Morphogenetic Expression of Bacteroides nodosus Fimbriae in Pseudomonas aeruginosa

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Type 4 fimbriae are found in a range of pathogenic bacteria, including Bacteroides nodosus, Moraxella bovis, Neisseria gonorrhoeae, and Pseudomonas aeruginosa. The structural subunits of these fimbriae all contain a highly conserved hydrophobic amino-terminal sequence preceding a variable hydrophilic carboxy-terminal region. We show here that recombinant P. aeruginosa cells containing the B. nodosus fimbrial subunit gene under control of a strong promoter (\(p_L\), from bacteriophage \(\lambda\)) produced large amounts of fimbriae that were structurally and antigenically indistinguishable from those produced by \(B.\) nodosus. This was demonstrated by fimbrial isolation and purification, electrophoretic and Western transfer analyses, and immunogold labeling and electron microscopy. These results suggest that type 4 fimbriated bacteria use a common mechanism for fimbrial assembly and that the structural subunits are interchangeable, thereby providing a basis for the development of multivalent vaccines.

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Bacteroides nodosus is the essential causative agent of ovine footrot (4, 11). This anaerobe contains numerous surface filaments, about 6 nm in diameter and ranging up to several micrometers in length (14, 46, 50), termed fimbriae (or common pili), which play a central role in both pathogenesis and immunity (for a recent review, see reference 29). Fimbriae have adherent functions and appear to be a mechanism for the colonization of epithelial tissues in eucaryotic hosts. The properties of \(B.\) nodosus fimbriae (14) suggest that they belong in the category of type 4, as proposed by Ottow (38), citing Pseudomonas aeruginosa (6) as a prototype. Fimbriae of this type have a polar location on the cell and appear to be involved in surface translocation by a phenomenon known as twitching motility (21). The same characteristics are also observed in the fimbriae found in a broad range of gram-negative species classified within the genera Acinetobacter, Alteromonas, Bacteroides, Eikenella, Moraxella, Neisseria, and Pseudomonas, among others (6, 17, 20, 21).

This grouping is supported by recent protein and DNA sequence analyses of the structural subunits of the fimbriae of \(B.\) nodosus (12, 31), Moraxella nonliquefaciens (16), Moraxella bovis (28), Neisseria gonorrhoeae (22, 33, 44), Neisseria meningitidis (22, 36), and \(P.\) aeruginosa (42). These subunits, which range in size from about 145 to 160 amino acids among different species and serotypes, all share the distinctive feature of an unusual modified amino acid, methlyphenylalanine (MeF), as the first residue in the mature protein, as well as a striking degree of sequence conservation throughout the amino-terminal region. This region is highly hydrophobic and exhibits at least 90% homology with the following 32-amino-acid consensus sequence:

\[
\text{MeF T L I E M I V (I/V) A I (I/V) G I L A A (I/V)}
\]

\[
A (I/L) P A Y (Q/N) D Y (I/V) (A/S) (R/K) (A/S) Q
\]

Further pockets of homology are evident up to the glycine residue at position 54/55 (Fig. 1). In addition, the subunits also seem to share a similar and unusual 6- to 7-amino-acid positively charged leader sequence in the primary translation product, M(K/N)(S/T/-)(A/L)QKG, which is cleaved from the protein at some point prior to incorporation into the mature fimbrial strand. Interspecies divergence is seen primarily in the more hydrophilic carboxy-terminal two-thirds of the molecule. Structural and antigenic variation within species also occur in this region, especially in hypervariable domains in which the immunodominant serological epitopes appear to be located (19, 28, 32, 42, 44, 49). This variation involves not only amino acid substitutions, but also small insertions and deletions (19, 28, 32, 42) (Fig. 1). In some cases at least, variant forms appear to be actively generated by recombination between active and silent gene loci (18, 19, 45).

The genes encoding the fimbrial subunits of \(N.\) gonorrhoeae, \(P.\) aeruginosa, \(M.\) bovis, and \(B.\) nodosus appear to be located on the bacterial chromosome, but little is known about the morphogenesis of type 4 fimbriae. Studies of type 1 chromosomally encoded and plasmid-encoded fimbrial systems found in uropathogenic and enterotoxigenic strains of Escherichia coli (and other Enterobacteriaceae) have shown that biosynthesis of these fimbriae involves the participation of several genes, which are normally clustered (24, 35, 37). One of these genes codes for the structural subunit itself, another for a basal protein which appears to act as the site for attachment or extrusion of the fimbrial strand at the cell surface, and the remainder for other polypeptides whose exact functions remain to be determined, but which are required for fimbrial assembly or affect the level of fimbrial expression (24, 35, 37). An analogous basal protein appears to be associated with the fimbriae of \(B.\) nodosus (30). However, there is no obvious homology between the structural subunits of type 4 fimbriae and those found in \(E.\) coli. There are in fact significant differences, and these systems appear to be distinct. Type 4 fimbrial subunits expressed from cloned genes in \(E.\) coli are not assembled into mature fimbriae, but rather are found embedded in the inner membrane of the cell (1, 13, 28, 33).
For N. gonorrhoeae and P. aeruginosa, it has been suggested that the hydrophobic amino-terminal portion of the fimbrial subunit is involved in subunit-subunit interactions within the fimbrial strand (44, 51), and this may well be the case. However, the extremely strong conservation of this sequence, including the small leader peptide, across the range of different bacterial species and genera which possess type 4 fimbriae suggested to us that this region may also contain important signals for fimbrial morphogenesis (29), in terms of the interaction of the structural subunit with other factors involved in the assembly system. If so, the fimbrial subunits containing these signals might be interchangeable throughout the type 4 group. This possibility was tested by inserting the B. nodosus fimbrial subunit gene into P. aeruginosa, and we show here that such recombinants will produce, after appropriate molecular genetic manipulation, high levels of mature B. nodosus-type fimbriae. P. aeruginosa was selected from the available type 4 fimbriated bacteria as the (prototype) recipient host simply on the basis that this species is a genetically well-characterized aerobe for which a range of plasmid shuttle vectors are available for gene cloning and transfer.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** B. nodosus VCS1001 (ATCC 25549), formerly known as strain 198, was used for this study. This strain is the designated prototype of serogroup A (8). P. aeruginosa PAK/2Pf (ATCC 53308), a phage-resistant, nonretractile multifimbriated mutant of P. aeruginosa PAK (5), was used as the host for morphogenetic expression. The plasmids used in the recombinant DNA constructions (Fig. 2) were pBA121 (1), which contains the B. nodosus VCS1001 fimbrial subunit gene; pPL-λ (P-L Biochemicals), which contains the bacteriophage λ promoter, pL; and pKT240 (3), a broad-host-range vector capable of being maintained in both E. coli and P. aeruginosa. All constructions involving the pL promoter were carried out in E. coli E148 (N. Murray, strain W1485, λIr5857 Sam7; obtained from B. Egan, University of Adelaide), grown at the permissive temperature of 30°C. The pL promoter was derepressed, when required, by shifting the culture to 42°C.

**Recombinant DNA constructions.** Plasmid DNA was purified by cesium chloride-ethidium bromide isopycnic ultracentrifugation following either the small-scale alkaline dodecyl sulfate (SDS) procedure (27) or the large-scale Triton X-100 procedure (1). All restriction endonuclease digestions and DNA ligations were carried out under standard conditions (27). Restriction digests were analyzed on 0.6% agarose gels in a Tris-borate-EDTA buffer system, containing 0.5 μg of ethidium bromide per ml (1, 27). The DraI and BamHI restriction fragments were gel purified prior to insertion into pPL-λ and pKT240, respectively (Fig. 2). These DNA fragments were recovered by electroelution and chromatography on NACS-52 minicolumns (Bethesda Research Laboratories).

**DNA transformation.** P. aeruginosa cells were made competent for transformation by treatment with MgCl₂ as follows. Cells were grown overnight at 43°C (23) in KNO₃ broth (2.5% Oxoid nutrient broth no. 2, 0.5% yeast extract, and 0.4% KNO₃) and then diluted 1:25 in fresh KNO₃ broth and incubated with shaking at 37°C until the culture had reached an A₅₆₀ of 0.5. The culture was then chilled quickly on ice, and the cells were recovered by centrifugation (6,000 × g, 6 min). All subsequent steps were carried out at 4°C. The cells were washed twice with suspension in one-fifth volume of 0.1 M MgCl₂ and then suspended in one-eighth volume of 0.15 M MgCl₂ and left on ice for 20 to 30 min. The cells were finally washed in one-twentieth volume of 0.15 M MgCl₂ and kept on ice ready for transformation. Approximately 200 ng of DNA was used to transform 0.1 ml of competent cells. E.
coli cells were made competent for transformation by conventional treatment with CaCl₂ (27).

For both P. aeruginosa and E. coli, transformation was carried out by standard procedures (27), including the heat shock step. Transformants were selected and maintained on Luria (L) broth (27) agar plates, containing 50 μg of ampicillin per ml (for E. coli) or 1 mg of carbenicillin per ml (for P. aeruginosa) (3).

Isolation of fimbriae. B. nodosus VCS1001 cells were grown on hoof agar plates (47) for 4 days at 37°C in an atmosphere of 90% H₂–10% CO₂ and harvested by scraping into cold phosphate-buffered saline (PBS) (140 mM NaCl, 10 mM sodium phosphate, pH 7.2) (30). B. nodosus fimbriae were purified from the supernatant of PBS-suspended cells by isoelectric precipitation with 0.1 M sodium acetate, pH 4.5, as described previously (30).

Normal P. aeruginosa fimbriae were isolated from PAK/2Pfs cells cultured aerobically at 37°C overnight on nutrient (L broth) agar plates. The cells were harvested by scraping into cold PBS, and the resulting suspension was blended for 1 min with a Silverson mixer-emulsifier. All steps were carried out at 4°C. The cells and cell debris were removed by centrifugation (24,000 × g, 30 min), and the supernatant was adjusted to pH 4.5 by the addition of acetic acid. After standing overnight, the fimbriae were collected by centrifugation (24,000 × g, 30 min). The fimbriae were then further purified by a second round of dissolution in PBS and precipitation with sodium acetate, pH 4.5.

Fimbriae were isolated from recombinant P. aeruginosa PAK/2Pfs cells following overnight culture at 37°C on L broth agar plates supplemented with carbenicillin (1 mg/ml). The cells were harvested in PBS and subjected to mechanical blending as described above (see Fig. 4). The fimbriae were recovered from the (cell-free) supernatant by either isoelectric precipitation with sodium acetate, pH 4.5, as described above, or by the independent method of precipitation with 0.1 M MgCl₂ (30).

Electrophoretic display. Samples were analyzed by electrophoresis on SDS-urea 8 to 15% polyacrylamide gradient gels with the modified Laemmli buffer system described previously (30). Protein bands were visualized by staining with Coomassie blue R250.

Western transfer. Unstained gel displays were electrophoretically transferred to nitrocellulose paper (48), which was then incubated with antifimbrial antiserum (diluted 1:1,000), followed by 125I-protein A (0.1 μCi/ml), as detailed in Anderson et al. (2).

Antisera. Antisera specific for B. nodosus VCS1001 fimbriae and P. aeruginosa PAK/2Pfs fimbriae were raised in rabbits following vaccination with purified fimbrial preparations, as described previously (30).

Immunogold labeling and electron microscopy. Whole cells of P. aeruginosa PAK/2Pfs, with or without pJSM202, were diluted in H₂O and looped onto parlodion-carbon-coated gold grids. The grids were dried, treated with 5% bovine serum albumin in PBS for 2 min, and then incubated for 1 h with either anti-P. aeruginosa PAK/2Pfs fimbrial antiserum or anti- B. nodosus VCS1001 fimbrial antiserum, diluted 1:500 and 1:100 in PBS, respectively. The grids were washed and then incubated for 1 h with protein A labeled with 15-nm colloidal gold particles (Janssen Pharmaceutica, Belgium) diluted 1:10 in 140 mM NaCl–10 mM Tris hydrochloride, pH 8.2, containing 0.1% bovine serum albumin. The grids were again washed and then negatively stained with 2% sodium phosphotungstate (pH 7.0) and examined in a Jeol 100CX electron microscope at 60 kV.

RESULTS

Genetic constructions. The B. nodosus fimbrial subunit gene used in this study was derived from strain VCS1001, which is the designated prototype of serogroup A (8). The
DraI, which cleaves subunit gene in placing this fractions pBA121 were plasmid from pBA121 into the corresponding site of the resulting promoter fragment and is expressed in the most highly purified fractions (data not shown). However, these fractions were composed almost entirely of the host P. aeruginosa subunit, and it was not possible to determine the exact distribution of the trace amounts of B. nodosus antigen in the fimbrae.

The problem of the low expression of the B. nodosus fimbral subunit gene in P. aeruginosa was overcome by placing this gene under the transcriptional control of the strong promoter, $p_L$, from bacteriophage $\lambda$ as follows. pBA121 was digested with the restriction endonuclease DraI, which cleaves at convenient sites flanking the fimbral subunit gene, one 30 nucleotides upstream from the initiation codon and the other 69 nucleotides downstream from the termination codon, to yield a blunt-ended 576-base-pair gene cartridge. This cartridge, which included the Shine-Dalgarno sequence as well as a downstream region of hypenated dyad symmetry which may function as a transcription termination signal (12), was inserted into the HpaI site located downstream from the $p_L$ promoter in pPL-\lambda (Fig. 2). The entire gene-promoter construction was then transferred as a BamHI fragment into the corresponding site of pKT240 to generate pJS202 (ATCC 40203) (Fig. 2). All constructions involving the $p_L$ promoter were carried out in an E. coli \lambda lysogen (E418) containing the temperature-sensitive cI857 repressor gene, grown at 30°C. Viable transformants could not be obtained in E. coli cells lacking a functional cI repressor, presumably because these cells are unable to cope with the large amount of B. nodosus fimbral subunit being produced and deposited into the membrane (1). On the other hand, we found that pJS202 was not lethal for P. aeruginosa, even though the $p_L$ promoter was active in this host (see below). We also found that there was no advantage to be gained from regulation of the $p_L$ promoter in P. aeruginosa by inserting the cI857 repressor gene into pJS202, although this system was functional (data not shown).

Morphogenetic expression. Analysis of P. aeruginosa PAK2Pfs transformants containing pJS202 showed that not only were large amounts of the B. nodosus fimbral subunit produced, but also that these subunits were assembled into mature fimbral structures on the surface of the cells. Although B. nodosus-type fimbrae are physically very similar to those naturally produced by the P. aeruginosa host strain, they may be distinguished immunologically and by electrophoretic differences between the structural subunits. The apparent molecular weight of the B. nodosus VCS1001 subunit in SDS-polyacrylamide gels is about 17,000, whereas that of the P. aeruginosa PAK2Pfs subunit is about 16,000; their actual molecular weights (calculated from the sequence) are 16,218 (151 amino acids) (12) and 15,082 (145 amino acids) (42), respectively.

Morphogenetic expression of B. nodosus-type fimbrae in recombinant P. aeruginosa cells was first indicated by the presence of substantial amounts of this antigen in the extracellular environment. In many fimbrinated bacteria, including B. nodosus and P. aeruginosa, a proportion of the fimbrae appear to be shed into the medium (or suspension buffer) and remain there after removal of the cells by centrifugation. Additional fimbrae may be removed by mechanical blending of the cell suspension. Figure 3 shows a Western transfer analysis of the cell pellet and derived supernatant fractions from recombinant cells cultured on solid medium. First, the B. nodosus fimbral subunit was transcribed very actively from the $p_L$ promoter in P. aeruginosa cells containing pJS202, at a level at least two orders of magnitude higher than that in the same host containing pJS125, in which the gene is transcribed from an associated promoter within the original cloned segment (1) of B. nodosus DNA. The activity of the $p_L$ promoter in P. aeruginosa seemed to be similar to that in E. coli (Fig. 3). Second, a large proportion of the fimbral subunit antigen was found in the supernatant fraction obtained from recombinant P. aeruginosa cells, in contrast to E. coli E418 cells containing pJS202, in which the antigen was entirely located in the cell (pellet) fraction. A substantial amount of B. nodosus fimbral subunit also remained with the recombinant P. aeruginosa cells, but it is not known to what extent this represents fimbrae not dislodged from the cell (cf. Fig. 4) or (as yet) unassembled subunits accumulated within the cell (52). Third, the fimbral subunit found in E. coli appeared to be slightly larger than that found in P. aeruginosa or in B. nodosus itself. This reflects the inability of E. coli to cleave the short leader sequence from the primary translation product (12), which presumably is part of the normal process of morphogenetic expression.

The assembly of B. nodosus fimbral subunits into mature fimbrae in recombinant P. aeruginosa cells was also dem-
onstrated by the presence of this antigen in purified fimbiae isolated under different conditions. Fimbriae may be recovered from cell-free supernatants either by isoelectric precipitation at pH 4.5 or by precipitation with MgCl₂ (30). These are standard procedures applicable to the isolation of fimbiae from both P. aeruginosa and B. nodosus. With either method, high yields of relatively pure fimbiae were obtained from P. aeruginosa cells containing pJSM202 (Fig. 4). This was confirmed by electron microscopy of negatively stained samples. Electrophoretic analysis showed that these fimbiae were composed primarily of a structural subunit which comigrated in the gel with that obtained from B. nodosus (Fig. 4). These fimbiae could also be further purified by isopycnic banding in CsCl gradients (14, 30). Quite unexpectedly, there was little or no evidence of the fimbral subunit characteristic of the host strain, PAK/2Pfs.

These results were confirmed by Western transfer analysis of the fimbiae produced by the recombinant cells in comparison with those obtained from B. nodosus VCS1001 and P. aeruginosa PAK/2Pfs (Fig. 5). This experiment clearly showed that not only did the fimbiae produced by P. aeruginosa cells containing pJSM202 consist of a structural subunit which had the same electrophoretic mobility as that found in B. nodosus itself (Fig. 5A), but also that this protein was recognized by antibodies directed against B. nodosus fimbiae (Fig. 5C). The reciprocal Western assay with anti-P. aeruginosa fimbral antiserum confirmed that the fimbiae produced by these recombinant cells contained little, if any, of the structural subunit naturally expressed by the host (Fig. 5B).

The production of B. nodosus-type fimbiae in the recombinant P. aeruginosa cells was also independently verified by immunogold labeling and electron microscopy (Fig. 6). Antibody binding was indicated by the presence of electron-
dense spherical gold particles, as well as by clumping together of the fimbral strands. As expected, the fimbiae produced by the host strain PAK/2Pfs reacted strongly with the homologous antiserum (Fig. 6A), but not with antiserum against B. nodosus fimbiae (Fig. 6B). This pattern was completely reversed in the recombinant P. aeruginosa cells containing pJSM202. Fimbriae produced by these cells were not recognized by antibodies directed against P. aeruginosa fimbiae (Fig. 6C), but rather reacted strongly with those against B. nodosus fimbiae (Fig. 6D). This was the same profile exhibited by B. nodosus itself.

Several other criteria were also used to verify the production of B. nodosus fimbiae by P. aeruginosa cells containing pJSM202. These included demonstrations that antiserum raised against the fimbiae prepared from recombinant cells agglutinate (8, 10) B. nodosus VCS1001 cells and reciprocally that antisera raised against B. nodosus VCS1001 cause agglutination of the recombinant P. aeruginosa cells. These agglutination reactions were (B. nodosus) serotype specific. Control experiments showed that anti-B. nodosus antiserum did not agglutinate host P. aeruginosa cells and that antiserum raised against normal P. aeruginosa fimbiae did not agglutinate B. nodosus cells. Similar results were obtained in standard solid-phase antibody-antigen binding assays, such as enzyme-linked immunosorbent assays (data not shown).

These results demonstrate that P. aeruginosa PAK/2Pfs cells containing the recombinant plasmid pJSM202 produce mature fimbral structures which are physically, structurally, and antigenically indistinguishable from those produced by the B. nodosus strain from which the fimbral subunit gene was originally derived. B. nodosus-type fimbiae were also obtained when other finmbrial P. aeruginosa strains were
FIG. 6. Immunogold labeling of host and recombinant fimbriae. Whole cells of the host *P. aeruginosa* PAK/2Pfs (A and B) or the same strain containing pSM202 (C and D) were placed onto parlodion-carbon-coated gold grids and treated with either anti-*P. aeruginosa* PAK/2Pfs fimbrial antiserum (A and C) or anti-*B. nodosus* VCS1001 fimbrial antiserum (B and D) and then with protein A labeled with 15-nm colloidal gold particles. The grids were then negatively stained and examined in an electron microscope at 60 kV. Each micrograph is shown at the same magnification. Bar, 0.1 μm.
used as the host for pJSM202 (data not shown). These included \textit{P. aeruginosa} PAK (ATCC 25210), the parent of PAK/2Fts, as well as \textit{P. aeruginosa} PAK1 (ATCC 25247) and its derived multifimbriated mutant DB-2 (25), which normally expresses a form of fimbrial subunit structurally and serologically distinct from that found in PAK strains.

**DISCUSSION**

The insertion of pJSM202 into a fimbriated \textit{P. aeruginosa} strain effectively resulted in complete substitution of the \textit{B. nodosus} fimbrial subunit for that normally produced by the \textit{P. aeruginosa} cells. This seemed to be largely due to the relatively high level of expression of the \textit{B. nodosus} fimbrial subunit, but it also suggests that these cells have little, if any, preference for assembly of the homologous over the heterologous subunit in fimbrial strands. We also found, however, in control experiments (data not shown) that the level of expression of the endogenous fimbrial subunit was substantially reduced in recombinant cells containing either pJSM202 or pKT240, apparently as a consequence of the physiological effects of antibiotic selection or the expression of genes on the vector plasmid itself.

The experiments described herein support the proposition that type 4 fimbrial subunits from different bacterial species are assembled by a common mechanism and may be interchanged among compatible hosts. This is certainly the case for \textit{B. nodosus} and \textit{P. aeruginosa} and should be equally true for other type 4 species retaining the conserved amino-terminal region of the fimbrial subunit (see below). From sequence data, \textit{B. nodosus} and \textit{P. aeruginosa} appear to be no more or less closely related in this respect than other members of the group. Moreover, the large differences in the carboxy-terminal two-thirds of these proteins do not appear to have affected the process of fimbrial assembly in the heterologous host. These differences include almost complete divergence in primary structure, as well as changes in the location and span of the internal disulfide loop, which occurs in the middle of the molecule in \textit{B. nodosus} and at the end in \textit{P. aeruginosa}. In this respect \textit{B. nodosus} would appear to be less related to \textit{P. aeruginosa} than are \textit{N. gonorrhoeae} and \textit{M. bovis}, which also have disulfide bridges near the carboxy terminus of the subunit, although the lengths of the loops are different (Fig. 1). In any case it would seem that there are few constraints imposed on variation in the latter part of the subunit, except perhaps for the overall size of the molecule. On the other hand, the facts that fimbriae from different type 4 species are physically very similar and that one subunit may be successfully substituted for another in the same host suggest that these polypeptides must have similar tertiary and quaternary structures. In support of this, there appear to be similarities in the secondary structure (B. Dalrymple and J. S. Matick, manuscript in preparation). These subunits are probably arranged with the conserved hydrophobic amino-terminal regions stacked in an overlapping helical array around the long axis of the fimbrial strand (15) and the variable carboxy-terminal regions displayed outwards. The exposed faces of the latter include the immunodominant epitopes, which divert attention from more conserved surface binding sites (41), possibly for glycoproteins on the host cell. These sites, which are not well defined, may themselves vary from species to species, depending on the target host and tissue. For example, \textit{B. nodosus} colonizes the epidermis of the ungulate hoof, \textit{M. bovis} the conjunctiva of cattle, and \textit{N. gonorrhoeae} the human urogenital tract. \textit{P. aeruginosa} itself is an opportunistic pathogen of epithelial surfaces in a variety of compromised hosts. In one case at least, two isolates of the same species (\textit{N. gonorrhoeae}) have been reported to express variant fimbriae with altered binding properties (25). It is also worth noting that one species which appears to have type 4 fimbriae (\textit{P. solanacearum}) is in fact a plant pathogen (17).

The results presented above add weight to the idea that type 4 fimbriae, despite the diversity of species which appear to possess them, represent a cohesive group, which are structurally, functionally, and evolutionarily related. Type 4 fimbriae may be characterized by their polar location on the cell, the twitching-motility phenotype, and the conserved amino-terminal sequence of the structural subunit. The latter characteristic has been detailed in only a few cases thus far, but it would seem relatively safe to predict that this conserved sequence will be found in most, if not all, species which exhibit the former characteristics. Assuming that this holds true, type 4 fimbriae would appear to be very widely distributed among gram-negative bacteria and presumably represent a relatively ancient mechanism for the colonization of eucaryotic tissue. This system is clearly distinct in both sequence and organization from the type 1 and related fimbrial systems found in members of the \textit{Enterobacteriaceae} (9, 24) which probably have a similar biological role. Although the structural subunits of the various families of enterobacterial fimbriae may exhibit greater overall sequence homology among themselves (24, 35, 40) than is observed among type 4 proteins, substitution appears to be possible only between closely related variants (9, 35, 39). This suggests that a significant degree of morphogenetic drift has occurred in the enterobacterial systems, which also implies that their assembly and structure are fundamentally different from that of type 4. In view of the large evolutionary distances between different species which express type 4 fimbriae, the strong conservation of the amino-terminal sequence of the subunit is striking indeed. This would appear to be the consequence of powerful constraints related to the assembly, the structure, and, ultimately, the function of the system. The corollary, substantiated by the present study, is that there should be few, if any, barriers to the morphogenetic expression of type 4 fimbrial subunits in heterologous hosts, and this (i.e., genetic exchange) is one possible route by which variation could be generated in natural populations. In this context there may be no real distinction between intra- and interspecific variants of the type 4 fimbrial subunit, apart from differences in antigenic profile and target specificity.

**Vaccine development**. Fimbriae are major surface antigens, and antibodies against fimbriae generally reduce or prevent infection. This is well documented in the case of \textit{B. nodosus}, in which the fimbriae are known to be the primary serological and protective antigens against footrot (29). Preliminary evidence suggests a similar situation with \textit{N. gonorrhoeae} (7, 41) and \textit{M. bovis} (26). The development of practical vaccines against these pathogens has been hampered by the difficulty in culturing the organisms and the range of serological variants. Again the best-characterized case is \textit{B. nodosus}, of which field isolates have been grouped into eight or nine major serogroups for the purposes of immunological coverage (8). Conventional multivalent whole-cell vaccines against footrot are available, but are relatively crude and expensive. The production of \textit{B. nodosus}-type fimbriae in \textit{P. aeruginosa} provides an alternative route for vaccine production. Vaccination trials have shown that the fimbriae produced by \textit{P. aeruginosa} containing pJSM202 are just as

This system has several biotechnological advantages for the production of type 4 fimbrial vaccines. Serotypic variants may be produced simply by substituting the relevant structural subunit gene into a host-vector expression system as described here. Such genes are relatively easy to clone, and, in the case of *B. nodosus*, must have already been obtained (29). At this stage there is no a priori reason to expect that this system will not be more generally applicable to the production of similar vaccines against other type 4 fimbriated pathogens. The fimbriae may be produced in higher yields than obtained in the natural host, which in many cases have fastidious growth requirements or exhibit unstable or low fimbrial expression. Consequently, representatives for inclusion in a vaccine may be selected solely on their immunological profile rather than on the growth yield properties of the original host strain. Finally, since the variation in the carboxy-terminal region of the structural subunit does not appear to interfere with the process of fimbrial assembly or the characteristics of the fimbrial strand, there is the attractive possibility that coinserion of two or more type 4 fimbrial subunit genes (under appropriate promoter controls) into the same compatible host cell would result in the formation of mixed or hybrid fimbriae, which may then provide a multivalent preparation active against more than one serotype or even perhaps against more than one species.

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