGE and by gel filtration as 22·5 kDa. The optimum temperature was 45°C. The pH range of high activity was between 6·5 and 8·5, with an optimum at pH 8·0. Activity was strongly inhibited by Hg^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{2+} and Fe^{3+}, EDTA and glutardialdehyde, but was less affected by Ni^{2+} and Cd^{2+}. Li^{+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, *N*-ethylmaleimide, carbodiimide and phenyl methyl sulphonyl fluoride had almost no affect. The K_m (45°C, pH 8) for starch hydrolysis was $2{\cdot}0 \times 10^{-3}$ g l^{-1}. Comparison of the blue value-reducing curves with the time of appearance of maltose identified the enzyme produced by *M. coralloides* D as an α-amylase.

INTRODUCTION

Myxococcus coralloides D is a soil-dwelling, Gram-negative bacterium with two striking characteristics: its cells migrate by gliding on a semisolid surface (Burchard 1984) and undergo a developmental cycle upon nutrient starvation (Shimkets 1990). During differentiation under starvation conditions, the cells move as a swarm and migrate together to an aggregation centre where they eventually form a fruiting body, within which the cells differentiate into myxospores. Although it is clear that the fruiting bodies of myxobacteria constitute the resistant or resting stage, they have another significance as well. This is to ensure the adequate function of the multicellular inoculum for the initiation of growth on a fresh substrate by organisms possessing a co-operative feeding habit (Dworkin 1972).

These micro-organisms typically produce extracellular enzymes able to hydrolyse macromolecules such as proteins, nucleic acids, fatty-acid esters and various polysaccharides. Most of them are capable of lysing other eukaryotic and prokaryotic micro-organisms (McCurdy 1989). Myxobacteria can use starch and polysaccharides (Beebe 1943; Norén 1955; Madi and Antranikian 1989) but, to date, there have been no studies to identify the factors affecting the production of amylase by these bacteria, or to characterize the enzymatic activities involved in the use of starch and polysaccharides.

Several myxobacteria have been tested for extracellular amylase activity and *M. coralloides* D was found to produce the largest quantities of this enzyme. In a previous paper (Fárez-Vidal *et al.* 1990) the precise quantities of amylase secreted by this myxobacterium during the various stages of its life cycle were described. The enzyme was characterized as an α-amylase according to the products released from the hydrolysis of starch. The optimal conditions for amylase production (pH, temperature, C and N sources) have also been reported (Fárez-Vidal *et al.* 1992)

In this paper the purification of the enzyme from *M. coralloides* D is reported. Optimum activity conditions, starch hydrolysis rate and significance of this amylase are described.

Correspondence to: J.M. Arias, Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain.

MATERIALS AND METHODS

Micro-organism and culture conditions

Myxococcus coralloides D, isolated in this laboratory (Arias and Montoya 1978), was grown in CTY liquid medium containing (g l^{-1}): trypticase peptone (BBL), 5; $MgSO_4$. $7H_2O$, 1; in 10 mmol l^{-1} potassium phosphate buffer, pH 6·5 (Arias *et al.* 1983). Soluble starch (1%), from Merck, was added to the medium. Growth was measured by optical density (O.D.) at 650 nm. Cultures were grown at 28°C and stirred at 200 rev min^{-1}.

Enzyme assay

The amylase assay was based on the reduction in blue colour intensity resulting from enzyme hydrolysis of starch