**Staphylococcus aureus** Cap5O Has UDP-ManNAc Dehydrogenase Activity and Is Essential for Capsule Expression

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The *Staphylococcus aureus* serotype 5 capsular polysaccharide (CP5) has a repeating unit composed of \((\rightarrow 4)-3-O\text{-acetyl-}\beta-D\text{-ManNAcA}-\((1\rightarrow 4)\alpha-L\text{-FucNAc}\text{H}-(1\rightarrow 3)\beta-D\text{-FucNAc}-(1\rightarrow n_1)\). Sixteen chromosomal genes (cap5A through cap5P) are involved in the synthesis of CP5. We recently demonstrated that Cap5P, a 2-epimerase, catalyzes the conversion of UDP–\(N\)-acetyl glucosamine (UDP-GlcNAc) to UDP–\(N\)-acetylmannosamine (UDP-ManNAc). In this study, we show that UDP-ManNAc is oxidized to UDP–\(N\)-acetylmannosaminuronic acid (UDP-ManNAcA) by a UDP-ManNAc dehydrogenase encoded by *S. aureus* cap5O. We expressed Cap5O in *Escherichia coli* and purified the recombinant protein. The UDP-ManNAc dehydrogenase activity of purified Cap5O was assessed by incubating Cap5P and UDP-GlcNAc (to produce UDP-ManNAc), together with Cap5O, NAD\(^+\), and a reducing agent. Enzymatic activity was quantitated indirectly by measuring the increase in absorbance at 340 nm resulting from NADH formation. The product of the reaction was confirmed as UDP-ManNAcA by gas chromatography-mass spectroscopy. A cap5O mutation, created by deletion of 727 bp in the 5’ end of the gene, was introduced by allelic replacement into *S. aureus* Reynolds, rendering it CP5 negative. Mice inoculated intravenously or subcutaneously with the wild-type strain Reynolds had greater numbers of *S. aureus* recovery from their kidneys (\(P = 0.019\)) or their subcutaneous abscesses (\(P = 0.0018\)), respectively, than did animals inoculated with the cap5O mutant. The results of this study indicate that *S. aureus* cap5O is essential for capsule production and that capsule promotes staphylococcal virulence in mouse models of abscess formation.

**Materials and Methods**

**Bacterial strains, plasmids, and growth media.** The bacterial strains and plasmids used in this study are listed in Table 1. Luria-Bertani medium was used for growth of *E. coli*. *S. aureus* strains were grown in tryptic soy broth or on Columbia agar (Difco Laboratories, Detroit, Mich.) plates supplemented with 2% NaCl. The culture medium contained chloramphenicol (Cm) at 10 \(\mu\)g/ml, erythromycin (Em) at 5 \(\mu\)g/ml, or kanamycin (Km) at 25 \(\mu\)g/ml when required. Chemicals. Reagents used in enzyme purification and activity assays were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise noted. Ultrapure reagents used for methanolysis, reduction, and derivatization of sugars were obtained from T. Baker, Inc. (Phillipsburg, N.J.); Sigma; or ICN Biomedicals, Inc. (Aurora, Ohio). Avidin was obtained from NEN Life Science Products, Inc., Boston, MA.
selecting for Cm-resistant colonies at 30°C. The mutation was introduced into the chromosome by allelic exchange. In brief, plasmid integrants in the chromosome were selected by plating of cells on tryptic soy agar containing Cm (5 μg/mL) at 37°C. Single colonies were then passaged three times at 30°C without antibiotic selection. Clones were then acetylated with 0.1 mL of acetic anhydride and 0.1 mL of pyridine for 1 h to complete conversion of aldital to acetylated aldital, and labeled the anomeric carbon-1 with one deuterium atom. The aldital was treated with 1 mL of 95% ethanol at 70°C for 10 min to precipitate carbohydrates by methanolysis with 0.5 mL of 1 M methanol containing 35% of sodium chloride for 24 h at 85°C. The methyl glycosides were reduced overnight at room temperature with 3 mL of sodium borodeuteride in 0.3 mL of 50 mM Tris-Cl buffer (pH 8.5). All the reagents except for NAD+ were mixed and placed in a dry bath at 37°C. NAD+ was then added, and the absorbance at 340 nm was read at 0, 10, 20, 30, and 60 min in a Beckman UV-visible spectrophotometer. NADH formation was expressed in nanomoles, determined according to the Lambert-Beer law with an extinction coefficient for NADH of 6,220 M−1 cm−1.

The effect of EDTA (2, 5, and 10 mM), magnesium (2 mM MgCl2 or MgSO4), and monovalent ions (150 mM KCl, NaH2Cit, or (NH4)2SO4) on enzyme activity was determined. These reagents were included in the assay mixture prior to the addition of NAD+. Identification of UDP-ManNAcA by GC-MS. Dehydrogenase reaction mixtures were treated with 1 mL of 95% ethanol at 70°C for 10 min to precipitate proteins. UDP-amino sugars in the supernatant were converted to methyl glycosides by methanolation with 0.5 mL of 1 M methanol containing 35 μL of acetyl chloride for 24 h at 85°C. The methyl glycosides were reduced overnight at room temperature with 3 mL of sodium borodeuteride in 0.3 mL of 50 mM ammonium hydroxide. This step converts the sugars into the corresponding alditols and labels the anomeric carbon-1 with one deuterium atom. The alditols were then acetylated with 0.1 mL of acetic anhydride and 0.1 mL of pyridine for 20 min at 100°C. Samples were washed with distilled water, partitioned with 0.5 mL of ethyl acetate, and analyzed by GC-MS (Saturn 2000; Varian, Palo Alto, Calif.) on a DB-17 column (30-m by 0.25-mm inner diameter by 0.25-μm df). Electron impact ionization was used in all MS methods.

Mouse infection studies. Male ICR and Swiss-Webster mice (6 to 7 weeks old) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.). The mice were housed (up to four animals per cage) in a modified barrier facility under viral antibody-free conditions. Food and water were provided to the mice ad libitum. Animals were handled according to Brigham and Women's Hospital and Harvard Medical School institutional guidelines.

Staphylococci were cultivated on Columbia salt agar plates at 37°C for 24 h, and bacterial colonies were suspended in phosphate-buffered saline (0.01 M phosphate, 0.15 M NaCl; pH 7.2 to 7.4). The optical density of the bacterial suspensions was measured, and the samples were diluted to yield the appropriate numbers of CFU per milliliter.

### TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant genotype or phenotype</th>
<th>Source or reference</th>
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<tr>
<td>E. coli BL21(DE3)</td>
<td>F−ompT1 ton hsdS rB (rB mB) DE3</td>
<td>Novagen, Inc.</td>
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<td>Reynolds CP5</td>
<td>S. aureus</td>
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<td>JLO22</td>
<td>cap50 deletion in cap50 gene of Reynolds, CP5 negative</td>
<td>This study</td>
</tr>
<tr>
<td>RN4240</td>
<td>Capsule negative, restriction negative</td>
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Plasmids

<table>
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<th>Plasmid</th>
<th>E. coli expression vector (Km)</th>
<th>Source or reference</th>
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<td>pET-24a+</td>
<td>9.1-kb EcoRI fragment from S. aureus</td>
<td>Novagen, Inc.</td>
</tr>
<tr>
<td>pJCL24</td>
<td>9.1-kb EcoRI fragment from S. aureus</td>
<td>Novagen, Inc.</td>
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<td>pKBK4</td>
<td>9.1-kb EcoRI fragment (cap50H to cap50P) from pJCL24 in pUC19</td>
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<td>727-bp HpaI deletion (Δcap50) of pKBK9</td>
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<td>pKBK9-2</td>
<td>4.1-kb Avai-EcoRI fragment (cap50M to cap50P) from pKBK5 in pUC19</td>
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<td>pLJSO</td>
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<td>pUC19</td>
<td>E. coli cloning vector (Ap')</td>
<td>New England Biolabs, Inc.</td>
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In the renal abscess model (18), groups of four to six mice were injected in the tail vein with \(4 \times 10^6\) or \(4 \times 10^7\) CFU of \(S.~aureus\) in a 0.2-ml inoculum. The number of CFU per milliliter of inoculum was verified by plate counts. Five days after bacterial challenge, mice were euthanized, and the kidneys were excised, weighed, and homogenized in 1 ml of tryptic soy broth. Serial dilutions of the homogenates were plated in duplicate on tryptic soy agar plates, and the results were expressed as the log CFU of \(S.~aureus\)/gram of tissue. The lower limit of detection by culture was 1.1 log CFU/g of tissue. Two separate experiments were performed with each mouse strain.

In the subcutaneous model (7), bacterial suspensions were mixed with equal volumes of dextran beads (Cytodex-1 microcarriers; Sigma) prepared according to the manufacturer’s instructions. Groups of three mice were injected subcutaneously in each flank with 0.2 ml of \(S.~aureus\) suspensions ranging from \(10^7\) to \(10^1\) CFU/ml. The numbers of CFU per milliliter of inoculum were verified by plate counts. Four days after bacterial challenge, mice were euthanized, and the abscesses were excised and homogenized in 1 ml of tryptic soy broth. Serial dilutions of the homogenates were plated in duplicate on tryptic soy agar plates, and the results were expressed as the log CFU of \(S.~aureus\)/abscess. The lower limit of detection was 1.0 log CFU/abscess. In two separate experiments, Swiss-Webster mice were coinjected with a bacterial suspension containing equal numbers of the parental strain Reynolds and the \(cap5O\) mutant JLO22 mixed with dextran beads. The number of CP5-positive versus CP5-negative colonies recovered in each abscess was assessed by a colony immunoblot method (20).

In the subcutaneous abscess model, data from coinfection experiments were analyzed by the unpaired Student’s \(t\) test for comparing Gaussian populations with unequal standard deviations. A semiparametric weighted least-squares method (32) was used to compare the results of quantitative bacterial cultures performed over a range of doses in the subcutaneous abscess model. Data from coinfection experiments were analyzed by the unpaired Student’s \(t\) test.

**RESULTS**

**Purification and properties of Cap5O.** Cap5O was overexpressed from pNB1 in \(E.~coli\) BL21(DE3). As shown in Fig. 1, most of the protein was recovered from the 39,000 × g supernatant, an indication that the protein was in a soluble form. The hydropathy plot of the deduced amino acid sequence of the protein confirmed its hydrophilic nature (data not shown). The histidine-tagged Cap5O, purified over a Ni\(^{2+}\) affinity column, showed a single band by SDS-PAGE, with a mass of \(-45.9\) kDa (Fig. 1). This result is in agreement with the predicted mass of 45.6 kDa deduced from the nucleotide sequence of the \(cap5O\) gene. The isoelectric point predicted from the amino acid sequence of Cap5O was 4.8. N-terminal protein sequencing of purified Cap5O yielded the sequence MKLTVVGLGY, which confirmed the translational start site predicted by the nucleotide sequence (29). Purified Cap5O, at a concentration of \(-3.5\) mg/ml, was stable for at least nine months when stored at \(-70°C\) in 50 mM Tris-HCl–250 mM ammonium sulfate (pH 8.5).

**Enzymatic function of Cap5O.** Because the substrate of Cap5O (UDP-ManNAc) is not commercially available, we produced UDP-ManNAc by incubating purified \(S.~aureus\) Cap5P with its substrate UDP-GlcNAc as previously described (14). Purified Cap5O and the cofactor NAD\(^+\) were added, and the mixture was incubated at 37°C. As an indirect determination of Cap5O activity, we measured the increase in the absorbance at 340 nm resulting from NADH formation. A reaction buffer with a basic pH (ranging from 8 to 9) and a reducing agent (either dithiothreitol or β-mercaptoethanol) were both required for maximal production of NADH (data not shown). Furthermore, no enzymatic activity was detected if NAD\(^+\), Cap5O, Cap5P, or the substrate UDP-GlcNAc was omitted from the assay mixture. No NADH formation was observed if GlcNAc or UDP-N-acetylgalactosamine (UDP-GalNAc) was substituted for UDP-GlcNAc as the substrate in the assay.

![](http://iai.asm.org/)

**FIG. 1.** SDS-PAGE analysis of \(S.~aureus\) Cap5O expression and purification in \(E.~coli\) BL21(DE3). Lane 1, cell lysate from uninduced cells of \(E.~coli\); lane 2, cell lysate from IPTG-induced cells; lane 3, supernatant resulting from centrifugation at 39,000 × g of cell lysate; lane 4, column effluent reflecting unbound proteins; lane 5, purified Cap5O; lane M, molecular mass markers (expressed as kilodaltons).

**FIG. 2.** Time course of NADH formation with different amounts of Cap5O added (A) or with different concentrations of NAD\(^+\) added (B). The results are from three separate experiments. The error bars indicate the standard deviations.
The reaction mixture lacking Cap5P was used as a negative control sample for most experiments. To determine the influence of Cap5O concentration on NADH production, we added increasing amounts of purified Cap5O to the enzyme reaction mixture containing 1.5 mM NAD$_1$H. As shown in Fig. 2A, NADH formation increased in a linear fashion with increasing amounts of Cap5O up to 33.75 mg. Similarly, when we added various concentrations of NAD$_1$H (0 to 6 mM) to a reaction mixture containing 70 mg of Cap5O, the amount of NADH increased proportionally up to 1.5 mM NAD$_1$H (Fig. 2B). We used 70 mg of Cap5O and 1.5 mM NAD$_1$H in our standard assay. Under the standard conditions, the rate of NADH formation was 50 μmol/min/mg of Cap5O.

Cap5O activity was unaffected by the addition of 2, 5, or 10 mM EDTA to the reaction mixture, indicating that exchangeable divalent cations were not required for enzyme activity (data not shown). Similarly, the addition of Mg$^{2+}$ had no significant effect on the kinetics of the assay. The addition of 150 mM ammonium sulfate or ammonium chloride to the assay mixture produced no effect, whereas potassium chloride at the same concentration caused a modest inhibition (~30%) of enzyme activity.

**Identification of UDP-ManNAcA.** According to our proposed pathway for UDP-ManNAcA biosynthesis, UDP-GlcNAc is epimerized by Cap5P to UDP-ManNAc, which is then oxidized by Cap5O to UDP-ManNAcA. In the reaction products, all three sugars were expected to be present.

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The products of the enzyme assay mixtures were reduced with sodium borodeuteride, hydrolyzed, and converted to alditol acetate derivatives. Analysis of gas chromatograms revealed two peaks with retention times of approximately 17.4 and 17.9 min (Fig. 3A), values corresponding to those for alditol acetate derivatives of authentic GlcNAc and ManNAc standards, respectively. Chromatograms from negative control samples with no Cap5P contained only the 17.4-min peak; MS analysis confirmed this peak to represent GlcNAc (data not shown). The peak at 17.9 min includes the derivatives of ManNAc and reduced ManNAcA. During the first reduction step with sodium borodeuteride, the carbon-6 of ManNAcA, but not ManNAc, is labeled with two deuterium atoms. Consequently, ManNAcA is two atomic mass units heavier than ManNAc. Thus, it was possible to distinguish the two enzymatic products accurately by MS analysis. The mass spectrum of the 17.9-min GC peak closely resembles that of a ManNAcA standard (peaks at 43, 85, 141, 196, 215, 258, 318, and 377 m/z; Fig. 3B). However, superimposed on the spectrum are peaks associated with ManNAc (peaks 43, 85, 139, 258, 318, and 375 m/z). By integration of the mass associated with the products of the reaction, the ratio of GlcNAc to ManNAc to ManNAcA was determined to be 1:3:22.

Essential role of the cap5O gene in CP5 expression. A cap5O mutation was created by deletion of 727 bp in the 5′ end of the cap5O gene and subcloning the mutated fragment in a temperature-sensitive shuttle vector. The cap5O deletion was introduced by allelic exchange into the chromosome of S. aureus serotype 5 strain Reynolds, yielding mutant JLO22. To confirm the deletion in the chromosomal copy of cap5O in the mutant, genomic DNA from Reynolds and JLO22 was digested with HindIII, electrophoresed in an agarose gel, and analyzed by Southern blotting. Labeled pKBK4 (cap5O gene in pUC19) hybridized to 6.2- and 1.4-kb DNA bands from Reynolds and a single 6.9-kb DNA band from JLO22. The band sizes reflect the deletion of the 727-bp Hpal fragment (including an internal HindIII site) from the cap5O gene in the mutant JLO22. Mutant JLO22 was negative for CP5 production as determined by immunodiffusion and colony immunoblots with CP5-specific antiserum. Its growth rate in vitro was identical to that of the parental strain (data not shown).

Effect of cap5O deletion on staphylococcal virulence. Two different strains of outbred mice were challenged intravenously with either S. aureus Reynolds or JLO22 to compare their virulence in the renal abscess model of infection. Swiss-Webster mice challenged with 4 × 10^5 CFU strain Reynolds had significantly (P = 0.019) higher numbers of CFU recovered per gram of kidney than mice challenged with mutant JLO22 (Table 2). However, this difference in infectivity could be overcome by increasing the inoculum to 4 × 10^6 CFU/mouse, in which case both groups of animals had similar numbers of staphylococci recovered from the kidney (data not shown). Significant differences in virulence were not seen at either inoculum when similar experiments were performed with ICR mice (Table 2).

We challenged Swiss-Webster mice subcutaneously with S. aureus inocula ranging from 10^3 to 10^5 CFU. As shown in Fig. 4, animals challenged with the large inocula (10^5 or 10^6 CFU) showed similar bacterial densities (~10^7 CFU/abscess) independent of the challenge strain. However, mice inoculated with 10^3, 10^2, or 10^1 CFU of the cap5O mutant JLO22 had significantly fewer CFU per abscess than mice inoculated with the wild-type strain Reynolds (P = 0.0018; Fig. 4). Significant differences between the strains were not observed when ICR mice were challenged with 10^5, 10^3, or 10^1 CFU S. aureus (data not shown).

We challenged an additional group of 10 mice with a mixed inoculum containing equal numbers of strain Reynolds and JLO22 (either 10^3 or 10^2 total CFU). As shown in Table 3, between 74 and 81% of the bacteria recovered from the abscesses on day 4 were capsule positive. These data suggest that encapsulation promotes bacterial growth and/or survival within the abscess and confirm the results depicted in Fig. 4.

**DISCUSSION**

DNA sequence analysis of the S. aureus cap5 and cap8 genes revealed that 16 genes [cap5(8)A through cap5(8)P], clustered on the bacterial chromosome, are involved in capsule biosynthesis (29). This information allowed us to compare the predicted amino acid sequences of cap5 and cap8 with sequences in the public databases and to assign putative functions to most of the genes (19, 29). The cap5 and cap8 gene clusters are almost identical in their flanking sequences (capA through capG and capL through capP), but they differ in the central serotype-specific gene region (capH through capK) (29). The function of only a few of the biosynthetic genes has been proven. We showed that the gene product of cap5H, one of the CP5-specific genes, O acetylates the third carbon on the ManNAcA residues of CP5 (5). In addition, we showed that the purified product of the cap5P gene is a UDP-GlcNAc 2-epimerase that catalyzes the conversion of UDP-GlcNAc to UDP-ManNAc (14). In this study, we characterized the S. aureus cap5O gene product as a UDP-ManNAc dehydrogenase.

The enzymatic activity of Cap5O was quantitated indirectly by mixing Cap5O with Cap5P and UDP-GlcNAc and measuring NADH production. The oxidation product was confirmed to be a UDP-ManNAcA by reduction of the reaction products with sodium borodeuteride and analysis of the derivatized products by GC-MS. Thus, we propose that the synthesis of the 2-epimerase reaction depletes the intermediate product by mixing Cap5O with Cap5P and UDP-GlcNAc and measuring NADH production. The oxidation product was confirmed to be a UDP-ManNAcA by reduction of the reaction products with sodium borodeuteride and analysis of the derivatized products by GC-MS. Thus, we propose that the synthesis of the 2-epimerase reaction depletes the intermediate product.
introduction of the wild-type cap5O

Moreover, Cap5O was unaffected by EDTA or divalent cations.

The biochemical properties of S. aureus Cap5O are similar to those described for other microbial dehydrogenases. For example, both S. aureus Cap5O and an E. coli UDP-ManNAc dehydrogenase require a basic pH and a reducing agent to be present for maximal activity (12). Like other dehydrogenases with specificity for UDP-ManNAc (11, 22) or UDP-glucose (3), S. aureus Cap5O was unaffected by EDTA or divalent cations. Moreover, S. aureus UDP-ManNAc dehydrogenase contains the N-terminal NAD-binding domain (GXGXXG) typical of other dehydrogenases (33) requiring NAD+ as a cofactor.

The deletion of cap5O in S. aureus Reynolds yielded a CP5-negative mutant. Similarly, Sau et al. (30) showed that the serotype 8 strain Becker with a chemically induced mutation in cap8O was negative for CP8 production. Recombinant plasmids containing intact cap8O complemented the function of the mutated cap8O gene in trans. Similarly, we showed that introduction of the wild-type cap5O gene on a plasmid restored CP5 expression to a cap5O mutant of strain Newman (28).

The role of the S. aureus capsule in the pathogenesis of staphylococcal infections has been examined in a number of test systems. Serotype 5 and 8 strains of S. aureus were shown to resist opsonophagocytic killing by human polymorphonuclear leukocytes (8, 31). In addition, CP5 enhanced virulence in mouse models of lethality (31), bacteremia (31), and septic arthritis (25) and promoted long-term nasal colonization by S. aureus in mice (15). In contrast, both CP5 and CP8 attenuated staphylococcal virulence in a rat model of catheter-induced endocarditis (4). In 1991, we challenged inbred C57BL/6J mice intravenously with \(5 \times 10^6\) CFU of the wild-type strain Reynolds, a capsule-deficient mutant created by transposon mutagenesis, or a chemically induced capsule-negative mutant (1). Because all of the mice developed renal abscesses and the numbers of bacteria recovered from the kidneys were similar, we concluded that CP5 did not influence renal abscess formation. The staphylococci in that study were harvested from logarithmic-phase broth cultures, in which little capsule is expressed (28, 31).

In this study, we reexamined the role of capsule in renal abscess formation by challenging two different strains of mice with S. aureus cultivated under conditions known to optimize capsule expression (31). At a challenge inoculum of \(4 \times 10^5\) CFU, significantly greater numbers of the parental strain Reynolds were recovered from the kidneys of Swiss-Webster mice compared with those challenged with the CP5-negative mutant JLO22. This effect on virulence was modest, however, since no differences between the two groups of animals were observed at a 10-fold-greater inoculum. Similar experiments carried out in ICR mice revealed no differences in virulence at either challenge dose. Mice are highly resistant to S. aureus infection and, in this model, an inoculum \(>10^5\) CFU is essential for infectivity.

The subcutaneous abscess model proved to be a more sensitive model of infection since inocula as low as 10 CFU could provoke an infection by the wild-type S. aureus strain. Significantly fewer organisms were recovered from the subcutaneous abscesses of Swiss-Webster mice challenged with \(\leq 10^3\) CFU mutant JLO22 compared with the wild-type strain. Moreover, in coinfection experiments, 75 to 80% of the organisms recovered after 4 days were CP5 positive. This result is in agreement with our findings that the encapsulated wild-type strain is more virulent than the acapsular mutant in this model. However, no differences in virulence were observed when ICR mice were challenged subcutaneously with \(10^7, 10^5,\) or \(10^3\) CFU. Taken together, these results suggest that the role of the capsule in the pathogenesis of staphylococcal infections is dependent not only on the bacterial growth conditions and inoculum size but also on the genetic background of the host.

The pathogenesis of the S. aureus subcutaneous infection model is clearly different from that of the renal abscess model. To induce subcutaneous abscesses, the bacteria are injected directly into the subcutaneous tissue in the presence of a foreign body (cytodex beads). It is likely that encapsulated staphylococci avoid uptake by phagocytes recruited to the site of infection, and thus capsule-positive S. aureus have a survival and growth advantage over staphylococci lacking a capsule. In contrast, a bolus dose of staphylococci is delivered intravenously to mice to provoke renal abscesses. The liver and spleen clear the majority of organisms, and only a small number of blood-borne organisms seed the kidney. We did not observe a significant difference in the number of parental or mutant S. aureus in mice (15).

![FIG. 4. Results of quantitative abscess cultures from groups of three to seven Swiss-Webster mice challenged subcutaneously with S. aureus Reynolds (solid symbols) or mutant JLO22 (open symbols). Curve fitting according to the statistical analysis for comparison of Reynolds and JLO22 groups is shown.](http://iai.asm.org/)

![FIG. 5. Synthesis of UDP-ManNacA in S. aureus.](http://iai.asm.org/)
cap5P is essential for CP5 expression, and a mutant lacking this enzyme is less virulent in two Swiss-Webster mice recovered from the kidney 24 h after bacterial challenge (unpublished observations). However, an S. aureus cap5SO mutant lacking CP5 expression showed greater adherence to endothelial cells in vitro compared with the parental strain (28). It is likely that capsule expression augments staphylococcal survival within the kidney by enhancing its resistance to phagocytic uptake and killing.

In conclusion, we have purified the S. aureus cap5SO gene product and demonstrated that it has UDP-ManNAc dehydrogenase activity. Cap5SO is essential for CP5 expression, and a mutant lacking this enzyme is less virulent in two Swiss-Webster mouse models of abscess formation. Our findings are consistent with the observation that antibodies that neutralize the S. aureus capsule show some protection against S. aureus infections.

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REFERENCES


8. Karakawa, W. W., A. Sutton, R. Schneerson, A. Karpas, and W. F. Vann. 1988. Capsular antibodies induce type-specific phagocytosis of capsulated S. aureus cells recovered from the kidney 24 h after bacterial challenge (unpublished observations). However, an S. aureus cap5SO mutant lacking CP5 expression showed greater adherence to endothelial cells in vitro compared with the parental strain (28). It is likely that capsule expression augments staphylococcal survival within the kidney by enhancing its resistance to phagocytic uptake and killing.

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REFERENCES


