# Use of Vi Phage Lysates in Genetic Transfer. (20338)

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Recent investigations by Zinder and Lederberg(1) have indicated that genetic exchange in Salmonella is mediated by a bacterial product which they have called FA (filterable agent). While the active filtrates utilized in these experiments possess the ability to transfer (transduce) many hereditary traits from one strain to another, indications are that for the most part factors are transferred singly by this mechanism. It has also become evident that the effectiveness of the initial filtrates depends upon the presence and activation of lysogenic phage. There seems, in addition, to be good reason to suspect these phage particles as the actual transducing agents, especially if one considers the interpretation of variation in bacterial viruses proposed by Bertani and Weigle(2). These workers have assumed the existence of a phage structure the specificity of which is completely or almost completely under control of the host cell.

Preliminary investigations in this laboratory have revealed an analogous system for genetic transfer which makes use of Vi phage lysates, thereby eliminating the necessity for induction of lysogenicity as a prerequisite in the preparation of active filtrates.

*Materials and methods.* Lysates are produced by the action of specific Vi phage on strains of *Salmonella typhosa* employing the usual methods described by Adams(3). For these experiments, sterile stocks of Vi phage $E_1$ were prepared by lysis of the donor strain, a streptomycin resistant mutant of *S. typhosa* strain Ty2, which is xylose positive and antigenically typical. The serotype of this culture is represented as Vi, IX, XII:d. Genotypically, the factors under study may be depicted as follows: Xyl+, $S^r$, designating the ability to ferment xylose and resistance to streptomycin. The number of phage particles present in the lysate can be accurately determined by the plaque count method and in general, prepared stocks are usually found to contain $10^9$ to $10^{10}$ phage particles per ml. Receptor strains consist of a number of Vi cultures of *S. typhosa* which are able to absorb the phage from the lysate without the occurrence of lysis. Assays of lysate supernates have indicated that more than 90% of phage particles originally present are absorbed by receptor strains. The basic experimental procedure involves the incubation of receptor cells in the presence of a phage lysate prepared from the donor strain. As controls, equal portions of the original suspension are suspended in both nutrient broth and boiled filtrate. After a 30 minute incubation period, the suspensions are washed, taken up in a few ml of saline and assayed for cell count. These suspensions are then examined for the presence of xylose positive and streptomycin resistant cells.

*Results.* *S. typhosa* strain 643 possesses an antigenic structure comparable to the donor strain, but is xylose negative and streptomycin sensitive (Xyl–, $S^s$). Cell suspensions of this strain after treatment with $E_1$ phage lysates of the donor culture are plated on Eosine-Methylene Blue (EMB) xylose plates to select cells transduced for the ability to ferment xylose. Approximately $10^9$ cells spread on EMB xylose plates have given rise to varying numbers of xylose positive cells depending upon the number of phage particles present in the original lysate. No positives have ever been observed on the control plates although uninhibited negatives appear in comparatively small number on both control and experimental plates (Fig. 1). The great majority of cells of strain 643 are completely inhibited when plated on EMB xylose plates. As a further check on spontaneous mutability of the xylose factor, fermentation tubes containing xylose broth with phenol red as an indicator were inoculated with control suspensions. Tubes were held at least 30 days and despite the use of dense suspensions of untreated cells no positive tubes were observed in over 200 fermentation tests. On the other hand, all tubes inoculated with phage treated

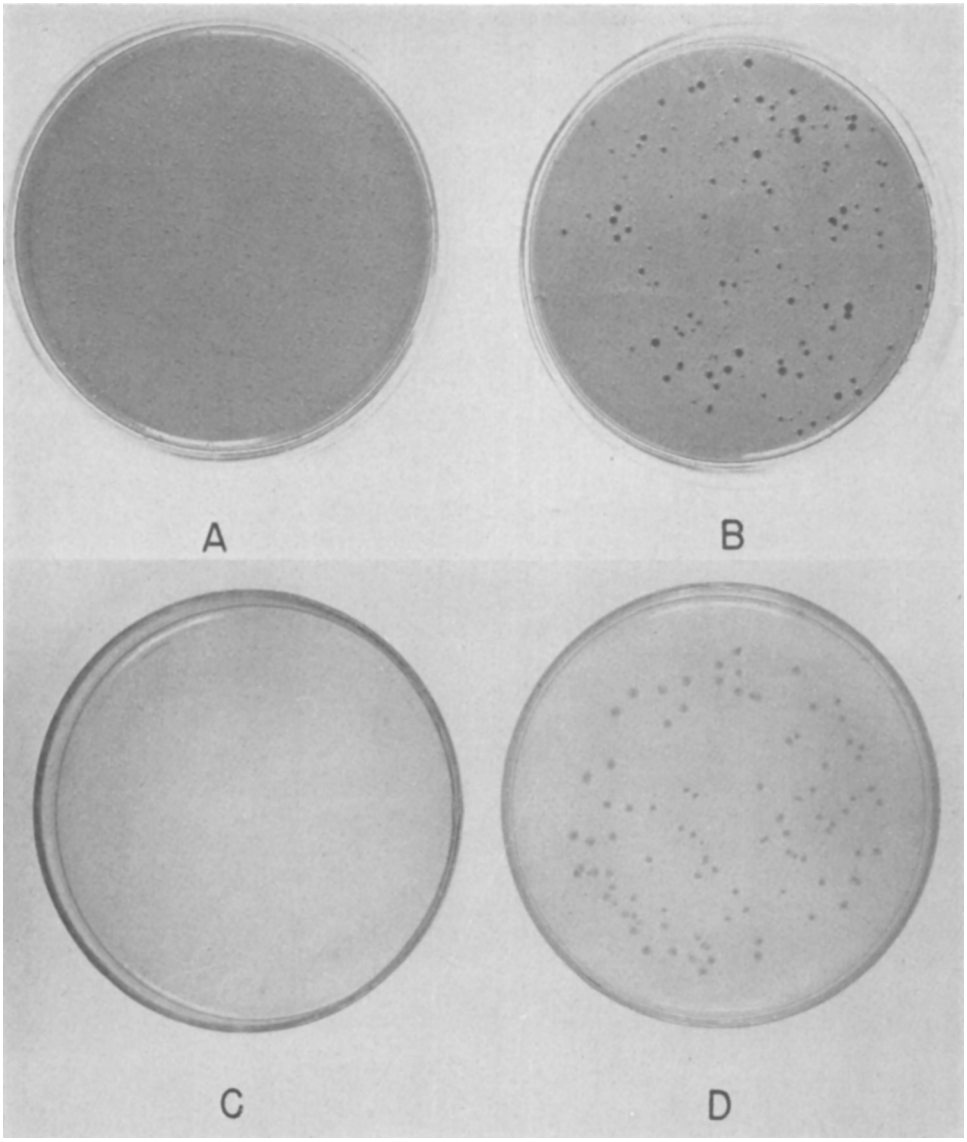


FIG. 1 (top). *Transfer of xylose fermenting ability.* Demonstrated by plating (A) cells treated with heat inactivated phage lysates, (B) cells treated with active phage lysates, on EMB xylose plates.

FIG. 2 (bottom). *Transfer of streptomycin resistance.* (C) cells treated with heat inactivated phage lysates, (D) cells treated with active phage lysates.

cells of the normally xylose negative strain 643 became positive in 2-3 days. Diluted suspensions of treated cells calculated to contain about 10 to 20 transduced positives fermented xylose in a majority of the tubes. All tubes were further checked by streaking on EMB xylose plates. In addition, large numbers of positive clones from EMB xylose plates were tested by streaking on nutrient agar plates containing 0.5 mg of streptomycin per ml and were uniformly found to remain streptomycin sensitive.

Experiments were undertaken designed to demonstrate a transfer of streptomycin resistance from the donor culture, using phage lysates, to strain 643 as well as to a serologi-

TABLE I. Transfer of Streptomycin Resistance to *S. typhosa* Strain 643. Streptomycin resistant clones.

| Control cells | Phage treated cells |
|---|---|
| 0 | 110 |
| 0 | 160 |
| 0 | 108 |
| 1 | 98 |
| 0 | 108 |
| 1 | 115 |
| 0 | 99 |

$10^9$ cells spread on nutrient agar plates and incubated 2 hr at 37°C. After incubation, plates were overlaid with .7% agar containing .5 mg streptomycin/ml.

cally rough culture, *S. typhosa* strain Vi I (Bhatnagar) which is Vi positive, non-motile and essentially devoid of the typhoid somatic antigens. Experimental and control suspensions prepared as previously described were spread on nutrient agar plates and incubated for two hours to allow for phenomic lag (4,5). At the end of this period, all plates were overlayed with 0.7% agar containing 0.5 mg of streptomycin per ml and incubated for two days. Results of a typical experiment are shown in Table I. The appearance of control and experimental plates at the completion of the experiment is illustrated in Fig. 2. All streptomycin resistant clones were streaked on EMB xylose plates and were found to remain xylose negative. Phage lysates prepared from streptomycin sensitive as well as xylose negative strains were used as controls in earlier experiments and served to indicate that only factors present in the genotype of the donor strain could be transferred.

Using standard cell suspensions, an attempt was made to relate the number of cells transduced for the xylose marker to the number of phage particles used in treatment. The semilogarithmic graph (Fig. 3) demonstrates a straight line relationship between numbers of transduced positives and numbers of phage particles. The possible presence of a transforming factor liberated by the action of the phage and comparable to the genetically active desoxyribonucleic acid preparations reported by Hotchkiss(6) and many other investigators, was determined by means of a desoxyribonuclease preparation (25 mg/ml).* Results obtained after treatment of the phage lysates with this enzyme indicated no effect on the transducing ability of the lysates.

The experiments set forth here have been repeated with another of the Vi phages (Phage K). Other Vi phages in the series as well as other genetic factor transductions are now being examined.

*Discussion.* One might interpret the remarkable findings of Zinder and Lederberg (1) as a form of transformation using bacteriophages, symbiotic in the donor cells, which after appropriate treatment become part of the genetic constitution of the recipient cells. Whether the phage is the intrinsic transferring agent or merely serves as the carrier of the genetically active material has not as yet been clearly established.

The experiments reported here indicate that phage lysates of one typhoid strain can have a genetic effect on other strains in the series. It is pertinent at this point, to consider the origin of these Vi typing phages and their

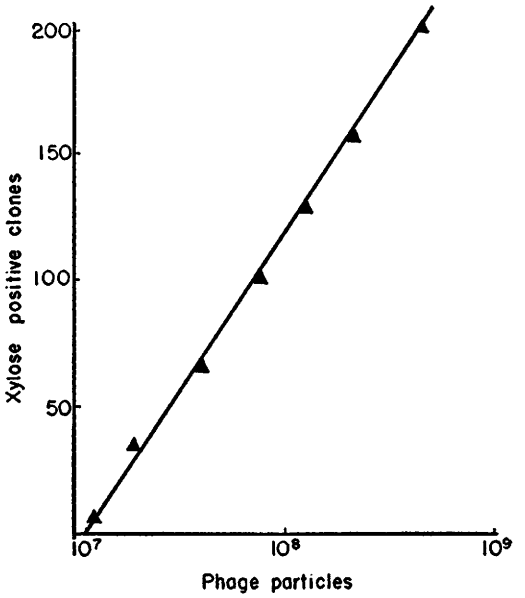


FIG. 3. *Effect of varying number of phage particles.* $10^8$ cells of *S. typhosa* strain 643 treated with varying amounts of Vi phage $E_1$ and plated on EMB xylose agar. Xylose positive clones were counted after 48 hr.

* Desoxyribonuclease preparations were tested for activity on purified thymus nucleic acid (kindly supplied by Dr. A. Saz) prior to use in experimental procedures.

practical applications. Craigie and Yen(7) developed the Vi bacteriophage method of typing strains of *S. typhosa* by starting with one phage and through a mechanism of 'phage adaptation' produced a series of specific Vi phages. These phages are now used in the routine typing of typhoid strains according to a revised scheme and standardized technic suggested by Craigie and Felix(8). Felix and Anderson(9) refer to a classification of strains of typhoid on the basis of differential fermentation of l-arabinose and xylose and state that a xylose negative strain retained this biochemical characteristic during more than eight years maintenance in the laboratory. While the obvious genetic significance of transduction by phage is of paramount importance, since the situation presents many problems with far-reaching implications, it is nevertheless worthwhile to be aware of the practical problems likely to be encountered (see Felix and Anderson(9)). It is possible to conceive of symbiotic relationships in the Vi phage series wherein a number of phages may be carried by typhoid strains, for Rountree(10) has shown that a single bacterial strain can carry as many as 5 different latent phages. As far as the geneticist is concerned, the attempts at 'phage adaptation' in the production of the Vi phage typing series by Craigie and Yen(7) may afford an unparalleled tool for the study of symbiotic and lysogenic relationships between bacteria and phage. The presence of the Vi antigen in strains of non-typhoid organisms such as *Escherichia coli, S. ballerup* and *S. paratyphi C* offers the opportunity for possible inter-strain transductions and perhaps, ultimately, an explanation for the presence of this antigen in these strains. Further studies with these objectives in mind are now being conducted in this laboratory, along with attempts at the elucidation of the underlying mechanisms involved.

*Summary.* Lysates prepared by the action of specific Vi phage $E_1$ on *S. typhosa* strain Ty2 possess the ability to transfer (transduce) single hereditary traits to other Vi strains of *S. typhosa*. Fermentative ability and drug resistance have been transferred to strains lacking these factors. These experiments have been repeated using Vi phage type K with the same results. The data obtained showed considerable similarity to the findings of Zinder and Lederberg with the advantage that induction of lysogenicity is not a necessary factor in the initial preparation of active filtrates. The practical and genetic implications of this system and further avenues of analysis have been discussed.

1. Zinder, N. D., and Lederberg, J., *J. Bact.*, 1952, v64, 679.

2. Bertani, G., and Weigle, J. J., *J. Bact.*, 1953, v65, 113.

3. Adams, M., *Methods in Medical Research,* 1950, v2, 1.

4. Davis, B. D., *Experientia,* 1950, v6, 41.

5. Demerec, M., and Cahn, E., *J. Bact.*, 1953, v65, 27.

6. Hotchkiss, R. D., *Cold Spring Harbor Symposia Quant. Biol.,* 1951, v16, 457.

7. Craigie, J., and Yen, C. H., *Can. Pub. Hlth. J.,* 1938, v29, 448, 484.

8. Craigie, J., and Felix, A., *Lancet,* 1947, v1, 823.

9. Felix, A., and Anderson, E. J., *J. Hyg.,* 1951, v49, 349.

10. Rountree, P. M., *J. Gen. Microbiol.,* 1949, v3, 153.

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