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Phage Typing Scheme for *Salmonella braenderup*

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A phage typing scheme for *Salmonella braenderup* based on both phage sensitivity and lysogenicity patterns is presented. Three *S. braenderup* symbiotic phages (br A, br B, and br C) were used in the phage sensitivity test, and three indicator strains were used for the lysogenicity test. The 424 strains examined were grouped in 15 of 64 possible types within this scheme. The most frequent types were: A_1 (32%), G_5 (21%), F_2 (14%), and H_1 (8%). All of the strains isolated successively from the same person belonged to the same type, as did the strains isolated from groups of individuals related to a common source.

Among the great number of *Salmonella* serotypes (apart from *S. typhi* and *S. paratyphi* A and B), it is difficult and even hazardous to establish differences in the degree of pathogenicity. As more serotypes, initially isolated from cold-blooded animals or sewage, are being identified in human infections, it seems likely that any serotype is capable of producing human disease. In this respect, two apparently contradictory facts may be cited. First, certain widespread serotypes are constantly prevalent in many countries, whereas others remain limited within small areas and are permanently isolated only in very rare cases (7, 9). On the other hand, a serotype of minor frequency often becomes a major one and may reach the first rank of isolations in any particular country (7, 9, 10, 13; I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*). Such a shift in frequency may be observed in the course of time in the same country or concurrently in different countries by comparison of the frequency of *Salmonella* serotypes.

Serological diagnosis is quite sufficient for epidemiological purposes when a serotype of minor frequency is involved, but this method should be supplemented by other methods, such as phage typing or biochemical typing, in the case of a prevalent serotype.

Phage typing schemes were naturally carried out first for *S. typhi* (5), *S. paratyphi B* (6), *S. typhimurium* (2, 6, 11, 12), and other widespread serotypes (2, 3, 8). It is, however, necessary to carry out phage typing schemes for all *Salmonella* serotypes that are, or may become, prevalent in any country.

S. braenderup, a frequently found serotype in Israel, belongs to the first 10 serotypes in order of frequency and constitutes 2.4 to 8.8% of all *Salmonella* isolations in Israel within the last 20 years (7). In a review of *S. braenderup* strains (9) isolated in 17 countries (from 1940 to 1964), 1,285 strains were registered in Israel as compared with 1,295 strains in the other 16 countries. This organism also caused a number of outbreaks. Therefore, elaboration of a phage typing scheme for this serotype was considered necessary.

MATERIALS AND METHODS

The *S. braenderup* strains were isolated in Israel between 1964 and 1968 and were identified in the National Salmonella Center (Government Central Laboratories, Ministry of Health), Jerusalem. Most of the strains originated from sporadic cases of gastroenteritis or food poisoning; other strains were obtained from nonhuman sources (poultry, cattle, rats, etc.).

The screening method used for detecting lysogenic strains and for obtaining a provisional grouping, based on lysogenic properties and phage sensitivity patterns, was as follows. Groups of 30 strains, issued from single, smooth colonies, were studied together. Each strain was seeded on a well-dried nutrient-agar plate by flooding the surface of the agar with about 0.5 ml of a 6-hr broth culture, draining off the excess, and allowing it to dry (about 15 min) uncovered in the thermostat. After drying, drops from chloroform-killed 18-hr broth cultures of all 30 strains were deposited on all of the seeded plates with the aid of a mechanical dropping machine. The plates were incubated for 8 hr and then read, or were refrigerated until the next morning and then read.

The methods used for isolating and purifying symbiotic phages were those described by Atkinson (3). Antisera were prepared for the symbiotic phages, as described by Adams (1), and the percentage of phage inactivation was determined as described previously (I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*).

Tests for heat sensitivity were performed in broth at 60, 70, 80, and 90 C; tests for sodium citrate sensi-

tivity were performed on nutrient agar with 0.05 M sodium citrate.

The phage sensitivity test was carried out (I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*) by streaking a strip, about 4 cm long and 1 cm wide, of a 4-hr broth culture of each strain to be examined on the surface of a well-dried nutrient-agar plate. After drying for about 10 min uncovered in the thermostat, three drops, each from one of the test phages in the routine test dilution (RTD), were dropped onto the strips; the plates were incubated for 8 hr at 37 C, refrigerated until the next morning, and then read.

The lysogenicity test was performed by the phage induction method with mixed culture as previously described (I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*). A broth tube was seeded with one drop of a young broth culture of the strain to be tested, together with one drop of a young culture of the sensitive, nonlysogenic strain br 6. The 20-hr mixed culture was killed with chloroform and drops were deposited on agar plates seeded with the three indicator strains.

RESULTS AND DISCUSSION

Phage sensitivity test. A total of 160 *S. braenderup* single colony cultures were submitted to the screening test and 36 lysogenic strains were selected. The symbiotic phages were isolated, multiplied on a nonlysogenic strain (br 6), purified by two successive single-plaque isolations, and finally multiplied on the same nonlysogenic strain. The 36 phage preparations were tested in 10-fold dilutions against all of the 160 *S. braenderup* strains. On the basis of host range activity, 5 phages were grouped together (representative type: phage br 5′ = br A), 22 phages formed another group (representative type: phage br 7′ = br B), and 9 phages formed a third group (representative type: phage br 9′ = br C).

Characterization of the three representative symbiotic phages of S. braenderup: host range activity. As in our previous work on *S. blockley* (I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*), three phages were used to identify eight possible sensitivity types, designated A to H (Table 3). Testing our collection of *S. braenderup* with the three phages, br A, br B, and br C, in RTD, types A, B, D, F, G, and H have thus far been encountered.

Specificity of lytic action. The three symbiotic phages of *S. braenderup* were tested against 42 strains belonging to 16 *Salmonella* serotypes of group C_1 (*S. bloemfontain, S. bonn, S. choleraesuis, S. concord, S. edinburg, S. infantis, S. lille, S. montevideo, S. ness-ziona, S. oranienburg, S. oslo, S. paratyphi C, S. richmond, S. tennessee, S. thompson, S. virchow*), 60 strains belonging to 5 serotypes of group C_2 (*S. glostrup, S. hadar, S. manhattan, S. newport, S. tallahassee*), 20 strains belonging to 3 serotypes of group C_3 (*S. emek, S. kentucky, S. virginia*), 6 strains of group C_4 (*S. jerusalem*), and 80 strains belonging to all the other O groups of *Salmonella* (A, B, D, E . . . to O_{60}).

TABLE 1. *Percentage of inactivation of the three S. braenderup phages, br A, br B, and br C, by the corresponding anti-phage sera*

Anti-phage serum	Percentage of inactivation of bacteriophages		
	br A	br B	br C
Anti-br A	99.5	96.0	0.5
Anti-br B.....	95.0	99.2	0.1
Anti-br C	0.5	2.1	72.0

TABLE 2. *Percentage of inactivation of the three S. braenderup symbiotic phages, br A, br B, and br C, in broth after 30 min of exposure at 60, 70, 80, and 90 C*

Temp	Percentage of inactivation of bacteriophages		
	br A	br B	br C
C			
60	1.0	0	88.9
70	1.5	12.5	99.9
80	5.0	14.0	100
90	99.9	99.9	100

The three phages were inactive in RTD against all but one strain of *S. infantis*. In lower dilutions, they were active against two of five *S. oranienburg* strains. In general, of 208 strains tested, they were active on only 3 strains other than *S. braenderup*.

Serological study. Cross-inactivation tests were carried out with the three *S. braenderup* symbiotic phages and the corresponding anti-phage sera (Table 1). The two phages br A and br B are serologically closely related and the br C phage appears to be completely distinct.

Heat sensitivity. The three phages were exposed for 30 min at 60, 70, 80, and 90 C, and the percentage of inactivation of plaque-forming particles was determined (Table 2). Phage br C was very heat-sensitive and was almost completely inactivated at 70 C; the br A and br B phages were more heat-resistant.

Sodium citrate sensitivity. The number of plaques produced by phages br A and br B on sodium citrate-containing medium was reduced by only 20 and 10%, respectively, as compared to those obtained on nutrient-agar. The lytic ac-

TABLE 3. *Phage typing scheme for S. braenderup*[a]

Type	Sensitivity to			Lysogeny on			Type	Sensitivity to			Lysogeny on			Type	Sensitivity to			Lysogeny on			Type	Sensitivity to			Lysogeny on		
	br A	br B	br C	br 6	br 1	br 7		br A	br B	br C	br 6	br 1	br7		br A	br B	br C	br 6	br 1	br 7		br A	br B	br C	br 6	br 1	br 7
A_1	CL	CL	CL	−	−	−	B_1	CL	CL	−	−	−	−	C_1	CL	−	CL	−	−	−	D_1	−	CL	CL	−	−	−
A_2	CL	CL	CL	+	−	−	B_2	CL	CL	−	+	−	−	C_2	CL	−	CL	+	−	−	D_2	−	CL	CL	+	−	−
A_3	CL	CL	CL	−	+	−	B_3	CL	CL	−	−	+	−	C_3	CL	−	CL	−	+	−	D_3	−	CL	CL	−	+	−
A_4	CL	CL	CL	−	−	+	B_4	CL	CL	−	−	−	+	C_4	CL	−	CL	−	−	+	D_4	−	CL	CL	+	−	+
A_5	CL	CL	CL	+	+	−	B_5	CL	CL	−	+	+	−	C_5	CL	−	CL	+	+	−	D_5	−	CL	CL	+	+	−
A_6	CL	CL	CL	+	−	+	B_6	CL	CL	−	+	−	+	C_6	CL	−	CL	+	−	+	D_6	−	CL	CL	+	−	+
A_7	CL	CL	CL	−	+	+	B_7	CL	CL	−	−	+	+	C_7	CL	−	CL	−	+	+	D_7	−	CL	CL	−	+	+
A_8	CL	CL	CL	+	+	+	B_8	CL	CL	−	+	+	+	C_8	CL	−	CL	+	+	+	D_8	−	CL	CL	+	+	+
E_1	CL	−	−	−	−	−	F_1	−	CL	−	−	−	−	G_1	−	−	CL	−	−	−	H_1	−	−	−	−	−	−
E_2	CL	−	−	+	−	−	F_2	−	CL	−	+	−	−	G_2	−	−	CL	+	−	−	H_2	−	−	−	+	−	−
E_3	CL	−	−	−	+	−	F_3	−	CL	−	−	+	−	G_3	−	−	CL	−	+	−	H_3	−	−	−	−	+	−
E_4	CL	−	−	−	−	+	F_4	−	CL	−	−	−	+	G_4	−	−	CL	−	−	+	H_4	−	−	−	−	−	+
E_5	CL	−	−	+	+	−	F_5	−	CL	−	+	+	−	G_5	−	−	CL	+	+	−	H_5	−	−	−	+	+	−
E_6	CL	−	−	+	−	+	F_6	−	CL	−	+	−	+	G_6	−	−	CL	+	−	+	H_6	−	−	−	+	−	+
E_7	CL	−	−	−	+	+	F_7	−	CL	−	−	+	+	G_7	−	−	CL	−	+	+	H_7	−	−	−	−	+	+
E_8	CL	−	−	+	+	+	F_8	−	CL	−	+	+	+	G_8	−	−	CL	+	+	+	H_8	−	−	−	+	+	+

[a] Framework for 64 theoretical possible types.

tivity of phage br C was completely inhibited by the presence of sodium citrate in the medium.

Lysogenicity test. Three strains were chosen for use as indicators in the lysogenicity test; one strain (br 6) was nonlysogonic and highly phage-sensitive, and the two other strains (br 1 and br 7) had different phage sensitivity patterns. As previously shown (I. Sechter and C. B. Gerichter, Ann. Inst. Pasteur, *in press*), eight possible lysogenicity types, designated 1 to 8 (Table 3), can thus be identified.

Testing our collection of *S. braenderup* strains against the three indicator strains, lysogenicity types 1, 2, 4, 5, and 8 have thus far been identified.

Phage typing scheme. Combining the 8 possible sensitivity patterns with the 8 types obtainable in the lysogenicity test, a framework of 64 theoretically possible types was obtained (Table 3).

Results obtained with the phage typing scheme. As many as 424 strains of *S. braenderup* from the collection of the National Salmonella Center, Jerusalem, Israel, were examined by this method. Of these, 315 were isolated from human begins and 109 were isolated from fowl, cattle, and various meat products.

The types observed were A_1, A_2, A_8, B_8, D_2, F_2, F_5, F_8, G_2, G_5, G_8, H_1, H_2, H_5 and H_8. Among the 64 possible types of *S. braenderup*, only 15 have thus far been identified; others may, no doubt, be found by examining a larger collection. However, even those types remaining unidentified can be said to possess significance, by indicating positive or negative correlations among the three phages and the three indicator strains used.

Table 4 gives the relative frequency of the types in our collection. The most frequent was the wholly sensitive, nonlysogenic A_1 type. Other frequent types were G_5, F_2, and H_1.

TABLE 4. *Relative frequency of the different phage types of S. braenderup in Israel (1964 to 1968)*

Type	Per cent
A_1	32
A_2	5
A_8	1
B_8	1
D_2	3
F_2	14
F_5	1
F_8	2
G_2	1
G_5	21
G_8	6
H_1	8
H_2	1
H_5	2
H_8	4

Epidemiological data. Three outbreaks of gastroenteritis caused by *S. braenderup* were investigated. In one outbreak, 9 strains were isolated and all belonged to type A_1; in a second outbreak, the 12 isolated strains belonged to type F_2; and, in the third outbreak, 7 strains were isolated (5 from human patients and 2 from fowl) and all belonged to type G_5. In six cases, two or more strains were successively isolated from the same person and belonged to the same type. It may thus be assumed that the phage types identified by this scheme are practically stable and may be useful in epidemiological studies.

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