Phosphatase activity during development cycle of *Myxococcus xanthus*

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Cell-bound and extracellular acid and alkaline phosphatase activity have been studied in *Myxococcus xanthus* strains DK101, DK1050, DK2834, and DK2836. Both phosphatases were released into a liquid medium during vegetative growth and the levels were similar in all strains. On solid media, *M. xanthus* DK101 showed maximum activity at the end of the developmental process, when mature myxospores appeared. An increase in phosphatase activity was also observed in glycerol-induced myxospores. A transitory increase in phosphatase activity occurred during the germination of both glycerol-induced and fruiting-body myxospores, although the activity of both phosphatases in fruiting-body myxospores was greater than that in glycerol-induced ones.

Key words: acid phosphatase, alkaline phosphatase, *Myxococcus xanthus*.

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L'activité des phosphatases acides et alcalines, tant extracellulaire que liée aux cellules, a été étudiée chez les souches de *Myxococcus xanthus* DK101, DK1050, DK2834, DK2836. Au cours de la croissance végétative de ces souches dans un milieu liquide, les deux phosphatases ont été libérées et ce à des concentrations similaires pour toutes les souches. L'activité de la souche DK101, croissant sur des milieux solides, a été maximale à la fin du processus de développement, lorsque les myxospores sont apparues. Une augmentation de l'activité phosphatasique a aussi été observée chez des myxospores induites dans du glycérol. Un accroissement transitoire de l'activité phosphatasique est survenue au cours de la germination des myxospores, qu'elles aient été produites par les structures de fructification ou qu'elles aient été induites dans du glycérol. Toutefois, l'activité des deux phosphatases des myxospores issues des fructifications a été supérieure à celle provenant des myxospores induites dans du glycérol.

Mots clés : phosphatase acide, phosphatase alcaline, *Myxococcus xanthus*.

[Traduit par la Rédaction]

Introduction

The myxobacteria are one of the few prokaryotic organisms to exhibit social behaviour patterns, consisting of vegetative growth, aggregation, fruiting-body formation, myxospore formation, and germination. This unique life cycle makes them extremely interesting for developmental studies in the prokaryotic kingdom. It is of prime necessity in such studies to have markers to characterize the various steps in their development. *Myxococcus xanthus* is the most fully studied myxobacterium and a number of proteins have been defined as specific markers, such as protein S (Inouye et al. 1979) and hemagglutinin (Cumsky and Zusman 1979) in their myxospores and during the aggregation process, respectively.

An additional characteristic of myxobacteria is their ability to produce a wide variety of enzymes, some of which vary during their life cycle (Orlowski et al. 1972; Filer et al. 1977). Nevertheless, the precise relationship of these enzymes to the development cycle is still not fully understood. Phosphatase activity has been used as a developmental marker in *Bacillus subtilis* (Hulett 1985), *Physarum polycephalum* (Hüttermann et al. 1979), and *Dictyostelium discoideum* (Killick and Wright 1974), and in a previous paper we have reported that phosphatase activity patterns changed according to the stage in the life cycle of *Myxococcus coralloides* D (Gonzalez et al. 1987).

Protein S and hemagglutinin are not easily assayed, and so we have chosen to use phosphatases instead. In this paper we describe acid and alkaline phosphatase activities during the different stages in the life cycle of *Myxococcus xanthus* and show that the activity of these enzymes increased significantly during certain stages in their multicellular development.

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Materials and methods

Organisms and culture conditions

Myxococcus xanthus strains used in this work (DK101, DK1050, DK2834, and DK2836) were given to us by Francisco Murillo (Department of Genetics, University of Murcia, Spain). DK101 and DK1050 are *M. xanthus* standard strains. The pigmentation of the strain DK101 is not always a stable character and both tan and yellow colonies may arise from a single colony (Burchard and Dworkin 1966). Strain DK1050 was isolated from DK101 as a spontaneous stable yellow, since it produces only yellow colonies (Martinez-Laborda et al. 1986). Strains DK2834 and DK2836 are mutant strains. DK2834 and DK2836 were obtained by Francisco Murillo after UV-induced mutgenesis (DK2834) or by isolating Tn*5* insertions (DK2836). Both strains derived from the DK101 strain. Strain DK2834 formed red colonies and strain DK2836 formed yellow colonies. Myxobacteria cells were grown at 33°C in a CT liquid medium (Bretscher and Kaiser 1978). Liquid cultures were shaken at 200 rpm. Growth was measured by following optical density (OD) at 650 nm. CTA solid medium was used for vegetative colony formation and yeast agar (YA) medium for fruiting-body formation (Muñoz et al. 1984).

Myxospores, fruiting bodies, and vegetative colonies

Myxospores were obtained by the addition of glycerol (0.5 M final concentration) to exponentially growing cells. The myxospores were washed once with 10 mM potassium phosphate buffer (pH 6.5) and then resuspended (10^8 myxospores/mL) in CT medium for germination.

Samples of an exponentially growing cell culture were applied as droplets (8 droplets of 50 μL per plate) to a YA solid medium or CTA solid medium. The plates were incubated at 28°C, and the fruiting body and vegetative colonies were scraped at different times from the solid medium and washed with a phosphate buffer. To prepare fruiting-body myxospores the mature fruiting bodies were suspended in 0.1% SDS; this treatment lysed the remaining vegetative cells but left the myxospores intact. Morphological examination during myxospore induction and germination was made by phase-contrast microscopy.

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