THE INFLUENCE OF TEMPERATE BACTERIOPHAGE Ø105 ON TRANSFORMATION AND TRANSFECTION IN BACILLUS SUBTILIS

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SUMMARY

The frequency of transformation in derivatives of Bacillus subtilis 168 is drastically reduced when the bacteria are made lysogenic for bacteriophage Ø105. This reduction in the frequency of transformation is not paralleled by a similar reduction in the frequency of: (a) transfection with DNA from bacteriophages Ø29 and SP01, or (b) transduction by PBS1 and SP10. The results indicate that there are significant differences between the processes of transformation and transfection.

INTRODUCTION

To characterize the steps involved in DNA-mediated transformation and transfection, we have isolated a number of mutants deficient in transformation. One of these, RUB19, is a nontransformable mutant (tfm-1), which cannot be transfected with DNA isolated from bacteriophage SP01, but is transfected at reduced levels with DNA isolated from bacteriophage Ø29 (1). This locus, tfm-1, maps near the attachment site (2) for bacteriophage Ø105 (R. E. Yasbin and F. E. Young, unpublished observation). Because tfm-1 is also near recB (3) and the general region where a previously studied noncompetent mutant (4) was localized, we have initiated an extensive investigation of the segment of the chromosome which is between the azlA and lys-3 (5). Studies with the temperate bacteriophage Ø105, initially isolated by B. E. Reilly, have revealed that its circular vegetative map is inserted linearly into the host chromosome between phe-1 and ilvC (2). Bacteria lysogenic for

Ø105 transform at reduced levels (6). Furthermore, this decrease in transformation is not confined to markers in any particular region of the chromosome. Peterson and Rutberg (6) also observed a decrease in the frequency of DNA-mediated transfection with bacteriophage Ø1 in bacteria lysogenic for Ø105. Because our recent studies with RUB19 demonstrated that phages vary in their capacity to transfect mutants, we have studied transfection in lysogenic bacteria with other viruses. The data to be presented demonstrate that lysogeny with Ø105 does not significantly alter the frequency of transduction with PBS1 or SP10 or the frequency of transfection with DNA isolated from bacteriophages Ø29 and SPO1, despite a marked decrease in transformation with bacterial DNA.

MATERIALS AND METHODS

Strains:

B. subtilis 168, an isogenic multiple auxotrophic derivative, BR151 (carrying *lys*-3, *trp*C2 and *met*B10), *B. subtilis* 168 carrying Ø105 (obtained from Dr. J. Hoch), *B. subtilis* HSR (obtained from B. E. Reilly), and *B. subtilis* W23 (obtained from K. Bott) were used in this study. Bacteriophages Ø29, SPO1, PBS1 and SP10 were propagated on *B. subtilis* HSR, *B. subtilis* 168, *B. pumilus*, and *B. subtilis* W23, respectively.

Procedures for Genetic Exchange:

The development of competence, the method of interaction with DNA, and the selection of transformants were similar to those procedures previously utilized in our laboratory (7). To determine transfection, competent cells were incubated with bacteriophage DNA for 30 min, the reaction terminated with DNase (50 μg/ml), and infectious centers assayed in an overlay of semi-solid agar (1% tryptone, 0.8% NaCl, 0.5% glucose and 0.6% agar) on Tryptose Blood Agar Base (Difco) with 168 (Ø105) as the indicator strain.

The DNA was prepared with slight modifications of the procedure described by Saito and Miura (8). Cultures of bacteria grown in Antibiotic

Medium 3 (Difco) were concentrated to one-tenth the original volume in a TRIS-EDTA buffer [0.15 M tris(hydroxymethyl) aminomethane (Tris)hydrochloride, 0.1 M EDTA, pH 8.0], incubated for 1 hr at 37°C with lysozyme (1 mg/ml, Calbiochemical Co.), frozen and thawed several times, and incubated with pronase (1 mg/ml, Calbiochemical Co.) for 1 hr at 37°C prior to the addition of 1% sarkosyl NL-79 (Geigy Co.) and 1% sodium dodecyl sulfate (Sigma). After 30 min at 37°C, the DNA was extracted with an equal volume of phenol (Mallinckrodt Co.) buffered with Tris (pH 8.1, 0.1 M). After 10 min of gently rocking, the phenol layer was discarded. This procedure was repeated twice. The DNA in the aqueous phase was dialyzed first against potassium phosphate buffer (pH 7.4, 0.1 M) containing 1.0 M NaCl for 6 hr and then against 0.015 M sodium citrate containing 0.15 M NaCl twice for a total of 6 to 8 hrs.

Lysates of Ø29 and SP01 were prepared by infecting the appropriate bacterial host during logarithmic growth (10^7 cells/ml) at a multiplicity of infection of 0.5. After lysis, the cultures were centrifuged for 20 min at 14,000 x g to remove debris. The phage were then pelleted from the 14,000 x g supernatant fraction by centrifugation at 50,000 x g for 2.5 hr. For preparation of DNA from bacteriophages, the pellet was covered with potassium phosphate buffer (pH 7.4, 0.1 M), was allowed to stand for 8 hr at 4°C, and the DNA extracted from the bacteriophage by the previously described phenol procedure.

The methods developed by Rutberg, et al. for induction of Ø105 with mitomycin C and the propagation of Ø105 were utilized (2, 6).

Transduction with PBS1 (9) and SP10 (10) were performed by standard procedures on the selective media which were utilized for transformation.

RESULTS AND DISCUSSION

In order to investigate the effect of lysogeny on transformation and transfection, BR151 was infected with bacteriophage Ø105, lysogenic clones were isolated and subcultures of these were grown to competence. The

TABLE 1. Transfection of BR151 and BR151 (Ø105)

DNA Preparation	Transfectants per 10^8 cells in BR151	BR151 (Ø105)	Ratio[1]
Ø29			
Expt. 1	4.7×10^5	5.0×10^5	1.9
Expt. 2	3.4×10^5	1.0×10^6	
SP01			
Expt. 1	2.2×10^5	1.0×10^5	0.5
Expt. 2	6.2×10^4	2.7×10^4	

[1] Ratio of the frequency of transfection in BR151 (Ø105) and BR151.

TABLE 2. Transformation of BR151 and BR151 (Ø105)

DNA Preparation	Transformants[1] per 10^8 cells in BR151	BR151 (Ø105)	Ratio[2]
168			
Expt. 1	1.0×10^6	1.3×10^3	0.005
Expt. 2	7.5×10^5	7.8×10^3	

[1] Transformation of the Trp^- to Trp^+

[2] Ratio of the frequency of transformation in BR151 (Ø105) and BR151.

results of transfection and transformation are reported in Tables 1 and 2. Table 1 clearly demonstrates that the level of transfection is not significantly altered despite a drastic reduction in transformation in recipients which are lysogenic for Ø105. The frequency of transformation of BR151 carrying Ø105 is 0.005 that of the nonlysogenic cultures (Ratio, Table 2). We found, as did Peterson and Rutberg (6), that transformants of the lysogenic bacteria remained lysogenic for Ø105. The pronounced difference between transfection and transformation is probably due to the fact that lysogeny alters either the uptake or integration of the bacterial

DNA but not of the bacteriophage DNA. This conclusion is consistent with the following observations: (a) DNA isolated from cultures which are lysogenic for Ø105 also transform the lysogenic recipient at a reduced frequency. Thus, a phage induced modification of the genome cannot explain this phenomenon. (b) As shown in Table 3, bacteria lysogenic for Ø105 are transduced at wild-type levels by bacteriophages PBS1 and SP10. Therefore, the deficiency probably does not reside in a defective recombination system. (c) Bacteria lysogenic for Ø105 can be transfected by DNA isolated from both Ø29 and SP01 even though transfection with DNA from SP01 requires genetic recombination or repair (11, 12), while DNA from Ø29 has no such requirement (13). Thus, these results indicate that at least one functional recombination system is present in bacteria lysogenic for bacteriophage Ø105. At present, however, it is not possible to exclude a defect in some step of recombination in those bacteria lysogenic for Ø105. Hoch *et al.* (14) previously demonstrated that *recA* mutants of *B. subtilis* undergo genetic modification by DNA-mediated transformation and SP10-mediated transduction at reduced levels, but that transduction with PBS1 is normal. These authors suggest that there may be two distinct recombination systems in *B. subtilis* (3, 14). One of these systems is necessary for PBS1 mediated transduction and

TABLE 3. Transduction of BR151 and BR151 (Ø105)

Bacteriophage	Transductants/ml	
	BR151	BR151 (Ø105)
PBS1		
Experiment 1	5060	5970
Experiment 2	1270	1940
SP10		
Experiment 1	1050	1260
Experiment 2	870	1200

[1] Transduction of the Trp^- marker to Trp^+

the other for SP10 mediated transduction and transformation. Dubnau et al. (15) reached a similar conclusion in studies on genetic exchange between B. subtilis 168 and B. subtilis W23. If lysogeny with Ø105 does produce an abnormality in a recombination system, then this defect is capable of discriminating between the DNA introduced by transformation and that introduced by transduction.

Recent evidence suggests that there is a greater difference between the expression of transforming and transfecting DNA than merely the integration of the bacterial DNA into the host genome. Wilson and Bott (16) noted that at high concentrations, nalidixic acid inhibited transformation and transfection with SP01, but not transfection with Ø29. Oostindier-Braaksma and Epstein (17) have shown that the peak of competence for DNA from bacteriophage SP82 is later than that for bacteriophage SP02 or bacterial DNA. We have found that the nontransformable mutant of B. subtilis RUB19 (1), which is capable of transfection with DNA from Ø29 but not from SP01, irreversibly binds less than 2% of bacterial DNA that is bound by the parental transformable strain. These observations, together with data presented in this study, demonstrate that there are significant differences between pathways involved in transfectior and transformation. Experiments are in progress to determine the precise role of recombinational events in the transfection of lysogenic cultures of B. subtilis.

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