Shared Antigenicity and Immunogenicity of Type 4 Pilins Expressed by *Pseudomonas aeruginosa*, *Moraxella bovis*, *Neisseria gonorrhoeae*, *Dichelobacter nodosus*, and *Vibrio cholerae*

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Immunoblotting with polyclonal rabbit antibodies raised against pilins expressed by *Pseudomonas aeruginosa*, *Moraxella bovis*, *Neisseria gonorrhoeae*, *Dichelobacter nodosus*, and *Vibrio cholerae* was used to demonstrate that these polypeptides display conserved antigenic and, in most cases, immunogenic determinants. These determinants appear to be localized to the highly homologous amino-terminal domains (residues 1 to 25).

The type 4 (N-methyl-Phe) class of bacterial pili is found on a wide variety of gram-negative bacteria (3), including *Moraxella bovis* (9), *Moraxella nonliquefaciens* (6), *Neisseria gonorrhoeae* (7), *Neisseria meningitidis* (7), *Bacteroides nodosus* (12) (now named *Dichelobacter nodosus* [4]), *Pseudomonas aeruginosa* (14), and *Vibrio cholerae* (15). The subunits of these pilus (pilins) share extensive amino-terminal amino acid sequence homology (3), and all the organisms named above except *V. cholerae* contain the modified amino acid N-methyl-phenylalanine as the first residue of the mature protein (7, 12). The functional significance of this conservation has been demonstrated by the ability of *P. aeruginosa* containing plasmids expressing the *D. nodosus* pilin gene to process and assemble those subunits into pilus which are structurally and immunologically indistinguishable from *D. nodosus* pili (5, 11). Similar experiments with *P. aeruginosa* that expressed an *M. bovis* pilin gene resulted in the elaboration of chimeric pilins containing both *M. bovis* and *P. aeruginosa* pilins (1).

Watts et al. (18) demonstrated that *P. aeruginosa* PAK pilus antisera cross-reacted with *N. gonorrhoeae* pilin in immunoblot experiments. More recently, it has been confirmed that the cross-reactive epitopes of gonococcal pilins map to the N-terminal 39 amino acid residues and that proper proteolytic processing at the Gly-3/Phe-4 junction is required to expose or create those epitopes (8). In this study, we extended these findings by examining the ability of antisera raised against purified type 4 pili of *D. nodosus*, *M. bovis*, *N. gonorrhoeae*, *P. aeruginosa*, or *V. cholerae* to cross-react with related pili.

The bacterial strains used in this study were *M. bovis* Epp63 Q and Epp63 I (formerly termed Epp63 beta and Epp63 alpha, respectively), *D. nodosus* 1001 A1, *N. gonorrhoeae* VD302 P++, *P. aeruginosa* PAK, and *V. cholerae* O395. Pili from the following organisms were purified as previously described: *M. bovis* (13), *D. nodosus* (10), *P. aeruginosa* (16), *V. cholerae* (17), and *N. gonorrhoeae* (8).

Antisera were obtained by immunizing rabbits with purified nondenatured pili from the following bacteria by previously described methods: *M. bovis* (9), *D. nodosus* (10), *P. aeruginosa* (16), *V. cholerae* (17), and *N. gonorrhoeae* (8).

Purified pili preparations were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels (Fig. 1A) and visualized by Coomassie blue staining or transferred to nitrocellulose filters for immunoblotting analysis. A 50-ng

![FIG. 1. SDS-polyacrylamide gel electrophoresis and immunoblotting of type 4 pilins. Lanes: 1. *V. cholerae* pili; 2. *M. bovis* Q pili; 3. *M. bovis* I pili; 4, *N. gonorrhoeae* pili; and 5. *P. aeruginosa* PAK pili. (A) Coomassie blue-stained gel; (B) immunoblot with PAK plus rabbit serum; (C) immunoblot with *M. bovis* Q pili rabbit serum; (D) immunoblot with gonococcal plus rabbit serum. Sera were used at a 1:500 dilution.](http://iai.asm.org/Downloaded from http://iai.asm.org)
sample of purified pili was electrophoresed in each lane of a 15% polyacrylamide gel. However, only 10 ng of purified pili was loaded when homologous antiserum was being used, and in the case of M. bovis, only 10 ng of Q pili was used when I antiserum was used and vice versa. Procedures for immunoblotting have been previously detailed (2).

PAK pilus antiserum was tested against the pili described above in immunoblots. Figure 1B shows that while PAK pilus antiserum reacted most strongly with PAK pilin, there was significant cross-reactivity with each of the other 4 pilins. In the case of V. cholerae toxin-coregulated pilin (TCP), only the more slowly migrating of the two bands present in the Coomassie blue-stained gel reacted with PAK pilus antiserum. The reactive species is a mature, processed TCP, while the other species is a degradation product lacking an as-yet-undefined domain of the amino terminus.

Similarly, M. bovis Q pilus antiserum reacted most strongly with Q pilin but cross-reacted with each of the other pilins (Fig. 1C). In contrast, gonococcal pilus antiserum reacted with the homologous pilin and weakly with M. bovis Q pilin but not with other pilins (Fig. 1D). The ability of antiserum raised against D. nodosus 1001 pilin to cross-react with the type 4 pilins described above (Fig. 2A) was also examined. Cross-reactivity varied substantially, with M. bovis Q pilin reacting with the strongest intensity of the nonidentical pilins, with P. aeruginosa, M. bovis I, and gonococcal pilins reacting weakly, and with V. cholerae TCP reacting faintly. Figure 2B shows that antiserum directed against M. bovis Q pilin cross-reacted substantially with D. nodosus pilin. Similar immunoblot experiments revealed that PAK pilus antiserum also cross-reacted with D. nodosus pilin, but antiserum specific for M. bovis I, gonococcal, and V. cholerae TCP all failed to react with D. nodosus pilin. Also, V. cholerae TCP antiserum cross-reacted weakly with M. bovis Q and I pilins but not the other type 4 pilins. Table 1 shows a summary of the results.

It has previously been demonstrated that PAK antiserum fails to react with gonococcal S pilin (a secreted pilin lacking the first 39 amino acids of mature pilin) and with gonococcal mutant pilin that is not proteolytically processed at the Gly\(^{39}\)/Phe\(^{40}\) junction (8). We tested whether this was also true for the antiserum directed against M. bovis Q pilin, which cross-reacted against wild-type gonococcal pilins, and we found that it also failed to cross-react with those altered gonococcal pilin polypeptides (data not shown). Thus, as in the case of PAK pilus antiserum reactivity with gonococcal pilins, the cross-reactive epitopes observed here most likely map to the hydrophobic amino terminus of the pilins. However, the cross-reactivity of several of the other type 4 pilin antiserum with the V. cholerae TCP suggests that the modified amino-terminal residue is not a critical component of these epitopes. While most of these results can be explained by the antigenic cross-reactivity of the amino-terminal domains postulated above, the exclusive reactivity of gonococcal pilus antiserum with M. bovis Q pilin suggests that cross-reactive epitopes mapping outside the conserved amino terminus may also exist. Whether the unilateral nature of some of the cross-reactions results from methodological differences in pilus purification and immunization regimens or from true differences in immunogenicity remains to be determined. These results reveal that the amino-terminal domains of type 4 pilins are antigenically conserved and, in most cases, immunogenic. It is not yet clear whether antibody responses directed at these conserved domains are biologically relevant (i.e., whether these antibodies react with native pili). While not constituting a dominant portion of the immune response, such cross-reactivity may facilitate analysis of pilin processing and the identification of N-methyl-Phe-like proteins in other bacterial species. In addition, the demonstrated conserved antigenicity and immunogenicity of these pilins may be relevant to studies examining type 4 pilin-related immune responses in colonized or infected hosts.

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REFERENCES

TABLE 1. Immunoblot cross-reactions between pili and pilus antiserum*  

<table>
<thead>
<tr>
<th>Pilus antiserum</th>
<th>P. aeruginosa PAK</th>
<th>M. bovis Q</th>
<th>M. bovis I</th>
<th>N. gonorrhoeae VD302</th>
<th>V. cholerae O395</th>
<th>D. nodosus 1001 A1</th>
</tr>
</thead>
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<td>P. aeruginosa</td>
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<td>+</td>
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<td>+</td>
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</tr>
<tr>
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<td>++ ++</td>
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</tr>
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<tr>
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<td>+</td>
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<td>+ /-</td>
<td>+ +</td>
</tr>
</tbody>
</table>

* ++ +, strong reaction; ++, reaction; +, weak reaction; +/-, faint reaction; -, no reaction; ND, not determined.


