GENETIC TRANSDUCTION IN BACILLUS SUBTILIS[X]

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In 1952, Zinder and Lederberg first demonstrated genetic transduction in Salmonella. Since then such genetic interaction has been described for a number of bacterial genera. Thorne (1961) reported that prototrophy could be transduced to various auxotrophic mutants of Bacillus subtilis. Transduction of streptomycin resistance (Sm^r), prototrophy (Prot), and sporogenesis (Sp) has been demonstrated independently in our laboratory with the same organism.

Temperate phage (PBS1) was isolated from soil by the method of Ivanovics (1958). Phage PBS1 forms turbid plaques with peripheral rings on all Marburg strains[XX] tested and three other strains of B. subtilis isolated from various sources. Stable lysogenic bacteria could be readily obtained from turbid plaques formed on soft agar plates seeded with phage and sensitive bacteria. The stability of the lysogenic strains thus obtained was shown by the fact that they retained their phage-producing capacity and immunity to homologous phage even after heat treatment of cultures at 85° C for 15 minutes and after many subcultures. High titer lysates were prepared either from two-day old lysogenic cultures without further treatment or from UV-irradiated cultures. In order to destroy any transforming DNA present in cultures,

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lysates were treated with DNase (10 μg/ml) at 37° C for 15 minutes prior to filtration. Recipient cells were grown for 4 hours in a minimal medium supplemented with 0.1% each of yeast extract and vitamin-free acid-hydrolysed casein. All incubations were carried out at 37° C. Transduction experiments were conducted in a 37° C water bath by mixing 1.5 ml of lysate (1×10^9 plaque-forming particles/ml) with 1.5 ml of recipient culture (3×10^8 cells/ml). After 30 minutes' incubation, cells were spun down and resuspended in minimal medium, 0.1 ml samples were then spread on minimal agar to detect prototrophic transductants. For transduction of streptomycin resistance, 0.1 ml samples were plated with tryptose blood agar base (Difco) and incubated for 3 hours before challenging with the same medium containing 2000 μg/ml of streptomycin. Colony counts were made after 40 hours.

The results of transduction experiments in which strain 19 (Sm^r, Prot) was used as donor and various non-lysogenic mutants as recipients, are summarized in Table 1. Transduction frequency per phage particle is about 10^{-6} for prototrophy and 10^{-7} for streptomycin resistance, and is comparable with rates encountered in other transduction systems. Reciprocal transductions between auxotrophic mutants which yielded prototrophs was also observed in *B. subtilis*. The results of a typical experiment of this type are shown in the last column of Table 1. It may be seen that when lysates of strain 168 (Indole$^-$) were mixed with cultures of auxotrophic mutants and plated on minimal agar, prototrophic colonies developed from all mutants except from strains 168 and W5.

Schaeffer, *et al.* (1959) reported that both reversible and irreversible asporogenous (Sp$^-$) strains of *B. subtilis* could be made sporogenous by transformation with DNA extracted from a wild-type strain. On the other hand, Spizizen (1960) stated that some Indole$^-$ Sp$^-$ strains could not be transformed at all for prototrophy. We have isolated a Sp$^-$ strain (Sp$^-$1) from a wild-

TABLE 1

Transduction of Streptomycin Resistance and Prototrophy in *Bacillus subtilis*

	Donor		
	19 (Sm^r Prot)		168 (Indol$^-$)
Recipient	$Sm^{r x}$/ml	Prototrophs/ml	Prototrophs/ml
H12 (Ph-alanine$^-$)	80	800	2,500
S10 (Glutamic ac.$^-$)	70	500	1,600
N2 (Serine$^-$)	< 10	310	540
W5 (Indole$^-$)	20	260	< 10
168 (Indole$^-$)	80	440	< 10
W3 (leucine$^-$)	70	630	1,000
P7 (Leucine$^-$)	60	810	1,600
C14 (Cysteine$^-$)	< 10	40	1,700
Sp$^-$1	80	-	-

x Number of colonies resistant to 1000 μg/ml of streptomycin. No streptomycin-resistant or prototrophic revertants developed when recipient cultures were plated without lysates.

type Marburg strain by UV-irradiation; spores were not observed on any of the several culture media tested. Thus strain Sp$^-$1 is an irreversible asporogenous strain according to Schaeffer's definition. Transduction of sporogenesis to strain Sp$^-$1 could be readily demonstrated with lysates of strain 19, although DNA derived from this strain was unable to confer either sporogenesis or streptomycin resistance to strain Sp$^-$1. Strain Sp$^-$1 treated with lysates of strain 19 and grown overnight on potato agar showed about 5% sporulation as determined by direct microscopic count of malachite green-stained samples, whereas untreated cultures or cultures

treated with DNA extracted from strain 19 contained no spores. Minimal agar medium supplemented with 0.01% vitamin-free acid hydrolysed casein and 5 μg/ml $MnSO_4$ was used for determining the frequency of transduction. After 3 days' incubation on this medium brown colonies of sporogenous bacteria can be differentiated from white colonies of asporogenous bacteria. The frequency for transduction of sporogenesis determined in this way was approximately 10^{-6} per phage particle.

Since culture filtrates of B. subtilis contain a measurable amount of transforming DNA, the question may be raised as to whether the appearance of new characters among recipient cells is due to phage-mediated transduction or to transformation by extracellular DNA. Control experiments were consequently carried out. Treatment with DNase, as described above, completely destroyed transforming activity (both Sm^r and Prot) in culture filtrates of the non-lysogenic strain 19. Reduction of phage titer to less than 10^6/ml by high speed centrifugation or by neutralization with rabbit antiserum inhibited transduction. It is also interesting to note that strain Sp^-1, which was not transformable to streptomycin resistance by DNA, could be transduced for streptomycin resistance by lysate of strain 19 (Table 1). When lysates of strain 19 were plated without recipient cells no colonies developed either on minimal agar or on potato agar. It may therefore be concluded that the results presented above are not due to transformation by extra-cellular DNA or bacterial contamination of lysates used but to transduction.

Full details of this work will be published elsewhere. The technical assistance of Miss A. I. MacIntosh is appreciatively acknowledged.

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