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Bacteriocin Typing of *Serratia marcescens* Isolates of Known Serotype/-Group

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The number of isolates of *Serratia marcescens* that could be typed by sensitivity to bacteriocins was compared with the nature of the serotype/-group of each of the isolates. Ninety-four of 101 isolates (93.1%) could be bacteriocin-typed; this compares with 80 of the isolates (79.2%) that had been serotyped, and with 91 of the isolates (90.1%) that carried determinable O antigens. It is recommended that bacteriocin typing of *S. marcescens* be adopted by reference laboratories, because this technique is simple, inexpensive, and appears to be of somewhat higher epidemiological resolution than classic serological procedures.

Recently we developed a simple, inexpensive, apparently reproducible technique for typing clinical isolates of *Serratia marcescens* based on bacteriocin sensitivity (4, 5). Unfortunately, specific antisera were not available to us; therefore, it was not possible to determine whether given bacteriocin types of this organism correspond to certain serotypes/-groups, or whether certain serotypes can be further divided into different bacteriocin types analogous to colicin typing of *Shigella sonnei* isolates (1). To date, 15 somatic (O) antigens and 13 flagellar (H) antigens have been defined among isolates of *S. marcescens* (2). For purposes of this study, B. Davis and G. J. Hermann of the Center for Disease Control (CDC), Atlanta, Ga., kindly provided us with isolates of *S. marcescens* of known serotype/-group from their collection. Here we wish to report comparative results obtained with our technique of bacteriocin typing.

MATERIALS AND METHODS

Bacteria. A total of 101 isolates of *S. marcescens* were received from B. Davis and G. J. Hermann, Enterobacteriology Unit, CDC, Atlanta, Ga., in August 1971. The isolates had been submitted to the CDC from various states during the period of 1969 to 1971. The isolates had been coded. After receipt, the isolates were checked for viability and purity by subculture to MacConkey agar (Fisher Scientific Co., Raleigh, N.C.). The isolates were screened for deoxyribonuclease production, employing deoxyribonuclease test agar (Difco Laboratories, Detroit, (Mich.); the plates were flooded with 1 N HCl after incubation at 33 C for 18 hr (3). All isolates proved deoxyribonuclease-positive. The isolates were maintained on Brain Heart Infusion agar slants (Difco) at 4 C.

Bacteriocin typing. The isolates were typed according to their sensitivity to bacteriocins as previously described (5). After completion of the experiments, the results obtained were sent to the CDC; in turn, we were informed as to the nature of the serotype/-group of each of the isolates.

RESULTS

All but 7 of the 101 isolates (93.1%) could be typed with our routine bacteriocin typing procedure. Shown in Table 1 are the distributions of the various serotypes/-groups of *S. marcescens* into bacteriocin types. Of the 101 isolates, one isolate carried indeterminate O and H antigens, and this isolate proved refractory to the killing action of all 10 bacteriocins employed. Nine additional isolates were O-nontypable; of these, six isolates were bacteriocin-typable, and three were tolerant to all bacteriocins. Twenty of the isolates were H-nontypable, but 19 of these proved susceptible to one or more bacteriocins.

The majority of the isolates were of bacteriocin types 1, 4, 9, 14, 15, 16, 18, and 21, a finding in agreement with the data for isolates of *S. marcescens* from our clinical material (4), except that we had encountered only one previous isolate of *S. marcescens* of bacteriocin type 15.

The majority of the isolates examined were of serogroups O2, 3, 4, 5, 6, and 14. Yet isolates of any one of these six serogroups could be further subdivided into at least four or more bacteriocin types, a rate comparing favorably with that achieved through the determination of

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TABLE 1. *Distribution of serotypes/-groups of Serratia marcescens into bacteriocin types*

Bacteriocin type	Serotypes/-groups	Total
1	O2:H1 (9 isolates); O15:H5	10
4	O5:H1; O undet.:H1 (2 isolates); rel. O5:H13; O5:H11; O5:H13; rel. O5:H1 (2 isolates); O14:H12	9
7	O4:H1 (2 isolates)	2
9	O14:H10 (3 isolates); O14:H4 (2 isolates); O14:H12 (2 isolates); O14:H undet.; rel. O14:H8; O6:H1	10
10	O4:H1	1
12	O14:H5	1
13	O3:H12; O undet.:H12	2
14	rel. O2:H undet.; O3:H5; O3:nonmotile; O6:H undet.; O undet.:H1; O14:H5 (3 isolates); O14:H undet. (2 isolates)	10
15	O undet.:H5; O2:H undet.; O2:nonmotile; rel. O2:H4; rel. O2:nonmotile; O2:H5 (2 isolates); O3:H5 (3 isolates); O3:H undet.; O3:nonmotile; O6:nonmotile; O14:H5 (2 isolates); O14:H undet.; O14:nonmotile	17
16	O1:H5 (2 isolates); O5:H6; O5:H undet.	4
17	O3:H1 (3 isolates)	3
18	O14:H4 (2 isolates); O14:H12 (4 isolates)	6
21	O5:H1; O5:nonmotile; O6:H2 (2 isolates); O6:H undet.; O14:H11	6
23	O14:H3	1
24	O4:H4; O13:H4	2
25	O undet.:H10	1
33	O1:H undet.	1
37	O1:H4; rel. O2:H1	2
39	O14:H11	1
43	O14:H undet.	1
44	O4:H12; rel. O4 + O15:H12	2
45	O5:H2 (2 isolates)	2
Nontypable	O12:H undet.; rel. O13:H10; O14:H5; O undet.:H6 (2 isolates); O undet.:H undet.; O undet.:H11	7
Total		101

flagellar antigens, i.e., serotyping. Serogroup O14 consisted of 31 isolates; with the exception of one nontypable isolate, the isolates could be subdivided into 10 different bacteriocin types, as compared with 7 serotypes (H antigens 3, 4, 5, 8, 10, 11, and 12). Comparison of the bacteriocin sensitivity/tolerance patterns (4) of bacteriocin types 4, 9, and 21, as well as 14 and 15, disclosed that isolates of the respective former and latter bacteriocin types appeared to be related, since the bacteriocin patterns differed only with regard to sensitivity or tolerance to one of the ten bacteriocins. Furthermore, it was noted that isolates of serogroup O3 that comprised bacteriocin types 14 and 15 differed in sensitivity to one bacteriocin, as was true for isolates of serogroups 6 and 14 that were of bacteriocin type 14 and 15, respectively. Likewise, isolates of serogroup 1 and bacteriocin types 33 and 37, as well as isolates of serogroup O5 and bacteriocin type 4 and 21, appeared to be closely related as judged by the similar bacteriocin sensitivity/tolerance patterns.

It was of interest to note that 9 of 10 isolates comprising bacteriocin type 1 were of identical serotype (O2:H1). Similarly, the two isolates of serotypes O4:H1 and O5:H2 were found to belong to bacteriocin types 7 and 45, respectively. However, in most instances the number of isolates of specific serotype/-group was too small to allow any definitive conclusions.

DISCUSSION

The purpose of this study was to determine how bacteriocin typing of isolates of *S. marcescens* compared with serologic procedures and whether isolates of *S. marcescens* of known serotype/-group could be further divided into different bacteriocin types. The number of isolates of known serotype/-group at hand was limited; we were not able to examine isolates of *S. marcescens* carrying O antigens 7, 8, 9, 10, or 11. For instance, isolates of serotype O11:H4 and O11:H13 had been found to be among the more prevalent at Boston City Hospital (6). Similarly, flagellar antigens H7 and 9 were not represented among the isolates studied. Nevertheless, certain conclusions could be drawn.

With the aid of the bacteriocin typing procedure, 94 of the 101 isolates (93.1%) could be typed. Serological tests permitted typing of 80 of the isolates (79.2%) and grouping of 91 (90.1%). It appears that a larger number of isolates of *S. marcescens* can be typed according to their sensitivity/tolerance to 10 selected bacteriocins than through serological methods. In some instances there appears to exist a correlation between certain serotypes and bacteriocin types. Also, certain serogroups appeared to be related to given bacteriocin types. However, in most cases the number of isolates of known serotype/-group received was too small to permit any further conclusions.

It is recommended that reference laboratories adopt the bacteriocin technique for typing isolates of *S. marcescens*, because this proce-

dure is simple, inexpensive, and apparently of higher "epidemic resolution" than hitherto employed classic serological procedures.

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