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Identification of Chromosomal Location of *mupA* Gene, Encoding Low-Level Mupirocin Resistance in Staphylococcal Isolates

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Low- and high-level mupirocin resistance have been reported in *Staphylococcus aureus*. The expression of plasmid-encoded *mupA* is responsible for high-level mupirocin resistance. Low-level mupirocin-resistant strains do not contain plasmid-encoded *mupA*, and a chromosomal location for this gene has not previously been reported. We examined high- and low-level mupirocin-resistant *S. aureus* strains to determine if *mupA* was present on the chromosome of low-level-resistant isolates. Southern blot analysis of DNA from four mupirocin-resistant strains identified *mupA* in both high- and low-level mupirocin-resistant strains. Low-level mupirocin-resistant strains contained a copy of *mupA* on the chromosome, while the high-level mupirocin-resistant isolate contained a copy of the gene on the plasmid. PCR amplification of genomic DNA from each mupirocin-resistant strain resulted in a 1.65-kb fragment, the predicted product from the intragenic *mupA* primers. This is the first report of a chromosomal location for the *mupA* gene conferring low-level mupirocin resistance.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a major cause of nosocomial infections and have been isolated with increasing frequency from patients in hospitals and nursing homes (1, 17, 26). Many different antibiotics have been used in an attempt to eradicate *S. aureus* carriage. Mupirocin (Bactroban; SmithKline Beecham Pharmaceuticals), a topical antibiotic used for the treatment of impetigo, is also effective for eradication of nasal carriage of *S. aureus* (4, 11, 20). With increased use of mupirocin, staphylococcal strains with high and low levels of resistance have been reported (3, 11–13, 24). Most commonly, low-level (MICs between 8 and 256 μg/ml) mupirocin-resistant strains are isolated, while high-level (MICs greater than 500 μg/ml) mupirocin-resistant strains are infrequently isolated (2, 7, 10, 23). High-level, but not low-level, mupirocin resistance has been associated with clinical and microbiological failure.

The antibacterial activity of mupirocin is due to competitive inhibition with isoleucine for isoleucyl tRNA synthetase (9). The native staphylococcal enzyme is encoded by the gene *ileS* (8). High-level mupirocin resistance in staphylococci is associated with an isoleucyl tRNA synthetase that is different in structure and is encoded by a novel plasmid-encoded gene, *mupA* (5–7). Further characterization of a high-level mupirocin-resistant plasmid carrying the *mupA* gene determined that this gene is flanked by direct repeat copies of IS*431*-IS*257* insertion sequence elements, similar to other staphylococcal antibiotic resistance genes and the conjugative transfer region (15, 16). Low-level mupirocin-resistant *S. aureus* strains do not contain plasmid-encoded *mupA* (10, 19). In vitro studies suggest that low-level mupirocin resistance emerged as the result of independent, spontaneous mutational events in response to increasing concentrations of mupirocin (23). A chromosomal location for *mupA* encoding for low-level mupirocin resistance in *S. aureus* has not been reported. In this study, we examined clinical isolates with high- and low-level mupirocin resistance phenotypes to determine if *mupA* could be located on the chromosomes of low-level-resistant isolates. We found that the low-level mupirocin-resistant strains contained a copy of *mupA* on the chromosome, while high-level mupirocin-resistant isolates contained a copy of the gene on the plasmid. This is the first report of a chromosomal copy of *mupA* encoding low-level mupirocin resistance in staphylococci.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Four mupirocin-resistant *S. aureus* isolates (three low level and one high level) were obtained from patients during a study of the use of 2% mupirocin ointment in a polyethylene glycol base for the eradication of nasal and wound carriage of MRSA in a long-term care facility (11) (Table 1). Two low-level strains (LZ-8 and LZ-9) and the high-level strain (LZ-1) were similar by contour-clamped homogeneous electric field (CHEF) electrophoresis patterns, and the third low-level strain (LZ-10) was different (19). LZ-8 and LZ-9 were determined to be clonal isolates. One isolate, ENV (environmental) that is mupirocin susceptible was obtained during an environmental survey of this long-term care facility. *S. aureus* NCTC 8325 was included as an epidemiologically unrelated mupirocin-susceptible control for studies with CHEF electrophoresis (18). The high-level mupirocin-resistant strain was capable of conjugative transfer of mupirocin resistance, while low-level strains were capable of conjugative transfer of gentamicin resistance (19). All strains and plasmids are listed in Table 1 along with their respective phenotypic markers.

DNA isolation and analysis. Plasmid and chromosomal DNAs were extracted from the *S. aureus* strains by ethidium bromide-cesium chloride density gradient centrifugation after cell lysis with lysostaphin (Applied Microbiology, Tarrytown, N.Y.) (21). Purified plasmid and chromosomal DNAs from each strain were digested separately with *Eco*RI or *Hin*dIII (GIBCO-BRL, Bethesda, Md.). The fragments were then separated by conventional agarose electrophoresis with 0.7% agarose according to the manufacturer's specifications.

For subsequent experiments with CHEF electrophoresis, genomic DNA was isolated after cell lysis and deproteinization in agarose plugs by the method of Struelens et al. (25). The cell density was adjusted to an optical density at 540 nm of between 0.2 and 0.3 prior to making agarose plugs. After restriction endonu-

TABLE 1. *S. aureus* strains and plasmids used in this study[a]

Strain	Mupirocin MIC (μg/ml)	Plasmid profile	Source
LZ-1 (MRSA)	>5,000	51.3 kb conjugative, Mup[r]	Clinical isolate (10, 11, 19)
LZ-9 (MRSA)	62.5	50 kb conjugative, Gm[r]	Clinical isolate (10, 11, 19)
LZ-10 (MRSA)	25	50 kb conjugative, Gm[r]	Clinical isolate (10, 11, 19)
LZ-8 (MRSA)	12.5	50 kb conjugative, Gm[r]	Clinical isolate (10, 11, 19)
ENV (MSSA)	3.1	38.5 and 1.8 kb	Environmental isolate (10, 11, 19)
NCTC 9325 (MRSA)	<0.5		Well characterized by PFGE (18)

[a] MSSA, methicillin-susceptible *S. aureus*; Mup, mupirocin; Gm, gentamicin; ENV, environmental; PFGE, pulsed-field gel electrohpresis.

clease digestion with *Sma*I (Promega Corp., Madison, Wis.), fragments were separated by CHEF electrophoresis as described previously (22).

PCR amplification. The oligonucleotide primers Mup1 (5′ CCC ATG GCT TAC CAG TTG A) and Mup2 (5′ CCA TGG AGC ACT ATC CGA A), corresponding to the *Nco*I sites of the *mupA* gene (8), were used to generate the *mupA* probe from LZ-1 and in the PCR amplifications of strains LZ-1, LZ-8, LZ-9, LZ-10, and ENV. Genomic DNA extracted by cesium chloride density gradient centrifugation was used as the template DNA for these strains. Twenty-eight cycles of PCR amplification were completed with *Taq* DNA polymerase (GIBCO-BRL). Annealing reactions were done at 5°C below the predicted melting temperature of the primers, and extension reactions were done at 5°C above the melting temperature.

DNA probes and Southern blot analysis. The PCR-amplified product of the intragenic 1.65-kb *mupA* fragment from LZ-1 was used for the mupirocin resistance gene probe. DNA probes and the molecular weight ladders, the 1-kb DNA ladder, and the lambda bacteriophage DNA digested with *Hin*dIII (GIBCO-BRL) were labeled by random priming and incorporation of digoxigenin-labeled dUTP with the Genius kit (Boehringer Mannheim, Indianapolis, Ind.). After electrophoresis, DNA was transferred to nitrocellulose membranes (Sigma, St. Louis, Mo.) as described by Sambrook et al. for Southern blot analysis (21). DNA hybridization and detection by Southern analysis were also carried out with the Genius kit system according to the manufacturer's specifications.

RESULTS

Plasmid DNA analysis. Analysis of the *Eco*RI-digested plasmid DNA revealed that the *mupA* probe hybridized to a 4.05-kb fragment obtained from the high-level mupirocin-resistant strain LZ-1. The *mupA* probe did not hybridize to DNA from the mupirocin-susceptible strain ENV or the low-level mupirocin-resistant strains (Fig. 1). Additional Southern blot analysis with *Hin*dIII-digested plasmid DNA confirmed that only the high-level mupirocin-resistant strain contained *mupA*, which was present on a 9.1-kb *Hin*dIII fragment (data not shown).

Genomic DNA analysis. Southern analysis of *Eco*RI-digested genomic DNA with the *mupA* probe identified a 4.05-kb fragment in the high-level mupirocin-resistant strain LZ-1 and in the low-level mupirocin-resistant strains LZ-8 and LZ-9 (Fig. 2). In LZ-10, the *mupA* probe hybridized to an *Eco*RI fragment greater than 12 kb in size. This larger *Eco*RI fragment was also identified in strains LZ-1, LZ-8, and LZ-9. The *mupA* probe did not hybridize to the mupirocin-susceptible strain ENV. Additional Southern blot analysis with *Hin*dIII-digested chromosomal DNA confirmed the presence of *mupA* on the chromosome of the same strains. In strains LZ-1, LZ-8, LZ-9, and LZ-10, the gene was present on a 9.1-kb *Hin*dIII fragment (data not shown). Again, the *mupA* probe did not hybridize with ENV DNA.

The *mupA* probe hybridized to a large *Sma*I fragment with a size of approximately 341 kb in both high- and low-level mupirocin-resistant clinical strains in Southern analysis of genomic DNA separated by CHEF electrophoresis (data not shown). In the high-level mupirocin-resistant strain LZ-1, the *mupA* probe hybridized to an approximately 50-kb *Sma*I fragment, corresponding to the predicted plasmid copy of *mupA*.

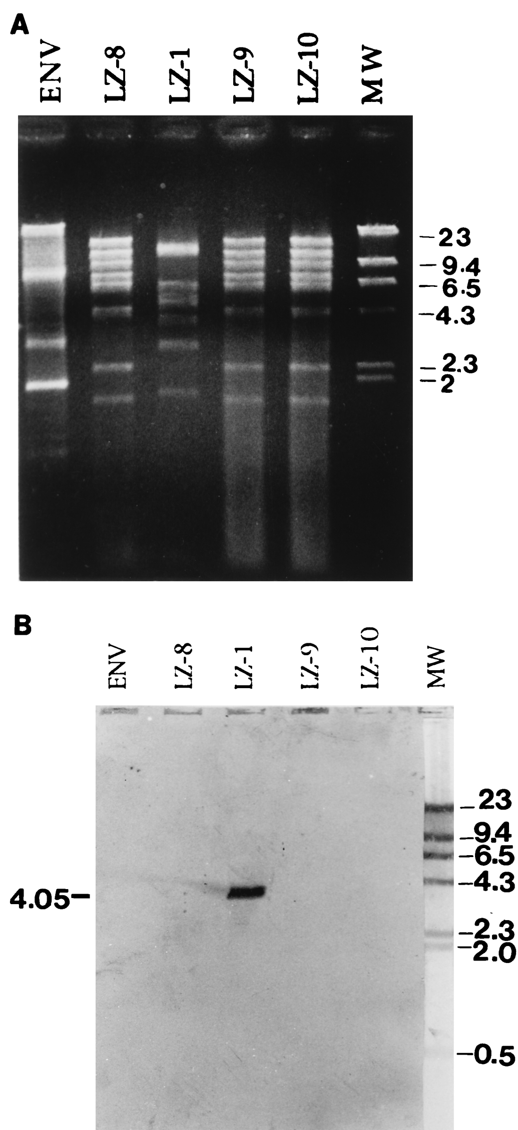

FIG. 1. (A) *Eco*RI restriction endonuclease analysis of *S. aureus* plasmid DNAs from the high-level mupirocin-resistant strain LZ-1; the low-level mupirocin-resistant strains LZ-8, LZ-9, and LZ-10; and the mupirocin-susceptible strain ENV separated by conventional electrophoresis. Lanes are labeled according to strain number. MW designates the lambda *Hin*dIII molecular weight marker (GIBCO-BRL). (B) Southern hybridization analysis of the gel in panel A with a digoxigenin-labeled *mupA*-specific probe. Lanes are labeled according to strain number.

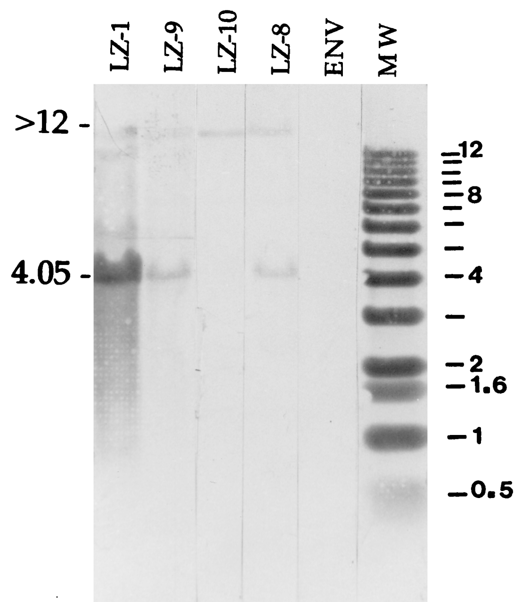

FIG. 2. Southern hybridization analysis of *S. aureus* chromosomal DNAs from the high-level mupirocin-resistant strain LZ-1; the low-level mupirocin-resistant strains LZ-8, LZ-9 and LZ-10; and the mupirocin-susceptible strain ENV after *Eco*RI restriction endonuclease analysis and conventional electrophoresis. Lanes are labeled according to strain number. MW designates the 1-kb DNA molecular weight marker (GIBCO-BRL). The digoxigenin labeled *mupA*-specific probe was used.

Neither ENV nor NCTC 8325 hybridized with the *mupA* probe.

PCR generation of *mupA* fragments. Electrophoretic analysis of the PCR amplification from LZ-1, LZ-8, LZ-9, LZ-10, and ENV with the primers Mup1 and Mup2 is shown in Fig. 3.

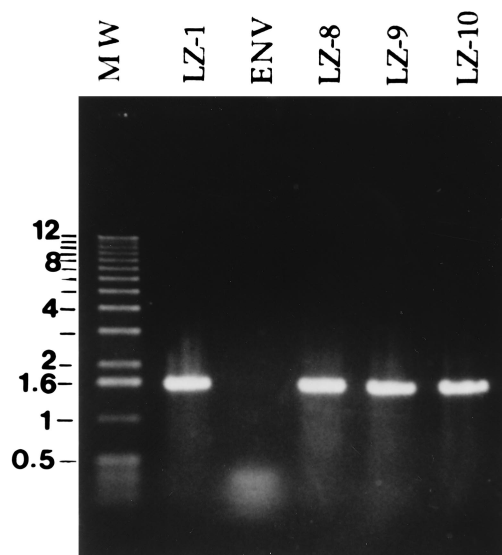

FIG. 3. Chromosomal DNAs from the high-level mupirocin-resistant strain LZ-1; the low-level mupirocin-resistant strains LZ-8, LZ-9, and LZ-10; and the mupirocin-susceptible strain ENV were used as template DNA for PCR amplification with the primer pair Mup 1 and Mup 2. The PCR products of this reaction are shown. Lanes are labeled according to strain number. MW designates the 1-kb DNA molecular weight marker (GIBCO-BRL).

The mupirocin-resistant strains, both low and high level, but not the mupirocin-susceptible strain ENV, contained the necessary cDNA to be amplified, as evidenced by the generation of the 1.65-kb intragenic fragment of *mupA*.

DISCUSSION

The DNA hybridization data presented herein identify a chromosomal location for *mupA* in three low-level mupirocin-resistant *S. aureus* isolates. *mupA* was found on plasmid DNA only in the high-level mupirocin-resistant strain. Neither plasmid DNA nor chromosomal DNA from the mupirocin-susceptible isolate hybridized with the *mupA* gene probe.

A physical map of the staphylococcal chromosome of representative strain NCTC 8325 has been characterized by Pattee et al. (18). This map includes the estimated sizes of *Sma*I fragments and the location of the native *ileS* gene within *Sma*I fragment F, which is approximately 208 kb. The *mupA* probe hybridized with a larger *Sma*I fragment (fragment C [341 kb]), but it did not hybridize with the native *ileS* contained in *Sma*I-digested fragment F from any of the strains, including NCTC 8325.

Further support for the presence of *mupA* on chromosomal DNA from the low-level mupirocin-resistant strains was provided by PCR amplification experiments with Mup1 and Mup2, primers specific for an intragenic portion of the *mupA* gene. PCR amplification produced a 1.65-kb fragment from chromosomal DNA from the high- and low-level mupirocin-resistant strains, while amplification failed to produce any fragment from the mupirocin-susceptible strain ENV.

Precedence for a chromosomal location for resistance determinants previously thought to reside only in a plasmid location has been established for gentamicin resistance in *S. aureus*. Although the majority of gentamicin-resistant *S. aureus* strains possess a plasmid copy of the resistance determinant for gentamicin, other strains have been shown to contain a chromosomally encoded gentamicin resistance determinant that shares homology with the plasmid-encoded copy in most strains (14).

The identification of the *mupA* gene on the chromosome of low-level mupirocin-resistant strains perhaps makes their isolation from clinical specimens more important than previously thought. It is possible that high-level mupirocin resistance differs from low-level mupirocin resistance by virtue of the location and/or copy number of *mupA*. It is also possible that high-level mupirocin resistance and low-level mupirocin resistance are mediated by different but closely related genes which hybridize with the *mupA* probe. More *S. aureus* strains from diverse geographic regions should be studied to determine if a chromosomal location for *mupA* is commonly associated with low-level mupirocin resistance.

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