TRANSDUCTION OF PROTOTROPHY TO AUXOTROPHIC MUTANTS OF PROTEUS MIRABILIS

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Genetic investigations in Proteus mirabilis were handicapped up to now by the absence of recombination between different auxotrophic mutants and such mutants and the wildtype. Recently we succeeded in transducing several prototrophic markers into various auxotrophic mutants of P.mirabilis by means of a temperate phage.

The temperate phage π 1 used in these experiments, was isolated from the lysogenic Proteus mirabilis strain R 11+. It was not yet possible to induce the phage with UV-irradiation, however, one gets high titer lysates (10^{10}/ml) with the agar-layer enrichment method (Adams 1959). For transduction experiments the phage was grown in the respective donor-strain. The transduction method was that generally applied in transduction experiments with Salmonella (see Demerec et al. 1956). It consists in mixing 1 ml of an overnight broth culture of the recipient strain with 2 ml of the respective phage lysate (multiplicity of infection 5.0), keeping the mixture in a water-bath at 37°C for 15 minutes and then plating 0.05 ml on each of four plates with minimal medium (Böhme 1961) or in the case of streptomycin-dependent recipient strains on streptomycin-free nutrient agar. Colonies were scored

+ This lysogenic strain was kindly supplied by Dr.U. Taubeneck, Jena.

after four days incubation at 37°C. The survival of the recipient bacteria was 15 to 30% in the different experiments.

The results of some typical experiments are given in table 1. The auxotrophic mutants used were either of spontaneous origin or induced by EMS treatment (Böhme 1962). The table shows that the auxotrophic mutants can be transduced to prototrophy by phage grown in the wild-type strain as well as by reciprocal transduction between different auxotrophic mutants. The frequency of transduction per phage particle was between 10^{-6} and 10^{-7}. Up to now no case of joint transduction of closely linked genes was observed in experiments with several different multiply marked mutants of P.mirabilis.

TABLE 1

Recipient	Donor	Transductants	
		Genotype	number/10^8 Survivors
lys^-	wildtype	lys^+	901.3
$met\text{-}49^-$	wildtype	met^+	1391.0
tyr^-	wildtype	tyr^+	312.5
str-d	wildtype	str-s	332.0
his^-lys^-	wildtype	his^+lys^- his^-lys^+	365.4 958.5
$met\text{-}29^-lys^-$	wildtype	met^+lys^-	1144.0
arg^-lys^-	wildtype	arg^+lys^-	1118.0
$met\text{-}49^-lys^+$	$met\text{-}49^+lys^-$	met^+lys^+	380.0
tyr^-lys^+	tyr^+lys^-	tyr^+lys^+	383.3

Generally the transductant colonies in these experiments consist of lysogenic bacteria which can not be used as recipients in further experiments. In order to obtain phage-sensitive, non-lysogenic transductants we used a virulent phage-mutant as

transducing phage (Zinder 1955; Starlinger 1958). π1c is a virulent mutant of phage π1, which produces clear plaques and does not lysogenize the infected bacteria. After UV-irradiation this phage can be used as transducing vector (Garen and Zinder 1955; Goldschmidt and Landman 1962). In these experiments phage π1c was irradiated to a survival of about 10^{-4} - 10^{-5} and the irradiated lysate was used in the same way as in the transduction method described above. The yield of transductants was only insignificantly less as compared with the experiments with non-irradiated temperate phage. The resultant transductants are phage-sensitive.

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