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# Transduction with Integration-Defective Mutants of *Salmonella typhimurium* Bacteriophage KB1

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Phage KB1 has gained use as a generalized transducing phage for *Salmonella typhimurium*, including strains resistant to phage P22. Integration-defective mutants of KB1 have now been isolated; one of these, *int-1*, is recommended for transduction when nonlysogenic recombinants are desired.

Phage KB1 was introduced as being particularly useful for the generalized transduction of *Salmonella typhimurium* strains lysogenic for phage P22 (3). In our work, we have found a number of *S. typhimurium* strains not involving P22, which, nevertheless, are refractory to transduction with that phage. These strains have generally evolved after multiple auxotroph selection or as partial diploids involving F'T80 (2). As with P22 lysogens, we have observed that these strains also accept KB1 for generalized transduction. However, such KB1 transductants are themselves lysogenic and therefore unsuitable for sequential transduction.

The analogous problem with P22 may be circumvented by transducing with P22 integration-defective mutants (2). Accordingly, we have isolated integration-defective mutants of phage KB1. One of these mutants, designated KB1 *int-1*, is now being used routinely to obtain nonlysogenic transductants of *S. typhimurium*.

The phage mutants were induced (with nitrosoguanidine) and isolated following essentially the same procedure Smith and Levine used for their isolation of P22 *int* mutants (6). Use of phage antiserum was omitted and the level of unadsorbed phage was reduced by collecting and washing the infected cells on membrane filters (Millipore Corp.). Three mutants were isolated from the first five parallel cultures examined.

Transductions with KB1 *int-1* are performed by the direct co-plating of 0.1-ml portions of cell and phage suspensions (B. Ely, personal communication). Nonlysogenic transductants are readily obtained upon primary isolation at 37 C. Representative data are summarized in Table 1. Stocks to be transduced were chosen at random and included one of strain LT7 and nine of strain LT2. All stocks transduced with KB1 *int-1* yielded at least one nonlysogenic colony among three colonies tested, compared to only one nonlysogen observed from the 10 transduced with KB1. Transductants nonlysogenic with respect to KB1 have been obtained with the same facility when the recipient *S. typhimurium* are P22 lysogens or are otherwise P22 resistant.

TABLE 1. *Efficiency of obtaining phage-sensitive transductants after use of KB1 and KB1 int-1*[a]

| Transducing phage | No. of transductions yielding 0, 1, 2, or 3 sensitive clones/3 isolates | | | |
|---|---|---|---|---|
| | 0[b] | 1 | 2 | 3 |
| KB1 | 9 | 1 | None | None |
| KB1 *int-1* | None | 2 | 4 | 4 |

[a] Ten distinct auxotrophs (3 *arg*, 2 *leu*, 2 *met*, 2 *trp*, 1 *pro*) were transduced to prototrophy with the two phages (multiplicity of infection = 10). From each transduction, three colonies were single streaked and one subclone of each of these was examined for KB1 plaque formation by the soft agar overlay method.

[b] Number of sensitive clones.

Phage *int-1* is routinely propagated by the soft agar overlay method (1) using L-agar (5). After overnight incubation, the plates are flooded with 3 ml of T2 buffer (4) and left for 1 to 2 h at room temperature. The recovered phage suspensions are stable for at least 4 months when stored at 4 C over chloroform. Plating of $10^5$ PFU by this method yields approximately $10^{11}$ PFU/ml. The integration-defective property of KB1 *int-1* has shown no evidence of reversion through five propagations over a 4-month period.

Availability of KB1 *int-1* greatly extends the utility of the KB1 transducing system.

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