Molecular Structure of the Replication Origin of a Bacillus subtilis (natto) Plasmid, pUHI

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The structure of ^a 2.0-kb BstEII DNA sequence necessary and sufficient for the replication of ^a 5.7-kb Natto plasmid, pUH1, which is responsible for γ -polyglutamate production by Bacillus subtilis (natto), has been characterized by using a trimethoprim resistance gene derived from B. subtilis chromosomal DNA as a selective marker. The 2.0-kb DNA sequence contains an open reading frame, rep, stretching for ⁹⁹⁹ bp; ^a promotor region for rep expression; and a possible replication origin for the plasmid upstream of the promotor. The predicted Rep protein has highly homologous amino acid sequences with rep_{14} of pFTB14 in B. amyloliquefaciens, RepB of pUB110, and protein A, which is necessary for pC194 replication in staphylococci throughout the protein molecule, but is not homologous with RepC of staphylococcal plasmid pT181.

In a previous paper (5) , we reported that a 5.7-kb plasmid designated pUH1, which encodes the γ -glutamyltranspeptidase gene responsible for γ -polyglutamate production, was detected in a Bacillus subtilis (natto) strain isolated from a

FIG. 1. Derivation of plasmids used in the present study. The chain of solid circles in the diagram indicates the DNA segment containing the Tmp^r gene of B. subtilis TTK24. Heavy and thin lines represent the regions of pBR322 and pTL12, respectively. Double lines represent the DNA fragment of pUH1. A, AatI; Bg, BglII; Bs, BstEII; E, EcoRI; H, HindIII; P, PstI.

fermented soybean food, natto. Recently, electron microscope studies of the hybridization products of pLS11, which is detected in B. subtilis IFO 3022 and is 8.6 kb in size, with pUH1 revealed ^a heteroduplex molecule containing 1.11 and 2.14-kb double-stranded DNA termini (6). We thought that these double-strand regions might play an important role in polyglutamate production and DNA replication.

This communication reports that the 2.0-kb BstEII DNA fragment corresponds to the replication origin of Natto plasmid pUH1 and contains a 999-bp open reading frame, a promoter for the open reading frame, and a possible replication origin upstream of the promoter. Significant homologies were observed between the amino acid sequence predicted from the 999-bp open reading frame and those of the similar putative proteins encoded by the B . amyloliquefaciens plasmid pFTB14 (13) and Staphylococcus aureus plasmid pC194 (3, 7).

To facilitate identification of the replication region of

FIG. 2. Structure and replication activity of the modified fragments of the 2.0-kb ori fragment. The open arrow in the restriction map indicates an open reading frame found in the sequence data (see Fig. 3). $+$ and $-$ indicate, respectively, ability and inability to replicate in the B. subtilis host. Modifications were made by deletion with restriction enzymes or insertion of a few base pairs (closed triangle), using the Klenow fragment, after digestion with the indicated restriction enzyme or by $Bal31$ digestion (B) from the $BgIII$ site of pBB2.

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BstE II
5' -ggtaaccGGACCGTAGGGA
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GGATTAAGGAAGTTGACTCGCTCAGCGCCACCCGAACCCTTTCCGCACTCt.AACAAACCCGTTTGTTTGACGCCAACCG ⁵⁰ 1TA °° 150 GGCAGGGAGCCCCCCGAAGAAGCGGGGGTTGGGGGGATTGAATGCTGGCATCCAACGGCCGTCCGTTGGTGGGTTTGGG 200 250 CAAAGCCAAGAACTGTTGCAAGGCTCGTTGAGAATAAAGAATGCTTTTCAGGATGCTTAGAATCGTTTCTGAGAGCTTC 300 AAATAAAAAAGATGACCTTTTATAGGGGGAAGCTCTTAAAATTGAATGTAGGGGCATTTAAACACGTTTAAAAATAAAA 350
AAAGCAGACTCTTTAGAGTCCGCCTTGTTATTTTTAACCCAGTGCTCCATTTTCGGCTGTTTGGAAATCTTTTGAGATG 450
CCATCCATTTTCTTTTGTTCCATGAAAAAGTGCTTTTGGATGCTTAAAAAGGCTTTTTCGTATAAAAAAGCC. 500
GATTTTTGAAAAAAAAATCTCCCTGCGGGGGAAGAATGGTTTTGATCTTTGGGTTTTAGGTTTTAAAAAAACCGGGCTG TTTTCAGCCGGCTTTTTTTCGATTTTGGCGGAGCCGAAATCGGGTCTTTTCTTATCTTGATACTATATAGAAACATCTC 12 ² ³⁶ AAGGCGAAAAAATAGCCCCCATCCCTTATTTGTCAAGGGTTTGACGGCTTTGTTAGAAACTCCTTCCGCffl SOO 800
AAAGTGCCCACTAAAATAATAGAATGCTAGATTACTAGCTCAGAAGAGAGTTTTTTTGTTCATGTATTCAAA ATG ATT ATA TCA TCC TTG AGG ACA AGA CCG CAA CAG GTA
Met Ile Ile Ser Ser Leu Arg Thr Arg Pro Gln Gln Val Lys Ser Gly Ile Gly Arg Gly 900 AAA AGA GAC 6GA CGA ATC TTA TGG CTG AGC ACT ATG AAG CTT TAC AGA GTA AAA CTG GTA Lys Arg Asp Giy Arg Ile Leu Trp Leu Ser Thr Met Lys Leu Tyr Arg Vai Lys Leu Vai .
TAC CTT ACT ATG CCA AAA AAG CTG AGA AAT TGT GCA GTT GTG CGG AAT GTC TTT CGT - TTA
Tyr Leu Thr Met Ala Lys Lys Leu Arg Asn Cys Ala Val Val Arg Asn Val Phe Arg Leu .
AAC GAG ACC CGG AGA CGC AAA TTA AAG TTG TAT CAA GCT CAG TTT TGT AAA GTG AGG TTA
Asn Glu Thr Arg Arg Arg Lys Leu Lys Leu Tyr Gln Ala Gln Phe Cys Lys Val Arg Leu 0
TGC CCG ATG TGT GCG TGG CGT AGG TCT TTA AAA ATT GCT TAT CAT AAT AAA TTA ATC GTT
Cys Pro Met Cys Ala Trp Arg Arg Ser Leu Lys Ile Ala Tyr His Asn Lys Leu Ile Val GAG GAA GCG AAT CGG CAG TAC GGT TGT GGA TGG ATT TTT CTC ACA LISO
Glu Glu Ala Asn Arg Gln Tyr Gly Cys Gly Trp Ile Phe Leu Thr Leu Thr Vai Arg Asn .
GTC GAG GGT GAC GGA TTA AAA CCC ATG ATT GCT GAC ATG ATG AAA GGA TGG AAC CGC CTT
Val Glu Gly Asp Gly Leu Lys Pro Met Ile Ala Asp Met Met Lys Gly Trp Asn Arg Leu ITC GCA TAT AAA CGA GTT AAG 1250
TTC GCA TAT AAA CGA GTT AAG GTA GCG ACT TTA GGT TAT TTC AGA GCT TTA GAG ATT ACC
Phe Gly Tyr Lys Arg Val Lys Val Ala Thr Leu Gly Tyr Phe Arg Ala Leu Glu Ile Thr .
AAA AAT CAC GAA GAA GAT ACA TAT CAT CCG CAT TTT CAT GTG TTG TTG CCT GTG AAG AAA
Lys Asn His Glu Glu Asp Thr Tyr His Pro His Phe His Val Leu Leu Pro Val Lys Lys 0 1400
AGC TAT TTT ACT CAC AAT TAC ATT AAG CAG TCT GAG TGG ACG AGC TTA TGG AAA AGG GCG
Ser Tyr Phe Thr His Asn Tyr Ile Lys Gln Ser Glu Trp Thr Ser Leu Trp Lys Arg Ala 1450 ATG AAA CTG GAC TAC ACG CCG ATT GTT GAT ATC CGA AGA GTC AAG GGA AGA OCT AAA ATT Met Lys Leu Asp Tyr Thr Pro Ile Val Asp Ile Arg Arg Vai Lys Gly Arg Ala Lys Ile .
GAT GCC GAA CAG ATT GAG AGC GAT GTG CGG GAA GCC ATG ATG GAG CAA AAA GCT GTT CTT
Asp Ala Glu Gln Ile Glu Ser Asp Val Arg Glu Ala Met Met Glu Gln Lys Ala Val Leu .
GAA ATC TCT AAA TAT CCG GTT AAA GAT ACG GAT GTT GTG CGC GGC AAT AAG GTG ACA GAA
Glu Ile Ser Lys Tyr Pro Val Lys Asp Thr Asp Val Val Arg Gly Asn Lys Val Thr Glu 1600 165 GAC AAT CTG AAC ACG GTG TTT TAT TTG OAT OAT GCG CTT TCT CGC CGC COO CTT ATT GOT Asp Asn Leu Asn Thr Vai Phe Tyr Leou Asp Asp Ala Leu Ser Arg Arg Arg Lou Ile Gly 0
TAC GGT GGC ATC TTG AAG GAA ATT CAT AAA GAA CTA'AAC CTC GGT GAT GCG GAG GAC GGC
Tyr Gly Gly Ile Leu Lys Glu Ile His Lys Glu Leu Asn Leu Gly Asp Ala Glu Asp Gly AN AND LUE Ser Lys
AC AAT CTG AAC
AC AAT CTG AAC
AC GGT GGC ATC
AC GGT GGC ATC
AT CTC GTC AAG
SP Leu Val Lys
SP Leu Val Lys .
GAT CTC GTC AAG ATT GAG GAA GAA GAT GAC GAG GTG GCG AAC GAA GAT TTT GAA GTT ATG
Asp Leu Val Lys Ile Glu Glu Glu Asp Asp Glu Val Ala Asn Glu Ala Phe Glu val Met 1800
GCT TAC TGG CAT CCA GGC ATT AAA AAT TAC ATA ATC AGA TAA "TCAGATAAAAAGCAGGCGTTGTT"
Via Tyr Trp His Pro Gly Ile Lys Asn Tyr Ile Ile Arg *** 1850 1900 CCTGCTTTTTTTATACTCTAATAGTCAAATCAAGAGTTAATTTTAGATGTAATTGTGAGAATTAGAGTGGCTGACCAGT 1950
ATTTGAAACTTCTTGGGCTACTTTCTTAACTTTATATAAAACTATGTATATATGTGTTGTTTTTCTATTTTGAT 2000 BstEII 2044 ATTATTTACAAGTATTGAAATTTTGCTAGGAGGGAAAGTTTTTATggttacc-3'

FIG. 3. Nucleotide sequence of the 2.0-kb BstEII DNA fragment. -10 , -35 , and SD indicate, respectively, plausible sequence for the -10 and -35 sequences and the ribosome binding site. The nucleotide sequences indicated by lowercase latters at the two termini originate from the **BstEII** site.

pUH1, we used the trimethoprim-resistant (Tmp^r) dihydrofolate reductase gene of B. subtilis 168. A schematic presentation of the constructed plasmids is given in Fig. 1. The dihydrofolate reductase-coding gene was derived from a Tmp^r strain, TTK24, of B. subtilis 168 (20) and has been cloned in plasmid pBR322 of Escherichia coli (2). DNAs from pTL12, carrying the Tmp^r dihydrofolate reductase gene, which was constructed by Tanaka and Kawano (18), and pBR322 were both treated with EcoRI and HindIII, mixed, and ligated with T4 ligase; then pATE1 was constructed. Natto plasmid pUH1 was digested with BstEII, and then the ends were filled in with Klenow fragment to generate ^a blunt end. The DNA fragments were mixed and ligated at the AatI site of pATE1 with T4 ligase and then added to B. subtilis MI112 (19) protoplasts. Several Tmp^r colonies were obtained on AA agar plates (18) containing trimethoprim (1 μ g/ml), and one of them was used for further study. Plasmid pBB2 carried in such a Tmp^r colony had a molecular mass of 8.4 kb (Fig. 1). The physical map of pBB2, using various restriction enzymes, is shown in Fig. 1.

To define the boundaries of ^a functional unit of pUH1 replication, a 2.0-kb BstEII fragment of pUH1 was digested with selected restriction endonucleases to obtain a set of overlapping DNA fragments. Digests modified with Klenow fragment were ligated with pATE1, followed by introduction into E. coli by transformation and successive selection for ampicillin resistance. The plasmid DNA preparations containing each generated fragment were tested for replication activity in B. subtilis. The results are summarized in Fig. 2. Plasmid pBB2, which corresponds to fragment ¹ in Fig. 2, and a derivative containing fragment 7 specified Tmp^r in B . subtilis, but attempts to transform B. subtilis with recombinant plasmid preparations containing the small fragments (fragments 2 to 6 in Fig. 2) and inserted fragments (fragments 8 and 9) were unsuccessful.

The nucleotide sequence of the 2.0-kb BstEII fragment was determined by the method of Sangar et al. (14). Though the strategy is not shown, the nucleotide sequence was determined for both strands by using numerous restriction fragments to give enough overlapping regions. By examination of possible open reading frames, we found only one large frame (Fig. 2), designated rep, which consists of 999 bp and encodes a protein molecule with 333 amino acids and an M_r of 39,074. The 5' upstream region of rep contains a 5'-AAGGAG-3' sequence (indicated as SD in Fig. 3) complementary to the ³' end of 16S rRNA (3'-OH-UCUUUC CUCCAGUAG-5') of B. subtilis (11) at nucleotides 777 to 782 for translation initiation. There is a 5'-TATTAT-3' sequence $(-10$ in Fig. 3) resembling a Pribnow box (12) at nucleotides 727 to 732, and at a site 24 bp upstream of this -10 sequence, there is a 5'-TTGACA-3' sequence resembling the -35 consensus sequence (12) of the B. subtilis gene. The observed distance (18 bp) between the -10 and -35 sequences accords well with that observed generally in B. subtilis genes $(17$ to 18 bp) (12) .

Plasmid pBB2 was digested with BgIII and then treated for 20 min with exonuclease Bal31 under conditions in which about ⁵⁰ bp was removed per min from each end of the DNA molecule. After ligation by T4 ligase, the DNA was introduced into B . *subtilis* by protoplast transformation. Tmp^r transformants, which contained fragment 11 in Fig. 2, were obtained at high efficiencies with Bal31-generated deletions of 581 bp (as determined by nucleotide sequencing), while no transformants were obtained with a similar 653-bp deletion (fragment 10 in Fig. 2), suggesting that the ⁵' upstream portion is dispensable for replication. The interaction of the

rep rep _k ReoB Protein A	10	30 20 --------	60 50 40 ------MIISSLRTRPQQVKSGIGRGKRDGRILWLSTMKLYRVKLVYLTMAKKLRNCAVV MYSSENDYRILEDKTATGKKRDWRGKKRRANLMAEHYEALEKRIGAPYYGKKAERLSECA
rep $\boldsymbol{rep}_{\mathcal{H}}$ RepB Protein A	70	90 80	120 100 110 RNVFRLNETRRRKLKLYQAQFCKVRLCPMCAWRRSLKIAYHNKLIVEEANRQYGCGWIFL EHLSFKRDPETGRLKLYQAHFCKVRLCPMCAWRRSLKIAYHNKLIIEEANRQYGCGWIFL TFLSFVADKTLEKQKLYKANSCKNRFCPVCAWRKARKDALGLSLMMQYIKQQEKKEFIFL
rep rep _u RepB Protein A	130	150 140	180 170 160 TL TVRNV-EGDGL KPMIADMMKGWNRLFGYKRVKVATLGYFRALEITKNHEEDTYHPHFHV TLTVRNV-KGERLKPQISEMMEGFRKLFQYKKVKTSVLGFFRALEITKNHEEDTYHPHFHV TL TVKNVYDGEELNKSL SDMAQGFRRMMQYKKINKNLVGFMRATEVTINNKDNSYNQHMHV TLTTPNVMSDE-LENEIKRYNNSFRKLIKRKKVGSVIKGYVRKLEITYNKKDNSYNOHMHV
rep rep _u RepB Protein A	190	200 - 200 - 200 - 200 - 200 - 200 - 200 - 200 - 200 - 200 - 201 - 201 - 202 - 2 210	220 230 240 LLPVKKSYF--THNYIKQSEWTSLWKRAMKLDYTPIVDIRRVKGRAKIDAEQIESDVREAMM LLPVKRNYF--GKNYIKOAEWTSLWKRAMKLDYTPIVDIRRVKGRVKIDAEQIESDVREAMM LVCVEPTYFKNTENYVNGKQWIQFWKKAMKLDYDPNV-----------KVQMIRPKNKYKSD LIAVNKSYFTDKRYYISQQEWLDLW-----RDVTGISEITQVQVQKIRQNNNKELYEMAKYS
rep rep _k RepB Protein A	250	260 270 GKDSDYLINKSKSL--------------------------------	280 290 300 EQKAVLEISKYPVKDTDVVRGNKVTEDNLNTVFYLDDALSRRRLIGYGGILKEIHKELNL EQKAVLEISKYPVKTDDVVRGSKVTDDNLNTVFYLDDALSARRLIGYGGILKEIHKELNL IQSAIDETAKYPVKDTD--FMTDDEEKNLKRLSDLEEGLHRKRLISYDGLLKEIHKKLNL
rep rep _M RepB Protein A	310	330 320 GDAEDGDLVKIEEEDDEVANEAFEVMAYWHPGIKNYIIR- GDAEGGDLVKIEEEDDEVANGAFEVMAYWHPGIKNYILK- DDTEEGDL-IHTDDDEKADEDGFSIIAMWNWERKNYFIKE	

FIG. 4. Comparison of the amino acid sequences of rep of pUH1, rep_{14} of pFTB14, RepB of pUB110, and protein A of pC194. Amino acid identities with rep of pUH1 are indicated (*). Amino acid numbers follow the sequence of the rep protein from the amino-terminal methionine to the carboxyl terminus. Gaps have been inserted to gain maximum matching. The one-letter amino acid code has been used.

initiator protein with its binding site on the DNA is ^a key step in the replication initiation process (4, 21). Therefore, the structure of the 2.0-kb ori sequence that is necessary and sufficient for the replication of the Natto plasmid pUH1 molecule contains a 999-bp open reading frame, rep, a promotor region for rep expression, and a putative replication origin for the plasmid, which is located upstream of the promoter between positions 581 and 653.

The amino acid sequence of the rep protein coding region of pUH1 was compared with ^a number of protein sequences registered in GenBank by using the homology search system of GENAS (10). Homologies between the predicted amino acid sequence of the rep protein of pUH1 and those of the rep protein of pFTB14, RepB of pUB110, and protein A of pC194 are illustrated in Fig. 4. A high similarity (71.8%) was found between the rep protein of pUH1 and rep_{14} of pFTB14 in B. amyloliquefaciens. The structure of the replication origin sequence of pFTB14 contains an open reading frame (rep), stretching for 1,017 bp, a promoter region for rep expression, and a possible replication origin for rep expression, which is located upstream of the promoter. The rep product is trans active and essential for plasmid replication (13). Compared with rep of pUH1, a low similarity (39.7%) was found for RepB of pUB110 (1) and for protein A (29.2%) of pC194 (8) in S. aureus. Khan et al. (9) determined the start site of pT181 DNA synthesis within ^a 127-bp segment and showed that a 168-bp segment containing the replication start site is enough to initiate unidirectional replication. Furthermore, like RepC protein of pT181 (9), the protein of E. coli plasmid R6K (4, 17, 18) does not have any significant homology in its amino acid sequences with those of the rep proteins of pUH1 and pFTB14 deduced from the open reading frame (data not shown). The Rep protein of Natto plasmid pUH1 shows high homology with replication proteins encoded by pFTB14, pC194, and pUB110, which originate from various gram-positive bacteria such as B. amyloliquefaciens and S. aureus. Taxonomic studies (based on the 16S rRNA similarities) revealed that Bacillus and S. aureus strains are more related than Streptococcus and Lactobacillus strains (16). The plasmid homologies suggest an exchange of plasmid replicons by recent horizontal transfer through the different genera, including "natto" Bacillus species.

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REFERENCES

- 1. Ano, T., T. Imanaka, and S. Aiba. 1986. The copy number of Bacillus plasmids pRBH1 is negatively controlled by RepB protein. Mol. Gen. Genet. 202:416-420.
- 2. Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene 2:95-113.
- 3. Dagert, M., I. Jones, A. Goze, S. Romac, B. Niaudet, and S. D. Ehrlich. 1984. Replication functions of pC194 are necessary for efficient plasmid transduction by M13 phage. EMBO J. 3:81-86.
- 4. Germino, J., and D. Bastia. 1983. Interaction of the plasmid R6K-encoded replication initiator protein with its binding sites on DNA. Cell 34:125-134.
- 5. Hara, T., A. Aumayr, Y. Fujio, and S. Ueda. 1982. Elimination of plasmid-linked polyglutamate production by Bacillus subtilis (natto) with acridine orange. Appl. Environ. Microbiol. 44: 1456-1458.
- 6. Hara, T., A. Ishizaki, and S. Ueda. 1986. Formation of heteroduplex molecules between plasmids pUH1 and pLS11 in polyglutamate-producing Bacillus strains. Agric. Biol. Chem. 50: 2391-2394.
- 7. Horinouchi, S., and B. Weisblum. 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J. Bacteriol. 150:815-825.
- 8. Iordanescu, S., and M. Surdeanu. 1983. Isolation and complementation of temperature-sensitive replication mutants of Staphylococcus aureus plasmid pC194. Mol. Gen. Genet. 191: 201-206.
- 9. Khan, S. H., S. M. Carlton, and R. P. Novick. 1982. Functional origin of replication of pT181 plasmid DNA is contained within

^a 168-base-pair segment. Proc. Natl. Acad. Sci. USA 79:4580- 4584.

- 10. Kuhara, S., F. Matsuo, S. Futamura, A. Fujita, T. Shinohara, T. Takagi, and Y. Sakaki. 1984. GENAS: a database system for nucleic acid sequence analysis. Nucleic Acids Res. 12:89-99.
- 11. McLaughlin, J. R., C. L. Murray, and J. C. Rabinowitz. 1981. Unique features in the ribosome binding site sequence of the gram-positive Staphylococcus aureus P-lactamase gene. J. Biol. Chem. 256:11283-11291.
- 12. Moran, C. P., Jr, N. Lang, S. F. J. LeGrice, G. Lee, M. Stephans, A. L. Sonenshein, J. Pero, and R. Losick. 1982. Nucleotide sequence that signal the initiation of transcription and translation in Bacillus subtilis. Mol. Gen. Genet. 186:339- 346.
- 13. Murai, M., H. Miyashita, H. Araki, T. Seki, and Y. Oshima. 1987. Molecular structure of the replication origin of a Bacillus amyloliquefaciens plasmid pFTB14. Mol. Gen. Genet. 210:92- 100.
- 14. Sangar, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5476.
- 15. Scott, J. R. 1984. Regulation of plasmid replication. Microbiol. Rev. 48:1-23.
- 16. Stackebrandt, E., and M. Teuber. 1988. Molecular taxonomy and phylogenetic position of lactic acid bacteria. Biochimie 70:317-324.
- 17. Stalker, D. M., R. Kolter, and D. R. Helinski. 1982. Plasmid R6K DNA replication. I. Complete nucleotide sequence of an autonomously replicating segment. J. Mol. Biol. 161:33-43.
- 18. Tanaka, T., and N. Kawano. 1980. Cloning vehicles for the homologous Bacillus subtilis host-vector system. Gene 10:131- 136.
- 19. Tanaka, T., and K. Sakaguchi. 1978. Construction of a recombinant plasmid composed of B. subtilis leucine genes and a B. subtilis (natto) plasmid: its use as cloning vehicle in B. subtilis 168. Mol. Gen. Genet. 165:269-276.
- 20. Wainscott, V. J., and J. F. Kane. 1976. Dihydrofolate reductase in *Bacillus subtilis*, p. 208–213. *In* D. Schlessinger (ed.), Microin Bacillus subtilis, p. 208-213. In D. Schlessinger (ed.), Microbiology-1976. American Society for Microbiology, Washing-ton, D.C.
- 21. Yamaguchi, K., and M. Yamaguchi. 1984. The replication origin of pSC101: the nucleotide sequence and replication functions of the ori region. Gene 29:211-219.