JOURNAL OF BACTERIOLOGY, Mar. 1974, p. 1306-1311

Vol. 117, No. 3
Printed in U.S.A.

Retraction of F Pili

CHARLES P. NOVOTNY AND PAULA FIVES-TAYLOR

Department of Medical Microbiology, The University of Vermont College of Medicine, Burlington, Vermont 05401

Received for publication 27 December 1973

The disappearance of F pili on *Escherichia coli* cells in the presence of 10^{-2} M NaCN was studied by electron microscopy and serum-blocking power. The pili which disappeared from the cell did not appear as free pili in the culture medium, suggesting that the pili had retracted into the cell. New pili were produced at a normal rate approximately 3 min after NaCN was removed. The adsorption of either F pili antibody or R17 bacteriophage to the sides of pili and temperatures below 24 C prevented retraction. The disappearance of pili was accompanied by a loss in the ability of cells to adsorb R17 phage and the type of F pili antibody that inhibits R17 phage infection and mating. The ability to adsorb M13 phage and the type of F pili antibody that inhibits M13 phage infection was not affected. This suggests that the tips of retracted pili are exposed.

Marvin and Hohn reported that F pili disappeared from the cell surface when cells were infected with a filamentous bacteriophage Ff and that the loss of pili paralleled the appearance of infected cells (8). They proposed that attachment of phage Ff to the pilus tip triggers retraction of the pilus which brings phage Ff to the cell surface where penetration occurs. Subsequently, it was shown that F pili with M13 phage on their tips became shorter with time, as predicted by the "retraction hypothesis," and, as pili disappear, M13 phage appear attached directly to the cell surface (6). The degree of shortening of *Pseudomonas aeruginosa* pili after adsorption of ribonucleic acid phage to the sides suggests that they retract to the point of attachment of the phage (2).

The concept of retraction has been incorporated into a model for conjugation which imagines that a pilus retracts and pulls the female to the surface of the male cell where deoxyribonucleic acid (DNA) transfer occurs (5). However, the report that gene transfer sometimes occurs in the absence of wall-to-wall contact indicates that retraction is not necessary for conjugation (13).

In a previous study, we reported that F pili disappeared in the presence of NaCN (11), and it has also been shown that cells poisoned with arsenate lose their pili (12). One explanation for this loss is that pili retract in the presence of energy poisons (11), and another is that pili fall off the cell when energy production is inhibited (10-12).

In this paper we present evidence that F pili retract in the presence of NaCN and show that retraction can be inhibited by attachment of either R17 phage or F pili antibody (FAB) to pili and by temperatures below 25 C.

MATERIALS AND METHODS

Bacteria and bacteriophages. Several strains of *Escherichia coli* were used in this study. Their pertinent characteristics are listed in Table 1. R17, a spherical RNA phage, and M13, a filamentous DNA phage, were used to study male phage infection.

Media. Bacteria were grown in an enriched medium supplemented with thymine (ZT medium) or without thymine (Z medium). TB agar medium was used in plates for phage assays (10). A glucose-salts medium and a glucose-salts-agar medium containing 200 μg of streptomycin per ml were used in mating experiments. The composition of these media are described in detail elsewhere (10, 11; (P. Fives-Taylor and C. Novotny, manuscript in preparation).

Growth conditions. Cells were grown overnight in Z medium, except for PT3 cells which were always grown in ZT medium. Overnight cultures containing approximately 1×10^9 cells/ml were diluted 1:10 (vol/vol) in homologous media and incubated aerobically at 37 C for approximately 100 min until the cell concentration was approximately 5×10^8 cells/ml. Cell concentrations were determined from turbidity measurements as previously described (10).

Growth of new F pili. A 50 to 100-ml volume of cultures containing 5×10^8 cells/ml in the exponential phase of growth was rapidly chilled to 0 C. F pili were removed by mechanical agitation (blending) of 50-ml samples at 0 C (10). Samples (20 ml) of blended cultures were centrifuged at 5,000 × *g* for 15 min at 0 C; cell pellets were resuspended in 20 ml of homolo-

TABLE 1. *Strains of Escherichia coli*

Cells	Strain	Genotype	F pili	Source
W1895	K-12	HfrC *met*	+	L. S. Baron
ERW	K-12	F^+his^+/his^-	+	E. Raizen
PT3	K-12	$F^+lac^+/lac^-thy^-thr^-leu^-met^-$	+	P. Taylor
B380	K-12	$F^-his^-str^r$	−	E. Raizen
HB11	B/r	F^+lac^+/lac^-	+	H. Boyer
JC3273	K-12	$F^+lac^+/lac^-gal^-his^-try^-lys^-Str^rT_6{}^rSu^-$	+	K. Ihler
M110	K-12	$F^+_{traG_{79}}lac^+/lac^-gal^-his^-try^-lys^-Str^rT_6{}^rSu^-$	+	K. Ihler
JC6296	K-12	$F^+_{traI_{61}}lac^+/lac^-gal^-his^-try^-lys^-Str^rT_6{}^rSu^-$	+	K. Ihler
JC6449	K-12	$F^+_{traD_{60}}lac^+/lac^-gal^-his^-try^-lys^-Str^rT_6{}^rSu^-$	+	K. Ihler

gous growth medium at 37 C and incubated aerobically at 37 C to allow for the growth of new F pili. The growth of pili was stopped at various times by pipetting 1-ml samples into test tubes containing 0.1 ml of 37% formaldehyde kept cold at 4 C.

Effect of NaCN on phage adsorption. Blended cultures of W1895 and HB11 cells containing approximately 5×10^8 cells/ml were incubated for 10 min in the presence and absence of 1×10^{-2} M NaCN at 37 C. The cultures were chilled to 0 C, and NaCN (1×10^{-2} M) was added to the cultures that did not contain cyanide. R17 and M13 phage were added (final concentration 2.5×10^6 plaque-forming units [PFU]/ml), and the phage were allowed to adsorb to the cells for 60 min at 0 C. Cells and adsorbed phage were removed by centrifugation at 5,000 × *g* for 15 min, and the supernatant fluids were assayed for PFU by a standard method described previously (10).

Preparation of samples for electron microscopy. Samples containing cells to be assayed for attached F pili were prepared and negatively stained with 1% uranyl acetate as previously described (11). The same procedure was used to prepare culture supernatants to be assayed for free pili, except that the free pili were allowed to settle on the specimen grid for 60 min instead of the 30 min allowed for cells.

Measurements of attached and free F pili. Attached F pili were assayed by electron microscopy as previously described (11). The number of free F pili in culture supernatants was estimated by counting all the F pili in one square of a standard 200-mesh copper electron microscope grid and listing the result as F pili per grid.

Serum-blocking power. The effect of NaCN on the ability of HB11 cells to adsorb FAB was determined by serum-blocking power. The procedure for using serum-blocking power to measure F pili is described in detail elsewhere and will only be outlined here (10). Blended HB11 cells were incubated at 0 and 37 C in the presence and absence of 1×10^{-2} M NaCN. These cultures were centrifuged, pellets were suspended in cold F pili antiserum diluted 1:300 (vol/vol) with Z medium, and the mixtures were incubated for 60 min at 0 C to allow for adsorption of FAB. Cells and adsorbed FAB were removed by centrifugation at 10,000 × *g* for 15 min. Supernatant fluids containing unadsorbed FAB were used as reaction mixtures for R17 infection of W1895 cells, M13 infection of W1895 cells, and as mating mixtures for conjugation between ERW and B380 cells.

The methods for studying phage infection have been described previously (10). Mating experiments were performed as follows: ERW (F^+his^+/his^-str^s) and B380 ($F^-his^-str^r$) cells were grown in Z medium to a concentration of 5×10^8 cells/ml. A 0.05-ml amount of ERW cells was mixed with 0.5 ml of supernatant fluid, and the unadsorbed FAB was allowed to adsorb to the F pili on ERW cells for 5 min at 37 C. Then 2.5 ml of B380 cells were added, and after 5 min of mating at 37 C, the mixtures were diluted 1:500 (vol/vol) into a medium consisting of glucose-salts and Z (4:1 vol/vol) at 37 C. The mating pairs were separated 30 min later by mechanical agitation, and samples were plated on glucose-salts-agar medium containing 200 μg of streptomycin per ml to allow for the growth of his^+str^+ recombinant colonies.

RESULTS

Effect of NaCN on the distribution of free and attached F pili on cell cultures. Depiliated PT3 cells grown and resuspended in ZT medium for 10 min at 37 C have approximately 0.7 F pili per cell (Table 2). Approximately 90% of these pili disappeared within 3 min when NaCN was added to the culture at 37 C. When the culture was chilled to 0 C and blended in an Omnimixer at 2,200 to 2,500 rpm, a speed that does not shear pili into small fragments (9), approximately 85% of the F pili disappeared. What happened to the F pili that were removed by blending and those that disappeared after exposure to cyanide? With blended cells, it is clear that the pili which are sheared from the cell persist in the culture fluid as free pili where they can be seen in electron micrographs. Supernatants prepared from these blended cultures contained many more free F pili (74 F pili/grid) than the supernatants prepared from cultures of nonblended cells (4 F pili/grid). In contrast, the supernatants prepared from the cultures of cyanide-treated cells had few F pili (5 F pili/cell), just like the supernatants of nonblended cultures. We feel the most likely explanation for this "cyanide effect" (the loss of attached F pili with no increase in free F pili) is that the F pili had retracted into the cell.

TABLE 2. *Disappearance of attached F pili and appearance of free pili after exposure to NaCN or blending*

Cultures and supernatants examined for F pili	F pili/cell		F pili/grid[a]	
	Expt 1	Expt 2	Expt 1	Expt 2
Nonblended cells	0.64	0.83		
Nonblended cells + 10^{-2} M NaCN[b]	0.06	0.07		
Blended cells[c]	0.15	0.12		
Supernatant nonblended cells			6	2
Supernatant nonblended cells + 10^{-2} M NaCN			3	7
Supernatant blended cells			76	72

[a] F pili/grid is the number of pili found in one square of a 200-mesh copper electron microscope grid.

[b] Cultures were incubated with 10^{-2} M NaCN for 3 min at 37 C.

[c] Cells were blended at low speed (2,200 to 2,500 rpm for 2 min). In this range most of the pili are removed, and the shearing of pili into pieces is minimized (8).

Inhibition of retraction by RNA phage and FAB. It has been suggested that F pili may grow in and out of the cell continuously in the absence of special signals such as phage adsorption and mating pair formation (6, 11) and that CN may only inhibit outgrowth or elongation (3, 11, 12). If this is true, one might predict that the adsorption of many R17 phage to the sides of F pili or the coating of the entire length of F pili with FAB would prevent retraction. Evidence that adsorption of these substances to F pili inhibits retraction is shown in Fig. 1. Adsorption of either R17 phage or FAB to F pili had no effect on the number of attached F pili, whereas the addition of NaCN caused the pili to disappear. However, if the pili were coated first with either R17 phage or FAB, they did not disappear. When the sequence of additions was reversed, so that NaCN was added first, R17 and FAB had no effect, and the pili disappeared. The data suggest that retraction can be inhibited by R17 and FAB and possibly by other substances that can bind to the sides of F pili.

Effect of NaCN on the adsorption of F pili antibody and male phage. Do the tips of retracted F pili remain exposed on the cell surface? One would expect that exposed pili tips would adsorb FAB specific for the tips of F pili and M13 phage, but not FAB specific for the sides of F pili and R17 phage which attach to the sides of pili. These expectations are supported by the data in Tables 3 and 4. Depiliated cells were incubated with NaCN and did not produce visible F pili. But they did possess a structure (the pilus tip?), which could adsorb the type of antibody that inhibited M13 phage infection (Table 3) and which could adsorb M13 phage (Table 4). A structure capable of adsorbing R17 phage, the type of antibody that inhibits R17 phage infection, and the type of antibody that inhibits bacterial conjugation were not produced. One conclusion that can be drawn from the data in Table 3 is that there are at least two types of F pili antibodies in our preparation of FAB. A purified preparation of intact F pili was the antigen for preparing FAB, so if a pilus has several antigenic determinants, our antibody preparations could contain antibody specific for the tip and antibody specific for the side. The results in Tables 3 and 4 are consistent with the idea that retracted pili have exposed tips which can adsorb FAB specific for the tip and M13 phage, but not R17 phage and FAB specific for the side.

Reappearance of F pili after removal of NaCN. PT3 cells were blended and allowed to grow new pili for 10 min and then given a 3-min pulse of NaCN (Fig. 2). There were approximately 0.65 F pili per cell at 10 min when NaCN was added and approximately 0.05 F pili per cell at 13 min when NaCN was removed. The number of F pili per cell remained at this level for approximately 2 min and then increased

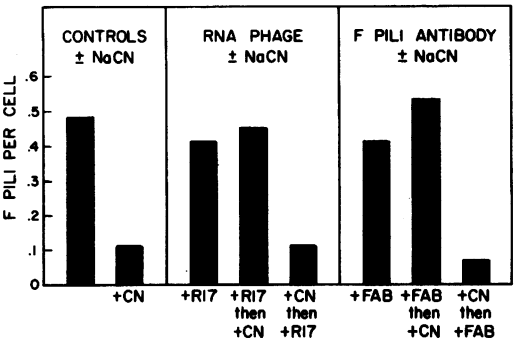

FIG. 1. *Effect of NaCN, R17 phage, and F pili antibody on the disappearance of F pili. PT3 cells were grown in ZT medium at 37 C and blended at 0 C, and pellets were suspended in ZT medium. Cells were allowed to grow new pili for 5 min at 37 C. NaCN (final concentration 1 × 10^{-2} M), R17 phage (final concentration 1 × 10^{10} PFU/ml) and F pili antiserum (FAB, final dilution 1:50 vol/vol) were added to portions of the culture singularly and in the sequences shown. After each addition the cells were incubated for 2 min at 37 C. Formaldehyde was added, and the cultures were chilled to 0 C at the end of each sequence.*

TABLE 3. *Effect of NaCN on the reappearance of serum-blocking power for male phage infection and mating*

Incubation[a]	Inhibition of phage infection and mating by F pili antibody remaining in supernatants of adsorption mixtures					
	M13[b] (infected cells/plate)		R17[b] (infected cells/plate)		*his*$^+$ [c] (colonies/plate)	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
At 0 C	107	24	30	145	104	93
At 37 C	200	43	117	295	254	269
At 37 C with 10^{-2} M NaCN	180	44	34	152	115	88
No HB11 cells added	111	21	34	155	26	27

[a] Adsorption mixtures contained F pili antibody (1/300) and blended HB11 cells which were incubated for 10 min.

[b] W1895 were used in mixtures for phage infection and as indicator bacteria.

[c] Mating between ERW (F$^+$ *his*$^+$/his$^-$) and B380 (F$^-$*his*$^-$).

TABLE 4. *Effect of NaCN on the reappearance of ability to absorb male phage*

Incubation temp of adsorption mixtures[a]	PFU remaining in supernatant fluid after 60 min adsorbtion at 0 C (%)				
	M13			R17	
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2
0 C	100[b]	100[b]	100[c]	100[b]	100[c]
37 C	90	72	60	32	23
37 C with 10^{-2} M NaCN	89	81	63	98	110

[a] Adsorption mixtures contained 5×10^8 cells/ml that were blended and incubated for 10 min at the indicated temperature prior to the addition of 6×10^6 M13 PFU/ml or 10^6 R17 PFU/ml.

[b] W1895 cells were used in adsorption mixtures.

[c] HB11 cells were used in adsorption mixtures.

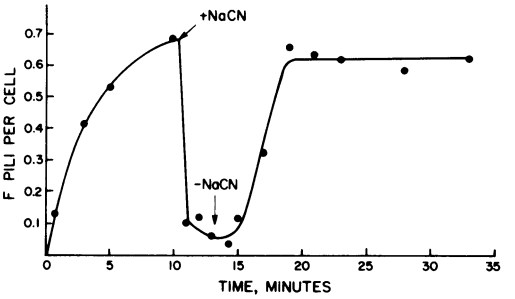

FIG. 2. *Disappearance and reappearance of F pili on PT3 cells after the addition and removal of 1 × 10^{-2} M NaCN. Cells were allowed to grow new pili for 10 min as described in Fig. 1. NaCN was added, and at 13 min the cells were removed from NaCN by filtration. Five ml of culture was filtered through a membrane filter in 0.25 min, and the cells were immediately washed off the filter pad with 5 ml of ZT medium at 37 C.*

rapidly. After 20 min, 6 min after the removal of NaCN, there were approximately 0.6 F pili per cell, which was equivalent to the number of F pili per cell at 10 min when NaCN was added. There is no significant difference in the rates of F pili production after blending (0 to 7 min) and after the removal of NaCN (13 to 20 min). Similar kinetics for the return of F pili after the removal of arsenate from the culture medium have been reported (12).

Effect of temperature on retraction. Because retraction occurs in the presence of NaCN, it was possible to block elongation with NaCN and study the effects of temperature on retraction alone. Cells were first allowed to grow new pili at 37 C. Then they were incubated at various temperatures for 3 min in the presence and absence of NaCN (Fig. 3). With NaCN present, there were approximately 0.5 F pili per cell when the temperature was below 24 C. At 25 C, approximately 50% of the pili disappeared, and at temperatures above 26 C almost all of the pili were gone. Because pili should only disappear as a result of retraction, the data suggest that retraction occurs at 26 C and at a slower rate at 25 C, but it is very slow or completely inhibited by temperatures below 24 C.

In mixtures without NaCN, there were approximately 0.5 F pili per cell at temperatures below 24 C, and from 24 to 25 C the number of pili per cell decreased as in the loss observed in mixtures with NaCN. However, as the temperature rises above 25 C, the number of pili per cell increases until the maximum level of piliation is attained at about 35 C. Interpretation of these results is complicated by the fact that at least two processes, elongation and retraction, can occur simultaneously and at different rates. Most likely, temperatures below 24 C inhibit both elongation and retraction, so that the pili are immobilized on the cell surface and there is no change in the number of pili per cell.

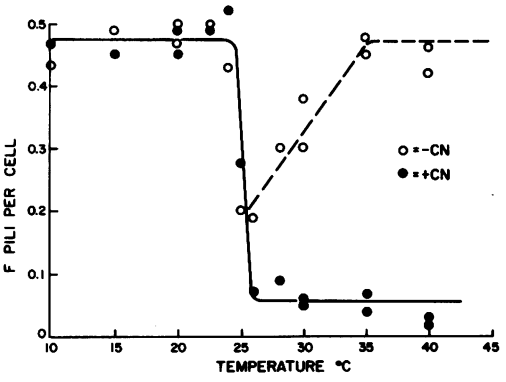

FIG. 3. *Number of F pili on cells which were incubated at different temperatures in the presence and absence of 1 × 10^{-2} M NaCN. PT3 cells were allowed to grow new pili for 10 min as described in Fig. 1. Culture was chilled to 0 C, and 1-ml samples were placed in test tubes with and without NaCN. Tubes were prewarmed to the temperature shown, and, after 3-min incubation at that temperature, formaldehyde was added to the cultures and they were chilled to 0 C.*

Elongation is probably inhibited at 24 to 25 C, but retraction occurs at these temperatures, so pili retract and the number of pili per cell decreases. At 25 C elongation may start, which begins to counteract the loss of pili due to retraction.

Effect of NaCN on F pili produced by transfer-deficient mutants. Transfer-deficient mutants of F, mutant in either the *tra D*, *tra G*, or *tra I* cistrons, produce F pili (1, 14). We studied the effects of NaCN on three *tra*$^-$ mutants (M110, *traG*; JC6296, *traI*; JC6449, *traD*) and the *tra*$^+$ parental strain (JC3273). Cultures were blended and allowed to grow new pili in ZT medium at 37 C, and 10^{-2} M NaCN was added. These strains produced new F pili like PT3 cells, and the pili disappeared within 3 min when NaCN was added. This suggests that mutations in the *tra D*, *tra G*, and *tra I* cistrons do not influence the outgrowth and retraction of F pili.

DISCUSSION

It is clear from the results reported here and elsewhere that F pili rapidly disappear when cells are exposed to 10^{-2} M NaCN and arsenate at temperatures above 25 C (11, 12). These pili have either withdrawn to the cell surface where they cannot be seen in electron micrographs, or they have fallen off the cell. If they detach from the cell, one would expect to find them in the culture supernatant. Because we did not find them there after exposure to NaCN, we conclude that the pili withdrew to the cell surface.

Consistent with this conclusion are the findings that the attachment of R17 phage and antibody to the sides of pili prevented the loss of pili in the presence of NaCN. One could conceive of how substances that bind to the sides of a pilus might preserve its structure and prevent the pilus from moving to the cell surface. It is more difficult to imagine how substances on the sides of pili would stop the pili from falling off.

We have already interpreted the sudden decrease and increase in pili which occurs between 24 and 35 C in terms of an elongation-retraction model for pili by assuming that temperature affects elongation and retraction differently (see Results). A previous report that some pili disappeared when cells were chilled to 20 C was interpreted in terms of pili falling off the cells (10). These results, however, are consistent with the elongation-retraction model which was not considered at that time.

Our observation that the antigenic properties and phage-adsorbing properties expected of pili tips persist in the presence of NaCN are also consistent with a withdrawal process that left the pilus tip intact and exposed on the cell surface. It can be argued, however, that after detachment a stub of a pilus remains on the cell, and the tip of this stub might have all the antigenic and phage-binding properties of the original tip. There is some evidence for this because one end of a fractured pilus can adsorb filamentous phage.

Some other observations seem to be more readily explained by retraction of pili than by a detachment process. For instance, cells with an I-like sex factor produced many more I pili in the presence of I pili antibody, and it was suggested that pili accumulated on the cell surface because antibody adsorbed to the sides of the pili prevented retraction (7). The observations that F pili become shorter and disappear after filamentous phage attach to their tips and that eventually phage appear to be attached directly to the cell surface also indicates that pili are able to retract with phage on their tips (6, 8). Our report that the modal length of F pili doubles when 5-bromouracil (5BU) is incorporated into DNA is also consistent with retraction (Fives-Taylor and Novotny, manuscript in preparation). It is not likely that 5BU-DNA could influence the length of F pili directly, because chemical analysis indicates that pili do not contain DNA (3) and DNA synthesis is not necessary for their production (11). Consequently, we suggested that pili might elongate and retract continuously, and that 5BU-DNA influenced the average length of pili indirectly

by changing the equilibrium between the rates of retraction and elongation (Fives-Taylor and Novotny, manuscript in preparation).

The presence of free pili in cultures and in culture supernatants led one of us to suggest that pili grow outward to a characteristic length and fall off the cell (9). However, since pili can be removed from the cell by mechanical agitation (blending), it is likely that some pili break off when cells are cultured and prepared for electron microscopy. We found that supernatants prepared from blended cultures contained about ten times as many pili as supernatants of nonblended cultures. Because pili in both supernatants were approximately the same length (data not shown), it is indicated that blending does not generate many small pili by shearing longer ones into pieces. This shows that there are more attached pili than free pili, which is exactly the opposite of what is expected if the pili elongate and fall off the cell (3, 9). We suggest, therefore, that the free pili present in our cultures represent pili that were broken off the cell by mechanical means, and that is biologically significant.

We have, until now, interpreted our results solely in terms of retraction. There is an alternate possibility. Conceivably, pili are not retracted in the presence of cyanide but are degraded instead. A degradation process that began at the tip of a pilus would account for the observation that pili are of different lengths, as some pili would be further degraded than others. The rapid loss of pili in the presence of cyanide can also be explained by degradation. If cyanide prevented the production of new pili without affecting degradation, the existing pili might be degraded into components that are not visible in electron micrographs of cell cultures and supernatant fluids. However, there is no direct evidence that pili are degraded under conditions ordinarily used to grow cells. Certainly those pili that have been removed from the cell by mechanical means are not degraded easily, because we see them in our electron micrographs. So if degradation occurs, free pili and attached pili must be degraded differently. However, the major reasons for our favoring the retraction hypothesis is that a degradation process does not readily account for other observations which are easily explained by retraction. For example, it is difficult to imagine how degradation could cause pili with filamentous phage attached to them to become shorter (6, 8), or how degradation could account for the increase in the average length of pili after 5BU is incorporated into DNA (11; Fives-Taylor and Novotny, manuscript in preparation). Both of these observations have been explained by retraction (6, 8; Fives-Taylor and Novotny, manuscript in preparation).

We think our data and other results are more consistent with F pili being structures that elongate and retract continuously (6, 11; Fives-Taylor and Novotny, manuscript in preparation). Confirmation of this model by other means and characterizing the mechanisms of elongation and retraction, particularly with regard to the role these processes may play in conjugation and male phage infection, will be important projects for future research.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service general research support grant no. 5 SO1 FR-05429 from the Division of Research Facilities and Resources, National Institutes of Health.

LITERATURE CITED

1. Achtman, M., N. Willetts, and A. J. Clark. 1972. Conjugational complementation analysis of transfer-deficient mutants of F*lac* in *Escherichia coli*. J. Bacteriol. **110**:831-842.
2. Bradley, D. E. 1972. Shortening of *Pseudomonas aeruginosa* pili after RNA-phage adsorption. J. Gen. Microbiol. **72**:303-319.
3. Brinton, C. C., Jr. 1971. The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. Critical Rev. Microbiol. **1**:105-160.
4. Caro, L. G., and M. Schnos. 1966. The attachment of male-specific bacteriophage f1 to sensitive strains of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **56**:126-132.
5. Curtiss, R., III. 1969. Bacterial conjugation. Annu. Rev. Microbiol. **23**:69-127.
6. Jacobsen, A. 1972. Role of F pili in the penetration of bacteriophage f1. J. Virol. **10**:835-843.
7. Lawn, A., and E. Meynell. 1972. Antibody-stimulated increase in sex pili in R^+ enterobacteria. Nature (London) **235**:441-442.
8. Marvin, D. A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33**:172-209.
9. Novotny, C., J. Carnahan, and C. C. Brinton, Jr. 1969. Mechanical removal of F pili, type I pili, and flagella from Hfr and RTF donor cells and the kinetics of their reappearance. J. Bacteriol. **98**:1294-1306.
10. Novotny, C., and K. Lavin. 1971. Some effect of temperature on the growth of F pili. J. Bacteriol. **107**:671-682.
11. Novotny, C. P., P. F. Taylor, and K. Lavin. 1972. Effects of growth inhibitors and ultraviolet irradiation on F pili. J. Bacteriol. **112**:1083-1089.
12. O'Callaghan, R. J., L. Bundy, R. Bradley, and W. Paranchych. 1973. Unusual arsenate poisoning of the F pili of *Escherichia coli*. J. Bacteriol. **115**:76-81.
13. Ou, J. T., and T. F. Anderson. 1970. Role of pili in bacterial conjugation. J. Bacteriol. **102**:648-654.
14. Willetts, N., and M. Achtman. 1972. Genetic analysis of transfer by the *Escherichia coli* sex factor F, using P1 transductional complementation. J. Bacteriol. **110**:843-851.