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NOTE

Relationship between Two Tetracycline Resistance Plasmids of *Staphylococcus aureus* in Korea

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To investigate the relationship between two tetracycline resistance (Tc^r) plasmids, the 24.82-kb pKH1 and the 4.44-kb pKH6, of *Staphylococcus aureus* isolated in Korea, cloning of the 4570-bp *Hin*dIII fragment into pBluescript II KS^+ after partial digestion of the Tc^r plasmid pKH1 with *Hin*dIII and sequence determination of that fragment were carried out. Analysis of the sequences revealed that the 4570-bp *Hin*dIII fragment contained a 4011-bp fragment of the small Tc^r plasmid pKH6 flanked by the partial sequences of IS*431*mec. It was concluded from the above result that the pKH1 was produced by integration of the partial sequence of the pKH6 into another plasmid via IS*431*mec.

Tetracycline resistance (Tc^r) in *Staphylococcus aureus* is commonly mediated by one of a family of closely related plasmids. pT181 is a prototype plasmid of this family (10) and pKH6 was a pT181-like plasmid of *S. aureus* isolated in Korea (8, 9). Both pT181 and pKH6 can be cleaved by *Hin*dIII into 3 fragments which are designated *Hin*dIII A(2.35-kb), *Hin*dIII B(1.53-kb), and *Hin*dIII C(0.56-kb), respectively. It was known that *Hin*dIII A had a *tet* gene conferring tetracycline resistance. Another 24.82-kb Tc^r plasmid pKH1 was also isolated in Korea and characterized by restriction enzyme mapping techniques (6). In a previous report (7), we described the complete sequence of the 2475-bp *Hin*dIII fragment (*Hin*dIII A') of pKH1 containing the *tet* gene and showed that *Hin*dIII A' consisted of two parts. One portion originated from IS*431*mec and the other portion originated from *Hin*dIII A fragment of pKH6. To find the *Hin*dIII B and C fragments in pKH1, partial digestion of pKH1 with *Hin*dIII and subsequent cloning into pBluescript II KS^+ was carried out. A recombinant plasmid containing the 4570-bp *Hin*dIII fragment (*Hin*dIII T) of pKH1 in the *Hin*dIII site of pBluescript II KS^+ was obtained. Complete *Hin*dIII digestion of *Hin*dIII T revealed that it was composed of *Hin*dIII A', *Hin*dIII C, and *Hin*dIII B-like fragments (*Hin*dIII B'). Subclonings and sequence determinations of *Hin*dIII C and *Hin*dIII B' were performed. From the sequences of *Hin*dIII A', *Hin*dIII B', and *Hin*dIII C, the complete sequence of *Hin*dIII T of pKH1 was determined (Fig. 1). The *Hin*dIII T contained a 4011-bp fragment of pKH6 flanked by the partial sequences of IS*431*mec. The location of *Hin*dIII T in pKH1 was determined by comparison of the restriction map of *Hin*dIII T with that of pKH1 (Fig. 2). IS*431*mec (2) is also known as IS*257* (13) and to flank many resistance genes (1, 3-5, 11-15). Integrations of pKH6-like plasmid into chromosome or with other plasmids are well known in *S. aureus*. The pT181 was representative of that kind of plasmid. Two copies of the insertion sequences IS*257* were flanking an integrated copy of the plasmid pT181 in the ANS46 chromosome of methicillin resistant *S. aureus* ANS46 (14) and in the tetracycline and mupirocin resistance plasmid pJ3358 (11). Internal 8 bp repeats were seen at the ends of the integrated copies of pT181 in the ANS46 chromosome and in the plasmid pJ3358. But internal 8 bp repeats could not be seen at the ends of the integrated copy of pKH6 and only 4011 bp of pKH6 was seen in pKH1. It can be explained that some deletion occurred during the formation of pKH1 by integration of pKH6 with the other plasmid. Owing to the above mentioned deletion the Rep protein of pKH6 in pKH1 was a truncated protein that had only 99 amino acids (AAs) among 314 AAs of the Rep protein of pKH6 (Fig. 2). This type of integration was first observed in Tc resistance plasmids.

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*Hin*dIII

aagctttaaacttaaacctgactgtcattgtacatcgaaatatctgaataacctcattgagcaagatcaccgtcatattaaagtaagaaagacaagata 100

tcaaagtatcaatacggcaaagaatactttaaaaggtattgaatgtatttacgctctatataaaaagaaccgcaggtctcttcagatctacggattttcc 200

IR-r

ccatgccacgaaattagcatcatgctagcaagttaagcgaacactgacatgataaattagtggttagctatattttttactttgcaacagaaccACAAA 300

AATGATATATTTAAACTATTCTAATTTAGGAGGATTTTTTTATGAAGTGTCTATTTAAAAATTTGGGGAATTTATATGAGGTGAAAGAATAATTTACCCC 400

TATAAACTTTAGTCACCTCAAGTAAAGAGGTAAAATTGTTTAGTTTATATAAAAAATTTAAAGGTTTGTTTTATAGCGTTTTATTTTGGCTTTGTATTCT 500

TTCATTTTTTAGTGTATTAAATGAAATGGTTTTAAATGTTTCTTTACCTGATATTGCAAATCATTTTAATACTACTCCTGGAATTACAAACTGGGTAAAC 600

ACTGCATATATGTTAACTTTTTCGATAGGAACAGCAGTATATGGAAAATTATCTGATTATATAAATATAAAAAAATTGTTAATTATTGGTATTAGTTTGA 700

GCTGTCTTGGTTCATTGATTGCTTTTATTGGTCACAATCACTTTTTTATTTTGATTTTTGGTAGGTTAGTACAAGGAGTAGGATCTGCTGCATTCCCTTC 800

ACTGATTATGGTGGTTGTAGCTAGAAATATTACAAGAAAAAAACAAGGCAAAGCCTTTGGTTTTATAGGATCAATTGTAGCTTTAGGTGAAGGGTTAGGT 900

CCTTCAATAGGGGGAATAATAGCACATTATATTCATTGGTCTTACCTACTTATACTTCCTATGATTACAATAGTAACTATACCTTTTCTTATTAAAGTAA 1000

TGGTACCTGGTAAATCAACAAAAAATACATTAGATATCGTAGGTATTGTTTTAATGTCTATAAGTATTATATGTTTTATGTTATTTACGACAAATTATAA 1100

TTGGACTTTTTTAATACTCTTCACAATCTTTTTTGTGATTTTTATTAAACATATTTCAAGAGTTTCTAACCCTTTTATTAATCCTAAACTAGGGAAAAAC 1200

ATTCCGTTTATGCTTGGTTTGTTTTCTGGTGGGCTAATATTTTCTATAGTAGCTGGTTTTATATCAATGGTGCCTTATATGATGAAAACTATTTATCATG 1300

TAAATGTAGCGACAATAGGTAATAGTGTTATTTTTCCTGGAACCATGAGTGTTATTGTTTTTGGTTATTTTGGTGGTTTTTTAGTGGATAGAAAAGGATC 1400

ATTATTTGTTTTTATTTTAGGATCATTGTCTATCTCTATAAGTTTTTTAACTATTGCATTTTTTGTTGAGTTTAGTATGTGGTTGACTACTTTTATGTTT 1500

ATATTTGTTATGGGCGGATTATCTTTTACTAAAACAGTTATATCAAAAATAGTATCAAGTAGTCTTTCTGAAGAAGAAGTTGCTTCTGGAATGAGTTTGC 1600

TAAATTTCACAAGTTTTTTATCAGAGGGAACAGGTATAGCAATTGTAGGAGGTTTATTGTCACTACAATTGATTAATCGTAAACTAGTTCTGGAATTTAT 1700

AAATTATTCTTCTGGAGTGTATAGTAATATTCTTGTAGCCATGGCTATCCTTATTATTTTATGTTGTCTTTTGACGATTATTGTATTTAAACGTTCTGAA 1800

AAGCAGTTTGAATAGTTATATTATATTTTGGTTTAGAACTATGAGTGGCTAGCATTTTGCCACTCATTTTTTGCGTTAGCAAAAACAGGTTTAAGCCTCG 1900

CAGAGCACACGTATTAACGACTTATTAAAAATAAGTCTAGTGTGTTAGACTTAAACTATTAAATACACATGAAACCTTTGTGCTTAGGAGTGATTTTTAT 2000

ATGTCTTATTCCATTGTTAGAGTTTCAAAAGTTAAATCTGGAACAAATACAACGGGCATACAAAAACATGTTCAAAGAGAAAATAATAATTATGAAAATG 2100

AAGATATAGACCATAGTAAAACTTACTTAAATTATGATTTGGTAAATGCTAATAAACAGAATTTTAATAACTTGATTGATGAAAAAATCGAACAGAATTA 2200

TACAGGCAAAAGAAAAATTAGAACAGACGCGATTAAACACATTGATGGTTTAATTACATCAGACAATGATTTCTTTGATAATCAAACGCCAGAAGATACA 2300

AAGCAGTTTTTTGAATATGCTAAAGAGTTTTTAGAACAAGAATACGGTAAAGATAATTTATTATATGCAACAGTTCACATGGACGAAAAAACACCACATA 2400

*Hin*dIII

TGCATTATGGCGTTGTTCCAATAACTGATGATGGTCGTTTAAGTGCTAAAGAAGTTGTAGGTAATAAAAAAGCTTTAACAGCGTTTCAAGATAGATTTAA 2500

TGAGCATGTTAAACAACGAGGATATGATTTAGAACGTGGGCAATCAAGACAAGTAACAAATGCTAAACATGAGCAAATAAGTCAGTATAAACAAAAAACA 2600

GAATATCATAAGCAAGAATATGAACGTGAGAGCCAAAAAACAGACCATATAAAGCAAAAGAACGATAAATTAATGCAAGAGTACCAAAAATCGTTAAATA 2700

CGCTTAAAAAGCCTATAAATGTTCCGTATGAGCAAGAAACTGAAAAAGTAGGTGGTTTATTTAGCAAAGAAATACAAGAAGCTGGAAATGTTGTAATAAG 2800

CCAAAAAGATTTCAATGAATTTCAGAAACAGATAAAAGCTGCTCAAGATATTTCGGAAGATTACGAGTATATAAAGTCTGGTAGAGCCTTAGATGATAAA 2900

GATAAGGAAATACGAGAGAAAGATGATTTATTAAATAAAGCAGTTGAGCGTATTGAAAACGCAGACGATAATTTTAACCAACTTTACGAAAATGCAAAGC 3000

*Hin*dIII

CACTTAAAGAGAATATAGAAATAGCGTTAAAGCTTTTAAAAATCTTACTAAAAGAGTTAGAACGAGTTTTAGGAAGAAATACCTTTGCGGAAAGAGTTAA 3100

TAAGTTAACAGAAGATGAACCAAAACTAAATGGTTTAGCAGGAAACTTAGATAAAAAAATGAATCCAGAATTATATTCAGAACAGGAACAGCAACAAGAA 3200

CAACAAAAGAATCAAAAACGAGATAGAGGTATGCACTTATAGAACATGCATTTATGCCGAGAAAACTTATTGGTTGGAATGGGCTATGTGTTAGCTAACT 3300

TGTTAGCGAGTTGGTTGGACTTGAATTGGGATTAATCCCAAGAAAGTACCAACCCAACAACACATAAAGCCCTGTAGGTTCCGACCAATAAGGAAATTGG 3400

AATAAAGCAATAAAAGGAGTTGAAGAAATGAAATTCAGAGAAGCCTTTGAGAATTTTATAACAAGTAAGTATGTACTTGGTGTTTTAGTAGTTTTAACTG 3500

TTTACCAGATAATACAAATGCTTAAATAAAAAAAGACTTGATCTGATTAGACCAAGTCTTTTGATAGTGTTATATTAATAACAAAATAAAAAGGAGTCGC 3600

TCACGCCTTGACCAAAGTTTGTGAACGACATCATTCAAAGAAAAAAACACTGAGTTGTTTTTATAATCTTGTATATTTAGATATTAAACGATATTTAAAT 3700

ATACATCAAGATATATATTTGGGTGAGCGATTCCTTAAACGAAATTGAGATTAAGGAGTCGATTTTTTATGTATAAAAACAATCATGCAAATCATTCAAA 3800

TCATTTGGAAAATCACGATTTAGACAATTTTTCTAAAACCGGCTACTCTAATAGCCGGTTGGACGCACATACTGTGTGCATATCTGATCCAAAATTAAGT 3900

TTTGATGCAATGACGATCGTTGGAAATCTCAACCGAGACAACGCTCAAGCCCTTTCTAAATTTATGAGTGTAGAGCCCCAAATAAGACTTTGGGATATTC 4000

TTCAAACAAAGTTTAAAGCTAAAGCACTTCAAGAAAAAGTTTATATTGAATATGACAAAGTGAAAGCAGATAGTTGGGATAGACGTAATATGCGTATTGA 4100

ATTTAATCCAAACAAACTTACACGAGATGAAATGATTTGGTTAAAACAAAATATAATAAGCTACATGGAAGATGACGGTTTTACAAGATTAGATTTAGCC 4200

TTTGATTTTGAAGATGATTTGAGTGACTACTATGCAATGTCTGATAAAGCAGTTAAGAAAACTATTTTTTATGGTCGTAATGGTAAGCCAGAAACAAAAT 4300

IR-l

ATTTTGggttctgttgcaaagttgaatttatagtataattttaacaaaaaggagtcttctgtatgaactatttcagatataaacaatttaacaaggatgt 4400

tatcactgtagccgttggctactatctaagatatgcattgagttatcgtgatatatctgaaatattaagggaacgtggtgtaaacgttcatcattcaacg 4500

*Hin*dIII

gtctaccgttgggttcaagaatatgccccaattttgtatcaaatttggaagaaaaagcataaaaaagctt 4570

Fig. 1. Complete nucleotide sequence of 4570-bp *Hin*dIII fragment of tetracycline resistance plasmid, pKH1.

IS*431*mec related sequences (1-295 and 4307-4570 bp) are indicated by small letter. Inverted repeat sequences in IS*431*mec are underlined. The sequence of the 4570-bp *Hin*dIII fragment of pKH1 has been registered in GenBank (NCBI) with the accession number U38656.

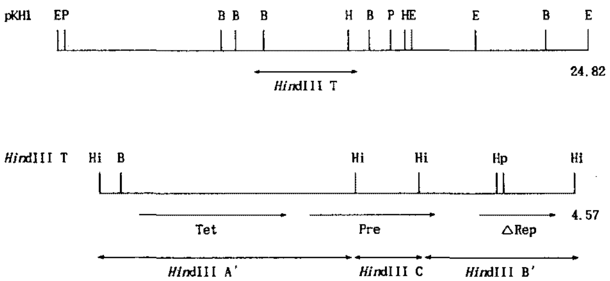

Fig. 2. Restriction endonucleases maps of pKH1 and pKH1T.
Restriction sites are indicated by B(*Bgl*II), E(*Eco*RI), H(*Hpa*II), Hi (*Hin*dIII), and P(*Pvu*II). Tet, Pre, and ΔRep indicate Tet protein, recombination protein, and truncated replication protein, respectively. Map coordinates are expressed in kilobases.

REFERENCES

1. Barberis-Maino, L., B. Berger-Bächi, H. Weber, W. D. Beck, and F. H. Kayser. 1987. IS*431*, a staphylococcal insertion sequence-like element related to IS*26* from *Proteus vulgaris. Gene* **59**: 107-113.
2. Barberis-Maino, L., R. Cristina, F. H. Kayser, and B. Berger-Bächi. 1990. Complete nucleotide sequence of IS *431*mec in *Staphylococcus aureus. Nucleic Acids Res.* **18**: 5548.
3. Bhuiyan, M. Z. A., K. Ueda, Y. Inouye, and M. Sugiyama. 1995. Molecular cloning and expression in *Escherichia coli* of bleomycin-resistance gene from a methicillin-resistant *Staphylococcus aureus* and its association with IS *431*mec. *Appl. Microbiol. Biotechnol.* **43**: 65-69.
4. Gillespie, M. T., B. R. Lyon, L. S. L. Loo, P. R. Matthews, P. R. Stewart, and R. A. Skurray. 1987. Homologous direct repeat sequences associted with mercury, methicillin, tetracycline and trimethoprim resistance determinants in *Staphylococcus aureus. FEMS Microbiol. Lett.* **43**: 165-171.
5. Gillespie, M. T., J. W. May, and R. A. Skurray. 1986. Detection of an integrated tetracycline resistance plasmid in the chromosome of methicillin-resistant *Staphylococcus aureus. J. Gen. Microbiol.* **132**: 1723-1728.
6. Kim, K. H., J. M. Kim, and K. H. Moon. 1992. Characterization of tetracycline resistant plasmid in *Staphylococcus aureus* by restriction enzyme mapping techniques. *Yakhak Hoeji* **36**: 255-258.
7. Kim, W. K., S. J. Yoon, J. M. Kim, C. K. Shin, S. H. Im, and K. H. Moon. 1996. Association of *tet* gene with partial sequence of IS*431*mec in tetracycline resistance plasmid pKH1. *Arch. Pharm. Res.* **19**: 171-172.
8. Lee, D. W. and K. H. Moon. 1995. Characterization of tetracycline resistance plasmid of multi-drug resistant *Staphylococcus aureus. Yakhak Hoeji* **39**: 6-9.
9. Lee, D. W., S. J. Yoon, W. K. Kim, C. K. Shin, S. H. Im, and K. H. Moon. 1996. Complete nucleotide sequence of pKH6, a tetracycline resistance plasmid from multi-drug resistant *Staphylococcus aureus* SA2. *Kor. J. Appl. Microbiol. Biotechnol.* in press.
10. Lyon, B. R. and R. A. Skurray. 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol. Rev.* **51**: 88-134.
11. Needham C., M. Rahman, K. G. H. Dyke, and W. C. Noble. An investigation of plasmids from *Staphylococcus aureus* that mediate resistance to mupirocin and tetracycline. *Microbiol.* **140**: 2577-2583.
12. Rouch, D. A., L. J. Messeroti, L. S. L. Loo, C. A. Kackson, and R. A. Skurray. 1989. Trimethoprim resistance transposon Tn4003 from *Staphylococcus aureus* encodes genes for a dihydrofolate reductase and thymidylate synthetase flanked by three copies of IS*257*. *Mol. Microbiol.* **3**: 161-175.
13. Rouch, D. A. and R. A. Skurray. 1989. IS*257* from *Staphylococcus aureus*: a number of an insertion sequence superfamily prevalent among Gram-positive and Gram-negative bacteria. *Gene* **76**: 192-205.
14. Skinner, S., B. Inglis, P. R. Matthews, and P. R. Stewart. 1988. Mercury and tetracycline resistance genes and flanking repeats associated with methicillin resistance on the chromosome of *Staphylococcus aureus. Mol. Microbiol.* **2**: 289-297.
15. Stewart, P. R., D. T. Dubin, S. G. Chikramane, B. Inglis, P. R. Matthews, and S. M. Poston. 1994. IS*257* and small plasmid insertions in the *mec* region of the chromosome of *Staphylococcus aureus. Plasmid* **31**: 12-20.

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