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NOTES

Occurrence of Protein A in *Staphylococcus aureus* and Closely Related *Staphylococcus* Species

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All but 1 of 143 strains of *Staphylococcus aureus* were positive for protein A, whereas all 34 strains of *Staphylococcus hyicus* and 123 of 127 strains of *Staphylococcus intermedius* were devoid of this cell wall component.

The presence of protein A on the cell wall and the production of coagulases and thermonucleases are presently accepted criteria for the identification of *Staphylococcus aureus* (1). These criteria are based for the most part on studies of strains isolated from humans (2, 5, 10). Recent studies with isolates from various nonhuman animals showed heterogeneity among groups of staphylococci closely related to *S. aureus*. Hájek and Marsálek (9) described four biotypes or ecotypes of *S. aureus*: biotype A (human origin), B (poultry and swine), C (cow), and D (hare). A group of homologous staphylococci isolated from dogs, pigeons, minks, and foxes has been reclassified to a new species, *Staphylococcus intermedius* (7). Another group of homogeneous staphylococci isolated from swine, cows, and poultry has been classified to another new species, *Staphylococcus hyicus* (3). These investigators reported that the production of coagulases and thermonucleases are not unique features of *S. aureus* but are shared by *S. intermedius* and *S. hyicus*.

There is a paucity of data on the occurrence of protein A among staphylococci of nonhuman animal origin. Intensive studies of staphylococci from Czechoslovakia indicated that *S. intermedius* strains are devoid of protein A, whereas the frequency of occurrence among *S. aureus* originating in nonhuman animals varied from none among strains of biotype D to 40% among biotype C (9). We examined the occurrence of protein A among strains of *S. hyicus* and reassessed its occurrence among strains of *S. aureus* and *S. intermedius*. The method used for the detection of protein A on the cell surface was the fluorescent-antibody technique which was originally designed to detect staphylococcal enterotoxins (6). This is a simple and sensitive technique in which fluorescein isothiocyanate-labeled immunoglobulin G binds to protein A-positive cells nonspecifically. A similar method was described by Lind (12). Young cultures were examined to detect cell-bound protein A before a large portion was released into the growth medium (4); fluorescent-antibody technique would be ineffective for those occasional strains of *S. aureus* which release protein A rapidly into the medium, leaving undetectable levels on the cell wall (5). Accordingly, each culture was grown in brain heart infusion broth (Difco) for 18 h at 37°C and examined at least twice. Under fluorescent microscopy, a fluorescent ring is discerned around each protein A-positive cell. *S. aureus* strain Wood 46 was used as a negative control. This technique enabled the detection of protein A among enterotoxinogenic cocci of uncertain identity (13).

The staphylococcal strains which were included in the study are summarized in Table 1. The strains were described in a previous study (11) except for the 67 enterotoxinogenic strains and the 30 nonpigmented strains isolated from cow milk. The latter were identified as *S. hyicus* (3): all were positive for thermonuclease and hyaluronidase but were nonhemolytic and negative for clumping factor; only 17 were positive for coagulase. Two strains from cows (United States) and two from poultry (Japan), originally identified as *S. epidermidis* biotype 2, had been identified as *S. hyicus* by Devriese et al. (3). By using a battery of tests suggested by Hájek and Marsálek (8), all of the enterotoxinogenic strains

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TABLE 1. *Origins of strains of Staphylococcus spp. which were examined*

Organism	Biotype	No. of strains	Origin	Country	Source
S. aureus	A	5	Human	Czechoslovakia	V. Hájek
	A	5	Dog	United States	S. S. Jang
	A	67	Food/human[a]	United States	C. Geningeorgis
	B	6	Poultry	Japan	G. Sato
	C	5	Cow	Czechoslovakia	V. Hájek
	C	50	Cow	United States	R. V. F. Lachica
	D	5	Hare	Czechoslovakia	V. Hájek
S. intermedius		5	Dog	Czechoslovakia	V. Hájek
		5	Pigeon	Czechoslovakia	V. Hájek
		117	Dog	United States	S. S. Jang
S. hyicus		2	Cow	United States	R. W. Brown
		2	Poultry	Japan	G. Sato
		30	Cow	United States	R. V. F. Lachica

[a] Known enterotoxinogenic strains.

which we studied were identified as *S. aureus* biotype A (data not shown). The *S. aureus* strains were also differentiated from the strains of *S. intermedius* and *S. hyicus* by the thermonuclease seroinhibition test (11).

Strains of both human and nonhuman animal origin of *S. aureus* were observed to contain protein A except for one strain of biotype D. The intensity of fluorescence varied among the strains. A similar observation, indicating variation in the amount of protein A coating staphylococci, was reported by Lind (12). On the other hand, the strains of *S. hyicus* and *S. intermedius* were devoid of the protein A cell wall component with the exception of four strains of *S. intermedius*.

Our observation of a high frequency of occurrence of protein A among strains of *S. aureus* of nonhuman animal origin differs markedly from the low frequency found by Hájek and Marsálek (9) among similar strains of *S. aureus*. Possibly, this reflects the higher sensitivity of fluorescent-antibody technique as compared with the double gel diffusion technique (14) used in the study of strains from Czechoslovakia (9). Strain variation by geographic origin as an explanation was ruled out since we observed that 9 out of 10 strains of *S. aureus* of nonhuman animal origin from Czechoslovakia were protein A positive. The absence of protein A among 10 strains of *S. intermedius* from Czechoslovakia was substantiated in the present study.

Our results reaffirm the view that the presence of protein A is a characteristic of *S. aureus* (1). On the other hand, the production of coagulases and thermonucleases cannot be regarded as unique to *S. aureus*.

LITERATURE CITED

1. **Baird-Parker, A. C.** 1974. Genus II. *Staphylococcus* Rosenbach, p. 483-489. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
2. **Barry, A. L., R. V. F. Lachica, and F. D. Atchison.** 1973. Identification of *Staphylococcus aureus* by simultaneous use of tube coagulase and thermonuclease tests. Appl. Microbiol. **25**:496-497.
3. **DeVriese, L. A., V. Hájek, P. Oeding, S. A. Meyer, and K. H. Schleifer.** 1978. *Staphylococcus hyicus* (Sompolinsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogens* subsp. nov. Int. J. Syst. Bacteriol. **28**:482-490.
4. **Forsgren, A.** 1969. Protein A from *Staphylococcus aureus* VIII. Production of protein A by bacterial and L-forms of *S. aureus*. Acta Pathol. Microbiol. Scand. **75**: 481-490.
5. **Forsgren, A.** 1970. Significance of protein A production by staphylococci. Infect. Immun. **2**:672-673.
6. **Genigeorgis, C., and W. W. Sadler.** 1966. Immunofluorescent detection of stappylococcal enterotoxin B. I. Detection in culture media. J. Food Sci. **31**:441-449.
7. **Hájek, V.** 1976. *Staphylococcus intermedius*, a new species isolated from animals. Int. J. Syst. Bacteriol. **26**: 401-408.
8. **Hájek, V., and E. Marsálek.** 1971. The differentiation of pathogenic staphylococci and a suggestion for their taxonomic classification. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A. **217**: 176-186.
9. **Hájek, V., and E. Marsálek.** 1976. Evaluation of classificatory criteria for staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl., p. 11-21.
10. **Kronvall, G., J. H. Dossett, P. G. Quie, and R. C. Williams, Jr.** 1971. Occurrence of protein A in staphylococcal strains: quantitative aspects and correlation to antigenic and bacteriophage types. Infect. Immun. **3**: 10-15.
11. **Lachica, R. V. F., S. S. Jang, and P. D. Hoeprich.** 1979. Thermonuclease seroinhibition test for distinguishing *Staphylococcus aureus* and other coagulase-positive staphylococci. J. Clin. Microbiol. **9**:141-143.
12. **Lind, I.** 1968. Non-specific absorption of FITC-labelled serum globulins to *Staphylococcus aureus*. Acta Pathol. Microbiol. Scand. **73**:613-629.
13. **Lotter, L. P., and C. A. Genigeorgis.** 1975. Deoxyribonucleic acid base composition and biochemical properties of certain coagulase-negative enterotoxigenic cocci. Appl. Microbiol. **29**:152-158.
14. **Oeding, P., A. Grov, and B. Myklestad.** 1974. Immunochemical studies on antigen preparation from *Staphylococcus aureus*. Precipitating and erythrocyte-sensitizing properties of protein A (antigen A) and related substances. Acta Pathol. Microbiol. Scand. **62**:117-127.