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"Frizzy" Mutants: a New Class of Aggregation-Defective Developmental Mutants of *Myxococcus xanthus*

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During fruiting-body formation in *Myxococcus xanthus*, cells aggregate into raised mounds, where they sporulate. A new class of aggregation-defective developmental mutants was identified within a collection of nonfruiting mutants of *M. xanthus*. The mutants failed to aggregate into discrete mounds, but rather aggregated into "frizzy" filaments. Many cells within the filaments sporulated normally. Pairwise mixtures of representative frizzy mutants were unable to stimulate each other to aggregate normally. Two strains of *M. xanthus* were isolated which contained transposon Tn*5* insertions mapping near one frizzy mutation. A search through 36 mutants exhibiting the frizzy phenotype showed that all were linked to the same Tn*5* insertion sites. Three-factor cross-analysis of 22 of these mutants allowed the mapping of these mutations into many loci. The localization of Tn*5* inserts adjacent to this region make possible further manipulation of these genes.

Myxococcus xanthus is a gram-negative bacterium that has a complex life cycle (8, 15). Under certain conditions of nutritional stress (10), cells form fruiting bodies. Fruiting-body formation has two components, aggregation and sporulation. As indicated from the study of mutants (11), aggregation and sporulation appear to be controlled by two parallel and, to some extent, independent pathways. The aggregation pathway directs the movement of cells inward towards aggregation centers, where 10^5 to 10^6 cells form raised mounds. The sporulation pathway controls the conversion of individual rod-shaped cells to ovoid, environmentally resistant, resting cells called myxospores. In *M. xanthus*, the raised mounds of myxospores are termed fruiting bodies.

To study particular steps within the developmental program, Morrison and Zusman (11) isolated 1,865 nonfruiting mutants by visually screening 1.5×10^6 mutagenized clones on a clone-fruiting medium in a top agar overlay (3). The mutants exhibited a very limited variety of phenotypes. Most of the mutants were rough; i.e., groups of cells showed some reassortment but very little aggregation (sporulation usually is observed in these mutants, even in the absence of aggregation). Another large class of mutants were normal in aggregation but defective in sporulation. These mutants formed translucent mounds. Two intermediate phenotypes (swirls and flat mounds) were also described.

Recently, we were reviving a large number of the mutants and noted that the nonconditional mutants recorded originally as swirl mutants had a different morphology than the temperature-sensitive swirl mutants described previously (11). Although they did form some swirls, particularly at low cell densities, the predominant phenotype was "frizzy" filaments. In this paper, the frizzy phenotype is examined in more detail. The mapping procedure of Kuner and Kaiser (9) was used which involves the use of a bank of strains containing independent insertions of Tn*5* (1), a transposon imparting kanamycin resistance (Kan^r), at multiple points in the chromosome. Two strains which contain Tn*5* insertions were located near a frizzy mutation. The linkage of other mutations to these insertions was then examined. These studies indicated that all of the mutations showing the frizzy phenotype map within a group of tightly linked loci.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *M. xanthus* DZF1 (an FB strain derived from DK101 [11] was used as the parental wild-type fruiting competent strain (Fru^+). The nonfruiting (Fru^-) mutants used in this study were isolated previously (11) and are described in Table 1. Transductions were done with phage MX4 *ts27 hft hrm* (5), a generalized transducing phage for *M. xanthus*, unless otherwise indicated.

Media and growth conditions. Vegetative cultures were grown in Casitone-yeast extract (CYE) broth (15) aerated by shaking at 175 to 200 rpm at 30°C. For analysis of fruiting morphologies, vegetative cultures (10^9 cells per ml) were collected by centrifugation (5,000 × *g* for 20 min) and suspended at 3,000 Klett units (about 15×10^9 cells per ml). The cells were then spotted on CF agar (6) with a multipronged inoculator

TABLE 1. Description of strains of *M. xanthus*

Strain designation	Relevant characteristics[a]	Fruiting phenotype[b]	Source or reference
DZ1	Nonmotile	Smooth	5
DZF1	*sglA*	Fruiting	11
DZF1207	Fru (*ts*)	Swirl	11
DZF1279	Fru (*ts*)	Swirl	11
DZF1454	Fru (*ts*)	Swirl	11
DZF1084	Fru$^-$	Frizzy	Spontaneous mutation
DZF1165	Fru$^-$	Frizzy	ICR mutagenesis
DZF1227	Fru$^-$	Frizzy	EMS mutagenesis
DZF1229	Fru$^-$	Frizzy	EMS mutagenesis
DZF1262	Fru$^-$	Frizzy	EMS mutagenesis
DZF1273	Fru$^-$	Frizzy	EMS mutagenesis
DZF1281	Fru$^-$	Frizzy	EMS mutagenesis
DZF1313	Fru$^-$	Frizzy	EMS mutagenesis
DZF1352	Fru$^-$	Frizzy	EMS mutagenesis
DZF1359	Fru$^-$	Frizzy	EMS mutagenesis
DZF1366	Fru$^-$	Frizzy	EMS mutagenesis
DZF1418	Fru$^-$	Frizzy	EMS mutagenesis
DZF1421	Fru$^-$	Frizzy	EMS mutagenesis
DZF1434	Fru$^-$	Frizzy	EMS mutagenesis
DZF1444	Fru$^-$	Frizzy	EMS mutagenesis
DZF1451	Fru$^-$	Frizzy	EMS mutagenesis
DZF1468	Fru$^-$	Frizzy	EMS mutagenesis
DZF1511	Fru$^-$	Frizzy	EMS mutagenesis
DZF1536	Fru$^-$	Frizzy	EMS mutagenesis
DZF1555	Fru$^-$	Frizzy	EMS mutagenesis
DZF1571	Fru$^-$	Frizzy	EMS mutagenesis
DZF1634	Fru$^-$	Frizzy	EMS mutagenesis
DZF1643	Fru$^-$	Frizzy	EMS mutagenesis
DZF1681	Fru$^-$	Frizzy	EMS mutagenesis
DZF1751	Fru$^-$	Frizzy	EMS mutagenesis
DZF1766	Fru$^-$	Frizzy	EMS mutagenesis
DZF1887	Fru$^-$	Frizzy	ICR mutagenesis
DZF1961	Fru$^-$	Frizzy	ICR mutagenesis
DZF2071	Fru$^-$	Frizzy	ICR mutagenesis
DZF2176	Fru$^-$	Frizzy	ICR mutagenesis
DZF2920	Fru$^-$	Frizzy	ICR mutagenesis
DZF2923	Fru$^-$	Frizzy	ICR mutagenesis
DZF2924	Fru$^-$	Frizzy	ICR mutagenesis
DZF2928	Fru$^-$	Frizzy	ICR mutagenesis
DZF2950	Fru$^-$	Frizzy	ICR mutagenesis
DZF3080	Tn*5* (Kan^r)	Fruiting	12
DZF3147	Tn*5* (Kan^r)	Fruiting	By transduction of DZF1227 with phage MX4 grown on pools of DZ1 containing Tn*5*
DZF3150	Tn*5* (Kan^r)	Fruiting	By transduction of DZF1227 with phage MX4 grown on pools of DZ1 containing Tn*5*

[a] *sglA*, Locus involved in the S motility system of *M. xanthus* (7); Fru (*ts*), temperature sensitive for fruiting body formation; Fru$^-$, nonfruiting phenotype at 28 or 34°C; ICR, ICR-191.

[b] The fruiting phenotypes were described by Morrison and Zusman (11). The frizzy phenotype is described in this paper.

which delivers 86 spots per plate (about 4 μl per spot, 7 mm apart) and incubated at 28°C for 5 to 7 days.

Analysis of sporulation. After 5 to 7 days of incubation at 28°C on CF agar, cells were loosened from the agar by gently scraping with a small glass spreader and suspended in 1 ml of TM buffer (10 mM Tris-hydrochloride buffer [pH 7.6] containing 8 mM $MgSO_4$) per plate. The suspension was sonicated twice for 30 s to disrupt spore clumps and to break vegetative cells. Appropriate dilutions were placed in a Petroff-Hausser counting chamber for determination of spore counts. At least 1,000 spores were counted for each sample.

Extracellular complementation of mutants. Eight representative frizzy mutants and DZF1 were grown vegetatively to 10^9 cells per ml, concentrated by centrifugation as described above, and spotted on CF agar. Each spot (4 μl) was applied with a sterile micropipette. The spots were allowed to dry for 10 min. Each spot was then overlaid with a second spot (4 μl) of a different mutant or DZF1. The plates were incubated for 7 days at 28°C and then photographed.

Isolation of Tn*5* insertion strains and nomenclature. Pools of Tn*5*-containing *M. xanthus* strains were isolated after P1::Tn*5* infection of DZ1. Strain DZ1, although nonmotile and Fru$^-$, was used for these experiments because it is a much better host for phage MX4, yielding titers as high as 2×10^{11} particles per ml in liquid lysates. Phage P1::Tn*5* was grown on *Escherichia coli* C600 as liquid lysates. The phage were irradiated with UV light to about 0.1% survival and used to infect *M. xanthus* DZ1 at a multiplicity of about 1.9 in CYE broth containing 2.5 mM $CaCl_2$ at 30°C. After 1 h of incubation, the cells were collected by centrifugation (5,000 × *g* for 10 min at 0°C) and suspended in TM buffer. The cells were then plated in soft agar overlays (CYE broth plus 0.6% agar [Difco Laboratories]) on CYE plates containing 20 μg of kanamycin (Sigma Chemical Co.) per ml. At this concentration of kanamycin, spontaneously resistant mutants were not observed ($<10^{-9}$). *M. xanthus* transductants were observed after 5 days of incubation at 34°C at a frequency of 10^{-7} per input phage or 5×10^{-7} per viable recipient. The Tn*5*-containing transductants showed large morphological variations: altered pigments (red, yellow, brown), motility, and growth characteristics. Independent transductants (1,250) were picked onto a grid pattern (48 per plate) and tested on A1 minimal medium (3) for growth. Fifty isolates were unable to grow on this medium. A total of 16 auxotrophs were identified from these isolates (7 arginine, 1 thymidine, 1 glycine, 1 biotin, 2 histidine, 2 threonine, 1 tryptophan, and 2 cytidine). Thirteen pools of independent transductants were made by scraping all of the cells from two plates (96 colonies) into a single culture. The pools were then used for the growth of liquid lysates with the phage mutant MX4 *ts*18 *ts*27 *htf-1* described previously (5).

The identification of Tn*5* insertions linked to frizzy mutations was performed as follows. Exponential-phase cells of strain DZF1227 (0.2 ml of 5×10^8 to 1×10^9 cells per ml) were mixed with about 0.02 ml of phage lysate (MX4 grown on DZ1 Kan^r; 10^{11}/ml) and plated in a CF agar overlay (without kanamycin) on CF agar containing 15 μg of kanamycin per ml. The plates were incubated at 34°C (the nonpermissive temperature for the phage) for 10 days. The plates contained 500 to 800 colonies, some of which con-

tained fruiting bodies. Fruiting clones were picked onto CYE agar containing kanamycin (20 μg/ml), and single colonies were purified. Phage MX4 *ts27 htf hrm* (which can plaque on strains derived from DZF1 [FB]) was used to grow phage stocks on the Fru^+ Kan^r clones as described by Torti and Zusman (12). The phage stocks were then used to transduce other Fru^- strains to Fru^+ Kan^r.

The location of the Tn*5* transposons were named in terms of the strain in which they were first identified. Thus, DZF3150, the kanamycin-resistant derivative of strain DZF1227 with phage from pool 114, contained the insert Ω3150 (4), and the strain DZF3147 contained the kanamycin-resistant derivative of strain DZF1227 with phage from pool 103 and was designated Ω3147.

RESULTS

Isolation and characterization of frizzy mutants. Morrison and Zusman (11) assembled a collection of nonfruiting mutants of *M. xanthus* after ethyl methane sulfonate (EMS) or ICR mutagenesis. Mutagenized colonies were plated in a soft agar overlay on a clone-fruiting medium (6) which has sufficient nutrients to permit colony formation but is insufficient to sustain colony growth. The starving colonies form fruiting bodies in situ. Potential nonfruiting mutants were picked and retested at least three times before storage in glycerol at −76°C. The morphology of each mutant was carefully recorded.

Since nonfruiting mutants of *M. xanthus* show only a limited number of phenotypes (11), we became interested in a relatively infrequent but distinctive aggregation-defective mutant which had been originally recorded as a swirl mutant but which upon further examination differed morphologically from the swirl mutants reported previously (11). Figure 1 shows the terminal morphology of one of these mutants, DZF1227, in comparison to the parental wild-type strain, DZF1. While the mutants frequently showed doughnut-shaped swirls at the perimeter of colonies, most of the cells appeared as frizzy filament-like aggregates which usually contained darkened patches. To avoid confusion with the swirl mutants, these mutants are now called frizzy mutants. For comparison, Fig. 2 shows the morphology of the three swirl mutants described previously. It should be noted that, although these mutants showed some resemblance to the frizzy mutants, they lacked the extensive filamentous networks which are characteristic of frizzy mutants. The colony appearance of the frizzy mutants suggested that on fruiting agar they are much more motile than the swirl mutants. This was even more apparent

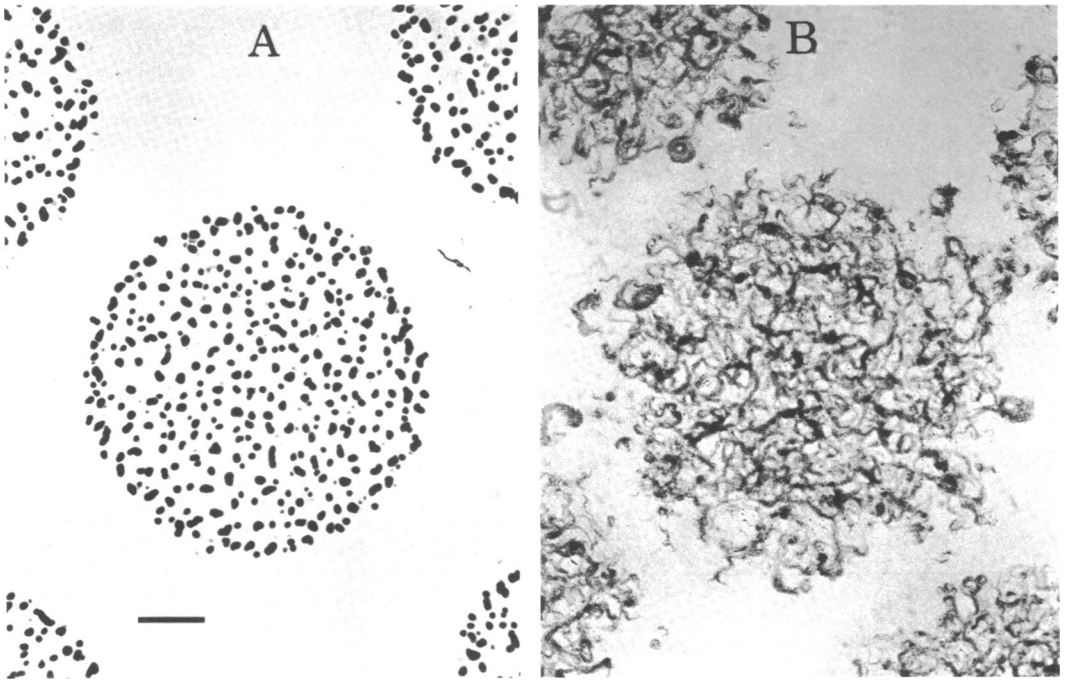

FIG. 1. Morphology of frizzy mutants. Exponential-phase liquid cultures of *M. xanthus* DZF1, the fruiting parental strain, and DZF1227, a frizzy mutant, were collected by centrifugation and spotted on CF agar as described in the text. After 7 days of incubation at 28°C, the spots were photographed at ×8.6 magnification. Bar, 1 mm. (A) Strain DZF1; (B) strain DZF1227.

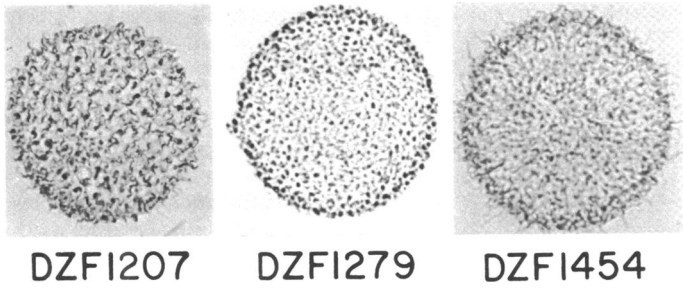

FIG. 2. Morphology of swirl mutants. Exponential-phase liquid cultures of the temperature-sensitive swirl mutants DZF1207, DZF1279, and DZF1454 were spotted on CF agar. After 7 days of incubation at 34°C, the nonpermissive temperature, the spots were photographed at ×5 magnification.

when cells were spotted at lower cell densities. Figure 3 shows the sequence of morphological changes which occurred when cells were spotted on CF agar. Strain DZF1 showed the wild-type sequence of events: smooth spots (0 h), rough spots (6 h), translucent mounds (24 h), and fruiting bodies (48 h). The frizzy mutants DZF1281 and DZF1536 showed defective aggregation. At 24 h, they aggregated into numerous lumps. By 48 h, the lumps degenerated into frizzy filaments with darkened patches (reflecting the onset of sporulation). At later times (96 h), the filaments appeared to extend themselves and darken further. The morphology of the mutants suggested that the cells fail to establish stable foci for mound formation. All of the frizzy mutants examined showed similar phenotypes, although some mutants were more filamentous than others (Fig. 3).

Sporulation in 22 frizzy mutants was mea-

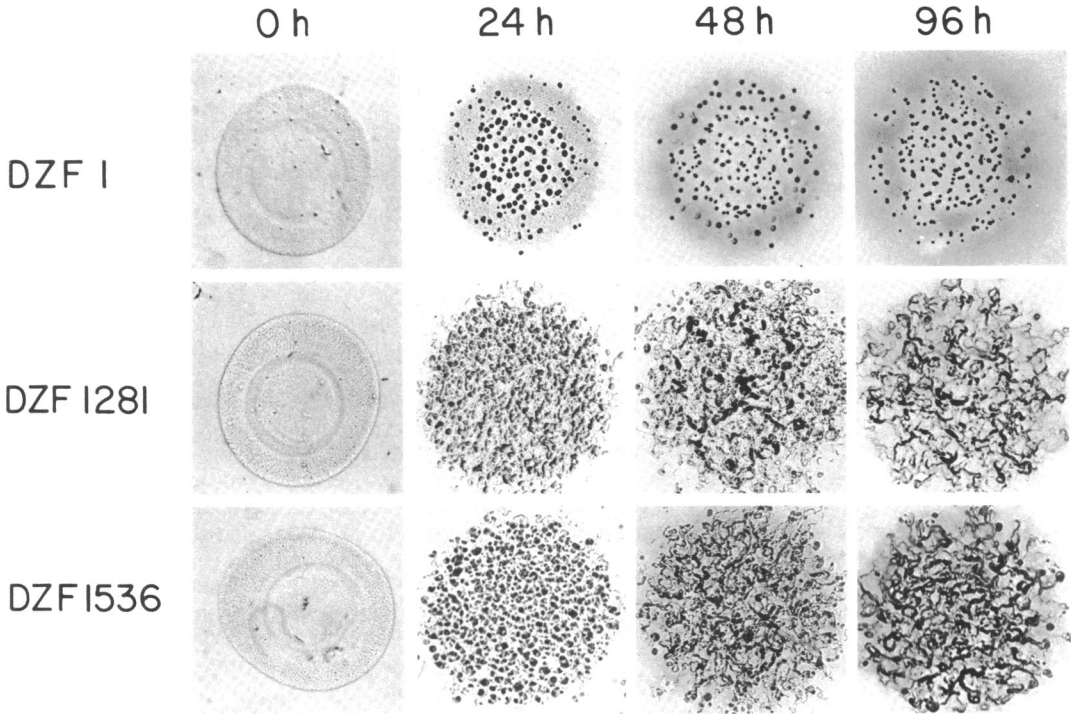

FIG. 3. Changes in frizzy mutant morphology with time on CF agar. Exponential-phase liquid cultures of DZF1 and the frizzy mutants DZF1281 and DZF1536 were spotted on CF agar and incubated at 28°C. At 24-h intervals, sample plates were removed from the incubator and photographed at ×4.2 magnification. (It should be noted that, at each time interval, equivalent but different spots were photographed.)

TABLE 2. Sporulation of frizzy mutants[a]

Strain	No. (% control, DZF1) of myxospores per plate in expt[b]:	
	A	B
DZF1	100	100
DZF1084	30	78
DZF1281	54	94
DZF1273	35	61
DZF2071	93	94
DZF1571	49	122
DZF1751	28	111
DZF1536	63	56
DZF1961	17	50
DZF1165	37	106
DZF1555	23	106
DZF1262	35	61
DZF1352	49	56
DZF1444	55	89
DZF1227	8	61
DZF1366	134	94
DZF1887	56	89
DZF1418	18	111
DZF1359	18	106
DZF1421	31	106
DZF1434	31	17
DZF1229	39	67
DZF1313	65	

[a] Cells were harvested and spotted on CF agar as described in the text. After 5 to 7 days of incubation at 28°C, duplicate plates were scraped with a glass spreader, and the cells were suspended in TM buffer. The spores were dispersed by sonication and counted under the microscope, using a Petroff-Hausser counting chamber. At least 1,000 spores were counted for each determination.

[b] The spore counts in experiment A were made on the original isolates. The spore counts in experiment B were made on isogenic strains constructed by transducing each of the frizzy alleles into DZF1, using Ω3150 as a linked marker. About 2×10^8 spores per plate were obtained for DZF1. It should be noted that cells grew on the plates before sporulation, so that some variability in the numbers was observed.

sured by harvesting cells from plates, disrupting vegetative cells by sonication, and counting spores with a Petroff-Hausser counting chamber. The results (Table 2) showed that all strains were able to sporulate, although some differences between strains were observed. It should be noted that, under laboratory conditions, *M. xanthus* (wild type) usually shows only 10 to 20% sporulation (13), with the remainder of the cells eventually lysing.

Since some nonfruiting mutants of *M. xanthus* have been shown to stimulate other nonfruiting mutants to form fruiting bodies (6), representative frizzy mutants were tested in pairwise mixtures to determine whether they can cross-feed each other. None of the eight mutants tested could cross-feed each other. However, when the mutants were spotted with DZF1 (the parental strain), fruiting bodies were observed (data not shown). In the latter experiment, the frizzy mutants continued moving, as evidenced by the formation of frizzy fringes around the fruiting center of the spots.

Identification of a Tn*5* insertion linked to a frizzy mutant. By the method of Kuner and Kaiser (9), a collection of *M. xanthus* DZ1 strains were assembled which contained independent insertions of the transposon Tn*5*. Pools of these strains, derived from 96 independent transductants, were used for growing stocks of the generalized transducing phage MX4. The phage stocks were then used to infect a frizzy mutant (Fru^- Kan^s), and the cells were plated on CF agar containing kanamycin (15 μg/ml). On this medium, transductants to Kan^r give rise to colonies which, if fruiting proficient (Fru^+), form fruiting bodies (12). Fru^+ transductants are clearly distinguishable from Fru^- transductants even on petri dishes containing as many as 500 transductants per plate.

When this procedure was performed with strain DZF1227, 4 of 11 independent pools showed Fru^+ Kan^r clones (pool 103, 5 of 2,400 colonies; pool 106, 1 of 2,400 colonies; pool 111, 3 of 1,500 colonies; pool 114, 3 of 1,500 colonies). One transductant from pool 114 (designated Ω3150) and one from pool 103 (designated Ω3147) were single-colony purified and saved for further experiments. These clones had presumably become Fru^+ through acquisition of a *fru*$^+$ allele linked to a Tn*5* insert carried by one of the original donor strains present in the pools. This was confirmed by using these Fru^+ Kan^r colonies for the preparation of MX4 phage stocks. These phages were then used to transduce a fresh culture of DZF1227 to Kan^r and Fru^+. The inserts Ω3150 and Ω3147 were 60 and 50% linked, respectively, to the *fru* mutation in DZF1227.

Linkage of Ω3150 and Ω3147 to other frizzy mutations. Our mutant collection contained 47 isolates listed as having the swirl phenotype. These strains were revived, and almost all (except for three) appeared to have a similar frizzy phenotype to DZF1227. To determine whether any of these frizzy mutations were linked to the same Tn*5* insert (and consequently linked to each other), phage grown on strains DZF3150 and DZF3147 (which are Fru^+ and Kan^r) were used to transduce the 44 mutants to Kan^r. As a control, phage grown on DZF3080 was also used. This strain carries Ω3080, which is closely linked to many of the temperature-sensitive rough mutations (12). The results of 36 such transductions are shown in Table 3. Ω3150 and Ω3147 were clearly linked to all of these muta-

TABLE 3. Linkage of Ω3150, Ω3147, and Ω3080 to frizzy mutants[a]

Strain	Frequency (%) of cotransduction of Fru$^+$ allele with:		
	Ω3150	Ω3147	Ω3080
DZF1084	63	64	<0.3
DZF1165	55	55	<0.3
DZF1227	60	50	<0.3
DZF1229	32	33	<0.3
DZF1262	56	46	<0.2
DZF1273	66	49	<0.4
DZF1281	74	56	<0.5
DZF1313	28	25	<0.2
DZF1352	48	48	<0.5
DZF1359	36	35	<0.2
DZF1366	41	39	<0.5
DZF1418	36	27	<0.6
DZF1421	41	30	<0.3
DZF1434	44	35	<0.1
DZF1444	44	42	<0.4
DZF1451	65	55	<0.5
DZF1468	58	44	<0.5
DZF1511	25	9	<0.5
DZF1536	19	7	<0.3
DZF1555	47	36	<0.2
DZF1571	66	51	<0.5
DZF1634	33	35	<0.5
DZF1643	37	37	<0.5
DZF1681	36	25	<0.5
DZF1751	61	43	<0.2
DZF1766	20	20	<0.5
DZF1887	34	20	<0.5
DZF1961	44	49	<0.2
DZF2071	29	30	<0.2
DZF2176	13	4	<0.5
DZF2920	41	20	<0.5
DZF2923	40	21	<0.5
DZF2924	14	8	<0.5
DZF2928	19	15	<0.5
DZF2950	31	17	<0.5

[a] Phage MX4 was grown on DZF3150, DZF3147, and DZF3080 and used to transduce the frizzy mutants to Kan^r. The transductants were plated on CF agar containing 15 μg of kanamycin per ml. After 10 days of incubation at 34°C, the frequency of fruiting clones among all Kan^r colonies was determined by using a colony counter equipped with a magnifying glass. A minimum of 100 colonies were scored in each transduction.

tions, whereas Ω3080 was not detectably linked. The eight strains which were not listed in this table were ambiguous. Many of these Kan^r transductants looked different from the parental strain (for example, some formed translucent mounds rather than frizzy filaments) but did not form fruiting bodies. Since these mutants are likely to be double mutants, they were not studied further. It should be noted that the three temperature-sensitive swirl mutations in DZF1207, DZF1279, and DZF1454 were not linked to Ω3150.

Three-factor cross-analyses of mutants. Three-factor crosses were performed as described previously (11). Phage MX4 was grown on Fru$^-$ Ω3150 mutants (obtained from the experiment described in Table 3) and used to infect recipient Fru$^-$ Kan^s strains. The cells were then plated on CF agar containing kanamycin (15 μg/ml), and the fraction of Kan^r Fru$^+$ transductants was scored after 10 days of incubation at 34°C. The results of these experiments with 22 frizzy mutants are shown in Table 4. These mutants were chosen because they could be transduced to morphologically clear fruiting clones. The data were arranged so that recombinant classes requiring four crossover events appear below the boxes; productive crosses requiring two crossover events appear above the boxes. Most of the mutations were not allelic, since wild-type recombinants arose from the majority of crosses at a frequency above the reversion frequency. These mutations were ordered in accordance with the number of recombinants observed. Several of the mutations may be allelic (for example, DZF1444 and DZF1227), since wild-type recombinants were not observed between these mutations. Some of the mutations were sufficiently close so that accurate ordering of the genes was not possible with the number of recombinants scored (for example, DZF1281 and DZF2071; DF1961 and DZF1165; DZF1262, DZF1352, DZF1444, and DZF1227; DZF1359, DZF1421, and DZF1434; and DZF1229 and DZF1313). All of the frizzy mutations tested appeared to be tightly linked, however, since the general frequency of wild-type recombinants was low. The recombinational distances spanned by the mutations in DZF1084 and DZF1313 suggest a relatively large region of DNA.

DISCUSSION

In this paper, a new class of developmental mutants of *M. xanthus* is described which has a distinctive and characteristic morphology: the formation of frizzy filaments. After placement on fruiting medium, the mutants appeared to show some aggregation properties, since they initially formed lumps or flat mounds. However, on subsequent incubation, these initial aggregates degenerated into ribbon-like filaments with a tangled or fizzy appearance. On still further incubation, the filaments elongated as the cells continued moving outwards. The frizzy mutants are described as aggregation defective in this paper, since the gross morphology of the mutants did not show discrete raised mounds typical of wild-type *M. xanthus*. The mutants did not appear to be defective in motility, since they showed continued movement under fruiting conditions (they showed more movement on fruiting

TABLE 4. Three-factor crosses of frizzy mutants[a]

Recipient Fru⁻ Strains	Frequency (%) of Fru⁺ Recombinants Among Transductants Carrying Ω3150 — Donor Fru⁻ Ω3150 Strains																					
	DZF 1084	DZF 1281	DZF 2071	DZF 1273	DZF 1571	DZF 1751	DZF 1536	DZF 1961	DZF 1165	DZF 1555	DZF 1262	DZF 1352	DZF 1444	DZF 1227	DZF 1366	DZF 1887	DZF 1418	DZF 1359	DZF 1421	DZF 1434	DZF 1229	DZF 1313
DZF1084	<0.2	1.5	1.5	3.2	6.8	6.1	5.4	9.9	9.3	15.4	9.0	13.2	11.3	14.4	13.8	14.6	20.3	18.3	23.2	20.8	24.5	27.7
DZF1281	<0.4	<0.2	0.3	0.8	3.2	2.6	8.4	14.3	10.0	11.0	11.7	12.6	8.9	11.6	14.3	13.1	10.6	19.0	18.3	17.3	23.4	21.7
DZF2071	<0.2	<0.1	<0.1	1.2	2.7	1.6	8.8	2.6	7.8	9.5	7.1	7.7	6.5	10.1	11.8	10.2	12.5	14.1	12.9	15.4	18.7	17.9
DZF1273	<0.2	<0.1	0.2	<0.1	1.9	2.4	4.5	6.1	6.2	11.4	9.2	8.1	6.9	9.8	11.4	12.8	13.1	14.7	17.6	16.8	19.4	22.2
DZF1571	0.3	<0.2	<0.2	<0.2	<0.5	1.4	2.5	3.4	4.6	10.0	6.7	5.9	5.8	7.2	9.7	10.8	9.9	14.5	19.2	15.1	22.6	16.0
DZF1751	0.8	<0.2	<0.2	<0.2	<0.3	<0.8	1.7	<3	2.8	5.1	3.1	5.0	6.6	5.5	6.9	6.4	4:8	10.7	8.1	13.5	14.7	12.8
DZF1536	0.4	<0.2	<0.2	<0.2	<0.4	<0.3	<0.3	1.2	2.6	5.5	4.6	5.5	4.9	7.1	8.1	7.7	6.8	9.8	12.2	10.6	17.1	16.0
DZF1961	0.6	0.1	<0.2	0.5	<0.4	<0.2	0.3	<2	0.4	1.6	3.4	2.1	2.2	3.3	4.2	3.3	8.8	6.7	8.2	6.1	9.3	14.9
DZF1165	<0.2	0.4	0.1	<0.2	<0.5	<0.5	<1.3	<0.9	<0.2	<1	3.2	0.6	<3	2.1	3.2	7.2	3.1	6.9	8.2	9.0	9.6	9.8
DZF1555	0.6	0.8	0.8	1.5	0.3	<0.8	1.1	0.6	0.4	<0.2	1.3	0.8	1.7	1.9	3.2	4.2	2.1	8.9	5.9	9.2	12.9	10.0
DZF1262	<0.2	0.2	0.1	0.5	0.3	<0.8	0.5	<0.4	0.2	<0.3	<0.1	<0.3	0.4	0.2	2.6	3.0	5.1	5.4	8.6	8.1	8.0	12.2
DZF1352	0.8	0.4	<0.1	0.4	<1	<0.2	<0.4	<0.5	0.2	<0.2	0.1	<0.2	0.3	<0.2	1.4	4.0	4.4	5.8	6.0	5.5	8.9	8.2
DZF1444	<0.2	0.4	<0.3	0.2	<0.5	<0.2	0.3	1.7	0.2	<0.2	0.3	<0.3	<0.2	<0.1	1.5	1.3	2.6	5.5	2.5	5.8	7.1	5.6
DZF1227	0.8	0.3	<0.2	0.2	<0.5	<0.5	<0.3	<0.7	0.3	<0.2	<0.1	<0.2	<0.2	<0.2	1.2	3.1	3.4	7.1	6.7	5.6	9.5	10.1
DZF1366	0.2	<0.2	<0.2	0.2	<0.5	<0.3	<0.2	<0.7	0.4	0.5	<0.3	<0.2	<0.1	<0.2	<0.2	1.4	2.4	3.1	2.9	4.0	5.0	7.2
DZF1887	0.6	<0.2	0.4	<0.2	<0.6	<0.6	<0.3	<1.2	0.3	0.2	<0.3	<0.2	<0.2	<0.2	0.2	<0.2	0.6	1.4	4.4	1.4	3.8	4.4
DZF1418	1.7	0.4	0.2	<0.2	0.6	0.2	<0.3	<0.6	0.2	0.8	0.3	<0.2	0.6	0.2	<0.2	<0.2	<0.5	0.2	<0.2	0.4	4.4	3.5
DZF1359	0.2	<0.2	<0.2	0.4	<0.5	<0.9	0.3	<0.5	0.3	0.1	<0.3	<0.3	<0.2	0.1	0.2	0.2	<0.5	<0.2	0.3	0.4	1.0	2.9
DZF1421	<0.2	0.2	0.3	0.4	1.0	<0.6	0.3	<0.4	0.5	0.4	1.0	0.2	0.2	<0.1	<0.2	<0.2	<0.6	<0.2	<0.3	0.5	2.5	2.5
DZF1434	0.2	<0.2	<0.2	<0.1	<0.8	<0.3	<0.2	<0.6	0.7	0.2	0.2	0.3	0.2	<0.2	0.2	<0.2	<0.5	<0.2	<0.3	<0.2	2.2	2.0
DZF1229	0.2	<0.1	0.1	0.3	0.4	<0.8	0.6	0.4	0.4	1.2	0.2	<0.2	0.7	0.2	<0.2	<0.1	<0.3	0.2	0.2	<0.1	<0.2	0.3
DZF1313	<0.2	0.2	0.2	<0.1	0.7	<0.2	0.3	<0.9	0.2	0.4	0.3	0.4	0.2	0.3	<0.2	<0.2	<0.5	0.2	<0.4	0.8	<0.2	<0.2

[a] Phage MX4 was grown on DZF3150 and used to transduce the Fru⁻ mutants listed above to Kan^r. Transductants which were Kan^r and Fru⁻ were single-colony purified and used for the preparation of MX4 phage stocks. Transducing phage (which carries the donor DNA) was used to infect each of the recipient Fru⁻ mutants and then plated on CF agar containing kanamycin (15 μg/ml). After 10 days of incubation at 34°C, the frequency of fruiting clones among the Kan^r transductants was determined. These numbers represent the average of two separate experiments; 200 to 1,800 transductants were analyzed for each determination. Mutants transduced with phage grown on strains containing the same allele gave no recombinants (data in boxes). The data have been arranged so that recombinant classes requiring four crossover events appear below the boxes; productive crosses (requiring two crossover events) appear above the boxes. In some cases, the number of recombinants was insufficient to order the mutations. In these cases, the ordering of the mutations was arbitrary (see text).

agar than did wild-type cells). There was no obvious defect in cell growth under developmental conditions and normal amounts of several developmental proteins (for example, protein *S* and myxobacterial hemagglutinin [15] are observed [unpublished data]). Furthermore, the number of spores made in most strains is normal. The nature of the aggregation defect in the mutants is unknown. However, the lack of aggregation towards specific sites or foci (which occurs in the wild-type strain during mound formation) suggests that the mutants may be defective in chemotaxis towards a developmental attractant. Since so little is known about developmental aggregation in *M. xanthus*, the frizzy mutants may provide a useful tool for dissecting this process.

The identification of a Tn*5* insert which is linked to one of the frizzy mutations allowed the mapping of these mutations. Most of the mutations showing the frizzy phenotype mapped at a single region of the *M. xanthus* chromosome, since they showed high linkage frequencies (13 to 74%) to Ω3150, one of at least two Tn*5* inserts linked to the first frizzy mutant studied, DZF1227; the remaining frizzy mutants behaved as double mutants, since they could be transduced to a non-frizzy phenotype but not to a fruiting phenotype. Therefore, it is possible that this region is the only one which can give rise to frizzy mutants when mutated.

Three-factor crosses were performed to determine the relative allele positions of the frizzy mutations and to determine the recombinational distances between the loci. The mutation which appeared to be closest to the Tn*5* insert Ω3150 was in DZF1084; the mutation which was farthest from the insert was in DZF1313. Since the

cotransduction frequency between Ω3150 and DZF1084 was about 63% and that between Ω3150 and DZF1313 was about 28%, the distance between these mutations should be sufficiently large to accommodate many genes. By using the model of Wu (14) to relate contransduction frequency to DNA length in a random, generalized transduction system with a phage the size of MX4 (39×10^6 daltons [5]), one can estimate the distance between Ω3150 and DZF1084 as 5×10^6 daltons and that between Ω3150 and DZF1313 as 13×10^6 daltons. Thus, the size of the frizzy region may be about 8×10^6 daltons, or 12 kilobase pairs. Since a Tn*5* insert has been located adjacent to these loci, it should now be possible to clone these genes in a plasmid vector and to determine the exact size of this region (2). It is hoped that such a clone would also help in the identification and characterization of the gene products coded for by these genes. It is noteworthy that all of the 22 frizzy mutants studied in detail appeared to have the same general phenotype (frizzy aggregation; production of spores), even though the proximal marker (in DZF1084) was genetically distant from the distal marker (in DZF1313). This suggests that the mutations may represent steps in a common pathway.

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