JOURNAL OF BACTERIOLOGY, May 1982, p. 815–825
0021-9193/82/050815-11$02.00/0

Vol. 150, No. 2

Nucleotide Sequence and Functional Map of pC194, a Plasmid That Specifies Inducible Chloramphenicol Resistance

SUEHARU HORINOUCHI† AND BERNARD WEISBLUM*

Pharmacology Department, University of Wisconsin Medical School, Madison, Wisconsin 53706

Received 16 July 1981/Accepted 13 January 1982

The nucleotide sequence of pC194, a small plasmid from *Staphylococcus aureus* which is capable of replication in *Bacillus subtilis*, has been determined. The genetic determinant of chloramphenicol (CAM) resistance, which includes the chloramphenicol acetyl transferase (CAT) structural gene, the putative promoter and controlling element of this determinant, have been mapped functionally by subcloning a 1,035-nucleotide fragment which specifies the resistance phenotype using plasmid pBR322 as vector. Expression of CAM resistance is autogenously regulated since the 1,035-nucleotide fragment containing the CAT gene sequence and its promoter cloned into pBR322 expresses resistance inducibly in the *Escherichia coli* host. A presumed controlling element of CAT expression consists of a 37-nucleotide inverted complementary repeat sequence that is located between the −10 and ribosome-loading sequences of the CAT structural gene. Whereas the composite plasmid containing the minimal CAT determinant cloned in pBR322 could not replicate in *B. subtilis*, ability to replicate in *B. subtilis* was seen if the fragment cloned included an extension consisting of an additional 300 nucleotides beyond the 5′ end of the single pC194 *Msp*I site associated with replication. This 5′ extension contained a 120-nucleotide inverted complementary repeat sequence similar to that found in pE194 *Taq*I fragment B which contains replication sequences of that plasmid. pC194 was found to contain four open reading frames theoretically capable of coding for proteins with maximum molecular masses, as follows: A, 27,800 daltons; B, 26,200 daltons; C, 15,000 daltons; and D, 9,600 daltons. Interruption or deletion of either frame A or D does not entail loss of ability to replicate or to express CAM resistance, whereas frame B contains the CAT structural gene and frame C contains sequences associated with plasmid replication.

Plasmid pC194 is one of several small R-plasmids found in *Staphylococcus aureus* reported by Iordanescu et al. (10, 11). pC194 belongs to incompatibility group 8 and specifies chloramphenicol-induced resistance to chloramphenicol (CAM) mediated by the enzyme chloramphenicol acetyl transferase (CAT), an enzyme shown by Shaw (18) to inactivate CAM by converting it successively to the inactive 3-acetyl and 1,3-diacetyl derivatives. This plasmid is of special interest because (i) it can apparently replicate in a wide range of bacterial hosts, including *Bacillus thuringiensis* (14) and *Escherichia coli* (6), (ii) it has served as a useful vector for analytical cloning of determinants of inducible resistance and replication in conjunction with studies of another small plasmid, pE194, as described in the accompanying publication (9), and (iii) it can serve as a useful system for studies of gene expression control mechanisms since the synthesis of CAT appears to be under autogenous control. By studying pC194 and pE194 jointly, it has been possible to design experiments which reveal details of the biological properties of both plasmids, particularly of requirements for plasmid replication common to both systems.

Ehrlich (3, 4) introduced pC194 into *Bacillus subtilis* by transformation, making it possible to use the large number of mutant strains of this bacterial host in functional studies of the plasmid. Ehrlich (4), Lofdahl et al. (12, 13), and Gryczan et al. (7, 8) have reported methods for use of this plasmid as an effective cloning vehicle in *B. subtilis*. We present here the DNA sequence of this plasmid in its entirety, including data pertinent to the mapping of biological functions and determinants of their regulation.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used are listed in Table 1 of the accompanying paper (9). Preparation of plasmid DNA, use of restriction endonucleases, and DNA sequence studies

† Present address: Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

were likewise performed as described in the accompanying paper (9).

RESULTS

A physical map of pC194 showing critical restriction endonuclease cleavage sites and four open reading frames deduced from computer analysis of the sequence are shown in Fig. 1. Pending identification of the polypeptides encoded by pC194 with their respective reading frames, we adopted the convention of referring to open reading frames (i.e., DNA sequences which when translated in a particular reading frame lack termination codons in that phase) alphabetically, in order of decreasing size, and assigning a polypeptide molecular mass as if the entire open reading frame were used. Schematic representations of recombinant plasmids that were constructed to elucidate biological functions of pC194 are shown in Fig. 2.

A more detailed physical map of pC194 including additional restriction sites and the extent to which sequencing was performed from each of the labeled ends is presented in Fig. 3. All restriction sites shown were identified in overlapping sequence determinations except for the *Mbo*I site at residue 1, the *Mbo*I B-A junction; about 60% of the pC194 sequence was determined in both strands. The complete nucleotide sequence, including potential polypeptides encoded by open reading frames as well as inverted complementary repeat sequences, is presented in Fig. 4.

Determinant of CAM resistance. Analysis of the pC194 sequence revealed the presence of four open reading frames potentially capable of encoding proteins, labeled in order of decreasing size, A, B, C, and D (Fig. 1, 3, and 4). First we tentatively identified the CAT structural gene as the polypeptide encoded by part of open reading frame B, residues 1260 (Met) to 1907 (Leu). The coding sequence is capable of specifying a polypeptide containing 216 amino acids, 83 residues of which are identical to respective residues in the Tn*9*-associated CAT of *E. coli*, which number 219 (1, 19; Fig. 5). The first three amino acid residues of pC194 CAT, Met Asn Phe, are identical to the CAT amino-terminal sequence

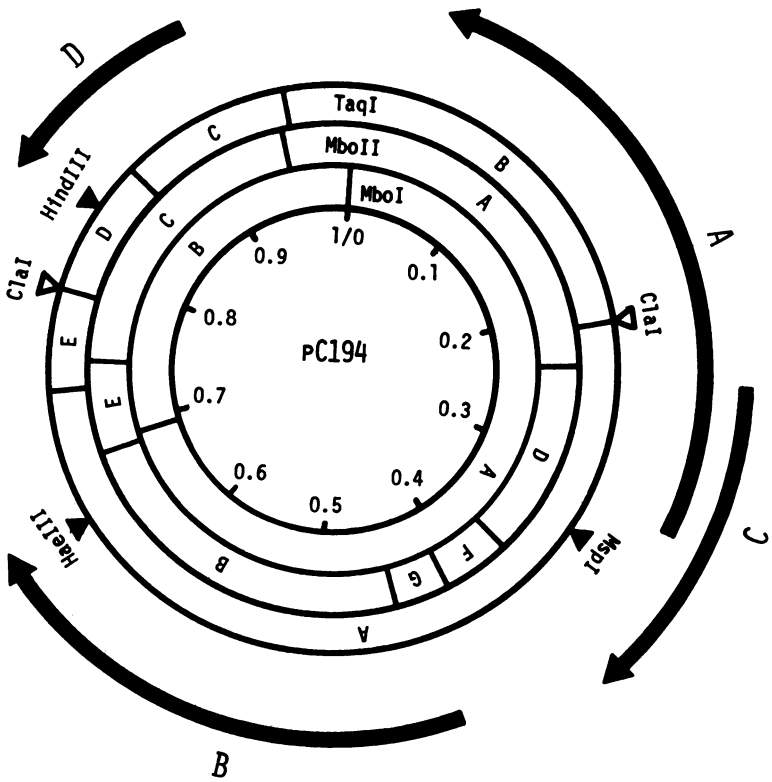


FIG. 1. Physical map of pC194 showing four open reading frames and restriction sites for *Mbo*I, *Mbo*II, and *Taq*I, as well as for the enzymes *Msp*I, *Hae*III, *Hin*dIII, and *Cla*I. Arrows indicate open reading frames and their respective 5′ to 3′ orientations. The open reading frames are labeled alphabetically in order of decreasing size. See text for details.

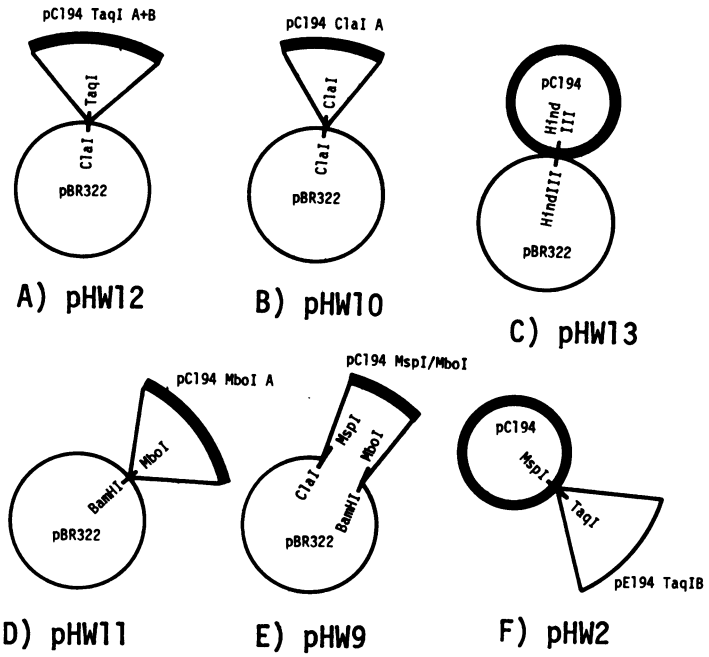


FIG. 2. Schematic summary of plasmid constructions. These include: (A) pHW12, obtained by cloning the two contiguous fragments *Taq*I A+B of pC194 into the unique *Cla*I site of pBR322, resulting in a plasmid which expresses CAM resistance and is capable of replication in *B. subtilis*; (B) pHW10, obtained by cloning pC194 *Cla*I fragment A into the *Cla*I site of pBR322, resulting in a plasmid which expresses CAM resistance and is capable of replication in *B. subtilis*; (C) pHW13, obtained by ligating full-length pC194 digested with *Hin*dIII with full-length pBR322 digested with *Hin*dIII, resulting in a plasmid capable of replication in either *E. coli* or *B. subtilis*; (D) pHW11, obtained by cloning pC194 *Mbo*I fragment A into the *Bam*HI site of pBR322, resulting in a plasmid which expresses CAM resistance and is capable of replication in *B. subtilis*; (E) pHW9, obtained by insertion of the pC194 *Msp*I-*Mbo*I fragment into pBR322 digested with *Cla*I and *Mbo*I, resulting in a plasmid which expresses inducible CAM resistance but is incapable of replication in *B. subtilis*; and (F) pHW2, obtained by insertion of pE194 *Taq*I fragment B into the unique *Msp*I site of pC194.

from *Haemophilus parainfluenzae*, but differ from other amino terminal sequences, including three from various *S. aureus* strains (24). Comparison with other amino acid residues at the amino end shows identity to CATs from either gram-positive or gram-negative organisms. Shaw et al. (19) have reported that the sequence His His Ala Val Cys (amino acid residues 192 to 196) represents part of the CAM binding site, and indeed strong conservation is seen in the comparison between the Tn9 and pC194 CAT sequences in this region. The single *Hae*III site in pC194 was found within the CAT structural gene sequence (amino acid residues 210 and 211, Arg Pro) six amino acids from the carboxy-terminus.

If our assignment regarding frame B is correct, cloned fragments containing this DNA sequence should confer resistance to CAM. Indeed, we found that the 1,035-nucleotide fragment between nucleotide residues 973 and 2008 when subcloned into pBR322 (pHW9, Fig. 2E) conferred CAM resistance on *E. coli* transformants. Moreover, the resistance phenotype was found to be inducible (Fig. 6).

Regulation of CAM resistance. pC194 was digested with selected restriction endonucleases to obtain a set of overlapping DNA fragments of increasing size covering the *Msp*I site. Digests of pC194 were ligated with pBR322 in suitable combinations (as described in the legend to Fig. 2), followed by introduction into *E. coli* by transformation, selection successively for ampicillin and CAM resistance, and screening as indicated. After checking the structure of each of these plasmids by digestion with restriction endonucleases, the plasmid DNA preparations were further tested for expression of CAM resistance in *E. coli* and ability to grow in *B. subtilis*. The results are summarized in Fig. 7.

The smallest cloned pC194 fragment (pHW9), *Msp*I-*Mbo*I, between residues 973 and 2005, specified CAM resistance in *E. coli*; pHW9 covalently closed circular DNA was readily re-

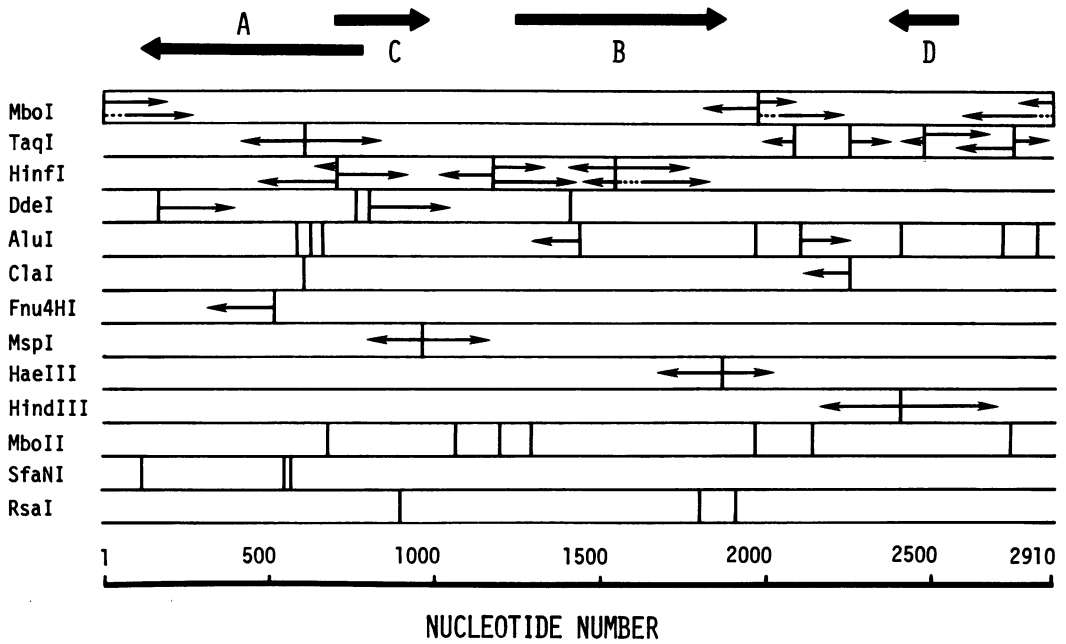


FIG. 3. Sequencing strategy for pC194. The sites used for end labeling of DNA fragments obtained by digestion with restriction endonucleases are indicated with the thinner arrows whose length and direction indicate the extent of sequence determination from these sites. Other restriction sites deduced from the sequence are also included. The thicker arrows representing open reading frames are labeled as in Fig. 1.

introduced into *E. coli* by transformation, but attempts to transform *B. subtilis* with the same DNA preparation were unsuccessful. In *E. coli*, pHW9 specified resistance to at least 20 μg of CAM per ml (Fig. 6), and expression of the resistance phenotype required induction by growth for 30 min in 0.5 μg of CAM per ml as described by Winshell and Shaw (23). Similar results have been obtained by W. V. Shaw and his associates (personal communication), using plasmid pC221, a small CAM resistance-determinant plasmid from *S. aureus* belonging to incompatibility group 4 (11).

Examination of the pC194 DNA sequence between residues 973 and 2005, the *Msp*I-*Mbo*I fragment, thus contains the entire CAT structural gene, including identifiable −35 and −10 sequences (15, 17), as well as a ribosome-loading (20) sequence similar to those found in other systems (16, 22). The results are summarized in Fig. 8. The most noteworthy feature of this region is the presence of an inverted complementary repeat sequence spanning a 37-nucleotide sequence, residues 1216 through 1252, located between the −10 and ribosome-loading sequences, which is reminiscent of the organization of the *lac* operator (5). The region which we propose as the promoter does not appear to contain any open reading frames or additional inverted complementary repeat sequences that might function as part of an attenuator.

Replication function of pC194 associated with the *Msp*I site. The single pC194 *Msp*I site (at residue 973) was located by Chang and Cohen (2) in a region essential for plasmid replication since insertion of a replicator fragment obtained by digestion of pSC101 (from *E. coli*) with *Hpa*II into the pC194 *Msp*I (*Hpa*II) site yielded plasmids capable of replication in *E. coli* but not *B. subtilis*. The *Msp*I recognition sequence in pC194 overlaps the single *Bgl*I recognition sequence (residues 967 to 977) also present. To map the DNA sequences involved in replication function, we subcloned fragments of pE194 into the *Msp*I site which permitted autonomous replication in *B. subtilis* and compared the DNA sequence surrounding the *Msp*I site of pC194 with that of the cloned pE194 subfragment.

Positive results were obtained as shown in the accompanying paper (9) by digestion of pE194 with *Taq*I (which cuts at three sites), followed by ligation of the pE194 *Taq*I digest with a pC194 *Msp*I digest, transformation of *B. subtilis*, and selection for resistance to CAM. Transformant clones obtained in this way were screened for erythromycin sensitivity, followed by preparation of covalently closed circular DNA and analysis of the DNA preparation with restriction

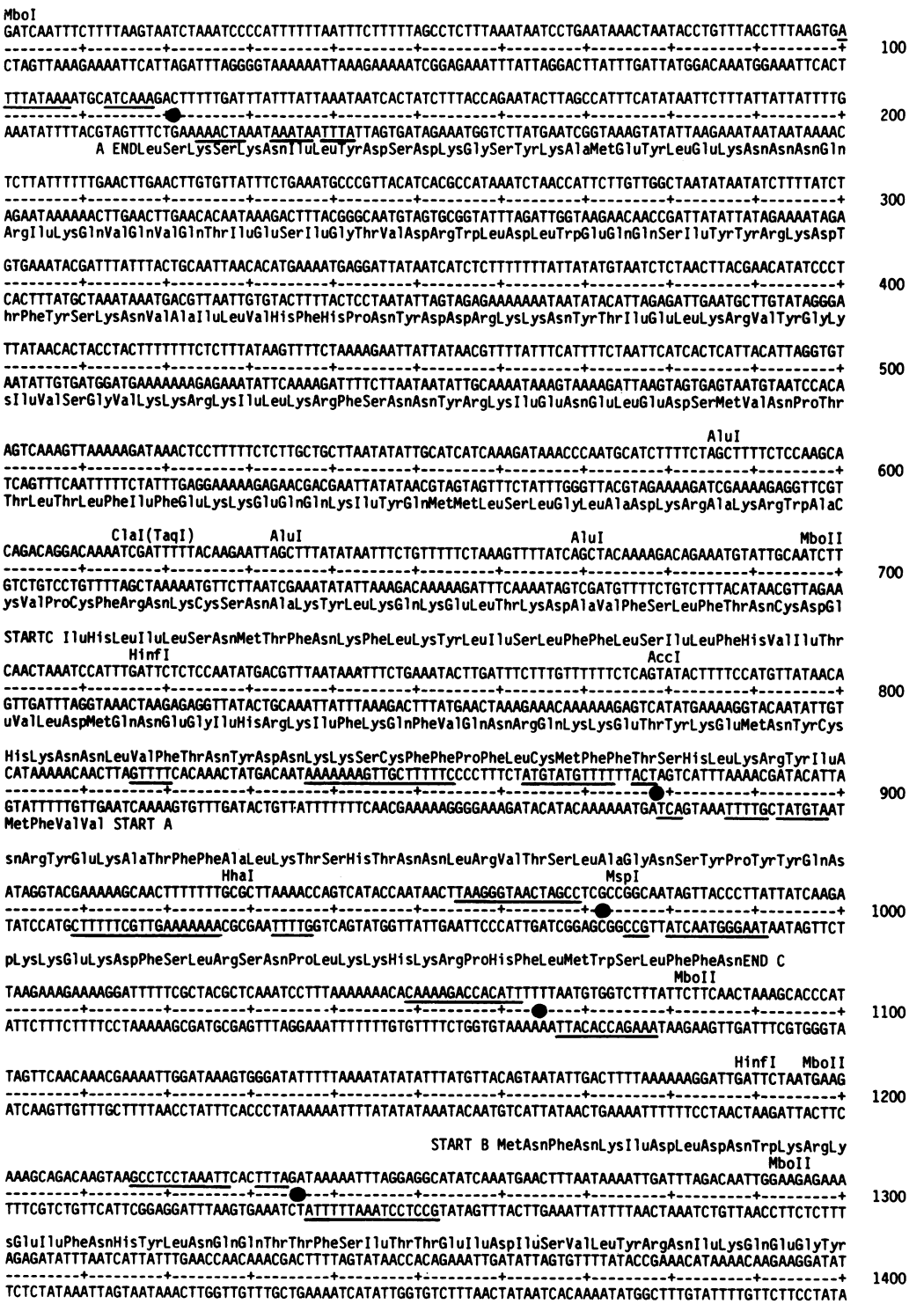


FIG. 4. The nucleotide sequence of pC194, numbered from the *Mbo*I B-A junction as reference point, is shown together with potential amino acid sequences specified by open reading frames A, B, C, and D, respectively. Also shown are the major inverted complementary repeat sequences with their respective centers of symmetry indicated by solid black circular markers.

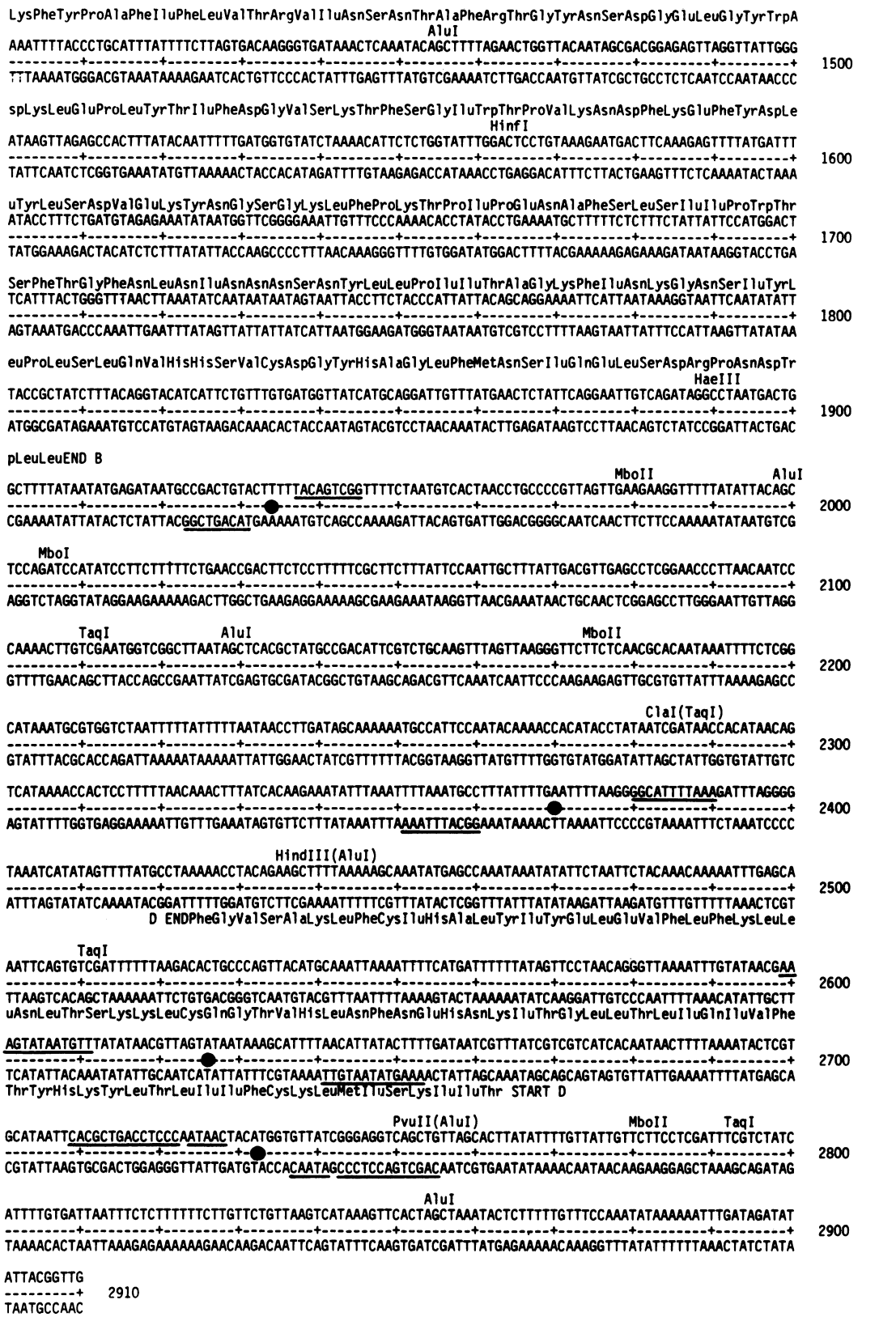


FIG. 4—*Continued*

```
          1        10        20        30        40        50        60        70        80        90       100
          :         :         :         :         :         :         :         :         :         :         :
pC194 MNFNKIDLDN'''''WKRKEIFNHYLNQ'QTTFSITTEIDISVLYRNIKQEGYKFYPAFIFLVTRVINSNTAFRTGYNSDGELGYWDKLEPLYTIFDGVSKTFSGIWTPVK
      =  ==    = === =   = =  =  ==    =  =======   =  =  ==    ====  ==   = == =   ===  =
Tn9   MEK'KITGYTTVCISQWHRKEHFEAFQSVAQCTYNQTVQLDITAFLKTVKKNKHKFYPAFIHILARLMNAHPEFRMAMK'DGELVIWDSVHPCYTVFHEQTETFSSLWSEYH
          :         :         :         :         :         :         :         :         :         :         :
          1        10        20        30        40        50        60        70        80        90       100       110

         110       120       130       140       150       160       170       180       190       200       210   216
          :         :         :         :         :         :         :         :         :         :         :     :
pC194 NDFKEFYDLYLSDVEKYNGSGKLFPKTPIPENAFSLSIIPWTSFTGFNLNINNNSNYLLPIITAGKFINKGNSIYLPLSLQVHHSVCDGYHAGLFMNSIQELSDRPNDWLL
      ==  =  =  ==  =     ===  = == =  =  == === = ==  =  =  =  = = ==   =    ==  ==== ==== = =  =  =  =
Tn9   DDFRQFLHIYSQDVACYGENLAYFPKGFI'ENMFFVSANPWVSFTSFDLNVANMDNFFAPVFTMGKYYTQGDKVLMPLAIQVHHAVCDGFHVGRMLNELQNYCDEWQGGA
          :         :         :         :         :         :         :         :         :         :         :     :
         120       130       140       150       160       170       180       190       200       210       219
```

FIG. 5. Comparison of *S. aureus* (pC194) and *E. coli* (Tn9) CATs. The amino acid sequence of CAT deduced from the nucleotide sequence of pC194 is compared with the *E. coli* CAT sequence determined both chemically (19) and deduced from the nucleotide sequence of Tn*9* (1). The pC194 CAT sequence contains 216 amino acid residues, whereas that of Tn*9* contains 219. If the two sequences are aligned as shown, 83 amino acid residues are identical. The symbol "'" indicates spaces added to bring the two amino acid sequences into alignment. Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

endonucleases. One such transformant, pHW2, was found to contain pE194 *Taq*I fragment B only. We conclude that some sequence(s) in the cloned pE194 *Taq*I fragment B could support or replace replication function in pC194 and might therefore bear some structural similarity to the region surrounding the pC194 *Msp*I site. Whether the inserted *Taq*I fragment B can function in both possible orientations has not been determined.

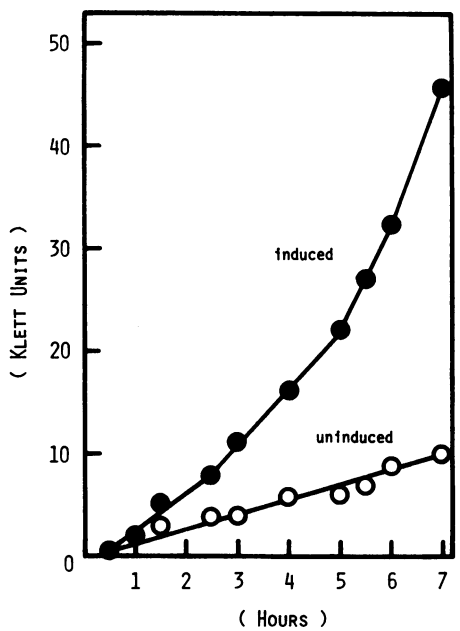
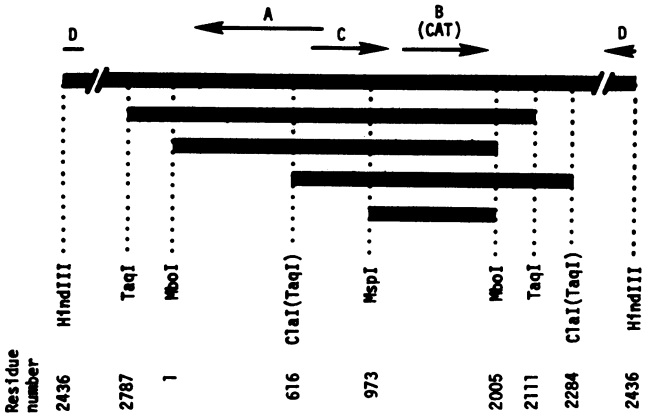


FIG. 6. Inducibility of CAM resistance in *E. coli* carrying pHW9. Conditions used for induction were based on those devised by Winshell and Shaw (23). An early-log-phase culture of *E. coli* cells carrying pHW9 was induced by addition of CAM to the growth medium (final concentration, 0.5 μg/ml). After incubation for 30 min, additional CAM was added to a final concentration of 20 μg/ml, and growth was followed by measurement of turbidity using a Klett photometer. The turbidity of the induced culture and of an uninduced control are plotted as a function of time.

To define the bounds of a functional unit of pC194 replication in terms of our sequence determination around the *Msp*I site, we constructed a set of plasmids using pBR322 as host vector into which a series of pC194 fragments of increasing size which contain the *Msp*I site were inserted. Recombinant plasmids were selected in *E. coli* using pBR322 resistance markers (resistance to ampicillin or tetracycline) plus CAM resistance, followed by a check of plasmid structure by agarose and polyacrylamide gel electrophoretic analysis of appropriate restriction endonuclease digests, preparation of covalently closed circular DNA, and an attempt to introduce the recombinant plasmid into *B. subtilis* by transformation and selection for CAM resistance. The results are summarized in Fig. 7.

Whereas pHW9 cannot replicate in *B. subtilis*, pHW10 which contains pC194 *Cla*I fragment A, residues 616 to 2284, can replicate. The *Mbo*I-*Cla*I sequence, residues 2005 to 2284, may have no direct relation to the replication function, since pHW11 which lacks this sequence and pHW12 in which the sequence is interrupted at residue 2111 can also replicate in *B. subtilis*.

The ability of pHW10 to replicate in *B. subtilis*, in spite of the fact that most of open reading frame A, i.e., residues 614 to 116, have been deleted, suggests that a polypeptide product encoded by this open reading frame is not required for replication in *B. subtilis*. This is also true for pHW13, where interruption of open reading frame D at its single *Hin*dIII site (residue 2436) has no obvious effect on the ability of the pC194-pBR322 composite plasmid to replicate in



PLASMID	REPLICATION IN B.subtilis	CHLORAMPHENICOL RESISTANCE
pHW13	YES	YES
pHW12	YES	YES
pHW11	YES	YES
pHW10	YES	YES
pHW9	NO	YES

FIG. 7. Schematic diagram summarizing properties of pC194-pBR322 composite plasmids. Plasmids constructed as described in Fig. 2 by insertion of the indicated pC194 fragments into pBR322 were tested for ability to express CAM resistance (in either *B. subtilis* or *E. coli*) and the ability to replicate in *B. subtilis*.

B. subtilis. These findings are consistent with results reported by Goze and Ehrlich (6) using pC194-pBR322 composite plasmids, whose studies suggested that pC194-encoded proteins were not required for replication.

In view of the fact that pE194 *Taq*I fragment B could be cloned into the pC194 *Msp*I site with retention of ability to replicate in *B. subtilis* (9), we searched for possible sequence similarity between the region immediately surrounding the *Msp*I site and some part of pE194 *Taq*I fragment B. We therefore compared the two sequences and found a striking similarity (Fig. 9). The pC194 DNA sequence (residues 816 to 937) contains a 121-nucleotide inverted complementary repeat region which resembles in its organization a comparably long 124-nucleotide inverted complementary repeat region (residues 656 to 780) in pE194 *Taq*I fragment B. Both sets of repeat sequences appear to have a neighboring guanine-cytosine-rich inverted complementary repeat sequence, which in the case of pC194 contains the *Msp*I site (residue 973), whose integrity is required for replication.

In addition to their symmetry properties, both sets of replication-associated sequences overlap open reading frames (pC194 frame C [126 amino acid residues] and pE194 frame D [105 amino acid residues]), which might encode polypeptides required for replication. The function of these potential coding sequences as well as their possible roles as priming sites for RNA or DNA synthesis associated with replication, or as template sequences for transcription products, remains to be tested.

DISCUSSION

To correlate structure with function in pC194, we determined the complete nucleotide sequence and compared interpretations based on examination of the sequence with experimental results derived from cloning studies in which pC194 and pE194 subfragments obtained by digestion with restriction endonucleases were used. Four open reading frames in pC194 were identified which could potentially encode polypeptides at least 100 amino acid residues in length; one of these, reading frame B, specifies the CAM resistance determinant of pC194. In previous studies of pC194-coded peptides, Shivakumar et al. (21), using a minicell system, reported that a major polypeptide product with molecular mass (based on electrophoretic mobility) estimated at 22,000 daltons was synthesized in a CAM-dependent fashion. This would correspond to the CAT deduced from the DNA sequence which would have a predicted molecular mass of 25,900 daltons. A second small peptide observed by Shivakumar et al. (21) with a molecular mass of 11,000 daltons could be the

FIG. 8. The CAT promoter and regulator sequences showing putative RNA polymerase recognition and binding sites (−35, −10) as well as the 37-nucleotide inverted complementary repeat sequence postulated to act as a regulatory element (operator) in the control of CAT expression, and the ribosome-loading sequence GGAGG (SD).

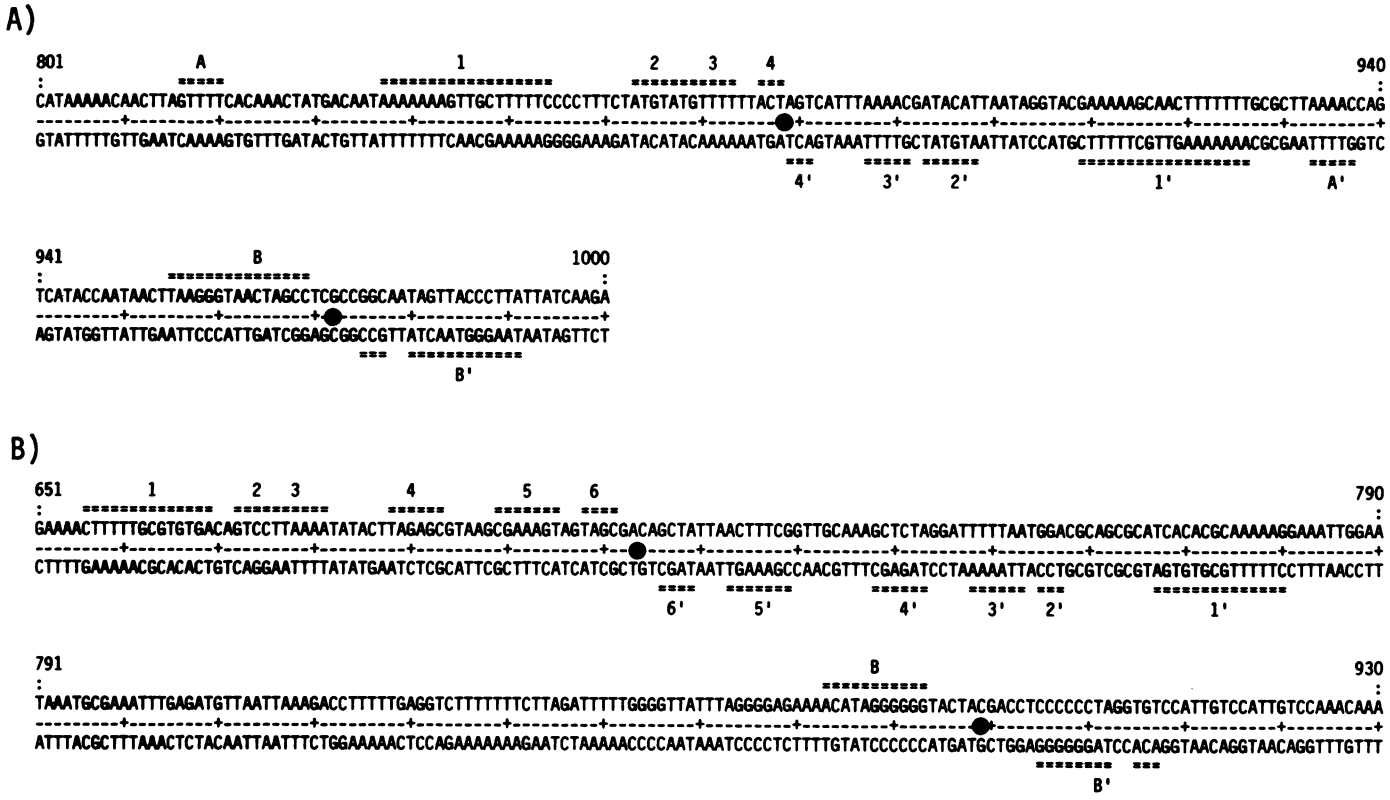


FIG. 9. Comparison of the inverted complementary repeat sequences of pC194 and pE194 associated with replication function. Solid black circular markers indicate centers of symmetry. (A) pC194 residues 801 to 1000, and (B) pE194 residues 651 to 930. For both pE194 and pC194, a pair of inverted complementary repeat regions are shown. The two longer sets of the pair are labeled numerically to indicate corresponding complementary elements. The larger of the two symmetric regions comprises, respectively, 121 bases for pC194 (residues 937 to 816) and 124 bases for pE194 (residues 656 to 780). For both large sequences, a flanking set of relatively longer inverted complementary repeat sequences labeled 1 and 1′ are shown. Additional complementary sequences centering on the same axis are labeled sequentially in similar fashion. A second neighboring smaller set of inverted complementary repeat sequences with their own symmetric centers labeled B and B′ are also shown. A and A′ designate part of the larger set of inverted complementary repeat sequences present in the pC194 sequence. The critical *Msp*I site of pC194 located in the BB′ set begins at residue 973.

product of pC194 open reading frames C or D; a definitive correlation has not yet been established.

Each of the four open reading frames in pC194 contains a methionine residue within several residues from the beginning of the respective frame which could serve as the start codon for that frame. Thus the A, B, C, and D reading frames starting at residues 812, 1248, 708, and 2663, respectively, have methionine codons at residues 791 or 713, 1260, 729, and 2645, respectively. It is pertinent to ask, in the cases of pC194 reading frames A and D, which appear capable of encoding polypeptides with molecular masses of 27,000 and 8,800 daltons, respectively, whether they too are synthesized. The potential translation products of these reading frames appear to be dispensable since interruption of these reading frames at the unique *Hin*dIII site or at the *Cla*I site (at nucleotide 616) by ligation to pBR322 did not appear to alter ability to replicate in *B. subtilis*. In the case of frame D, it is possible that a polypeptide encoded by this region begins with the methionine codon, ATG, starting at residue 2704 and that the sequence GGAGG starting at residue 2721 serves as the ribosome loading site for the frame D product.

Formally, the open reading frames are found by computer analysis as sequences uninterrupted by stop codons; such sequences do not always start with Met codons. The precise nucleotide residue at which translation is initiated requires direct evidence based on peptide analysis. In the case of the frame B product, we infer that the Met codon at residue 1260 is probably used because of the similarity with Tn*9*-associated CAT and the proximity of the sequence GGAGG at residue 1247, capable of serving as a ribosome-loading site (20).

The presence of a 37-nucleotide inverted repeat sequence interposed between the −10 and ribosome-loading sites of the CAT determinant suggests that this sequence may play a role in the regulation of inducible resistance. It is our working hypothesis, by analogy to the sequence organization of the *lac* operon (5), that this inverted repeat sequence serves as the binding site for a regulatory protein, and our cloning studies suggest that the regulator is CAT itself.

If CAT functions as a negative regulatory element, we would expect to find a class of CATs altered by mutation, in which the altered CAT was overproduced. We are currently testing this possibility by examination of the level of polypeptide products synthesized by altered CAT structural genes. We find that *S. aureus* carrying pC194 treated with rifampin (0.05 μg/ml) during a 30-min period of induction with CAM (0.5 μg/ml) remain uninduced, whereas viability is only partially affected (data not shown).

In studies of incompatibility groups of the small staphylococcal plasmids, Iordanescu et al. (10, 11) have reported four different incompatibility groups for the CAM plasmids, namely, Inc 4, 8, 9, and 10, corresponding to pC221, pC194, pUB112, and pC223, respectively. Moreover, the two small erythromycin resistance plasmids pE194 and pE1764 have been assigned to incompatibility groups 11 and 12, respectively. We expect that these sequencing studies will facilitate a directed approach to plan recombinant plasmid construction which will permit identification of sequence determinants of these plasmid functions, as well.

ACKNOWLEDGMENTS

We thank W. Reznikoff and R. H. Rownd for stimulating critical discussions of this work. J. Schroeder, F. Blattner, J. Devereux, P. Haeberli, and O. Smithies were very helpful to us with computer-aided analysis of the DNA sequence. P. Jasczewski and M. Mayford provided vital technical assistance.

This research was supported by The National Science Foundation (Research Grant PCM-719390) and by research grants from The Upjohn Co., Kalamazoo, Mich., and Eli Lilly & Co., Indianapolis, Ind.

LITERATURE CITED

1. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence of the chloramphenicol resistance transposon Tn*9*. Nature (London) **282**:864–869.
2. **Chang, S., and S. N. Cohen.** 1979. High frequency transformation of *Bacillus subtilis* protoplasts by plasmid DNA. Mol. Gen. Genet. **168**:111–115.
3. **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.
4. **Ehrlich, S. D.** 1978. DNA cloning in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **75**:1433–1436.
5. **Gilbert, W., N. Maizels, and A. Maxam.** 1978. Sequences of controlling regions of the lactose operon. Cold Spring Harbor Symp. Quant. Biol. **38**:845–855.
6. **Goze, A., and S. D. Ehrlich.** 1980. Replication of plasmids from *Staphylococcus aureus* in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:7333–7337.
7. **Gryczan, T. J., and D. Dubnau.** 1978. Construction and properties of chimeric plasmids in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **75**:1428–1432.
8. **Gryczan, T., A. G. Shivakumar, and D. Dubnau.** 1980. Characterization of chimeric plasmid cloning vehicles in *Bacillus subtilis*. J. Bacteriol. **141**:246–253.
9. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **150**:804–814.
10. **Iordanescu, S., and M. Surdeanu.** 1980. New incompatibility groups for *Staphylococcus aureus* plasmids. Plasmid **4**:256–260.
11. **Iordanescu, S., M. Surdeanu, P. Della Latta, and R. Novick.** 1978. Incompatibility and molecular relationships between small staphylococcal plasmids carrying the same resistance marker. Plasmid **1**:468–479.
12. **Löfdahl, S., J. Sjöström, and L. Philipson.** 1978. Characterization of small plasmids from *Staphylococcus aureus*. Gene **3**:149–159.

13. **Löfdahl, S., J. Sjöström, and L. Philipson.** 1978. A vector for recombinant DNA in *Staphylococcus aureus*. Gene 3:161–172.
14. **Martin, P. A. W., J. R. Lohr, and D. H. Dean.** 1980. Transformation of *Bacillus thuringiensis* protoplasts by plasmid deoxyribonucleic acid. J. Bacteriol. 145:980–983.
15. **Pribnow, D.** 1975. Nucleotide sequence of an RNA polymerase binding site at an early T7 promoter. Proc. Natl. Acad. Sci. U.S.A. 72:784–788.
16. **Rosenberg, M., and D. Court.** 1980. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. 13:319–353.
17. **Schaller, H., C. Gray, and K. Herrmann.** 1975. Nucleotide sequence of an RNA polymerase binding site from the DNA of bacteriophage fd. Proc. Natl. Acad. Sci. U.S.A. 72:737–741.
18. **Shaw, W. V.** 1967. The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant *Escherichia coli*. J. Biol. Chem. 242:687–693.
19. **Shaw, W. V., L. C. Packman, B. D. Burleigh, A. Dell, H. R. Morris, and B. S. Hartley.** 1979. Primary structure of a chloramphenicol acetyltransferase specified by R plasmids. Nature (London) 282:870–872.
20. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequence of *Escherichia coli* ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. U.S.A. 71:1342–1346.
21. **Shivakumar, A. G., J. Hahn, and D. Dubnau.** 1979. Studies on the synthesis of plasmid-coded proteins and their control in *Bacillus subtilis* minicells. Plasmid 2:279–289.
22. **Siebenlist, U., R. B. Simpson, and W. Gilbert.** 1980. *E. coli* RNA polymerase interacts homologously with two different promoters. Cell 20:269–281.
23. **Winshell, E., and W. V. Shaw.** 1969. Kinetics of induction and purification of chloramphenicol acetyltransferase from chloramphenicol-resistant *Staphylococcus aureus*. J. Bacteriol. 98:1248–1257.
24. **Zaidenzaig, Y., J. E. Fitton, L. C. Packman, and W. V. Shaw.** 1979. Characterization and comparison of chloramphenicol acetyltransferase variants. Eur. J. Biochem. 100:609–618.