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A Macrolide-Lincosamide-Streptogramin B Resistance Determinant Gene (*ermJ*) Cloned from *B. anthracis* 590

Hee-Sun Kim, Eung-Chil Choi, Byong-Kak Kim and Young-In Park*

College of Pharmacy, Seoul National University, Seoul 151-742,
Department of Genetic Engineering, Korea University,
Seoul 136-701, Korea

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Abstract □ *Bacillus anthracis* 590 having an inducible resistance determinant to MLS antibiotics was isolated from a soil sample in Korea. The resistance gene (*ermJ*) was cloned by Southern blotting of chromosomal DNA fragment digested by various restriction enzymes and coloy hybridization method and the cloned plasmid was named as pBA423. The size of inserted DNA fragment to pBS42 vector was about 2.9 kb and the DNA sequence of the subcloned fragment (Hinc II-Hinc II, 1.4 kb) was determined. The DNA sequence of *ermJ* was composed of 357 bp for leader region and 861 bp for the strcuctural gene. Because the leader sequence of *ermJ* was homologous to that of *ermK*, the expression of *ermJ* is also thought to be controlled by a transcriptionl attenuation mechanism.

Keywords □ MLS antibiotics, inducible resistance, gene cloning, *B. anthracis*.

The MLS resistance phenotype refers to cross-resistance to three groups of antibiotics; the macrolides, the lincosamides and the streptogramin B. The classical phenotype was described for four *Staphylococcus aureus* isolates, in which resistance to the macrolide spiramycin was shown to be inducible by low concentration of erythromycin[1]. The phenotype has been found to occur in a variety of baterial genera[2], but the *S. aureus* system has been subjected to the most intensive scrutiny and is understood in greatest detail[3-4]. The induction process entails activation of an mRNA that encodes a 23S rRNA methylase, the *erm* methylase, which in turn renders newly synthesized ribosomes resistant to the MLS agents by methylating a specific adenosine residue (equivalent to *Esherichia coli* 23S rRNA A2058[5] of the rRNA component of the peptidyl transferase center). The activation involves alteration in the secondary structure of mRNA, resulting from a stall of an erythromycin-ribosome complex on the leader region(s) encoding short polypeptides. Constitutive variants readily arise *via* mutational changes in these leader regions.

In constrast to translational attenuation mechanism found in most MLS resistance genes, *ermK*, a MLS resistance element isolated from *B. lichenifomis* is regulated at transcriptional level[6]. The *ermK* mRNA leader sequence can fold in either of two mutually exclusive conformation, one of which is postulated to form in the absence of induction and to contain two rho factor independent terminators. It is suggested that the synthesis of *ermK* message is initiated consitutively upstream of the proposed terminator but completed inducibly downstream of this site.

We have isolated an MLS resistance strain from a soil sample in Korea. It was identified as *Bacillus anthracis*[7]. By colony hybridization method, the MLS resistance element, designated as *ermJ*, was cloned from the chromosomal DNA of that strain. The *ermJ* DNA sequence was determined. The leader sequence of *ermJ* was compared with those of other *erm* genes and the possible control mechanism of expression of *ermJ* was considered.

MATERIALS AND METHODS

Bacteria and plasmid

Bacillus anthracis 590[7], *E. coli* CSH26 (lac-, CAM^r)[8], *B. subtilis* UOTO277 (EM^r, CAM^r)[9] and pBS42

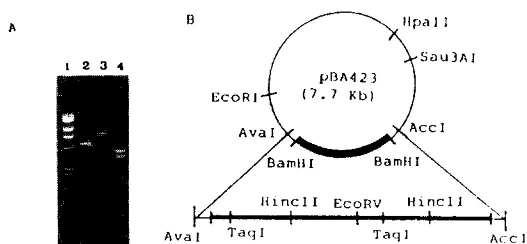

Fig. 1. (a) Characterization of pBA423 plasmid.
lane 1: HindIII digested lamda DNA
lane 2: BamHI digested pBS42 plasmid
lane 3: EcoRI digested pBA423 plamsid
lane 4: AvaI and AccI digested pBA423 plmsid (2.9 kb insert)
(b) Restriction endonuclease map of pBA423 plasmid containing *ermJ* DNA fragment indicated by solid bar.

plasmid vector (EM^r, CAM^r) were used. For DNA sequencing, pGEM 3Zf(+), (−) phagemid vector[10], *E. coli* K12 JM109[11] and R408, M13K07 helper phage were purchased from Promega.

Antibiotics and enzymes

Erythromycin(EM), chloramphenicol(CAM), ampicillin and other antibiotics were purchased from Sigma Chemical Co. and various restriction enzymes were purchased from New England Biolabs, Boehringer Mannheim, Promega and KOSCO Chemical Co. Ltd.

Cloning of MLS resistance determinant from Bacillus anthracis 590 by clony hybridization method

Total chromosomal DNA isolated from the culture of *B. anthracis* 590[7] was digested with various restristion enzymes and by Southern blotting[13], it was identified that BclI digested fragments of about 3.4 kb have homology with the probe, MspI-MspI fragment (660 bp) of *ermK*[6]. The BclI digested fragment of 3.4 kb was electroeluted and ligated with pBS42 shuttle vector in BamHI site. *E. coli* CSH26 was transformed with the recombinant plasmids and the transformants were screened by clony hybridization method[4] using the same probe in the Southern blot.

Restriction enzyme site mapping of pBA423 and subcloning of MLS resistance determinant

The pBA423 plasmid composed of the MLS re-

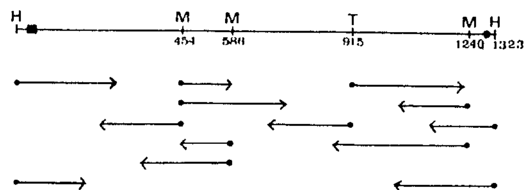

Fig. 2. DNA sequencing strategy of *ermJ*.
The arrows indicates the direction of sequencing. The position of the *ermJ* promoter and probable transcriptional terminator are shown by a filled square and a circle, respectively.
H: HincII, M: MspI, T: TaqI.

sistance determinant cloned from *B. anthracis* 590 and pBS42 plasmid vector was digested with various restriction enzymes. After electrophoresis, restriction enzyme site map was determined. For subcloning, HincII-HincII (1.4 kb), TaqI-TaqI (2.0 kb), BamHI-EcoRV (1.8 kb) fragments were isolated by electroelution. The eluted fragments were ligated with pBS 42 shuttle vector, respectively and *B. subtilis* UOTO 277 was transformed with the recombinant plamids[15]. After 2 days incubation on LB agar plate containing erythromycin (10 μg/m*l*) and chloramphenicol (10 μg/m*l*), the transformants were selected.

DNA sequencing of MLS resistance determinant (ermJ) cloned from B. anthracis 590

By Sanger's dideoxy nucleotide chain termination method[16], DNA sequencing was performed. The sequencing strategy is shown in Fig. 2 and Klenow sequencing system using pGEM-3Zf phagemid vector (Promega) was used.

RESULTS AND DISCUSSION

Cloning of MLS resistance determinant from B. anthracis 590

By colony hybridization, four colonies showing the positive signal were selected. The recombinant plasmid with the cloned gene was isolated from the culture of selected transformants and retransformed to *B. subtilis* UOTO277[15]. The transformed *B. subtillis* resistant to both erythromycin and chloramphenicol were selected. The inducibility patterns of resistance determined by agar disk method were identical with that of orignal 590 strain. The recombinant plamid was designated as pBA423. Fig . 1(a)

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HincII                          MboI              60   MnII
GTTGACGTTTTTCCAAAGAATGCCCTACAATGAGATCGTAACTTTAACTTTTCAGGAGGATTATTAAAAA
-35                       -10  100                        SD-1
ATGACACACTCAATGAGACTTCGTTTCCCAACTTTGAACCAGTAATTAAATACGTTCAAAGGCTCTGTT
MetThrHisSerMetArgLeuArgPheProThrLeuAspGln
           150       MboI                                     200
TGTGTATGCAGAGTAAACGGGATCAGTCTGTCCTTTTTTAATAATCTTCAATTTGCATATAAAATTTGT
               MboI                             250              DdeI
AATTTGTAGAAAGCCGGATCGTTCTGCCTTTCTTTTTTGTTTAATCCTTTTTTTCTAACGGCTGAGAAA
                        300                                        HinfI
GGCTTATTTGGTTATGGAAATACCGCGGCTTCATCCTTAAAGGATGGCTCTTCCCTTTACTCTGAATCA
   350                          MnII                    400
CAGGCAGACCGCCTGTGATTTTTTATGATGAGAGGAAGAGGAAACATGAAGAAAAAAAATCATAAGTAC
                                SD-2            MetLysLysLysAsnHisLysTyr
                                          450   HpaII
AGAGGAAAAAAGTTAAACCGCGGGGAATCTCCGAATTTTTCCGGACAGCATTTGATGCATAATAAAAAA
ArgGlyLysLysLeuAsnArgGlyGluSerProAsnPheSerGlyGlnHisLeuMetHisAsnLysLys
                 MboI                                               550
TTAATTGAAGAAATTGTGGATCGGGCAAATATTAGCATAGACGATACGGTTTTAGAGTTAGGAGCGGGA
LeuIleGluGluIleValAspArgAlaAsnIleSerIleAspAspThrValLeuGluLeuGlyAlaGly
                        DdeI       HpaII         600
AAAGGGGCTTTGACAACTGTCCTAAGTCAAAAAGCCGGTAAGGTATTGGCAGTGGAAAACGATTCTAAA
LysGlyAlaLeuThrThrValLeuSerGlnLysAlaGlyLysValLeuAlaValGluAsnAspSerLys
                              650                              EcoRV
TTCGTTGATATACTCACACGTAAAACGGCACAGCATTCAAATACGAAAATTATTCATCAAGATATCATG
PheValAspIleLeuThrArgLysThrAlaGlnHisSerAsnThrLysIleIleHisGlnAspIleMet
          700                                                750
AAGATTCATTTACCAAAAGAAAAGTTTGTGGTGGTCTCTAATATTCCCTATGCCATCACAACCCCCATC
LysIleHisLeuProLysGluLysPheValValValSerAsnIleProTyrAlaIleThrThrProIle
                                                 MboI
ATGAAAATGCTTTTGAACAATCCTGCAAGCGGATTTCAAAAAGGGATCATCGTAATGGAAAAAGGGGCT
MetLysMetLeuLeuAsnAsnProAlaSerGlyPheGlnLysGlyIleIleValMetGluLysGlyAla
                         850
GCTAAACGTTTCACATCAAAATTCATTAAAAATTCCTATGTTTTAGCTTGGAGAATGTGGTTTGATATT
AlaLysArgPheThrSerLysPheIleLysAsnSerTyrValLeuAlaTrpArgMetTrpPheAspIle
 900            TaqI                                  950
GGCATTGTCAGAGAAATATCGAAAGAGCATTTTTCTCCCCCTCCAAAAGTGGACTCGGCAATGGTCAGA
GlyIleValArgGluIleSerLysGluHisPheSerProProProLysValAspSerAlaMetValArg
                                       1000
ATAACACGAAAAAAAGACGCGCCTCTATCACATAAACATTATATTGCGTTTCGGGGACTTGCCGAATAC
IleThrArgLysLysAspAlaProLeuSerHisLysHisTyrIleAlaPheArgGlyLeuAlaGluTyr
                 1050                                             1100
GCCGCTAAAGGAGCCGAATATCCCTCTCTGTGTTCGTTTACGCGGAATTTTTACCCCGCGTCAAATGAAA
AlaLeuLysGluProAsnIleProLeuCysValArgLeuArgGlyIlePheThrProArgGlnMetLys
                                                    1150
CACTTAAGAAAAAGTCTAAAAATCAACAATGAAAAAACCGTTGGAACGCTCACCGAAAACCAATGGGCG
HisLeuArgLysSerLeuLysIleAsnAsnGluLysThrValGlyThrLeuThrGluAsnGlnTrpAla
                              1200                                HpaII
GTTATTTTTAACACGATGACTCAGTATGTAATGCATCACAAATGGCCAAGAGCAAATAAGCGAAAACCC
ValIlePheAsnThrMetThrGlnTyrValMetHisHisLysTrpProArgAlaAsnLysArgLysPro
      1250                                  HindIII        1300
GGAGAAATATAAAGAAAAAGCTGCTGACTTCCCGTCAGCAGCTTTAGCTATTTCTCGAGGGATTCAGAT
GlyGluIle
     HincII
GTCCCTGTCAAC
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Fig. 3. DNA sequence of *ermJ*. The positions of the *ermJ* promoter (−35 and −10), the probable transcriptional terminator, relevent restriction endonuclease recognition sites, SD-1 and SD-2 and their associated initiation codons, and the leader peptide and methylase termination codons are all underlined.

shows digested pattern of pBA423 with four restriction endonucleases; lane 3 shows a 7.7 kb linear fragment after EcoRI digestion, whereas lane 4 shows two fragments after AccI and AvaI digestion. Therefore, the pBA423 contains about the 2.9 kb insert.

Restriction enzyme site mapping of pBA423 and subcloning of MLS resistance determinant

Using various restriction enzymes, restriction endonuclease map of pBA423 was determined (Fig. 1(b)). Based on the map, various fragments were electroeluted and ligated with pBS42 vector. As the result of *B. subtilis* UOTO277 transformation with the ligated plasmid, it was identified that MLS resistance gene is located on the HincII-HincII (1.4 kb) fragment.

Table I. Differences of DNA base sequences and amino acid codons between *ermJ* and *ermK*

Position		Base(a,a) substitution	
ermJ	*ermK*	*ermJ*	*ermK*
90	471	T(Leu)	G(Leu)
305	686	C	A
443	824	C(Ser)	A(Tyr)
571	952	G(Val)	A(Met)
558	939	G(Gly)	T(Gly)
629	1010	A(Asp)	C(Ala)
658	1039	T(Ser)	C(Pro)
1008	1389	T(Tyr)	C(Tyr)
1019-20	1400-1	G, G(Arg)	T, T(Leu)
1023	1404	A(Gly)	G(Gly)
1035	1416	C(Tyr)	T(Tyr)
1051-3	1432-4	A, A(Asn)	C, A(Gln)
1054-5	1435-6	A, T(Ile)	G, C(Ala)
1060	1441	C(Leu)	T(Phe)
1069-70	1450-1	C, G(Arg)	G, C(Ala)
1089	1470	C(Thr)	T(Thr)
1128	1509	C(Ile)	T(Ile)
1203	1584	A(Val)	G(Val)
1209	1590	T(His)	C(His)
1271	1652	T	G
1274	1655	C	T
—	1671	—	A
1292	1675	A	T

DNA sequencing of MLS resistance determinant (ermJ)

DNA sequence of HincII-HincII fragment was determined by Sanger's dideoxy chain termination method (Fig. 3) and the sequence was analyzed by IBM PC DNA SEQUENCE ANALYSIS PACKAGE. The 1325 bp DNA fragment contains 357 leader sequence and 861 bp methylase coding sequence of *ermJ* mRNA. Small open reading frame (ORF) found in #70 to #110 base encodes leader peptides composed of 14 amino acids. And Shine-dalgarno (SD-1) region was located in upstream region of that ORF. Large ORF from #391 to #1252 base is thought to be the structural gene of *ermJ* encoding the methylase. Another Shine-Dalgarno (SD-2) region was also located upstream of this large ORF. When the DNA sequence of *ermJ* was compared with that of *ermD*[17] or *ermK*[6], the MLS resistance element both cloned from *B. licheniformis*, the degree of homology was 99% between *ermJ* and *ermD*, and 97% between *ermJ* and *ermK*. Therefore it is suggested that the MLS resistance are phylogenetically related. It was demonstrated that *ermK* ex-

pression is controlled by transcriptional attenuation in contrast to translational attenation in other inducible *erm* genes[6]. The *ermK* mRNA leader sequence can fold in either of two mutually exclusive conformations, one of which is postulated to form in the absence of induction and to contain two rho factor-independent terminators. It is suggested that the synthesis of the *ermK* message is initiated consistitutively upstream of the proposed terminator but completed inducively downstream of this site. Because the leader sequence of *ermJ* was homologous to that of *ermK*, the expression of *ermJ* is also thought to be controlled by transcriptional attenuation mechanism.

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LITERATURE CITED

1. Chabbert, Y.: Antagonism *in vitro* entre l'-erythromycine et la spiramycine. *Ann. Inst. Pasteur* (Paris). **90**, 787 (1956).
2. Duval, J.: Evolution and epidermiology of MLS resistance. *J. Antimicrob. Chemother.* **16**(Suppl. A), 137 (1985).
3. Novick, R. P. and Murphy, E.: MLS-reistance determinants in *Staphylococcus aureus* and their molecular evolution. *J. Antimicrob. Chemother.* **16** (Suppl. A), 101 (1985).
4. Weisblum, B.: Inducible resistance to macrolides, lincosamides and streptogramin type B antibitiotics: The resistance phenotype, its biological diversity, and structural elements that regulate expression-a review. *J. Antimicrob. Chemother.* **16**(Suppl. A), 63 (1985).
5. Thakker-Varia, S., Ranzini, A. R. and Dubin, D. T.: Mechanism of erythymicin-resistance in *Staphylococcus aureus* and *E. coli.* The "MLS", oligonuleotide of 23S RNA. *Plasmid* **14**, 152 (1985).
6. Kwak, J. H., Choi, E. C. and Weisblum, B.: Transcriptional attenuation control of *ermK*, a Macrolide-Lincosamide-Streptogramin B resistance determinant from *Bacillus licheniformis. J. Bacteriol.* **173**, 4725 (1991).
7. Choi, E. C., Woo, K. W., Woo, J. S., Kwak, J. H. and Kim, B. K.: The cloning of MLS antibiotics inducible resistance gene. *Arch. Pharm. Res.* 12(3), 176 (1989).
8. Miller, J. H.: Exeperiment in molecular genetics, Cold Spring Harbor U.S.A. (1972).
9. Band, L. and Henner, D. J.: *Bacillus sublitis* requires a stringent Shine-Dalgarno region for gene expression. *DNA* **3**, 17 (1984).
10. Mead, D. A. and Kemper, B.: Vectors, A suvery of molecular cloning vector and their uses (R. L. Rodriguez and D. T. Denhardt, eds.), Butterworth Publishing Co., Stonham, MA. (1987).
11. Yanish-Perron, C., Vieira, J. and Messing, J.: Improved M13 phage cloning vectors and host strains. Nucleotides sequences of the M13 mp18 and 19 vectors. *Gene* **33**, 103 (1985).
12. Selzer, G., Som, T., Itoh, T. and Tomizawa, J.: The origin of regulation of replication of plasmid p15A and comparative studies on the nucleotides sequences around the origin of related plasmids. *Cell* **32**, 119 (1983).
13. Southern, E.: Detection of specific sequences among DNA fragments separated by electrophoresis. *J. Mol. Biol.* **98**, 503 (1975).
14. Davis, L. G., Dibner, M. D. and Battey, J. F.: Methods in molecular biology. Elsevier Science Publishing Co., In. U.S.A. (1986).
15. Gryczan, T. J., Contents, S. and Dubnau, D.: Characterization of *Staphylococcus aureus* plasmid introduced by transformation into *Bacillus subtilis. J. Bacteriol.* **134**, 318 (1978).
16. Sanger, F., Nicklen, S. and Coulson, A. R.: DNA sequencing with chain termination inhibitors, *Pro. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
17. Gryczan, T., Israeli-Reches, M., Del Bue, M. and Dubnau, D.: DNA sequence and regulation of *ermD*, a macrolide-linosamide-streptogramine B resistance element from *Bacillus licheniformis. Mol. Gen. Genet.* **194**, 349 (1984).