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Incidence of the Highly Conserved *fib* Gene and Expression of the Fibrinogen-Binding (Fib) Protein among Clinical Isolates of *Staphylococcus aureus*

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We have recently described a 19-kDa fibrinogen-binding protein, termed Fib, which is secreted into the extracellular medium by *Staphylococcus aureus*. In this study, the presence of the Fib protein and the *fib* gene among clinical isolates of *S. aureus* and among other staphylococcal species known to interact with fibrinogen was investigated. This task was pursued at the DNA, mRNA, and protein levels. It was found that the *fib* gene was unique to *S. aureus* and highly conserved at the nucleotide level. The Fib protein was produced by all *S. aureus* strains investigated but was not detected in all bovine mastitis strains, because of proteolytic degradation by simultaneously produced staphylococcal proteases. It was concluded that the *fib* gene was unique to *S. aureus* and that it could be used in the identification of *S. aureus*.

Despite the development of sophisticated antimicrobial agents, *Staphylococcus aureus* continues to be one of the world's major pathogens, and the emergence of methicillin-resistant strains is a severe problem in hospitals (19). This bacterium produces a large number of extracellular proteins, many of which are considered to be important virulence factors (9). For example, the ability of several *S. aureus* cell surface proteins to mediate the binding to extracellular matrix proteins is believed to contribute to the colonization of host tissue (14). *S. aureus* interacts in several ways with fibrinogen through both secreted and cell surface proteins, but the clinical significance of this is not known. Coagulase induces the polymerization of fibrinogen into fibrin by binding and activating prothrombin (12). This type of coagulation can also be mediated by staphylococcal metalloprotease (33). Coagulase, which also binds fibrinogen (2), was earlier believed to be an important virulence factor, but it has recently been shown that coagulase is not important for the virulence of *S. aureus* in experimental infections in mice (27). In a similar manner, staphylokinase binds and activates plasminogen and thereby induces the proteolytic degradation of fibrin. However, staphylokinase does not bind fibrin (24). It is well known that *S. aureus* clumps in the presence of fibrinogen (16) and binds to fibrinogen-coated surfaces (6, 21). This ability has been suggested to facilitate staphylococcal colonization of intravascular catheters (32) and of endothelial cells (7). On the other hand, binding to fibrinogen can also enhance neutrophil activity against bacteria (13). It has been suggested that a cell surface protein is responsible for this binding (16). Recently, an *S. aureus* gene which confers fibrinogen-binding and clumping activity when introduced into *S. aureus* clumping factor-negative mutants and *Escherichia coli* was cloned (26). However, this protein has not been demonstrated to be exposed on the *S. aureus* cell surface. In addition, *S. aureus* produces at least three different fibrinogen-binding proteins that are secreted into the culture medium (3).

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There are other staphylococcal species that interact with fibrinogen. For example, *Staphylococcus intermedius*, which is found mainly in dogs, exerts coagulase activity (19) and *Staphylococcus hyicus*, which is found mainly in pigs, has been described as coagulase variable (23). In addition, *Staphylococcus lugdunensis* clumps in the presence of fibrinogen (19).

We have previously purified and sequenced a fibrinogen-binding protein with a molecular mass of 19 kDa, which is secreted by *S. aureus* (4). This protein, termed Fib, shows sequence homologies with the C-terminal domain of coagulase from *S. aureus*. This domain, which is conserved among *S. aureus* coagulases of different serotypes, consists of 27 amino acids repeated five to eight times (17, 18, 27). The C-terminal domain has been implicated in fibrinogen binding (25). The homologous region of the Fib protein consists of a 22-amino-acid domain which is repeated twice.

The aim of this study was to investigate the presence of the Fib protein and its corresponding gene among *S. aureus* strains and other staphylococcal species interacting with fibrinogen.

MATERIALS AND METHODS

Bacterial strains, cloning vectors, and culture conditions. The staphylococcal strains used are indicated in Table 1. Staphylococcal strains were grown overnight in brain heart infusion medium (Difco Laboratories, Detroit, Mich.). After centrifugation, the bacterial pellet was resuspended in 5 culture volumes of freshly prepared brain heart infusion and grown at 37°C for 3 h with constant shaking. Staphylococci producing large amounts of proteases were grown in brain heart infusion to which 1 mM phenylmethylsulfonyl fluoride was added every hour and 10 mM *N*-ethylmaleimide and 10 mM EDTA were added after 2 h. *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.), used with the cloning vector pGEM-T (Promega Co., Madison, Wis.), was grown in Luria-Bertani broth.

Characterization of strains. Tests for nuclease were performed on DNase test agar (Difco). Coagulase tests were performed in Coagulase Plasma (Difco). The tests were read after 1, 2, 3, 4, 6, and 24 h, and any indication of coagulation but not precipitation was considered positive (19). Typing of coagulase-negative staphylococci (CoNS) was performed with API-Staph (Bio Mérieux SA, Marcy-l'Etoile, France). Protease production was assessed by the casein agar method (1).

Affinity chromatography. Staphylococcal Fib protein was affinity purified essentially as described previously (2). Briefly, fibrinogen-Sepharose was prepared by coupling human fibrinogen (IMCO, Stockholm, Sweden) to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden), using the procedure recommended by the manufacturer. The Sepharose was equilibrated with phosphate-buffered saline (PBS; 145 mM NaCl, 10 mM phosphate, pH 7.4) containing

TABLE 1. Bacterial strains

Organism (source of isolates)	Origin	No. of isolates	Result of test for[a]:		Source
			DNase	Coagulase	
S. aureus FDA486	Human	1	+	+	Clontech[b]
S. aureus Newman	Human	1	+	+	M. Lindberg[c]
S. aureus (wound)	Human	39	+	+	This study[d]
S. epidermidis (wound)	Human	3	−	−	This study
CoNS (wound)	Human	6	−	−	This study
S. aureus (mastitis)	Bovine	19	ND	+	SVA[e]
S. aureus (mastitis)	Bovine	8	ND	+	R. Hummel[f]
S. hyicus	Porcine	5	ND	+/−	H. Wegener[g]
S. hyicus	Porcine	5	ND	−	H. Wegener
S. lugdunensis	Human	10	ND	−	Å. Ljungh[h]
S. intermedius	Canine	7	ND	+	M. Thore[i]
S. intermedius	Equine	2	ND	+	M. Thore
S. intermedius	Human	1	ND	+/−	M. Thore

[a] +, positive result; −, negative result; +/−, weak reaction; ND, not determined.
[b] Palo Alto, Calif. (purchased).
[c] Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[d] Clinical isolates from the Department of Clinical Bacteriology, Huddinge Hospital, Huddinge, Sweden.
[e] National Veterinary Institute (Statens Veterinärmedicinska Anstalt), Uppsala, Sweden.
[f] Veterinär- und Lebensmittelinstitut, Jena, Germany.
[g] National Veterinary Laboratory, Copenhagen, Denmark.
[h] Department of Medical Microbiology, Lund University, Lund, Sweden.
[i] Purchased from Clinical Microbiology Laboratory, Centrallasarettet, Västerås, Sweden.

0.05% Nonidet P-40 (NP-40). Staphylococcal culture supernatants supplemented with 1 mM phenylmethylsulfonyl fluoride, 10 mM *N*-ethylmaleimide, 10 mM EDTA, and 0.05% NP-40 were loaded onto the column. The resin was washed four times in PBS containing 0.05% NP-40. The absorbed material was eluted with 0.7% acetic acid containing 0.05% NP-40. Alternatively, elution was performed by boiling the fibrinogen-Sepharose, which contained absorbed material, in running buffer. The samples were subsequently loaded onto sodium dodecyl sulfate (SDS)-polyacrylamide gels.

SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting. SDS-PAGE and subsequent diffusion blotting were performed with the Phast System (Pharmacia) as described previously (2). Nitrocellulose filters were incubated for 2 h at room temperature with rabbit anti-Fib protein antiserum, which is directed against the Fib protein from *S. aureus* Newman (3), and which was diluted 1:1,000 in PBS supplemented with 0.05% Tween 20. Alkaline phosphatase-conjugated swine anti-rabbit immunoglobulin G antibodies (Dakopatts, Glostrup, Denmark) were diluted 1:1,000 and incubated with the filters for 1 h. The alkaline phosphatase reaction was developed in 100 mM Tris hydrochloride (pH 8.0) containing 10 mM $MgCl_2$, 0.02 mg of α-naphtylphosphate (E. Merck AG, Darmstadt, Germany) per ml, and 0.02 mg of Fast Blue (Merck) per ml for 10 to 20 min.

PCR amplification of the *fib* gene. Two oligonucleotide primers (see Fig. 1), S.fib, with the sequence 5′-GCGAAGGATACGGTCCAAGAGA-3′, and M.fib, with the sequence 5′-CAATTCGCTCTTGTAAGACCATT-3′, were synthesized on the basis of the *fib* gene sequences from *S. aureus* FDA486 and *S. aureus* Newman (4). The predicted melting temperatures are 68 and 64°C, respectively, according to the 4°C × (G+C) + 2°C × (A+T) method (31). The *fib* gene was amplified from whole bacterial cells in a 10-μl volume. Each reaction mixture contained 5 pmol of each primer, 0.2 mM deoxynucleoside triphosphates, 0.5 U of *Taq* polymerase (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) or AmpliTaq DNA polymerase (Perkin-Elmer Cetus Inc., Norwalk, Conn.), and the incubation buffer supplied with the enzymes. PCR amplification by 30 repeated cycles was performed on a DNA Thermal Cycler 9600 (Perkin-Elmer) with a thermal step program that included denaturation at 94°C for 10 s, annealing at 67°C for 20 s, and primer extension at 72°C for 10 s. Amplified material was analyzed on 1.5% agarose gels.

Northern (RNA) blots. Total RNA was prepared according to the method of Janzon et al. (15). RNA samples were separated on 1% agarose gels containing 15% formaldehyde and subsequently blotted onto Hybond membranes (Amersham International plc, Little Chalfont, United Kingdom) in 20× SSC (3 M NaCl, 0.3 M sodium citrate). The filters were prehybridized overnight at 42°C in a buffer containing 5× SSC, 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 50% formamide, 3× Denhardt's solution, 0.5 mg of baker's yeast RNA per ml, and 0.5% SDS. A 378-bp fragment, generated by PCR with the S.fib and M.fib primers, was labelled with [^{32}P]dCTP, using the Megaprime labelling system (Amersham). The probe was incubated with the filters in the same hybridization buffer overnight. The filters were washed twice for 15 min each time in 2× SSC and for 20 min in 1× SSC. All incubations were at 42°C. The filters were analyzed by using autoradiography and in a Bio-Imaging Analyzer Bas2000 (Fuji Photo Film Co., Ltd., Japan).

DNA sequencing and sequence analysis. *fib* gene fragments were amplified, using the F.fib primer with the sequence 5′-GTAAGATAATAATTTGGAG GAT-3′ and the M.fib primer described above. PCR-amplified fragments were ligated into the pGEM-T vector. DNA sequences were determined using fluorescent dye-labelled dideoxy-nucleotide terminators and cycle sequencing kits (Applied Biosystems Inc., Foster City, Calif.). Computer-assisted analysis of DNA sequences was performed with the Genetics Computer Group software package (10).

RESULTS

Characterization of staphylococcal strains. Clinical isolates from human wound infections were tested for nuclease and coagulase production (Table 1). Thirty-nine of the 48 isolates were positive for nuclease and coagulase production and were subsequently regarded as *S. aureus*. Nine isolates that failed to produce nuclease or coagulase were defined as CoNS. Three of these were typed as *Staphylococcus epidermidis* by API-Staph. In addition, all strains from other sources were tested for coagulase production. All *S. aureus* bovine mastitis strains and all *S. intermedius* strains were positive by coagulase tests. The *S. hyicus* strains either were negative or gave weak coagulation results.

PCR analysis of the *fib* gene from staphylococcal strains. A PCR analysis method was developed to determine the prevalence of the *fib* gene. In this method, the *fib* gene was amplified by using the S.fib and M.fib primers (Fig. 1). When the annealing temperature was set at 67°C, positive reactions showing a 378-bp band were recorded in 38 (97%) of the human *S. aureus* isolates and in 27 (100%) of the bovine *S. aureus* isolates (Table 2). Figure 2 shows PCR results for a selection of *S. aureus* strains. However, 19 (49%) of the human isolates showed weaker but distinct bands (Table 2 and Fig. 2, lanes 2, 3, and 5). These bands may be an indication of a limited sequence difference within the primer region. Further supporting this theory, the number of positive isolates increased to 39 (100% of the human *S. aureus* isolates), when the annealing temperature was lowered to 65°C (data not shown). No PCR product could be detected regardless of the annealing temperature, in samples from the human CoNS, *S. hyicus*, *S. lugdunen-*

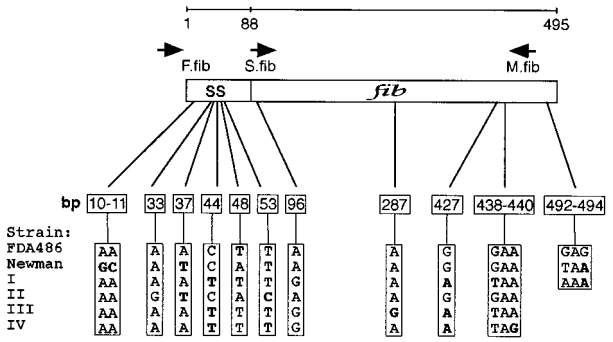

FIG. 1. Schematic model of the cloned *fib* gene. The size, in base pairs, of the DNA is indicated. SS, signal sequence; *fib*, structural gene for the mature Fib protein. The primers used for PCR amplification are indicated by arrows. I to IV, clinical isolates of *S. aureus*. Lines extending down from SS and *fib* indicate the positions at which the other *fib* genes differ from the *fib* gene from strain FDA486. The precise locations of these differences are presented in boxes prefaced by bp. The differences noted in the DNA sequences are described in the boxes below, and the nucleotide changes giving rise to changes in amino acids are shown in boldface type. The DNA sequences for the last three strains were only determined up to nucleotide 442, which comprises the 3′ end of the M.fib primer.

sis, *S. intermedius* strains included in this study. Thus, the *fib* gene was shown to be present only in *S. aureus*.

Isolation of Fib protein from staphylococcal strains. To evaluate whether the *fib* gene, which was shown to be present in all investigated *S. aureus* strains, was also expressed in these strains, the Fib protein was purified from *S. aureus* cultures. The Fib protein was isolated by affinity purification on fibrinogen-Sepharose from 10-ml cultures grown to late exponential phase. The resulting protein preparations were analyzed on immunoblots. Figure 3 shows immunoblot results for the same selection of *S. aureus* strains as shown in Fig. 2. In addition to the 19-kDa Fib protein band, breakdown products as well as dimers of the Fib protein can be seen. Higher-molecular-weight bands are probably due to the presence of protein A and coagulase. All human *S. aureus* isolates expressed the Fib protein. Among the bovine *S. aureus* mastitis isolates only 14 (52%) expressed Fib protein in this assay. However, when these strains were grown in the presence of protease inhibitors, the Fib protein could be detected in all the bovine samples (data not shown), suggesting that the Fib protein actually was produced by these strains but was degraded by simultaneously produced proteolytic enzymes. Protease production by mastitis strains was assessed by the ability to form a characteristic precipitate on casein agar. There was a correlation between

TABLE 2. Presence of *fib* gene and production of Fib protein by staphylococcal strains

Staphylococcal species (origin of isolates)	No. of isolates	No. of isolates with PCR result		No. of isolates expressing Fib protein
		+++[a]	+[b]	
S. aureus (human)	39	19	19	39
CoNS (human)	9	0	0	0
S. aureus (bovine)	27	27	0	27[c]
S. hyicus (porcine)	10	0	0	0
S. lugdunensis (human)	10	0	0	0
S. intermedius (mixed)[d]	10	0	0	0

[a] Strong bands at 67°C, annealing temperature.
[b] Weak bands at 67°C, annealing temperature.
[c] Thirteen strains were positive only after the addition of protease inhibitors.
[d] Seven canine, two equine, and one human.

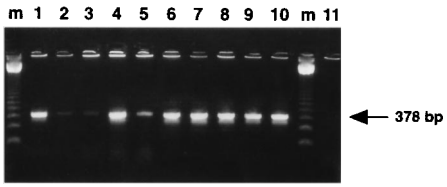

FIG. 2. PCR amplification (at 67°C, annealing temperature) of a 378-bp fragment, using the S.fib and M.fib primers, separated by electrophoresis in an 1.5% agarose gel. Lanes: m, 123-bp ladder; 1 to 5, human *S. aureus* isolates; 6 to 8, bovine *S. aureus* isolates; 9, bovine *S. aureus* 2128; 10, bovine *S. aureus* 2114; 11, *S. intermedius* isolate.

absence of Fib protein and production of protease and vice versa ($P < 0.001$; data not shown). In Fig. 4, two bovine *S. aureus* mastitis strains are compared: strain 2128, which was negative for protease on casein agar and produced large amounts of Fib protein (Fig. 4, lane 2), and strain 2114, which gave a large precipitate on casein agar but was negative for Fib protein in the immunoblot assay (Fig. 4, lane 5). When the two cell-free culture supernatants were mixed, the Fib protein produced by strain 2128 was degraded by proteases produced by strain 2114 (Fig. 4, lane 4). Adding protease inhibitors to the culture supernatants before mixing them gave a good yield of Fib protein (Fig. 4, lane 3). To obtain Fib protein from strain 2114, the presence of the serine protease inhibitor phenylmethylsulfonyl fluoride, added every hour during growth, was essential. Moreover, including thiol- and metalloprotease inhibitors during the last hour of growth increased the yield significantly (Fig. 4, lane 6).

To determine whether fibrinogen-binding proteins reacting with the anti-Fib serum existed in other staphylococcal species, similar experiments were performed with the CoNS, *S. hyicus*, *S. lugdunensis*, and *S. intermedius* strains. However, these strains did not produce Fib-like proteins under the conditions described above.

It was concluded that the Fib protein is expressed by all *S. aureus* strains investigated, and thus silent *fib* genes are not likely to exist. No fibrinogen-binding proteins reacting with anti-Fib serum were detected from other staphylococcal species. However, preliminary experiments suggest that other fibrinogen-binding proteins may be present in the culture supernatant of some of these species (data not shown).

Northern blot analysis of *fib* gene transcript. Since the presence of protease inhibitors also interferes with the growth of *S. aureus*, the amounts of Fib protein detected in cultures containing protease inhibitors may not be comparable to amounts produced in the absence of proteases. To evaluate whether the absence of Fib protein production was due entirely to differences in protease production or was, rather, a consequence of

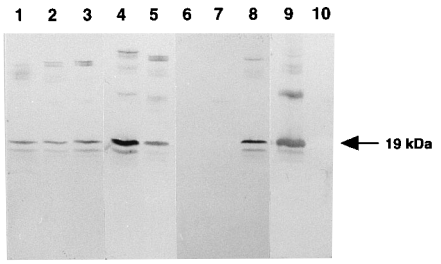

FIG. 3. Analysis of affinity-purified material from fibrinogen-Sepharose. Immunoblot probed with anti-Fib serum. *S. aureus* strains in lanes 1 to 10 are the same as in Fig. 2. The 19-kDa Fib protein is indicated.

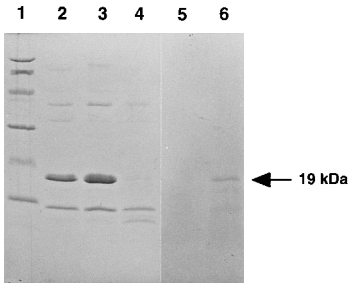

FIG. 4. Analysis of fibrinogen-Sepharose affinity-purified material from *S. aureus* bovine mastitis strains 2128 and 2114. Coomassie blue-stained SDS-PAGE gel. Lanes: 1, molecular weight markers (94, 67, 43, 30, 20.1, and 14.4 kDa, respectively); 2, strain 2128; 3, strain 2128 and strain 2114 with protease inhibitors added after growth; 4, strain 2128 and strain 2114; 5, strain 2114; 6, strain 2114 grown with protease inhibitors. The 19-kDa Fib protein is indicated.

fib mRNA levels, two bovine mastitis strains that differed in Fib protein levels were compared. Total RNA was prepared from (i) strain 2128, an *S. aureus* bovine mastitis strain which gave a strong band in the PCR analysis (Fig. 2, lane 9) and produced a large amount of Fib protein (Fig. 3, lane 9, and Fig. 4, lane 2) and (ii) strain 2114, an *S. aureus* bovine mastitis strain which gave a strong band in the PCR analysis (Fig. 2, lane 10) but did not produce Fib protein unless protease inhibitors were present during the incubation (Fig. 3, lane 10, and Fig. 4, lanes 5 and 6). The *fib* mRNA levels in these strains were almost identical (Fig. 5), which indicates that the difference in protein production between these two strains was due only to the higher protease production by strain 2114.

To further investigate the possibility that a *fib*-like sequence was produced by staphylococci other than *S. aureus*, Northern blots were performed with total RNA preparations from *S. epidermidis*, *S. hyicus*, *S. lugdunensis*, and *S. intermedius* strains (Fig. 6, lanes 5 to 12). No hybridization of the *fib* gene probe to these preparations could be detected.

To determine whether mRNA levels differed between strains with differences in Fib protein production but comparable protease production, two representative strains, *S. aureus* Newman and *S. aureus* FDA486, from which the *fib* gene and flanking regions had previously been sequenced, were studied. In a Northern blot of total RNA from these strains it was shown that the amount of *fib* mRNA reflected the amount of protein produced by a specific strain. Strain Newman, which produces large amounts of exoproteins, including the Fib protein, had high levels of *fib* mRNA (Fig. 6, lanes 1 and 2), whereas strain FDA486, previously shown to produce small amounts of Fib protein, had correspondingly lower levels of *fib*

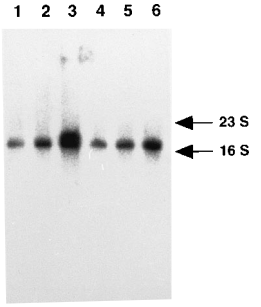

FIG. 5. Northern blot of total RNA. The [^{32}P]dCTP-labelled PCR-generated 378-bp fragment was used as a probe. Lanes: 1 to 3, strain 2128 (2.5, 5, and 10 μg of RNA, respectively); 4 to 6, strain 2114 (2.5, 5, and 10 μg of RNA, respectively).

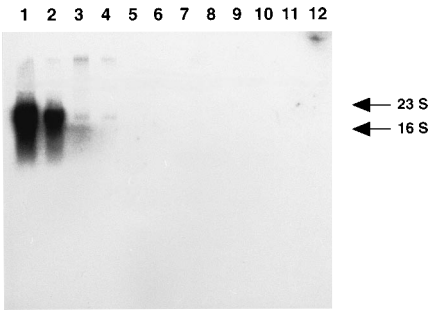

FIG. 6. Northern blot of total RNA. The [^{32}P]dCTP-labelled PCR-generated 378-bp fragment was used as a probe. Lanes: 1 to 2, *S. aureus* Newman (20 and 10 μg of RNA, respectively); 3 to 4, *S. aureus* FDA486 (20 and 10 μg of RNA, respectively); 5 to 6, *S. epidermidis* (20 and 10 μg of RNA, respectively); 7 to 8, *S. hyicus* (20 and 10 μg of RNA, respectively); 9 to 10, *S. lugdunensis* (20 and 10 μg of RNA, respectively); 11 to 12, *S. intermedius* (20 and 10 μg of RNA, respectively).

mRNA (Fig. 6, lanes 3 and 4). The size of the *fib* mRNA transcript from all the investigated *S. aureus* strains is the same, approximately 2 kb. This is in accordance with our previous findings that the *fib* gene is situated on a polycistronic message. An additional hybridization band in the vicinity of the sample wells was caused by contaminating DNA in the RNA preparations.

It was concluded that levels of *fib* mRNA influence the amounts of Fib protein produced by a particular *S. aureus* strain but that the Fib protein formed can also be degraded by simultaneously produced proteases in the culture medium, irrespective of *fib* mRNA levels.

DNA sequence of the *fib* gene from different *S. aureus* strains. To confirm previous preliminary findings that the *fib* gene is highly conserved, four isolates were picked at random and their *fib* genes were amplified by PCR, using the F.fib and M.fib primers. The DNA fragments were introduced into the pGEM-T vector and subsequently sequenced and compared with previously sequenced *fib* genes. All of the analyzed *fib* gene sequences were highly homologous (Fig. 1). The DNA base changes leading to a change in amino acid sequence were preferentially located in the signal sequence and in the very C terminus of the protein. Only one strain had an amino acid change from a basic lysine to a likewise basic arginine in a central domain of the Fib protein. The sequence recognized by the S.fib primer was identical, except for an A to G change in some strains. This change does not give rise to weak bands, since this difference occurs in a central part of the primer and strains containing this sequence gave strong bands in the PCR assay.

Thus, the *fib* gene was found to be unique to *S. aureus* and highly conserved at the nucleotide level.

DISCUSSION

The purpose of this study was to investigate the presence of the Fib protein and the *fib* gene among clinical isolates of *S. aureus* and other staphylococcal species known to interact with fibrinogen. This was pursued at the DNA level by using PCR analysis and sequencing, at the RNA level by using Northern blot analysis, and at the protein level by using affinity purification and immunoblot analysis.

It was found that the *fib* gene was present in all of the *S. aureus* strains but not in any other staphylococcal species investigated in this study (Table 2). All of the *S. aureus* strains isolated from bovine mastitis samples were positive for a strong 378-bp band following PCR amplification at 67°C (Fig. 2, lanes

6 to 10). However, 19 (49%) of the human *S. aureus* isolates produced weak bands in the PCR assay, using the same stringent annealing temperature (Fig. 2, lanes 1 to 5). It has been shown that mismatches in the 3′ terminus of primers can cause absence of amplification in PCR at stringent annealing temperatures (22). Evidence supporting this explanation is also strengthened by the fact that the intensity of these bands increased as the annealing temperature was lowered. In fact, one strain did not produce a band unless the annealing temperature was lowered by 2°C to 65°C (data not shown).

The *fib* gene DNA sequences from four randomly picked clinical *S. aureus* isolates were compared with two previously sequenced *fib* genes (4) (Fig. 1). All six sequences show a very high degree of homology (Fig. 1). The differences in DNA sequences that gave rise to amino acid sequence variation were confined to the signal sequence or to the very C-terminal part of the protein. There is only one exception, one strain which has the lysine in position 67 changed to an arginine. This position is at the end of the second of the two repeated domains which have been suggested to be involved in fibrinogen binding (4). The M.fib primer region seemed to have more sequence divergences than the S.fib primer region (Fig. 1), suggesting that the former region contained the sequence differences responsible for the reduced PCR yields. Interestingly, all of the bovine *S. aureus* strains had the consensus sequence derived from *S. aureus* Newman and FDA486, since these produced strong bands even at the higher annealing temperature.

The finding that the *fib* gene is highly conserved among *S. aureus* strains is intriguing. Other exoproteins that are produced exclusively by *S. aureus* have been shown to have homologous regions but also variable domains. Protein A has been suggested to form seven different variants on the basis of peptide mapping (5), and diversity in molecular masses of protein A from different strains could be due to variations in the number of immunoglobulin G-binding repeats (8). *S. aureus* coagulase genes have variable N-terminal domains, which are probably responsible for the differences in immunological response by different serotypes, and conserved C-terminal domains consisting of several repeated sequences. The number of repeats varies among coagulases, and this feature can be used in the typing of *S. aureus* strains (11).

The mechanism of regulation of the *fib* gene is not known; however, this gene seems to belong to the class of genes not regulated by the *agr* locus (4, 20). To see whether mRNA levels are involved in the regulation of the *fib* gene, two representative strains, of high and low Fib protein production, were studied. These strains, Newman and FDA486, from which the *fib* genes were previously sequenced (4), produced comparably low amounts of proteases. It was shown that the amounts of protein produced were correlated to the levels of mRNA (Fig. 6, lanes 1 to 4). Whether the mRNA levels were an effect of transcriptional level or mRNA stability was not possible to deduce from these experiments. Putative transcription initiation sites of the *fib* gene in these strains were homologous (4).

However, low mRNA levels were not the only reason for low Fib protein yields. Similar levels of mRNA were detected in two bovine mastitis strains that produced large amounts of Fib protein or no Fib protein at all (Fig. 5). The difference in protein production between these strains was shown to be an effect of protease production. In fact, half of the bovine mastitis strains analyzed in this study did not yield a positive Fib protein band in immunoblots (Fig. 3, lanes 6 to 10) unless protease inhibitors were present during growth. Cell-free culture supernatants from a protease-producing strain were shown to degrade Fib protein in a culture supernatant from a strain which produced large amounts of Fib protein (Fig. 4, lane 4). These results confirm earlier findings that bovine isolates generally produce very large amounts of serine protease compared with human isolates (1).

Since the *fib* gene and its gene product were found in all *S. aureus* strains investigated and as the *fib* gene was homologous to the coagulase gene, strains producing coagulases and/or interacting with fibrinogen were subsequently investigated for the presence of *fib* genes. There are several examples of genes that are homologous among staphylococcal species. For example, there are several plasmid-borne antibiotic resistance genes that exhibit a high degree of similarity between species, e.g., the chloramphenicol acetyltransferase gene *cat* (30) and the tetracycline resistance genes *tet*(K) and *tet*(L) (29). Such genes are believed to be subject to intergeneric exchange through natural plasmid transfer and hence are not confined to a particular species or to all members of a species. Another highly conserved gene is the chromosomal *mecA*, coding for a penicillin-binding protein, which has been shown to be present in both *S. aureus* and *S. epidermidis* (28). However, the *mecA* gene is only present in methicillin-resistant staphylococci, and it is believed to be transferred via an associated transposon (34).

The coagulase-producing species *S. intermedius* and *S. hyicus* and the fibrinogen-binding species *S. lugdunensis* were chosen for this study since they interact with fibrinogen. However, the structures responsible for these interactions have not been identified. It is likely that these putative fibrinogen-binding proteins are not very homologous to their *S. aureus* counterparts. As anticipated, there was no Fib-like protein produced by these strains nor was there a homologous gene or mRNA transcript present in these strains (Table 2 and Fig. 6). However, from the results in this study one cannot exclude the possibility that there are other staphylococcal species that contain sequences similar to the *fib* gene. We have mainly been concerned with discriminating *S. aureus* from CoNS in human infection, and it is feasible that this can be achieved by using the *fib* gene as a probe. The data from the limited number of other staphylococcal strains investigated in this study suggest that coagulase- or clumping-positive strains other than *S. aureus* do not contain a gene similar to *fib*. Further investigations are required in order to develop a diagnostic method based on the *fib* gene.

In conclusion, the presence of the *fib* gene was investigated at the DNA, mRNA, and protein levels. The *fib* gene and its corresponding gene product were found only in *S. aureus* isolates and were highly conserved at the nucleotide level. Thus, there must be a selective pressure for maintaining this nucleotide sequence, and the function of the *fib* gene or its gene product, a 19-kDa fibrinogen-binding protein, must be essential to *S. aureus*. The sequences generated here can be used to design PCR primers matching highly conserved sequences of the *fib* gene, for the identification of strains belonging to the *S. aureus* species.

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