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Biofilms: understanding physiology for developing new therapeutic strategies

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&

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- Introduction to biofilms
- Biofilms in infections and device-related infections (DRI)
- Staphylococcus spp. and staphylococcal biofilms in DRI
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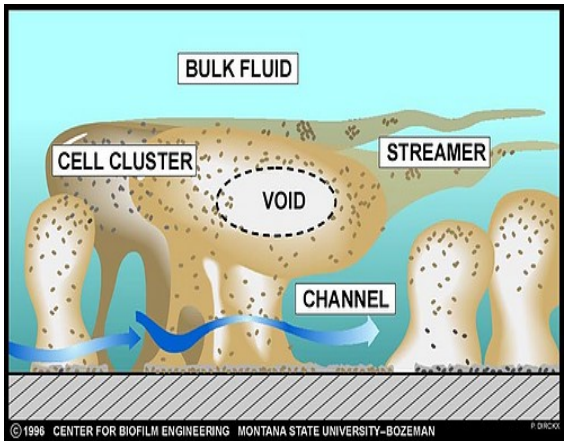
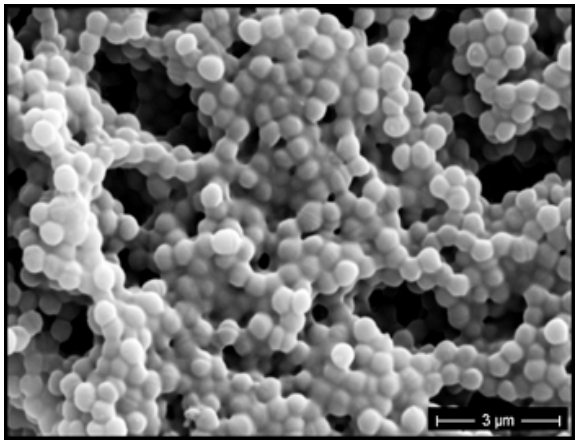
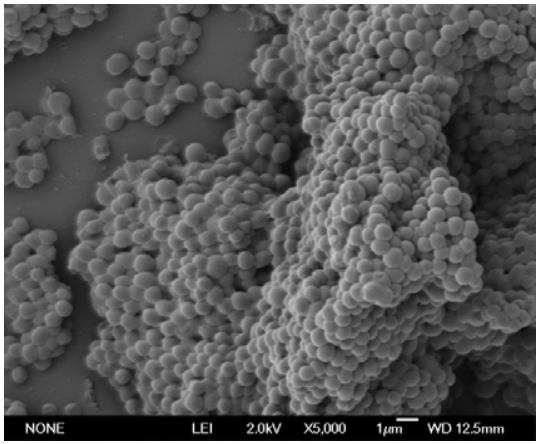
What is a biofilm?

Structured communities of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or a living surface.

A biofilm is like a tiny city in which microbial cells form towers. The "streets“ between the towers are fluid-filled channels that bring in nutrients, oxygen and other necessities for live biofilm communities.







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Key characteristics of biofilms

- **Biofilms are heterogeneous, complex, dynamic structures, responsive to their environment**
- **Biofilm cells have altered gene and protein expression profiles and patterns compared to their planktonic counterparts**
- **Biofilm cells can coordinate behavior via intercellular communication using biochemical signaling molecules (Quorum Sensing)**
- **Biofilms are less susceptible to antimicrobial agents**

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Mechanisms of biofilm resistance

- Barrier properties of the matrix (restricted penetration)
- Low metabolic activity, slow growth and stress response
- Antimicrobial destroying enzymes and gene transfer
- Quorum sensing (QS) and heterogeneity
- Persisters, phenotypic subpopulation of bacteria that survives antibiotic treatment

Clinical importance of biofilms

- **Notoriously resistant to immune system attack and antimicrobial agents (up to 1500 times more resistant)**
- **Biofilms have been found to be involved in a wide variety (up to 80%) of microbial infections**
  - **Biofilms lead to ≈5 million infections and ≈150,000 deaths in USA and EU annually**
- **Regularly, antimicrobial therapy fails without removal of the implanted device**

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Biofilms in infections

Infectious processes in which biofilms have been implicated include:

- **urinary tract infections**
- **catheter infections**
- **middle-ear infections**
- **sinusitis**
- **formation of dental plaque, gingivitis**
- **coating contact lenses**
- **endocarditis**
- **infections in cystic fibrosis**
- **infections of permanent indwelling devices such as joint prostheses and heart valves**

Device-related infections (DRI)

Table 1. The magnitude of the problem of device-associated infections.

Device	Estimated no. inserted in the United States per year	Rate of infection, %	Attributable mortality[a]
Bladder catheters[b]	>30,000,000	10–30	Low
Central venous catheters[b,c]	5,000,000	3–8	Moderate
Fracture fixation devices[b]	2,000,000	5–10	Low
Dental implants[d]	1,000,000	5–10	Low
Joint prostheses[b]	600,000	1–3	Low
Vascular grafts[b]	450,000	1–5	Moderate
Cardiac pacemakers[b,d]	300,000	1–7	Moderate
Mammary implants, in pairs[e]	130,000	1–2	Low
Mechanical heart valves[d]	85,000	1–3	High
Penile implants[b,d]	15,000	1–3	Low
Heart assist devices[d]	700	25–50	High

[a] Semiquantitative scale for attributable mortality: low, <5%; moderate, 5%–25%; high, >25%.
[b] Numbers estimated by analysis of market reports.
[c] Numbers estimated by review of the medical literature.
[d] Numbers estimated by personal communication with personnel from device manufacturing companies.
[e] Numbers estimated by review of data provided by medical associations.

linical Infectious Diseases 2001; 33:1567–72

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Device-related infections (DRI)

- ***Staphylococcus aureus*** **and coagulase-negative staphylococci (CoNS), in particular, *S. epidermidis*, have emerged as major nosocomial pathogens associated with DRI, due to the facts that:**
  - **they are the most abundant skin-colonizing bacteria**
  - **they are able to adhere to the surface and form a biofilm**
- **Biofilm formation is one of the major virulence factor for Staphylococcus spp.**

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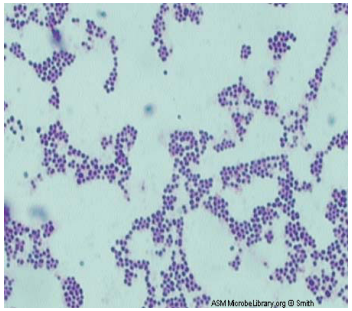
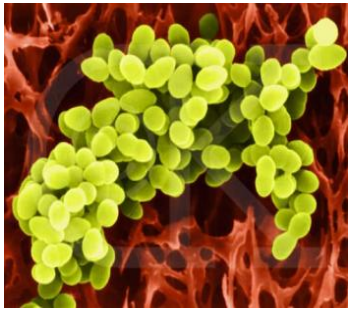
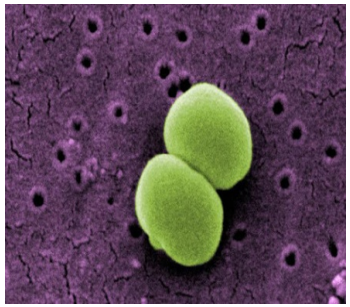
Staphylococcus spp.

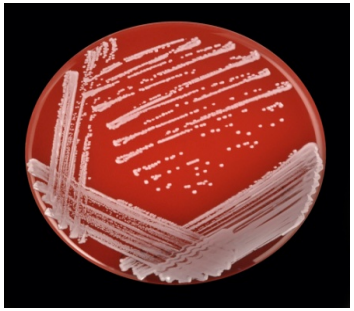
- Gram-positive, non motile, non-spore forming, spherical bacterium, coagulase negative or positive
- Arrange grape-like clusters
- Form white colonies ≈ 1-2 mm Ø after 24 h



- Most are harmless and normal inhabitant of human skin and mucous membranes







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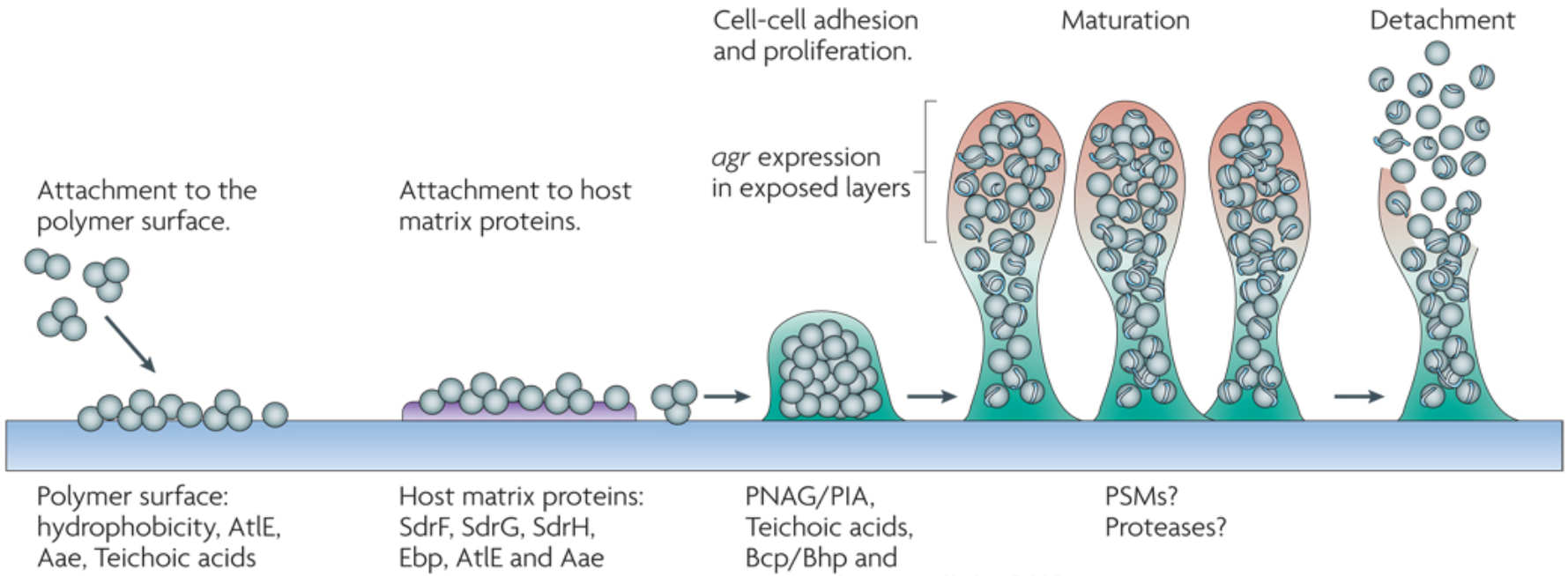
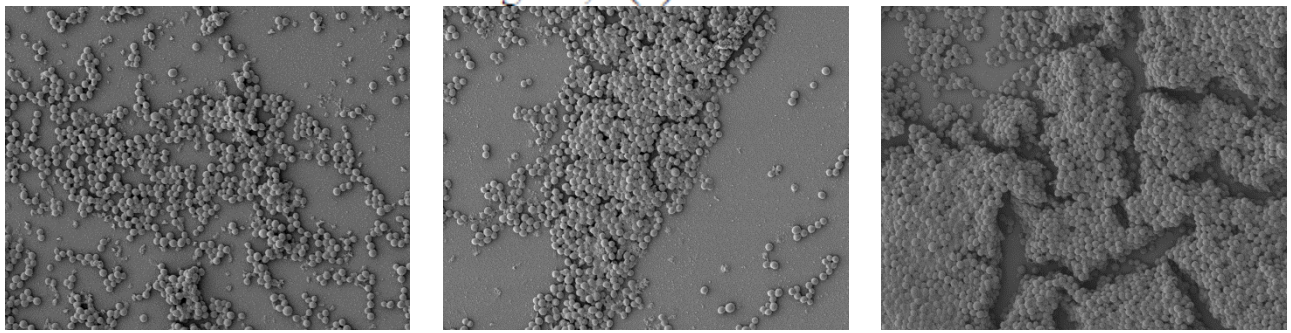
Biofilm development in *Staphylococcus* spp.



Nat Rev Microbiol. 2009 August ; 7(8): 555–567



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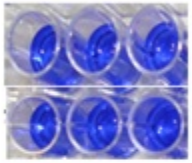
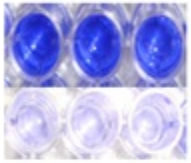
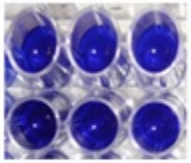
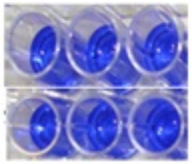
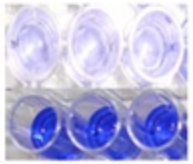
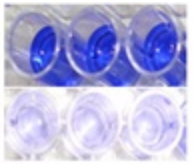
Biofilm development in *Staphylococcus* spp.

- Effect of NaCl and glucose on biofilm formation

Strain	Biofilm phenotype	BHI	BHI+NaCl (4%)	BHI+Glucose (1%)
8325-4	PIA-dependent			
BH1CC	proteinaceous			

- Effect of dispersal agents on established biofilms

Strain	Biofilm phenotype	BHI	SM	PK
8325-4	PIA-dependent			
BH1CC	proteinaceous			

SM: Sodium Metaperiodate, PK: Proteinase K

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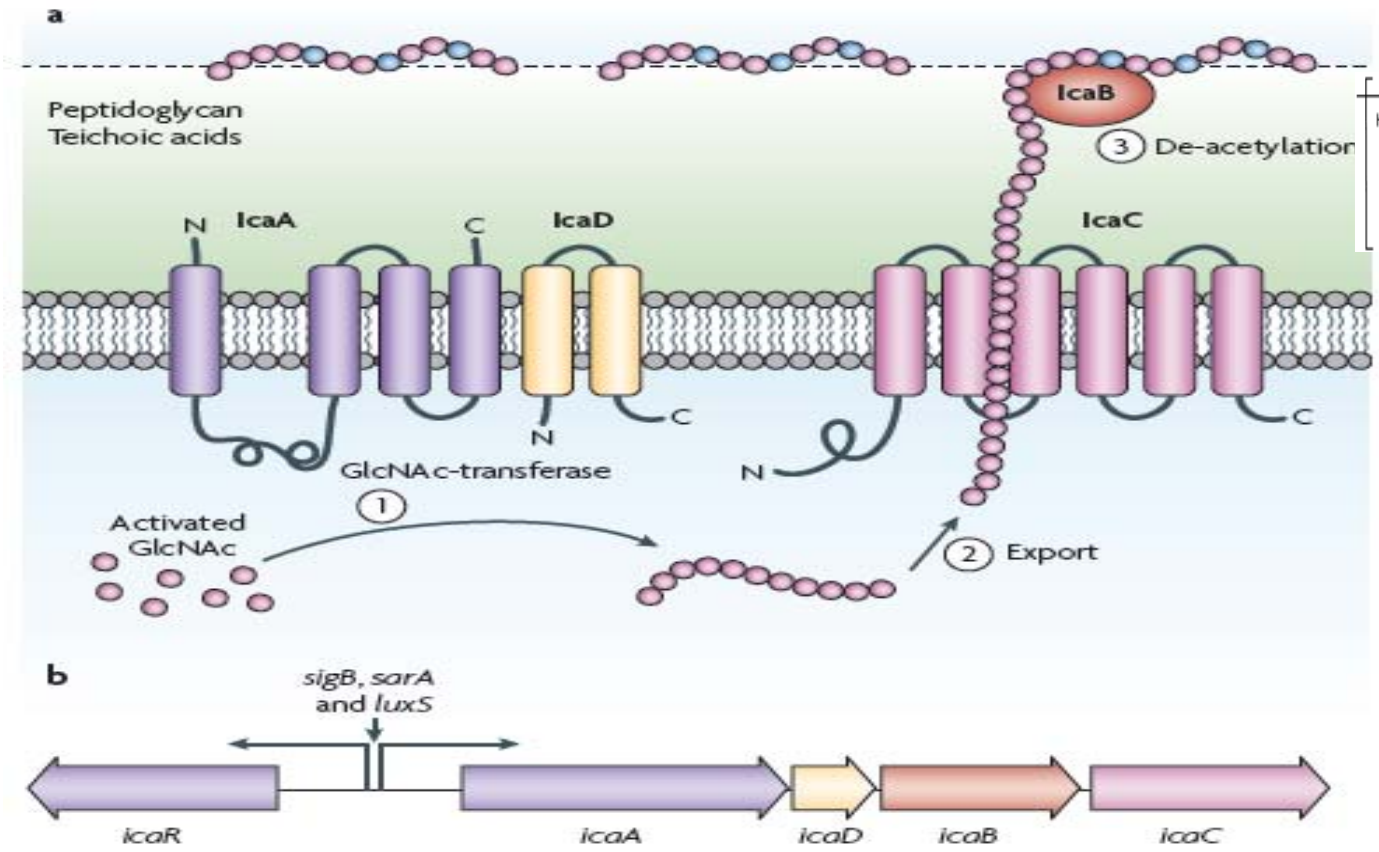
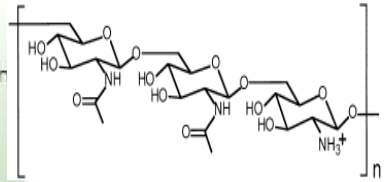
Role of *ica* operon in staphylococcal biofilms

Schematic procedure of PIA synthesis (a) and the gene arrangement in the *ica* operon (b)





β-1, 6-linked N-acetylglucosamine

Nat Rev Microbiol. 2009 August ; 7(8): 555–567

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Role of *ica* operon in staphylococcal biofilms

- **PIA is synthesized by enzymes encoded by *ica* operon**
- **PIA play a role in attachment and accumulation phases**
- **Most of clinical isolates of CoNS and *S. epidermidis* are *ica*$^{+}$, PIA-dependent biofilm-forming strains**
- **So far, all MRSA (methicillin-resistant *Staphylococcus aureus*) have been shown to be *ica*$^{+}$, proteinaceous (PIA-independent) biofilm-forming strains, whereas MSSA (methicillin-resistant *Staphylococcus aureus*) can be *ica*$^{-/+}$, PIA independent/ dependent biofilm forming**

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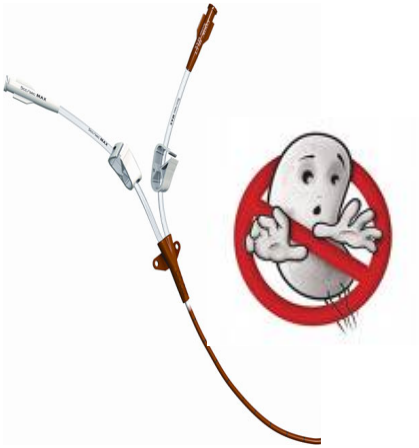
Preventive strategies

- Improvement of specific clinical practice guidelines
  - can decrease the incidence of DRI
- Antimicrobial biomaterial
  - induction, generation and selection of resistance
- Antimicrobial prophylaxis
  - high prevalence of antimicrobial resistance
- Targeting essential biofilm factors
  - inhibition of enzymes involved in biofilm biosynthesis
  - Immunoprophylaxis (need a vaccine)



Treatment of biofilms

- **Traditional approach is administration of antimicrobial agents**
  - **Currently, the only effective treatment for biofilm infections is to remove the implant, fight the infection with antibiotics, and replace the implant, a risky, costly and stressful procedure**
- **QS perturbation to revert established biofilms**
  - **In a biofilm, *agr* expression is limited to surface-exposed area and *agr* mutants occur naturally in deeper layers**
- **Immunological approaches**

S. aureus and *S. epidermidis* vaccines

- Active immunization
  - Current and finished clinical vaccine trials using active immunization
    - Merck V710 vaccine
    - StaphVax developed by Nabi
    - ExPAX™
  - Vaccines in pre-clinical development using active immunization
    - Alpha-toxin
    - Panton-Valentine leukocidin (PVL)
    - Pentastaph (Nabi)
    - Multi-component adhesin vaccine
    - Poly-N-acetylglucosamine (PNAG)
    - Als3p (Novadigm):
    - Iron-regulated proteins (Syntiron/Sanofi Pasteur)
- Passive immunization/therapeutic antibodies
  - Passive immunization strategies in clinical trials
    - Altastaph from individual treated with Nabi's StaphVax
    - Clumping factor (ClfA) targeted antibodies
    - Aurograb
    - Pagibaximab
  - Passive immunization strategies in pre-clinical development
    - Alpha toxin
    - PVL
    - Superantigens

All active and passive immunization strategies have thus far failed to show efficacy in humans, despite being protective against staphylococcal infection in animal models.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Novel targeted immunotherapy approaches for staphylococcal infection

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Immunoprophylaxis and immunotherapy of *Staphylococcus epidermidis* infections: challenges and prospects

Lieve Van Mellaert, Mohammad Shahrooei, Dorien Hofmans and Johan Van Eldere


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Inferring reasons for the failure of *Staphylococcus aureus* vaccines in clinical trials

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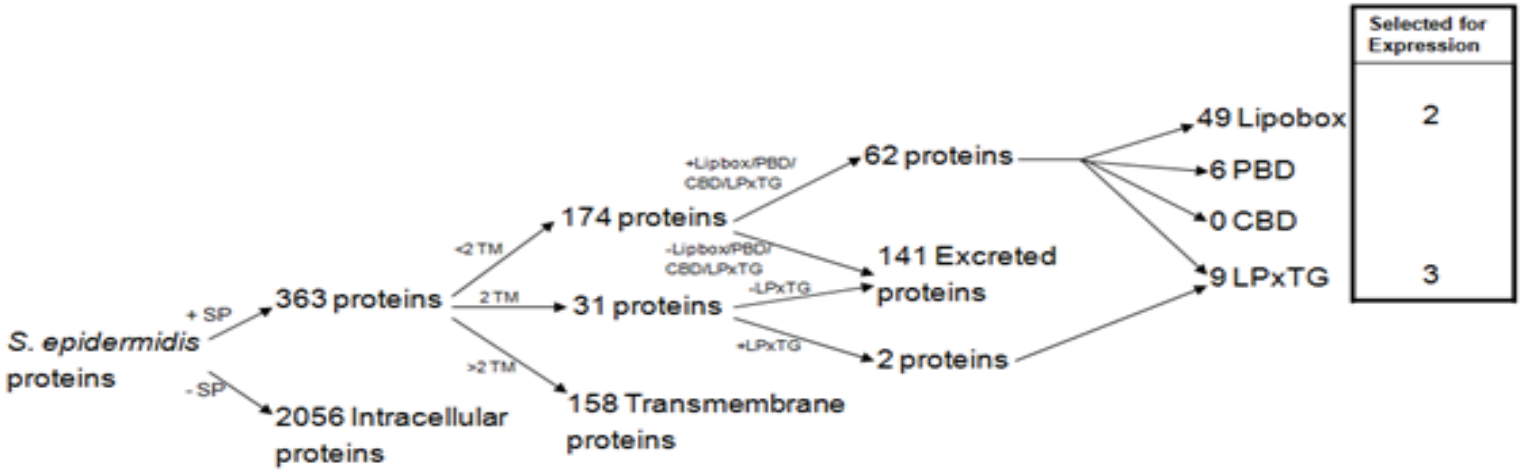
Identification of potential vaccine targets for vaccination against *S. epidermidis* biofilm formation

- ***In silico* selection of *S. epidermidis* surface (Ses) proteins. SP, signal peptide; TM, transmembrane helix; PBD, peptidoglycanbinding domain; CBD, choline-binding domains**



Ideal anti-biofilm vaccine targets are surface components that were conserved across the species, in particular those which are highly expressed in the bloodstream and in biofilms, with a possible role in biofilm formation or an essential function

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Selection of best potential vaccine targets

- **Five Ses proteins were selected based on the protein size, the number of antigenic determinants and the importance of the protein family, to which the candidate protein belongs, in *S. epidermidis* biofilm formation and pathogenesis**

Locus Name	Putative product name	Protein accession number	Protein size (amino acid)	Motif	No. of antigenic determinants
SE2232	conserved hypothetical protein (SesC)	NP_765787.1	676	LPXTG	20
SE1106	ABC transporter _membrane spanning protein (SesL)	NP_764661.1	564	Lipobox	16
SE1981	nickel ABC transporter/ nickel binding protein (SesM)	NP_765536.1	491	Lipobox	18
SE1501	hypothetical protein (SesK)	NP_765056.1	415	LPXTG	11
SE2152	hypothetical protein (SesB)	NP_765707.1	196	LPXTG	7

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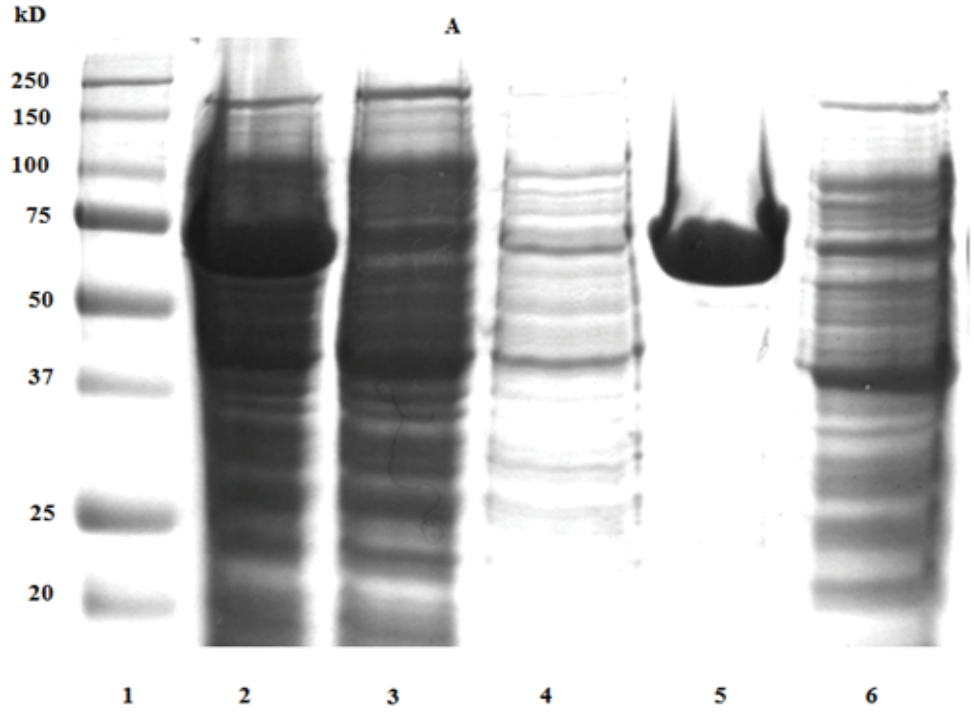
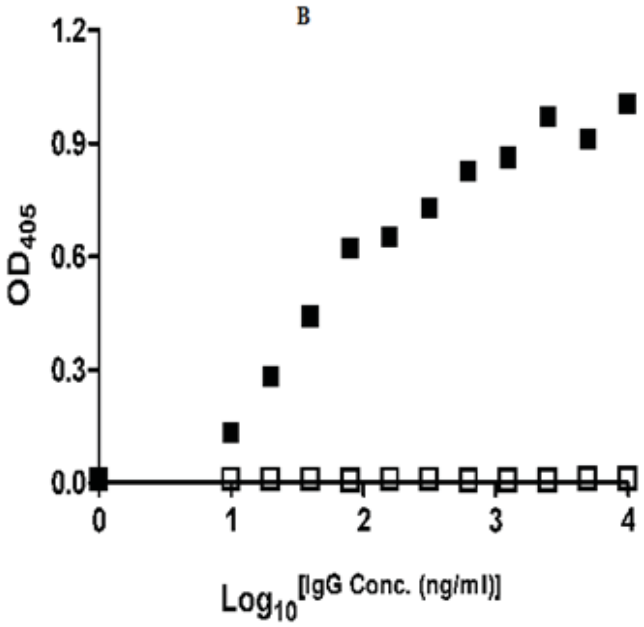
Recombinant Ses and anti-Ses antibody production

- **Surface-exposed part of Ses proteins were recombinantly expressed in *E. coli* and polyclonal anti-Ses antibodies were raised against them and specific anti-Ses antibodies were purified using antigen-affinity purification**





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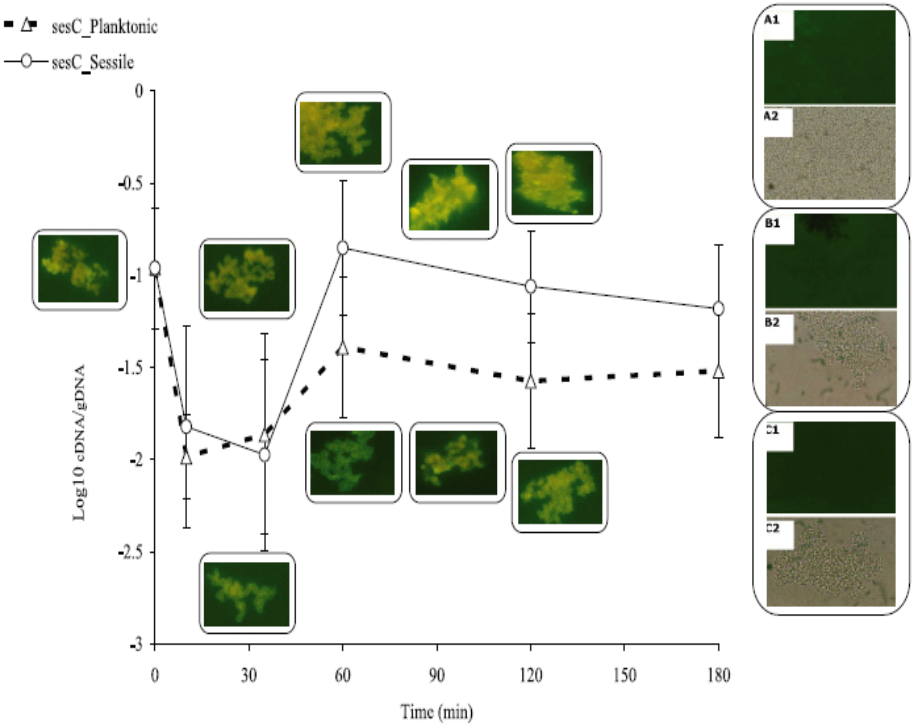
Validation of expression of Ses proteins on the surface

Western blot and ELISA data using immune sera against recombinant Ses proteins and whole cell *S. epidermidis* ATCC 12228.

Antiserum	on respective rSes protein		on *S. epidermidis* lysate		on WC *S. epidermidis*
against	Western blot	ELISA	Western blot	ELISA	ELISA
SesC	+	+	+	+	+
SesL	+	+	+	+	+
SesM	+	+	+	+	-
SesK	+	+	-	+	-
SesB	+	+	+	+	+
WC	+*	+	+	+	+

*: Western blot with whole cell antiserum on recombinant proteins was positive for all proteins except SesK

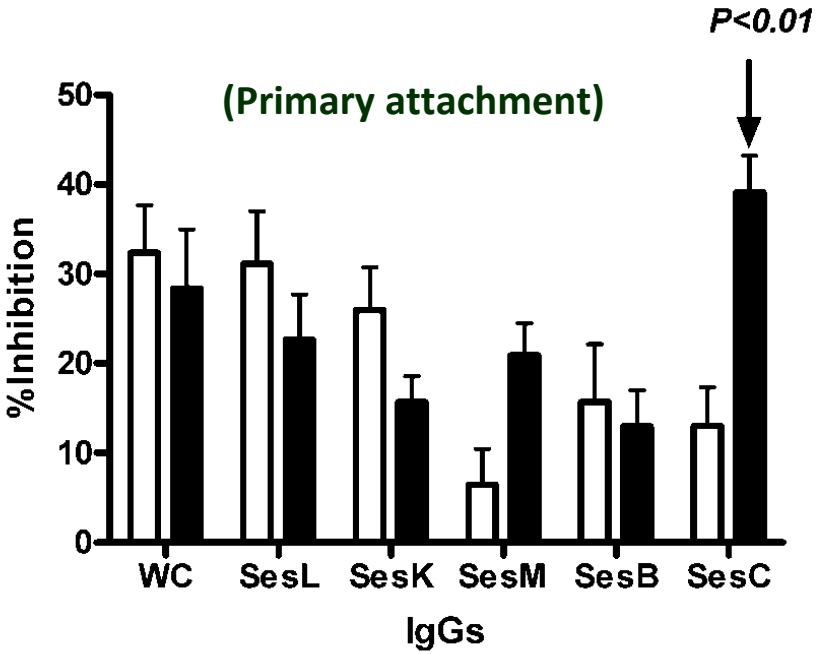
WC: whole cell

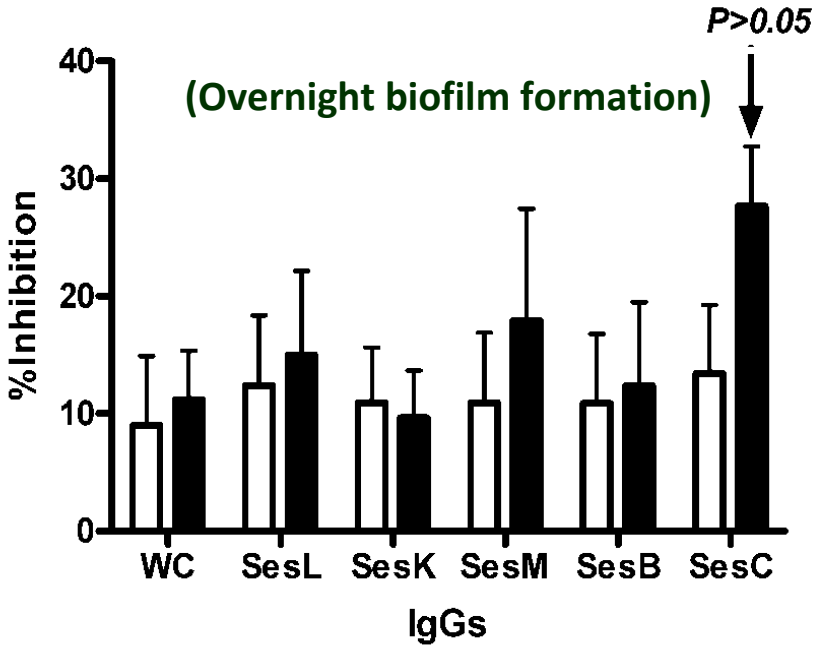


Selection of best potential vaccine target

- **Biofilm inhibition was assessed *in vitro*, using the microtiter plate assay**

Pre-immune (□) Post-immune (■)





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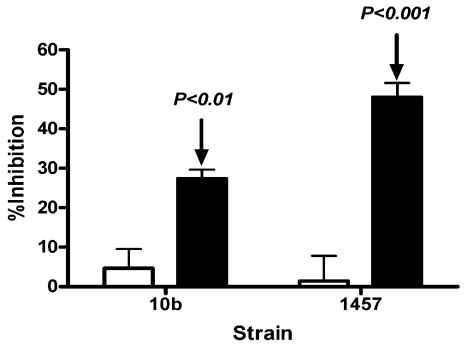
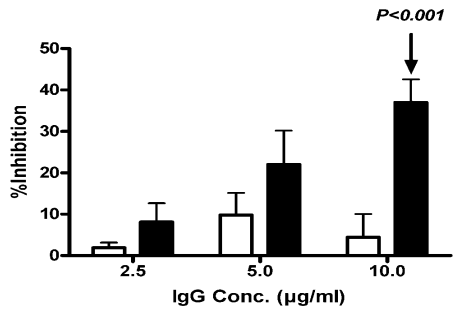
Effect of anti-SesC IgG's on *S. epidermidis* biofilms *in vitro*

Pre-immune (□) Post-immune (■)

Primary attachment

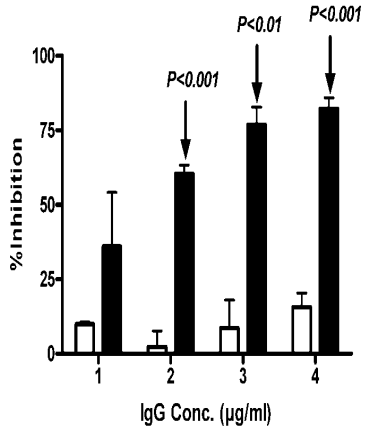


Effect on 1-day established biofilms

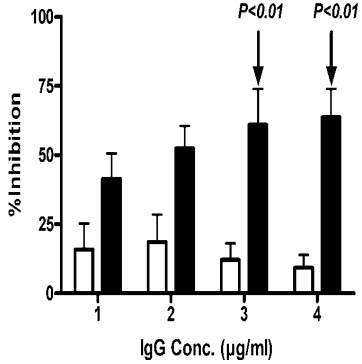


Specific and dose-dependent effect

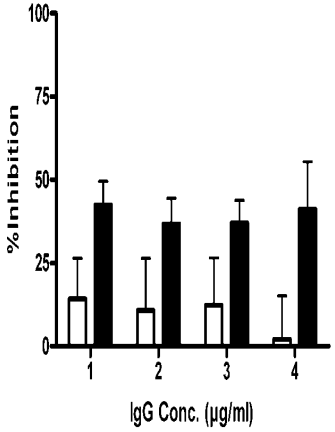
***S. epidermidis* 10b**



***S. epidermidis* 1457**



S. warneri



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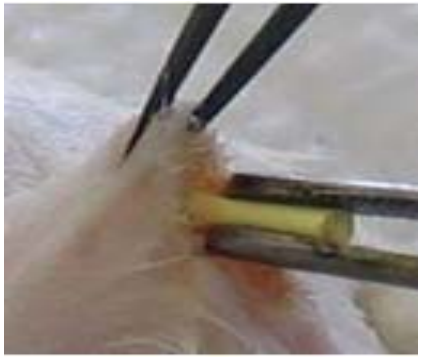
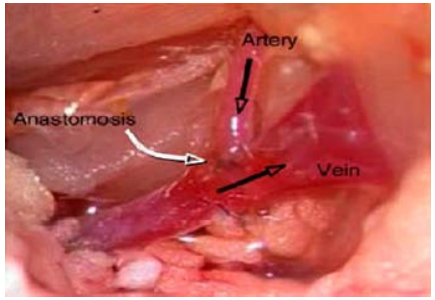
In vivo models

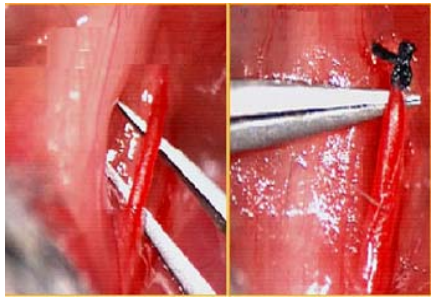
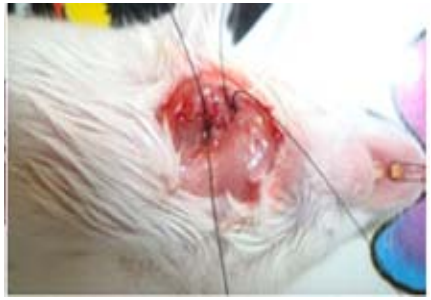
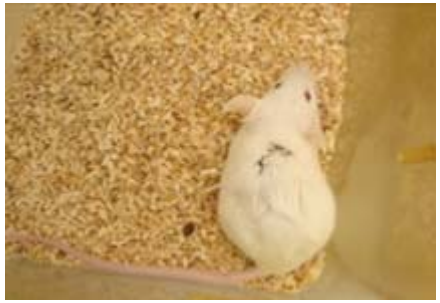
Subcutaneous catheter (SC) rat model





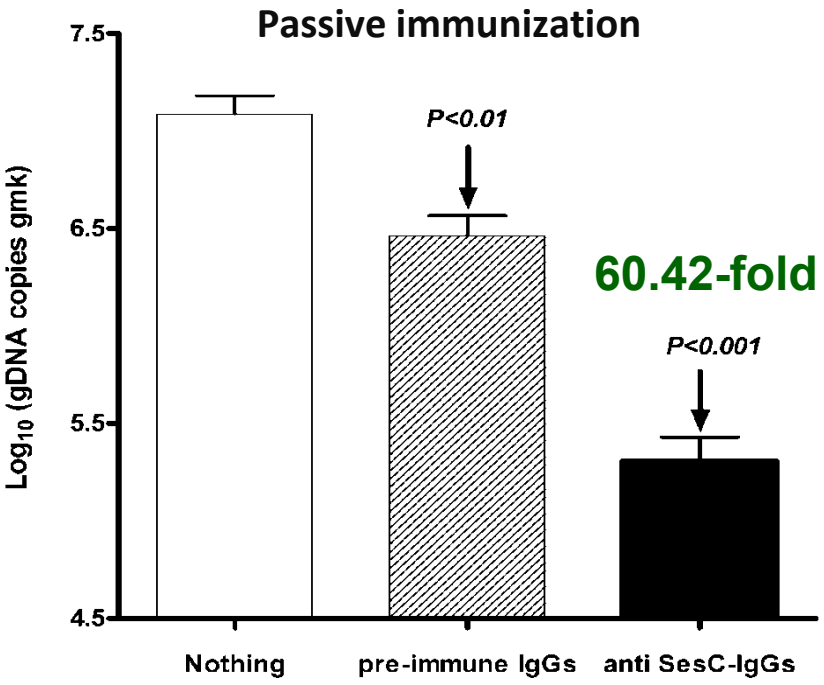

Jugular vein catheterized (JVC) mouse model

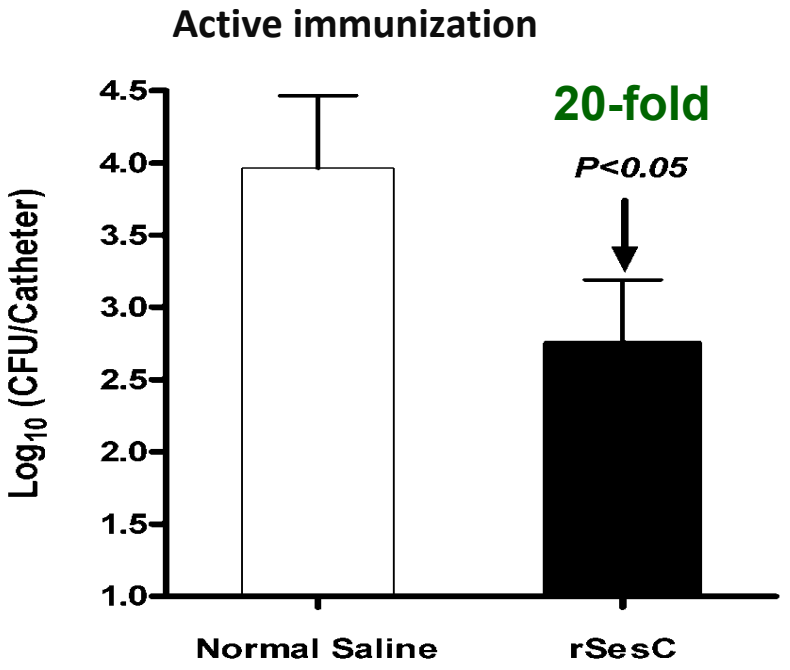


Active and passive immunization

- Effect of αSesC-IgGs on 1-day old biofilms *in vivo* (passive immunization)
- Effect of immunization of rats with rSesC on biofilm formation (active immunization)





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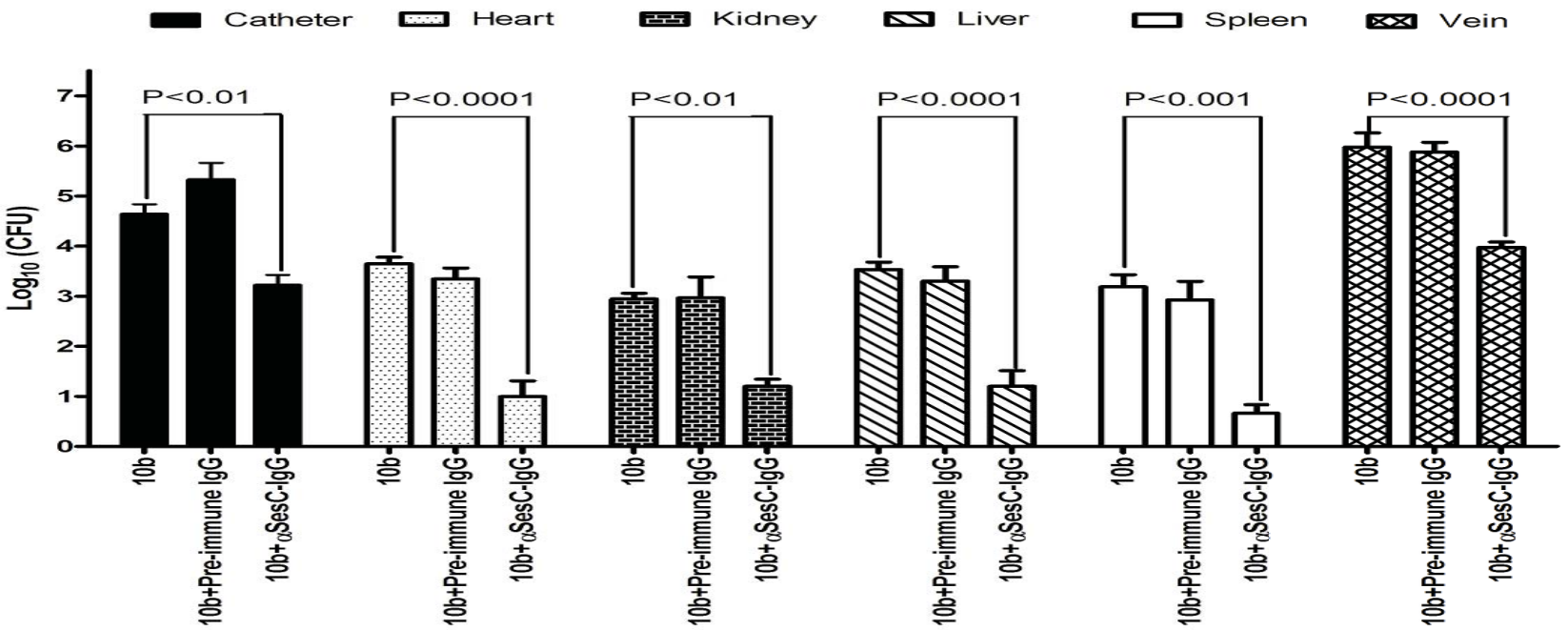
Effect of anti-SesC on DRI in JVC model

24 h after the implantation, JVC mice were inoculated with 1.0E+8 CFU 10b pre-incubated with pre-immune or αSesC-IgG's. 5 days after inoculation, the number of bacteria colonizing the catheter, organs or in blood stream was quantified by CFU counting. * P<0.05; ** P<0.01; *** P<0.001



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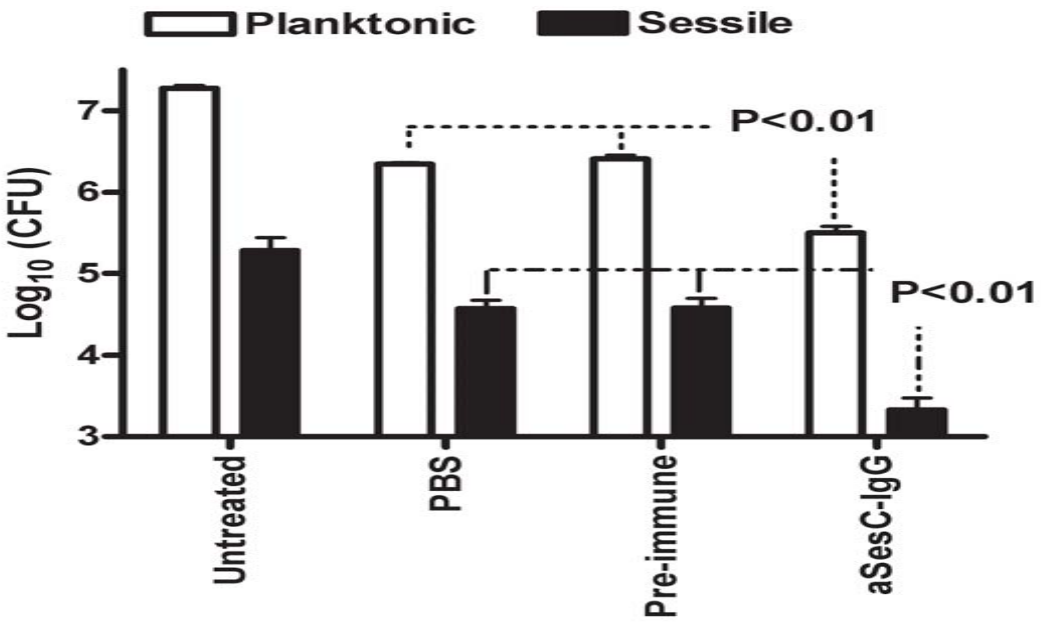
Mechanism of function of anti-SesC IgG's

Semi-quantitative microtiter plate

In vitro → neutralization

In vivo opsonophagocytosis assay

In vivo → opsonization



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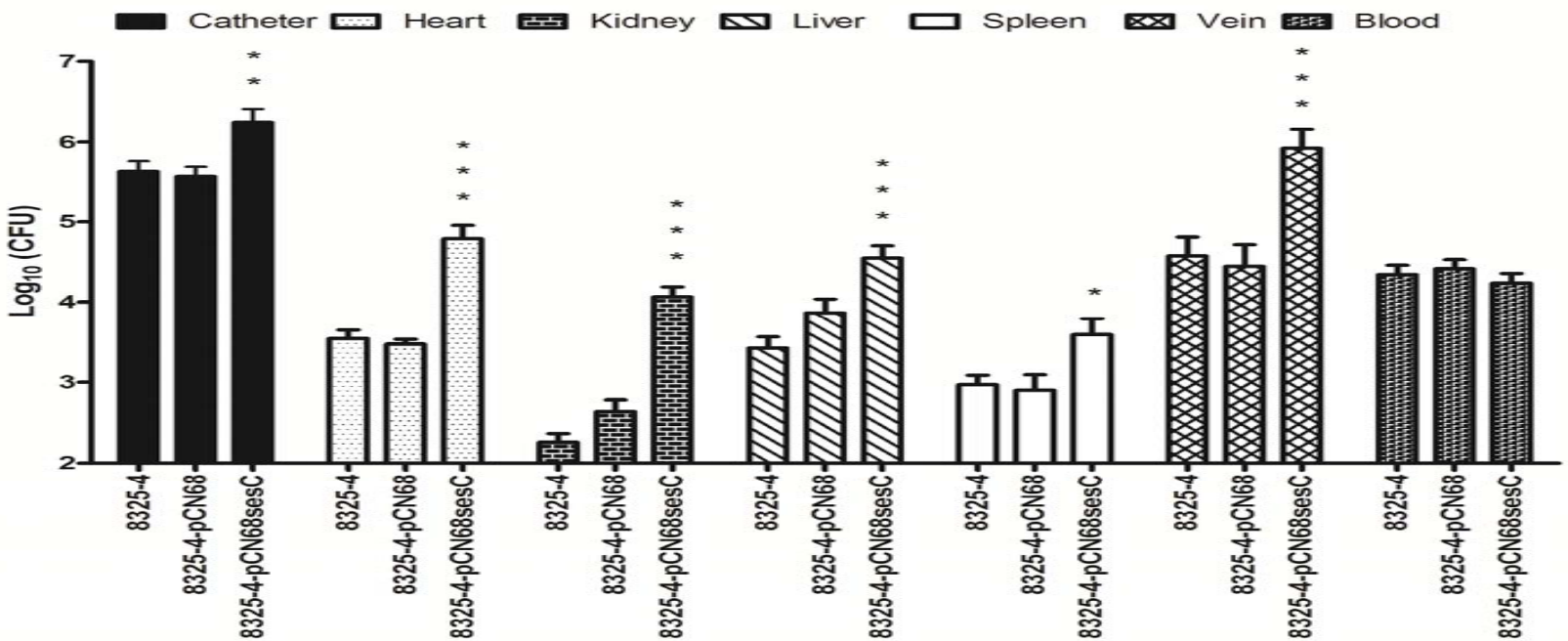
SesC is associated with DRI *in vivo*

24 h after the implantation, JVC mice were inoculated with 1.0E+7 CFU *S. aureus* via the catheter lumen, 5 days after inoculation, the number of bacteria colonizing the catheter, organs or in blood stream was quantified by CFU counting. The error bars indicate the standard errors of the mean. * P<0.05; ** P<0.01; *** P<0.001



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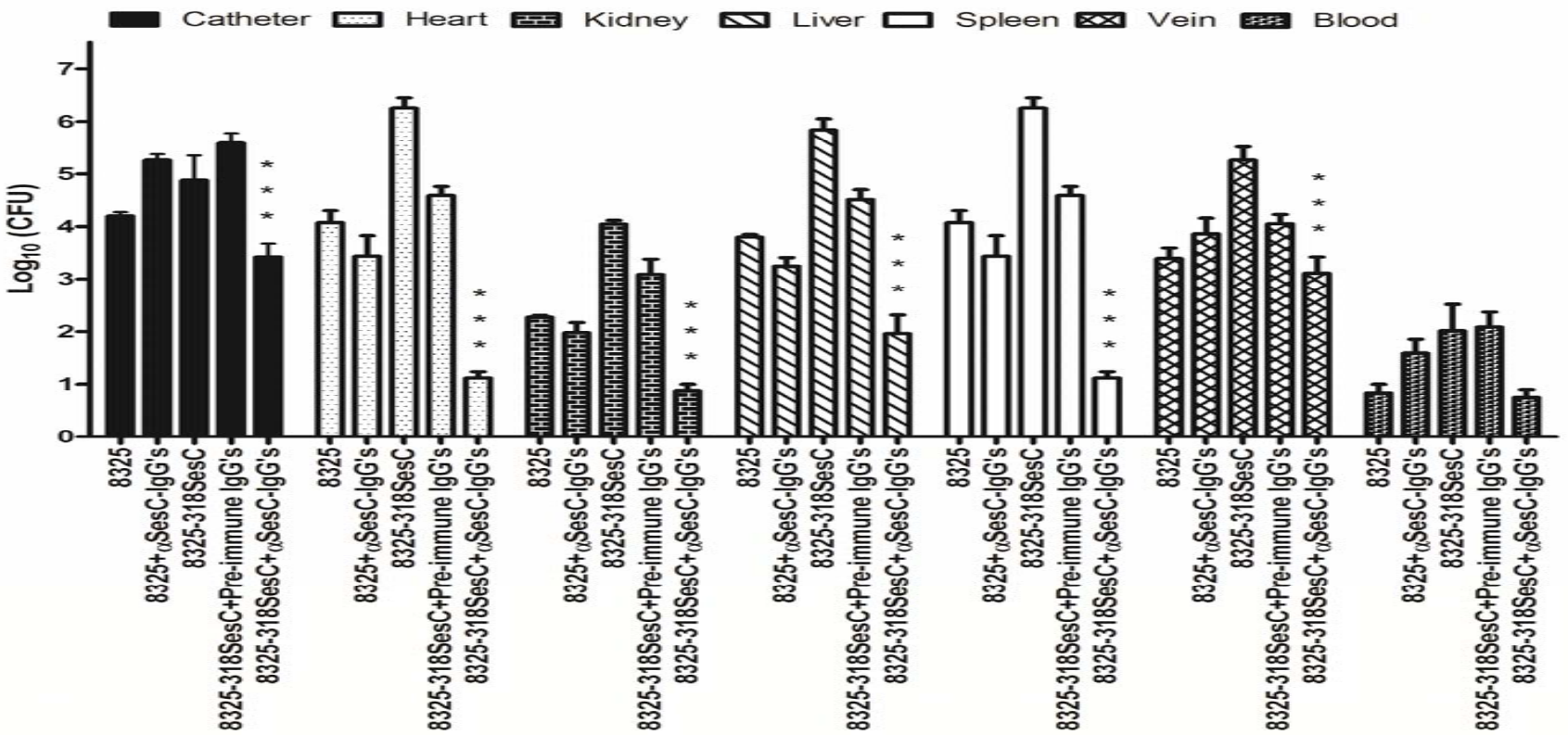
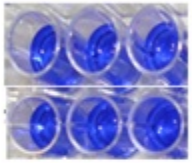
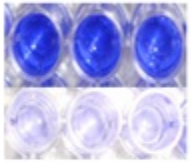
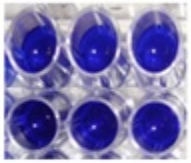
SesC is associated with DRI *in vivo*

Effect of pre-incubation with pre-immune or αSesC-IgG's on S. aureus 8325-4 strain and its sesC-positive transformant

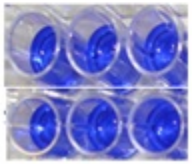
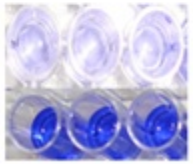
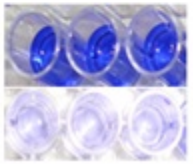


Biofilm development in *Staphylococcus* spp.

- Effect of NaCl and glucose on biofilm formation

Strain	Biofilm phenotype	BHI	BHI+NaCl (4%)	BHI+Glucose (1%)
8325-4	PIA-dependent			
BH1CC	proteinaceous			

- Effect of dispersal agents on established biofilms

Strain	Biofilm phenotype	BHI	SM	PK
8325-4	PIA-dependent			
BH1CC	proteinaceous			

SM: Sodium Metaperiodate, PK: Proteinase K

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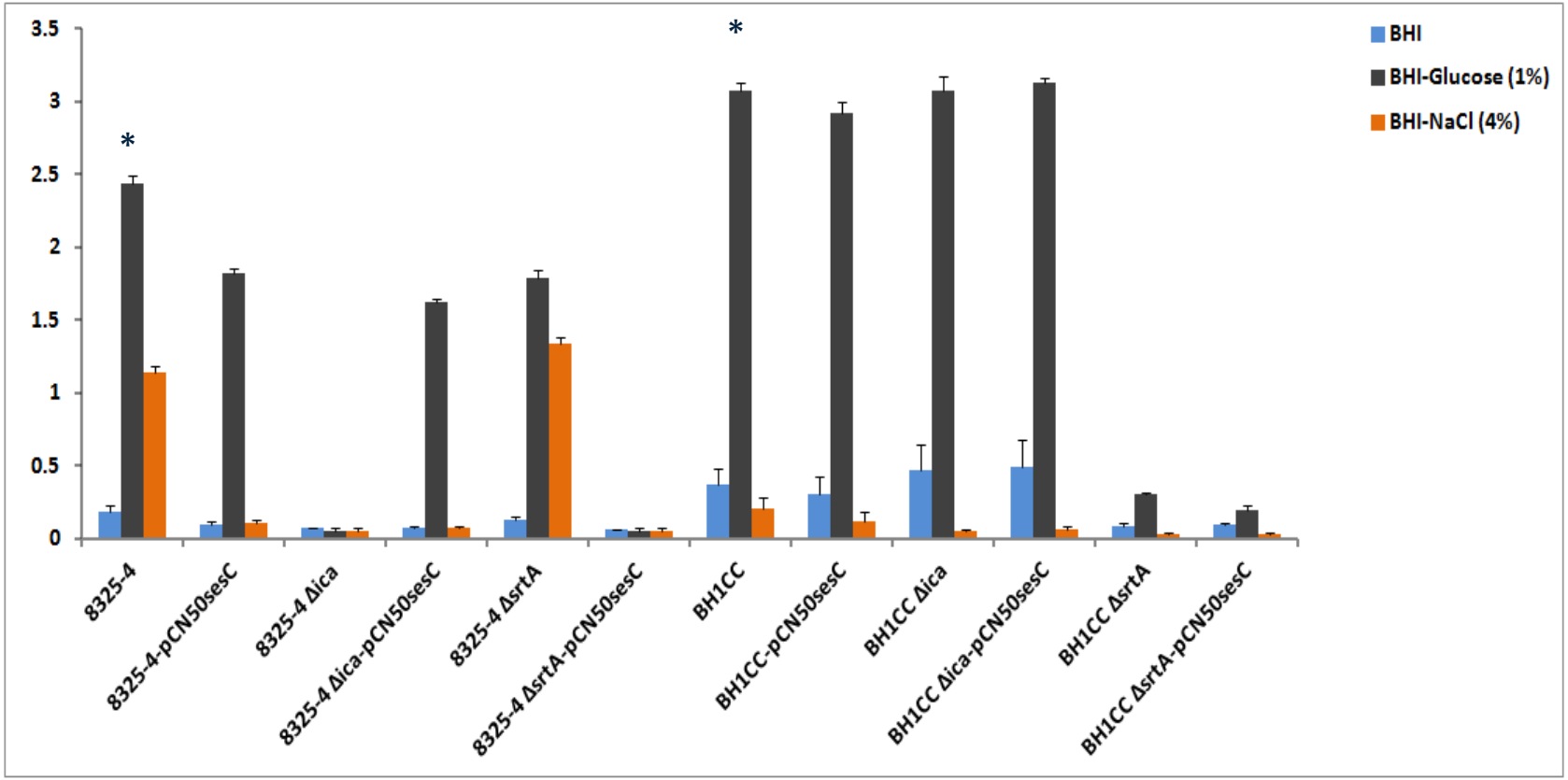
SesC switches mechanism of biofilm formation *in vitro*

Transformation with *sesC* changes the phenotype of biofilm formation of PIA-dependent biofilm-forming strains



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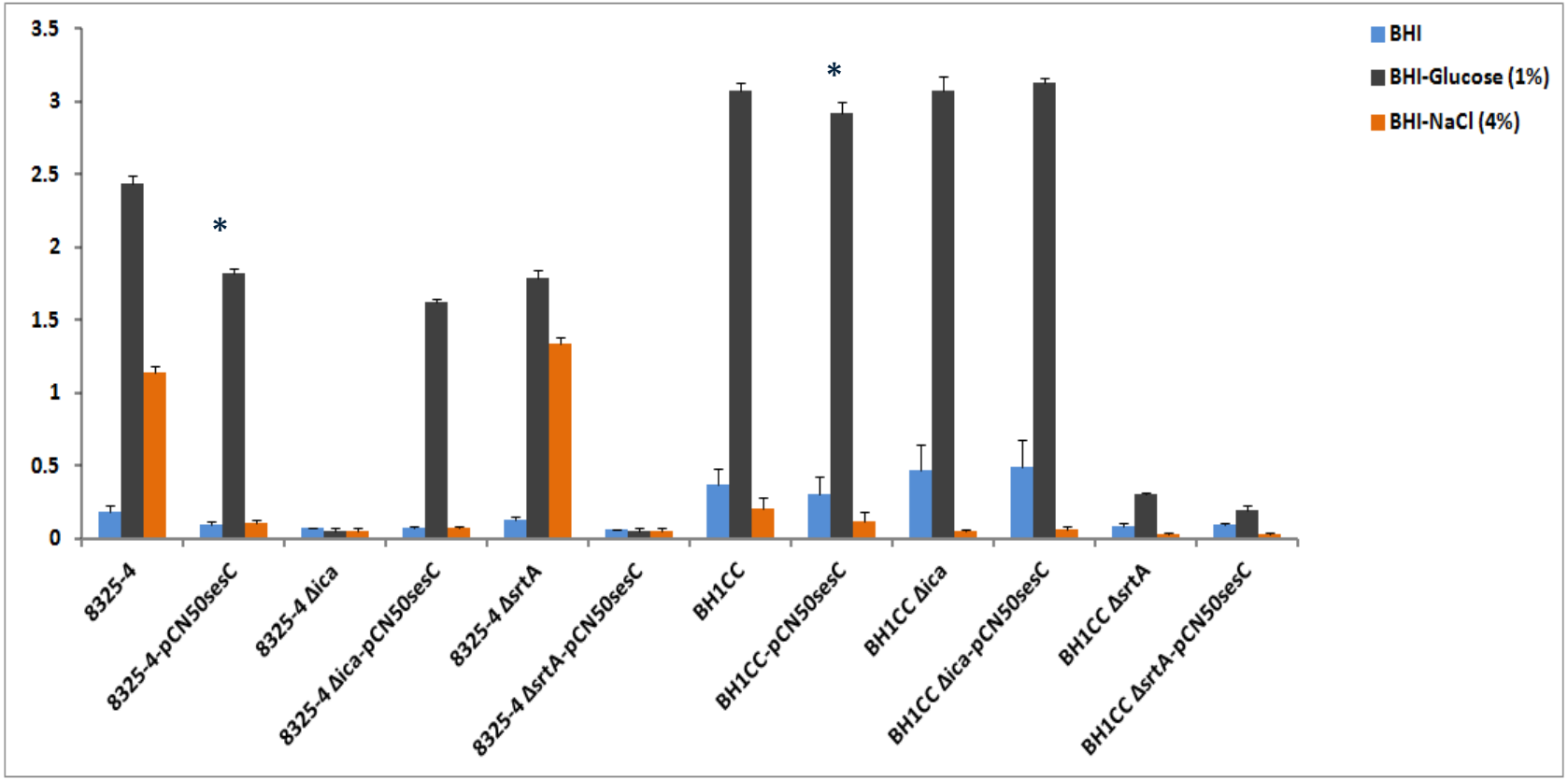

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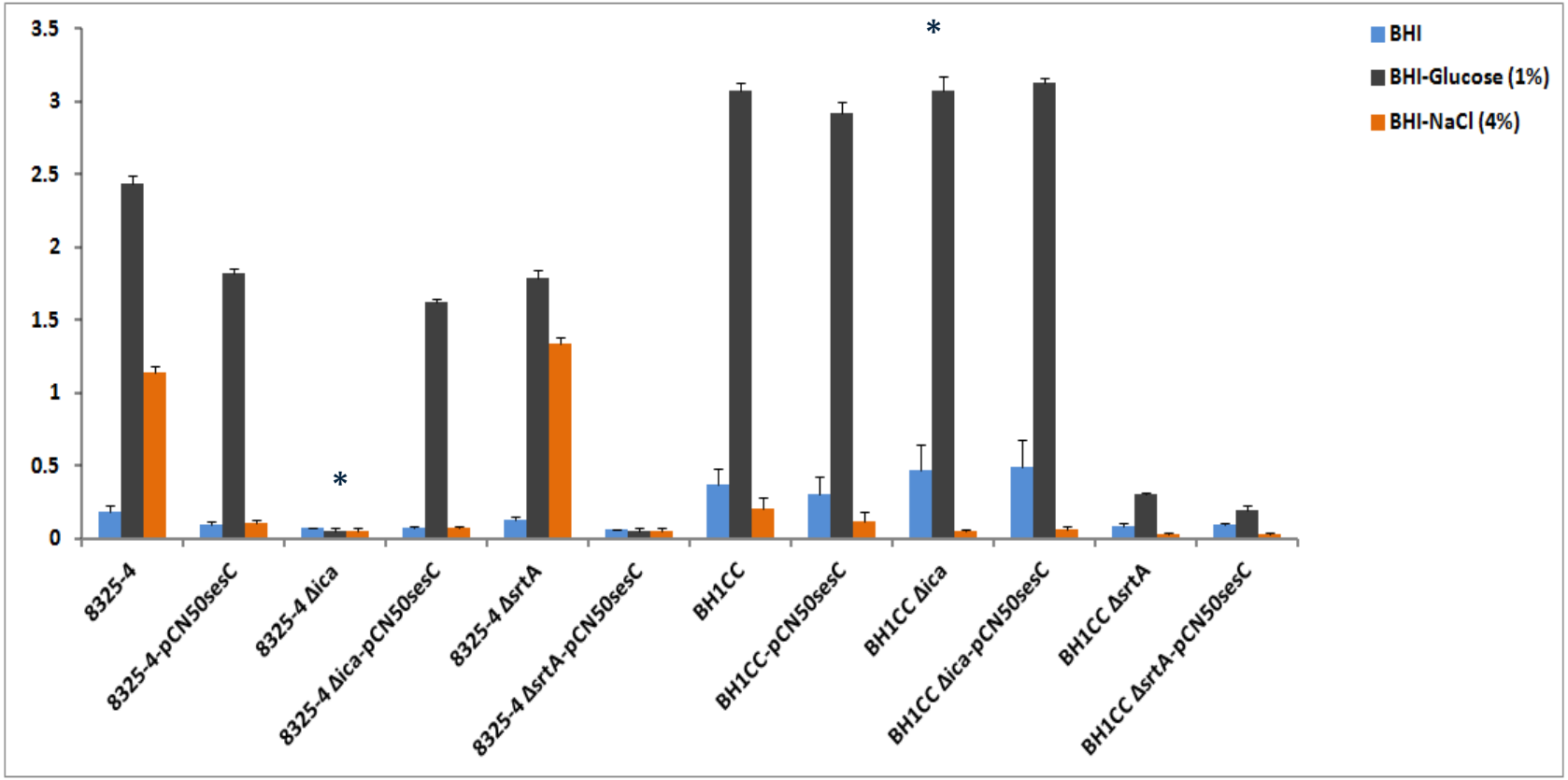
SesC switches mechanism of biofilm formation *in vitro*

Transforamtion with *sesC* changes the phenotype of biofilm formation of PIA-dependent biofilm-forming strains



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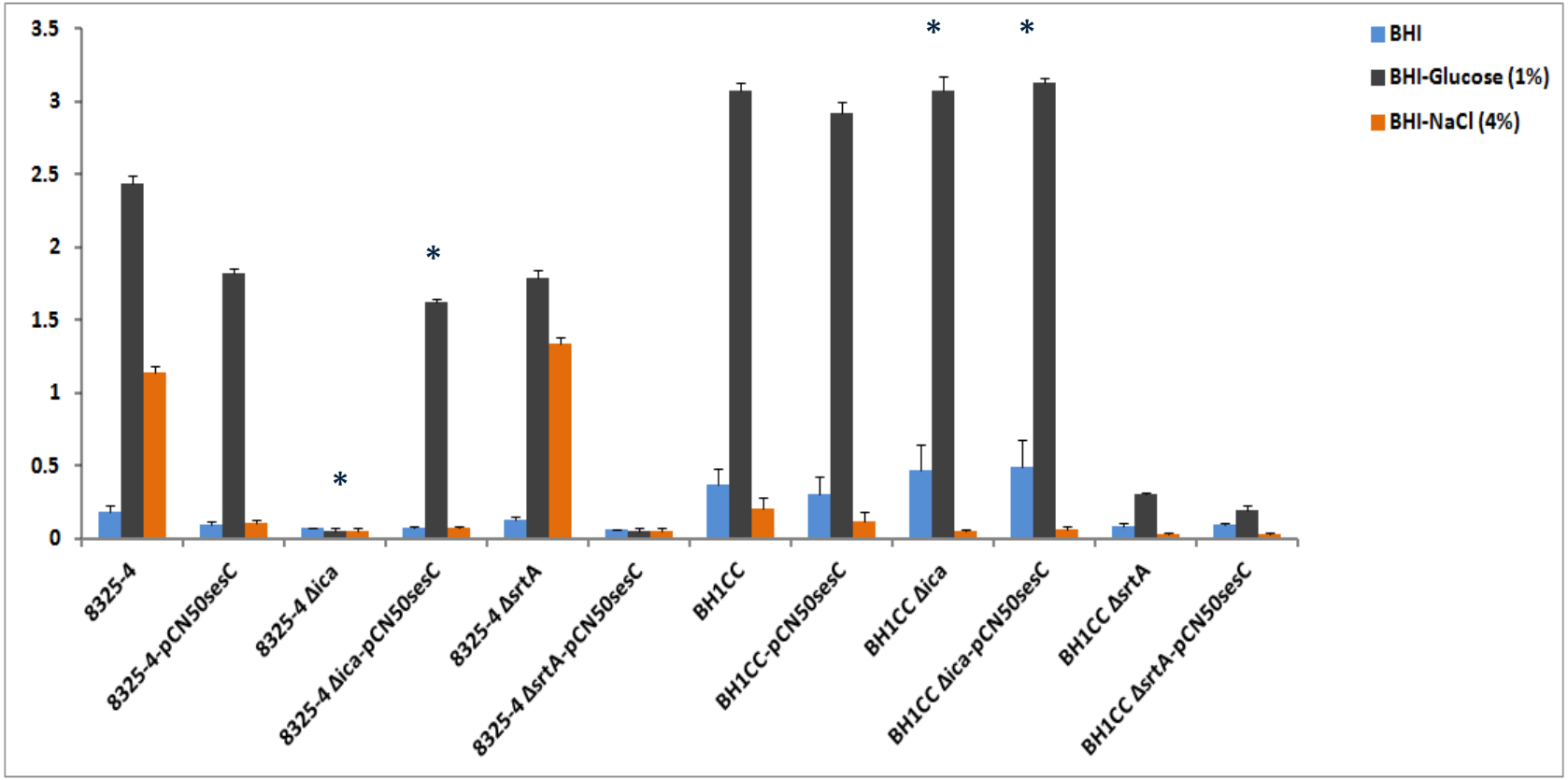
SesC switches mechanism of biofilm formation *in vitro*

Transformation with *sesC* changes the phenotype of biofilm formation of PIA-dependent biofilm-forming strains



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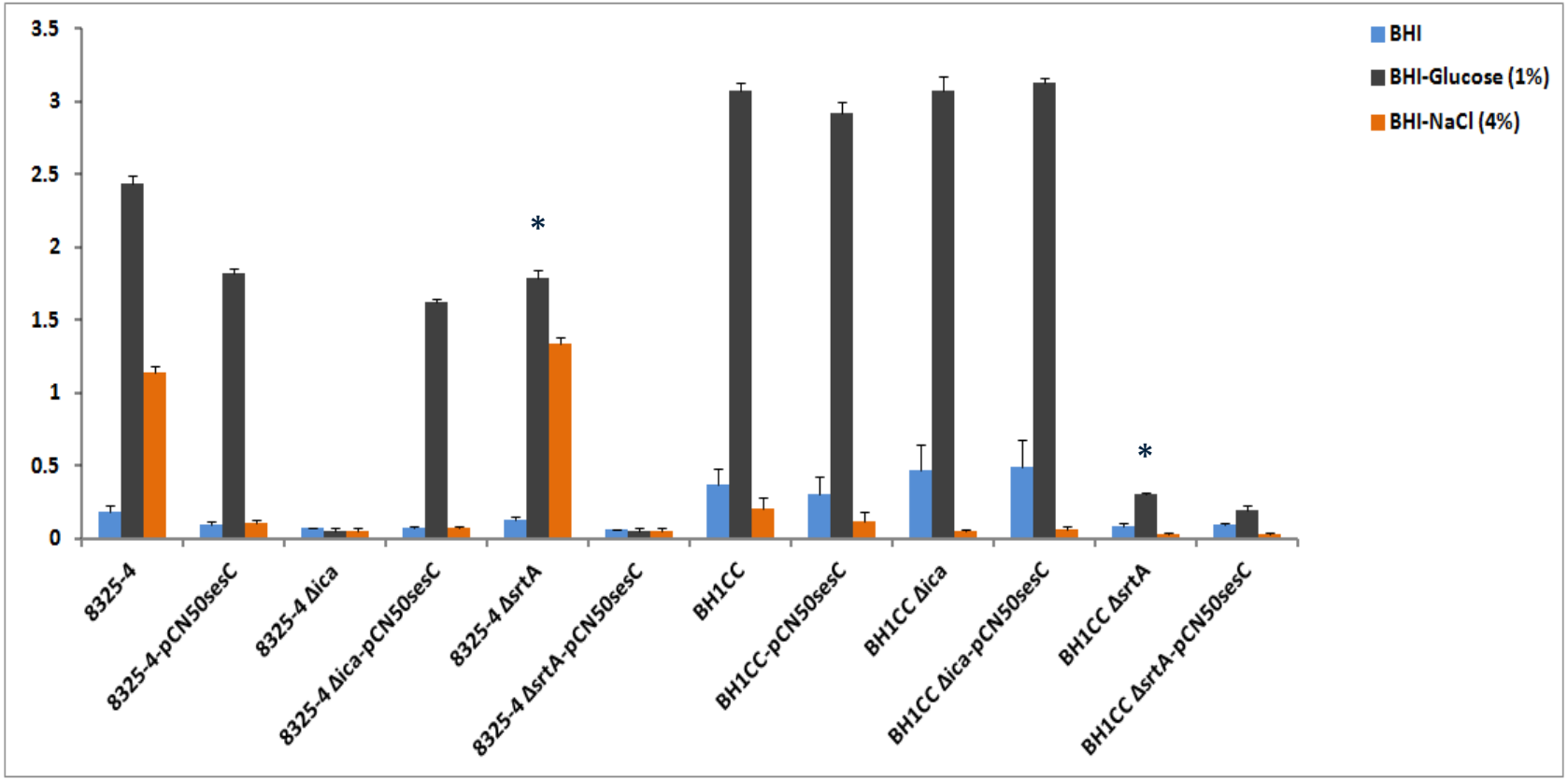
SesC switches mechanism of biofilm formation *in vitro*

Transformation with *sesC* changes the phenotype of biofilm formation of PIA-dependent biofilm-forming strains



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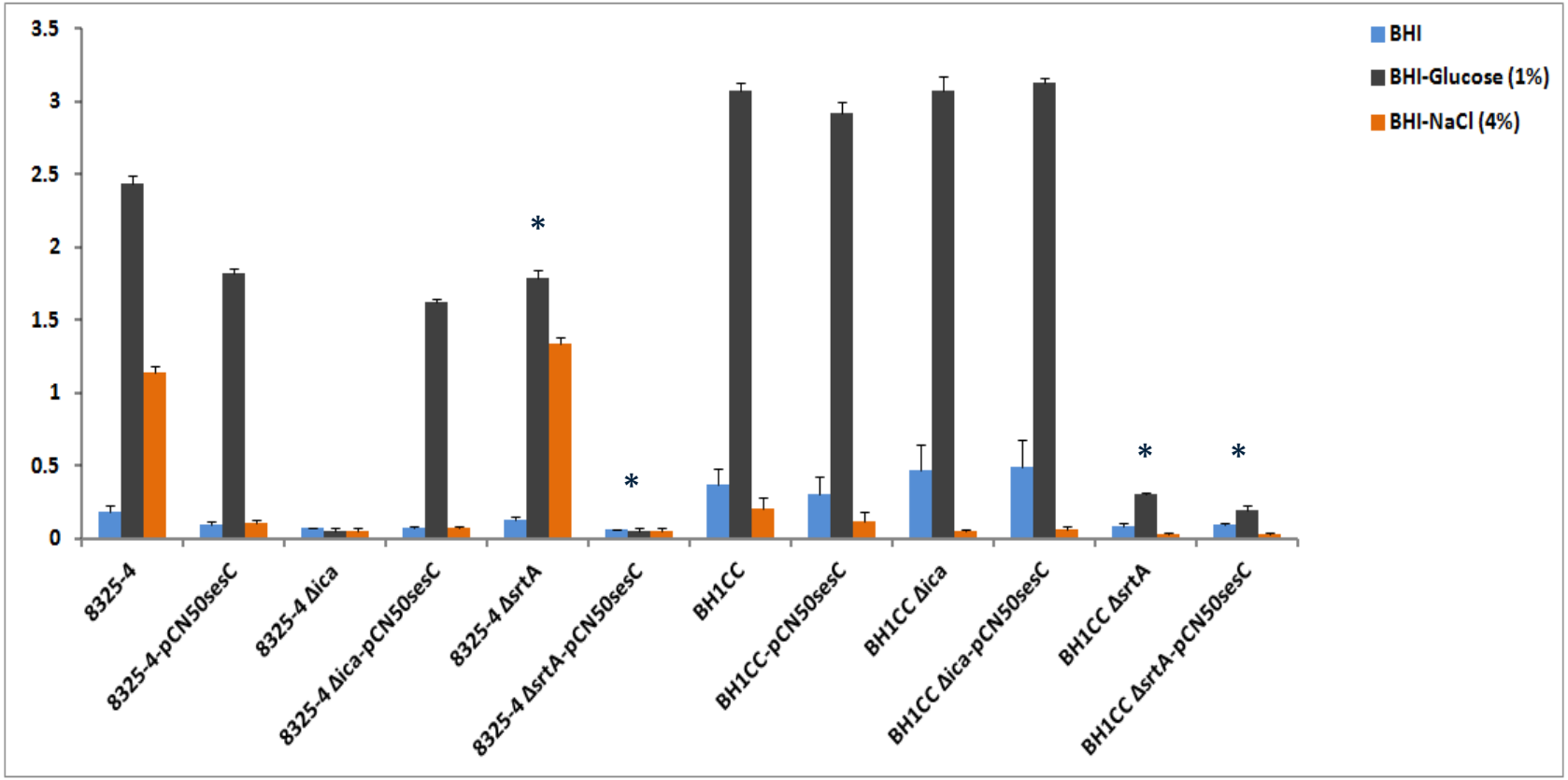
SesC switches mechanism of biofilm formation *in vitro*

Transformation with *sesC* changes the phenotype of biofilm formation of PIA-dependent biofilm-forming strains



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Conclusions

- **SesC plays a role in *S. epidermidis* biofilm formation**
- **SesC might encode an essential function in *S. epidermidis***
- **SesC might be a promising target for vaccine development against *S. epidermidis* biofilm formation**

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- Prof. Johan Van Eldere
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Questions