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Transduction of Rifampin Resistance in Group A Streptococci

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Rifampin-resistant strains of group A streptococci were isolated as spontaneous mutants. Transduction analyses employing phage A25 showed the rifampin marker to be transferred with high frequency. The mutations conferring resistance to rifampin and streptomycin are not co-transducible.

A genetic approach to the study of group A streptococci became possible when Leonard, Colon, and Cole described the first successful transduction experiment involving a transfer of streptomycin resistance to streptomycin-sensitive organisms (3). Since group A streptococci are fastidious organisms requiring a complex medium for optimal growth, the isolation of auxotrophic markers has not been practicable, and consequently only a few markers have been obtained which show transfer from one strain to another. These include resistance to antibiotic inhibitors of ribosome functions (such as erythromycin, kanamycin, lincomycin, spectinomycin, and streptomycin) as well as transfer of the *uvr*$^+$ allele to *uvr* bacteria (3, 6). In the present study we report the transfer of rifampin resistance in two strains of *Streptococcus pyogenes*.

Rifampin is a broad-spectrum antibiotic which inhibits the growth of several bacterial species by specifically binding to the bacterial deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase, resulting in a blocking of the initiation of RNA synthesis (1, 2). In some instances, rifampin resistance is known to be due to changes in permeability to rifampin (7, 8). In this study, four spontaneous rifampin-resistant mutants of *S. pyogenes* 9440 *str-10* (type 6) were obtained after incubation of an overnight lawn of bacteria on Todd-Hewitt agar plates containing 120 μg of rifampin per ml. The transducing phage utilized was the double temperature-sensitive phage mutant A25 ts 1-2, isolated by Malke (4). Transduction experiments were performed by the agar overlay procedure described by Malke (4), employing as a base serum Todd-Hewitt agar plates as described by McKane and Ferretti (manuscript in preparation). Table 1A shows that all four rifampin-resistant markers were transduced with high frequency to a type 12 rifampin-sensitive recipient (K56), employing, as a donor, phage grown on the type 6 rifampin-resistant strains. When type 6 rifampin-sensitive, streptomycin-resistant strains were used as the recipients, rifampin-resistant recombinants were found, but at consistently lower frequencies. The rifampin-resistant recombinants selected in this experiment all retained the *str*^{R} marker as evidenced by the fact that over 100 single-colony isolates were all capable of growing on agar plates containing rifampin and streptomycin.

When phage grown on type 12 rifampin-resistant strains were used as donors, no transduction was observed with either type 6 or type 12 bacterial recipients. This lowered ability of the type 12 strain to serve as an efficient donor in transduction was also observed in transduction experiments selecting for streptomycin-resistant recombinants (Table 1B). Whereas the type 6 strain as a donor gave high frequencies of transduction, very low frequencies of transduction were observed with phage grown on the type 12 strain. These results agree with the observations of Malke (5) that K56 and its derivatives are poor donors in A25-mediated transductions. Conversely, the data in Table 1A suggest that the type 12 strain (K56) serves as a better recipient in transduction experiments than the type 6 strain.

A co-transduction experiment of the rifampin and streptomycin markers was attempted employing phage grown on a type 6 bacterial strain resistant to these antibiotics as a donor, and a

TABLE 1. *Transduction experiments*[a]

Phage grown on donor strains	Bacterial recipient	Transductants[b]	
		Per plate	Per PFU
A. Selection for rifampin-resistant recombinants			
9440 *str-10 rif-1* (6)[c]	K56 (12)	700	9.4×10^{-6}
9440 *str-10 rif-2* (6)	K56 (12)	137	1.4×10^{-6}
9440 *str-10 rif-3* (6)	K56 (12)	145	3.2×10^{-6}
9440 *str-10 rif-4* (6)	K56 (12)	302	4.4×10^{-6}
9440 *str-10 rif-1* (6)	9440 *str-10* (6)	103	1.0×10^{-6}
9440 *str-10 rif-2* (6)	9440 *str-10* (6)	88	8.3×10^{-7}
9440 *str-10 rif-3* (6)	9440 *str-10* (6)	72	1.5×10^{-6}
9440 *str-10 rif-4* (6)	9440 *str-10* (6)	44	5.8×10^{-7}
K56 *rif-4* (12)	9440 *str-10* (6)	0	0
K56 *rif-4* (12)	K56 (12)	0	0
B. Selection for streptomycin-resistant recombinants			
9440 *str-10* (6)	K56 (12)	600	2.5×10^{-6}
K56 *str-10* (12)	K56 (12)	10	1.0×10^{-9}
C. Selection for streptomycin- and rifampin-resistant recombinants			
9440 *str-10 rif-1* (6)	K56 (12)	0	0
9440 *str-10 rif-4* (6)	K56 (12)	–	–

[a] Transduction experiments were performed by the agar overlay procedure described by Malke (4), and the recipients (1×10^8 CFU/plate) were grown into late log phase before use. Serum Todd-Hewitt agar plates (McKane and Ferretti, manuscript in preparation) were employed and contained 3% Todd-Hewitt broth (Difco), 1% agar, 0.38% Na_2HPO_4, 0.02% $CaCl_2$, and 5% horse serum. The concentration of rifampin and streptomycin in the agar overlay was 120 μg/ml and 750 μg/ml, respectively. The number of colonies obtained from control plates for spontaneous mutation was subtracted from the data given above, and represented, on the average, 4 colonies on streptomycin and 14 colonies on rifampin-supplemented plates.

[b] PFU, Plaque-forming units; CFU, colony-forming units.

[c] Numbers in parentheses represent strain types.

type 12 sensitive strain as a recipient. As shown in Table 1C, no recombinants were obtained utilizing either of the two rifampin- and streptomycin-resistant mutant donors. These results indicate that the streptomycin and rifampin markers are located in regions of the *S. pyogenes* chromosome too distant from one another to be co-transduced.

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