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Genetic Analysis of Ribonucleic Acid Polymerase Mutants of *Bacillus subtilis*

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Deoxyribonucleic acid-dependent ribonucleic acid polymerase mutants of *Bacillus subtilis* strain Marburg were isolated after mutagenesis of spores with ethyl methane sulfonate. Genetic analysis by PBS1-mediated transduction and by transformation indicated that mutations responsible for all of the four phenotypic classes studied (rifampin resistance, streptovaricin resistance, streptolydigin resistance, and temperature sensitivity) were clustered close to the *cysA14* locus. Three-factor transformation analysis has indicated the most probable marker order as follows: $Rif^R(Stv)^R$-Std^R-Ts_{418}-Ts_{427}. In addition, further characterization of the classical group I reference marker, *cysA14*, is reported.

Deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase (RNA nucleotidyl transferase EC 2.7.7.6) control of differential gene transcription during sporulation has been suggested by Losick and Sonenshein (12), and additional data to support this hypothesis have been published by Losick et al. (11) and by Leighton et al. (5, 11). The importance of the RNA polymerase in the control of this morphogenic process, as well as its role in the control of normal transcription of messenger and stable forms of RNA, suggests that physiological and genetic studies on altered polymerase molecules may be of benefit. Since specific drugs are available which inhibit the mode of action of the RNA polymerase molecule, the direct selection of mutations in the gene coding for RNA polymerase has been possible (11, 18). Screening of large numbers of such mutants has shown pleiotropic effects resulting from alterations of the strucutre of the RNA polymerase molecule (5, 11, 12). These secondary phenotypes include failure to sporulate, oligospore formation, and production of spores with altered morphology (5, 9). To better classify the mutants, genetic analysis has been carried out by using two-factor transduction and two- and three-factor transformation crosses. One locus for rifampin resistance has been genetically mapped in *Bacillus subtilis* by Harford and Sueoka (8) who were able to show rifampin resistance closely linked to a group I marker, *cysA14*.

We have isolated large numbers of RNA polymerase mutants resistant to the three drugs known to act on prokaryotic RNA polymerase and report here the genetic mapping of streptolydigin-resistant, streptovaricin-resistant, and rifampin-resistant mutants of *B. subtilis* and some of the properties of these various mutants. In addition, we have mapped conditional mutations in the DNA adjacent to the RNA polymerase genes which may code for another subunit of the polymerase not known to be associated with the drug-resistant phenotype.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. Strain used are listed in Table 1.

A genetic cross by transduction or transformation is depicted in the following manner: OSB "z" (Rif^R) × OSB "y" (Std^R). The strain on the left is the donor, and the strain on the right is the recipient. The phenotype of each strain is included in parentheses. (The following abbreviations will be used when referring to the phenotype of a strain: rifampin resistant, Rif^R; rifampin sensitive, Rif^S; streptovaricin resistant, Stv^R; streptovaricin sensitive, Stv^S; streptolydigin resistant, Std^R; streptolydigin sensitive, Std^S; temperature sensitive, Ts; thermal stable, Ts+; unable to grow without cysteine supplement, Cys^-; able to grow without cysteine supplement, Cys^+.)

Constructed strains. OSB 3 (Cys^-Rif^R) was constructed by bacteriophage PBS1-mediated transduction by the genetic cross OSB 2 (Rif^R) × OSB 154 (Cys^-).

OSB 11 (Cys^-Rif^R) was constructed by PBS1-

TABLE 1. *Bacterial strains and bacteriophage*

Strain designation	Phenotype	Source
OSB 2	Prototroph Rif^R	L. R. Brown, nitrosoguanidine mutagenesis
OSB 3	Cys$^-$ Rif^R	Construction
OSB 10	Prototroph Rif^R	L. R. Brown, nitrosoguanidine mutagenesis
OSB 11	Cys$^-$ Rif^R	Construction
OSB 17	Prototroph Rif^R	L. R. Brown, nitrosoguanidine mutagenesis
OSB 114	Prototroph Stv^{R}Rif^R	L. R. Brown, cytidine-5-^3H decay
OSB 122	Prototroph Stv^R	L. R. Brown, cytidine-5-^3H decay
OSB 144	Prototroph Stv^R	L. R. Brown, cytidine-5-^3H decay
OSB 154	Cys$^-$	R. H. Doi
OSB 158	Prototroph *B. subtilis* Marburg	N. Sueoka
OSB 159	Prototroph *B. pumilus*	F. Young
OSB 192	Cys$^-$	Construction
OSB 400	Prototroph Std^R	EMS Mutagenesis
OSB 401	Prototroph Std^R	Spontaneous
OSB 402	Prototroph Std^R	Spontaneous
OSB 403	Prototroph Std^R	EMS Mutagenesis
OSB 404	Prototroph Std^R	EMS Mutagenesis
OSB 405	Prototroph Std^R	EMS Mutagenesis
OSB 406	Prototroph Std^R	EMS Mutagenesis
OSB 407	Prototroph Std^R	EMS Mutagenesis
OSB 408	Prototroph Std^R	EMS Mutagenesis
OSB 409	Prototroph Std^R	EMS Mutagenesis
OSB 410	Prototroph Std^R	EMS Mutagenesis
OSB 412	Prototroph Std^R	EMS Mutagenesis
OSB 413	Prototroph Std^R	EMS Mutagenesis
OSB 414	Prototroph Std^R	EMS Mutagenesis
OSB 415	Prototroph Std^R	EMS Mutagenesis
OSB 416	Prototroph Std^R	EMS Mutagenesis
OSB 417	Prototroph Std^{R}Ts	EMS Mutagenesis
OSB 418	Prototroph Std^{R}Ts	EMS Mutagenesis
OSB 419	Prototroph Std^{R}Ts	EMS Mutagenesis
OSB 420	Prototroph Rif^R	EMS Mutagenesis
OSB 421	Prototroph Rif^R	EMS Mutagenesis
OSB 422	Prototroph Rif^R	EMS Mutagenesis
OSB 423	Prototroph Rif^R	EMS Mutagenesis
OSB 424	Prototroph Rif^R	EMS Mutagenesis
OSB 425	Prototroph Rif^R	EMS Mutagenesis
OSB 426	Prototroph Rif^R	EMS Mutagenesis
OSB 427	Prototroph Rif^{R}Ts	EMS Mutagenesis
OSB 428	Prototroph Rif^{R}Ts	T. Leighton EMS Mutagenesis
OSB 429	Prototroph Rif^{R}Ts	T. Leighton EMS Mutagenesis
OSB 431	Prototroph Rif^{R}Std^{R}Ts	Construction
OSB 432	Prototroph Rif^{R}Std^{R}Ts	Construction
OSB 433	Prototroph Rif^{R}Std^{R}Ts	Construction
OSB 439	Prototroph Rif^{R}Std^R	Construction
OSB 440	Prototroph Rif^{R}Std^R	Construction
PBS1 bacteriophage	*B. subtilis* generalized transducing phage	J. Hoch

mediated transduction by the genetic cross OSB 10 (Rif^R) × OSB 154 (Cys$^-$).

OSB 192 (Cys$^-$) was constructed by transformation by the cross OSB 154 (Cys$^-$) × OSB 418 (Std^{R}Ts).

OSB 431 (Rif^{R}Std^{R}Ts) was constructed by transformation by the cross OSB 423 (Rif^R) × OSB 418 (Std^{R}Ts). A Rif^{R}Std^{S}Ts recombinant was selected on rifampin and used as the recipient in the cross OSB 404 (Std^R) × Rif^{R}Std^{S}Ts. OSB 432 (Rif^{R}Std^{R}Ts) and OSB 433 (Rif^{R}Std^{R}Ts) were constructed in exactly the same way except that OSB 405 (Std^R) and OSB 415 (Std^R), respectively, were used as donors in the cross with the Rif^{R}Std^{S}Ts recombinant.

OSB 439 (Rif^{R}Std^R) was constructed by PBS1-mediated transduction in the cross OSB 406 (Std^R) × OSB 3 (Rif^R).

OSB 404 was constructed by PBS1-mediated transduction in the cross OSB 405 (Std^R) × OSB 11 (Rif^R).

Culture media. Unless otherwise stated, all culture media were sterilized by autoclaving. Glucose was sterilized separately and was added aseptically to a medium just prior to its use. Media which were filter sterilized were passed through a 0.22-μm membrane filter (Millipore Corp.).

The commonly prepared dehydrated media used were Pennassay Broth (PAB) or antibiotic medium no. 3 (Difco, Detroit, Mich.) and Tryptose-blood-agar base (TBAB; Difco). M medium was the minimal medium described by Anagnostopoulos and Spizizen (3). MG medium was M medium except that D-glucose was supplemented to 0.5%. MG agar was MG medium solidified with 1.5 g of agar per liter. Double-strength M medium and double-strength agar were sterilized separately and then combined aseptically before distribution into petri dishes. When this agar was used to select for *cysA14* transformants, it was supplemented with the filter-sterilized enhancing amino acid mixture described by Wilson and Bott (17). SG medium was 2× Schaeffer medium as modified by Maia et al. (13). SSM medium was a filter sterilized, defined sporulation medium as described by Freese and Fortnagel (6) except that the tryptophan and methionine supplements were omitted.

Reagents. Liquified phenol (J. T. Baker Chem. Co., Phillipsburg, N.J.) was mixed with 10% (vol/vol) 0.05 M Na_2HPO_4 and adjusted to neutral pH with NaOH before use in DNA extraction. Chloroform-isoamyl alcohol solution was a 1:24 mixture of isoamyl alcohol-chloroform. Saline-EDTA contained 0.15 M NaCl plus 0.1 M ethylenediaminetetraacetic acid with the pH adjusted to 8.0. Concentrated saline citrate solution (10 × SSC) contained 1.5 M NaCl plus 0.15 M trisodium citrate and was adjusted to pH 7.0. This solution was diluted 1:10 to achieve standard saline citrate concentration (1 × SSC) and was diluted 1:100 to achieve the dilute saline citrate concentration (0.1 × SSC). Acetate-EDTA contained 3.0 M sodium acetate plus 0.001 M EDTA and was adjusted to pH 7.0.

Scintillation fluid contained, in an appropriate volume of reagent-grade toluene, 0.4% 2,5-diphenyloxaxole; scintillation grade; (Packard Instrument Co., Downers Grove, Ill.) and 0.01% 1,4-bis-[2-(5-phenyloxazolyl)]-benzene; scintillation grade; (Packard).

Mutagen. Ethyl methane sulfonate (EMS) was purchased from Eastman Kodak Chemical Co., Rochester, N.Y.

Enzymes. Deoxyribonuclease (DNase) I and 5 × crystalline, salt-free, A-grade bovine pancreatic ribonuclease (RNase) were purchased from Calbiochem, Los Angeles, Calif. RNase was dissolved in 0.15 M NaCl (pH 5.0) to a final concentration of 0.2% and was heated at 80 C for 10 min to destroy any residual DNase activity.

Egg white lysozyme was purchased from Sigma Chemical Co., St. Louis, Mo.

Antibiotics. Chloramphenicol (CAP; lot Rx291064A) was a gift from Parke-Davis Co., Detroit, Mich.

Rifampin (lot D-1281) was a gift from Justus Gelzer, CIBA Pharmaceutical Co., Summit, N.J. Additional rifampin (lot OD1760) was purchased from Calbiochem, Los Angeles.

Streptovaricin U-7750 (complex; lot 11560-5) and streptolydigin U-5482 (sodium; lot 4969-WTP-54) were kindly donated by G. B. Whitfield, the Upjohn Co., Kalamazoo, Mich.

Rifampin, streptovaricin, and streptolydigin stock solutions were prepared in M medium. The final concentrations used in the growth media were rifampin, 10 μg/ml; streptovaricin, 30 μg/ml; and streptolydigin, 100 μg/ml.

Preparation of spore stocks. SG medium was inoculated with a 5% inoculum of log-phase OSB 158 and incubated on a rotary shaker for 24 to 30 h. The resulting spores were harvested by centrifugation at 13,348 × *g* for 20 min at 4 C, and the pellet was washed exhaustively with sterile, distilled water. Successive centrifugations were at 337 × *g* for 15 min at 4 C. The clean spores were stored at 4 C in sterile, distilled water.

Selection of drug-resistant or thermal-resistant mutants. Cells to which resistance to rifampin, streptovaricin, or streptolydigin had been conferred by mutagenesis, transduction, or transformation were allowed an expression time (Table 2), during which new, altered RNA polymerase could by synthesized, before they were challenged with the appropriate drug. The same technique was used for temperature-sensitive strains that had been transformed to thermal stability.

Routinely, the spores or cells were first plated on an 86-mm diameter 0.45-nm membrane filter (Millipore Corp.) and washed once with 6 ml of M medium. The membrane was transferred immediately to a TBAB plate, incubated for an appropriate time (0 to 4.0 h, Table 2) at 37 C, transferred to another TBAB plate containing an appropriate drug, and incubated at 37 C for 24 h.

Temperature-sensitive recipient cells transformed to thermal resistance were plated directly onto TBAB agar, incubated for an appropriate time at 37 C, and then shifted to 50 C for 24 h.

Maximal recovery of drug-resistant mutants from EMS-mutagenized spores was obtained by a 3.5-h delayed selection time. Drug-resistant or thermal-resistant transformants were delayed 2 h before challenging with drug or shifting to 50 C.

Mutagenesis. *B. subtilis* spores were diluted into sterile, distilled water at 37 C and equilibrated for 5 min. EMS was added to a final concentration of 0.3%, and, after 20 min, 0.1-ml samples were removed and added to 9.9 ml of sterile, distilled water. The entire diluted sample was filtered through an 86-mm 0.45-nm membrane filter (Millipore Corp.), and drug-resistant mutants were then selected.

TABLE 2. *Expression of drug-resistant phenotype after mutagenesis*

Time (h)	No. of mutants/ml	
	Std^R	Rif^R
0.0	9	1
1.0	600	300
3.0	1,200	725
3.5	1,280	850
4.0	1,140	860

The isolation of temperature-sensitive mutants was identical except that mutants were first selected for drug resistance and then tested for the ability to grow at 37 C and inability to grow at 50 C.

Stock and donor lysate production. A 20-ml sample of PAB was inoculated to visible turbidity from an overnight plate of *B. pumilus* or appropriate donor strain and incubated with shaking at 37 C on a rotary shaker. When the culture reached peak motility, a sample was diluted 1:4 into fresh PAB. PBS1 bacteriophage were added to a multiplicity of infection (MOI) of 1 to 2, and the cells were shaken for 1 h at 37 C.

CAP was added to a final concentration of 5 μg/ml. The culture was shaken for 2 h at 37 C and then was placed in a 37 C stationary incubator overnight. DNase was added to a final concentration of 2 μg/ml. The culture was centrifuged at 2,308 × *g* for 15 min, filtered through a 0.45-μm membrane filter (Millipore Corp.), and then stored at 4 C. The lysate was checked for sterility on TBAB plates.

An alternate method of lysate production was achieved by plating approximately 1,000 PBS1 bacteriophage with an appropriate bacterial strain by using the standard overlay technique described by Adams (1). After 12 h of incubation at 37 C, 3 to 5 ml of PAB was pipetted onto the confluently lysed bacterial lawn and allowed to stand at room temperature for 15 to 20 min. The broth and overlay were removed and centrifuged at 2,309 × *g* for 15 min. DNase was added to a final concentration of 2 μg/ml, and the suspension was filtered through a 0.45-nm Millipore membrane filter and stored at 4 C.

Bacteriophage assay. PBS1 was assayed as described by Adams (1). Top agar was PAB plus 0.4% agar, and bottom agar was TBAB. If fresh, moist TBAB plates are not used, minute plaques will result which make quantification difficult. With this system, both OSB 158 and OSB 159 as hosts gave highly reproducible plaque counts.

Transduction procedure. A 10-ml amount of PAB was inoculated to visible turbidity with a recipient strain. When the culture reached peak motility, 1 ml of cells was mixed with 1 ml of phage of sufficient titer to give an MOI of 1 and was shaken at

37 C for 30 min. The culture was then combined with 5 ml of M medium, centrifuged for 15 min at 1,610 × *g*, and resuspended in 2 ml of M medium. Appropriate dilutions were plated on the required selection plates.

Preparation of transforming DNA. A 250-ml amount of log-phase cells was centrifuged at 3,337 × *g* for 15 min and suspended in 5 ml of saline-EDTA. Lysozyme (10 mg/ml) was added to a final concentration of 900 μg/ml, and the suspension was incubated at 37 C, resulting in extensive lysis in approximately 1 h. Sodium dodecyl sulfate (2%) was added to a final concentration of 0.07%, and the suspension was incubated at 37 C for 5 min with shaking. Sodium perchlorate (5 M) was added to the lysed suspension to a final concentration of 1 M. The mixture was then combined with an equal volume of chloroform isoamyl alcohol, shaken for 2 min, and centrifuged at 2,309 × *g* for 10 min. The aqueous top layer was removed with a pipette and transferred to a 30-ml Corex centrifuge tube to which two volumes of cold 95% ethanol were pipetted slowly down the wall of the tube. The precipitate was wound onto a glass rod, rinsed with 70 and 100% ethanol, and transferred to 5 ml of 0.1 M NaCl. The dissolved precipitate was pipetted into 20 ml of cold 95% ethanol, and the resulting precipitate was collected with a sterile, wire loop and transferred to 5 ml of 2 M NaCl. The DNA concentration was estimated by ultraviolet absorption at 260 nm.

Transformation procedure. Preparation of competent cells was performed essentially as described by Anagnostopoulos and Spizizen (3). The recipient strain was incubated overnight on a rotary shaker at 37 C in PAB and transferred to MG medium (~ 10^8 cells/ml) containing 50 μg of L-tryptophan per ml and 0.2% casein hydrolysate. For auxotrophic recipients, required amino acids were supplemented at 50 μg/ml. A 2.5-ml amount of this cell suspension was placed in a screw-cap tube (19 by 150 mm), shaken vigorously on a reciprocal shaker at 37 C for 4 h, centrifuged at 3,020 × *g* for 5 min, and diluted 1:10 in MG medium containing 5 μg of L-tryptophan per ml, 0.01% casein hydrolysate, an additional 5 μmol of $MgSO_4$ per ml, and 5 μg of any other required amino acid per ml. A 0.9-ml amount of this cell suspension and 0.1 ml of a DNA solution (0.1 μg/ml) were placed in a screw-cap tube (16 by 125 mm) which was placed at 37 C for 90 min on a reciprocal shaker. DNase I (100 μg/ml) was added to a final concentration of 33 μg/ml, and the cells were plated on selective medium. Viable cell counts were done on TBAB.

RESULTS

Mutagenesis. EMS mutagenesis of *B. subtilis* spores was used to isolate rifampin-resistant and streptolydigin-resistant mutants or conditional mutants which contained both drug resistance and temperature sensitivity. The use of spores facilitates the selection of independent mutants, and a large number of rifampin-resistant and streptolydigin-resistant mutants were isolated. The spontaneous mutation rate was 1.2×10^{-8} for streptolydigin resistance and 1.5×10^{-8} for rifampin resistance. The induced mutation rate by a 20-min exposure to EMS was 5.9×10^{-6} for streptolydigin resistance and 1.5×10^{-6} for rifampin resistance; there was no loss in viability after 20 min of exposure to EMS as measured on TBAB plates.

Attempts to recover mutants resistant to both rifampin and streptolydigin at spore concentrations on the membrane up to 10^{11} were not successful. Increased concentrations of EMS or longer exposure to EMS yielded lower survival rates without appreciably increasing the recovery of drug-resistant mutants. A representative sample of mutants was picked for further study.

Transductional and transformational analysis. Resistance to rifampin and streptovaricin has been shown to be linked by co-transformation and co-transduction to the *cysA14* locus in *B. subtilis*. Resistance to streptolydigin in *E. coli* has been linked by co-transduction to rifampin resistance (15), and evidence is available that the loci for rifampin resistance and streptolydigin resistance were located in the DNA segment coding for the β subunit of RNA polymerase in *Escherichia coli*. To examine the possibility that rifampin resistance and streptolydigin resistance might be linked by co-transduction in *B. subtilis*, two- and three-factor crosses were conducted with PBS1-mediated transduction. The data in Tables 3 and 4 confirm that streptolydigin resistance, rifampin resistance, streptovaricin resistance and the *cysA14* locus are linked by co-transduction. Three-factor crosses indicated that *cysA14* is an outside marker and that the *cysA14* Rif^R (Stv)-Std^R Ery^r is a probable marker order. It is apparent that, within the genetic distances involved in these experiments, there is a bias towards incorporation of all the donor markers. Due to its greater resolving power, transformation was used to analyze further this small region of the *B. subtilis* chromosome.

The primary reference marker used in these experiments, *cysA14*, behaved as a complex locus in transformation experiments. Harford and Sueoka (8) reported two classes of Cys^+ transformants when selection was made on MG agar. The first class formed large, readily observable colonies. Upon close inspection, however, a second class of very minute colonies was also observed and represented 60 to 80% of the total transformants. Normal development of the minute colonies was achieved by supplementing the MG agar with a mixture of nine

enhancing amino acids described by Wilson and Bott (17). DNA from the minute colony class yielded only minute transformants, whereas DNA from the large colony class yielded both large and minute transformants. They also reported that no spontaneous Cys$^+$

TABLE 3. *Two-factor transductional analysis*

Donor		Recipient		Selected marker	Total colonies tested	Recombinant classes		Co-transduction (%)
Strain designation	Phenotype	Strain designation	Phenotype			11[a]	10	
OSB 17	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	208	186	22	89.4
OSB 2	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	175	162	13	92.5
OSB 404	Cys$^+$ Std^R	OSB 154	Cys$^-$ Std^S	Cys$^+$	632	584	48	91.8
OSB 405	Cys$^+$ Std^R	OSB 154	Cys$^-$ Std^S	Cys$^+$	632	584	48	91.8
OSB 23	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	236	224	12	94.9
OSB 7	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	200	192	8	96.0
OSB 10	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	200	192	8	96.0
OSB 36	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	270	256	14	94.8
OSB 21	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	290	275	15	94.8
OSB 17	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	218	192	26	88.1
OSB 22	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	203	182	21	89.7
OSB 2	Cys$^+$ Rif^R	OSB 154	Cys$^-$ Rif^S	Cys$^+$	200	171	29	85.5
OSB 125	Cys$^+$ Stv^R	OSB 154	Cys$^-$ Stv^S	Cys$^+$	206	198	8	96.1
OSB 122	Cys$^+$ Stv^R	OSB 154	Cys$^-$ Stv^S	Cys$^+$	192	182	10	94.8
OSB 126	Cys$^+$ Stv^R	OSB 154	Cys$^-$ Stv^S	Cys$^+$	240	230	10	95.8
OSB 129	Cys$^+$ Stv^R	OSB 154	Cys$^-$ Stv^S	Cys$^+$	237	229	8	96.6
OSB 112	Cys$^+$ Stv^R	OSB 154	Cys$^+$ Stv^S	Cys$^+$	200	172	18	86.0
OSB 113	Cys$^+$ Stv^R	OSB 154	Cys$^+$ Stv^S	Cys$^+$	200	168	32	84.0
OSB 115	Cys$^+$ Stv^R	OSB 154	Cys$^+$ Stv^S	Cys$^+$	226	194	32	85.8
OSB 120	Cys$^+$ Stv^R	OSB 154	Cys$^+$ Stv^S	Cys$^+$	206	193	13	93.7

[a] "1" and "0" refer to donor and recipient phenotypes, respectively.

TABLE 4. *Three-factor transductional analysis*

Donor		Recipient		Selected marker	Total colonies tested	Recombinant classes						
Strain designation	Phenotype	Strain designation	Phenotype			111[a]	110	100	101	010	011	001
OSB 439	Cys$^+$Rif^{R}Std^R	OSB 154	Cys$^-$Rif^{S}Std^S	Cys$^+$	512	475	6	38	1			
				Rif^R	457	375	1			7	74	
				Std^R	486	406			1		51	28
OSB 440	Cys$^+$Rif^{R}Std^R	OSB 154	Cys$^-$Rif^{S}Std^S	Cys$^+$	520	469	3	47	1			
				Rif^R	472	434	1			2	35	
				Std^R	521	474			0		30	17
OSB 406	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	798	421	343	29	5			
				Std^R	208	199			1		4	4
OSB 400	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	658	454	165	33	5			
				Std^R	510	486			17		14	3
OSB 401	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	785	392	358	30	5			
				Std^R	260	254			1		2	3
OSB 402	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	510	328	147	30	5			
				Std^R	161	145			4		5	7
OSB 403	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	850	404	414	30	2			
				Std^R	260	253			1		4	2
OSB 404	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	1,029	606	373	47	5			
				Std^R	506	400			4		5	7
OSB 405	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	521	336	159	23	3			
				Std^R	156	153			0		1	2
OSB 407	Cys$^+$Rif^{S}Std^R	OSB 3	Cys$^-$Rif^{R}Std^S	Cys$^+$	846	261	548	36	1			

[a] "1" and "0" refer to donor and recipient phenotypes, respectively.

revertants were ever observed from the *cysA14*$^-$-carrying strain.

The *cysA14* locus carried by OSB 154 behaved in a similar manner. The minute colony class comprised over 90% of the total yield of transformants recovered on MG agar and did not require the amino acid supplement for normal development. On the amino acid-supplemented plates the two classes of transformants were still readily distinguishable in that the large colony class produced larger and more opaque colonies than did the minute colony class. Of 178 large-colony Cys$^+$ transformants examined from various Rif^R × *cysA14*$^-$ genetic crosses, 39 were rifampin resistant for a co-transformation frequency of 21.9%. Of 164 large colony Cys$^+$ transformants examined from various Std^R × *cysA14* genetic crosses, 17 were streptolydigin resistant for a co-transformation frequency of 10.4%. No spontaneous Cys$^+$ revertants were ever observed from OSB 154. In addition, some other interesting behavior of OSB 154 strain was observed.

Mapping studies with OSB 427, a rifampin-resistant, temperature-sensitive strain, indicated that rifampin resistance and temperature sensitivity in that strain were linked by 18% co-transformation when OSB 158 rifampin-resistant recombinants were scored for temperature sensitivity. However, when OSB 154 was the recipient strain and OSB 427 was again the donor strain, no temperature-sensitive recombinants were recovered. Instead, when either Cys$^+$ or Rif^R transformants were scored at 50 C for temperature sensitivity, two easily distinguishable colony types were observed. The most numerous class displayed normal colony morphology. However, the second recombinant class yielded much smaller "leaky" colonies whose temperature "sensitivity" and rifampin resistance were linked by co-transformation. The 19.4% co-transformation obtained was consistant with the rifampin-resistant temperature sensitivity co-transformation frequency observed when OSB 158 was the recipient. There is a suppression of the Ts phenotype in OSB 154.

When the *cysA14*$^-$ locus was transferred into the *B. subtilis* strain Marburg chromosome, the new strain, OSB 192, exhibited some new properties. Cys$^+$ transformants of this strain were no longer of two classes, and the amino acid supplement in the MG agar was no longer required for normal development of Cys$^+$ transformants. The colony morphology of this strain was very much like that of the recipient parent, and spontaneous Cys$^+$ revertants could now be recovered from minimal agar plates. In addition, when OSB 192 was the recipient in a transformation experiment with OSB 427 as donor, the rifampin-resistant, temperature-sensitive recombinants no longer exhibited the "leaky" temperature-sensitive phenotype. Rifampin resistance linkage of various strains by co-transformation with the *cys*$^-$ locus of OSB 192 was essentially the same as that found for the *cysA14*$^-$ locus of OSB 154 when it was the recipient strain (Table 5).

Transformation experiments were conducted to determine the relative location on the *B. subtilis* chromosome of the mutation(s) from which the Rif^R, Stv^R, Std^R, and Ts phenotypes result. When Cys$^+$ transformants were selected, co-transformation frequencies of rifampin resistance with the *cysA14* locus varied from 21.7 to 36.1% (Table 6). However, when recombinants were selected on rifampin, the Cys$^+$ Rif^R co-transformation frequencies varied from 10.6 to 19.8%. The one streptovaricin-resistant strain tested behaved in a similar manner. The data in Table 7 show that co-transformation frequencies of streptolydigin resistance with the *cysA14* locus vary from 8.9 to 23.6% when Cys$^+$ transformants were selected. However, when recombinants were selected on streptolydigin, the co-transformation frequencies dropped to a range of 5.4 to 13.5%.

The two-factor transformation data indicate that the mutation(s) that confers resistance to rifampin resides much closer to the *cysA14* locus than does the mutation(s) conferring resistance to streptolydigin. The one streptovaricin-resistant strain tested by two-factor transformation mapped as closely to the *cysA14* locus as did the rifampin-resistant strains. Among the rifampin-resistant strains tested, the co-transformation frequency was twice as great when Cys$^+$ transformants were selected as when rifampin-resistant transformants were selected. This non-reciprocality was also present but was not nearly so pronounced among the streptolydigin-resistant strains.

Drug-resistant, temperature-sensitive strains were genetically analyzed by transformation to determine whether a single-step mutation had conferred both drug resistance and temperature sensitivity. If the phenotype was the result of a single mutation, then drug resistance and temperature sensitivity should be co-transformed 100% of the time. Of the six strains examined, only two strains displayed any linkage of drug resistance and temperature sensitivity by co-transformation. OSB 427

showed an 18% rifampin resistance, temperature sensitivity co-transformation frequency and OSB 418 demonstrated a co-transformation frequency of 51.9% for streptolydigin resistance and temperature sensitivity. These data indicate that the drug-resistant phenotype and the temperature-sensitive phenotype are separable by genetic manipulation.

Even though the two temperature-sensitive mutations co-transform with the *cys* locus at almost identical frequencies, they are probably two different mutations since one mutant yields "leaky" temperature-sensitive recombinants from OSB 154, and the other one yields true temperature-sensitive recombinants. If the two mutations are identical, then no thermal-stable recombinants should result at 50 C from an OSB 427 × OSB 418 genetic cross. However, if they are separate mutations, then thermal-stable recombinants should be recovered at 50 C. As the data in Table 8 indicate, thermal-stable recombinants were recovered when selected at 50 C. When transformants from this cross were selected on rifampin at 37 C, the rifampin-resistant, thermal-stable classes were diminished in number compared to the usual recovery of these classes. Since OSB 427 had never attained the competent state, the reciprocal of the above cross was not conducted.

Appropriate three-factor crosses were performed to determine the probable sequence of markers which yield the Cys$^-$, Rif^R, Stv^R, Std^R, Ts phenotypes. In Table 9 the markers are listed in the most probable order as determined by two-factor crosses. The double recombinant class is always represented in the "101" column. In all cases, the data clearly confirm the suggested order. The marker order indicated by the three-factor data is *cysA14*-Rif^R (Stv)^{R}Std^{R}Ts$_{418}$(Ts$_{427}$). Rifampin resistance and streptovaricin resistance could not be ordered by three-factor crosses because all rifampin-resistant strains selected on rifampin were also resistant to streptovaricin. However, only two-thirds of the streptovaricin-resistant strains that were originally selected on streptovaricin were resistant to rifampin.

TABLE 5. *Genetic crosses comparing OSB 154 and OSB 192*

Cross	Total tested	Selected marker	Co-transformation (%)	
			Cys$^+$-Rif^R	Rif^R-Ts
OSB 427 × OSB 154	856	Rif^R	12.7	21.3
OSB 427 × OSB 192	208	Rif^R	9.6	20.7
OSB 420 × OSB 154	416	Rif^R	17.6	
OSB 420 × OSB 192	104	Rif^R	11.5	
OSB 423 × OSB 154	416	Rif^R	13.9	
OSB 423 × OSB 192	104	Rif^R	19.2	
OSB 424 × OSB 154	406	Rif^R	18.2	
OSB 424 × OSB 192	104	Rif^R	14.4	

TABLE 6. *Two-factor transformational analysis of rifampin-resistant mutants*

Donor		Recipient		Selected marker	Total colonies tested	Recombinant classes			Co-transformation (%)
Strain designation	Phenotype	Strain designation	Phenotype			11[a]	10	01	
OSB 10	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	780	234	546		30.0
				Rif^R	416	64		348	15.4
OSB 420	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	435	157	278		36.1
				Rif^R	416	74		342	17.8
OSB 421	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	364	91	273		25.0
				Rif^R	416	48		368	11.5
OSB 422	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	364	86	278		23.6
				Rif^R	416	44		372	10.6
OSB 423	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	447	110	337		24.6
				Rif^R	416	58		358	13.9
OSB 424	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	412	104	308		25.2
				Rif^R	406	74		328	18.2
OSB 425	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Rif^R	364	70		294	19.3
OSB 426	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	717	156	561		21.7
				Rif^R	780	154		626	19.8
OSB 427	Rif^{R}Ts	OSB 158	Rif	Rif^R	572	105		467	18.4
OSB 428	Cys$^+$Rif^R	OSB 154	Cys$^-$Rif^S	Cys$^+$	416	88	328		21.1
				Rif^R	416	46		370	11.1

[a] "1" and "0" refer to donor and recipient phenotypes, respectively.

TABLE 7. *Two-factor transformational analysis of streptolydigin-resistant mutants*

Donor		Recipient		Selected marker	Total colonies tested	Recombinant classes			Co-transformation (%)
Strain designation	Phenotype	Strain designation	Phenotype			11[a]	10	01	
OSB 400	$Cys^+Std^{R'}$	OSB 154	Cys^-Std^S	Cys^+	416	98	318		23.6
OSB 401	$Cys^+Std^{R'}$	OSB 154	Cys^-Std^S	Cys^+	416	83	333		20.0
OSB 402	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	76	340		18.3
OSB 403	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	91	325		21.9
OSB 404	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	832	152	680		18.3
				Std^R	654	74		580	11.2
OSB 405	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	389	79	310		16.9
				Std^R	501	55		446	9.9
OSB 406	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	85	331		20.4
OSB 407	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	91	325		21.9
OSB 408	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	312	38	274		12.1
				Std^R	260	14		246	5.4
OSB 409	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	52	364		12.5
				Std^R	416	33		383	7.9
OSB 410	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	292	27	265		9.3
				Std^R	346	32		314	9.2
OSB 412	Cys^+Std^R	OSB 154	Cys^-Std^R	Cys^+	404	80	324		19.8
				Std^R	398	32		366	8.0
OSB 413	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	398	46	352		11.9
				Std^R	416	46		370	11.1
OSB 414	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	63	353		15.1
				Std^R	416	49		367	11.8
OSB 415	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	37	379		8.9
				Std^R	364	22		342	6.0
OSB 416	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	416	55	361		13.2
				Std^R	416	46		370	11.1
OSB 417	$Cys^+Std^{R'}$	OSB 154	Cys^-Std^S	Cys^+	416	54	362		12.9
				Std^R	416	56		360	13.5
OSB 419	Cys^+Std^R	OSB 154	Cys^-Std^S	Cys^+	859	142	717		16.5
				Std^R	562	75	487		13.3

[a] "1" and "0" refer to donor and recipient phenotypes, respectively.

TABLE 8. *Comparison of recombinant classes resulting from an OSB427 × OSB418 genetic cross and an OSB420 × OSB418 genetic cross*

Recombinant classes selected	Cross	
	OSB427 × OSB418	OSB420 × OSB418
At 50 C		
$Rif^RStd^RTs^+$	0	10
$Rif^RStd^STs^+$	2	66
$Rif^SStd^RTs^+$	47	139
$Rif^SStd^STs^+$	3	39
On rifampin		
Rif^RStd^R+	7	3
Rif^RStd^RTs	214	176
Rif^RStd^S+	0	34
Rif^RStd^STs	83	47

DISCUSSION

A primary objective of this study was to isolate and genetically map new classes of *B. subtilis* RNA polymerase mutants. Harford and Sueoka (10) genetically mapped three rifampin-resistant mutants of *B. subtilis* by transformation, and Brown (Bacteriol. Proc., p. 131, 1970) genetically mapped over 40 rifampin-resistant and streptovaricin-resistant mutants of *B. subtilis* by PBS1-mediated transduction.

We obtained large numbers of streptolydigin-resistant mutants by EMS mutagenesis and genetically mapped 18 of them by transduction or transformation. Two temperature-sensitive mutants which are also RNA polymerase mutants were also isolated by EMS mutagenesis and genetically mapped by trans-

TABLE 9. *Three-factor transformation analysis*

Determination	Selected marker	Total recombinants tested	Recombinant classes						
			111[a]	110	101	100	010	011	001
Cross ordering: Cys$^-$-Rif^R-Std^R									
OSB 440 × OSB 154	Cys$^+$	406	51	30	9	316			
	Std^R	306	51		8			149	98
Cross ordering: Cys$^-$-Rif^R-Ts$_{427}$									
OSB 427 × OSB 154	Cys$^+$	558	26	124	11	396			
	Rif^R	852	19	78			564	147	
Cross ordering: Cys$^-$-Std^R-Ts$_{418}$									
OSB 418 × OSB 154	Cys$^+$	406	16	32	6	352			
	Std^R	416	20	21			162	213	
OSB 154 × OSB 418	50 C	416	28		5			193	190
Cross ordering: Stv-Std-Ts$_{418}$									
OSB 144 × OSB 418	Stv^R	260	37	178	3	42			
	50 C	312	100		5			65	142
OSB 122 × OSB 418	Stv^R	312	90	81	2	139			
	50 C	234	56		9			50	119
Cross ordering: Rif^R-Std^{R}Ts$_{418}$									
OSB 420 × OSB 418	Rif^R	260	34	47	3	176			
	50 C	254	66		10			39	139
OSB 423 × OSB 418	Rif^R	207	46	32	7	122			
	50 C	208	95		5			33	75

[a] "1" and "0" refer to donor and recipient phenotypes, respectively.

formation. A genetic map of these mutations has been constructed from two- and three-factor transformation data. In vitro assays of RNA polymerase isolated from these mutants (to be published elsewhere) confirms the genotype of both the drug-resistant and temperature-sensitive mutants as RNA polymerase mutants.

That controlled mutagenic conditions resulted in the isolation of a majority of mutants of RNA polymerase rather than large numbers of double mutants can be inferred from several observations. Of the mutants examined, all of the rifampin-resistant and the streptolydigin-resistant mutants were prototrophic, none were cross-resistant, and only three streptolydigin-resistant mutants were also temperature sensitive.

Temperature-sensitive RNA polymerase mutants undoubtedly could be isolated in the same manner as were the rifampin-resistant and streptolydigin-resistant mutants. However, no selection pressure was available for specifically isolating temperature-sensitive RNA polymerase mutants. Yura and Igarashi (18) had shown that a class of RNA polymerase mutants in *E. coli* could be isolated that had obtained temperature sensitivity and resistance to streptovaricin by a single-step mutation. Therefore, in our isolation procedure, mutants were first selected for streptolydigin resistance and then tested for temperature sensitivity.

Genetic analysis of three Std^R Ts mutants and three Rif^R Ts mutants which had been obtained by the above selection procedure indicated that in only two of the strains, OSB 418 and OSB 427, were drug resistance and temperature sensitivity genetically linked by co-transformation. In these strains, a single-step mutation did not result in both drug resistance and temperature sensitivity since the two phenotypes could be separated by

genetic manipulation and by shearing of transforming DNA (results to be reported later).

Genetic analysis also indicated that the temperature-sensitive phenotype of OSB 418 and OSB 427 is the result of at least two different mutations.

cysA14 locus. In our transformation system, the *cysA14* locus behaves in a manner similar to that described by Harford and Sueoka (8). However, instead of the reported 60 to 80%, we find in our system that over 90% of the Cys^+ transformants are of the minute colony class. Harford and Sueoka also reported, for three spontaneous rifampin-resistant mutants, an average co-transformation frequency with *cysA14* of 63% when *cys* was the selected marker. This frequency is over twice as great as that found for our rifampin-resistant strains. The reason for this discrepancy is unknown.

As calculated from two-factor transformation data, when *cys* is the selected marker, the average co-transformation frequency of the Cys^+ Rif^R phenotype is 28% and that for the Cys^+ Std^R phenotype is 15.5%. However, when only the large-colony class of Cys^+ transformants is considered, the Cys^+ Rif^R phenotypes are co-transformed only 21.9% of the time and the Cys^+ Std^R phenotypes are co-transformed only 10.4% of the time. These data suggest that the *cysA14* locus is composed of two separate mutations which will be referred to as x^- and y^-. The genotype of the large colony Cys^+ transformant class is x^+y^+ and that of the small colony class is x^-y^+. The mapping data are consistent with the x mutation being located to the left of y, (with respect to a given marker, left refers to the chromosome origin direction and right refers to the chromosome terminus direction). In the genetic cross OSB 154 × OSB 418 where the Cys^- phenotype was transferred into essentially an OSB 158 chromosome (OSB 418 is a mutant derived from OSB 158), the method of selection of transformants made it probable that only the y^- mutation was integrated. Until a detailed study of the complexities of the *cysA14* locus is conducted, the reasons for its behavior can only be surmised.

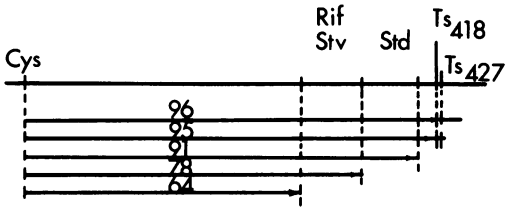

FIG. 1. *Purposed genetic map for the RNA polymerase region of the Bacillus subtilis.*

The non-reciprocality of genetic crosses in *B. subtilis* has been described by other workers (4). Generally, when the marker on the right in a given pair is the selected marker, the co-transformation frequency is lower than when the marker on the left is the selected marker. Although this discrepancy makes it difficult to assign recombinational distances to the intervals between mutations, it does not affect the ordering of the mutational sites. In three-factor crosses, although the apparent linkage by co-transformation is different for the markers, depending on whether the right-hand or the left-hand marker is the selected marker, both sets of data are consistent with only one marker order. Spatz and Trautner (16) and Bresler et al. (4) have presented evidence for the existence of a heteroduplex correction system in *B. subtilis*, but this kind of mechanism may or may not explain the polarity of the recombination events.

Behavior of OSB 427 temperature-sensitive mutation in OSB 154 recombinants. When Cys^+ or rifampin-resistant recombinants from an OSB 427 × OSB 154 genetic cross were tested at 50 C for temperature sensitivity, no temperature-sensitive recombinants were observed. Instead, the "leaky" temperature-sensitive phenotype resulted. This behavior suggests that the temperature sensitivity mutation is being suppressed by OSB 154, but it seems unlikely that this is a case of nonsense suppression.

A similar situation has been described in *E. coli* where a suppressor Q can suppress a Ts mutation in the *pheS* gene (14). The suppressor did not suppress several known amber or ochre mutations and was not gene specific.

Further analysis will be required to determine by what mechanism the OSB 427 temperature-sensitive phenotype is being suppressed.

Location of RNA polymerase on the B. subtilis chromosome. Genetic mapping by transduction and transformation indicates that all of the Rif^R, Stv^R, Std^R, and two of the Ts phenotypes are the result of tightly clustered mutations that co-transduce or co-transform with the *cysA14* locus. With the exception of the Rif^R Stv^R phenotype, all of the different phenotypes appear to be the result of different classes of mutations that can be genetically and physically separated, e.g., a Rif^RStd^s strain can be transformed to a Rif^sStd^s phenotype as well as to a Rif^RStd^R phenotype. Mutants within each phenotypic class display a range of co-transformation frequencies with the *cysA14* locus, but the actual number of different mutations represented by these mu-

tants cannot be determined from existing data. Fine-structure mapping by the recombination index method should answer this question.

Finally, a genetic map has been constructed from two- and three-factor transformation data. Map distances were calculated from data in which *cys* was the selected marker (Fig. 1). Co-transformation frequencies were converted to map units by the following relationship: 100 – percent co-transformation = map units.

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages, p. 592. Interscience Publishers, New York.
2. Anagnostopoulos, C., and I. P. Crawford. 1961. Transformation studies on the linkage of markers in the tryptophan pathway in *B. subtilis*. Proc. Nat. Acad. Sci. U.S.A. **47**:378–390.
3. Anagnostopoulos, C., and J. Spizizen. 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
4. Bresler, S. E., R. A. Kreneva, and V. V. Kushev. 1971. Molecular heterozygotes in *B. subtilis* and their correction. Mol. Gen. Genet. **113**:204–213.
5. Doi, R. H., L. R. Brown, G. Rodgers, and Y. Hsu. 1970. *Bacillus subtilis* mutants altered in spore morphology and in RNA polymerase activity. Proc. Nat. Acad. Sci. U.S.A. **66**:404–410.
6. Freese, E., and P. Fortnagel. 1967. Analysis of sporulation mutants. I. Response of uracil incorporation to carbon sources, and other mutant properties. J. Bacteriol. **94**:1957–1969.
7. Goldthwaite, C., D. Dubnau, and I. Smith. 1970. Genetic mapping of antibiotic resistance in markers *Bacillus subtilis*. Proc. Nat. Acad. Sci. U.S.A. **65**:96–103.
8. Harford, N., and N. Sueoka. 1970. Chromosomal location of antibiotic resistance markers in *Bacillus subtilis*. J. Mol. Biol. **51**:267–286.
9. Korch, C. T., and R. H. Doi. 1971. Electron microscopy of the altered spore morphology and a ribonucleic acid polymerase mutant of *Bacillus subtilis*. J. Bacteriol. **105**:1110–1118.
10. Leighton, T. J., P. K. Freese, R. H. Doi, R. A. J. Warren, and R. A. Kelln. 1972. Initiation of sporulation in *Bacillus subtilis:* requirement for serine protease activity and ribonucleic acid polymerase modification, p. 238–246. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
11. Losick, R., R. G. Shorenstien, and A. L. Sonenshein. 1969. Structural alteration of RNA polymerase during sporulation. Nature (London) **227**:910–193.
12. Losick, R., and A. L. Sonenshein. 1969. Change in template specificity of RNA polymerase during sporulation of *Bacillus subtilis*. Nature (London) **244**:35–37.
13. Maia, J. C. C., P. Kerjan, and J. Szulmajster. 1971. DNA dependent RNA polymerase from vegetative cells and spores of *Bacillus subtilis*. FEBS Lett. **13**:269–274.
14. Russell, R. R. B., and A. J. Pittard. 1971. New suppressor in *Escherichia coli*. J. Bacteriol. **107**:736–740.
15. Schleif, R. 1969. Isolation and characterization of a streptolydigin resistant RNA polymerase. Nature (London) **223**:1068–1069.
16. Spatz, H. C., and T. A. Trautner. 1970. One way to do experiments on gene conversion. Transfection with heteroduplex SPP1 DNA. Mol. Gen. Genet. **109**:84–106.
17. Wilson, G., and K. Bott. 1968. Nutritional factors influencing the development of competence in the *Bacillus subtilis* transformation system. J. Bacteriol. **95**:1439–1449.
18. Yura, T., and K. Igarashi. 1968. RNA polymerase mutants of *E. coli*. I. Mutants resistant to streptovaricin. Proc. Nat. Acad. Sci. U.S.A. **61**:1313–1319.