

# Genetic Mapping of a Defective Bacteriophage on the Chromosome of *Bacillus subtilis* 168

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A genetic marker responsible for the killing activity of PBSX, a defective phage carried by *Bacillus subtilis* 168, has been located on the bacterial chromosome. Two mutant strains of *B. subtilis* 168, which produced tailless phage particles upon mitomycin C induction, were shown to carry lesions, designated *xtl-1* and *xtl-2*, which were linked by transformation and PBS1-mediated transduction to *metC*. The linkage relationship between *xtl* and adjacent auxotrophic markers was determined by three-factor PBS1 transduction, the suggested order of markers being *argO1 metA metC xtl*.

All strains of *Bacillus subtilis* examined thus far produce noninfectious phage-like particles upon induction. The phages contain almost exclusively host deoxyribonucleic acid (DNA) and can kill related strains with an activity spectrum analogous to certain bacteriocins (13, 24). A number of bacterial strains have now been shown to produce defective phage-like particles, either spontaneously or by induction, and have been characterized by electron microscopy. These include: *B. licheniformis* (15); *B. mycoides* (37); *Acetobacter* (3); *Haemophilus influenzae* (31); *Escherichia coli* (4, 11, 12, 23, 29); *Pseudomonas aeruginosa* (4, 17, 20); *Proteus vulgaris* (7); *P. mirabilis* (36); *Clostridium botulinum* (16); *C. tetani* (26); *Mycobacterium tuberculosis* (27, 28); *Thermomacrinomyces vulgaris* (1); *Listeria monocytogenes* (4); *Enterobacter cloacae* (9); and *Vibrio cholerae* (21).

Because of the traits some of these particles (4, 7, 11, 20, 21, 24) share in common with certain bacteriocins, one of the initial problems, in their genetic analysis, is to establish whether their genetic determinants are carried as extra-chromosomal episomes or plasmids similar to the colicinogenic factors (14) or are integrated on the bacterial chromosome. In this report, we describe a chromosomal location for a genetic marker responsible for the killing activity of PBSX, a defective phage carried by *B. subtilis* 168.

## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used are listed in Table 1. 44A0 and BS10 are mutant strains

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of *B. subtilis* 168 which were kindly supplied by K. Bott and J. Gross, respectively. Upon induction, these independently derived nonkilling strains (kill<sup>-</sup>) produce PBSX head structures as revealed by electron microscopy (J. Mangan, unpublished observations), but fail to make observable phage tails which are responsible for the PBSX-killing activity (24). We have referred to the mutations responsible for the kill<sup>-</sup> phenotype in 44A0 and BS10 as *xtl-1* and *xtl-2*, respectively.

**Media and chemicals.** The minimal medium used was described previously by Copeland and Marmur (8). Veal-Yeast Extract (VY) broth contained 2.5% Veal Infusion (Difco) and 0.5% Yeast Extract (Difco) in distilled water. Overlay plates used in the PBSX killing assay consisted of 2.5 ml of top agar described by Okubo and Romig (25), seeded with a PBSX-sensitive strain of *B. subtilis*, layered onto Tryptose Blood Agar Base (Difco).

Mitomycin C (MC) was purchased from the Kyowa Hakko Kogyo Co., Ltd. Stock solutions of 200 µg/ml in water were prepared fresh weekly and stored in the dark at 4 C.

**Transduction and transformation.** The procedure described by W. B. Pritkin (Ph.D. Thesis, University of California, 1967) was used to prepare transducing lysates of phage PBS1. This phage is capable of transducing large segments of the *B. subtilis* genome amounting to approximately 8% of the overall chromosome length (10). DNA for transformation was isolated by the Marmur method (22), and competent cells were prepared by the procedure described by Stewart (32).

**Mapping procedure.** Since it was not possible to select for either the acquisition or the loss of the kill<sup>-</sup> phenotype, prototrophic transductants or transformants were selected first and then tested for linkage to the *xtl-1* and *xtl-2* mutations. Prior to scoring for recombinants, transductants or transformants were purified by streaking on selective media and single colonies were tested for their ability

TABLE 1. List of strains

Strains	Previous designation	Genotype	Source
44A0 <sub>1</sub>		<i>ad16 thy xtl-1</i>	K. Bott
44A0 <sub>2</sub>		<i>thy trp xtl-1</i>	Transformation
BS 10		<i>thy ind xtl-2</i>	D. Karamata
MB 47	SB-1	<i>hisA1 trp2</i>	E. Nester
MB 54	SB-26	<i>metC<sup>a</sup></i>	D. Comstock
MB 156	BD-81	<i>cysA14</i>	D. Dubnau
MB 181	BS-71	<i>his1 argC ura-1</i>	D. Dubnau
MB 251		<i>metC xtl-1 ura-1</i>	<i>metC</i> from MB 54 introduced by PBS1 transduction; <i>xtl</i> from 44A0 by transformation
MB 269	BP-18	<i>argO1 metA1</i>	F. Young
W23/PBS1	W23M8	—	Spontaneous mutant of W23M8

<sup>a</sup> We have utilized the classification of C. Anagnostopoulos (*personal communication*) for the *met* mutants; *metC*, formerly called *metA*, responds only to methionine; *metA* mutants respond to cystathione as well as homocysteine and methionine.

to produce PBSX and for linkage to other nutritional markers.

**PBSX induction.** To handle the large numbers of transformants and transductants being examined, the following procedure was used to bring all the cultures to early log phase, the period for optimum MC induction of defective phage. Single colonies were inoculated into VY broth, and the cultures were grown overnight to stationary phase. These cultures were then diluted 1:40 with fresh VY and aerated by vigorous shaking at 37 C for 2 hr. MC was added to a final concentration of 0.5 µg/ml, and the incubation continued for 3 hr. The resulting lysates were spotted on overlay plates seeded with an indicator strain of *B. subtilis* (W23/PBS1) which was sensitive to PBSX and resistant to the transducing phage PBS1. A PBS1-resistant strain was used for the indicator lawn to avoid the possibility of artifactual killing by PBS1 which may be present in the transduced cultures in a carrier state (34). The presence of PBSX in a lysate resulted in a clear zone in the confluent lawn of indicator bacteria after overnight incubation at room temperature.

RESULTS

**Two-factor crosses.** To determine what region of the *B. subtilis* chromosome carried the *xtl* mutations, a number of markers were selected from different chromosomal linkage groups to test for linkage to the *xtl-1* mutation. When the prototrophs, selected after transduction with PBS1 grown on the kill<sup>-</sup> 44A0 strain, were tested for their ability to kill the PBSX-sensitive strain, the kill<sup>-</sup> phenotype was found to cotransduce with both the *argC* and *metC* loci (Table 2). *ArgC* and *metC* are known to be linked to each other by PBS1-mediated transduction (10) and are located on the same linkage group (Fig. 1) of the *B. subtilis* genetic map (10, 38).

Since the fragment of DNA incorporated into the resident genome of recombinants is shorter

TABLE 2. Linkage values obtained by two-factor crosses

PBS1-mediated transduction		Transformation <sup>a</sup>	
Selected markers	Per cent of co-transfer of <i>xtl-1</i>	Selected markers	Per cent of co-transfer of <i>xtl-1</i>
<i>ad16</i>	0 (0/200)	<i>argC</i>	0 (0/100)
<i>ery</i>	0 (0/200)	<i>metA3</i>	0 (0/150)
<i>hisA1</i>	0 (0/200)	<i>metC</i>	11 (34/300)
<i>argC</i>	14 (27/200)		
<i>metC</i>	58 (58/100)		
<i>ura-1</i>	0 (0/200)		
<i>thy</i>	0 (0/200)		
<i>trp2</i>	0 (0/200)		

<sup>a</sup> All transformations were performed with non-saturating concentrations of DNA (0.01 µg/ml) isolated from 44A0.

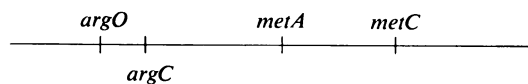


FIG. 1. Partial map of the *B. subtilis* chromosome from Young et al. (1969).

in DNA-mediated transformation than PBS1-mediated transduction (2, 35), the markers *argC*, *metC* (and a marker falling between them, *metA*) were tested by transformation for linkage to *xtl-1*. The results indicated that among the markers tested the *xtl-1* mutation cotransformed only with *metC* (Table 2).

**Three-factor crosses.** An attempt was then made to order the *xtl-1* and *xtl-2* mutations with respect to the adjacent auxotrophic markers by a series of three-factor crosses. The results obtained in the first cross involving the markers *argO1 metA* and *xtl-1* (Table 3A) indicate that the *xtl-1* mutation is to the right of *metA*. Simi-

larly the *xtl-2* mutation, carried by BS10 (the other kill<sup>-</sup> strain), also mapped to the right of *metA* with approximately the same frequency of cotransduction as *xtl-1* (Table 3B).

The markers *argC* and *metC* were then used to determine whether the *xtl* mutations were to the right or the left of *metC*. The results of this

cross (Table 3C) unfortunately do not define an unequivocal map position for the *xtl-1* mutation but suggest that *xtl-1* maps to the right of *metC*.

## DISCUSSION

We have demonstrated a chromosomal map position for a genetic determinant, *xtl*, responsible for PBSX phage tail production. At the present time we do not know if the mutations responsible for the tailless particles produced by the two mutated strains, 44A0 and BS10, involve the tail structural genes or genes responsible for phage assembly. The PBSX phage tail is a complex contractile structure implying the interaction of several proteins and, although electron microscopy does not reveal intact phage tails in kill<sup>-</sup> strains (J. Mangan, unpublished observations), some unassembled tail proteins might be made.

Siegel and Marmur (30) have recently described a temperature-sensitive mutant of *B. subtilis* which induces PBSX at the nonpermissive temperature. This mutation, which presumably resides in the phage-specific DNA and may involve the PBSX repressor, was found to map in the *purB6* region of the *B. subtilis* chromosome; furthermore, it is not linked by PBSI transduction to any of the markers used in the present study. One piece of evidence which suggested that the mutation may be located in the phage repressor was the inability of DNA from two related strains of *B. subtilis*, which do not produce PBSX but rather the related defective phages PBSY and PBSZ (33), to transform the temperature-sensitive mutation to wild type. These phages, although morphologically similar to PBSX, have different killing spectra and may represent a situation analogous to the different repressor immunity types found among the lambdoid phages of *E. coli* (5). Similar attempts by us to transform the *xtl* mutations with DNA from the *B. subtilis* strain carrying PBSZ have also failed. This failure to detect transformants involving the exchange of phage-specific information between strains carrying different defective phages may be due to a lethal event similar to zygotic induction (18). If the incoming donor DNA carries phage genes which are not sensitive to the repressor present in the recipient cells, the expression of the nonrepressed genes could lead to cell death and the loss of these cells from the transformed population.

If the temperature-sensitive mutation does indeed involve a phage-coded repressor, its distance from the *xtl* mutations suggests that a contributory factor in the defective nature of PBSX may be its inability to be excised as a

TABLE 3. Analysis of three-factor crosses

### (A) *argO1 metA xtl-1*

Donor: 44A0 (*adI6 thy xtl-1*)  
 Recipient: BP 18 (*argO1 metA3*)  
 Selected phenotype: *arg*<sup>+</sup>

<i>argO1</i>	<i>metA3</i>	<i>xtl-1</i>	No. of recombinants
1 <sup>a</sup>	0	0	50
1	1	0	78
1	0	1	0
1	1	1	36
Total			164

Frequency of cotransduction of *xtl-1* with *argO1*  
 = 22%

Frequency of cotransduction of *metA3* with *argO1*  
 = 70%

Suggested order of markers: *argO1 metA xtl-1*

### (B) *argO1 metA xtl-2*

Donor: BS 10 (*thy ind xtl-2*)  
 Recipient: BP 18 (*argO1 metA3*)  
 Selected phenotype: *arg*<sup>+</sup>

<i>argO1</i>	<i>metA3</i>	<i>xtl-2</i>	No. of recombinants
1	0	0	34
1	1	0	59
1	0	1	0
1	1	1	11
Total			104

Frequency of cotransduction of *xtl-2* with *argO1*  
 = 10%

Frequency of cotransduction of *metA3* with *argO1*  
 = 67%

Suggested order of markers: *argO1 metA xtl-2*

### (C) *argC metC xtl-1*

Donor: MB 251 (*metC xtl-1 ura-1*)  
 Recipient: MB 181 (*his-1 argC ura-1*)  
 Selected phenotype: *arg*<sup>+</sup>

<i>argC</i>	<i>metC</i>	<i>xtl-1</i>	No. of recombinants
1	0	0	422
1	1	0	38
1	0	1	30
1	1	1	46
Total			536

Frequency of cotransduction of *xtl-1* with *argC*  
 = 14%

Frequency of cotransduction of *metC* with *argC*  
 = 16%

Suggested order of markers: *argC metC xtl-1*

<sup>a</sup> Values of 1 and 0 refer to donor and recipient phenotype, respectively.

single unit from the bacterial chromosome. An analogous situation may be found in the regulation of the structural genes of the arginine biosynthetic pathway in *E. coli*. Genetic analysis has shown that these genes are noncontiguous yet are under the control of a single regulatory protein (19).

To determine whether the PBSX genome is scattered on the *B. subtilis* chromosome, attempts to isolate additional phage-specific mutants and determine their map location are now in progress. Such mutants, however, are difficult to select since they lack an easily assayable biological activity such as the killing function of the phage tails.

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