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# NOTES

## Infection of *Serratia marcescens* by Bacteriophage χ

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Bacteriophage χ has attracted attention because of its characteristic host range: it attacks motile cells of both *Salmonella* and *Escherichia* species (V. Sertic and N. A. Boulgakov, Compt. Rend. Soc. Biol. **123**:951, 1936; E. W. Maynell, J. Gen. Microbiol. **25**:253, 1961; S. M. Zottu and J. Adler, *personal communication*). The receptor site of χ has been shown to be on the flagella of these bacteria. Sensitivity to χ is determined not only by the presence of motile flagella but also by the specificity of flagellar surface structure. For example, bacteria with flagellar antigens of the *g*-complex are resistant to χ. This note reports that phage χ is also able to infect motile strains of *Serratia marcescens*.

The *Serratia* strains used were B-181-2 through B-181-10, B-182-1 through B-182-9, B-183-2, and B-183-3 from the culture collection at the Institute of Applied Microbiology of the University of Tokyo. One of these 20 strains, B-181-6, was nonflagellate and, consequently, nonmotile. The remaining 19 strains were motile, having peritrichous flagella with normal curvature. The fraction of the motile cells in broth culture differed among the strains, ranging from 50 to 100%.

Sensitivities of these *Serratia* strains to phage χ and its host-range mutant M8, which is able to attack *Salmonella* possessing the *g*-complex antigen [I. Sasaki, Virus (Osaka) **12**:168, 1962], were examined by the spotting method of E. W. Meynell (J. Gen. Microbiol. **25**:253, 1961). The bacteriophage used was propagated on *Salmonella abortivoequina* strain SJ241, which is sensitive to both χ and M8 and has been used as a standard indicator strain of χ. It was found that the nonmotile strain, B-181-6, was completely resistant to both χ and M8, whereas the remaining 19 motile strains were lysed by both χ and M8. The plaques formed with the latter strains were clear and indistinguishable in size and shape from those formed with *S. abortivoequina* SJ241. When the culture contained some fraction of nonmotile cells, clearing was not complete, and turbid, but discernible, spots appeared.

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The electron micrographs of the mixture of phage χ and χ-sensitive *Serratia* cells showed that particles of χ were adsorbed to flagella (Fig. 1). A spontaneous nonflagellate mutant was isolated from motile strain B-181-8 by screening on semisolid nutrient agar medium. The mutant was resistant to both χ and M8.

Phage χ can be used as a selective agent for the isolation of nonmotile mutants from actively motile strains of *Serratia* by the same procedure which has been applied to *Salmonella* species and *Escherichia coli* (B. A. D. Stocker et al., J. Gen. Microbiol. **9**:410, 1953; M. Enomoto and T. Iino, J. Bacteriol. **86**:473, 1963). Among the eight nonmotile mutants obtained from strain B-181-7 after selection by treatment with χ, six were nonflagellate and the remaining two possessed paralyzed flagella.

Strain B-181-7, which, of the *Serratia* strains examined, showed the most complete lysis by phage χ, was adopted as the standard host strain of phage χ for the χ-*Serratia* system, and further experiments were carried out.

Several χ phages were propagated in *S. marcescens* B-181-7, *S. abortivoequina* SJ241, and *E. coli* W3110, and the stocks were designated χ(Ser), χ(Sal), and χ(Ec), respectively. The host range of χ(Ec) was distinct quantitatively from χ(Sal); χ(Ser) was much the same as χ(Sal) (Table 1). The combinations of χ(Sal) and *Serratia*, and χ(Ser) and *Salmonella*, gave efficiencies of plating (EOP) not significantly different from the homospecific combinations, i.e., the combinations in which the indicator strain used was the same as the propagating one. However, when χ(Sal) or χ(Ser) was tested with *Escherichia*, the EOP decreased markedly. The infection by χ(Ec) of either *Salmonella* or *Serratia* also resulted in decreased EOP as compared with the homospecific combinations, although the decrease was not as remarkable as that in the reciprocal combinations. When χ(Ec) was grown on *S. abortivoequina* SJ241 or *S. marcescens* B-181-7, the progeny phages then exhibited high EOP on these bacteria, whereas the EOP on *E. coli* W3110 decreased markedly. This

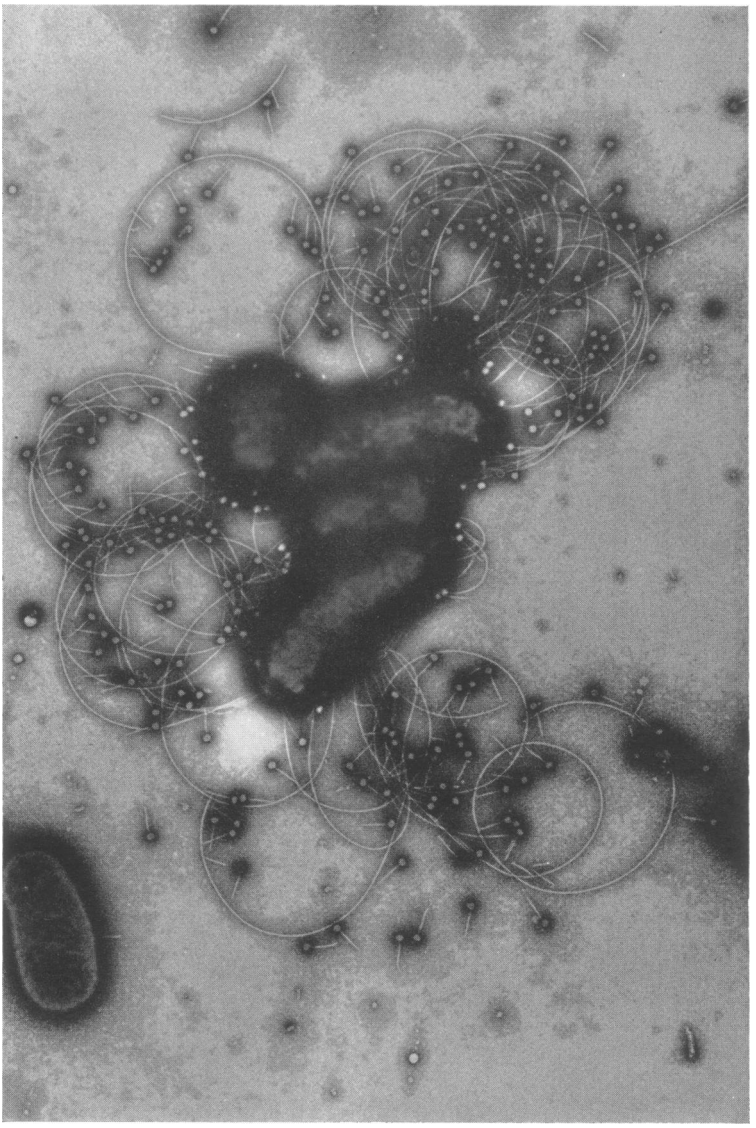

FIG. 1. *Electron micrograph of bacteriophage χ absorbed to the flagella of Serratia marcescens B-181-6. Phage χ was propagated from a strain of Salmonella abortivoequina. The sample was fixed in 2.7% osmium tetroxide (pH 6.2) and 0.4% Formalin, and negatively stained by phosphotungstic acid by the method of S. Brenner and R. W. Horne (Biochim. Biophys. Acta* **34**:*103, 1959).*

TABLE 1. *Efficiency of plating of bacteriophage χ on the bacteria belonging to three different genera of Enterobacteriaceae*[a]

| Indicator bacteria | Bacteriophages | | |
|---|---|---|---|
| | χ (Sal) | χ (Ser) | χ (Ec) |
| *Salmonella abortivoequina* SJ241 | 1.00 | 0.81 ± 0.39 | 0.38 ± 0.29 |
| *Serratia marcescens* B-181-7 | 1.06 ± 0.49 | 1.00 | 0.18 ± 0.16 |
| *Escherichia coli* W3110 | 0.0091 ± 0.0075 | 0.0076 ± 0.0056 | 1.00 |

[a] Values on the homospecific combinations are taken as 1.00, and the relative efficiencies of plating on the heterospecific indicator bacteria are shown with the 95% confidence intervals.

suggests the presence of host-induced modification in the system.

Anti-$\chi$ serum was prepared by immunization of a rabbit with a phage stock of $\chi$(Sal). The serum was absorbed with the propagating strain and diluted 1,000-fold, and neutralization velocity constants (G. M. Kalmanson, A. D. Hershey and J. Bronfenbrenner, J. Immunol. **45**:1, 1942) with $\chi$(Sal), $\chi$(Ser), and $\chi$(Ec) were measured at 37 C. The constants found were 495 $\pm$ 69, 497 $\pm$ 61, and 521 $\pm$ 73, respectively (95% confidence interval). Thus, the efficiency of inactivation of $\chi$(Ec) by anti-$\chi$(Sal) serum was not significantly different from those of both $\chi$(Sal) and $\chi$(Ser).

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