Serological Typing of 31 Achromogenic and 40 Melanogenic Pseudomonas aeruginosa Strains

EIKO YABUUCHI, NORIKO MIYAJIMA, HISAKO HOTTA, AND YOICHI FURU
Kansai Medical School, Department of Microbiology, Moriguchi-city, Osaka 570, Japan

Received for publication 29 June 1971

Thirty-one achromogenic and 40 melanogenic Pseudomonas aeruginosa strains were studied with 10 monovalent typing sera (3). Twenty-one of the achromogenic (67.7%) and seven of the melanogenic (17.5%) strains were agglutinated by one of the 10 typing sera. Ten achromogenic and 33 melanogenic strains were not agglutinated by any of the 10 typing sera. As far as this set of antisera is concerned, the typability of achromogenic and melanogenic P. aeruginosa strains appears to be much lower than that of the chromogenic, nonmelanogenic strains of the species reported previously.

In an attempt to standardize the serological typing of Pseudomonas aeruginosa for epidemiological studies, Homma et al. (3) selected 10 strains to prepare a set of 10 monovalent antisera. Antisera were distributed among laboratories, including hospital laboratories, in Japan. A summary of results recorded in various laboratories indicated that 87.2% of 915 chromogenic, nonmelanogenic P. aeruginosa strains were typable. The purpose of this report is to describe the typability of achromogenic and melanogenic strains of P. aeruginosa with Homma’s set of 10 antisera, because neither achromogenic nor melanogenic strains were included in the study by Homma et al. (personal communication; 3).

MATERIALS AND METHODS

The histories and corresponding strain numbers of 71 P. aeruginosa strains (31 achromogenic and 40 melanogenic) are listed in Tables 1 and 2. They were reidentified morphologically and biochemically as P. aeruginosa by the criteria of Hugh (4). Strains which produced no pigment on Pseudomonas agar P (Difco catalog no. 0449) and Pseudomonas agar F (Difco catalog no. 0448) were regarded as achromogenic. The ability to produce melanin was determined by inoculating a glutamate-tyrosine agar slant. The medium contained: sodium glutamate, 10 g; L-tyrosine, 2 g; potassium phosphate (monobasic), 2 g; sodium chloride, 5 g; agar, 15 g; and distilled water, 1 liter; the pH was adjusted to 7.0 with sodium hydroxide. When the medium was heavily inoculated from a 24-hr slant culture of a melanogenic P. aeruginosa strain, a water-soluble red color developed throughout the entire slant within 4 hr at 37°C. The medium became coffee-brown after 18 to 24 hr of incubation. Nonmelanogenic, pyorubin-producing P. aeruginosa strains do not produce a red color in the medium within 24 hr.

A set of 10 monovalent antisera (T1-T10) was supplied by J. Y. Homma, Institute of Medical Science, University of Tokyo, Japan.

Antigen for the tube agglutination test was prepared from an infusion slant culture incubated at 30°C for 24 hr. Growth was suspended in 3 ml of 0.15 M phosphate-buffered saline (pH 7.0) and autoclaved at 120°C for 90 min. The autoclaved suspension was centrifuged, supernatant fluid was decanted, and the sediment was resuspended in 1 ml of buffered saline. One drop of heated antigen suspension was added to 0.2 ml of each antiserum. Tubes were incubated at 37°C and examined for agglutination after 2 and 20 hr (3).

RESULTS

Twenty-one (67.7%) of the 31 achromogenic strains were agglutinated by one of the 10 antisera, and 10 strains failed to be agglutinated by any of the 10 antisera. The serotypes of the 21 strains are listed in Table 3. Seven (17.5%) of the 40 melanogenic strains were agglutinated by one of the 10 antisera; the serotypes of these 7 strains and the data reported by Homma et al. (3) are also listed in Table 3. None of the strains agglutinated spontaneously, and none was agglutinated by two or more antisera.

DISCUSSION

The achromogenic and melanogenic strains of P. aeruginosa were serotyped less frequently than the chromogenic, nonmelanogenic P. aeruginosa strains described by Homma et al. (3). Habs and Habs (2) reported that the 12 melanogenic strains of P. aeruginosa share thermostable O-group 7 antigen. These 12 strains were included in our studies and were not agglutinated by any of the 10 monovalent antisera.
Table 1. Histories and corresponding strain numbers of 31 achromogenic Pseudomonas aeruginosa strains

| KM | ATCC | NRRL | CDC | Other | Received from |
|----|------|------|-----|-------|---------------|
| 058 | 25005 |      |     |       |   | Osawa |
| 065 |      |      |     |       |   | Osawa |
| 142 |      |      |     |       |   | Osawa |
| 158 |      |      |     |       |   | Homma |
| 168 |      |      |     |       |   | Homma |
| 170 |      |      |     |       |   | Homma |
| 176 |      |      |     |       |   | Homma |
| 325 |      |      |     |       |   | Takeda |
| 326 |      |      |     |       |   | Jessen, Caselitz |
| 327 |      |      |     |       |   | Jessen |
| 330 | 256b | B-248 |     |       |   | Haynes, Hugh |
|     | 17434b |      |     |       |   | Stanier |
| 656 |      |      |     |       |   | Stanier |
| 745 |      |      |     |       |   | Wada |
| 841 |      |      |     |       |   | Caselitz |
| 842 |      |      |     |       |   | Caselitz |
| 843 |      |      |     |       |   | Caselitz |
| 844 |      |      |     |       |   | Caselitz |
| 845 |      |      |     |       |   | Caselitz |
| 849 |      |      |     |       |   | Caselitz |
| 850 |      |      |     |       |   | Caselitz |
| 853 | 142  | B-12  |     |       |   | Haynes, Hugh |
| 858 | 262  | B-250 |     |       |   | Hugh |
| 860 |      |      |     |       |   | Hugh |
| 869 |      |      |     |       |   | Naito |
| 903 |      |      |     |       |   | Naito |
| 909 |      |      |     |       |   | Naito |
| 910 |      |      |     |       |   | Naito |
| 919 |      |      |     | 4601 (1) |   | Weaver |
| 920 |      |      |     | 6990 (1) |   | Weaver |
| 921 |      |      |     | 9905   |   | Weaver |
| 922 |      |      |     | A1466  |   | Weaver |

*Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CCTM, Centre de Collection de Types Microbiens, Institut Pasteur de Lille (Nord); CDC, Center for Disease Control, Atlanta, Ga.; IFO, Institute for Fermentation, Osaka, Japan; KM, Kansai Medical School Culture Collection, Osaka, Japan; NCTC, National Collection of Type Cultures, London, England; NRRL, Northern Utilization Research and Development Division, Peoria, Ill.; PJ, Ove Jessen, Copenhagen, Denmark; RH, Rudolph Hugh, George Washington University, Washington, D.C.

b Two ATCC numbers for the same strain (personal communication from H. D. Hatt).
Strain KM330 was agglutinated by T7 antiserum; it was first received from Stanier in January 1968 and was preserved in semisolid motility medium (4) at room temperature. A slant culture of this strain was received from Hugh in November 1970, and a lyophile was received from Haynes in December 1970. Strain KM325, agglutinated by T8 antiserum, was supplied by Jessen as a lyophile in December 1967, and a stock culture was maintained at room temperature. Caselitz sent the same strain in stabbed culture in December 1970. To avoid changes in serotype resulting from frequent subculture, Homma et al. (3) strongly recommended that the strains be preserved in a frozen or freeze-dried state. Serological variation was not demonstrable between lyophilized specimens and room temperature stock cultures of strains KM330 and KM325.

Since 1957, several investigators have studied the serology of *P. aeruginosa* (1–3, 5–9). It is desirable that each of the serotypes of *P. aeruginosa*, like those of salmonellae and shigellae, be fixed by international agreement.

ACKNOWLEDGMENTS

We are grateful to those who supplied strains, and we also thank J. Y. Homma for the antisera.

---

**Table 2. Histories and corresponding strain numbers of 40 melanogenic *Pseudomonas aeruginosa* strains**

| KM | ATCC | RH | NCTC | CDC | Other | Received from |
|----|------|----|------|-----|-------|---------------|
| 117 | 23993 | 1711 |      |      |       | Inoue         |
| 233 | 23994 | 1419 |      |      |       | Hugh          |
| 255 | 23268 | 2582 | 10780|      |       | Habs          |
| 256 | 23267 | 2583 | 10781|      |       | Habs          |
| 257 | 23996 | 1712 |      |      |       | Habs          |
| 272 | 23997 | 2409 |      |      |       | Habs          |
| 273 | 23998 | 2537 |      |      |       | Habs          |
| 303 | 23999 |      |      |      |       | Habs          |
| 350 | 25000 | 2682 |      |      |       | Habs          |
| 357 | 25001 | 2684 |      |      |       | Habs          |
| 374 | 25002 | 2686 |      |      |       | Habs          |
| 386 |      | 2693 |      |      |       | Habs          |
| 400 |      |      |      |      |       | Habs          |
| 419 |      |      |      |      |       | Habs          |
| 432 |      |      |      |      |       | Habs          |
| 433 |      |      |      |      |       | Habs          |
| 434 |      |      |      |      |       | Habs          |
| 435 |      |      |      |      |       | Habs          |
| 436 |      |      |      |      |       | Habs          |
| 437 |      |      |      |      |       | Habs          |
| 438 |      |      |      |      |       | Habs          |
| 439 |      |      |      |      |       | Habs          |
| 440 |      |      |      |      |       | Habs          |
| 441 |      |      |      |      |       | Habs          |
| 442 |      |      |      |      |       | Habs          |
| 443 |      |      |      |      |       | Habs          |
| 658 |      | 2817 | 10783|      |       | Habs          |
| 678 |      |      |      |      |       | Habs          |
| 727 |      |      |      |      |       | Habs          |
| 728 |      |      |      |      |       | Habs          |
| 729 |      |      |      |      |       | Habs          |
| 730 |      |      |      |      |       | Habs          |
| 731 |      |      |      |      |       | Habs          |
| 732 |      |      |      |      |       | Habs          |
| 733 |      |      |      |      |       | Habs          |
| 735 |      |      |      |      |       | Habs          |
| 736 |      |      |      |      |       | Habs          |
| 760 | 19582 | 6749 |      |      | NRRL B-997 | Lapage, Haynes |
| 873 |      |      |      |      |       | von Graevenitz |
| 901 |      |      |      |      |       | Wada          |

* See Table 1 for key to abbreviations.
TABLE 3. Distribution of serotypes of 21 achromogenic, 7 melanogenic, and 656 chromogenic, nonmelanogenic* P. aeruginosa strains

| Strains                     | Serotype | Total no. and percentages |
|-----------------------------|----------|---------------------------|
|                             | T1  | T2  | T3  | T4  | T5  | T6  | T7  | T8  | T9  | T10 | Typed | Not typed |
| Achromogenic                |     |     |     |     |     |     |     |     |     |     |        |            |
| KM strain numbers           | 849 | 158 | 058 | 065 | 170 | 656 | 845 | 903 |     |     | 21     | 10          |
| Total no. of strains        | 2   | 2   | 7   | 5   | 3   | 2   | 0   | 0   |     |     | 67.7   | 32.3        |
| Total serotyped (%)         | 6.5 | 6.5 | 22.5| 16.1| 9.7 | 6.5 | 0   |     |     |     |        |            |
| Melanogenic                 |     |     |     |     |     |     |     |     |     |     |        |            |
| KM strain numbers           | 678 |     | 727 |     | 255 |     | 729 |     |     |     |        |            |
| Total no. of strains        | 0   | 2.5 | 0   | 5   | 0   | 7.5 | 0   | 2.5 |     |     | 17.5   | 72.5        |
| Total serotyped (%)         | 0   | 2   | 0   | 3   | 1   | 7    |     |     |     |     |        |            |
| Chromogenic, non-melanogenic*|     |     |     |     |     |     |     |     |     |     | 656    | 117         |
| Total no. of strains        | 60  | 7   | 15  | 41  | 149 | 23  | 99  | 88  | 74  | 100 |        |            |
| Total serotyped (%)         | 6.6 | 0.8 | 1.6 | 4.5 | 16.3| 2.5 | 10.8| 9.6 | 8.1 | 10.9| 71.7   | 12.8        |

* Data from Homma et al. (3).

LITERATURE CITED
1. Habs, I. 1957. Untersuchungen über die O-Antigen von Pseudomonas aeruginosa. Z. Hyg. Infektionskr. 144:218-228.
2. Habs, H., and M. Habs. 1968. Ueber das thermostabile Antigen der melaninbildenden Stimme von Pseudomonas aeruginosa. Zentralbl. Bakteriol. Abt. 1 Orig. 208:283-288.
3. Homma, J. Y., K. S. Kim, H. Yamada, M. Ito, H. Shionoya, and Y. Kawabe. 1970. Serological typing of Pseudomonas aeruginosa and its cross-infection. Jap. J. Exp. Med. 40:347-359.
4. Hugh, R. 1970. Pseudomonas and Aeromonas, p. 175-190. In J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
5. Lányi, B. 1966. Serological properties of Pseudomonas aeruginosa. Acta Microbiol. Acad. Sci. Hung. 13:295-318.
6. Lányi, B., M. Gregács, and M. M. Adám. 1966. Incidence of Pseudomonas aeruginosa serogroups in water and human faeces. Acta Microbiol. Acad. Sci. Hung. 13: 319-326.
7. Matsumoto, H., T. Tazaki, and T. Kato. 1968. Serological and pyocine types of Pseudomonas aeruginosa from various sources. Jap. J. Microbiol. 12:111-119.
8. Muraschi, T. F., D. M. Bolles, C. Moczulski, and M. Lindsay. 1966. Serologic types of Pseudomonas aeruginosa based on heat-stable O antigens: correlation of Habs' (European) and Verder and Evans' (North American) classifications. J. Infec. Dis. 116:84-88.
9. Verder, E., and J. Evans. 1961. A proposed antigenic schema for the identification of strains of Pseudomonas aeruginosa. J. Infec. Dis. 109:183-193.