JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 295-300
0021-9193/79/01-0295/06$02.00/0

Vol. 137, No. 1

Rifampin-Resistant Mutants of *Myxococcus xanthus* Defective in Development

KENNETH RUDD AND DAVID R. ZUSMAN*

Department of Bacteriology and Immunology, University of California, Berkeley, California 94720

Received for publication 6 September 1978

Rifampin, an antibiotic which is known to bind to and inhibit RNA polymerase, was used to probe the molecular regulation of development in *Myxococcus xanthus*. Rifampin-resistant mutants were screened for defects in fruiting-body formation. About 20% of the isolates in the initial screenings showed major defects in developmental aggregation or sporulation. Eleven independent mutants with wild-type growth rates and stable phenotypes were analyzed by transduction. In these strains, the rifampin-resistant and nonfruiting phenotypes showed cotransduction frequencies equal to or greater than 99.0 to 99.9%. The RNA polymerase activities were resistant to rifampin in vitro, indicating that the RNA polymerase is altered in these strains. Although their fruiting phenotypes are heterogeneous, these strains can be divided into two classes based on the level of aggregation. The results suggest that RNA polymerase plays a significant role in the regulation of development in *M. xanthus* since mutations which cause no apparent changes in vegetative growth result in striking defects in fruiting-body formation.

Myxococcus xanthus is a gram-negative bacterium which grows vegetatively in soils on decaying organic material, on the bark of living trees, or by preying upon other microorganisms (7). When nutrients are depleted from a solid culture medium, cells aggregate to form mounds. Within the mounds, the rod-shaped vegetative cells convert to round or ovoid spores. Mounds of mature myxospores are referred to as fruiting bodies.

We have recently investigated protein synthesis during aggregation and fruiting-body formation in *M. xanthus* and have found that many proteins showed significant changes in their amounts and patterns of synthesis during development (M. Inouye, S. Inouye, and D. R. Zusman, Dev. Biol., in press). These results suggest a complex but precise program of gene expression. It is likely that the synthesis of at least some of these proteins is regulated at the transcriptional level. To study this possibility, we searched for developmental mutants with defects in RNA polymerase. RNA polymerase mutations are easily obtained in many bacteria by selecting for resistance to the antibiotic rifampin (6, 10, 11, 15), a specific inhibitor which is known to bind to the β subunit of RNA polymerase (11, 14). We found that a significant class of these rifampin-resistant mutants in *M. xanthus* were, in fact, defective in fruiting-body formation; in at least 11 of these mutants the two phenotypes are probably caused by a single mutation. The heterogeneous terminal phenotypes observed in these mutants suggests that transcriptional specificities may be altered in several different ways during development.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. Three different strains that are "wild type" for fruiting-body formation were used: DZ2 (5), DZF1 (FB) obtained from D. Kaiser, who obtained it from M. Dworkin, and DZF6 (4). DZ1 (18), a nonfruiting strain, was used as a phage indicator. The phage strain MX4*ts27htfhrm* (4) was used for all transductions.

Media and cultural conditions. Bacteria were routinely grown in CT broth (4), CYE broth (4), or TYE broth (CYE broth which contains Trypticase [BBL] instead of Casitone [Difco]). Cultures were grown at 30°C with gyratory shaking at 175 to 200 rpm. Fruiting-body formation was induced by inoculating cells on coli agar (plastes containing 8×10^8 cells of autoclaved *Escherichia coli* HfrH per ml, 0.1% $MgSO_4 \cdot 7H_2O$, and 1.6% Difco agar) or on CF agar (2, 9).

Isolation of rifampin-resistant mutants. Spontaneous rifampin-resistant (Rif^r) mutants were obtained by plating rifampin-sensitive strains on CF, CT, CYE, or TYE agar containing rifampin (25 μg/ml; Sigma Chemical Co.). Single colonies were repurified on CT, CYE, or TYE agar without rifampin. When the use of independent cultures is indicated, each culture was grown from a single colony to midlog phase in the absence of rifampin and then plated on media containing rifampin (25 μg/ml).

Phenotypic analysis of Rif^r mutants. Rifampin-resistant strains were grown on CT, CYE, or TYE agar for 3 to 6 days and then transferred by toothpick to coli agar to test for fruiting-body formation. After at least 10 days at 30°C, fruiting phenotypes were ex-

amined under a dissecting microscope. Strains which failed to form fruiting bodies on three subsequent tests were considered stable mutants. Fruiting phenotypes were analyzed further by harvesting exponentially growing cells ($\leq 1.2 \times 10^9$ cells per ml) by centrifugation (10 min at 7,000 × *g* at 4°C) and resuspending the pellets in TM buffer [0.01 M tris(hydroxymethyl)aminomethane buffer, pH 7.6, containing 0.01 M $MgCl_2$] at low (1.6×10^9 cells per ml) or high (8×10^9 cells per ml) density. The cells were then spotted on CF agar and incubated at 30°C. Fruiting phenotypes were observed and photographed after 5 days, using a dissecting microscope equipped with a Polaroid camera.

Growth rates in TYE broth were determined by measuring turbidity, using a Klett-Summerson photoelectric colorimeter. Spore samples were prepared by harvesting cells after 5 days on CF agar. The cells were resuspended in TM buffer, heated at 55°C for 1 h, and sonically oscillated for 1 min. (The probe was sterilized in chloroform and rinsed in 95% ethanol between samples to prevent cross-contamination.) This procedure reduced viability of vegetative cells by $>10^9$-fold but did not affect spore viability. Samples were plated on CYE or TYE agar, using a CF agar overlay. Sporulation frequencies were normalized to 1.0 for wild type.

The effect of wild-type cells on the sporulation of mutant strains was determined by mixing wild-type and mutant cells in a 1:1 ratio and spotting at high density on CF agar. Spore counts were determined on plates containing no rifampin and plates containing 10 μg of rifampin per ml.

Glycerol inductions were performed by adding glycerol (0.5 M) to exponential-phase cultures (8).

Bacteriophage growth and transductions. Stock lysates of bacteriophage were prepared by infecting DZ1 at a multiplicity of 1 in CYE broth, incubating at 28°C overnight, treating with chloroform, and clearing lysates by centrifugation. DZ1 was used as an indicator for all phage lysates.

Transducing phage lysates were prepared by inoculating CT plates with donor bacteria and bacteriophage at a multiplicity of infection of 0.005. Plates were incubated for 2 to 4 days at 28°C and harvested by leaching overnight in 5 ml of 0.1% NaCl per plate. Bacteriophage yields were between 10^9 and 10^{10} phage per ml.

Transductions were carried out by mixing phage and recipient bacteria (multiplicity of infection, 2 to 10) and plating directly on CF agar at 35°C. After 24 h of outgrowth, rifampin was added in an agar overlay to a final concentration of 10 μg/ml. This procedure ensured that all transductants were independently derived. DZF1 was the recipient strain in all transduction experiments.

In vitro assay of DNA-dependent RNA polymerase activity. The procedure of Burgess (3) was used to assay in vitro DNA-dependent RNA polymerase activities, with modifications as noted. Buffers G and A contained 1.0 mM ethylenedinitrilotetraacetic acid and 1.0 mM phenylmethanesulfonyl fluoride to inhibit protease activity; the pH of buffer G was 7.5, and buffer A contained 10% glycerol. Cells were grown in TYE broth at 30°C to 1.0×10^9 cells per ml. The cells were centrifuged at 7,000 × *g* for 10 min, washed once in buffer G, and frozen as pellets at −76°C. Thawed cells were resuspended in 2 ml of buffer G per g (wet weight) and sonically oscillated for a total of 3 min in 30-s bursts. The extracts were maintained below 4°C until assayed. Three volumes of buffer G were added to the sonic extracts, and solid ammonium sulfate was added to bring the final concentration to 25% saturation. The extracts were centrifuged at 27,000 rpm for 2 h (using a Spinco no. 30 rotor) to remove ribosomes and cell debris. The supernatant was brought to 65% saturated ammonium sulfate, stirred for 45 min, and centrifuged at 40,000 × *g* for 25 min. The top of the pellet was washed three times and resuspended in a minimal volume of buffer A. The extracts were dialyzed for 4 h against buffer A and stored in 50% glycerol at −20°C. Protein concentration was determined by the method of Lowry et al. (12).

The RNA polymerase assay mixture of Burgess was modified to contain 1.0 mM ethylenedinitrilotetraacetic acid, 1.0 mM phenylmethylsulfonyl fluoride, and 40 μg of salmon testes DNA per ml. Newly synthesized RNA was labeled with [^{3}H]UTP and [^{3}H]CTP (Amersham Corp., 53 mCi/mmol) at 35°C, and 100-μl samples were trichloroacetic acid precipitated on Whatmann 3MM filters, using a batch-washing procedure. Filters were washed four times in cold trichloroacetic acid, twice in acetone, and once in ethyl ether. Samples were air dried and counted in a liquid scintillation counter. One unit of activity incorporates 1 pmol of CMP or UMP into trichloroacetic acid-precipitable material per min per ml.

RESULTS

Isolation of rifampin-resistant mutants defective in fruiting-body formation. *M. xanthus* was extremely sensitive to rifampin; the minimum inhibitory concentration was only 0.2 μg/ml, as determined by cell viability on CYE agar containing various drug concentrations. Therefore, spontaneous mutants resistant to rifampin (Rif^r) were selected by simply plating cultures of strains DZ2, DZF1, and DZF6 on growth media containing 25 μg of rifampin per ml, a concentration 100 times higher than the minimum inhibitory concentration and therefore sufficiently high to reduce the probability of selecting for permeability mutations. The Rif^r mutants appeared at a frequency of 10^{-7} to 10^{-8}. In an initial experiment, 1325 Rif^r colonies derived from two independent cultures of strain DZ2 were isolated and then tested for their ability to fruit on coli agar. Of these, 84 were unable to form fruiting bodies on the first screening. In a control experiment, 1,000 rifampin-sensitive colonies were tested for their ability to form fruiting bodies, and all did. These results suggested that nonfruiting mutants (Fru$^-$) may be found as a subclass among Rif^r mutants.

Since these Fru$^-$ mutants were not derived from independent cultures, they could not be

used to determine the frequency of Rif^r Fru$^-$ mutations. In Fig. 1, 64 independent cultures of *M. xanthus* were used to select Rif^r colonies. Eighteen of these colonies from each independent culture were tested for their ability to form fruiting bodies. The results showed significant differences between the independent cultures. For example, in several cultures none of the 18 colonies were defective in fruiting; in others, as many as 15 of 18 colonies were Fru$^-$. These results probably reflect the large numbers of siblings present in the independent cultures so that the earliest spontaneous mutant to Rif^r predominates. In Fig. 1, the average spontaneous frequency of Fru$^-$ phenotypes among many independent cultures was about 20% of the initial Rif^r isolates.

It should be noted that about half of the initial Rif^r Fru$^-$ isolates showed Rif^r Fru$^+$ phenotypes on subsequent subculturing and retesting (each strain was subcultured on CYE agar and retested for fruiting on coli agar at least three times). In all cases, the Fru$^-$ phenotype but not the Rif^r phenotype reverted. These results suggest either that the original double phenotype (Rif^r Fru$^-$) was due to two separate mutations or that the Fru$^-$ phenotype (but not the Rif^r phenotype) was suppressed by a second site mutation in the "revertants."

Genetic analysis of the Rif^r Fru$^-$ mutants. Sixty-seven stable Rif^r Fru$^-$ strains were infected with bacteriophage MX4*ts27htfhrm*, a temperature-sensitive, high-frequency generalized transducing phage with wide host range for strains of *M. xanthus* (4). The phage lysates were used to transduce a "wild-type" strain, DZF1, to Rif^r. The transductants were then tested for their ability to form fruiting bodies on coli agar. About one-half of the strains used showed very high cotransduction of the *rif fru*$^-$ allele(s); the remaining strains showed no cotransduction or, in a few cases, low cotransduction. Since many of these crosses were not isogenic, it is possible that the original Rif^r Fru$^-$ phenotypes were dependent upon the particular genetic backgrounds of the initial Rif^r isolates. In other cases where the crosses were isogenic, it is clear that the original strains, although spontaneously induced mutants, were actually double mutants.

The strains which showed very high cotransduction frequencies were studied further. Eleven DZF1 transductants from independently derived mutants were used to prepare the phage lysates, and their mutations were transduced again into DZF1 (Table 1). The transductants were plated in a CF agar overlay. After 24 h of outgrowth at 35°C, a second overlay containing rifampin was placed above the microcolonies. Thus, in each

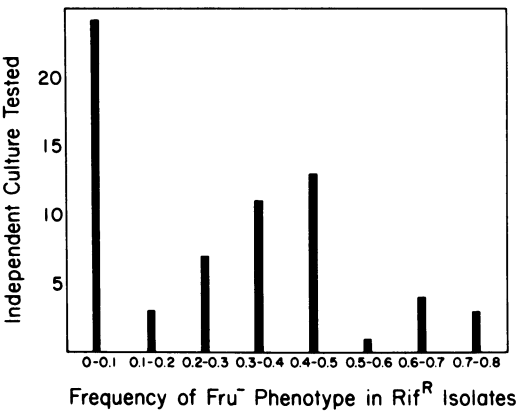

FIG. 1. *Distribution of Rif^r Fru$^-$ phenotypes in independent cultures. Sixty-four independent cultures of M. xanthus strain DZF6 were concentrated and plated on CF plates containing rifampin (25 μg/ml). After 10 days of incubation at 30°C, 18 Rif^r colonies were isolated, and their fruiting phenotypes were determined on coli agar. The histogram shows the frequency of Rif^r Fru$^-$ phenotypes observed in the 18 colonies.*

experiment, the Rif^r clones are independent transductants; furthermore, the medium is a fruiting medium so that the Fru$^+$ phenotype can be screened directly. In this manner, over 900 independent transductants were analyzed for each mutant. In each case the cotransduction frequencies were ≥99%. The resolution of the transduction data was limited by the number of transductants screened and by the low but finite level of new spontaneous mutations to Rif^r (10^{-7} to 10^{-8}). The transduction frequency of the *rif* locus was 100- to 1,000-fold higher than this spontaneous rate. The high cotransduction frequencies in these strains support the hypothesis that the Rif^r and Fru$^-$ phenotypes are the result of a single mutation.

Phenotypic analysis of the Rif^r Fru$^-$ mutants. Figure 2 shows the morphological changes which occur when the wild-type strain DZF1 is spotted at high density on CF agar. The spots undergo a series of transitions which include: smooth spots (0 h), wrinkled aggregates (4 to 8 h), translucent mounds (8 to 15 h), and morphologically mature fruiting bodies (28 to 32 h). All the Rif^r Fru$^-$ mutants analyzed appear to show terminal morphological phenotypes which roughly correspond to intermediate stages of wild-type development. We therefore divided the mutants into two groups on the basis of their terminal phenotypes: the class I mutants appear to be blocked early. They show very little tendency to aggregate at high density (8×10^9 cells per ml) but some tendency to aggregate into swirls or flat mounds at low density (1.6×10^9

TABLE 1. *Phenotypic and genetic analysis of independent Rif^r Fru$^-$ mutants*

Phenotypic class[a]	Strain	Sporulation frequency[b]		Growth rate[c] (% wild type)	No. of independent Rif^r transductants tested[d]	Cotransduction (%)
		Alone	Plus wild type			
I. Low-level aggregation	DZF1020	10^{-2}-10^{-4}	1.0	100	1,024	≥99.0
	DZF3042	10^{-3}-10^{-5}	1.0	100	1,669	≥99.9
	DZF3030	10^{-1}-10^{-2}	10^{-1}	100	1,193	≥99.9
	DZF3039	$<10^{-7}$	10^{-1}	100	985	≥99.0
	DZF3028	10^{-7}	10^{-2}	100	939	≥99.0
	DZF1782	$<10^{-7}$	10^{-1}	100	908	≥99.0
	DZF3041	10^{-6}	10^{-6}	100	916	≥99.0
II. Translucent mounds	DZF3031	10^{-2}-10^{-3}	10^{-1}	95	1,585	≥99.9
	DZF3044	10^{-2}	10^{-1}	90	1,903	≥99.9
	DZF3045	10^{-3}-10^{-5}	1.0	90	1,181	≥99.5
	DZF3043	10^{-7}	10^{-1}	100	1,375	≥99.9

[a] Phenotypes were determined by spotting cells at high density on CF agar and incubating at 30°C for 5 days.
[b] Sporulation frequencies were determined from the frequency of spores found in the 5-day-old spots described above. It should be noted that sporulation frequencies are normalized to 1.0 for wild type (DZF1).
[c] Growth rates were determined in TYE broth at 30°C.
[d] Independent Rif^r transductants were selected as described in the text.

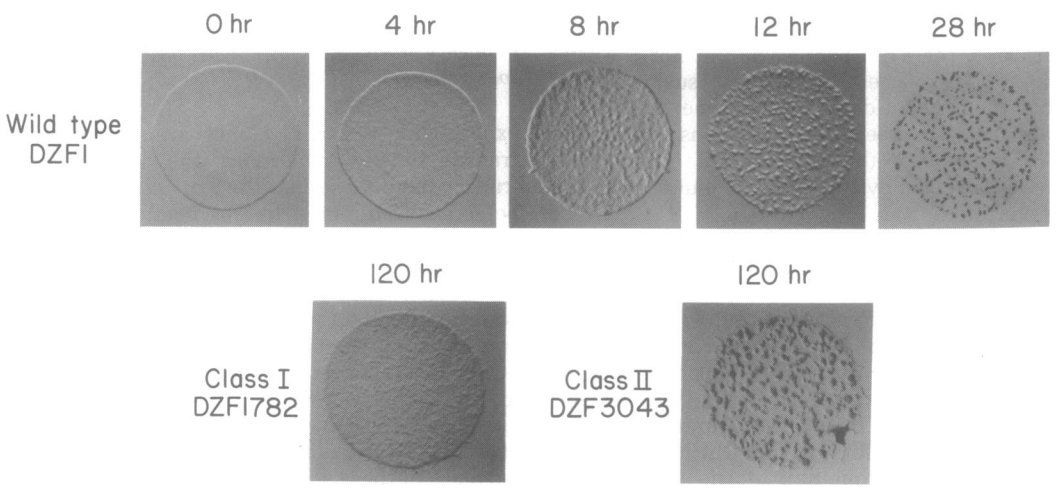

FIG. 2. *Morphology of wild type and Rif^r Fru$^-$ mutants on a fruiting medium. Wild type (DZF1) and Rif^r Fru$^-$ mutants (DZF1782 and DZF3043) were concentrated and spotted at high density on CF agar as described in the text. The spot diameter is 5 mm.*

cells per ml). The class II mutants form tight aggregates (translucent mounds) at both high and low density. Many of the mutants within each class show small but distinctive morphological differences.

The ability of the Rif^r Fru$^-$ mutants to sporulate was also examined. All mutants could be induced to form glycerol spores by the addition of 0.5 M glycerol to exponential-phase cultures. Under these conditions, Dworkin and Gibson (8) showed that vegetative cells synchronously convert to spherical sporelike cells in liquid broth in the absence of aggregation. In contrast, all of the mutants showed low ($<10^{-2}$) or very low ($<10^{-7}$) levels of sporulation on fruiting medium (Table 1). The small number of spores obtained with the mutants gave rise to mutant clones when plated on growth medium and therefore were not revertants.

Hagen et al. (9) found that many nonfruiting mutants, when mixed with wild-type cells, can be stimulated to sporulate, although usually at a reduced level. We therefore mixed equal numbers of Rif^r Fru$^-$ mutants with DZF1 and spotted the cells on fruiting medium. After 5 days, the cells were removed from the plates and analyzed for Rif^r spores. Table 1 shows that 10 of 11 strains tested in this manner could be stimulated to sporulate by wild-type cells. In several strains (DZF3039, DZF1782, and DZF3043), sporulation was 10^6-fold higher than that obtained by the mutant strain alone. These

results suggest that most of the Rif^r Fru$^-$ strains have the capacity to sporulate but for some reason do not receive the signal to sporulate in the absence of wild-type cells.

Table 1 also includes the growth rates of the strains in rich medium (TYE broth). In most cases the growth rates were 3.75 h at 30°C, a value identical to the parental wild-type strain, DZF1. We estimate the error in these measurements at about 5%. These strains also show normal motility (compared to DZF1) and are in the "yellow"-phase variant form (17). Therefore, the mutations in the *rif* locus do not detectably interfere with vegetative functions.

In vitro RNA polymerase assays of the mutants. The RNA polymerase activity of *M. xanthus* DZF1 was assayed in extracts and found to be sensitive (50% inhibition) to 0.02 μg of rifampin per ml. The strains listed in Table 1 were studied in vitro by preparing extracts and assaying them in the presence and absence of 4 μg of rifampin per ml. The results of a typical experiment are shown in Fig. 3. Whereas extracts of DZF1 were almost completely inhibited by rifampin, extracts of the Rif^r Fru$^-$ mutant DZF3039 were completely resistant to the drug. The extracts were rendered DNA dependent by extensive sonic treatment. To eliminate the possibility that the mutants have acquired a detoxification activity, rifampin from the mutant extracts was tested on the RNA polymerase activity of DZF1 and found to be active. Thus, the mutations of Rif^r in the strains tested are likely to be caused by alterations in the RNA polymerase itself rather than in changed cell permeability or increased detoxification activity.

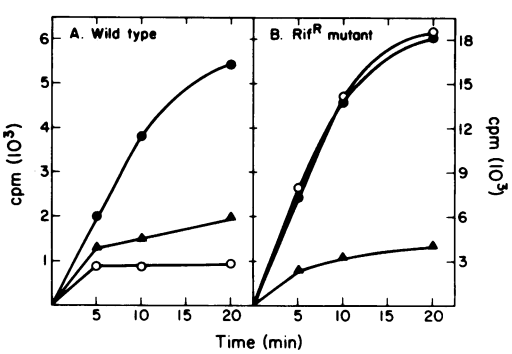

FIG. 3. *In vitro RNA polymerase assays of extracts from wild type and a Rif^r Fru$^-$ mutant. Extracts were prepared from wild-type (DZF1) and mutant (DZF3039) vegetative cultures and assayed as described in the text. Symbols: (●) complete, no rifampin added; (○) complete plus rifampin (4 μg/ml); (▲) complete minus salmon testes DNA, no rifampin added.*

DISCUSSION

We have isolated a collection of Rif^r mutants which are defective in fruiting-body formation. The mutants appear to be altered in their RNA polymerases since in vitro studies showed rifampin-resistant RNA polymerase activity but no detectable detoxification activity. Since the Rif^r and Fru$^-$ phenotypes cotransduce at very high frequency (in some strains ≥99.9%), it is likely that a single mutation confers both drug resistance and impaired ability to form fruiting bodies. The data presented, however, cannot rule out the possibility that two very closely linked mutations arose spontaneously in each of the strains examined. Reversion analysis would be useful in strengthening the argument. Unfortunately, we have so far been unsuccessful in the selection of *fru*$^+$ revertants.

It was curious that many of our initial isolates of spontaneous *rif*^{r} mutants clearly contained at least two mutations: one responsible for the drug resistance and another one for the defect in fruiting-body formation. One possible explanation for this finding is that the initial *rif*^{r} mutation might confer a selective disadvantage on the cell, allowing compensatory mutations to arise during the growth of a single clone that might affect both fruiting and vegetative growth. Fortunately, these secondary mutations can readily be separated by transduction.

The mutants studied show normal vegetative growth rates and morphology but exhibit serious defects in their ability to form fruiting bodies. The mutants can be divided into two phenotypic classes on the basis of their progress in the development pathway. The most likely explantion of these findings is that mutations in the RNA polymerase molecule are responsible for changing the transcriptional specificities of the enzyme. The alterations must be subtle since the detectable vegetative functions remain unchanged. However, developmental transcription may be seriously impaired. This could occur by altering the ability of the enzyme to bind a regulatory factor or, alternatively, by directly changing its ability to recognize certain promoter sites. The fact that at least two phenotypic classes of Rif^r Fru$^-$ mutants were found suggests that the transcriptional specificities may be altered more than once during development. Altered transcriptional specificities have been reported during phage development (1, 16) and have been suggested during sporulation of *Bacillus subtilis* (13). Changes in transcriptional specificities during development of *M. xanthus* can best be determined by in vitro studies on defined templates. This work is currently in progress.

The developmental defects of the Rif^r mutants of *M. xanthus* differ significantly from those found in *B. subtilis* (6, 10, 15). The mutants described here appear to be blocked primarily in developmental aggregation or cell-cell communication, and only as a consequence, in sporulation. Presumably, the cells do not sporulate because they never receive the aggregation-dependent signal for sporulation. This conclusion is supported by the very dramatic (10^6-fold) higher sporulation frequency observed for several mutants when they are stimulated by wild-type cells. Furthermore, all of the mutants were able to form heat-resistant and sonic treatment-resistant, sporelike cells in the presence of glycerol. In contrast, the Rif^r developmental mutants isolated in *B. subtilis* appear to be defective in sporulation. The coupling of sporulation to cell-cell communication in *M. xanthus* is an interesting area for future research.

ACKNOWLEDGMENTS

This work was supported by grants from the Public Health Service National Institute of General Medical Sciences (GM 20509) and the National Science Foundation (PCM 75-09231 A01) One of us (K.R.) was a Public Health Research Predoctoral Trainee (5-T32-GM07232).

We thank C. Morrison for critical reading of the manuscript.

LITERATURE CITED

1. **Bolle, A., R. H. Epstein, and E. P. Geiduschek.** 1968. Transcription during bacteriophage T4 development: synthesis and relative stability of early and late RNA. J. Mol. Biol. **31**:325–348.
2. **Bretscher, A. P., and D. Kaiser.** 1978. Nutrition of *Myxococcus xanthus*, a fruiting myxobacterium. J. Bacteriol. **133**:763–768.
3. **Burgess, R. R.** 1969. A new method for the large scale purification of *E. coli* DNA-dependent RNA polymerase. J. Biol. Chem. **244**:6160–6168.
4. **Campos, J. M., J. Geisselsoder, and D. R. Zusman.** 1978. Isolation of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119**:167–178.
5. **Campos, J. M., and D. R. Zusman.** 1975. Regulation of development in *Myxococcus xanthus*: effect of 3':5'-cyclic AMP, ADP, and nutrition. Proc. Natl. Acad. Sci. U.S.A. **72**:518–522.
6. **Doi, R. H., L. R. Brown, G. Rogers, and Y. Hsu.** 1970. *Bacillus subtilis* mutant altered in spore morphology and in RNA polymerase activity. Proc. Natl. Acad. Sci. U.S.A. **66**:404–410.
7. **Dworkin, M.** 1972. The myxobacteria: new direction in studies of procaryotic development. Crit. Rev. Microbiol. **2**:435–452.
8. **Dworkin, M., and S. M. Gibson.** 1964. A system for studying microbial morphogenesis: rapid formation of microcysts in *Myxococcus xanthus*. Science **146**: 243–244.
9. **Hagen, D. C., A. P. Bretscher, and D. Kaiser.** 1978. Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev. Biol. **64**:284–296.
10. **Leighton, T. J.** 1973. An RNA polymerase mutation causing temperature-sensitive sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **70**:1179–1183.
11. **Linn, T. G., R. Losick, and A. L. Sonenshein.** 1975. Rifampin resistance of *Bacillus subtilis* altering the electrophoretic mobility of the beta subunit of ribonucleic acid polymerase. J. Bacteriol. **122**:1387–1390.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Pero, J., J. Nelson, and R. Losick.** 1975. In vitro and in vivo transcription by vegetative and sporulating *Bacillus subtilis*, p. 202–212. *In* P. Gerhardt, R. N. Costilow, and H. L. Sadoff (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
14. **Rabussay, D., and W. Zillig.** 1969. A rifampicin resistant RNA-polymerase from *E. coli* altered in the β subunit. FEBS Lett. **5**:104–106.
15. **Sonenshein, A. L., and R. Losick.** 1970. RNA polymerase mutants blocked in sporulation. Nature (London) **227**:906–909.
16. **Talkington, C., and J. Pero.** 1977. Restriction fragment analysis of the temporal program of bacteriophage SP01 transcription and its control by phage-modified RNA polymerases. Virology **83**:365–379.
17. **Wireman, J. W., and M. Dworkin.** 1975. Morphogenesis and developmental interactions in myxobacteria. Science **189**:516–523.
18. **Zusman, D. R., D. M. Krotoski, and M. Cumsky.** 1978. Chromosome replication in *Myxococcus xanthus*. J. Bacteriol. **133**:122–129.