INFECTION AND IMMUNITY, Feb. 2001, p. 712–718 Vol. 69, No. 2
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.2.712–718.2001

Staphylococcus caprae Strains Carry Determinants Known To Be Involved in Pathogenicity: a Gene Encoding an Autolysin-Binding Fibronectin and the *ica* Operon Involved in Biofilm Formation

JEANINE ALLIGNET,[1] SYLVIE AUBERT,[1] KEITH G. H. DYKE,[2] AND NEVINE EL SOLH[1]*

Unité des Staphylocoques, Centre National de Référence des Staphylocoques, Institut Pasteur, Paris, France,[1] and Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom[2]

Received 9 August 2000/Returned for modification 2 October 2000/Accepted 23 October 2000

The *atlC* gene (1,485 bp), encoding an autolysin which binds fibronectin, and the *ica* operon, involved in biofilm formation, were isolated from the chromosome of an infectious isolate of *Staphylococcus caprae* and sequenced. AtlC (155 kDa) is similar to the staphylococcal autolysins Atl, AtlE, Aas (48 to 72% amino acid identity) and contains a putative signal peptide of 29 amino acids and two enzymatic centers (*N*-acetylmuramoyl-L-alanine amidase and endo-β-*N*-acetylglucosaminidase) interconnected by three imperfect fibronectin-binding repeats. The glycine-tryptophan (GW) motif found in the central and end part of each repeat may serve for cell surface anchoring of AtlC as they do in *Listeria monocytogenes*. The *S. caprae ica* operon contains four genes closely related to *S. epidermidis* and *S. aureus icaA*, *icaB*, *icaC*, and *icaD* genes (≥ 68% similarity) and is preceded by a gene similar to *icaR* (≥70% similarity). The polypeptides deduced from the *S. caprae ica* genes exhibit 67 to 88% amino acid identity to those of *S. epidermidis* and *S. aureus ica* genes. The *ica* operon and *icaR* gene were analyzed in 14 *S. caprae* strains from human specimens or goats' milk. Some of the strains produced biofilm, and others did not. All strains carry the *ica* operon and *icaR* of the same sizes and in the same relative positions, suggesting that the absence of biofilm formation is not related to the insertion of a mobile element such as an insertion sequence or a transposon.

Staphylococcus caprae (13) is the predominent species among the staphylococci recovered from mastitis-free goats' milk (5). It is also increasingly recognized as a human pathogen infecting implanted foreign bodies (1, 6, 14, 44, 46, 52). Despite the amended description of this species (25), its phenotypic identification remains difficult. Therefore, molecular identification methods such as the analysis of ribotypes (1, 5, 12, 52), DNA-DNA hybridization (25), sequencing of the 16S rRNA gene (46), or analysis of the banding patterns on gels of penicillin-binding proteins (24) have been used for ecological studies and investigation of the involvement of *S. caprae* in infections. Some *S. caprae* strains from human specimens and goats' milk form biofilms (1, 4). Other strains do not, although the genomes of all strains tested carry nucleotide sequences hybridizing, at low stringency, with the *S. epidermidis* genes involved in initial adherence (*atlE*) and biofilm accumulation (the *ica* operon) (1). *S. caprae* adherence to fibronectin- and gelatin-coated coverslips is very weak. Nevertheless, surface proteins binding fibronectin have been detected on all *S. caprae* strains tested (1). The N-terminal part of the 175-kDa fibronectin-binding protein released from the surface of *S. caprae* clinical isolate 96007 has more than 50% amino acid identity (1) to the N-terminal part of the staphylococcal autolysins Atl (38), AtlE (18), and Aas (20). The aim of this study was to isolate the *atlC* autolysin gene of isolate 96007 to check whether the purified protein encoded by this gene binds fibronectin. We also characterized the *ica* operon to determine whether the absence of biofilm production in some strains is due to the integration of a mobile element as reported for the *S. epidermidis ica* operon (56).

* Corresponding author. Mailing address: Unité des Staphylocoques, Centre National de Référence des Staphylocoques, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: 33-(0)1 45-68-83-63. Fax: 33-(0)1-40-61-31-63. E-mail: nelsolh@pasteur.fr.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The relevant characteristics of the 14 *S. caprae* strains isolated from specimens from four infected patients and from milk samples have been described in a previous paper (1). *S. aureus* strain DU5883(pFNBA4) (17) was used as a positive control in fibronectin-binding experiments. *Micrococcus luteus* strain ATCC 9341 was used for the detection of bacteriolytic activity. *Escherichia coli* strain M15 harboring pREP4, which constitutively expresses the Lac repressor protein encoded by the *lacI* gene (QiaExpress System; Qiagen, Hilden, Germany), was used as a recipient.

Plasmid pQE31 (Qiagen) was used as a vector to produce a fusion protein with the His_6 tag at the N terminus of the protein. Plasmid pIP1818 (this study) was constructed by cloning into pQE31 a 1,884-bp fragment amplified from within *atlC* with primers Atl5 and Atl6 (Table 1). The recombinant plasmids pIP1781, pIP1789, pIP1807, pIP1808, and pIP1823 used for sequencing *atlC* and *ica* genes (this study) are pUC18 carrying chromosomal restriction fragments from *S. caprae* strain 96007. Staphylococcal and *M. luteus* strains were grown on brain heart infusion (Difco, Detroit, Mich.) or Trypticase soy broth (Difco). *E. coli* strains were cultured in Luria-Bertani medium supplemented with 100 μg of ampicillin per ml and, as required, 25 μg of kanamycin per ml.

DNA isolation and analysis. Total cellular DNA was isolated from staphylococcal strains and was purified using the QIAamp tissue kit from Qiagen. Plasmid DNA was extracted and purified from *E. coli* using the QIA-prep Spin plasmid kit from Qiagen.

Restriction endonucleases were obtained from Amersham-Pharmacia Biotech, Inc. (Piscataway, N.J.), and were used as specified by the manufacturer. Native or digested DNA was analyzed by electrophoresis in a 0.7% (wt/vol) agarose gel. DNA fragments of less than 1 kb amplified by PCR were separated by electrophoresis in 4% (wt/vol) Nusieve agarose gels (FMC Products, Rockland, Maine).

TABLE 1. Oligonucleotides used for PCR experiments

Primer	Sequence (5′-3′)	Gene	Accession no.	Position
Atl5	CTGGTCAAGT**GGATCC**TTGG *Bam*H1	*atlC*	AF244123	1883–1902
Atl6	GTATCT**CTGCAG**CATGAGC *Pst*I	*atlC*	AF244123	3784–3766
RF	CAATTCTTATTTTCTTCAATAACC	*icaR*	AF246926	442–466
AR	AACTCTTGTTGCGAATACGTGGG	*icaA*	AF246926	1604–1627
BF	CAATTATCAGAAATGTATCGTACAGG	*icaB*	AF246926	3112–3138
LR	CAAGACAGTTCAGATACAGTACGC	*gehC*	AF246926	4832–4855
CF	CATTAAGTGAAAAAGCTGTCACTCC	*icaC*	AF246926	3368–3393

Blotting and hybridization. DNA was transferred to Hybond-N^+ membranes (Amersham) and hybridized under stringent conditions (65°C) as previously described (9) or at lower stringency, i.e., 42°C.

PCR. PCR experiments were performed at high stringency (initial cycle of 5 min at 95°C followed by 30 cycles of 1 min at 60°C, 1 min 30 at 72°C, and 45 s at 95°C and a final extension step of 10 min at 72°C). The primers used are listed in Table 1 and were prepared by the phosphoramidite method with an Applied Biosystems (Foster City, Calif.) model 380B DNA synthesizer.

Sequencing. An Applied Biosystems automated 373A DNA sequencer and the protocol described by the manufacturer were used for sequencing. The amino acid sequences deduced from the nucleotide sequences were analyzed with the GCG, Inc., package and compared with those deduced from nucleotide sequences in the GenBank/EMBL Database.

Detection of bacteriolytic enzyme activity. Bacteriolytic activity was detected using renaturing gels as described by Sugai et al. (47). Dried cells of *M. luteus* strain ATCC 9341 or *S. caprae* 96007 (1) were incorporated into sodium dodecyl sulfate (SDS)-polyacrylamide gels (1 mg · ml^{-1}). After electrophoresis, the gels were washed in distilled water to remove the SDS and incubated in renaturating buffer (100 mM phosphate buffer [pH 7]) at 37°C for 1 h to overnight with gentle shaking. When lytic bands appeared, photographs were taken under oblique translucent light from the back against a black background. Thus, lytic bands appear as dark zones on the photographs.

Protein production. For generation of recombinant protein, plasmid pQE31 was used. Production and purification steps were performed as specified by the manufacturer (QIAexpress system; Qiagen).

Extraction of cell surface-associated proteins, SDS-PAGE, and Western affinity blotting. LiCl extractions were carried out as described by Komatsuzawa et al. (27). Briefly, the pellet of a 40-ml cell culture (6 h at 37°C) was suspended in 200 μl of 3 M LiCl (Sigma, Aldrich Chemie, Deisenhofen, Germany) in an ice bath for 15 min. After centrifugation (10,000 × *g* for 15 min), the supernatant was extensively dialyzed against distilled water (4°C). SDS-polyacrylamide gel electrophoresis (PAGE) of the proteins, their transfer onto polyvinylidene difluoride membranes (Hybond-P; Amersham), and incubation of the membranes with 3 μg of fibronectin (Chemicon, Temecula, Calif.) per ml were carried out as described previously (1). Binding proteins were detected with mouse anti-human fibronectin N-terminal monoclonal antibody (Chemicon). The ECL kit (Amersham) was used for antibody detection by chemiluminescence.

Nucleotide sequence accession numbers. The nucleotide sequences of the *atlC* gene and the *ica* operon from *S. caprae* strain 96007 have been submitted to GenBank under accession numbers AF244123 and AF246926, respectively. The nucleotide sequence of the *icaC* gene from *S. caprae* strain 89318 is registered under accession number AF246927.

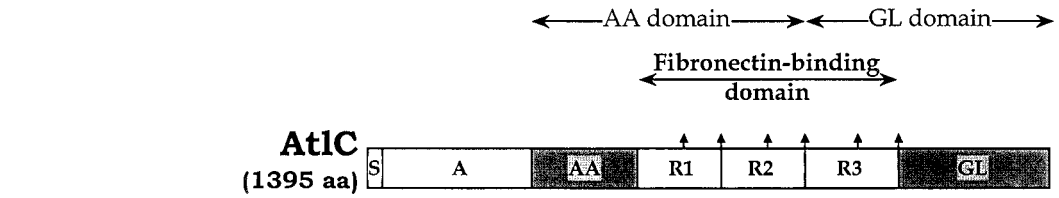
RESULTS

Characterization of the *atlC* autolysin gene from the *S. caprae* isolate 96007. In *S. caprae* clinical isolate 96007 (1), the nucleotide sequences hybridizing at low stringency with the *S. epidermidis atlE* probe were found on 8.9-kb *Hin*dIII and 4.3-kb *Hin*cII fragments having a 1.6-kb overlapping region (results not shown). Each of these two restriction fragments was inserted into pUC18. The resulting recombinant plasmids, pIP1781 and pIP1808, respectively, were used to determine the sequence of the regions exhibiting similarity to *atlE* (18). The sequence (accession number AF244123), contains a 4,185-bp putative gene, *atlC*, delimited by the ATG start codon at nucleotides (nt) 271 to 273 and the TAA stop codon at nt 4456 to 4458. This gene exhibits at least 55% similarity to the three sequenced staphylococcal autolysin genes, *atl*, *atlE*, and *aas*. The ATG start codon of *atlC* is preceeded, 10 nt upstream, by a 6-nt putative ribosome-binding site. The ΔG of the interaction of the most stable structure between this putative ribosome-binding site and the 3′ terminus of the 16S rRNA, calculated by the method of Tinocco et al. (51), is -60.2 kJ · mol^{-1}. The DNA sequence upstream from the start codon contains a putative promoter sequence, $AATACAN_{17}TAT$ AAT (nt 206 to 234). This sequence has 9 of 12 possible matches with the consensus sequence of the *Bacillus subtilis* vegetative T-factor promoter, σ^A ($TTGACAN_{18}TATAAT$) (36) and the putative promoter of *S. aureus atl* (CGCACA N_{18}TATAAT) (39) There is a putative transcriptional terminator consisting of inverted repeats (nt 4503 to 4538) followed by an AT-rich sequence downstream from the stop codon. The G+C content of *atlC* is 33.5%. This is similar to the value for the staphylococcal genome (32 to 36%) (26). The gene encodes a 1,395-amino-acid putative protein of 155 kDa.

Analysis of the AtlC sequence. The N-terminal part of the deduced amino acid sequence is a putative signal peptide of 29 amino acids as assessed by the method of von Heijne (53). The overall sequence exhibits significant similarities to the staphylococcal Atl-related autolysins: Atl (38), AtlE (18), and Aas (20) (61, 72, and 48% amino acid identity, respectively). Three imperfect repeats are present in the central region of each of these autolysins (Fig. 1). A glycine-tryptophan (GW) dipeptide motif is present in the central part and at the end of each repeat.

The two enzymatic domains of AtlC (Fig. 1), *N*-acetylmuramoyl-L-alanine amidase and endo-ß-*N*-acetylglucosaminidase, were deduced from the sequence similarities to the staphylococcal autolysins Atl (2, 27, 38, 39, 48), AtlE (18), and Aas (20). The repeats are required for the active enzymatic centers of these autolysins to be expressed on the bacterial surface.

Cell-associated bacteriolytic enzymes. The proteins released from LiCl extracts of *S. caprae* clinical isolate 96007 were assayed for bacteriolytic activity by using renaturating gels containing either *S. caprae* strain 96007 (Fig. 2) or *M. luteus* strain ATCC 9341 (results not shown). The bacteriolytic banding profiles were similar on the two gels: there were major bands of ≈175, 130, 116, 56, and 35 kDa. Two of these bacteriolytic bands (175 and 116 kDa) gave a strong fibronectin-binding signal (1), whereas the signals observed for the three other



Amino acids (aa)		29	303	214	160	169	193	326
% aa identity with								
Atl (1256 aa)	(38)		28	83	73	64	50	63
AtlE (1335 aa)	(18)		37	88	89	81	69	72
Aas (1430 aa)	(20)		42	74	54	31	54	57
Ami (918 aa)	(22)			56	29	30	40	

FIG. 1. Organization of the *S. caprae* autolysin AtlC and percent amino acid identity to the corresponding domains of *S. aureus* Atl (38), *S. epidermidis* AtlE (18), and *S. saprophyticus* Aas (20). ↑, Glycine-tryptophan (GW) dipeptide; ■, active enzymatic center. The enzymatic domains (AA and GL) were deduced from the similarities to those of the staphylococcal Atl-related autolysins. Abbreviations: AA, *N*-acetylmuramoyl-L-alanine amidase; GL, *N*-acetylglucosaminidase; R, repeats.

bands were weak and not consistently reproducible. The N terminus of the larger bacteriolytic band binding fibronectin was sequenced (1). It appeared to be a secreted form of the unprocessed product of *atlC* (150 kDa, deduced from the sequence); hence the molecular mass of ≈175 kDa previously attributed to this protein (1) on the basis of mobility was overestimated. The 116-kDa band may correspond to the two enzymatic domains (57 and 59 kDa), and the 56-kDa bacteriolytic band may correspond to either or both these two enzymatic domains.

Characterization of the AtlC fibronectin-binding domain. The repeats of the *S. saprophyticus* autolysin Aas are involved in fibronectin binding (20). Therefore, we checked whether the repeats of AtlC express fibronectin-binding activity. Primers Atl5 and Atl6 (Table 1) were used to amplify an 1,884-bp fragment from *atlC* by PCR. The amplified fragment was cleaved with *Bam*HI and *Pst*I and inserted into pQE31 (Qiagen) cut with the same restriction endonucleases. The resulting construct (pIP1818) encodes a fusion protein with His_6 tag at the N terminus. pIP1818 was introduced into *E. coli* strain M15(pREP4). The transformant was grown in the absence or presence of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to induce the expression of the His-tagged protein. Aliquots were removed 1, 2, 3, and 4 h after induction and 4 h after growth without induction. The bacterial proteins were purified on Ni-nitrilotriacetic acid resin (Qiagen) under denaturating conditions. A single 66-kDa protein band was visualized by Coomassie staining in all the induced samples but was not detected in the uninduced culture (results not shown). The purified 66-kDa protein bound fibronectin (Fig. 3, lane 3) but not fibrinogen or collagen (results not shown). The protein binding fibronectin was detected in bacterial lysates of the IPTG-induced cultures (results not shown) but not in the non-induced cultures (lane 2).

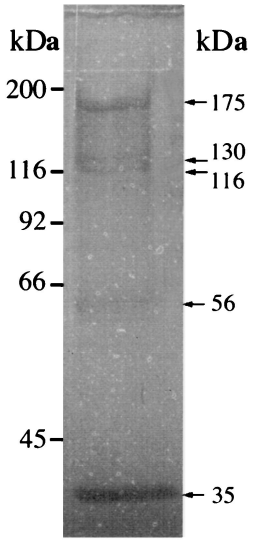


FIG. 2. Bacteriolytic enzyme profile of *S. caprae* strain 96007 on an SDS-polyacrylamide gel containing dried *S. caprae* 96007 cells (1 μg · ml^{-1}) as a substrate. Bands with lytic activity were observed as clear zones in the opaque gel and as dark bands after photography against a dark background. The sizes of marker proteins (in kilodaltons) are indicated on the left, and those of the bacteriolytically active proteins are shown on the right.

Characterization of the *ica* locus in *S. caprae* strains. Nucleotide sequences hybridizing at low stringency with an *S. epidermidis ica* probe were detected in the genomes of all the *S. caprae* strains tested (1). In clinical isolate 96007, the hybridizing sequences were carried by contiguous *Hae*III fragments of 4 and 4.3 kb overlapping a 1.8-kb *Hin*dIII fragment (Fig. 4). Each of the three fragments was inserted into pUC18 cleaved with *Sma*I or *Hin*dIII. The recombinant plasmids, pIP1807, pIP1823, and pIP1789 (Fig. 4), were used to sequence the regions similar to *S. epidermidis* and *S. aureus ica* operons (11, 19). The sequence of these regions in 96007 (accession number AF246926) contains three putative genes closely related (≥68% similarity) to *icaA*, *icaB*, and *icaD* of the *S. epidermidis* and *S. aureus ica* operons and a region similar to *icaC* (≥73% similarity) which is interrupted by a stop codon (nt 3743 to 3745) (Fig. 4). The organization of the locus is identical to those of *S. epidermidis* and *S. aureus ica* operons. A gene starting with a TTG codon and similar to *icaR* (≥70%

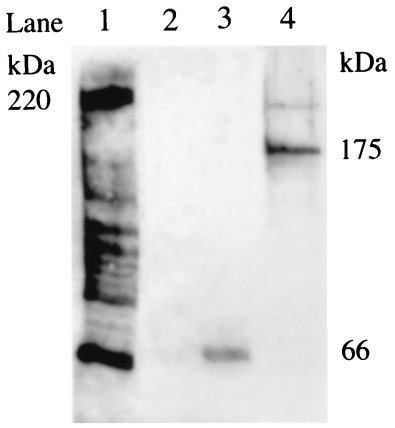


FIG. 3. Screening for fibronectin-binding proteins according to the Western affinity blotting technique described previously (1). Lanes: 1, surface proteins released from an LiCl extract of *S. aureus* strain DU5883 (pFNBA4) (17) used as positive control; 2, proteins released from uninduced culture of *E. coli* M15 harboring pREP4 and pIP1818; 3, 66-kDa protein resulting from purification on Ni-nitrilotriacetic acid resin (Qiagen) of the proteins released from an IPTG-induced culture of *E. coli* M15 harboring pREP4 and pIP1818; 4: proteins released from an LiCl extract of *S. caprae* isolate 96007.

similarity) was found upstream from the *S. caprae ica* operon and transcribed in the opposite direction from the four genes of the operon. The sequence of the incomplete open reading frame downstream from the *S. caprae ica* operon is similar to that of the end part of the *S. epidermidis* lipase gene *gehC* (accession number M95577), adjacent to the *S. epidermidis* and *S. aureus ica* operons. Isolate 96007, in which *icaC* is interrupted by a stop codon, does not produce biofilm. Therefore, primers CF and LR (Table 1) were designed to amplify *icaC* from an *S. caprae* isolate producing biofilm, 89318 (1). The amplicon of the expected size (1.5 kb) was sequenced (accession number AF246927): the *icaC* gene in strain 89318 was 99% identical to that in 96007 but was not interrupted by a stop codon. The sizes of the predicted polypeptides deduced from the *S. caprae* genes and the amino acid identities to the polypeptides encoded by the *S. epidermidis* and *S. aureus ica* genes are reported in Table 2.

TABLE 2. Sizes of the polypeptides deduced from the sequenced *S. caprae ica* genes and the amino acid identity to those encoded by *ica* genes from *S. epidermidis* ATCC 35984 and *S. aureus* ATCC 35556 strains

Polypeptide designation	Size (amino acids)[a]	% Identity to polypeptides of[b]:	
		S. epidermidis	*S. aureus*
IcaR	190	77	68
IcaA	412	88	82
IcaD	102	73	65
IcaB	289	72	66
IcaC	358	84	78

[a] For all the predicted polypeptides except IcaC, the sizes reported are those of 96007 (accession number AF246926). Since 96007 *icaC* is interrupted by a stop codon (nt 3743 to 3745), the size of the predicted polypeptide IcaC of strain 89318 (accession number AF246927) is given.

[b] Accession numbers are U43366 for the *S. epidermidis ica* gene and AF086783 for the *S. aureus ica* gene.

We tested whether the absence of *ica* expression in some strains was due to the integration of an insertion sequence as reported for the *S. epidermidis ica* operon (56). The 14 *S. caprae* isolates tested, including 96007, carry a *Hin*dIII fragment of 1.8 kb hybridizing with the *S. epidermidis ica* probe (1). Two pairs of primers, RF-AR and BF-LR (Table 1), were designed to test whether the regions flanking this central fragment in the 96007 *ica* operon are present in the other *S. caprae* isolates. Fragments of 1.2 and 1.7 kb were amplified from the genomes of all *S. caprae* strains tested including 96007 (results not shown). Thus, in all the strains, the *ica* operon and *icaR* have the same sizes, suggesting that no mobile elements are inserted in them.

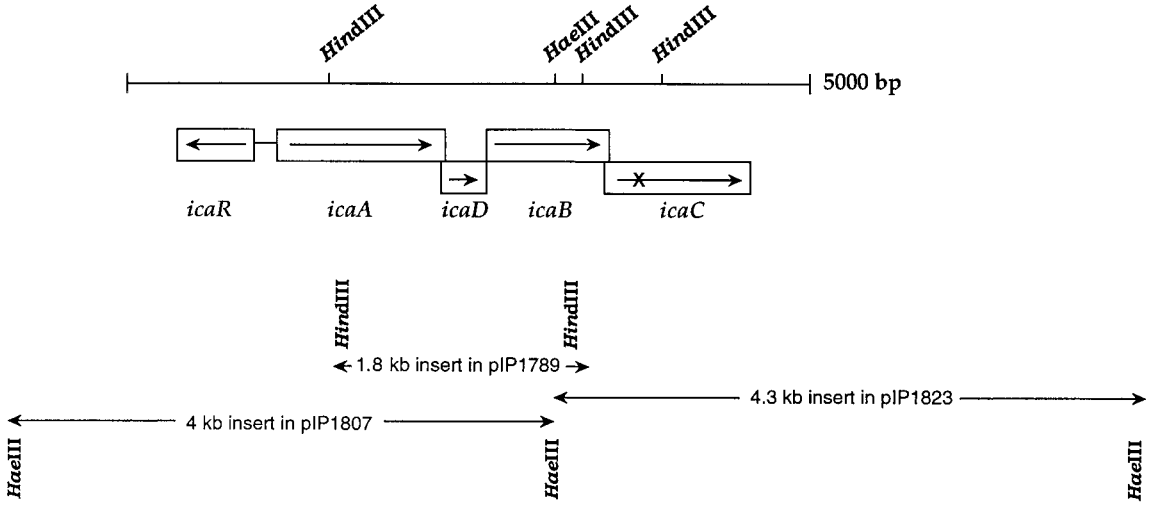


FIG. 4. Map of the *ica* locus and surrounding chromosomal region in *S. caprae* strain 96007 (accession number AF246926). The IcaC product is truncated. The fragments reported below the map were cloned into pUC18 for sequencing; their sizes and the designation of the recombinant plasmids are given.

DISCUSSION

We describe the *S. caprae* autolysin gene *atlC* and the *ica* operon. The inactivation of similar genes in other staphylococcal species is associated with the loss of biofilm production (11, 19) or a surface adhesin (18) and/or a decrease in virulence in animal infection models (31, 42, 43). It is therefore likely that these genes are involved in *S. caprae* virulence.

The *S. caprae* surface autolysin AtlC has the same overall organization as the three staphylococcal surface autolysins Atl (38), AtlE (18), and Aas (20), which are necessary for cluster dispersion during cell division. The presence in these autolysins of two enzymatic domains has probably resulted from the fusion of two genes. None of these autolysins contains the motifs typical of gram-positive surface proteins including the LPXTG motif (37) recognized by the sortase (32). However, the three imperfect repeats connecting the two enzymatic active centers are required for the exposure of proteins on the bacterial surface (2, 18, 20, 39). The *L. monocytogenes* InlB and Ami proteins also contain tandem repeats including GW dipeptides (7, 22). These repeats were shown to be responsible for displaying proteins on the cell surface by associating with lipoteichoic acids. Such GW repeats, also found in the staphylococcal autolysins LytA (54), Atl (39), AtlE (18), Aas (20), and AtlC (this study), may serve for cell surface anchoring in staphylococci as they do in *L. monocytogenes*. Synthetic 10- to 30-mer oligopeptides derived from repeat 1 of Atl were synthesized, and their effects on the autolysis of *S. aureus* cells were studied (50). All active peptides were located on the C-terminal side of repeat 1 and are suspected to affect the interaction between the autolytic enzymes and lipoteichoic acid.

In addition to the bacteriolytic activity, some staphylococcal autolysins have adhesive functions. The adhesion properties of the *S. aureus* autolysin Atl (38) have not been investigated. The *S. epidermidis* autolysin AtlE mediates the primary attachment to polystyrene and binds vitronectin, whereas its binding to fibronectin is very weak and not reproducible (19). The *S. saprophyticus* autolysin Aas binds fibronectin and sheep erythrocytes, and the binding domain has been mapped to the central region containing the repeats (20). Analysis of the ability of the Atl and AtlE regions containing the repeats to bind fibronectin may help to elucidate the correlation between the amino acid sequence of the repeats and binding. The repeats connecting the two enzymatic centers of the staphylococcal Atl-related autolysins are different from those involved in the fibronectin-binding activity of the described LPXTG surface proteins in staphylococcal and streptococcal strains (37, 40). Moreover, the three motifs GGXX(I/V)DF (33, 49), VETEDT (28), and HFDNXXP (21, 40), which are critical or important for the fibronectin-binding activity of the LPXTG proteins, were not found in the repeats of the staphylococcal Atl-related autolysins. The different fibronectin-binding proteins may recognize different parts of the fibronectin molecule. Moreover, the recombinant fusion proteins containing the repeats of the *S. aureus* FnBPA and FnBPB proteins (23, 45), which have been proposed as vaccines (8, 49), are not expected to inhibit the fibronectin-binding activity of the autolysins. Analysis of the contribution of *S. caprae* AtlC and in particular of its fibronectin-binding activity to infections associated with the implantation of foreign bodies will require the construction of isogenic *S. caprae* mutants in which *atlC* or part of this gene is deleted or modified. Such variants would also be useful to check whether an autolysin(s) or fibronectin-binding protein(s) other than AtlC is produced by *S. caprae*.

Some human and goat *S. caprae* strains produce biofilm, but production in vitro is variable (1, 4), despite the presence of the *ica* operon and *icaR* which are not interrupted by detectable mobile elements. In *S. epidermidis*, such variations have been attributed to the spontaneous insertion of IS*256* in *icaB* (56) and to mutations triggered by insertion of Tn*917* into the *ica* operon and three other loci contributing to the regulation of transcription of the genes involved in biosynthesis of the polysaccharide intercellular adhesin (PIA) (30). This regulation has not been elucitated and may also be involved in biofilm formation in *S. caprae*.

Biofilm may contribute to the virulence of *S. caprae* and protect the bacterium against antibiotics by preventing access, as it does in *S. epidermidis* (10, 41). Biofilm formation is thought to be a two-step process that requires the adhesion of bacteria to a substrate surface followed by cell-cell adhesion, forming the multiple layers of the biofilm. In *S. epidermidis*, *atlE* (18) is involved in initial adherence and the formation of multiple layers is due to the PIA (19, 29) also named PS/A (34). PIA is composed of linear ß-1,6-linked glucosaminylglycans. The genes *icaA* and *icaD* mediate the synthesis of the oligomers in vitro (16). The *N*-acetylglucosaminyltransferase activity, due to IcaA and IcaD, together with IcaC, is associated with the in vitro formation of a product that is recognized by an antibody raised against PIA. The *ica* locus of *S. aureus* (11, 35) was sequenced and is similar to *S. epidermidis ica* (19). It is present in all *S. aureus* strains tested and is required for biofilm formation (11). The biofilm polysaccharide, also named PNSG (poly-*N*-succinyl-ß1-6-glucosamine), is produced in vivo during human and animal infection by *S. aureus* and is a target for protective antibodies (35).

ica DNA probes from both *S. epidermidis* and *S. aureus* were used in hybridization experiments at low stringency to screen for related genes or operons in strains belonging to 21 staphylococcal taxa (1, 11). Cross-hybridizations with the probes have been detected in seven taxa including *S. caprae*. The organization of the *S. caprae ica* operon and flanking regions is identical to that in *S. epidermidis* and *S. aureus*, with close relatedness between the *ica* operon genes and the flanking genes, i.e., *icaR* and *geh*. The *ica* operon is significantly more prevalent among *S. epidermidis* isolates responsible for catheter-related and joint prosthesis infections than among those isolated from normal skin and mucosa of healthy controls (3, 15, 55). In contrast, *ica* was detected in all *S. caprae* isolates tested regardless of their source, whether from infected human specimens or mastitis-free goats' milk (1).

It is not known whether *S. caprae* strains produce PIA that reacts with antibodies directed against *S. epidermidis* PIA or *S. aureus* PNSG (35). If there is a cross-immunosensitivity with *S. aureus* PNSG, protection against *S. aureus* infection (35) may also be effective against *S. caprae* infection. In various other staphylococcal taxa, tests to detect PIA production by using the antibodies directed against *S. epidermidis* PIA were shown to be inconclusive since strains belonging to the same species (*S. aureus* or *S. epidermidis*) behave very differently (11). The

immune system may recognize sugar moieties in the biofilm, and these moieties may be differently modified in different strains (deacetylated or succinylated). Therefore, an analysis of staphylococcal biofilm polysaccharides may help in the design of vaccines protecting against foreign body infections caused by *S. aureus* and coagulase-negative staphylococci.

ACKNOWLEDGMENT

We thank C. Tran for secretarial assistance.

REFERENCES

1. **Allignet, J., J.-O. Galdbart, A. Morvan, K. G. H. Dyke, P. Vaudaux, S. Aubert, N. Desplaces, and N. El Solh.** 1999. Tracking adhesion factors in *Staphylococcus caprae* strains responsible for human bone infections following implantation of orthopaedic material. Microbiology **145:**2033–2042.
2. **Baba, T., and O. Schneewind.** 1998. Targeting of muralytic enzymes to the cell division site of Gram-positive bacteria: repeat domains direct autolysin to the equatorial surface ring of *Staphylococcus aureus*. EMBO J. **17:**4639–4646.
3. **Barbier-Frebourg, N., S. Lefebvre, S. Baert, and J.-F. Lemeland.** 2000. PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. J. Clin. Microbiol. **38:**877–880.
4. **Bedidi-Madani, N., T. Greenland, and Y. Richard.** 1998. Exoprotein and slime production by coagulase-negative staphylococci isolated from goats' milk. Vet. Microbiol. **59:**139–145.
5. **Bedidi-Madani, N., A. Kodjo, L. Villard, and Y. Richard.** 1998. Ribotyping of *Staphylococcus caprae* isolated from goat milk. Vet. Res. **29:**149–158.
6. **Blanc, V., J. Picaud, E. Legros, M. Bes, J. Etienne, D. Moatti, and M. F. Raynaud.** 1999. Infection sur prothèse totale de hanche à *Staphylococcus caprae*. Pathol. Biol. **47:**409–413.
7. **Braun, L., S. Dramsi, P. Dehoux, H. Bierne, G. Lindahl, and P. Cossart.** 1997. InIB: an invasion protein of *Listeria monocytogenes* with a novel type of surface association. Mol. Microbiol. **25:**285–294.
8. **Brennan, F. R., T. D. Jones, M. Longstaff, S. Chapman, T. Bellaby, H. Smith, F. Xu, W. D. O. Hamilton, and J.-I. Flock.** 1999. Immunogenicity of peptides derived from a fibronectin-binding protein of *S. aureus* expressed on two different plant viruses. Vaccine **17:**1846–1857.
9. **Chesneau, O., J. Allignet, and N. El Solh.** 1993. Thermonuclease gene as a target nucleotide sequence for specific recognition of *Staphylococcus aureus*. Mol. Cell. Probes **7:**301–310.
10. **Christensen, G. D., L. Baldassarri, and W. A. Simpson.** 1994. Colonization of medical devices by coagulase-negative staphylococci, p. 45–78. *In* A. L. Bisno and F. A. Waldvogel (ed.), Infections associated with indwelling medical devices. American Society for Microbiology, Washington, D.C.
11. **Cramton, S. E., C. Gerke, N. F. Schnell, W. W. Nichols, and F. Götz.** 1999. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. **67:**5427–5433.
12. **De Buyser, M. L., A. Morvan, S. Aubert, F. Dilasser, and N. El Solh.** 1992. Evaluation of a ribosomal RNA gene probe for the identification of species and subspecies within the genus *Staphylococcus*. J. Gen. Microbiol. **138:**889–899.
13. **Devriese, L. A., B. Poutrel, R. Killper-Bälz, and K. H. Schleifer.** 1983. *Staphylococcus gallinarum* and *Staphylococcus caprae*, two new species from animals. Int. J. Syst. Bacteriol. **33:**480–486.
14. **Elsner, H.-A., G. P. Dahmen, R. Laufs, and D. Mack.** 1998. Intra-articular empyema due to *Staphylococcus caprae* following arthroscopic cruciate ligament repair. J. Infect. **37:**66–67.
15. **Galdbart, J.-O., J. Allignet, H.-S. Tung, C. Rydèn, and N. El Solh.** 2000. Screening for *Staphylococcus epidermidis* markers discriminating between skin flora strains and those responsible for joint prosthesis infections. J. Infect. Dis. **182:**351–355.
16. **Gerke, C., A. Kraft, R. Süßmuth, O. Schweitzer, and F. Götz.** 1998. Characterization of the *N*-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. J. Biol. Chem. **273:**18586–18593.
17. **Greene, C., D. McDevitt, P. Francois, P. E. Vaudaux, D. P. Lew, and T. J. Foster.** 1995. Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of *fnb* genes. Mol. Microbiol. **17:**1143–1152.
18. **Heilmann, C., M. Hussain, G. Peters, and F. Götz.** 1997. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. Mol. Microbiol. **24:**1013–1024.
19. **Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz.** 1996. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol. Microbiol. **20:**1083–1091.
20. **Hell, W., H.-G. W. Meyer, and S. G. Gatermann.** 1998. Cloning of *aas*, a gene encoding a *Staphylococcus saprophyticus* surface protein with adhesive and autolytic properties. Mol. Microbiol. **29:**871–881.
21. **Jaffe, J., S. Natanson-Yaron, M. G. Caparon, and E. Hanski.** 1996. Protein F2, a novel fibronectin-binding protein from *Streptococcus pyogenes*, possesses two domains. Mol. Microbiol. **21:**373–384.
22. **Jonquières, R., H. Bierne, F. Fiedler, P. Gounon, and P. Cossart.** 1999. Interaction between the protein InlB of *Listeria monocytogenes* and lipoteichoic acid: a novel mechanism of protein association at the surface of Gram-positive bacteria. Mol. Microbiol. **34:**902–914.
23. **Jönsson, K., C. Signäs, P. Müller, and M. Lindberg.** 1991. Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. Eur. J. Biochem. **202:**1041–1048.
24. **Kanda, K., E. Suzuki, K. Hiramatsu, T. Oguri, H. Miura, T. Ezaki, and T. Yokota.** 1991. Identification of a methicillin-resistant strain of *Staphylococcus caprae* from a human clinical specimen. Antimicrob. Agents Chemother. **35:**174–176.
25. **Kawamura, Y., X.-G. Hou, F. Sultana, K. Hirose, M. Miyake, S.-E. Suh, and T. Ezaki.** 1998. Distribution of *Staphylococcus* species among human clinical specimens and emended description of *Staphylococcus caprae*. J. Clin. Microbiol. **36:**2038–2042.
26. **Kloos, W. E., and D. W. Lambe, Jr.** 1991. *Staphylococcus*, p. 222–237. *In* A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
27. **Komatsuzawa, H., M. Sugai, S. Nakashima, S. Yamada, A. Matsumoto, T. Oshida, and H. Suginaka.** 1997. Subcellular localization of the major autolysin, Atl, and its processed proteins in *Staphylococcus aureus*. Microbiol. Immunol. **41:**469–479.
28. **Lindmark, H., K. Jacobsson, L. Frykberg, and B. Guss.** 1996. Fibronectin-binding protein of *Streptococcus equi* subsp. *zooepidemicus.* Infect. Immun. **64:**3993–3999.
29. **Mack, D., W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, and R. Laufs.** 1996. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear ß-1,6-linked glucosaminoglycan: purification and structural analysis. J. Bacteriol. **178:**175–183.
30. **Mack, D., H. Rohde, S. Dobinsky, J. Riedewald, M. Nedelmann, J. K-M. Knobloch, H.-A. Elsner, and H. H. Feucht.** 2000. Identification of three essential regulatory gene loci governing expression of *Staphylococcus epidermidis* polysaccharide intercellular adhesin and biofilm formation. Infect. Immun. **68:**3799–3807.
31. **Mani, N., L. M. Baddour, D. Q. Offutt, U. Vijaranakul, M. J. Nadakavukaren, and R. K. Jayaswal.** 1994. Autolysis-defective mutant of *Staphylococcus aureus*: pathological considerations, genetic mapping, and electron microscopic studies. Infect. Immun. **62:**1406–1409.
32. **Mazmanian, S. K., G. Liu, H. Ton-That, and O. Schneewind.** 1999. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. Science **285:**760–763.
33. **McGavin, M. J., S. Gurusiddappa, P.-E. Lindgren, M. Lindberg, G. Raucci, and M. Höök.** 1993. Fibronectin receptors from *Streptococcus dysgalactiae* and *Staphylococcus aureus*. Involvement of conserved residues in ligand binding. J. Biol. Chem. **268:**23946–23953.
34. **McKenney, D., J. Hübner, E. Muller, Y. Wang, D. A. Goldmann, and G. B. Pier.** 1998. The *ica* locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. Infect. Immun. **66:**4711–4720.
35. **McKenney, D., K. L. Pouliot, Y. Wang, V. Murthy, M. Ulrich, G. Döring, J. C. Lee, D. A. Goldmann, and G. B. Pier.** 1999. Broadly protective vaccine for *Staphylococcus aureus* based on an *in vivo*-expressed antigen. Science **284:**1523–1527.
36. **Moran, C. P., Jr.** 1993. RNA polymerase and transcription factors, p. 653–667. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other Gram-positive bacteria. American Society for Microbiology, Washington, D.C.
37. **Navarre, W. W., and O. Schneewind.** 1999. Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Mol. Biol. Rev. **63:**174–229.
38. **Oshida, T., M. Sugai, H. Komatsuzawa, Y.-M. Hong, H. Suginaka, and A. Tomasz.** 1995. A *Staphylococcus aureus* autolysin that has an *N*-acetylmuramoyl-L-alanine amidase domain and an endo-ß-*N*-acetylglucosaminidase domain: cloning, sequence analysis, and characterization. Proc. Natl. Acad. Sci. USA **92:**285–289.
39. **Oshida, T., M. Takano, M. Sugai, H. Suginaka, and T. Matsushita.** 1998. Expression analysis of the autolysin gene (*atl*) of *Staphylococcus aureus*. Microbiol. Immunol. **42:**655–659.
40. **Rocha, C. L., and V. A. Fischetti.** 1999. Identification and characterization of a novel fibronectin-binding protein on the surface of group A streptococci. Infect. Immun. **67:**2720–2728.
41. **Rupp, M. E., and G. L. Archer.** 1994. Coagulase-negative staphylococci: pathogens associated with medical progress. Clin. Infect. Dis. **19:**231–243.
42. **Rupp, M. E., J. S. Ulphani, P. D. Fey, K. Bartscht, and D. Mack.** 1999. Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. Infect. Immun. **67:**2627–2632.
43. **Rupp, M. E., J. S. Ulphani, P. D. Fey, and D. Mack.** 1999. Characterization

of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. Infect. Immun. **67:**2656–2659.
44. **Shuttleworth, R., R. J. Behme, A. McNabb, and W. D. Colby.** 1997. Human isolates of *Staphylococcus caprae*: association with bone and joint infections. J. Clin. Microbiol. **35:**2537–2541.
45. **Signas, C., G. Raucci, K. Jonsson, P. E. Lindgren, G. M. Anantharamaiah, M. Hook, and M. Lindberg.** 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. Proc. Natl. Acad. Sci USA **86:**699–703.
46. **Spellerberg, B., K. Steidel, R. Lütticken, and G. Haase.** 1998. Isolation of *Staphylococcus caprae* from blood cultures of a neonate with congenital heart disease. Eur. J. Clin. Microbiol. Infect. Dis. **17:**61–62.
47. **Sugai, M., T. Akiyama, H. Komatsuzawa, Y. Miyake, and H. Suginaka.** 1990. Characterization of sodium dodecyl sulfate-stable *Staphylococcus aureus* bacteriolytic enzymes by polyacrylamide gel electrophoresis. J. Bacteriol. **172:** 6494–6498.
48. **Sugai, M., H. Komatsuzawa, T. Akiyama, Y.-M. Hong, T. Oshida, Y. Miyake, T. Yamaguchi, and H. Suginaka.** 1995. Identification of endo-ß-*N*-acetylglucosaminidase and *N*-acetylmuramyl-L-alanine amidase as cluster-dispersing enzymes in *Staphylococcus aureus*. J. Bacteriol. **177:**1491–1496.
49. **Sun, Q., G. M. Smith, C. Zahradka, and M. J. McGavin.** 1997. Identification of D motif epitopes in *Staphylococcus aureus* fibronectin-binding protein for the production of antibody inhibitors of fibronectin binding. Infect. Immun. **65:**537–543.
50. **Takano, M., T. Oshida, A. Yasojima, M. Yamada, C. Okagaki, M. Sugai, H. Suginaka, and T. Matsushita.** 2000. Modification of autolysis by synthetic peptides derived from the presumptive binding domain of *Staphylococcus aureus* autolysin. Microbiol. Immunol. **44:**463–472.
51. **Tinoco, I., Jr., P. N. Borer, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature New Biol. **246:**40–41.
52. **Vandenesch, F., S. J. Eykyn, M. Bes, H. Meugnier, J. Fleurette, and J. Etienne.** 1995. Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. J. Clin. Microbiol. **33:**888–892.
53. **von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
54. **Wang, X., B. J. Wilkinson, and R. K. Jayaswal.** 1991. Sequence analysis of a *Staphylococcus aureus* gene encoding a peptidoglyccan hydrolase activity. Gene **102:**105–109.
55. **Ziebuhr, W., C. Heilmann, F. Götz, P. Meyer, K. Wilms, E. Straube, and J. Hacker.** 1997. Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect. Immun. **65:**890–896.
56. **Ziebuhr, W., V. Krimmer, S. Rachid, I. Lößner, F. Götz, and J. Hacker.** 1999. A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. Mol. Microbiol. **32:**345–356.

Editor: E. I. Tuomanen