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T-related Bacteriophages Isolated from *Shigella sonnei*

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The properties of three T-related phages—35, 55, and 3201—isolated from *Shigella sonnei* were studied. They were similar with respect to morphology of plaques, duration of the latent periods, lysis inhibition effect, and serological characteristics. These phages closely resembled the T-even phages. Phages 3201, 35, and 55 had the same host range and receptor specificity as phage T2.

The T-even phages are characterized by their high virulence. They form the group of so-called "autonomous virulent" phages (10). The genetic and biosynthetic activity of the bacterial cell, not participating in the phage reproduction, is blocked immediately upon infection with T-even phages (4). This explains the absence of lysogenic systems with these phages.

The source of the T phages is not known. It has not been proved whether these phages could originate from temperate phages as virulent mutants, as is the case with other virulent phages. Despite the fact that all attempts to isolate temperate phages related to the T phages have been unsuccessful, the possibility that they exist cannot be excluded.

The present paper reports a study of the properties of three T-related phages isolated from *Shigella sonnei* strains.

MATERIALS AND METHODS

Phages 35, 55, and 3201 were isolated from *S. sonnei* strains in the course of an examination for lysogeny (5). The strains originated from dysentery patients and were isolated from different sources of infection. The T-even phages T2, T4, and T6 were used for comparative studies. *S. sonnei* strain 2 served as a phage-sensitive indicator. The strains used to test the host range of the phages are listed in Table 1. The receptor specificity of the phages was studied by examining their host range on resistant mutants obtained from *S. sonnei* 2 against each of the six phages tested (Table 1).

The latent period of the phages was determined in one-step growth experiments (6), the multiplicity of the phage infection being 0.1. A higher multiplicity in the range of 10 phages per bacterium was applied to visibly turbid cultures to test whether the phenomenon of lysis inhibition occurs with the *S. sonnei* phages (5).

Antiphage sera were obtained from rabbits (9). Serum samples were taken before phage immunization. The neutralization test was carried out according to the method described by Adams (1). The serum dilutions used were of the order of 10^{-1} to 10^{-3}, and the incubation period was from 5 to 30 min. The velocity constant K for all sera was calculated.

RESULTS

Phages 35, 55, and 3201 gave plaques similar in form and size with diameters between 1 and 2 mm. The plaques were clear with turbid halos on an agar plate. The minimal latent periods of *S. sonnei* phages 35, 55, and 3201 were 28, 24, and 26 min, respectively; those of T2, T4, and T6 phages, in our experiments, were 25, 27, and 28 min, respectively. When the cultures were infected with an excess of *S. sonnei* phages, no lysis occurred after the same latent periods observed in the one-step growth experiments, but about 5 hr later there was some lysis, as judged by visual observation. However, complete clearing of the cultures did not ensue; they remained opalescent.

The host range of the *S. sonnei* phages and the T-even phages is presented on Table 1. All phages showed lytic activity to the *S. sonnei* strains tested. Phages 35 and 55 were also active on *S. dysenteriae* 2 and *S. flexneri* 6. The *Escherichia coli* strains were not sensitive to these phages. Phages 3201 and T2 produced lysis of all the strains tested, with the exception of *S. dysenteriae* 2. A similar host range was found with phage T6, which differed from 3201 and T2 in that it was not active on *S. flexneri* 6. The host range of T4 was limited to *E. coli* B and K-12 and to *S. sonnei* strains; T4 did not show lytic activity on the remaining strains of the genus *Shigella*. It is noteworthy that the lytic activities of the phages on *S. dysenteriae* 1 and on *S. flexneri* strains 1a, 2a, 3, 4a, and 5 were the same, whereas activity on *S. flexneri* 6 was different.

The cross-resistance of *S. sonnei* to the T-even

TABLE 1. *Host range of the S. sonnei and the T-even phages*[a]

Host	Phage					
	35	55	3201	T2	T4	T6
S. sonnei	+	+	+	+	+	+
S. flexneri						
1a	−	−	+	+	−	+
2a	−	−	+	+	−	+
3	−	−	+	+	−	+
4a	−	−	+	+	−	+
5	−	−	+	+	−	+
6	+	+	+	+	−	−
S. dysenteriae	−	−	+	+	−	+
S. dysenteriae	+	+	−	−	−	−
E. coli B	−	−	+	+	+	+
E. coli K-12	−	−	+	+	+	+
So/35[b]	−	−	+	+	+	+
So/55	−	−	+	+	+	−
So/3201	+	+	−	−	+	+
So/T2	+	+	−	−	+	+
So/T4	+	+	+	+	−	+
So/T6	+	+	+	+	+	−

[a] Symbols: + = sensitivity to phage; − = nonsensitivity to phage.

[b] So/35 = mutant of *S. sonnei* 2 resistant to phage 35; So/55 = mutant of *S. sonnei* 2 resistant to phage 55; etc.

phages was also studied (Table 1). The mutants resistant to phage 35 were also resistant to phage 55. Cross-resistance to phages T2 and 3201 was established as well. *S. sonnei* 2 mutants resistant to phage 55 were resistant to phage T6, but *S. sonnei* 2 mutants resistant to T6 were not resistant to phage 55.

The results of the cross-neutralization are given in Table 2. It must be pointed out that the normal rabbit sera taken before the immunization did not show neutralizing activity to either the *S. sonnei* phages or the T-even phages. It is apparent that the antiphage sera neutralize to the homologous phages the highest titers; K values ranged from 60 to 52%. A different degree of cross-neutralization was established in all phage antisera. The antiserum of phage 35 neutralized phage 55, the value of the velocity constant being higher than half of the homologous K value. The same serum manifested a weaker neutralization with phage 3201, the K value being 32. In the case of reciprocal neutralization, antiserum 55 neutralized phage 35 with a K value of 23, as opposed to a K value of 121 for the homologous phage. It is apparent from Table 2 that antiserum to 3201 neutralizes phages 3201, 35, and 55 with K values of 198, 23, and 91, respectively. Antisera of *S. sonnei* phages neutralize the T-even phages to a

TABLE 2. *Cross neutralization of S. sonnei phages and T-even phages*

Antiserum	Phage					
	35	55	3201	T2	T4	T6
35	527[a]	358	32	6	9	4
55	23	121	11	2	6	3
3201	23	91	198	4	4	18
T2	15	20	24	60	30	15
T4	30	34	38	34	451	31
T6	22	38	40	18	39	153

[a] Value of K in min^{-1}.

lesser degree, the K values varying from 2 to 18. The latter value was observed with antiserum 3201 and phage T6. The antisera of the T-even phages showed higher neutralization activity to *S. sonnei* phages. Antiserum T2 neutralized the *S. sonnei* phages to the extent of about one-fourth to one-third of the homologous constant. The same degree of neutralization was observed with T4 and T6. The K values obtained with antiphage serum T6 and phages T6, 35, 55, and 3201 are listed in Table 2. In the case of antiserum T4 with all the heterologous T-even and *S. sonnei* phages, the velocity constants were about one-twelfth of the homologous constant.

DISCUSSION

Phages 35, 55, and 3201 isolated from *S. sonnei* strains possessed similar properties, which closely resembled those of the T-even phages. Their plaques were almost undistinguishable from each other and manifested a similarity with respect to clarity and turbid halos when compared with the T-even phages, but were of a smaller size than the T-even phages. The latent periods of phages 35, 55, and 3201 were similar to those of the T-even phages. Like the T-even r^+ phages, the *S. sonnei* phages 35, 55, and 3201 had a lysis inhibition effect on multiply-infected cultures. The absence of complete clearing of the cultures has been observed also with T_2r^+ (5).

It is known from the literature and is seen from our results that the T-even phages are active against *E. coli* B and K and against some *Shigella* strains. The *S. sonnei* phages 3201 and T2, as well as 35 and 55, had the same host range and receptor specificity. Unlike phage 3201, phages 35 and 55 did not lyse *E. coli* strains. A certain relationship was established between phages 55 and T6, which may be ascribed to an incomplete homology between the two phage receptors. On the basis of the lytic activity of *S. flexneri* 1a, 2a, 3, 4a, and 5 phages and *S. dysenteriae* 1 phage,

a closer relationship with respect to phage receptor specificity may be presumed to exist between these strains than between *S. flexneri* 1a, 2a, 3, 4a, and 5 and *S. flexneri* 6, which belongs to the same subgroup.

It must be noted that in an earlier study the phages 35, 55, and 3201 were included in a common group on the basis of a serological investigation of 10 *S. sonnei* phages (9). An antigenic relationship was found to exist among these phages and with the T-even phages. No serological relationship could be established with the remaining T phages (T1, T3, T5, and T7) nor with other temperate enteric phages.

The study on the cross-neutralization discussed in the present paper confirms our previous results. The absence of neutralization activity in the serum samples taken before phage immunization indicates that no phage antibodies are found in normal rabbit sera. This suggests that the cross-neutralization between the *S. sonnei* phages and the T-even phages is due to the presence of common antigens. It is noteworthy that the antisera of the T-even phages neutralize the *S. sonnei* phages to a higher degree than is the case with the reciprocal assay. Similar asymmetrical relationships in phage cross-neutralization have also been observed by other workers (2, 3, 7). Phage 3201 shows the same host range and receptor specificity as phage T2, but the antigenic relationship between them is less close than that between 3201 and phages 35 and 55, which leads us to consider that the phylogenetic relationship of phages 3201 and phages 35 and 55 is closer than the relationship between 3201 and T2. We agree with the opinion of some authors (1, 3, 7) that the most important taxonomic criterion of phages is their antigenic relationship.

It is known that T-related phages could be bound in sewage or feces, but their isolation from bacterial strains is not usual, because of their high virulence. The question whether the production of T-related phages from *S. sonnei* strains is due to pseudolysogeny or to true lysogeny will be the subject of a future study.

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