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Bacteriophage Interference in *Bacillus subtilis* 168

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Strains of *Bacillus subtilis* lysogenic for temperate bacteriophage SPO2 inhibit the development of bacteriophage $\phi 1$. After infection by bacteriophage $\phi 1$, DNA and RNA synthesis in the lysogenic host terminates, culminating in cell death. Bacteriophage SPO2 also prevents the production of bacteriophage $\phi 105$. Mechanisms for these two types of bacteriophage interference are discussed.

Successful infection by bacteriophage requires attachment, injection of its nucleic acid into a bacterium, transcription, translation and replication of bacteriophage genetic information, as well as release of infectious progeny. Many factors may cause abortion of the infection such as alteration of growth media, prophage interference, and preinfection of the bacteria with identical or different bacteriophages, or both (1, 2, 8). In *Bacillus subtilis*, sporulation profoundly influences viral replication. For example, the genetic information of bacteriophage ϕe cannot be transcribed in sporulating cells (19, 20) and bacteriophages $\phi 2$ and $\phi 15$ can infect but not lyse cultures of *B. subtilis* 168, whereas they are capable of lysing nonsporulating mutants (14, 15). Abortive infection can also be the result of lysogenic conversion or prophage interference (1, 2). Prophage interference can be accomplished by a restriction and modification system (6) with alteration of the cell envelope (16, 21) or by the repression and/or inhibition of bacterial enzymes (18, 25). In *Escherichia coli*, bacteriophage lambda kills bacteria lysogenic for bacteriophage P2 without producing progeny phage (18, 25, 26), whereas lambda lysogens exclude the successful development of bacteriophages T2, T4, and T6 that carry the rII mutation (2, 12). The ability of a prophage to subsequently interfere with the development of other bacteriophages is often dependent on interactions between the bacterial host and the resident prophage. Specifically, temperate bacteriophage ω will prevent bacteriophages T2 and T4 from successfully infecting lysogenic strains of *E. coli* W and *E. coli* Ws, but not *E. coli* K-12 (21). In addition, the inability of bacteriophage SP10 to grow under normal conditions in certain strains of *B. subtilis* also has been attributed to prophage interference (10, 11).

$\phi 105$ (isolated by Reilly) and SPO2 (isolated by Okubo) are two temperate bacteriophages of *B. subtilis* which have approximately the same molecular weight and the same morphology (3, 5) and similarly decrease the levels of transformation in lysogenic cultures (28-30). However, these two bacteriophages (i) are heteroimmune (4), (ii) have different receptor sites, (iii) integrate at different sites on the bacillus chromosome (13, 23, 27), (iv) show about 10% homology in DNA-DNA hybridization studies (7), and (v) differ in their requirements for host DNA polymerases (24). Our studies of interference by bacteriophages $\phi 105$ and SPO2 on infection with nine bacillus bacteriophages have revealed that bacteriophage SPO2 inhibits the virulent cycle of bacteriophage $\phi 1$. Additionally, this report will show that bacteriophage $\phi 105$ is depressed in its development by SPO2 when both bacteriophages are in a lytic cycle of infection.

MATERIALS AND METHODS

Strains and methods of propagation. Strain BR151 (carrying *lys*-3, *trp*C2, *met*B10) is an auxotrophic derivative of *B. subtilis* 168, originally obtained from B. Reilly. BR151 ($\phi 105$), BR151 (SPO2), and BR151 ($\phi 105$, SPO2) are lysogenic strains of BR151 carrying the prophages of $\phi 105$, SPO2, and both $\phi 105$ and SPO2, respectively. *B. subtilis* HSR was obtained from B. Reilly.

The bacteriophages used in this study are listed in Table 1 along with their method of propagation. All of the bacteriophages were propagated on strain BR151 except for $\phi 29$ which was grown on *B. subtilis* HSR. Three methods of propagation were employed. (A) The temperate bacteriophages were induced from the lysogenic cultures by the methods previously described (29). (B) Most of the phage lysates were

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TABLE 1. *Bacteriophages used and method of propagation*

Bacteriophage	Method of propagation[a]	Indicator strain	Media utilized[b]	Source
φ105	A	BR151	M agar, M broth	J. Hoch
φ105cl-z	B	BR151	M agar, M broth	S. Zahler
SPO2	A	BR151	M agar, M broth	W. Romig
SPO2c1-2	B	BR151	M agar, M broth	S. Zahler
φe	B	BR151	M agar, Penassay broth[c]	C. Yehle
φ1	B	BR151	M agar, Penassay broth	B. Reilly
φ25	B	BR151	M agar, Penassay broth	B. Reilly
φ29	B	HSR	TBAB, Penassay broth	B. Reilly
SP82	B	BR151	M agar, Penassay broth	M. Green
SPO1	B	BR151	M agar, Penassay broth	K. Bott
SPP1	B, C	BR151	M agar, M broth	T. Trautner

[a] Procedures discussed in Materials and Methods.
[b] Media are described in Materials and Methods.
[c] Penassay broth supplemented with 0.5% glucose and 0.1% yeast extract at the time of inoculation.

prepared by the addition of phage to exponentially growing cultures of bacteria (optical density of 50 Klett units on a Klett-Summerson colorimeter, filter no. 66) at an MOI of 1 at 37 C. (C) Bacteriophage SPP1 was collected from agar plates of bacteria having approximately 1,000 plaques by placing the top soft agar in Penassay broth (2 ml/plate), mixing and then centrifuging at 1,200 rpm in a Sorvall type SP centrifuge. Except for bacteriophage φ29, all of the phages were assayed in a semisolid overlay of modified M agar on modified M agar plates as previously described (29). Bacteriophage φ29 was assayed in a semisolid overlay of 1.0% Tryptone, 0.8% NaCl, 0.6% agar, and 1.0% glucose on TBAB agar plates.

Measurement of RNA and DNA synthesis. A 20-μCi amount of [^{3}H]uridine (New England Nuclear Corp., Boston, Mass., 42.1 Ci/mmol) or 3 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass., 6.7 Ci/m mole) was added to 2 ml of exponentially growing cells (optical density of 50 Klett units, Klett-Summerson spectrophotometer, filter no. 66) in Penassay Broth (Difco antibiotic medium 3). Samples of 0.3 ml were transferred at various times into 1.0 ml of cold 10% trichloroacetic acid. After 20 min of incubation the samples were poured onto filters (HAWP 025, HA, 0.45 μm pore size; Millipore Corp. Bedford, Mass.) that had been previously soaked in 0.2 M sodium pyrophosphate. The sample tubes were washed three times with 3 ml of 0.015 M NaCl containing 1.5 mM sodium citrate and 0.014% trichloroacetic acid, and the washes were poured over the filters. The filters were dried, placed in 10 ml of Aquasol (Nuclear Chicago, Chicago, Ill.), and the radioactivity was determined in a Tri-Carb scintillation counter.

RESULTS

Effect of lysogeny on bacteriophage infection. Nine bacteriophages were used to infect *B. subtilis* BR151 and its lysogenic derivatives. As expected, bacteriophages φ105 and SPO2 did not successfully infect strains BR151 (φ105) and BR151 (SPO2), respectively (Table 2). This inability to infect the lysogenic strains is presumably attributable to the presence of repressor products produced by the resident prophages. Strains lysogenic for bacteriophage SPO2 were found to severely restrict bacteriophage φe and to totally prevent the development of bacteriophage φ1. The results with bacteriophage φe are similar to those reported by Rettenmier and Hemphill (22). The prophage SPO2 mediated interference of bacteriophage φ1 is not related to the inability of φ1 to attach to the cell wall of a lysogen of SPO2 (manuscript in preparation). Lysogeny does not appear to interfere with infection by SPO1, SP82, SPP1, φ29, or φ25.

TABLE 2. *Efficiency of plating*

Bacteriophages studied	Bacterial strains[a]		
	BR151	BR151 (φ105)	BR151 (SPO2)
φ105	100	*0*	100
SPO2	100	100	*0*
φe	100	100	*3*
φ1	100	100	*0*
SPO1	100	100	100
SP82	100	100	100
SPP1	100	100	100
φ29	100	100	100
φ25	100	100	100

[a] The percentage of the bacteriophages plating on strains BR151 (SPO2) or BR151 (φ105) as compared with BR151.

To determine if bacterial death resulted from an infection of strain BR151 (SPO2) by bacteriophage φ1, the number of colony forming units (CFU) was determined prior to and after infection at an MOI of 20. The results (Table 3)

clearly show that bacteriophage $\phi 1$ infection is lethal to bacteria lysogenic for SPO2. Control experiments involved infecting BR151 (SPO2) with SPO2c1-2 (a clear plaque mutant of SPO2) at an MOI of 20. In this case there was little if any cell death due to bacteriophage infection. These results clearly indicate that bacteriophages $\phi 1$ and SPO2 are prevented from developing in strains of *B. subtilis* lysogenic for SPO2 by different mechanisms.

Co-infection of B. subtilis with bacteriophages $\phi 1$ and SPO2. Exponentially growing cultures of strain BR151 were infected simultaneously with bacteriophages $\phi 1$ and SPO2. These infected cultures completely lysed approximately 60 min after infection. However, the number of bacteriophage produced was severely decreased compared to the single infections (Table 4). These results were also obtained when bacteriophage SPO2c1-2 was used instead of SPO2. This reduction in the numbers of progeny bacteriophage could be the result of a depressor effect type of phenomenon (1). Mutual exclusion and depressor effect phenomena have been previously identified in *B. subtilis* with bacteriophages SPO2 (17). Although simultaneous infection of strain BR151 with bacteriophages $\phi 1$ and SPO2 result in decreased bacteriophage progeny, the interaction is different than that observed when strain BR151 (SPO2) is infected by bacteriophage $\phi 1$. The former represents bacterial lysis with the production of bacteriophage, whereas the latter results in the death of the bacteria without production of any bacteriophage or cell lysis.

Macromolecular synthesis in $\phi 1$ infected lysogens of SPO2. A determination of DNA and RNA synthesis was made on SPO2 lysogens infected with bacteriophage $\phi 1$. Strain BR151 (SPO2) was infected with $\phi 1$ (MOI of 20) simultaneously with the addition of radioactive uridine or thymidine. The results in Fig. 1 and 2 demonstrate that shortly after infection, the synthesis of both RNA and DNA was terminated. The abrupt cessation of RNA and DNA synthesis was not observed when nonlysogenic cultures were infected with $\phi 1$. Additionally, preincubation of the SPO2 lysogen in 200 μg of chloramphenicol per ml for 5 min prior to the addition of bacteriophage $\phi 1$ prevents the shut

TABLE 3. *Infection of BR151 (SPO2) with bacteriophages $\phi 1$ and SPO2*[a]

Infecting bacteriophages	After infection	
	CFU/ml	Survival (%)
$\phi 1$	6.0×10^5	0.9
SPO2c1-2	6.1×10^7	71.8

[a] Bacterial strain BR151 (SPO2) at 8.5×10^7 colony forming units/ml (CFU/ml) was infected with either SPO2c1-2 or $\phi 1$ (MOI = 20), and cultures were incubated at 37 C for 15 min.

TABLE 4. *Infection of BR151 with bacteriophages $\phi 1$ and SPO2*[a]

Bacteriophages added	Bacteriophage progeny (PFU/ml)	
	SPO2	$\theta 1$
$\phi 1$	ND[b]	1.1×10^{10}
SPO2	5.2×10^9	ND
SPO2 + $\phi 1$	1.0×10^8	1.2×10^7
SPO2c1-2	4.7×10^9	ND
SPO2c1-2 + $\phi 1$	1.0×10^8	1.8×10^7

[a] Bacterial strain BR151 (1.2×10^8 colony forming units per ml) was infected with bacteriophages $\phi 1$ and SPO2 at an MOI of 20. Fifteen minutes after infection, the cultures were centrifuged at 1,200 rpm in a Sorvall type SP centrifuge, resuspended in M media, and incubated until lysis.

[b] ND, None determined.

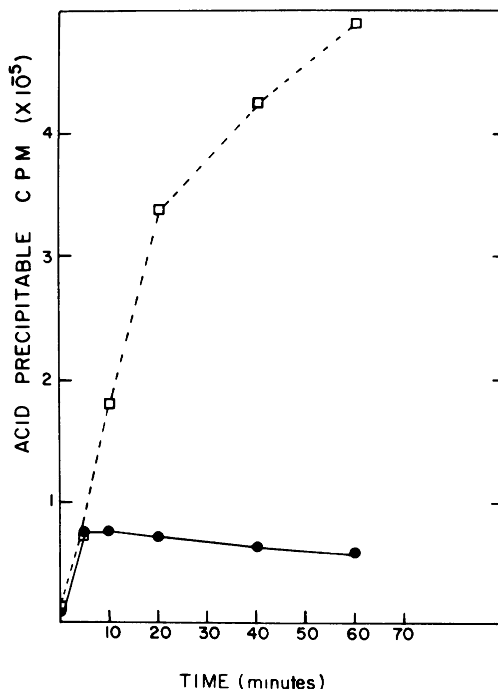

FIG. 1. *Incorporation of [³H]uridine into acid-precipitable counts in growing cultures of strains BR151 (SPO2) (□) and BR151 (SPO2) infected with $\phi 1$ at an MOI of 20 (●) as described in Materials and Methods.*

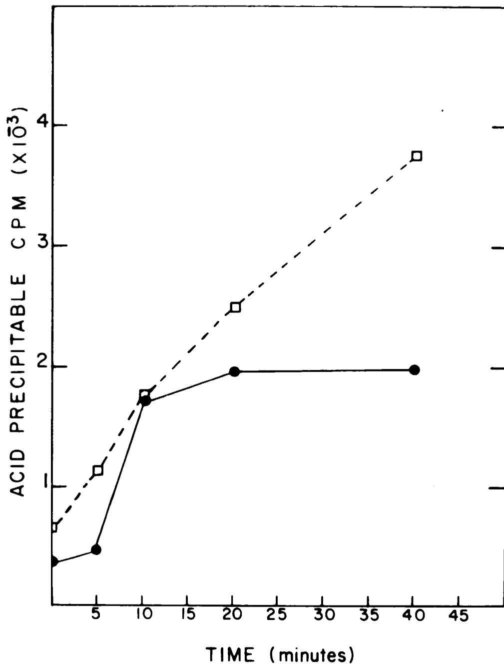

FIG. 2. *Incorporation of [³H]thymidine into acid-precipitable counts in growing cultures of strains BR151 (SPO2) (□) and BR151 (SPO2) infected with φ1 at an MOI of 20 (●) as described in Materials and Methods.*

off of DNA synthesis (unpublished observation). This could be interpreted as an indication that some protein is made either by bacteriophage φ1 or by the lysogen after infection with bacteriophage φ1 that adversely affects both host and bacteriophage DNA synthesis.

Effect of bacteriophage SPO2 on development of bacteriophage φ105. Bacteriophage φ105 plates with equal efficiency on bacterial strains BR151 and BR151 (SP02) (Table 2). However, we observed that after the induction (with mitomycin C) of bacterial strain BR151 (φ105, SPO2) 100- to 1,000-fold more bacteriophage SPO2 were produced than bacteriophage φ105 (Table 5). The two bacteriophages were distinguished by use of BR151, BR151 (φ105), and BR151 (SPO2) as indicator strains. Additionally, plaques formed on strain BR151 after induction of strain BR151 (φ105, SPO2) were picked and tested for their ability to lyse strains BR151 (φ105) and BR151 (SPO2). Of the 208 plaques tested, 206 contained only bacteriophage SPO2, whereas 2 were only bacteriophage φ105. Furthermore, simultaneous infection of BR151 by bacteriophages SPO2cl-2 and φ105cl-z (a clear plaque mutant of φ105) again resulted in 100 times the number of bacteriophage SPO2 released, compared to bacteriophage φ105 (Table 6). These results are similar to those obtained by L. B. Boice (personal communication).

A shorter eclipse period for bacteriophage SPO2 than for φ105 might explain the results seen in a mixed infection. Bacterial strains BR151 (SPO2), BR151 (φ105), and BR151 (SPO2, φ105) were induced with mitomycin C to establish one step growth curves for these bacteriophages. Under the conditions utilized in our laboratory, bacteriophage SPO2 does have a shorter latent period than does bacteriophage φ105 (Fig. 3). Bacteriophage SPO2 begins to be released from the induced lysogen approximately 20 min before bacteriophage φ105 is released. The latent period is the same in the single and double lysogen.

DISCUSSION

Prophage interference. The establishment of lysogeny can result in vast physiological changes in bacteria (2, 9). Among these changes are the production of restriction enzymes (6), the alteration of cell envelope (16, 21), and the interference with the biosynthesis and activity of enzymes (18, 25, 26). Because of these physiological changes, lysogenic cultures often are unable to support the propagation of bacterio-

TABLE 5. *Induction of BR151 (φ105, SPO2)*[a]

Time after induction (min)	Bacteriophage released (PFU/ml)	
	SPO2	φ105
15	1.1×10^8	9.0×10^5
120[b]	4.3×10^9	6.1×10^6

[a] Strain BR151 (φ105, SPO2) was induced with mitomycin C as described in Materials and Methods.
[b] Complete lysis had occurred by 120 min.

TABLE 6. *Infection of BR151 with bacteriophages SPO2cl-2 and φ105cl-z*[a]

Bacteriophages added	Bacteriophage progeny (PFU/ml)	
	SPO2	φ105
SPO2cl-2	2.5×10^9	ND[b]
φ105cl-z	ND[b]	8.3×10^9
SPO2cl-2 + φ105cl-z	2.1×10^9	2.3×10^7

[a] Bacterial strain BR151 was infected with bacteriophages SPO2cl-2 and/or φ105cl-z at an MOI of 20 as described in Table 4. The cultures were incubated at 37 C until lysis.
[b] ND, None determined.

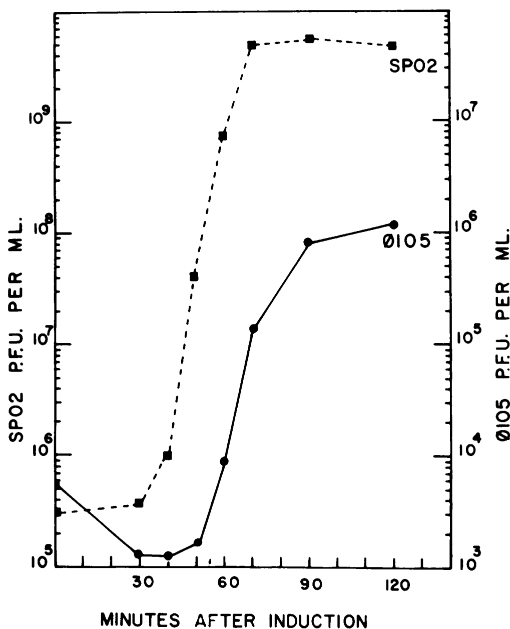

FIG. 3. *Induction of lysogenic cultures with mitomycin C as described in Materials and Methods. After addition of mitomycin C, samples were removed from strain BR151 (φ105, SPO2), centrifuged in a Brinkmann 3,200 centrifuge (8,000 × g) for 1 min, and the number of PFU in the supernatant fluid was determined.*

phages. In this report we have identified two types of prophage interference that exist in *B. subtilis*. Bacteria lysogenic for bacteriophage SPO2 severely restrict virulent bacteriophage φe, whereas these same lysogenic bacteria totally prevent the development of virulent bacteriophage φ1 (Table 2). The type of interaction that exists between bacteriophage φ1 and prophage SPO2 appears to resemble the inability of temperature bacteriophage lambda to successfully infect strains of *E. coli* lysogenic for P2 (18). This prophage interference has been shown to be the result of an interaction of the old^+ gene product of the prophage P2 and the three gene products of the bacteriophage lambda ($gamma^+$, $delta^+$, and $red^+\alpha$ or $red^+\beta$) (26, 31, 32). When both of the bacteriophages are wild type they interact to cause cell death without lysis (26). This phenomenon appears to be the result of a requirement for the *rec*B, C enzyme for survival in P2 lysogens and the fact that lambda infection inactivates this enzyme (25, 26, 31, 32). Infection of a *B. subtilis* lysogenic for SPO2 with bacteriophage φ1 similarly results in cell death without lysis (Table 3), with a concomitant termination of DNA and RNA synthesis (Fig. 1 and 2). Presently, it cannot be determined if the termination of DNA and RNA synthesis in bacteriophage φ1 infected strains of BR151 (SPO2) is synchronous or asynchronous. However, Fig. 1 and 2 suggest the cessation of RNA synthesis precedes cessation of DNA synthesis. Determination of the mechanism of prophage interference which inhibits the development of bacteriophage φ1 in bacterial cultures lysogenic for SPO2 will depend on the identification of those genes controlling this prophage interference system.

Effect of bacteriophage SPO2 on bacteriophage φ105. As shown in Table 2, bacterial cultures lysogenic for either bacteriophages φ105 or SPO2 can be successfully infected by either SPO2 or φ105, respectively. However, bacteriophage SPO2 seems to inhibit the release of bacteriophage φ105 in simultaneous mixed infections or induced cultures (Tables 5 and 6). This depression of the number of bacteriophage φ105 produced in a mixed infection could be the result of a shorter latent period for SPO2 than φ105 (Fig. 3). On the other hand, this depression could be the result of bacteriophage SPO2 interfering with the development of bacteriophage φ105. It has already been shown that bacteriophage φ105 is more dependent on the bacterial DNA polymerase than is bacteriophage SPO2 (24). Conceivably, during the development of bacteriophage SPO2, host enzyme systems which are essential for the development of bacteriophage φ105 could be inhibited. In any case, an important problem has arisen by the finding that both bacteriophages φ105 and SPO2 cannot develop in the same bacterium. Rutberg and co-workers attempted to demonstrate that genetic recombination or complementation did not occur between bacteriophages φ105 and SPO2 (24). However, their experiments were carried out by infecting bacteria with various mutants of bacteriophage φ105 and a particular strain of bacteriophage SPO2, and then by scoring the number of bacteriophage φ105 produced under restrictive conditions. Because they chose to look at only the number of φ105 bacteriophages produced, their conclusion that genetic recombination or complementation did not exist between these two temperate bacteriophages should be reexamined. Chow et al. have recently reported 14% homology between the DNA of bacteriophages φ105 and SPO2, and have concluded that this homology might represent the genes coding for antigen specificity (7). This conclusion is currently being tested by performing the appropriate genetic recombination and com-

plementation studies with bacteriophages ϕ105 and SPO2.

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Bertani, G. 1958. Lysogeny. Advan. Virus Res. **5**:151-193.
3. Birdsell, D. C., G. M. Hathaway, and L. Rutberg. 1969. Characterization of temperate bacillus bacteriophage ϕ105. J. Virol. **4**:264-270.
4. Boice, L. B. 1969. Evidence that *Bacillus subtilis* bacteriophage SPO2 is temperate and heteroimmune to bacteriophage ϕ105. J. Virol. **4**:47-49.
5. Boice, L. B., F. A. Eiserling, and W. R. Romig. 1969. Structure of *Bacillus subtilis* phage SPO2 and its DNA; similarity of *Bacillus subtilis* phages SPO1, ϕ105, and SPP1. Biochem. Biophys. Res. Commun. **34**:398-403.
6. Boyer, H. W. 1971. DNA restriction and modification mechanisms in bacteria. Ann. Rev. Microbiol. **25**:153-176.
7. Chow, L. T., L. B. Boice, and N. Davidson. 1972. Map of the partial sequence homology between DNA molecules of *Bacillus subtilis* bacteriophages SPO2 and ϕ105. J. Mol. Biol. **68**:391-400.
8. Delbruck, M. 1945. Interference between bacterial viruses. III. The mutual exclusion effect and the depressor effect. J. Bacteriol. **50**:151-170.
9. Echols, H. 1972. Developmental pathways for the temperate phage: lysis vs lysogeny. Ann. Rev. Genet. **6**:157-190.
10. Goldberg, I. D., and T. Bryan. 1968. Productive infection of *Bacillus subtilis* 168, with bacteriophage SP-10, dependent upon inducing treatments. J. Virol. **2**:805-812.
11. Gwinn, D. D., and W. D. Lawton. 1968. Alteration of host specificity in *Bacillus subtilis*. Bacteriol. Rev. **32**:297-301.
12. Howard, B. D. 1967. Phage lambda mutants deficient in rII exclusion. Science **158**:1588-1589.
13. Inselburg, J. W., T. Eremenko-Volpe, L. Greenwald, W. L. Meadow, and J. Marmur. 1969. Physical and genetic mapping of the SPO2 prophage on the chromosome of *Bacillus subtilis* 168. J. Virol. **3**:627-628.
14. Ito, J., G. Mildner, and J. Spizizen. 1971. Early blocked asporgenous mutants of *Bacillus subtilis* 168. I. Isolation and characterization of mutants resistant to antibiotics produced by sporulating *Bacillus subtilis* 168. Mol. Gen. Genet. **112**:104-109.
15. Ito, J., and J. Spizizen. 1971. Abortive infection of sporulating *Bacillus subtilis* 168 by ϕ2 bacteriophage. J. Virol. **7**:515-523.
16. Jonasson, J., L. Rutberg, and F. E. Young. 1969. Lysogenic conversion in *Bacillus amyloliquefaciens* H affecting viral adsorption. J. Virol. **4**:309-310.
17. Kolenbrander, P. E., H. E. Hempfill, and H. R. Whiteley. 1973. Mixed infections of *Bacillus subtilis* involving bacteriophage SPO2-c1. J. Virol. **11**:25-34.
18. Lindahl, G., G. Sironi, H. Bialy, and R. Calendar. 1970. Bacteriophage lambda; abortive infection of bacteria lysogenic for phage P2. Proc. Nat. Acad. Sci. U.S.A. **66**:587-594.
19. Losick, R., R. G. Shorenstein, and A. L. Sonenshein. 1970. Structural alteration of RNA polymerase during sporulation. Nature (London) **227**:910-913.
20. Losick, R., and A. L. Sonenshein. 1969. Change in the template specificity of RNA polymerase during sporulation of *Bacillus subtilis*. Nature (London) **224**:35-37.
21. Pizer, L. I., H. S. Smith, M. Miovic, and L. Pylkas. 1968. Effect of prophage ω on the propagation of bacteriophages T2 and T4. J. Virol. **2**:1339-1345.
22. Rettenmier, C. W., and H. E. Hemphill. 1973. Prophage-mediated interference affecting the development of *Bacillus subtilis* bacteriophage ϕe. J. Virol. **11**:372-377.
23. Rutberg, L. 1969. Mapping of a temperate bacteriophage active on *Bacillus subtilis*. J. Virol. **3**:38-44.
24. Rutberg, L., R. W. Armentrout, and J. Jonasson. 1972. Unrelatedness of temperate *Bacillus subtilis* bacteriophages SPO2 and ϕ105. J. Virol. **9**:732-737.
25. Sakaki, Y., A. E. Karu, S. Linn, and H. Echols. 1973. Purification and properties of the γ-protein specified by bacteriophage λ: an inhibitor of the host Rec B C recombination enzyme. Proc. Nat. Acad. Sci. U.S.A. **70**:2215-2219.
26. Sironi, G., H. Bialy, H. A. Lozeron, and R. Calendar. 1971. Bacteriophage P2: interaction with phage lambda and with recombination deficient bacteria. Virology **46**:387-396.
27. Smith, I., and H. Smith. 1973. Location of the SPO2 attachment site and the bryamycin resistance marker on the *Bacillus subtilis* chromosome. J. Bacteriol. **114**:1138-1142.
28. Yasbin, R. E., M. J. Tevethia, G. A. Wilson, and F. E. Young 1973. Analysis of steps in transformation and transfection in transformation-defective mutants and lysogenic strains of *Bacillus subtilis*, p. 3-26. *In* L. Archer (ed.), Bacterial transformations. Academic Press Inc., New York.
29. Yasbin, R. E., G. A. Wilson, and F. E. Young. 1973. Transformation and transfection in lysogenic strains of *Bacillus subtilis* 168. J. Bacteriol. **113**:540-548.
30. Yasbin, R. E., and F. E. Young. 1972. The influence of temperate bacteriophage ϕ105 on transformation and transfection in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. **47**:365-371.
31. Zissler, J., E. Signer, and F. Schaefer. 1971. The role of recombination in growth of bacteriophage lambda. I. The gamma gene, p. 455-468. *In* A. O. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Monograph Series, Cold Spring Harbor, N.Y.
32. Zissler, J., E. Signer, and F. Schaefer, 1971. The role of recombination in growth of bacteriophage lambda. II. Inhibition of growth by prophage P2, p. 469-475. *In* A. O. Hershey (ed.), The bacteriophage lambda, Cold Spring Harbor Monograph Series, Cold Spring Harbor, N.Y.