JOURNAL OF BACTERIOLOGY, July 1969, p. 344-346
Vol. 99, No. 1

Infection of *Bacillus stearothermophilus* with Bacteriophage Deoxyribonucleic Acid

ULDIS N. STREIPS AND N. E. WELKER

Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201

Received for publication 21 April 1969

Early stationary-phase cells are most susceptible to infection with deoxyribonucleic acid from bacteriophage TP-1C. Transfection is destroyed by deoxyribonuclease and unaffected by phage antiserum.

The development of a genetic system in *Bacillus stearothermophilus*, such as transformation or transduction, is of interest in connection with our studies on the biochemical basis of thermophily. In this note we present our preliminary studies on the infection of *B. stearothermophilus* 4S with deoxyribonucleic acid (DNA) from bacteriophage TP-1C (6). Information obtained from these studies will be used to develop a transformation system in this organism. The use of bacteriophage DNA as a valid assay for studying the development of competence has already been established for *B. subtilis* (2, 3, 5).

A 500-ml Erlenmeyer flask (baffled bottom) containing 100 ml of TYG medium (6) was inoculated with 2.5 ml of a culture grown in the same medium for 2 hr at 55 C. The flask was shaken at 55 C in a New Brunswick gyratory water-bath shaker (model G-76). Phage were added in the early logarithmic phase of growth (2×10^8 cells/ml) at a phage-to-cell ratio of 2:1. Lysis was complete after 3 to 4 hr with a phage yield of 3×10^{10} to 5×10^{10} plaque-forming units (PFU) per ml. The lysate was centrifuged at $3{,}020 \times g$ for 15 min to remove cell debris and then at $134{,}229 \times g$ for 4 hr to pellet the phage. The phage pellets were gently suspended in one-tenth the original volume of PNM buffer (6), *p*H 7.0. The combined phage suspension was further purified by banding in 7.5 M CsCl - 0.02 M tris(hydroxymethyl)aminomethane buffer (*p*H 7.2). After centrifugation for 20 hr at $134{,}229 \times g$, the layer of phage was removed with a syringe fitted with a no. 20 gauge needle, and dialyzed against several changes of PNM buffer. The phage suspension was diluted to 3×10^{11} to 5×10^{11} PFU/ml with PNM buffer, and DNA was extracted by the method described by Kaiser and Hogness (4), with the exception that phenol was saturated with PNM buffer. The aqueous layer was dialyzed against several changes of 0.15 M NaCl - 0.015 M trisodium citrate, *p*H 7.0. Purified phage DNA gave typical absorption ratios at 260, 230, and 280 nm of 1.00:0.59:0.52, respectively. The concentration of DNA was determined by absorption at 260 nm.

Infectious DNA and mature phage were assayed by mixing 0.5 ml of cells (grown in TYG medium at 55 C) and 0.1 ml of DNA solution or phage suspension in tubes containing 2.0 ml of prewarmed Trypticase (BBL)-soft agar (6). The mixture was poured onto the surface of a Trypticase-agar plate (6) and incubated overnight at 55 C.

Phage antiserum, sufficient to inactivate > 99% of the mature phage in the control, had no effect on DNA infection (Table 1). However, preincubation with deoxyribonuclease (2.5×10^{-2} μg) completely inhibited DNA infectivity and had no effect on phage infection. Preincubation of phage DNA and mature phage with ribonuclease or trypsin had no effect on infectivity. These results indicate that DNA and not mature phage or disrupted phage present in the phenol extract is the infectious component in this system. Figure 1 shows that cells are the most susceptible to DNA infection in the early stationary phase of growth. Infection with DNA is probably limited to a small number of cells competent for DNA uptake at the time of DNA addition. In contrast, phage TP-1C infection is not restricted to competent cells. These results are similar to the uptake of infectious phage DNA in *B. subtilis* where the appearance of competence can be identified with a specific stage of the growth cycle (2). The percentage of transfection cannot be calculated from these data because the number of cells on the plate at the time of DNA uptake is unknown. The data indicate that only a small fraction of the cells was infected with phage DNA.

The effect of phage DNA concentration on the number of infectious centers is shown in Fig. 2. The number of infectious centers is directly proportional to the concentration of DNA. The

TABLE 1. *Sensitivity of bacteriophage DNA and mature bacteriophage to various treatments*

Method[a]	Treatment	Phage TP-1C DNA (infectious centers/ml)	Phage TP-1C (PFU/ml)[b]
A	None	1.4×10^3	2.2×10^7
	TP-1C antiserum	1.4×10^3	1.2×10^5
B	None	1.4×10^3	2.1×10^3
	Deoxyribonuclease (0.025 μg)	0	1.9×10^3
	Ribonuclease (10 μg)	1.4×10^3	2.0×10^3
	Trypsin (50 μg)	1.6×10^3	1.7×10^3

[a] (A) Phage antiserum (diluted 1/500 in TYG medium and heated at 70 C for 45 min) was mixed with an equal volume (0.2 ml) of phage DNA (20 μg/ml) or phage suspension (1.1×10^9 PFU/ml) and held at 37 C for 30 min. Controls were treated in the same manner, except that phage antiserum was replaced by TYG medium. Samples (0.1 ml) were mixed with 0.5 ml of cells in 2.0 ml of prewarmed Trypticase-soft agar, poured onto Trypticase-agar plates, and incubated overnight at 55 C. (B) Each enzyme was added to an equal volume (0.2 ml) of phage DNA (20 μg/ml) or phage suspension (1.1×10^5 PFU/ml) and incubated at 37 C for 30 min. Samples (0.1 ml) were assayed as described in method (A).

[b] Plaque-forming units per milliliter.

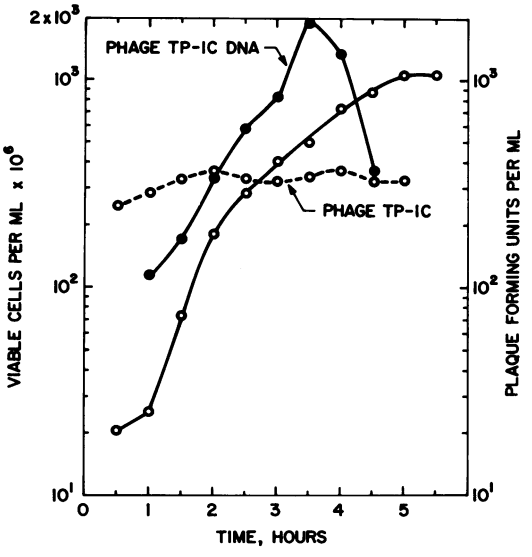

FIG. 1. *Effect of culture age on phage TP-1C DNA and phage TP-1C infection of Bacillus stearothermophilus 4S. Samples (0.5 ml) of a culture growing in TYG medium at 55 C were removed at the indicated times and mixed with 0.1 ml of phage DNA (20 μg/ml) or phage suspension (8.3×10^8 PFU/ml) in 2.0 ml of prewarmed Trypticase-soft agar. The mixture was poured onto the surface of a Trypticase-agar plate and incubated overnight at 55 C. Viable cell counts were determined by diluting in TYG medium and spreading (0.1 ml) onto the surface of Trypticase-agar plates. The plates were incubated overnight at 55 C.* (○——○). *Viable cells;* (●——●) *phage TP-1C DNA;* (○- - -○) *phage TP-1C.*

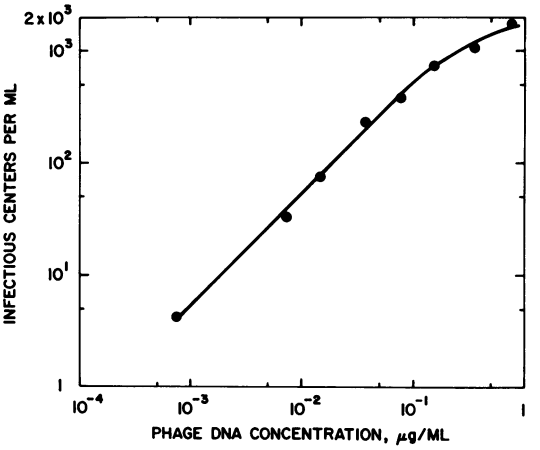

FIG. 2. *Effect of phage DNA concentration on infectious center formation. Phage DNA (0.1 ml) and cells (0.5 ml) from a culture of B. stearothermophilus 4S grown for 3.5 hr (5.4×10^8 viable cells/ml) in TYG medium at 55 C were mixed in 2.0 ml of prewarmed Trypticase-soft agar. The mixture was poured onto the surface of a Trypticase-agar plate and incubated overnight at 55 C.*

dose-response curve is similar to that reported by Reilly and Spizizen (5) for infectious DNA from *B. subtilis* phage φ29. The DNA from phage TP-1C (6) and phage φ29 (1) is similar in that the molecular weight is 1.2×10^7 and 1.1×10^7 daltons, respectively. The leveling off of the dose-response curve is not shown because concentrations of phage DNA greater than 1 μg/ml resulted in complete lysis of cells on the plate.

These results indicate that *B. stearothermophilus* 4S is capable of developing competence. Experiments are now in progress to determine the cultural conditions required for the development of competence for transfection and transformation in broth culture.

This work was supported by Public Health Service research grant AI 06382 from the National Institute of Allergy and In-

fectious Diseases. The senior author is a trainee under Developmental Physiology Training Grant GM00903 of the National Institute of General Medical Sciences to the Department of Biological Sciences, Northwestern University.

LITERATURE CITED

1. Anderson, D. L., D. D. Hickman, and B. E. Reilly. 1966. Structure of *Bacillus subtilis* bacteriophage ϕ29 and the length of ϕ29 deoxyribonucleic acid. J. Bacteriol. **91**:2081–2089.
2. Bott, K. F., and G. A. Wilson. 1967. Development of competence in the *Bacillus subtilis* transformation system. J. Bacteriol. **94**:562–570.
3. Bott, K. F., and G. A. Wilson. 1968. Metabolic and nutritional factors influencing the development of competence for transfection of *Bacillus subtilis*. Bacteriol. Rev. **32**:370–378.
4. Kaiser, A. D., and D. S. Hogness. 1960. The transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λdg. J. Mol. Biol. **2**:392–415.
5. Reilly, B. E., and J. Spizizen. 1965. Bacteriophage deoxyribonucleate infection of competent *Bacillus subtilis*. J. Bacteriol. **89**:782–790.
6. Welker, N. E., and L. L. Campbell. 1965. Induction and properties of a temperate bacteriophage from *Bacillus stearothermophilus*. J. Bacteriol. **89**:175–184.