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Monoclonal Antibody for an Outer Membrane Lipoprotein of the *Pseudomonas fluorescens* Group of the Family *Pseudomonadaceae*

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Monoclonal antibody MA1-6 directed against *Pseudomonas aeruginosa* strain PAO1 outer membrane lipoprotein H2 also interacted with a protein having an identical molecular weight from 50 of 52 strains of *P. aeruginosa* tested. This antibody interacted with the *P. fluorescens* group of the family *Pseudomonadaceae* and with *Azotobacter vinelandii* but not with several other species of gram-negative bacteria. A colony blotting assay performed with this antibody allowed rapid detection of the *P. fluorescens*-*A. vinelandii* group.

Currently, the identification of *Pseudomonas* species in clinical laboratories requires a large number of biochemical tests (12, 13). These methods are time consuming, and some tests, especially those used to differentiate glucose-oxidizing *Pseudomonas* species, are not entirely satisfactory (6, 12, 13). In recent years techniques that involve determinations of deoxyribonucleic acid base composition (4), deoxyribonucleic acid-deoxyribonucleic acid hybridization (10), ribonucleic acid homology (1, 11), or fatty acid composition (6, 7) have been developed. Although these techniques have provided useful information for taxonomic grouping of *Pseudomonas* species, they involve expensive reagents or equipment or both and therefore are not readily applicable in clinical laboratories. Thus, there is need for new methods for rapid identification of groups of these bacteria. In this paper we describe the use of a monoclonal antibody specific for a single antigenic epitope on outer membrane protein H2 of *Pseudomonas aeruginosa* as an identification tool.

Monoclonal antibody MA1-6 was obtained from fusion of NS-1 myeloma cells and spleen cells from a mouse immunized with *P. aeruginosa* PAO1 outer membranes (3). This antibody interacted in enzyme-linked immunosorbent assays with strain PAO1 outer membranes and in Western immunoblots with outer membrane peptidoglycan associated-lipoprotein H2 of *P. aeruginosa* (2) (Fig. 1A). The antigenic epitope recognized by monoclonal antibody MA1-6 was present on lipoprotein H2 in the outer membranes from all 17 International Antigen Typing Scheme serotype strains tested (Fig. 1A) and from 28 isolates of *P. aeruginosa* from cystic fibrosis patients. (*P. aeruginosa* type strain ATCC 10145 was not examined in this study.)

To test for the conservation of antigenic epitopes recognized by monoclonal antibody MA1-6 among *Pseudomonas* species and other gram-negative bacteria, the antibody was tested against outer membranes isolated from several other species by both the enzyme-linked immunosorbent assay (9) and the Western immunoblot technique (9) (Table 1).

The cross-reactivity of monoclonal antibody MA1-6 was demonstrated with an outer membrane protein having a molecular weight similar to that of lipoprotein H2 of *P. aeruginosa* in Western immunoblots of outer membranes from *Pseudomonas chlororaphis* ATCC 9446^T (T = type strain), *Pseudomonas fluorescens* ATCC 949 and ATCC 13525^T, *Pseudomonas putida* ATCC 4359 and ATCC 12633^T, and *Pseudomonas syringae* ATCC 19310^T, with a protein having a higher molecular weight in *Pseudomonas stutzeri* ATCC 17588^T, *Pseudomonas anguilliseptica* ET2 and ET7601, and *Azotobacter vinelandii* OP, and with a protein having a slightly lower molecular weight in *Pseudomonas aureofaciens* ATCC 13985^T outer membranes (Fig. 1B). There was no interaction with outer membranes from *Pseudomonas cepacia* ATCC 25416, *Pseudomonas solanacearum* ATCC 11696^T, *Pseudomonas acidovorans* ATCC 9353, *Pseudomonas maltophilia* ATCC 13637^T, *Escherichia coli* CGSC 6044 and PC0479, or *Salmonella typhimurium* LT2 SGSC 205 and SGSC 206 or with outer membranes from any strain belonging to the *Vibrionaceae* or *Aeromonas* group (Table 1).

TABLE 1. Cross-reactivities of monoclonal antibody MA1-6 specific for *P. aeruginosa* outer membrane lipoprotein H2

Antigen from:	Cross-reactivity		
	ELISA reading (absorbance at 405 nm)[a]	Western immunoblot	Colony blot
P. aeruginosa (50 strains)	1.2-0.4	+	+
P. putida ATCC 4359 and ATCC 12633^T	0.4, 0.6	+	+
P. syringae ATCC 19310^T	0.4	+	+
P. chlororaphis ATCC 9446^T	0.3	+	+
P. aureofaciens ATCC 13985^T	0.1	+	+
P. stutzeri ATCC 17588^T	0.3	+	+
P. fluorescens ATCC 949 and ATCC 13525^T	0.5, 0.5	+	+
P. anguilliseptica ET2 and ET7601	0.1, 0.15	+	+
Azotobacter vinelandii OP	0.1	+	+
P. maltophilia ATCC 13637^T	–	–	–
P. acidovorans ATCC 9353	–	–	–
P. solanacearum ATCC 11696^T	–	–	–
Pseudomonas pseudomallei ATCC 23343^T	–	–	–
P. cepacia ATCC 25416	–	–	–
Escherichia coli CGSC 6044 and PC0479	–	–	–
S. typhimurium LT2 SGSC 205 and SGSC 206	–	–	–
Edwardsiella tarda E79054	–	–	–
Vibrio anguillarum ET208 and HT7602	–	–	–
Aeromonas salmonicida NCMB 2020	–	–	–
Aeromonas hydrophila ET2	–	–	–

[a] ELISA, Enzyme-linked immunosorbent assay.

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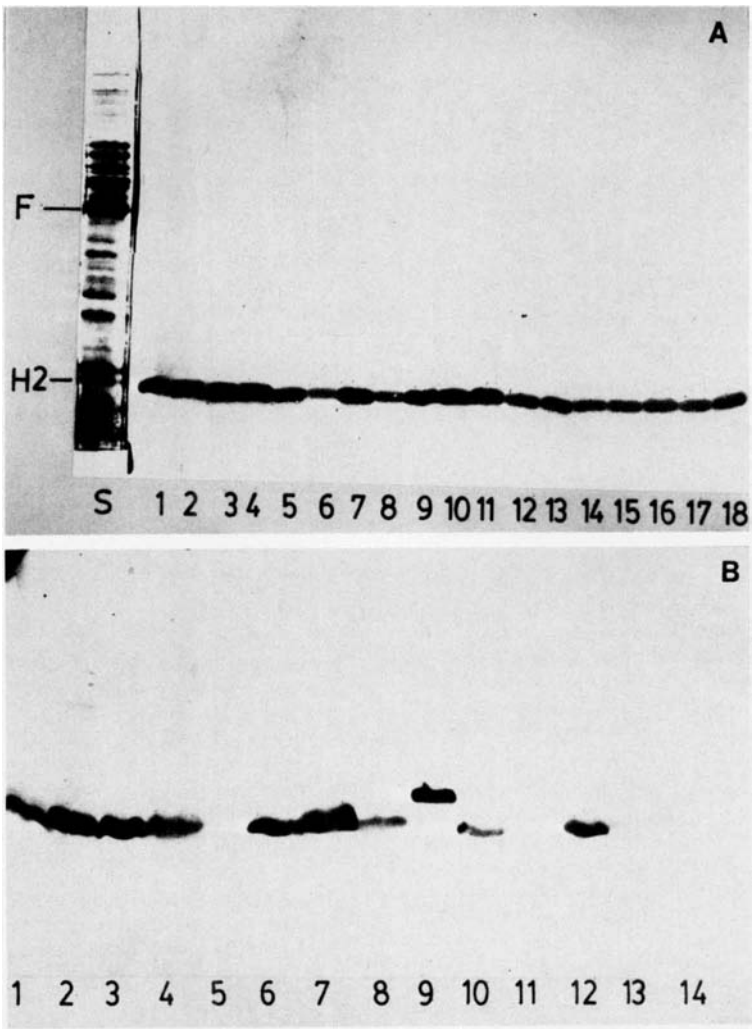

FIG. 1. (A) Western electrophoretic blots of outer membranes of the serotyping strains of *P. aeruginosa*. The blots were treated with antibody MA1-6, followed by goat anti-mouse alkaline phosphatase-conjugated antibody and addition of the substrate (napthol AS MX phosphoric acid and Fast Red TR salt). Lane S, amido black-stained outer membrane profile of our laboratory wild-type strain H103; lane 1, serotype 1; lane 2, serotype 2; lane 3, serotype 3; lane 4, serotype 4; lane 5, serotype 5; lane 6, serotype 6; lane 7, serotype 7; lane 8, serotype 8; lane 9, serotype 9; lane 10, serotype 10; lane 11, serotype 11; lane 12, serotype 12; lane 13, serotype 13; lane 14, serotype 14; lane 15, serotype 15; lane 16, serotype 16; lane 17, serotype 17; lane 18, purified lipoprotein H2. Only one band was labeled. Lane S is a sodium dodecyl sulfate-polyacrylamide gel electrophoretogram of *P. aeruginosa* strain H103 outer membrane proteins stained with Coomassie blue. (B) Western electrophoretic blot showing interaction of monoclonal antibody MA1-6 with outer membranes from *P. anguilliseptica* ET2 (lane 1), *P. aeruginosa* strain H103 (lane 2), *P. fluorescens* ATCC 949 (lane 3), *P. syringae* ATCC 19310^T (lane 4), *P. aeruginosa* CF46nm (lane 5), *P. putida* ATCC 12633^T (lane 6), *P. stutzeri* ATCC 17588^T (lane 7), *P. aeruginosa* ATCC 8689 (lane 8), *Azotobacter vinelandii* OP (lane 9), *P. aureofaciens* ATCC 13985^T (lane 10), *P. cepacia* ATCC 25416 (lane 11), *P. chlororaphis* ATCC 9446^T (lane 12), *P. pseudomonallei* ATCC 23343^T (lane 13), and *P. aeruginosa* ATCC 9721 (lane 14). The blot was developed as described above.

Similar results were obtained by using a colony immunoblot technique (Fig. 2 and Table 1). In this technique, bacterial colonies were transferred from agar plates onto prewashed nitrocellulose paper by contact. After incubation of the filters at 30°C for 30 min, the colony blots were incubated for 45 min at 37°C with tris(hydroxymethyl)aminomethane-buffered saline [TBS; 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.4, 2 mM $MgCl_2$, 100 mM NaCl] containing 3% (wt/vol) gelatin and then for 2 h with the test antibody (MA1-6) diluted in TBS containing 1% (wt/vol) gelatin. The blots were subsequently washed three times by incubation for 15 min in TBS containing 0.1% (wt/vol) gelatin and then were incubated for 2 h at 37°C with a goat anti-mouse immunoglobulin coupled to horseradish peroxidase (Flow Laboratories, Inc., McLean, Va.). After washing as described above, the blots were developed by using a histochemical substrate for peroxidase (5 mg of 4-chloro-1-napthol dissolved in 1.67 ml of ice-cold absolute methanol and mixed at 23°C with 10 ml of TBS containing 5 μl of 30% [vol/vol] H_2O_2). Cell surface-localized components could be differentiated from nonsurface accessible components (which included protein H2) by varying the concentration of NaCl in the TBS from 1 to 100 mM (9a). The colony immunoblot technique provided a rapid and sensitive screening procedure by which a large number of bacterial strains could be tested in a short time.

In agreement with the data described above, Mizuno and Kageyama (5) demonstrated immunoprecipitation of a lipoprotein from the outer membranes of *P. fluorescens* and *P. putida* by using a polyclonal antiserum against *P. aeruginosa*

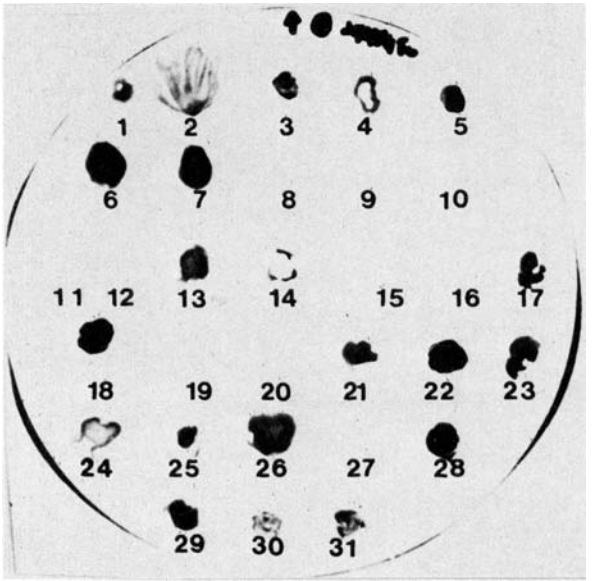

FIG. 2. Colony immunoblot showing interaction of monoclonal antibody MA1-6 specific for protein H2 of *P. aeruginosa* with the following strains: 1, *P. aeruginosa* strain P1 nonmucoid; 2, *P. aeruginosa* strain P1 mucoid; 3 and 4, *P. fluorescens* ATCC 949 and ATCC 13525^T, respectively; 5, *P. aeruginosa* PAO1 strain H103; 6, *P. aeruginosa* strain C1 mucoid; 7, *P. aeruginosa* strain C1 nonmucoid; 8, 9, and 10, *Escherichia coli* strains CGSC 6041, CGSC 6044, and PC0479, respectively; 11, *P. pseudomallei* ATCC 23343^T; 12, *P. solanacearum* ATCC 11696^T; 13 and 14, *P. putida* ATCC 4359 and ATCC 12633^T, respectively; 15 and 16, *S. typhimurium* LT2 strains SGSC 206 and SGSC 227, respectively; 17, *P. aeruginosa* ATCC 8689; 18, *P. aeruginosa* PAO1 strain H103; 19, *Aeromonas salmonicida* NCMB 2020; 20, *Aeromonas hydrophila* ET2; 21, *P. chlororaphis* ATCC 9446^T; 22, *P. aeruginosa* ATCC 19305^T; 23, *P. aeruginosa* strain Z61; 24, *P. aureofaciens* ATCC 13985^T; 25, *P. syringae* ATCC 19310^T; 26, *P. aeruginosa* CF4349; 27, *P. maltophilia* ATCC 13637^T; 28, *P. stutzeri* ATCC 17588^T; 29, *P. aeruginosa* strain H223; 30 and 31, *P. aeruginosa* strains AK1012 and AK1282, respectively. Colonies were transferred from agar plates onto nitrocellulose paper and incubated with 3% gelatin and then with the monoclonal antibody; this was followed by treatment with goat anti-mouse immunoglobulin coupled to horseradish peroxidase and addition of the substrate (4-chloro-1-napthol and H_2O_2).

lipoprotein H2. Recently, Palleroni et al. and DeVos and DeLey suggested that the family *Pseudomonadaceae* consists of a number of distantly related taxonomic groups of bacteria. The bacteria with which monoclonal antibody MA1-6 interacted were placed in the *P. fluorescens* group (also called group 1 pseudomonads); the other *Pseudomonas* species which did not interact with the antibody were placed in taxonomically distinct groups (1, 10, 11). In comparison with these data, monoclonal antibody MA5-8, which is specific for outer membrane protein F, interacted only with *P. aeruginosa* strains (9a), whereas lipopolysaccharide lipid A-specific monoclonal antibody 5E4 interacted with almost all of the gram-negative bacteria tested (8).

The results presented here support the taxonomic data obtained from ribosomal ribonucleic acid homology studies suggesting that *Azotobacter vinelandii* is taxonomically related to the *P. fluorescens* group of the family *Pseudomonadaceae* (1). Thus, monoclonal antibody MA1-6 showed specific interaction with outer membranes and colonies of strains belonging to a single taxonomic subdivision, the group 1 pseudomonads (*P. fluorescens* group), indicating that this antibody has a potential role in the rapid identification of these strains and in taxonomic studies. However, it should be noted that such a method cannot serve as the sole criterion for species identification since a point mutant resulting in loss of the specific antigenic site on protein H2 could cause inaccurate identification. Indeed, we have identified two *P. aeruginosa* strains, strain ATCC 9721 (Fig. 1B, lane 14) and an isolate from a patient with cystic fibrosis, which lacked protein H2 on sodium dodecyl sulfate-polyacrylamide gels and failed to interact with antibody MA1-6. Nevertheless, these 2 strains provided the only exceptions among the 52 *P. aeruginosa* strains examined to date. Thus, we feel that monoclonal antibody MA1-6 provides a useful screening tool for the *P. fluorescens* group of the family *Pseudomonadaceae*. We are currently attempting to deposit this antibody with the American Type Culture Collection.

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