

*Staphylococcus and biofilms
(and foreign body infections)*

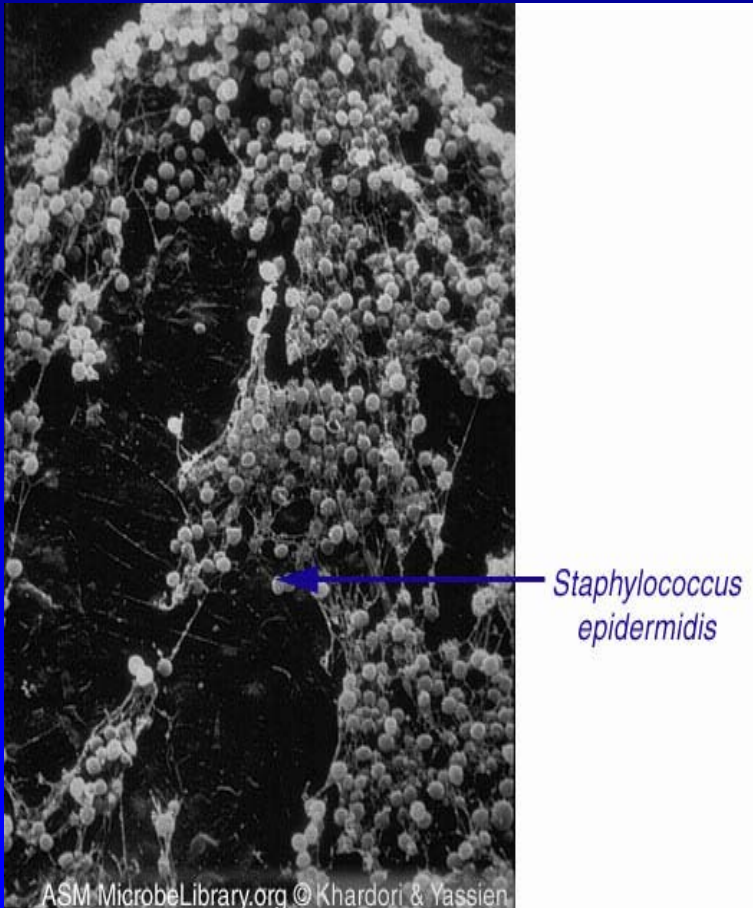
Johan Van Eldere

K.U.Leuven

Staphylococci and staphylococcal biofilms in foreign body infections

- CoNS are the leading cause of nosocomial bloodstream infections
 - 20-32% of all bloodstream isolates
- CoNS most frequent isolates in implanted devices associated infections
- High level of antibiotic resistance in FBI associated CoNS

Staphylococci and staphylococcal biofilms in foreign body infections



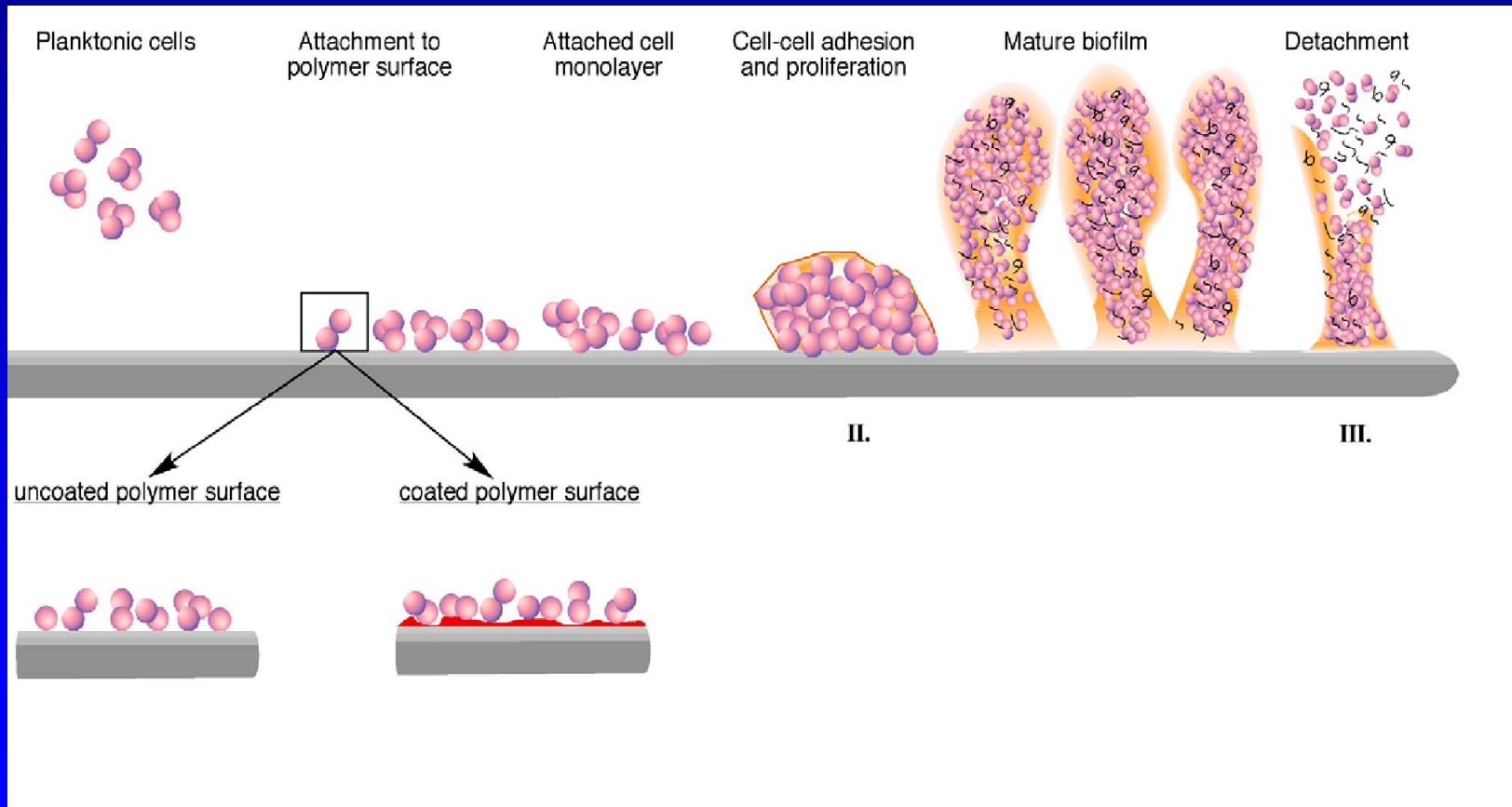
- Biofilms represent a protected growth mode
 - Against changing environmental conditions (pH, hypoxia, ...), against disinfectants and disinfecting techniques, against host defence mechanisms, against antibiotics

» Costerton, Science, 99, 284, 1318; De Beer, AEM, 94, 60, 4339; Cochran, J Med Microbiol., 92, 36, 255; Khoury, Am Soc Artif Int Organs J, 92, 38, 174; Chen, Environ Sci Technol, 96, 30, 2078; Bolister, JAC, 89, 24, 619; Dunne, AAC, 93, 37, 2522; Anderl, AAC, 00, 44, 1818

Molecular events in staphylococcal biofilm formation

- Three stages:
 - Attachment:
 - Uncoated, coated material
 - Biofilm formation
 - Intercellular adhesion and accumulation of multi-layered cell clusters
 - Generation of slime glycocalix
 - Persistence and detachment (phase variation)

Molecular events in staphylococcal biofilm formation



Molecular events in staphylococcal biofilm formation

- Initial attachment to uncoated plastic material
 - primary adhesion: within seconds after exposure, aspecific, dependent on physicochemical interactions and surface properties of foreign body surface
 - Main bacterial parameter: hydrophobicity of bacterial surface
 - Role of AtlE (autolysin) in surface hydrophobicity
 - » Direct binding, loss of surface proteins, binding to host-matrix proteins
 - Role of lipoteichoic-like acids?
 - » Altered surface charge, decreased binding of surface proteins
 - Role of fimbria-like polymers
 - » SSP1 and SSP2
- Significance for biofilm formation considering rapid (seconds) coating of foreign body?

» Ferreiros, FEMS Microbiol Lett, 89, 51, 89; Vacheethasanee, J Biomed Mat, 98, 42, 425; Vacheethasanee, J Biomed Mat, 00, 50, 302; Heilmann, Infect Immun, 96, 46, 277; Gross, Infect Immun, 01, 69, 3423; Lambert, FEMS Immun Med Micro, 00, 29, 195

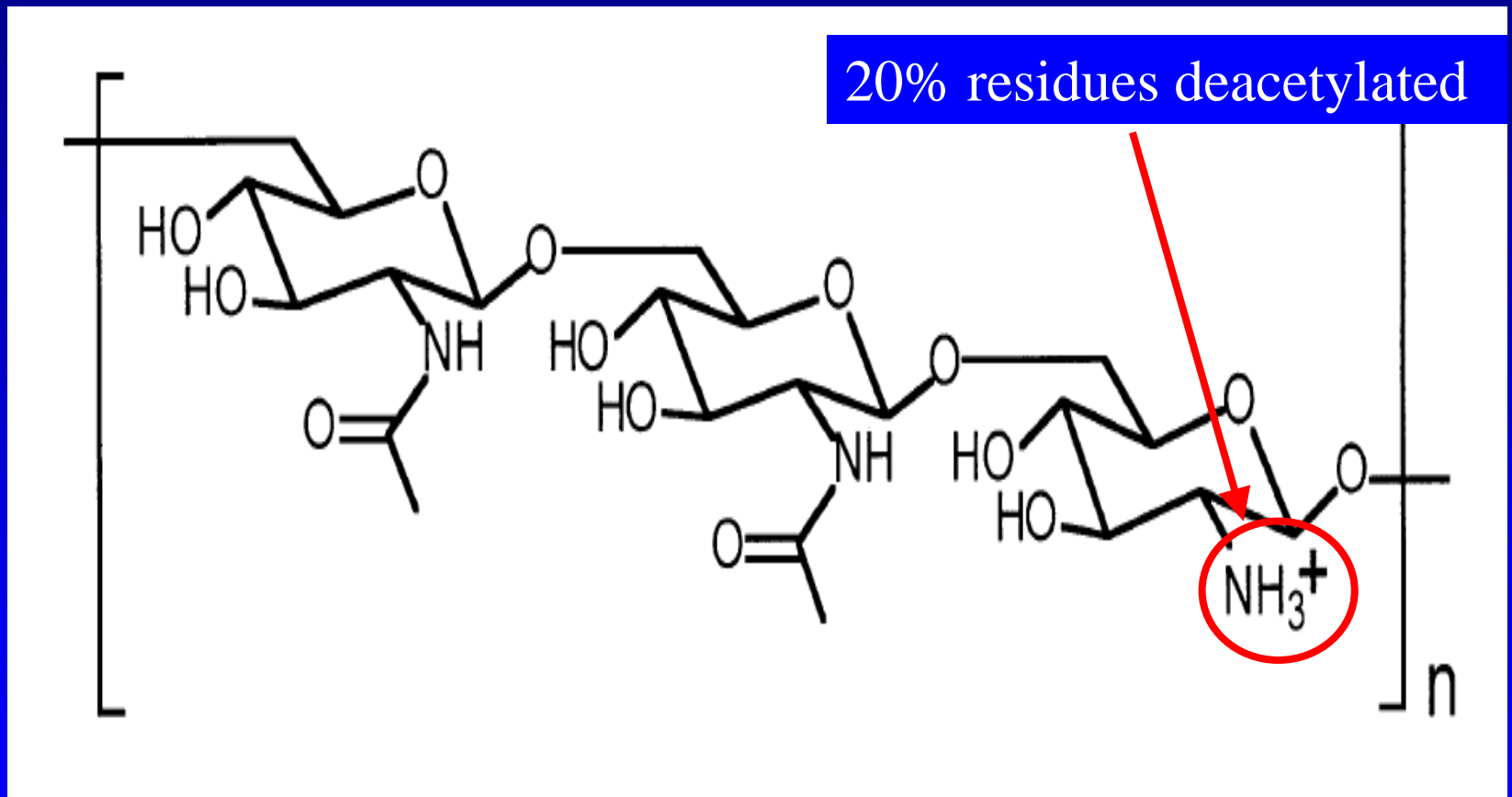
Molecular events in staphylococcal biofilm formation

- (Secondary) attachment to material coated with host-derived proteins
 - Promoted by surface irregularities, host-derived substances (fibronectin, collagen, laminin, vitronectin, fibrinogen, fibrin thrombi, activated platelets)
 - MSCRAMM's:
 - fibrinogen-binding protein (Fbe)
 - Few peptidoglycan-bound surface proteins
 - Role of non-covalently linked surface proteins (AtlE)
 - Polysaccharide Intercellular Adhesin (PIA) or PNAG (*S. aureus*)
 - Encoded by *icaABCD*
 - » Gross, Infect Immun, 01, 69, 3423; Franson, JCM, 84, 20, 500;; Heilman, Mol Microbiol, 97, 24, 1013; Timmerman, Infect Immun, 91, 59, 4187; Tojo, JID, 88, 157, 713; Mc Kenney, Infect Immun, 98, 66, 4711; Dunne, CMR, 02, 15, 155; Nilsson, Infect Immun, 98, 66, 2666

Molecular events in staphylococcal biofilm formation

- Intercellular adhesion and accumulation
 - Within 40 to 60 min after adhesion
 - Formation of multi-layered clusters of interconnected cells
 - Several polymeric carbohydrates and proteins involved
 - Accumulation Associated Protein (AAP)
 - Polysaccharide Intercellular Adhesin (PIA) or PNAG (*S. aureus*)
 - encoded by *icaABCD*
 - » Mack, J Bac, 96, 178, 175; Heilmann, Mol Microbiol, 97, 24, 1013; Husain, Infect Immun, 97, 65, 519; Stewart, Lancet, 01, 358, 135; Zimmerli, JID, 82, 146, 487

Molecular events in staphylococcal biofilm formation

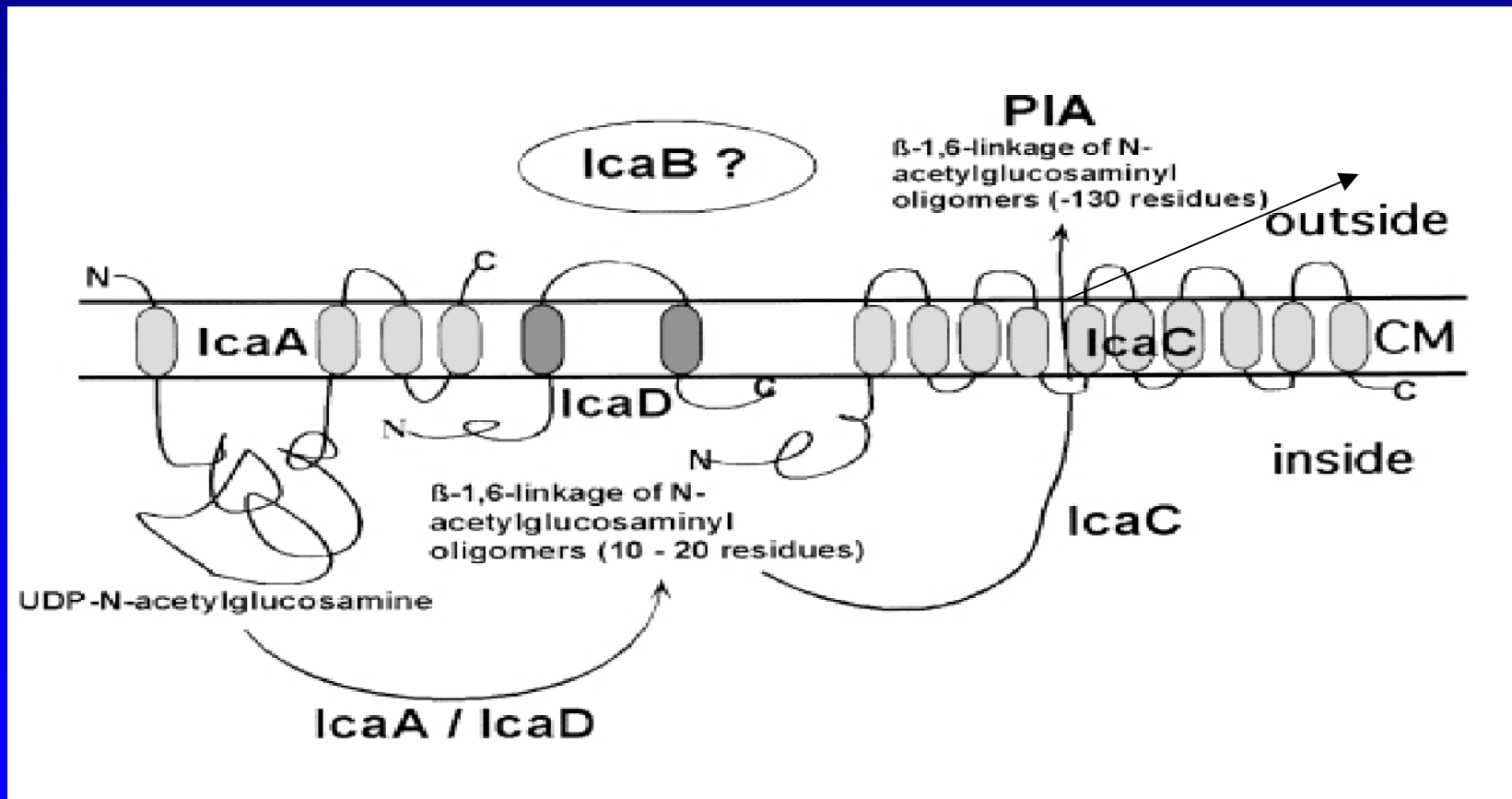


PIA: linear homoglycan of β -1,6-linked *N*-acetylglucosamine

Molecular events in staphylococcal biofilm formation

- Generation of extra cellular slime
 - Composition extra cellular slime
 - Teichoic acid
 - Bacterial proteins
 - Host proteins
 - Polysaccharide Intercellular Adhesin (PIA)

Molecular events in staphylococcal biofilm formation



Model for PIA – PNAG biosynthesis

Gotz, Mol Micro, 02, 43, 1367;

McKenney, Infect Immun, 98, 66, 4711

Regulation of *ica* operon expression

- Anaerobic growth conditions
- Low concentrations of antibiotics
- Osmotic stress
- Environmental regulation via *icaR*
- *TcaR* in *S. aureus*

Molecular events in staphylococcal biofilm formation

- Persistence:
 - Deficiencies in local host immune response
 - Due to bacterial products
 - Due to the foreign body
 - » Chuard, JID, 91, 163, 1369; Chuard, AAC, 93, 37, 625; Baddour JID, 88, 157, 757; Zimmerli, JID, 82, 146, 487; Francois, 9, 17, 514;
 - Intrinsic resistance to antimicrobial compounds

Molecular events in staphylococcal biofilm formation

Development of an *in vivo* rat model of FBI

- First generation offspring from ex-germfree inbred Fisher rats
- Subcutaneous implantation of catheter fragments inoculated with defined number of *S. epidermidis* CFU
- Explantation of catheters after varying time intervals and study of adherent staphylococci
- Use of low (physiological) inocula of *S. epidermidis* and pre-implantation inoculation
- Development of FBI with *S. epidermidis* in 90-100% if no prophylaxis given

» Van Wijngaerden, JAC, 99, 44, 669; Van Eldere, Micro Ther, 99, 28, 307

Molecular events in staphylococcal biofilm formation

persistence of CoNS biofilms:

- resistance against host immune defences

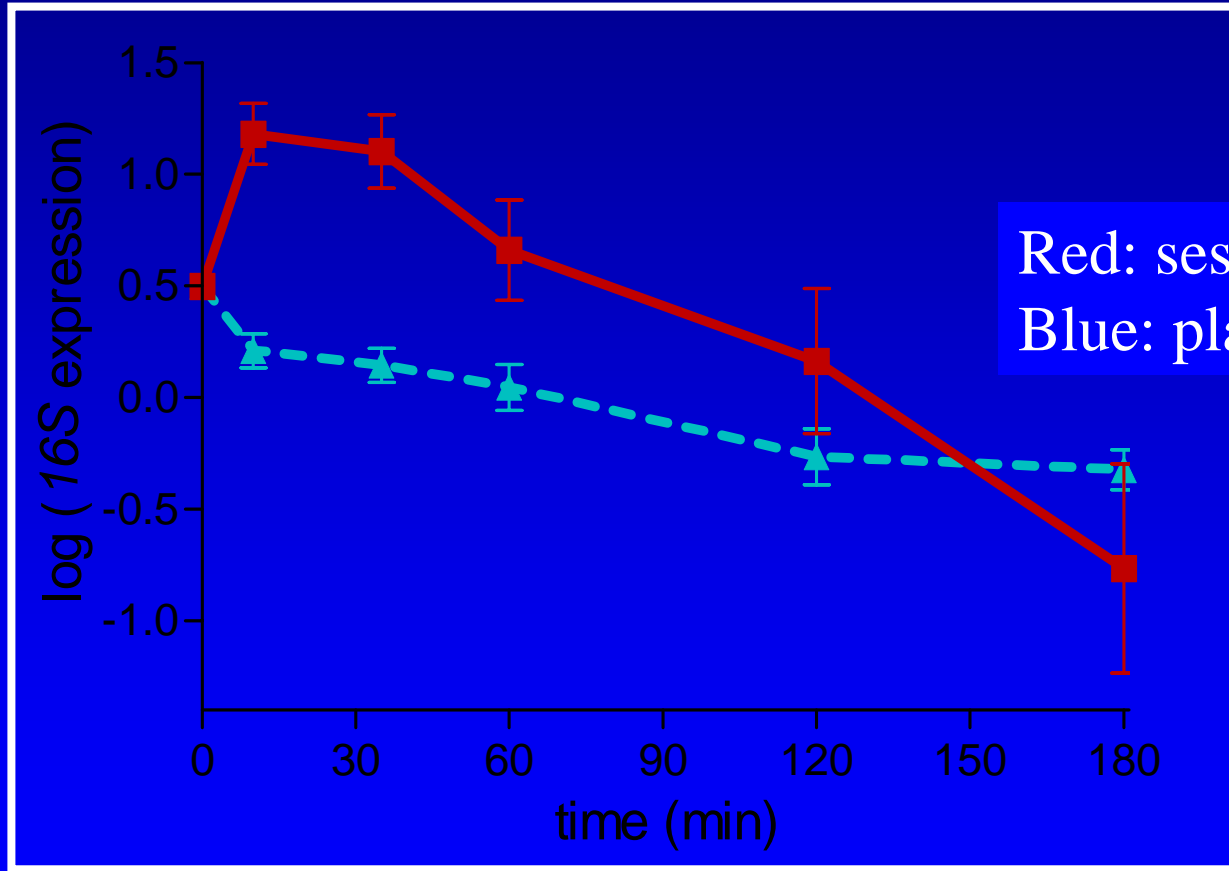
	blood	foreign body
Numbers \log_{10} CFU	6.3 / 1.7	6.2 / 2.1
Chemotactic index	2.4 / 0.2	2.0 / 0.5
phagocytosis	410 / 74	60 / 8.9
respiratory burst activity	12.2 / 0.5	4.4 / 2.8
expression of ICAM-1	3.7 / 2.4	43 / 9.8

Molecular events in staphylococcal biofilm formation

- Quantification of gene expression in FBI-associated *S. epidermidis*
 - rapid disruption and isolation nucleic acids (< 3 min)
 - ⇒ use of instant mechanical disruption (FastPrep™) in presence of lytic solution combined with Qiagen RNeasy kit + RNase treatment to reduce gDNA contamination
 - Cheung, Anal.Biochem,1994,222,511-514; Vandecasteele, J Bac, 01, 183, 7094
 - use of gDNA as a measure of the initial amount of bacteria

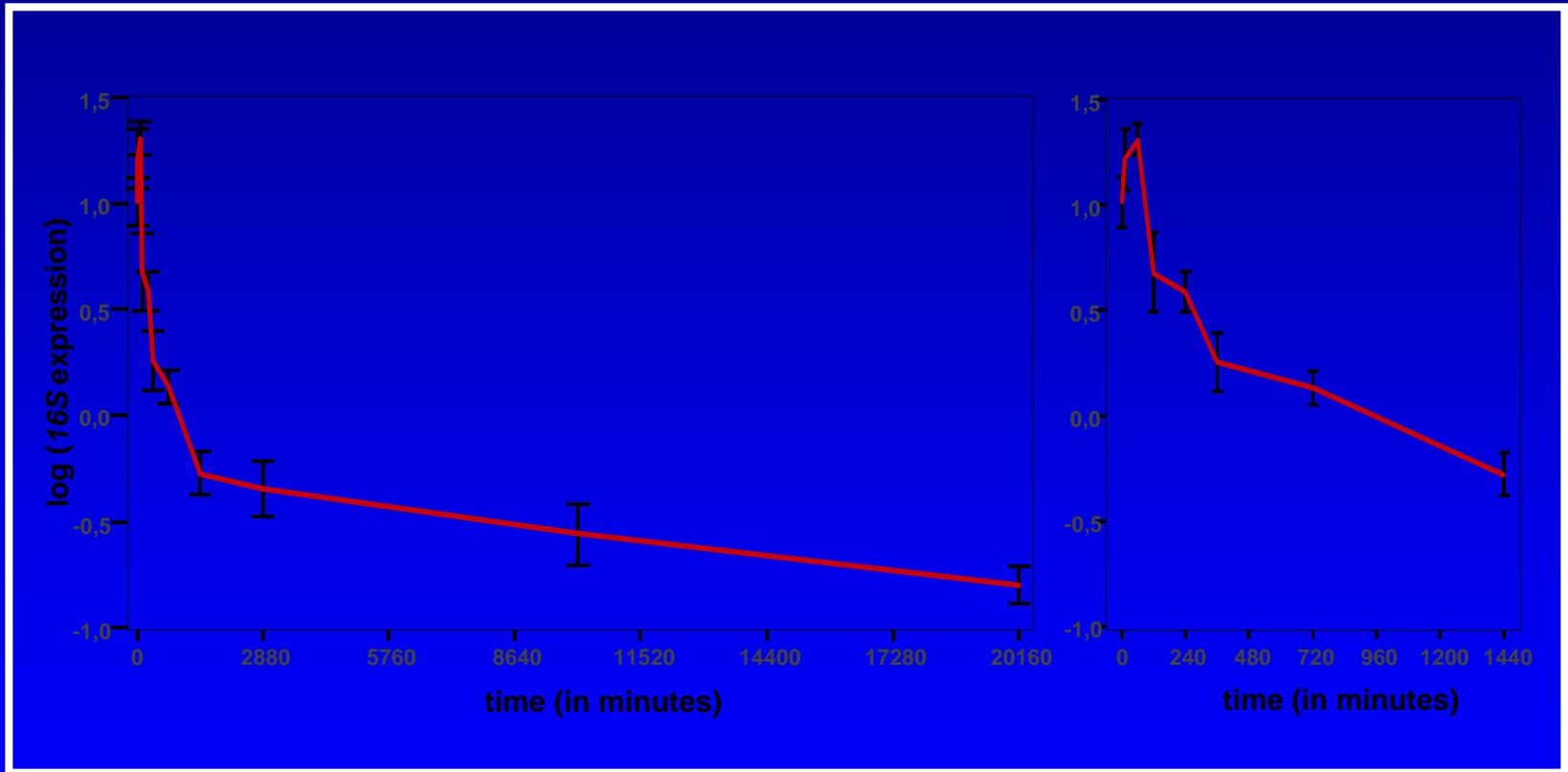
- Vandecasteele,BBRS 02, 291, 528

Molecular events in staphylococcal biofilm formation



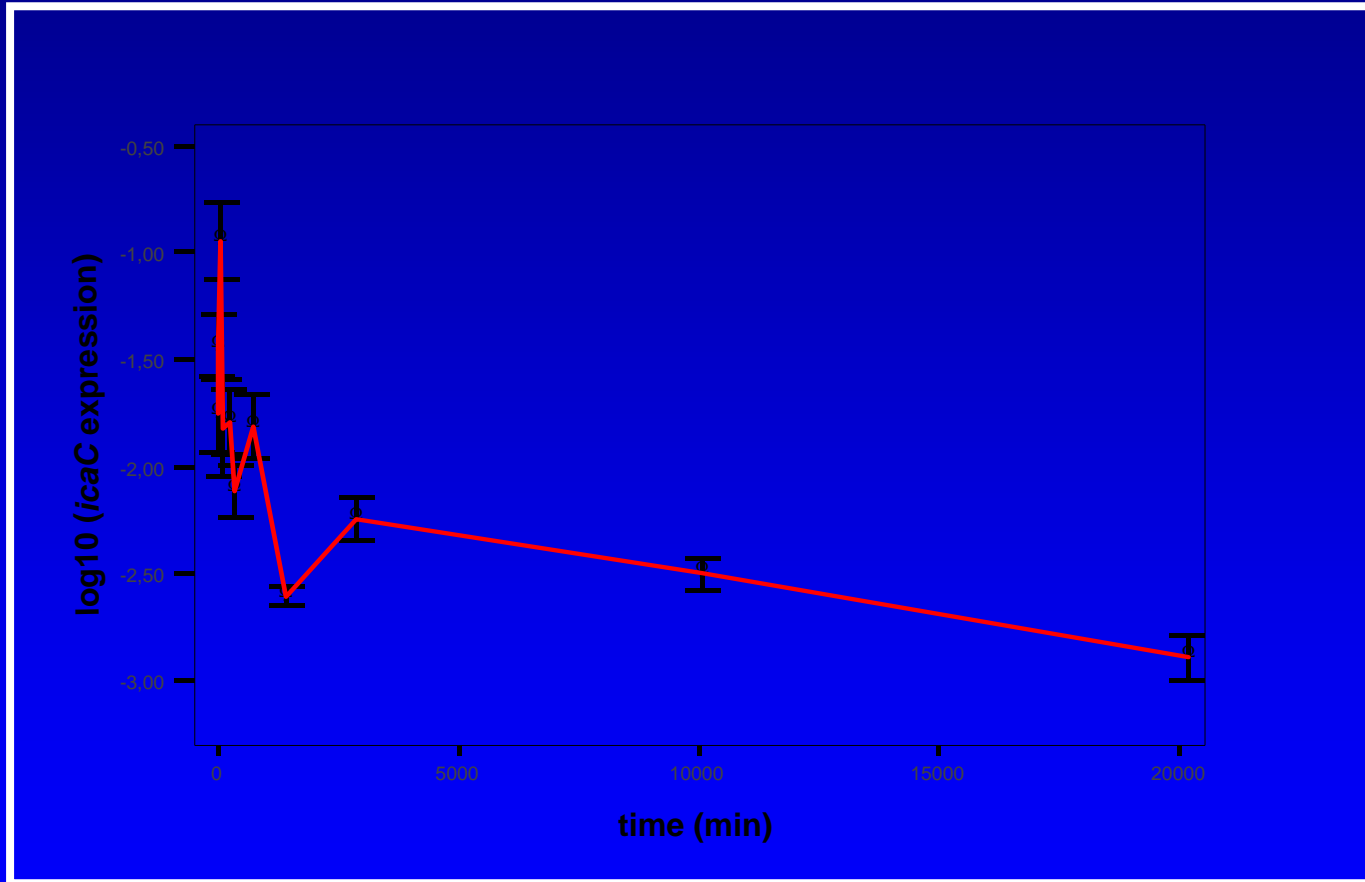
16S expression *in vitro*

Molecular events in staphylococcal biofilm formation



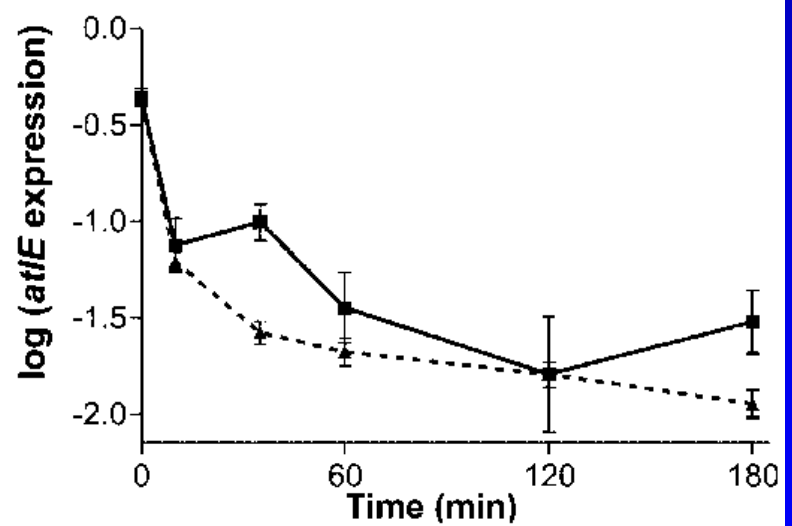
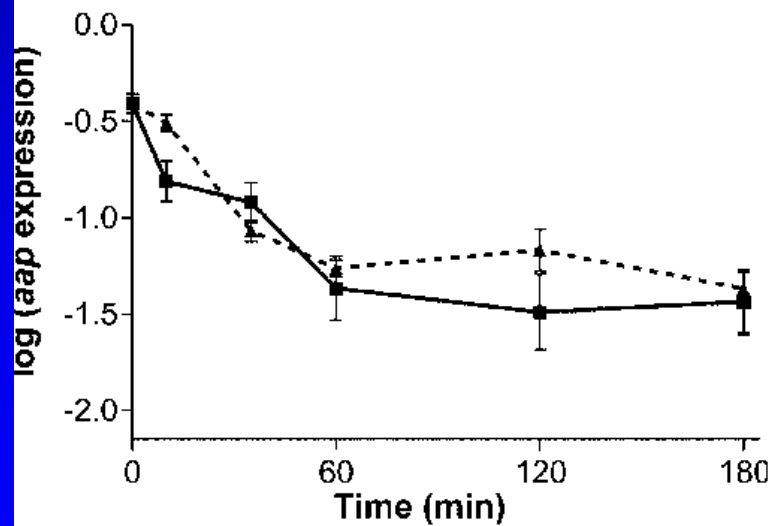
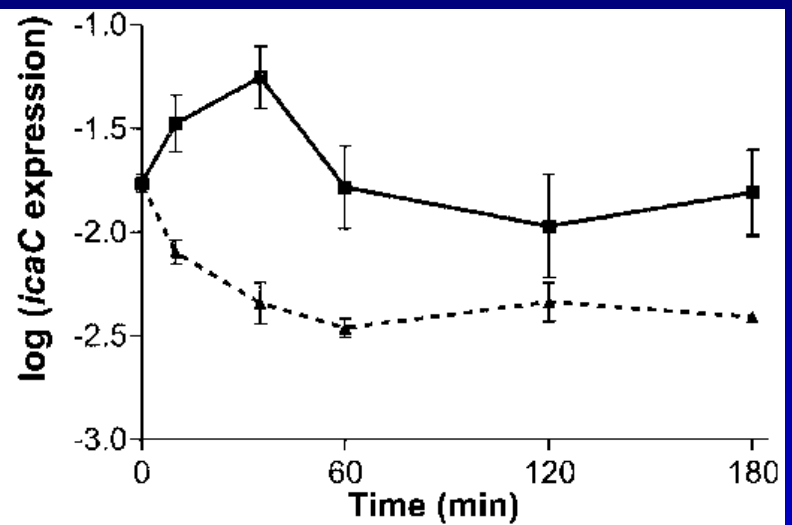
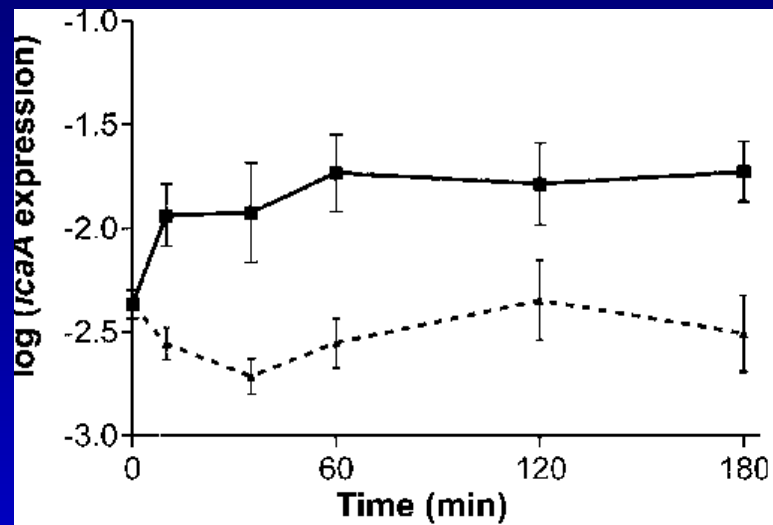
16S expression *in vivo*

Molecular events in staphylococcal biofilm formation



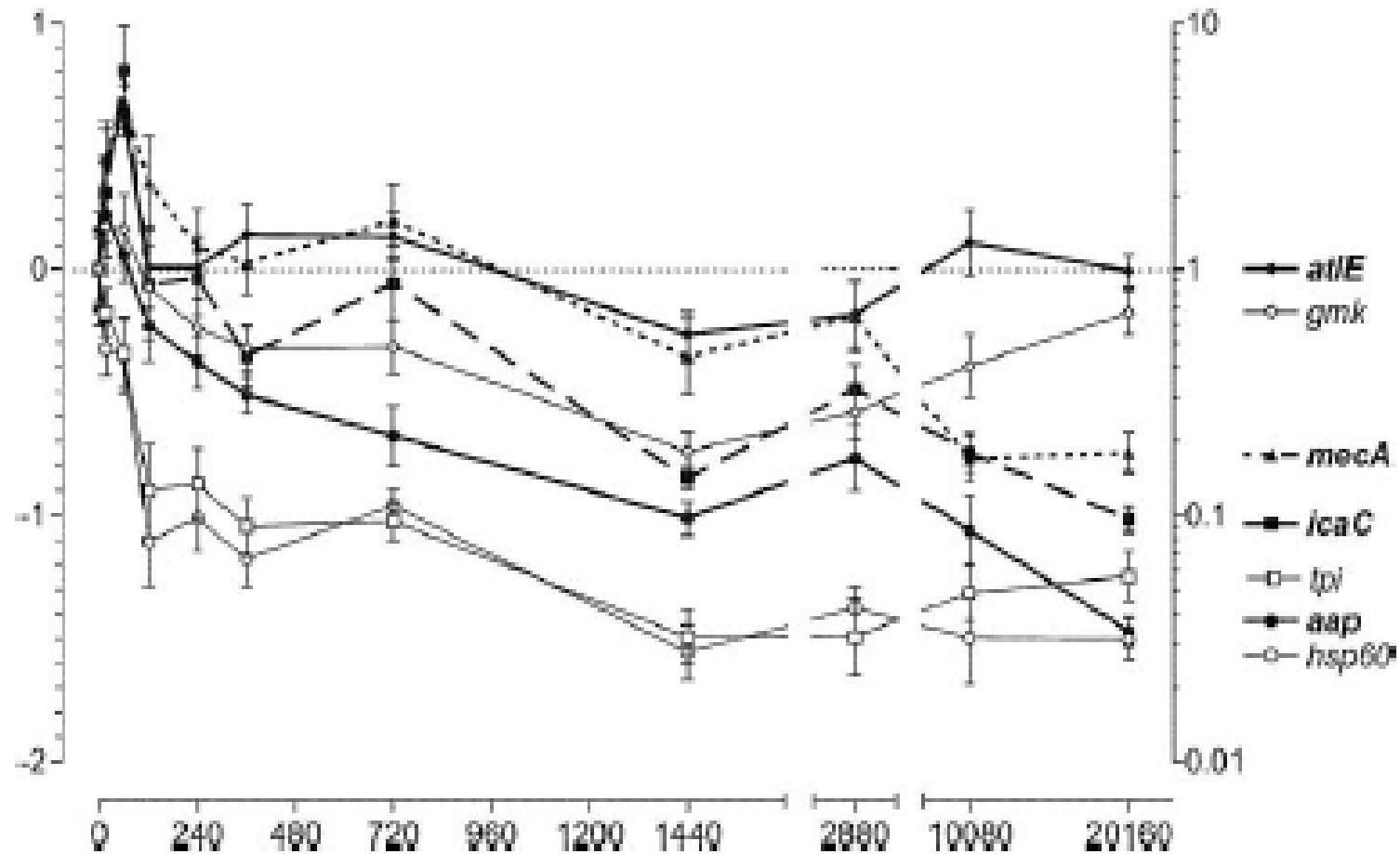
icaC expression in vivo

In vitro expression of biofilm-associated genes



In vitro expression of biofilm-associated genes

In vivo expression of biofilm-associated genes

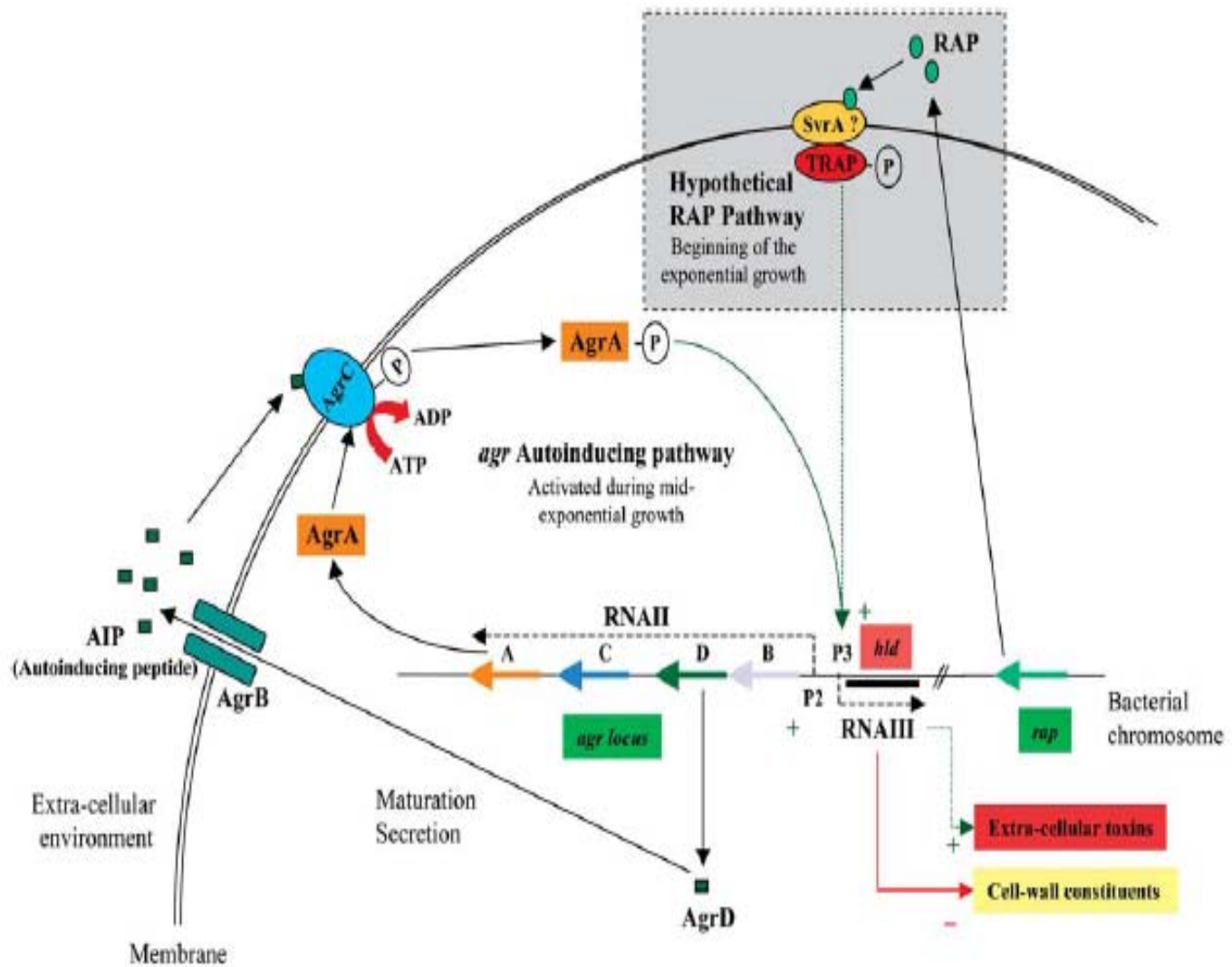


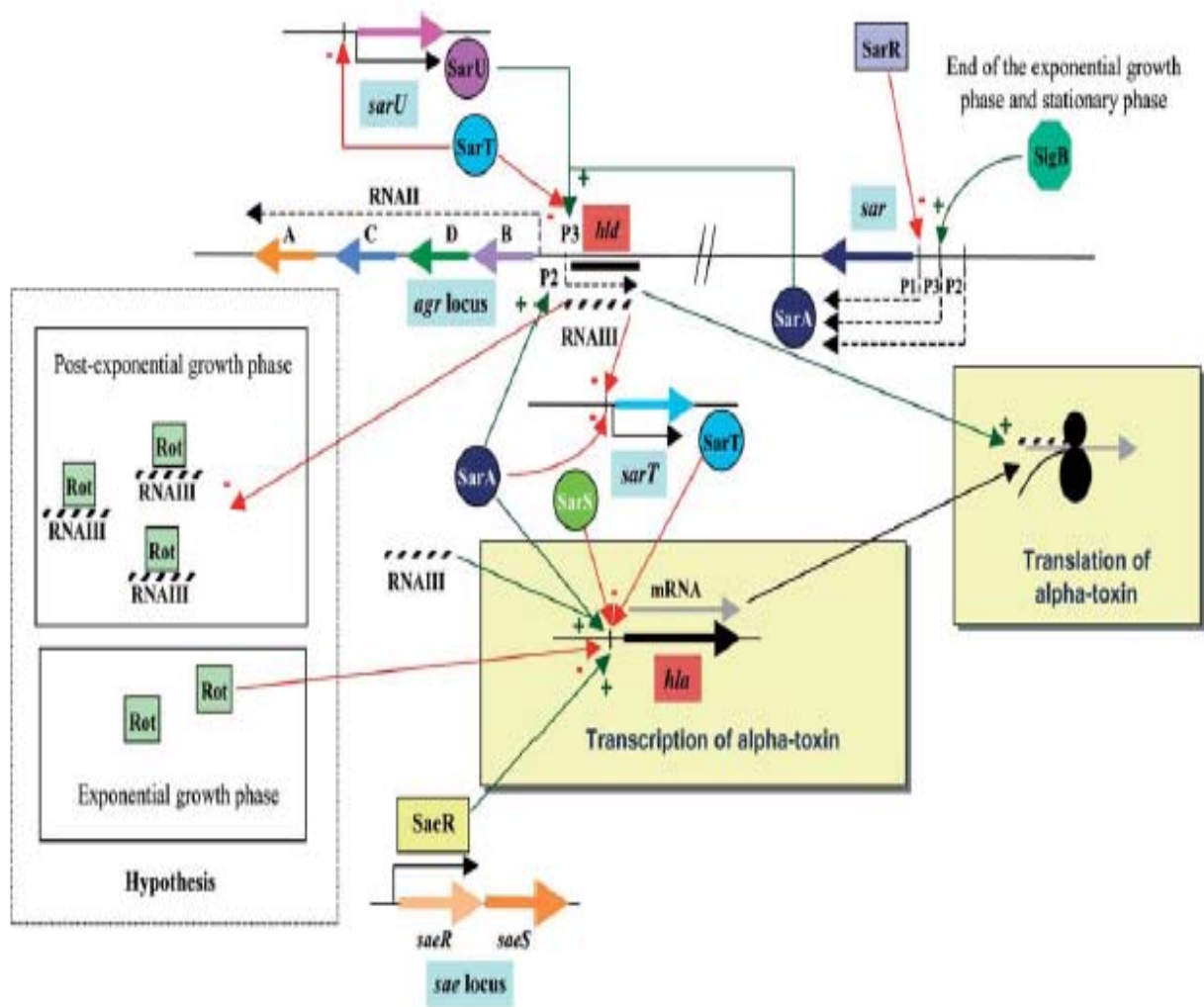
Contents

- Foreign body infections and role of staphylococci
- **Molecular events in staphylococcal biofilm formation**
 - Different stages in biofilm formation
 - **Cellular control of biofilm formation**

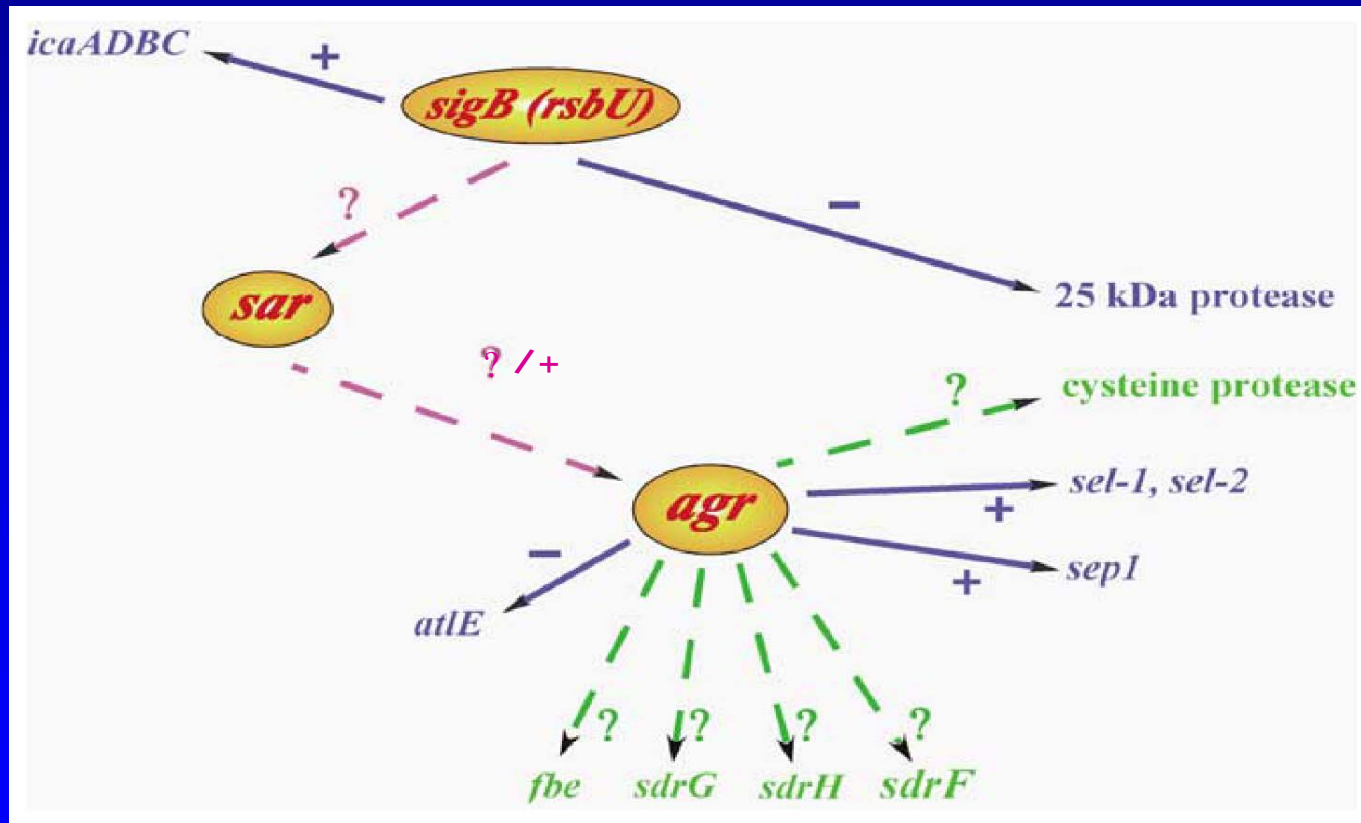
Molecular events in staphylococcal biofilm formation

- Cellular control of biofilm formation
 - Role of *agr* quorum-sensing system:
 - Stimulates expression virulence factors
 - Down regulates expression of surface –proteins (including AtlE)
 - Role of additional regulatory loci:
 - *sar*: SarA co-stimulates with AgrA~P transcription RNAIII
 - SigB: stimulates PIA and biofilm production
 - » Otto, FEBS Lett, 98, 424, 89; Rachid, AAC, 00, 44, 3357; Fluckiger, Infect Immun, 98, 66, 2871; Vuong, Infect Immun, 00, 68, 1048; Otto, Pept, 01, 22, 1603

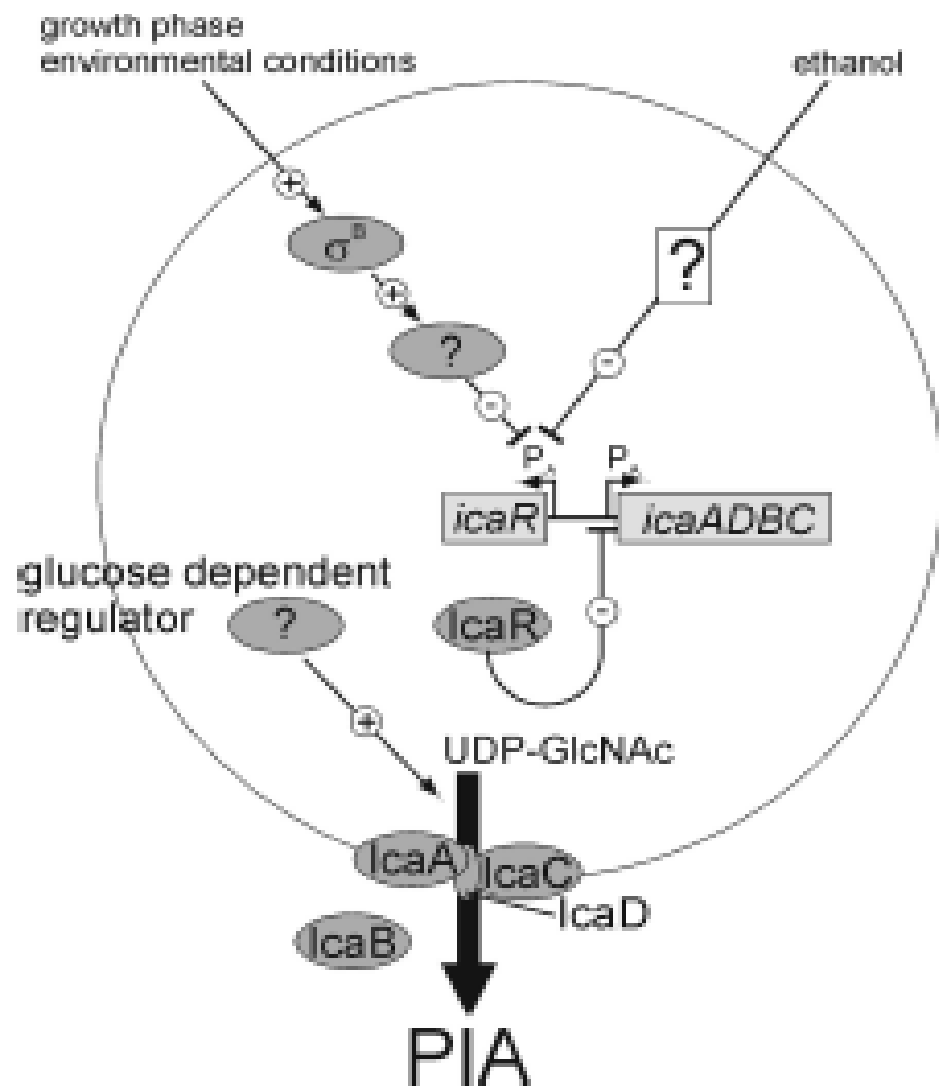




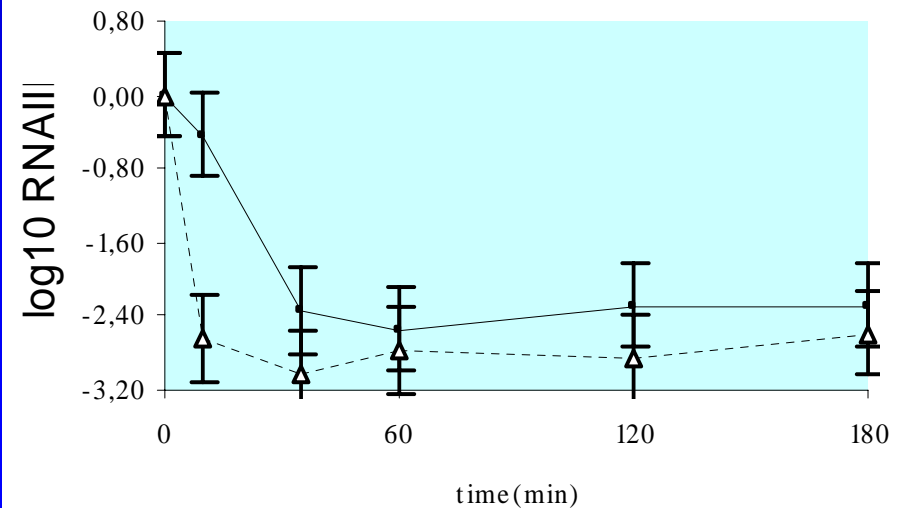
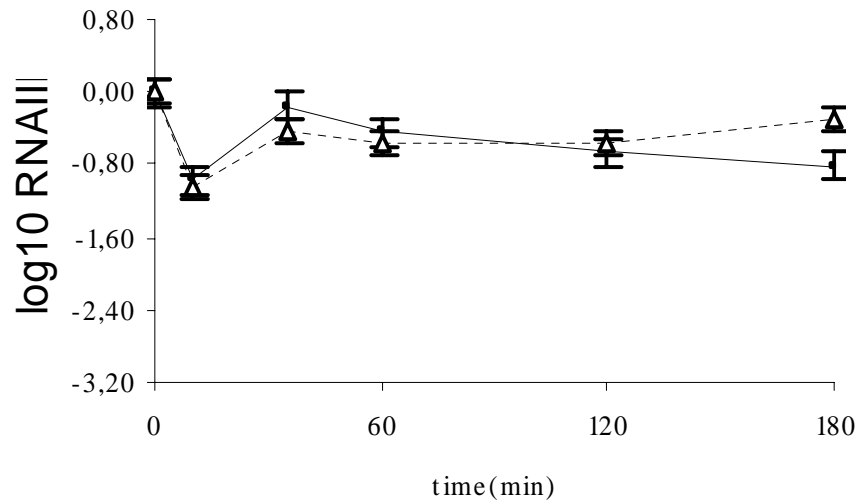
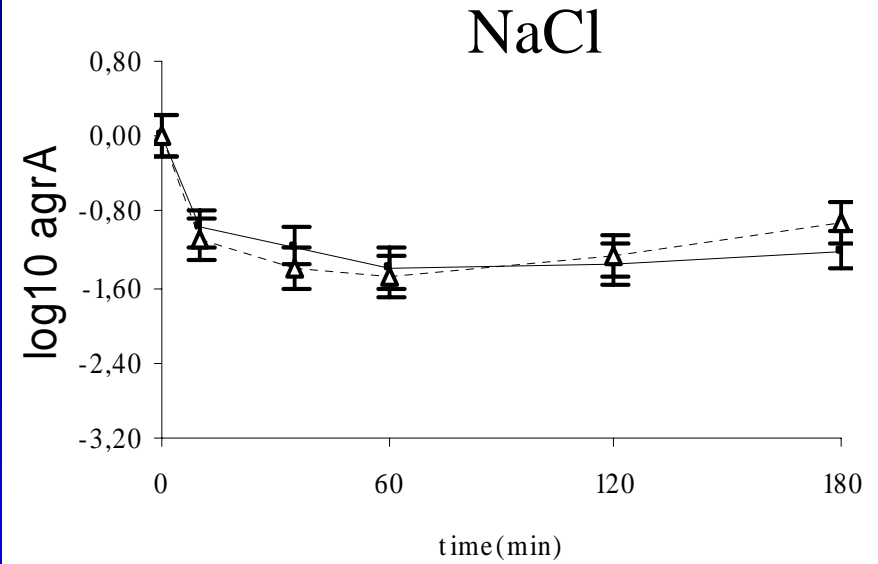
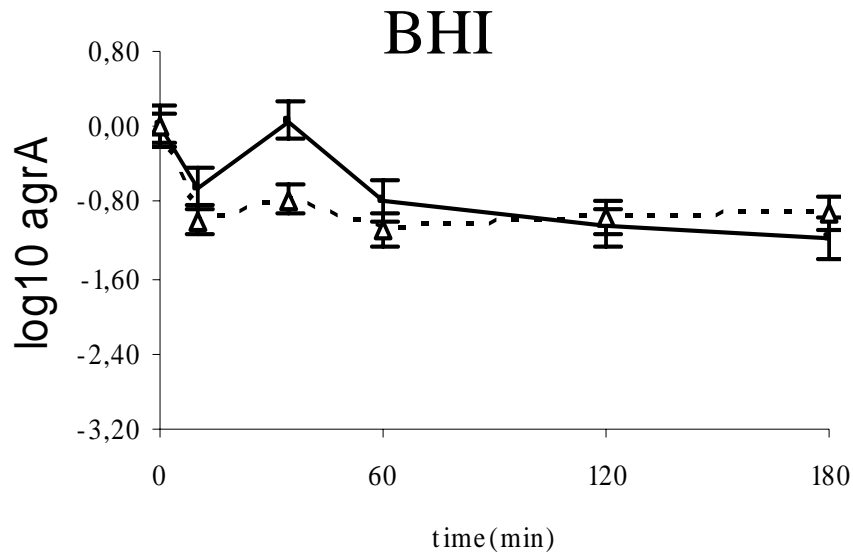
Molecular events in staphylococcal biofilm formation



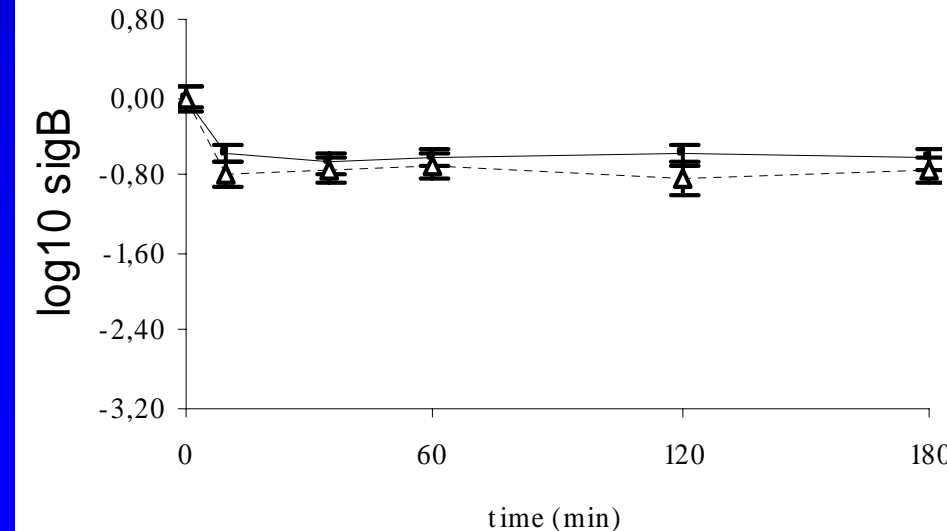
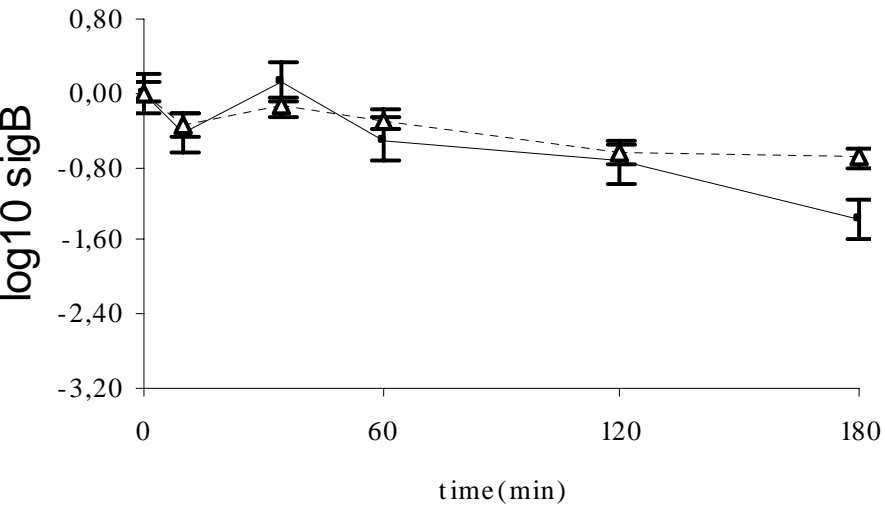
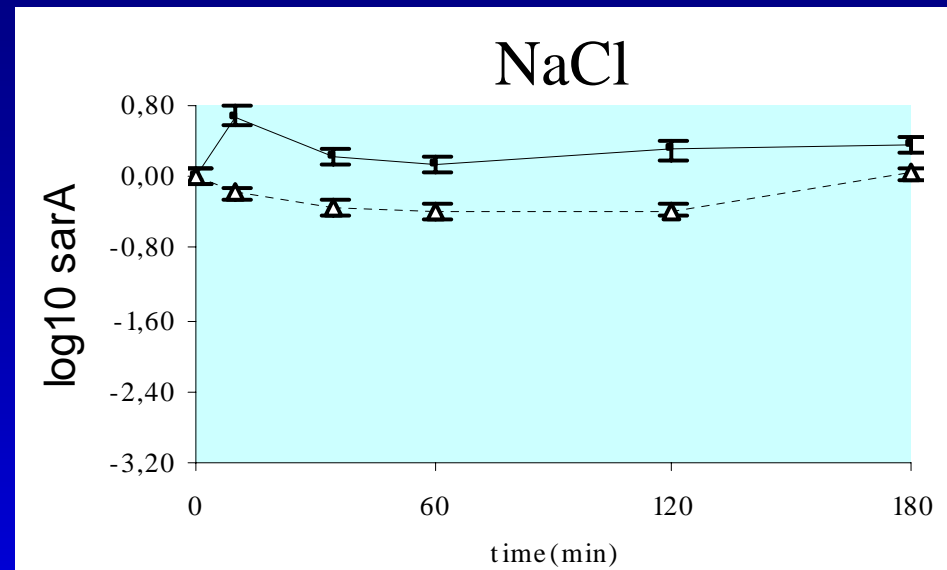
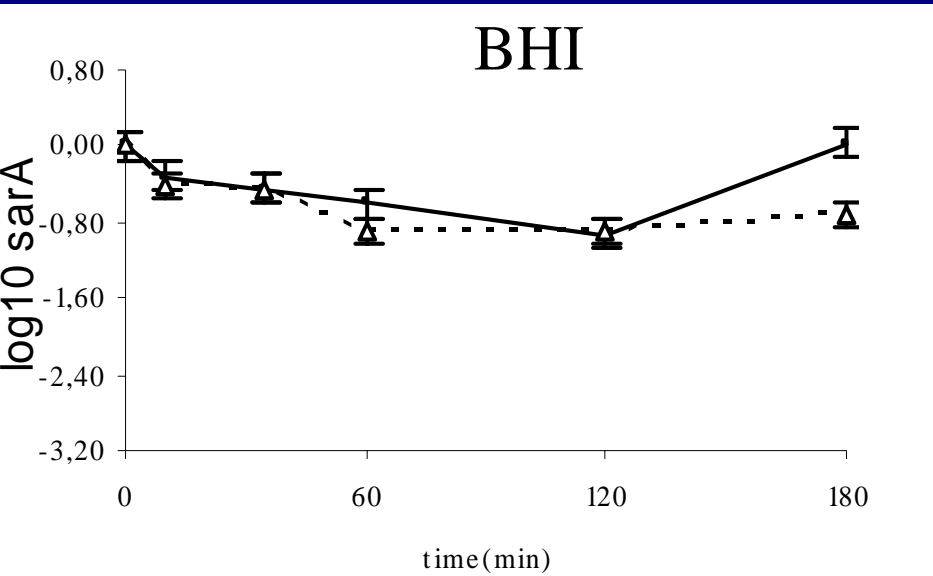
Global regulation of protein expression in *S. epidermidis*



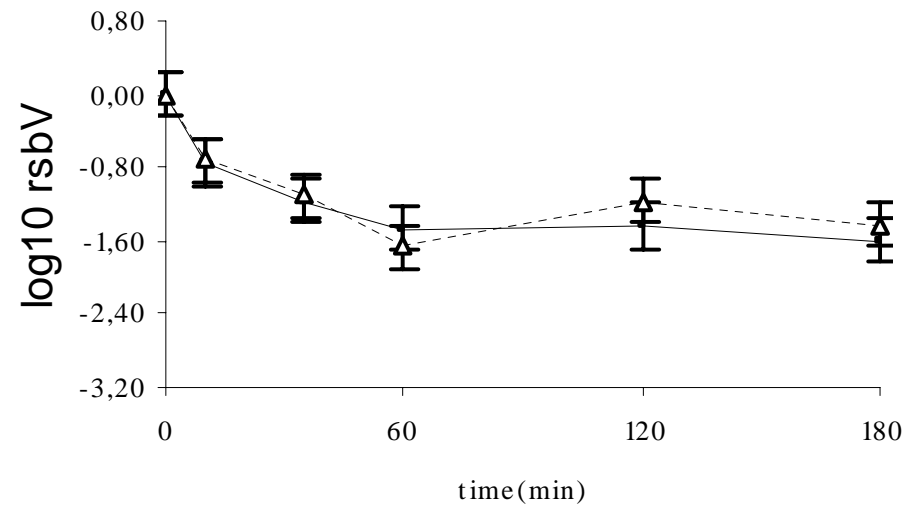
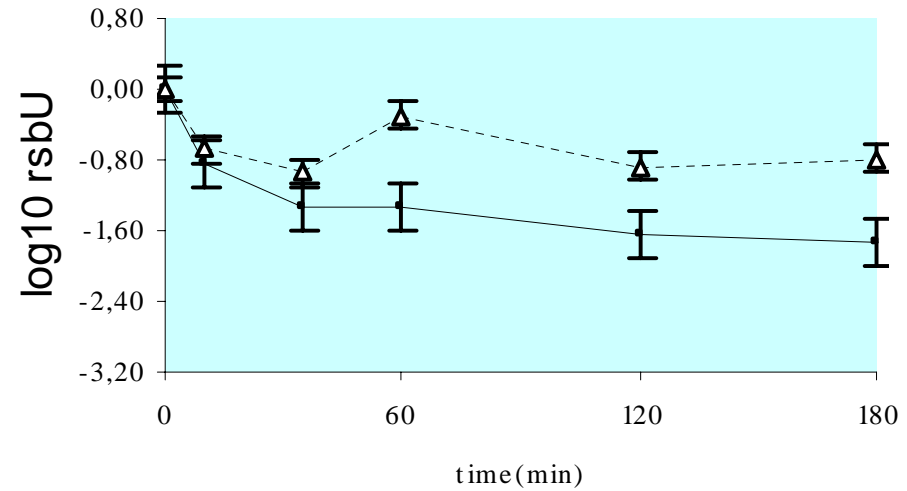
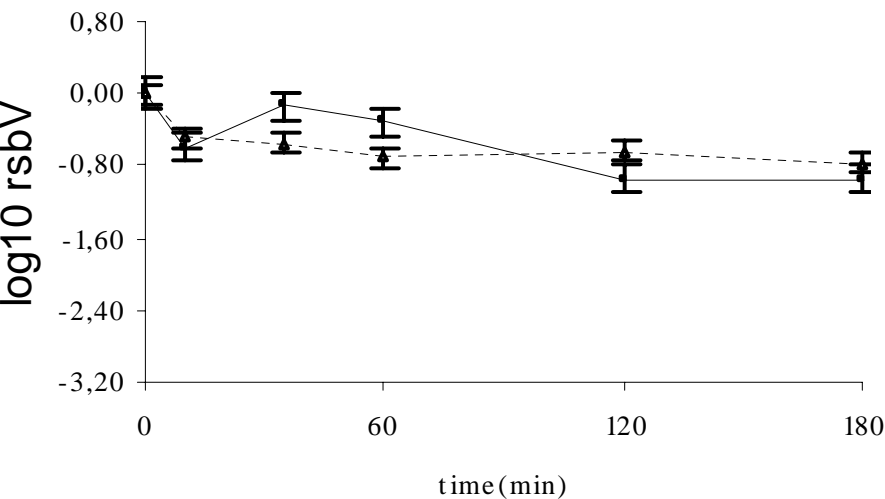
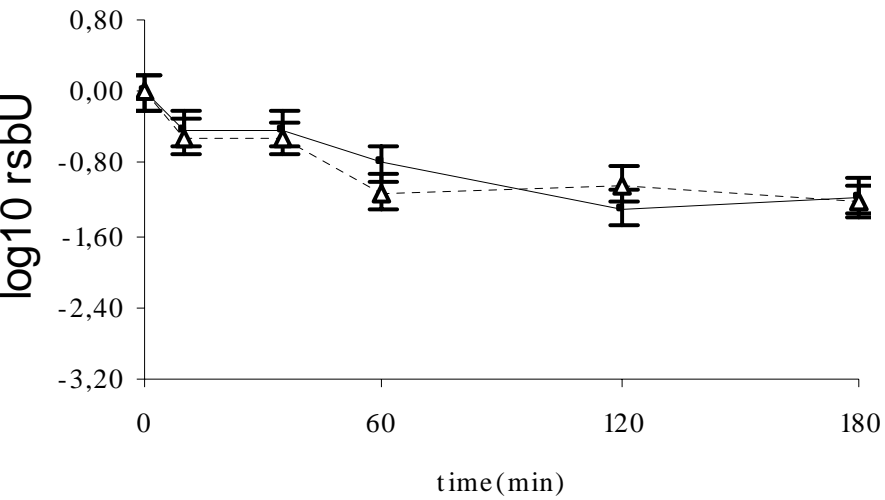
In vitro expression of *S. epidermidis* genes in sessile versus planktonic bacteria



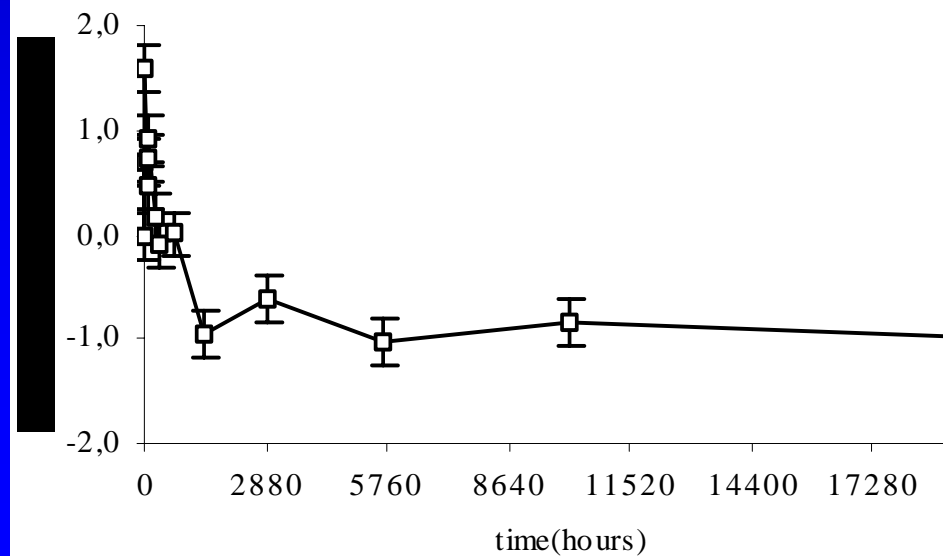
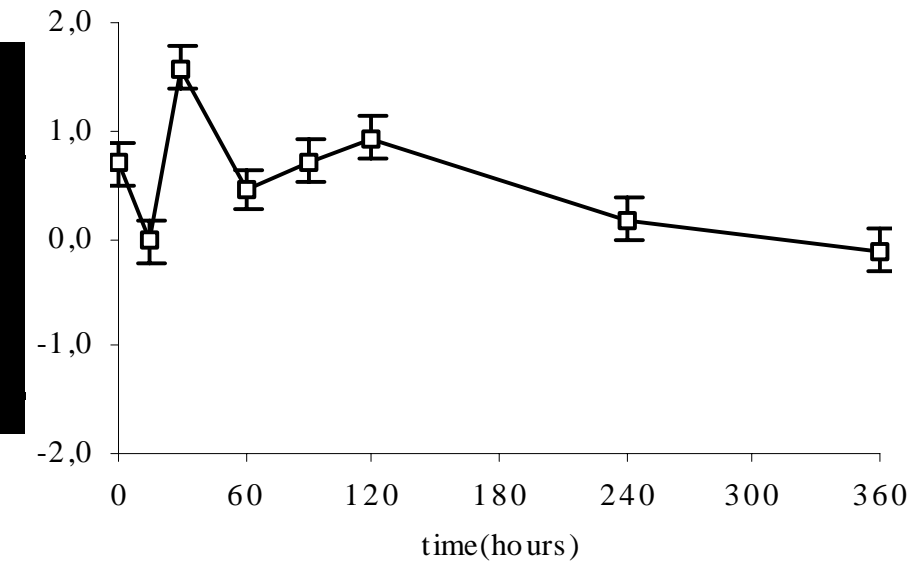
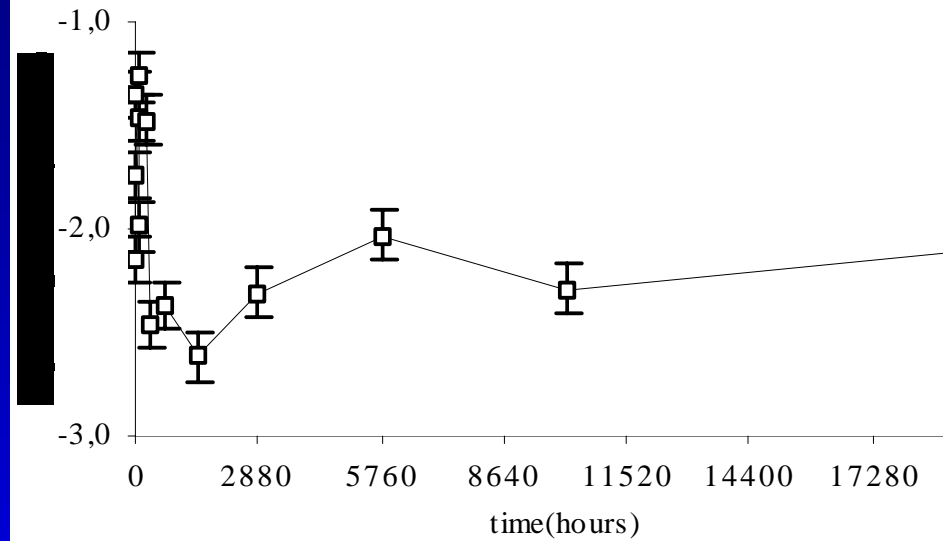
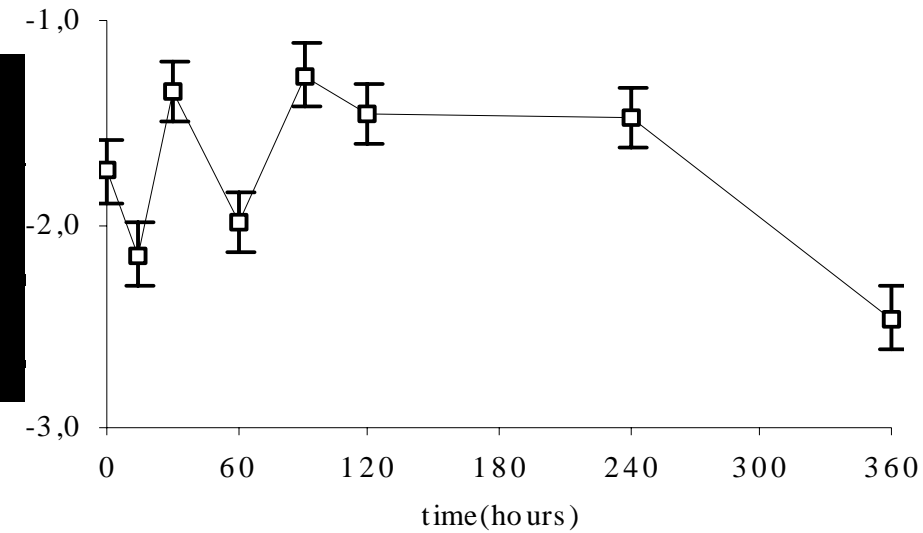
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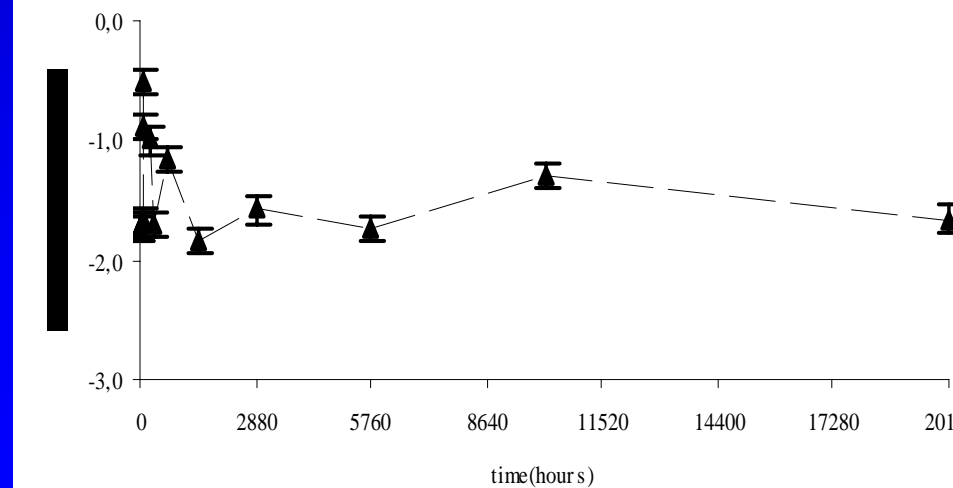
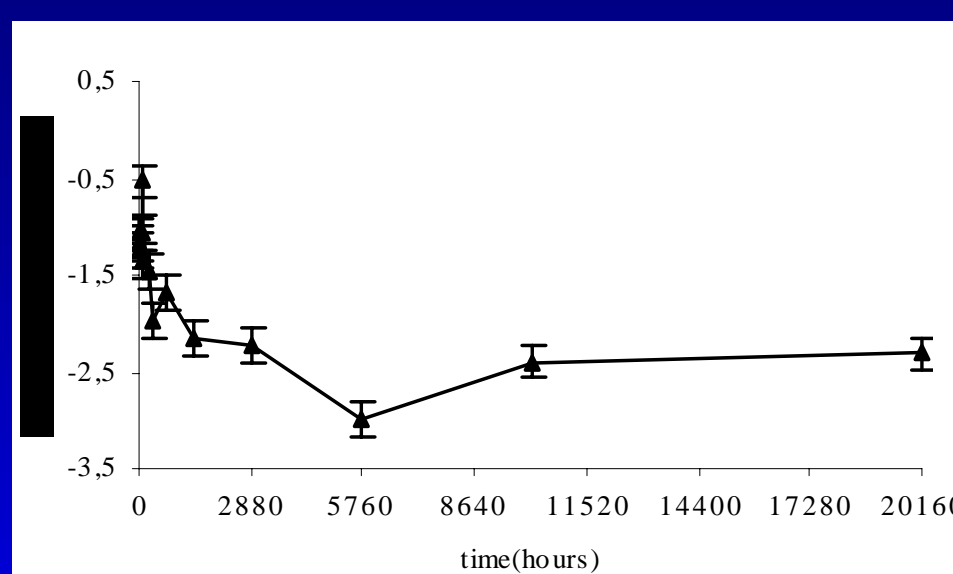
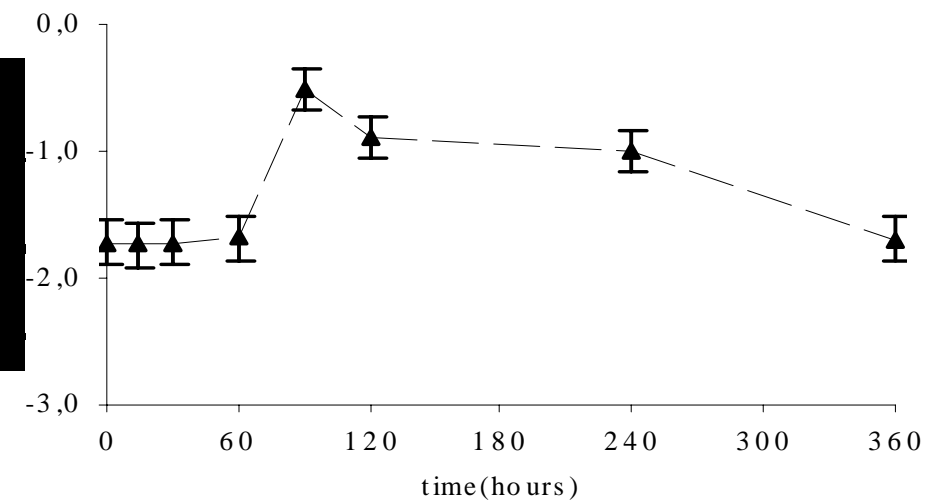
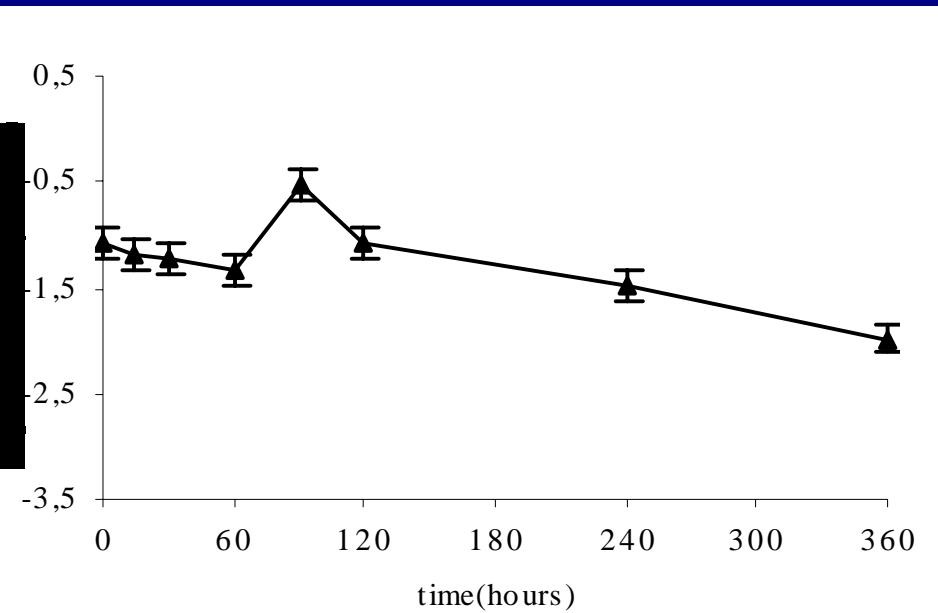
In vitro expression of *S. epidermidis* genes in sessile versus planktonic bacteria

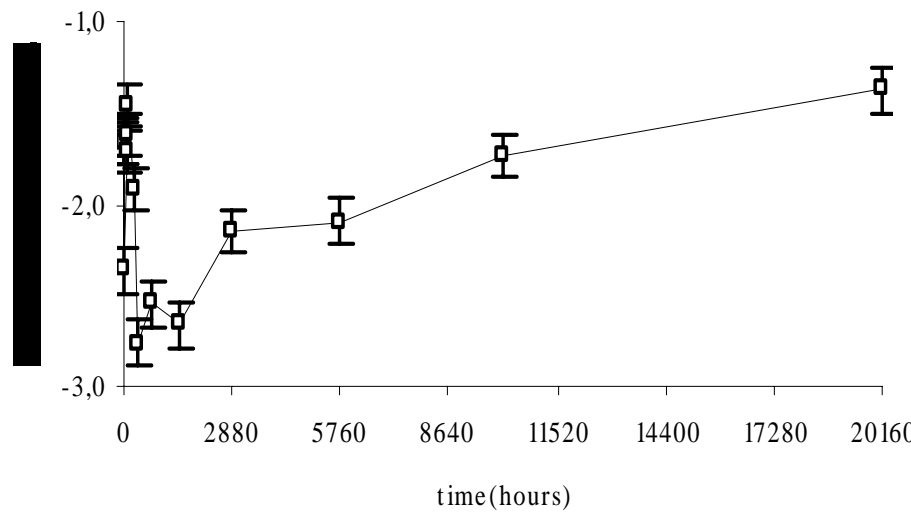
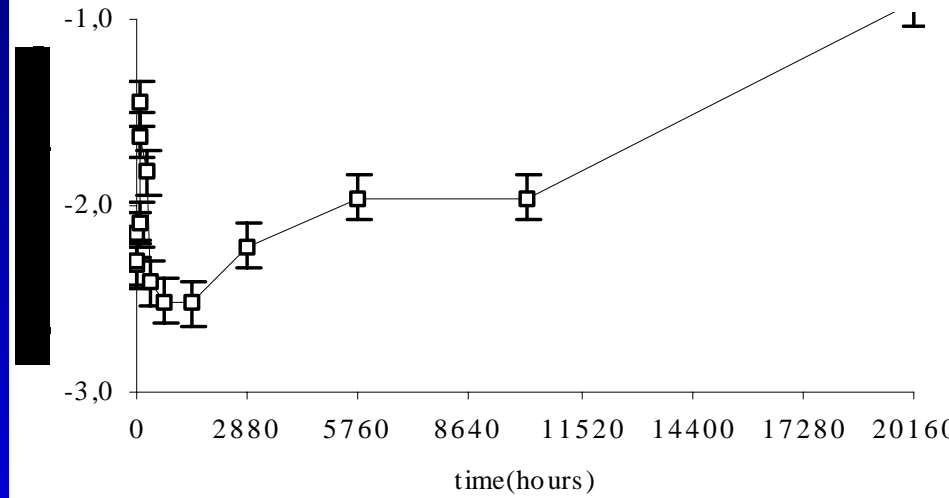
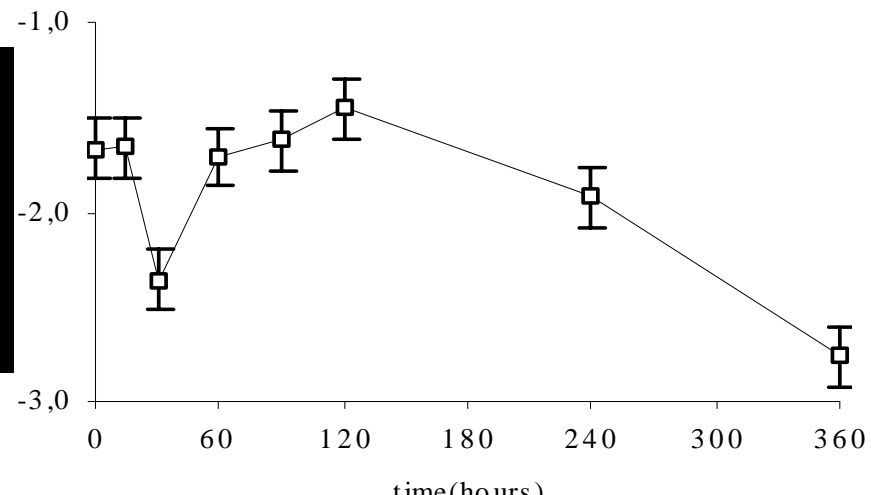
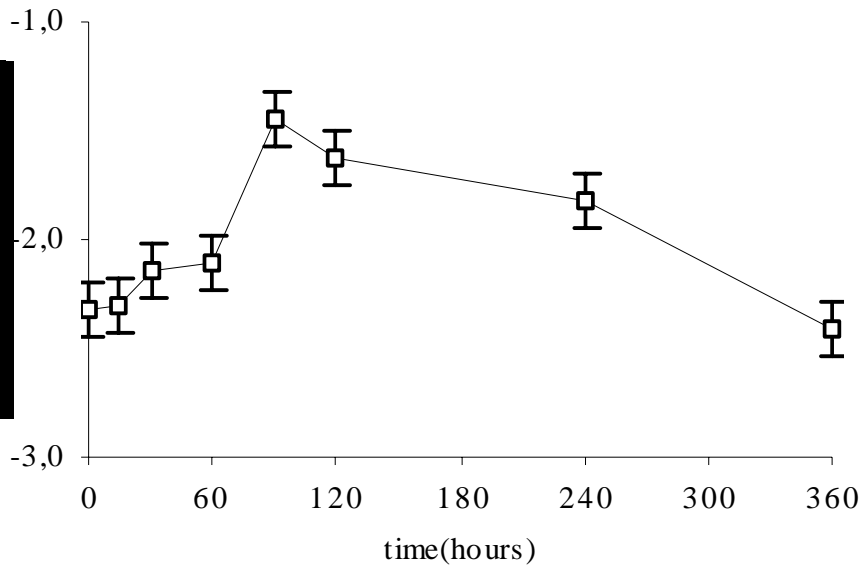


In vivo expression of *S. epidermidis* genes in sessile bacteria



In vivo expression of *S. epidermidis* genes in sessile bacteria





- E. Van Wijngaerden
- J. Vandersmissen
- S. Vandecasteele
- Valerie Pintens
- Caroline Massonet
- R. Merckx