# *In situ* assay of *Myxococcus coralloides* D phosphatases

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## Abstract

Vegetative cells, glycerol myxospores and fruiting body myxospores from *Myxococcus coralloides* D were permeabilized with numerous agents. Acid and alkaline phosphatases were measured *in situ* after permeabilization treatment. Conditions are presented for the *in situ* assay of these enzymes in vegetative cells and both myxospore types. Toluene at 10% (v/v) was optimum for the *in situ* assay of both phosphatases in vegetative cells. In glycerol myxospores and fruiting body myxospores, toluene at 10% (v/v) and dimethylsulphoxide at 15% (v/v) were the most effective agents for the *in situ* assay of acid and alkaline phosphatases.

## Introduction

Permeabilization is a useful technique for the assaying of various enzymes. The porosity of cell membranes can be increased by several agents which allow substrates to enter the cells. These conditions, called *in situ* by Reeves and Sols (1973), have been used with bacteria and yeasts for measuring several enzymes (Kornberg and Reeves, 1972; Notario, 1982; Felix, 1982).

Myxobacteria produce a wide variety of products, such as enzymes, polysaccharides and antibiotics (Kaiser *et al.*, 1979). A number of products produced by *Myxococcus coralloides* D have been studied, including a bacteriocin (Muñoz *et al.*, 1984), antibiotics (Arias *et al.*, 1979) and phosphatases (González *et al.*, 1987). Acid and alkaline phosphatases have been detected associated with the cells, both being released into the liquid medium during the exponential growth phase. Phosphatases have also been found in glycerol myxospores and fruiting body myxospores (González *et al.*, 1987).

In this paper, the optimum conditions for permeabilizing and the assay of these enzymes of *Myxococcus coralloides* D are described in different cell types, namely vegetative cells, glycerol myxospores and fruiting body myxospores.

## Materials and methods

### Strain and culture methods

*Myxococcus coralloides* D, isolated in our laboratory (Arias and Montoya, 1978) was grown in a TT liquid medium, containing 0.75% trypticase peptone (BBL), 0.1% $MgSO_4.7H_2O$ and 10 mM potassium phosphate buffer, pH 6.5. The cultures were incubated at 28°C with orbital shaking at 200 rpm. Myxospore formation was induced by the technique of Dworkin and Gibson (1964). Fruiting body myxospores were obtained according to González *et al.* (1987).



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