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# Purification of Sex Pili from *Escherichia coli* Carrying a Derepressed F-Like R Factor

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A procedure for the purification of sex pili is described. *Escherichia coli* K-12 carrying R1*drd*19 was grown in nutrient broth and blended at the time of peak sex pilus production. The cells were removed by centrifugation, and the supernatant fraction was concentrated, dialyzed, and clarified in an ultrafiltration system. After an additional blend and a clearing spin, the material was centrifuged in a CsCl gradient, and the fractions containing the sex pili were subjected to isoelectric focusing. About 5 mg of intact pili of approximately 98% purity were obtained by this method from about 100 g (wet weight) of cells.

The ability of a bacterial cell to act as a genetic donor is often correlated with the presence of sex pili on the cell surface (16). Most sex factors specify sex pili which belong to one of two main classes, F-like or I-like (14), but other classes have recently been observed (6). F-like and I-like sex pili characteristically adsorb F-type (e.g., MS2 and M13) and I-type (e.g., If1) male-specific bacteriophages, respectively (14). F-like R pili resemble F pili, although some can be distinguished serologically (11) and by density (1*a*). The I-like pili, on the other hand, are morphologically and serologically quite distinct from F and F-like sex pili.

Purification of F pili has often been attempted but has been hindered because of clumping of sex pili to the more numerous common pili, and because some of the procedures used resulted in alteration of the phage-binding properties of sex pili, which interfered with their assay, (4, 19). Brinton (*personal communication*) has recently purified F pili by selectively precipitating them as crystalline aggregates in tris(hydroxymethyl)aminomethane (Tris)-saline buffer at *p*H 8.5, and then redissolving them in 30% (w/v) sucrose. These preparations were further purified by cycles of low-speed centrifugation and reprecipitation.

This paper describes the isolation of intact sex pili, in a form still capable of binding phage, from *Escherichia coli* carrying a derepressed F-like R factor. The method overcomes the problem of additional filamentous appendages on the cell surface.

## MATERIALS AND METHODS

Strain UB1025 (*E. coli* K-12; *lac leu* Str$^r$ Fla$^-$) was used as the host for the R factor R1*drd*19, which is derepressed (13) and which confers resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, and sulphonamides. Male-specific bacteriophage MS2 was titrated and propagated on strain UB1057 (*E. coli* K-12; HfrH, FPA$^r$) by a modification of the soft-agar plate method of Adams (1).

Ampholytes were obtained from LKB Produkter, Bromma, Sweden. All other reagents were of analytical grade.

**Abbreviations.** Abbreviations used in this paper are: EDTA, ethylenediaminetetraacetic acid; Fim$^-$, phenotypically nonfimbriate (lacking common pili); Fla$^-$, phenotypically nonflagellate; FPA$^r$, resistant to 3 mg of fluorophenylalanine per ml; SDS, sodium dodecyl sulfate; Str$^r$, resistant to 200 $\mu$g of streptomycin per ml; and PFU, plaque-forming units.

**Media.** The media used were as follows (percent, w/v): nutrient broth (nutrient broth [Difco], 1.6; NaCl, 0.5), nutrient agar (nutrient broth plus agar [Difco], 1.3), and soft agar (nutrient broth plus agar [Difco], 0.7). The *p*H of each was adjusted to 7.5.

IC broth consisted of (percent, w/v): $K_2HPO_4$, 0.7; $KH_2PO_4$, 0.3; $CaCl_2$, 0.0147; sodium citrate, 0.05; $NH_4Cl$, 0.1; glucose, 0.5; and $MgCl_2 \cdot 6H_2O$, 0.1, *p*H 7.0. SIC broth was as for IC broth, but substituting $(NH_4)_2SO_4$ for $NH_4Cl$, and $MgSO_4 \cdot 7H_2O$ for $MgCl_2 \cdot 6H_2O$. Buffer A consisted of Tris, 1.453; $MgCl_2 \cdot 6H_2O$, 0.2033; and 2-mercaptoethanol, 0.078 (v/v), *p*H 7.3. Buffer B consisted of Tris, 1.453; NaCl, 0.5844; and EDTA, 0.3723, *p*H 7.3. SSC consisted of NaCl, 0.877; and sodium citrate, 0.441, *p*H 7.0.

**Assay for sex pili.** The assay for sex pili was modified from that of Ippen and Valentine (9). $^{35}$S-labeled MS2 phage was prepared in a vigorously aerated flask at 37 C, containing 1 liter of IC broth,

40 ml of 40% (w/v) glucose, 20 ml of SIC broth, 30 mCi of carrier-free $^{35}S$-sulfate, and an inoculum of MS2 obtained from a fresh plaque on UB1057 grown on a soft agar plate. The phage was harvested by lysing the cells with 50 ml of 0.1 M EDTA containing 1 mg of crystalline lysozyme per ml and 10 ml of chloroform, and then was precipitated by adding 28 g of $(NH_4)_2SO_4$ per 100 ml, at 4 C. The precipitate was collected and suspended in 150 ml of buffer A containing 500 $\mu$g of deoxyribonuclease, and was centrifuged at 15,000 $\times$ $g$ for 30 min, and the phage in the clear supernatant fraction from that spin was sedimented at 100,000 $\times$ $g$ for 15 hr. The pellet was suspended in 50 ml of buffer B; CsCl was added to a final concentration of 42.88% (w/w), and the solution was centrifuged at 100,000 $\times$ $g$ for 72 hr. Fractions from this gradient were assayed for PFU on soft agar plates, and the fractions containing the phage were pooled and dialyzed against 100 volumes of SSC with two changes in 48 hr and then were diluted 1:10 in SSC to give about 50 ml of phage with a titer of about $10^{11}$ PFU/ml and a specific activity of $10^{-5}$ counts per min per PFU.

This material was used in a modification of the filtration assay (5) using 20 $\mu$liters of $^{35}S$-MS2 in a mixture of 1 ml of SSC and up to 1 ml of the culture or fraction to be assayed. The mixture was incubated at 4 C for 30 min to permit adsorption and then was filtered by negative pressure through Gelman-Hawksley triacetate metricel filters (type G.D., pore size 0.45 $\mu$m). The filters were washed with three 5-ml portions of SSC and then dried at 80 C for 30 min. They were counted in a Packard Tri-Carb scintillation spectrometer in butyl PBD/toluene (4 g/liter). Fractions from CsCl gradients could be assayed directly; fractions from the isoelectric focusing column were assayed after dialysis against SSC.

**Protein estimations.** Protein estimations were carried out by the method of Lowry et al. (12).

**Diafiltration.** Diafiltration (7) was performed in an Amicon TC1D ultrafiltration module at 4 C by using the XM-300 membranes which retain molecules above $3 \times 10^5$ daltons.

**Ultracentrifugation.** Ultracentrifugation was performed in a Superspeed 50-TC centrifuge (Measuring and Scientific Equipment Ltd.) at a temperature setting of 5 C.

**Isoelectric focusing.** Isoelectric focusing was carried out by the method of Vesterberg and Svensson (21), with very little modification. Material to be investigated was dialyzed for 4 days against three changes of 1% (w/v) glycine in "Elgastat" deionized water, and then incorporated into the heavy (50%, w/v, sucrose) solution in an LKB gradient mixer (model 8121), which also contained 5% (w/v) ampholyte in the *p*H range 3 to 8. The light solution was Elgastat deionized water. The electrode solutions contained concentrated $H_2SO_4$ and solid NaOH, respectively, with the anode at the bottom of the column (LKB model 8101). The column was filled directly from the gradient mixer, and a voltage of 350 v was applied. After 24 hr the current had fallen to about 3 ma, and the voltage was increased to 450 v for about 5 hr to tighten the bands. Fractions were collected in an LKB Ultrorac by using a Watson-Marlow peristaltic pump at a flow rate of 2 ml/min. Samples (0.1 ml) of each fraction were added to 1 ml of SSC at 4 C and allowed to equilibrate for 4 days before filtration assay. The *p*H of the fractions was read directly on an EIL 23A *p*H meter with a microelectrode. The whole operation was carried out at 4 C. The fractions from preparative runs were collected after being passed through a continuous-flow cell in a Unicam SP1800 recording spectrophotometer at 280 nm.

**Electron microscopy.** Electron microscopy was performed by a modification of the method of Lawn (10). Samples were labeled with MS2 phage at 4 C, Formalin was added to a final concentration of 0.2% (v/v), and the mixture was centrifuged at 10,000 $\times$ $g$ for 30 min. The pellet was suspended in 2% (w/v) ammonium acetate, and drops were placed on grids and stained with 0.2% (w/v) uranyl acetate.

## RESULTS

**Choice of producer strain.** Purification of sex pili is greatly simplified if the host strain produces no other filamentous appendages such as flagella or common pili. We have studied Fim$^-$ Fla$^-$ derivatives of both *E. coli* K-12 and *E. coli* B/r as hosts for R1*drd*19, but we have been unable to isolate clones which produce R pili in sufficient quantity to allow successful purification, even though these strains produce many sex pili when carrying an F factor. Subsequent experiments have, therefore, been carried out with a nonflagellate isolate (8) of a strain of *E. coli* K-12 which produced numerous R pili as determined by electron microscopy. Attempts to isolate a nonfimbriate mutant from this Fla$^-$ variant were unsuccessful, so the major technical difficulty in the purification of R pili has been their separation from common pili. The method described here overcomes this problem and can be used to study sex pili, both F and F-like, formed by other strains.

**Purification of sex pili: stage one, growth of producer strain.** A 24-hr shaken culture of *E. coli* K-12 (R1*drd*19) grown in nutrient broth at 38 C was diluted 1:100 into fresh prewarmed nutrient broth and incubated at 38 C for 20 hr with high aeration. The extent of piliation was determined at intervals by electron microscopy or radioactive MS2 phage assay. After 20 hr the culture was cooled rapidly to 4 C and taken to stage two.

Figure 1 illustrates the production of sex pili during the growth cycle of the host under the conditions described above. The rapid loss of piliation when the culture reaches early stationary phase has also been reported for F pili (2, 19), and this was the reason for rapidly

cooling the culture before harvesting the pili. The height of the peak in Fig. 1, but not the time of its occurrence, was proportional to the amount of aeration. Under exceptional conditions of high aeration, minimal foaming, and freshly subcultured inocula, up to nine pili per cell of average length ca. 10 $\mu$m were observed in the electron microscope at the time of peak production. Observations on the effect of the temperature of incubation, which have recently been confirmed for F pili in greater detail (15), indicated that a temperature of 37 to 40 C was required for maximal piliation.

The piliation of the host was also studied during growth in three other types of nutrient broth and two types of minimal broth, all in regular use in this department. Nutrient broth (Difco) was outstanding under all conditions.

**Stage two: blending.** The chilled culture obtained in stage one was blended by pumping it, at a rate of 50 ml/min, through a continuous-flow cell on an MSE homogenizer fitted with 3-cm blades. The homogenizer was run at 2,000 rev/min and 4 C, and the volume of the flow cell was 100 ml.

Electron microscopy of samples from the continuous-flow cell revealed that a decrease in the flow rate (thus increasing the duration of blending) led to a progressively marked decrease in the length of both cell-attached and free sex pili. Also, the degree of aggregation of the longer pili increased with the time of blending, and they were thus lost with the pellet during the subsequent centrifugation (stage three). An increase in the flow rate, on the other hand, gave less efficient removal of pili. Blade speed was directly proportional to the efficiency of removal of pili, and was therefore set at the maximum compatible with steady flow (ca. 2,000 rev/min).

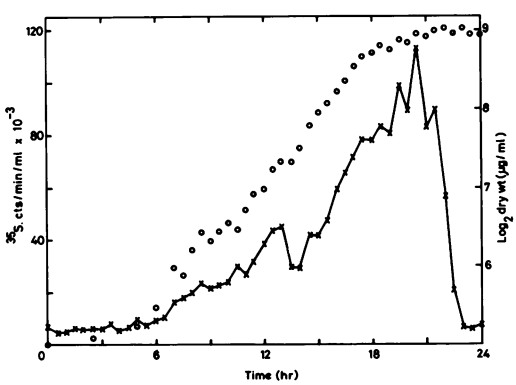


FIG. 1. *Production of R pili during the growth cycle of host in vigorously aerated nutrient broth (Difco) at 37 C. Samples were removed from the culture at intervals, and the dry weight of the cells, and the total cell-bound and free phage-binding material determined. Symbols: ×, radioactive male-specific phage assay; O, $log_2$ dry weight.*

**Stage three: low-speed spin.** Cells and cellular debris were cleared from the chilled, blended culture obtained in stage two in an M.S.E. HS18 centrifuge by using the continuous-action rotor at a speed of 15,000 rev/min. The culture was gravity-fed at a rate of 3 liters/hr, and the supernatant fluid was collected into a vessel surrounded by ice.

Examination of centrifuged samples in the electron microscope showed that maximal clearing of cells and cellular debris, without concomitant removal of the sex pili from the supernatant fluid, was achieved at low speeds for long periods. In small-scale runs, centrifugation at 10,000 × *g* for 1 hr provided equivalent conditions.

**Stage four: ultrafiltration.** The supernatant fraction from stage three was diafiltered against five volumes of SSC buffer in an Amicon TC1D ultrafiltration module and was concentrated 100-fold. The apparatus was maintained at 4 C. The concentrated material was then reblended to break up pilus-cell complexes and given an additional clearing spin at 10,000 × *g* for 1 hr.

Studies on the supernatant fraction before and after diafiltration and concentration showed that almost all the sex pili were recovered by this method, with very little breakage or aggregation. Cells were inevitably concentrated by this process, however, and became enmeshed in a tangle of pili. An additional blend and clearing spin resulted in a 4,000-fold higher yield compared to an unblended control.

**Stage five: density gradient centrifugation.** The concentrated, clarified material obtained in stage four was centrifuged at 70,000 × *g* for 72 hr after addition of cesium chloride to a final concentration of 32.05% (w/w). On large-scale (20-liter) runs, an angle rotor (8 by 50) was employed for this purpose. Fractions (ca. 1.2 ml) were collected, and samples (0.1 ml) from each fraction were assayed for phage-binding activity. The fractions containing the sex pili were pooled, and this material was used in stage six.

Figure 2 illustrates a typical sedimentation profile in cesium chloride. The peak of phage-binding activity at a density of 1.309 g/cm$^3$ was shown to contain sex pili and common pili, but no other structures, by electron microscopy (Fig. 3). The presence of both sex pili and common pili in a single band in CsCl could be explained in three ways. They could have similar density and either be complexed

together or unassociated, or they could have different densities and be complexed in a fixed ratio and thus have an intermediate density. Control experiments on the R⁻ variant of the producer strain showed that common pili, in the absence of sex pili, banded at a density of 1.309 g/cm³. Furthermore, the two types of pilus were not complexed together since sex pili prelabeled with radioactive MS2 phage formed a homogeneous band in CsCl, whereas common pili remained at the top of the gradient (Fig. 4). Attempts to purify sex pili by prelabeling followed by density gradient centrifugation failed because the drastic methods required to break up phage-pilus complexes disrupted the pilus structure.

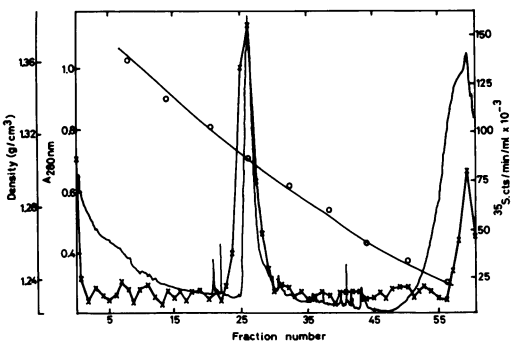


FIG. 2. *Equilibrium sedimentation profile of R pili in CsCl. Symbols:* ×, *radioactive male-specific phage assay;* ——, *absorbance at 280 nm;* O, *density at 5 C. The solution was centrifuged at 5 C in an angle rotor (8 by 50) at 70,000 × g for 72 hr. Fractions were collected after the tube contents were pumped through a continuous-flow cell in a Unicam SP1800 recording spectrophotometer. A 0.1-ml portion of each fraction was assayed for phage-binding ability. The densities were calculated from measurements of the refractive index of samples from the fractions (17).*

**Stage six: isoelectric focusing.** Isoelectric focusing (see Materials and Methods) of the peak from the CsCl gradient showed that it had two main components: (i) a protein peak (pI 3.50) which showed phage binding activity after extensive dialysis against SSC; and (ii) a protein peak (pI 5.75) which did not show phage-binding activity after extensive dialysis against SSC. The fractions containing the material of pI 3.5 were pooled and dialyzed at 4 C against three changes of 100 volumes of SSC over a period of 4 days, and this material was stored at −20 C for use in further experiments.

The technique (18) of isoelectric focusing requires that the protein under test is present in solution at low ionic strength (<0.5 mM). No buffers can be used, and therefore problems of protein denaturation can arise. The use of El-

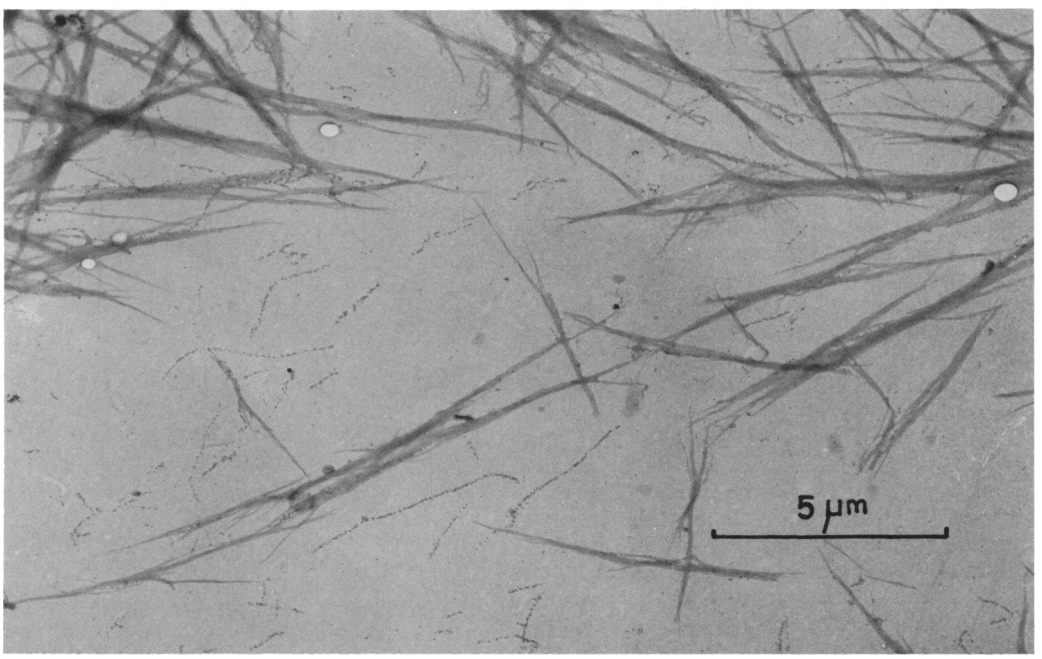


FIG. 3. *Electron micrograph of the material in the peak in Fig. 2. Samples were MS2-labeled and prepared for the microscope by a modification of the method of Lawn (10), by using uranyl acetate as a negative stain.*

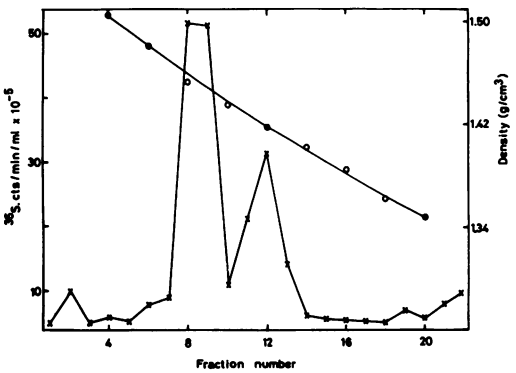


FIG. 4. *Equilibrium sedimentation profile in CsCl of R pili prelabeled with $^{35}$S-MS2 phage. Symbols: ×, radioactive male-specific phage; O, density at 5 C. A 20-ml amount of pilus material from stage four was mixed with 0.1 ml of $^{35}$S-MS2 and incubated for 30 min at 37 C to permit adsorption. Formalin was added to a final concentration of 1% (v/v), and CsCl to a density of 1.42. The mixture was added to one tube of an SW rotor. Controls of $^{35}$S-MS2 alone and pilus material alone were placed in the other tubes. The rotor was centrifuged as described in Fig. 2; fractions were collected and, where the tubes contained the phage, counted directly in 30% (v/v) Triton X-100 in toluene containing 6 g of butyl PBD per liter. The control tube containing pilus material alone was phage assayed in the normal way, and confirmed that unlabeled pili remained at the top of the gradient. Electron microscopy of samples of each fraction demonstrated the presence of common pili at the top of the gradient, and confirmed the identity of the phage-binding material with sex pili. The left-hand peak is free $^{35}$S-MS2, and the right-hand peak is prelabeled sex pili which are free from common pili.*

gastat deionized water resulted in loss of phage-binding ability of sex pili, in accord with the observation (5) that binding requires monovalent cations at about 0.1 M. Furthermore, electron microscopy showed that the integrity of the pili was lost, and the protein was present as a random bundle of fibers which tended to precipitate. Redialysis of this material against SSC restored about 30% of original phage-binding ability, but the characteristic structure was never regained. Proteins can be protected in deionized water by monoamino monocarboxylic amino acids (22), and a 1% (w/v) solution of glycine in Elgastat was therefore tested as a dialysis medium for sex pili. The phage-binding ability of the pili was still lost, but the integrity of their structure was not, and on redialysis against SSC they regained almost all their ability to adsorb phage and became indistinguishable in the electron microscope from untreated pili. Dialysis of pili against 1% (w/v) glycine in SSC showed that the glycine was not itself responsible for inhibition of phage binding.

The two peaks on isolectric focusing were obtained whether or not glycine was present, the only difference being that focusing was a little less sharp in the presence of glycine. Furthermore, the pI values of the peaks were unchanged in runs where the polarity of electrodes was reversed, indicating that precipitation effects were negligible.

**Criteria of purity.** $^{3}$H-labeled R$^{-}$ cultures and $^{14}$C-labeled R$^{+}$ cultures (Fig. 5) were mixed at the start of a preparative run. $^{3}$H and $^{14}$C labels were detectable together at all stages in the procedure up to, and including, the CsCl gradient. On isoelectric focusing (Fig. 5), no $^{3}$H was associated with the peak at *p*H 3.5, and no R$^{+}$-specific label ($^{14}$C) was detected in the absence of $^{3}$H, except at *p*H 3.5. Control runs in which only one of the two cultures was labeled showed that there was no artifact involved in the reduced efficiency of counting in double-label experiments. Calculations of the yield from the peaks in the column (in terms of both micrograms of protein and count per minute) indicated that this procedure allowed almost 100% recovery of protein, and that any other sex-specific component comprised less than 2% of the total pilus protein. Figure 6 illustrates the appearance of the materials in the peak at *p*H 3.5. The total yield of sex pili from this procedure was 3 to 6 mg of protein per 20-liter culture.

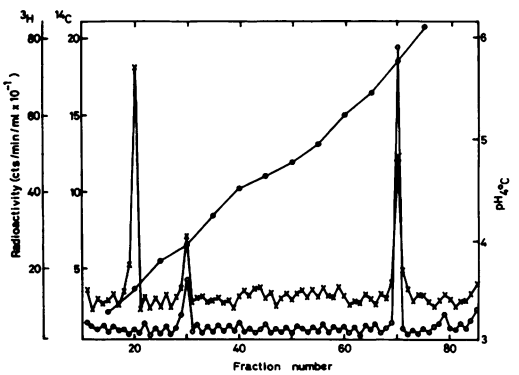


FIG. 5. *Isoelectric focusing of pilus material from $^{14}$C-labeled R$^{+}$ and $^{3}$H-labeled R$^{-}$ cells. Symbols: ×, $^{14}$C (R$^{+}$) counts/min; O, $^{3}$H (R$^{-}$) counts/min; ●, pH at 4 C. One-liter cultures of the R$^{+}$ and R$^{-}$ variants were grown in nutrient broth (Difco) in the presence of 0.1 mCi of $^{14}$C- and $^{3}$H-phenylalanine, respectively. At the time of harvest, the cultures were mixed, and sex pili were prepared as described in Results. Fractions from the column were counted directly as described in Fig. 4.*

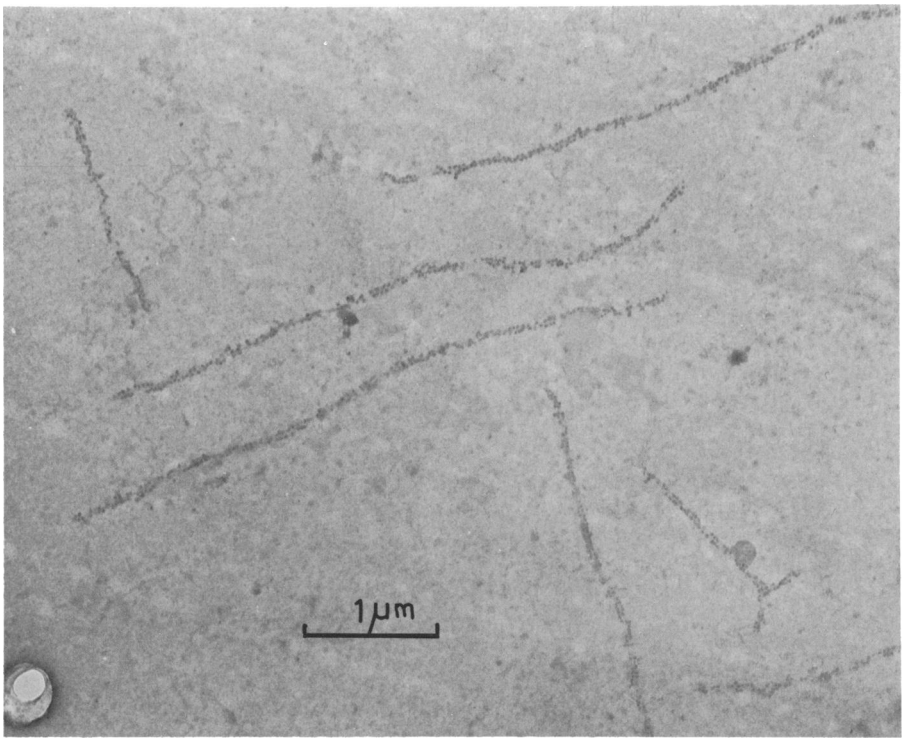


FIG. 6. *Electron micrograph of isoelectrically focused material at pH 3.5. Samples were prepared as in Fig. 3.*

## DISCUSSION

The technique of isoelectric focusing described here facilitates the separation of intact sex pili from a mixture of macromolecules, as judged by the male-specific phage assay and electron microscopy. This is important because subunits obtained after treatment with SDS and 6 M urea are unrecognizable by these assay procedures (*unpublished observations*). Isoelectric focusing is also a sensitive indicator of purity (20) and is the only well developed method for the study of macromolecules. Other methods are appropriate only for homogeneous diffusible molecules of low molecular weight.

It is conceivable that the material in the peak at *p*H 3.5 contains a contaminating nonprotein component which is either bound to the pilus protein or which has a pI of 3.5. Preliminary analysis of the material in the peak indicates that this is unlikely. Similarly, the peak at *p*H 3.5 could be a complex of pilus and non-pilus proteins coded for by the R factor. Polyacrylamide-gel electrophoresis in the presence of SDS indicates the presence of a single protein, but elucidation of this point must await further analysis. The electron micrographs demonstrate that pilus proteins are present in large quantities, but they do not exclude the presence of other components.

The total yield of R pili from this procedure (3–6 mg per 20-liter culture) represents a recovery of about 10 to 20% of the total amount of pilus protein in an average culture at the time of harvest (estimated from the density and mean dimensions of the pili). The precipitation procedure of Brinton (*personal communication*) allows a fourfold higher yield of F pili (1 mg/liter) by use of a strain producing large numbers of F pili/cell. Our studies indicate that R factor-carrying cells rarely produce sex pili in large numbers, and there is no evidence at present that R1*drd*19-determined pili can be precipitated by the method of Brinton. The purification method described here is independent of any particular chemical characteristic and can therefore be used to study sex pili specified by a number of R factors in a variety of strains.

Early investigations on the nature of sex pili have produced conflicting results. F pili have been described (23) which have a buoyant density of 1.197 g/cm$^3$ in CsCl and which are extremely sensitive to heat and lipid solvents. Brinton (3) observed a buoyant density of

1.257 g/cm$^3$ for F pili and found that there is no evidence for lipid in their structure, but that they consist of protein subunits of molecular weight 11,800, with two phosphate groups and one D-glucose residue per molecule. The F pili which we have studied (1*a*) have a buoyant density of 1.296 g/cm$^3$, which renders a high lipid content improbable. Conceivably, R1*drd*19-determined sex pili may contain lipid, carbohydrate or nucleic acid, although their buoyant density again rules out a high proportion of lipid. Preparation of large batches of R pili for chemical and physical analysis is in progress.

It has been proposed that sex pili are composed of identical protein subunits assembled in a helical manner to form a biotubule (4, 19), which suggests that each subunit has sites concerned with adsorption of deoxyribonucleic acid- and ribonucleic acid-containing male-specific phages, attachment to a recipient cell, polymerization to other subunits (both proximal and distal), and nucleic acid uptake and transport. Purification of intact F and F-like R pili is a prerequisite for the elucidation of these properties, and for a detailed study of the mechanisms of sex factor-mediated gene transfer and male-specific phage infection.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
1*a*. Beard, J. P., T. G. B. Howe, and M. H. Richmond. 1972. Centrifugation properties of sex pili. J. Mol. Biol. **66**:311-313.
2. 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.
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. Crit. Rev. Microbiol. **1**:105-160.
4. Brinton, C. C., Jr., and H. Beer. 1967. The interaction of male-specific bacteriophages with F pili, p. 251-289. *In* J. S. Colter and W. Paranchych (ed.), Molecular biology of viruses. Academic Press Inc., New York.
5. Danziger, R. E., and W. Paranchych. 1970. Stages in phage R17 infection. II. Ionic requirements for phage R17 attachment to F pili. Virology **40**:547-553.
6. Datta, N., and R. W. Hedges. 1971. Compatibility groups among fi$^-$ R factors. Nature (London) **234**:222-223.
7. Henderson, L. W., A. Besarab, A. Michaels, and L. W. Bluemle, Jr. 1967. Blood purification by ultrafiltration and fluid replacement (diafiltration). Trans. Amer. Soc. Artif. Intern. Organs. **13**:216-222.
8. Howe, T. G. B., and J. P. Beard. 1972. The selection of non-flagellate mutants of *Escherichia coli*. Mutat. Res. **15**:80-81.
9. Ippen, K. A., and R. C. Valentine. 1965. An assay for the male substance (F pili) of *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **21**:21-27.
10. Lawn, A. M. 1966. Morphological features of the pili associated with *Escherichia coli* K-12 carrying R factors or the F factor. J. Gen. Microbiol. **45**:377-383.
11. Lawn, A. M., E. Meynell, and M. Cooke. 1971. Mixed infections with bacterial sex factors: sex pili of pure and mixed phenotype. Ann. Inst. Pasteur (Paris) **120**: 3-8.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
13. Meynell, E., and M. Cooke. 1969. Repressor-minus and operator-constitutive derepressed mutants of F-like R factors: their effect on chromosomal transfer in HfrC. Genet. Res. **14**:309-313.
14. Meynell, E., G. G. Meynell, and N. Datta. 1968. Phylogenetic relationships of drug-resistance factors and other transmissible plasmids. Bacteriol. Rev. **32**:55-83.
15. Novotny, C. P., and K. Lavin. 1971. Some effects of temperature on the growth of F pili. J. Bacteriol. **107**: 671-682.
16. Novotny, C., E. Raizen, W. S. Knight, and C. C. Brinton, Jr. 1969. Functions of F pili in mating-pair formation and male bacteriophage infection studied by blending spectra and reappearance kinetics. J. Bacteriol. **98**:1307-1319.
17. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**:430-443.
18. Svensson, H., 1962. Isoelectric fractionation, analysis, and characterisation of ampholytes in natural pH gradients. III. Description of apparatus for electrolysis in columns stabilised by density gradients and direct determination of isoelectric points. Arch. Biochem. Biophys. **1**:132-138.
19. Valentine, R. C., P. M. Silverman, K. A. Ippen, and H. Mobach. 1969. The F pilus of *Escherichia coli*. Advan. Microb. Physiol. **3**:1-51.
20. Vesterberg, O. 1971. Isoelectric focusing and separation of proteins, p. 595-613. *In* J. Norris and D. W. Ribbons (ed.), Methods in microbiology, 5B. Academic Press Inc., New York.
21. Vesterberg, O., and H. Svensson. 1966. Isoelectric fractionation, analysis, and characterisation of ampholytes in natural pH gradients. IV. Further studies on the resolving power in connection with separation of myoglobins. Acta Chem. Scand. **20**:820-834.
22. Wadström, T., 1968. Studies on extracellular proteins from *Staphylococcus aureus*. IV. Separation of $\alpha$-toxin by isoelectric focusing. Biochim. Biophys. Acta **168**: 228-242.
23. Wendt, L. W., K. A. Ippen, and R. C. Valentine, 1966. General properties of F pili. Biochem. Biophys. Res. Commun. **23**:375-380.