INFECTION AND IMMUNITY, Aug. 2006, p. 4615–4623 Vol. 74, No. 8
0019-9567/06/$08.00+0 doi:10.1128/IAI.01885-05

SdrI, a Serine-Aspartate Repeat Protein Identified in *Staphylococcus saprophyticus* Strain 7108, Is a Collagen-Binding Protein

Türkan Sakinc, Britta Kleine, and Sören G. Gatermann*

Abteilung für Medizinische Mikrobiologie, Institut für Hygiene und Mikrobiologie, Ruhr-Universität Bochum, D-44780 Bochum, Germany

Received 17 November 2005/Returned for modification 21 March 2006/Accepted 24 April 2006

A gene encoding a serine-aspartate repeat protein of *Staphylococcus saprophyticus*, an important cause of urinary tract infections in young women, has been cloned and sequenced. In contrast to other SD repeat proteins, SdrI carries 21 additional N-terminal repeats with a consensus sequence of (P/A)ATKE(K/E)A(A/V)(T/I)(A/T/S)EE and has the longest $SD(AD)_{(1-5)}$ repetitive region (854 amino acids) described so far. This highly repetitive sequence contains only the amino acids serine, asparagine, and a distinctly greater amount of alanine (37%) than all other known SD repeat proteins (2.3 to 4.4%). In addition, it is a collagen-binding protein of *S. saprophyticus* and the second example in this organism of a surface protein carrying the LPXTG motif. We constructed an isogenic *sdrI* knockout mutant that showed decreased binding to immobilized collagen compared with wild-type *S. saprophyticus* strain 7108. Binding could be reconstituted by complementation. Collagen binding is specifically caused by SdrI, and the recently described UafA protein, the only LPXTG-containing protein in the genome sequence of the type strain, is not involved in this trait. Our experiments suggest that, as in other staphylococci, the presence of different LPXTG-anchored cell wall proteins is common in *S. saprophyticus* and support the notion that the presence of matrix-binding surface proteins is common in staphylococci.

Attachment of microorganisms to host tissues is commonly regarded as the crucial initial step in the pathogenesis of microbial infections. *Staphylococcus saprophyticus*, an important cause of urinary tract infections, binds fibronectin (7, 42), laminin (33), and collagen (33, 35) and hemagglutinates sheep erythrocytes (10, 16).

Two major surface proteins have been characterized, the *S. saprophyticus* surface-associated protein (Ssp), a 95-kDa protein (6, 35) which has recently been identified as a surface-associated lipase, and an autolysin (Aas) (15), a multifunctional protein responsible for agglutination of sheep erythrocytes, fibronectin binding, and autolysis. In addition, the genomic sequence of the type strain of *S. saprophyticus* has been published recently (20). The sequence contains only one protein that is predicted to be covalently linked to the cell wall via an LPXTG motif (20). This protein, UafA, seems to mediate attachment to a bladder carcinoma cell line and causes hemagglutination. However, it cannot be the sole factor responsible for attachment and/or pathogenesis since nonhemagglutinating strains may cause infections (10, 16) and still adhere to collagen (35). In addition, all other sequenced staphylococcal genomes contain more than one cell wall-anchored protein.

In staphylococci, binding to extracellular matrix (ECM) proteins is often mediated by surface receptors designated MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) (30) that interact with proteins of the ECM such as fibrinogen (5, 25, 27, 43), collagen (31, 41), fibronectin (17, 39), vitronectin (21), or elastin (29).

Many MSCRAMMS are organized as SD repeat (Sdr) proteins that are characterized by a common organization comprising an amino-terminal signal sequence, a functional domain often labeled the A region, the SD repeat region, a cell wall-spanning region, an LPXTG motif, and a hydrophobic membrane-spanning domain, followed by a series of positively charged residues (19, 22, 23). The LPXTG motif is the target of a transpeptidase that cleaves the motif between threonine and glycine residues and anchors the protein to the peptidoglycan of the cell wall (37).

Some Sdr proteins contain additional repeats, termed B repeat regions, located between the A regions and SD repeat regions (18).

In this report, we describe the cloning and characterization of an Sdr protein of *S. saprophyticus*, which is also a wall-anchored protein of this species. It binds collagen and represents a description of an Sdr protein of *S. saprophyticus*. In addition, it contains N-terminal repeats of unknown function.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *S. saprophyticus* strain 7108, a hemagglutinating, fibronectin-binding, and collagen-binding clinical isolate, has been described previously (10, 15, 35). For cloning, a lambda ZAP Express library kindly provided by W. Hell was used (15). *Escherichia coli* XL1 Blue MRF′ (Stratagene, La Jolla, CA) was the host for the phage and the phagemids. *E. coli* XLOLR (Stratagene) was used for in vivo excision. *E. coli* DH5 was the host for expression experiments and the intermediate host during construction of the plasmids for allelic replacement (12). Shuttle plasmid pBT2 (4) contains the temperature-sensitive replicon of pE194, the chloramphenicol resistance determinant of pC194, and the multiple cloning site of pUC18. Plasmid pEC4 (4) was used as the *ermB* source. Plasmid pQE (QIAGEN, Hilden, Germany) was used for recom-

* Corresponding author. Mailing address: Institut für Hygiene und Mikrobiologie, Abteilung für Medizinische Mikrobiologie, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany. Phone: 49-234-3226467. Fax: 49-234-3214197. E-mail: soeren.gatermann@ruhr-uni-bochum.de.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Species or vector	Size (kb)	Description[a]	Reference or source
Strains				
XL1 Blue MRF′	*E. coli*		Δ(*mrcA*)*183* Δ(*mrcCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}*Z* ΔM15 Tn*5* (Kan^r)]; strain for construction of phage library	Stratagene
XLOLR	*E. coli*		Δ(*mrcA*)*183* Δ(*mrcCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}*Z* ΔM15 Tn*5* (Kan^r)] Su$^-$ λ^r; strain for in vivo excision	Stratagene
DH5	*E. coli*		F$^-$ (λ$^-$) (Φ80d *lacZ* ΔM15) Δ(*lacZYA argF*)*U169 genV44 deoR gyrA relA91 endA1 thi-1 hsdR17*; cloning host	12
M15	*E. coli*		*lac ara gal mtl* F pRep4; strain for propagation of pQE constructs	M. R. Villarejo and I. Zabin
CCM883	*S. saprophyticus*		Hemagglutination-positive type strain	F. Götz
7108	*S. saprophyticus*		Wild-type isolate; fibronectin binding	10
7108Δ*sdrI*	*S. saprophyticus*		Isogenic knockout mutant of 7108, *sdrI* gene interrupted by *ermB* cassette at bp 2002	This study
7108sΔ*sdrI*(pBTSA11)	*S. saprophyticus*		Isogenic *sdrI* knockout mutant of 7108 containing pBTSA11	This study
Plasmids				
pBT2		6.97	Staphylococcal shuttle vector; Cm^r Em^r	4
pQE31		3.40	Ap^r *E. coli* expression vector which introduces an N-terminal hexahistidyl sequence	Qiagen
pCRII TOPO		3.90	Ap^r Km^r	Invitrogen
pUC18		2.69	Ap^r	Pharmacia
pBK-CMV		4.51	Km^r Nm^r	Stratagene
pBSA1	pBK-CMV	8.58	Km^r Nm^r plasmid containing *S. saprophyticus sdrI* fragment	This study
pBSA2	pCRII TOPO	6.90	Ap^r Km^r plasmid containing *S. saprophyticus sdrI* (promoter region with *sdrI* fragment)	This study
pBTSA1	pBT2	10.96	Cm^r plasmid containing *S. saprophyticus sdrI* fragment	This study
pBTSA2	pBT2	12.40	Cm^r Em^r plasmid containing *ermB* cassette in *S. saprophyticus* SdrI	This study
pEC8	pCRII TOPO	5.40	Ap^r Em^r Km^r plasmid containing *ermB* cassette with PstI restriction sites on both sides	This study
pEC4	pBluescript	4.41	Em^r Ap^r donor of *ermB* cassette	4
pBSA4	pQE 31	4.65	Ap^r expression plasmid containing *S. saprophyticus sdrI* fragment (aa 338-754)[b]	This study
pBSA11	pCRII TOPO	9.58	Complete *sdrI* gene with own promoter	This study
pBTSA11	pBT2	13.37	Complete *sdrI* gene with own promoter	This study
pBSA12	pUC18	5.82	Ap^r plasmid containing *sdrI* fragment (aa 858-1893)	This study
pBSA13	pUC18	7.00	Ap^r Km^r pBSA12 containing transposon (Tn7) insertion	This study

[a] Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Cm^r, chloramphenicol resistance; Nm^r, neomycin resistance.
[b] aa, amino acid.

binant protein expression. Also, we used the pCRII TOPO vector (Invitrogen, Karlsruhe, Germany).

Bacterial growth media and antibiotics. *E. coli* strains harboring plasmids were grown in Luria broth or on L agar. *S. saprophyticus* strain 7108 was grown in peptone yeast extract broth, in tryptic soy broth (TSB; Oxoid, Wesel, Germany), or on agar plates (6). Bacteria were usually incubated at 37°C, but in some experiments 30°C and 42°C were also used. Concentrations of 100 μg/ml ampicillin, 25 μg/ml kanamycin, and 12.5 μg/ml tetracycline were used for selection of plasmids in *E. coli*. For selection of plasmids or chromosomal markers in *S. saprophyticus*, 10 μg/ml chloramphenicol and 5 μg/ml erythromycin were used.

Antisera. For screening of the library, a polyclonal antiserum toward *S. saprophyticus* surface proteins was used (6). A polyclonal antiserum directed to the SdrI A region was generated by subcutaneous immunization of New Zealand White rabbits with the cloned and expressed SdrI A fragment (see below) by using an adjuvant (MPL + TDM + CWS adjuvant system; Sigma, Taufkirchen, Germany). The secondary antiserum was swine anti-rabbit serum (1:20,000) conjugated with alkaline phosphatase (DAKO, Hamburg, Germany) (2). The specificity of the antiserum for SdrI A was verified by inhibition experiments using soluble recombinant antigen to inhibit binding of the antibody to the immobilized antigen.

DNA techniques. (i) DNA manipulation. Restriction, ligation, and transformation were performed by standard techniques as described elsewhere (36). All restriction enzymes were obtained from Roche. T4 ligase (Roche, Mannheim, Germany) was used for ligation.

(ii) DNA preparation. Chromosomal DNA of *S. saprophyticus* strain 7108 was prepared from cleared lysates and purified by double cesium chloride density centrifugation as described previously (8).

(iii) Southern blot hybridization. Southern blot hybridization was performed as previously described (36). Chromosomal DNA was digested with EcoRI, resolved on 0.8% Tris-borate-EDTA gels, and transferred onto positively charged nylon membranes (Roche). Probes were prepared with a PCR labeling kit (Roche). For construction of an *ermB* cassette, primers ermB4R (5′ TCTA GAACTAGTGGATCCC 3′) and ermB4/PstI (5′ tattgtCTGCAGCCGAGAGT GATTGGTCTT 3′, where the lowercase letters indicate nonhomologous parts of the primers with incorporated restriction sites) were used. For construction of the *cat* probe, primers CAT-forw. (5′ GTTACAGTAATATTGACT 3′) and CAT2-rev. (5′ CATAAACAATCCTGCATG 3′) were used. Hybridization and washing were carried out under stringent conditions. Hybridization was done with 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS)–50% formamide at 42°C, and washing was done three times for 20 min each time with 2× SSC–0.1% SDS at 68°C. For detection of hybridized DNA, a digoxigenin luminescence detection kit (Roche) was used. The membrane was subjected to autoradiography with Amersham Hyperfilm MP (Amersham, Freiburg, Germany).

(iv) DNA sequencing. Both strands of the cloned DNA were automatically sequenced (LI-COR DNA Sequencer 4000; LI-COR, Bad Homburg, Germany). For initial sequencing, the standard pUC/M13 primers (labeled with IRD800;

TABLE 2. Cloning and PCR primers used in this study

Primer	Sequence (5′-3′)[a]	Use(s) (location [bp])
N	ACTTTATTGTCATAGTTTAGATCTATTTTG	Sequencing of transposon mutant
S	ATAATCCTTAAAAACTCCATTTCCACCCCT	Sequencing of transposon mutant
95.1.21	ATTCAGTTCATTTATAGC	Inverse PCR (1057-1074)
95.1.22	TTTACAAACATTATCAGC	Inverse PCR (1212-1229)
95.1.25/HindIII	tgataagcttTCATTGAGCAAATATAAAGACTACCTAT	Cloning (5695-5722)
95.1.28	TATCAGCTATTGAACCTT	Cloning (1224-1240)
95.1.29/BamHI	tatggatccGGATAAAAATAGCACAATCGACGAA	Cloning (1011-1035)
95.1.31	ATAGCTTGTTTTGCATAA	Cloning (1044-1061)
95.1.33/HindIII	tgaaaagcttATTATCTCCTTGTGCTGTT	Cloning (2244-2262)
95.1.45/HindIII	tgataagcttCAAGGCTATATTTAGGTGTTTCATA	Cloning (2611-2635)
95.1.54/BamHI	cgcggatccATAGTGCGTAGGAATGCA	Cloning (744-726 [rev])
S.sa_SSP0135_seq3	GAATGCTACAGCTAGTGATGC	PCR of *uaf* gene (884-905)
S.sa_SSP0135_rev2	CCTCAATCTCATCAGGAGATG	PCR of *uaf* gene (1768-1789)
ermB4R	TCTAGAACTAGTGGATCCC	PCR, digoxigenin probe synthesis, cloning
ermB/PstI	tattgtctgcagCCGAGAGTGATTGGTCTT	PCR, digoxigenin probe synthesis, cloning
CAT-forw.	GTTACAGTAATATTGACT	PCR, digoxigenin probe synthesis (183-201)
CAT-rev.	CATAAACAATCCTGCATG	PCR, digoxigenin probe synthesis (872-890)

[a] Restriction sites in the 5′ extensions of primers are underlined. Lowercase letters indicate nonhomologous sequences with added restriction sites.

MWG, Ebersberg, Germany) were used. Extension of DNA sequences was achieved by primer walking.

(v) In vitro transposon mutagenesis. Since the $SD(AD)_{(1-5)}$ repeats were highly conserved, they could not be sequenced by primer walking because it was impossible to find new sequencing primers. The GPS-1 genome priming system (New England Biolabs, GmbH, Frankfurt am Main, Germany) contains a Tn7 transposon-based in vitro system that uses TnsABC transposase to insert a transposon (Transprimer) randomly into the DNA target (40). Primers that bind to sequences of this transposon can then be used to sequence the target. The repeat region was excised from pBSA11 with PstI und HindIII to obtain a 3.2-kb DNA fragment which included the complete $SD(AD)_{(1-5)}$ repeats of the *sdrI* gene. This insert was ligated into pUC18. The resulting plasmid (pBSA12) was incubated with the transposon mixture. After transformation, clones with an inserted transposon cassette were detected by selection with ampicillin and kanamycin. Plasmids that contained the transposons were digested with restriction endonucleases, and clones with centrally inserted transposons were sequenced. Sequencing was performed with primers from both transposon ends (primer N and primer S) and with M13 primers. Sequencing was performed by Seqlab Laboratories (Göttingen, Germany) with a long sequencing gel.

(vi) Screening of the expression library. The lambda ZAP Express library was screened with a polyclonal antibody directed toward *S. saprophyticus* surface proteins (6). Positive clones were converted into phagemids by coinfection of *E. coli* XLOLR and ExAssist helper phage. Phagemids were transformed back into *E. coli* XL1 Blue MRF′.

(vii) Construction of an insertion mutant by allelic replacement. The *sdrI* gene was interrupted by insertion of the *ermB* resistance gene, and pBT2 (4) was used as the replacement vector. For construction of pBTSA2, the *ermB* cassette was amplified from pEC4 with primers ermB4R (5′ TCTAGAACTAGTGGATCCC 3′) and ermB4/PstI (5′ tattgtctgcagCTGCAGCCGAGAGTGATTGGTCTT 3′, where the lowercase letters indicate nonhomologous sequences with added restriction sites), which yielded a PCR product that had PstI sites on both sides. The insert of pBSA1 was excised from the phagemid with SalI and HindIII and ligated into temperature-sensitive replacement vector pBT2. This plasmid, pBTSA1, was digested with PstI and ligated with the erythromycin resistance cassette (*ermB*). This plasmid was designated pBTSA2. By this method, the *sdrI* gene was interrupted at bp 2002.

The pBTSA2 constructs were purified from *E. coli* DH5 by cesium chloride density ultracentrifugation and transformed into *S. saprophyticus* strain 7108 by protoplast transformation (15). Chloramphenicol- and erythromycin-resistant clones were grown in the presence of erythromycin (5 μg/ml at 30°C for 24 h) and used to inoculate 1,000 ml of prewarmed (42°C) broth containing erythromycin (5 μg/ml). After overnight incubation, appropriate dilutions were plated onto P agar containing erythromycin (5 μg/ml). Clones that grew on erythromycin but not on chloramphenicol had lost the plasmid and were checked for SdrI expression.

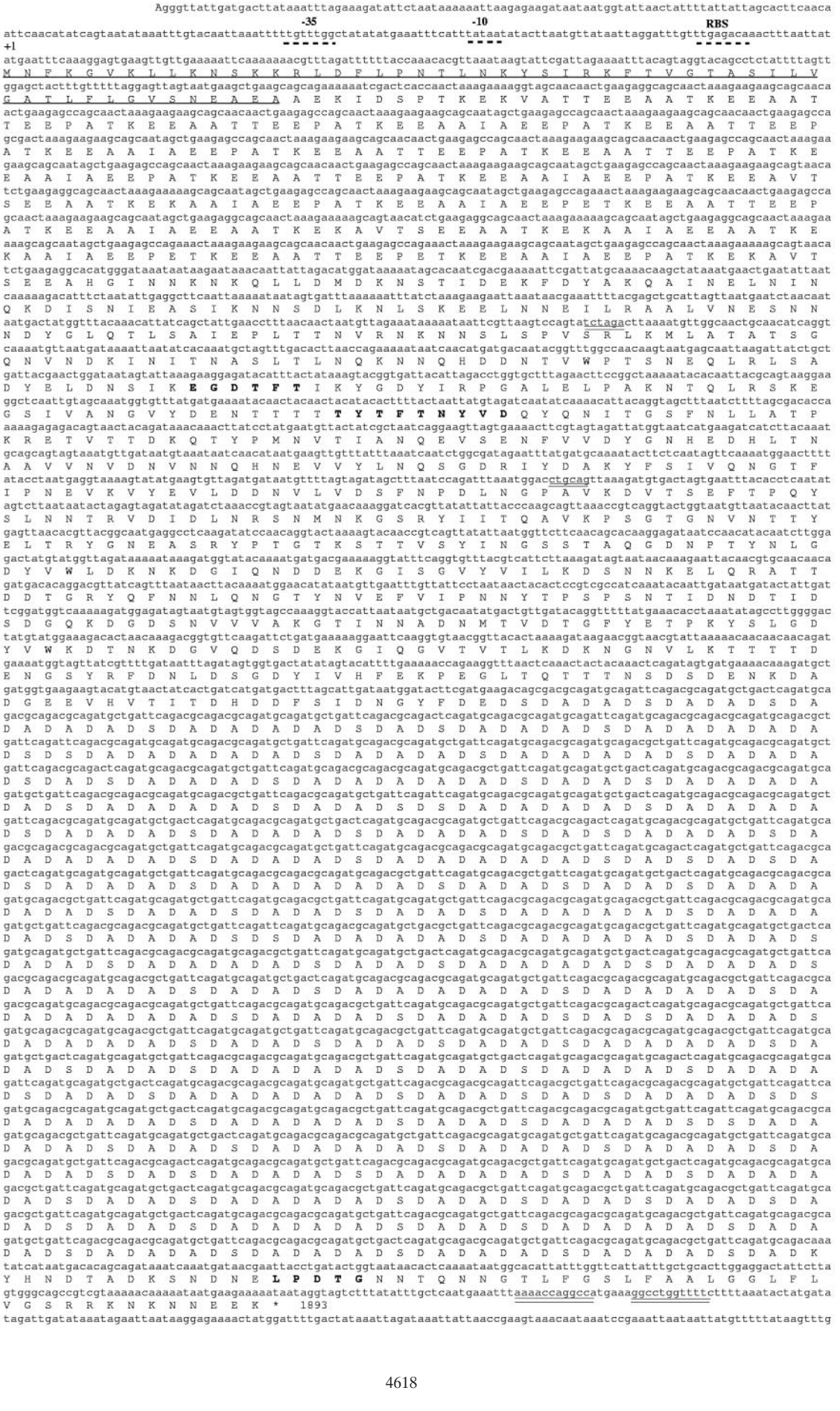
(viii) Complementation of the SdrI-negative mutant. The *sdrI* gene with its own promoter was excised from pBSA11 with BamHI and HindIII and cloned into the pBT2 vector. The resulting plasmid (pBTSA11) was transformed into *E. coli*, purified from this strain, and introduced into the SdrI-negative mutant by protoplast transformation.

Protein techniques. (i) Expression and purification of recombinant SdrI A fragment. The SdrI A domain was amplified by PCR with primer 95.1.29/BamHI and primer 95.1.33/HindIII and with chromosomal DNA from *S. saprophyticus* strain 7108 as the template. The fragment were expressed by induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Gibco-BRL, Karlsruhe, Germany) for 2 to 4 h in *E. coli* strain M15 (pRep4; QIAGEN). Purification of the six-His-tagged fusion protein was performed according to the manufacturer's (QIAGEN) guide.

(ii) PCR for *sdrI* and *uafA*. To test whether the recently described *uafA* gene and/or the *sdrI* gene were present in the *S. saprophyticus* strains used, we used a PCR with specific primers S.sa_SSP0135_seq3 and S.sa_SSP0135_rev2 for *uafA* and 95.1.29/BamHI and 95.1.33/HindIII for *sdrI* (Table 2). As the template, we used genomic DNA prepared from overnight cultures with a QIAamp DNA kit (QIAGEN, Hilden, Germany). The PCR was carried out with *Taq* polymerase for 30 cycles at 95°C for 30 s, 52°C (for *uafA*) or 50°C (for *sdrI*) for 30 s, and 72°C for 1 min. In addition to strains 7108 and CCM 883, we also tested 123 clinical isolates of *S. saprophyticus* which originated from urinary tract infections.

(iii) Binding of bacteria to immobilized collagen. Microtiter plates (Immulon 4; Dynex Technologies, Chantilly, VA) were coated with 1 μg of collagen I (Vitrogen; Cohesion, Palo Alto, CA) in 100 μl of PBS (140 mM NaCl, 0.27 mM KCl, 0.43 mM Na_2HPO_4, 0.147 mM KH_2PO_4, 0.02% NaN_3, pH 7.4) per well overnight at 4°C. Wells were then washed three times with PBS and blocked with 1% bovine serum albumin in PBS for 1 h before the addition of bacteria. Bacteria were grown in 25 ml of PY broth to the mid-logarithmic phase or to the stationary phase, pelleted, washed with PBS, and adjusted to an optical density at 600 nm of 6.0. Bacterial suspensions were added to the wells (100 μl of cells/well), and the plates were incubated for 2 h at room temperature. The bacterial suspensions were carefully aspirated, and the wells were washed twice with 200 μl of PBS. Adherent cells were fixed with 100 μl of 25% aqueous formaldehyde and incubated at room temperature for 30 min. Wells were gently washed two times with 200 μl of PBS, stained with carbol fuchsin for 5 min, washed again with PBS, and read with an enzyme-linked immunosorbent assay plate reader at 550 nm. *Staphylococcus aureus* Cowan I, which is known to express Cna (41), served as the positive control, and *S. aureus* RN 6390 (Cna negative) (11) was used as the negative control. We used *S. saprophyticus* CCM883, which does not bind to collagen (35), as an additional control.

(iv) Flow cytometry. The expression of SdrI by *S. saprophyticus* cells was determined by flow cytometry with a FACScalibur flow cytometer (B-D Biosciences, San Jose, Calif.) equipped with an argon ion laser (488 nm). Overnight cultures (20 ml of TSB) were pelleted and resuspended in PBS. A dilution of bacteria (1:100 in PBS) was incubated with the antiserum against the SdrI A fragment (1:1,000 in PBS) for 1 h at 37°C. After being washed once with PBS, cells were incubated with fluorescein isothiocyanate-conjugated swine anti-rabbit antibody at a 1:20 dilution for 1 h at 37°C. The cells were pelleted and washed twice with PBS. The labeled cell suspensions were aspirated through the flow


-35
-10
RBS
+1

cytometer, and a fluorescence emission measurement was performed in which at least 20,000 events were collected and analyzed with the Cell Quest Pro software provided with the flow cytometer. In some experiments, the bacterial cells were preincubated (2 h) with different concentrations of collagen, SdrI A antiserum was added (diluted 1:1,000 in PBS), and the mixture was incubated (1 h, 37°C) and further processed as described above to show that the antiserum and collagen competed for the same receptor.

Computer analysis. Database and homology searches were carried out by the EMBL services (34) and the NIH BLAST program (1).

SignalP (28, 44) was used for signal sequence prediction.

Nucleotide sequence accession number. The DNA sequence reported in this article has been deposited in the GenBank nucleotide sequence database under accession no. AF402316.

RESULTS

Cloning of *sdrI*. Several plaques that reacted with the antiserum were found, and the DNA of these clones was converted into phagemids. One of these clones was designated pBSA1. Sequencing of the DNA of clone pBSA1 showed an incomplete open reading frame that coded for a protein with similarities to Sdr proteins. For isolation of the complete gene encoding the SdrI protein of *S. saprophyticus*, inverse PCR was used as described previously (35). We religated XbaI-digested chromosomal DNA and used it as a template for inverse PCR with primers 95.1.21 and 95.1.22 (Table. 2). The resulting PCR fragment of 3 kb was reamplified with primers 95.1.28 and 95.1.31, subcloned into the pCRII TOPO vector (Invitrogen), and then sequenced (Fig. 1). By this method, the N-terminal part of the *sdrI* gene was obtained.

Since the SD repeats were very long, we wanted to verify their length and exclude any cloning artifacts (e.g., due to slipped-strand mispairing). We used a primer C terminally and one N terminally of the repeats to amplify the DNA fragment from wild-type strains, the cloning donor, and the phagemid. In all cases, a size of approximately 2.6 kb for the $SD(AD)_{(1-5)}$ repeats was found. Complete sequencing of the $SD(AD)_{(1-5)}$ repeats by primer walking turned out to be impossible because the coding sequence was highly conserved (Fig. 1). To complete the sequence, we constructed clones with in vitro-generated transposon insertions at different positions in the $SD(AD)_{(1-5)}$ repeats and sequenced from primers binding within this transposon. Finally, we determined a size of 854 amino acids for the $SD(AD)_{(1-5)}$ repeats.

Sequence analysis of the SdrI protein. The deduced amino acid sequence of the *S. saprophyticus* SdrI protein predicts a polypeptide of 1,893 amino acids (Fig. 1). The calculated molecular mass of the primary translation product is 195.06 kDa. The primary amino acid sequence organization of the deduced *S. saprophyticus* Sdr protein is similar to that of the Sdr protein family. Also, it shows features typical of cell surface proteins of gram-positive bacteria. These features include positively charged residues (with the amino acid sequence RRKNKNN EEK) at the extreme C terminus, preceded by a hydrophobic membrane-spanning region and an LPXTG motif (Fig. 1). SdrI possesses a signal sequence (54 amino acids) followed by 21 N-terminal repeats with a highly conserved 12-amino-acid-long sequence (Fig. 2). C terminally of the repeat sequence, an A region of 432 amino acids is found. The A region of SdrI contains the TYTFTDYVD motif (Fig. 1).

```
1     AATKEEAATTEE
2     PATKEEAATTEE
3     PATKEEAAIAEE
4     PATKEEAATTEE
5     PATKEEAAIAEE
6     PATKEEAATTEE
7     PATKEEAATTEE
8     PATKEEAAIAEE
9     PATKEEAATTEE
10    PATKEEAAIAEE
11    PATKEEAVTSEE
12    AATKEKAAIAEE
13    PATKEEAAIAEE
14    PETKEEAATTEE
15    PATKEEAAIAEE
16    AATKEKAVTSEE
17    AATKEKAAIAEE
18    AATKEKAAIAEE
19    PETKEEAATTEE
20    PETKEEAAIAEE
21    PATKEKAVTSEE
      . ***:*. :**
```

FIG. 2. Alignment of the 21 N-terminal repeats. The 21 highly conserved N-terminal repeats of SdrI are shown. Conserved residues are in bold type with asterisks below. The sequences were aligned by using CLUSTAL W. Colons, conserved substitutions; periods, semiconserved substitutions.

SdrI possesses two B repeat regions (Fig. 1). The repeats are composed of 110 and 119 amino acids. Finally, it has long, highly conserved $SD(AD)_{(1-5)}$ repeats with a size of 854 amino acids.

Construction of an *sdrI*-isogenic mutant of *S. saprophyticus*. To investigate the function of SdrI, an *sdrI* knockout mutant was constructed. Plasmid construct pBTSA2, which contains the *sdrI* gene interrupted by an *ermB* cassette, was transformed into *S. saprophyticus* strain 7108, cells were cured of the plasmid by cultivation at 42°C, and insertion mutants were selected with erythromycin. Insertion of *ermB* and loss of *cat* were verified by Southern hybridization and PCR as shown in Fig. 3c. Also, the correct position of the insertion was verified by sequencing.

Loss of SdrI expression in the knockout mutant was verified by fluorescence-activated cell sorting with antiserum against the A region of SdrI. In contrast to the wild type, the knockout mutant showed no expression of the SdrI protein (Fig. 4). The reaction of the mutant with the specific antiserum was virtually identical to that of the wild type with preimmune rabbit serum (Fig. 4).

FIG. 1. SdrI nucleotide and amino acid sequences. The putative promoter region (−10 and −35) and the RBS (ribosome-binding site) are underlined with broken lines. The hypothetical signal sequence is underlined with a solid line. The conserved amino acid motifs TYTFTNYVD and LPXTG are in bold. Domains A, B1, B2, and R start at residues 324 (A), 755 (B1), 874 (B2), and 984 (R). The N-terminal repeats are found between residues 72 and 323. The XbaI and PstI restriction sites are also indicated by double underlining. Termination sites for transcription are indicated by double underlining after the stop codon. The numbers on the left show the nucleic acid and amino acid numbers.

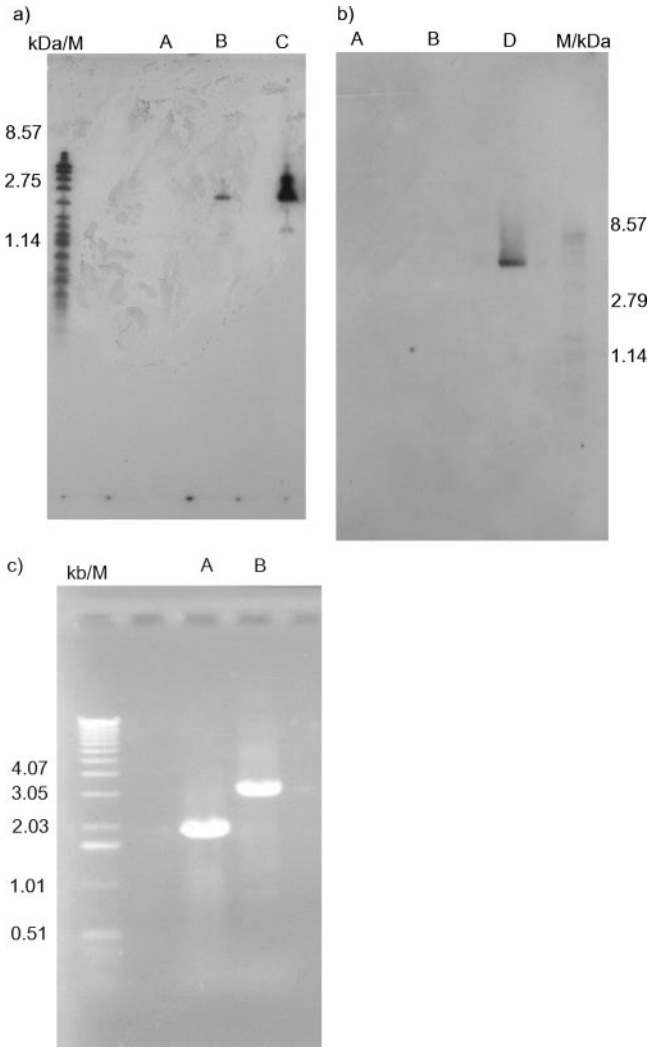


FIG. 3. The mutant contains one *ermB* insertion only. Southern hybridization of the knockout mutant and wild-type strain 7108 with *ermB* and *cat* sequences (a and b). Lanes M contained molecular weight marker II (labeled with digoxigenin; Roche, Mannheim, Germany). Lanes A, chromosomal DNA of *S. saprophyticus* strain 7108 digested with EcoRI; lanes B, chromosomal DNA of *sdrI* knockout mutant digested with EcoRI; lane C, *ermB* cassette of pEC4 digested with EcoRI (used as a positive control); lane D, *cat* cassette of pBT2 digested with EcoRI (used as a positive control). Blot a was hybridized with the *ermB* probe, and blot b was hybridized with the *cat* probe. (c) PCR analysis of chromosomal DNAs of wild-type strain 7108 (lane A) and of the *sdrI* mutant (lane B) with primers 95.1.29/BamHI and 95.1.30/HindIII. The size difference amounts to that of the inserted cassette (*ermB*). Lanes M, molecular size standards.

***S. saprophyticus sdrI* mutant shows decreased binding to collagen.** Since binding to collagen has been described for some *S. saprophyticus* strains (33, 35), we analyzed the binding of strain 7108, the mutant, and the complemented strain to collagen-coated microtiter wells (Fig. 5). The binding of the mutant was drastically reduced compared with that of the wild-type strain. This difference was highly significant ($P < 0.0001$, *t* test). Reintroduction of the gene into the mutant reconstituted collagen binding (Fig. 5). The positive control (*S. aureus* Cowan I) (41) showed the expected binding to the

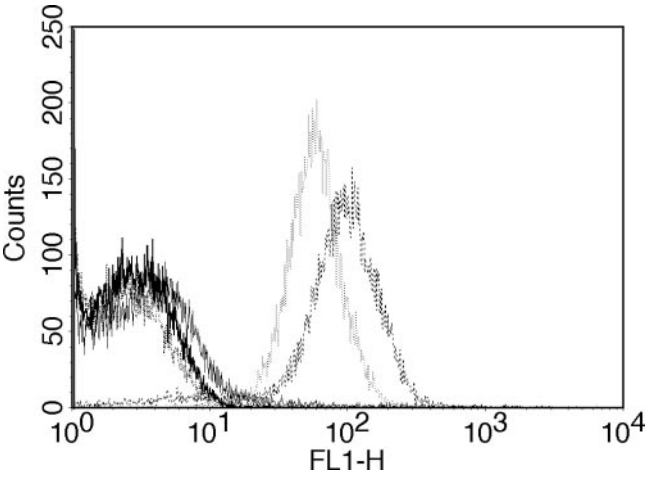


FIG. 4. Fluorescence-activated cell sorter analysis of wild-type, mutant, and complemented strains. Wild-type *S. saprophyticus* strain 7108, the *sdrI* knockout mutant, and the complemented mutant were grown overnight in TSB. The bacteria were incubated with antiserum against the SdrI A region, and binding of the antiserum was detected with fluorescein isothiocyanate-labeled anti-rabbit immunoglobulin G. Fluorescence intensity (FL1-H) of particles was counted. The wild-type and complemented strains show high proportions of labeled cells, whereas the mutant does not bind the specific antiserum, as shown by the similarity of the reaction to that obtained with preimmune rabbit serum.

matrix protein, whereas the negative control, *S. aureus* RN 6390 (11), did not bind to collagen-coated wells. In addition, type strain CCM 883, which does possess *uafA* but not *sdrI* (20), did not attach to collagen. Strain 7108 contained both *uafA* and *sdrI*, as analyzed by PCR (data not shown). Binding of strain 7108 to immobilized collagen could be inhibited by

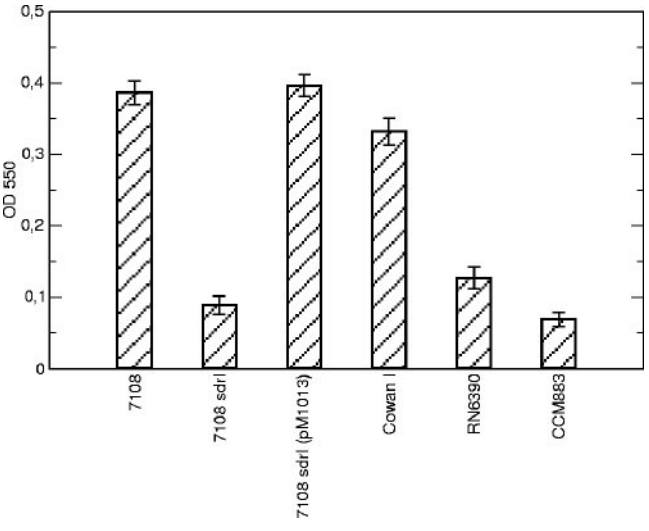


FIG. 5. Adherence of *S. saprophyticus* wild-type strain 7108 and the SdrI mutant to immobilized collagen. Cell suspensions of both strains were added to microtiter wells coated with collagen and incubated for 3 h at 37°C. The adherent bacterial films were detected photometrically (405 nm) with an enzyme-linked immunosorbent assay plate reader. The mean of at least four repetitions for each strain is depicted along with its standard deviation. The difference in binding to collagen between the mutant and wild type was highly significant (*t* test, $P < 0.0001$). OD, optical density.

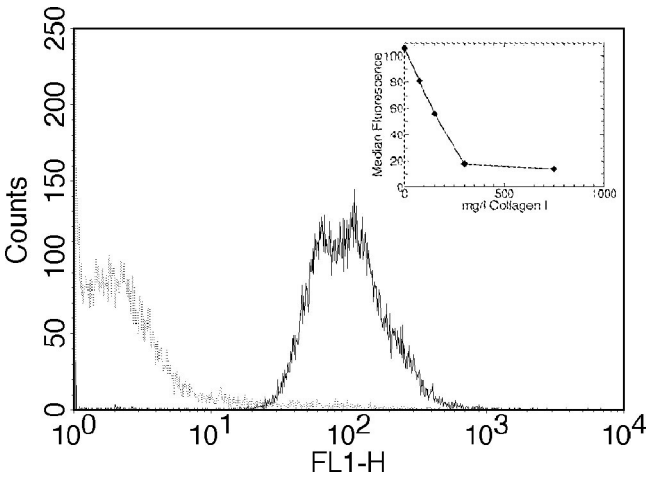


FIG. 6. Collagen inhibits binding of SdrI A antiserum to *S. saprophyticus*. Binding of anti-SdrI A antiserum to SdrI was inhibited by increasing concentrations of collagen. Reactions of the antiserum with the wild-type strain without collagen and with 1,500 μg/ml collagen are shown. The inset shows the effects of different collagen concentrations.

the antiserum to SdrI A (data not shown). In addition, binding of the SdrI A antiserum to the wild-type strain could be inhibited by preincubation with collagen (Fig. 6).

Prevalence of the *sdrI* gene in *S. saprophyticus*. Clinical isolates of *S. saprophyticus* (123 strains), strain 7108, and type strain CCM883 were tested by PCR for the presence of the *sdrI* and *uafA* gene sequences (primers 95.1.29/BamHI and 95.1.45/HindIII for *sdrI* and S.sa_SSP0135_seq3 and S.sa_SSP0135_rev2 for *uafA*). We found *sdrI* in 11% of the strains and *uafA* in 94%. Strain 7108 contained *uafA* and *sdrI*, whereas strain CCM 883 harbored solely *uafA*.

DISCUSSION

S. saprophyticus is an important cause of urinary tract infections, especially in young female outpatients. In earlier experiments, it has been shown that the urease of this organism acts as an important virulence factor (8, 9) by contributing to pathogenicity in the bladder. Also, hemagglutination of sheep erythrocytes (10, 16), adherence to uroepithelial cells (26), and binding to laminin (33), fibronectin (7, 9, 42), and collagen (33, 35) have been described for *S. saprophyticus*.

Two major surface proteins have been described, an autolysin (Aas), a multifunctional protein without an LPXTG motif that is responsible for cell wall turnover and fibronectin binding and may be involved in the agglutination of sheep erythrocytes (7, 9, 15), and the *S. saprophyticus* surface-associated protein (Ssp), a 95-kDa protein (6) that exhibits lipase activity (35). In contrast to GehD (3), the lipase of *Staphylococcus epidermidis*, Ssp, is not involved in collagen binding (35). Interestingly, the type strain of *S. saprophyticus*, CCM883 (the same as recently sequenced strain ATCC 15305), hemagglutinates sheep erythrocytes but does not bind to collagen (20, 35). Hemagglutination is apparently caused by UafA, the only protein with a cell wall anchor that is present in the genomic sequence of this strain (20). We therefore sought to identify an additional collagen-binding factor.

For cloning of the *sdrI* gene, we used a polyclonal antibody directed toward *S. saprophyticus* surface proteins to screen a lambda ZAP Express library of hemagglutinating and collagen-binding strain 7108 (15, 35). The inserts of the clones obtained by this screening showed an N-terminally incomplete open reading frame. We used inverse PCR to complete the N terminus. Sequencing showed that the reading frame of the *sdrI* gene of *S. saprophyticus* codes for a 1,893-amino-acid-long polypeptide with a deduced molecular mass of 195.06 kDa. Homology analysis suggested that the gene is a member of the Sdr surface protein family (19, 23). It contains motifs typical of gram-positive surface proteins (LPXTG, membrane-spanning region, charged C terminus), as well as an A region containing the TYTFTDYVD motif, two B repeats, and a highly repetitive sequence containing only the amino acids serine, asparagine, and alanine.

Sequencing of the $SD(AD)_{(1-5)}$ repeats was technically demanding and necessitated the use of in vitro transposon mutagenesis to insert Tn*7* into the conserved repeats. The sequence of the repeat region was completed with primers from the vector and primers from the transposon. SdrI of *S. saprophyticus* contains one of the longest $SD(AD)_{(1-5)}$ amino acid sequences described so far, comprising 854 amino acid residues (18, 19, 23, 24).

In addition, the amino acid composition of this repeat sequence is different from that of other SD repeats. This region contains a very high percentage of alanine residues (37%). The presence of alanine has been described in other Sdr proteins but at lower percentages (2.3% for ClfA [14, 24, 25] and 4.7% for SdrF [23]). The second known cell wall-anchored protein of *S. saprophyticus*, UafA, also contains a C-terminal repeat region which is composed of conserved SESESL repeats spanning 1,451 amino acids (20).

The lengths of the SD repeat regions differ considerably among SD proteins, with only 56 for SdrG (23), 308 for ClfA (24, 25), 558 for SdrF (23), and 854 for SdrI. Although the function of the SD repeat region and of its variation is not understood, experiments with ClfA showing that at least 72 residues of the SD repeats are needed for efficient binding of *S. aureus* to fibrinogen (14) indicate that a minimal length of this region is required for correct surface presentation of ECM-binding domain A.

In contrast to the Sdr proteins identified so far, SdrI possesses 21 12-amino-acid-long repeats with a consensus sequence of (P/A)ATKE(K/E)A(A/V)(T/I)(A/T/S)EE located between the signal sequence and A region. Most interestingly, Aas, an autolysin of *S. saprophyticus*, also contains N-terminal repeats but with a distinctively different amino acid sequence (15); UafA, however, does not contain such repeats (20). To date, the function(s) of these repeats is unknown.

The 432-amino-acid A region of *S. saprophyticus* SdrI is located between the 21 N-terminal repeats and the B repeats. Comparisons revealed that the A region of SdrI is 55% identical to the A region of the SdrF protein of *S. epidermidis* (23). The TYTFTDYVD motif, whose function is unknown and which is present in all of the Sdr proteins described so far (19), is also found in the A region of SdrI (Fig. 1).

The two B repeat regions of SdrI are located between the A region and the $SD(AD)_{(1-5)}$ repeats. The repeats are composed of 110 and 119 amino acids, and each contains a Ca^{2+}-binding

EF-hand motif with a 12-amino-acid consensus sequence [DX(N/D)X(D/N)GXX(D/N/G)XX(E/D)] (18). Although it has been shown in vitro for SdrD that the EF hands are functional (18), their physiological role remains elusive. For Cna, the collagen-binding MSCRAMM of *S. aureus* (11, 13, 31, 41, 32), it was shown that binding of the ECM is not dependent on the presence of the B repeats (13, 32).

For further analysis of the function of *S. saprophyticus* SdrI, an SdrI knockout mutant of strain 7108 was constructed by inserting a single *ermB* cassette at amino acid position 667. In adherence assays with immobilized collagen, the mutant showed less binding than the wild type, which could be restored by introduction of a plasmid carrying *sdrI*. This result indicates that SdrI is responsible for collagen binding by *S. saprophyticus*. Binding of whole bacteria to immobilized collagen could be inhibited by the antiserum directed toward the A domain of SdrI, and binding of this antibody to *S. saprophyticus* could be inhibited by collagen. These results indicate that the SdrI A region is specifically involved in collagen binding. CCM883, which does express UafA and is SdrI negative, does not bind to collagen, which indicates that UafA is not involved in collagen binding. Our experiments with wild-type strain 7108 (*sdrI* and *uafA* positive) and its SdrI-negative mutant support this notion since the *sdrI* mutant did not bind to collagen. In *E. coli*, collagen binding is thought to play an important role in the pathogenesis of urinary tract infections (38), and it is tempting to speculate that it acts in a similar manner in *S. saprophyticus*. Sequences encoding SdrI were found at a comparatively low percentage (11%) only. It is not known if the protein is associated with increased pathogenicity or is another example of a redundantly encoded adhesin in staphylococci.

In this study, we characterized an Sdr protein of *S. saprophyticus*. In contrast to other SD repeat proteins, SdrI carries 21 additional N-terminal repeats and has the longest SD repetitive region described so far. In addition, it is the first characterized collagen-binding protein of *S. saprophyticus* and the second example of a surface protein of *S. saprophyticus* carrying the LPXTG motif.

Our experiments suggest that, similar to other staphylococci, binding to matrix proteins and adhesion are redundantly encoded in *S. saprophyticus* and support the notion that the presence of Sdr proteins is common in staphylococci.

ACKNOWLEDGMENTS

Parts of this work were supported by the Deutsche Forschungsgemeinschaft (grants DFG Ga 352/5-1 and Ga 352/5-2) and by the Ruhr-Universität Bochum (FoRUM F2 11/00).

The excellent technical assistance of S. Ortmann and S. Friedrich is gratefully acknowledged.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Blake, M. S., G. J. Johnston, G. J. Russel-Jones, and E. C. Gotschlich.** 1984. A rapid sensitive method for the detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136:**175–179.
3. **Bowden, M. G., L. Visai, C. M. Longshaw, K. T. Holland, P. Speziale, and M. Höök.** 2002. Is the GehD lipase from *Staphylococcus epidermidis* a collagen binding adhesin? J. Biol. Chem. **277:**43017–43023.
4. **Brückner, R.** 1997. Gene replacement in *Staphylococcus carnosus* and *Staphylococcus xylosus*. FEMS Microbiol. Lett. **151:**1–8.
5. **Cheung, A. I., S. J. Projan, R. E. Edelstein, and V. A. Fischetti.** 1995. Cloning, expression, and nucleotide sequence of a *Staphylococcus aureus* gene (*fbpA*) encoding a fibrinogen-binding protein. Infect. Immun. **63:**1914–1920.
6. **Gatermann, S. G., B. Kreft, R. Marre, and G. Wanner.** 1992. Identification and characterization of a surface-associated protein (Ssp) of *Staphylococcus saprophyticus*. Infect. Immun. **60:**1055–1060.
7. **Gatermann, S., and H. G. Meyer.** 1994. *Staphylococcus saprophyticus* hemagglutinin binds fibronectin. Infect. Immun. **62:**4556–4563.
8. **Gatermann, S., and R. Marre.** 1989. Cloning and expression of *Staphylococcus saprophyticus* urease gene sequences in *Staphylococcus carnosus* and contribution of the enzyme to virulence. Infect. Immun. **57:**2998–3002.
9. **Gatermann, S., H. G. Meyer, and G. Wanner.** 1992. *Staphylococcus saprophyticus* hemagglutinin is a 160-kilodalton surface polypeptide. Infect. Immun. **60:**4127–4132.
10. **Gatermann, S., R. Marre, J. Heesemann, and W. Henkel.** 1988. Hemagglutinating and adherence properties of *Staphylococcus saprophyticus*: epidemiology and virulence in experimental urinary tract infection of rats. FEMS Microbiol. Immunol. **47:**179–186.
11. **Gillaspy, A. F., C. Y. Lee, S. Sau, A. L. Cheung, and M. S. Smeltzer.** 1998. Factors affecting the collagen binding capacity of *Staphylococcus aureus*. Infect. Immun. **66:**3170–3178.
12. **Grant, S. G., J. Jessee, F. R. Bloom, and D. Hanahan.** 1990. Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. Proc. Natl. Acad. Sci. USA **87:**4645–4649.
13. **Hartford, O., D. McDevitt, and T. J. Foster.** 1999. Matrix-binding proteins of *Staphylococcus aureus*: functional analysis of mutant and hybrid molecules. Microbiology **145:**2497–2505.
14. **Hartford, O., P. Francois, P. Vaudaux, and T. J. Foster.** 1997. The dipeptide repeat region of the fibrinogen-binding protein (clumping factor) is required for functional expression of the fibrinogen-binding domain on the *Staphylococcus aureus* cell surface. Mol. Microbiol. **25:**1065–1076.
15. **Hell, W., H. G. 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.
16. **Hovelius, B., and P. A. Mardh.** 1979. Haemagglutination by *Staphylococcus saprophyticus* and other staphylococcal species. Acta Pathol. Microbiol. Scan. Sect. B **87:**45–50.
17. **Jönsson, K., C. Signäs, H. P. Müller, and M. Lindberg.** 1991. Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. Eur. J. Biochem. **202:**1041–1048.
18. **Josefsson, E., D. O′Connell, T. J. Foster, I. Durussel, and J. A. Cox.** 1998b. The binding of calcium to the B-repeat segment of SdrD, a cell surface protein of *Staphylococcus aureus*. J. Biol. Chem. **273:**31143–31152.
19. **Josefsson, E., K. W. McCrea, D. Ni Eidhin, D. O′Connell, J. Cox, M. Höök, and T. J. Foster.** 1998. Three new members of the serine-aspartate repeat protein multigene family of *Staphylococcus aureus*. Microbiology **144:**3387–3395.
20. **Kuroda, M., A. Yamashita, H. Hirakawa, M. Kumano, K. Morikawa, M. Higashide, A. Maruyama, Y. Inose, K. Matoba, H. Toh, S. Kuhara, M. Hattori, and T. Ohta.** 2005. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc. Natl. Acad. Sci. USA **102:**13272–13277.
21. **Liang, O. D., J. I. Flock, and T. Wadstrom.** 1995. Isolation and characterization of a vitronectin-binding surface protein from *Staphylococcus aureus*. Biochim. Biophys. Acta **1250:**110–116.
22. **Liu, Y., B. Ames, E. Grovits, B. D. Prater, P. Syribeys, J. H. Vernachio, and J. M. Patti.** 2004. SdrX, a serine-aspartate repeat protein expressed by *Staphylococcus capitis* with collagen VI binding activity. Infect. Immun. **72:**6237–6244.
23. **McCrea, K. W., O. Hartford, S. Davis, D. Ni Eidhin, G. Lina, P. Speziale, T. J. Foster, and M. Höök.** 2000. The serine-aspartate repeat (Sdr) protein family in *Staphylococcus epidermidis*. Microbiology **146:**1535–1546.
24. **McDevitt, D., and T. J. Foster.** 1995. Variation in the size of the repeat region of the fibrinogen receptor (clumping factor) of *Staphylococcus aureus* strains. Microbiology **141:**937–943.
25. **McDevitt, D., P. Francois, P. Vaudaux, and T. J. Foster.** 1994. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. Mol. Microbiol. **11:**237–248.
26. **Meyer, H.-G. W., U. Wengler-Becker, and S. G. Gatermann.** 1996. The hemagglutinin of *S. saprophyticus* is a major adhesin for uroepithelial cells. Infect. Immun. **64:**3893–3896.
27. **Ni Eidhin, D., S. Perkins, P. Francois, P. Vaudaux, M. Hook, and T. J. Foster.** 1998. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. Mol. Microbiol. **30:**245–257.
28. **Nielsen, H., J. Engelbrecht, S. Brunak, and G. von Heijne.** 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. **10:**1–6.
29. **Park, P. W., D. D. Roberts, L. E., Grosso, W. C. Parks, J. Rosenbloom, W. R. Abrams, and R. P. Mecham.** 1991. Binding of elastin to *Staphylococcus aureus*. J. Biol. Chem. **266:**23399–23406.
30. **Patti, J. M., B. L. Allen, M. J. McGavin, and M. Höök.** 1994. MSCRAMM-mediated adherence of microorganisms to host tissues. Annu. Rev. Microbiol. **48:**585–617.
31. **Patti, J. M., H. Jonsson, B. Guss, L. M. Switalski, K. Wiberg, M. Lindberg, and M. Höök.** 1992. Molecular characterization and expression of a gene

encoding a *Staphylococcus aureus* collagen adhesin. J. Biol. Chem. **267:**4766–4772.
32. **Patti, J. M., K. House-Pompeo, J. O. Boles, N. Garza, S. Gurusiddappa, and M. Höök.** 1995. Critical residues in the ligand-binding site of the *Staphylococcus aureus* collagen-binding adhesin (MSCRAMM). J. Biol. Chem. **270:**12005–12011.
33. **Paulsson, M., A. Ljungh, and T. Wadström.** 1992. Rapid identification of fibronectin, vitronectin, laminin, and collagen cell surface binding proteins on coagulase-negative staphylococci by particle agglutination assays. J. Clin. Microbiol. **30:**2006–2012.
34. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
35. **Sakinc, T., M. Woznowski, M. Ebsen, and S. G. Gatermann.** 2005. The surface-associated protein of *Staphylococcus saprophyticus* is a lipase. Infect. Immun. **73:**6419–6428.
36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
37. **Schneewind, O., A. Fowler, and K. F. Faull.** 1995. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. Science **268:**103–106.
38. **Selvarangan, R., P. Goluszko, J. Singhal, C. Carnoy, S. Moseley, B. Hudson, S. Nowicki, and B. Nowicki.** 2004. Interaction of adhesin with collagen type IV is a critical step in *Escherichia coli* renal persistence. Infect. Immun. **72:**4827–4835.
39. **Signas, C., G. Raucci, K. Jonsson, P. E. Lindgren, M. Anantharamaiah, M. Höök, 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.
40. **Stellwagen, A. E., and N. L. Craig.** 1997. Gain-of-function mutations in TnsC, an ATP-dependent transposition protein that activates the bacterial transposon Tn7. Genetics **145:**573–585.
41. **Switalski, L. M., P. Speziale, and M. Höök.** 1989. Isolation and characterization of a putative collagen receptor from *Staphylococcus aureus* strain Cowan 1. J. Biol. Chem. **264:**21080–21086.
42. **Switalski, L. M., C. Ryden, K. Rubin, A. Ljungh, M. Höök, and T. Wadström.** 1983. Binding of fibronectin to *Staphylococcus* strains. Infect. Immun. **42:**628–633.
43. **Wann, E. R., S. Gurusiddappa, and M. Höök.** 2000. The fibronectin binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. J. Biol. Chem. **275:**13863–13871.
44. **Wilkins, M. R., E. Gasteiger, R. D. Appel, and D. F. Hochstrasser.** 1999. Protein identification and analysis tools in the ExPASy server. Methods Mol. Biol. **112**:531–552

Editor: V. J. DiRita