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Temperature-Sensitive Induction of Bacteriophage in *Bacillus subtilis* 168

ELI C. SIEGEL[1] AND JULIUS MARMUR

Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

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In a temperature-sensitive mutant of *Bacillus subtilis* 168, induction of the defective phage PBSX occurred at 48 C. Cell lysis began after 90 min of growth at 48 C, and cell viability began to decrease after 10 to 30 min. The loss in viability at the nonpermissive temperature was prevented by azide or cyanide. Deoxyribonucleic acid (DNA), ribonucleic acid, and protein synthesis were not inhibited at 48 C. Temperature induction of the temperate phage SPO2 also occurred in this mutant. The temperature-sensitive mutation, designated *tsi-23*, was linked by transduction to *purB6* and *pig*, the order being *purB6 pig tsi-23*. Mutation *tsi-23* was transformable to wild type by *B. subtilis* 168 DNA but not by DNA from the closely related strains W23 or S31. DNA from the latter two strains transformed auxotrophic markers of strain 168 at frequencies close to those found with 168 donor DNA. Upon temperature induction, cellular DNA was broken to a size of 22*S*, characteristic of DNA in PBSX particles. The DNA isolated from temperature-induced PBSX did not give an increased Ade^+/Met^+ transformant ratio relative to cellular DNA nor contain preferential break points as determined by transformation of four closely linked markers.

Temperature-sensitive induction of prophage has been studied primarily in the *Escherichia coli* K-12 phage λ system. Such induction can occur in two distinct ways. The product of the phage C_I gene, the lambda repressor (19), is inactivated above 35 C in temperature-sensitive C_I mutants, resulting in the eventual induction of the prophage (12, 13, 26). Induction of prophage at a nonpermissive temperature can also occur as the result of a mutation in the host genome (5, 11). In this case, any ultraviolet-inducible phage carried by the host becomes temperature-inducible. This temperature-sensitive host mutation resulted in filament formation at the nonpermissive temperature in cured strains and also appeared to be involved with deoxyribonucleic acid (DNA) repair.

We have found a temperature-sensitive mutant of *Bacillus subtilis* 168 in which the defective phage PBSX (6, 17, 24, 25) and the temperate phage SPO2 (2, 9, 22) are temperature-inducible. The relation of SPO2 to the host cell is very similar to that of phage λ. However, the *B. subtilis* 168 PBSX system has many novel properties. All derivatives of strain 168 are lysogenic for PBSX which is induced by treatment with ultraviolet irradiation or mitomycin C (17, 24, 25). The phage cannot form plaques on any host, and the DNA within its head cannot be distinguished from host DNA (6, 7, 17). From the work of Okamoto et al. (16, 17) and Haas and Yoshikawa (6, 7), a general picture of PBSX induction has developed. Upon induction, phage-specific proteins are formed, and the host chromosome is broken down to pieces of a uniform size of 22*S*. This DNA is then packaged within the phage head. Induction and phage development can occur in the absence of DNA synthesis (16), and there is no evidence for the replication of phage-specific DNA following induction (7). PBSX can be recognized by its colicin-like killing activity against *B. subtilis* strains W23 and S31 (17). These strains, which are closely related to 168, also form defective phages having an appearance similar to PBSX (J. Mangan, *personal communication*). Among these three strains, it was found that the defective phage formed by any one strain was only adsorbed to and killed the two heterologous strains (17).

Temperature-sensitive induction of PBSX has many interesting aspects and has raised questions about the genetic relationships among the defective phages of different lines of *B. subtilis*.

[1] Present address: Department of Biology, Tufts University, Medford, Mass. 01155.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1.

Media. The minimal medium was that described by Copeland and Marmur (3). Spizizen I medium was that of Anagnostopoulos and Spizizen (1). Veal-yeast extract (VY) broth and Brain Heart Infusion (BHI) agar were previously described (17). DSP diluent contained 0.87% (w/v) NaCl, 0.348% K_2HPO_4, 0.05% peptone, and 10^{-6} M $MnSO_4$. It was adjusted to *p*H 7.5 with HCl. Phage SPO2 was grown and assayed by using the media of Okubo and Romig (18). Pigmentation of sporulated colonies was determined on AK Agar no. 2 (BBL).

Preparation of cultures. Spores of temperature-sensitive strains were prepared by growth in sporulation medium (23) for 3 days at 34 C. The spores were repeatedly washed and then stored in distilled water at 4 C. To start a culture, spores were germinated on BHI agar at room temperature, and a resulting colony less than 1 day old was used as an inoculum. This procedure was necessary because of the instability of the temperature-sensitive mutation. All liquid cultures were vigorously aerated by shaking. Permissive and nonpermissive temperatures were 34 and 48 C, respectively. To follow the decrease in viability after transfer to 48 C, a culture of MB500 was grown at 34 C in VY broth to a Klett reading (540 nm) of 80 to 100, which corresponds to about 2×10^8 cells per ml, and 1 ml was transferred to 9 ml of VY broth at 48 C. Samples were taken at intervals, diluted into DSP at room temperature, and plated on BHI agar at 34 C.

Transduction and transformation. The procedure for PBS1 transduction was previously described (4). Competent cells were prepared by the method of C. R. Stewart (Ph.D. Thesis, Stanford University, Stanford, Calif., 1967). All transformants or transductants were purified by single-colony isolation before testing for unselected markers.

Induction of mutations. A PBS1 lysate was prepared on MB400 and concentrated 100-fold by differential centrifugation. The concentrated lysate was mutagenized with hydroxylamine by the procedure of Kahan (10). The reaction was stopped by diluting the treated phage 20-fold into cold VY broth containing 10% acetone. The preparation was then dialyzed in the cold against VY broth and finally sterilized by filtration through an HA filter (Millipore Corp., Bedford, Mass.). The mutagen-treated phage were used to transduce a *purB6* recipient, MB12, to Pur$^+$. Of 1,800 transductants, 9 were temperature-sensitive as determined by growth on BHI agar at 34 C but not 48 C. Eight of these were found to lyse when grown at 48 C in VY broth. One such temperature-sensitive transductant, MB500, was used for further study. The method described had been developed to find linked mutations. The finding of a mutant that lysed at 48 C was fortuitous.

Temperature induction of SPO2. MB500 lysogenic

TABLE 1. *List of strains*[a]

Strain	Previous designation	Genotype	Source or reference
MB1	Mu8u5u16	*metB5 leu-8 purA16*	N. Sueoka
MB7	Mu8u5u5 Mot$^+$	*metB5 leu-8 thr-5*	N. Sueoka
MB12	Mu8u5u6 Mot$^+$	*metB5 leu-8 purB6*	N. Sueoka
MB67	SB202	*aro-2 trp-2 hisB2 tyr-1*	Nester et al. (15)
MB79		*thyA thyB trp-2*	J. L. Farmer
MB134		*purB6 leu-8*	Transduction of MB12 to Met$^+$
MB221		*purA16 ery-1 argC4 leu-8 metB5*	
MB227	UT683	*pig*	Rogalsky (21)
MB400	SB19SEM	*str-1 mic-1 ery-1*	Dubnau et al. (4)
MB500	6TR23	*metB5 leu-8 tsi-23*	
MB501		*purB6 leu-8 tsi-23*	Transformation of MB500 with MB134 DNA (congression)
MB502		*tsi-23*	Transformation of MB500 to Leu$^+$ and Met$^+$ (congression)
MB1070	W23 *thr*$^-$	*thr ery*	
MB2030	S31 *ery*^{r}	*ery*	Subbaiah et al. (25) (*ery* spontaneous)
BC102		*purA16 leu-8 metB5 tms-26*	Copeland & Marmur (3)

[a] Gene symbols indicating requirement for and sensitivity or resistance to the following: *arg* (arginine), *aro* (shikemic acid), *ery* (erythromycin), *his* (histidine), *leu* (leucine), *met* (methionine), *mic* (micrococcin), *pur* (adenine or guanine), *str* (streptomycin), *thr* (threonine), *tms* (temperature-sensitive), *tsi* (temperature-sensitive induction), *thy* (thymine), *trp* (tryptophan), *tyr* (tyrosine), *pig* (spore pigmentation).

for SPO2 was grown at 34 C in VY broth to a Klett reading (540 nm) of 80. The culture was centrifuged in the cold, resuspended in VY broth, and incubated at 48 C. At intervals, 0.5-ml samples were transferred into 2.0 ml of cold VY broth saturated with chloroform. After standing for 4 hr at 4 C, the samples were centrifuged to remove debris and unlysed cells, and assayed for plaque-forming units by using MB12 as an indicator.

Detection of PBSX killing activity. The method used was that of Okamoto et al. (17).

Isotope incorporation. Isotope incorporation was previously described (3). The cells were grown to log phase at 34 C in Spizizen I medium and prelabeled for 30 min at this temperature before the start of the experiment.

Preparation of PBSX DNA. The methods were basically those of Okamoto et al. (17). MB502 was grown at 34 C in VY broth to a Klett reading (540 nm) of 60. The culture was then transferred to 48 C and incubation was continued until the turbidity fell to a mimimum. PBSX phage was then concentrated by differential centrifugation. The phage suspension obtained was dialyzed against TMK buffer (17) and then subjected to CsCl density gradient centrifugation. DNA from phage in the peak fractions of the gradient was extracted with phenol. DNA from mitomycin C-induced PBSX was isolated by the same procedure. The conditions of mitomycin induction were described by Okamoto et al. (17).

Isolation of bacterial DNA. The method of Marmur (14) or a gentler procedure previously described (17) was used.

Shearing of DNA. DNA in standard saline citrate (0.15 M NaCl plus 0.015 M sodium citrate), at a concentration of 24 to 36 μg/ml, was sheared for 5 min at a power setting of 56 in a homogenizer (Virtis, model 23). The microhomogenizing assembly was used with the blades bent vertically. DNA size was then determined by sedimentation analysis in an ultracentrifuge (Spinco, model E).

Sedimentation velocity centrifugation. The procedures of Okamoto et al. (17) were used.

RESULTS

Growth characteristics. Stationary-phase cultures of MB12, the parent of the temperature-sensitive MB500, form colonies with equal efficiency at 34 and 48 C on BHI agar. MB500 was recognized by its inability to form colonies at 48 C. Figure 1 shows the growth of MB500 in VY broth at 34 and 48 C. After 90 min of growth at 48 C, the culture began to lyse. Similar lysis occurred at 48 C in the less rich Spizizen I medium. The change in turbidity after the shift to 48 C was similar to that observed when *B. subtilis* 168 was induced with mitomycin C. As observed microscopically, cells of MB500 at 48 C appeared normal in size and shape until lysis began. Electron microscopy showed that the lysate contained phage particles similar to PBSX (J. Mangan, *personal communication*). The killing spectrum of the lysate as determined by spot tests on strains W23 and S31 was characteristic of PBSX (17, 25). Sucrose gradient sedimentation, with PBSX induced by mitomycin C from MB79 used as a standard, demonstrated that the phage particle released at 48 C from MB500 was the same size as PBSX. It was concluded that induction of PBSX occurred at 48 C in MB500. This induction was specific; it did not occur at the nonpermissive temperature in a series of temperature-sensitive strains blocked in other functions (J. C. Copeland, *personal communication*).

Effect of metabolic inhibitors on viable count. Figure 2 gives the viable count of an MB500 culture after transfer to VY broth at 48 C. Under these conditions, the viable count began to decrease after 10 to 30 min and then fell exponentially. The presence of sodium azide at a concentration of 10^{-2} M prevented cell death at 48 C. The azide was eliminated before plating by dilution. Similar results were found with 10^{-2} M sodium cyanide. When chloramphenicol was used

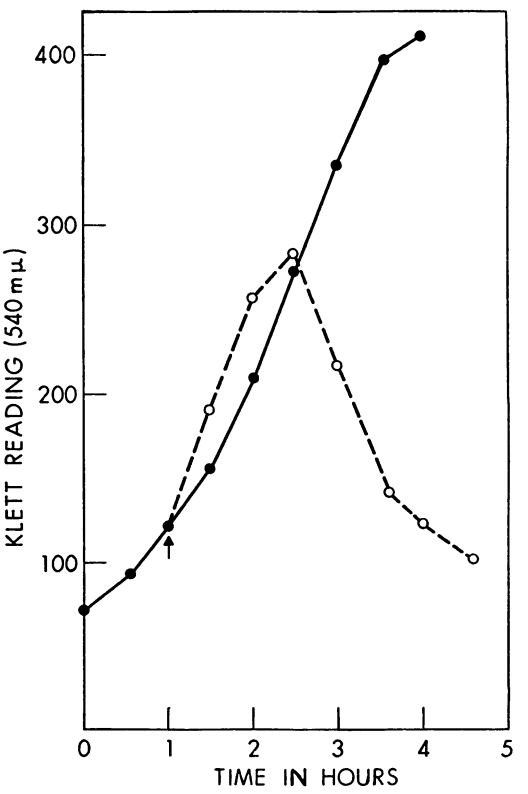

FIG. 1. *Growth of MB500 in VY broth at 34 and 48 C. At the time indicated by the arrow, one half of the culture was transferred to 48 C. Symbols:* ○, *48 C;* ●, *34 C.*

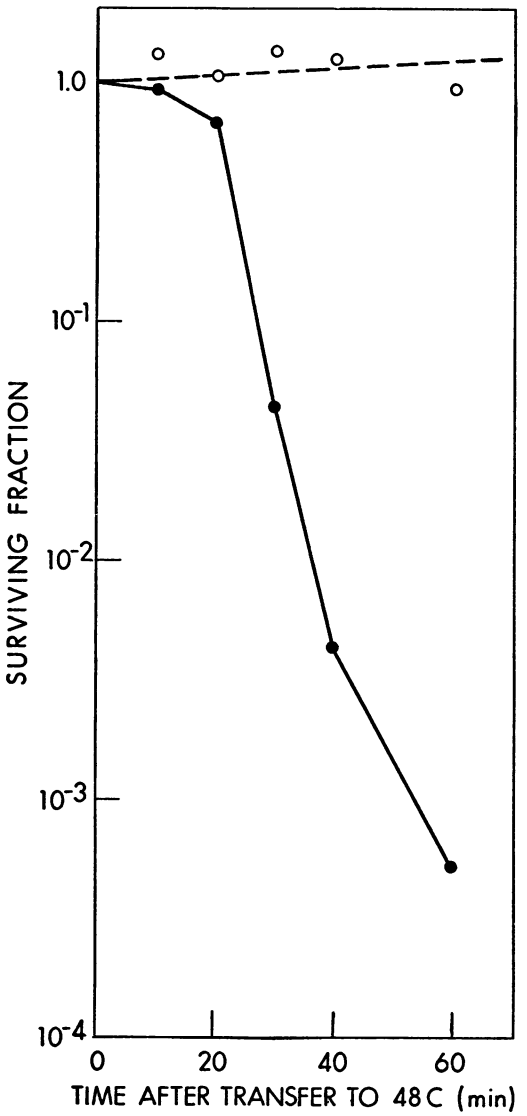

FIG. 2. *Viable count of MB500 in VY broth at 48 C in the presence and absence of sodium azide (10^{-2} M). Symbols: ○, azide; ●, no azide.*

at a concentration of 100 μg/ml, MB500 died as rapidly at 48 C as in the absence of the drug. However, the parental strain, MB12, died at a similar rate at 48 C in the presence of chloramphenicol, indicating an additional effect.

When sodium azide was added at various times after transfer to 48 C (Fig. 3), there was a transient increase in viable count followed by a continuing decrease. A possible explanation is that addition of azide immediately, but only temporarily, stopped death. The apparent increase in viability can be attributed to the time lag before cell death stopped when samples were simply plated at 34 C.

Induction of Tsi$^+$ revertants. Forty Tsi$^+$ revertants of MB500 were induced with mitomycin C and tested for production of PBSX by killing activity against W23. All 40 formed PBSX.

Macromolecular synthesis. Synthesis of DNA, ribonucleic acid (RNA), and protein at 34 and 48 C was measured by the incorporation of the radioactive precursors thymidine, uridine, and phenylalanine, respectively, into acid-insoluble material. The results (Fig. 4) indicate that DNA synthesis was not inhibited in the temperature-sensitive strain at 48 C. There was, however, a slight decrease in RNA and protein synthesis. Since agents which interfere with the structure or metabolism of DNA can induce PBSX (25), these experiments support the idea that the *tsi-23* muta-

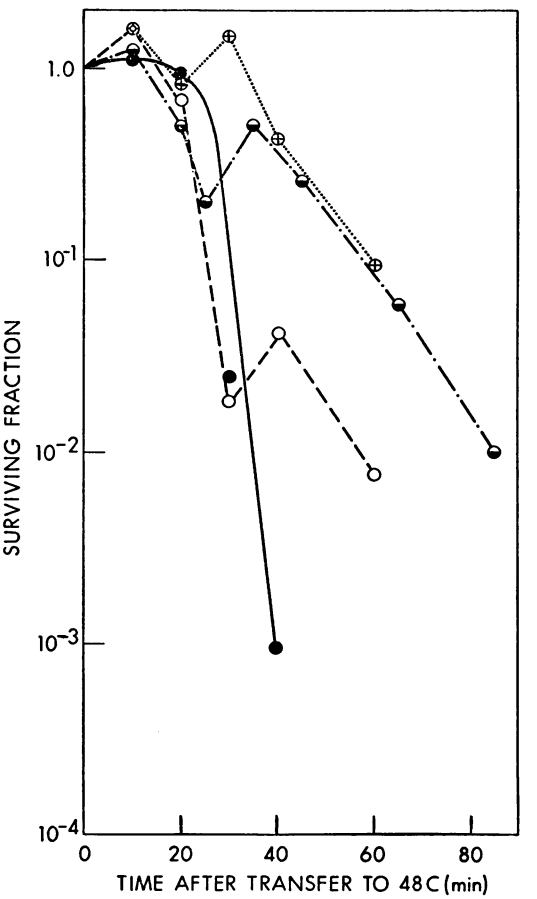

FIG. 3. *Viable count of MB500 in VY broth at 48 C. Sodium azide added at various times. Symbols: ●, no azide; ⊕, azide added at 20 min; ◑, azide added at 25 min; ○, azide added at 30 min.*

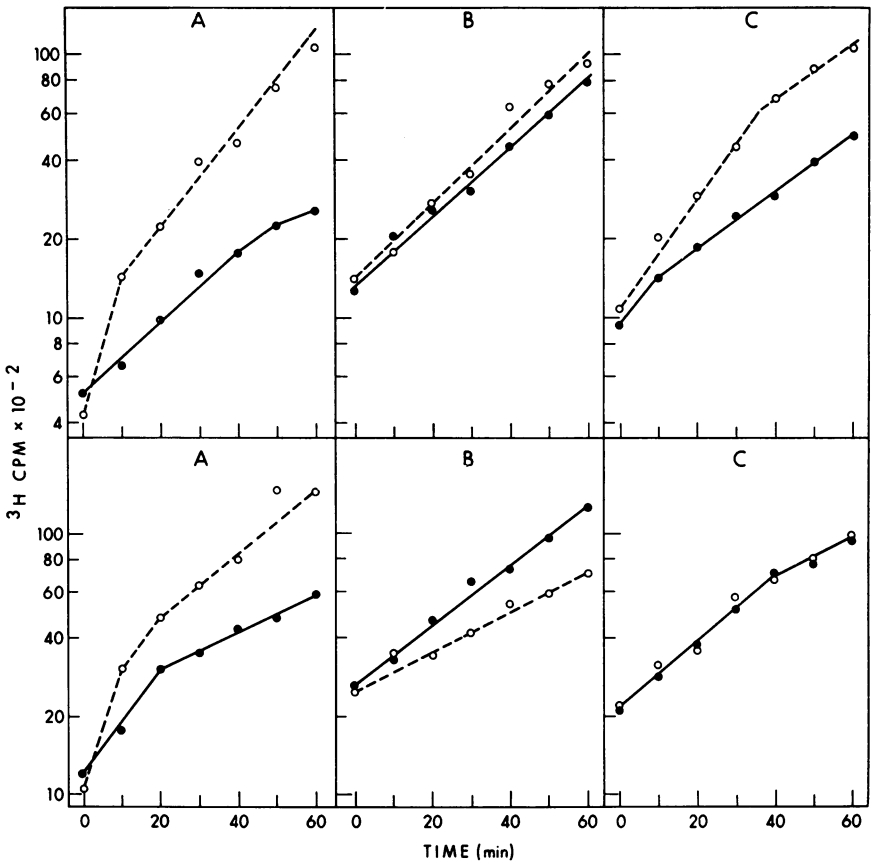

FIG. 4. *Macromolecular synthesis in MB12 and MB500 at 34 and 48 C. Top panel, MB12; lower panel, MB500. (A) ^{3}H-thymidine; (B) ^{3}H-uridine; (C) ^{3}H-phenylalanine. Symbols:* ○, *48 C;* ●, *34 C.*

tion is most likely phage-specific and not a mutation affecting host DNA synthesis.

Induction of SPO2. When MB500 lysogenic for SPO2 was transferred to VY broth at 48 C, induction of SPO2 occurred (Fig. 5). The transient decreases in SPO2 titer at 48 C were attributed to inactivation of a fraction of the phage at the high temperature. No increase in PBSX production, as detected by killing activity on W23, was found in the SPO2 lysate as compared with the supernatant fluid of a culture of the same strain grown at 34 C. MB12 lysogenic for SPO2 was not induced at 48 C.

Genetic analysis. Because the temperature-sensitive mutation *tsi-23* was selected by cotransduction of *purB6* to Pur$^+$ by mutagen-treated PBS1, *tsi-23* was expected to be cotransducible with *purB6*. This was confirmed, and a three-factor cross was done to order *purB6*, *tsi-23* and *pig*, which is also cotransducible with *purB6* (21). The results (Table 2) indicate that the order is *purB6 pig tsi-23*; *tsi-23* was not linked by transduction to *thr-5* or *ery-1*, the genes on either side of the *purB* linkage group (4).

Mutant *tsi-23* could be transformed to wild type by DNA from MB400, also a derivative of 168. The results of the transformation of MB500 by DNA from strain 168 and the related strains W23 and S31 are given in Table 3. The frequency of transformants to Tsi$^+$ with 168 DNA was approximately that of MetB$^+$ transformants. DNA from W23 or S31 gave a similar number of MetB$^+$ transformants but no significant number of Tsi$^+$ transformants above that of the control figure in repeated experiments. Furthermore, the spontaneous reversion frequency of *tsi-23* to Tsi$^+$ could not be reduced below the level seen in Table 3. W23 and S31 were not themselves temperature-sensitive and functioned well as donors in the transformation to wild type of *tms-26*, a mutant of strain 168 blocked in cell wall formation at 48 C (3).

The results of the transformation of the temperature-sensitive marker involved in the induc-

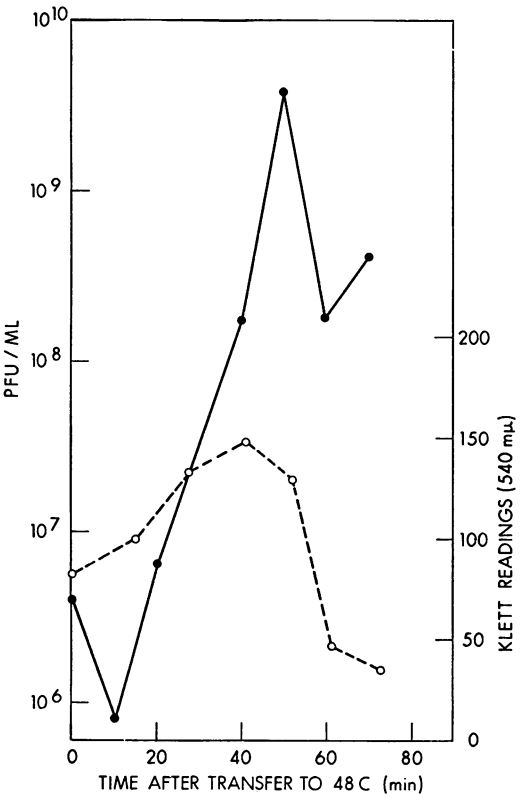

FIG. 5. *Temperature induction of phage SPO2 in strain MB500. Symbols: ○, Klett reading; ●, plaque-forming units per ml.*

TABLE 2. *Analysis of the three-factor transduction involving the pig, purB6, and tsi-23 markers*[a]

Marker			No. of transductants
purB6	*pig*	*tsi-23*	
1[b]	1	1	175
1	1	0	40
1	0	0	41
1	0	1	2

[a] Donor, MB227; recipient, MB501. Pur$^+$ transductants selected.
[b] Donor (1) phenotype; recipient (0) phenotype.

tion of PBSX contrast with those of Copeland and Marmur (3), who found that *tms-26* and a temperature-sensitive mutation of cross wall formation, *tms-12*, were transformed to wild type with DNA from such heterologous strains as *B. niger* or *B. licheniformis*.

Characteristics of PBSX DNA. When PBSX is induced, the *B. subtilis* 168 chromosome is broken into pieces with a defined sedimentation coefficient of 22*S*, which is the size of the DNA incorporated into PBSX particles (16). It is conceivable that breaks may occur preferentially at certain points. Hirokawa and Kadlubar (8), using DNA extracted from a PBSX-like defective phage of *B. subtilis* induced by 4-nitroquinoline-1-oxide, found no linkage by transformation between *his-31* and *trp-2* which are ordinarily closely linked by transformation. DNA isolated from mitomycin C and temperature-induced PBSX, as well as cellular DNA obtained from the same culture just before induction and sheared to a size of approximately 22*S*, was used to transform MB67. This strain contains the four linked markers *hisB2*, *trp-2*, *aro-2*, and *tyr-1* (15). Cellular and PBSX DNA were used at a final concentration of 0.2 μg/ml. The results of this transformation are given in Table 4. The percentages of transformants in the several classes of recom-

TABLE 3. *Transformation of MB500 with W23, S31, and 168 DNA*[a]

DNA	Transformants (per ml)	
	Met$^+$	Tsi$^+$
None	5×10^1	4.2×10^2
168	1.6×10^5	9.7×10^4
S31	4.6×10^4	4.4×10^2
W23	4.6×10^4	5.7×10^3

[a] Source of 168 DNA was MB400, source of S31 DNA was MB2030, and source of W23 DNA was MB1070. The final DNA concentration was 2 μg/ml.

TABLE 4. *Frequency of recombinant classes found with PBSX and cellular DNA*[a]

Marker				Percentage in class		
aro-2	*trp-2*	*hisB2*	*tyr-1*	Temp-induced PBSX DNA	MC-induced PBSX DNA	Cellular DNA
0	1[b]	0	0	43	38	31
0	1	1	0	12	12	7.6
0	1	1	1	19	24	19
0	1	0	1	<0.32	1.1	<0.35
1	1	0	0	22	21	29
1	1	0	1	0.32	<0.37	<0.35
1	1	1	0	2.2	1.8	6.6
1	1	1	1	0.95	1.8	6.6
				316[c]	271[c]	289[c]

[a] MB502 was the source of temperature- and mitomycin C (MC)-induced PBSX and cellular DNA. MB67 was the recipient. Trp$^+$ transformants were selected.
[b] Donor (1) phenotype; recipient (0) phenotype.
[c] Total transformants tested.

binants were similar for PBSX and cellular DNA, except for the 1110 and 1111 classes. The higher frequencies of these recombinants found with cellular DNA might indicate incomplete shearing. No selective break points seem to exist in the region examined.

Okamoto et al. (17) and Haas and Yoshikawa (7) found that the ratio of Ade^+/Met^+ transformants was higher with DNA isolated from mitomycin C-induced PBSX compared with DNA from noninduced cells. PBSX DNA was isolated from mitomycin C and temperature-induced PBSX formed by strain MB502. These DNA types and DNA extracted from cells just before induction were used to transform MB221. As described, the cellular DNA was sheared to a size of approximately 22*S*. All DNA was used at a saturating concentration of 1 μg/ml. The results, which were not normalized to transformation with spore DNA, are given in Table 5. Because the mitomycin C- and temperature-induced PBSX, which were the sources of phage DNA, were obtained in two separate experiments, each was compared with its own cellular DNA control. The ratio of Ade^+/Met^+ transformants obtained with mitomycin C-induced PBSX DNA was two times that of the cellular DNA as previously found by Okamoto et al. (17). No increase over the ratio found with cellular DNA was found with temperature-induced PBSX DNA. The higher Ade^+/Met^+ ratio of the control from the temperature induction experiment compared to that with mitomycin C induction can probably be attributed to a more rapidly growing culture. The unusual Leu^+/Met^+ ratios (0.20 to 0.36) appear to be a function of MB502. They were also found when MB502 DNA was used to transform MB1. DNA from other strains did not give this result.

The size of DNA in temperature-induced cells was followed by sedimentation velocity centrifugation of lysed ^{3}H-thymidine-labeled cells as described by Okamoto et al. (16, 17). The results were similar to those previously found with mitomycin C induction (16). Before and just after a culture of MB500 was transferred to 48 C, the DNA had a uniform size of approximately 50*S*. The amount of 50*S* bacterial DNA decreased as induction proceeded with a concomitant increase in 22*S* DNA, which is the size of the DNA contained in PBSX particles. Before lysis was complete, all DNA was 22*S*. The 16*S* DNA observed during mitomycin C induction by Okamoto et al. (16) was not seen.

DISCUSSION

Temperature induction of the defective phage PBSX has permitted an analysis of induction in the absence of agents such as mitomycin C or ultraviolet irradiation. The cell lysis and formation of 22*S* DNA were similar to the findings of Okamoto et al. (16, 17) using mitomycin C induction. However, no 16*S* DNA formed after temperature induction. In addition, it was possible to observe the lethal effect of induction in the absence of a bacteriocidal agent. As found by Lieb for λ induction (13), cell death was the first indication of induction. When cellular metabolism was arrested by azide or cyanide, no induction occurred.

The Ade^+/Met^+ ratio found by transformation when using temperature-induced PBSX DNA was actually lower than that found with cellular DNA extracted before induction. Mitomycin C induction of the temperature-sensitive strain resulted in an increased Ade^+/Met^+ ratio, as was found by Okamoto et al. (17) and Haas and Yoshikawa (7). The results suggest that an increased Ade^+/Met^+ ratio might be more a function of mitomycin C treatment than PBSX induction. The lack of an increase found with temperature-induced PBSX DNA may also be attributed to the relatively slow lysis that occurred after the transfer of the large volume (900 ml) of culture needed for phage and DNA isolation to 48 C. Haas and Yoshikawa (7) found a low Ade^+/Met^+ ratio when using PBSX DNA from cultures that lysed slowly.

TABLE 5. *Transformant ratios obtained with PBSX and cellular DNA*[a]

DNA	Ratio		
	Ade^+/Met^+	Arg^+/Met^+	Leu^+/Met^+
Cellular	4.6 ± 0.6	1.6 ± 0.25	0.23 ± 0.07
PBSX (Temp)[b]	2.3 ± 0.4	1.5 ± 0.5	0.20 ± 0.10
Cellular	2.7 ± 0.6	1.5 ± 0.6	0.36 ± 0.13
PBSX(MC)[b]	6.1 ± 0.9	2.1 ± 0.3	0.35 ± 0.16

[a] MB502 was the source of PBSX and of cellular DNA. MB221 was the recipient (*purA16 ery-1 argC4 leu-8 metB5*).

[b] Temp = temperature-induced; MC = mitomycin C-induced.

The failure to find any cured Tsi$^+$ revertants was expected. Mutant *tsi-23* is very unstable and, even if some Tsi$^+$ revertants were cured, the majority would represent back mutations. In addition, unsuccessful attempts were made to cure MB12 by repeated induction with mitomycin C (*unpublished data*). The absence of autonomous replication of PBSX DNA has been cited by Haas and Yoshikawa (7) as an explanation for the failure to find strains of *B. subtilis* 168 cured of PBSX.

SPO2 is a truly temperate phage of *B. subtilis* resembling λ in that it exists as an integrated prophage and is inducible (2, 9, 22). The temperature-sensitive induction of SPO2 would appear to indicate that the mutation, *tsi-23*, is in the host genome, not in the hypothetical PBSX repressor gene, and resembles the T-44 mutant of Kirby, Jacob, and Goldthwait (11). However, if we were dealing simply with such a mutation, transformation to wild type (Tsi$^+$) would be expected to occur with W23 or S31 DNA. The lack of transformation cannot be attributed to delayed expression of donor wild-type DNA, because Tsi$^+$ transformants were found with strain 168 as the donor. A possible explanation is that strains S31 and W23 do not contain DNA homologous to that of 168 in which the mutation to temperature inducibility occurred. The defective phages of strain W23 and S31 closely resemble PBSX but have different killing ranges (17, 25). The DNA regions lacking homology with 168 may be those coding for the repressors of these phages. The immunity regions of λ and the closely related phages 21 and 434, in which the C_I repressor gene is found, are not homologous by genetic (27) and physical evidence (28). A second explanation is that overall homology of the defective phage repressor genes among the 168, W23, and S31 bacterial strains is close and adequate for recombination, but small differences in the primary structures of the three repressors prevent binding of repressors to heterologous operators. This would assume that the operator(s) of the defective phage, unlike lambda (20), is not closely linked to the repressor. The induction of SPO2 by exposure of lysogenized strains to the nonpermissive temperature would then be attributed to some event occurring early in PBSX induction that results in inactivation of the SPO2 repressor.

It has been shown recently by A. J. Garro (*unpublished data*) that a mutant defective in tail formation of PBSX is linked to the host marker *metA3*, which in turn is unlinked to *purB6* by PBS1-mediated transduction. If indeed *tsi-23*, which is linked to *purB6*, is a mutation in the repressor gene of the phage-specific DNA, then the genetic analysis would indicate that the PBSX phage genome is integrated in a scattered manner in the host chromosome. This would then account for some of the unusual properties and defective nature of phage PBSX.

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