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Expression and Inducibility in *Staphylococcus aureus* of the *mecA* Gene, Which Encodes a Methicillin-Resistant *S. aureus*-Specific Penicillin-Binding Protein

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A β-lactam-sensitive strain of *Staphylococcus aureus* could be converted to methicillin resistance by the introduction of a plasmid carrying the 4.3-kilobase *Hin*dIII chromosomal DNA fragment which encoded the *mecA* gene from a methicillin-resistant *S. aureus*. Transformant cells produced methicillin-resistant *S. aureus*-specific penicillin-binding protein constitutively, and additional insertion of an inducible penicillinase plasmid caused production of the penicillin-binding protein to become inducible.

Methicillin-resistant *Staphylococcus aureus* (MRSA), a serious clinical pathogen (8), is capable of producing a new penicillin-binding protein (PBP) that has a relatively low affinity for most β-lactam antibiotics. The production of the PBP, called PBP2′ (12, 18, 19), PBP-2a (3, 4, 14), or MRSA-PBP (16), has been supposed to be the major if not the sole cause of the high resistance of staphylococci to β-lactams. Loss of the resistance to β-lactams has been found to accompany the disappearance of this PBP (18). Moreover, induction of the MRSA-PBP by exposing the staphylococci to a β-lactam (12, 14, 18) may favor the survival of cells that have acquired such resistance. The mechanism involved in induction of the MRSA-PBP seems to be shared with induction of penicillinase (16, 18). The gene encoding a MRSA-PBP has been cloned as a 4.3-kilobase (kb) *Hin*dIII fragment from a clinically isolated MRSA strain, TK784, into *Escherichia coli* (10). This fragment, when introduced into the *E. coli* cells, caused constitutive production of MRSA-PBP. However, the transformant did not show resistance to β-lactams, because the structure of the peptidoglycan which is the product of the biosynthetic reaction carried by PBPs (13) is different in these organisms (10). To determine whether MRSA-PBP is actually responsible for the β-lactam resistance of MRSA, we had to demonstrate the transformation of β-lactam-sensitive *S. aureus* cells to resistance by the introduction of a DNA fragment containing the coding frame of this PBP. We report here the results of this transformation experiment and show that the inducibility of the MRSA-PBP encoded by the cloned *mecA* gene is controlled by a gene(s) in the penicillinase plasmid.

Table 1 shows the strains and plasmids used in these experiments. Chromosomal DNA from *S. aureus* and recombinant plasmids from *E. coli* were isolated by methods described previously (10). Transformation procedures of *E. coli* TG1 and *S. aureus* SA113 were performed by methods described previously (references 9 and 2, respectively).

Figure 1 shows the construction of recombinant plasmid pSP53 carrying the *mecA* gene. A 4.3-kb *Hin*dIII fragment (16) from the chromosomal DNA of an MRSA strain, TK784, that contained the *mecA* gene encoding MRSA-PBP was ligated with vector plasmid pUC19 at the *Hin*dIII site (10). The plasmid constructed, pSP51, was amplified in *E. coli* TG1 and cut at one of the two *Hin*dIII sites, and the resulting 7.0-kb DNA fragment was ligated with plasmid pSU40 (originated from staphylococcal plasmid pSU20) at the *Hin*dIII site. Then, transformants that contained plasmid

TABLE 1. Bacterial strains and plasmids

Strains and plasmids	Relevant genotype and phenotype	Source or reference
Strains		
S. aureus		
TK784	Mc^r (*mec*) Pc^r (*blaZ*) Tm^r (*aadD*) Em^r	Clinical isolate (18)
TK388E	Mc^r (*mec*) Em^r	This laboratory (18)
SA113	$r^- m^-$	L. L. Jänosi
KU201	SA113(pSP53)	This study
KU201E	Derivative strain cured pSP53 from KU201	This study
KU203	SA113(pSP53 plus pTU512)	This study
E. coli TG1	K-12 Δ(*lac-pro*) *supE thi hsD5/F′ traD36 proA$^+$B$^+$ lacI^q lacZΔM15*	M. Kanno
Plasmids		
pUC19	Ap^r	20
pSU20	Cm^r (*cat*) *repD*	Clinical isolate, Teikyo University Hospital
pSU40	Cm^r (*cat*) *repD* (insertion of *Eco*RI-*Hin*dIII multiple cloning site into pSU20)	This study
pSU4	*mec* Tm^r (*aadD*) Cm^r (recombination of *Bam*HI fragment of staphylococcal chromosome and *E. coli* vector plasmid pACYC184)	This study
pSP51	*mec* Ap^r	This study
pSP52	*mec* Ap^r Cm^r	This study
pSP53	Mc^r (*mec*) Cm^r (*cat*) *repD*	This study
pTU512	Pc^r (*blaZ*) Km^r (*aphA*) Em^r (*ermC*)	This laboratory (7)

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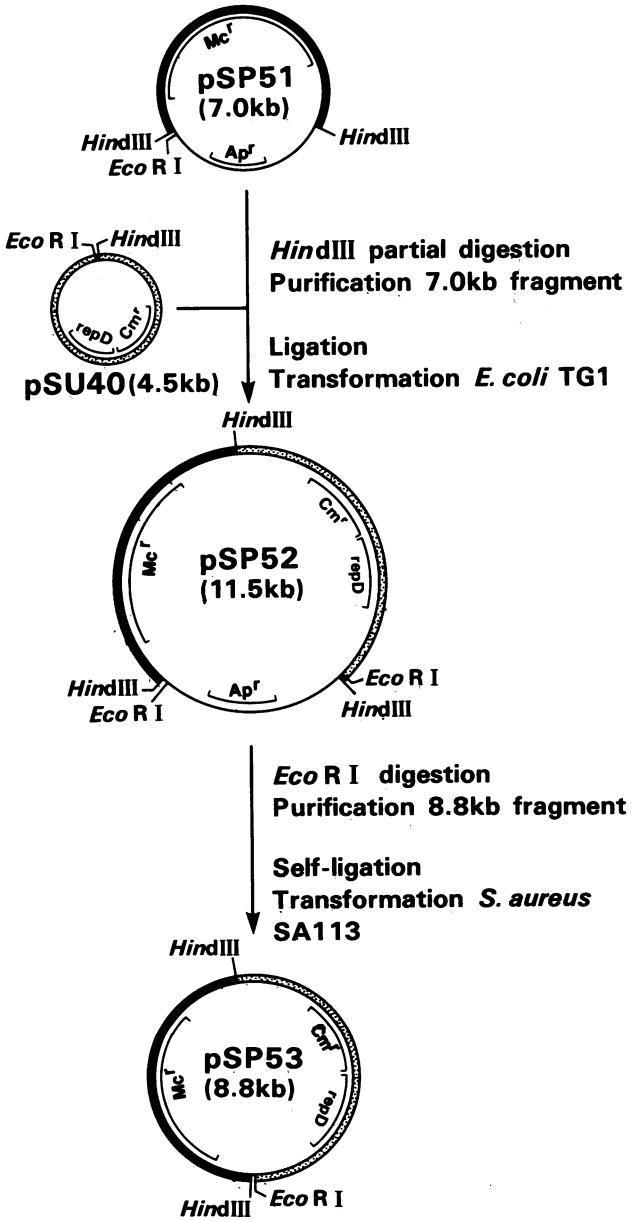

FIG. 1. Construction of recombinant plasmid pSP53 carrying the *mecA* gene coding for MRSA-PBP. Recombinant plasmid pSP51 was constructed with a 4.3-kb *Hin*dIII fragment from recombinant plasmid pSU4, which contained the *mecA* gene coding for MRSA-PBP, and vector plasmid pUC19. A 7.0-kb *Hin*dIII fragment derived from pSP51 was ligated into the *Hin*dIII site of the pSU40 and used to transform cells of *E. coli* TG1. The resulting recombinant plasmid pSP52 was digested with *Eco*RI, self-ligated after the elimination of most pUC19 DNA, and transformed the cells of *S. aureus* SA113. The final resulting recombinant plasmid, pSP53, contains methicillin resistance (*mecA* gene) and most of pSU40, which codes for chloramphenicol resistance (*cat* gene) and replication functions (*repD*). Abbreviations: Mc^r, methicillin resistance; Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance.

pSP52, in which multiple cloning sites of pSU40 were positioned close to the Ap^r site of the pSP51 portion, were isolated. To eliminate most of the *E. coli*-derived plasmid pUC19 DNA, plasmid pSP52 was cut at the *Eco*RI site, self-ligated, and used to transform *S. aureus* SA113. Colonies grown on selective agar plates containing 5 μg of chloramphenicol per ml were tested for the plasmid, and a transformant containing an 8.8-kb DNA fragment was selected. The resulting plasmid, pSP53, was composed of the chromosomal fragment encompassing the *mecA* gene and the greater part of the *S. aureus* vector plasmid pSU40 that encompassed chloramphenicol resistance (*cat* gene) and the replication functions (*repD*).

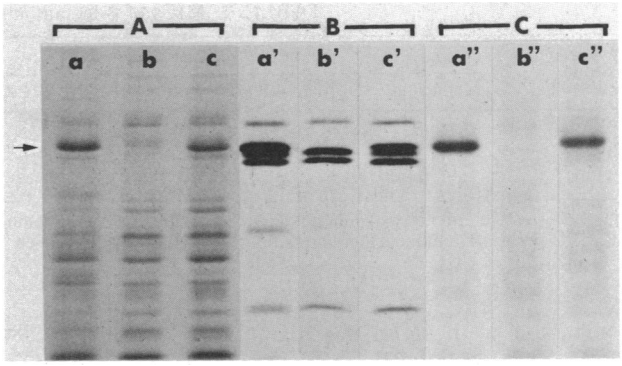

FIG. 2. Formation of MRSA-PBP in the transformant cell. The binding reaction was carried out for 10 min at 30°C with radiolabeled antibiotics. Membrane proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a separation gel of 8.4% acrylamide and 0.06% bisacrylamide. (A) Coomassie brilliant blue-stained gel; (B) fluorogram of PBPs bound with [^{14}C]benzylpenicillin; (C) fluorogram of PBPs bound with [^{14}C]cefzonam. Lanes: a, a', and a'', intrinsic methicillin-resistant strain TK388E cured of penicillinase plasmid; b, b', and b'', recipient strain SA113 of methicillin sensitivity; c, c', and c'', transformant strain KU201 of methicillin resistance. Arrow indicates MRSA-PBP.

Susceptibilities of the recipient strain SA113 and transformant KU201 to several β-lactam antibiotics are shown in Table 2. With the exception of the cloxacillin MIC, the MICs of the β-lactam antibiotics for strain KU201 were 32 to 1,000 times higher than those for the recipient strain, which were

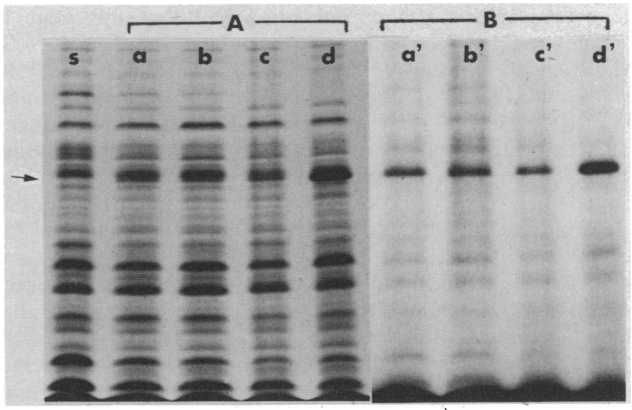

FIG. 3. Inducibility of MRSA-PBP in the double-transformant strain KU203 involving plasmids pSP53, containing the *mecA* gene, and pTU512, containing the penicillinase gene. Each strain was cultured with and without 0.5 μg of cefmetazole per ml. (A) Coomassie brilliant blue-stained polyacrylamide gel; (B) fluorogram of the PBPs bound with [^{14}C]cefzonam. Lanes: a and a', membrane proteins from uninduced strain KU201; b and b', induced strain KU201; c and c', uninduced strain KU203; d and d', induced strain KU203; s, recipient strain SA113. The production of MRSA-PBP was converted to inducibility in KU203 after incubation of additional cefmetazole but not in strain KU201. Arrow indicates MRSA-PBP.

TABLE 2. MICs of β-lactam antibiotics for SA113, KU201, and KU201E

Strain	MIC (μg/ml) of[a]:							
	Methicillin	Cloxacillin	Cefazolin	Cefmetazole	Cefpiramide	Ceftizoxime	Cefzonam	Flomoxef
SA113	0.78	0.39	0.1	0.39	0.39	6.25	0.39	0.39
KU201	100	1.56	100	25	100	100	50	12.5
KU201E	0.78	ND	0.2	ND	ND	ND	ND	ND

[a] MICs were determined by using serial twofold dilutions of the antibiotics in sensitivity test agar. Bacteria were cultured overnight, inoculated onto the agar (10^5 CFU per spot), and incubated for 48 h at 32°C. ND, Not determined.

comparable to those for the original MRSA strain, TK784, from which the cloned fragment originated. The MIC of cloxacillin was strongly affected by inoculum size and rose to 12.5 μg/ml at 10^6 CFU per spot. Strain KU201E, derived from the transformant KU201 by culturing at 42°C to eliminate pSP53, showed the original susceptibilities to methicillin and cefazolin of the recipient strain.

Figure 2 shows the results of the production of MRSA-PBP. MRSA-PBP, which was not present in the recipient SA113 cells, was found in the transformant KU201 cells. This MRSA-PBP was indistinguishable in electrophoretic mobility from that present in the intrinsically β-lactam-resistant TK388E cells. Coomassie blue staining showed that this new PBP in the transformant-cell membranes was produced in an amount comparable to that produced by the TK388E cells; moreover, the production of MRSA-PBP was constitutive. To determine whether the inducibility of the MRSA-PBP could be recovered equally well with clinical isolates of MRSA, a penicillinase plasmid, pTU512 (7), was introduced into strain KU201. The production of MRSA-PBP in the transformant strain KU203, which contains both plasmids pSP53 and pTU512 (Fig. 3), was induced in the presence of 0.5 μg of cefmetazole per ml but not in its absence. The double transformant KU203 also showed inducibility of resistance to β-lactam antibiotics; MICs for this strain were elevated two- to fourfold.

Recent molecular genetics studies in our laboratories showed that MRSA-specific PBP is encoded by the *mecA* gene (10, 16) on the chromosome and that this gene is present in MRSA strains from worldwide sources (15; S. Maesaki, M. D. Song, M. Matsuhashi, K. Ubukata, N. Itoh, M. Konno, K. Okonogi, A. Imada, H. F. Chambers, and J. F. Richardson, Abstr. Int. Symp. Bacterial Cell Surfaces Biosci., p. 22–23, 1987; manuscript submitted). The coding frame of *mecA* was preceded by possible promoter and Shine-Dalgarno sequences and overlapped one of the possible promoter sequences of a large palindrome sequence (15, 16) in which smaller repeated palindrome sequences are seen. Similar palindrome sequences are seen in the promoter region of a classical pI258 penicillinase gene (11) and are also shown as one of the consensus sequences for the repressor-binding site of the penicillinase gene of *Bacillus licheniformis* (5). Thus, these palindrome sequences probably function as binding sites of the repressor for the *mecA* gene. Introduction of the 4.3-kb DNA fragment containing the *mecA* gene into the methicillin-sensitive *S. aureus* strain SA113 caused constitutive production of MRSA-PBP. Furthermore, insertion into the cell of a plasmid encoding an inducible penicillinase rendered the expression of MRSA-PBP inducible. These results may indicate that penicillinase plasmid contains some gene(s) essentially involved in the mechanism of induction of the *mecA* gene but not contained in the 4.3-kb chromosomal DNA fragment. It is necessary to demonstrate which gene(s) on the penicillinase plasmid pTU512 is essential for the induction of both the penicillinase and the MRSA-PBP.

Cloning of staphylococcal chromosomal DNA fragments expected to be involved in expression of methicillin resistance has also been reported by other investigators (1, 6, 17). Moreover, transformation of a methicillin-sensitive *S. aureus* cell to methicillin resistance by the 4.3-kb *Hin*dIII fragment, probably identical to that isolated previously by the present authors (16), has also been reported (6). It may be necessary to transform sensitive cells with a shorter DNA fragment that contains only the *mecA* gene in order to establish that the MRSA-PBP is responsible for the β-lactam resistance of the MRSA cell.

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