NUTRITIONAL REGULATION OF MORPHOGENESIS IN *MYXOCOCCUS XANTHUS*

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ABSTRACT

DWORKIN, MARTIN (University of Minnesota, Minneapolis). Nutritional regulation of morphogenesis in *Myxococcus xanthus*. J. Bacteriol. **86**:67–72. 1963.—Fruiting-body formation by *Myxococcus xanthus* can be induced by omitting phenylalanine and tryptophan from the chemically defined growth medium. This effect was specific for these two amino acids and was not attributable to a nonspecific lowering of the growth rate. A complex pool of amino acids is present in vegetative cells of *M. xanthus*. Nutritional environments leading to morphogenesis also result in a decrease in the level of this pool. These changes have been described.

It is a common observation that the formation of fruiting bodies and microcysts by myxobacteria is a response to the nutritional environment. Specifically, growth in a rich medium seems to favor vegetative growth and to inhibit morphogenesis (Vahle, 1909; Oetker, 1953; Kühlwein, 1953). A similar relationship has been established for perithecium formation in fungi (Asthana and Hawker, 1936) and pseudoplasmodium formation in the myxomycetes (Raper, 1940). In general then, the formation of microcysts, perithecia, and other types of resting stages may indeed represent a response to a less favorable nutritional environment rather than an inexorable part of a normal developmental cycle. This notion has been discussed and expanded by Foster (1956).

A recent investigation defined the nutritional requirements for the vegetative growth of *Myxococcus xanthus*, a fruiting myxobacterium (Dworkin, 1962). As a result, it has been possible to define the nutritional conditions which supress or induce fruiting-body formation. This report deals with both a description of this nutritional regulation as well as an attempt to obtain some information as to the mechanism of this regulation.

MATERIALS AND METHODS

Organism. M. xanthus strain FB was used throughout this investigation. This culture was obtained in 1960 from the stock culture collection of the Bacteriology Department, University of California, Berkeley, and was originally isolated by E. Ordal. It will go through the entire developmental cycle, forming fruiting bodies and microcysts.

Media. Casitone-salts (CT) medium (Dworkin, 1962) was used for routine cultivation of vegetative cell cultures. Coli agar (CA) medium (Dworkin, 1962) was used for storing stock cultures as microcysts. The composition of the synthetic medium used for obtaining vegetative growth (medium S) is listed in Table 1 (Dworkin, 1962). All amino acids were the L isomers.

Preparation of starved cells. Vegetative cells grown on liquid CT medium to a Klett reading of about 300 (using a no. 54 filter) were centrifuged and inoculated into 20 ml of a mixture of 0.1% $MgSO_4$ and 0.01 M K_2HPO_4-KH_2PO_4 (pH 7.6). The initial Klett reading was about 100. The culture was shaken in a 250-ml Erlenmeyer flask for 16 to 18 hr, during which time the Klett reading dropped to about 80. Plate counts performed during this period did not indicate any significant decrease in viable cells, nor did total counts indicate any significant changes in the number of cells.

Details of cultivation. All cultures were incubated at 30 C (±1 C). Liquid cultures were incubated on a rotary shaking machine (240 strokes/min) as shallow layers in Erlenmeyer flasks (20 to 30 ml per 250-ml flask).

Stock cultures were stored at 4 C as fruiting bodies on CA slants.

On CT agar there was almost 100% germination of microcysts, and there was relatively poor germination on CA agar. Therefore, to prevent selection of nonmicrocyst-forming vegetative cells by serial transfer on CA medium, cultures were alternately transferred to CT slants.

TABLE 1. *Composition of defined medium for vegetative growth of Myxococcus xanthus FB*

Component	Amount
	mg/ml
Tyrosine	0.6
Asparagine	0.5
Leucine	1.0
Isoleucine	0.5
Proline	0.5
Arginine	0.1
Histidine	0.05
Glycine	0.05
Lysine	0.25
Methionine	0.05
Phenylalanine	1.0
Tryptophan	1.0
Serine	0.1
Threonine	0.1
Valine	0.1
Djenkolic acid	0.1
Alanine	1.0
$MgSO_4$	1.0
K_2HPO_4-KH_2PO_4 (pH 7.6)	0.01 M

For fruiting-body formation on the defined medium, starved cells were centrifuged, adjusted to a Klett reading of about 1,000 with the $MgSO_4$-phosphate mixture, and a 0.025-ml drop was pipetted onto the surface of the fruiting agar. For this, plastic petri dishes (60 mm diameter) containing 10 ml of agar medium were used. The drop was allowed to dry, and the plates were then incubated in a moist chamber at 30 C. Observations of fruiting-body formation were made using a dissecting microscope at 20 × magnification.

Amino acid pools. The internal free amino acids were extracted from the cells according to the method of Gale (1947). Cells were starved as described above. These cells were used to inoculate 1-liter Erlenmeyer flasks containing 200 ml of media of various compositions. Where the medium was of such a nature that it would not support significant amounts of growth (i.e., media, which when solidified would support fruiting-body formation), an extremely large inoculum was used. These were then, in essence, replacement cultures. After 24 hr of incubation with shaking, microscopic examination revealed no significant amount of spheroplast formation or autolysis. The cells were then centrifuged, washed once with distilled water, made up to 20 ml with distilled water, and heated to boiling for 10 min. The cells were removed by centrifugation, and the supernatant fluid was passed through a Millipore filter. The filtrate was reduced in volume by gentle heating to about 3 ml, and 50% trichloroacetic acid was added to a final concentration of 5%. The precipitate was removed by centrifugation and washed with 5% trichloroacetic acid. The washings and supernatant fluid were pooled, and the resultant material was analyzed for individual amino acids with a Spinco model 120 automatic amino acid analyzer.

RESULTS

When a heavy suspension of vegetative cells is placed on the appropriate fruiting agar medium, the cells will aggregate into centers, forming relatively undifferentiated mounds. The surface of the agar between these mounds becomes cleared of all but a thin layer of cells. Within these mounds, the vegetative cells will convert to microcysts. These raised mounds, within which conversion to microcysts has taken place, are referred to as fruiting bodies.

Fruiting-body formation on defined media. When starved vegetative cells are placed on the surface of S agar, they divide by binary transverse fission and form colonies with typical myxobacterial edges (Fig. 1). Only vegetative rods can be observed in the colony. However, when starved vegetative cells are placed on the surface of S agar from which L-phenylalanine and L-tryptophan have been omitted (SF agar), fruiting bodies are formed within 30 hr (Fig. 2).

One can obtain fruiting-body formation with washed cells taken directly from a CT culture. However, the fruiting bodies obtained when these cells are placed on the SF agar are not sharply delineated and frequently coalesce after short periods of time. Often the immature fruiting bodies never culminate in microcyst formation. However, when the vegetative cells are starved prior to being placed on the fruiting medium, fruiting body formation takes place more rapidly; the fruits are more sharply delineated and conversion of vegetative cells to microcysts invariably takes place.

It was next of interest to determine whether the triggering of the morphogenetic sequence by the absence of phenylalanine and tryptophan was specific for this pair of amino acids or could be duplicated by the omission of other constitu-

ents of the medium. Accordingly, each amino acid was omitted singly from the medium, and all possible pairs of amino acids were also omitted. In no case was it possible to duplicate the effect which was obtained by omitting phenylalanine and tryptophan. When, however, the over-all level of amino acids in the S medium was reduced in a stepwise fashion, fruiting-body formation occurred on the medium diluted to 0.6 of the original concentration of amino acids. To determine whether this could be a simple consequence of reducing the levels of phenylalanine and tryptophan, the amounts of these two amino acids in the medium were similarly reduced in a stepwise fashion. Again, fruiting-body formation took place, but at a concentration of phenylalanine and tryptophan of 0.4 mg/ml each (normal concentration of each in the S medium was 1.0 mg/ml) as shown in Table 2. These figures varied slightly from experiment to experiment. In general, however, to obtain fruiting body formation, it was necessary to dilute the phenylalanine and tryptophan in medium S to a somewhat greater extent than was necessary for the S medium itself.

Growth of vegetative cells on the liquid SF medium was extremely poor (Fig. 3). Equally poor growth was obtained by omitting pairs of amino acids other than phenylalanine and tryptophan. It appears, then, that morphogenesis in *M. xanthus* is not simply a response to a decrease in the rate of biosynthesis of cellular constituents, but clearly reflects the external level of phenylalanine and tryptophan.

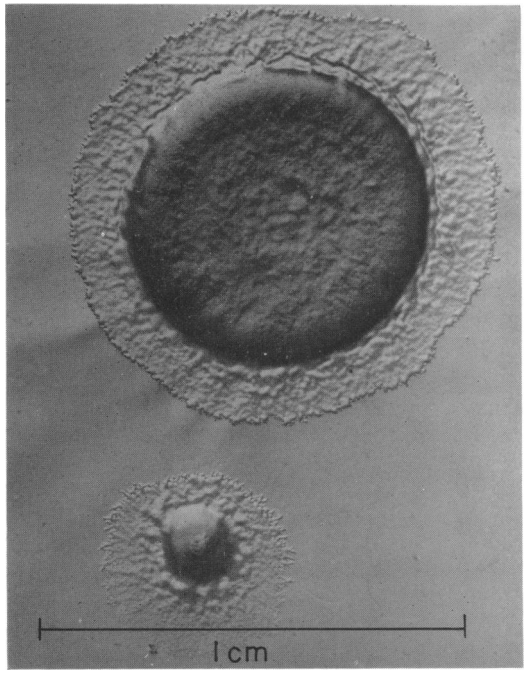

FIG. 1. *Vegetative colony of Myxococcus xanthus FB.*

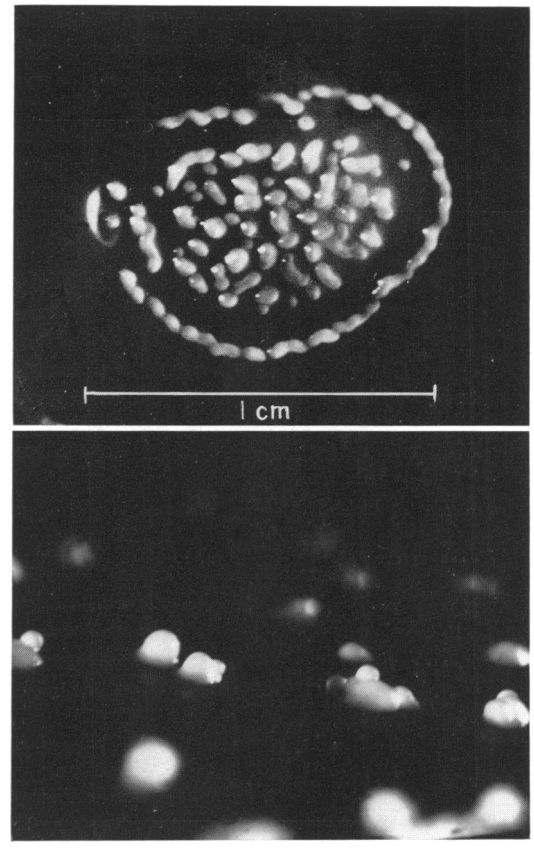

FIG. 2. *Fruiting bodies of Myxococcus xanthus FB. (top) Top view; (bottom) side view.*

Amino acid pool. The effect of starvation on fruiting-body formation suggested the involvement of the amino acid pool in the process. A relationship between amino acid pool levels and aggregation in the slime mold, *Dictyostelium discoideum*, has already been demonstrated (Wright and Anderson, 1960). Vegetative cells of *M. xanthus* grown on a rich medium (CT) were found to contain a large pool of amino acids consisting of 16 identified constituents (Table 3). Starvation reduced the pool level drastically. The pool level of starved cells replaced on a

TABLE 2. *Induction of fruiting-body formation by diluting entire medium or by reducing levels of phenylalanine and tryptophan in medium S*

Medium	Results	Phenylalanine and tryptophan in medium S (mg/ml each)	Results
1.0	Vegetative growth	1.0	Vegetative growth
0.9	Vegetative growth	0.9	Vegetative growth
0.8	Vegetative growth	0.8	Vegetative growth
0.7	Vegetative growth	0.7	Vegetative growth
0.6	Fruiting bodies	0.6	Vegetative growth
0.5	Fruiting bodies	0.5	Vegetative growth
0.4	Fruiting bodies	0.4	Fruiting bodies
0.3	Fruiting bodies	0.3	Fruiting bodies
0.2	Fruiting bodies	0.2	Fruiting bodies
0.1	Fruiting bodies	0.1	Fruiting bodies
		0	Fruiting bodies

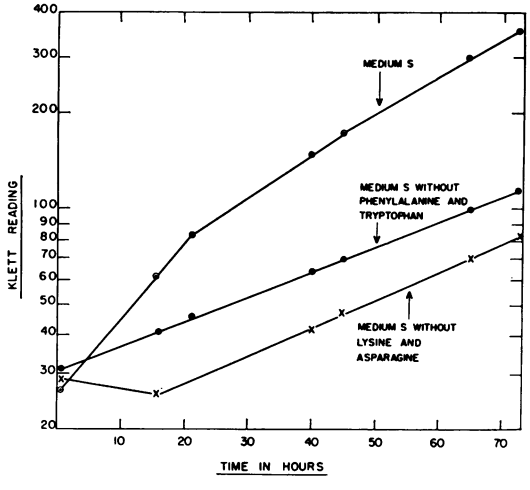

FIG. 3. *Vegetative growth of Myxococcus xanthus FB in liquid media.*

medium supporting vegetative growth (medium S) was compared with the pool level found in cells replaced in a synthetic fruiting medium (Table 3). One can see that, as a result of the

TABLE 3. *Amino acid pools in vegetative cells of Myxococcus xanthus FB placed under different nutritional conditions**

Amino acid	CT	S	SF	¼ × S	$MgSO_4$-phosphate buffer
Lysine	14.0	16.0	7.0	5.6	3.8
Histidine	4.0	<0.1	<0.2	<0.2	<0.1
Arginine	15.2	7.0	<0.2	<0.2	<0.1
Aspartic acid	9.2	5.0	2.6	1.6	0.6
Threonine	8.4	4.4	<0.2	1.0	<0.1
Serine	16.2	8.8	5.4	3.4	1.2
Glutamic acid	24.2	27.6	13.2	6.6	1.2
Proline	12.6	<0.1	<0.2	<0.2	<0.1
Glycine	16.0	9.8	5.4	2.6	0.8
Alanine	24.2	15.4	17.0	7.4	3.2
Valine	13.2	6.4	<0.2	<0.2	<0.1
Methionine	5.8	2.6	<0.2	<0.2	<0.1
Isoleucine	6.6	4.2	3.0	0.6	<0.1
Leucine	16.4	7.8	7.2	1.6	0.4
Phenylalanine	10.8	4.2	<0.2	<0.2	<0.1
Tyrosine	9.2	<0.1	<0.2	<0.2	<0.1
Total	206.0	119.2	60.8	30.4	11.2
Per cent	100	58	30	15	5

* Results expressed as μmoles of amino acid per 10 g (dry weight) of cells.

starvation, the over-all level of amino acids in the pool was reduced to 5% of the level obtained when the cells were grown in CT medium. When starved cells were replaced on medium S (which supports vegetative growth), the level rose to 58% of the original level. The pool from cells replaced on SF medium (fruiting medium) was considerably lower than that of cells on medium S, rising to only 30% of the original level. Table 3 also indicates the level of individual amino acids in the pool under these various conditions. With some exceptions (e.g., alanine and leucine), levels of the individual pool amino acids were considerably lower when the cells were incubated on the fruiting medium (SF) than when they were grown on S or CT media.

DISCUSSION

It is now possible, by manipulating specific components of a chemically defined medium, to induce or suppress the life cycle of a fruiting myxobacterium. It was hoped initially that this would provide an opportunity to investigate the basis of the induction of morphogenetic change by clarifying the relationship between the ex-

ternal milieu and a morphogenetic trigger. The discovery, however, that *M. xanthus* possesses an amino acid pool of relatively large, complex, and variable proportions made it clear that such a direct relationship was unlikely. We must now shift our attention to the pool itself with two questions in mind. One, how does the presence or absence of phenylalanine and tryptophan in the external medium regulate the level of at least 14 other amino acids in the pool? Two, if the level of the pool or of a specific component of the pool is causally related to the initiation of morphogenesis, what is the mechanism by which this occurs? Regarding the first question, preliminary results with C^{14}-labeled amino acids suggest that many of the amino acids penetrate into the cell somewhat more rapidly in the presence of phenylalanine and tryptophan than in their absence. The possibility that the phenylalanine and tryptophan are acting as energy sources, and are thereby permitting the active transport of the other amino acids, is ruled out by manometric and isotopic experiments which indicate that numerous other amino acids are oxidized more rapidly than are phenylalanine and tryptophan (*unpublished results*). Additional work is under way to clarify this situation. Regarding the second question, one may only make vague suggestions: e.g., the repressible biosynthesis of a chemotactic substance is regulated by the level of a component or components of the amino acid pool. That such a chemotactic substance exists has been suggested by the experiments of Lev (1954), Jennings (1961), and McVittie and Zahler (1962). It becomes immediately apparent, however, that it is premature to discuss specific mechanisms of repression and control before one can describe fruiting-body formation in biochemical terms.

It is interesting to consider the differences between microcyst formation and endospore formation. Microcyst formation differs fundamentally from endospore formation in a number of ways. One of these is that, whereas endospore formation requires the presence of an amino acid pool as precursors for spore protein (Hardwick and Foster, 1952) and involves drastic alterations in the physical and chemical nature of the cell (Doi and Halvorson, 1961; Young and Fitz-James, 1962), the formation of the microcyst seems to be stimulated by the reduction of the pool level. A recent study of the fine structure of *M. xanthus* during morphogenesis (Voelz and Dworkin, 1962) suggests that, morphologically, the microcyst is merely a vegetative cell with a lowered surface-to-volume ratio, with a surrounding dense slime capsule. The lack of a drastic structural reorganization is consistent with the presumed lack of function of the pool as a source of microcyst precursors.

The rapid response of the vegetative cells to the external level of amino acids is consistent with the ecological notion of the microcysts as a resting stage, even though the metabolic and resistance properties of microcysts have not yet been clearly defined.

Acknowledgments

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