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Outer Membranes of Environmental Isolates of *Pseudomonas aeruginosa*

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The outer membrane composition of 30 environmental isolates of *Pseudomonas aeruginosa* was examined. Other than variations in the amounts of lipoprotein H2, there were no major differences in the outer membrane protein or lipopolysaccharide patterns when compared with those of previously studied clinical isolates.

The outer membranes of gram-negative bacteria have significant medical and environmental importance since they constitute a size-dependent permeability barrier which can influence the uptake of antibiotics and substrates (11) and since they often constitute the cellular layer which is in direct contact with the environment. We have previously demonstrated that the outer membrane proteins of *Pseudomonas aeruginosa* are strongly conserved among the 17 different serotype strains (9) and the 30 different clinical isolates of *P. aeruginosa* (3, 7, 8).

In contrast, the outer membrane proteins of *Neisseria* spp. (2, 14), Haemophilus influenzae (1), and *Escherichia coli* (12) show substantial strain-to-strain variation. This raised the question of whether clinical isolates represent a clonotype of *P. aeruginosa* or whether all *P. aeruginosa* strains have similar outer membrane protein patterns. Therefore, we examined the patterns by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and the immunochemistry of the outer membrane proteins of *P. aeruginosa* strains isolated previously from river water in Japan (5).

The environmental isolates were received from J. Y. Homma, Kitasato Institute, Tokyo, Japan, via B. Iglewski, Oregon Health Sciences University, Portland. The 30 serologically distinct strains represented isolates that were collected from 25 rivers in Japan (5). When received by us, all strains proved to be motile, nonmucoid, pigment-producing, gram-negative, oxidase-positive rods. Strains were grown overnight at 37°C in 1% proteose peptone no. 2 medium, diluted 1:20 in a similar medium, and grown to an optical density at 600 nm of 0.6 to 0.9. Outer membranes were isolated and examined by SDS-PAGE as described previously (9).

The outer membrane protein patterns were strongly conserved in all 30 isolates; and proteins E, F, G, H1, H2, and I were identified by SDS-PAGE (Fig. 1A). As described previously for the different serotype strains (9), proteins with mobilities similar to that of protein D2 were observed, but the apparent molecular weight of these bands varied between 46,000 and 49,000. Of the outer membrane proteins listed above, the protein found to vary the most in content was lipoprotein H2. This was confirmed by Western immunoblotting (6) by using a monoclonal antibody specific for protein H2 (8) (Fig. 1C). In total, 15 of the 30 strains underexpressed protein H2 relative to our laboratory wild-type strain H103, a phenomenon that has been observed for only 2 of 50 clinical isolates studied to date (8). In contrast,

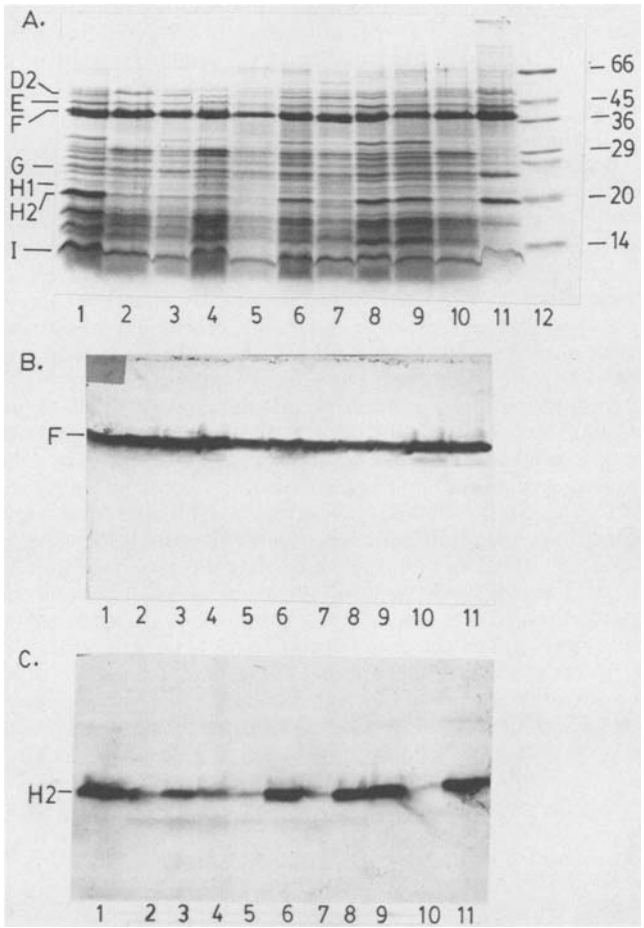

FIG. 1. Outer membrane protein profiles of environmental isolates of *P. aeruginosa* and their reactivities with specific monoclonal antibodies. (A) SDS-polyacrylamide gel electrophoretogram.. (B) Western immunoblot with protein F-specific monoclonal antibody MA5-8 (6) after transfer of separated outer membranes to nitrocellulose. (C) Western immunoblot with protein H2-specific monoclonal antibody MA1-6 (8). Lanes 1 to 10 represent outer membranes from 10 representative environmental isolates. Lanes 11, Outer membrane from our laboratory wild-type *P. aeruginosa* PAO1 strain H103 (6); lane 12, molecular weight markers (in thousands), as indicated to the right of the gel. The positions of major outer membrane proteins are indicated on the left.

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the major outer membrane porin protein F was highly expressed in all of the environmental isolates, as confirmed by Western immunoblotting with the protein F specific (6) monoclonal antibodies MA4-10, MA4-4, and MA5-8 (Fig. 1B). It should be noted that there were some differences in the amounts of other outer membrane proteins (Fig. 1A), but these were not analyzed exhaustively since these proteins have not been described or studied in detail previously.

The outer membrane lipopolysaccharide (LPS) patterns were examined by the method of Hitchcock and Brown (4), which involved periodate treatment and silver staining of SDS-PAGE-separated LPS in outer membrane preparations digested with proteinase K. The LPSs revealed the pattern typical of smooth *P. aeruginosa* strains (13). The majority of the LPS was unsubstituted with O-antigen side chains, whereas approximately 10 to 15% of the LPS species were variably substituted with O-antigen subunits, resulting in a typical ladder pattern (data not shown). These strains represented a variety of serotypes (5), suggesting that their O antigens had different chemical compositions. Nevertheless, the only readily observed differences in LPS patterns were slight alterations in the spacing of the ladder patterns or in the relative mobilities of the most prominent smooth LPS bands. Similar variations have been observed previously for the smooth LPS of clinical strains (3).

These data are in agreement with previous observations which suggest that environmental isolates are not substantially different from those of clinical origin. Thus, there were no substantial differences between clinical and environmental *P. aeruginosa* strains based on the distribution of serotypes (5); production of the extracellular virulence factors phospholipase C, exotoxin A, alkaline protease, and elastase (10); outer membrane protein patterns; antigenicity of proteins F and H2; and LPS patterns. To date, the only marked differences in these properties among clinical *P. aeruginosa* isolates are found in those from the lungs of patients with cystic fibrosis. In these patients, the isolates tend to be nonserotypable or multiagglutinable and contain rough LPS (3). In addition, such isolates often have an unusual mucoid phenotype (3). However, since patients with cystic fibrosis tend to suffer from chronic, long-term *P. aeruginosa* infections, it has been argued that these unusual isolates and the production of mucoid exopolysaccharide result from selective pressures in the lungs during chronic infection (3).

In conclusion, it appears that environmental isolates of *P. aeruginosa* do not differ substantially from most clinical isolates with respect to outer membrane properties.

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