The Glycosyltransferases *sdgA* and *sdgB* Expression in *Staphylococcus Epidermidis* Depends On The Conditions of Biofilm Formation.

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Research Article

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Abstract

The *Staphylococcus aureus*’ SdrG protein is glycosylated by SdgA and SdgB for their protection against its degradation by the neutrophil’s cathepsin G. So far, there is not information about the role of *Staphylococcus epidermidis*’ SdgA nor SdgB in the production of biofilm, therefore the main of this work was to determine the distribution and expression of sdrG, sdgA and sdgB genes in *S. epidermidis* in conditions of biofilm. The frequency of the genes sdrG, sdgA and sdgB were evaluated by PCR in a collection of 75 isolates. The isolates were grown in dynamic conditions (in agitation) or static conditions (biofilm producer: planktonic or sessile cells). The expression of sdrG, sdgA and sdgB were determined by RT-qPCR in cells grown under dynamic conditions (CGDC), as well as planktonic and sessile cells, and in cells adhered to a catheter (*in vivo*). The genes sdrG and sdgB were detected in 100% of isolates, meanwhile the gene sdgA was detected in 71% of the samples (*p*<0.001). The CGDC did not expressed the sdrG, sdgA and sdgB mRNAs. The planktonic and sessile cells expressed sdrG and sdgB, and the same was seen in cells adhered to the catheter. In particular, one isolate, able to induce biofilm under cathepsin G treatment, expressed sdrG and sdgB in planktonic, sessile and in cells adhered to the catheter. This suggests that the state of cells adherence is an important factor for the transcription of sdgA, sdgB and sdrG.

Introduction

Biofilms are bacterial communities adhered to a biotic or abiotic surface, where bacteria are embedded inside of extracellular polymeric substances produced by the same bacteria (Watters et al. 2016). Indeed, biofilms contribute to the success of *S. epidermidis* in causing infection (Costerton et al. 1999), as the correlation between the formation of biofilm and persistence of infection in implanted medical devices has been demonstrated (Römling and Balsalobre 2012). Biofilms diminish dramatically the possibility of treatment for the infection through the action of the immune system of the host or the dosing of antibiotics. Biofilm formation is commonly divided into three steps: initial adhesion, cellular accumulation, and biofilm disruption (Wolska et al. 2016).

One critical condition in the first step of biofilm formation, initial adhesion, is the strong adhesion of bacteria to surface of the implanted medical devices, which results in a successful infection. The surface of *S. epidermidis* is covered with a variety of cell wall proteins engaged to the peptidoglycan (Foster and Höök 1998). The adhesion of the bacteria to the surface of the implanted medical device is driven by the hydrophobicity of the bacterial cell membrane in which certain specific proteins are involved such as AtlE, Bhp (Heilmann et al. 1997; Tormo et al. 2005) and SSP-1 and SSP-2 (Heilmann et al. 1996; Veenstra et al. 1996; Qin et al. 2007). When medical device is implanted within an organism, the extracellular matrix proteins such as the fibronectin, fibrinogen, vitronectin and collagen from the host quickly cover the surface of device (Baier et al. 1984). *Staphylococcus aureus* and *S. epidermidis* possess a type of cell wall proteins called Microbial Surface Recognizing Adhesive Matrix Molecules (MSCRAMMs) that specifically recognize to extracellular matrix proteins allowing an interaction and bind to them (Bowden et al. 2005; Mack et al. 2006). The Sdr proteins are members of MSCRAMM family, and they have been
named as such due to the content of a region of repeated dipeptide serine – aspartate (SD) (McDevitt and Foster 1995). The proteins Sdr can interact specifically with the extracellular matrix proteins that cover the surface of the implanted medical device (Josefsson et al. 1998).

Three proteins Sdr have been identified in *S. epidermidis*: SdrF that binds to collagen, SdrG that binds to fibrinogen and SdrH, with a ligand still not elucidated (Nilsson et al. 1998; Lei et al. 1999). The Sdrs participate in the *S. epidermidis* virulence (Cheng et al. 2009), SdrG is involved in the *S. epidermidis* adherence to osteoblasts, generating a colonization and infection of the implant leading to an osteomyelitis (Claro et al. 2015), furthermore, serologic antibodies against the Sdr proteins have been isolated from patients infected by *S. epidermidis*, which suggests that these are expressed during the course of the infection (McCrea et al. 2000).

Hazenbos et al. 2013, demonstrated that the SD region of the proteins ClfA, ClfB, SdrC, SdrD and SdrE of *S. aureus* USA300 strain, and the proteins SdrG, SdrF and SdrH of *S. epidermidis* 85W1740 strain, are highly glycosylated (Hazenbos et al. 2013). These authors identified two new glycosyltransferases, SdgA and SdgB, which are members of the TarM glycosyltransferases family (Hazenbos et al. 2013). In addition, the genes that codify for these glycosyltransferases in *S. aureus* are adjacent to the *sdrCDE* loci, and this array is completely conserved in different strains of *S. aureus*.

The lack of glycosylation in the SD region of Sdr proteins by SdgA or SdgB provokes the Sdr proteolysis by the neutrophil cathepsin G protease (Hazenbos et al. 2013). Also, the degradation of the nonglycosylated Sdr proteins has as consequence the loss of the bacterial capability to adhere to human fibrinogen (Hazenbos et al. 2013). Moreover, it has been demonstrated that the glycosyltransferases SdgA and SdgB are involved in the glycosylation of the plasmin-sensitive protein (Pls) and these Pls glycosyl residues can stimulate biofilm formation in *S. aureus* (Bleiziffer et al. 2017). The presence of the three genes *aggA*, *aggB* (*sdgA*), and *aggC* (*sdgB*) have been detected in *S. aureus*, which shows that AggA and SdgB contribute to staphylococcal agglutination with fibrin fibrils in human plasma (Thomer et al. 2014).

So far, the distribution and expression of *S. epidermidis* *sdgA* and *sdgB* genes have not been explored in detail, therefore in this work we determined the expression of *sdgA* and *sdgB* genes in planktonic and sessile cells (grown in biofilm), in cells grown under dynamic conditions (CGDC), and in cells adhered to a catheter.

**Materials And Methods**

*S. epidermidis* isolates

We worked with 29 isolates from ocular infection (OI) with capacity to generate biofilm (13 isolates; 44.8%) or not (16 isolates; 55.2%), and 46 isolates from healthy skin (HS), also with capacity to generate biofilm (2 isolates; 4.3%) or not (44 isolates; 95.7%). The HS60 isolate was used as non-biofilm producer control; in this last one, the biofilm was induced with cathepsin G protease treatment.
For the expression assays, the strain type RP62A was used as a biofilm producer control.

PCR amplification of the genes sdrG, sdgA, sdgB.

The isolates were cultured in 3 ml of Tryptone soy-broth (TSB; Sigma-Aldrich, Estado de Mexico, Mexico) and incubated overnight at 37°C. The cells were obtained by centrifugation at 12000 rpm for 3 min, then 200 µl of Whinston solution (2% Triton X-100, 1% SDS, 10 nM NaCl, 10 mM Tris base at pH 8.0 and 1 mM EDTA) were added. A mechanical disruption was conducted for 1 min at 3000 rpm followed by incubation in ice for 30 s (this was done in 5 cycles), then another 200 µl of the Whinston solution were added. The DNA was extracted with phenol-chloroform-isoamlylic alcohol (25:24:1) and precipitated with one volume of isopropanol. The genes sdrG, sdgA and sdgB were amplified using the oligonucleotides shown in Table 1. The PCR amplifications were carried out with MyTaq™ DNA polymerase (Invitrogen, MA, USA) according to the manufacturer’s instructions.

| Gene  | Sequence                          | Expected size (bp) |
|-------|-----------------------------------|--------------------|
| sdrG  | Fw: CAGTCAGAACAGATGAAGAGGG        | 146 bp             |
|       | Rv: ATTCGCTTCTGAGTCTAGTGC         |                    |
| sdgB  | Fw:GGTCCAGGTGAATTGATACG           | 146 bp             |
|       | Rv:TGTGCAGTTTCAATACATGG           |                    |
| sdgA  | Fw:ATTGACAAGTAAAATGGAAGGTC        | 102 bp             |
|       | Rv:AACTCAGAAGGTCCGTATTTGG         |                    |
| 16S rRNA| Fw: AGGAGTCTGGACCGTGCTTC         | 201 bp             |
|       | Rv: GCGTAGCGACCTGAGAG            |                    |

Analysis of the genomic disposition of sdrG, sdgA and sdgB

The analysis was performed with 30 fully sequenced Staphylococcus genomes that were downloaded from NCBI (www.ncbi.nlm.nih.gov/genomes) via FTP (Supplementary Table 1). Gene products were aligned with BLAST genes to obtain homology. The SdgA (SAUSA300_0549) and SdgB (SAUSA300_0550) gene products from Staphylococcus aureus USA300_FPR3757 were used as reference. To determine the genomic arrangement the azoreductase gene (SAUSA300_0545) was used as a reference point, this gene is orthologous in all genomes. The phylogenetic tree was obtained with the PhyML program (www.atgc-montpellier.fr/PhyML) using the concatenation of five proteins (RecA, RpoB, AtpD, GyrB and ClpB) with the LG evolutionary model and a bootstrap of 100.

Biofilm formation
Isolates were inoculated in TSB (Sigma-Aldrich) and incubated for 24 h at 37°C. Then, in 6-well tissue culture plates (Nunc, Thermo Fisher Scientific, MA, USA) the cells were diluted 1:200 in TSB medium. In the case of HS60 isolate, TSB medium was supplemented with 0.1 µg/mL of cathepsin G (Gibco, Thermo Fisher Scientific). Plates were incubated for 24 hr at 37°C without stirring (static conditions). In the dynamic growing conditions assays, the plates were incubated for 24 hr at 37°C and kept shaking at 2.6 Hz, as Stepanovic et al. (2001) described. The biofilm formation was determined as described by Christensen et al. (1985).

Expression by RT-qPCR evaluated under dynamic conditions (non-biofilm condition)

The strain S. epidermidis RP62A and the biofilm producers clinical and commensal isolates were inoculated in 6 well plates (Nunc, Thermo Fisher Scientific) containing 3 ml of TSB (Sigma-Aldrich) and were incubated in dynamic conditions for 13 h at 37°C shaking at 2.6 Hz, as described by Stepanović et al. (2001); under these conditions the formation of biofilm was also tested as indicated above. The cell culture was transferred to a sterile tube and the no adherent cells were recovered by centrifugation at 12000 rpm and washed with DEPC treated water. The RNA purification and RT-qPCR were performed as previously described by Martínez-García et al. (2019). The expression of 16S rRNA was used as a control, and sdrG, sdgA and sdgB were determined using the primers listed in Table 1. Relative expression was determined by the $2^{-\Delta\Delta Ct}$ method.

Expression by RT-qPCR in biofilm cells (static growing conditions)

The strain S. epidermidis RP62A (biofilm producer control), the clinical and commensal isolates with biofilm phenotype, and the strain S. epidermidis HS60 were inoculated in TSB (sigma-Aldrich) and incubated at 37°C and shaking overnight. From those cultures, an inoculum was taken and transferred to a 6 flat bottom wells plate (Nunc) and adjusted with TSB to a 1:200 dilution. Each strain was inoculated in quadruplicate, human cathepsin G (Gibco) was added only to the strain HS60 at a final concentration of 0.1 µg/ml to induce the biofilm formation. The plate was incubated at 37°C for 24 h, without stirring, afterwards, the floating planktonic cells were taken and the sessile cells were obtained by scratching and resuspended in 1 mL of 1x PBS. Total RNA was extracted from all the samples and RT-qPCR were performed as previously described by Martínez-García et al. (2019).

Expression by RT-qPCR in a model of catheter (in vivo)

Female Balb/c mice were used in a model of subcutaneous implanted device-related infection according to Sander et al. (2012). This study was carried out following the recommendations of the bioethics review board of the “Escuela Nacional de Ciencias Biológicas-IPN.” The mice were weighed and anesthetized by intraperitoneal injection with filocain (100 mg/g of weight). The hair was removed from the back using electric hair clippers, and a small incision was made. Then, 1 cm of a sterile 14-gauge teflon intravenous catheter (Exel International, FL, USA) was inserted, and the incision was sutured. Five mice were used as control (no bacterial inoculum) and the rest of the mice were injected through the catheter with a bacterial inoculum of $1.5 \times 10^8$ CFU in 20 µL of sterile PBS. The distribution of the strains utilized as inoculum for
the catheters was as follows: five mice were inoculated with the strain type RP62A, five mice with the strain HS60 and five mice with the strain HS60 supplemented with cathepsin G (0.1 µg/ml), five mice with biofilm producer IO7 isolate and five mice with biofilm producer HS6 isolate. After 7 days post-infection, the animals were sacrificed. The catheters were removed, and each one was put in 1 mL of 1x PBS and sonicated at 200 Hz for 5 min twice. On one hand, the CFU/mL for each catheter was determined by decimal dilutions, and on the other hand, a bacterial pellet was obtained by centrifugation in order to extract the total RNA and carry out the RT-qPCR as described previously.

**Statistical analysis**

To determine the proportion analysis, the accurate Fisher test was conducted. In order to analyze the expression levels, a two way ANOVA and Tuckey tests were conducted. These analyses were carried out with the software GraphPad Prism version 7.0.

**Results And Discussion**

**Distribution of sdrG, sdgA, sdgB genes and genotypes**

A total of 75 isolates were tested and they were grouped in biofilm producers and non-biofilm producers. The sdrG and sdgB genes were detected indistinctly in all the isolates tested (100%), except for the gene sdgA, which was only detected in 71% (Table 2), showing a significant statistical difference (p<0.001) in relation to sdrG and sdgB genes, however there was not a difference between biofilm producers and non-biofilm producers. The gene sdrG was found in all isolates analyzed, which is in agreement with the former findings, since the presence of the gene sdrG is reported between 78-91% of S. epidermidis strains isolated from orthopedic infections and from central venous catheter-associated infections (Arciola et al. 2004).

| Isolates | sdrG (%) | sdgB (%) | sdgA (%) |
|----------|----------|----------|----------|
| Total (n= 75) | 75 (100%) | 75 (100%) | 53 (71%)*** |
| OI (n=29) | 29 (100%) | 29 (100%) | 23 (79.3%)* |
| HS (n= 46) | 46 (100%) | 46 (100%) | 30 (65.2%)*** |

Detection of the genes was carried out by PCR as described in materials and methods. *(p=0.0235), *** (p<0.001).

The genotypes identified in the isolates were two; 1) sdrG⁺, sdgA⁺, sdgB⁺ and 2) sdrG⁺, sdgA⁻, sdgB⁺, where the genotype 1 was predominant (71%) over the genotype 2 (29%) considering all the isolates (p<0.001). Analyzing the frequency of genotypes within each source of isolation (IO and HS) genotype 1 is also predominant in both cases (OI p<0.001 and HS p<0.05) (Table 3).
Table 3
Frequency of the genotypes present in isolates of *S. epidermidis* from OI and HS.

| Isolates (n=75) | Genotype 1 (%) | Genotype 2 (%) |
|----------------|----------------|----------------|
| *sdrG*, *sdgA*, *sdgB* | 23 (79.3%) | 6 (20.7%)*** |
| *sdrG*, *sdgA*, *sdgB* | 30 (65.2%) | 16 (34.8%)** |

***(p<0.001), **(p=0.0064).**

**Genomic array of the genes** *sdrG*, *sdgA* and *sdgB* in *S. epidermidis*

Several *Staphylococcus* genomes were analyzed in order to determine the diversity in genomic organization and conservation of the mentioned genes. It was found that there was some diversity in the presence and organization of these genes. Analyzing the *Staphylococcus* species, it was observed that one or more homologues of *sdrG* were present and the glycosylases *sdgAB* have been disorganized. In the figure 1 it can be observed that, in other species, the *sdgAB* genes are immediately contiguous, in the opposite direction, but in the *S. epidermidis* strains this organization was lost and only *sdgB* conserved its position with respect to *sdrG* (Fig. 1). The other glycosylase, *sdgA*, appeared at a considerable distance, between 200 and 365 kb (as is the case with ATCC12228). In other strains, it happened that *sdrG* is found at more distant positions (191 and 660 kb), as in 13T0028, JMUB898 and NCTC13838, that do not appear in the figure. *sdgB* is normally contiguous with the *sdrG* gene in all *S. epidermidis* strains analyzed; these two genes are in a opposite direction. Strains ATCC14990, CDC121, FDAARGOS_153 and FDAARGOS_161 presented another gene homologous to *sdrG*, annotated as *sesJ* (belonging to MSCRAMM family, cell wall-anchored protein), in chromosome positions unrelated to this cluster. The *S. aureus* strain USA300_FPR3757 showed similar organization as the MSHR1132 strain, three contiguous *sdrCDE* homologues. The phylogenetic tree showed representative *Staphylococcus* species and it was useful to associate phylogeny with the genetic conservation and diversity of these genes.

From this analysis we can infer that there was a translocation event in the glycosylase *sdgA* segment in *S. epidermidis*. On the other hand, there was conservation of the *sdrG* and *sdgB* positions, showing a greater functional or regulatory commitment. Given the diversity of these components and the loss of organization, it is possible that these are factors that influence the pathogenicity of the strains.

**Relative expression of *sdgA* and *sdgB* genes under dynamic growth conditions (non-biofilm condition)**

From all the isolates grown under dynamic conditions no one formed biofilm (data no shown). The cells grown under dynamic conditions (CGDC) expressed only the 16S rRNA, and the expression of *sdrG*, *sdgA* and *sdgB* was not detected in the CGDC coming from the clinical and commensal isolates biofilm producers (data no shown), nor in the CGDC coming from the non-biofilm producers isolates (data no shown).
The *sdrG* expression in *S. epidermidis* has been scarcely studied. The expression of the *sdrG* gene do not occur in *vitro* nor under certain conditions such as: iron depletion, bacterial growth in the presence of 5% of CO₂, conditions stimulated with whole human blood or with 70% of human serum (Sellman et al. 2008). The expression of *sdrG* gene has been only detected under *in vivo* conditions through the systemic infection of *S. epidermidis* in mice (Sellman et al. 2008). Moreover, it has been demonstrated that the abundance of the gene *sdrG* on the cell surface of *S. epidermidis* dramatically improves their ability to bind to fibrinogen-coated implanted medical devices (Vanzieleghem et al. 2015) implicating a role of SdrG in the *in vivo* adhesion conditions. The assays for the expression of *sdrG* in CGDC did not demonstrate that the induction of this gene can be achieved, as occur in *sdgA* y *sdgB* genes under these conditions. Our results confirm that there is a correlation between the expression of the gene *sdrG* and the expression of the genes *sdgA* and *sdgB*.

**Relative expression of the sdrG, sdgA and sdgB in biofilm growth conditions (static growth)**

The expression of the genes of interest was assayed in *S. epidermidis* RP62A strain (biofilm producer). The biofilm was produced in static growth conditions, and there was absence of biofilm in the dynamic growth conditions (Fig. 2a). In the biofilm conditions the expression of *sdrG* and *sdgB* were observed in the planktonic and in sessile cells, but *sdgA* was not expressed (Fig. 2b). In the dynamic growth conditions, the CGDC did not expressed *sdrG*, *sdgB* nor *sdgA* (Fig. 2b). Also we did not find statistical difference among the samples tested (*p*>0.05).

Afterwards, the relative expression of the genes of interests was tested in the clinical (13 isolates) and commensal (2 isolates) *S. epidermidis* isolates with biofilm producers phenotype. In the static growth condition, all the isolates produced biofilm (Fig. 3a), and both planktonic (Fig. 3b) and sessile cells (Fig. 3c) expressed *sdrG* and *sdgB*, and in some isolates the levels of expression of *sdrG* were higher than *sdgB* (*p*<0.05). Concerning the gene *sdgA*, both planktonic and sessile cells coming from three clinical isolates expressed *sdgA* (Fig. 3b and c).

The *in silico* analysis of 20 genomes of *S. epidermidis* demonstrated differences in the genomic arrays reported for *S. aureus* (Hazembos et al. 2013). In the analysis, 13 genomes of *S. epidermidis* the sequence of the gene *sdgA* was not found. This confirms the reports on *S. aureus*, in which is stated that the gene *sdgA* is not essential for the protection of the Sdr proteins against the proteolysis caused by the cathepsin G (Hazembos et al. 2013; Thomer et al. 2014). In the present work, we suggest that in the case of *S. epidermidis*, the gene *sdgA* may not be essential because its expression was absent in planktonic and sessile cells in the biofilm producers OI isolates, in which case only 3 out of 13 expressed the gene *sdgA*.

We found that the *in vitro* growth conditions for the production of biofilm promote the expression of *sdrG* and glycosyltransferases *sdgA* and *sdgB* in planktonic cells as well as in sessile cells indicating a correlation between the expression of *sdrG* gene and glycosyltransferases. In a recent report it was demonstrated that umbelliferone, a natural product of the coumarin family, exerts an anti-biofilm effect
on *S. epidermidis* by turning down the initial adhesion and cellular accumulation, due to the reduced expression of adhesion encoding genes (*icaD, atLE, aap, bhp, ebh, sdrG*, and *sdrF*) (Swetha et al. 2019). To the best of our knowledge, there are no reports on the expression of the genes *sdgA* and *sdgB* of *S. epidermidis* under biofilm conditions.

On the other hand, we also analyzed if the correlation between the expression of *sdrG* gene and glycosyltransferases also happen in isolates with non-biofilm forming phenotype. The isolate *S. epidermidis* HS60 has a non-biofilm forming phenotype, and to promote its biofilm formation it is necessary the presence of the cathepsin G. In this isolate, the presence of cathepsin G induced the biofilm formation (Fig. 4a) and the genes *sdrG, sdgB*, and *sdgA* were expressed (Fig. 4b) in the planktonic and the sessile cells, but not in absence of cathepsin G. There were no significant differences in the comparison of each gene (*p*>0.05). Rhode et al. (2005) demonstrated that the non-biofilm forming strains of *S. epidermidis* can induce the production of biofilm in the presence of a protease such as cathepsin G, just like strain HS60 did. The strain HS60 was important in our work because the planktonic and sessile cells, induce the expression of the genes *sdrG, sdgA* and *sdgB*. These results suggest that any isolate can induce the expression of *sdrG* gene and glycosyltransferases when grown under appropriate conditions that favor the adhesion, supporting the hypothesis of a correlation in the expression of these genes.

**Relative expression of the *sdgA* and *sdgB* genes in a model of catheter (in vivo)**

The catheters inoculated with different strains of *S. epidermidis* (RP62A, HS60, IO7, HS6) were implanted in the back of Balb/c mice. Seven days post implantation the mice were sacrificed and the catheters were recovered. For each catheter the CFU/mL was quantified and the bacterial pellet was used for RT-qPCR. In the case of *S. epidermidis* RP62A, which is the model strain for biofilm production, 1.4x10^7 CFU/mL were obtained, and the same number was for IO7 isolate, and HS6 isolate was 1.0x10^7 CFU/mL. In the case of *S. epidermidis* HS60, which is not a biofilm producer, we got 2.5x10^6 CFU/mL, however in this same strain supplemented with cathepsin G reached a count of 9.3x10^6 CFU/mL (Fig. 5a). There was a significant difference between the strain *S. epidermidis* HS60 that was not supplemented with cathepsin G and the strain that was supplemented with it (*p*<0.05).

From the bacteria recovered from the catheters, the expression of *sdrG, sdgA* and *sdgB* were evaluated. In the *S. epidermidis* RP62A, IO7 and HS6 isolates there was the expression of the *sdgB* and *sdrG* mRNAs. In the *S. epidermidis* HS60 isolate *sdgB* and *sdrG* mRNAs were expressed, either treated or not treated with cathepsin G, meanwhile *sdgA* was only expressed in the isolate that was not treated with cathepsin G (Fig. 5b). In both isolates there was no significant difference among the different treatments (*p*>0.05).

Regarding *S. epidermidis* HS60 (non-biofilm producer phenotype), we observed that in the absence of cathepsin G treatment (biofilm inducer in this strain) HS60 adhered to the catheter and expressed the *sdrG* gene and the glycosyltransferases *sdgA* and *sdgB* genes, suggesting that the adherence of the bacteria to the catheter is enough stimuli for the expression of the genes. This is related to the fact that SdrG provides a strong binding force and slow dissociation with the fibrinogen and clustering of SdrG,
and these biophysical features provide a molecular foundation for the ability of *S. epidermidis* to colonize to implanted biomaterials and to withstand physiological shear forces (Herman et al. 2014).

**Conclusion**

It was demonstrated that *sdrG* is expressed under biofilm conditions (*in-vitro* and *in-vivo*), at the same time, the genes *sdgB* and *sdgA* are also expressed indicating an association between *sdrG* and the glycosyltransferases. Moreover, the *sdgA* gene was expressed at a low proportion among the isolates, which suggests that it is not an essential gene for the SdrG glycosylation. So, strains with non-biofilm forming phenotype can be potentially biofilm producers since in their genome are the *sdrG* and *sdgB* genes and their expression can contribute to the adhesion to the catheter.

**Declarations**

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**Author contributions:** All authors contributed to the study conception, design, analysis and manuscript. Material preparation, data collection and analysis were performed by Itzia S. Gómez-Alonso, Ilse D. Estrada-Alemán, and Sergio Martínez-García. Data analysis were also performed by Humberto Peralta, Erika T. Quintana, and Cipriano Chávez-Cabrera. The first draft of the manuscript were written by Claudia Guerrero-Barajas, and Sandra Rodríguez-Martínez. All authors read and approved the final manuscript. Design, and the final draft of the manuscript were written by Mario E. Cancino-Diaz, and Juan C. Cancino-Diaz.

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**Conflicts of interest.** The authors have no conflicts of interest to declare.

**Ethical approval:** This study was reviewed and approved by IACUC of the IPN number ZOO-002-2021.

**Availability of data and materials.** All data generated or analysed during this study are included in this published article and its supplementary information files.

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**Figures**

**Figure 1**

*Genomic arrays found in Staphylococcus spp.* The analysis was performed with 30 fully sequenced *Staphylococcus* genomes. The SdgA (SAUSA300_0549) and SdgB (SAUSA300_0550) gene products from *Staphylococcus aureus* USA300_FPR3757 were used as reference. To determine the genomic arrangement the azoreductase gene (SAUSA300_0545) was used as a reference point, this gene is orthologous in all genomes. The phylogenetic tree was obtained with the PhyML program with the LG evolutionary model and a bootstrap of 100.

**Figure 2**

*Levels of sdrG, sgdA and sgdB expression in planktonic cells and in sessile cells of Staphylococcus epidermidis RP62A.* (a) biofilm production in static growth and dynamic growth. (b) Levels of expression in different cells. Cells grown under dynamic conditions (CGDC). Results obtained from three independent replicates.

**Figure 3**
Levels of \textit{sdrG}, \textit{sdgA} and \textit{sdgB} expression in the clinical and commensal isolates that produce biofilm. (a) Biofilm production in the isolates. (b) Levels of expression in the planktonic cells obtained from the biofilm. (c) Levels of expression in the sessile cells obtained from the biofilm. Results obtained from three independent replicates.

Figure 4

Levels of \textit{sdrG}, \textit{sdgA} and \textit{sdgB} expression in \textit{Staphylococcus epidermidis HS60}. (a) Biofilm production in presence of cathepsin G (CatG(+)) and absence of cathepsin G (CatG(-)). Cathepsin G protease concentration used to induce biofilm formation 0.1 µg/ml. (b) Levels of expression in planktonic, sessile and cells grown under dynamic conditions (CGDC). Results obtained from three independent replicates.

Figure 5

Assay of the catheter inoculated with \textit{Staphylococcus epidermidis} and implanted to the back of a mouse. (a) Viable bacterial count in the catheters after 7 days of incubation. (b) Levels of expression in the bacterial cells obtained from the catheter after 7 days of incubation. CatG: treated with cathepsin G at a final concentration of 0.1µg/ml. Results obtained from three independent replicates.

Supplementary Files

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