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Serological Classification of *Xanthomonas maltophilia* (*Pseudomonas maltophilia*) Based on Heat-Stable O Antigens

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Twenty-six serotypes of *Xanthomonas maltophilia* were defined by using 15 antisera described by Hugh and Ryschenkow (R. Hugh and E. Ryschenkow, J. Gen. Microbiol. 26:123–132, 1961) and 11 new antisera. The antisera were prepared by immunizing rabbits with bacterial strains heated at 100°C for 2 h. Twelve antisera required adsorptions with cross-reacting heterologous immunizing strains. We tested 275 clinical and environmental strains of *X. maltophilia* with 26 antisera by the slide agglutination technique. A total of 259 (94.2%) strains were typeable, with 137 (49.8%) strains agglutinating in three antisera.

Xanthomonas maltophilia is an important nosocomial pathogen which is isolated from a wide variety of clinical sources, including blood, the respiratory tract, urine, wounds, and spinal fluid, and from environmental sources such as hospital water supplies, faucets, sink drains, respirators, and disinfectant solutions (3–5, 7, 9, 14, 17, 19, 21, 23). *X. maltophilia* is also associated with malignant lesions (16). The rate of isolation is increasing (14, 15), and it is often resistant to antimicrobial agents that are commonly used initially to treat gram-negative infections (2, 6, 13, 14, 18, 22, 23).

This study continues the work begun by Hugh and Ryschenkow (10), in which they described 15 serotypes of *X. maltophilia* on the basis of somatic antigens. These 15 serotypes were among those detected in 26 strains of *X. maltophilia*. We used the antisera that were produced by Hugh and Ryschenkow (10) and prepared 11 additional antisera to new O antigens. The purpose of this study was to develop a serological typing scheme for *X. maltophilia* that could be used in the future as an epidemiological typing screen. We present here the results of serologic reactions of 275 clinical and environmental isolates of *X. maltophilia*.

MATERIALS AND METHODS

Cultures. Two hundred seventy-five strains of *X. maltophilia* were obtained from diverse geographical areas of the United States. Of these strains, 211 were from human and 16 were from environmental sources; the sources of 48 strains were not known. The immunizing strains used by Hugh and Ryschenkow (10) were from the culture collection of one of us (R.H.). Strains were stored at −70°C in defibrinated rabbit blood. Identification of the isolates was confirmed by using conventional methods (1).

Preparation of antigens. Immunizing antigens for the production of O antisera were prepared as described previously (10), except that broth cultures were incubated at 35°C. Strains selected for production of O antisera were not agglutinated in antisera prepared by Hugh and Ryschenkow (10).

O antigens for slide agglutination were prepared as follows. The strains to be serotyped were removed from the freezer, streaked on Trypticase soy agar–5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) for isolation, and incubated at 35°C for 18 to 24 h. Several morphologically similar colonies were inoculated into 10 ml of veal infusion broth (Difco Laboratories, Detroit, Mich.). The inoculated broth was incubated at 35°C for 18 to 24 h. After incubation, the broth culture was centrifuged at 1,126 × *g* for 15 min, the supernatant was discarded, and 2 ml of 0.01 M phosphate-buffered saline (pH 7.20) was added to the sedi-

TABLE 1. Antisera used in typing *X. maltophilia*

Proposed serotype	Immunizing strain	Source of antiserum[a]	Adsorbing strain(s)	Titer
1	873-3[b]	CDC	None	16
2	447[b]	CDC	611, 229, 788-3	64
3	PM-56	CDC	None	16
4	363-4[b]	CDC	PM-97, 557	16
5	229	CDC	447, 788-3	32
6	557[b]	CDC	None	16
7	PM-97	CDC	363-4	32
8	810-2[b]	HR	None	16
9	556[b]	CDC	None	16
10	558[b]	HR	None	32
11	294[b]	HR	None	64
12	555[b]	CDC	None	16
13	109-4[b]	HR	None	8
14	653-4[b]	HR	None	8
15	611[b]	CDC	None	32
16	560[b]	CDC	788-3	32
17	PM-61	CDC	447, 229, 558, PM-56	8
18	788-3[b]	CDC	447, 810-2, 611	32
19	609[b]	HR	None	32
20	PM-143	CDC	PM-97	64
21	PM-41	CDC	None	64
22	PM-62	CDC	810-2, 556	16
23	PM-94	CDC	556, 611	32
24	PM-283	CDC	None	32
25	PM-219	CDC	873-3, 447, PM-56, 611, 560	32
26	PM-225	CDC	611	16

[a] Abbreviations: CDC, Centers for Disease Control; HR, Hugh and Ryschenkow.
[b] *X. maltophilia* strains used by Hugh and Ryschenkow to prepare antisera.

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TABLE 2. Results of O agglutination of 275 *X. maltophilia* isolates from human and environmental sources

Serotype	Immunizing strain	No. of isolates from source[a]:										% of total
		BL	WO	UR	RE	CSF	TI	Misc.	EN	UK	Total	
1	873-3	0	0	0	0	0	0	0	0	0	0	0
2	447	0	1	0	0	1	0	0	0	0	2	0.7
3	PM-56	33	3	3	7	0	2	0	8	1	57	20.7
4	363-4	2	0	0	2	0	1	0	0	0	5	1.8
5	229	0	0	1	0	0	0	1	1	1	4	1.5
6	557	1	0	2	0	0	0	1	1	2	7	2.5
7	PM-97	0	0	0	0	0	0	0	1	1	2	0.7
8	810-2	1	2	1	2	0	3	1	0	0	10	3.6
9	556	0	0	1	1	0	0	1	0	5	8	2.9
10	558	7	7	1	17	0	1	0	1	8	42	15.3
11	294	0	0	0	0	0	0	0	0	0	0	0
12	555	2	0	1	3	0	0	1	0	1	8	2.9
13	109-4	1	2	0	4	0	1	3	0	1	12	4.4
14	653-4	1	0	3	1	0	0	0	0	0	5	1.8
15	611	0	0	1	2	0	0	0	1	4	8	2.9
16	560	1	0	1	4	0	0	0	0	2	8	2.9
17	PM-61	2	0	1	0	0	0	1	0	1	5	1.8
18	788-3	1	1	1	3	0	0	0	0	4	10	3.6
19	609	10	3	3	9	1	0	2	0	10	38	13.8
20	PM-143	0	1	2	3	0	0	0	0	0	6	2.2
21	PM-41	0	0	0	0	0	0	0	0	1	1	0.4
22	PM-62	1	3	0	2	0	0	1	0	3	10	3.6
23	PM-94	0	0	0	0	0	0	0	1	1	2	0.7
24	PM-283	0	0	1	0	0	1	1	0	0	3	1.1
25	PM-219	0	0	0	0	0	0	0	0	0	0	0
26	PM-225	2	0	0	3	1	0	0	0	0	6	2.2
Autoagglutination		0	0	0	0	0	0	1	2	0	3	1.1
Polyagglutination		2	1	0	1	0	0	0	0	0	4	1.5
Nontypeable		2	0	1	3	0	0	1	0	2	9	3.3
Total		69	24	24	67	3	9	15	16	48	275	99.9

[a] Abbreviations: BL, blood; WO, wound; UR, urine; RE, respiratory tract; CSF, spinal fluid; TI, tissue; Misc., miscellaneous; EN, environmental; UK, unknown.

ment. The contents of the tube were vortexed for several seconds and then placed in flowing steam (100°C) for 2 h. The tube was cooled, and the suspension was thoroughly mixed. The turbidity of the cell suspension was adjusted to approximately equal a no. 8 McFarland standard. If the slide agglutination test could not be performed on the same day the antigen was prepared, 6 μl of commercial 40% (wt/vol) formaldehyde was added to the antigen, which was then stored at 4°C, and the strain was serotyped on the following day.

Preparation of antisera. Antisera were produced in New Zealand White rabbits, which were injected intravenously at 4-day intervals with 0.5, 1.0, 2.0, 2.0, and 2.0 ml of cell suspensions. Six days after the last injection, the animals were bled. Preimmunization serum was obtained from each animal and tested for agglutination with the immunizing strain. To preserve antisera, thimerosal (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1:10,000. The undiluted antisera were stored at −20°C, and the diluted antisera were stored at 4°C.

Titration of antisera. The slide agglutination technique was used to titrate the antisera. Serial twofold dilutions starting at 1:2 were prepared with 0.5% phenolized saline. Each antiserum was titrated with the homologous immunizing strain, and the reciprocal of the highest dilution that gave a strong positive reaction within 1 min was determined to be the antibody titer. The working dilution of antisera was one dilution less than the endpoint titer.

Slide agglutination technique. Tests were performed by adding one drop of antigen suspension to single drops of each of the 26 antisera. The slide was tilted back and forth for 1 min and then examined for evidence of agglutination. All strains used in this study were serotyped at least two times on different dates.

Adsorption of O antisera. Antisera that reacted with heterologous immunizing strains were adsorbed with the cross-reacting strains. The adsorbing strain was grown in veal infusion broth incubated at 35°C for 18 to 24 h. The entire surface of each of five plates (100-mm diameter) of veal infusion agar (Difco Laboratories, Detroit, Mich.) was inoculated with a swab dipped in the broth culture. The plates were incubated at 35°C for 18 to 24 h. Then, approximately 3 ml of 0.01 M phosphate-buffered saline (pH 7.20) was added to each of the plates, and the growth was suspended with the aid of a cell scraper (Costar, Cambridge, Mass.). The cell suspension was transferred to a 50-ml test tube and vortexed for several seconds. The suspension was placed in flowing steam (100°C) for 2 h, cooled, and centrifuged at 7,700 × *g* for 15 min. The supernatant was discarded. Five milliliters of the antiserum to be adsorbed was diluted 1:2 with sterile 0.85% saline and then added to the sedimented cells. The resulting adsorption mixture contained approximately 10% (vol/vol) bacterial cells. (Antisera requiring adsorptions with two or more cross-reacting strains were adsorbed in a single step in a tube containing sedimented cells of all the required strains.) After thorough mixing, the antiserum and cells were incubated in a 48°C water bath for 2 h and then incubated overnight at 4°C. Following centrifugation (12,000 × *g*), the antiserum was filter sterilized by using a 0.22-μm-pore-size membrane filter and then tested by slide agglutination with the homologous and adsorbing strain(s).

RESULTS AND DISCUSSION

Twenty-six antisera (Table 1) were used to type 275 isolates of *X. maltophilia*. Six of the antisera were included in the study by Hugh and Ryschenkow (10), and 20 were prepared in this study. Twelve of the antisera exhibited a cross-reaction with one or more of the heterologous immunizing strains and were adsorbed to remove the cross-reacting factors. Designations of the antisera, immunizing strains, and adsorbing strains are listed in Table 1. The titers of the antisera in this study ranged from 8 to 64 by using the slide agglutination test. The titers of antisera prepared by R. Hugh in 1961 (10) were comparable to the titers of antisera prepared in this study. By using adjuvants or changing the immunization schedule, future studies are planned to reduce the number of cross-reactions and to increase titers. An unfortunate consequence of increased titers of antisera reported by some investigators is a corresponding increase in the number of cross-reactions (11, 12).

Strains 609 and 229 agglutinated in antiserum 609 (10). We produced an antiserum to strain 229 (serotype 5) and were not able to detect cross-reactivity between these strains. We therefore believe that 229 and 609 were shown to be distinct serotypes by the slide agglutination technique. We postulate that perhaps in some manner the antigenic structure of strain 229 has changed.

The results of slide agglutination of 275 *X. maltophilia* strains from human and environmental sources are presented in Table 2. Overall, 259 (94.2%) of the strains could be typed. Nine (3.3%) strains did not agglutinate in the 26 antisera when tested a minimum of three times. Three (1.1%) strains isolated from the same institution could not be typed because they autoagglutinated. Two of them were from solutions for injection and one was from an adrenal cortex. Four (1.5%) cultures agglutinated in two or more antisera (polyagglutination). Of the 259 typeable strains, 163 (62.9%) agglutinated in one of the antigenic types described by Hugh and Ryschenkow (10), and the remaining 96 (37.1%) were typeable with the new antisera.

One hundred thirty-seven (49.8%) of the *X. maltophilia* strains agglutinated in antisera PM-56 (serotype 3), 558 (serotype 10), and 609 (serotype 19). Serotype 3 was most frequently encountered and included 57 (20.7%) strains, over half of which were isolated from blood. Thirty of the serotype 3 strains were isolated during an epidemiological investigation of pyrogenic reactions in a hemodialysis unit. No isolates representing serotypes 1 (873-3), 11 (294), or 25 (PM-219) were found among the 275 *X. maltophilia* strains examined.

The reproducibility of agglutination reactions was 100% on at least two different dates (data not shown), with intervals between repeat agglutination reactions ranging from 2 weeks to over 1 year.

This typing set of 26 antisera was primarily intended to serve as a screen to differentiate between strains of *X. maltophilia*. Its value as an epidemiological tool needs to be determined by further application to strains from outbreaks. With more sophisticated techniques such as rRNA typing (8) or isoenzyme electrophoretic typing (20), the subdivision of strains within serotypes could be possible. Our future plans include the study of additional strains and identification of new serotypes of *X. maltophilia*.

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