TRANSFORMATION OF GENETIC TRAITS BY THE DNA PREPARED FROM A LYSOGENIC PHAGE S-1

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An experimental system involving both transformation and transduction was recently established in Bacillus subtilis Marburg (1,2,3), but there appears to be no published account of the transformation by the DNA prepared from the transducing phage (SP-10). The bacteriophage S-1 described in this paper is a temperate phage different in some respects from the SP-10. Details of the experimental methods and the specificities of the phage are to be reported elsewhere.

Preparation of phage S-1 A substrain of Marburg was lysogenized by the phage, and heat-shocked spores of 23(S-1) were inoculated into nutrient broth in a two liter flask. Cultivation was carried out at 37°C with vigorous aeration for 16 hours. Bacterial cells were spun down at 4,000 and 8,000 rpm and infective phages (about 10^9/ml) in the supernatant were salted out with $(NH_4)_2SO_4$ at a concentration of 45%. The precipitate appeared by the treatment was dissolved in 40 ml of 0.1 M Tris-buffer (pH 7.4) and the solution was subjected to centrifugation to remove residual cells. The last procedure was repeated several times. Finally, the phage particles collected by ultracentrifugation (40,000 g, 45 min.) were suspended in 5 ml Tris-buffer (pH 9.0).

Such phage suspension was prepared twice, P-1 and P-2, each independently, and it was evidenced by the plating method and the microscopic

observation that each milliliter of the suspensions contained about 10^{11} infective phages, less than 10 viable cells, and less than 100 cell debris.

Preparation of DNA from phage S-1 DNA was extracted from the phage suspension by a modified phenol method (4). The DNA precipitated in 95% ethanol was dissolved in 5 ml of 0.15 M NaCl-citrate solution (pH 7.0). The solution prepared from the P-1 contained 53 μg/ml of DNA and that from the P-2 contained 42 μg/ml (DNA-I and DNA-II). At the concentration used in the transformation experiments, these solutions gave no colony and plaque on plates charged with complete medium or inoculated with sensitive cells. Therefore, the solutions are free of viable cells and infective phages.

Transduction by phage S-1 Strain 30 ($ind^{-}his_{30}^{-}$) was used as a recipient. An aliquot of the phage suspension was added to $3x10^{8}$ cells in one milliliter and the mixture was placed at 37°C for 15 minutes for adsorption. The cells were washed and 0.1 ml of the suspension was plated onto minimal medium supplemented with either L histidine or L tryptophan to select transductants independent on one amino acid. The transductants were scored after two days' incubation at 37°C. Because the frequencies of transduction to each genetic marker were equal regardless of the presence or absence of DNase, the result in Table 1 may be taken as an evidence of transduction by the phage S-1.

Transformation by the DNA prepared from phage S-1 Using the DNA mentioned above as a donor and the three auxotrophs indicated in Table 2 as recipients, transformation experiments were carried out by the method reported previously (5). The results are shown in Table 2. As the numbers of transformants appeared were too large to consider them as contaminants and, moreover, the DNase treated samples gave no transformant, it is concluded that the DNAs prepared from the S-1 are able to transform, like the DNAs of the donor strain (23W) which are shown in Table 2 as index DNAs, the genetic traits of the recipients.

Table 1. Transduction by phage S-1

Recipients	Phage prepn.	m.o.i.	Transductants/ml ind^+his^-	ind^-his^+
$30(ind^-his^-_{30})$	P-1	0.3	16,500	640
" "	P-2	0.3	32,200	1,900
			try^+arg^-	try^-arg^+
171-15 (try^-arg^-)	#	0.25	3,120	1,465
	##	0.1	4,750	1,900

and ## indicate phage suspensions prepared independently. It is calculated that the numbers of transducing phages carrying ind^+ in P-1 and P-2 are roughly 10^8 and 10^9 and those of phages carrying his^+ 10^7 and 10^8 respectively at the least.

Table 2. Transformation by the DNA prepared from S-1

Recipients	DNA prepn.	DNA* ug/ml	DNase (10ug/ml)	Transformants/ml ind^+his^-	ind^-his^+	ind^+his^+
$30(ind^-his^-_{30})$	index	0.1	-	208,000	200,000	
" "	DNA-I	0.047	-	3,250	2,330	
" "	DNA-I	0.047	+	0	0	
" "	DNA-II	0.014	-	9,750	8,190	
" "	DNA-II	0.014	+	0	0	
$31(ind^-his^-_{31})$	index	0.1	-	23,600	18,000	14,400
" "	DNA-I	0.047	-	2,400	1,550	1,440
" "	DNA-II	0.014	-	1,770	1,630	1,030
				try^+arg^-	try^-arg^+	
171-15 (try^-arg^-)	index	0.1	-	16,000	16,000	
" "	DNA-I	0.047	-	1,300	460	
" "	DNA-II	0.014	-	3,220	1,620	

* Final concentration
It is calculated that the numbers of DNA molecules carrying ind^+ and his^+ in the solutions corresponding to the P-1 and P-2 are $2\text{-}9\times10^7$ and $1\text{-}7\times10^7$ respectively.
Strains 30 and 31 were provided by Dr. S. Zamenhof and strain 171-15 was produced from 160 (try^-) of Dr. J. Spizizen by X-ray irradiation.

Discussion The attempt to perform transformation by the use of disrupted phage particles instead of the DNA of S-1 was successful in the first two experiments but not in the later. The disrupted phage particles were prepared by the method described in the Guthrie and Shinsheimer's paper (6). It appears therefore that delicate conditions are necessary for the performance. The success reported in this paper might have been due to the high titer of transducing phages in the suspensions and/or the improved method for extracting DNA from the phage suspensions.

Next, we wish to call reader's attention to the following point. When transformation is performed between DNAs extracted from a wild-type strain (23W) and cells of strain 171-15, the ratio, try^+arg^- to try^-arg^+, in the transformants are almost 1 : 1 (Table 2). Meanwhile, the ratio becomes invariably 2 : 1 when the DNAs prepared from the S-1 are used as donors, showing a good agreement with the transduction data shown in Table 1. It is presumable, therefore, that in the phage suspension the number of try^+ carrying phages is not equal to the number of arg^+ carrying phages. Such unequal distribution might have been caused by preferential attachment of phage particles to a special region of chromosome or unequal incorporation of chromosomal fragments in transducing phages.

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