Journal of Applied Bacteriology 1994, **77**, 567–573

Partial purification and biochemical properties of acid and alkaline phosphatases from *Myxococcus coralloides* D

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4754/12/93: received 3 December 1993, revised 27 May 1994 and accepted 7 July 1994

F. GONZÁLEZ, M.E. FÁREZ-VIDAL, J.M. ARIAS AND E. MONTOYA. 1994. Acid phosphatase and alkaline phosphatase from vegetative cells of *Myxococcus coralloides* D were purified by two chromatographic steps. The molecular weights were estimated by gel filtration and SDS-PAGE. Optimum pH, stability, optimum temperature and thermal inactivation studies were made for both enzymes. EDTA and other chelating agents inhibited alkaline but not acid activity. Mg^{2+} activated the alkaline phosphatase, while the acid phosphatase was inhibited by fluoride. Both enzymes degraded a number of phosphomonoesters, but were unable to hydrolyse either polyphosphates or cAMP. The K_m values of the acid and alkaline phosphatases for *p*-nitrophenylphosphate were $5{\cdot}0 \times 10^{-3}$ mol l^{-1} and $1{\cdot}5 \times 10^{-3}$ mol l^{-1}, respectively.

INTRODUCTION

Myxobacteria have a unique life cycle for procaryotes, consisting of vegetative growth, aggregation, fruiting-body formation, myxospore formation and germination (Shimkets 1990). These bacteria are soil micro-organisms which move by gliding and can forms swarms on surfaces. They are very common, frequently isolated from soils, being able to colonize other habitats, such as rhizospheres, dung of herbivorous animals, rotting wood and decaying organic materials in general (Reichenbach 1984).

Various sporulate micro-organisms, such as *Bacillus subtilis* (Hulett 1987) and *Dictyostelium discoideum* (Killick and Wright 1974), express higher levels of alkaline phosphatase in spores than in vegetative cells. Alkaline and acid phosphatases hydrolyse a wide variety of phosphate esters. They are apparently ubiquitous in nature, occurring in many animal tissues, plants and micro-organisms (Hollander 1971).

Myxococcus coralloides D produces alkaline and acid phosphatases during its vegetative growth in a liquid medium and high concentrations of inorganic phosphate have no effect (González *et al.* 1987, 1989a). Phosphatase activities during the fruiting-body formation reached one single maximum and a transitory increase in both alkaline and acid phosphatase activity during myxospore induction and germination has also been described (González *et al.* 1987).

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Weinberg and Zusman (1990) and González *et al.* (1991) have studied the pattern of phosphatase activities during development in *Myxococcus xanthus* and suggest that phosphatases can be used as reliable and easy markers during development. However, there have not been any studies on phosphatase characterization from myxobacteria. In this paper the partial purification and some biochemical characteristics of alkaline and acid phosphatases from vegetative cells of *M. coralloides* D are described.

MATERIALS AND METHODS

Micro-organism and growth conditions

Myxococcus coralloides D was from laboratory stock. It was grown in TT liquid medium, containing (g l^{-1}): trypticase peptone (BBL), 5·5; $MgSO_4 . 7H_2O$, 1; in KH_2PO_4–K_2HPO_4 buffer 10 mmol l^{-1}, pH 6·5. The cultures were incubated at 28°C with shaking at 200 rev min^{-1} for the production of alkaline and acid phosphatases.

Purification of the enzymes

All the purification steps were carried out at 4°C. Fractions collected in the chromatographic steps were assayed for