Effects of In Vitro and In Vivo Growth Conditions on Expression of Type 8 Capsular Polysaccharide by *Staphylococcus aureus*

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Type 8 capsular polysaccharide (CP8) is widely prevalent among clinical isolates of *Staphylococcus aureus*, but the role that the capsule plays in the pathogenesis of staphylococcal infections is unclear. This study was performed to identify growth conditions that would optimize the production of CP8 and to determine whether enhanced CP8 expression would influence staphylococcal virulence. *S. aureus* Becker grown in a chemically defined broth medium with $<1\ \mu\text{M}$ ferric nitrate produced up to eightfold more CP8 per milligram of biomass than did bacteria cultivated in the same medium containing $20\ \mu\text{M}$ ferric nitrate. The bacteria produced >350 -fold more cell-associated CP8 per milligram of biomass when grown on the surface of Columbia agar than when grown in Columbia broth. Most of the CP8 produced by broth-grown cells was secreted into the culture medium. *S. aureus* cultivated on the surface of nitrocellulose membranes floating on Columbia broth produced levels of CP8 similar to those produced by cells grown on Columbia agar. Similarly, bacteria harvested from endocardial vegetations of rabbits infected with *S. aureus* produced high levels of CP8. These results indicate that staphylococci grown on surfaces, both in vitro and in vivo, produce larger quantities of cell-associated CP8 than those grown in liquid cultures. However, no differences were observed in the 50% lethal dose for mice of strain Becker grown on solid medium (high levels of capsule expression) or in liquid medium (low levels of capsule expression).

Capsular polysaccharides are produced by approximately 90% of *Staphylococcus aureus* isolates (16, 17, 27, 30). Although 11 capsular serotypes have been described, most isolates of *S. aureus* belong to serotype 5 or 8 (2, 4, 16, 17, 27, 30). Microcapsules elaborated by serotype 5 or 8 isolates are extracellular, uronic acid-containing polysaccharides that are not visualized by negative stains such as India ink. Serotype 5 and 8 isolates are referred to as microencapsulated because their capsules are smaller than the capsules produced by serotype 1 and 2 isolates. Isolates of the latter serotypes are highly encapsulated and virulent in animal models of infection (10, 18, 19, 32) but are rarely encountered among commensal or pathogenic isolates (4, 20, 30).

That capsular polysaccharide type 1 (CP1) promotes staphylococcal virulence was shown by studies comparing a highly encapsulated strain with transposon-induced, isogenic nonmucoid mutant strains that were either microencapsulated or nonencapsulated (19). The wild-type strain had a 50% lethal dose (LD_{50}) that was $>3,000$ -fold lower than those of the mutant strains. Moreover, the highly encapsulated parent strain was cleared less readily from the bloodstream and kidneys of infected animals than the nonmucoid mutant strains. Conversely, type 5 and 8 microcapsules have not been shown to contribute to *S. aureus* virulence, since wild-type strains were not more virulent than mutant strains lacking capsular polysaccharide expression (1, 7). In fact, strains expressing type 5 or 8 microcapsules were less virulent than nonencapsulated mutant strains in a rat model of catheter-induced endocarditis (7).

In the virulence studies cited above, animals were inocu-

lated with *S. aureus* harvested from mid-logarithmic-phase cultures in Columbia broth supplemented with 0.1% glucose, 1% yeast extract, and 0.5% NaCl (17). Columbia medium has a low phosphate content, and certain bacteria demonstrate enhanced capsule production when cultivated in medium with limiting phosphate levels (12, 13). Preliminary studies in our laboratory suggested that serotype 5 and 8 *S. aureus* cultivated in Columbia broth (low phosphate) produced little cell-associated capsular polysaccharide. To identify culture conditions that might optimize capsule expression by *S. aureus*, we measured the effects of specific environmental parameters on bacterial growth and type 8 capsular polysaccharide (CP8) production in vitro. In addition, we evaluated CP8 production in vivo by analyzing bacteria harvested from the endocardial vegetations of rabbits experimentally infected with *S. aureus*. The relative virulence of *S. aureus* grown under conditions of high and low levels of capsule expression was assessed in a mouse model of *S. aureus*-induced mortality.

MATERIALS AND METHODS

Bacteria, capsular antigens, and antibodies. *S. aureus* Reynolds and Becker are prototypic capsule type 5 and 8 strains, respectively (17). Strain SA1 mucoid is a capsule type 1 strain (19), and strain JL252 is a capsule-negative mutant derived from strain Becker (7). All bacteria were maintained in skim milk at -70°C . Chemically defined medium (CDM) was prepared as described by Mills et al. (26) and supplemented with either a low (0.41 mM) or a high (8.2 mM) concentration of magnesium acetate and a trace ($<1\ \mu\text{M}$) or a high (20 μM) concentration of ferric nitrate. Columbia broth (Difco Laboratories, Detroit, Mich., or Becton Dickinson Microbiology Systems, Cockeysville,

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Md.) was supplemented with 0.1% D-glucose, 1% yeast extract, and 0.5% NaCl (17) and solidified with 1.5% (wt/vol) agar (Bitek; Difco). All bacterial cultures were incubated at 37°C for 24 h, and broth cultures were incubated with reciprocal shaking. *S. aureus* CP1 was purified from *S. aureus* SA1 mucoid as described previously (21). CP8 was purified as described by Fournier et al. (14), except that teichoic acid was eliminated by oxidation with sodium metaperiodate (21). Rabbit antiserum raised to formalin-killed *S. aureus* was prepared as previously described (33). Rabbit antibodies to type 8 strain Becker were kindly provided by R. D. Arbeit, Veterans Affairs Medical Center, Boston, Mass.

Preparation of capsular polysaccharide. *S. aureus* grown in liquid cultures was sedimented at $11,800 \times g$ for 20 min, and the bacteria were washed in 1 volume of 10 mM sodium phosphate–0.85% sodium chloride (pH 7.3) (phosphate-buffered saline [PBS]). Staphylococci were transferred from solid medium to PBS with a wire loop. Bacterial cells were either washed in 100 ml of PBS or processed without being washed. Staphylococci prepared by either method were suspended in a minimal amount (≤ 1 ml) of PBS such that a 1:500 dilution of the suspension yielded an optical density at 650 nm of ~ 0.4 to 0.8. For dry-weight determinations, 10- μ l aliquots of undiluted cell suspensions were trapped on preweighed filters (Metricel; Gelman Sciences, Inc., Ann Arbor, Mich.), which were then dried in a 60°C desiccating oven overnight and reweighed. The number of CFU in the suspensions was determined by plating in duplicate serial 10-fold dilutions of the samples on tryptic soy agar plates. The bacterial suspensions were autoclaved at 121°C for 60 min to extract capsular polysaccharide (16). This process released $\sim 80\%$ of cell-associated CP8 from *S. aureus* Becker. Following centrifugation, the autoclaved extracts were filtered (0.45- μ m pore size; Millipore Corp., Bedford, Mass.) and stored at -20°C . Culture supernatants were filter sterilized, autoclaved, and concentrated by either ultrafiltration or dialysis and lyophilization.

Quantitation of capsular polysaccharide. The amount of CP1 or CP8 present on the surface of intact *S. aureus* or in concentrated culture supernatants was measured by two methods: rocket immunoelectrophoresis (RIE) and an enzyme-linked immunosorbent assay (ELISA) inhibition method. RIE was performed with glass slides covered with 1% agarose (Indubiose; Accurate Chemical and Scientific Corp., Westbury, N.Y.) in 0.05 M barbital buffer (pH 8.6) containing 0.4 to 0.5% rabbit serum raised to killed *S. aureus*. CP1 or CP8 standards and dilutions of the staphylococcal extracts were electrophoresed through the gel at 20 V/cm and 4°C until precipitin lines were visible. Following electrophoresis, the gel was washed in several changes of saline, dried, and stained with Coomassie blue. A standard curve relating capsular polysaccharide concentration to migration distance was used to estimate the amount of capsule in each sample. CP1 or CP8 concentrations in the bacterial extracts were normalized to bacterial dry weights or to CFU.

The ELISA inhibition method, described previously (1), is based on the ability of encapsulated bacteria (trypsinized to remove protein A) or soluble capsular polysaccharide to absorb capsular antibodies from immune serum. Rabbit serum raised to strain Becker was diluted 1:10,000 or 1:40,000 and incubated overnight at 4°C with serial dilutions of bacteria (10^{11} to 10^2 CFU/ml), concentrated culture supernatants, or purified CP8 (20 to 0.001 $\mu\text{g/ml}$). Absorbed serum samples were added to wells of microtiter plates coated with purified CP8 (4 $\mu\text{g/ml}$) coupled to poly-L-lysine

by the cyanuric chloride method (15), and the ELISA was carried out as described previously (1). The concentration of a sample that resulted in 50% inhibition of antibody binding was determined, and the CP8 content of the sample was calculated from the standard curve.

Animal experiments. Female CD-1 mice were obtained from Charles River Laboratories (Kingston, Mass.) at 6 to 10 weeks of age; they received food and water ad libitum. Five groups of eight or nine mice were challenged intraperitoneally with ~ 3.2 -fold dilutions of unwashed cells of strain Becker. The inocula, ranging from 2.2×10^{10} to 1.1×10^8 CFU per mouse, were harvested from 24-h cultures of Columbia broth or Columbia agar medium. Groups of 6 to 10 mice were challenged with 10-fold dilutions of broth-grown strain SA1 mucoid ranging from 1.4×10^7 to 1.4×10^5 CFU per mouse. Lethality was assessed 72 h after bacterial challenge. LD_{50} s were calculated by the method of Reed and Muench (28).

We used the rabbit model of catheter-induced *S. aureus* endocarditis described by Sande and Johnson (29). Female New Zealand White rabbits (Millbrook Farms, Amherst, Mass.) weighing ~ 3 kg were anesthetized with ketamine (40 mg/kg of body weight; Ketalar; Parke-Davis, Morris Plains, N.J.) and xylazine (10 mg/kg; Rompun; Mobay, Shawnee, Kans.), and the right carotid artery was exposed and ligated. A polyethylene catheter (outside diameter, 1 mm) was inserted into the left ventricular cavity via the right carotid artery. The catheter was tied in place, and the incision was closed. Rabbits were injected in the marginal ear vein with *S. aureus* Becker grown to the logarithmic phase in Columbia broth; the inocula ranged from 1.8×10^5 to 6.7×10^5 CFU. In an effort to obtain vegetations that were maximal in size and contained the largest number of staphylococci possible, we varied both the time between catheterization and bacterial challenge from 2 to 6 days and the time between bacterial challenge and necropsy from 3 to 5 days. The aorta and left ventricle were opened, and the vegetations were excised, weighed, and homogenized in ~ 0.5 ml of PBS. Dilutions of the homogenates were prepared in sterile brain heart infusion broth, and quantitative plate counting was performed as described above.

RESULTS

Bacterial growth and CP8 production in CDM. Previous studies revealed an inverse relationship between capsule expression by *Neisseria meningitidis* and iron concentration in the culture medium (24). Likewise, Mills et al. (26) reported that magnesium deprivation retarded bacterial growth and enhanced total exoprotein production by *S. aureus*. For determination of the influence of iron or magnesium limitation on bacterial growth and capsule expression by *S. aureus*, the bacteria were cultivated in CDM with variable concentrations of iron and magnesium. As shown in Fig. 1, magnesium limitation markedly reduced the bacterial growth rate and final cell yield. In fact, bacterial multiplication was not observed in CDM supplemented with <0.4 mM magnesium acetate. In CDM containing 8.2 mM magnesium acetate, the growth rate was independent of the iron content of the medium. Unsupplemented CDM contained <1 μM iron, as determined by the ferrozine assay (31).

For quantitation of the effects of iron or magnesium limitation on CP8 production, bacterial extracts prepared from each of the CDM cultures were subjected to RIE. As shown in Fig. 2, RIE specifically measured CP8, since extracts prepared from strain Becker but not extracts made

FIG. 1. Representative growth curve for *S. aureus* Becker cultivated in CDM with variable concentrations of Mg^{2+} and Fe^{3+} . The graph demonstrates that magnesium but not iron limitation significantly affected the bacterial growth rate and final cell yield. No growth was observed in CDM without magnesium supplementation (data not shown). Symbols: ●, 8.2 mM Mg and <1 μM Fe; ○, 8.2 mM Mg and 20 μM Fe; ▲, 0.4 mM Mg and <1 μM Fe; △, 0.4 mM Mg and 20 μM Fe.

from capsule type 5 strain Reynolds or capsule type 8-negative mutant strain JL252 reacted with our antiserum. *S. aureus* typically produced elevated CP8 concentrations when cultivated under conditions of iron limitation, whereas magnesium deprivation had little effect on capsule expression (Fig. 2). In five independent experiments, CP8 levels were normalized to the dry weights of cells from which the bacterial extracts were prepared (Fig. 3). This method was

FIG. 2. RIE slide containing 0.5% serum raised in rabbits to capsule type 8 *S. aureus* Becker. Samples included CP8 and extracts from *S. aureus* Becker grown in CDM containing either 0.4 or 8.2 mM Mg^{2+} and <1 or 20 μM Fe^{3+} . Extracts from strains Reynolds (capsule type 5) and JL252 (capsule type 8 negative) were prepared from cells grown on Columbia agar plates.

FIG. 3. Quantitative effects of iron and magnesium on CP8 expression by *S. aureus* (assessed by RIE; mean of five experiments). CP8 production is expressed as the amount of CP8 produced per milligram of cell dry weight.

preferred over normalization to CFU, since *S. aureus* cells grown under conditions of magnesium limitation were enlarged and showed abnormal clumping patterns. Depending on the magnesium content of CDM, cultures without added iron produced four- to eightfold more capsular polysaccharide than did cultures containing 20 μM iron. Thus, *S. aureus* showed enhanced capsule production when the iron content of the culture medium was low; in contrast, magnesium had a marked effect on bacterial multiplication but little effect on CP8 production.

Capsule production in complex medium. Much of the biological characterization of microencapsulated *S. aureus* types 5 and 8 was performed with bacteria cultivated in Columbia broth (17), which contains low levels of phosphate. Organisms harvested from Columbia agar plates produced 2.3 μg of CP8 per mg of biomass (Fig. 4), a value >3-fold higher than that of cells grown in CDM without added iron. *S. aureus* cultivated on another solid medium, such as tryptic soy or Mueller-Hinton agar, had a CP8 content similar to that of cells grown on Columbia agar (data not shown). Washing agar-grown *S. aureus* once or twice in PBS released 28 and 35%, respectively, of the cell-associated capsular polysaccharide.

Very little CP8 (0.006 μg per mg of biomass) was associated with *S. aureus* cells grown in Columbia broth (Fig. 4). Moreover, staphylococci harvested in either the logarithmic or the stationary phase of growth had similar levels of CP8 per mg of biomass (data not shown). Because staphylococci may release soluble capsular antigens during growth in broth cultures, we measured the CP8 concentration in culture supernatants and compared it with the concentration of cell-associated CP8. Per milliliter of culture, only 0.005 μg of CP8 (2%) was bound to the bacterial cells, whereas 0.22 μg of CP8 (98%) was recovered in the culture supernatants. Nonetheless, the total amount of CP8 produced by cells grown in liquid medium was much smaller than that produced by cells grown on solid medium. These differences were corroborated by data obtained with ELISA inhibition experiments showing that agar-grown *S. aureus* produced 21.8 μg of CP8 per 10^{10} CFU but that broth-grown cells produced only 0.04 μg of CP8 per 10^{10} CFU. As measured by

FIG. 4. Capsular polysaccharide production by *S. aureus* grown on solid or in liquid Columbia medium. Organisms harvested from Columbia agar were assayed without being washed or after one or two washes in PBS. CP8 was measured by RIE as described in the text.

the ELISA, the CP8 concentration in Columbia broth culture supernatants was 0.25 µg/ml.

We considered that differences in capsule expression between cells grown on solid medium and those grown in liquid medium might be due to components in the agar used to solidify the medium. Therefore, we inoculated *S. aureus* on the surface of nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) floating on Columbia broth. After 24 h of incubation, the staphylococci were harvested without washing, bacterial extracts were prepared, and CP8 was measured by RIE. As shown in Fig. 4, the CP8 content of these surface-associated bacteria was 260-fold higher than that of broth-grown cells and similar to that of cells cultivated on solid medium.

Capsule production by *S. aureus* in vivo. Because of the differences observed in capsule expression by *S. aureus* cultivated in vitro on solid and in liquid media, we sought to measure the amount of CP8 produced by *S. aureus* in vivo. Extracts from 10^9 to 10^{10} CFU of *S. aureus* are necessary for CP8 quantitation by RIE. In the rabbit model of catheter-induced staphylococcal endocarditis, these cell numbers can be achieved in infected vegetations (29). Therefore, this animal model of staphylococcal infection was chosen to assess in vivo capsule production by *S. aureus*.

Catheterized rabbits were challenged with *S. aureus* grown to the logarithmic phase in Columbia broth. At the time of necropsy, three of five rabbits had $>10^9$ CFU/ml of vegetation homogenate, an amount yielding sufficient bacterial biomass for CP8 quantitation by RIE (Table 1). *S. aureus* harvested from the endocardial vegetations of rabbits produced 8.7 µg of CP8 per 10^{10} CFU. In comparison, staphylococci cultivated in vitro on agar and in broth yielded 6.9 and 0.02 µg of CP8 per 10^{10} CFU, respectively.

Virulence of *S. aureus* with high or low levels of capsule expression. As indicated above, *S. aureus* cultivated on Columbia agar had levels of cell-associated CP8 that were 2 orders of magnitude higher than those of cells grown in Columbia broth (Fig. 4). For determination of whether staphylococci grown under conditions of high or low levels

TABLE 1. In vivo production of CP8 by *S. aureus* Becker in a rabbit model of catheter-induced endocarditis

Rabbit	Inoculum (log ₁₀ CFU)	Log ₁₀ CFU/ml of homogenate	CP8 (µg/10 ¹⁰ CFU ± SD) ^a
1	5.83	9.11	13.9 ± 2.0
2	5.46	9.45	5.3 ± 1.5
3	5.26	9.02	6.9 ± 3.9

^a Mean ± SD, 8.7 ± 2.6.

of capsule expression would differ in virulence, we inoculated mice intraperitoneally with organisms grown under the two culture conditions. The LD₅₀s of strain Becker cultivated on agar and in broth were 9.3×10^8 and 5.9×10^8 CFU, respectively. Thus, differences in virulence were not observed, despite the fact that >350 -fold more CP8 per mg of biomass was associated with cells grown on solid medium than with cells grown in liquid medium.

We also examined CP1 production by highly encapsulated strain SA1 mucoid cultivated under these growth conditions and correlated our findings with bacterial virulence for mice. Like type 8 strain Becker, strain SA1 mucoid also produced more capsule on solid medium (130 µg of CP1 per mg of biomass) than in liquid medium (30 µg of CP1 per mg of biomass). Even when grown in broth cultures, however, strain SA1 mucoid was heavily encapsulated, compared with strain Becker. Strain SA1 mucoid grown in broth cultures had an LD₅₀ for mice of 3.9×10^4 CFU (19), a value 4 orders of magnitude lower than that of strain Becker.

DISCUSSION

Microcapsules are prevalent among *S. aureus* isolates from both commensal and pathogenic sources. Although capsule type 5 and 8 isolates make up ~22 and 53%, respectively, of isolates examined, these organisms have not been shown to be more virulent in animal models of staphylococcal infection than capsule-deficient or capsule-negative mutants (1, 7). The role, if any, that these capsular polysaccharides play in the pathogenesis of staphylococcal infections has remained elusive.

We considered that if capsule expression were influenced by environmental growth conditions, we might be able to identify culture conditions that would augment the production of the capsular polysaccharide. We examined three environmental parameters for their potential effects on capsule expression. First, the effect of growth under conditions of iron limitation was examined, since these conditions enhance *N. meningitidis* capsule production and virulence (24). Second, we investigated whether magnesium deprivation affected CP8 production, since Mills et al. showed that total exoprotein production by *S. aureus* was regulated by magnesium (26). Third, we determined whether capsule expression was influenced by cultivation on solid versus liquid media, since cell wall proteins of *S. aureus* are variably expressed by cells depending on the physical state of the culture medium (9).

Our results indicate that capsule expression by *S. aureus* is regulated by the microenvironment of the culture. Like *N. meningitidis* (24), *S. aureus* showed enhanced capsule production when cultivated in CDM without added iron. Siderophore production by *S. aureus* was recently reported (25), but no information is available on siderophore expression by strain Becker. Virtually nothing is known about the regulation by iron of staphylococcal protein or polysaccharide

expression. Nutrient limitation alone could not account for the effect of iron depletion on capsule expression, since magnesium deprivation had a marked effect on the bacterial growth rate but a minimal effect on capsule expression. *S. aureus* cultured in CDM with or without added iron produced more CP8 per milligram of biomass than cells cultured in Columbia broth medium. Similar results were obtained recently by Dassy et al. (11), who reported higher type 5 capsular polysaccharide production by *S. aureus* Reynolds grown in a semisynthetic medium than by the same strain grown in Columbia or other complex media.

Most striking among our findings was the observation that staphylococcal cells grown on a solid support yielded quantities of cell-associated CP8 >300-fold higher than those yielded by cells grown in liquid cultures. Most of the capsular material produced by cells grown in Columbia broth was released into the culture medium. The release of soluble CP8 by *S. aureus* has also been detected in vivo. Arbeit and colleagues (3, 5) detected circulating CP8 not only in the blood of animals with staphylococcal bacteremia but also in the blood of rats with subcutaneous staphylococcal infections. Dassy et al. (11) reported that the physical state of the culture medium did not influence type 5 capsule production; however, we observed that *S. aureus* Reynolds demonstrated enhanced type 5 capsule production when grown on agar versus when grown in broth (6). It is clear that liquid and solid culture media present disparate microenvironments to the bacterial cell and that the environmental signal(s) that triggers enhanced capsule expression by *S. aureus* is not known. Agar-grown cells not only have limited oxygen and nutrient availability but also are subject to more local changes in pH and metabolite concentrations than cells grown in liquid cultures (22). In addition to enhanced capsule production by surface-associated staphylococci, other phenotypic differences exist between bacterial cells grown on solid versus in liquid media. *S. aureus* cultivated on a solid support shows considerably thickened cell walls and cross walls (23), shows enhanced production of high-molecular-weight proteins (9), and is poorly adherent to fibroblasts (6) in comparison with cells grown in liquid cultures. The identification of in vitro culture conditions that augment capsule expression by *S. aureus* should facilitate the isolation and purification of CP8 from staphylococcal cultures. More interesting, however, are questions raised by this study concerning the regulation of capsule expression and physiologic responses of the bacterium to iron deprivation and growth on solid or in liquid culture medium. These questions represent fertile areas for future studies.

Previous ultrastructural studies of bacteria grown in vivo revealed that they more closely resemble bacteria grown in vitro on a solid support rather than in liquid cultures (22). The expression of the *S. aureus* capsule in vivo has been reported (2, 3, 5), but efforts to quantitate capsule production by cells grown in vivo have not been made. We evaluated in vivo capsule expression by inoculating rabbits with broth-grown *S. aureus* that expressed little cell-associated CP8. Bacteria recovered from the endocardial vegetations 3 to 5 days later produced high levels of CP8, similar to those produced by bacteria grown in vitro on solid medium. Because of the partial saturation of the iron-binding proteins lactoferrin and transferrin, available iron concentrations are very low in vivo, on the order of 10^{-18} M (8). Limiting iron concentrations and bacterial growth on a surface may both contribute to the enhancement of CP8 production in vivo. Whether capsule expression in vivo differs when bacteria multiply in a liquid milieu (e.g., blood) or on other solid

supports (e.g., the surface of mammalian cells or prosthetic devices) is not known.

Although agar-grown bacteria produced >300-fold more CP8 per milligram of biomass than cells grown in liquid cultures, the LD₅₀s of the two cultures in mice were very similar and quite high (> 10^8 CFU per mouse). Likewise, in the rat model of catheter-induced endocarditis, the virulence of type 5 strain Reynolds was similar whether the bacteria were cultivated on solid or in liquid Columbia medium (6). These results are consistent with those of our previous studies showing that type 5 and 8 microcapsules do not enhance staphylococcal virulence (1, 7).

Strain SA1 mucoid, a capsule type 1 isolate, harvested from Columbia broth cultures had an LD₅₀ for mice of only 3.9×10^4 CFU (19). This strain produced 10- to 60-fold more capsular polysaccharide than did strain Becker grown under optimal conditions. In contrast to our findings with strain Becker, ~80% of the capsular polysaccharide produced by strain SA1 (mucoid) in broth cultures is cell associated (21). That the large capsule is responsible for the virulence of strain SA1 mucoid was suggested by the fact that isogenic microencapsulated or nonencapsulated mutant strains produced from strain SA1 mucoid by Tn551 mutagenesis have LD₅₀s of ~ 10^8 CFU per mouse (19). It is clear that there are biologically relevant differences between *S. aureus* CP1 and *S. aureus* CP8. Whereas the type 1 capsule enhances bacterial virulence, presumably because of its antiphagocytic properties (19, 21, 33), the present study failed to show a correlation between enhanced type 8 capsule expression and staphylococcal virulence. Nonetheless, our data underscore the importance of controlling environmental parameters that markedly affect the physiology of the bacterial cell in response to its environment. Future studies using in vitro growth conditions that more closely resemble those likely to be encountered by organisms in vivo should allow for a better evaluation and understanding of the functional role of the *S. aureus* microcapsule.

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