Detection of Capsular Antigen Production in Unencapsulated Strains of *Staphylococcus aureus*

KOSAKU YOSHIDA, AYAKO NAKAMURA, TOSHICHIKA OHTOMO, AND SACHIYE IWAMI

Department of Microbiology, St. Marianna University School of Medicine, Takatu-ku, Kawasaki 213 Japan,
and Department of Microbiology and Immunology, Nippon Medical School, Bunkyo-ku, Tokyo 113, Japan

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Of 91 compact-type strains of *Staphylococcus aureus* in regular serum soft agar (SSA), 82 converted diffuse-type growth in serum soft agar (pH adjusted to 6.0). With the addition of four different rabbit anticapsular sera (anti-type A, B, C, and D sera) in low pH (6.0) SSA, 21 strains of *S. aureus* showed compact-type colonial morphologies. Eleven, one, and one strains of *S. aureus* reacted singly with rabbit anticapsular sera types A, B, and C, respectively, and no strain reacted with rabbit anticapsular type D. Eight of these strains reacted with both rabbit anticapsular sera types A and B. When the ability to absorb the converting activities of the antisera (changes of colonial morphologies of anticapsular sera in SSA) was quantitatively tested, 7- to 27-fold of these organisms were capable of absorbing the activities compared with the Smith diffuse organisms. These results suggest that even unencapsulated *S. aureus* strains are capable of producing capsular substance, although the capability is quantitatively different from strain to strain.

Previously, Yoshida et al. (7) proposed a hypothesis that *Staphylococcus aureus* strains possessed a life cycle as exhibited by the unencapsulated to encapsulated forms through pseudocapsulation in vivo.

Yoshida (4) recently described a diffuse type of growth by originally compact-forming colonies of *S. aureus* strains in low-pH serum soft agar (SSA). Serological application of the SSA technique to pH-dependent colonial morphologies of the compact-type staphylococcal strains suggests the possibility of detecting the existence of antigen production in unencapsulated organisms. Therefore, attempts were made to investigate whether a capsular substance could be found in unencapsulated *S. aureus* strains.

MATERIALS AND METHODS

Strains. Ninety-one strains of *S. aureus* were obtained from the clinical Laboratory, Nippon Medical School Hospital, Tokyo. These were positive for coagulase, deoxyribonuclease, acid phosphatase, and the clumping factor reaction determined by a method described elsewhere (5). They also exhibited the compact type of growth in the regular SSA technique of Finkelstein and Sulkin (2). Strains Smith diffuse, NS58D, NS41D, and NS68D were also utilized.

SSA technique with low-pH media. To 100 ml of distilled water, 1.0 g of peptone (Kyokuto Chem Co. Ltd., Tokyo), 0.5 g of yeast extract (Difco), 1.0 g of glucose, and 3.0 g of NaCl were added. Several lots of this medium were adjusted to pH values of 6.0, 6.2,

6.4, 6.6, 6.8, and 7.0 with 10 N HCl. One-tenth volume of a 0.1 M phosphate buffer of the corresponding pH and of 0.15% agar (Difco) were added to these media. The media were then autoclaved at 121 C for 15 min. By using these prepared media, the SSA technique was performed as described above.

Determination of the capsular-type antigen. One unit of converting activity (activity to convert the diffuse type of growth to compact type of growth by rabbit anticapsular sera in SSA as described elsewhere [3]) of four different anticapsular sera was added to 10 ml of 0.15% soft agar media which had been previously adjusted to pH 6.0 with 10 N HCl and 0.1 M phosphate buffer of similar pH was further supplemented as noted above. One-tenth milliliter of a cell suspension, obtained from a brain heart infusion broth culture at 37 C overnight and diluted 10^{-6} with sterile saline, and 0.1 ml of normal rabbit serum were added to each tube. These were incubated overnight at 37 C, and their colonial morphologies were recorded.

Absorption of anticapsular activities in sera. Five, 10, 20, 40, and 80 mg of lyophilized organisms were added to 1.0 ml of anticapsular rabbit sera prepared by the method described previously (3). These mixtures were kept at 37 C for 2 h and then centrifuged at $5,000 \times g$ for 20 min to remove the organisms, and supernatants were applied to the SSA technique. The growth types in SSA (which contained one unit of antisera absorbed with unencapsulated organisms) strains Smith diffuse, NS58D, NS41D, and NS68D, which were representative strains of A, B, C, and D capsular types, respectively, were determined.

Determination of phage and serotype. Phage-

and serotyping, Oeding and Pillet method, of *S. aureus* strains were performed by the procedure described elsewhere (5).

RESULTS

Growth types of *S. aureus* in low-pH SSA technique. Primarily, the appropriate pH of a medium for producing long-tail compact to diffuse forms, as designated in a previous paper, (4) was investigated to determine the routine medium for use in these experiments. Results indicated that strain 46, which showed the compact-type growth at pH 7.0, converted to the diffuse type at pH 6.0 through a short- to a long-tail compact-type growth (Fig. 1). When the pH of the medium was adjusted to below 6.0, cell growth was remarkably inhibited although prolonged tailing was exhibited by the colonies. Therefore, a medium adjusted to pH 6.0 was subsequently used for further experiments. With this medium, the colonial morphologies of 82 of 91 strains (90.0%) of the compact-type *S. aureus* converted from the long-tail- to the diffuse-type growth in SSA, although significant changes of colonial morphologies were not observed with the remaining strains.

Growth types of *S. aureus* strains in low-pH SSA containing anticapsular sera. Eighty-two strains of *S. aureus* exhibiting long-tail compact to diffuse forms in pH 6.0 SSA were subjected to the anticapsular factor sera for observation of any conversion of their colonial morphologies in SSA. Results showed that 21 strains reacted with one or two anticapsular rabbit sera (Fig. 2). Eleven, one, and one strains of the 21 investigated reacted singly with anticapsular type A, B, and C sera, respectively. Eight showed both A and B type capsules

(Table 1). However, none of the strains tested reacted with the type D anticapsular serum.

Absorption of the converting activities in anticapsular sera with unencapsulated organisms. Twenty-one strains of *S. aureus* which reacted to one or two anticapsular factor sera were tested to determine whether they absorb the converting activity of the anticapsular sera. In these experiments, 3 mg of lyophilized cells of the Smith strain was required to absorb one unit of anti-Smith serum activity, whereas 20 to 80 mg of the other organisms were required to absorb this activity (Table 2, Fig. 3). For the absorption of the converting activities of the anti-58D and anti-41D sera, the minimal amount of organism to absorb this activity with the homologous organism was 5 mg; however, even 80 mg of the heterologous organisms was not capable of absorbing the converting activity from the antisera.

Phage and serotype. *S. aureus* strains whose capsular antigen types could be determined were tested for their patterns of phage and serotypes. Various phage patterns as well as serotypes, by both the Oeding and Pillet methods, were demonstrated by these strains

FIG. 1. Colonial morphologies of the *S. aureus* strain 21 in serum soft agar of different pH values. Tubes 1 through 6 show pH values of 6.0, 6.2, 6.4, 6.6, 6.8, and 7.0, respectively.

FIG. 2. Colonial morphologies of *S. aureus* strain 28 in serum soft agar, pH 6.0. Serum soft agar tubes 1 through 5 contain anti-Smith diffuse, anti-NS58D, anti-NS41D, anti-NS68D, and normal rabbit sera, respectively.

TABLE 1. Capsular type patterns of 21 unencapsulated strains of *S. aureus*

Capsular type	No. of strain	%
A	11	52.4
B	1	4.8
A/B	8	38.0
C	1	4.8
D	0	0
Total	21	100.0

TABLE 2. Amount of lyophilized cells necessary for absorption of one unit of converting activity from anticapsular sera by 11 unencapsulated strains of *S. aureus*

Strain no.	Amount (mg [dry wt])
13	40
28	80
31	40
44	20
45	40
48	20
60	40
69	80
97	20
109	40
111	40
Smith diffuse	3

FIG. 3. Colonial morphologies of the Smith diffuse strain in serum soft agar containing anti-Smith diffuse rabbit serum absorbed with 40 mg of *S. aureus* 28 (1), anti-Smith diffuse serum absorbed with 3 mg of homologous organisms (2), anti-Smith diffuse serum unabsorbed (3), anti-Smith diffuse serum absorbed with 80 mg of *S. aureus* strain 81 (4), and normal rabbit sera (5).

(Table 3). No specificities among these strains could be demonstrated with these experiments.

DISCUSSION

Previously, Ekstedt and Yoshida (1) noted a conversion of antigen production by living organisms of unencapsulated strains of *S. aureus* in an experimental infection in mice, suggesting a transition of the organism from unencapsulated to encapsulated form in vivo. Also, Yoshida et al. (5) described the conversion from a compact to a diffuse type of growth through a pseudo-compact type in SSA from an experimental

abscess formation in mice. These experiments suggest that unencapsulated staphylococcal organisms produce capsules in vivo. Thus, a methodology for investigating the ability of unencapsulated organisms to produce the capsular substance has been investigated. Recently, Yoshida (4) observed that the compact-type *S. aureus* strains, as determined by the regular SSA technique, exhibited a diffuse type of growth in low-pH SSA. The experiments were then designed to investigate the capsular substance in unencapsulated staphylococcal organisms by applying our capsule typing method (3) to such diffuse colonies which developed in the low-pH SSA. The experimental results showed that 21 of 91 unencapsulated strains of *S. aureus* reacted with anticapsular sera, indicating a capsular substance production by these organisms. This was further demonstrated by the absorption of the anticapsular antibody with unencapsulated organisms.

Regarding the relationship of colonial morphologies of strains in SSA, Yoshida and Takahashi (6) recently described a large, round-form, Smith diffuse variant that contained almost one-third of the capsular substance compared with that of the streaming-form variant of the Smith diffuse organisms. It was presumed that a majority of the compact-type *S. aureus* strains had already lost their capsular antigen production activities in these experiments; however, this activity still existed in some strains. The amount of capsular substance in these strains was assumed to be almost one-seventh to one-twentieth of that produced by the Smith diffuse organism, indicating that they could have been Smith-like strains in the past. Regarding the other strains which reacted with the anticapsular types B and C sera, even 80 mg of these organisms was not capable of absorbing the specific antibody activity. These results were anticipated since almost twice the amounts of NS58D and NS41D organisms compared with that of the Smith diffuse organisms were required for the absorption of the specific activities of the homologous antisera.

The strains which showed the bivalent capsule types have not been described thus far. Whether the Smith-type strain possessed cross-antigenic substance with strain NS58D was clarified in a previous paper (3). However, it was made clear that they had both types A and B capsular substances, although these experimental results were not included in this text.

Regarding the protection-inducing antigen, it is not known at this time whether the capsular type-specific material is identical to the protection-inducing substance. However, the protective antibody in rabbit anti-Smith serum was

TABLE 3. Relation between phage types, serotypes, and capsular types of 21 unencapsulated strains of *S. aureus*

Strain no.	Phage pattern	Serotype		Capsular type
		Oeding	Pillet	
11	42E/83A	b, c, h2, mi	II, 69, 10, 11, 14, 16, 17, 18, ck3	A
13	52/52A/80/81	h2, mi, 263-2	11, 17, 18	A/B
25	NT ^a	NT	NT	C
28	29	NT	NT	A
31	53	k2, mi, 263-2	10	A
44	29	hi, h2	I, 17, 18	A
45	29/52A/80/81	h2, mi	I, 17, 18, ck3	A
48	NT	NT	NT	A
52	52A	c, h2	I, 6, 7, 11, 13, 16, 17, 18, ck3	A
60	79/83A/52	b, c, k2, 263-2	13, 18	A
62	52/52A/80/81	h2, mi	10, 17	B
69	29	h2, mi	10, 11, 17	A
73	3B/3C/55	h2, 263-2	NT	A
88	NT	NT	I, 11, 17, ck3	A/B
93	29	NT	I, 11, 17, ck3	A/B
97	NT	c, h2	II, 16, 17, 18, 67, 11	A/B
98	79	c, h2, m, 263-2	III, 6, 19, 11, 16, 17, 18	A
103	NT	NT	NT	A/B
109	6/7/47/53/54/75	a4, bc, h2	6, 10, 11, 13, 16, 18	A/B
110	6/53/83A	c, h2	10, 13, 18, ck3	A
115	6/53/83A	b, c, k2, m	III, 6, 10, 13, ck3	A/B

^a NT, Not tested.

absorbed with a large amount of the original Smith-type strains described in these experiments (unpublished observation). This indicates that they had serological properties similar to those of the Smith diffuse organisms.

The phage and serotypes of these strains represented various patterns similar to those mentioned in our previous report (5), suggesting that these strains were not specific organisms among strains of *S. aureus*. These results support our hypothesis regarding the life cycle of unencapsulation to encapsulation of staphylococcal organisms.

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