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Preliminary Report: a System for Typing *Salmonella thompson*

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A system is described for the phage typing of *Salmonella thompson*. The system is based upon a number of bacteriophages that were isolated from sewage.

There are now approximately 1,600 species of salmonellae, and new isolates continue to augment this numerically impressive collection. The number of existing species in itself is not particularly disturbing because it can be epidemiologically useful. However, if a particular species is fairly common and widely disseminated, it becomes difficult, if not impossible, to assess its role in an outbreak of disease. Indeed, different "types" must first be delineated to be of value to the health authorities engaged in tracing the origin and spread of a given infection.

Serological methods exist for the delineation of various bacterial isolates; however, the number of types distinguishable in this manner is usually inadequate for epidemiological application.

In 1938 Craigie and Yen introduced a phage-typing scheme for *Salmonella typhi* (4, 5). Its success led to the development and acceptance of the phage-typing technique as a reliable laboratory procedure. One of the most important properties of phages is their host specificity. Phages may be so selective with respect to a single species, or even to a certain type or subdivision of that species, that phage-typing will distinguish varieties among apparently identical bacteria.

Phage-typing schemes have been devised for many pathogens (2, 3, 6, 7, 9). In keeping with our own immediate interests and circumstances, we are developing a phage-typing potential for *S. thompson*.

The cultures used in this project were obtained from our own diagnostic service and the National Animal Disease Laboratory, Ames, Iowa.

Untreated sewage samples were obtained locally from a number of treatment plants. Samples (100 ml) were inoculated with a 1.5-hr nutrient broth culture of one of many *S. thompson* cultures collected for this investigation. The samples were incubated for 18 hr at 37 C and passed through a 0.45-mm membrane filter. The filtrates were then assayed for the presence of phage by plating them onto the cultures initially used as an inoculum. Phage isolates were purified by serial, single-plaque passages and brought to titer by the procedure described by Swanstrom and Adams (8).

Phages of sufficiently high titer were diluted and tested against the *S. thompson* cultures in our collection. The phage dilution used for phage-typing is referred to as the routine test dilution (RTD). The RTD is the highest phage dilution that produces confluent lysis on its propagating strain. Its use minimizes the occurrence of confusing indiscriminate cross-reactions. All of the phages employed in this study were used at an RTD of not less than 10^{-3}. Phage isolates were selected and maintained for regular employment if they were stable and potentially suitable for type differentiations.

Cultures to be typed were lightly inoculated

Table 1. *Method for recording degrees of lysis*

Symbol	Characteristic
CL	Confluent lysis
OL	Opaque lysis (opacity due to heavy secondary growth)
SCL	Semiconfluent lysis
<SCL	Less than semiconfluent lysis
+++	120 Plaques
++±	81-120 Plaques
++	61-80 Plaques
+±	41-60 Plaques
+	21-40 Plaques
±	6-20 Plaques
−	0-5 Plaques

TABLE 2. *Reactions of type strains of Salmonella thompson with typing phages at routine test dilutions*

Type strain	Typing phages							
	1	2	3	4	5	6	7	8
1	CL[a]	—	CL	—	—	±	—	—
2	—	CL	—	—	<SCL	++±	—	CL
3	+++	SCL	CL	—	±	<SCL	OL	OL
4	<SCL	—	<SCL	CL	—	—	—	—
5	+±	—	CL	—	CL	CL	CL	CL
6	<SCL	—	CL	—	SCL	CL	SCL	CL
7	++	CL	CL	—	CL	CL	CL	CL
8	—	—	—	—	SCL	+++	<SCL	CL

[a] See Table 1 for explanation of symbols.

into 3 ml of nutrient broth and incubated at 37 C for 1.5 hr or until turbidity was barely detectable. A small quantity of the broth culture was then flooded onto a nutrient agar plate, allowed to dry for approximately 15 min, and then spotted with phages by using a 1-ml syringe with a 26-gauge needle. The plates were incubated overnight at 37 C and read the following day. The cultures were examined with the aid of an ×10 aplanat hand lens and viewed through the bottom of the plate. Susceptibility to a phage was demonstrated by areas of clearing that range from isolated plaques to confluent lysis. While some strains may be susceptible to only a single phage, lysis by several phages was most common. Phage activity was recorded on the basis of the reactions described in Table 1 (1).

Eight phages were isolated. The lytic pattern of these phages is described in Table 2. Each phage exhibits a characteristic spectrum of activity against the cultures of *S. thompson* that are used in propagating the various phages in this typing battery. This lytic pattern remains quite constant and is frequently employed to ascertain the purity of new phage lots.

By using these isolates we have thus far been able to establish 13 distinct phage types. It is conceivable that more types exist and will be revealed as new cultures are examined. It is our intention to expand our basic set of phages in an effort to develop an even greater potential for delineating *S. thompson* types.

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