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Inducible Phages of *Bacillus subtilis**

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A group of prophages induced in *Bacillus subtilis* has been studied. The evidence presented indicates that these phages are genetically defective. DNA isolated from the phages exhibits many properties that are similar to host DNA, and is capable of transforming *B. subtilis* with respect to host genetic markers.

Bacteria are known to harbor a variety of latent particles ranging in complexity between bacteriocins and bacteriophages. The induction of these particles, either spontaneously or by chemical or physical means, may or may not result in the killing or lysis of the host bacterium (Lwoff, 1953; Hartman and Goodgal, 1959). In this communication we wish to report the induction, by mitomycin C, of a phage particle found in several *Bacillus* strains whose production is accompanied by cell lysis. This particle contains protein and DNA, is capable of killing and lasing sensitive (non-particle-producing) strains of *B. subtilis*, but would appear to be incapable of self-reproduction in the recipient strain. We have named this particle PBSX, following the nomenclature for *B. subtilis* phages suggested by Takahashi, (1961).

MATERIALS AND METHODS

Strains Used and Growth Conditions.—The following strains were used in this study: *B. subtilis* 168^6 (168 wild type resistant to six antibiotics), *B. subtilis* var *aterrimus* ATCC 6460, *B. natto* MB 275, *B. polymyxa* ATCC 842, *B. licheniformis* NRS 243, *B. lentus* ATCC 10840, *B. cereus* MB 19, *B. macerans* ATCC 7069, *B. subtilis* SMY Ind^- and *B. subtilis* 168^6 X^s (a cured strain derived from *B. subtilis* 186^6).

All cultures were stored in the frozen state in Difco brain-heart infusion broth containing 10% glycerol. They were grown on tryptose blood agar base (Difco) slants overnight and inoculated into antibiotic medium no. 3 (Penassay, Difco) for induction or assay experiments.

Induction of PBSX Particles.—Bacterial cultures were grown in Penassay broth to a density of 1–3 $\times$ 10^7/ml. Three μg/ml of mitomycin C (Sigma or Kyowa Hakko Kogyo) were added and the culture was incubated at 37° with shaking for 10 minutes. The mitomycin C was removed after 10 minutes, contact by harvesting the cells and resuspending at the original concentration in fresh Penassay broth. The incubation was continued at 37° with shaking until lysis occurred (usually 2–3 hours after resuspension).

Processing of Lysates.—The lysates were cleared of bacterial debris by low-speed centrifugation. The supernatant was treated with 1 μg/ml deoxyribonuclease (Worthington) in the presence of 5×10^{-3} M $MgCl_2$ for 60 minutes at 37° and the PBSX particles were harvested in the Spinco Model L ultracentrifuge for 90 minutes at 28 K. The pellets were suspended in a synthetic medium described by Nomura *et al.* (1962) and stored at 4° in the presence of chloroform. The lysates were found to be stable in the presence of chloroform.

Isolation of B. subtilis 168^6 X^s.—*B. subtilis* 168^6, which is lysogenic for PBSX, was cured in order to render it sensitive to the phage. Bacteria were grown in Penassay to a density of 2×10^7/ml and induced with 3 μg/ml mitomycin C in the presence of 20 μg/ml acridine orange. The cells were centrifuged after 10 minutes of contact and resuspended at the original concentration in fresh Penassay containing 20 μg/ml acridine orange. They were incubated overnight with aeration. The culture was then diluted and plated on brain-heart infusion broth agar plates. The colonies were cross-streaked against PBSX. One out of approximately 100 colonies tested was sensitive to PBSX. This new strain was designated X^s.

Assay of PBSX.—X^s was grown in Penassay to a density of 3–4 $\times$ 10^7/ml. The culture was diluted in Penassay to yield 3–4 $\times$ 10^3 bacteria/ml. Aliquots (0.9 ml) of the culture were added to 0.1 ml lysate to be assayed. The tubes were incubated at 37° for 10 minutes and 0.05-ml samples were plated in duplicate on brain-heart infusion broth agar plates for determination of surviving bacteria.

The immunological cross-reactivity of PBSX induced from different strains of *Bacillus* was determined as follows. Lysates were treated with antiserum prepared against PBSX induced from *B. subtilis* 168.

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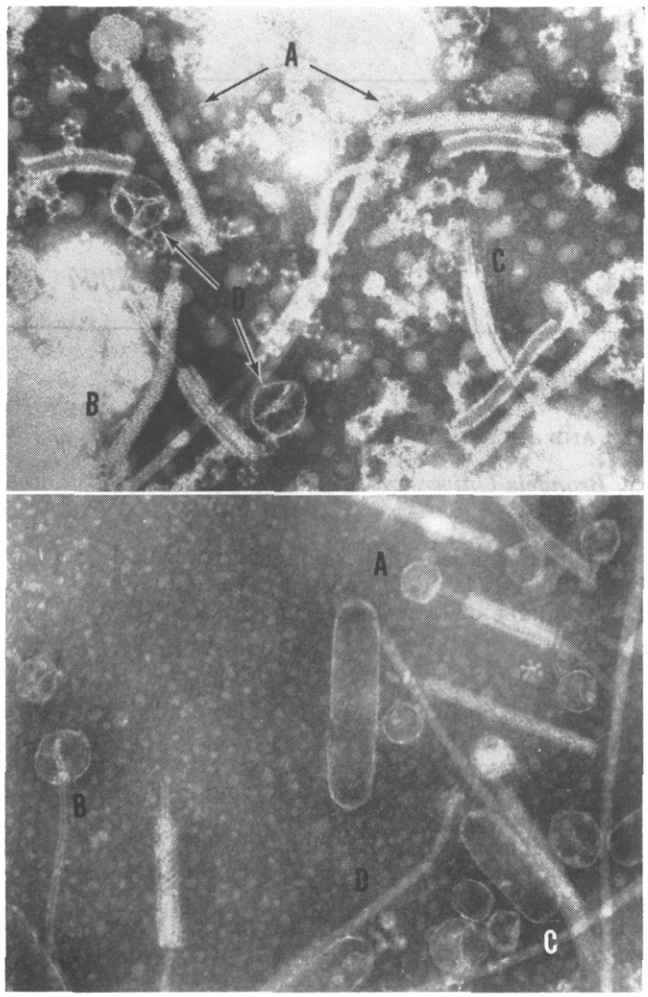

FIG. 1.—Electron micrograph of PBSX × 190,000. (a, upper) Shown are two intact small phages (A) together with several headless tails with normal (B) and contracted (C) sheaths. At (D) are seen two empty heads of a larger phage, seen with tail still attached in (b). In the background can be detected a number of small hexagonal structures, perhaps segments of the tail sheath. In this and the other picture the longest fibers are bacterial flagella. (b, lower) At (A) are seen the empty head and contracted sheath of the small phage; at the end of the sheath is seen a distinct plate. The hexagonal structure below this phage may be a detached plate seen end-on. At (B) is seen a larger phage with a long tail; the head is empty. At (C) is the still larger empty head of another phage; an unusually long head is also visible. The detached tail at (D) probably belongs to this oval-headed phage.

The lysates were incubated with antiserum for 10 minutes at 37° and the remaining killing activity of the neutralized lysates was assayed as described earlier.

DNA Isolation.—DNA was isolated from the concentrated lysates following the procedure of Mandell and Hershey (1960). Two or three phenol extractions were carried out followed by extensive dialysis against 0.15 M saline solution containing 0.015 M Na citrate.

Determination of Sedimentation Coefficients.—The sedimentation coefficient $s_{20,w}$ of DNA dissolved in saline citrate was determined in a Spinco Model E ultracentrifuge at concentrations of 20 μg/ml. A 12-mm centrifuge cell was used, fitted with a Kel-F centerpiece. Molecular weights were calculated from the following equation: $s_{20,w} = 0.080\ M^{0.35}$ (Burgi and Hershey, 1963).

CsCl *Density-Gradient Centrifugation.*—The method employed was similar to that described by Meselson *et al.* (1957). The DNA was centrifuged at 44,770 rpm for 24 hours and the banded DNA was photographed using ultraviolet optics. A sample of DNA of known buoyant density was added as a density marker. All buoyant densities in CsCl are referred to that of *Escherichia coli* DNA which is taken to be 1.710 g/cc. The band pattern was traced using a Joyce-Loebl microdensitometer.

Purification of PBSX in a CsCl *Gradient.*—Fresh concentrated lysates were added to 63% (w/w) CsCl and adjusted to a density of 1.268 g/cc. The lysates were then centrifuged in the Spinco Model L ultracentrifuge in the SW39 rotor at 24,000 rpm for 15 hours. The bottoms of the tubes were pierced and 3-drop fractions were collected.

Transformation of B. subtilis.—Transformation was performed according to the method of Spizizen (1959) and Mahler *et al.* (1963). The following genetic markers were used in transformation: $A^- \rightarrow A^+$ (arginine); $H^- \rightarrow H^+$ (histidine); $Bry^s \rightarrow Bry^r$ (Bryamycin). Comparisons of transformation efficiencies were made at equivalent concentrations of DNA.

RESULTS

Induction of PBSX—Induction of *B. subtilis* with mitomycin C results in the lysis of the culture accompanied by the release of PBSX phage particles. Induction of a similar particle can be accomplished by ultraviolet irradiation of the lysogenic cells (Kellenberger and Kellenberger, 1952; P. Schaeffer, personal communication).

Lysates from induced cells are not viscous, whereas autolysates of *Bacillus* kept under unfavorable (e.g., anaerobic) conditions are highly viscous. The electron micrograph (Fig. 1) reveals the presence of three distinct phage types. The smallest phage consists of a mixture of complete particles and ghosts as well as detached heads and tails. A larger phage always appears as a ghost with or without a tail. The third phage type is a still larger oval shaped empty head. The work described in this paper was performed with unfractionated PBSX lysates.

Several species and strains of *Bacillus* were tested for inducibility (Table I). It may be seen that all of the *B. subtilis* strains and most of the closely related species are lysogenic. *B. licheniformis* was the only genetically unrelated strain (Marmur *et al.*, 1963) which was inducible with mitomycin C and released a

TABLE I

INDUCTION BY MITOMYCIN C OF DIFFERENT *Bacillus* SPECIES

Name of Organism	Production of PBSX-like Particle	Ability of the Particle to Kill X^s	Neutralizing Activity of PBSX Antiserum
B. subtilis 168[6]	+	+	+
B. subtilis SMY Ind$^-$	+	+	+
B. subtilis var *aterrimus*	+	+	+
B. subtilis 168 T$^-$ Ind$^-$	+	+	+
B. subtilis 168 T$^-$	+	+	+
B. natto	+	+	+
B. licheniformis	+	−	−
B. lentus	−		
B. cereus	−		
B. macerans	−		
B. polymyxa	−		
B. megaterium	−		
B. thurigensis	−		
B. pumilus	−		

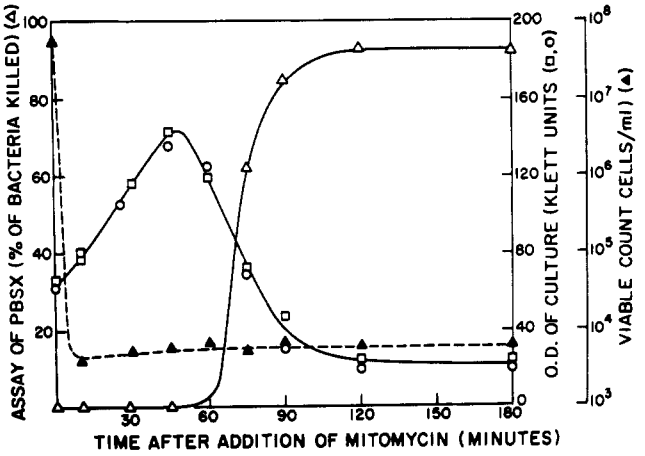

FIG. 2.—Kinetics of induction of *B. subtilis* var *aterrimus* by mitomycin C and the release of PBSX. PBSX was assayed as described under Materials and Methods.

PBSX-like particle. The properties of this particle, however, were quite different from PBSX (Table I) and will be discussed later.

The particles derived from the different strains were compared using the following two criteria: (1) the ability to kill the X^s indicator strain and (2) their reactivity with antiserum prepared against PBSX derived from *B. subtilis* 168^6. Since the induction of *B. subtilis* var *aterrimus* was found to be more rapid and reproducible, this strain was used to obtain PBSX lysates for all subsequent studies.

Figure 2 shows the kinetics of induction and release of PBSX. The induced bacteria lose their colony-forming ability within 10 minutes after the addition of mitomycin C. The turbidity of the induced culture increases for about 45 minutes and then drops. This drop is accompanied by the liberation of PBSX into the medium. The cell lysis and liberation of PBSX is complete at 90–120 minutes.

The induction process, as expected, is sensitive to chloramphenicol (Fig. 3). Bacterial cultures were induced with mitomycin C, and chloramphenicol at a concentration of 80 μg/ml was added at different times after induction. Chloramphenicol is fully effective in preventing lysis if added during the first 30 minutes. When added 45 minutes after induction it reduces the yield of PBSX and is completely ineffective if added at 60 minutes. The above results are interpreted to mean that there is no early protein-synthesizing activity but rather a continuous synthesis almost up to the time of lysis.

The rise in the turbidity of the culture during the first 45 minutes after addition of mitomycin C (Fig. 2), indicates that protein synthesis continues during induction. This point is supported by the chloramphenicol experiment. It can be seen (Fig. 3) that the increase in optical density is prevented by the addition of chloramphenicol. The effect of 5-fluorodeoxyuridine, which is known to inhibit DNA synthesis, on the induction of PBSX was also investigated. The induction was performed in synthetic medium (Nomura *et al.*, 1962). 5-Fluorodeoxyuridine (5 μg/ml) was added at the time of addition of mitomycin C. Lysis proceeded well in the presence of 5-fluorodeoxyuridine and there was no apparent reduction of phage yield indicating that a burst could be obtained in the absence of DNA synthesis.

This rather suprising result led us to examine the question whether the DNA contained in PBSX particles was a preexisting DNA, a prophage packaged

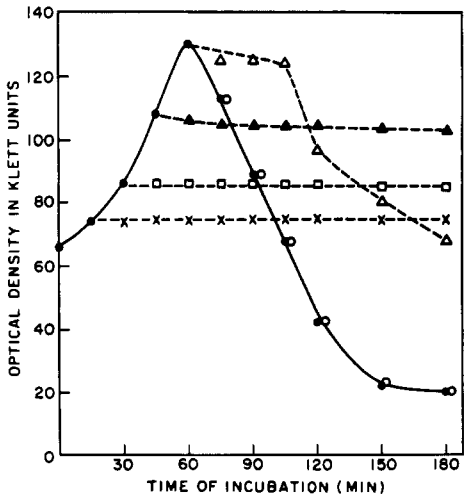

FIG. 3.—Effects of chloramphenicol on induction of PBSX. Mitomycin C was added at zero time and removed by centrifugation at 10 minutes. Chloramphenicol was removed after 60 minutes of contact. Time of chloramphenicol addition: x---x, 0 min; □---□, 15 min; ▲---▲, 30 min; △---△, 45 min; ○---○, 60 min; ●---●, control without chloramphenicol.

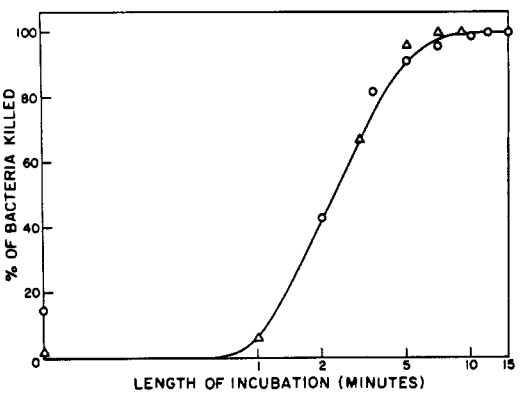

FIG. 4.—Kinetics of killing of X^s bacteria by PBSX. The points represent two experiments.

in a newly synthesized protein coat. *B. subtilis* var *aterrimus* cells were grown for many generations in a fully deuterated medium (Crespi *et al.*, 1962). Upon reaching a density of 1–3 × 10^7/ml they were collected by centrifugation, transferred to H_2O-Penassay, and induced. PBSX was harvested by the usual method and DNA was isolated. The density of this DNA in a CsCl gradient corresponded to the density of fully deuterated DNA, that is, DNA synthesized in heavy media before induction. There was no trace of hybrid or of light DNA.

Properties of PBSX—An intensive search for a host which would support the multiplication of PBSX failed to produce such a strain. Since no naturally occurring indicator could be found, an attempt was made to cure the *B. subtilis* 168^6 lysogenic strain and thus render it sensitive. The cured strain was found to be not inducible by mitomycin C. When cured X^s cells were incubated in liquid medium with PBSX, complete killing could be observed in 7–10 minutes (Fig. 4) and complete lysis of concentrated bacterial suspensions in 30 minutes (Fig. 5). No plaques were ever found on solid medium. PBSX was plated at high multiplicities in the hope of observing complementation or recombination but no phage production could be obtained.

TABLE II
TRANSFORMATION OF *B. subtilis*[a]

Source of Transforming DNA	Transformants/ml		
	Arginine	Histidine	Bryamycin
B. subtilis 168[s]	3.2×10^6	2.2×10^5	
	1.28×10^5	7.45×10^5	1.85×10^5
	2.1×10^5	1.2×10^5	
PBSX from *B. subtilis* 168[s]	2.5×10^5	1.25×10^5	2.7×10^5
PBSX from *B. subtilis* var *aterrimus*	1.1×10^5	1.8×10^5	
	1.15×10^5	3.9×10^4	
PBSX from *B. subtilis* var *aterrimus*; reconstruction expt. 1	9.2×10^4	9.7×10^4	
PBSX from *B. subtilis* var *aterrimus*; reconstruction expt. 2	1.1×10^5		0
PBSX-like particle from *B. licheniformis*	0	0	
PBSX-like particle from *B. licheniformis*; reconstruction expt. 1	0	0	0
PBSX-like particle from *B. licheniformis*; reconstruction expt. 2	0	0	0

[a] The recipient strain in all the transformation experiments was *B. subtilis* 168 H$^-$Ind$^-$A$^-$Bry^s.

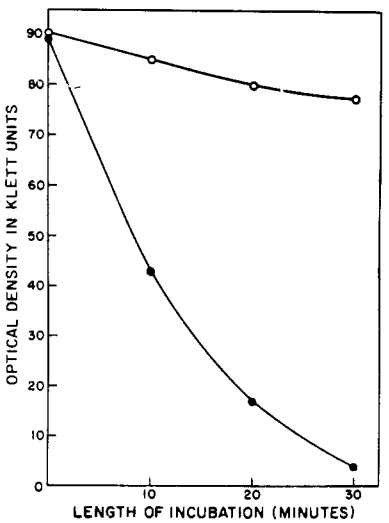

FIG. 5.—The lytic activity of PBSX as a function of time. O—O, X^s bacteria; ●—●, X^s bacteria incubated with PBSX.

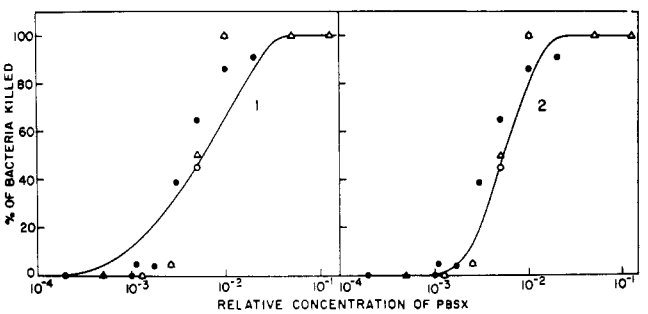

FIG. 6.—Multiplicity of killing of X^s by PBSX. Data from three concentration-dependence experiments were fitted into theoretical Poisson distribution curves for single-hit and double-hit events (theoretical curves from Lauffer and Price, 1945).

The absence of plaque-forming ability strongly suggests that the phage does not multiply in the infected bacterium; however, the possibility exists that PBSX is capable of undergoing only one vegetative cycle resulting in a burst of particles which in turn cannot infect. Such a situation would also result in the absence of plaques. In order to decide unequivocally whether any production of infectious particles occurred in the first cycle of infection, X^s cells were infected with PBSX and incubated for 15, 30, 60, and 120 minutes. Fresh X^s cells were then added to the same incubation tubes in order to determine whether the PBSX titer increased during the first cycle of kill. The number of killing particles did not increase, indicating that PBSX does not multiply on X^s cells in liquid medium.

Since lysis in liquid medium proceeded very rapidly it was thought that PBSX may have a lysozyme associated with it. Concentrated PBSX lysates were assayed for lysozyme activity using *Micrococcus lysodeikticus* as the test system but no lysozyme activity could be demonstrated. The presence of a lysozyme like enzyme which is specific for *B. subtilis* only (and therefore gives no reaction in the *M. lysodeikticus* system) was tested in the following way: X^s cells were grown in Penassay to 1–3 $\times 10^7$/ml. The cells were collected by centrifugation and suspended at the original concentration in a synthetic medium stabilized with 0.7 M sucrose. The suspension was treated with (a) 200 μg/ml lysozyme and (b) a PBSX lysate. Both preparations were examined microscopically for the presence of protoplasts. The lysozyme-treated cells were converted to protoplasts within 20 minutes, whereas the PBSX-infected cells were lysed and only large cellular debris could be seen under the microscope.

The concentration-dependent killing of sensitive cells by PBSX was determined by diluting PBSX serially in Penassay and assaying 0.1-ml aliquots on X^s cells as described above. The data from several such concentration curves were fitted into theoretical Poisson curves for 1-hit and 2-hit phenomena (Fig. 6). The experimental points do not fall on either of the theoretical curves, but rather indicate two separate modes of killing. Such behavior could be due to heterogeneity of the bacterial population or to heterogeneity of the phage. We favor the second possibility which seems well substantiated by the electron micrographs (Fig. 1).

The heat sensitivity of PBSX was studied by incubating the phage in Penassay for 10 minutes at temperatures ranging from 40 to 75° (Fig. 7). The phage is fully inactivated at 65° and the range of inactivation is fairly narrow (63–65°).

PBSX-DNA.—PBSX exists in many strains of *B. subtilis* as a stable prophage, yet it is unable to multiply in a sensitive bacterium. This rather perplexing situation prompted us to examine the DNA of PBSX. DNA was isolated by a gentle phenol

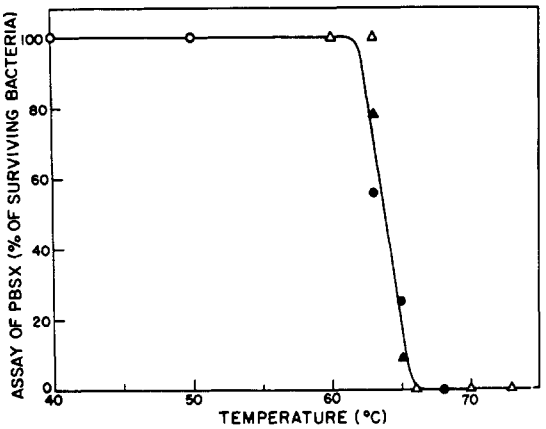

FIG. 7.—Heat sensitivity of PBSX. The different points represent independently heated samples.

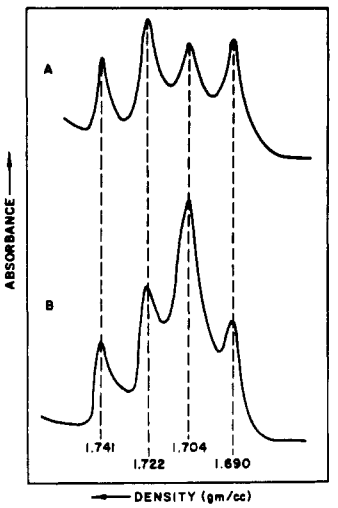

FIG. 8.—Hybridization of fully deuterated *B. subtilis* var *aterrimus* DNA with (A) light PBSX-DNA; (B) light *B. subtilis* var *aterrimus* DNA. The DNA samples were heated for 2 hours at 60° and cooled slowly to room temperature. Each DNA was present at a concentration of 5 μg/ml. The annealed mixtures were treated with dogfish-liver single-stranded endonuclease to remove any unrenatured material. DNA of fully deuterated *Ps. aeruginosa* (1.741 g/cc) was used as a density marker.

method in which care was taken to avoid any shearing. The yield of DNA per absorbance unit of lysate was very low, a fact which is in agreement with the micrographs which reveal a preponderance of empty heads. The T_m in saline citrate (87.5°) and buoyant density in CsCl (1.703 g/cc) of PBSX-DNA were identical to those of *B. subtilis* DNA (43% G + C) (Marmur and Doty, 1962; Schildkraut *et al.*, 1962) and there was no discrepancy between the two measurements. Molecular weight estimates were made from sedimentation coefficients giving $s_{20,w} = 24.2$ and mw $= 1.2 \times 10^7$. This molecular weight is considerably lower than molecular weights determined for DNA of other *B. subtilis* phages; it is also slightly lower than the molecular weight of *B. subtilis* DNA isolated by the procedure described by Marmur (1961).

On denaturation and fast cooling of PBSX-DNA, the band-width molecular weight of the denatured DNA in CsCl density gradient is lower than that expected from strand separation alone. No extensive measurements have been performed to determine the exact magnitude of the decrease in molecular weight, but we feel that the above observation indicates the presence of preformed single-chain breaks in the native structure.

Transforming activity of PBSX-DNA was tested in order to determine whether the phage contains any of the host genome. The transformation experiments are summarized in Table II. It can be seen that the DNA isolated from PBSX induced from *B. subtilis* 168^6 or from *B. subtilis* var *aterrimus* transforms with a similar efficiency as does the bacterial DNA. The DNA isolated from the particle induced from *B. licheniformis* has no transforming activity using *B. subtilis*-recipient cells. This was to be expected since *B. licheniformis* DNA does not transform *B. subtilis* (Marmur *et al.*, 1963).

PBSX-DNA transformed three randomly chosen unlinked markers suggesting a random incorporation of bacterial DNA into the phage coat. In order to exclude the possibility of bacterial DNA's adsorbing nonspecifically onto the phage during lysis, thus being responsible for the observed transformation, the following reconstruction experiments were performed: *B. subtilis* var *aterrimus* and *B. licheniformis* cultures were induced with mitomycin C. When the cultures were fully induced 1 mg of *B. subtilis*-transforming DNA was added per liter of lysate and incubated for 60 minutes. The lysates were then incubated for an additional 60 minutes with 1 μg/ml deoxyribonuclease and processed in the usual manner. In a second reconstruction experiment the transforming DNA was added at the beginning of the induction period. The results of the reconstruction experiments (Table II) demonstrate that there is no adsorption of DNA onto the phage particles and that the observed transformation must be an intrinsic property of the DNA contained within the PBSX particle.

A further test of the relatedness of PBSX-DNA to its host DNA was performed using the hybridization technique (Schildkraut *et al.*, 1961). Fully deuterated *B. subtilis* var *aterrimus* DNA was annealed with PBSX-DNA (induced either from *B. subtilis* 168^6 or *B. subtilis* var *aterrimus*), and the annealed mixtures were treated with dogfish-liver single-stranded endonuclease (Levine and Van Vunakis, 1963) and centrifuged in a CsCl density gradient. The hybrid DNA (Fig. 8) is a further proof of the homology of the DNA samples tested.

DISCUSSION

It is likely that a spectrum of phage types exists in nature which vary from virulent ones in which there is no genetic interaction between the phage DNA and host DNA to temperate phages whose DNA is intimately associated with host DNA and exhibits extensive regions of homology with the host nucleic acids (Cowie and McCarthy, 1963).

The data presented in this paper points to the existence of a group of very rudimentary phages for the genus *Bacillus*. All of the *B. subtilis* strains tested were found lysogenic for at least one and possibly more of the phages described above. We consider the group of particles referred to here as PBSX to represent a transitional evolutionary form between an episome and a phage. PBSX behaves as though it were a genetically defective phage in its inability to self-replicate. An alternative and admittedly much simpler explanation of the inability to demonstrate phage replication would be to assume that we have not found the natural host system for PBSX. This explanation is not very likely since in addition to the very extensive scanning of *Bacillus* strains we have succeeded in curing a lysogenic *B. subtilis* strain, render-

ing it sensitive to attack as measured by adsorption and killing by PBSX. Yet this strain could not support the growth of the phage.

The probable reason for the inability of PBSX to either lysogenize or transduce (in contrast to other genetically defective systems) is that the phage kills the sensitive bacterium upon contact, before the DNA can become expressed (if indeed the phage is capable of injecting its DNA). We are faced with a paradox of a defective prophage (temperate by definition) which upon induction acquires a virulent coat and subsequently behaves in a manner similar to a highly virulent entity incapable of self-reproduction. A precedent for a temperate phage containing killing (nonreplicating) particles exists in the PBS1 and PBS2 *B. subtilis* systems of Takahashi (1963 and personal communication). The proportion of killing particles present in the PBS1 and PBS2 lysates is much lower (30%) and the rest of the phage population behaves normally. The possibility that PBSX could contaminate the transducing lysates of PBS2 and thus account for the killing properties of the lysates was entertained. Such a contamination would also explain the presence of two DNA species (banding at densities of 1.703 g/cc and 1.722 g/cc) observed in CsCl density-gradient centrifugation of the DNA isolated from PBS2 lysates (I. Mahler, M. Cahoon, and J. Marmur, in preparation). However, such a possibility was eliminated by the lack of serological cross reactivity between PBSX and PBS2 lysates.

PBSX-DNA transforms *B. subtilis* for three randomly chosen bacterial markers. Two explanations can be offered for such a generalized transformation. (1) The classical case of a multiple prophage site (Bertani, 1955), and (2) the random incorporation of fragments of bacterial DNA into the phage coat (Zinder, 1953; Okubo *et al.*, 1963; Mahler *et al.*, 1963) The evidence for the latter is obtained from a PBSX-like particle isolated from *B. licheniformis*. The density of the PBSX-like DNA is 1.703 g/cc and that of *B. licheniformis* DNA 1.707 g/cc. When DNA is isolated from PBSX-like (*B. licheniformis*) phage and banded in CsCl, two bands are observed corresponding in densities to the PBSX and *B. licheniformis*, respectively. This is not due to contamination with *B. licheniformis* DNA during the isolation of phage (see under Results, reconstruction experiment), but rather to the presence of host DNA within the PBSX. This incorporation of host DNA into PBSX favors the hypothesis that there is no linkage between prophage and bacterial DNA. It is possible that mitomycin C induces the formation of an active intracellular deoxyribonuclease which destroys host DNA, part of which is randomly incorporated along with phage-specific DNA inside the PBSX protein coat.

One of the most intriguing properties of the PBSX system is the lack of DNA replication following induction. It has been demonstrated that mitomycin C stops the *in vivo* synthesis of bacterial DNA presumably by cross-linking it (Iyer and Szybalski, 1963), whereas it has no effect on the synthesis of phage DNA (Otsuji *et al.*, 1959). If the prophage or closely adjacent regions were cross-linked, DNA replication of that region could be prevented. We do not feel, however, that any significant extent of cross-linking occurs during the exposure of cells to mitomycin C in the course of the induction experiments. When PBSX-DNA was heated and rapidly cooled and banded in CsCl density gradient, all of the DNA had the density characteristic of denatured DNA and no residual native band could be observed. If any cross-linking had occurred during exposure of the bacteria to mitomycin C, the DNA isolated from PBSX would not denature upon heating and rapid cooling.

We propose the following description of the PBSX system: PBSX is a "protophage," a poorly evolved phage which lacks some of the essential functions mandatory to self-reproduction. The prophage DNA, which is that associated with the specific phagelike properties of PBSX, is capable of replication only in synchrony with the host chromosome. The prophage region is physically detached from the host chromosome during induction and thus no longer capable of replication (possibly contains no replicon; Jacob *et al.*, 1963). The prophage is capable of directing messenger RNA synthesis resulting in phage-specific protein synthesis. According to this hypothesis, each lysing cell releases one DNA-containing phage particle and numerous ghosts. Experiments are now in progress to study the kinetics of the synthesis of PBSX protein following mitomycin C induction of *B. subtilis* and determine the nature of the newly synthesized protein and the number of ghosts in the lysate.

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Optical Rotatory Dispersion of DNA and RNA*

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Optical rotatory dispersion (ORD) of salmon sperm DNA was measured over a wavelength range of 190–600 mμ. The dispersion curve obeyed a one-term Drude equation at wavelengths above 350 mμ. Strong Cotton effects occurred in the ultraviolet region with three peaks at 290, 228, and 200 mμ, two troughs at 257 and 215 mμ, and cross-overs (zero rotations) at about 274 and 248 mμ. Heat (90°) or alkaline (*p*H 12.3) denaturation of DNA depressed the Cotton effects; the profile below 250 mμ appeared to be particularly sensitive to the breaking up of the secondary structure. The ORD of rat liver RNA, which followed a simple Drude equation down to about 310 mμ wavelength, showed a strong peak and trough at 280 and 252 mμ, with a cross-over at 265 mμ. A weak peak and trough occurred at 228 and 218 mμ, followed by another very strong peak at 195 mμ. In alkaline solution or at high temperatures the 280 mμ peak and 252 mμ trough shifted to 290 and 260 mμ.

The ORD[1] of nucleic acids follows a simple Drude equation

$$[\alpha]_\lambda = k/(\lambda^2 - \lambda_c^2) \qquad (1)$$

in the visible region (Fresco, 1961; Ts'o *et al.*, 1962). At wavelengths below 350 mμ, however, equation (1) is no longer applicable to DNA (Fresco, 1961). The presence of strong absorption bands in the ultraviolet region makes measuring optical rotations of nucleic acids very difficult. Nevertheless, Simmons and Blout (1960) were able to show a positive Cotton effect for the RNA isolated from TMV with an inflection point around 260 mμ, which shifted to 272 mμ in 8 M urea. Fresco *et al.* (1961) further demonstrated a multiple Cotton effect in calf thymus DNA and calf liver RNA between 230 and 300 mμ, which contained considerable detail not evident in the corresponding absorption spectra; their study was, however, done with 0.02% solutions ($A_{260} = 4$). Later, Urnes and Doty (1961) reported that rotatory artifacts simulating Cotton effects can be produced easily in regions of high absorbance, and they suggested a critical limit of 2 for the absorbance of solutions prepared for ORD experiments. In view of these findings we reinvestigated the rotatory properties of two DNA (salmon and calf thymus) and one RNA (rat liver) solutions using a spectropolarimeter that extends measurements down to 190 mμ.

Experimental Procedures

Materials.—The DNA and RNA solutions were clarified by filtering through a medium sintered-glass filter before being measured. Their concentrations (in the native state) were determined spectrophotometrically, assuming $A^{1\%}_{1\,cm} = 200$ at 260 mμ.

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[1] Abbreviations used in this work: ORD, optical rotatory dispersion; TMV, tobacco mosaic virus; dAMP, deoxyadenosine-5′-phosphate; dCMP, deoxycytidine-5′-phosphate; dGMP, deoxyguanosine-5′-phosphate; dTMP, deoxythymine-5′-phosphate.

Optical Rotatory Dispersion.—The early ORD measurements were made with a Rudolph manual spectropolarimeter (Model MSP 4), the calibration of which has been described elsewhere (Yang and Samejima, 1963a). We repeated most of the experiments in the ultraviolet range using a Cary Model 60 recording spectropolarimeter. This instrument was also calibrated with a sucrose solution (National Bureau of Standards grade), the rotations of which obey a one-term Drude equation between 220 and 600 mμ: our values were $[\alpha]_\lambda = 21.65/(\lambda^2 - 0.0217)$; Harris *et al.* (1932) found $[\alpha] = 21.676/(\lambda^2 - 0.0213)$. (Below 220 mμ the rotation of sucrose became more dextrorotatory than that calculated from the Drude equation; for example, it was 33% higher at 195 mμ.) Virtually no band-width dependence of the rotations was detected for the Cary instrument, thus eliminating the laborious extrapolation to zero slit width (on the monochromator). The rotations remained unchanged when the sample cell was placed in series with another cell containing an absorbing material such as formamide-water and K_2CrO_4 solution, indicating that there was no significant stray light (Yang and Samejima, 1963a). The polarimeter tubes used varied from 10 to 0.01 cm in light path, the latter being calibrated with a solution of known rotations. In regions of the absorption bands, the concentration of the solution was so adjusted that the absorbance against empty reference cell was always much less than 2. The experimental data were reproducible, and the specific rotations showed no concentration dependence beyond normal experimental error. Data obtained below 210 mμ were less precise than those at longer wavelengths, mainly because of the very dilute solutions used (to reduce absorbance) and the increase in the noise level of the instrument near its limit of wavelength range.

Spectrophotometry.—Absorption spectra were measured with an Optica spectrophotometer.

Results

DNA.—Figures 1 and 2 show the ORD of native and denatured DNA. To reduce the absorbance