Reconstruction of the Phenotypes of Methicillin-Resistant Staphylococcus aureus by Replacement of the Staphylococcal Cassette Chromosome mec with a Plasmid-Borne Copy of Staphylococcus sciuri pbpD Gene

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The mecA gene, the central determinant of methicillin (meticillin)-resistant Staphylococcus aureus (MRSA), is not native to this bacterial species but may have originated in the animal commensal species Staphylococcus sciuri. All S. sciuri strains carry a close homologue of mecA in the form of pbpD, the genetic determinant of penicillin binding protein 4 (PBP 4) of S. sciuri. Here we describe an experimental system that could be used for additional tests for this proposition. The S. sciuri pbpD gene was cloned into a shuttle plasmid and introduced into methicillin-susceptible S. aureus strain COL-S derived from parental MRSA strain COL from which the resistance cassette staphylococcal cassette chromosome mec was excised. The S. sciuri pbpD determinant was transcribed and translated in the S. aureus transductants producing large amounts of the 84-kDa S. sciuri PBP 4 and was then deposited in the plasma membrane of the host bacterium. Transductants carrying the heterologous S. sciuri pbpD gene exhibited properties typical of those of parental MRSA strain COL, including broad-spectrum, high-level, and homogeneous resistance to structurally different β-lactams. Antibiotic resistance was dependent on the functioning of S. aureus PBP 2 and was suppressed by the specific regulatory genes mecI and mecR and by inhibitors of an early step in cell wall biosynthesis. S. sciuri PBP 4 was also able to replace the essential physiological function(s) of the native PBP 2 of S. aureus and produce peptidoglycan typical of that of parental MRSA strain COL. Our results provide further support for the proposition that the resistance determinant mecA of MRSA strains has evolved from S. sciuri pbpD.

Staphylococcus aureus is a major human pathogen responsible for several life-threatening infections, including septicemia, endocarditis, and toxic shock syndrome. Methicillin (meticillin)-resistant S. aureus (MRSA) strains were first reported in 1961, shortly after the introduction of methicillin in clinical practice. Since then, MRSA has become an increasing critical threat in hospital and community environments worldwide. The genetic determinant of β-lactam resistance, the mecA gene, is carried on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), and encodes a low-affinity penicillin binding protein (PBP), PBP 2A (8). Several lines of evidence suggest that the heterologous β-lactam resistance gene, mecA, which is resident in all MRSA strains, may have its evolutionary origin in a close homologue of this gene that is ubiquitous in both β-lactam-susceptible and -resistant isolates of the animal commensal species Staphylococcus sciuri (2, 3). The S. sciuri pbpD gene (the S. aureus mecA homologue) is the genetic determinant of PBP 4, one of the six PBPs recently identified in this species (20). Previous studies have shown that an upregulated form of the S. sciuri pbpD gene transduced into a susceptible S. aureus strain was able to produce a moderate but significant (twofold) increase in the oxacillin MIC of the transductants (19, 20). In that experiment, the source of the upregulated pbpD gene was the laboratory mutant K1M200, obtained by stepwise exposure of antibiotic-susceptible S. sciuri strain K1 to gradually increasing concentrations of methicillin. The recipient strain was S. aureus COL, in which the mecA resistance determinant was inactivated by a transposon insert.

The purpose of the studies described here was to construct a new experimental system that would allow one to further test the validity of the proposition that the resistance determinant of MRSA strains may have originated from the S. sciuri pbpD gene. The chromosomal resistance determinant SCCmec was excised from highly and homogeneously methicillin-resistant S. aureus strain COL to provide a methicillin-susceptible S. aureus (MSSA) strain, COL-S. In some of the new experiments, the essential S. aureus pbpB gene was also put under the control of an inducible promoter producing strain, COL-S/pac-pbpB. Both strains were then used as the recipient for plasmid-borne copies of the upregulated S. sciuri pbpD gene recovered from oxacillin-resistant clinical isolate SS37 of S. sciuri (3, 4). Transductants carrying the S. aureus mecA determinant on the same plasmid were used as controls. The physiological, genetic, and biochemical properties of the S. aureus COL-S transductants carrying the heterologous S. sciuri pbpD gene were then compared to the properties of original MRSA strain COL to determine to what extent the drug resistance-related phenotypes of the original MRSA strain were reconstructed in the transductants.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The characteristics of the bacterial strains and plasmids used in this study are described in Table 1. Bac-
TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Characteristics</th>
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<tr>
<td><strong>S. aureus strains</strong></td>
<td></td>
<td></td>
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<tr>
<td>COL</td>
<td>Clinical isolate; Ox' Em' Cm' Te'</td>
<td>RU collection</td>
</tr>
<tr>
<td>COL-spac::phpB</td>
<td>COL derivative in which phpB is under the control of spac promoter/phpBP2i; Em'</td>
<td>13</td>
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<tr>
<td>COL-S</td>
<td>COL with SColneC excised; Ox' Em' Cm' Te'</td>
<td>11</td>
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<td>COL-S-spac::phpB</td>
<td>COL-S derivative in which phpB is under the control of spac promoter/phpBP2i; Em'</td>
<td>This study</td>
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<tr>
<td>COL-SspC6pD</td>
<td>COL-S carrying the plasmid-borne S. aureus mecA region/pSTSW2C; Te'</td>
<td>This study</td>
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<tr>
<td>COL-SspC6pD</td>
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<td>This study</td>
</tr>
<tr>
<td>S. sciuri strain SS37</td>
<td>Clinical isolate recovered from the nasopharynx of a patient; Ox'</td>
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</table>

**Plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Characteristics</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>pPB2i</td>
<td>S. aureus integrational vector with phpB ribosome-binding site and first 171 codons fused to PspB promoter; Em'</td>
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<tr>
<td>pSTSW2C</td>
<td>pSP7181C with 3,737-bp PCR product of S. aureus COL mecA region; Te'</td>
<td>19</td>
</tr>
<tr>
<td>pSS37MA</td>
<td>pSP181C with 1,410-bp PCR product of S. sciuri SS37 pdB region; Te'</td>
<td>20</td>
</tr>
<tr>
<td>pGC2::mec-l-mecR1</td>
<td>pGC2 with 2,466-bp PCR product of S. aureus N315 mec-l-mecR1 region; Cm'</td>
<td>D. C. Oliveira</td>
</tr>
<tr>
<td><strong>S. sciuri strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL</td>
<td>Clinical isolate from the nasopharynx of a patient; Ox'</td>
<td>4</td>
</tr>
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</table>

**Antibiotic susceptibility testing.** The susceptibilities of the S. aureus strains to β-lactam antibiotics were determined by population analysis, as described previously (17).

**Peptidoglycan purification and analysis by HPLC.** Peptidoglycan was purified from 1-liter cultures grown at 30°C to mid-exponential phase, as described previously (15). The detection of PBPs was performed by incubating the membrane preparations (150 μg of proteins) with a saturating concentration (20 μg/mL) of [3H]-labeled peptidoglycan (Sigma-Aldrich), separated by high-performance liquid chromatography (HPLC) on a C18 column (3 μm, 4.6 by 250 mm; ODS-Hypersil; Thermo Electron Corporation), and detected by measurement of the absorbance at 206 nm, as described previously (5).

**Membrane purification and PBP assay.** Membrane preparations were purified from cultures grown to the late exponential phase, as described previously (15). The detection of PBPs was performed by incubating the membrane preparations (150 μg of proteins) with a saturating concentration (20 μg/mL) of [3H]-labeled peptidoglycan (Sigma-Aldrich), separated by high-performance liquid chromatography (HPLC) on a C18 column (3 μm, 4.6 by 250 mm; ODS-Hypersil; Thermo Electron Corporation), and detected by measurement of the absorbance at 206 nm, as described previously (5).

**Detection of S. aureus PBP 2A and S. sciuri PBP 4 by Western blotting.** Membrane preparations (80 μg of proteins) were separated by SDS-PAGE as described above. Transfer and blotting were done according to the instructions accompanying the ECL Western blotting analysis system (GE Healthcare). The ChromPure human immunoglobulin G Fe fragment (Jackson ImmunoResearch Laboratories) was used at 3 μg/mL to eliminate nonspecific hybridization with protein A. The primary antibody was a monoclonal antibody against S. aureus PBP 2A (dilution, 1:20,000) obtained by incubating a rabbit with the synthetic peptide sequence CDKFNFKQVKDSSYSKSDNG conjugated to keyhole limpet hemocyanin (a gift from JoAnn Hoskins, Eli Lilly, Indianapolis, IN), and the secondary antibody was the anti-rabbit antibody included in the kit (dilution, 1:5,000). S. sciuri PBP 4 has previously been shown to react also with the monoclonal antibody raised against S. aureus PBP 2A (4, 19).

**RNA isolation and Northern blot analysis.** Total RNA was extracted from cultures grown up to an optical density at 600 nm (OD600) of 0.7. RNA (5 μg) was resolved by electrophoresis on 1.2% agarose–0.66 M formaldehyde gels in morpholinepropanesulfonic acid running buffer. RNA was blotted onto Hybond-N+ membranes (GE Healthcare) with a turbo blotter alkaline transfer system (Schleicher & Schuell) with 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The PCR-amplified DNA probes were labeled with [α-32P]dCTP (GE Healthcare) by using a Ready-To-Go labeling kit (GE Healthcare) and hybridized under high-stringency conditions. The blots were subsequently washed and autoradiographed.

**Introduction of a plasmid-borne S. aureus COL mecA gene and S. sciuri SS37 phpB gene into S. aureus strain COL-S and into a phpB conditional mutant of COL-S.** Plasmid pPB2i (13) was transduced into oxacillin-susceptible S. aureus strain COL-S (11) to produce strain COL-SspC::phpB, in which the expression of the chromosomal phpB gene is under the control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible spac promoter. A PCR-amplified sequence of the 3,737-bp region of S. aureus COL mecA was ligated into shuttle plasmid pSP181C to form pSTSW2C (19). A 1,410-bp fragment that included the promoter region of phpB gene with a complete copy of IS256 inserted 109 bp upstream of the start codon and the entire phpB gene was amplified from S. sciuri SS37 by PCR and was cloned into shuttle plasmid pSP181C to form pSS37MA (20). Recombinant plasmids pSPSW2C and pSS37MA were subsequently introduced into S. aureus strain RN420 by electroporation and were then transduced by phage 80s into S. aureus strain COL-S to yield the transductants COL-SspC::phpB and COL-SspC::phpB::pGC2::mec-l-mecR1, respectively.

**Cloning of mec and mecR regulatory genes and introduction into S. aureus.** The 2,466-bp mec-l-mecR1 region from S. aureus strain N315 was amplified with primers mecIP5 (5'-AGAGGGGATCTCAGACGATGGTACCATGTTTCC-3') and mecRP6 (5'-GGGAAATTCGGTTACTCAGGCTC-3') and ligated into the BamHI and EcoRI sites of high-copy-number plasmid pGC2 (D. C. Oliveira, unpublished data). The resulting recombinant plasmid, pGC2::mec-l-mecR1, was electroporated into RN420 and then transduced into COL-SspC::phpB and COL-SpC::phpB::pGC2::mecl-l-mecR1, respectively.

**RESULTS**

The experimental system: construction of MSSA strain COL-S and introduction of plasmid-borne copies of S. sciuri phpD and S. aureus mecA genes. The chromosomal SCCmec type I cassette was removed from MRSA strain COL by pre-
cise excision, producing strain COL-S, which was fully susceptible to all beta-lactam antibiotics tested (Table 2) (11). A \( \text{pbpB} \) conditional mutant of strain COL-S (COL-\( \text{spac}\!\!\!\text{::pbpB} \)) was constructed by placing the genetic determinant of PBP 2 under the control of the IPTG-inducible \( \text{spac} \) promoter (13).

The source of the \( \text{S. sciuri pbpD} \) gene was clinical isolate \( \text{S. sciuri SS37} \), which was recovered from the nasopharynx of a patient (3). Strain SS37 exhibited heterogeneous resistance to oxacillin and carried a \( \text{pbpD} \) gene that was overexpressed due to the insertion of IS256 upstream of the gene (3, 4). The upregulated \( \text{pbpD} \) gene was cloned into a shuttle plasmid (20) and introduced into \( \text{S. aureus} \) COL-S and COL-\( \text{spac}\!\!\!\text{::pbpB} \) to produce transductants COL-S SS\( \text{pbpD} \) and COL-\( \text{spac}\!\!\!\text{::pbpB/SSpbpD} \), respectively. As a control, the same shuttle plasmid carrying the \( \text{mecA} \) gene from MRSA strain COL (19) was also introduced into the same \( \text{S. aureus} \) backgrounds, yielding transductants COL-S \( \text{mecA} \) and COL-\( \text{spac}\!\!\!\text{::pbpB/SAmeC} \), respectively.

**Transcription and translation of plasmid-borne \( \text{S. aureus mecA} \) and \( \text{S. sciuri pbpD} \) in \( \text{S. aureus} \).** Figure 1 shows the results of Northern blot analysis, performed to estimate the degree of expression of \( \text{S. aureus mecA} \) and \( \text{pbpB} \) and \( \text{S. sciuri pbpD} \) in the different \( \text{S. aureus} \) backgrounds. No \( \text{mecA} \) transcript was detected in COL-S or its \( \text{pbpB} \) conditional mutant, COL-\( \text{spac}\!\!\!\text{::pbpB} \). \( \text{S. aureus mecA} \) and \( \text{S. sciuri pbpD} \) were both effectively and highly expressed in transductants COL-S \( \text{SAmeC} \) and COL-\( \text{spac}\!\!\!\text{::pbpB/SSmeC} \), respectively.

**The inhibition of \( \text{pbpB} \) transcription was confirmed in cultures of strains COL-\( \text{spac}\!\!\!\text{::pbpB/SAmeC} \) and COL-\( \text{spac}\!\!\!\text{::pbpB/SSpbpD} \) grown in the absence of the IPTG inducer.**

The PBP patterns were determined by incubating membrane preparations in the presence of radioactive penicillin (Fig. 2A),

**FIG. 1.** Transcription of \( \text{S. aureus mecA} \) and \( \text{pbpB} \) genes and \( \text{S. sciuri pbpD} \) gene. RNA was purified from cultures grown at 30°C to an \( \text{OD}_{620} \) of 0.7. RNA (5 \( \mu \)g) was resolved by electrophoresis on agarose-formaldehyde gels. After transfer, the membranes were hybridized with \( ^{32}\text{P} \)-labeled \( \text{S. aureus mecA} \) and \( \text{pbpB} \) and \( \text{S. sciuri pbpD} \) DNA probes.

**TABLE 2. Susceptibility to \( \beta \)-lactam antibiotics**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>COL</th>
<th>COL-S</th>
<th>COL-( \text{SAmeC} )</th>
<th>COL-( \text{SSpbpD} )</th>
<th>COL-( \text{SAmeC/SSmeC} )</th>
<th>COL-( \text{SPspbD/SSmeC} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>800</td>
<td>0.8</td>
<td>400</td>
<td>400</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>800</td>
<td>0.8</td>
<td>800</td>
<td>400</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>800</td>
<td>0.8</td>
<td>800</td>
<td>200</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cephradine</td>
<td>400</td>
<td>6</td>
<td>400</td>
<td>100</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>800</td>
<td>3</td>
<td>200</td>
<td>200</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**FIG. 2.** PBP patterns and detection of protein products of \( \text{S. aureus mecA} \) (PBP 2A) and \( \text{S. sciuri pbpD} \) (PBP 4). (A) Membrane preparations (150 \( \mu \)g of proteins) were incubated with a single saturating concentration of \( ^{14}\text{C} \)-benzylpenicillin. After SDS-PAGE, the gel was exposed to a tritium storage phosphor screen for 2 weeks. (B) Membrane preparations (80 \( \mu \)g of proteins) were tested by Western blot analysis for the production of proteins that react with a monoclonal antibody raised against \( \text{S. aureus PBP 2A} \). The results for \( \text{S. aureus COL} \) and \( \text{S. sciuri SS37} \) are provided as positive controls and size markers for PBP 2A and PBP 4, respectively. (C) Protein patterns on an SDS-polyacrylamide gel stained with Coomassie blue.
and a Western blot analysis was performed with an antibody raised against *S. aureus* PBP 2A (Fig. 2B), which has previously been shown to also react with *S. sciuri* PBP 4 (4, 19, 20). In accordance with the results of the Northern blot analysis, these experiments confirmed that PBP 2 was absent from the membrane preparation of COL-Spac pbpB/SspbpD grown in the absence of IPTG and that PBP 2A was absent from COL-S and its derivative, COL-Spac pbpB. Large amounts of PBP 2A were detected in the membrane preparations of COL-Spac pbpB/S.S. The foreign *S. sciuri* PBP 4 was also correctly translocated and deposited in large amounts in the plasma membrane of *S. aureus* host cells. As shown in Fig. 2C, *S. aureus* PBP 2A (78 kDa) and *S. sciuri* PBP 4 (84 kDa) were already evident from the SDS-polyacrylamide gel stained with Coomassie blue.

Similarly, high levels of transcription of *S. aureus* mecA and *S. sciuri* pbpD and large amounts of PBP 2 and PBP 4 were also observed for transductants COL-S S. S. mecA and COL-S S. mecA, respectively (data not shown).

**Phenotypic expression of β-lactam resistance.** Table 2 shows that the introduction of both the *S. sciuri* pbpD gene and the *S. aureus* mecA gene into MSSA strain COL-S was able to provide virtually identical levels of resistance to a group of β-lactam antibiotics which have considerably different degrees of selective binding to the four *S. aureus* PBPs. Transductants COL-S S. mecA and COL-S S. mecA produced high-level and homogenous resistance to cefotaxime and cefoxitine (selective affinity for PBP 2), cephradine (PBP 3), cepfotaxin (PBP 4), and oxacillin.

**Inhibition of β-lactam resistance in transductants COL-S S. mecA and COL-S S. mecA by subinhibitory concentrations of D-cycloserine and by the mecI-mecR1 regulatory genes.** It has previously been shown that inhibitors of the early steps of cell wall synthesis can reduce the level of methicillin resistance and change the homogeneous resistance phenotype of strain COL to a heterogeneous one (16). Susceptibility to oxacillin in the presence of subinhibitory concentrations (0.25× MIC) of the cell wall synthesis inhibitor D-cycloserine was determined by population analysis. While the oxacillin MIC of strain COL-S remained unchanged, the resistance levels were drastically reduced in the presence of D-cycloserine, from MICs of 800 to 400 μg/ml for strains COL, COL-S S. mecA, and COL-S S. mecA to MICs as low as 1, 0.75, and 0.5 μg/ml, respectively. In addition, the homogeneous expression of oxacillin resistance in COL-S S. mecA and COL-S S. mecA was converted to heterogeneous expression (data not shown), as previously described for COL (16).

The *S. aureus* mecA gene has been shown to be transcriptionally regulated in some clinical isolates by mecRI and mecI, cotranscribed chromosomal genes that encode a signal transducer and a repressor, respectively (9). The mecI-mecRI region from *S. aureus* strain N315 was cloned in a high-copy-number plasmid and introduced into COL, COL-S S. mecA, and COL-S S. mecA. The mecI and mecRI regulatory genes repressed the phenotypic expression of β-lactam resistance in COL, as described previously (10), and well as in COL-S S. mecA (Table 2). Most interestingly, the level of β-lactam resistance was also extensively repressed in strain COL-S S. mecA, which expressed the plasmid-borne *S. sciuri* pbpD gene (Table 2), suggesting that the mecI repressor can bind to the promoter region of *S. sciuri* pbpD similar to the way in which it binds to the promoter region of *S. aureus* mecA (14).

**Replacement of the normal (essential) physiological function(s) of the *S. aureus* host PBP 2 by *S. sciuri* PBP 4.** Previous studies have shown that *S. aureus* PBP 2, the protein product of the *pbpB* gene, is essential for growth in β-lactam-susceptible *S. aureus* strains. However, the additional PBP 2A present in MRSA strains can replace the essential function(s) of PBP 2 (7, 13). In order to test if *S. sciuri* PBP 4 was also able to support growth in PBP 2-deprived *S. aureus* cells, the plasmid-borne *S. sciuri* pbpD gene was introduced into *S. aureus* strain COL-S S. mecA, in which the *pbpB* gene was put under the control of an IPTG-inducible promoter. As a control, the plasmid-borne *S. aureus* mecA gene was also introduced in the same background. As previously described for a β-lactam-susceptible *S. aureus* strain, the absence of IPTG prevented the growth of COL-S S. mecA, but only slowed the growth of the *pbpB* conditional mutant of MRSA strain COL, COL-S S. mecA.
Composition of transductants carrying the plasmid-borne essential function(s) of the native PBP 2 of S. aureus.

The rate was comparable to that of COL-S.

S. aureus mecA and COL-S, plasmid-borne genes provide the normal (essential) function(s) of the native PBP 2 of S. aureus.

The numbers of CFU were counted after incubation for 72 h at 30°C. The oxacillin susceptibility profiles were determined for COL (○), COLS::pBPB grown in the presence (▲) and absence (△) of IPTG, COL-S::pBPB grown in the presence of IPTG (◇), COL-S::pBPB::S. anncA grown in the presence (●) and absence (□) of IPTG, and COL-S::pBPB::S. anncA grown in the presence (●) and absence (□) of IPTG.

Effect of suppression of mecA::spac in the absence of IPTG, and COL-S::pBPB::SS growing in the presence of IPTG and COL-S::pBPB::SS growing in the presence of IPTG.

Effect of introduction of the plasmid-borne S. aureus mecA or S. sciuri pBPB genes and suppression of pBPB transcription on the expression of oxacillin resistance in S. aureus. Aliquots of overnight cultures were plated on tryptic soy agar containing increasing concentrations of oxacillin. The muropeptide compositions of the peptidoglycans were analyzed by HPLC (Fig. 4). The suppression of pBPB transcription in cultures of COL-S::pBPB::SSanncA (Fig. 4B) resulted in the production of peptidoglycan with a moderate decrease in the relative proportion of the highly cross-linked muropeptides with retention times of over 100 min and a parallel slight increase in the proportion of the monomeric muropeptide 5 compared to the peptidoglycan composition of COL as shown in Fig. 4A. Similar variations in the muropeptide composition were observed in cultures of strain COL in which pBPB transcription was suppressed (7). The HPLC elution profile of COL-S::pBPB::SSanncA grown in the absence of IPTG (Fig. 4C) clearly differed from that of S. sciuri strain SS37 (Fig. 4D) but was identical to that of COL-S::pBPB::S. anncA. These results indicate that S. sciuri PBP 4 was able to replace the essential enzymatic activity of S. aureus PBP 2 and catalyze the biosynthesis of peptidoglycan to produce an S. aureus type of cell wall with the cell wall precursors of the S. aureus host.

Effect of suppression of pBPB transcription on the oxacillin resistance level of transductants carrying the plasmid-borne S. sciuri pBPB or S. aureus mecA gene. High-level and homogeneous resistance to oxacillin in MRSA strain COL is known to depend on the level of transcription of the S. aureus pBPB gene (7). As shown in Fig. 5, the inhibition of pBPB transcription in the conditional mutant of strain COL (COLS::pBPB::spac) reduced the oxacillin resistance levels of the majority of the cells and converted the homogeneous phenotype to a heterogeneous one. In the presence of IPTG, the introduction of either the
plasmid-borne \textit{S. sciuri} \textit{pbpD} gene or the \textit{S. aureus} \textit{mecA} gene into the \textit{pbpB} conditional mutant \textit{COL-S}_{pbpB::\textit{pbpA}} produced high-level and homogeneous resistance that was close to that of \textit{S. aureus} strain \textit{COL} (Fig. 5). However, in the absence of IPTG, the oxacillin MICs for the majority of the cells decreased 16-fold and the population analysis profiles became heterogeneous (Fig. 5). These results indicate that even in the presence of large amounts of either \textit{S. sciuri} \textit{PBP 4} or \textit{S. aureus} \textit{PBP 2A} in the transductants, the host \textit{PBP 2} was still required for the optimal expression of antibiotic resistance in \textit{S. aureus}, as was already shown for MRSA strain \textit{COL} (7, 12).

**DISCUSSION**

The genetic determinant of \(\beta\)-lactam resistance, \textit{mecA}, is not native to \textit{S. aureus} but was acquired from an extraspecies source (1). The \textit{S. sciuri} \textit{pbpD} gene, which is uniformly present in both \(\beta\)-lactam-susceptible and -resistant isolates of this widely spread animal commensal species, was first identified on the basis of its high degree of structural similarity with the \textit{S. aureus} \textit{mecA} gene (2, 18). Previous studies have proposed that \textit{S. sciuri} \textit{pbpD} may represent the evolutionary precursor of the \textit{S. aureus} \textit{mecA} gene, mainly on the basis of epidemiological and genetic evidence. The protein product of \textit{pbpD} was subsequently identified as PBP 4, one of the six PBPs detected in \textit{S. sciuri} (20). Recombinant \textit{S. sciuri} \textit{PBP 4} was purified and was shown to share several biochemical properties with \textit{S. aureus} \textit{PBP 2A} (6).

In this report, a new experimental system was designed to further test the validity of the proposition that the \textit{mecA} resistance determinant present in all MRSA strains may have originated from the \textit{S. sciuri} \textit{pbpD} gene. The resistance cassette \textit{SCCmec} was excised from extensively studied MRSA strain \textit{COL} to generate MSSA strain \textit{COL-S}, which was subsequently used as the recipient of a plasmid-borne copy of an upregulated \textit{mecA} gene. The transductants were able to transcribe and translate this \textit{mecA} gene, which is uniformly present in high-level and homogeneous and broad-spectrum \(\beta\)-lactam antibiotics, similar to the properties of MRSA strain \textit{COL}. Also, similarly to strain \textit{COL}, \textit{pbpD} was excised from extensively studied MRSA strains isolated from humans. The \textit{mecA}-lactam resistance determinant \textit{mecA} carried by all MRSA strains has evolved from the \textit{S. sciuri pbpD} gene.

**ACKNOWLEDGMENTS**

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**REFERENCES**


