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Cloning and Expression of Bacteriophage SP02 DNA Polymerase Gene L in *Bacillus subtilis*, Using the *Staphylococcus aureus* Plasmid pC194

LARS RUTBERG,* BENNY RÅDÉN, AND JAN-INGMAR FLOCK†

Department of Bacteriology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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*Hin*dIII restriction endonuclease fragments of DNA from temperate *Bacillus subtilis* bacteriophage SP02 were cloned in *B. subtilis* by using the plasmid pC194. Three hybrid plasmids which permit growth of the mutant SP02 *susL244* in suppressor-negative bacteria were isolated. SP02 gene *L* is thought to code for a DNA polymerase essential for autonomous replication of SP02 DNA. Extracts of bacteria carrying one of these hybrid plasmids, pC194-96, had 10- to 30-fold increased DNA polymerase activity. The plasmid-induced DNA polymerase activity differed from that of the known *B. subtilis* DNA polymerases in several respects. The results of the experiments support the idea that phage SP02 codes for a new DNA polymerase.

SP02 is a temperate bacteriophage of *Bacillus subtilis*. It contains double-stranded DNA with a molecular weight of 24×10^6 to 26×10^6 (2, 23); SP02 DNA has cohesive ends (23). SP02 is unusual among temperate phages in that it does not seem to require the host DNA polymerase III (pol III) for replication. This suggestion is based on the following observations: (i) SP02 replicates in the presence of 6-(*p*-hydroxyphenylazo)-uracil (HPUra), a known inhibitor of pol III (3, 20); (ii) infection of a pol I-deficient *B. subtilis* mutant with SP02 is followed by a strong increase in in vitro DNA polymerase activity (19); (iii) a *B. subtilis* mutator pol III gives an increased frequency of mutations in phage SPP1 but not in SP02 (15); (iv) SP02 will grow at high temperature in pol III *ts* mutants (20; unpublished data); and (v) SP02 gene *L* mutants are DNA negative (22) and do not induce an increased polymerase activity after infection of nonpermissive bacteria (19).

Besides gene *L*, several other SP02 genes are needed for replication of SP02 DNA. Only one bacterial gene, *dnaA*, which is believed to be involved in ribonucleotide reduction (18), is known to be essential for SP02 replication (20). In the present experiments we used the *Staphylococcus aureus* plasmid pC194 (10, 13) to clone *Hin*dIII restriction endonuclease fragments of SP02 DNA in *B. subtilis*. Hybrid plasmids which permit growth of the mutant SP02 *susL244* in a nonpermissive host have been isolated. One of these plasmids gives rise to a large increase in DNA polymerase activity as measured in crude extracts. This activity can be distinguished from that of the bacterial DNA polymerases by several criteria. These results strongly indicate that SP02 codes for a DNA polymerase which is distinct from the DNA polymerases of its host strain.

MATERIALS AND METHODS

Bacteria and phage. The bacterial strains employed are listed in Table 1. Phage SP02c$_1$ was obtrained from J. Marmur. *sus* mutants of SP02 (22) were obtained from T. A. Trautner.

The bacteria were kept on tryptic blood agar base plates (Difco). Liquid cultures were grown in NY broth (17). For strains containing plasmid pC194 or derivatives thereof, 20 μg of chloramphenicol (CAP) per ml was added to all media.

Stocks of SP02c$_1$ were prepared by infecting sensitive bacteria in liquid culture as described (20). Stocks of SP02 *sus* mutants were prepared by induction of lysogenic derivatives of strain SR135 or as plate stocks using SR135 as indicator bacteria. Phage was assayed on NY plates as described previously (20). Phage was purified by CsCl centrifugation, and DNA was prepared from these purified stocks by phenol extraction as described previously (20).

Plasmids. pC194 is a 2-megadalton (2.7-kilobase) plasmid originally isolated from *S. aureus* (10). It was prepared from *S. aureus* SA 25 as described by Löfdahl et al. (13). For preparation of plasmid DNA from *B. subtilis* the method of Canosi et al. (4) was used. DNA concentrations were determined by reading the absorbancy at 260 nm, taking one unit to equal 50 μg of DNA per ml with a 1-cm light path. Electrophoresis of plasmid DNA was done on a vertical slab gel ap-

† Present address: Searle Research and Development, High Wycombe, Bucks HP124 HL, England.

TABLE 1. *B. subtilis strains used*

Strain	Relevant properties	Source
3G18	*ade met trpC2*	G. Venema
BD294	*trpC2 thr-5 polA59 polC25*	T. A. Trautner
W168	Prototrophic	J. Spizizen
SR135	*trp-7 spoA9*, Su^{+3}	J. Hoch
1306	*met leu*, pol I	J. Gross

paratus. The gels contained 0.8% agarose. The buffer system contained 0.09 M Tris-hydrochloride, 2.5 mM EDTA, and 0.09 M boric acid (pH 8.3). The gels were stained with ethidium bromide (1 μg/ml), and the bands were visualized under UV light.

Cloning of SP02 DNA fragments. SP02 DNA at 20 μg/ml was partially cleaved with restriction endonuclease *Hin*dIII. The DNA was reextracted with phenol, and 0.7 μg was mixed with 0.7 μg of *Hin*dIII-cleaved pC194 DNA. Ligation with T4 ligase was then done in a volume of 40 μl. All enzymatic steps were performed according to the recommendations of the manufacturer (Bethesda Research Laboratories). The ligase-treated DNA was used to transform competent cells of strain 3G18. After 60 min of incubation in broth at 37°C for phenotypic expression, CAP-resistant transformants were selected on tryptic blood agar base plates with 8 μg of CAP per ml. From these plates 312 colonies were picked for colony hybridization (9), using nick-translated SP02 DNA as a probe. A total of 116 positive colonies were isolated. These colonies were checked in spot tests for their ability to allow growth of SP02 *susL244*, a mutant which is deficient in SP02 DNA polymerase activity (19). Three positive clones were found, and these hybrid plasmids were named pC194-74, pC194-91, and pC194-96.

Preparation of bacterial extracts and assay of DNA polymerase activity. For preparation of extracts the bacteria were grown in 100 ml of NY broth in 1,000-ml flasks with shaking at 37°C to an absorbancy at 600 nm of 0.8. CAP was added to the medium at 20 μg/ml for all plasmid-carrying strains. The bacteria were centrifuged at 6,000 × *g* for 10 min, and the pellets were suspended in 1/20 volume of 50 mM Tris-hydrochloride (pH 7.5) containing 5 mM 2-mercaptoethanol and 2 mM EDTA with 600 μg of lysozyme per ml. The bacteria were incubated at 37°C for 60 min, sonicated, and then centrifuged at 14,500 × *g* for 20 min. The resulting supernatant fraction was used for assay of DNA polymerase activity. It contained 4 to 6 mg of protein per ml as determined by the method of Lowry et al. (14).

The mixture for assay of DNA polymerase contained (in 87 μl): 15% glycerol, 10 μg of bovine serum albumin, 65 mM Tris-hydrochloride (pH 7.5), 5 mM $MgCl_2$, 3 mM 2-mercaptoethanol, 40 μM each of dATP, dGTP, and dCTP, 10 μM of [^{3}H]TTP (specific activity, 20 mCi/mmol), and 30 μmol of activated calf thymus DNA. To this mixture was added 20 μl of bacterial extract. Incubation was done for 30 min at 37°C. The reaction was terminated by placing the assay tube in ice water and adding 0.1 ml of calf thymus DNA (0.3 mg/ml) dissolved in 0.1 M sodium pyrophosphate and 0.4 ml of $HClO_4$ (10%) in 0.1 M sodium pyrophosphate. The samples were then filtered through Whatman GF/A filters. The filters were rinsed with 30 ml of 0.1 M HCl in 0.01 M sodium pyrophosphate, followed by 10 ml of ice-cold ethanol. The filters were dried in air and then put into vials with 5 ml of toluol scintillation fluid (5 g of PPO [2,5-diphenyloxazole], 65 mg of POPOP [1,4-bis-(5-phenyloxazolyl)benzene], and 500 ml of toluene), and the radioactivity retained on the filter was determined.

Chemicals. Calf thymus DNA, polydeoxyadenylate-polydeoxythymidylate, deoxyribonucleotides, ethidium bromide, bovine pancreas DNase, lysozyme, and 2-mercaptoethanol were obtained from Sigma Chemical Co. [^{3}H]TTP was obtained from New England Nuclear Corp. Dextran 500 and DEAE-Sephacel were obtained from Pharmacia Fine Chemicals. Agarose was obtained from Bio-Rad Laboratories. Restriction enzymes and T4 ligase were obtained from Bethesda Research Laboratories. All other chemicals were of the purest commercially available quality.

RESULTS

Expression of SP02 gene *L* in pC194-SP02 hybrid plasmids. Experiments were first performed to see whether any of the three hybrid plasmids which permit growth of SP02 *susL244* also express the phage gene *L*. This would presumably manifest itself as an increased DNA polymerase activity in extracts of bacteria which harbor these plasmids. Strain BD294, which carries the mutations *polA59* and *polC25*, has the lowest in vitro DNA polymerase activity of any *B. subtilis* strain that we have tested. Plasmids pC194, pC194-74, pC194-91, and pC194-96 were transformed into BD294 protoplasts (5). The protoplasts were regenerated on plates containing CAP. From 5 to 10 transformants were tested for each plasmid. They all permitted growth of SP02 *susL244* in spot tests. One transformant of each class was kept for further experiments.

Extracts were next prepared from each plasmid-carrying strain of BD294, and in vitro DNA polymerase activity was assayed. Roughly similar activities were found in extracts of strains BD294(pC194), BD294(pC194-74), and BD294-(pC194-91), whereas at least 10 times higher activities were consistently found in strain BD294(pC194-96) (Table 2). This latter strain was next grown overnight at 37°C in NY broth without CAP, and dilutions were plated on tryptic blood agar base plates without CAP. Single colonies from these plates were tested for CAP resistance. About 20% of the colonies were sensitive to CAP, suggesting that they had lost the original plasmid. The CAP-sensitive bacteria no longer permitted growth of SP02 *susL244*. Extracts were prepared from two independently isolated CAP-sensitive derivatives of strain BD294- (pC194-96). These extracts were tested for DNA polymerase activity together with extracts from strains BD294(pC194) and

BD294(pC194-96). The DNA polymerase activity of the CAP-sensitive bacteria was similar to that of strain BD294(pC194). A simultaneously prepared extract of strain BD294(pC194-96) had about 17 times higher DNA polymerase activity (Table 3). The DNA polymerase activity measured was proportional to the amount of extract protein added in the protein concentration range used (Fig. 1). Less than 10% activity was found when the exogenous template was omitted from the assay mixture. When extracts from strains BD294(pC194) and BD294(pC194-96) were mixed in various proportions, the DNA polymerase activity measured was proportional to the activity found in each extract (data not shown).

Characterization of pC194-96. Restriction endonuclease *Hin*dIII cleaves SP02 DNA into 21 fragments which we have labeled A through U. The hybrid plasmid pC194-96 contains fragments F, and G or H (Fig. 2). We are presently studying which fragment contains the wild-type allele of the *susL244* mutation.

Comparison of some properties of the DNA polymerase activity in extracts of strains BD294(pC194) and BD294(pC194-96). The three known DNA polymerases of *B. subtilis* can be distinguished from each other by several criteria, e.g., salt sensitivity, heat stability, and template specificity (7). Furthermore, in the scheme devised by Okazaki and Kornberg (16) for purification of pol I, it was later found that pol II and pol III preferentially partition into the polyethyleneglycol (PEG) phase upon addition of 20% $(NH_4)_2SO_4$ to the first PEG phase. This fact has been used by Gass and coworkers (8) to characterize a pol I-deficient mutant of *B. subtilis*.

TABLE 2. *DNA polymerase activity in extracts of BD294 and its pC194-carrying derivatives*[a]

Strain	Extract protein in assay (μg)	Acid-insoluble cpm
BD 294	12	1,940
BD294(pC194)	10	1,260
BD294(pC194-74)	10	3,200
BD294(pC194-91)	8	2,550
BD294(pC194-96)	10	52,700

[a] Extracts were prepared and assayed for DNA polymerase activity as described in the text.

TABLE 3. *DNA polymerase activity in extracts of BD294(pC194) and BD294(pC194-96) and in strains cured of pC194-96*[a]

Strain	Extract protein in assay (μg)	Acid-insoluble cpm
BD294(pC194)	10	3,970
BD294(pC194-96)	11	68,030
BD294.R1	11	2,440
BD294.R2	9	2,250

[a] Extracts were prepared and assayed for DNA polymerase activity as described in the text. BD294.R1 and BD294.R2 are derivatives of strain BD294(pC194-96) that had been cured of the plasmid.

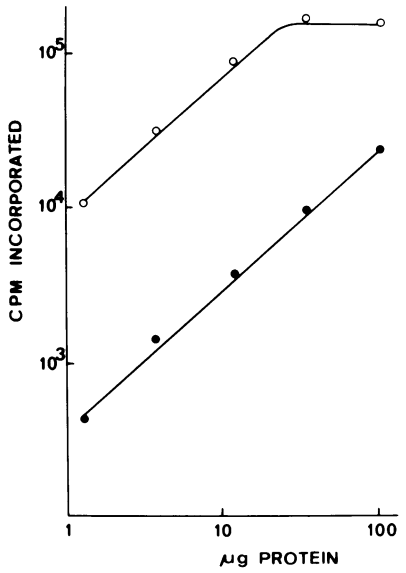

FIG. 1. *DNA polymerase activity at various dilutions of an extract prepared from strain BD294-(pC194)* (●) *and BD294(pC194-96)* (○).

Extracts were prepared from strains BD294(pC194) and BD294(pC194-96). The extracts were chromatographed on DEAE-Sephacel to remove endogeneous template. Elution was done with a linear NaCl gradient. All DNA polymerase activity in both extracts eluted as a single peak at 0.15 to 0.20 M salt. The peak fractions were pooled and used in the following experiments. The activity was measured in the standard DNA polymerase assay at 37, 45, and 55°C. At 55°C about 70% of the strain BD294(pC194) activity remained. In contrast, strain BD294(pC194-96) activity was more temperature sensitive, with 40% activity remaining at 45°C and only about 5% remaining at 55°C (Table 4). DNA polymerase activity was next measured in the standard assay with various amounts of KCl added. The results of these experiments clearly show that the strain BD294(pC194-96) activity is less sensitive to high salt than is the activity of BD294(pC194) (Table 5). The two preparations were also tested in the standard assay with polydeoxyadenylate-polydeoxythymidylate as a template; both preparations gave about 20% of the activity found

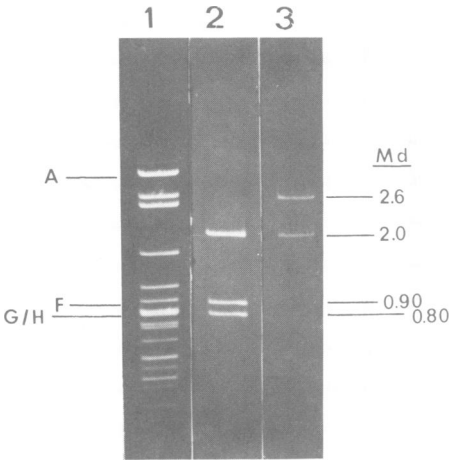

FIG. 2. *Gel electrophoresis pattern of HindIII-cleaved DNA. Slot 1, Mature SP02 DNA; slot 2, pC194-96; slot 3, pHV14, which is a composite of pBR322 (2.6 × 10^6 molecular weight) and pC194 (2.0 × 10^6 molecular weight). The molecular weights of the SP02 fragments were determined by using Bacillus phage φ105 EcoRI fragments as a standard.*

with activated calf thymus DNA as the template (data not shown).

Extracts of strains BD294, BD294(pC194), and BD294(pC194-96) were next subjected to PEG-salt fractionation as described by Gass et al. (8) (Table 6). As expected for pol I-deficient mutants, most of the activity in BD294 (76%) and in BD294(pC194) (80%) was found in the upper (PEG) phase. In strain BD294(pC194-96) the activity found in the upper phase was about twice that found in the other strains. However, the activity in the lower phase was about 20 times higher in strain BD294(pC194-96) than in the two other strains, and it represented 74% of the total activity found in BD294(pC194-96).

Finally, plasmid pC194-96 was transformed into the pol I-deficient, HPUra-sensitive strain 1306. Extracts of strain 1306(pC194-96) had about a 10-fold increased DNA polymerase activity. When the reaction mixture contained 200 μM HPUra (reduced by dithionite), DNA polymerase activity in strain 1306 extracts was inhibited to about 75%, whereas at most 5% inhibition was found in strain 1306(pC194-96) extracts.

These results show that most of the DNA polymerase activity induced by pC194-96 differs from that found in the bacteria in heat sensitivity, salt sensitivity, and HPUra sensitivity, and how it partitions in a two-phase system.

DISCUSSION

The present work is part of a study in which we are attempting to use DNA recombinant technology to characterize genes and gene products required for phage SP02 DNA replication. A number of plasmids originating from various *Bacillus* species (12, 21) or from *S. aureus* (6, 11) have been used for DNA cloning in *B. subtilis*. In this work we used the *S. aureus* plasmid pC194, which contains a single *Hin*dIII site (13).

The main finding to discuss in this paper is that strain BD294 when carrying the hybrid plasmid pC194-96 had substantially increased levels of DNA polymerase activity detectable in vitro. Strain BD294 is deficient in pol I and has an HPUra-resistant, temperature-sensitive pol III (7). As a consequence BD294 is more sensitive than wild type to, e.g., mitomycin C. This is true also for strains BD294(pC194) and BD294(pC194-96) (unpublished data). When pC194-96 is carried by bacteria which have a HPUra-sensitive pol III, they are still unable to replicate in the presence of this drug. The DNA polymerase activity determined by the SP02 *Hin*dIII fragments of pC194-96 thus cannot substitute for either pol I in repair or pol III in replication of bacterial DNA. This is true also for the DNA polymerase activity induced upon SP02 infection (1). pC194-96 does not replicate in the presence of HPUra (unpublished data).

The pC194-96 DNA polymerase activity dif-

TABLE 4. *Heat sensitivity of DNA polymerase from strains BD294(pC194) and BD294(pC194-96)*[a]

Assay temperature (°C)	Percent activity	
	BD294(pC194)	BD294(pC194-96)
37	100	100
45	108	39
55	69	5

[a] Extracts were prepared as described in the text and chromatographed on DEAE-Sephacel. The peak activity fractions were pooled, and the pooled samples were assayed in the standard DNA polymerase assay. Activity at 100% was 27,940 cpm for strain BD294(pC194) and 55,970 cpm for strain BD294(pC194-96).

TABLE 5. *Salt sensitivity of DNA polymerase from strains BD294(pC194) and BD294(pC194-96)*[a]

KCl (mmol) added to standard assay mixture	Percent activity	
	BD294(pC194)	BD294(pC194-96)
333	0	43
167	4	76
83	22	82
42	48	91
21	63	91
0	100	100

[a] The same samples as in Table 3 were used. Activity at 100% was 32,260 cpm for strain BD294(pC194) and 60,430 cpm for strain BD294(pC194-96).

TABLE 6. *Phase fractionation of DNA polymerase from strains BD294, BD294(pC194), and BD294(pC194-96)*[a]

Strain	Polymerase activity (cpm/mg of protein)	
	$(NH_4)_2SO_4$ Upper phase	$(NH_4)_2SO_4$ Lower phase
BD294	1.6×10^6 (76%)	5.1×10^5 (24%)
BD294(pC194)	2.1×10^6 (79%)	5.5×10^5 (11%)
BD294(pC194-96)	4.3×10^6 (26%)	1.2×10^7 (74%)

[a] Bacterial extracts were prepared as described in the text. Dextran 500 was added to a final concentration of 1.6% (wt/vol), and PEG was added to 6.4% (wt/vol). NaCl was then slowly added to 23.5% (wt/vol) final concentration. Two phases were formed. Essentially all polymerase activity partitioned into the upper (PEG) phase. The PEG phase was dialyzed against Tris-mercaptoethanol-EDTA-glycerol buffer. $(NH_4)_2SO_4$ was then added to 20% final concentration (wt/vol). Two phases formed with PEG partitioning in the upper phase. The two phases were separated and dialyzed against buffer. The DNA polymerase activity of each phase was then assayed.

fers from the activity found in extracts of strains carrying only the vector pC194 in several respects. It is more temperature sensitive and is less sensitive to high salt, and in a PEG-salt two-phase system, most of the DNA polymerase activity of strain BD294(pC194-96) partitions differently from the activity found in strain BD294(pC194). These facts and the manyfold increased activity found in strain BD294 carrying pC194-96 strongly support the idea that the SP02 *Hin*dIII fragments carried by the hybrid plasmid code for a DNA polymerase which is distinct from the known bacterial DNA polymerases rather than a "factor" which modifies, e.g., pol III. A definite solution to this problem requires purification of the proposed SP02 DNA polymerase. Such work is in progress in our laboratory.

In the present screening of pC194-SP02 hybrid plasmids we found three plasmids which permit growth of the DNA polymerase-negative mutant SP02 *susL244* in an Su^- host. Only one of these plasmids, pC194-96, expresses the SP02 DNA polymerase gene. All three plasmids carry SP02 *Hin*dIII fragments F, and G or H (manuscript in preparation). The "nonexpressing" plasmids contain one or two additional SP02 *Hin*dIII fragments. In pC194-96, transcription of the cloned DNA may start from a promotor located on the plasmid or on the SP02 fragments. The order of the SP02 fragments in the respective hybrid plasmids is not known. Physical mapping of these fragments together with recloning of the fragments should enable us to get some understanding of the control of transcription of cloned DNA in the respective plasmids. Identification of an efficient promotor for inserted foreign DNA on pC194 or on a pC194 hybrid plasmid should be of more general interest with respect to DNA cloning in *B. subtilis*.

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LITERATURE CITED

1. **Arwert, F., and L. Rutberg.** 1974. Induction of prophage SP02 in *Bacillus subtilis* by 6-(*p*-hydroxyphenylazo)-uracil. J. Virol. **14**:1470–1475.
2. **Boice, L. B., F. A. Eiserling, and W. A. Romig.** 1969. Structure of *Bacillus subtilis* phage SP02 and its DNA; similarity of *Bacillus subtilis* phages SP02, ϕ105, and SPP1. Biochem. Biophys. Res. Commun. **34**:398–403.
3. **Brown, N. C.** 1970. 6-(*p*-Hydroxyphenylazo)-uracil: a selective inhibitor of host DNA replication in phage-infected *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **67**:1454–1461.
4. **Canosi, U., G. Morelli, and T. A. Trautner.** 1978. The relationship between molecular structure and transformation efficiency of some *S. aureus* plasmids isolated from *B. subtilis*. Mol. Gen. Genet. **166**:259–267.
5. **Chang, S., and S. N. Cohen.** 1979. High frequency transformation of *Bacillus subtilis* protoplasts by plasmid DNA. Mol. Gen. Genet. **168**:111–114.
6. **Ehrlich, S. D.** 1977. Replication and expression of plasmids from *Staphylococcus aureus* in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **74**:1680–1682.
7. **Gass, K. B., and N. R. Cozzarelli.** 1973. Further genetic and enzymological characterization of the three *Bacillus subtilis* deoxyribonucleic acid polymerases. J. Biol. Chem. **248**:7688–7700.
8. **Gass, K. B., T. C. Hill, M. Goulian, B. S. Strauss, and N. R. Cozzarelli.** 1971. Altered deoxyribonucleic acid polymerase activity in a methyl methanesulfonate-sensitive mutant of *Bacillus subtilis*. J. Bacteriol. **108**:364–374.
9. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
10. **Iordanescu, S.** 1975. Recombinant plasmid obtained from two different compatible staphylococcal plasmids. J. Bacteriol. **124**:597–601.
11. **Keggins, K. M., P. S. Lovett, and E. J. Durall.** 1978. Molecular cloning of genetically active fragments of *Bacillus* DNA in *Bacillus subtilis* and properties of the vector plasmid pUB 110. Proc. Natl. Acad. Sci. U.S.A. **75**:1423–1427.
12. **Kreft, J., K. Bernhard, and W. Goebel.** 1978. Recombinant plasmids capable of replication in *B. subtilis* and *E. coli*. Mol. Gen. Genet. **162**:59–67.
13. **Löfdahl, S., J. E. Sjöström, and L. Philipson.** 1978. Characterization of small plasmids from Staphylococcus aureus. Gene **3**:149–159.
14. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. **Montenegro, M. A., H. Esche, and T. A. Trautner.** 1976. Induction of mutations in *B. subtilis* phage SPP1 by growth on host cells carrying a mutator DNA polymerase III. Mol. Gen. Genet. **149**:131–134.
16. **Okazaki, T., and A. Kornberg.** 1964. Enzymatic synthesis of deoxyribonucleic acid. XV. Purification and properties of a polymerase from *Bacillus subtilis*. J. Biol. Chem. **239**:259–268.
17. **Okubo, S., and W. R. Romig.** 1966. Impaired transform-

ability of *Bacillus subtilis* mutant sensitive to Mitomycin C and ultraviolet irradiation. J. Mol. Biol. **15:** 440–454.

18. **Rima, B. K., and I. Takahashi.** 1978. Deoxyribonucleoside-requiring mutants of *Bacillus subtilis*. J. Gen. Microbiol. **107:**139–145.
19. **Rutberg, L., and R. W. Armentrout.** 1972. Deoxyribonucleic acid polymerase activity in a deoxyribonucleic acid polymerase I-deficient mutant of *Bacillus subtilis* infected with temperate bacteriophage SP02. J. Virol. **10:**658–660.
20. **Rutberg, L., R. W. Armentrout, and J. Jonasson.** 1972. Unrelatedness of temperate *Bacillus subtilis* bacteriophages SP02 and ϕ105. J. Virol. **9:**732–737.
21. **Tanaka, T., and N. Kawano.** 1980. Cloning vehicles for the homologous *Bacillus subtilis* host-vector system. Gene **10:**131–136.
22. **Yasanuka, K., H. Tsukamoto, S. Okubo, and T. Horiuchi.** 1970. Isolation and properties of suppressor-sensitive mutants of *Bacillus subtilis* bacteriophage SP02. J. Virol. **5:**819–821.
23. **Yoneda, Y., S. Graham, and F. E. Young.** 1979. Restriction fragment map of the temperate *Bacillus subtilis* bacteriophage SP02. Gene **7:**51–68.