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Genetic Analysis of Autolysin-Deficient and Flagellaless Mutants of *Bacillus subtilis*

H. M. POOLEY AND D. KARAMATA*

Institut de Génétique et de Biologie Microbiennes, 1005 Lausanne, Switzerland

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Three mutants with an autolysin-deficient and flagellaless phenotype (*lyt*) were genetically analyzed and compared with three thermosensitive flagellaless mutants. In view of the near indistinguishability of their phenotypes, all six mutations were assigned to *fla* loci. They were distributed into four linkage groups, designated *flaA* through *flaD*. *flaA* and *flaB* map between *pyrD* and *thyA*, *flaD* maps between *aroD* and *lys*, and, in agreement with a previous report, *flaC* maps near *hisA*. A locus associated with hypermotility, *ifm-3*, maps near the latter marker. Introduction of *ifm-3* into *lyt-1*- and *flaA4*-containing strains led to partial suppression of the nonmotile phenotype. We discuss the possibility that the cellular concentration of autolysins is regulated by the expression of *fla* genes. Discrepancies with respect to previous mapping of *flaA* and *flaB* are accounted for.

The pleiotropic phenotype of a *Bacillus subtilis* mutant, i.e., overproduction of protease and α-amylase, absence of flagella, and autolytic deficiency, was shown to be due to a single mutation (1) subsequently mapped with the *sacU* locus (16). This observation, together with the nonmotility observed in several chain-forming, autolysin-deficient, genetically uncharacterized *lyt* mutants of *B. subtilis* (5, 6, 14, 15), led Fein (6) to propose that autolysin activity might be necessary to allow the flagella to penetrate the thick cell wall layer.

The association of autolysin deficiency with a flagellaless phenotype (6, 15) prompted us to compare *lyt* mutations with several previously isolated thermosensitive flagellaless mutants (7). We report the genetic analysis of three *lyt* mutations, *lyt-15* (14), *lyt-1*, and *lyt-2* (5); three *fla* mutations, *flaA4*, *flaB2*, and *flaC51* (7); and a locus (*ifm*) associated with increased motility. We suggest that the regulation of the bulk of the autolysin activity is under the control of *fla* genes in *B. subtilis*.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1.

Media. SPIZ-I, SPIZ-II, SA, and VIB media and L and TS plates were as described previously (9). Supplemented BO medium contained, per liter, 8 g of Nutrient Broth (Difco Laboratories, Detroit, Mich.), 5 g of Trypticase peptone (BBL Microbiology Systems, Cockeysville, Md.), and 5 g of NaCl to which 10 mM $MgCl_2$, 10 mM $CaCl_2$, and 0.08 mM $MnSO_4$ were added (final concentrations). Semisolid medium contained Spizizen salts at 1.2 times the original concentration and 0.1% Casamino Acids and was solidified with 8% gelatin and 0.4% agar (7). When necessary, media were supplemented with amino acids (20 μg/ml) and adenine, uracil, and thymine, all at 100 μg/ml.

Isolation of *ifm*-like mutants. Spontaneous mutants showing a zone of motility on semisolid plates with a diameter approaching four times that produced by the motile parent strain in the same lapse of time were isolated essentially as described previously (7). A semisolid plate, inoculated in the center from a colony of the parent strain, was incubated for 16 h at 37°C. Cells from the edge of the visible motile zone were used to inoculate a second soft agar plate, which was also incubated at 37°C. After one further cycle of selection, cells at the edge of the motile zone were streaked out onto LA plates. Individual colonies, seeded into soft agar plates, spread up to four times faster than the parent. Colonies of these *ifm*-like mutants were rather small, flat, and shiny with a clear, smooth edge.

Transformation. The methods used were essentially as described by Karamata and Gross (9). DNA was extracted by the method of Marmur (11) from exponentially growing cultures in SA medium at 37°C harvested at a cell density equivalent to between 50 and 70 μg (dry weight) per ml. A typical preparation of a competent culture was as follows. An overnight culture of the recipient, grown at 20 to 25°C in SA medium, was diluted into SPIZ-I medium to a concentration equivalent to about 5 to 7 μg (dry weight) per ml and incubated at 37°C. Growth was followed nephelometrically, and ca. 45 min after the end of exponential growth, cells were diluted 10-fold into SPIZ-II medium and incubated for a further 90 min with vigorous aeration. For transformation, DNA (0.1 ml) and $MgSO_4$ (20 mM, final concentration) were added to 0.9 ml of the cells, and the mixture was incubated with shaking for 60 min before being spread onto TS selection plates. For transformation under conditions of saturating DNA, 1 to 5 μg of DNA per ml was employed.

Transduction. Stocks of PBS1-transducing particles were prepared as previously described (9). Transduction was performed as follows. Supplemented BO medium (15 ml) was inoculated with 10 to 20 colonies of the recipient grown on L plates for 18 h at 30°C. The culture was incubated at 37°C with aeration and examined by phase contrast until a vigorous motility was observed. Strains nonmotile at higher temperatures were incubated overnight at room temperature. PBS1 stock (0.1 ml) was added to 0.4 ml of the motile culture in a tube at a nearly horizontal position. After 30 min at room temperature, the mixture was plated onto selection plates, which were incubated at 30°C. Transducing particles on thermosensitive flagellaless mutants TS2, TS4, and TS51 were prepared at 37°C, at which temperature the strains were flagellated (7).

* Corresponding author.

Test for motility. Freshly grown colonies of recombinants, purified by restreaking on selection plates, were transferred to semisolid medium and incubated for 6 to 10 h at the appropriate temperature. Motility was scored either immediately or, if necessary, after a further 16 h at room temperature.

RESULTS

Attempt to cross out *fla* mutations from strains with a thermosensitive flagellaless phenotype by cotransduction with a *hisA* marker.

To facilitate comparison of *lyt* and *fla* mutations, we decided to transfer to a common genetic background three mutations assigned to linkage groups *flaA* through *flaC*, present in the original strains TS4, TS2, and TS51, respectively, since they had been obtained by an elaborate selection procedure after mutagenesis (7). As these *fla* markers were reported to be cotransducible with the *hisA* locus, we set out to transfer them to a *hisA* recipient by means of PBS1-transducing particles obtained on strains TS2 and TS4 and a *his*$^+$ derivative of TS51. The linkage (35%) of *flaC51* to *hisA* was confirmed (data not shown), and a three-factor transduction cross gave the order *hisA-tag-1-flaC* (Table 2) in agreement with a previous report (7). Surprisingly, in crosses with donors TS2 and TS4, no nonmotile recombinants were found among over 100 *his*$^+$ transductants examined in each case. Again, when a *hisA* derivative of TS4 was transduced to *his*$^+$ with a wild-type (*fla*$^+$) donor, not a single motile recombinant was obtained among 100 transductants examined.

These results lead to the conclusion that the mutations responsible for the thermosensitive flagellaless phenotype in TS2 and TS4 do not map in the *hisA* region, in apparent contradiction with previous findings (7) (see below for an explanation of this discrepancy).

TABLE 1. *B. subtilis* strains

Strain	Genotype (or phenotype)	Source, reference, or construction
BD54	*leuA8 ilvA1 metB5 mot*	4
BD68	*argC4 ura-26 trpC2*	4
BD70	*metC3 trpC2*	4
BD92	*hisA1 trpC2 cysB3*	4
M22	*purA16 leuA8 metB5 ilvA1*	9
QB935	*aroD120 lys-1 trpC2*	3
QB943	*pyrD1 thyA thyB ilvA1 trpC2*	3
GB80	*metC3 pyrA*	Garro
172ts-200B	*leuA8 metB5 tag-1*	10
TS2	*lys trpC2 flaB2* Ifm	7
TS4	*lys trpC2 flaA4* Ifm	7
TS51	*hisA1 argC4 ura-1 flaC51*(Ts)	7
Ni15	*xin-15 flaA15 (lyt-15)*[a] *thyA thyB trpC2*	14
FJ3	*metC3 flaD1 (lyt-1)*[a]	5
FJ6	*metC3 flaD2 (lyt-2)*[a]	5
GSY1441	*cysC7*	Anagnostopoulos
L1440	prototroph	BD54 → BD70[b]
L5027	*hisA1 leuA8 argC4 ilvA1*	BD68 → L5086
L5028	*hisA1 argC4 metC3 pyrA*	GB80 → L5027
L5086	*hisA1 leuA8 metB5 ilvA1*	BD92 → M22
L5256	*purA16 flaA15 ilvA1*	Ni15 → M22
L5278	*hisA1 flaA15 ilvA1*	BD92 → L5256
L5281	*purA16 leuA8 flaB2* Ifm	TS2 → M22
L5303	*lys cysC7 flaB2* Ifm	GSY1441 → TS2
L5311	*purA16 flaC51*(Ts) *leuA8 metB5*	TS51 → M22
L5343	*purA16 ifm-3 leuA8 metB5 ilvA1*	Spontaneous mutant (see the text)
L5347	*purA16 ifm-3 hisA1 metB5 ilvA1*	L5028 → L5343
L5348	*ifm3 hisA1 pyrA flaA4*	L5347 → L5404
L5401	*hisA1 lys flaA4* Ifm	L5028 → TS4
L5402	*pyrD1 flaA4 thyB ilvA1 trpC2*	TS4 → QB943[c]
L5404	*hisA1 argC4 pyrA flaA4*	L5402 → L5028
L5410	*lys-1 cysC7 flaA4* Ifm	GSY1441 → TS4
L5415	*flaD1 lys-1 metB5 ilvA1*	QB935 → L5417
L5416	*aroD120 flaD1 lys-1 ilvA1*	QB 935 → L5415
L5417	*purA16 flaD1 metB5 ilvA1*	FJ3 → M22
L5418	*hisA1 flaD1 metB5 ilvA1*	FJ3 → L5086
L6148	*hisA1 leuA8 argC4 ilvA1 gtaB515*	NTG[d] treatment of 5027 and selection for resistance to Ø25
L6331	*purA16 leuA8 ilvA1 gtaB515*	L6148 → M22
L6440	*hisA1 argC4 leuA8 tag-1*	172ts-200B → L5027

[a] Previous designation.
[b] Arrow points from donor to recipient. Unless stated otherwise, crosses were performed by transformation: markers being transferred by congression with saturating concentrations of DNA (1 to 5 μg/ml).
[c] Cross performed by PBS1-mediated transduction.
[d] Nitrosoguanidine mutagenesis.

Mapping of *flaA4* and *flaB2* mutations. To map *fla* mutations in strains TS4 and TS2, we looked for linkage to the reference markers in Dedonder kits (3). When a PBS1 stock prepared on strain TS4 was crossed to all Dedonder reference markers, clear *fla* recombinants were found only among transductants at *pyrD* and *thyA* loci, with frequencies of 70 and 6%, respectively (Table 3). In addition, a very leaky *fla* phenotype was found among *aroD*$^+$ and *lys*$^+$ recombinants. To eliminate the possibility that the rare *thy*$^+$ *fla* colonies were an artifact due to the use of PBS1 (13), we crossed a *thy*$^+$ *fla* recombinant to a *pyr*$^+$ *fla* recombinant by transformation. The absence of *fla*$^+$ recombinants (data not shown) shows that both derivatives harbor the same *fla* mutation, designated *flaA4*. The absence of linkage in crosses to outside markers, *metC* and *gltA*, confirms that *flaA* lies between *thyA* and *pyrD*. Analogous crosses performed with a derivative of TS2 (Table 3) revealed the presence of a single mutation, *flaB2*, also mapping between *thyA* and *pyrD*; the linkage to the latter marker was about 50%. These results were confirmed by reciprocal crosses with a *fla*$^+$ donor in which the *fla* phenotype of strains TS2 and TS4 was eliminated by exchanging the *pyrD-cysC* region (Table 3); in each case, the proportion of *fla*$^+$ among *cys*$^+$ recombinants was in good agreement with the figures obtained above. *flaA4* and *flaB2* were not linked by transformation (data not shown).

It is noteworthy that, when crossed out, neither *flaA4* nor *flaB2* retained the thermosensitive phenotype of the parent strains; derivatives were nonmotile on semisolid medium at 30 and 37°C as well as at 47°C, with the sole exception of *flaA4*, which was poorly motile at 30°C. This observation strongly suggests that the *fla* mutations in TS2 and TS4 are suppressed at 37°C by an additional unlinked mutation. Indeed, crossing out the mutations from TS2 and TS4 by transformation (Table 4) yielded, in addition to *fla* derivatives, uncharacterized hypermotile *ifm*-like (7) recombinants. Neither of these phenotypes was obtained when a wild-type (*fla*$^+$) donor was used (data not shown). The contrasting phenotypes of these mutations raise the possibility that the *ifm*-like marker could be responsible for the suppression of the *fla* mutation (see below).

TABLE 2. Mapping of *flaC*: three-factor transduction cross involving *hisA* and *tag-1* loci[a]

Donor[b]	Recipient[b]	Recombinants			
		Selected marker	Classes	No.[c]	Suggested order
L5311 *flaC51* (Ts)	L6440 *hisA1 tag-1*	*his*$^+$	*his*$^+$ *tag*$^+$ *flaC51*	53	*hisA-tag-flaC*
			his$^+$ *tag*$^+$ *fla*$^+$	40	
			his$^+$ *tag-1 fla*$^+$	57	
			his$^+$ *tag-1 flaC51*	0	

[a] See reference 2.
[b] Relevant markers are shown.
[c] Numbers represent the sum of two crosses.

Genetic characterization of a mutant with a supermotile phenotype. A spontaneous mutant (*ifm*) showing a three- to fourfold-faster migration than the parent strain in semisolid plates was isolated (see above). To identify the mutation(s) responsible, linkage was sought to *hisA* which is close to a previously reported mutation associated with an Ifm phenotype. Reciprocal three-factor transduction crosses with a *gtaB* marker (17) clearly indicated the order *hisA-ifm-gtaB* (Table 5). However, the locus designated *ifm-3* seems to contain more than one mutation, as suggested by the existance of a minority class of *ifm* recombinants with an intermediate motility. A similarly complex genotype was found associated with other Ifm mutants (Table 4; some data not shown), suggesting that it may be characteristic of such mutants.

Mapping of mutations with an autolysin-deficient phenotype. Pairwise crosses by transformation revealed that three *lyt* mutations were distributed into two linkage groups, one containing *lyt-1* and *lyt-2* and the second containing *lyt-15* (Table 6). Their genetic relationship to the *fla* mutants mapped above was assessed by transformation.

lyt-15 was found to be closely linked to *flaA4* (Table 7) and was accordingly renamed *flaA15*. This mapping was confirmed by a 6% cotransformation of *flaA15* with a *pyrA* marker (Table 8). A comparable linkage of *flaA4* to the *pyrA* locus was also found (data not shown). No evidence for linkage of *lyt-1* to any *fla* marker was obtained by transformation. Since *lyt-1* is flagellated at room temperature (5, 6), PBS1 transduction could be exploited. However, as attempts to obtain PBS1-transducing particles on this strain at 20°C were unsuccessful, the mutation was introduced into all the Dedonder reference strains by congression. Derivatives thus obtained were transduced with a wild-type donor, and recombinants were scored for the *lyt* locus. Linkage was found to the following markers only: *aroD*, *lys*, *ilvA*, and *trpC*. Three factor crosses revealed the order *aroD-lyt-1-lys* (Table 9).

Thermosensitive suppression of the nonmotile phenotype of *flaA4* and *lyt-1* by an *ifm* marker associated with supermotility. The finding that a mutant with a lytic-deficient phenotype is closely linked to a *fla* linkage group prompted us to seek evidence that might relate the *lyt* mutations belonging to the remaining linkage group (*lyt-1* and *lyt-2*) to *fla* markers. The observation that *fla* mutations were conditionally suppressed in strains TS2 and TS4, possibly by *ifm*-like mutations, led to the question of whether *lyt-1* and *lyt-2* could be suppressed in an analogous way. A positive result would indicate that these *fla* and *lyt* markers have much more in common than

TABLE 3. Location of *flaA* and *flaB* markers on the *B. subtilis* chromosome by PBS1 transduction

Donor[a]	Recipient[a]	Recombinants				
		Selected marker	Classes	No.[b]	Cotransfer index	Suggested order
TS *flaA4*	QB943 *pyrD1 thyA1 thyB1*	*pyr*$^+$	*pyr*$^+$ *fla*^{c}	132	0.69	*pyrD-flaA-thyA*
			pyr$^+$ *fla*$^+$	54		
		thy$^+$[d]	*thy*$^+$ *fla*^{c}	18	0.06	
			thy$^+$ *fla*$^+$	284		
L5281 *flaB2*	QB943 *pyrD1 thyA1 thyB1*	*pyr*$^+$	*pyr*$^+$ *fla*^{c}	49	0.46	*pyrD-flaB-thyA*
			pyr$^+$ *fla*$^+$	59		
		thy$^+$[d]	*thy*$^+$ *fla*^{c}	4	0.04	
			thy$^+$ *fla*$^+$	94		
L1440 *fla*$^+$	L5410 *flaA4 cysC*	*cys*$^+$	*cys*$^+$ *fla*$^+$	86	0.65	
			cys$^+$ *fla*^{e}	39		
L1440 *fla*$^+$	L5303 *flaB2 cysC*	*cys*$^+$	*cys*$^+$ *fla*$^+$	87	0.49	
			cys$^+$ *fla*^{d}	93		

[a] Relevant markers are shown.
[b] Numbers represent the sum of at least two crosses.
[c] All *fla* recombinants were nonmotile at 30, 37, and 47°C. *flaA4* recombinants were weakly motile at 30°C.
[d] *thy*$^+$ recombinants, selected at 47°C, are *thyA*$^+$ *thyB*, since the *thyB* gene product is inactive at this temperature (12).
[e] All *cys*$^+$ *fla* recombinants retained the thermosensitive phenotype of the recipient strain.

TABLE 4. Crossing out by transformation of *fla* mutations from strains TS2 and TS4

Donor[a]	Recipient	Recombinants		
		No. of selected *ilv*$^+$ examined	Classes for unselected markers	No. (%)
TS4	M22 *leuA8 ilvA1*	80	*ilv*$^+$ *leu*$^+$	9 (11)
			ilv$^+$ *fla*	6 (7.5)
			ilv$^+$ Ifm	3 (3.8)
TS2	M22 *leuA8 ilvA1*	80	*ilv*$^+$ *leu*$^+$	21 (26)
			ilv$^+$ *fla*	8 (10)
			ilv$^+$ Ifm	3 (3.8)

[a] Saturating concentrations of DNA were used.

TABLE 5. Mapping of an *ifm* locus by PBS1 transduction: three-factor crosses

Donor[a]	Recipient[a]	Selected marker	Recombinant classes for unselected markers	No.	Indicated order
L5343 *ifm-3 gta*$^+$	L6148 *hisA1 ifm*$^+$ *gtaB515*	*his*$^+$	*his*$^+$ *ifm*$^+$ *gtaB*	34	*hisA-ifm-gtaB*
			his$^+$ *ifm*$^+$ *gta*$^+$	0	
			his$^+$ *ifm gtaB*	8	
			his$^+$*ifm gta*$^+$	21	
L6331 *ifm*$^+$ *gtaB515*	L5347 *hisA1 ifm-3 gta*$^+$	*his*$^+$	*his*$^+$ *ifm*$^+$ *gta*$^+$	22	*hisA-ifm-gtaB*
			his$^+$ *ifm*$^+$ *gtaB*	51	
			his$^+$ *ifm gta*$^+$	42[b]	
			his$^+$ *ifm gtaB*	1	

[a] Relevant markers only are shown.
[b] About 20% of *ifm* recombinants had a motility phenotype intermediary between *ifm* and *fla*$^+$ (wild type).

simply being flagellaless. To obtain strains with both *fla* and *ifm-3* mutations, we introduced the latter mutation directly by transduction into strains carrying the *lyt-1* or *flaA4* marker, which at low temperature (20 and 30°C, respectively) are motile and thus transducible (see above). The motility phenotype of selected *his*$^+$ recombinants, a high proportion of which should have inherited the *ifm* marker, was examined to determine whether suppression of the *fla* marker occurred at 37°C, at which temperature the *fla* or *lyt* mutation alone has a nonmotile phenotype (Table 10). In each case, analysis of at least 60 *his*$^+$ transductants revealed that between one-third and two-thirds were motile at 37°C but not at 47°C, clearly indicating suppression of both *flaA4* and *lyt-1* by *ifm-3*. The conditional character of the suppression appears very alike for both mutations. Comparable results were obtained when another *ifm* marker, crossed out of strain TS4, was used instead of *ifm-3* (data not shown). Suppression of *lyt-2* has not been examined. The apparently identical motility phenotypes of *ifm fla* and *ifm lyt* double mutants strongly support the argument that the *lyt-1* linkage group contains mutations analogous to *flaA*. The former locus has been accordingly designated *flaD*, and *lyt-1* and *lyt-2* renamed *flaD1* and *flaD2*.

DISCUSSION

The genetic analysis of six mutants with a flagellaless phenotype presented above reveals the existence of at least four *fla* linkage groups, *flaA* through *flaD*, localized in widely separated regions of the chromosome (Fig. 1). Nevertheless, the majority of the known loci affecting motility are found in two chromosome segments. The first, near

TABLE 6. Distribution of *lyt* mutations into linkage groups[a]

Donor[a]	Recipient[a]	Recombinants					
		Selected marker	Classes	No. of *pur*$^+$ or *lyt*$^+$ recombinants/no. of *met*$^+$ recombinants examined	%	R[b]	Recombination index (R/R$_o$)
L5278 *lyt-15*	L5417 *purA16 metB5 lyt-1*	*met*$^+$	*met*$^+$ *pur*$^+$	28/294	9.5	0.5	1.5
			met$^+$ *lyt*$^+$	11/200	5.5		
FJ6 *lyt-2*	L5417 *purA16 metB5 lyt-1*	*met*$^+$	*met*$^+$ *pur*$^+$	63/524	11	0	0
			met$^+$ *lyt*$^+$	0/200	0		
L5027 *lyt*$^+$	L5417 *purA16 metB5 lyt-1*	*met*$^+$	*met*$^+$ *pur*$^+$	31/435	7	0.33 (= R$_o$)	1
			met$^+$ *lyt*$^+$	4/171	2.3		

[a] Crosses were performed by transformation with saturating concentrations of DNA. Relevant markers only are shown.
[b] R, Ratio of the concentration of *met*$^+$ *lyt*$^+$ to that of *met*$^+$ *pur*$^+$ recombinants.

TABLE 7. Linkage of *lyt-15* and *flaA4* by transformation

Donor[a]	Recipient	Recombinants					
		Selected marker	Classes	No.	%	R[b]	Recombination index (R/R_o)
L5278 *lyt-15*	L5402 *thyA thyB flaA4 trpC2*	*trp*$^+$	*trp*$^+$ *thy*$^+$	112	4.5	0.24	0.16
			trp$^+$ *thy*	2,358			
		trp$^+$	*trp*$^+$ *fla*$^+$	3	1.1		
			trp$^+$ *fla*	273			
L1440 *fla*$^+$	L5402 *thyA thyB flaA4 trpC2*	*trp*$^+$	*trp*$^+$ *thy*$^+$	203	8.1	1.54 (= R_o)	1
			trp$^+$ *thy*	2,287			
		trp$^+$	*trp*$^+$ *fla*$^+$	31	12.5		
			trp$^+$ *fla*	218			

[a] Saturating concentrations of DNA were used.
[b] R, Ratio of the frequency of *trp*$^+$ *fla*$^+$ to that of *trp*$^+$ *thy*$^+$ recombinants.

TABLE 8. Linkage of *flaA15* (*lyt-15*) to *pyrA* by transformation

Donor[a]	Recipient	Recombinants				
		Selected marker	Classes	No.	%	Cotransfer index for *flaA* marker
L5278 *flaA15*	L5028 *argC4 pyrA*	*pyr*$^+$	*pyr*$^+$ *fla*	12	6.3	0.06
			pyr$^+$ *fla*$^+$	180	93.7	
		pyr$^+$	*pyr*$^+$ *arg*$^+$	0	0	
			pyr$^+$ *argC*	1,600	100	

[a] Nonsaturating concentrations of DNA (0.01 μg/ml) were used.

hisA, contains *ifm* (7), *sacU* (1, 16), *hag* (7), and *flaC*; the previously reported location for an *ifm* locus (i.e., between *hisA* and *gtaA* markers) has been confirmed by reciprocal three-factor crosses on an independently isolated mutant. The reported position for the *hag* locus has been similarly confirmed (data not shown). The second segment, between *pyrD* and *thyA*, includes *flaA*, *flaB*, and *che* (13); cotransduction frequencies obtained from two-factor crosses suggest that *flaB* (50% linked to *pyrD*) may be close to the cluster of *che* mutations (34% cotransducible with *pyrD*), with *flaA* located between the *flaB-che* region and *pyrD*. The remaining markers, *flaD* and *cheR*, map near *aroD* and *trpC*, respectively (13). The distribution of *fla* genes in several widely separated clusters recalls the situation in the family *Enterobacteriacae* in which ca. 30 genes involved in flagellation have been identified (8). The fact that no *fla* linkage group so far identified in *B. subtilis* contains more than two mutations strongly suggests that many more *fla* genes remain to be identified.

Thermosensitive flagellaless mutants TS4 and TS2 (7) were shown here to harbor mutations *flaA4* and *flaB2*, respectively. Both mutations, belonging to separate linkage groups, map in the *pyrD-thyA* region. However, no *fla* markers linked to *hisA* could be identified in either of these strains. This observation is in apparent conflict with an earlier study (7) in which transduction of a *hisA* recipient to *his*$^+$ was performed with 25 independently isolated, thermosensitive, flagellaless mutants (including TS2 and TS4). In every case, ca. 70% of the *his*$^+$ recombinants had a thermosensitive, nonmotile phenotype. However, when we transduced the *hisA* recipient used by the previous authors but with a wild-type donor, we found that about 70% of the *his*$^+$ recombinants again exhibited a thermosensitive nonmotile phenotype (data not shown). This figure, indistinguishable from those previously obtained (7) shows that the earlier results can be accounted for by a singular recipient genotype and are unlikely to be related to that of the donor. The explanation for the existence of a hidden determinant for nonmotility in the recipient may well lie in the fact that it is a W23/168 hybrid of the *hag* locus; indeed, the latter is 70% cotransducible with the *hisA* marker. Conspicuously, the only one of the 26 mutations mapped by Grant and Simon (7) which did not map close to the *hag* locus, *flaC51*, was isolated in a strain containing the *hisA* marker, which

TABLE 9. Mapping of *flaD1* (*lyt-1*): three-factor transduction cross involving *aroD* and *lys-1*

Donor[a]	Recipient[a]	Recombinants				
		Selected marker	Classes	No.	%	Suggested order
L1440 *fla*$^+$	L5416 *aroD120 lys-1 flaD1*	*lys*$^+$	*lys*$^+$ *fla*$^+$ *aroD*	168	28	*aroD-flaD-lys*
			lys$^+$ *flaD1 aroD*	419	70	
			lys$^+$ *fla*$^+$ *aro*$^+$	12	2	
			lys$^+$ *flaD1 aro*$^+$	1	0.2	
		aro$^+$	*aro*$^+$ *fla*$^+$ *lys-1*	45	47	*aroD-flaD-lys*
			aro$^+$ *flaD1 lys-1*	49	51	
			aro$^+$ *fla*$^+$ *lys*$^+$	2	2	
			aro$^+$ *flaD1 lys*$^+$	0	0	

[a] Relevant markers only shown.

TABLE 10. Construction of *fla ifm* and *lyt ifm* double mutants[a]: thermosensitive suppression of *lyt-1* and *flaA4* markers by the *ifm-3* locus

Donor[b]	Recipient[b]	Selected marker	Recombinant classes (no.)	No. motile at 37°C	No. motile at 47°C	Motility phenotype	Effect on Fla (Lyt) phenotype
L5343 *ifm-3*	L5418 *hisA1 ifm*$^+$ *lyt-1*	*his*$^+$	*his*$^+$ *ifm*$^+$ *lyt-1* (36)	0	0	Fla$^-$	
			his$^+$ *ifm-3 lyt-1* (54)	54[c]	0	Fla(Ts)	Thermosensitive suppression
L5343 *ifm-3*	L5404 *hisA1 ifm*$^+$ *flaA4*	*his*$^+$	*his*$^+$ *ifm*$^+$ *flaA4* (40)	0	0	Fla$^-$	
			his$^+$ *ifm-3 flaA4* (27)	27	0	Fla(Ts)	Thermosensitive suppression
L1440 *fla*$^+$	L5348 *ifm-3 pyrA flaA4*	*pyr*$^+$	*pyr*$^+$ *ifm-3 flaA4* (21)	21	0	Fla(Ts)	Thermosensitive suppression
			pyr$^+$ *ifm-3 fla*$^+$ (39)	39[d]	39[d]	Ifm	

[a] PBS1 transduction.
[b] Relevant markers only are shown.
[c] At 37°C, the majority (38) of the motile recombinants had a motility phenotype similar to that of an *fla*$^+$ strain. The remainder were intermediary between *fla*$^+$ and *ifm*.
[d] Ifm-like motility.

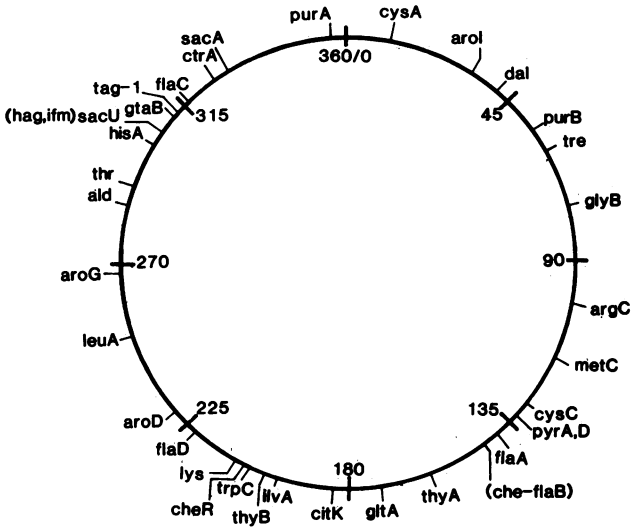

FIG. 1. Position of *fla* and other markers affecting motility on *B. subtilis* genetic map.

therefore could not have been used as a donor in a cross with the hybrid recipient. As the discrepancy between previous and present results is now resolved for TS2 and TS4, it is most likely that the remaining strains isolated by Grant and Simon (7) harbor mutations mapping in yet undiscovered loci.

Of the three mutations previously designated *lyt*$^-$ and mapped here, one, *lyt-15*, is closely linked to *flaA4*; the remaining two, *lyt-1* and *lyt-2*, like *lyt-15*, affect almost certainly regulatory functions rather than autolysin structural genes (5) and determine a flagellaless phenotype which in the case of *lyt-1* has been shown to be suppressible by an *ifm* mutation. This behavior is common to several Fla$^-$ and Lyt$^-$ mutants. That such mutations can be suppressed by other mutations associated with a hypermotile phenotype suggests that this behavior may be characteristic of many *fla* loci and could therefore be exploited for mapping purposes. However, since the procedure used to select for thermosensitive Fla$^-$ mutants (7) is likely to have favored the survival of mutations that are suppressible, we believe that *ifm* suppressibility is unlikely to be common to all *fla* mutations, such as, for example, nonsense mutations affecting flagellar structural proteins.

Our observations, including genetic linkage and suppressibility by *ifm* loci, raise the question of the nature of the genes affected by *fla* and *lyt* mutations. Indeed, when examined for autolytic activity, *flaA4* was found, like *lyt-15* (*flaA15*), to be lytic deficient, as were all of the other *fla* mutations studied here (15; manuscript in preparation). Since, in view of results presented here, there is no way of distinguishing between *fla* and *lyt* mutations at this stage, we were led to replace previous designations *lyt-1*, *lyt-2*, and *lyt-15* by *flaD1*, *flaD2*, and *flaA15*, respectively. Nevertheless, Fla$^-$ mutants selected for thermosuppressibility (7) may correspond to a special class of *fla* loci; the Lyt$^-$ phenotype may not be common to all *fla* mutations.

Association of the autolytic-deficient phenotype with *fla* mutations suggests the possibility that the cellular autolysin activity is under the control of *fla* genes and places in a new light the proposal that autolytic activity might be necessary for flagellation (5).

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LITERATURE CITED

1. **Ayusawa, D., Y. Yoneda, K. Yamane, and B. Maruo.** 1975. Pleiotropic phenomena in autolytic enzyme(s) content, flagellation, and simultaneous hyperproduction of extracellular α-amylase and protease in a *Bacillus subtilis* mutant. J. Bacteriol. **124**:459–469.
2. **Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young.** 1972. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. J. Bacteriol. **110**:281–290.
3. **Dedonder, R. A., J.-A. Lepesant, J. Lepesant-Kejzlarová, A. Billault, M. Steinmetz, and F. Kunst.** 1977. Construction of a kit of reference strains for rapid genetic mapping in *Bacillus subtilis* 168. Appl. Environ. Microbiol. **33**:989–993.
4. **Dubnau, D., and C. Goldthwaite.** 1967. Genetic mapping in *Bacillus subtilis*. J. Mol. Biol. **27**:163–185.
5. **Fein., J. E.** 1979. Possible involvement of bacterial autolytic enzymes in flagellar morphogenesis. J. Bacteriol. **137**:933–946.
6. **Fein, J. E., and H. J. Rogers.** 1976. Autolytic enzyme-deficient mutants of *Bacillus subtilis* 168. J. Bacteriol. **127**:1427–1442.

7. **Grant, G. F., and M. I. Simon.** 1969. Synthesis of bacterial flagella. II. PBS1 transduction of flagella-specific markers in *Bacillus subtilis*. J. Bacteriol. **99**:116–124.
8. **Iino, T.** 1977. Genetics of structure and function of bacterial flagella. Annu. Rev. Genet. **11**:161–182.
9. **Karamata, D., and J. D. Gross.** 1970. Isolation and genetic analysis of temperature-sensitive mutants of *B. subtilis* defective in DNA synthesis. Mol. Gen. Genet. **108**:277–287.
10. **Karamata, D., M. McConnell, and H. J. Rogers.** 1972. Mapping of *rod* mutants of *Bacillus subtilis*. J. Bacteriol. **111**:73–79.
11. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
12. **Neuhard, J., A. R. Price, L. Schack, and E. Thomassen.** 1978. Two thymidylate synthetases in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **75**:1194–1198.
13. **Ordal, G. W., D. O. Nettleton, and J. A. Hoch.** 1983. Genetics of *Bacillus subtilis* chemotaxis: isolation and mapping of mutations and cloning of chemotaxis genes. J. Bacteriol. **154**:1088–1097.
14. **Pooley, H. M.** 1976. Turnover and spreading of old wall during surface growth of *Bacillus subtilis*. J. Bacteriol. **125**:1127–1138.
15. **Pooley, H. M., and D. Karamata.** 1983. Correlation of cell wall turnover and autolytic activity in fla$^-$ and supermotile mutants of *Bacillus subtilis*, p. 279–284. *In* The target of penicillin. Walter De Gruyter, Inc., Berlin.
16. **Steinmetz, M., F. Kunst, and R. Dedonder.** 1976. Mapping of mutations affecting synthesis of exocellular enzymes in *Bacillus subtilis*. Mol. Gen. Genet. **148**:281–285.
17. **Young, F. E., C. Smith, and B. E. Reilly.** 1969. Chromosomal location of genes regulating resistance to bacteriophage in *Bacillus subtilis*. J. Bacteriol. **98**:1087–1097.