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Pseudocompact-Type Growth and Conversion of Growth Types of Strains of *Staphylococcus aureus* In Vitro and In Vivo

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Some strains of *Staphylococcus aureus* grew as compact colonies in Brain Heart Infusion–serum-soft agar but as diffuse colonies in a modified Staphylococcus 110–serum-soft agar. These strains were designated "pseudocompact." Strains showing compact-type colonial morphology in both media were designated "compact," whereas strains showing diffuse-type growth in both media were designated "diffuse." It was observed that the most recently isolated strains of *S. aureus* were of the pseudocompact type, whereas most stock culture strains tested were of the compact type. Using cultures recently isolated from clinical material, it was shown that pseudocompact strains convert to compact-type growth after prolonged incubation. Interconversion of compact, diffuse, and pseudocompact growth forms could be induced in vitro by appropriate cultural conditions, and conversion of growth type was also observed in vivo. Femoral abscesses produced in mice by four different compact-type strains showed conversion to diffuse or pseudocompact-type growth during the course of the infection.

In a previous publication, Yoshida and Ekstedt (12) reported that some strains of *Staphylococcus aureus* which grow as compact-type colonies in Brain Heart Infusion (BHI)–serum-soft agar (SSA) exhibit diffuse type growth in modified Staphylococcus 110-SSA. These strains are more virulent for mice and resemble, in several respects, the Smith diffuse-type organisms. They also observed that with some strains there was a mixture of compact and diffuse growing variants in the same culture. It was considered of interest to study a larger number of strains in an attempt to correlate the type of growth observed under these conditions with the history of the culture. The possibility of inducing the conversion of the growth type by appropriate conditions in vitro or in vivo was considered, and the results of these studies are presented in this paper.

MATERIALS AND METHODS

Organisms. The following strains of *S. aureus* were obtained from R. D. Ekstedt, Department of Microbiology, Northwestern University Medical School, Chicago, Ill.: Smith diffuse OSU, Smith diffuse Koenig, Smith compact Koenig, 334, 258, Foggie, SK4473, and M.D. Thirty strains, NM-1 to NM-30, were randomly selected from stock cultures in our laboratory, and 30 strains, YT-1 to YT-30, were recent isolates from clinical sources. All strains were positive for free coagulase and for clumping factor with the exception of the Smith diffuse OSU and Koenig strains, which were free coagulase-positive but clumping factor-negative.

Media. BHI (Difco) broth or agar was prepared according to the manufacturer's directions. The modified Staphylococcus 110 medium was prepared as follows. A 10-g amount of peptone (Difco) and 2.5 g of yeast extract (BBL) were dissolved in 50 ml of 3% NaCl in boiling water, transferred to a dialysis bag, and dialyzed against 1,000 ml of 3% NaCl for 4 days at 4 C. To 850 ml of the dialysate we added 5 g of K_2HPO_4, 50 ml of 1 M phosphate buffer (*p*H 7.6), and either 1.5 or 20 g of agar (Difco) to prepare soft or regular agar, respectively. The media were autoclaved at 115 C for 10 min. A 10-g amount of mannite (Wako Chemical Industries, Osaka) and 2 g of lactose (Wako Chemical Industries, Osaka) were dissolved in 100 ml of 3% NaCl and sterilized by membrane filtration (Millipore Corp., Bedford, Mass.; pore size, 0.45 nm). These two solutions were then combined.

SSA technique. SSA was prepared according to Finkelstein and Sulkin (2), with BHI or modified Staphylococcus 110 medium and pooled normal rabbit serum. Colonial morphology of the inoculated strains was observed after 24 hr of incubation at 37 C. Those strains of *S. aureus* which showed a diffuse-type colonial morphology in both media were designated diffuse, those strains growing as compact-type colonies in both media were designated compact, and those strains which grew as compact colonies in BHI-

SSA but as diuffse colonies in modified Staphylococcus 110-SSA were designated pseudocompact. The Smith diffuse OSU and the NM-3 strains of *S. aureus* were used as typical diffuse- and compact-type strains.

Conversion of the growth types in vitro. For isolation of colonial variants, tubes of modified Staphylococcus 110-SSA containing less than 10 compact colonies were incubated at 37 C for 18 to 20 hr and then were transferred to an incubator at 30 C for an additional 2 weeks. During this prolonged incubation, part of the compact colony which had grown up during the incubation at 37 C began to stream down, and the growth reached the bottom of the tube in about 2 weeks. Compact and diffuse portions of single colonies were transferred by using sterile capillary pipettes, and their colonial morphology in modified Staphylococcus 110 and BHI-SSA was determined.

Conversion of the growth types in vivo. Femoral abscesses were produced in mice with the 334 compact, Foggie, SK4473, and 258 strains of *S. aureus* by the procedure of Okonogi et al. (8). The colonial morphology of the organisms cultured from the abscesses was determined by using BHI and modified Staphylococcus 110-SSA. Clinical manifestations of the abscesses were recorded for correlation with the cultural characteristics of the organisms isolated during the course of the infection.

RESULTS

Thirty stock cultures of *S. aureus* and the same number of fresh isolates were studied to determine their colonial morphology in BHI and modified Staphylococcus 110-SSA. The results of this survey are shown in Table 1. The majority (73.3%) of the fresh isolates showed a pseudocompact-type growth, whereas the majority (80.0%) of the stock culture strains exhibited a compact-type colonial morphology in both media. Figure 1 shows typical diffuse, compact, and pseudocompact strains as determined by their colonial morphology in BHI and modified Staphylococcus 110-SSA.

Conversion of growth types in vitro. These results suggested that pseudocompact-type organisms converted to compact type during subculture on routine media in the laboratory. To test this hypothesis, nine pseudocompact strains were subcultured daily for 10 days on both BHI and modified Staphylococcus 110 agar slants. Under these conditions, there was no significant conversion to the compact-type growth (Table 2). If, however, subculture was continued at 2-week intervals, a majority of the strains converted to the compact-type colonial morphology, and, after 2 months at 4 C, all of the strains had become compact when tested in BHI and modified Staphylococcus 110-SSA. It also appeared that, during the conversion of diffuse- or pseudocompact-type organisms to the compact form, there was an intermediate stage in which the colonies in SSA showed a small amount of streaming. This form is designated "short tail-type growth" in Table 2.

In an attempt to isolate organisms exhibiting different colonial morphology from a single colony, the 334 compact strain was inoculated into modified Staphylococcus 110-SSA and allowed to incubate at 37 C for 18 to 20 hr and at 30 C for 2 weeks. During this period of incuba-

TABLE 1. *Growth types of 30 stock cultures of S. aureus and 30 fresh isolates*

Growth type	Stock cultures	Fresh isolates
Diffuse	1 (3.3)[a]	3 (10.0)
Compact	24 (80.0)	5 (16.7)
Pseudocompact	5 (16.7)	22 (73.3)
Total	30 (100.0)	30 (100.0)

[a] Numbers in parentheses represent percentages.

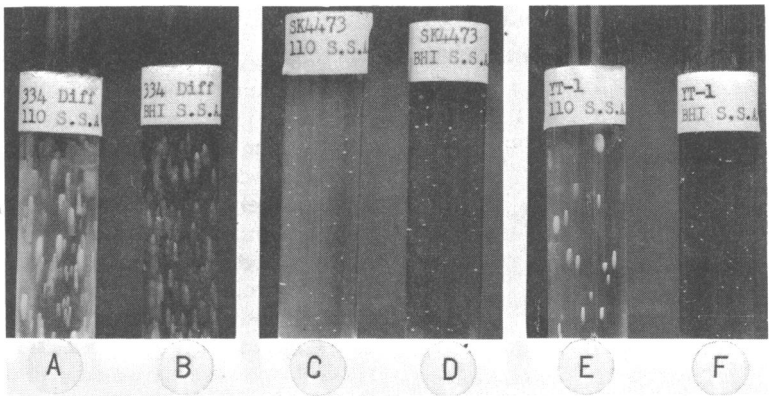

FIG. 1. *Growth types of three strains in SSA. A, C, and E: 334 diffuse variant, SK4473, and YT-1 strains grown in SSA prepared with modified 110 media. B, D, and F: 334 diffuse variant, SK4473, and YT-1 strains grown in SSA prepared with BHI media.*

TABLE 2. *Conversion of nine strains of the pseudocompact type during subculture on BHI and modified Staphylococcus 110 slant agar*[a]

Incubation time	Culture age	BHI									Modified Staphylococcus 110								
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
	days																		
1 day	1	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	2																		
	3																		
	4																		
	5																		
	6																		
	7																		
	8																		
	9																		
	10	P	S	P	P	P	P	P	S	P	P	P	P	P	P	P	P	S	P
2 weeks	11																		
	12	S	C	C	C	P	P	S	C	C	P	S	P	P	P	P	P	S	P
	13	C	C	C	C	P	P	C	C	C	P	C	S	S	P	P	P	C	S
2 months	14[b]	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

[a] Abbreviations: P, pseudocompact type; C, compact type; S, short tail-type growth in modified staphylococcus 110-SSA.

[b] Subcultures of original strains after 2 months of incubation at 4 C.

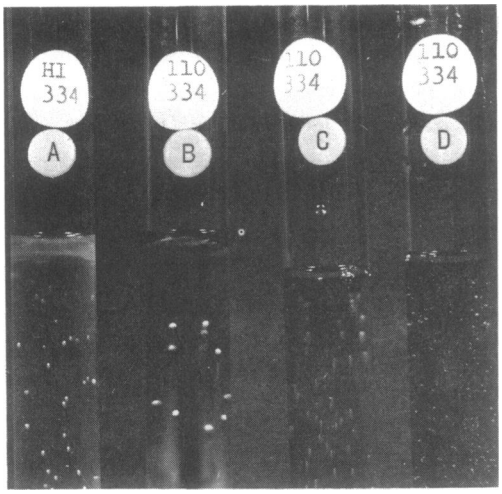

FIG. 2. *A, overnight culture of original 334 compact strain grown in BHI-SSA. B, 2-week culture of 334 strain grown in modified Staphylococcus 110-SSA. C and D, overnight culture of variants isolated from a compact and streaming portion of a single colony in B grown in modified Staphylococcus 110-SSA.*

tion, a portion of the colony began streaming down from the original compact-type growth seen after incubation at 37 C. Figure 2A shows the compact colonial morphology of this strain in BHI-SSA, and Fig. 2B shows the culture after 2 weeks in modified Staphylococcus 110-SSA. Subculture of the streaming and compact portion of a single colony at this time gave rise to diffuse- and compact-type growth as shown in Fig. 2C and D. Similar experiments with the 258 strain of *S. aureus* gave identical results.

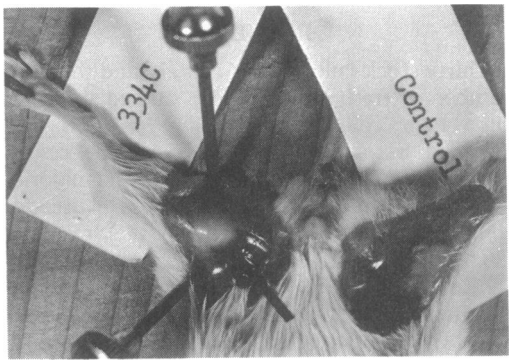

FIG. 3. *Femoral abscess produced 2 days after injection with 10^6 organisms of the 334 compact variant strain.*

Conversion of growth types in vivo. Femoral abscesses were produced in mice by injecting approximately 10^6 organisms of four different compact strains of *S. aureus*. Figure 3 shows a typical abscess 48 hr after injection of the 334 compact strain. Cultures taken at daily intervals from the abscesses were studied for their colonial morphology in BHI and modified Staphylococcus 110-SSA and were correlated with the intensity of the infection. Table 3 shows the results of these experiments, and Fig. 4 shows the colonial morphology of the 258 strain isolated at 2 and 4

TABLE 3. *Conversion of growth type of S. aureus strains from experimentally produced abscesses in SSA*[a]

Time after injection	Strain							
	334		258		Foggie		SK4473	
	Abscess formation	Growth type	Abscess formation	Growth type	Abscess formation	Growth type	Abscess formation	Growth type
days								
1	+	D	±	−	−	−	±	C
2	++	D	++	D	++	C	+	C
3	+++	D + C	+++	P	+++	P	++	P
4	+++	C	+++	P	+++	P	+++	P
5	+++	C	+++	P	+++	P	++	P + C
6	+++	ND	+++	P	+++	P	++	C
7	++	ND	++	P	+++	P	++[b]	C
8	++	C	+	P	+++	P	+[b]	C

[a] Strains 334, 258, Foggie, and SK4473 were used for abscess production and were of the compact type. Abbreviations: C, compact-type growth; D, diffuse-type growth; P, pseudocompact-type growth; ND, not done. Plus and minus signs indicate intensity of the abscess formation.

[b] Fistule formation.

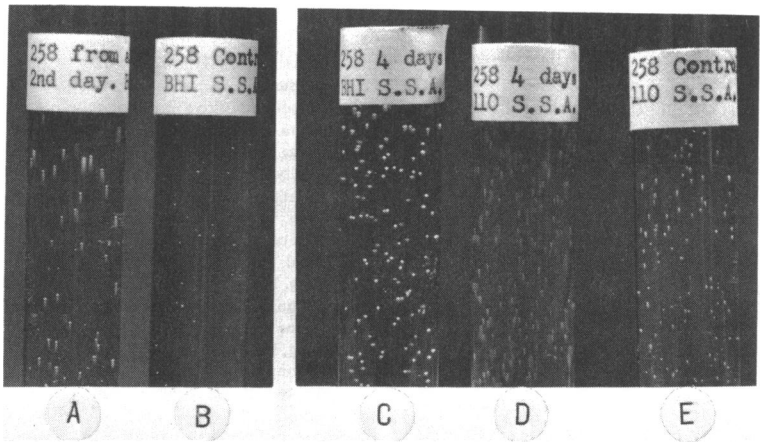

FIG. 4. *A and B show strain 258 isolated from an abscess, 2 days after injection, and a control grown in BHI-SSA. C and D show strain 258 isolated from abscess, 9 days after injection, grown in BHI and modified Staphylococcus 110-SSA. E shows a control grown in modified Staphylococcus 110-SSA.*

days from an abscess. At 2 days, the organisms showed diffuse-type growth, whereas at 4 days they would be considered pseudocompact, since they grew as diffuse colonies in modified Staphylococcus 110-SSA but as compact colonies in BHI-SSA.

The number of organisms isolated from the pus at the height of the infection was approximately 10^8 per ml. The organisms were primarily within phagocytic cells and many appeared to be surrounded by a "halo," suggesting encapsulation. Barber (1) described a similar phenomenon. The time of conversion of the organisms from compact to diffuse or pseudocompact varied depending on the strain of *S. aureus* used but, in general, correlated reasonably well with the intensity and course of the infection. The pseudocompact-type growth appeared primarily at the beginning of abscess formation or during the period of recovery, depending upon the strain used.

DISCUSSION

Encapsulation of *S. aureus* has been studied in the Smith strain by Koenig and co-workers (3, 4, 5) and in the "wound strains" described by Wiley (10) and Wiley and Maverakis (11). Recently, Yoshida and Ekstedt (12) reported that some strains of *S. aureus* show compact-type growth in BHI-SSA but diffuse-type growth in modified Staphylococcus 110-SSA. These strains are more virulent for mice and synthesize large amounts of a polysaccharide slime layer material which can

be seen surrounding the organisms as a "capsule." In this paper, such strains have been designated pseudocompact. It is of considerable interest that the majority of strains of *S. aureus* isolated recently from an infectious process exhibit this dual type morphology when cultured under appropriate conditions, whereas strains which have been maintained in stock culture for longer periods of time are usually of the compact-type colonial morphology when tested in SSA prepared either with BHI or with modified Staphylococcus 110 media. It appears that the nonencapsulated organisms are selected for under routine culture conditions in the laboratory, whereas the use of a medium such as modified Staphylococcus 110 favors the maintenance of the organisms in the phase capable of synthesizing slime layer or capsular material. It has been found (*unpublished data*) that five of eight stock strains can be converted from the compact-type growth to the diffuse or pseudocompact type by culture in modified Staphylococcus 110-SSA. The genetic or nutritional mechanism of this conversion remains to be clarified.

The observations on the conversion of compact strains of *S. aureus* to diffuse or pseudocompact strains during an infectious process are also of considerable interest. They add further experimental evidence to the suggestions of Rogers (9) and Koenig et al. (6) that strains of *S. aureus*, when growing in the tissues of an infected animal, elaborate antiphagocytic surface antigens similar to the "Smith Surface Antigen" described by Morse (7) but lose this capacity when cultured in artificial media. Koenig and Melly (4) have also reported a rapid decrease in mouse virulence of recently isolated strains after culture in the laboratory in routine culture media.

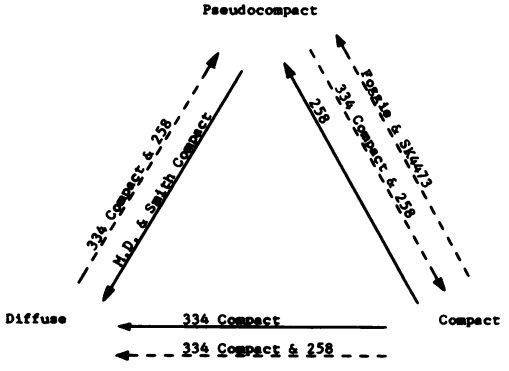

FIG. 5. *Conversion of growth types, in SSA, of six strains of S. aureus in vitro and in vivo. Solid and dashed lines indicate course of the conversion of growth types of the organisms in vitro and in vivo, respectively.*

Figure 5 shows a summary of the interconversion of several strains of staphylococci to diffuse, compact, and pseudocompact forms both in vitro and in vivo. The mechanism of this phenomenon and its importance in experimental and natural staphylococcal infections are being investigated further in this laboratory.

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