Region of the Streptococcal Plasmid pMV158 Required for Conjugative Mobilization

SCOTT D. PRIEBE AND SANFORD A. LACKS*

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Received 13 February 1989/Accepted 26 May 1989

The nonconjugative streptococcal plasmid pMV158 can be mobilized by the conjugative streptococcal plasmid pIP501. We determined the sequence of the 1.1-kilobase *Eco*RI fragment of pMV158 to complete the DNA sequence of the plasmid. We showed that an open reading frame, *mob* (able to encode a polypeptide of 58,020 daltons), is required for mobilization of pMV158. An intergenic region present in the *Eco*RI fragment contains four lengthy palindromes that are found also in one or more of the staphylococcal plasmids pT181, pE194, and pUB110. One palindromic sequence, *palD*, which is common to all four plasmids, also appeared to be necessary for mobilization. Circumstantial evidence indicates that this sequence contains both an *oriT* site and the *mob* promoter. The Mob protein is homologous in its amino-terminal half to Pre proteins encoded by pT181 and pE194 that were shown by others to be essential for site-specific cointegrative plasmid recombination; their main biological function may be plasmid mobilization.

A variety of conjugative and nonconjugative plasmids are found in streptococcal species (for a review, see reference 6). The conjugative plasmid pIP501 confers erythromycin resistance (Em^r) and chloramphenicol resistance (Cm^r). This 30-kilobase (kb) plasmid was found by Horodniceanu and co-workers in a strain of *Streptococcus agalactiae* (15) and shown to pass conjugatively into various streptococcal species (13). The 5.5-kb nonconjugative plasmid pMV158 confers tetracycline resistance (Tc^r); it was found by Burdett in another strain of *S. agalactiae* (5). Guild and co-workers transferred the two plasmids into *Streptococcus pneumoniae* by transformation (32). They showed that when both plasmids were present in the same cell, pIP501 was able to mobilize pMV158 for conjugative transfer. In this work, we inquired into the molecular basis of such mobilization.

A derivative of pMV158 called pLS1, which was constructed by removal of a 1.1-kb EcoRI fragment, replicates normally in S. pneumoniae (33). Both pMV158 and pLS1 also replicate in other gram-positive bacteria, such as Bacillus subtilis (11), and in gram-negative bacteria, such as Escherichia coli (20). The entire nucleotide sequence of pLS1 was determined, and genes and proteins for two surmised replication functions and for tetracycline resistance were identified (20). Several replication signals were subsequently reported (9, 28). Neither a protein nor a function was attributed to another open reading frame, called OrfD, which may have been truncated at its 5'terminal end by removal of the EcoRI fragment from pMV158. After determining the entire sequence of pMV158, we found this to be the case. Furthermore, the sequence of the EcoRI fragment showed significant homologies with portions of several other plasmids of gram-positive bacteria origin.

Novick and co-workers found that recombination between two staphylococcal plasmids, pT181 and pE194, to give a cointegrate product occurred within closely related 70-basepair (bp) sequences (called RS_A) in these plasmids (12, 25). This site-specific recombination was independent of a *recA* mutation, but it required a protein called Pre (for plasmid recombination), encoded by a plasmid gene, *pre* (12). The pre gene product of either pT181 or pE194 could promote recombination, and the pT181 protein was shown to act in *trans*, that is, when the active *pre* gene was present on a plasmid not participating in the cointegrate formation (12). The Pre proteins predicted from the DNA sequence of pT181 and pE194 appeared to be homologous, and it was pointed out that another staphylococcal plasmid, pUB110, contains a sequence identifical to part of RS_A and encodes a protein with a sequence similar to that of Pre (12). It appeared to us that if the Pre-mediated recombination was due to nicking at the RS_A site, then the primary function of the *pre* system could be conjugative mobilization of the plasmid that encodes it.

Inasmuch as the 1.1-kb *Eco*RI segment of pMV158 was found to share considerable homology with pT181 and pE194 in the vicinity of *pre*, we examined the role of this region of pMV158 in conjugative mobilization. Various derivatives of pMV158 were tested for their abilities to be mobilized by pIP501.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains of *S. pneumoniae* used are derivatives of the nonencapsulated wild-type strain R6 or Rx. Novobiocin resistance (Nov^r) and streptomycin resistance (Str^r) markers were introduced into the Rx derivative strain 778 (*malDXMP581*) by transformation with DNA from strain 533 (*sul str nov ery*) to give strains 1131 and 1132, respectively. The plasmid used for DNA sequencing was pLS141 (2), which contains the entire sequence of pMV158. Plasmid pIP501 was kindly provided by W. Guild in strain DP3201 and passed into strain 1131 by conjugation. Plasmids pMV158 (5), pLS1 (33), and the derivatives made in the present work were transferred into strain 1131(pIP501) by transformation. The source of the *Dpn*II fragment containing the *cat* gene was pJS3 (3) grown in *S. pneumoniae* 708.

Growth, transformation, and conjugation of bacteria. Cultures of *S. pneumoniae* were grown with sucrose in semisynthetic medium (19) and transformed as previously described (1). Transformants or transconjugants were selected in 1% agar medium with novobiocin at 10 μ g/ml, streptomycin at 100 μ g/ml, erythromycin at 1 μ g/ml, tetracycline at 1 μ g/ml, and chloramphenicol at 2.5 μ g/ml.

^{*} Corresponding author.



FIG. 1. Functional map of pMV158 and derivatives altered in the mobilization region. Restriction maps are shown linearized at a *PstI* site. Symbols: \Box , coding regions of open reading frames; r^{\bullet} , direction of transcription from putative promoters; *pal*: *A B C D*, inverted repeat sequences within the 1.1-kb EcoRI fragment; ---, deleted portions of plasmids; \bullet , loss of restriction sites by filling in of overlapping sequences; \blacktriangle , insertion of *Bam*HI linker; \triangle , *cat* gene insert. The Mob phenotype is summarized from Table 1.

For conjugal plasmid transfer, filter matings were carried out in CAT medium as described by Smith and Guild (31). A mixture of 2 ml of recipient culture and 0.2 ml of donor culture (each at 5×10^8 CFU/ml) was filtered onto sterile 13-mm filters (composed of mixed cellulose esters; GN-6; Gelman Sciences, Inc., Ann Arbor, Mich.). After the filters were embedded cell-side down in agar medium and incubated at 37°C for 4 h, they were removed and the cells were washed off with CAT medium containing 10 mM MgCl₂, bovine serum albumin at 2 mg/ml, bovine DNase I at 10 μ g/ml, and 10% (vol/vol) glycerol. The resuspended cells were stored at -70° C until plated in semisynthetic medium. After 90 min at 37°C to allow for phenotypic expression, the plates were overlaid with medium containing antibiotics to give the final concentrations indicated above.

Preparation and manipulation of plasmid DNA. Purified plasmids were prepared by the procedure of Currier and Nester (8). Crude plasmid preparations were made from S. *pneumoniae* by a modification (33) of the method of Birnboim and Doly (4).

DNA manipulations, such as cleavage with restriction endonucleases, gel electrophoresis, filling in of DNA strands by synthesis with the Klenow fragment, and ligation of DNA, were carried out by standard methods (21).

DNA sequence determination. Trace amounts of RNA were removed from plasmid samples by treatment with pancreatic RNase and gel filtration. After cleavage with restriction enzymes, the DNA fragments were treated with alkaline phosphatase and labeled at their 5' ends with $[\gamma^{-32}P]ATP$ and T4 polynucleotide kinase. Nucleotide sequences were determined by the chemical method of Maxam and Gilbert (22).

RESULTS

Conjugative mobilization of pMV158. A restriction map of pMV158 is shown in Fig. 1. Previously identified functions outside of the *Eco*RI segment (between 3.2 and 4.3 kb) are the genes *repA* and *repB*, which encode proteins presumably involved in replication; *tet*, which encodes a membrane protein that confers tetracycline resistance; and the putative promoters P_1 to P_4 (20). Origins of replication, by what appears to be a rolling-circle mechanism, of the leading plus strand (28) and the lagging minus strand (9) were also identified. These features appear to be adequate for stable

replication of the plasmid and expression of tetracycline resistance in a variety of bacterial hosts (20).

All of the features described above were present in pLS1, which lacks the 1.1-kb EcoRI fragment of pMV158. Five other constructs, which also were apparently normal in replication, are depicted in Fig. 1. Plasmid pLS199 was made by removing the 0.7-kb Tth111I-HindIII fragment, filling in the 5' extensions by extending the 3' ends with the Klenow fragment of DNA polymerase I, and ligating the blunt ends; pLS164 was made similarly by removing the 0.2-kb DraI-Tth1111 fragment; pLS198 was made by opening the plasmid with *HindIII*, filling in the overlapping ends with Klenow fragment, and ligating. To make pLS166, pMV158 was cut with *Tth*1111, the ends were made blunt as described above, and a BamHI oligodeoxynucleotide linker (5'-CGCGGATC CGCG) was ligated in to give a total insertion of 13 bp. A 1.1-kb DpnII fragment of pJS3 containing the cat gene was introduced into the BamHI site of pLS166 to give pLS167.

Plasmid pMV158 by itself is not transferred conjugatively by cell-to-cell contact, but it can be mobilized for such transfer by the presence of the conjugative plasmid pIP501 in the same cell (32). The results of such mobilization are shown in Table 1, in which it is seen that Tc^r cells resulting

 TABLE 1. Conjugative mobilization of pMV158 and its derivatives

Donor plasmids"	No. of transconjugants per 10 ⁷ donor cells ^b		Ratio of Tc ^r /Em ^r
	Em ^r	Tc ^r	transconjugants
pIP501 + pMV158	6,900	710	0.10
pIP501 + pLS1	3,100	<1	< 0.0003
pIP501 + pLS199	3,600	<1	< 0.0003
pIP501 + pLS198	36,000	<1	< 0.0001
pIP501 + pLS164	7,000	<1	< 0.0002
pIP501 + pLS166	6,400	5,000	0.78
pIP501 + pLS167	40,000	81	0.002

" Donor strain was 1131 (Nov"). pIP501 carried the $\rm Em^r$ marker; other plasmids were $\rm Tc^r.$

^b Recipient strain was 1132 (Str^r). Transconjugants were selected for Str^r and the plasmid marker; the transformation control (Nov^r Str^r) was $<10^{-6}$ in all cases.

AGGGATTTCTAAATCGTT<u>AÅGGGATCAACTT</u>GGGAGAGÅGTTCA<u>AAATTGGATCCTT</u>TTTTTATAACAGGAATTCAAATČTTTTTGTTCCATTAAAGGGC 3200 GCGATTGCTĠAAT<u>AAAAGAŤACGAGAGACĊTCTCTTGTAŤCTTT</u>TATŤTTGAGTGGTŤTTGTCCGTTÁCACTAGAAAÅCCGAAAGACÅATAAAAATTT 3300 TATTCTTGCTGAGTCTGGCTTTCGGTAAGCTAGACAAAACGGACAAAATAAAAATCAGCAAGGGACAGGTAGTATTTTTTGAGTGATCTTCTCAAAAAAT 3400 ACTACCTGTCCCTTGCTGATTTTTAAACGAGCACGAGAGCAAAACCCCCCTTTGCTGAGGTGGCAGAGGGCAGGTTTTTTTGTTTTTTCTCGTAAA 3500 <u>AAAAAGAAA</u>ĠGTCTTAAAGĠTTTTATGGTŤTTGGTCGGCÁCTGCCGACAĠCCTCGCAGAĠCACACACTŤATGAATA<u>TAÀAGTATA</u>GTGŤGT<u>TATACTTŤ</u> 3600 AAAGACAAGTCTT<u>AAGGAGG</u>GAAATCTATGAGTTACATGGTTGCAAGAATGCAGAAGATGAAAGCTGGCAATTTGGGAGGGCTTTTAAGCATAATGAAC 3800 S.D. fMetSerTyrMetValAlaArgMetGlnLysMetLysAlaGlyAsnLeuGlyGlyAlaPheLysMisAsnGlu GTGTTTTTGÅGACGCACTCÅAATAAAGATÅTAAACCCGAÅCAGGTCACAČCTCAACTACGÅGTTGACGGÅTCGTGACCGĊTCGGTGTCGTATGAAAAGCA 3900 ArgValPheGluThrHisSerAsnLysAspIleAsnProSerArgSerHisLeuAsnTyrGluLeuThrAspArgAspArgSerValSerTyrGluLysGln GATTAAAGAĊTATGTGAACĠAAAATAAGGŤTTCTAATCGÅGCAATCCGAÅAAGATGCAGŤTCTATGTGÅŤGAGTGGATTÅTCACATCTGÅTAAAGATTTT 4000 IleLysAspTyrValAsnGluAsnLysValSerAsnArgAlalleArgLysAspAlaValLeuCysAspGluTrpIleIleThrSerAspLysAspPhe TTTGAAAAGTTGGATGAGGAACAGACGAGAACGTTTTTTGAAACGGCTAAAAATTATTTTGCAGAGAACTATGGCGAGTCAAATATTGCTAATGCGAGTG 4100 PheGluLysLeuAspGluGluGluThrArgThrPhePheGluThrAlaLysAsnTyrPheAlaGluAsnTyrGlyGluSerAsnIleAlaTyrAlaSer TTCACTTGGÅTGAAAGCACĊCCTCACATGĊACATGGGAGTAGTAGTACTTTTGAGAACGGTÅAATTGTCATĊAAAAGCAATĠTTTGACCGTĠAGGAGCTAAA 4200 ValHisLeuAspGluSerThrProHisMetHisMetGlyValValProPheGluAsnGlyLysLeuSerSerLysAlaMetPheAspArgGluGluLeuLys ACATATCCAÅGAGGACTTGĊCTAGATACAŤGAGCGACCAĊGGTTTTGAGŤTGGAACGTGĠCAAGCTGAAŤAGTGAAGCTÁAGCATAAGAĊTGTAGCTGAA 4300 HislleGluGluAspLeuProArgTyrMetSerAspHisGlyPheGluLeuGluArgGlyLysLeuAsnSerGluAlaLysHisLysThrValAlaGlu TTCAAGAGGĠĊAATGGCTGÅTATGGAACTĊAAAGAGGAAĊTTCTTGAAAÅATATCATGCÀCCGCTTTTTĠTTGATGAGAĠAACAGGCGAĠTTGAACAATG 4400 PheLysArgAlaMetAlaAspMetGluLeuLysGluGuLeuCeuGluLysTyrHisAlaProLeuPheValAspGluArgThrGlyGluLeuAsnAsn ACACGGAAGCTTTTTTGGCATGAAAAAGAGTTTGCTGATATGTTTGAAGTTCAATCTCCGATACGTGAAACAACTAACCAAGGAAAAAATGGACTGGTTAAG 4500 AspThrGluAlaPheTrpHisGluLysGluPheAlaAspMetPheGluValGluSerProIleArgGluThrThrAsnGluGluLysMetAspTrpLeuArg AAAACAGTAĊCAAGAAGAGĊTGAAAAAACĊAGAATCGTCĊAAAAAGCCCĊTAGAAGACGÀTTTAAGCCAĊTTTAGAAGAGTTGCTTGATAÀAAAGACCAAG 4600 LysGlnTyrGlnGluGluLeuLysLysLeuGluSerSerLysLysProLeuGluAspAspLeuSerHisLeuGluGluLeuLeuAspLysLysThrLys GAATATATTAAAATCGATTCTGAGGCCTCTGAGAGGGCCTCAGAGCTATCTAAAGCCGAGGGATATATAAATCCCTAGAAAATCATTCGAAGAGCTTAG 4700 GluTyrIleLysIleAspSerGluAlaSerGluArgAlaSerGluLeuSerLysAlaGluGlyTyrIleAsnThrLeuGluAsnHisSerLysSerLeu AAGCGAAAATAGAGTGTTTTÄGAGAGTGATÅATCTACAATTGGAAAAACAÅAAGGCGACAÅAACTCGAAGČGAAAGCGTTĠAACGAGTGAGTGAGTGAGAGA 4800 GluAlaLysIleGluCysLeuGluSerAspAsnLeuGlnLeuGluLysGlnLysAlaThrLysLeuGluAlaLysAlaLeuAsnGluSerGluLeuArgGlu ACTAAAGCCTAAGAAGAATTTTCTAGGAAAAGAGCATTATGAGTTAAGTCCTGAACAATTTGAAGGGTTGAAGGCAGAAGTTTATCGTAGTAGAACTCTA 4900 LeuLysProLysLysAsnPheLeuG1yLysG1uHisTyrG1uLeuSerProG1uG1nPheG1uG1yLeuLysA1aG1uVa1TyrArgSerArgThrLeu TTGCACCACÀAAGATATTGÀACTGGAGCAÀGCAAAACGTĊAAGTATCTCÌGAGAGCCTCÌAAAAACTATÌTTACAGCTAĠTTTAGAGCGÀGCTAAGGAAA 5000 LeuHisHisLysAspIleGluLeuGluGInAlaLysArgGluValSerLouArgAlaSerLysAsnTyrPheThrAlaSerLeuGluArgAlaLysGlu AAGCTAAAGĠTGAGAGTATĂGACCGTCTTĂAAAGCGAAŤAAAGCGACTĂAAAACGAAĂATTCAATTTĂCGTCAGCAĂAATGACAAGĂTGCTAGGGAA 5100 LysA1aLysG1yG1uSerIleAspArgLeuLysSerG1uIleLysArgLeuLysAsnG1uAsnSerIleLeuArgG1nG1nAsnAspLysMetLeuG1yLys ATTAAGAGAĠTTAATGCCTĠATAAAGCCTÌTAAGAATTTĠTTATCAGAAĊTTAAGGCGAÌTAAGCCAATĊGTGAATATAÀTTAAAAAGGĊTATTGAAAAG 5200 LeuArgGluLeuMetProAspLysAlaPheLysAsuLeuLeuSerGluLeuLysAlaIleLysProIleValAsuIleIleLysLysAlaIleGluLys AGCTTGTTCTGAGCGATTTÅTGCCGTGAAÅGCTATTTGAČAATAAGCAGTGACAGAGTAČGCTAGGACGTGCCGAGCCGÅAAGGCTTTAGCGTTTCGGAC 5300 Sellenphe GGACACGGAČAAAGGACGGČAGTCACTGGŤTACTTGTTGŤCAAATAGACČATGGAATAAÅAAGCGTCAAÅAGTCTTGAGŤGGATGATACČCTATGGTACT 5400 CTATTCGCCTTTTGACTTTTTTGCTATAATTTAAGTGTCGCCAGTTCTTCCGCCAGGTAATGCGAACTTAGACTGGAGGTGAGCGTTGTGAAGACATTCC 5500 TCGAGCITGTCTTTGTCCCTTTTGTGGTTGGCGTTG

FIG. 2. Nucleotide sequence of pMV158 containing the mobilization region. The sequence of the 1.1-kb EcoRI fragment is inserted into the pLS1 sequence previously reported, and numbering from the *Pst1* reference site is identical to that of pLS1 until nucleotide 3176. One DNA strand is shown with its 5' end beginning at 3101 (upper left). Inverted repeats are underlined; they correspond successively to the putative *tet* transcript terminator and palindromes *palA* through *palD*. The predicted amino acid sequence of the Mob polypeptide is shown together with the Shine-Dalgarno sequence preceding it.

from conjugative transfer of pMV158 were generally found approximately 1/10 as frequently as Em^r cells resulting from conjugative transfer of pIP501. Removal of the *Eco*RI fragment to give pLS1 or the slightly smaller deletion in pLS199 abolished mobilization of the plasmid, as did the 4-bp insertion at the *Hind*III site in pLS198 (Table 1). Removal of the segment between *Dra*I and *Tth*1111 in pLS164 also blocked mobilization. However, a 13-bp insertion at the *Tth*1111 site did not interfere with mobilization of pLS166. Insertion of the *cat* gene segment, which also contained a transcription terminator at its right end (26), severely reduced but did not abolish conjugative mobilization of pLS167. These effects will be interpreted below in light of the DNA sequence of the region.

The frequency of transfer of both donor plasmids to the same recipient cell was determined for the mobilizable plasmids by selecting Em^r Tc^r transconjugants. With pMV158 this frequency was 5% of Tc^r cells, and with pLS166 it was 16% in the experiments of Table 1. These results indicate that most mobilization events of pMV158 or its derivatives neither coincide with nor require cointegration of the donor plasmids.

Nucleotide sequence of the 1.1-kb *Eco*RI segment of pMV158. The DNA sequence of the 1.1-kb *Eco*RI segment

of pMV158 was determined on both strands and with overlapping of the previously determined pLS1 portion. When the new sequence was incorporated into the nucleotidenumbering format used for pLS1 (20), the *Eco*RI recognition sites (5'-GAATTC) bordered the newly sequenced segment of pMV158 at nucleotides 3170 and 4298 (Fig. 2). Numbering of pMV158 from 1 (the distal *Pst*I site) to 3175 was identical to that of pLS1, while nucleotides 3176 to 4408 of pLS1 became 4304 to 5536 of pMV158.

At the beginning of the sequence shown in Fig. 2 are the three final codons of the *tet* gene and its stop codon at position 10. They are followed by the underlined palindrome representing the putative transcription terminator (3119 to 3157) of the *tet* mRNA, which presumably is initiated at P_4 (20). Four subsequent palindromes within the newly sequenced *Eco*RI segment occur between coordinates 3214 and 3245, 3349 and 3425, 3483 and 3509, and 3578 and 3601. They will be referred to as *palA*, *palB*, *palC*, and *palD*, respectively.

A large open reading frame is present in the right half (as drawn in Fig. 1) of the *Eco*RI segment, and it extends into what was called OrfD in pLS1 (20). The protein translated from the first ATG codon in the open reading frame would contain 492 amino acid residues, which are indicated in Fig.

рМV158: 3110-ТАЛАТССТТАЙССААСТТТСССАССТТТСССААЛТССААЛТССААЛТССААЛТС 3180 рUB110: 1583-ТАЛААТСТАТТАТ 1570

pWV158: TTTTTGTTCCATTAAAGGGGCGCGATTGCTGAAAAAAGATACGAGAGACCTCTCTTGTATCTTTTATTTTGAGTGGTTTTGTCCGTTACACTAGAAAA 3280
pUB110: AATCTGTTCAGCAATCGGGGCGCGATTGCTGAATAAAAGATACGAGAGACCTCTCTTGTATCTTTTTTTT
pE194: 2858-ATTITTTCCTCCTTATAAAAT 2879
pNV158: CCGAAAGACAATAAAAATTTTTATTCTTGCTGAGTCTGGCTTTCGGTAAGCTAGACAAAAACGGACAAAAA <u>TAAAAATCAGCAAGGGACAGGTAGT-ATT</u> 3376
pUB110: CCGAAAGACAATAAAAATTTTATTCTTGCTGAGTCTGGCTTTCGGTAAGCTAGACAAAACGGACAAAAATAAAAATTGGCAAGGGTTTAAAGGTGGAGATT 1370
pT181: 2183-TAGCCATGGCTAACCTTATTATTTAAGTTGTCTTTTGACGATTATTGTATTTAAAAACGTTCTGAAAAGCAGTTTGAATAGTTATATTAT 2094
pE194: TAGTATAATTATAGCACGAGCTCTGATAAATATGAACATGATGAGTGATCGTTAAATTTATACTGCAATCGGATGCGATTATTGAATAAAAGATATGAGA 2979
pNV158: TTTTGAGTGATCTTCTCAAAAAATACTACCTGTCCCTTGCTGATTTTTAAACGÅGCACGAGAGCACAAAACCCCCCCTTTGCTGAGGTGGCAGAGGGGCAGGGT 3476
pUB110: TTTTGAGTGATCTTCTCAAAAAATACTACCTGTCCCTTGCTGATTTTTAAACGAGCACGAGAGCAAAAACCCCCCTTTGCTGAGGTGGCAGAGGGCAGGTT 1270
pT181: ATTTTGGTTTAGAACTATGAGTGGCTAGCATTTTGCCACTCATTTTTTGCGTTAGCAAAAAACAGGTTTAAGCCTCGCAGAGCACACGTATTAACGACTTA 1994
pE194: GATITATCTAATITCTTTTTTTTTTTTTGTAAAAAA-GAAAGTTCTTAAAGGTTTTATAGTTTTTGGTCGTAGAGCACACGGCTTTAACGACTTA 3068
pWV158: TTTTTGTTTCTTTTTTCTCGTAAAAAAAGAAAGGTCTTAAAGGTCTTTATGGTTTTGGTCGGCACTGCCGACAGCCTCGCAGAGCACACACTTTA 3571
pUB110: TTTTTGTTTCTTTTTTCCCGTAAAAAAAAGAAAGGTCTTAAAGGTTTTATGGTTTTGGTCGGCACTGCCGACAGCCCCGCAGAGCACACCTTTA 1175
$ {}_{p} {}_{181:}TTATAAAATAAGTCTAGTGTGTGTTAGAGTCTAAAAACTATTAAAAAACCTTTGTGCTTAGGAGTGATTTTTAT 1918 } $
pE194: ATTACGAAGTAAAT <u>AAGTCTA</u> GTGTGT <u>TAGACTT</u> TATGAAATCTATATACGTTTATATATATTTATTATCCGGAGGTGTAGC 3150
pWV158:TGAATATAAGTATAGTOTGTTATACTTTACATGGAAGTTATACCGAAATTGTGCTAAACTACCAGTTAAGATGTTGCTGATTAAGACGAGCAA 3665
pUB110:TGAATA <u>TAAAGTATA</u> GTGTGT <u>TATACTTTA</u> CTTGGAAGTGGTTGCCGGAAAGAGCGAAAATGCCTCACATTTGTGCCACCTAAAAAGGAGCGAT 1081
PMV158: TAGACTGTGTCGGCTCGTCTGTCTTTCGGTAATCGAAAGACAAGTCTTAAGGAGGGAAATCT 3727
pUB110: TTACAT 1075

FIG. 3. Comparison of DNA sequences 5' to putative mobilization genes in plasmids from gram-positive bacteria. Segments shown are from the DNA strand corresponding to the mRNA; they begin (upper left) just past the start or end of the nearest upstream coding region, and they terminate (lower right) just before the *mob* (or *pre*) gene. Their polarity is 5' to 3' from left to right. The plasmids are numbered according to the following references: pMV158 (this work), pT181 (18), pE194 (14), and pUB110 (23, 24). Dots over the pMV158 sequence indicate 10-bp intervals in this plasmid. Asterisks indicate pairwise identity of bases. Inverted repeat sequences corresponding to *palA*, *palB*, *palC*, and *palD* are underlined. The start site of pT181 *pre* mRNA (12) is shown by the exclamation point, and its putative promoter is overlined. The dotted underline corresponds to the membrane-binding fragment BA3 of pUB110 (23). Dashes within the sequence represent breaks made in the alignment to maximize similarity.

2. Upstream from the start site is an excellent potential ribosome-binding sequence in which seven contiguous bases are complementary to 16S rRNA (30). The 4-bp insertion in pLS198 interrupts the gene after nucleotide 4411, which alters the protein after amino acid residue 226 and terminates the protein at a stop codon four residues downstream. Inasmuch as this alteration blocked conjugative transfer of the pMV158 derivative (Table 1), the protein product of this gene must be required for conjugative mobilization. Therefore, we called the gene *mob* and its protein product Mob.

The potentially frameshifting insertion in pLS166 did not affect mobilization, presumably because the site of insertion was upstream from the *mob* structural gene. However, the severe reduction caused by the *cat* insert in the same site can be attributed to termination of the *mob* transcript by the *cat* terminator. This implies that the *mob* promoter lies upstream from the *Tth*1111 site. Such a location could explain the absence of mobilization of pLS164, in which that upstream region is deleted.

Examination of the DNA sequence 5' to the *mob* gene for possible promoter sequences reveals the first potential -10promoter site (not differring by more than one base from the procaryotic consensus 5'-TATAAT; 29) at nucleotides 3593 to 3598. This putative promoter site fell within *palD*, a palindrome that is homologous to a palindrome present upstream from the *pre* gene in both pT181 and pE194, as shown below. The latter palindrome corresponds to the conserved "core" of the RS_A sites at which site-specific recombination mediated by the pT181 Pre protein occurs (12, 25). The -10 site of the promoter for transcription of the pT181 *pre* gene falls within this core palindrome (12).

Homology of pMV158 to other plasmids. Comparison of the pLS1 portion of pMV158 to pT181 previously revealed homology between the *tet* genes and an adjacent controlling element but no similarity outside the tet region (20). However, the EcoRI segment of pMV158 showed significant similarity to pT181 in two places: in the mob gene, as discussed below, and in palD (Fig. 3). In Fig. 3, segments of four plasmids, which correspond in each case to the stretch of DNA between the gene upstream and the mob gene or its homolog, are aligned to maximize pairwise correspondence. The extensive correspondence between the RS_A sites of pT181 and pE194 is evident at the 3' end of the stretch. Also apparent is the similarity of the *palD* region to the core region of the RS_A sites. Although the RS_A palindromes are slightly shorter than palD, their arms differ by only single bases, and their central portions are identical. The initiation site for transcription of pre mRNA from pT181 and its putative promoter (12) are indicated in Fig. 3. Although pMV158 and pUB110 have putative -10 promoter sites that are even closer to the consensus than pT181, none of the putative -35 sites are close to the consensus (TTGACA; 29), and the plasmids diverge in sequence at this point. It may be that these promoters lack a -35 RNA polymerase-binding site.

In addition to the homology at *palD*, pMV158 and pE194 showed a nearly perfect match over a 55-bp sequence that includes *palC* (Fig. 3). The greatest homology observed in

pMV158:	MSYMVARMQKMKAGNLG-GAFKHNERVFETHSNKDINPSRSHLNYELTDRDRSVSYEKQIKDYVNENKVSNRAIRKDAVLCDEWIITSDKDFFEKLDEEQ	99
pUB110:	ŃŚŸAŸĊŔŃQŔŸŔŚĂĢĹĸ–ĠMQFĖŇQŔERKŚŔŤŇDĎĬĎHEŔŤŘEŇŶĎĹKŇ–ĎŔŇĬĎŸŇERŸŔĔĬĬĖŚQŔŦĢŦŔKŦŔŔĎĂŸĹVŇĔĹĽŸŤŚĎŔĎĔŕĔQĹĎŖĠĖ	98
pT181:	MSYSİVRŸSKVKSĞTNTTĞIQKHVQRENNNYENEDIDHSKTYLNYDLVN-ANKQNFNNLIDEKIEQNYTĞKRKIRTDAİKHIDĞLİTSDNDFFDNQTPED	99
pE194:	MSHSILRVARVKGSSNTNGIQRHNQRENKNYNNKDINHEETYKNYDLIN-AQNIKYKDKIDETIDENYSGKRKIRSDAIRHVDGLVTSDKDFFDDLSGEE	99
•		
pMV158:	ŤRTFFETAKNÝFAENYGESNÍ AYASVHLDEŠTPHMHMGVVPFEN-GKLSSKÁMF-DREELKHÍ QEDLPRYMSDHGFELERGKLNSEAKHKTVÁKRAMA	195
pUB110:	QKRĚFĚESYKLĚSĚRÝČKONÍ ÁŽÁTVENDĚQTPHMILČVVPMRD-ČKLQGKNVĚ-ŇŘČELLVLÓDKĚPEHMKKQČĚELKŘČERGŠDRKH I ETÁKFKKQ	194
pT181:	TKQFFEYAKEFLEQEYGKDNLLYATVHMDEKTPHMHYGVVPITDDGRLSÅKEVVGNKKALTAFQDRFNEHVKQRGYGLERGQSRQVTNAKHEQISQYKQK	199
- nE194:	I ERFFKD SLEFLENEYGKENMLYATVHLDERVPHMHFGFVPLTEDGRLSAKEOLGNKKDFTOLODRFNEYVNEKGYELERGTSKEVTEREHKAMDOYKKD	199
P === · · ·		
pMV158:	DMELKEELLEKYHAPLFVDERTGELNNDTEAFWHEKEFADMFEVQSPIRETTNQEKMDWLRKQYQEELKKLESSKKPLEDDLSHLEELLDKKTKEYIKID	295
pUB110:	ŢĹĖĸĿĮĎŗĹĖĸŊĹĂ–ŸĸĸĎĖŴŢĂ–ŸSĎĸVĸSDĹEVPAĸŖĦŃĸŚVĖVŶŢĠĔĸŚMŗĢĿĢĸĿĬMŔŢĔĸĸĸŖŢĸŊŶvĬŚĔŔĎŸĸŇĿŸŢAAŖĎŊŊŖĿŔQĤVŖŊĿŃS	292
pT181:	Ť-ĚYHKQEÝĚŘĚSQKTDHIKQKNDKLMQEYQKSLNTLKKPINVPYEQETĚKVGGLFSKĚIQEAGNVVISQKDFNĚFQKQIKAAQDISEDYEYIKSGRA	296
- рЕ194:	T-VFHKQELQEVKDELQKANKQLQSGIEHMRSTKPFDYENER-TGLFSGR-EETGRKILTADEFERLQETI-SSAERIVDDYENIKSTDY	286
1		
pMV158:	SEASERASELSKAEGYINTLENHSKSLEAKIECLESDNLQLEKQKATKLEAKALNESELRE	356
pUB110:	TDMAREYKKLSKEHGQVKEKY SGLVERFNENVNDYNELLEENKSLKSKI SDLKRDVSLIYESTKEFLKERTDGLKAFKNVFKGFVDKVKDKTAQFQEKHD	392
pT181:	LÖDKDKEI ŘEKDŮLLŇK AVERI EN AĎDNFŇQĽYĖN AKPLKENI E I ALKLLKILLKELERVLGRNTF AEŘVNKL TEDĚPKLŇGL AGNL	383
DE194:	YTENGELKKRRESL	368
-		
pMV158:	LKPKKNFLGKEHYELSPEQFEGLKAEVYRSRTLLHHKDIELEQAKRQVSLRASKNYFTASLERÅKEKAKGESIDRLKSEIKRLKNENSILRQQNDKMLGK	456
pUB110:	LEPKKNEFELTHNREVKKERSRDQGMSL 420	
pT181:	DKKMNPELYSEQEQQQEQQKNQKRDRGMHL 413	
DE194:	DSKFERVGÖFMDVVÖDNVÖKVDRKREKORTDDLEM 403	
nMV158:	LRELMPDKAFKNLLSELKAIKPIŇNIKKAIEKŠLF 492	

FIG. 4. Comparison of putative mobilization proteins encoded by plasmids of gram-positive bacteria. Amino acid sequences are shown from the amino termini (upper left) to the carboxyl termini (lower right). They are aligned to give maximum correspondence. Dashes indicate gaps produced by this alignment in one or the other sequence. Numbers at right correspond to positions in the individual polypeptide sequences. Dots over only the pMV158 Mob sequence indicate every 10th residue. For pairwise comparisons, two dots indicate identical residues and one dot indicates amino acid residues with similar properties.

these intergenic regions, however, was between pMV158 and pUB110. The two plasmids shared a nearly identical stretch of 418 bp. This stretch included *palA* and *palB*. Curiously, most of the differences in this stretch occurred in the 5' arm of *palB*. In pMV158, the perfect inverted repeat of *palB* was 35 bp in length. We have no hint of the significance of this remarkable structure. The dotted underline of pUB110 in Fig. 3 indicates a fragment of DNA (BA3) that can bind to cellular membranes in vitro under low-salt conditions (23).

Considerations of sequence conservation among the plasmids, and of inverse repeat structure in the intergenic DNA of pMV158, suggest that this region may include as many as five distinct functional portions. Proceeding from 5' to 3' (Fig. 3), these potentially functional portions correspond to the following: (i) palA, (ii) a putative membrane binding site, (iii) palB, (iv) a sequence including palC common to three plasmids, and (v) the palD sequence common to all four. The last was shown in pT181 and pE194 to be a focus of recombinatory events (12).

Homology of Mob and Pre proteins. The genes located downstream from the *palD* sequence or its homologs in the different plasmids encoded proteins containing 403 to 492 residues. The Mob protein of pMV158 is homologous to the corresponding protein of pUB110 (β open reading frame; 23, 24) and to the Pre proteins of pT181 and pE194 (previously shown to be homologous to each other; 12), at least with respect to the amino-terminal halves of the proteins (Fig. 4). Comparison of the entire protein sequences by the ALIGN program obtained from the Protein Identification Resource (Georgetown University, Washington, D.C.) showed the pMV158 protein to be most closely related to the protein from pUB110, less closely related to that from pT181, and least closely related to that from pE194. The pUB110 protein was equidistant from the other three, and the pT181 and pE194 proteins showed the greatest similarity. The Mob protein of pMV158 showed 45% identity of residues with the amino-terminal half of the pUB110 protein and 14% identity with the carboxyl-terminal half. Corresponding values for the Pre protein of pT181 were 35 and 3%.

DISCUSSION

Studies of conjugative plasmid transfer in *E. coli*, principally with the sex factor F, showed that transfer is initiated when a plasmid-encoded protein nicks the DNA at a specific site, called *oriT* (for a review, see reference 36). Beginning at the nicked site, a single DNA strand is transferred into the recipient cell by a process involving a number of plasmid *tra* gene products. Small plasmids that cannot mediate their own transfer, such as ColE1, can be mobilized when a mobilization protein (Mob), which is encoded by the small plasmid, nicks at an *oriT* site in the plasmid (35). Transfer in this case also requires products of a conjugative plasmid present in the donor cell. Mobilizable plasmids sometimes form a "relaxation complex." in which the Mob protein binds to the DNA; if the protein is brusquely altered by treatment with chaotropic agents, it can leave the plasmid nicked (7).

Comparison of *oriT* sites from six gram-negative bacterial plasmids (two conjugative plasmids, F and RK-2, and four nonconjugative ones, ColE1, CloDF13, pSC101, and RSF1010) showed them all to contain at least one palindrome

in the vicinity of the nick site (36). However, the arrangements of the palindromes and the nucleotide sequences around the sites were distinctly different. In contrast, the putative *oriT* sites in the four gram-positive plasmids considered in Fig. 3 are virtually identical. Their *palD* sequences are distinct from the *oriT* sites of all of the gram-negative plasmids discussed above. Inasmuch as pIP501 failed to mobilize pLS198, in which only the Mob protein is altered, we expect that the mobilization protein of pIP501, and hence its *oriT* site, would be different from that of pMV158.

A group of five staphylococcal plasmids has been shown by Projan and Novick (27) to share a functional gene order not unlike that of pMV158 (Fig. 1), that is, (+)ori, copy control region, replication protein gene, antibiotic resistance gene, *pre* or *mob* gene, and (-)ori. Three of these staphylococcal plasmids form relaxation complexes and contain *mob* genes that are necessary for their conjugative mobilization (27). The other two, pT181 and pE194, contain the *pre* elements which, because of their homology to pMV158, we believe to be also responsible for plasmid mobilization. Aside from the *palD*-RS_A and Mob-Pre similarities and the previously demonstrated homology of the *tet* genes in the two plasmids, pMV158 and pT181 do not appear to be closely related.

It is generally believed that recombinatory events are initiated by a nick in at least one strand of DNA. With respect to cointegrate formation by pT181 and pE194, inasmuch as the strand junctions are predominantly within the core sequence of RS_A (12, 25), it is reasonable to assume that nicks occur within this sequence. We suggest that these nicks are produced by Pre in the first step of a pathway normally used for mobilization of the plasmid for conjugative transfer, which could occur only in the presence of a plasmid providing other tra functions. Thus, recombination to give cointegrates may be an incidental result of the system. An analogous situation is the promotion of cointegrate formation between pBR322 and phage lambda by E. coli DNA gyrase (17). Recombination at oriT sites can also occur during conjugative transfer of plasmids in gram-negative species (16; see also reference 10 and references therein). Although recombination mediated by Pre may have survival value in abetting the evolution of plasmids, as suggested by Gennaro et al. (12), the role of plasmid mobilization in survival of both plasmids and bacteria is obvious. In further discussion, therefore, we shall refer to the Pre proteins as Mob.

Arguing from homology, we propose that in pMV158, the corresponding *palD* sequence, which we shall tentatively call *oriT*, is nicked by Mob. The process of conjugative transfer would then require other functions provided by pIP501 or similar conjugative plasmids. Alternatively, other proteins provided in *trans* may be needed even to render the nicking irreversible. It is interesting that the putative *mob* promoter (P_5 in Fig. 1) coincides with the *oriT* site. Binding of the Mob protein may prevent transcription, thereby allowing Mob to regulate its own synthesis. Such autoregulation is a reasonable mechanism, because no more than one protein molecule at *oriT* would be needed for nicking. This Mob protein molecule could be a dimer, since its putative binding site is palindromic.

Our results relating insertions and deletions in the *mob* region of pMV158 to mobilizability of the plasmid derivatives were entirely consistent with the mechanism described above. Alteration of the *mob* structural gene by a frameshift insertion, as in pLS198, or by deletion, as in pLS1 or pLS199, prevented mobilization. Deletion of the *oriT*-cumpromoter sequence in pLS164 also blocked mobilization. A frameshift insertion between the *mob* promoter and structural gene, as in pLS166, did not affect mobilization, but introduction at that point of a transcription terminator (along with the *cat* gene) severely reduced mobilization in pLS167. The residual mobilizability of the latter plasmid probably resulted from some transcription continuing past this terminator. The effect of the *cat* terminator has been shown to be incomplete in another system also (26).

Because of the homology of the putative *oriT* sites and of the amino-terminal halves of the Mob proteins, we propose that pMV158, pT181, pE194, and pUB110 all have similar mobilization systems. The amino-terminal domains of the proteins may be responsible for recognizing and nicking at *oriT*. Since that site has been conserved, the protein domain has also been conserved. The carboxyl-terminal half of the Mob proteins may interact with membrane structures or other products of the conjugative plasmid *tra* system. Since these may be specific for particular conjugative plasmids, the carboxyl-terminal domains may have diverged more rapidly. Also, the Mob protein could represent a fusion of two independently evolved ancestral proteins. It would be interesting to see whether pT181, pE194, or pUB110 can be mobilized by a conjugative staphylococcal plasmid.

Since conjugation involves transfer of DNA through the cell membrane, the presence of DNA that can bind to the membrane in the vicinity of the mobilization region in both pMV158 and pUB110 may be significant. BA3, which is only one of four fragments of the pUB110 genome that can bind to cell membranes (34), occurs in this position. With the exception of 9 bp (all in the 5' arm of *palB*, which is at the 3' end of BA3), the entire BA3 membrane-binding sequence is present in pMV158 (Fig. 3). This includes palA, but the relevance of the palindrome for binding is unclear, since the membrane binding site may not cover the whole BA3 sequence. However, cleavage at the HinfI site (GAGTC) at nucleotide 1439 in pUB110 does eliminate binding (34). More-precise information on the functions of various elements in the mobilization region of pMV158 will be sought by further dissection of the region.

ACKNOWLEDGMENTS

We appreciate the expert assistance of Bill Greenberg in determining the DNA sequence reported in this work. We thank Noboru Sueoka for providing the sequence of pUB110 in computer-accessible form.

Research was conducted at Brookhaven National Laboratory under the auspices of the U.S. Department of Energy Office of Health and Environmental Research. This work was supported in part by U.S. Public Health Service grants AI14885 and GM29721.

LITERATURE CITED

- 1. Balganesh, T. S., and S. A. Lacks. 1984. Plasmid vector for cloning in *Streptococcus pneumoniae* and strategies for enrichment for recombinant plasmids. Gene **29:**221–230.
- Balganesh, T. S., and S. A. Lacks. 1985. Heteroduplex DNA mismatch repair system of *Streptococcus pneumoniae*: cloning and expression of the *hexA* gene. J. Bacteriol. 162:979–984.
- Ballester, S., P. Lopez, J. C. Alonso, M. Espinosa, and S. A. Lacks. 1986. Selective advantage of deletions enhancing chloramphenicol acetyltransferase gene expression in *Streptococcus pneumoniae* plasmids. Gene 41:153–163.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant DNA. Nucleic Acids Res. 7:1513–1523.
- 5. Burdett, V. 1980. Identification of tetracycline-resistant Rplasmids in *Streptococcus agalactiae* (group B). Antimicrob. Agents Chemother. 18:753–760.

- Clewell, D. B. 1981. Plasmids, drug resistance, and gene transfer in the genus *Streptococcus*. Microbiol. Rev. 45:409–436.
- Clewell, D. B., and D. R. Helinski. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. USA 62:1159–1166.
- 8. Currier, T. C., and E. W. Nester. 1976. Isolation of covalently closed circular DNA of high molecular weight from bacteria. Anal. Biochem. 76:431–441.
- 9. del Solar, G. H., A. Puyet, and M. Espinosa. 1987. Initiation signals for the conversion of single stranded to double stranded DNA forms in the streptococcal plamsid pLS1. Nucleic Acids Res. 15:5561-5580.
- Derbyshire, K. M., and N. S. Willetts. 1987. Mobilization of the nonconjugative plasmid RSF1010: a genetic analysis of its origin of transfer. Mol. Gen. Genet. 206:154–160.
- Espinosa, M., P. Lopez, M. T. Perez-Urena, and S. A. Lacks. 1982. Interspecific plasmid transfer between *Streptococcus pneumoniae* and *Bacillus subtilis*. Mol. Gen. Genet. 188:195– 201.
- 12. Gennaro, M. L., J. Kornblum, and R. P. Novick. 1987. A site-specific recombination function in *Staphylococcus aureus* plasmids. J. Bacteriol. **169**:2601–2610.
- Horaud, T., C. Le Bouguenec, and K. Pepper. 1985. Molecular genetics of resistance to macrolides, lincosamides and streptogramin B(MLS) in streptococci. J. Antimicrob. Chemother. 16(Suppl. A):111-135.
- 14. 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.
- Horodniceanu, T., D. H. Bouanchaud, G. Bieth, and Y. A. Chabbert. 1976. R plasmids in *Streptococcus agalactiae* (group B). Antimicrob. Agents Chemother. 10:795–801.
- 16. Horowitz, B., and R. C. Deonier. 1985. Formation of Δtra F' plasmids: specific recombination at *oriT*. J. Mol. Biol. 186: 267–274.
- Ikeda, H., K. Aoki, and A. Naito. 1982. Illegitimate recombination mediated in vitro by DNA gyrase of *Escherichia coli*. Structure of recombinant DNA molecules. Proc. Natl. Acad. Sci. USA 79:3724–3728.
- 18. Khan, S. A., and R. P. Novick. 1983. Complete nucleotide sequence of pT181, a tetracycline-resistance plasmid from *Staphylococcus aureus*. Plasmid 10:251–259.
- 19. Lacks, S. 1966. Integration efficiency and genetic recombination in pneumococcal transformation. Genetics **53**:207–235.
- Lacks, S. A., P. Lopez, B. Greenberg, and M. Espinosa. 1986. Identification and analysis of genes for tetracycline resistance and replication functions in the broad-host-range plasmid pLS1.

J. Mol. Biol. 192:753-765.

- 21. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. 65:499-560.
- 23. McKenzie, T., T. Hoshino, T. Tanaka, and N. Sueoka. 1986. The nucleotide sequence of pUB110: some salient features in relation to replication and its regulation. Plasmid 15:93–103.
- McKenzie, T., T. Hoshino, T. Tanaka, and N. Sueoka. 1987. A revision of the nucleotide sequence and functional map of pUB110. Plasmid 17:83-85.
- Novick, R. P., S. J. Projan, W. Rosenblum, and I. Edelman. 1984. Staphylococcal plasmid cointegrates are formed by hostand phage-mediated general rec systems that act on short regions of homology. Mol. Gen. Genet. 195:374–377.
- Priebe, S. D., S. M. Hadi, B. Greenberg, and S. A. Lacks. 1988. Nucleotide sequence of the *hexA* gene for DNA mismatch repair in *Streptococcus pneumoniae* and homology of *hexA* to *mutS* of *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. 170:190-196.
- 27. Projan, S. J., and R. Novick. 1988. Comparative analysis of five related staphylococcal plasmids. Plasmid 19:203-221.
- Puyet, A., G. H. del Solar, and M. Espinosa. 1988. Identification of the origin and direction of replication of the broad-host-range plasmid pLS1. Nucleic Acids Res. 16:115–133.
- 29. Rosenberg, M., and D. Court. 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. 13:319–353.
- Shine, J., and L. Dalgarno. 1975. Determinant of cistron specificity in bacterial ribosomes. Nature (London) 254:34–38.
- Smith, M. D., and W. R. Guild. 1980. Improved method for conjugative transfer by filter mating of *Streptococcus pneumoniae*. J. Bacteriol. 144:457–459.
- Smith, M. D., N. B. Shoemaker, V. Burdett, and W. R. Guild. 1980. Transfer of plasmids by conjugation in *Steptococcus pneumoniae*. Plasmid 3:70–79.
- 33. Stassi, D. L., P. Lopez, M. Espinosa, and S. A. Lacks. 1981. Cloning of chromosomal genes in *Streptococcus pneumoniae*. Proc. Natl. Acad. Sci. USA 78:7028–7032.
- Tanaka, T., and N. Sueoka. 1983. Site-specific in vitro binding of plasmid pUB110 to *Baccillus subtilis* membrane fraction. J. Bacteriol. 154:1184–1194.
- 35. Warren, G. J., A. J. Twigg, and D. J. Sherratt. 1978. ColE1 plasmid mobility and relaxation complex. Nature (London) 274:259-261.
- Willetts, N., and B. Wilkins. 1984. Processing of plasmid DNA during bacterial conjugation. Microbiol. Rev. 48:24–41.