Bacteriophages Mediating Somatic Antigenic Conversion in *Salmonella cholerae-suis*: Their Isolation from Sewage and Other *Salmonella* Serotypes Possessing the Somatic 6 Antigen

By P. A. BARROW

Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire PE17 2DA, UK

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Bacteriophages which mediate the conversion of the O somatic antigen of *Salmonella cholerae-suis* from the 6,7 to the 6,7 phenotype have been isolated from two strains of *S. newport* and one of *S. muenchen*, and also from sewage collected from two areas where there have been no reports of *S. cholerae-suis* infection for several years. The phages differed from each other by cross-resistance tests.

**INTRODUCTION**

Members of the C group of salmonella serotypes possess the O antigens 6 and 7. There are two variants of the 6 antigen, designated 6₁ and 6₂. Strains of *Salmonella cholerae-suis* exist in either the 6₁ or 6₂ form but do not possess both antigenic types simultaneously. Escobar & Edwards (1964) found that all the 6,7 strains of this serotype were lysogenized by a phage which could convert 6₁,7 strains to the 6₁ form. While lysogenizing phages were found in other 6, 7 serotypes (Escobar & Edwards, 1968), none of these mediated this type of conversion.

The inability to isolate converting phage from serotypes containing the 6 antigen other than *S. cholerae-suis* seemed unusual. The search was therefore extended to serotypes in the groups C₂ and C₄ (possessing the 0 antigens 6, 8 and 6, 7, 14 respectively) in addition to strains in the C₁ group. Sewage was also examined for the presence of converting phage.

**METHODS**

**Bacteria and cultural conditions.** Phages were enriched using cultures of *S. cholerae-suis* A58 (O antigens 6,7) and were detected by spotting onto a lawn of nalidixic acid resistant (Nal⁺) mutants of A58. Phages were also tested for lysis on Nal⁺ mutants of *S. cholerae-suis* A50 (O antigens 6,7), which is lysogenized by the phage which mediates the 6₁/6₂ conversion. The converting phages obtained were also tested for lysis on five *Citrobacter* strains from our culture collection. All broth cultures were made in 10 ml nutrient broth (Oxoid CM67) incubated at 37 °C for 24 h in a shaking water bath (100 cycles min⁻¹). They contained approximately 10⁶ c.f.u. ml⁻¹.

The serotypes from group C₁ (O antigens 6, 7) tested for the possession of converting phage included *S. montevideo* (six strains), *S. thompson* (seven strains), *S. menston* (four strains), *S. infantis* (three strains), *S. oranienburg* (three strains) and one strain each of *S. mbandaka*, *S. ohio* and *S. tennessee*. Serotypes from group C₂ (O antigens 6, 8) included *S. newport* (ten strains), *S. hadar* (20 strains), *S. bovis-morbificans* (nine strains), *S. muenchen* (five strains), two strains each of *S. blockley* and *S. dusseldorf*, and one strain each of *S. manchester*, *S. nagoya*, *S. gold-coast* and *S. takoradi*. One strain from group C₄ (O antigens 6, 7, 14), *S. eimshuettel*, was examined. The five *Citrobacter* strains were also tested in the same way.

**Isolation of lysogenic phages from bacteria and sewage.** A broth culture of the bacterial strain to be tested was inoculated (0.03 ml) into 10 ml nutrient broth with 0.03 ml of a broth culture of A58. After incubation the mixture was centrifuged at 1500 g for 30 min and the supernatant decanted.

Samples of sewage in 5 litre volumes were mixed with 75 g of nutrient broth powder, and 1 ml of a broth culture of A58 was added. After static incubation overnight at 37 °C, 10 ml was withdrawn and centrifuged at 1500 g for 30 min. The supernatant was decanted and heated at 58 °C for 30 min.

Both types of phage preparation were spotted onto lawns of neat and 1 in 10 diluted broth cultures of A58 Nal⁺ on Tryptose agar (Difco B64) containing 20 µg nalidixic acid ml⁻¹. Phage plaques were purified on identical lawns. Purified plaques were picked with a small amount of the surrounding bacterial growth and cultured...
overnight in nutrient broth. These cultures were centrifuged, and the supernatants were heated at 58 °C for 30 min and then stored at 4 °C.

Phage conversion. Phage preparations were inoculated into 10 ml nutrient broth with 0.03 ml of a broth culture of A58. Phages which produced confluent lysis on A58 were inoculated in 0.03 ml volumes; those that produced few isolated plaques were inoculated in 0.5 ml quantities. The cultures were incubated and were plated onto tryptose agar to produce well-separated colonies. These were tested with antisera prepared by the method of Williams Smith & Parsell (1974). Any phages that converted A58 to the 6₁ antigen type were tested for cross resistance against each other by spotting 0.03 ml of the phage preparation onto a lawn of cultures of A58 which had been lysogenized by the individual phages.

RESULTS

Isolation of converting phage from salmonellas of groups C₁, C₂ and C₄ and from sewage

No converting phages were isolated from the 26 salmonella strains from group C₁ and the one strain from group C₄. Of the 52 group C₂ salmonellas tested, phages which converted S. cholerae-suis A58 to the 6₁ phenotype were isolated from two out of ten strains of S. newport and one out of five strains of S. muenchen. Culture filtrates of one of the S. newport strains (no. 67) produced a greater degree of lysis on A58 than on A50. Several other salmonella strains produced phages lytic on A58 but not on A50 but none of these were converting phages.

Thirteen sewage samples from eight treatment plants in England and Wales and water samples from three rivers were examined. From these samples 113 phages were isolated of which four produced plaques on A58 but not on A50. None of these was a converting phage. Three phages from the remaining 109 converted A58 to the 6₁ form. These were isolated from sewage obtained from two areas where no animal or human infections of S. cholerae-suis had been reported for several years. Using the antisera available many other phages appeared able to change A58 so that it agglutinated with equal strength with antisera specific for both the 6₁ and 6₂ forms.

None of the five Citrobacter strains produced any visible lysis on S. cholerae-suis A58.

Relationships between the converting phages

All six phages produced plaques similar to each other and to the plaques produced by phage liberated by the 6₁ form of S. cholerae-suis (A50). The plaques were 1–1.5 mm in diameter with an irregular outline and no halo. The zones of lysis contained some secondary growth, except in the case of the phages produced by the two S. newport strains, where the zones were clearer.

All six phages produced lysis on both A50 and A58. The patterns of cross resistance of the strains of A58 lysogenized by the six phages towards each phage are shown in Table 1. Despite

Table 1. The production of lysis by converting phages on the 6₂ somatic form of S. cholerae-suis lysogenized by the same phages

<table>
<thead>
<tr>
<th>S. cholerae-suis strain used as lawn*</th>
<th>Phage:</th>
<th>S. newport</th>
<th>S. newport</th>
<th>S. muenchen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>130</td>
<td>135</td>
<td>67</td>
</tr>
<tr>
<td>A58</td>
<td>C</td>
<td>P</td>
<td>C/P</td>
<td>P</td>
</tr>
<tr>
<td>A58 (phage 60)</td>
<td>P</td>
<td>C</td>
<td>SC</td>
<td>SC</td>
</tr>
<tr>
<td>A58 (phage 130)</td>
<td>C</td>
<td>-</td>
<td>P</td>
<td>C</td>
</tr>
<tr>
<td>A58 (phage 135)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A58 (S. newport 67)</td>
<td>-</td>
<td>C</td>
<td>-</td>
<td>C/P</td>
</tr>
<tr>
<td>A58 (S. newport 1322)</td>
<td>-</td>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A58 (S. muenchen 225)</td>
<td>P</td>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* For the lysogenized strains, the phage used to lysogenize the strain, or the bacterial strain from which the phage used was recovered, is shown in parentheses.

† C, confluent lysis; SC, semi-confluent lysis; P, individual plaques; -, no plaques. Two readings separated by an oblique line indicates the presence of more than one type of phage in the preparation used.
the fact that two of the phage preparations used (phage 135 and *S. newport* 67) contained more than one phage the results suggest that the converting phages were not related to each other.

Phage 60 produced some lysis on one of the *Citrobacter* strains, phage 130 produced lysis on another but phage 135 had no effect on any of the five *Citrobacter* strains examined. Phages isolated from *S. newport* strains 1322 and 67 produced some lysis on one of the *Citrobacter* strains.

**DISCUSSION**

This paper reports the isolation of converting phages, previously isolated only from *S. cholerae-suis*, from sewage in two areas where this particular serotype had not been isolated from pigs or man for a number of years and also from *Salmonella* serotypes of group C2. It seemed likely that the phages found in sewage had arisen from sources other than *S. cholerae-suis*. Salmonellas from group C2 are not infrequently isolated from sewage and two of the three phages isolated from sewage were able to lyse selected *Citrobacter* strains, suggesting that other organisms closely related to *Salmonella* may also be a source. None of the *Citrobacter* strains, however, were lysogenized by phages which acted on *S. cholerae-suis* strain A58.

That these phages act on *Citrobacter* is not surprising since antisera prepared against the 6,7 antigens of *Salmonella* cross react strongly with *Citrobacter* strains possessing the 28, 1c antigens (Edwards & Ewing, 1972). It is therefore conceivable that transfer of the ability to produce antigenic determinants may also occur by transduction between closely related bacterial genera.

The phages found were obviously different from those recovered from the 61 form of *S. cholerae-suis*. While most of them produced similar plaques, they all produced lysis on the 61 (lysogenized) form of *S. cholerae-suis*, and the patterns of cross-resistance between the different phages were also different.

Williams Smith & Parsell (1974) raised the question of how lysogenic conversion within *S. cholerae-suis* could occur in the field, since this pathogen is very rare. Phages which could mediate this conversion have now been isolated from the environment and from other salmonellas.

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**REFERENCES**


