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Production of *Neisseria gonorrhoeae* Pili (Fimbriae) in *Pseudomonas aeruginosa*

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***Pseudomonas aeruginosa* K/2PfS, when transformed with an expression plasmid harboring the pilin gene (*pilE1*) of *Neisseria gonorrhoeae* MS11, was able to express and assemble gonococcal pilin monomers into surface-associated pili, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoblotting, and immunoelectron microscopy. Concomitant with the expression of gonococcal pili in *P. aeruginosa* was the virtual loss of production of *P. aeruginosa* K/2PfS pili normally associated with the host cell.**

The *N*-methylphenylalanine (mePhe) pili produced by a number of species of gram-negative bacteria, including *Dichelobacter nodosus* (formerly *Bacteroides nodosus* [5]), *Moraxella bovis*, *Moraxella nonliquefaciens*, *Moraxella lacunata*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Pseudomonas aeruginosa*, can be assigned to a common group on the basis of shared morphological and biochemical characteristics (for reviews, see references 6 and 30). Characteristically, pili of this group have a polar location on the cell, are 6 to 7 nm in diameter and up to 2.5 μm in length, and are composed predominantly of a repeated-subunit protein, pilin, of about 150 amino acid residues in length. Mature pilin is formed from a prepilin by removal of an unusually short leader sequence of six or seven amino acids and N methylation of the new N-terminal phenylalanine. The toxin-coregulated pilus of *Vibrio cholerae* is a distant member of the group, on the basis of limited amino acid sequence commonality, but possesses 25 amino acids in its cleaved leader sequence (11, 35), in contrast to shorter leader sequences of 6 or 7 amino acids common to mePhe pilins. Additionally, the N-terminal residue of pilin from toxin-coregulated pilus is *N*-methylmethionine rather than mePhe (18).

Comparison of amino acid sequences of mePhe pilins from different bacterial species reveals highly conserved N-terminal regions, C-terminal regions possessing lower but detectable sequence conservation, and central regions with little or no sequence commonality (6). Within a species, mePhe pili can display extensive interstrain variability, but a single strain of *P. aeruginosa* or *D. nodosus* is capable of producing only one type of mePhe pilus (30) whereas *M. bovis* can produce a maximum of two (8, 13). *N. gonorrhoeae* pili differ from mePhe pili of other bacterial species; the former are subject to a remarkable intrastrain variability through genomic rearrangement processes (for a review, see reference 25). While the amino terminus of gonococcal pilin is highly conserved for variant pilins, the variations are virtually all focused on six distinct segments (minicassettes) located in the central and carboxy-terminal domains. A second characteristic of gonococcal pilin is that the prepilin can be processed at two different sites, located in front of or behind a hydrophobic region at the amino terminus, to yield full-length pilin able to polymerize or truncated, soluble pilin (S pilin), respectively (14).

In addition to amino acid sequence conservation between mePhe pilins, the close relationship of the biosynthetic apparatus for pilus production is indicated by the ability of one bacterial species, *P. aeruginosa*, to express pili of *D. nodosus* (9, 23) or *M. bovis* (3, 8) when transformed with an expression plasmid carrying the appropriate foreign mePhe pilin gene. This expression of foreign mePhe pili in *P. aeruginosa* contrasts with the results obtained with *Escherichia coli* as host for the expression of *D. nodosus* (1, 7) and *M. bovis* pilins (8, 22). In *E. coli*, although *D. nodosus* and *M. bovis* prepilins are expressed, no pili are produced. In order to ascertain whether compatibility with the pilus assembly system of *P. aeruginosa* extends to other mePhe pilins, the present study examines the expression of *N. gonorrhoeae* pilin in *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains and media. *P. aeruginosa* K/2PfS (ATCC 53308) (PAK/2PfS), a hyperpiliate mutant produced by Bradley (4) from *P. aeruginosa* K, was used in all transformations.

Medium and culture conditions. PAK/2PfS was routinely grown at 37°C in yeast-tryptone (YT) broth (0.8% Bacto Tryptone, 0.5% yeast extract, and 0.5% sodium chloride) or on YT agar (1.6%). Strains harboring plasmids pME290 (15) or derivatives were grown on the same media supplemented with carbenicillin (500 μg/ml).

Plasmid constructions. The *Pseudomonas* plasmid pME290 and derivatives used in this study carry the Tn*801* *bla* gene specifying carbenicillin resistance, which was utilized as a selective marker. Plasmid pME290 was digested with *Bam*HI, dephosphorylated, and ligated to a 1.2-kbp *Bam*HI fragment containing the p_L promoter and *N* gene of phage lambda (Pharmacia P_L Promoter GenBlock [Pharmacia, Uppsala, Sweden]). The ligation mixture was used to transform PAK/2PfS made competent with $MgCl_2$ (9). Following isolation of a number of carbenicillin-resistant transformants, plasmid DNA was prepared by a small-scale alkali lysis method (34). A plasmid pPAH121, possessing a single GenBlock cartridge (Fig. 1), was selected on the basis of restriction digests of plasmid DNA. The plasmid pPAH121

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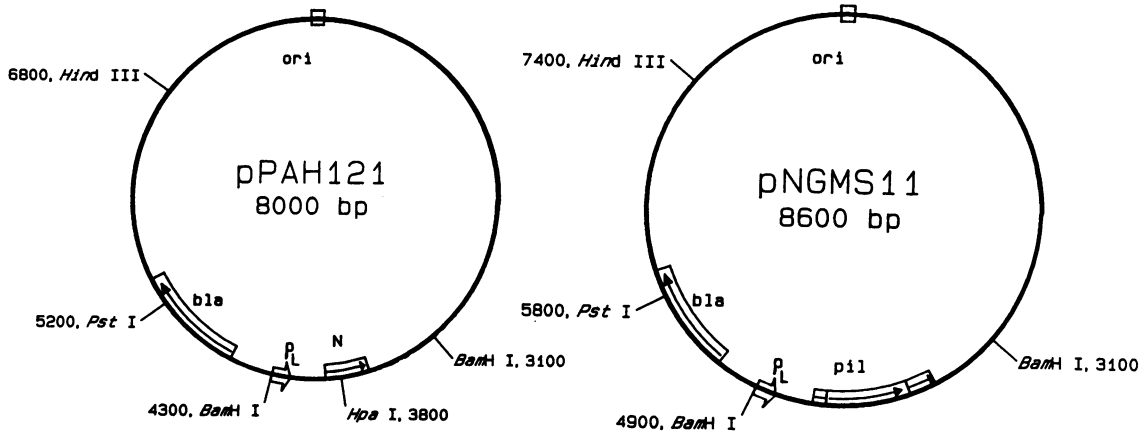

FIG. 1. *Pseudomonas* expression plasmid pPAH121 and the derived plasmid pNGMS11 holding a copy of the pilin gene of *N. gonorrhoeae* MS11. The 1.2-kbp *Bam*HI fragment of pPAH121 was derived from bacteriophage lambda and contains the p_L promoter (large arrow) and the *N* gene of phage lambda (N). Relevant restriction sites are indicated. Single-line arrows indicate directions of gene transcription. bla, β-lactamase resistance gene; ori, origin of replication; pil, pilin gene of *N. gonorrhoeae* MS11.

contained a unique *Hpa*I restriction site flanked upstream by the left promoter (p_L) of phage lambda and downstream by the rho-dependent t_L transcription terminator. A 668-bp *Bsu*36I-*Cla*I DNA fragment from plasmid pNG1320, a derivative of pBR322, was used as a source for the cloned pilin structural gene (*pilE1*) from *N. gonorrhoeae* MS11 (24) and incorporated the ribosome binding site and translation initiation and termination signals of *pilE1*. The 668-bp DNA fragment was end filled and ligated with *Hpa*I-cut and dephosphorylated pPAH121. Competent PAK/2PfS cells were transformed with the ligation mixture and plated on YT agar supplemented with carbenicillin.

Screening *P. aeruginosa* transformants for expression of *N. gonorrhoeae* MS11 pili. Colonies of *P. aeruginosa* resulting from the aforementioned ligation and transformation were grown at 37°C for 20 h in YT broth supplemented with carbenicillin. Formaldehyde (to a final concentration of 0.2%) was added to the cultures, and pili were isolated from the cells by shearing, essentially by the method described below for cells grown on solid medium. Pilus preparations were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (15% gel), and immunoblots were probed with rabbit antisera against whole piliated *N. gonorrhoeae* MS11. One of the transformants (PAK/2PfS-pNGMS11) containing the plasmid pNGMS11 (Fig. 1), which by restriction endonuclease analysis was shown to contain a single *N. gonorrhoeae* MS11 pilin gene in the desired orientation, was chosen for further study.

Isolation of pili from *P. aeruginosa*. Bacteria were harvested by being scraped from YT agar plates incubated for 20 h at 37°C. The bacterial cells were suspended in normal saline containing 0.2% formaldehyde, and pili were sheared from cells by passing (10 times) the suspension through a 25-gauge hypodermic needle. The cells were removed by centrifugation (12,000 × *g*, 4 min, 4°C). The supernatant was adjusted to 0.1 M $MgCl_2$, and the solution was held at 4°C for 3 h. Following centrifugation (12,000 × *g*, 15 min, 4°C), the pellet containing pili was suspended in TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and stored at −20°C.

Isolation and purification of pili from *N. gonorrhoeae*. Gonococci grown for 18 h at 37°C on GC medium base agar (Difco Laboratories, Detroit, Mich.) plates were suspended in ethanolamine buffer (0.15 M ethanolamine-HCl [pH 10.2]). Pili were sheared from cells in a Sorvall omnimixer (5,000 rpm, 2 min), and bacteria were removed by centrifugation (25,000 × *g*, 30 min, 4°C). The supernatant was adjusted to 10% $(NH_4)_2SO_4$ and held for 1 h at 4°C, and pili were collected by centrifugation (12,000 × *g*, 30 min). The pilus-containing pellet was suspended in ethanolamine buffer and centrifuged (25,000 × *g*, 30 min, 4°C) to remove contaminating membrane fragments. Finally, the pili were precipitated again with 10% $(NH_4)_2SO_4$ as described above, and the pellet was suspended in TE and stored at −20°C.

SDS-PAGE and immunoblotting. Pilus samples were denatured by being boiled for 3 min in 20 mM Tris-HCl (pH 6.8) containing 0.7% SDS and 1.8% (wt/vol) 2-mercaptoethanol. Samples were electrophoresed in 15% polyacrylamide gels (20) in a Bio-Rad Mini-Protean II gel apparatus, and proteins were stained with Coomassie brilliant blue R250 or were electrophoretically transferred to nitrocellulose membranes (37). Membranes were treated with 50 mM Tris-HCl-buffered saline (pH 8.0) containing 0.5% skim milk powder and incubated with either rabbit anti-PAK/2PfS pilus serum or rabbit anti-*N. gonorrhoeae* MS11 serum. After the membrane was washed with Tris-HCl-buffered saline, bound antibodies were detected with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Bio-Rad, Richmond, Calif.).

Immunogold labeling and electron microscopy. *P. aeruginosa* colonies grown for 24 h at 37°C on YT agar of *N. gonorrhoeae* colonies grown for 24 h at 37°C in 5% CO_2 on supplemented GC medium base agar (Difco Laboratories) were gently suspended in phosphate-buffered saline (PBS; pH 7.0) containing 1% bovine serum albumin (BSA). The bacterial suspension was applied to carbon-coated Formvar grids. After being rinsed by immersion in PBS containing 1% BSA, the grids were immersed in anti-*N. gonorrhoeae* MS11 pilus serum which had been raised in rabbits against authentic *N. gonorrhoeae* MS11 pili. The grids were rinsed as described before and immersed in protein A-gold (5-nm spheres; Jenssen Pharmaceutica, Beerse, Belgium). After a final rinsing in PBS containing 1% BSA, the grids were briefly treated with 1% ammonium molybdate, dried, and examined by transmission electron microscopy with a JEOL JEM 100S.

Protein analysis of pili from PAK/2PfS-pNGMS11. To

obtain N-terminal amino acid sequences of pili isolated from PAK/2PfS-pNGMS11, a sample of pili was subjected to 19 cycles of amino acid sequencing, with a model 470A protein sequencer from Applied Biosystems (Foster City, Calif.).

In order to show that pilin from PAK/2PfS-pNGMS11 did not terminate earlier than the C-terminal lysine residue predicted by the MS11 pilin gene sequence from pNG1100, pili isolated from PAK/2PfS-pNGMS11 were denatured and S carboxymethylated in 8 M urea. S-carboxymethylated protein was recovered by precipitation with ice-cold methanol. The resultant pellet was dissolved in 0.05 M NH_4HCO_3 and digested with trypsin for 16 h at 37°C at a 1:50 enzyme-to-protein ratio, and a search for the expected C-terminal tryptic peptide Ala-Ser-Asp-Ala-Lys-OH was made. Tryptic peptide fragments were loaded onto a Vydac C_{18} 5-μm reversed-phase column (The Separations Group, Hesperia, Calif.) connected to a Perkin-Elmer Series 4 liquid chromatography system (Perkin-Elmer Corporation, Norwalk, Conn.). The hydrophilic peptides not bound to the column were reacted with phenylisothiocyanate to render them hydrophobic, and then these peptides were rechromatographed over the Vydac column. Peptides eluted from the column were characterized by amino acid analysis and amino acid sequence determination.

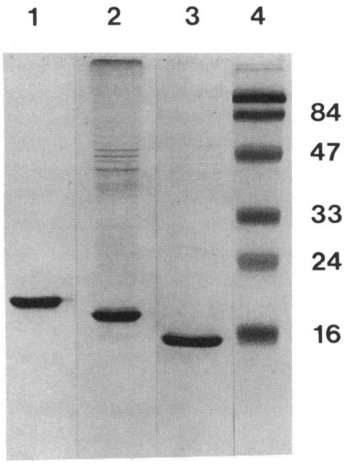

FIG. 2. Electrophoretic separation of pilus preparations in SDS-polyacrylamide gel (15%), stained with Coomassie brilliant blue R250. Lanes: 1, 2, and 3, pili isolated from *N. gonorrhoeae* MS11, PAK/2PfS-pNGMS11, and PAK/2PfS-pPAH121, respectively; 4, molecular mass standards. Molecular masses (in kilodaltons) are shown to the right of the figure.

RESULTS

Screening *Pseudomonas* transformants for production of *N. gonorrhoeae* MS11 pili. Pilus preparations from broth cultures of PAK/2PfS transformed to carbenicillin resistance were analyzed by SDS-PAGE and immunoblotting. These analyses indicated that most cultures were producing a protein, recognized by anti-*N. gonorrhoeae* MS11 serum, that had a substantially higher apparent molecular weight than PAK/2PfS pilin (data not shown). However, SDS-PAGE of pilus preparations from several clones exhibited a second band which had a mobility identical to that of PAK/2PfS pilin. The relative intensities of the two bands varied between individual clones. Inocula from broth cultures of clones showing two pilin bands were plated to solid medium containing carbenicillin. After 24 h of growth at 37°C, all culture plates displayed colonies with two distinct colony morphologies: a petite, high-domed type and a larger, spreading type. Single colonies representative of each morphological type were streaked on YT agar plates containing 500 μg of carbenicillin per ml, and after 20 h of growth at 37°C, bacteria were harvested and pili were isolated. In all cases, SDS-PAGE of pilus preparations revealed a single pilin band. The petite-colony type exhibited a pilin band with an apparent molecular weight higher than that of PAK/2PfS pilin, and the larger colony type gave a pilin band with a mobility identical to that of PAK/2PfS pilin. The large-colony phenotype, irrespective of whether grown on solid or liquid medium, remained true to original colony morphology and produced only PAK/2PfS pilin, whereas the petite-colony type, when grown in liquid medium, gave rise to mixed populations of large- and petite-colony types and produced the two types of pilin (data not shown). Streaking and growth of the petite-colony type on solid medium almost exclusively produced petite colonies expressing a single pilin with an apparent molecular weight higher than that of PAK/2PfS pilin. All subsequent studies utilized solid medium and clone PAK/2PfS-pNGMS11, which grew as the petite-colony type on solid medium. Restriction digests of plasmid DNA from this clone indicated that it contained a plasmid with a single MS11 pilin gene fragment oriented as shown in Fig. 1.

Analysis of pili. Pili isolated from PAK/2PfS-pNGMS11 grown on solid medium produced a major band on SDS-PAGE (Fig. 2) with an apparent molecular weight (apparent M_r, 17,500) higher than that of PAK/2PfS pilin (apparent M_r, 15,000) but with an apparent molecular weight lower than that of authentic pilin of *N. gonorrhoeae* MS11 (apparent M_r, 18,500). Immunoblot analysis of pili from PAK/2PfS-pPAH121, PAK/2PfS-pNGMS11, and the corresponding *N. gonorrhoeae* MS11 strain revealed that pilin from PAK/2PfS-pNGMS11 was recognized by antisera raised against *N. gonorrhoeae* MS11 (Fig. 3A) but not by antisera raised against PAK/2PfS pili (Fig. 3B). The yield of gonococcal

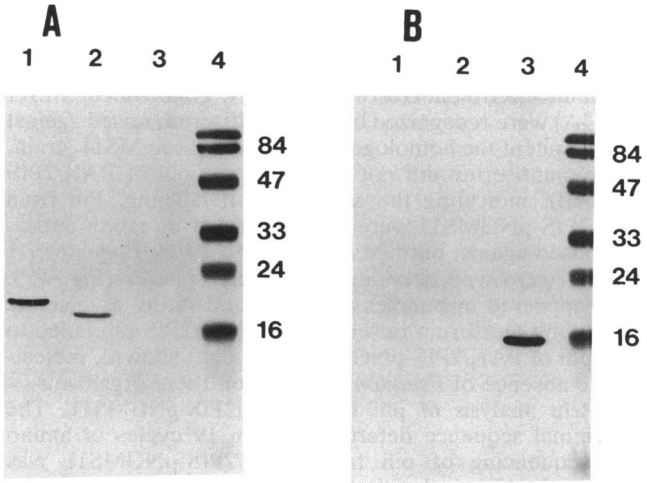

FIG. 3. Immunoblot analyses of pilus preparations with anti-*N. gonorrhoeae* MS11 serum (A) and anti-PAK/2PfS pilus serum (B). Lanes: 1, 2, and 3, pili isolated from *N. gonorrhoeae* MS11, PAK/2PfS-pNGMS11, and PAK/2PfS-pPAH121, respectively; 4, molecular mass standards. Molecular masses (in kilodaltons) are shown to the right of each panel.

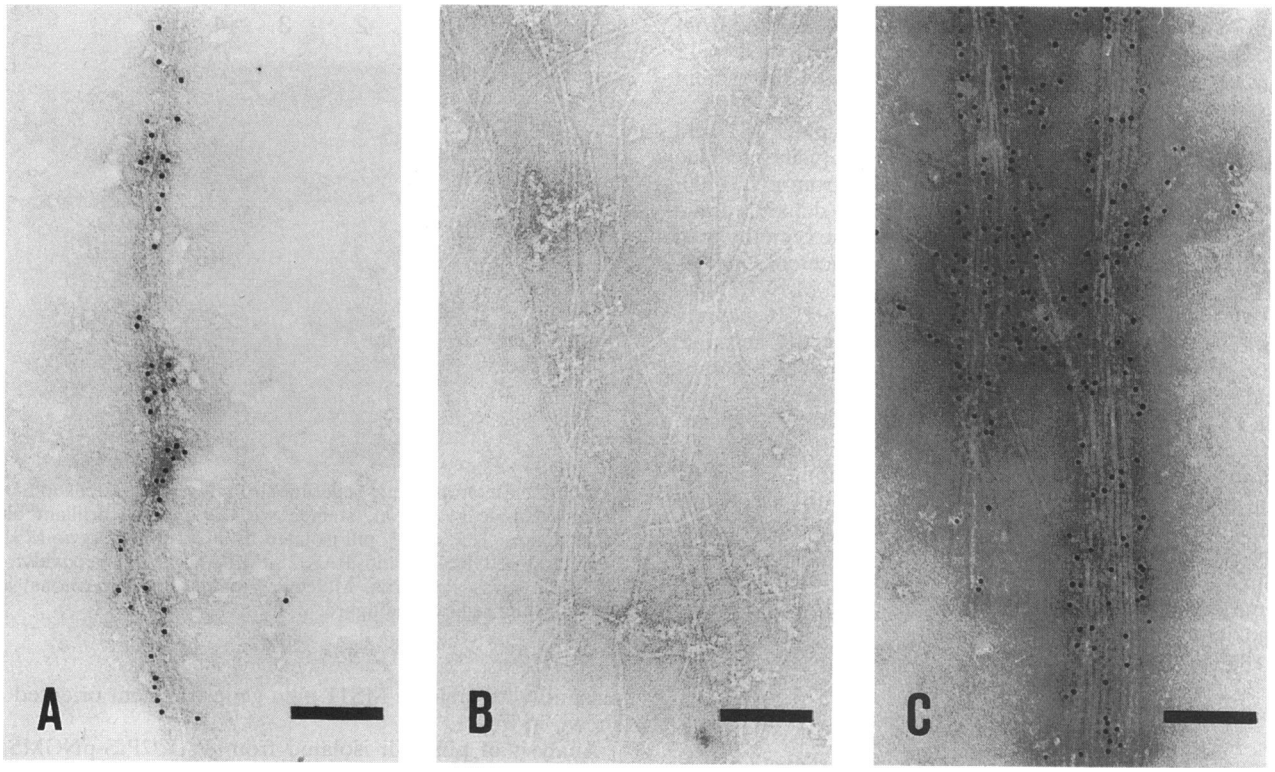

FIG. 4. Electron micrographs of pili from whole cells treated with anti-*N. gonorrhoeae* MS11 pilus serum followed by protein A conjugated to 5-nm gold particles. (A) *N. gonorrhoeae* MS11; (B) PAK/2PfS-pPAH121; (C) PAK/2PfS-pNGMS11. Size bars, 0.1 μm.

MS11 pili from PAK/2PfS harboring the MS11 pilin gene was approximately 1 μg/mg of wet cells, compared with PAK/2PfS, which produces approximately 2 μg of PAK pili per mg of wet cells. Following removal of pili by shearing, whole cells of PAK/2PfS-pNGMS11 were examined for residual pilin content. High levels of gonococcal pilin were detected (approximately 20% of total cellular protein), as determined by dye binding in SDS-PAGE. Immunoblotting with rabbit anti-*N. gonorrhoeae* MS11 serum and rabbit anti-PAK/2PfS pilus serum demonstrated the presence of high levels of MS11 pilin and the absence of PAK pilin (data not shown).

Immunoelectron microscopy. Pili of *N. gonorrhoeae* MS11 (Fig. 4A) were recognized by rabbit antiserum raised against purified pili of the homologous *N. gonorrhoeae* MS11 strain, but this antiserum did not recognize the pili of PAK/2PfS (Fig. 4B), indicating the specificity of labeling. Pili from PAK/2PfS-pNGMS11 were recognized by the rabbit antiserum raised against purified authentic *N. gonorrhoeae* MS11 pili and were well decorated with gold spheres (Fig. 4C). They appeared in bundles and rafts and rarely as discrete pili. Rabbit antiserum raised against PAK/2PfS pili failed to label pili of PAK/2PfS-pNGMS11 (data not shown), indicating the absence of *Pseudomonas* pili on these organisms.

Protein analysis of pili from PAK/2PfS-pNGMS11. The N-terminal sequence determined from 19 cycles of amino acid sequencing of pili from PAK/2PfS-pNGMS11 was shown to be identical with the sequence of the first 19 amino acids of mature pilin of *N. gonorrhoeae* MS11 predicted from the DNA sequence of pNG1100 (24).

A pentapeptide (Ala-Ser-Asp-Ala-Lys) was isolated from tryptic digestion of pili from PAK/2PfS-pNGMS11 and corresponded to the C-terminal pentapeptide sequence of MS11 pilin predicted from the pilin gene sequence in pNG1100 (24).

These combined results suggest that the pilin sequence predicted from the DNA sequence of pNG1100 is produced by PAK/2PfS-pNGMS11 and that the mature gonococcal pilin of PAK/2PfS-pNGMS11 is not proteolytically shortened compared with authentic, mature MS11 pilin.

DISCUSSION

The plasmid vector pPAH121 provides a means for rapid incorporation of a blunt-end DNA fragment holding a mePhe pilin gene into hyperpiliated PAK/2PfS for subsequent expression of foreign pili. In particular, this study demonstrates the capacity of *P. aeruginosa* to produce, at high levels, pili of *N. gonorrhoeae* MS11. This finding extends the documented ability of *P. aeruginosa* to synthesize and assemble mePhe pili of two other bacterial species, viz., *D. nodosus* (9, 23) and *M. bovis* (3, 8). In contrast, although *E. coli* harboring mePhe pilin genes of *D. nodosus* (1, 7), *M. bovis* (8, 22), *P. aeruginosa* (12), or *N. gonorrhoeae* (26) is able to synthesize mePhe prepilins at moderately high levels, it is unable to correctly process these to mature pilins and is totally unable to fabricate mePhe pili. These observed differences in expression of mePhe pilins in *P. aeruginosa* and *E. coli* may be explained by the presence of a set of accessory proteins required for mePhe pilus biogenesis which is present in *P. aeruginosa* but not in *E. coli*. Nunn et al. (29) have identified three genes (*pilB*, *pilC*, and *pilD*) in *P. aeruginosa* which are adjacent to *pilA* (the pilin subunit gene) and which encode accessory proteins required for pilus biogenesis. PilD of *P. aeruginosa* functions as an

endopeptidase for removal of the short (6-amino-acid) leader sequence from PAK prepilin and shows sequence similarity to PulO of *Klebsiella pneumoniae* (2) and to ComC of *Bacillus subtilis* (27), which are associated with secretion systems involving proteins showing homology to the conserved N terminus of prepilin. PilD, recently identified as XcpA of *P. aeruginosa* PAO (2), in addition to its leader peptidase activity on prepilin, is associated with the export of a number of other secreted proteins, including exotoxin A, elastase, alkaline phosphatase, and phospholipase C; however, its action in the secretion of these other exported proteins may be indirect, since these proteins lack prepilin-like leader sequences (36). The *V. cholerae tcp* gene cluster contains the gene *tcpJ* (18), whose open reading frame encodes a protein displaying sequence similarity (30%) to PilD and which also functions as an endopeptidase for processing prepilin. It is likely that bacterial species displaying mePhe pili possess a homologous set of assembly proteins for pilus biogenesis which, in *P. aeruginosa*, have been shown to be sufficiently compatible with a number of mePhe pilins from other species to enable the fabrication of foreign mePhe pili. Ultimately, the compatibility of foreign mePhe pilus production in host strains will depend on the extent of divergence of host and donor pilus assembly systems. The observed instability of PAK/2PfS expressing gonococcal pilin in liquid medium and the reduced growth rate on solid medium contrast with the stability of PAK/2PfS expressing *D. nodosus* pili and may indicate that the limits of interspecies expression of mePhe pili are being approached in this instance.

We have previously mistakenly reported (6a) the inability of *P. aeruginosa* to express *N. gonorrhoeae* pili. Beard et al. (3) reported similar results with an identical gonococcal pilin gene fragment and lambda p_L promoter in a different expression vector (pKT240). Our original report was based on the absence of observable gonococcal pili by SDS-PAGE analysis during primary screening of PAK/2PfS transformants harboring an *N. gonorrhoeae* MS11 pilin gene. Our subsequent studies revealed that gonococcal pili were produced by *P. aeruginosa* but were completely pelleted under centrifugation conditions (12,000 × *g*, 15 min) routinely employed for the removal of bacterial cells after pili were sheared from *P. aeruginosa*. Reduction in centrifugation time from 15 to 4 min resulted in gonococcal pili remaining in solution rather than being pelleted. These observations resulted in a revised centrifugation protocol (12,000 × *g*, 4 min) being adopted for separation of *P. aeruginosa* cells from the crude pilus-containing supernatant. Therefore, in hindsight, the centrifugation conditions (25,000 × *g*, 40 min) employed by Beard et al. (3) to remove cellular debris from pili in suspension would have also been sufficient to pellet MS11 pili obtained from *P. aeruginosa* and may account for their apparent failure to express pili of *N. gonorrhoeae* MS11 in *P. aeruginosa*.

Although pilin and pili expressed by PAK/2PfS-pNGMS11 are recognized by antibodies raised against *N. gonorrhoeae* MS11, the pilin produced has an apparent molecular weight lower than that of pilin obtained from *N. gonorrhoeae* MS11 possessing an identical pilin gene. This molecular weight discrepancy might have been a result of proteolytic shortening of MS11 pilin in *P. aeruginosa*, which has not been reported for other mePhe pilins expressed in *P. aeruginosa*, or it could have been due to differences in posttranslational processing of MS11 pilin in *N. gonorrhoeae* compared with in *P. aeruginosa*. The former possibility is eliminated by the identification of sequences in MS11 pilin isolated from *P. aeruginosa* which correspond to N and C termini of authentic MS11 pilin, while the later explanation is supported by results obtained from analysis of pili from *N. gonorrhoeae* P9 (33) indicating the presence of one to two phosphate groups and one to two hexose groups per pilin subunit. Anomalous behavior of some mePhe pili on SDS-PAGE and differences in apparent molecular weight between native and recombinant DNA-derived mePhe pilins have been noted in several studies. Fulks et al. (13) have suggested that post-translational modifications may account for the higher-than-expected apparent molecular weight of *M. bovis* EPP63 I pilin in SDS-PAGE. Koomey et al. (19) have shown by SDS-PAGE that pilin from *N. gonorrhoeae* VD302 P^{++} has a higher apparent molecular weight than pilin expressed in *E. coli* harboring the cloned pilin gene of VD302 P^{++}. Pilins from some strains of *M. bovis* expressed in *P. aeruginosa* exhibit differences in mobility compared with that of the native product, while others show identical mobility on SDS-PAGE. *M. bovis* Dalton 2d pilin shows a mobility identical to that of the equivalent *Pseudomonas*-derived product (8). On the other hand, an unexplained difference in mobility on SDS-PAGE between authentic *M. bovis* EPP63 Q pili and EPP63 Q pili expressed in *P. aeruginosa* has been reported elsewhere (3), the native pilin again having an apparent molecular weight higher than that of pilin derived from *P. aeruginosa*. In contrast to these observations for *M. bovis* pilins, no anomalies have been noted by SDS-PAGE between the mobilities of pilins of a number of *D. nodosus* strains and the equivalent pilins expressed in *P. aeruginosa* (9 and unpublished data). These combined results suggest variation in posttranslational processing of mePhe pilins between different bacterial genera; e.g., the genera *Neisseria* and *Moraxella* may possess sequence-dependent modification systems for mePhe pilins which are not present in the genera *Dichelobacter* or *Pseudomonas*.

PAK/2PfS-pNGMS11 grows on solid medium as petite colonies which produce only gonococcal pili, with no indication of the production of *P. aeruginosa* pili. However, in liquid culture and despite antibiotic selection, these organisms are moderately unstable, giving rise to faster-growing mutants which express *P. aeruginosa* pili rather than gonococcal pili. Therefore, from liquid culture a mixture of PAK and MS11 pilin may be isolated, which reflects the products of two distinct cell populations rather than the products of a single cell type producing a mixed population of PAK and MS11 pili or producing mosaic pili composed of two types of pilin subunit within each pilus. Without a fortuitous difference in colony morphology, strain instability resulting in reversion to *P. aeruginosa* pilus production by some organisms may have gone unrecognized. Superficially similar observations for *P. aeruginosa* cells which produce a mixture of two types of pilin have been reported elsewhere (3). The study just mentioned reports no observed differences in colony morphology and relies on immunoelectron microscopy with double labeling to draw a conclusion that there is expression of mosaic pili composed of *P. aeruginosa* and *M. bovis* pilin subunits in each pilus. In our opinion, however, interpretation of the published electron micrographs from that study is complicated by possible pilus bundle formation, making identification of a single pilus and therefore of pilus components difficult. The production of mosaic pili is also at variance with observations of the expression of pili from two strains of *D. nodosus* on the same *P. aeruginosa* cell (10) and the dual production of PAO and PAK pili on *P. aeruginosa* (32). It would appear from these two published studies that although mosaic pili can be produced under exceptional

circumstances, their formation is generally prevented. Johnson and Lory (16) have suggested that in *P. aeruginosa*, feedback regulation of *pilA* transcription occurs, probably through a positive activator linked to a pathway capable of sensing a demand for *pilA* transcription via the concentration of pilin or pilus assembly protein. The natural corollary of this proposed feedback regulation seems to be demonstrated by the present and previous observations (8, 9) that high levels of foreign mePhe pilin, generated from plasmid-borne pilin genes controlled by strong promoters, result in virtual loss of pili normally produced by *P. aeruginosa*.

The only detectable subunit of *P. aeruginosa* pili is pilin (32), unlike the Pap pili of uropathogenic *E. coli*, which in addition to the major pilin contains several minor pilins, a terminator protein, and an adhesin (PapG) (21). Within the Pap pilus, the tip-located adhesin (PapG) has been implicated in epithelial cell attachment. The pili of *N. gonorrhoeae*, like the Pap pili of *E. coli*, are associated with bacterial binding to mucosal epithelial cells and with hemagglutination of erythrocytes. Recent studies indicate that in addition to the major pilin subunit, gonococcal pili may possess small quantities of other pilus-associated proteins (28, 31); more recently, an *N. gonorrhoeae* outer membrane protein (PilC) which copurifies with pili has been identified (17) as essential for the biogenesis of gonococcal pili. Gonococcal pili produced in *P. aeruginosa* could assist in further studies to determine the functional role of minor proteins associated with the gonococcal pilus and to ascertain which pilus-associated components are responsible for adhesion to mucosal epithelial cells.

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