JOURNAL OF BACTERIOLOGY, May 1990, p. 2601–2607
0021-9193/90/052601-07$02.00/0

Vol. 172, No. 5

Morphogenetic Expression of *Moraxella bovis* Fimbriae (Pili) in *Pseudomonas aeruginosa*

M. K. M. BEARD,[1]* J. S. MATTICK,[2] L. J. MOORE,[1] M. R. MOTT,[3] C. F. MARRS,[4] AND J. R. EGERTON[1]

Department of Animal Health, The University of Sydney, Camden, New South Wales, 2570, Australia[1]; Centre for Molecular Biology and Biotechnology, The University of Queensland, St. Lucia, Queensland, 4067, Australia[2]; Division of Molecular Biology, Commonwealth Scientific and Industrial Research Organisation, North Ryde, New South Wales, 2113, Australia[3]; and School of Public Health, Department of Epidemiology, The University of Michigan, Ann Arbor, Michigan 48109[4]

Received 20 September 1989/Accepted 7 February 1990

Type 4 fimbriae (pili) are found in a wide variety of gram-negative bacteria and are composed of small structural subunits which share significant sequence homology among different species, especially at their amino-terminal ends. Previous studies demonstrating morphogenetic expression of *Bacteroides nodosus* fimbriae from cloned subunit genes in *Pseudomonas aeruginosa* suggested that there is a common mechanism for type 4 fimbriae assembly and that the structural subunits are interchangeable (J. S. Mattick et al., J. Bacteriol. 169:33–41, 1987). Here we have examined the expression of *Moraxella bovis* fimbrial subunits in *P. aeruginosa*. *M. bovis* subunits were assembled into extracellular fimbriae in this host, in some cases as a homopolymer but in others as a mosaic with the indigenous subunit, indicating structural equivalence. This result contrasts with other studies in which recombinant *P. aeruginosa* expressing different subunits produced fimbriae composed almost exclusively of one subunit or the other (T. C. Elleman and J. E. Peterson, Mol. Microbiol. 1:377–380, 1987). Both observations can be explained by reversibility of subunit-subunit interactions at the site of assembly, with the forward equilibrium favoring chain extension between compatible subunits.

Fimbriae, classified as type 4 by Ottow (20), are found in a wide range of bacterial pathogens, including *Moraxella bovis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Bacteroides nodosus*, and *Pseudomonas aeruginosa* (7, 14, 18, 19, 22). The characteristics of type 4 fimbriae include a predominantly polar location on the cell, association with a phenomenon known as "twitching motility," and certain conserved features of the structural subunit which composes the fimbrial strand (7, 18). The structural subunits vary from about 145 to 160 amino acids long (approximately 16,000 molecular weight) between different genera, species, and serotypes, but all contain a highly homologous and hydrophobic amino-terminal region. In addition, most contain an unusual modified amino acid, *N*-methylphenylalanine, as the first residue in the mature protein, which is produced from a preprotein by cleavage of a short (six- to seven-amino-acid) positively charged leader sequence. Intergenera, interspecies, and interserotype variations occur primarily in the carboxy-terminal two-thirds of the protein (7, 18).

The conserved amino-terminal region of the subunit is thought to contain important signals for the structure and assembly of the fimbrial strand. The high degree of sequence conservation would also suggest that these various type 4 fimbriate organisms use a common mechanism for fimbrial biosynthesis (18). This was supported by the demonstration of high-level morphogenetic expression of *B. nodosus* fimbriae from a cloned subunit gene (under appropriate promoter control) in *P. aeruginosa* (18) and the subsequent use of this material to vaccinate sheep against footrot (8).

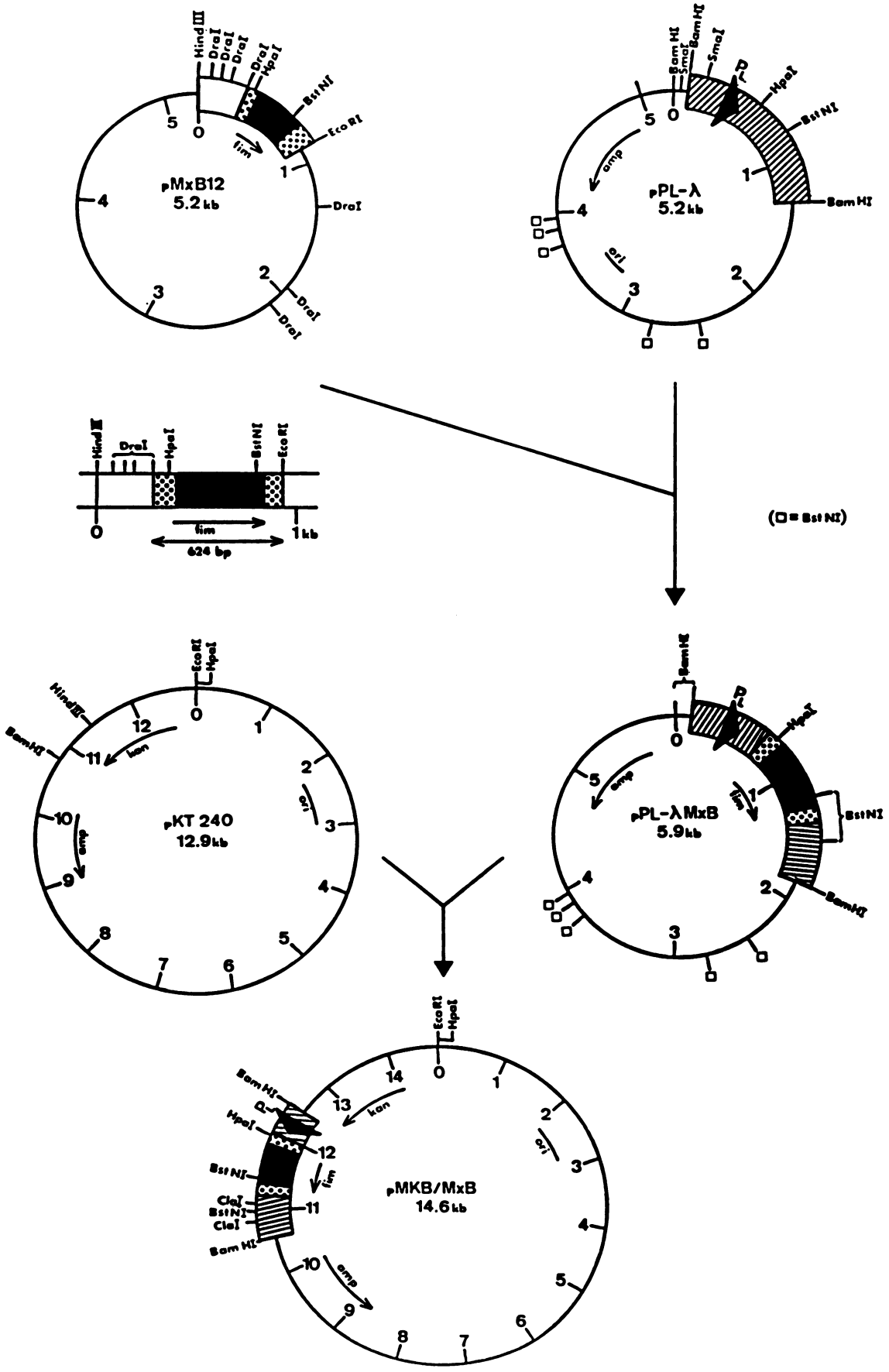
In this study we used a similar approach to demonstrate morphogenetic expression of *M. bovis* fimbriae from a cloned subunit gene in *P. aeruginosa*, although analogous experiments with an *N. gonorrhoeae* subunit gene were not successful. These results indicate that there is extended but perhaps not absolute interchangeability of type 4 fimbrial subunits among this group.

* Corresponding author.

MATERIALS AND METHODS

Plasmids and bacterial strains. Plasmids used in the recombinant DNA constructions (Fig. 1) were pMxB12, containing the *M. bovis* EPP63 Q-fimbrial subunit gene (previously termed β) (14), pPL-λ (P-L Biochemicals), which contains the bacteriophage λ promoter p_L, and pKT240 (3), a broad-host-range vector capable of being maintained in both *Escherichia coli* and *P. aeruginosa*. All constructions involving the p_L promoter were carried out in *E. coli* POP2136 cells (kindly provided by P. Lehrbach, Arthur Webster Pty. Ltd., Sydney) containing the temperature-sensitive *c*I857 repressor gene, grown at the permissive temperature of 30°C. The p_L promoter was derepressed, when required, by increasing the temperature at which the culture was grown to 42°C. *P. aeruginosa* PAK/2Pfs (ATCC 53308), a nonretractile (nontwitching) multifimbriate and phage-resistant mutant of *P. aeruginosa* PAK (5), was used as the host for morphogenetic expression.

Recombinant DNA constructions. Plasmid DNA was prepared by either the small-scale alkali-sodium dodecyl sulfate (SDS) procedure (13) or a direct scale-up of this method with 200 ml of overnight cell culture, in which case the DNA was further purified by cesium chloride-ethidium bromide isopycnic ultracentrifugation. Restriction endonuclease digestions and other manipulations such as phosphatase treatment of the vector (with calf intestinal alkaline phosphatase), end-filling (Klenow fragment of DNA polymerase I), nick translation reactions, and ligations were carried out under standard conditions (13). Restriction digests were analyzed on 0.6, 0.8, or 1.5% agarose gels in a Tris-borate-EDTA buffer system, containing 0.5 μg of ethidium bromide per ml (1, 13).


pMxB12
5.2 kb
pPL-λ
5.2 kb
(□ = BstNI)
624 bp
pKT 240
12.9 kb
pPL-λMxB
5.9 kb
pMKB/MxB
14.6 kb

The 624-base-pair (bp) *Dra*I-*Eco*RI fragment of plasmid pMxB12 containing the *M. bovis* EPP63 fimbrial subunit gene (14) was end-filled to generate blunt ends and agarose gel-purified by electroelution, followed by phenol extraction. These fragments were then ligated into the *Hpa*I site of pPL-λ and transformed into *E. coli* POP2136 cells (pPL-λMxB) as described below. The orientation of the gene with respect to the p_L promoter in the *Bam*HI segment was checked by restriction endonuclease mapping with reference to the internal *Hpa*I and *Bst*NI sites and confirmed by induction at 42°C (see below). The resultant *Bam*HI expression cartridge was purified as described above, ligated into pKT240, and retransformed into *E. coli* POP2136 cells (pKT240-MxB). Recombinant colonies were screened with the appropriate nick-translated DNA fragment at each step, with subsequent transformation of the entire gene-promoter *Bam*HI construction into *P. aeruginosa*(pMKB/MxB) as described below.

DNA transformation. *P. aeruginosa* cells were made competent for transformation by treatment with $MgCl_2$ following overnight growth at 43°C (11) in KNO_3 broth (2.5% nutrient broth no. 2 [Oxoid Ltd.], 0.5% yeast extract, and 0.4% KNO_3) by the method described previously (18). Approximately 500 ng of DNA was used to transform 0.2 ml of competent cells. *E. coli* cells were made competent for transformation by conventional treatment with $CaCl_2$ (13).

For both *P. aeruginosa* and *E. coli*, transformation was carried out by standard procedures (13), including the heat shock step. Transformants were selected and maintained on Luria (L) broth (13)-agar plates containing 750 μg of carbenicillin per ml (for *P. aeruginosa*) or 50 μg of ampicillin per ml (for *E. coli*).

Isolation of fimbriae. *M. bovis* EPP63 Q-fimbriae were purified by a method described previously (23) and supplied by W. Ruehl (Stanford University, Stanford, Calif.) and A. Lepper (Commonwealth Scientific and Industrial Research Organisation, Melbourne, Australia). Native fimbriae from *P. aeruginosa* were isolated from washed and blended cells and purified as described previously (17, 18). Fimbriae were isolated from recombinant *P. aeruginosa* PAK/2Pfs cells following overnight culture at 37°C on L-agar (2%) plates supplemented with carbenicillin (750 μg/ml). The cells were harvested in cold phosphate-buffered saline, and the resulting suspension was blended three times for 30 s each with a Sorvall Omnimixer. All steps were carried out at 4°C. The cells and cell debris were removed by centrifugation (25,000 × *g*, 40 min), and the fimbriae were recovered from the resultant supernatant by overnight precipitation with 0.1 M $MgCl_2$ (17) and subsequent centrifugation at 25,000 × *g* for 30 min.

Induction of fimbrial subunits under p_L promoter control in *E. coli* recombinant cells. A 1-ml amount of overnight culture of *E. coli* cells containing recombinant plasmid pPL-λMxB or pKT240-MxB with fimbrial genes under p_L promoter control was divided into two 3-ml volumes of sterile L-broth and incubated at 30°C for 30 min with vigorous agitation. One sample tube was then transferred to a waterbath at 42°C and incubated for 4 h to induce expression of the fimbrial subunit from the derepressed p_L promoter. The second sample was maintained at 30°C for 4 h. Whole-cell fractions (150 μl) were then prepared in duplicate for electrophoretic analysis, together with purified reference fimbriae, as described below.

Electrophoretic display. Samples were analyzed by electrophoresis on 8 to 15% or 13 to 15% polyacrylamide gradient–SDS–urea gels with the modified Laemmli buffer system described previously (17). Protein bands were visualized by staining with Coomassie blue R250.

Western immunoblot transfer. Unstained gel displays were transferred electrophoretically to nitrocellulose paper (25) and incubated with various antisera followed by ^{125}I-protein A (0.1 μCi/ml) as described previously (2). Antisera were used at the following dilutions: anti-*P. aeruginosa* PAK/2Pfs fimbrial antiserum, 1:5,000; anti-*M. bovis* EPP63 fimbrial antiserum, 1:20,000.

Antisera. Antiserum specific for *P. aeruginosa* PAK/2Pfs fimbriae was raised in rabbits following vaccination with purified fimbrial preparations, as described previously (17). Antiserum specific for *M. bovis* EPP63 Q-fimbriae was supplied by W. Ruehl and A. Lepper.

Immunogold labeling and electron microscopy. Double colloidal gold labeling was used to detect the expression of both host and recombinant fimbrial subunit genes. Whole cells of *P. aeruginosa* PAK/2Pfs, with or without cloned fimbrial subunit genes, and *M. bovis* EPP63R were diluted in distilled water and looped onto glow-discharged Formvar- and carbon-coated nickel grids (due to quarantine restrictions, isolate EPP63R was kindly provided by A. Lepper as a substitute for isolate EPP63 in these experiments, for use as an alternative control of the same fimbrial serotype). The grids were dried and incubated for 1 h at room temperature with anti-*P. aeruginosa* PAK/2Pfs fimbrial antiserum diluted 1:500 in TGOT buffer (0.5 M Tris buffer [pH 7.2] containing 0.1% [wt/vol] gelatin, 1% [wt/vol] ovalbumin, and 1% [wt/vol] Tween 20), a diluent known to reduce nonspecific immunolabeling (4). The grids were washed in distilled water and then incubated for 1 h at room temperature with protein A labeled with 14-nm colloidal gold particles (Probing and Structure, Australia), diluted 1:25 in TGOT buffer. The grids were then incubated for 5 min in excess unlabeled protein A in TGOT buffer to block residual binding sites, washed, and incubated for 1 h at room temperature with the anti-*M. bovis* fimbrial antiserum diluted 1:100 in TGOT buffer. The grids were washed and probed as before but with 6-nm colloidal gold particles. Finally the grids were washed, dried, negatively stained with 2% sodium phosphotungstate (pH 7.0), and examined in a Jeol 100 CX electron microscope at 60 kV.

RESULTS

The *M. bovis* EPP63 Q-fimbrial subunit gene was placed under p_L promoter control in a broad-host-range vector (Fig. 1) for expression in *P. aeruginosa*, using approaches similar to those developed in prototype studies for the *B. nodosus* fimbrial subunit (18). The gene cartridge was a 624-bp *Dra*I-*Eco*RI fragment spanning the subunit coding sequence

FIG. 1. Genealogy and construction of pMKB/MxB. The area detailed in pMxB12 indicates the fragment of genomic DNA cloned from *M. bovis* EPP63 (14) including the *Dra*I-*Eco*RI fragment (dotted area) which contains the fimbrial subunit gene (black area). The hatched sequences in pPL-λ, pPL-λMxB, and pMKB/MxB indicate the *Bam*HI segment which contains the p_L promoter and into which the *Dra*I-*Eco*RI fragment was cloned (at the *Hpa*I site). The numbers refer to the distances in kilobases (kb) around each plasmid. Arrows indicate the direction of transcription and approximate boundaries of the structural genes indicated. Relevant restriction endonuclease sites are shown. *fim*, Fimbrial subunit gene; *amp*, ampicillin (or carbenicillin) resistance; *kan*, kanamycin resistance; *ori*, origin of replication.

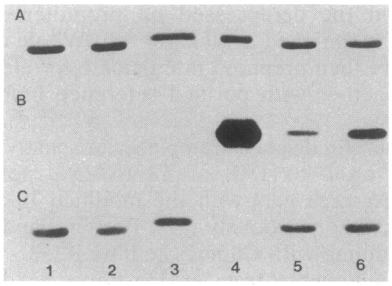


FIG. 2. Electrophoretic and immunological (B and C) analysis of fimbriae expressed by *P. aeruginosa* PAK/2Pfs containing the pMKB/MxB cartridge with *P. aeruginosa* PAK/2Pfs fimbrial antiserum (B) and *M. bovis* EPP63 fimbrial antiserum (C). Lanes 1, 2, 5, and 6 show fimbrial protein from the recombinant *P. aeruginosa* primary clones MKB/MxB6, MKB/MxB7, MKB/MxB2, and MKB/MxB4, respectively. Lanes 3 and 4 show fimbrial protein from *M. bovis* EPP63 and *P. aeruginosa* PAK/2Pfs, respectively. In both cases only the lower portion of the gel, containing the fimbrial subunit, is shown.

from 66 nucleotides upstream of the start codon to 87 nucleotides downstream of the stop codon (14). Constructions were first checked by restriction mapping and then by electrophoretic and immunological analysis of the induction of fimbrial subunit expression under p_L control in an *E. coli* host strain containing the temperature-sensitive *c*I857 repressor gene. The *M. bovis* fimbrial subunit induced well under these conditions in both pPL-λ and pKT240 (data not shown).

Introduction of pKT240 plasmids containing the *M. bovis* fimbrial subunit gene under p_L promotor control into *P. aeruginosa* produced two distinct types of tranformants. The first type was equivalent to those previously obtained with the *B. nodosus* fimbrial subunit gene in that the extracellular fimbriae of the transformants were found to be composed entirely of the heterologous *M. bovis* subunit. This was shown by reciprocal Western transfer analysis with specific anti-*P. aeruginosa* PAK/2Pfs and anti-*M. bovis* EPP63 fimbrial antisera. Data are presented for two representative clones (Fig. 2, lanes 1 and 2). *P. aeruginosa* and *M. bovis* fimbrial subunits cannot be readily distinguished by electrophoretic mobility, as both migrate in SDS-polyacrylamide gels with an apparent molecular weight of about 15,000 (Fig. 2A). The actual molecular weights of the mature subunits calculated from sequence data are 15,010 (145 amino acids [aa]) and 15,380 (151 aa) for *P. aeruginosa* and *M. bovis*, respectively. It should also be noted that in these experiments, the electrophoretic mobilities of the fimbrial subunits isolated from the transformants appeared to be marginally faster than those of the purified standards. No explanation can be provided for this at present, but the difference may simply reflect minor variations in the preparation of the samples. Nevertheless, despite the difficulty in electrophoretic discrimination between *M. bovis* and *P. aeruginosa* fimbrial subunits, it is clear from the immunological analysis that these transformants were producing fimbriae composed of the heterologous *M. bovis* subunit (Fig. 2C), with no evidence of any incorporation of the indigenous *P. aeruginosa* subunit (Fig. 2B). This was confirmed by electron micrographic analysis with immunogold double labeling, showing exclusive labeling with *M. bovis* antifimbrial antibodies (Fig. 3A); spurious labeling with *P. aeruginosa* antifimbrial antibodies was consistent with that observed upon double labeling of *M. bovis* controls (data not shown). The level of fimbrial production was high, on the order of 0.3 mg of purified fimbriae per standard agar plate culture, similar to that reported previously for equivalent *B. nodosus* fimbrial gene constructions in *P. aeruginosa* (18).

However, in other transformants the extracellular fimbriae were found to be composed of a mosaic of indigenous *P. aeruginosa* subunits and the cloned *M. bovis* subunits. Western transfer analysis of these fimbriae showed a positive reaction with both anti-*P. aeruginosa* and anti-*M. bovis* fimbrial antisera (Fig. 2B and C, lanes 5 and 6). These results were also confirmed by electron micrographic techniques. Whereas nonrecombinant *P. aeruginosa* controls showed exclusive fimbrial labeling with homologous antifimbrial antibodies and only spurious labeling with *M. bovis* antifimbrial antibodies (data not shown), these recombinant fimbriae showed true mixed labeling with both types of antibody on the same fimbrial strand (Fig. 3B).

The reason for the difference between transformants producing solely *M. bovis* fimbriae and those producing mosaic fimbriae appeared to be differences in the ratio of cloned to indigenous subunits in the different clones, with a downregulation in *M. bovis* fimbrial subunit production (to various degrees) in the latter leading to a more balanced production of subunits and the assembly of mixed fimbriae.

Although recombinant *P. aeruginosa* cells producing more than one type of fimbriae have been described previously (10), this is the first report of fimbriae composed of a mosaic of subunits from different species.

DISCUSSION

The results presented here demonstrate that it is possible to obtain high-level morphogenetic expression of *M. bovis*-type fimbriae from a cloned subunit gene in *P. aeruginosa*, consistent with earlier predictions (16) and results with the *B. nodosus* fimbrial subunit gene (18). However, unlike the previous study, we also obtained *P. aeruginosa* transformants which produced fimbriae containing both heterologous and indigenous subunits. This second category of transformants appeared to be a consequence of a (variable) downregulation in the level of expression of the cloned *M. bovis* fimbrial subunit gene, but we have not yet attempted to determine the precise nature of the secondary genetic changes in such clones.

However, and irrespective of the cause of this variability, the production of mixed fimbriae is quite different from the results reported by Elleman and Peterson (10), who found that *P. aeruginosa* recombinants containing fimbrial subunit genes from two structurally distinct *B. nodosus* serogroups (A and H) expressed two distinct types of fimbriae composed almost exclusively of one or other *B. nodosus* subunit type. Fimbriae containing both *B. nodosus* subunits were rare and segmented into relatively homogeneous blocks of the different subunits. There were also occasionally fimbriae composed exclusively of the indigenous *P. aeruginosa* subunit. No mosaics were encountered. These observations were interpreted to indicate close coupling between translation and fimbrial assembly, with each fimbrial strand being produced from the translation products of a single mRNA sequestered exclusively at the assembly site (10). This explanation is, however, difficult to reconcile with the fact that in these experiments both *B. nodosus* subunit genes were expressed on the same transcription unit (10).

This explanation is also inconsistent with our present observations, as well as those of Pasloske et al. (21), and we propose a simpler alternative based on the reversibility and

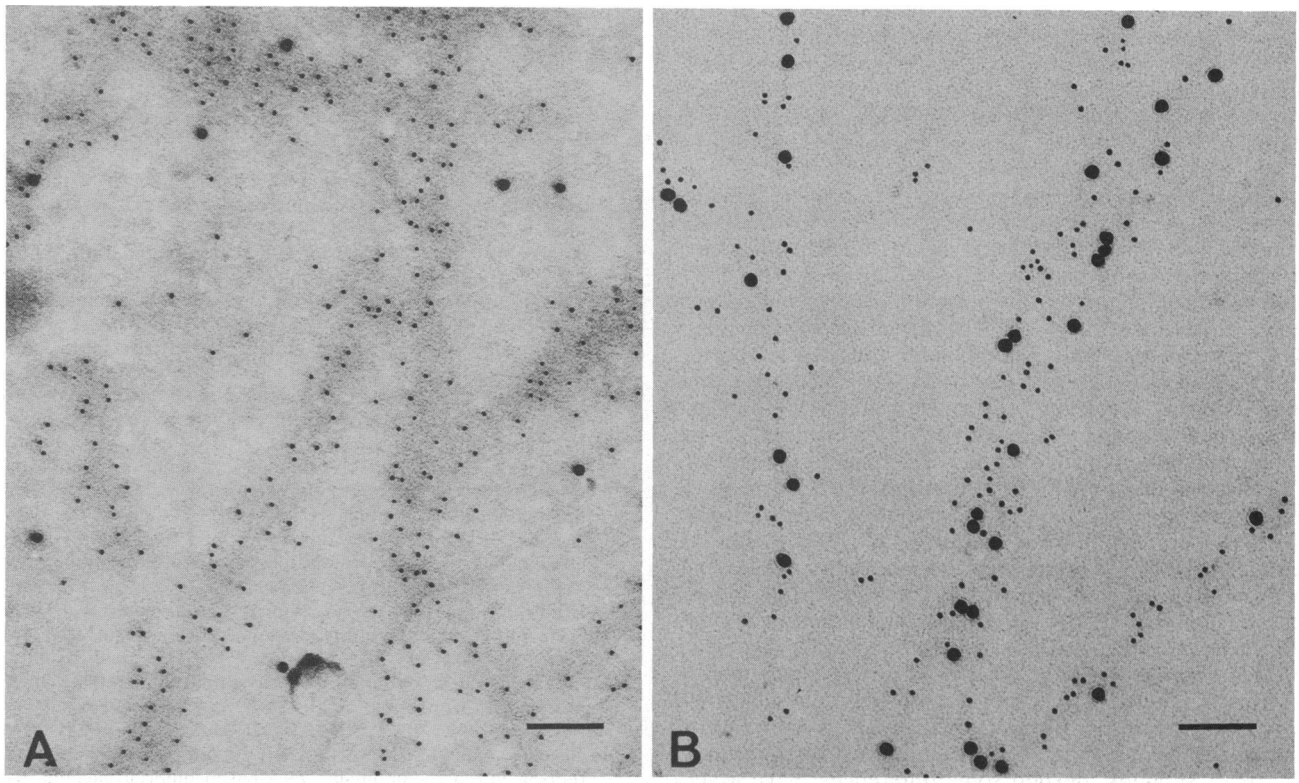


FIG. 3. Electron micrographic analysis of the fimbriae expressed by *P. aeruginosa* PAK/2Pfs containing the pMKB/MxB cartridge. Whole cells of recombinant *P. aeruginosa* primary clones MKB/MxB6 (A) and MKB/MxB4 (B) were treated with *P. aeruginosa* PAK/2Pfs fimbrial antiserum in association with 14-nm colloidal gold particles and then *M. bovis* EPP63 fimbrial antiserum in association with 6-nm colloidal gold particles. Each micrograph is shown at the same magnification. Bars, 0.1 μm

relative stability of subunit-subunit interactions in the fimbrial strand. This proposal is distinct from the idea of exclusive specificity of interaction between subunits which was considered and rejected by Elleman and Peterson (10). There is good evidence that fimbrial retraction is the mechanical basis of the twitching motility observed in type 4 fimbriate bacteria (6). It has been suggested that this occurs by assembly and disassembly of structural subunits in the fimbrial strand (12) and that there is a pool of subunits within the cell, associated with the inner membrane (26). Even though our studies, and those of others (10, 21), have employed nonretractile mutants of *P. aeruginosa* as the recombinant host, it seems perfectly feasible that there may be reversible entry of subunits into the fimbrial strand at the site of assembly, even if this is not switchable to unidirectional assembly or disassembly as would be required for extension and retraction. We propose that site-of-entry reversibility of subunit assembly could account for the different types of fimbriae produced in cells expressing more than one type of subunit, if interactions between homologous subunits are relatively stable, whereas interactions between heterologous subunits are only stable if the subunits are sufficiently similar in structure. Hence, stable interaction would favor the forward equilibrium and assembly of similar subunits at the expense of unstable interactions between dissimilar subunits.

In this model, the existence of fimbriae containing mosaics of *P. aeruginosa* PAK and *M. bovis* EPP63 subunits would indicate relatively stable stacking between the subunits, and indeed these proteins are relatively similar in primary sequence and secondary features, such as the size and location of putative disulfide loops (7, 14). Supporting evidence, at least in part, comes from the studies of Pasloske et al. (21), who observed that *P. aeruginosa* PAO cells which express a mutant PAK subunit together with the indigenous (PAO) subunit produced compound fimbriae, albeit with an altered morphology. However, the same cells containing the wild-type PAK fimbrial subunit gene were reported to produce only homopolymeric fimbriae (21). This apparent contradiction was not explained, but the data are difficult to assess because direct double-labeling experiments were not performed.

In the case of *B. nodosus*, the subunits from serogroup A and serogroup H in fact represent two quite distinct classes, with different primary structures (9, 15), despite their occurrence within the same species. The class II subunits (serogroups D and H) are much more closely related to the structural subunits of *M. bovis* and *P. aeruginosa* than to class I (serogroups A, B, C, E, F, G, and I) and appear to have been derived by lateral transfer from another species and replacement of most of the fimbrial gene region in class I strains (M. Hobbs, B. Dalrymple, and J. S. Mattick, manuscript in preparation; see also reference 15). The significant differences in these subunits might well affect the stability of their interaction and account for the overwhelming preponderance of fimbriae composed of one type or the other in recombinant *P. aeruginosa* cells expressing both types (10).

The compatibility of the *M. bovis* subunit and the *P. aeruginosa* assembly system supports the concept of the interchangeability of type 4 fimbrial subunits, but this interchangeability may not be absolute. In conjunction with the

present study, we also attempted without success to express *N. gonorrhoeae* fimbriae in *P. aeruginosa*, using a 668-bp *Dde*I-*Cla*I cartridge spanning the *N. gonorrhoeae* MS11 fimbrial subunit gene (from −32 to +138 relative to the start and stop codons) (see reference 19) (M. K. M. Beard, J. S. Mattick, M. So, and J. R. Egerton, unpublished). While this gene is expressed normally under p_L promoter control in *E. coli*, we have thus far been unable to obtain viable transformants in *P. aeruginosa* which produce *N. gonorrhoeae*-type fimbriae. Two lines of evidence suggest that the *N. gonorrhoeae* construction may be toxic to *P. aeruginosa*. First, large-scale comparative transformation experiments show a reduction of at least two orders of magnitude in the number of *N. gonorrhoeae* gene recombinants, relative to controls with the equivalent *B. nodosus* or *M. bovis* gene constructions. Second, the few transformants that were obtained grew very poorly and expressed extremely low levels of the fimbrial subunits. Similar difficulties in obtaining assembly of *N. gonorrhoeae* subunits in *P. aeruginosa* have been reported recently by others (T. C. Elleman and P. Hoyne, Proc. Aust. Biochem. Soc. 21, abstr. P67, 1989). Although negative results are difficult to interpret, this implies that there may be some incompatibility between the *N. gonorrhoeae* subunit and the *P. aeruginosa* assembly system. Comparative sequence studies (7) have shown that relative to the fimbrial subunits of *P. aeruginosa*, *M. bovis*, and *B. nodosus*, this protein has a significant deletion in the central region of the protein, with a compensating insertion in the carboxy-terminal region within the putative disulfide loop. The other subunits, despite some differences, have a very similar linear arrangement of variable and conserved regions, and even the *B. nodosus* class I subunits, which do not have the terminal disulfide loop, retain conserved features in this domain, indicating that no gross rearrangement has occurred (7). It is possible that the rearrangement of the *N. gonorrhoeae* subunit was sufficient to affect its assembly by the *P. aeruginosa* system and to cause lethal effects either from its inability to be exported or from blockage of the assembly pathway. Whether there is a significant or trivial reason for the difficulty in obtaining expression of *N. gonorrhoeae* fimbriae in *P. aeruginosa*, recent sequence analysis of the fimbrial subunit gene of *Vibrio cholerae* (24) suggests that there are even more divergent members of the type 4 group. Further studies are required to determine the true extent of the evolution, distribution, and interchangeability of the type 4 fimbrial system, which may require redefinition as subsets, although these may overlap. This may further necessitate a review of the whole question of phylogenetic interrelationships within the broad group currently referred to as type 4. Certainly, based on the criteria of their polar location and association with the phenomenon known as twitching motility, type 4 fimbriae are widely distributed in at least the β and γ subdivisions of the Proteobacteria, as well as possibly in some gram-positive species (7; B. Dalrymple and J. S. Mattick, manuscript in preparation).

ACKNOWLEDGMENTS

We thank Sue Livingstone, Noel Jones, and David Cox for technical assistance and Matthew Hobbs for helpful suggestions.

This work was supported in part by grants from the Australian Meat and Livestock Research and Development Corporation, the Australian Society for Microbiology, the National Health and Medical Research Council, and Public Health Service grant BM-1 5ROIEY07125 from the National Eye Institute, Bethesda, Md. M. K. M. Beard is supported by a Postgraduate Studentship from the Australian Meat and Livestock Research and Development Corporation.

LITERATURE CITED

1. **Anderson, B. J., M. M. Bills, J. R. Egerton, and J. S. Mattick.** 1984. Cloning and expression of *Escherichia coli* of the gene encoding the structural subunit of *Bacteroides nodosus* fimbriae. J. Bacteriol. **160**:748–754.
2. **Anderson, B. J., C. L. Kristo, J. R. Egerton, and J. S. Mattick.** 1986. Variation in the structural subunit and basal protein antigens of *Bacteroides nodosus* fimbriae. J. Bacteriol. **166**: 453–460.
3. **Bagdasarian, M. M., E. Amann, R. Lurz, B. Ruckert, and M. Bagdasarian.** 1983. Activity of the hybrid *trp-lac* (*tac*) promoter of *Escherichia coli* in *Pseudomonas putida*. Construction of broad-host-range, controlled-expression vectors. Gene **26**:273–282.
4. **Beesley, J. E., and M. P. Betts.** 1986. A quantitative investigation into the role of buffer components for reducing nonspecific immunolabelling. Histochem. J. **18**:55.
5. **Bradley, D. E.** 1974. The adsorption of *Pseudomonas aeruginosa* pilus-dependent bacteriophages to a host mutant with non-retractile pili. Virology **58**:149–163.
6. **Bradley, D. E.** 1980. A function of *Pseudomonas aeruginosa* PAO pili: twitching motility. Can. J. Microbiol. **26**:146–154.
7. **Dalrymple, B., and J. S. Mattick.** 1987. An analysis of the organization and evolution of type 4 fimbrial (MePhe) subunit proteins. J. Mol. Evol. **25**:261–269.
8. **Egerton, J. R., P. T. Cox, B. J. Anderson, C. Kristo, M. Norman, and J. S. Mattick.** 1987. Protection of sheep against footrot with a recombinant DNA-based fimbrial vaccine. Vet. Microbiol. **14**:393–409.
9. **Elleman, T. C., P. A. Hoyne, N. M. McKern, and D. J. Stewart.** 1986. Nucleotide sequence of the gene encoding the two-subunit pilin of *Bacteroides nodosus* 265. J. Bacteriol. **167**:243–250.
10. **Elleman, T. C., and J. E. Peterson.** 1987. Expression of multiple types of *N*-methyl-Phe pili in *Pseudomonas aeruginosa*. Mol. Microbiol. **1**:377–380.
11. **Holloway, B. W.** 1965. Variations in restriction and modification following increase of growth temperature of *Pseudomonas aeruginosa*. Virology **25**:634–642.
12. **Johnson, K., and S. Lory.** 1987. Characterization of *Pseudomonas aeruginosa* mutants with altered piliation. J. Bacteriol. **169**:5663–5667.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Marrs, C. F., G. Schoolnik, J. M. Koomey, J. Hardy, J. Rothbard, and S. Falkow.** 1985. Cloning and sequencing of a *Moraxella bovis* pilin gene. J. Bacteriol. **163**:132–139.
15. **Mattick, J. S.** 1989. The molecular biology of the fimbriae (pili) of *Bacteroides nodosus* and the development of a recombinant DNA-based vaccine, p 195-218. *In* J. R. Egerton, W. K. Yong, and G. G. Riffkin (ed.), Footrot and foot abscess of ruminants. CRC Press Inc., Boca Raton, Fla.
16. **Mattick, J. S., B. J. Anderson, and J. R. Egerton.** 1985. Molecular biology and footrot of sheep, p. 79–91. *In* R. A. Leng, J. S. F. Barker, D. G. Adams, and K. R. Hutchinson (ed.), Biotechnology and recombinant DNA technology in the animal production industries (Reviews in Rural Science, vol. 6). University of New England, Armidale, New South Wales, Australia.
17. **Mattick, J. S., B. J. Anderson, M. R. Mott, and J. R. Egerton.** 1984. The isolation and characterization of the fimbriae of *Bacteroides nodosus* structural subunit and basal protein antigens. J. Bacteriol. **160**:740–747.
18. **Mattick, J. S., M. M. Bills, B. J. Anderson, B. Dalrymple, M. R. Mott, and J. R. Egerton.** 1987. Morphogenetic expression of *Bacteroides nodosus* fimbriae in *Pseudomonas aeruginosa*. J. Bacteriol. **169**:33–41.
19. **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.

20. **Ottow, J. C. G.** 1975. Ecology, physiology, and genetics of fimbriae and pili. Annu. Rev. Microbiol. **29**:79–108.
21. **Pasloske, B. L., D. G. Scraba, and W. Paranchych.** 1989. Assembly of mutant pilins in *Pseudomonas aeruginosa*: formation of pili composed of heterologous subunits. J. Bacteriol. **171**:2142–2147.
22. **Sastry, P. A., B. B. Finlay, B. L. Pasloske, W. Paranchych, J. R. Pearlstone, and L. B. Smillie.** 1985. Comparative studies of the amino acid and nucleotide sequences of pilin derived from *Pseudomonas aeruginosa* PAK and PAO. J. Bacteriol. **164**: 571–577.
23. **Schoolnik, G. K., R. Fernandez, J. Y. Tai, J. Rothbard, and E. C. Gotschlich.** 1984. Gonococcal pili: primary structure and receptor binding domain. J. Exp. Med. **159**:1351–1370.
24. **Taylor, R. K., V. L. Miller, D. B. Furlong, and J. J. Mekalanos.** 1987. Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc. Natl. Acad. Sci. USA **84**:2833–2837.
25. **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.
26. **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.