JOURNAL OF BACTERIOLOGY, Apr. 1977, p. 506-511

Vol. 130, No. 1
Printed in U.S.A.

Genetic Complementation Analysis of *Escherichia coli* Type 1 Somatic Pilus Mutants

LOIS M. SWANEY,[1] YING-PING LIU,[2] KARIN IPPEN-IHLER,[3] AND CHARLES C. BRINTON, JR.*
Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Received for publication 12 January 1977

A genetic complementation analysis of 75 stable nonpiliated mutants of a type 1 piliated strain of *Escherichia coli* K-12, AW405, was performed. Strains containing pairs of *pil* mutations were constructed by the infectious transfer of an F101 plasmid containing one *pil* mutation into *E. coli* K-12 AW405 containing another *pil* mutation. The presence or absence of type 1 pili on the merodiploid strains was determined by agglutination with type 1 pilus antiserum. All 75 mutants fell into one of four complementation groups. The pattern of complementation defined three cistrons involved in pilus formation, *pilA*, *pilB*, and *pilC*. The fourth complementation group was composed of a large number of mutants defective in both *pilA* and *pilB* functions.

Pili, nonflagellar filamentous appendages that appear in large numbers on the cell surface, are associated with a broad spectrum of bacterial types. We refer to chromosomally determined pili as "somatic" to distinguish them from the conjugative pili associated with transfer genes of plasmids (see reviews by Brinton et al. [4, 5], Duguid and Gillies [9], and Ottow [12]). Type 1 pili of *Escherichia coli*, the prototype of somatic pili, have been extensively described and characterized and shown to be composed of a single repeating subunit, pilin, arrayed in a helical structure that self-assembles in vitro (3, 4, 5, 7).

Type 1 piliation of an *E. coli* strain can be readily observed in the electron microscope or detected by precipitation of cultured cells with antibody prepared against purified type 1 pili. In addition, piliated cells of *E. coli* have a selective growth advantage over nonpiliated cells when grown under conditions of limiting oxygen (11; P. Gemski, Jr., Ph.D. thesis, University of Pittsburgh, Pittsburgh, Pa., 1964). In a prior publication (13), we used these properties, as well as a subtle difference in colonial morphology which we discovered to be characteristic of nonpiliated derivatives of our strain, to isolate a series of stable *E. coli* K-12 mutants that are defective in pilus production. In this paper we describe the results of complementation analysis of these mutants.

[1] Present address: Plum Island Animal Disease Center, U.S. Department of Agriculture, Agricultural Research Service, Greenport, NY 11944.

[2] Present address: Department of Genetics, Medical School, Stanford University, Palo Alto, CA 94305.

[3] Present address: College of Medicine, Texas A&M University, College Station, TX 77843.

Previous genetic studies of type 1 piliation in *E. coli* showed (6, 8) that at least one chromosomal site affecting type 1 piliation could be located at a position corresponding to 98 min on the *E. coli* K-12 linkage map of Bachmann et al. (2). Although we did not know whether any of our pili-defective mutants had also resulted from lesions in this region of the map, it seemed reasonable to assume that at least one of them would be located at this site. We therefore used the following rationale for our experiments: (i) F′ plasmid, F101, which carries a region of the *E. coli* chromosome containing the markers *pil thr leu ara* (10), was transferred into a series of pili-deficient mutants. Matings resulting in a merodiploid with a Pil^+ phenotype could be interpreted as indicating that the mutation of the *pil* mutant was located in the chromosomal region carried by F101 and recessive to expression of the wild-type gene on the plasmid. (ii) Pil^- isolates were selected from the merodiploid strain to obtain homogenotes in which the original *pil* mutation on the chromosome also occurred, as a result of recombination, on the F101 plasmid. (iii) Such F101 derivatives were then transferred into the rest of the series of mutants defective in type 1 piliation. Transconjugants were tested for type 1 piliation to determine whether or not the chromosomal lesions could be complemented by the plasmid derivative. Our results allow us to conclude that each of the *pil* mutants tested bears a recessive mutation in the region of the *E. coli* chromosome carried by the F101 plasmid and is assignable to one of four complementation groups. These are interpretable as reflecting the activities of three *pil* cistrons.

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used are described in Table 1. The *pil* mutants and their characteristics were described previously, as were growth media (13).

Mating procedure. Donor and recipient strains were grown to log phase in Z medium and mixed in a ratio of approximately 1:5 in a total volume of 0.5 ml. They were allowed to stand at 37°C for 45 min and then vortexed for 30 s before dilution and plating. When larger volumes were used in the mating, small flasks were employed and shaken gently for the same time period.

Male-phage sensitivity tests. Approximately 0.05 ml of an overnight Z broth culture was added to 0.6 ml of soft Z agar containing 0.001 M $CaCl_2$, and the mixture was poured into one-fourth of a Z-agar plate. A drop of a male-specific phage (MS-2) lysate (10^{10} plaque-forming units/ml) was placed on each overlay. The plates were then incubated overnight. Sensitivity to the phage confirmed the presence of an F factor in the strain tested.

Agglutination test with type 1 pilus antiserum. Type 1 pilus antiserum was prepared as described previously (13). A 0.04-ml portion of a culture to be tested and 0.04 ml of a 1:500 dilution of antiserum in saline were gently mixed on slide agglutination plates for 15 min at room temperature (22 to 25°C). The plates were covered with large petri dish lids to prevent evaporation during the reaction. Results were recorded after the plates were examined under slight magnification. Control reactions consisting of 0.04 ml of cells and 0.04 ml of saline were performed for each culture tested.

RESULTS

Construction of episomes carrying the Pil⁻ mutations. To obtain F′ plasmids carrying a series of our Pil⁻ mutations that would be suitable for use in complementation analysis, UTH4109 was mated with 18 of the PL1000 series mutants. Thr⁺ Leu⁺ transconjugants were selected on minimal medium containing histidine and thiamine. Four typical colonies from each mating were purified on the same selective medium and tested with type 1 pilus antiserum to confirm that the presence of the plasmid resulted in the synthesis of type 1 pili by the recipient. In all cases, the antiserum tests were positive for the F′Pil⁺/Pil⁻ strains, establishing that all of the *pil* mutants used resulted from mutations located in the region of the chromosome carried by the F101 episome and were recessive to a wild-type allele.

F′Pil⁻/Pil⁻ homogenotes were then obtained for each of the 18 strains. Either the selective plates from the same mating, or colonies obtained by spreading the F′Pil⁺/Pil⁻ isolates on minimal medium, were examined under oblique lighting as described previously to distinguish colonies with the difference in transparency from the majority (F′Pil⁺/Pil⁻) of colonies typical of the Pil⁻ phenotype (13). These colonies were then tested with type 1 pilus antiserum. If a negative antiserum test was obtained, presence of the F101 plasmid was confirmed in the F′Pil⁻/Pil⁻ by testing sensitivity of the strain to male-specific phage.

F′Pil⁻/Pil⁻ isolates were readily obtained from some strains and more difficult to find for others. For a difference in colony transparency to be detectable, the plates had to be examined within 18 to 20 h after the cells were plated on minimal medium. In general, colonies with the unusual morphology were seen at a frequency of approximately 1/500.

F′Pil⁻/Pil⁻ strains were given PL3000 series numbers corresponding to the number of the PL1000 series mutant from which they were derived.

Complementation between Pil⁻ mutants. Each of the F′Pil⁻/Pil⁻ donors (series PL3000) were mated with the Pil⁻ Nal^R series of mutants (PL2000) and plated on minimal medium containing histidine, thiamine, and 10 μg of

TABLE 1. *Bacterial strains*

Strain	Genotype	Comments	Source
AW405	F⁻ *thr leu his thi lac gal ara xyl str ton tsx*	Wild-type Pil⁺ parent of the type 1 pili mutants	J. Adler
UTH4109	F′ *pil⁺ thr⁺ leu⁺/thr leu his arg pro rec str*	Contains the F101 plasmid described by K. B. Low (10)	T. Matney
PL1001-PL1100	F⁻ *thr leu his thi lac gal ara xyl str ton tsx pil*	Pil⁻ derivatives of AW405	Mutagenesis of AW405
PL2001-PL2100	F⁻ *thr leu his thi lac gal ara xyl str ton tsx pil nal*	Nal^R derivatives of PL1001-PL1100	Selection in the presence of nalidixic acid
PL3001-PL3100	F′ *pil⁻ thr⁺ leu⁺/thr leu his thi lac gal ara xyl str ton tsx pil*	Pil⁻ homogenotes of F101 and the PL1001-PL1100 series of *pil* mutants	UTH4109 crossed with PL1001, etc. followed by selection of *pil⁻*

nalidixic acid per ml. Ten Thr^+ Leu^+ Nal^R colonies were selected at random and purified on the same selective medium. Each of the ten was then tested for its reaction with type 1 pilus antiserum and for its sensitivity to the male-specific phage MS-2. For all strains that were MS-2 sensitive (and therefore maintained the F101), the antiserum tests were used as the criterion for complementation. For many of the strains, additional confirmation of Pil phenotype was made by electron microscopy.

The results of 718 complementation tests with 75 *pil* mutants are shown in Table 2. A plus indicates that all Thr^+ Leu^+ sexductants from the PL3000 series and the PL2000 series cross gave a positive type 1 pilus antiserum agglutination test. A minus indicates that no agglutination could be observed. Five to ten Thr^+ Leu^+ colonies were tested from each cross. Occasionally one or two colonies from a cross that otherwise yielded agglutinating cells failed to show agglutination. In each case, however, these proved also to be female, as judged by their resistance to bacteriophage MS-2. Therefore, they were considered to be chromosomal Thr^+ Leu^+ recombinants which no longer maintained the F101 episome carrying the *pil* heterogenote. For this reason, only colonies shown to be male were considered in recording agglutination results. Transconjugants from a few crosses appeared to give a poorer agglutination than would normally be expected, although the reaction was definitely positive. These are indicated on the table by an asterisk.

It is clear from Table 2 that all the stable mutants isolated fall into four complementation patterns. We have named these groups AB, A, B, and C. Group AB was represented by 33 mutants, A by 4, B by 19, and C by 19. Group AB fails to complement mutants in either group A or B and thus appears to be lacking two otherwise complementing activities. Therefore, the results can be summarized in terms of 3 functional activities for type 1 pili production as shown in Table 3.

DISCUSSION

The complementation pattern indicates that at least three functional activities are required by *E. coli* K-12 for type 1 pilus production. The cistrons determining these activities—A, B, and C—must all be located within the region of the bacterial chromosome, which is carried by F101, since all of the *pil* mutants tested could be complemented by at least one set of F101 derivatives. Preliminary results of interrupted mating experiments and cotransductional analysis employing the *deoA* marker (1) confirm this conclusion and indicate that mutations from all complementation classes are located at approximately 98 min.

One especially interesting aspect of our results is the presence of a large number of mutants isolated in complementation class AB. The mutants in this class were obtained with a variety of mutagens including ultraviolet light, 5-bromouracil, and nitrosoguanidine, and about half are revertible to a Pil^+ phenotype (13). Therefore, it seems unlikely that they should all be deletions or result from double mutations. In any case, the absence of a similar class of mutants that do not complement A and C or B and C suggests that the A and B activities can easily be affected simultaneously, whereas the C cistron may be somewhat independent in location and function. There are several possibilities that could have given this result. We shall mention only those we consider most likely.

One explanation of the AB class of mutants is the proposal that the A and B activities are products of contiguous genes under coordinate control, and are easily subject to simultaneous loss by polar mutation or by mutation in a *cis*-dominant control site, such as the operator locus of an AB operon under positive control. Transdominant mutations affecting the expression of two genes (as found in negative control systems) can be eliminated from consideration since all the mutants isolated are recessive to the wild type. If some of the mutants in the AB class result from mutations affecting the expression of A and B by alteration of a control site, it would seem that the C gene could not be a part of the same operon. All class AB, A, and B mutants complemented class C mutants. It is conceivable that there are two genes A and B under positive control and that the C gene product might be a positive control protein.

Variation in type 1 piliation according to growth condition and the phenomenon of phase variation (13) support the hypothesis that type 1 pilus production must be subject to regulation. We proposed that phase variation may reflect the capacity of cells to alternate between two physiological states in which piliation is only one of several properties affected (13). Expression of piliation could easily be subject to physiological regulation (through, for example, the accumulation of effector molecules) if the *pil* genes are in an operon such as that suggested above. The *pil* functions must be clarified if such a model is to be substantiated.

An alternative explanation is the possibility that the A and B functions belong to the same protein product determined by a gene AB; that

TABLE 2. *Complementation of pil mutants*

Recipient PL2000 strains (F^- *pil*, *nal*)	*pil* mutation in donor PL3000 strains (F' pil_x/pil_x)																	
	AB[a]						A[a]			B[a]							C[a]	
	5	6	15	19	21	58	1	16	94	4	12	17	20	24	60	90	14	48
AB[a]																		
3	−	−	−	−	−		−	−		−	−	−	−	−			+	
6	−	−	−	−	−		−	−		−	−	−	−	−			+	
15	−	−	−	−	−		−	−		−	−	−	−	−			+	
19	−	−	−	−	−		−	−		−	−	−	−	−			+	
21	−	−	−	−	−		−	−		−	−	−	−	−			+	
27	−	−	−	−	−		−	−		−	−	−	−	−			+	
29	−	−	−	−	−		−	−		−	−	−	−	−			+	
34	−	−	−	−	−		−	−		−	−	−	−	−			+	
35	−	−	−	−	−		−	−		−	−	−	−	−			+	
44				−		−	−		−		−				−		+	+
52				−		−	−		−		−				−		+	+
54				−		−	−		−		−				−		+	+
55				−			−				−						+	
56				−		−	−		−		−				−		+	+
57				−			−				−						+[b]	
58				−		−	−		−		−				−		+	+
61				−		−	−		−		−				−		+	+
65				−		−	−		−		−				−		+	+
66				−		−	−		−		−				−		+[b]	+
69				−		−	−		−		−				−		+	+
73				−		−	−		−		−				−		+	+
74				−		−	−		−		−				−		+	+
75				−		−	−		−		−				−		+	+
77				−		−	−		−		−				−		+[b]	+
81				−			−				−						+	
82				−		−	−		−		−				−		+	+
84				−		−	−		−		−				−		+	+
86				−			−	−			−						+	
87				−			−	−			−					−	+	
92				−			−	−			−					−	+	
98				−			−	−			−					−	+	
100				−			−	−			−					−	+	
A[a]																		
1	−	−	−	−	−		−	−		+	+	+	+	+			+	
16	−	−	−	−	−		−	−		+	+	+	+	+			+	
47				−		−	−		−		+[b]				+[b]		+	+
94				−			−	−	−		+					+	+	
B[a]																		
4	−	−	−	−	−		+	+		−	−	−	−	−			+	
12	−	−	−	−	−		+	+		−	−	−	−	−			+	
17	−	−	−	−	−		+	+		−	−	−	−	−			+	
20	−	−	−	−	−		+	+		−	−	−	−	−			+	
22	−	−	−	−	−		+	+		−	−	−	−	−			+	
24	−	−	−	−	−		+	+		−	−	−	−	−			+	
31	−	−	−	−	−		+	+		−	−	−	−	−			+	
32	−	−	−	−	−		+	+		−	−	−	−	−			+	
33	−	−	−	−	−		+	+		−	−	−	−	−			+	
40	−	−	−	−	−		+	+		−	−	−	−	−			+	
60				−		−	+		+		−				−		+	+
63				−		−	+		+		−				−		+	+
64				−		−	+		+		−				−		+	+
68				−		−	+		+		−				−		+	+
71				−		−	+		+		−				−		+	+
76				−		−	+[b]		+[b]		−				−		+[b]	+[b]
79				−		−	+		+		−				−		+	+
80				−		−	+		+		−				−		+	+
90				−			+	+[b]	+		−						+	

TABLE 2—*Continued*

Recipient PL2000 strains (F^- pil_y *nal*)	*pil* mutation in donor PL3000 strains (F' pil_x/pil_x)																
	AB[a]						A[a]			B[a]						C[a]	
	5	6	15	19	21	58	1	16	94	4	12	17	20	24	60	90	14	48
C[a]																		
7	+	+	+	+	+		+	+		+	+	+	+	+			−	
14	+	+	+	+	+		+	+		+	+	+	+	+			−	
30	+	+	+	+	+		+	+		+	+	+	+	+			−	
42				+	+	+	+		+		+				+		−	−
43				+	+	+	+		+		+				+		−	−
45				+	+	+	+		+		+				+		−	−
46				+	+	+	+		+		+				+		−	−
48				+	+	+	+		+		+				+		−	−
49				+	+	+	+		+		+				+		−	−
53				+	+	+	+		+		+				+		−	−
59				+			+				+				+		−	
62				+	+	+	+		+		+				+		−	−
70				+	+	+	+		+		+				+		−	−
72				+	+	+	+		+		+				+		−	−
78				+	+	+	+		+		+				+		−	−
83				+	+	+	+		+		+				+		−	−
88				+			+	+			+					+	−	
89				+			+	+			+						−	
93				+			+	+			+					+	−	

[a] Complementation class.
[b] Antibody precipitation test less positive than usual.

TABLE 3. *Complementation analysis of 75 pil mutants of E. coli K-12*

Complementation class; no. of mutants in each class	Complementation class; no. of mutants in each class			
	AB, 33	A, 4	B, 19	C, 19
AB, 33	−	−	−	+
A, 4	−	−	+	+
B, 19	−	+	−	+
C, 19	+	+	+	−

is, that complementation classes A and B could be the result of intragenic complementation. This would explain why mutants that fell into complementation class AB were obtained so frequently, in comparison with those for class A or class B. One might expect that intragenic complementation could sometimes result in only a partially active protein; that is, complementation between A and B class mutants should be less efficient than between A and C or between B and C mutants. Unfortunately, the antibody precipitation test used as our assay of complementation is not sufficiently quantitative to establish differences in piliation. However, pilus antibody precipitation that was poorer than usual was observed in transconjugants from crosses of the A mutant PL2047 with the B mutants PL3012 and PL3016, and in transconjugants from the cross PL3016 (A) with PL2090 (B). Once the biochemical nature of the complementing activities is known, it may be possible to measure the level of B activity in an A-type and a C-type mutant as a test of this model.

We expect that the mutants characterized in this paper should also provide us with the basis for development of a genetic system in which the relatedness of somatic pili produced by other *E. coli* strains may also be tested. The introduction by recombination of *pil* genes into the chromosome of *E. coli* strains producing other kinds of somatic pili should result in the loss of piliation if the two kinds of pili are controlled by the same genes. The introduction of *pil* mutations on the F101 plasmid into *pil* mutants of *E. coli* having other kinds of pili could show the extent of genetic relatedness, if any, by the complementation pattern obtained. Somatic pili related to type 1 pili (as determined by morphology, physical properties, and serological cross reactions) exist on many enteropathogenic strains of *E. coli* such as H10407. Unlike the pili produced by AW405, H10407 pili exhibit specific adhesion, twitching motility, and agar pitting and probably determine virulence. Isolation of mutants of H10407 affected in piliation are now in progress. The genetic analysis of these mutants and comparison of their properties with our AW405 mutants should

greatly elucidate the mechanisms by which somatic pili are produced and function.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI-03242 from the National Institute of Allergy and Infectious Diseases and by the U.S. Army Medical Research and Development Command Contract DAMD 1775C5005 to C.C.B. K.I.-I. was supported by Public Health Service grant AI-10768 from the National Institute of Allergy and Infectious Diseases.

We are grateful to Julius Adler and Thomas Matney for the gift of *E. coli* strains.

LITERATURE CITED

1. **Ahmad, S. I., and R. H. Prichard.** 1969. A map of four genes specifying enzymes involved in catabolism of nucleosides and deoxynucleosides in *Escherichia coli*. Mol. Gen. Genet. 104:351-359.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116-167.
3. **Brinton, C. C., Jr.** 1959. Non-flagellar appendages of bacteria. Nature (London) 183:782-786.
4. **Brinton, C. C., Jr.** 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans. N.Y. Acad. Sci. 27:1003-1054.
5. **Brinton, C. C., Jr.** 1967. Contributions of pili to the specificity of the bacterial surface, and a unitary hypothesis of conjugal infectious heredity, p. 37-70. *In* B. D. Davis and L. Warren (ed.), The specificity of cell surfaces. Prentice-Hall, Inc., Englewood Cliffs, N.J.
6. **Brinton, C. C., Jr., and L. S. Baron.** 1960. Transfer of piliation from *Escherichia coli* to *Salmonella typhosa* by genetic recombination. Biochim. Biophys. Acta 42:298-311.
7. **Brinton, C. C., Jr., A. Buzzell, and M. A. Lauffer.** 1954. Electrophoresis and phage susceptibility studies on a filament-producing variant of the *E. coli* B bacterium. Biochim. Biophys. Acta 15:533-542.
8. **Brinton, C. C., Jr., P. Gemski, Jr., S. Falkow, and L. S. Baron.** 1961. Location of the piliation factor on the chromosome of *Escherichia coli*. Biochem. Biophys. Res. Commun. 5:293-298.
9. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. 74:397-411.
10. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. 36:587-607.
11. **Old, D. C., and J. P. Duguid.** 1970. Selective outgrowth of fimbriate bacteria in static liquid medium. J. Bacteriol. 103:447-456.
12. **Ottow, J. C. G.** 1975. Ecology, physiology and genetics of fimbriae and pili. Annu. Rev. Microbiol. 29:79-108.
13. **Swaney, L. M., Y.-P. Liu, C.-M. To, C.-C. To, K. Ippen-Ihler, and C. C. Brinton, Jr.** 1977. Isolation and characterization of *Escherichia coli* phase variants and mutants deficient in type 1 pilus production. J. Bacteriol. 130:494-505.