Staphylococcus saprophyticus surface-associated protein (Ssp) is associated with lifespan reduction in Caenorhabditis elegans

Florian Szabados*, Amelie Mohner, Britta Kleine, and Sören G Gatemann

Institute for Hygiene and Microbiology; Department of Medical Microbiology; Ruhr-University Bochum; Bochum, Germany

Keywords: Staphylococcus saprophyticus, Ssp, lipase, C. elegans, lifespan reduction

Staphylococcal lipases have been proposed as pathogenicity factors. In Staphylococcus saprophyticus the surface-associated protein (Ssp) has been previously characterized as a cell wall-associated true lipase. A S. saprophyticus Δssp:ermB mutant has been described as less virulent in an in vivo model of urinary tract infection compared with its wild-type. This is the first report showing that S. saprophyticus induced a lifespan reduction in Caenorhabditis elegans similar to that of S. aureus RN4220. In two S. saprophyticus Δssp:ermB mutants lifespan reduction in C. elegans was partly abolished.

In order to attribute virulence to the lipase activity itself and distinguish this phenomenon from the presence of the Ssp-protein, the conserved active site of the lipase was modified by site-directed ligase-independent mutagenesis and lipase activity-deficient mutants were constructed. These results indicate that the Ssp is associated with pathogenicity in C. elegans and one could speculate that the lipase activity itself is responsible for this virulence.

Introduction

Staphylococcus saprophyticus causes mainly urinary tract infections, especially in young female outpatients.1 Severe systemic infections also occur rarely.2-4 The abundant surface associated protein of S. saprophyticus (Ssp) has been characterized as a non-covalently bound, surface-associated, true lipase.5-6 Even though some reports have been published with regard to other staphylococcal lipases and their contribution to virulence,7,8 no data concerning a function of a lipase in the urinary tract with regard to pathogenicity has been published yet. In addition, lipids have not been described in human urine. Lipases hydrolyze triacylglycerols into free fatty acids and glycerol.

Staphylococcal lipases have been proposed as pathogenicity factors in addition to having a processing role in the lipid metabolism. True lipases have been described for Staphylococcus aureus and Staphylococcus epidermidis isolated from human facial sebaceous skin.11 In previous experiments it has been shown that the lipase of S. saprophyticus (Ssp) is a surface-bound, true lipase,4 with similarities to lipases of S. aureus and S. epidermidis.5 In an in vivo model of urinary tract infection an S. saprophyticus Δssp:ermB mutant is less virulent compared with its wild-type,7 indicating an involvement of Ssp in the virulence of urinary tract infections due to S. saprophyticus.

Therefore the question was raised whether the lipase activity itself or the presence of the lipase protein is responsible for this previously described virulence. In uropathogenic Escherichia coli (UPEC), a lipase activity has not been described. UPECs adhere to uroepithelial cells via type 1 pili and interact with the uropilakin receptor, which is located within lipid rafts.13-15 It has been shown that UPECs invade into the human bladder epithelial cells and also into the human bladder carcinoma cell line 5637.15 Interestingly, S. saprophyticus also invades this cell line.16 Nevertheless, the pathways of the interaction of the gram-positive S. saprophyticus with the eukaryotic bladder host cells is widely unknown but likely different from that of the gram-negative UPECs since structures similar to that of type 1 pili of UPECs have not been described in S. saprophyticus.

We hypothesized that Ssp might modify the composition of lipid rafts or might be part of a phagosomal/phagolysosomal escape mechanism.

Whether this virulence is associated in staphylococci to the lipase activity or to the lipase protein has not been yet described in literature. In order to attribute the virulence to the lipase function, function-deficient mutants of Ssp were constructed.

In order to analyze if the lipolytic activity of Ssp is needed for virulence or if the presence of the protein is sufficient we constructed S. saprophyticus expressing an active protein. A Caenorhabditis elegans killing model was chosen, which has been extensively described as a virulence model for a variety of pathogens and also in staphylococci other than S. saprophyticus.17-19

In addition the suspected virulence of the Ssp shall be retested in an independent second model.
Results and Discussion

Construction of putative lipase function-deficient mutant. In order to construct lipase activity-deficient mutants, the putative active site of the lipase, the Ser_{482}^c, Asp_{673}^c, and His_{712}^c-catalytic triad was individually mutated. In addition a homologous ssp-knockout mutant was constructed (MB119). All three amino acids putatively involved in the active site were mutated. Using site-directed, ligase-independent mutagenesis, three lipase derivatives with mutations S482C (MB120), D673S (MB121), and H712P (MB122) were cloned. The mutated spc-genes were introduced into a lipase-deficient knockout mutant (S. saprophyticus 9325 Δssp::ermB) by protoplast transformation (Table 1).

The S. saprophyticus wild-type strain and the mutants were tested with regard to their lipase activity by use of a previously described lipase agar assay enhanced by supplementing gum arabic. The lipase activity of the wild-type S. saprophyticus 9325 was similar to that of S. saprophyticus 7108.

The lipase activity was present in the S. saprophyticus 9325 wild-type strain, abrogated in the S. saprophyticus 9325 Δssp::ermB mutant (MB119) and was also abrogated in this mutant harbouring plasmids with the ssp_{S482C} (MB120), ssp_{D673S} (MB121), or ssp_{H712P} (MB122) containing mutations in the catalytic triad (Fig. 1). The lipase activity was restored in the S. saprophyticus 7108 Δssp::ermB (pMB1108) complemented by the ssp gene as previously shown.

Lifespan reduction of C. elegans. S. saprophyticus strain 9325, a clinical isolate of human urinary tract infection, induced a lifespan reduction in C. elegans compared with the non-pathogenic feeding strain OP50 and heat-inactivated S. aureus RN4220. The lifespan reduction was similar to that of the live S. aureus RN4220 strain (Fig. 2).

Initially the wild-type strains of S. saprophyticus 7108 and 9325 were tested in the C. elegans model. Ours is the first study describing lifespan reduction of C. elegans by S. saprophyticus similar to that of S. aureus. In the wild-type S. saprophyticus 7108, a lifespan reduction of C. elegans was detected, but partly abolished in its Δssp::ermB mutant (Fig. 3). In addition to the results obtained with the strain 7108, lifespan was also reduced in the wild-type S. saprophyticus 9325, and partly abolished in its Δssp::ermB mutant (Fig. 4). The abolished lifespan reduction in S. saprophyticus 7108 Δssp::ermB was again restored in experiments using this previously described mutant bearing a plasmid with the wild-type ssp gene (Fig. 3).

This finding strongly suggests that Ssp is a virulence factor in C. elegans.

Table 1. Bacteria and plasmids used

| Species | Strain | Properties | Source |
|---------|--------|------------|--------|
| S. aureus | Cowan I | ATCC 12589 | 30 |
| E. coli | DH5a | F endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG-F'80dlacZΔM15 Δ[lacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r~ m~ λ~)] OP50 C. elegans feeding strain | |
| S. saprophyticus | 9325 | Wild-type, clinical isolate | 29 |
| MB110 | MB120 | 9325 Δssp::ermB+pMB1121 | This study |
| MB121 | MB122 | 9325 Δssp::ermB+pMB1122 | This study |
| 7108 | 7108 Δssp::ermB | 29 |
| MB123 | 7108 Δssp::ermB+pMB1108 | 4 |

Plasmids

| pMB1103 | Ssp gene with its own promoter | 4 |
| pMB1106 | Ssp gene interrupted by ermB used for homologous recombination | 4 |
| pMB1108 | Complete ssp gene (insert from pMB1103 cloned into vector part of pPS44) | 4 |
| pUC18 | General cloning-vector | |
| pRB473 | General shuttle-vector | 26 |
| pMB1109 | pUC18 + ssp_{S482C} and its own promoter | This study |
| pMB1110 | pUC18 + ssp_{D673S} and its own promoter | This study |
| pMB1111 | pUC18 + ssp_{H712P} and its own promoter | This study |
| pMB1121 | pRB473 + ssp_{S482C} and its own promoter | This study |
| pMB1122 | pRB473 + ssp_{D673S} and its own promoter | This study |
| pMB1123 | pRB473 + ssp_{H712P} and its own promoter | This study |

©2013 Landes Bioscience. Do not distribute.
Since staphylococcal lipases may possess additional activity, such as adhesion to collagen, we wanted to test whether the lipase activity or the Ssp-protein is responsible for pathogenicity. Therefore, lipase-deficient mutants harboring plasmids with the ssp\_S482C, ssp\_D673S, or ssp\_H712P genes containing mutations in the lipase catalytic triad were tested in a C. elegans killing model.

In all three cases (MB120, MB121, and MB122) the lifespan reduction of C. elegans was similar to that of the Δssp::ermB mutant and different from that of the wild-type (sign test, \( P = 0.01 \)). One might speculate that the lipase activity of S. saprophyticus itself is responsible for virulence in C. elegans (Fig. 4). Nevertheless, a drawback of this strain set is that a functionally complemented mutant of these lipase function-deficient mutants (MB120, MB121, and MB122) is lacking.

The mechanism of the virulence in C. elegans has not yet been published in staphylococci, even though multiple factors have been suspected similar to other species. Most isolates of S. saprophyticus has been described to produce a fatty acid modifying enzyme (FAME)-activity (esterification) in addition to the lipase activity. One might speculate that the Ssp lipase activity could be involved in lipid modification, not only in the degradation, but also in the production of toxic lipids. Another hypothesis is an involvement of extracellular matrix in the lifespan reduction in C. elegans. The capsule of S. saprophyticus strain 7108 has been recently characterized and differs from that of S. aureus and S. epidermidis, since different cup genes were expressed. In addition, the S. saprophyticus 9325 Δssp::ermB mutant and restored in the complemented mutant. In the S. saprophyticus 9325 Δssp::ermB mutant the lipase activity was abolished. In this mutant harboring plasmids with the ssp\_S482C, ssp\_D673S, or ssp\_H712P genes containing mutations in the lipase catalytic triad also did not show lipase activity. Experiment was repeated three times on different days. The opaque zone was determined in mm ± SD.
also relevant in human urinary tract infection is speculation, since no data in humans with regard to *S. saprophyticus* lipase activity has been published yet. Our results indicate that the lipase activity of Ssp was abolished when the putative catalytic triad in *S. saprophyticus* was modified. In addition, one might speculate that the lipase activity of *S. saprophyticus* itself and not only the presence of the Ssp protein is responsible for virulence in *C. elegans*.

Materials and Methods

Bacterial strains. The strains *S. saprophyticus* 9325 and *S. saprophyticus* 7108 were collected from human urinary tract infections and have been described previously (Table 1). The species-diagnosis was confirmed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry\(^2\) and amplification and sequencing of the *soda* gene,\(^2\) as described previously.

Growth conditions. *E. coli* DH5α used for cloning was grown in Luria Bertani (LB) medium (Invitrogen) at 37 °C. Staphylococci were grown at 37 °C in LB or PY-medium (10 g L\(^{-1}\) pepton (Oxoid), 5 g L\(^{-1}\) yeast extract (Oxoid), 1 g L\(^{-1}\) glucose, 5 g L\(^{-1}\) NaCl, 1.25 g L\(^{-1}\) Na\(_2\)HPO\(_4\)) unless described otherwise. Antibiotics were added at the following concentrations when appropriate: ampicillin 100 μg mL\(^{-1}\) (for *E. coli*), chloramphenicol 20 μg mL\(^{-1}\), or erythromycin 5 μg mL\(^{-1}\).

Manipulation and extraction of DNA. DNA manipulations were done using standard methods. Restriction enzymes were obtained from Fermentas (Fermentas) and used according to the manufacturer’s instructions. Plasmid DNA from *E. coli* was isolated using the NucleoSpin Plasmid Kit (Macherey Nagel). Chromosomal DNA of *S. saprophyticus* was isolated using 20 μL of a 5 mg mL stock-solution of lysostaphin (Ambicin L, WAK-Chemie Medical GmbH) and the NucleoSpin Tissue kit (Macherey Nagel). PCR products were purified using the Extract II kit (Macherey Nagel). Ligation into the restricted plasmids pUC18 and pRB473\(^\circ\) were performed with T4-Ligase (Life Technologies).

Construction of function-deficient *ssp* gene complementant strains. A knockout-mutant of the *ssp* gene of *S. saprophyticus* strain 9325 was constructed by transformation of the previously described shuttle-vector pMB1106 into the *S. saprophyticus* wild-type strain 9325. In this plasmid the *ssp* gene is interrupted by an *ermB* resistance gene. After homologous recombination, the temperature-sensitive plasmid pMB1106 was cured as described earlier.\(^5\) For the detection of the presence of the *ssp* gene primer *ssp_specific_F* and *ssp_specific_R* were used (Table 2).
reaction 4 PCR products of 5832 bp were obtained, two products each with one with an adaptor at one side, one product with two adaptors and one product without an adaptor (Table 2). The non-mutated DNA was restricted with DpnI, was denatured and annealed to obtain several heteroduplex forms. The DNAs with the (complementary) adaptors were more stable and were transformed in to XL1-Blue (Stratagene, Agilent). The obtained mutants (SspS482C, SspD673S, and SspH712P) were named pBM1109, pMB1110, and pMB1111 (Table 1) and were verified by sequencing. The active site of the lipase was then modified by replacement with distinct amino acids (SspS482C, SspD673S, and SspH712P). The mutated ssp-genes in pUC18 were amplified with primers ssp_SacI_F and ssp_XbaI_R (Table 2), restricted and cloned with their original promoter region into the pRB473 shuttle-vector, and named pMB1121, pMB1122, and pMB1123. The derived mutants were verified by sequencing using following primers ssp_SC_F, ssp_SC_R, ssp_DS_HP_F, and ssp_DS_HP_R. Preparations of cell wall proteins (including non-covalently bound Ssp) from S. saprophyticus 9325 Δssp::ermB mutants, harboring plasmids with mutated ssp-genes (sspS482C, sspD673S or sspH712P-genes each) were loaded in an SDS-PAGE gel and stained with Coomassie brilliant blue (Biorad) as previously shown and compared with purified wild-type Ssp protein. In the mutants a band similar to that of Ssp was detected, indicating that the mutated Ssp-variants are expressed.

The S. saprophyticus 9325 Δssp::ermB mutant was verified by PCR (isolate was ssp-negative and ermB-positive) and by sequencing of the region up- and downstream to the ssp gene and then named MB119 (Table 1).

In order to analyze if the lipolytic activity of Ssp is needed for virulence or if the presence of the protein is sufficient, the conserved active sites (catalytic triad) of the lipase was modified by site-directed ligase independent mutagenesis as described previously using a set of four primers and two adaptors (10 pmol each) for each mutation (Table 2). The amino acids Ser482, Asp673, and His712, putatively comprising the catalytic triad, were targeted and were mutated individually, not simultaneously.

Two short primers were constructed (e.g., for Ssp S482C mutation: Ssp_Ser_F and Ssp_Ser_R), which were up to 9 base pairs from the region to be mutated. Two (long) primers (e.g., for Ssp S482C mutation: Ssp_Ser482Cys_F Ssp_Ser482Cys_R) were used together with two adaptors (adaptor 1 and 2) bearing the desired mutation (Table 2).

As a template DNA the plasmid pMB1103 was used, where the ssp gene with its own promoter has been cloned into a pUC18. In addition, a 1:1 mixture of a Pfx and Taq-Polymerase (GE-Healthcare, Invitrogen) was included into this assay. For the inverse PCR a denaturation temperature of 94 °C for 30 sec, an annealing temperature of 61 °C for 20 sec, and an elongation temperature of 68 °C was used and repeated 25 times. In this case 4 PCR products of 5832 bp were obtained, two products each with one with an adaptor at one side, one product with two adaptors and one product without an adaptor (Table 2). The non-mutated DNA was restricted with DpnI, was denatured and annealed to obtain several heteroduplex forms. The DNAs with the (complementary) adaptors were more stable and were transformed in to XL1-Blue (Stratagene, Agilent). The obtained mutants (SspS482C, SspD673S, and SspH712P) were named pBM1109, pMB1110, and pMB1111 (Table 1) and were verified by sequencing. The active site of the lipase was then modified by replacement with distinct amino acids (SspS482C, SspD673S, and SspH712P). The mutated ssp-genes in pUC18 were amplified with primers ssp_SacI_F and ssp_XbaI_R (Table 2), restricted and cloned with their original promoter region into the pRB473 shuttle-vector, and named pMB1121, pMB1122, and pMB1123. The derived mutants were verified by sequencing using following primers ssp_SC_F, ssp_SC_R, ssp_DS_HP_F, and ssp_DS_HP_R. Preparations of cell wall proteins (including non-covalently bound Ssp) from S. saprophyticus 9325 Δssp::ermB mutants, harboring plasmids with mutated ssp-genes (sspS482C, sspD673S or sspH712P-genes each) were loaded in an SDS-PAGE gel and stained with Coomassie brilliant blue (Biorad) as previously shown and compared with purified wild-type Ssp protein. In the mutants a band similar to that of Ssp was detected, indicating that the mutated Ssp-variants are expressed.

Figure 3. S. saprophyticus wild-type strain 7108 in the C. elegans killing model. S. saprophyticus wild-type strain 7108 induced a lifespan reduction in C. elegans also similar to that of S. aureus RN4220. In the S. saprophyticus 7108 Δssp::ermB mutant the lifespan-reduction is partly abolished and restored again in the ssp-gene complemented mutant.
Protoplast transformation. *S. saprophyticus* strain 9325 \(\Delta \text{ssp::ermB}\) (MB119) was seeded from cryoculture on sheep blood agar and incubated for 18 h at 37 °C. Bacteria from this plate were inoculated into 5 mL PY-medium containing 5 \(\mu\)g mL\(^{-1}\) erythromycin and incubated for 6 h at 39 °C. One-hundred microliters of this culture was inoculated into 20 mL PY-medium containing 5 \(\mu\)g mL\(^{-1}\) erythromycin, and incubated for 18 h at 40 °C. The bacteria were harvested at 5400 \(\times\) g for 10 min and then transferred into 20 mL of SOMMP\(^{29}\) with the addition of 1 mL of 5% BSA (Invitrogen). Fifty microliters of lysostaphin of the 5 mg mL\(^{-1}\) stock-solution was added. The cultures were incubated at 30°C and 90 rpm until the OD600 had dropped to one-third of the initial value (between 30 and 60 min). Cells were pelleted at room temperature at 5400 \(\times\) g and washed in SOMMP before they were transferred into 2 mL of SOMMP and aliquots of 300 \(\mu\)L were prepared. For transformation a mixture of 300 \(\mu\)L protoplast suspension, 3–6 \(\mu\)g plasmid DNA and 2 mL of PEG 6000 40% (Merck) was prepared and incubated for 2 min at room temperature. Six milliliters of SOMMP was added and the cells were harvested at room temperature at 5400 \(\times\) g. The supernatant was discarded and cells were taken up in 2 mL SOMMP for plating on DM3-agar. The plates were incubated for 4 to 6 h at 30 °C, covered with CY-overlay agar containing chloramphenicol to select for transformants and incubated at 30 °C for up to 14 d.

**Caenorhabditis elegans** killing model. The *C. elegans* Bristol N2 was used in this killing model. *C. elegans* were propagated at 24 °C on NGM-agar with the feeding strain *E. coli* OP50. Ten microliters of a 1:10 diluted overnight-culture of the bacteria was plated on antibiotic containing TSB-agar plates (killing plates) and incubated at 30 °C for 12 h. The heat-inactivated and non-viable *Staphylococcus aureus* RN4220 was plated on the agar in a 200-fold concentration (200 \(\mu\)L of undiluted overnight-culture of bacteria) compared with the strains tested. The worms were synchronized and an estimated 30–50 worms in the L4 larval stage were plated on antibiotic and bacteria containing killing plates, as previously described for *S. aureus*,\(^{17,18}\) but with the addition of 5-fluoro-2’-desoxyuridin (FUDR) (25 mg L\(^{-1}\)) as an inhibitor of worm replication. Experiments were performed with and without the addition of nalidixic acid (5 \(\mu\)g mL\(^{-1}\)) where appropriate in order to kill remnant feeding *E. coli* OP50. The non-pathogenic feeding strain *E. coli* OP50 was used without nalidixic acid. *S. aureus* RN4220 was used as a control for lifespan-reduction. Non-viable, heat-inactivated (2 h at 95 °C) *S. aureus* RN4220 was used in addition to *E. coli* OP50 to evaluate background levels of worm death.
The plates were then incubated at 24 °C and scored for living and dead worms at the beginning and daily for up to 144 h (6 d). A worm was considered to be dead when it failed to respond to plate tapping or gentle touch with a hair. Worms that died as a result of getting stuck to the wall of the plate were excluded from the analysis. The experiments were performed in duplicate and repeated seven times.

**Lipase activity assay.** Lipase activity was determined by an agar plate assay using 20 g L⁻¹ tributyrin agar base (Merck) and a Ca²⁺-containing 1% tributyriglycerol solution (Merck) according to the manufacturer’s instructions. The agar plate assay was enhanced by adding 5% (w v⁻¹) gum arabic (Roth) to the tributyriglycerol solution (Merck). Bacteria were plated onto the media and the size of the opaque zone resulting from lipase activity was determined.

**Disclosure of Potential Conflicts of Interest**
No potential conflicts of interest were disclosed.

**Acknowledgments**
We thank Türkan Sakinc (Freiburg) for providing *S. saprophyticus* 9325 and 7108 ssp.*ermB* mutants.

**Table 2. Primer and adaptors used in this study**

| Name                | (5' to 3')                                      |
|---------------------|------------------------------------------------|
| Ssp_Ser482Cys_F     | GTCGGTCATCTGAGGGGAGGAAACTGT                   |
| Ssp_Ser482Cys_R     | TCCACTAATAGGCGCAGCTAGTATGCTTTTTT             |
| Adaptor 1           | CGGCAATTAG                                     |
| Ssp_Ser_F           | GGCAACAGCTGTCGGTCATAG                       |
| Ssp_Ser382Cys_R     | TAGGTGTTCTTGGGCGGAGTTG                       |
| Adaptor 2           | GGCCAGGTTG                                     |
| Ssp_Asp673Ser_F     | GAGAGAAAAACTCaAGATGTTCTG                      |
| Adaptor 3           | AATTTCATCCCATACACATC                        |
| Ssp_Asp_F           | TCTGTATTTTCTCACCACATC                        |
| Ssp_Asp673Ser_R     | AAAAAAGCTTGAATTGTTAATGAGAGTATG              |
| Adaptor 4           | CTTTCTAGGTGTTTAC                              |
| Ssp_Asp_R           | CTTGCTTCGGGTGGTTG                            |
| Ssp_His472Pro_F     | GGGACAGCATGAGTTTCTAGGACA                     |
| Adaptor 5           | AGATGATGAAAGATACAA                            |
| Ssp_His_F           | TAGGACAAGATAGAAGATACAA                       |
| Ssp_Asp672Pro_R     | CAAATACGAGTGAAGTGTG                        |
| Spp_His_R           | TTGGTGTTGACT                                  |
| ssp_specific_F      | AGCATCAACTACAGAACACT                          |
| ssp_specific_R       | AGACCCCTTATTTTCAACAGGT                      |
| ssp_SacR            | CGC GAGCTC TCTGTAAGTACCTTCTCT                |
| ssp_Xbair            | CGC TCTAGA TAATGGAGAATACACAGA                |
| ssp_SC_F             | CTTGAGTGCTACTTGGTGTACTT                      |
| ssp_SC_R             | GGGTTTATTAAAGATGGCAACCTGGGC                  |
| ssp_DS_HP_F         | AAAACAAACACTCGGAGAAACCTT                     |
| ssp_DS_HP_R         | TTGGTGGTGTGACGGT                             |

Mutated region and restriction sites are shown in bold.

**References**

1. Hovelius B, Mådh P, Bygren P. Urinary tract infections caused by *Staphylococcus saprophyticus*: recurrences and complications. J Urol 1979; 122:645-7; PMID:501819
2. Golledge CL. *Staphylococcus saprophyticus* bacteremia. J Infect Dis 1988; 157:215; PMID:3358033; http://dx.doi.org/10.1093/iai/157.1.215
3. Lee W, Carpenter RJ, Phillips LE, Furio S. Pyelonephritis and sepsis due to *Staphylococcus saprophyticus*. J Infect Dis 1987; 155:1079-80; PMID:3559281; http://dx.doi.org/10.1093/iai/155.5.1079-a
4. Sakinc T, Woznowski M, Ebsen M, Gattermann SG. The surface-associated protein of *Staphylococcus saprophyticus* is a lipase. Infect Immun 2005; 73:6419-28; PMID:16177313; http://dx.doi.org/10.1128/IAI.73.10.6419-6428.2005
5. Sakinc T, Kleine B, Gattermann SG. Biochemical characterization of the surface-associated lipase of *Staphylococcus saprophyticus*. FEMS Microbiol Lett 2007; 274:335-41; PMID:17645923; http://dx.doi.org/10.1111/j.1574-6968.2007.00857.x
6. Meyer H, Gattermann S. Surface properties of *Staphylococcus saprophyticus*: hydrophobicity, haemagglutination and *Staphylococcus saprophyticus* surface-associated protein (SpS) represent distinct entities. APMIS 1994; 102:538-44; PMID:7917723; http://dx.doi.org/10.1111/j.1699-0463.1994.tb05203.x
7. Kline KA, Ingersoll MA, Nielsen HV, Sakinc T, Henriques-Normark B, Gattermann S, et al. Characterization of a novel murine model of *Staphylococcus saprophyticus* urinary tract infection reveals roles for Ssp and Sfd in virulence. Infect Immun 2010; 78:1943-51; PMID:20176795; http://dx.doi.org/10.1128/IAI.01255-09
8. Lambe DW Jr., Ferguson RP, Keplinger JL, Gemmell CG, Kalbfleisch JH. Pathogenicity of *Staphylococcus lugdunensis*, *Staphylococcus schleiferi*, and three other coagulase-negative staphylococci in a mouse model and possible virulence factors. Can J Microbiol 1990; 36:455-63; PMID:2224644; http://dx.doi.org/10.1139/m90-080
9. Long JP, Hart J, Albers W, Kapral FA. The production of fatty acid modifying enzyme (FAME) and lipase by various staphylococcal species. J Med Microbiol 1992; 37:232-4; PMID:1404319; http://dx.doi.org/10.1099/00222615.37.4-232
10. Hu C, Xiong N, Zhang Y, Rayner S, Chen S. Functional characterization of lipase in the pathogenesis of *Staphylococcus aureus*. Biochim Biophys Acta 2012; 1829:216-29; PMID:2269949; http://dx.doi.org/10.1016/j.bbamcr.2012.02.057
11. Xie W, Khosavish S, Suwanto A, Kim HK. Characterization of lipases from *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from human facial sebaceous skin. J Microbiol Biotechnol 2012; 22:84-91; PMID:22297223; http://dx.doi.org/10.4014/jmb.1107.07060
12. Jacobsson B, Colque-Navarro P, Gustafsson E, Andersson R, Molily R. Antibody responses in patients with invasive *Staphylococcus aureus* infections. Eur J Clin Microbiol Infect Dis 2010; 29:715-25; PMID:20383551; http://dx.doi.org/10.1007/s10096-010-0919-x
13. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun 2001; 69:4572-9; PMID:11402001; http://dx.doi.org/10.1128/IAI.69.7.4572-4579.2001
14. Hung CS, Boukaert J, Hung D, Pinkner J, Widberg C, DePusco A, et al. Strain-specific basis of tropism of *Escherichia coli* to the bladder during urinary tract infection. Mol Microbiol 2002; 44:903-15; PMID:11941088; http://dx.doi.org/10.1046/j.1365-2958.2002.02315.x
15. Schilling JD, Hultgren SJ. Recent advances into the pathogenesis of recurrent urinary tract infections: the bladder as a reservoir for uropathogenic *Escherichia coli*. Int J Antimicrob Agents 2002; 19:457-60; PMID:12193832; http://dx.doi.org/10.1016/S0924-8579(02)00908-5
16. Azadshos A, Kleine B, Anders A, Kaase M, Sakinc T, Schmitz I, et al. *Staphylococcus saprophyticus* ATCC 15305 is internalized into human urinary bladder carcinoma cell line 5637. FEMS Microbiol Lett 2008; 285:163-9; PMID:18573154; http://dx.doi.org/10.1111/j.1574-6968.2008.01218.x
26. Brückner R, Wagner E, Götz F. Characterization of a sucrose gene from *Staphylococcus xylosus*. J Bacteriol 1993; 175:851-7; PMID:8423155
27. Farrell AM, Foster TJ, Holland KT. Molecular analysis and expression of the lipase of *Staphylococcus epidermidis*. J Gen Microbiol 1993; 139:267-77; PMID:8436947; http://dx.doi.org/10.1099/00221287-139-2-267
28. Chiu J, March PE, Lee R, Tillett D. Site-directed, Ligase-Independent Mutagenesis (SLIM): a single-tube methodology approaching 100% efficiency in 4 h. Nucleic Acids Res 2004; 32:e174; PMID:15585660; http://dx.doi.org/10.1093/nar/gnh172
29. Gatermann S, Marre R. Cloning and expression of *Staphylococcus saprophyticus* urease gene sequences in *Staphylococcus carnosus* and contribution of the enzyme to virulence. Infect Immun 1989; 57:2998-3002; PMID:2777370
30. Maze I, Rydén C, Wadström T, Rubin K. Specific attachment of *Staphylococcus aureus* to immobilized fibronectin. Infect Immun 1986; 54:695-704; PMID:3781623

17. Sifri CD, Begun J, Ausubel FM, Calderwood SB. *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. Infect Immn 2003; 71:2208-17; PMID:12654843; http://dx.doi.org/10.1128/IJAI.71.4.2208-2217.2003
18. Begun J, Sifri CD, Goldberg S, Calderwood SB, Ausubel FM. *Staphylococcus aureus* virulence factors identified by using a high-throughput *Caenorhabditis elegans* killing model. Infect Immun 2005; 73:872-7; PMID:15664928; http://dx.doi.org/10.1128/IJAI.73.2.872-877.2005
19. Sifri CD, Barthes-Bental A, Calderwood SB, von Eiff C. Virulence of *Staphylococcus aureus* small colony variants in the *Caenorhabditis elegans* infection model. Infect Immun 2006; 74:1091-6; PMID:16428756; http://dx.doi.org/10.1128/IJAI.74.2.1091-1096.2006
20. Lee MH, Oh KH, Kang CH, Kim JH, Oh TK, Ryu CM, et al. Novel metagenome-derived, cold-adapted alkaline phospholipase with superior lipase activity as an intermediate between phospholipase and lipase. Appl Environ Microbiol 2012; 78:4959-66; PMID:22554355; http://dx.doi.org/10.1128/AEM.00260-12
21. Bowden MG, Visai L, Longshaw CM, Holland KT, Speziale P, Hook M. Is the GehD lipase from *Staphylococcus epidermidis* a collagen binding adhesin? J Biol Chem 2002; 277:43017-23; PMID:12218064; http://dx.doi.org/10.1074/jbc.M207921200
22. Park S, Kelley KA, Vinogradov E, Solinga R, Weidenmaier C, Misawa Y, et al. Characterization of the structure and biological functions of a capsular polysaccharide produced by *Staphylococcus saprophyticus*. J Bacteriol 2010; 192:4618-26; PMID:20639341; http://dx.doi.org/10.1128/JB.00104-10
23. Begun J, Gaiani JM, Rohde H, Mack D, Calderwood SB, Ausubel FM, et al. *Staphylococcus* biofilm exopolysaccharide protects against *Caenorhabditis elegans* immune defenses. PLoS Pathog 2007; 3:e57; PMID:17447841; http://dx.doi.org/10.1371/journal.ppat.0030057
24. Szabados E, Wolozyn J, Richter C, Kaase M, Gatermann S. Identification of molecularly defined *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization time of flight mass spectrometry and the Biotypen 2.0 database. J Med Microbiol 2010; 59:787-90; PMID:20360398; http://dx.doi.org/10.1099/jmm.0.016733-0
25. Poyart C, Queene G, Bousada C, Trieu-Cuot P. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. J Clin Microbiol 2001; 39:4296-301; PMID:11724835; http://dx.doi.org/10.1128/JCM.39.12.4296-4301.2001

©2013 Landes Bioscience. Do not distribute.