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Cloning and Expression of the Pilin Gene of *Pseudomonas aeruginosa* PAK in *Escherichia coli*

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Many strains of *Pseudomonas aeruginosa* possess pili which have been implicated in the pathogenesis of the organism. This report presents the cloning and expression in *Escherichia coli* of the gene encoding the structural subunit of the pili of *P. aeruginosa* PAK. Total DNA from this strain was partially digested with *Sau*3A and inserted into the cloning vector pUC18. Recombinant *E. coli* clones were screened with oligonucleotide probes prepared from the constant region of the previously published amino acid sequence of the mature pilin subunit. Several positive clones were identified, and restriction maps were generated. Each clone contained an identical 1.1-kilobase *Hin*dIII fragment which hybridized to the oligonucleotide probes. Western blot analysis showed that all of the clones expressed small amounts of the *P. aeruginosa* pilin subunit, which has a molecular mass of ca. 18,000. This expression occurred independently of the orientation of the inserted DNA fragments in the cloning vector, indicating that synthesis was directed from an internal promoter. However, subclones containing the 1.1-kilobase *Hin*dIII fragment in a specific orientation produced an order of magnitude more of the pilin subunit. While the expressed pilin antigen was located in both the cytoplasmic and outer membrane fractions of *E. coli*, none appeared to be polymerized into a pilus structure.

Pili have been observed on a variety of clinical and laboratory strains of *Pseudomonas aeruginosa*, and recently, they have been implicated in pathogenesis as well. Woods et al. (34), using an in vitro binding assay, demonstrated that there was a direct interaction of purified pili and piliated organisms with mammalian buccal epithelial cells. Pseudomonas pili resemble those from other bacterial genera in their morphological appearance and subunit molecular weights. They are flexible structures with a diameter of 6 nm and a length of about 30 nm. The molecular mass of the pilin subunit from several strains is about 18,000 daltons.

Amino acid sequencing of pilin from *P. aeruginosa* PAK revealed the presence of an unusual amino acid, methylphenylalanine, at the amino terminus, followed by a stretch of predominantly hydrophobic amino acids (27). This sequence of about 30 amino acids appears to be a constant component of antigenically related pilin from various *P. aeruginosa* isolates, while the immunological differences are due to different epitopes in the C terminus. Furthermore, the same constant amino-terminal sequence is found in pilin from a number of rather diverse genera, such as *Neisseria gonorrhoeae* (14, 29), *Moraxella nonliquefaciens* (11), and *Bacteroides nodosus* (19). In addition to the common amino-terminal region, the protein coding sequence deduced from the nucleotide sequence of the cloned pilin genes from *N. gonorrhoeae* and *B. nodosus* revealed an unusual sequence of seven N-terminal amino acids not found in the protein obtained from purified pili. Apparently, this short amino-terminal peptide functions as a secretion signal and is removed during or immediately following translation of the mRNA. Subsequently, a methyl group is attached to the newly formed amino terminus. Most pili from *Escherichia coli* appear to be synthesized as true secretory precursors, with the characteristic hydrophobic signal sequences (2, 12) being cleaved without any additional posttranslational modification of the mature protein. Thus, pili of *N. gonorrhoeae*, *B. nodosus*, and probably *P. aeruginosa* may represent an evolutionarily distinct group of proteins with a conserved primary sequence and specific machinery for modification and assembly.

Despite the great amount of biochemical data available about the pilin of *P. aeruginosa* and the availability of a well-defined genetic system, relatively little work has been done on the elucidation of the regulation of pilin expression. Selecting for resistance to pilus-specific phages, Bradley (4) isolated several mutants of *P. aeruginosa* that appeared to overproduce pilin and thus are defective in an as yet undefined regulatory element. In addition, Bradley unequivocally established the location of the pilin structural gene on the chromosome. In this report, we describe the isolation of recombinant plasmids containing the pilin structural gene from *P. aeruginosa*. The pilin synthesized by the recombinants is localized in both inner and outer membrane fractions of the recombinants but does not appear to be assembled into a pilus.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *P. aeruginosa* strain used in this study (PAK) was a gift of David Bradley, Memorial University of Newfoundland, New Brunswick, Newfoundland, Canada. *E. coli* HB101 (F⁻ *hsdS20* [*hsdR hsdM*] *recA13 ara-14 proA2 lacY1 galK2 rpsL20* [Str^r] *xyl-5 mtl-1 supE44* λ⁻) and *E. coli* JM109(*recA*, Δ(*lac-proAB*) *endA1 gyrA96 thi-1 hsdR17 supE44 elA1* λ⁻ [F′ *traD36 proAB lacI^q*]) were the recipient strains for recombinant plasmids. All cultures were routinely grown on L medium (18) at 37°C with the appropriate antibiotics.

DNA isolation and analysis. Total DNA was isolated from washed (two times with 10 mM Tris [pH 8.0]) *P. aeruginosa* cells by first suspending the cells in 10 mM Tris (pH 8.0)–10 mM EDTA containing 1 mg of lysozyme per ml. After incubation for 15 min at 37°C, the cells were lysed by the addition of Sarkosyl (CIBA-GEIGY Corp., Summit, N.J.) to 1% and proteinase K to 50 μg/ml. The lysate was incubated an additional 30 min at 37°C, followed by three phenol and two chloroform extractions. The DNA was ethanol precipi-

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tated, spooled out, washed with 75% ethanol, and suspended in TE (10 mM Tris, 1 mM EDTA [pH 8.0]). After treatment with RNase A (100 μg/ml), the DNA was again extracted once each with phenol and chloroform, ethanol precipitated, and suspended in TE.

Large-scale preparations of plasmid DNA were obtained from chloramphenicol-treated *E. coli* cultures by a lysis procedure (7) with lysozyme–EDTA–Triton X-100. Plasmid DNA was further purified by cesium chloride-ethidium bromide density gradient centrifugation. For routine plasmid screening, the alkaline-sodium dodecyl sulfate (SDS) lysis procedure was employed (3).

Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories, Gaithersburg, Md., and International Biotechnology Inc., New Haven, Conn., respectively. Restriction enzyme digestion and ligations of DNA were carried out under specifications provided by the vendor. DNA was analyzed by electrophoresis in 1% agarose gels in Tris–acetic acid–EDTA buffer (18).

DNA was transferred from agarose gels to nitrocellulose filters by the method of Southern (30). Hybridizations were done at 37°C with 50% formamide as described by Portnoy et al. (26).

DNA cloning. *P. aeruginosa* PAK DNA was partially digested with the restriction enzyme *Sau*3A, and at various times the extent of the digestion was determined by agarose gel electrophoresis. To obtain the largest yield of fragments between 4 and 9 kilobase pairs (kbp), 100 μg of *P. aeruginosa* PAK DNA was digested with 2 U of *Sau*3A for 30 min at 37°C, after which the reaction was stopped by heating for 10 min at 65°C and adding EDTA to 10 mM. The digested DNA was electrophoresed on a 1% agarose gel containing 0.5 μg of ethidium bromide per ml, and the region of the gel containing fragments of 6 to 9 kbp was cut out. DNA was recovered by electroelution, which was followed by phenol-chloroform extraction of the eluate and a final ethanol precipitation before use. The DNA was then ligated into the *Bam*HI site of the plasmid cloning vector pUC18 (23) which had undergone prior dephosphorylation with calf intestinal phosphatase (Boehringer GmbH, Mannheim, Federal Republic of Germany) (18). Ligation with T4 DNA ligase was carried out for 12 to 16 h at 15°C at vector-to-insert ratios of 2:1. This ligation mixture was used to transform competent *E. coli* HB101 directly, and recombinant transformants were selected on L agar containing 100 μg of ampicillin per ml. Subcloning of DNA fragments in pUC18 to give pMS27 and pMS28 and in the broad-host-range vector pRK404 (8) to give pRK404-27 was carried out by analogous procedures.

Oligonucleotide probes. A mixed sequence oligonucleotide probe (14-mer) based on the amino acid sequence of *P. aeruginosa* PAK pilin from residues 23 to 27 in the constant region (27) was synthesized by the Custom DNA Synthesis Laboratory, Department of Chemistry, University of Washington. The mixed oligonucleotide DNA was end-labeled with [γ-^{32}P]ATP (New England Nuclear Corp., Boston, Mass.), and T4 polynucleotide kinase (Bethesda Research Laboratories) following the procedure outlined by Woods (33).

Screening of the DNA library for pilin sequences. Recombinant *E. coli* clones were plated on L agar containing ampicillin, and after incubation for growth, the colonies were lifted off onto nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.). The colonies on filters were lysed, and the DNA was denatured with NaOH before it was probed (18). Colony hybridizations were carried out as described by Woods (33), using approximately 0.03 μg of the radiolabeled oligonucleotide probe for each filter. Colonies containing DNA sequences that hybridized with the probe were visualized by autoradiography of filters.

Purification of pili and preparation of antisera. Pili from *P. aeruginosa* pf, a multipiliated mutant of PAK (4), were purified as described by Paranchych et al. (25). Antiserum against purified pili was raised by subcutaneous injection of female New Zealand rabbits with 100 μg of pili in complete Freund adjuvant, followed by a booster injection of 100 μg of pili in phosphate-buffered saline 1 month later. Blood was collected 10 days after the second injection, and immunoglobulin G was purified from the serum by ammonium sulfate precipitation and passage through a DEAE-cellulose column.

Western transfer. Cells were lysed or samples were dissolved by boiling for 10 min in a running buffer containing 2% SDS, 1% 2-mercaptoethanol, and 50 mM Tris (pH 7.5) before they were applied to the gel. Preparations were separated by electrophoresis on either 12.5% or on linear 10 to 17% gradient polyacrylamide gels, containing 0.1% SDS, using the system of Laemmli (16). Standard protein mixtures (Bethesda Research Laboratories) and purified *P. aeruginosa* PAK pilin were included for the determination of molecular weights.

The proteins in the gels were electrophoretically transferred to nitrocellulose filters by the method of Towbin et al. (31). The filters were first blocked with bovine serum albumin, treated with rabbit antipilin antisera, and then probed with [^{125}I]protein A (New England Nuclear), followed by autoradiography.

RESULTS

Construction of *P. aeruginosa* genomic library and identification of recombinant plasmids. Approximately 5,000 recombinant clones from the genomic library of *P. aeruginosa* DNA in *E. coli* HB101 were screened for the presence of sequences homologous with the oligonucleotide probe for pilin. A total of seven clones exhibited a reproducible reaction with the radiolabeled probe in colony hybridization assays; and by initial restriction site analysis, three clones, representing three different insert size classes, were selected for further study.

Restriction enzyme analysis of cloned pili sequences. The restriction maps for plasmids pMS2, pMS5, and pMS6 are shown in Fig. 1. The sizes of the inserts in these plasmids were 2.7, 6.2, and 4.2 kbp, respectively. Southern blot analysis of restriction digests of these plasmids with the radiolabeled oligonucleotide probe localized the hybridizing sequence to a common 1.1-kbp *Hin*dIII fragment that was present in all three plasmids, as well as to a 0.6-kbp *Pst*I fragment located entirely within the *Hin*dIII fragments. Based on this information, additional subclones were prepared. Clones pMS27A and pMS27B represented the 1.1-kbp *Hin*dIII fragment inserted in plasmid pUC18 in opposite orientations relative to the *lac* promoter, and pMS28A and pMS28B were constructs with the 0.6-kbp *Pst*I fragment in both orientations. In addition, the 1.1-kbp *Hin*dIII fragment was subcloned into the low-copy-number vector pRK404 (8) in both orientations, giving pRK404-27A and pRK404-27B.

Expression and location of *P. aeruginosa* pilin in *E. coli*. To determine whether the pilin gene from *P. aeruginosa* was expressed in *E. coli*, the recombinant plasmids pMS2, pMS27A, pMS27B, pMS28A, pMS28B, pMS5, pMS6, pRK404-27A, and pRK404-27B were introduced into *E. coli* JM109. Polypeptides synthesized by these cells were sub-

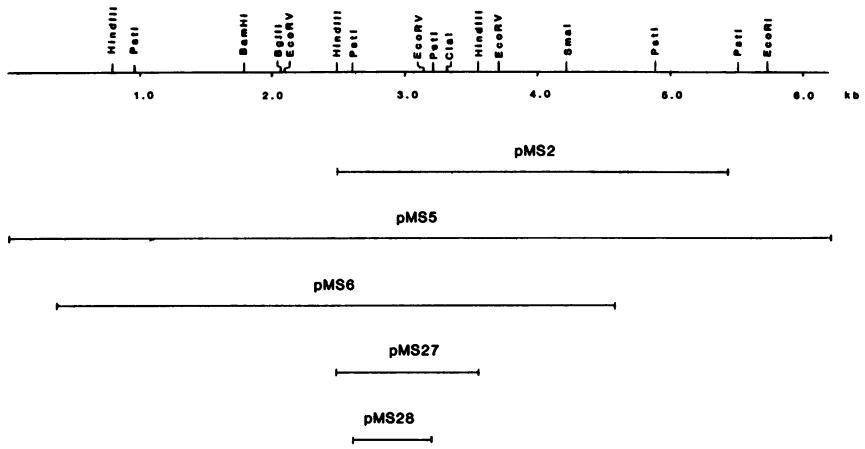

FIG. 1. Restriction map of the cloned *P. aeruginosa* DNA segments containing the pilin structural gene. The recombinant plasmid pRK404-27 (data not shown) contains the same 1.1-kbp *Hin*dIII fragment as pMS27.

jected to Western blot analysis, following growth in media with and without isopropyl β-D-thiogalactopyranoside (IPTG; 150 μg/ml), the inducer of the *lac* promoter. Following lysis of bacteria with SDS, proteins were electrophoresed, transferred to nitrocellulose and probed with antipilin antisera and [^{125}I]protein A.

Clones carrying pMS2, pMS27B, pMS5, and pMS6 produced a small amount of antigen which comigrated with the *P. aeruginosa* pilin (molecular mass, ca. 18,000), while clone pMS27A produced at least an order of magnitude more (Fig. 2). Cloning of the 1.1-kbp *Hin*dIII fragment in both orientations relative to the *lac* promoter in a low-copy-number vector (pRK404-27A and pRK404-27B) resulted in similar differential expression. Induction of the *lacZ* gene promoter with IPTG had no effect on the expression of pilin in any of the clones, and identical results were obtained in *E. coli* HB101 (data not shown) as well as in JM109, which contains the *lac* repressor encoded by *lacI*^{q}. These results suggest that synthesis of pilin is directed by the promoter of the pilin structural gene. However, overproduction of the pilin protein in one orientation and not the other in pMS27A and pMS27B and in pRK404-27A and pRK404-27B, respectively,

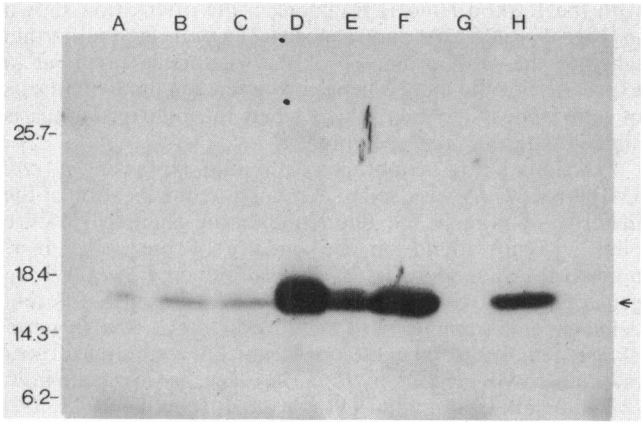

FIG. 2. Western blot analysis of the probe-positive *E. coli* clones. Lanes: A, JM109(pMS2); B, JM109(pMS5); C, JM109(pMS6); D, JM109(PMS27A); E, JM109(pMS27B); F, JM109(pRK404-27A); G, JM109(pRK404-27B); H, purified pilin from *P. aeruginosa* PAK (arrow). Molecular mass standards are shown at the left side, in kilodaltons.

indicates that the regulatory sequences of the cloning vector influence the expression of the pilin gene within the 1.1-kbp *Hin*dIII fragment. Preliminary nucleotide sequence information of the insert DNA in pMS27A indicates that the amino terminus of the structural gene lies between the two *Pst*I sites and that the carboxy terminus lies between the *Cla*I and *Eco*RV sites (data not shown). The direction of transcription proceeded from left to right in the orientation shown in Fig. 1, which was the same orientation as the direction of transcription directed by the promoter of the *lacZ* gene in pUC18.

Clones carrying pMS28A and pMS28B (the 0.6-kbp *Pst*I fragment insertion into pUC18) did not express antigen that reacted to pilin antisera on Western blots (data not shown). It therefore appears that some of the information necessary for expression of the pilin gene must lie outside the 0.6 kbp *Pst*I fragment.

Localization of pilin produced in *E. coli* (pMS27A). Although pili are readily released from *P. aeruginosa* (25), pilin was not present in appreciable amounts in culture supernatants of the recombinant clones. To determine the location of the expressed antigen in *E. coli*, proteins from the recombinant clone JM109(pMS27A) were separated into periplasmic, membrane, and cytoplasmic fractions by the method of Ito et al. (15). The majority of the pilin was located in the membrane of *E. coli* (Fig. 3). Subsequent separation of the total membrane fraction by density in isopycnic sucrose gradients (24, 28) indicated that most of the pilin was associated with the outer membrane, and a smaller proportion banded with the less dense cytoplasmic membrane (Fig. 4).

We attempted to determine the nature of the pilin-membrane association by detergent solubilization. Membranes from *E. coli* expressing the pilin polypeptide were isolated and treated with the detergent Sarkosyl. These conditions were shown to selectively solubilize the cytoplasmic membrane protein of *E. coli* (10). Similar treatment solubilized approximately 90% of the membrane-bound pilin (Fig. 4), leaving some antigen still associated with the Sarkosyl-insoluble membrane fraction. Thus, it appears that pilin, unlike the major outer membrane proteins, was associated less tightly with the membrane of *E. coli* and was readily released by treatment with a mild detergent.

Repeated shearing of the bacterial suspension by passage through a narrow gauge needle released >90% of pilin from *P. aeruginosa*. Similar treatment of *E. coli* (pMS27A) failed

to release any detectable pilin antigen. We failed to detect pilin on the surface of intact recombinant *E. coli* by radioimmunoassays, thus raising the possibility that the pilin antigen is present on the inner surface of the outer membrane or that it is on the outer surface in an immunologically nonreactive conformation. We are currently investigating the precise arrangement of the pilin polypeptide chain by probing it with solid-state radiolabeling reagents and by assessing its protease accessibility.

Southern blot analysis of restriction endonuclease-digested whole cell DNA. Total chromosomal DNA from *P. aeruginosa* PAK digested with a variety of restriction enzymes was Southern blotted and hybridized with a radiolabeled probe derived from the 1.1-kbp *Hin*dIII fragment of pMS27A. *Bam*HI, *Bgl*II, *Sma*I, and *Hin*dIII digests of PAK DNA contain a single major band which is homologous to the probe, while the two bands in the *Pst*I digest, a doublet at 1.5 kbp and a single band at 0.6 kbp, have molecular masses consistent with those predicted by the restriction map in Fig. 1 (Fig. 5). Comparable results were obtained with a variety of single and double digests with other restriction endonucleases. Since this strain of *P. aeruginosa* does not contain detectable plasmids, we conclude that this gene is present in a single copy on the bacterial chromosome. However, the *Bgl*II digest also contains a faint 4.4-kbp band, so the existence of other pilin-related sequences cannot be ruled out at this time.

DISCUSSION

Using oligonucleotide probes corresponding to a conserved region of *P. aeruginosa* pilin, we isolated the pilin structural gene from a genomic library of *P. aeruginosa* DNA in plasmid pUC18. We identified several recombinants that contained cloned sequences homologous to the oligonucleotide probe. By mapping restriction enzyme recognition sites of three randomly selected recombinant plasmids, we have shown that they represent different permutations of the same region of the *P. aeruginosa* chromosome (Fig. 1). Furthermore, all of the probe-reactive recombinant plasmids contained the same 1.1-kbp *Hin*dIII fragment that includes the pilin structural gene and its promoter. This conclusion is based on the presence of the gene product in the original recombinant cells with and without induction of the *lacZ* promoter of the cloning vector.

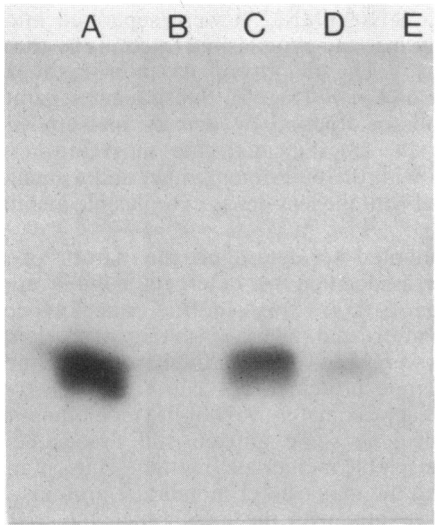

FIG. 3. Western blot analysis showing the cellular location of pilin in *E. coli* JM109(pMS27A). Lanes: A, purified pilin from *P. aeruginosa* PAK; B, cytoplasm; C, membrane; D, periplasmic space; E, supernatant.

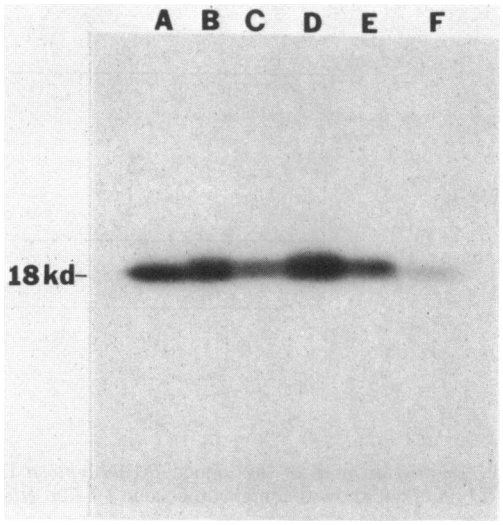

FIG. 4. Location of pilin in the membrane fraction of JM109(pMS27) and its solubility in Sarkosyl. The membrane fraction of the clone was separated into its cytoplasmic and outer membrane components by centrifugation in 0.75 to 2 M sucrose gradients at 35,000 rpm for 16 h in an SW41 rotor (26). Fractions were collected and the protein concentration was determined by reading the absorbance at 280 nm for selection of those fractions corresponding to the cytoplasmic and outer membranes. In addition, whole membrane was solubilized in 0.5% Sarkosyl (10) followed by separation into soluble and insoluble components by centrifugation through 15% (wt/vol) sucrose onto a 70% (wt/vol) sucrose shelf for 1 h at 40,000 rpm in an SW50.1 rotor. The samples were electrophoresed on a linear 10 to 17% polyacrylamide gel gradient containing 0.1% SDS and Western blotted as described in the text. Lanes: A, purified pilin from *P. aeruginosa* PAK; B, outer membrane fraction of JM109(pM27A); C, cytoplasmic membrane fraction; D, whole membrane; E, Sarkosyl-soluble fraction of whole membrane; F, Sarkosyl-insoluble fraction of whole membrane. Kd, Kilodaltons.

Substantial amounts of the pilin subunit were synthesized in *E. coli* especially in the clones that contained the plasmid with the 1.1-kbp *Hin*dIII fragment in the orientation shown in Fig. 1 (pMS27A and pRK404-27A). It is not certain whether the *lacZ* promoter of the vectors is involved in expression of the cloned gene; however, maximal synthesis of pilin appears to take place when the pilin promoter is aligned with the *lacZ* promoter.

Fractionation of membranes from pilin-expressing *E. coli* JM109(pMS27A) was used to demonstrate the location of the majority of pilin in the outer membrane fraction. Mature pilin was not found on the outside of the cells. It is noteworthy that products of several cloned *P. aeruginosa* genes that are expressed in *E. coli* are localized to different cellular compartments than in *P. aeruginosa*. The *P. aeruginosa* hemolysin and exotoxin A, which were both excreted into the growth medium by *P. aeruginosa*, are localized in *E. coli* outer membrane and cytoplasm, respectively (13, 17). Thus, the export machinery of *E. coli* fails to properly compartmentalize foreign proteins, despite the presence of the secretion signal on the primary translational product.

Since the majority of the pilin synthesized by the *E. coli* recombinants was found in both cytoplasmic and outer membrane fractions, it appears that the initial steps of export

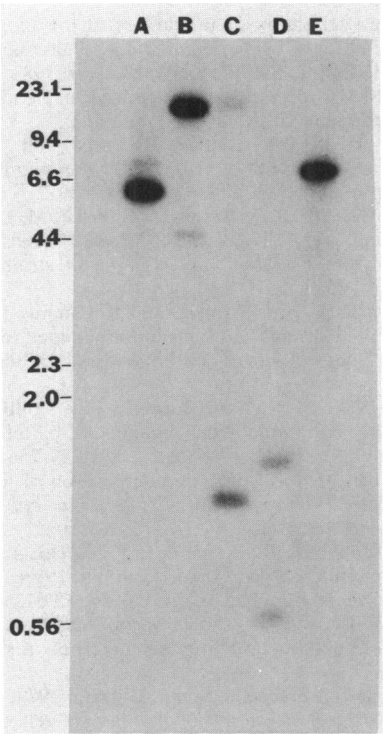

FIG. 5. Analysis of the pilin gene in chromosomal DNA digests of *P. aeruginosa* PAK. The DNA was digested with the restriction endonucleases listed below, electrophoresed on a 1% agarose gel, transferred to nitrocellulose, and probed with the radiolabeled insert from pMS27A as described in the text. Lanes: A, *Bam*HI; B, *Bgl*II; C, *Hin*dIII; D, *Pst*I; E, *Sma*I. Lambda phage DNA digested with *Hin*dIII served as size standards and are shown to the left of the gels in kilobase pairs.

of pilin took place in the cloning host, but the translocation and assembly process was not completed. Whether this block is due to differences in the apparatus of secretion between *E. coli* and *P. aeruginosa* or whether the localization differences are consequences of the different chemical composition of the cytoplasmic or outer membranes of these two gram-negative bacteria is not clear.

It is known that the process of assembly of K88 pili by *E. coli* (22) and of pili by *P. aeruginosa* (32) differs. Our results demonstrate that *P. aeruginosa* pilin is synthesized in the *E. coli* cytoplasm and is efficiently inserted into the cytoplasmic membrane, presumably utilizing the normal *E. coli* secretion apparatus. However, no organelle assembly takes place from this pool of membrane-associated subunits.

Pili on surfaces of most gram-negative bacteria are fairly uniform structures consisting of hollow tubes composed of a single subunit, pilin. It is therefore surprising that there appears to be two distinct mechanisms of membrane export and assembly. Initial stages of membrane traversal are probably identical for pili from a wide range of bacteria and may involve the general bacterial secretion apparatus, since secretion-deficient (*secA*) mutations of *E. coli* fail to process and assemble the common pili under nonpermissive conditions (9). However, complete assembly may involve additional specific membrane components, or it may be part of the structural information of pilin protein or its precursor. Signal sequences of various types of pilin from *E. coli* share structural sequences with a number of secreted proteins with respect to their size, high content of hydrophobic amino acids, and a characteristic signal-peptidase cleavage site. In contrast, the putative signal sequence of pilin from *N. gonorrhoeae* and *B. nodosus* are considerably different in that they are only seven amino acids in length and not very hydrophobic, and the processing involves cleavage between the glycine-phenylalanine residues, with subsequent modification of the phenylalanine to methylphenylalanine. It is likely that the processing of *P. aeruginosa* pilin is also similar, and the special signal sequence and possibly the conserved amino-terminal region directs the secretion and assembly of this class of pilin by mechanisms that differ from those used for the assembly of *E. coli* pili.

Recent reports by Meyer et al. (20, 21) indicate that in *N. gonorrhoeae* sequences homologous to the pilin gene are present in many copies. Chromosomal rearrangements among these copies, most of which are not involved in expression of pilin, are responsible for the antigenic heterogeneity detected among clonal isolates of a single strain. Since independent isolates of *P. aeruginosa* express antigenically distinct pili (5, 34), we searched for the presence of multiple pilin genes in the *P. aeruginosa* chromosome. However, results of Southern blot hybridizations with the pilin-specific probe were consistent with the presence of a single copy of the pilin gene in the chromosome of *P. aeruginosa* PAK. The source of the antigenic diversity of pilin in various *P. aeruginosa* strains does not appear to involve intrachromosomal rearrangement among highly homologous sequences. However, we cannot exclude the possibility that the chromosome of each strain contains silent pilin sequences which do not share substantial homology with the expressing gene; therefore, they are not detected by Southern hybridization.

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LITERATURE CITED

1. **Anderson, B. J., M. M. Bills, J. R. Egerton, and J. S. Mattick.** 1984. Cloning and expression in *Escherichia coli* of the gene encoding the structural subunit of *Bacteroides nodosus* fimbriae. J. Bacteriol. **160**:748–754.
2. **Baga, M., S. Normark, J. Hardy, P. O'Hanley, D. Lark, O. Olsson, G. Schoolnik, and S. Falkow.** 1984. Nucleotide sequence of the *papA* gene encoding the Pap pilus subunit of human uropathogenic *Escherichia coli*. J. Bacteriol. **157**:330–333.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:513–1523.
4. **Bradley, D. E.** 1974. The adsorption of *Pseudomonas aeruginosa* pilus-dependent bacteriophage of a host mutant with nonretractile pili. Virology **58**:149–163.
5. **Bradley, D. E., and T. L. Pitt.** 1975. An immunological study of the pili of *Pseudomonas aeruginosa*. J. Hyg. Camb. **74**:419–430.
6. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring vitamin B_{12}. J. Bacteriol. **60**:17–28.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Ditta, G., T. Schmidhauser, E. Yakobson, P. Lu, X. Liang, D. Finlay, D. Guiney, and D. Helinski.** 1985. Plasmids related to the broad host range vector, pRK290, useful for gene cloning and for monitoring gene expression. Plasmid **13**:149–153.
9. **Dodd, D. C., and B. I. Eisenstein.** 1984. Dependence of secretion and assembly of type 1 fimbrial subunits of *Escherichia coli* on normal protein export. J. Bacteriol. **159**:1077–1079.
10. **Filip, C., G. Fletcher, J. L. Wulff, and C. F. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli*

by the ionic detergent sodium lauryl sarcosinate. J. Bacteriol. **115**:717–722.
11. **Froholm, L. O., and K. Sletten.** 1977. Purification and N-terminal sequence of a fimbrial protein from *Moraxella nonliquefaciens*. FEBS Lett. **73**:29–32.
12. **Gaastra, W., F. R. Mooi, A. R. Stuitje, and F. K. de Graaf.** 1981. The nucleotide sequence of the gene encoding the K88ab protein subunit of porcine enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **12**:41–46.
13. **Gray, G. L., D. H. Smith, J. S. Baldridge, R. N. Harkins, M. L. Vasil, E. Y. Chen, and H. L. Heyneker.** 1984. Cloning, nucleotide sequencing, and expression in *Escherichia coli* of the exotoxin A structural gene of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **81**:2645–2649.
14. **Hermodson, M. A., K. C. S. Chen, and T. M. Buchanan.** 1978. *Neisseria* pili proteins: amino-terminal amino acid sequences and identification of an unusual amino-acid. Biochemistry **17**:442–445.
15. **Ito, K., T. Sato, and T. Yura.** 1977. Synthesis and assembly of the membrane proteins in *E. coli*. Cell **11**:551–559.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lory, S., and P. C. Tai.** 1983. Characterization of the phospholipase C gene of *Pseudomonas aeruginosa* cloning in *Escherichia coli*. Gene **22**:95–101.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **McKern, N. M., I. J. O'Donnell, A. S. Inglis, D. J. Stewart, and B. L. Clark.** 1983. Amino-acid sequence of pilin from *Bacteroides nodosus* (strain 198), the causative agent of ovine footrot. FEBS Lett. **164**:149–153.
20. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
21. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81**:6110–6114.
22. **Mooi, F. R., A. Wijfjes, and F. K. de Graaf.** 1983. Identification and characterization of precursors in the biosynthesis of the K88ab fimbria of *Escherichia coli*. J. Bacteriol. **154**:41–49.
23. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
24. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. J. Biol. Chem. **247**:3962–3972.
25. **Paranchych, W., P. A. Sastry, L. S. Frost, M. Carpenter, G. D. Armstrong, and T. H. Watts.** 1979. Biochemical studies on pili isolated from *Pseudomonas aeruginosa* strain PAO. Can. J. Microbiol. **25**:1175–1181.
26. **Portnoy, D. A., S. L. Mosley, and S. Falkow.** 1981. Characterization of plasmid and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31**: 775–782.
27. **Sastry, P. A., J. R. Pearlstorne, L. B. Smillie, and W. Paranchych.** 1983. Amino acid sequence of pilin from *Pseudomonas aeruginosa* PAK. FEBS Lett. **151**:253–256.
28. **Schnaitman, C.** 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890–901.
29. **Schoolnik, G. K., R. Fernandez, Y. T. Tai, J. Rothbard, and E. C. Gotschlich.** 1984. Gonococcal pili. Primary structure and receptor binding domain. J. Exp. Med. **159**:1351–1370.
30. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
31. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
32. **Watts, T. H., E. A. Worobec, and W. Paranchych.** 1982. Identification of pilin pools in the membranes of *Pseudomonas aeruginosa*. J. Bacteriol. **152**:687–691.
33. **Woods, D.** 1984. Oligonucleotide screening of cDNA libraries. BRL Focus **6**:1–3.
34. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146–1151.