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Preliminary Report of a New System for Typing *Salmonella typhimurium* in the United States

VIRGINIA R. WILSON, GEORGE J. HERMANN, AND ALBERT BALOWS

Bacteriology Section, Center for Disease Control, Atlanta, Georgia 30333

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A new system is described for the bacteriophage typing of *Salmonella typhimurium* cultures isolated in the United States. The system is based upon one phage adapted to different *S. typhimurium* strains.

The most prevalent *Salmonella* serotype is *typhimurium*. To date, we have not had a satisfactory system for phage typing strains of this serotype isolated in the United States. This paper is a preliminary report of a new system developed in the Enteric Unit of the Bacteriology Section, Center for Disease Control, Atlanta, Ga.

We have been using and reporting the phage types as typed by the original (and revised) thirteen *S. typhimurium* phages of Felix and Callow (2) provided by the International Reference Center for Enteric Phage Typing located in Colindale, England. However, a great number of the cultures sent to us for phage typing were either not lysed by any of these phages or did not give a pattern of lysis for a designated type.

This new system of phage typing is similar to that for *S. typhi* devised by Craigie and Yen (1). It is based upon the adaptation of one phage to a number of *S. typhimurium* cultures.

The strains of *S. typhimurium* used were stock cultures. The phage used for adaptation was one isolated from a canal by R. Th. Scholtens of the Netherlands. This phage was first grown on a number of strains of *S. typhimurium* to select one which would yield the highest titer yet lyse the fewest cultures.

Only single colonies of the parent strains of *S. typhimurium* grown on a nutrient agar slant were used. Nutrient broth for cultivation of *S. typhi* and for dilution of phage consisted of nutrient broth (Difco dehydrated), 15 g; sodium chloride, 7 g; and distilled water, 1,000 ml. After a 2- to 3-hr growth period in phage broth, 0.5 ml was pipetted into a 125-ml flask containing 20 ml of Veal Infusion Broth (Difco). One milliliter of the undiluted phage suspension was then added, and the flask was incubated at 37.5 C overnight. The following morning the material from the flask was poured into a 50-ml centrifuge tube and centrifuged at about 8,000 × *g* for 20 min. The supernatant was removed and placed in a 60 C water bath for 2 hr to kill the *S. typhimurium*. Serial passages were made, and a single plaque was picked each time to insure a pure phage.

We now have 20 *S. typhimurium* phages that give 26 distinct phage types (Table 1). The degree of lysis and its pattern are also shown for each phage type in the tables. Table 2 shows the reaction of the Felix and Callow phage type strains with the 20 new phages. As the work progresses, it is quite probable that there will be more phages added to the schema as a result of further adaptations, thus giving more patterns of lysis. There is also a possibility of obtaining different phage patterns from a wider examination of cultures.

The Enteric Unit plans to train people interested in the phage typing of *S. typhimurium* and to supply the necessary phages for use in their own laboratory. In this way we hope that, through the combined efforts of our laboratory and those whom we train, we will be able to phage type *S. typhimurium* cultures isolated from food, animals, feeds, and environment. This will give us an overall picture of the *S. typhimurium* phage types isolated in the United States and provide basic information which will be useful in epidemiological investigations of *S. typhimurium* outbreaks.

We express our appreciation of the criticism and suggestions of A. Bernstein, Marquette University School of Medicine, Milwaukee, Wis.

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2. Felix, A., and B. Callow. 1956. Phage typing of *Salmonella typhimurium*: its place in epidemiological and epizootiological investigations. J. Gen. Microbiol. 14:208-222.

TABLE 1. *Reactions of type strains of Salmonella typhimurium with test dilutions of the new typing phages*[a]

Strain	Strains																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	CL	CP	CL	CP	CP	CP	CP	CL	CL	CP	SCP	CL	++n	CL	CL	CL	CL	CP	+m	CL
2	—	CL	—	—	—	—	—	—	—	±n	CP	—	+n	—	—	—	—	++sm	—	—
3	—	—	CL	—	—	—	+s	—	—	±s	—	—	±n	—	—	—	—	SCP	—	—
4	—	—	—	CL	—	—	—	—	—	—	—	—	++++n	—	—	—	—	—	++m	—
5	—	—	—	—	CL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	CL	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	++++ SCR	—	CL	CL	CL	CL	—	—	—	CL	—	CL	—	CP	—	—
8	—	—	—	—	++	—	±n	CL	CL	CL	—	—	—	7l	—	—	—	CL	—	—
9	—	—	—	—	—	—	—	—	CP	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	CL	CL	—	—	6l	—	—	—	—	++s	—	—
11	—	—	—	—	—	—	—	—	—	—	CL	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	++s	—	—	CL	—	—	—	—	—	±m	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	CT	—	—	—	—	—	++m	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	CL	—	—	—	—	—	—
15	—	—	—	—	—	++s	—	—	—	—	—	—	++s	—	CL	—	—	—	+sm	+s
16	—	—	—	—	—	—	—	+s	SCT	CT	—	—	—	+++s	—	CT	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CL	—	—	—
18	—	—	—	—	—	—	+s	—	—	—	—	—	—	—	—	—	—	CTP	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CP	—
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CL
21	—	CL	—	—	—	—	—	—	—	—	CL	CL	—	—	—	—	—	—	—	—
22	—	CL	CL	CL	CL	CL	—	CL	CL	CL	CL	CL	—	—	CL	CL	CL	CL	—	CL
23	—	CL	CL	CL	CL	CL	—	CL	CL	—	CL	CL	—	—	CL	—	CL	CL	—	CL
24	—	CL	CL	CL	CL	CL	—	CL	CL	CL	CL	CL	CL	—	CL	—	CL	CL	—	CL
25	—	CL	CL	CL	CL	CL	—	CL	CL	CL	CL	CL	CL	—	CL	—	CL	CL	CL	CL
26	—	SCP	CP	CL	CL	CP	—	CL	CL	CL	CL	CL	—	—	CL	—	CL	CL	—	CP

[a] Method of recording degrees of lysis on *Salmonella* phage typing plates: CL, confluent lysis; CT, confluent translucency; CP, confluent plaques; CPT, confluent translucent plaques; SCP, semiconfluent plaques; ++++, less than SCP; +++, 81 to 120 plaques; ++, 61 to 80; +, 21 to 60; ±, 6 to 20; l, large plaque; n, normal; s, small; m, minute.

TABLE 2. *Reactions of type strains of Felix and Callow of S. typhimurium with test dilutions of the new typing phages*[a]

Type strain		Strains																			
Old	New	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	25	—	CP	CP/SCP	CP	CL	CL	—	CL	CL	CL	CL	CP	CP	—	CL	—	CP	CL	CL	CP
1a	26	—	CL	CL	CP	CL	CL	—	CL	CL	CL	CL	CL	—	—	CL	—	CL	CL	—	CL
1aV1	21	—	CL	—	—	—	—	—	—	—	—	CL	CL	—	—	—	—	—	—	—	—
1b	3	—	—	CL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CL	—	—
2	8	—	—	—	—	++/SCP	—	—	CL	CL	CL	—	—	—	—	—	—	—	CL	—	—
2a	10	—	—	—	—	—	—	—	—	CT	CTP	—	—	—	—	—	—	—	++++1	—	—
2b	9	—	—	—	—	—	—	—	—	CT	—	—	—	—	—	—	—	—	—	—	—
2c	9	—	—	—	—	—	—	—	—	CT	—	—	—	—	—	—	—	—	—	—	—
2d	6	—	—	—	—	—	CL	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	4	—	—	—	CL	—	—	—	—	—	—	—	—	SCP	—	—	—	—	—	CL	—
3a	5	—	—	—	—	CL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	7	—	—	—	—	++s	—	++++s	CP	CL	CL	—	—	—	CP	—	CP	—	CP	—	—
5	26	—	SCP	CP	CL	CL	CP	—	CL	CL	CL	CL	CL	—	—	CL	—	CL	CL	—	CP

[a] See footnote *a*, Table 1.