Cloning and Base Sequence Determination of Replication Initiation Gene (*rep*) Isolated from *Staphylococcus aureus* DH1 R-plasmid pSBK203

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A replication initiation gene was identified and its nucleotide sequence has been determined from a 3.8 kb, chloramphenicol acetyltransferase conferring R-plasmid pSBK203 of *Staphylococcus aureus*. Location of the replication related region on pSBK203 was determined by interuption with pUC119 at *Xba*I and *Msp*I sites which resulted in inactivation of replication in *Bacillus subtilis*. Base sequence of this region revealed an open reading frame of 942 base pairs, which encoded a 314 amino acid protein. Base sequence homology with other *rep* of pT181 family plasmids such as pT181, pC221, pC223, pS194, pUB112, and pCW7 was ranged from 78% to 97% and the predicted amino acid sequence homology was from 72% to 95%.

KEY WORDS □ R-plasmid, pSBK203, *rep* gene, *Staphylococcus aureus*

Among plasmids of gram positive bacteria, small multicopy plasmids of *Staphylococcus aureus* have been extensively studied in genomic organization, expression control of antibiotic resistance gene and regulation of replication. Trans-acting plasmid-specific replication initiation protein (Rep) has been identified from those staphylococcal plasmids and the base sequence of *rep* genes has been determined and analyzed (5, 11, 13). The main roles of *rep* gene product are specific recognizing, nicking and nick-closing of their own replication origin and initiating replication (6).

pSBK203 was initially identified from *Staphylococcus aureus* (3) and its inducible chloramphenicol acetyltransferase (CAT) gene was identified and characterized previously (7). As a part of the study understanding the mechanism of replication control of pSBK203, the *rep* gene was cloned and the base sequence was firstly determined.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Plasmid pSBK203 haboring *Staphylococcus aureus* DH1 was clinically isolated by J.S. Suk of the Seoul National University Hospital and kindly provided to us. *Bacillus subtilis* BD170 (*thr trp*) was obtained from B. Weisblum and used as host for maintaining pSBK203. Phage M13mp19 and M13mp18 were used as vectors for cloning and sequencing and *Escherichia coli* MV1190 was used as host.

Enzymes and Chemicals

All restriction endonucleases, Klenow fragment, and T4 ligase were purchased from New England Biolabs and Kosco. [α-^{35}S]dATP was purchased from Amersham and fine reagents such as lysozyme, X-gal, RNase, IPTG, urea, acrylamide and various antibiotics such as ampicillin, chloramphenicol, tetracyclin, erythromycin from Sigma Chemical Co. Media and of its components for culturing bacterial strains were purchased from Difco Lab.

DNA Base Sequencing

The recombinant M13 ssDNA which contain cloned *rep* fragment was isolated and sequenced by dideoxynucleotide chain termination method (9).

Transformation of *B. subtilis*

For transformation of *B. subtilis*, competent cells were obtained through the method of Dubnau *et al.* (4).

RESULTS AND DISCUSSION

Physical Map of pSBK203

The *Xba*I site has been chosen as the origin of map cooriginate and base number was read clockwise.

Identification of Replication Related Region

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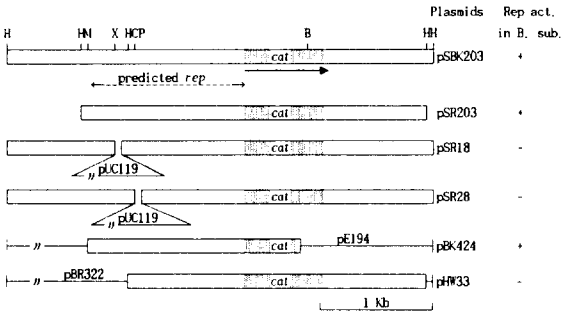

Fig. 1. *Physical structure of pSBK203 and confirming of the replication related region by analysis of several recombinant plasmids.*
Abb.: H, *Hin*dIII; M, *Mbo*I; X, *Xba*I; C, *Cfr*10I; P, *Msp*I.

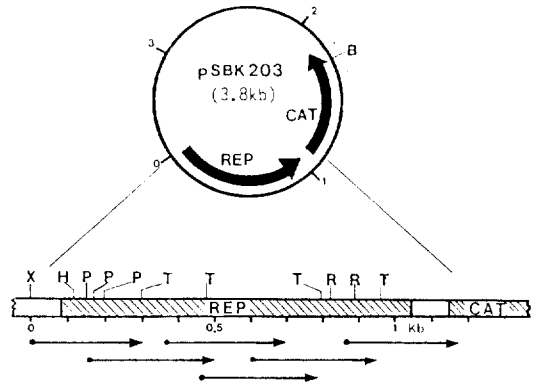

Fig. 2. *Sequencing strategy and restriction map of rep region.*
Abb.: X, *Xba*I; H, *Hin*dIII; P, *Msp*I; T, *Taq*I; R, *Rsa*I.

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XbaI
TCTAGATATTAAACGATATAAGTTTATTCTTCAAGATATATATTCGGGTGAGCGACTTCTTAAATTAAATTAAGGAGTTGTTTTTTT ATG ATT AAA
                                                                    S.D.          MET Ile Lys

AAA GCA GAA GAA ATT CAG GCA AAA CAA AGC TTA GAA AAC GAA AAC TTA AAT TTT TCT AAA ACC GGA TAC TCT AAT
Lys Ala Glu Glu Ile Gln Ala Lys Gln Ser Leu Glu Asn Glu Asn Leu Asn Phe Ser Lys Thr Gly Tyr Ser Asn

AGC CGG TTA AAC CGA CAT ACT ATG TAC ACC CCG GAA CCA AAA TTA AGT TTT GAC GCT ATG ACT ATT GTT GGA AAT
Ser Arg Leu Asn Arg His Thr MET Tyr Thr Pro Glu Pro Lys Leu Ser Phe Asp Ala MET Thr Ile Val Gly Asn

CTT AAT AAA AAT AAT GCT CAC AAA CTA TCT GAA TTT ATG AGT GTC GAG CCA CAA ATT CGA CTT TGG GAT ATA CTA
Leu Asn Lys Asn Asn Ala His Lys Leu Ser Glu Phe MET Ser Val Glu Pro Gln Ile Arg Leu Trp Asp Ile Leu

CAA ACT AAA TTT AAA GCT AAA GCT CTA CAA GAA AAA GTT TAT ATC GAA TAT GAC AAA GTA AAA GCA GAT ACG TGG
Gln Thr Lys Phe Lys Ala Lys Ala Leu Gln Glu Lys Val Tyr Ile Glu Tyr Asp Lys Val Lys Ala Asp Thr Trp

GAT AGA CGT AAT ATG CGT GTT GAA TTT AAT CCA AAT AAA CTT ACG CAT GAA GAA ATG CTT TGG TTA AAA CAA AAC
Asp Arg Arg Asn MET Arg Val Glu Phe Asn Pro Asn Lys Leu Thr His Glu Glu MET Leu Trp Leu Lys Gln Asn

ATT ATC GAC TAC ATG GAA GAC GAT GGT TTT ACA AGA TTA GAT TTA GCT TTT GAT TTT GAA TAT GAT TTA AGT GAT
Ile Ile Asp Tyr MET Glu Asp Asp Gly Phe Thr Arg Leu Asp Leu Ala Phe Asp Phe Glu Tyr Asp Leu Ser Asp

TAT TAT GCA ATG ACT GAT AAA TCA GTT AAG AAA ACT ATT TTT TAT GGT CGT AAC GGT AAA CCA GAA ACG AAA TAT
Tyr Tyr Ala MET Thr Asp Lys Ser Val Lys Lys Thr Ile Phe Tyr Gly Arg Asn Gly Lys Pro Glu Thr Lys Tyr

TTT GGT GTT CGT GAC AGT GAT AGA TTT ATT AGA ATT TAT AAT AAA AAA CAG GAA CGC AAA GAT AAT GCA GAT ATT
Phe Gly Val Arg Asp Ser Asp Arg Phe Ile Arg Ile Tyr Asn Lys Lys Gln Glu Arg Lys Asp Asn Ala Asp Ile

AAA ATT ATG TCT GAA CAC TTA TGG CGT GTA GAA ATT GAA TTA AAA AGA GAT ATG GTT GAT TAT TGG AAC GAT TGT
Lys Ile MET Ser Glu His Leu Trp Arg Val Glu Ile Glu Leu Lys Arg Asp MET Val Asp Tyr Trp Asn Asp Cys

TTT AAT GAT TTA CAT ATA TTA CAA CCA GAT TGG AAA ACT ATC GAA CGT ACT TCT GAT AGA GCA ATG GTT TTT ATG
Phe Asn Asp Leu His Ile Leu Gln Pro Asp Trp Lys Thr Ile Glu Arg Thr Ser Asp Arg Ala MET Val Phe MET

TTG TTG AAT GAT GAA GAA GAA TGG GGA AAA TTA GAA AGA CGT ACT AAG AAT AAA TAT AAA AAA TTA ATT AAA GAA
Leu Leu Asn Asp Glu Glu Glu Trp Gly Lys Leu Glu Arg Arg Thr Lys Asn Lys Tyr Lys Lys Leu Ile Lys Glu

ATA TCT CTA ATT GAT TTA ACT GAT TTA ATG AAA TCG ACT TTA AAA GCG AAC GAA AAA CAA TTG CAA AAG CAG ATT
Ile Ser Leu Ile Asp Leu Thr Asp Leu MET Lys Ser Thr Leu Lys Ala Asn Glu Lys Gln Leu Gln Lys Gln Ile

GAT TTT TGG CAA CGT GAA TTT AGA TTT TGG AAG TAA
Asp Phe Trp Gln Arg Glu Phe Arg Phe Trp Lys ...
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Fig. 3. *Nucleotide sequence of the rep gene and deduced amino acid sequence of Rep protein from plasmid pSBK203.*
The nucleotide sequence has been submitted in the GenBank Nucleotide Sequence Database with accession no. M90090.

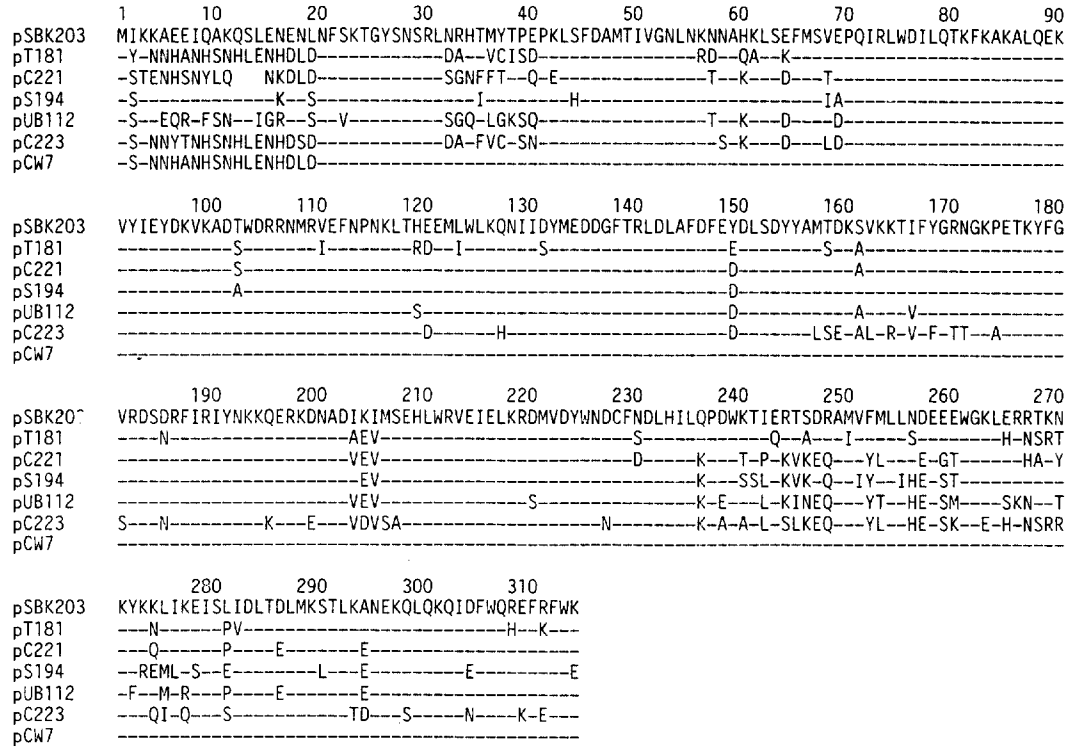

Fig. 4. *Amino acid alignment of seven Rep variants from staphylococcal pT181 family plasmids, pSBK203 (this study, Fig. 3), pT181 (5), pC221 (2), pS194 (12), pUB112 (13), pC223 (13), and pCW7 (1).* "-" designates identical amino acids as pSBK203-Rep.

Physical location of replication related region on pSBK203 was determined by interuption with pUC119 at *Xba*I and *Msp*I sites. Resulted two pSBK203::pUC119 cointegrates, pSR18 and pSR28 were unable to replicate in *B. subtilis*. Another recombinant plasmid, pHW33, which was constructed by recombining the *Hin*dIII-A fragment of pSBK203 with pBR322 did not replicate in *B. subtilis* either (8). On the other hand, plasmid pSR203 which is the *Hin*dIII-B fragment deleted and composed only with the *Hin*dIII-A, and C fragment of pSBK203 has been replicated normally in *B. subtilis*. Formerly constructed *E. coli*−*B. subtilis* shuttle vector plasmid pBK424 (8) which contains *Hin*dIII-C and part of *Hin*dIII-A replicated normally in *B. subtilis* too (Fig. 1).

It was deduced that the replication related gene locates within about 1.3 kb region which includes *Hin*dIII-C and a part of the *Hin*dIII-A fragment.

Coding Sequence of *rep* gene

The nucleotide sequence of the *rep* gene region was determined by the dideoxynucleotide chain termination method. Sequencing strategy and partial restriction map of *rep* region are shown in Fig. 2. Single open reading frame of 942 base pair was deduced and the predicted number of amino acids of the Rep protein is 314 (Fig. 3). Staphylococcal plasmids are classified into three types−class I, II, and III (10). Class I is divided further into pT181, pC194, pSN2, and pE194 families. Analysis of the base sequence of pSBK 203-*rep* revealed that pSBK203 belongs to the pT181 family along with pT181 (5), pC221 (2), pS 194 (12), pUB112 (13), pC223 (13), and pCW7 (1). Base numbers of other *rep* genes of pT181 family are also 942 bases except that of C221 (933 bases). The base sequence homology of pSBK203-*rep* with those of pT181, pC221, pC223, pS194, pUB112, and pCW7 are 84%, 84%, 78%, 89%, 85%, and 97%, respectively.

Analysis of Amino Acid Sequence of pSBK203-Rep

Amino acid sequence of Rep proteins of pT181 family plasmids were compared and shown in Fig. 4. Rep protein of pSBK203 showed the highest homology with that of pCW7 (95%) and the lowest with that of pC223 (72%). The N-terminal amino acid sequence of pSBK203-Rep is very similar with that of pS194 (16 out of 19 amino acids) and the rest part of the Rep is exactly same as that of pCW7.

사 사

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REFERENCES

1. **Balson, D.F. and W.V. Shaw,** 1990. Nucleotide sequence of the *rep* gene of Staphylococcal plasmid pCW7. *Plasmid* **24**, 74-80.
2. **Brenner, D.G. and W.V. Shaw,** 1985. The use of synthetic oligonucleotides with universal templates for rapid DNA sequencing: Results with staphylococcal replicon pC221. *EMBO J.* **4**, 561-568.
3. **Byeon, W.-H., Y.S. Kim, E.H. Cho, D.H. Kwon, H.Z. Lea, and S.J. Hong,** 1985. R-plasmids in *Staphylococcus aureus*. *Kor. J. Microbiol.* **23**, 282-290.
4. **Dubnau, D., R. Davidoff-Abelson, B. Scher, and C. Cirigliano,** 1973. Fate of transforming deoxyribonucleic acid after uptake by competent *Bacillus subtilis*: Phenotypic characterization of radiation-sensitive recombination-deficient mutants. *J. Bacteriol.* **114**, 273-286.
5. **Khan, S.A. and R.P. Novick,** 1983. Complete nucleotide sequence of pT181, a tetracyclin-resistance plasmid from *Staphylococcus aureus*. *Plasmid* **10**, 251-259.
6. **Koepsel, R.R., R.W. Murray, W.D. Rosenblum, and S.A. Khan,** 1985. The replication initiator protein of plasmid pT181 has sequence-specific endonuclease and topoisomerase-like activites. *Proc. Natl. Acad. Sci. USA* **82**, 6845-6849.
7. **Kwon, D.H. and W.-H. Byeon,** 1989. Nucleotide sequence and inducibility analysis of chloramphenicol acetyltransferase gene from *Staphylococcus aureus* R-plasmid pSBK203. *Kor. J. Microbiol.* **27**, 194-200.
8. **Kwon, D.H., J.S. Suk, and W.-H. Byeon,** 1987. Cloning of *ori* region of R-plasmid pSBK203 and construction of new shuttle-vectors for *E. coli* & *B. subtilis* using cloned fragments. *Kor. J. Microbiol.* **25**, 262-273.
9. **Messing, J.,** 1979. A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. *Recomb. DNA Tech. Bull.* **2**, 43-48.
10. **Novick, R.P.,** 1989. Staphylococcal plasmids and their replication. *Ann. Rev. Microbiol.* **43**, 537-565.
11. **Projan, S.J., J. Kornblum, S.L. Moghazeh, I. Edelman, M.L. Gennaro, and R.P. Novick,** 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *S. aureus*. *Mol. Gen. Genet.* **199**, 452-464.
12. **Projan, S.J., S. Mogazeh, and R.P. Novick,** 1988. Nucleotide of pS194, a streptomycin resistance plasmid from *Staphylococcus aureus*. *Nucleic Acids Res.* **16**, 2179-2187.
13. **Projan, S.J. and R.P. Novick,** 1988. Comparative analysis of five related Staphylococcal plasmids. *Plasmid* **19**, 203-221.

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초 록: *Staphylococcus aureus* DH1에서 분리된 R-plasmid pSBK203의 복제 개시 유전자(*rep*) 분리 및 염기서열 결정

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*Staphylococcus aureus*에서 분리된 R-plasmid인 pSBK203의 복제관련 유전자를 확인하고 그 염기서열을 결정하였다. 이 plasmid의 복제조절 관련 부위 내에서 942 base pair로 구성된 open reading frame이 발견되었고 이것과 여타 *rep* 유전자와의 homology를 분석한 결과 염기서열은 78%에서 97%, 아미노산 서열은 72%에서 95% 정도의 상동성을 갖는 것으로 밝혀졌다.