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Restriction and Modification of Bacteriophage in *Bacillus stearothermophilus*

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Host-controlled restriction and modification of TP-1C phage and infectious phage DNA occurs in *Bacillus stearothermophilus* and is subject to control by TP-8 or TP-12 prophage.

Our understanding of the restriction and modification of DNA in molecular terms comes for the most part from studies on phage-host systems of *Escherichia coli* (5). The genetic determinants of restriction and modification activities can reside on the bacterial chromosome or are associated with a plasmid (5). Restriction and modification activities have been found in strains of *Salmonella* (3), *Pseudomonas* (4), *Haemophilus* (7), *Staphylococcus* (6, 11), and *Streptococcus* (2). Hemphill (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 237) suggested that phage development in certain strains of *Bacillus subtilis* may be restricted by defective prophages. Wilson and Young (13) proposed that host-specific restriction and modification can account for both high efficiency and low efficiency intergenotic transformation in *B. subtilis*. In this note we present our preliminary studies on the restriction and modification of phage by *Bacillus stearothermophilus*.

Welker and Eager (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 201) reported that the strain of *B. stearothermophilus* used in transfection studies (8–10) was lysogenized with temperate phages TP-8 and TP-12, and is now designated 4S(8,12). A cured strain (4S) was isolated after N-methyl-N′-nitro-nitrosoguanidine treatment. Strains 4S(8) and 4S(12) are lysogenized with phage TP-8 and TP-12, respectively.

Modified phage stocks were prepared by repeated single-plaque isolations. Phage obtained from strains 4S, 4S(8), 4S(12), and 4S(8,12) were designated TP-1C, TP-1C·(8), TP-1C·(12), and TP-1C·(8,12), respectively. The medium, growth conditions, and the conditions for phage assay were described previously (12). Restriction-negative bacterial mutants (r^-) were isolated after treating growing cultures of 4S or 4S(8,12) with 10 μg of nitrosoguanidine per ml for 30 min. Cells were removed by centrifugation, washed twice, suspended in fresh TYG medium (8), and grown for 2 h at 55 C. Surviving cells were plated on Trypticase-agar (BBL) plates seeded with 2×10^5 plaque-forming units of phage TP-1C·(8). Colonies that were partially lysed were freed of phage by repeated single-colony isolation. Mutants exhibiting no restricting activity for phage TP-1C·(8) were

TABLE 1. *Efficiency of plating of phage TP-1C grown and tested on cured and lysogenic strains of B. stearothermophilus 4S*[a]

Indicator host	Efficiency of phage plating			
	TP-1C	TP-1C·(8)	TP-1C·(12)	TP-1C·(8, 12)
4S	1.0	7.4×10^{-4}	2.4×10^{-4}	1.0
4S (8)	0.5	0.8	0.8	0.4
4S (12)	0.7	1.0	1.0	0.6
4S (8, 12)	0.3	6.8×10^{-4}	4.6×10^{-4}	0.2

[a] Host bacteria were grown in TYG medium for 2 h at 55 C. Phage were assayed by mixing 0.5 ml of cells and 0.1 ml of phage suspension in tubes containing 2.0 ml of prewarmed Trypticase soft agar (BBL) (12). The mixture was poured onto the surface of a Trypticase-agar plate and incubated overnight at 55 C. Efficiency of plating is expressed as the quotient of the number of plaques counted on each restricting host divided by the number of plaques counted on a nonrestricting host.

designated as 4S r⁻ or 4S r⁻(8,12). For the purposes of this investigation, relatively small differences in plating efficiency (0.1 to 1.0) are not considered to be significant.

Strain 4S is capable of restricting phage propagated on strain 4S(8) or 4S(12) but does not modify (Table 1). Strains 4S(8) and 4S(12) modify phage TP-1C such that it is restricted by strains 4S or 4S(8,12). The singly lysogenic strains do not exhibit restricting activity. The double lysogen resembles the cured strain in both the restriction and modification activities. The relative efficiency of plating of phage TP-1C on r⁻ mutants of strains 4S and 4S(8,12) is shown in Table 2. Although the four representative mutants were selected for their inability to restrict phage TP-1C·(8), they also do not restrict phage TP-1C·(12). With the exception of strain 4S r_{19}^-, none of the r⁻ mutants modify phage TP-1C (Table 3). The restriction of phage TP-1C·(r_{19}^-) by strains 4S and 4S(8,12) was unexpected and indicates that the r_{19}^- mutation in strain 4S changes the modification phenotype from m⁻ to m⁺. Of the nine 4S r⁻ mutants examined, two behaved like r_{19}^- and seven as r_{52}^-. Only two r⁻ mutants of strain 4S(8,12) were

TABLE 2. *Efficiency of plating of phage TP-1C on nonrestricting mutants of B. stearothermophilus 4S and 4S(8,12)*[a]

Indicator host	Efficiency of phage plating			
	TP-1C	TP-1C·(8)	TP-1C·(12)	TP-1C·(8, 12)
4S r_{52}^-	1.0	1.0	1.0	1.0
4S r_{19}^-	0.9	1.0	0.9	0.7
4S r_2^- (8, 12)	0.2	0.4	0.5	0.3
4S r_3^- (8, 12)	0.3	0.1	0.9	0.3

[a] Growth conditions and procedure for the phage assay are described in Table 1.

TABLE 3. *Efficiency of plating of phage TP-1C grown on nonrestricting mutants and tested on cured and lysogenic strains of B. stearothermophilus 4S*[a]

Indicator host	Efficiency of phage plating			
	TP-1C·(r_{52}^-)	TP-1C·(r_{19}^-)	TP-1C·[r_2^-(8, 12)]	TP-1C·[r_3^-(8, 12)]
4S	1.0	1.0×10^{-4}	1.0	1.0
4S (8)	0.7	1.0	0.4	0.4
4S (12)	0.7	1.0	0.6	0.8
4S (8, 12)	0.1	2.8×10^{-4}	0.2	0.2

[a] Growth conditions and procedure for the phage assay are described in Table 1. Phage obtained from strains 4S r_{52}^-, 4S r_{19}^-, 4S r_2^- (8, 12), and 4S r_3^- (8, 12) are designated TP-1C·(r_{52}^-), TP-1C·(r_{19}^-), TP-1C·[r_2^-(8, 12)], and TP-1C·[r_3^-(8, 12)], respectively.

TABLE 4. *Efficiency of plating of phage TP-1C grown and tested on lysogenic derivatives of B. stearothermophilus 4S and 4S r⁻*[a]

Indicator host	Efficiency of phage plating			
	TP-1C·[r_{52}^-(12)]	TP-1C·[r_{19}^-(12)]	TP-1C·(8)	TP-1C
4S	1.0	1.1×10^{-4}	7.4×10^{-4}	1.0
4S (8)	0.4	0.9	0.8	0.5
4S (12)	0.7	1.0	1.0	0.7
4S (8, 12)	0.3	3.8×10^{-4}	6.8×10^{-4}	0.3
4S r_{52}^-(12)	0.8	0.9	0.5	0.7
4S r_{19}^-(12)	0.8	0.8	0.6	0.7

[a] Growth conditions and procedure for the phage assay are described in Table 1. Strains of 4S r⁻ lysogenized with phage TP-12 were isolated by picking a phage-resistant colony from the center of a plaque and freed of phage by repeated single colony isolation. The lysogenic nature of each strain was established by the spontaneous liberation of phage, induction with mitomycin C (0.1 μg/ml), and immunity to infection by phages TP-12 or TP-12 C (clear plaque mutant). Lysogenized strains are designated 4S r⁻ (12), and phage obtained from 4S r⁻ (12) mutants are designated TP-1C·[r⁻(12)].

TABLE 5. *Infection of lysogenic strains of B. stearothermophilus 4S with DNA of phage TP-1C and TP-1C·(8)*[a]

Phage DNA[b]	Recipient strain	Efficiency of transfection[c]
TP-1C	4S (12)	4.4×10^3
TP-1C·(8)	4S (12)	2.6×10^3
TP-1C	4S (8, 12)	4.2×10^3
TP-1C·(8)	4S (8, 12)	1
TP-1C	4S r_2^- (8, 12)	9.6×10^3
TP-1C·(8)	4S r_2^- (8, 12)	5.5×10^3

[a] Welker and Eager (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 201) reported that the infection of 4S(8) cells with TP-1C DNA depends on the presence of helper phage TP-12 or TP-12C. Helper phage do not have to be added to the transfection assay if the recipient strain is lysogenized with phage TP-12. The transfection assay consists of cells (0.1 ml) grown in TYG medium for 1.5 h at 55 C and phage DNA (0.05 ml). After the volume was adjusted to 0.25 ml with TYG medium, 0.1 ml was spread onto a Trypticase-agar (TA) plate, and incubated overnight at 55 C. Each plate contains 8.0×10^6 to 1.8×10^7 viable cells and sufficient phage DNA to give 30 to 500 infectious centers (IC) per plate.

[b] Phage were isolated according to the procedure described by Yamamoto et al. (14). Phage DNA was isolated according to the procedure described by Streips and Welker (8) with the exception that phenol was saturated with 0.05 M of Tris-hydrochloride buffer (pH 7.5) containing 0.01 M NaCl and 0.001 M $CaCl_2$.

[c] The number of IC per plate was adjusted to IC/4.35×10^{-3} μg DNA (IC_A), and the efficiency of transfection was defined as $IC_A/10^6$ helper phage. The number of helper phage was estimated by adding the number of TP-12 phage in the supernatant fluids to that fraction of the recipient culture that liberates TP-12 phage during growth on the TA plate. Free phage were assayed on strain 4S r_{52}^- as described in Table 1, and the number of cells that liberates phage on TA plates was estimated by using the standard phage assay, except that the 0.1 ml of phage suspension was replaced with appropriately diluted recipient culture (100 to 200 recipient cells per plate).

used in this investigation. Lysogenic derivatives of strains 4S r_{52}^- and 4S r_{19}^- exhibit the same restriction and modification activity as the nonlysogenized parental strains (Table 4). Infectious phage DNA is also sensitive to the same restricting mechanism as the phage in the various lysogenic recipients (Table 5). The relevant phenotype of each strain is shown in Table 6. The host-specificity notations r_{TP} and m_{TP} refer to thermophilic phage (TP)-specific restriction and modification, respectively. Strains 4S and 4S(8,12) do not exhibit TP-specific modification but do possess TP-specific restriction activity. Lysogenization of strain 4S with phage TP-8 or TP-12 results in a change of phenotype from r^+m^- to r^-m^+. A similar change in phenotype has occurred in 4S r_{19}^- as a result of mutation. Five of the seven r^- mutants of strain 4S and the two 4S r^- (8, 12) mutants retain the m^- phenotype. The TP-specific modification and restriction activities of the 4S r^- mutants were not changed after lysogenization with phage TP-12. When the two prophage are present in the same cell, the TP-specific modification activity is not expressed. Phages TP-8, TP-12, and TP-84 (Welker, unpublished data) are also subject to TP-type restriction and modification.

The data presented in this study indicate that TP-specific modification and restriction is a property of the host cell (*B. stearothermophilus* 4S) and is subject to control by TP-8 or TP-12

TABLE 6. *Phenotype of strains used in this study*

Strain	Restriction and modification phenotype[a]	Origin
4S (8, 12)	$r_{TP}^+m_{TP}^-$	Wild type; Welker and Campbell (12)
4S	$r_{TP}^+m_{TP}^-$	Cured strain; isolated after treatment of strain 4S(8,12) with NG
4S (8)	$r_{TP}^-m_{TP}^+$	Spontaneous loss of TP-12 prophage from strain 4S(8,12)
4S (12)	$r_{TP}^-m_{TP}^+$	Lysogenic derivative of strain 4S
4S r_{52}^-	$r_{TP}^-m_{TP}^-$	Restriction-deficient mutant of strain 4S
4S r_{19}^-	$r_{TP}^-m_{TP}^+$	Restriction-deficient mutant of strain 4S
4S r_{52}^- (12)	$r_{TP}^-m_{TP}^-$	Lysogenic derivative of strain 4S r_{52}^-
4S r_{19}^- (12)	$r_{TP}^-m_{TP}^+$	Lysogenic derivative of strain 4S r_{19}^-
4S r_2^- (8, 12)	$r_{TP}^-m_{TP}^-$	Restriction-deficient mutant of strain 4S(8,12)

[a] Host-specificity designation conforms to the recommendations of Arber and Linn (1).

prophage. Definitive proof as to the nature of the restriction and modification functions as well as their genetic determinants will be provided by the results of experiments currently under way in this laboratory.

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