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Rapid Methods for Generalized Transduction of *Salmonella typhimurium* Mutants

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A procedure has been developed that allows the propagation of generalized transducing phage directly on cells growing on solid media. After the donor cells are killed with chloroform, the phage can be transferred directly to recipient cells and transductants can be selected.

Generalized transduction has facilitated the genetic analyses of numerous microorganisms, particularly the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*. We have developed a simple and rapid procedure for performing transductions that extends the capabilities of currently used methods. Other investigators have used rapid procedures for isolating phage mutants having altered transducing properties (8) and specialized transducing phages (4, 9). Our procedure entails the propagation of generalized transducing phage directly on cells forming colonies on solid media; this technique eliminates the need to culture and infect cells in liquid media. This technique allows a large number of genetic crosses to be performed on a single selection plate such that: (i) mutations causing similar phenotypes can be examined to determine whether they are closely linked; (ii) transductants can be selected on several different media by spotting or replica plating phage onto recipients on a variety of media; (iii) unmapped mutations can be rapidly screened to determine whether they cotransduce with known genetic markers; (iv) strains mutagenized with a Tn*10* transposon, which confers tetracycline resistance, can be efficiently surveyed to locate the region of insertion.

The procedure involves the spotting or gridding of donor cells onto Luria broth agar medium (3) which has been spread with a 0.1-ml sample of either P22*HT105/1int* (7) or KB1*int-1* (1, 6) phage preparations containing about 10^{10} plaque-forming units per ml. This master plate is incubated at 37°C for 9 to 12 h to allow sufficient phage infection and multiplication as the bacterial cells grow. The surviving cells are killed with chloroform vapor at room temperature by inverting the bottom of the master plate onto a glass petri plate bottom containing 3.0 ml of chloroform. After 1 h, the plates are separated, and the chloroform is evaporated from the master plate by leaving the lid slightly ajar for 1 h. Transductions are performed by transferring a mixture of killed cells and phage from the streaks to either a lawn or spots of recipient cells spread onto one or more selective media. Sterile toothpicks or replica plating have been used to perform the transfers discussed above. Transductants appear after 12 to 48 h of incubation at 37°C, depending on the composition of the selective media.

Figure 1 shows the results of reciprocal transductions with KB*int* phage, using four methionine auxotrophs having mutations in different genes and one histidine auxotroph (Table 1). Some areas appear as confluent growth rather than isolated colonies because of the large number of transductants obtained. The following controls verified that this growth is the result of transduction. A Luria broth agar plate inoculated with chloroform-treated cells alone did not give rise to colonies; therefore, the results in Fig. 1 are not attributable to the transfer of viable cells from the master plate. The diagonal of no growth represents homologous transductions. The absence of colonies demonstrates that the recipients did not revert to prototrophy and that few or no wild-type transducing phage were transferred from the donor phage plate. Similar experiments were performed with uninfected cells which had been chloroform treated. No colonies arose on the selective plate, indicating the results shown in Fig. 1 are not due to transformation. Analogous experiments with P22*HTint* phage as the transducing phage give the same results but yield even more transductants. This higher yield is consistent with the increased transducing ability of the P22 HT mutant (8) as compared with other P22 phage derivatives or KB. Figure 1 represents 25 distinct transductions on one section of a petri plate; clearly, use of the entire plate would allow additional crosses to be performed.

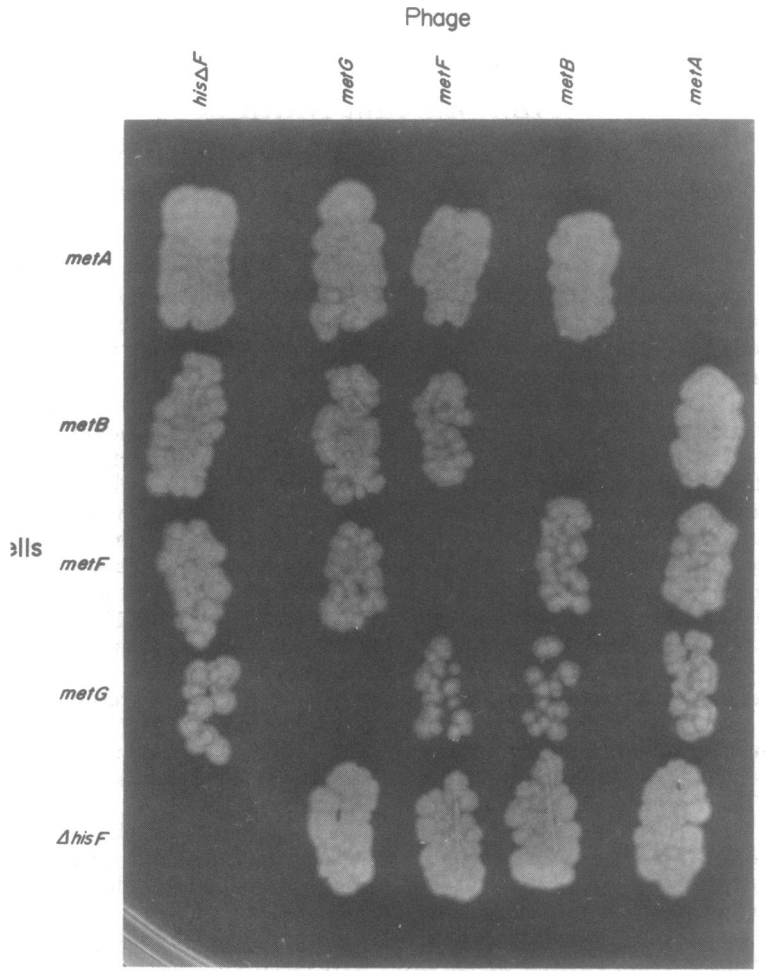

FIG. 1. *Transductions with methionine and histidine auxotrophs. The KB1int-1 phage was produced by donor cells growing on solid media as described in the text. After chloroform treatment, the phage were spotted onto a glucose-minimal agar plate. A small volume (10 to 20 μl) of freshly grown recipient cells (10^9 cells per ml) was spotted onto the streaks of donor phage, and the plate was incubated for 48 h at 37°C.*

TABLE 1. *Bacterial strains used in this investigation*

Strain	Relevant genotype	Source
JL308	*metA309*	This laboratory
JB516	*metB63*	This laboratory
JB518	*metF96*	This laboratory
JB519	*metG319*	This laboratory
TA831	*ΔhisF645*	S. Kustu

Using the procedure described for Fig. 1, we found that reciprocal crosses with mutants having closely linked mutations near or within the *glnA* gene yielded significantly fewer wild-type recombinants than did crosses with a *gln*$^+$ donor. Thus, closely linked mutations can be detected by the reduction in the number of transductants produced with this technique. In addition, the number of transductants can be easily varied by altering the quantity of material transferred from the chloroform-treated cells. For example, in cotransduction experiments it is useful to obtain isolated colonies so that the transductants can be scored for an unselected marker. This can be accomplished by transferring a smaller amount of material from the master plate and by streaking it over a wider area of recipient cells.

The recent investigations with translocatable drug resistance elements have provided additional genetic tools for mutant selection and characterization (2, 5). Using our transduction procedure, we have established linkage of the

Tn*10* insertion element, which confers tetracycline resistance, with *glnA* and *gdh* genes in different strains of *S. typhimurium*. Modifications of these transduction methods would extend the utility of Tn*10*-mediated in vivo mutagenesis experiments by allowing a more rapid screening of the colonies becoming tetracycline resistant. With this procedure, generalized transducing phage can be grown directly on hundreds of mutagenized colonies, each having the Tn*10* inserted at a different site and the resulting transducing particles spotted onto numerous recipients or on various selective media to identify the Tn*10* insertion sites. Thus, this is a rapid method that uses only a few agar plates.

In addition to the ease of performing a large number of genetic crosses, these procedures are useful for transducing recipient strains having markers that revert or mutate at a high frequency. By conventional transduction methods, such revertants are indistinguishable from transductants. For example, transductants receiving mutations for amino acid analog resistance have been difficult to identify due to the high frequency of recipient cells becoming spontaneously resistant to a particular analog. These complications may be overcome with our method of patching phage from a master plate onto a lawn of recipient cells. Although spontaneous mutations in the recipient cells still occur on the selection plate, the numbers are few when compared with the large number of transductants clustered in the small area where the phage are applied. Thus, it is highly probable that a colony growing in this cluster is the result of transduction.

The basic procedure described here can be modified to be useful under other experimental conditions. Mutations requiring time for expression may be easily transduced by spotting the donor phage on nonselective media containing recipient cells. After time for transduction and expression of the mutation is allowed, the transduction plate can be printed onto selective media. A sufficient number of transductants can be transferred to the selective media and are readily detectable.

We have used these procedures with *S. typhimurium*, but the basic protocol should be applicable to any genetic system in which the phage propagate sufficiently on cells growing on solid media. The preparation of transducing phage on solid media is a useful addition to other methods for genetic analysis and strain construction. Extensions and modifications of these techniques would be extremely beneficial and have a variety of applications.

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