

SHORT COMMUNICATION

Use of Membrane Filters to Study the Development of Fruiting Bodies of *Myxococcus xanthus*

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The use of cellulose acetate membrane filters to study fruiting body formation by *Myxococcus xanthus* is described. This method is used to measure loss and replacement of meso-diamino[¹⁴C]pimelic acid during development. Pulse-labelling experiments indicate that fruiting body myxospores resemble those induced by glycerol (Dawson & Jones, 1979) in that cellular morphogenesis is characterized by replacement of existing peptidoglycan by new material.

INTRODUCTION

Fruiting body formation by *Myxococcus xanthus* is a complex process involving several distinct stages (Wireman & Dworkin, 1975, 1977). Detailed investigation of this process has been limited by the fact that it will only occur when the organism is in contact with a solid surface, usually on agar medium under laboratory conditions. This restricts the ability of the investigator to alter the environmental conditions once fruiting has been initiated. One consequence of these difficulties has been the considerable use of induction techniques (Dworkin & Sadler, 1966) to study myxospore formation. However, some differences between induced and fruiting body myxospores have been observed (White, 1975; Shimkets & Seale, 1975; Inouye *et al.*, 1979) and application of results obtained with induced myxospores to those in the fruiting body must be made with caution (Gerth & Reichenbach, 1978). We now describe an improved method of studying fruiting body formation by *M. xanthus* which greatly extends the range of experiments that can be performed. This method has been used to compare recent results on cell wall turnover in glycerol-induced myxospores (Dawson & Jones, 1979) with myxospore formation in the fruiting body.

METHODS

Organism. *Myxococcus xanthus* FB (NCIB 9412) was grown in Casitone medium as previously described (Dawson & Jones, 1979).

Fruiting body formation. A culture growing exponentially was filtered through cellulose acetate filters (25 mm diam., 0.45 µm pore size; Oxoid) to give approx. 3×10^8 organisms per filter. After washing with 5 ml 0.2% (w/v) MgSO₄·7H₂O, filters were placed on the surface of fruiting medium containing 0.05% (w/v) Casitone (Difco), 0.2% (w/v) MgSO₄·7H₂O and 1.5% (w/v) Davis agar. Up to four filters were placed on 20 ml medium in each Petri dish (90 mm diam.) and incubated at 30 °C. Fruiting bodies were picked off the filters using a needle and crushed in 0.2% (w/v) MgSO₄·7H₂O to release the myxospores. Heat-fixed smears were stained with methylene blue, which allows darkly-stained intact vegetative cells and myxospores to be distinguished from lightly staining dead and lysed cells. Fruiting body formation was also initiated by spreading or spotting a cell suspension directly on fruiting medium (Jones, 1978).

Pulse-labelling. Filters were transferred from fruiting medium to an absorbent pad (two layers of Whatman no. 1 filter paper, 50 mm diam.) soaked with 4 ml 0.2% (w/v) MgSO₄·7H₂O containing 0.05% (w/v) Casitone and 2 µCi (DL + meso)-2,6-diamino[1,7-¹⁴C]pimelic acid (50 mCi mmol⁻¹; The Radiochemical Centre,

Table 1. Incorporation of *meso*-diamino^[14C]pimelic acid during fruiting body formation

Myxococcus xanthus on membrane filters was transferred from fruiting medium to pads soaked with *meso*-diamino^[14C]pimelic acid. After 3 h, filters were washed and the incorporated radioactivity was determined. Fruiting bodies were examined microscopically to determine the proportion of myxospores present. Mature fruiting bodies were at least 4 d-old.

Developmental stage	¹⁴ C incorporated during 3 h pulse (c.p.m.)	
	Expt 1	Expt 2
Vegetative cell swarm	22790	23800
Vegetative cell aggregates	16563	13491
Immature fruiting body:		
0–20 % myxospores	6538	7909
40–50 % myxospores	—	6193
50–60 % myxospores	1633	1997
80–90 % myxospores	10862	14741
> 90 % myxospores	15919	24542
Young fruiting body, 100 % myxospores	12084	—
Mature fruiting body (4 d-old)	2766	3202
Killed vegetative cells	91	81

Amersham). After 3 h incubation at 30 °C, filters were either washed with 10 ml 0.2 % (w/v) MgSO₄·7H₂O and dried at 80 °C or transferred to a fresh pad in which the ¹⁴C-labelled *meso*-diaminopimelic acid had been replaced by 2 mM unlabelled *meso*-diaminopimelic acid. The amino acid has no effect on fruiting at this concentration (Campos & Zusman, 1975) and is incorporated almost entirely into peptidoglycan (Dawson & Jones, 1979). After 1 h incubation, the 'pulse-chased' filters were returned to fruiting medium to allow development to continue. Dried filters were counted for radioactivity in toluene containing 2,5-diphenyl-oxazole (7 g l⁻¹) using an Inter technique SL30 liquid scintillation spectrophotometer.

Cell wall autolysis. A culture growing exponentially was labelled for three generations with *meso*-diamino^[14C]pimelic acid (0.1 μCi ml⁻¹). Filters were prepared as described above and removed from fruiting medium at intervals for determination of bound radioactivity. To estimate the radioactivity in myxospores alone, fruiting bodies were prepared by spreading 0.1 ml of a washed suspension of pre-labelled organisms on fruiting medium. After 6 d myxospores were recovered by the method of Wireman & Dworkin (1975). Vegetative cells and myxospore numbers were determined by counting in a Thoma chamber.

RESULTS AND DISCUSSION

Fruiting body formation on membrane filters

Myxococcus xanthus on cellulose acetate membrane filters was able to construct fruiting bodies on a suitable fruiting medium in the same time as organisms spotted directly on the agar surface. Provided care was taken to ensure that the organisms were evenly spread over the surface of the filter and that the filter was fully in contact with the agar surface, the fruiting bodies were of a regular size and shape and were evenly distributed over the filter. Transferring filters from one plate to another of the same medium had no effect on fruiting body formation. Filters could also be transferred to absorbent pads, soaked in suitable liquid media, for 1 to 6 h without deleterious effects on fruiting. This method can therefore be used to study the effect of inhibitors, or other compounds, on particular stages of fruiting body formation. The filters hold little liquid, and water-soluble compounds diffuse away rapidly into the agar. In particular, the use of membrane filters offers a considerable improvement on previously published methods for pulse-labelling during fruiting body formation where radioactive compounds are injected below the agar (Wireman & Dworkin, 1977). Filters may be dried and stained to provide a permanent record of the size and number of fruiting bodies formed under different conditions.

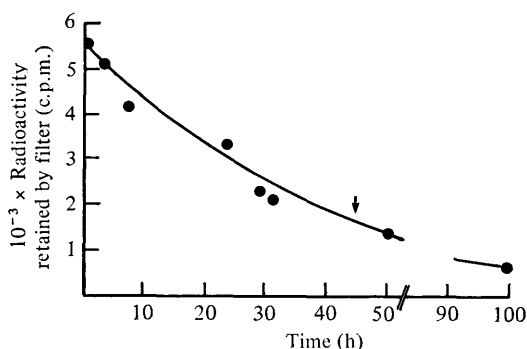


Fig. 1. Loss of *meso*-diamino¹⁴C]pimelic acid during fruiting body formation. The organism was grown in the presence of *meso*-diamino¹⁴C]pimelic acid, filtered and the washed filters were placed on fruiting agar. Duplicate filters were removed at intervals during fruiting body formation and the radioactivity remaining was determined. The arrow indicates the time when 50% myxospores were present.

Cell wall turnover during fruiting body formation

These experiments were aimed at establishing whether the breakdown and resynthesis of walls observed during glycerol-induced myxospore formation (Dawson & Jones, 1979) was an artefact resulting from glycerol addition, or whether this restructuring of the peptidoglycan was also essential to myxospore differentiation as it occurred within the fruiting body under conditions of nutrient depletion.

Developing fruiting bodies of *M. xanthus* on membrane filters were pulse-labelled with radioactive *meso*-diaminopimelic acid. In several experiments, two of which are shown in Table 1, a similar pattern of labelling was observed. Starting from vegetative cells, there was a fall in *meso*-diamino¹⁴C]pimelic acid incorporated per 3 h labelling period until 45 h, when the fruiting bodies contained 50% spherical cells (i.e. developing myxospores). Incorporation then increased considerably until myxospore formation was complete. Older fruiting bodies showed reduced incorporation although this was higher than that for control filters with an equivalent number of killed cells. This may be due to the presence of some vegetative organisms towards the edges of the filters or the mature fruiting body myxospores may not have been fully dormant. The period of maximum incorporation corresponds morphologically with the period of increasing cross-linkage in the peptidoglycan observed in glycerol-induced myxospores (White *et al.*, 1968).

The loss of *meso*-diaminopimelic acid from the peptidoglycan was investigated by pre-labelling the culture to be filtered, and then measuring the radioactivity remaining bound to the filters during fruiting body development. The results (Fig. 1) indicate that loss of *meso*-diaminopimelic acid from the walls occurs from the time of filtering (i.e. vegetative cells) until mature fruiting bodies are present.

Wireman & Dworkin (1975, 1977) have reported that a large part of the cell population lyses during fruiting body formation and that the remainder form myxospores. To show that the loss of *meso*-diaminopimelic acid shown in Fig. 1 was not due to these lysing vegetative cells alone but also occurred in developing myxospores, we recovered the myxospores from mature (6 d) fruiting bodies and determined the radioactivity remaining per cell. These myxospores retained only 6 to 7% of the radioactivity per cell of the initial vegetative organisms from which they developed. Thus it is clear that loss and resynthesis of peptidoglycan occurs during the formation of fruiting body myxospores.

The use of membrane filters facilitates the comparison of fruiting body myxospores with those induced by glycerol. The results obtained show that wall breakdown and resynthesis are common to the formation of both myxospore types, and we believe that the biochemical

process initiated by the inducer glycerol is probably the same as that in a mature fruiting body. Thus, so far, the study of induction systems seems to reflect mechanisms active in natural sporulation.

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