Generalized Transduction in *Corynebacterium renale*

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A phage, designated RP28, was obtained which carried out generalized transduction in *Corynebacterium renale*, and 11 different auxotrophic markers were all transduced.

Generalized transduction has been reported to occur in several species of bacteria (2). However, transduction has never been reported in the genus *Corynebacterium*. Evidence presented in this report shows that generalized transduction occurs in *C. renale* by a phage capable of restoring prototrophy to several amino acid auxotrophs derived from the parent bacterium.

Frequencies of transduction were low when minimal medium (MM) agar was used as the selective medium, but they were high when enriched minimal medium (EMM) agar was used for the same purpose. For instance, the average number of transductants recovered from a 0.1-ml sample of a 1:10 dilution of transduction mixture of phage RP28 and the 8-50 (arg-) mutant was 41 when MM was used, but 925 when EMM was used as the selective medium. Therefore, the use of EMM was necessary to the transduction experiment.

Table 1 shows the results of a transduction experiment in which the 8-50 (arg-) mutant was transduced to prototrophy by phage RP28 propagated on a wild-type strain. From the mixture made up of cells and phage, 1,340 arg+ colonies were obtained with transduction frequencies of $5 \times 10^{-4}$ per plaque-forming unit. The mixture containing cells but not phage produced no colonies; the mutant 8-50 (arg-) was quite stable, although a few revertants have appeared spontaneously. When phage was heated at 60°C for 30 min or inactivated with antiphage serum, no transductants were found. Incubation of phage with deoxyribonuclease did not affect the transduction results. When the phage alone were plated on nutrient agar, no prototrophs were found. Colonies of the transductants could not be distinguished from those of the prototroph.

Reciprocal transductions were done using the phage RP28 and 11 auxotrophic mutants. All 11 of the auxotrophic markers, *try*, *arg*, *his*, *ile*, *meth*,

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### Table 1. Inhibition of transduction by antiphage serum, heat, and deoxyribonuclease

<table>
<thead>
<tr>
<th>Treatment of phagea</th>
<th>aux+ Transductantsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phage omitted (control)</td>
<td>0</td>
</tr>
<tr>
<td>No treatment</td>
<td>1,340</td>
</tr>
<tr>
<td>Incubated with antiphage serumc</td>
<td>0</td>
</tr>
<tr>
<td>60 C for 30 min</td>
<td>0</td>
</tr>
<tr>
<td>Deoxyribonuclease treatmentd</td>
<td>1,015</td>
</tr>
</tbody>
</table>

a RP28 phage were induced by ultraviolet irradiation of lysogenic *C. renale* strain 28 (8). The phage was repeatedly cloned from the plaque produced on wild-type strain 8. Donor stocks of RP28 used in transduction experiments were prepared from lysis plates (1) and were sterilized with 1% chloroform.

b Auxotrophic derivatives of the wild-type strain were isolated by the replica-plating technique (6). Recipient cells were grown on nutrient agar for 24 hr at 37°C. The cell was suspended in 10 ml of nutrient broth (10^8 cells per ml), and 0.5 ml of this suspension was transferred to a small tube. To this suspension was added 0.5 ml of the transducing phage (5 x 10^8 plaque-forming units per ml). Adsorption was allowed to proceed for 30 min. A dilution (1:10) of phage and cells was prepared in phosphate-buffered saline. A portion (0.1 ml) of the diluted mixture was then spread onto duplicate EMM agar plates, which were incubated at 37°C. After 72 hr, transductant colonies were scored. The minimal medium described by Hirai and Yanagawa (3), and Hirai et al. (4) was used. Enriched minimal medium, which was minimal medium supplemented with vitamin-free Casamino Acid (0.025%), cystine (0.1 μg/ml), and tryptophane (0.1 μg/ml), was used for the selection of transductants.

c Phage was incubated with 1% antiphage serum for 15 min at 37°C before cells were added. The treatment with antiphage serum inactivated 99.9% of the plaque-forming units (8).

d Phage was incubated with deoxyribonuclease (30 μg/ml) for 15 min at 37°C before cells were added.
cys, lys, ser, thr, ade, and pro, were transduced by phage RP28 propagated on a wild-type strain. Phage propagated on each auxotroph transduced other auxotrophs but not the one on which it was propagated. The data provide additional evidence that phage RP28 is a general transducing phage.

Lysogenization is not a requisite of *C. renale*. The fact that most of arg+ transductants were sensitive to lysis by phage RP28 suggests that RP28 transducing particles are defective in at least some of its normal phage functions, as has been shown in transduction by phage P1 (5, 7).

Presumably, this is the first example of genetic exchange by transduction in the genus *Corynebacterium*. Since recombination by conjugation has not yet been observed in *C. renale*, direct mapping of the entire chromosome is not possible. Therefore, transduction seems to be a useful tool for genetic analysis in *C. renale*.

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**LITERATURE CITED**