Correlation Between Susceptibility to Bacteriophage PBS1 and Motility in *Bacillus subtilis*

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ABSTRACT

JOYS, TERENCE M. (University of Minnesota Medical School, Minneapolis). Correlation between susceptibility to bacteriophage PBS1 and motility in *Bacillus subtilis*. J. Bacteriol. **90**:1575-1577. 1965.—A correlation is shown to exist in *Bacillus subtilis* between susceptibility to phage PBS1 and motility, indicating that the receptor site for this phage is located on the flagellum.

During a preliminary study of transduction of motility in *Bacillus subtilis*, it was found that phage PBS1 (Takahashi, 1961) did not form plaques when assayed on strain SB 108, a non-motile mutant of *B. subtilis* prepared by Stocker (1963). Experiments are described which show a correlation between susceptibility to this phage and presence of motility, indicating that the receptor site for phage PBS1 is located on the flagellum.

MATERIALS AND METHODS

Phages. Phage PBS1 was obtained from C. Anagnostopoulos (Laboratoire de Genetique, Gif-sur-Yvette, Seine & Oise, France). Two virulent mutants, designated PBS1-V1 and PBS1-V2, were isolated from single clear plaques and purified.

Bacterial strains. *B. subtilis* SB 108 was obtained from E. W. Nester (University of Washington, Seattle), and was used to prepare various other strains (Table 1). Strains 168, MS 10, and SB 1 were obtained from the collection of John Spizizen. Stocker (1963) showed SB 108 to be non-flagellated and to mutate rarely to motility. However, the strain used in these experiments often reverted to the motile form and, because of this, could not be used as a recipient in transformation of motility. Genetic markers are indicated as follows: tryptophan, *try*; histidine, *his*; flagella production, *fla*; ability to grow in the presence of 1 mg/ml of streptomycin, *Sr*.

Preparation of phage lysates. Lysates of phage PBS1 and its virulent derivatives were made by the method of Takahashi (1963), and were assayed on the various strains by the overlay technique (Adams, 1959), with an overlay of Penassay Broth (Difco) containing 0.4% agar; this medium was

also used as a semisolid medium for motility tests. All bacterial strains were examined by phase-contrast microscopy before use to confirm presence or absence of motility.

Transformation. The methods described by Anagnostopoulos and Spizizen (1961) were used for isolation of deoxyribonucleic acid (DNA), preparation of competent cells, and the technique of transformation.

Phage-absorption tests. Log-phase cells, grown in either Penassay Broth or minimal tryptophan broth (Anagnostopoulos and Spizizen, 1961), were mixed with a phage suspension at a ratio of 1 phage infective unit per 100 bacterial cells, and were shaken for 30 min at 37°C; cells were removed by low-speed centrifugation, and the supernatant fluid was assayed for phage. Controls with corresponding sterile media were included.

RESULTS

Table 2 shows the results obtained when lysates of phage PBS1, and two of its virulent derivatives, were assayed on the strains listed in Table 1. A titer of about 4×10^8 infective units per ml indicated approximately 40 plaques from 0.1 ml of a 10^{-6} dilution of the lysate, with evidence for larger numbers of plaques when lower dilutions of the phage lysate were used.

These results demonstrate the correlation between motility and phage susceptibility. Phage PBS1 did not form plaques on SB 108, but did form plaques, with the same efficiency as with strains 168 and SB 1, on five spontaneous motile mutants isolated from SB 108. The phage also formed plaques on MS 10, but did not do so on five nonmotile transformants of MS 10, produced by treatment with DNA from SB 108 with selection for *his*⁺ and absence of motility; transformants which were *his*⁺ and motile remained susceptible to the phage. Two spontaneous motile

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TABLE 1. *Strains obtained from stock collections and derived from SB 108*

Strain	Genotype	Method of preparation
168	<i>try₂⁻ fla⁺</i>	Spizizen (1958)
SB 1	<i>try₂⁻ his₁⁻ fla⁺</i>	Nester (1964)
MS 10	<i>try₂⁻ his₂⁻ fla⁺ Sr</i>	
SB 108	<i>try₂⁻ fla⁻</i>	Stocker (1963)
SB 108 a, b, c, d, e	<i>try₂⁻ fla⁺</i>	Five spontaneous motile mutants of SB 108, each isolated from a different clone in semisolid medium
MS 14 a, b, c, d, e	<i>try₂⁻ fla⁻ Sr</i>	Nonmotile transformants of MS 10 treated with SB 108 DNA
MS 14a-1, MS 14b-1	<i>try₂⁻ fla⁺ Sr</i>	Two motile mutants of MS 14

TABLE 2. *Correlation between susceptibility to phage PSB1 and motility in various strains of Bacillus subtilis*

Strain	Motility in liquid medium	Titer* of a lysate of PSB1	Titer of a lysate of PSB1-V1	Titer of a lysate of PSB1-V2
168	Motile	4×10^8	5×10^8	3×10^8
SB 1	Motile	4×10^8	5×10^8	3×10^8
MS 10	Motile	4×10^8	5×10^8	3×10^8
SB 108	Non-motile	0	0	0
SB 108 a-e	Motile	4×10^8	5×10^8	3×10^8
MS 14 a-e	Non-motile	0	0	0
MS 14 a-1 MS 14b-1	Motile	4×10^8	5×10^8	3×10^8

* Phage titers are expressed in infective units per milliliter; 0 = no plaques formed with 10-fold dilutions of the lysates (from undiluted to 10^{-8} dilution).

mutants (MS 14a-1 and MS 14b-1) were isolated from these *his⁺ fla⁻* transformants, and were found to be phage-susceptible.

This correlation was confirmed by adsorption tests with SB 108 and one of its motile derivatives, SB 108a. The motile mutant adsorbed almost 100% of the phage in Pennassay Broth at 37 C (less in minimal tryptophan broth), whereas the nonmotile SB 108 did not adsorb any phage.

DISCUSSION

Stocker (1963) showed that SB 108 is nonmotile because it does not produce flagella; the correla-

TABLE 3. *Adsorption of phage PSB1 with motile and nonmotile strains of Bacillus subtilis**

Growth medium	Inoculum	Motility	Titer of phage after adsorption	Per cent adsorption
Pen-assay Broth	0	—	2×10^6	0
	SB 108	Nonmotile	2×10^6	0
	SB 108 a	Motile	3×10^4	98
Minimal tryptophan	0	—	2×10^6	0
	SB 108	Nonmotile	2×10^6	0
	SB 108 a	Motile	6×10^5	70

* Titer of the phage lysate before use was 4×10^6 infective units per ml; the absorption test diluted this 1:2. Cell concentration was about 10^8 viable cells per milliliter.

tion shown here to exist between motility and susceptibility to phage PSB1 indicates that the receptor site for this phage is located on the flagellum. A similar location for the receptor site has been shown for the χ phage of Sertic and Boulgakov (1936), which attacks *Salmonella* species. This phage has been shown to possess further specificity (Meynell, 1961), as it does not adsorb to serotypes possessing H antigens of the g series nor to serotypes with "paralyzed" flagella. Whether such specificity exists for phage PSB1 is not yet apparent.

Phage PSB1 is unlike the χ phage in that it has been shown to act as a transducing phage of the type yielding generalized transduction (Takahashi, 1961). The χ phage has not been reported as mediating in transduction.

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ADDENDUM IN PROOF

I. Takahashi has kindly examined the phage used in this investigation and has confirmed its serological identity with his standard phage PSB1.

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