

Association of *tet* Gene with Partial Sequence of IS431mec in Tetracycline Resistance Plasmid pKH1

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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 (Lyon and Skurray, 1987). pKH6, a pT181-like plasmid, was also found from multi-drug resistance *S. aureus* SA2 in Korea (Lee and Moon, 1995) and its complete nucleotide sequence was determined (Lee *et al.*, 1995). Both pT181 and

pKH6 can be cleaved by *Hind*III into 3 fragments which are designated *Hind*III A (2.35-kb), *Hind*III B (1.53-kb), and *Hind*III C (0.56-kb), respectively. It was known that *Hind*III A had a *tet* gene conferring tetracycline resistance. Unlike above plasmids, a large 24.82-kb Tc^r plasmid pKH1 was found in Tc^r *S. aureus* SA8 and characterized by the restriction enzyme mapping techniques (Kim *et al.*, 1992). To find out the relationship between pKH1 and pKH6, 2475-bp *Hind*III fragment containing *tet* gene was cloned from pKH1 into the phagemid pBluescript II KS⁺ and its complete sequence was determined. Fig. 1 showed that the fragment was consisted of two parts. One portion (1-295 bp) indicated by small letter was originated from IS431mec which was found in transposon and the other portion (296-2475 bp) containing *tet* gene was originated from *Hind*III A fragment of pKH6. It was shown that many resistance genes were flanked by IS431mec (Barberis-Maino *et al.*, 1987; Rouch *et al.*, 1989; Bhuiyan *et al.*, 1995). So there is a possibility that pKH1 is a large plasmid made by integrating pKH6 into another plasmid. Integration of pT181 into chromosome of methicillin resistant *S. aureus* was also reported (Gillespie *et al.*, 1986). Now, an experiment is going on to find out pKH6-derived *Hind*III B and *Hind*III C fragments in pKH1 and this will give a helpful information to elucidate the mechanism of *tet* gene transfer in Korea.

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aagcttttaaaccttaaacctgactgctatgtacatcgaaatactgaaataacctcattgagcaagatcacccgtcatataaagtaagaagaagacaagata 100
tcaaaagtatcaatacggcaagaataactttaaaaggattgaatgtatttacgctctatataaaaagaaccgaggctccttcagatctacggattttcc 200
ccatgccacgaanaatagcatcatgctagcaagttaagcgaacactgacatgataaattagtggttagctatattttttactttgcaacagaaccacAAA 300
AATGATATATTTAAACTATTCTAATTTAGGAGGATTTTTTATGAAGTGCTATTTAAAAATTTGGGGAATTTATATGAGGTGAAGAATAATTTACCCC 400
TATAAACTTTAGTCACCTCAAGTAAAGAGGTAATAATTTGTTTAGTTTATATAAAAAATTTAAAGGTTTGTGTTTATAGCGTTTTATTTTGGCTTTGTATTCT 500
TTCAATTTTTAGTGATTAATAAGAAATGGTTTTAAATGTTCTTTACCTGATATGGCAAATCATTTTAATACTACTCCTGGAATTACAACTGGGTAAC 600
ACTGCATATATGTTAACTTTTTCGATAGGAACAGCAGTATATGGAAAATTTATCTGATATATAAATAAAAAAATTTGTTAATTATTGGTATTAGTTTGA 700
GCTGCTTTGGTTCATTGATTGCTTTTTATTGGTCACAATCACTTTTTTATTGATTTTTGGTAGGTTAGTACAAGGAGTAGGATCTGCTGCATTCCTTC 800
ACTGATTATGGTGGTTGTAGCTAGAATATTACAAGAAAAAACAAGGCAAGCCCTTTGGTTTTATAGGATCAATTGTAGCTTTAGGTGAAGGGTTAGGT 900
CCTTCAATAGGGGGAATAATAGCACATTATATTCATTGGTCTTACCTACTTATACTTCTATGATTACAATAGTAACATATACCTTTTCTTATTAAGTAA 1000
TGGTACCTGGTAAATCAACAAAAATACATTAGATATCGTAGGTATGTTTTAATGCTATAAGTATTATATGTTTTATGTTATTTACGACAAATTATAA 1100
TTGGACTTTTTAACTACTCTTCACAATCTTTTTTGTGATTTTTTAAACATATTTCAAGAGTTTCTAACCCCTTTTAAATCCTAACTAGGGAAAAAC 1200
ATTCCGTTATGCTTGGTTTTGTTTTCTGGTGGCTAATATTTTCTATAGTAGCTGGTTTTATATCAATGGTGCCTTATATGATGAAAACATTTTATCATG 1300
TAAATGTAGCCACAATAGGTAATAGTGTTATTTTTCTGGAACCATGAGTGTATTGTTTTGGTTATTTTGGTGGTTTTTATGTTGATGAAAAGGATC 1400
ATTATTTGTTTTTATTTTAGGATCATTGCTATCTCTATAAGTTTTTAACTATTGCAITTTTTGTTGAGTTTATGATGTTGGTTGACTACTTTTATGTTTT 1500
ATATTTGTTATGGGGGATTATCTTTTACTAAAACAGTTATATCAAAAATAGTATCAAGTAGTCTTTCTGAAGAAGAAGTTGCTTCTGGAATGAGTTTGC 1600
TAAATTTACAAAGTTTTTATCAGAGGGAACAGGTATAGCAATTTGAGGAGTTTTATGTCACFACAATGATTAATCGTAAACTAGTTCTGGAATTTAT 1700
AAATTTCTTCTGGAGTGTATAGTAAATTTCTGTAGCCATGGCTATCCTTATTTATTTATGTTGCTTTTGGAGATTGTTTAAACGTTCTGAA 1800
AAGCAGTTTGAATAGTTATATTATTTTTGGTTTTAGAAGTATGAGTGGCTAGCATTTTGCCACTCATTTTTTGGCTTAGCAAAAACAGGTTTAAAGCCTCG 1900
CAGAGCACACGATTTAACGACTTATTAATAAATAGTCTAGTGTGTTAGACTTAACTATTAATAACACATGAAAACCTTTGTTGCTTAGGAGTGAATTTAT 2000
ATGCTTATTCCATTGTTAGAGTTTCAAAAGTTAAATCTGGAACAAATACAACGGGCATACAAAAACATGTTCAAAGAGAAAAATAAATATTATGAAAATG 2100
AAGATATAGCCATAGTAAAACCTACTTAAATTTAGATTTGGTAAATGCTAATAACAGAAATTTAATAAATGATGAAAATAATCGAACAGAAATTA 2200
TACAGGCAAAAGAAAATTAGAACAGACGCGATTAAACACATTGATGTTTAAATACATCAGACAATGATTTCTTTGATAATCAAACGCCAGAGATACA 2300
AAGCAGTTTTTTGAAATATGCTAAAGAGTTTTTAGAACAAGAATACGGTAAAGATAATTTATTATATGCAACAGTTTACATGGAGCAAAAAACACCACATA 2400
TGCATTATGGCGTTGTTCCAATAACTGATGATGGTCTGTTAAGTGCTAAAGAAGTTGTAGGTAATAAAAAAGCTT 2475

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Fig. 1. Nucleotide sequence of 2475-bp *Hind*III fragment (accession no. U38656) of tetracycline resistance plasmid pKH1. IS431mec related sequence (1-295 bp) is indicated by small letter. Start codon of *tet* gene (436-1815 bp) is TGC.

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