Universität Rostock Traditio et Innovatio

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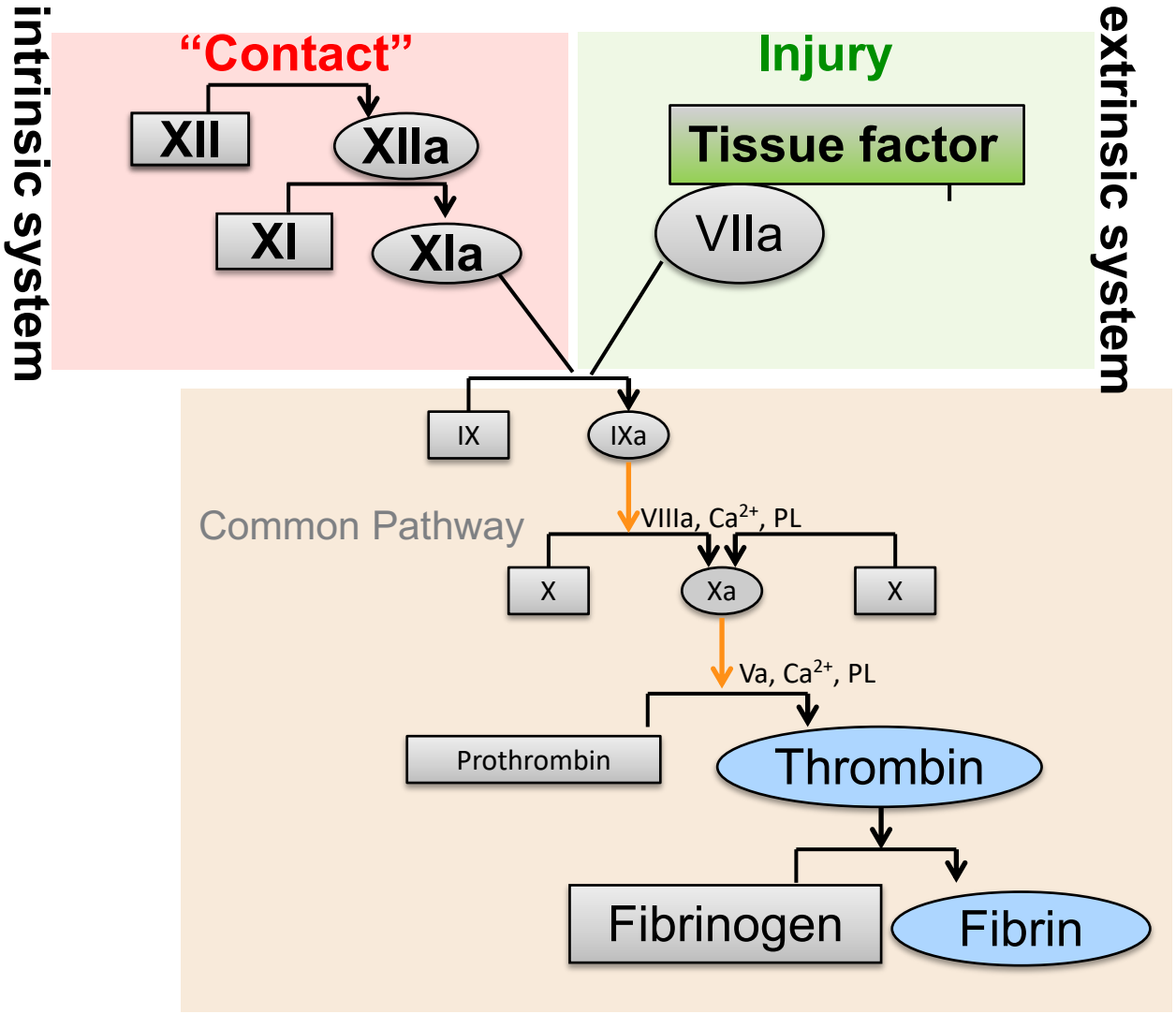
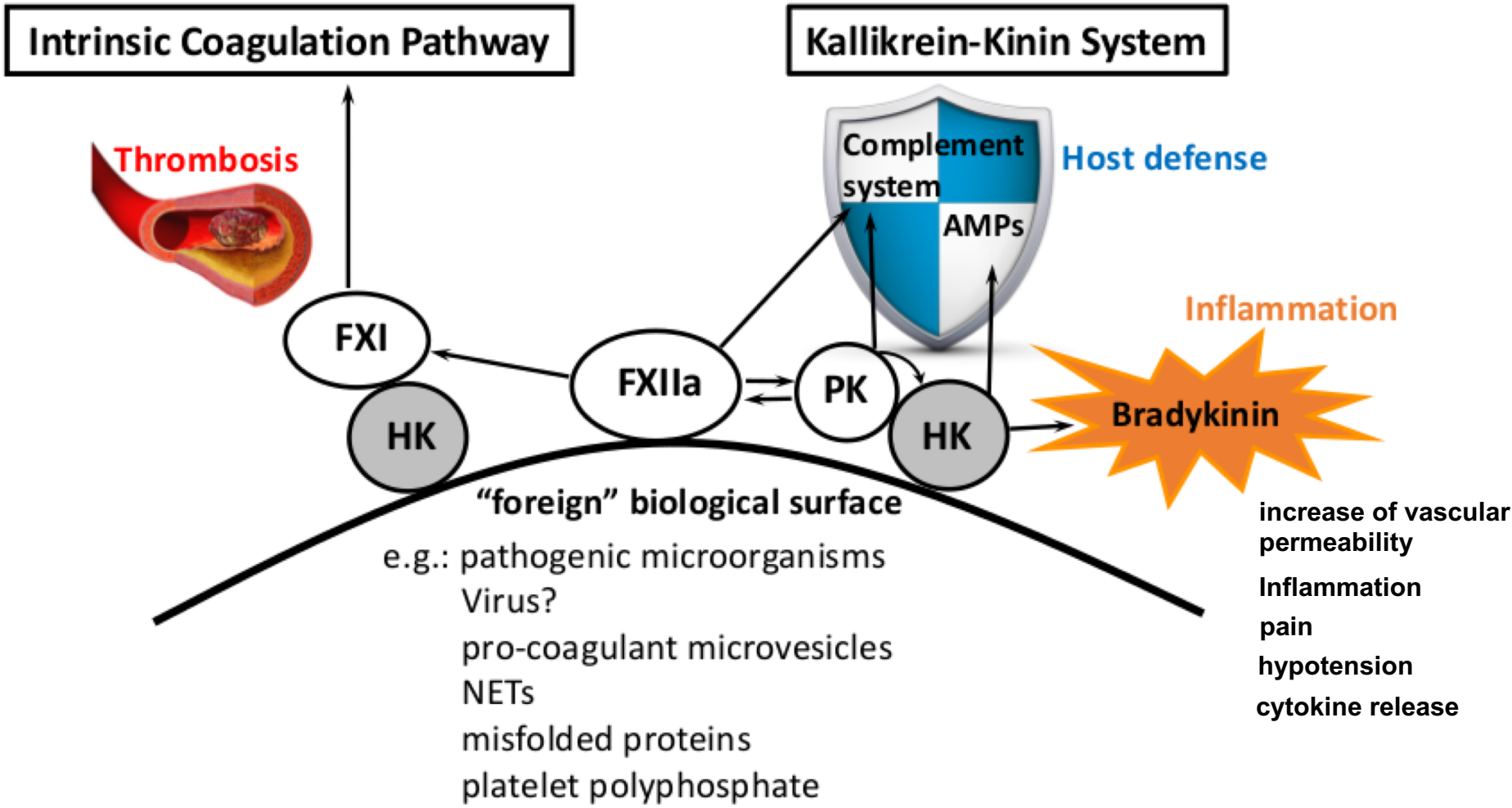
Streptococcus gallolyticus subsp. gallolyticus and the coagulation system

Sonja Oehmcke-Hecht

The coagulation system



The contact system



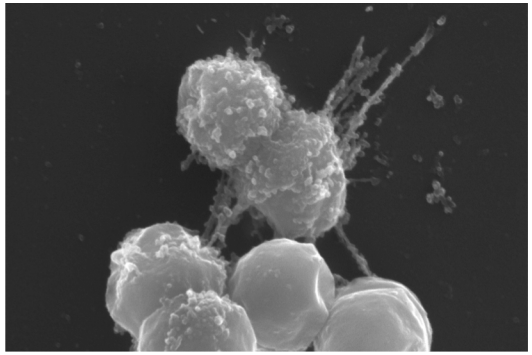
Microbial proteases that cleave contact factors

Species	Enzyme	Target	Reference
Bacteria			
Aeromonas sobria	Serine protease (ASP)	PK, HK, LK	(71)
Bacillus stearothermophilus	Thermolysin	FXII/PK	(74)
Bacillus subtilis	Subtilisin	FXII/PK	
Group A streptococcus (Streptococcus pyogenes)	Cysteine protease (SpeB)	HK	(67)
	Streptokinase activated plasmin	FXII/PK, HK	(75)
Porphyromonas gingivalis	Lysine - specific gingipain (Kgp)	HK	(91)
	Arginine-specific gingipains (RgpA, RgpB)	PK	(69)
Pseudomonas aeruginosa	alkaline Phosphatase	FXII	(74,92)
	Elastase	FXII	
Serratia marcescens	56-, 60-, and 73-kD proteinases	FXII	(74)
Staphylococcus aureus	Staphopains (ScpA, ScpB)	HK	(68)
	V8 proteinase	HK	(74)
Streptomyces caespitosus	Proteinase	HK	(74)
Vibrio cholerae	Protease	Not known	(93)
Vibrio parahaemolyticus	Serine protease	FXII/PK	(94)
Vibrio vulnificus	Metalloprotease	FXII/PK	(74,95)
Fungi			
Aspergillus melleus	Proteinase	FXII	(74)
Candida albicans	Carboxyl peptidase	FXII/PK	(96)
Candida spp.	Aspartic proteases	HK	(97-99)
Parasites			
Fasciola hepatica	Cysteine proteases	HK	(100)
Plasmodium chabaudi and *Plasmodium falciparum*	Falcipain-2	HK	(72)
	Falcipain-3		
Trypanosoma cruzi	Cysteinyl-Proteinase	HK	(70,101)
	(Cruzipain)		
Schistosoma mansoni	Secreted enzyme	FXII/PK, HK	(102)

Oehmcke-Hecht and Köhler, Front. Immu. (2018)

Microbial proteins that bind contact factors

Species	Binding protein	Contact factor	Reference
Bacteria			
Escherichia coli	Curli (CsgA)	HK, FXII, FXI, PK	(45)
Group A streptococcus (Streptococcus pyogenes)	M protein	HK, FXII, FXI	(57,88,89)
Group G streptococcus	FOG	HK, FXII, FXI, PK	(60)
	Protein G	HK, FXII, FXI, HK	
Porphyromonas gingivalis	Fimbriae (FimA),	HK, FXII, PK	(47)
	Gingipains (RgpA, Kgp)	HK, FXII, PK	
Salmonella typhimurium	Curli (CsgA, CsgB)	HK, FXII, FXI, PK	(45)
Streptococcus gallolyticus ssp. gallolyticus (Sgg)	Pili (Gallo2179)	FXII	(54)
Fungi			
Candida albicans	Agglutinin-like sequence protein3 (ALS3, adhesin)	HK, FXII	(90,91)
	Enolase 1 (Eno1)	HK, FXII, PK	
	Phosphoglycerate mutase1	PK	
	Triosephosphate isomerase1	HK, FXII, PK	
	Glucose-6-phosphate isomerase 1	HK, FXII, PK	
Candida parapsilosis	Agglutinin-like sequence proteins	HK	(92)
	Heat shock protein (Ssa2)	HK	
	6-phosphogluconate dehydrogenase 1	HK	



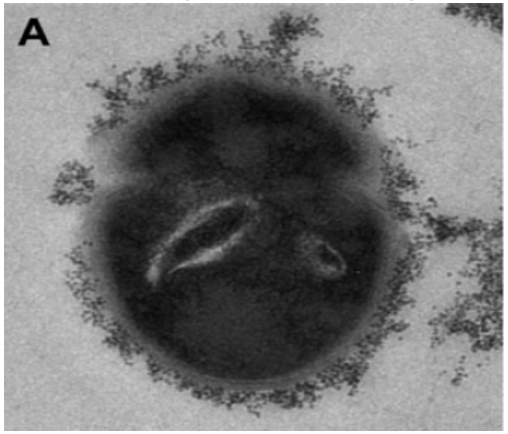
Sgg after incubation in plasma

Oehmcke-Hecht and Köhler, Front. Immu. (2018)

The *Streptococcus bovis/ equinus* complex (SBSEC)

Streptococcus gallolyticus subsp. gallolyticus (*Sgg*, formerly *S. bovis* biotype I)

- found in the gastrointestinal tract of animals and healthy humans (up to 15%)
- Opportunistic pathogen
- Associated with :
  - Infective endocarditis
  - 24 % of streptococcal endocarditis cases
  - Colon cancer
- Virulence factors: Polysaccharide capsule
  Pili expression (3 types)



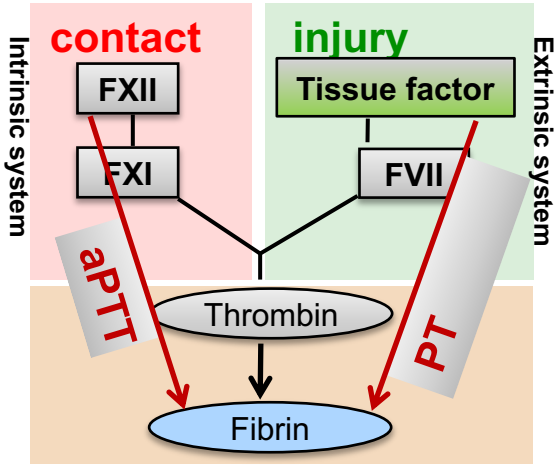
(Boleij et al., 2011)

Streptococcus infantarius subsp. infantarius (*Sii, formely S. bovis biotype II*)

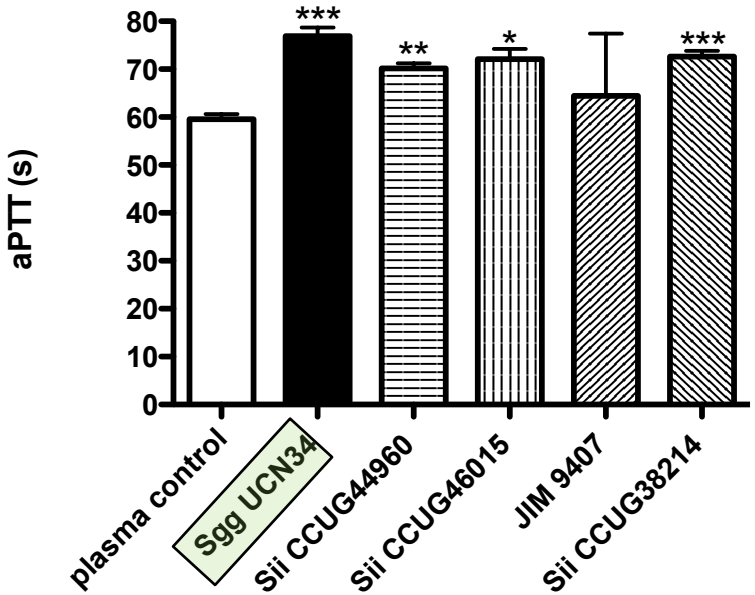
- commensals of the gastrointestinal tract of animals and humans
- important impact on African fermented diary food production and Greek cheese production
- Ingested in high numbers

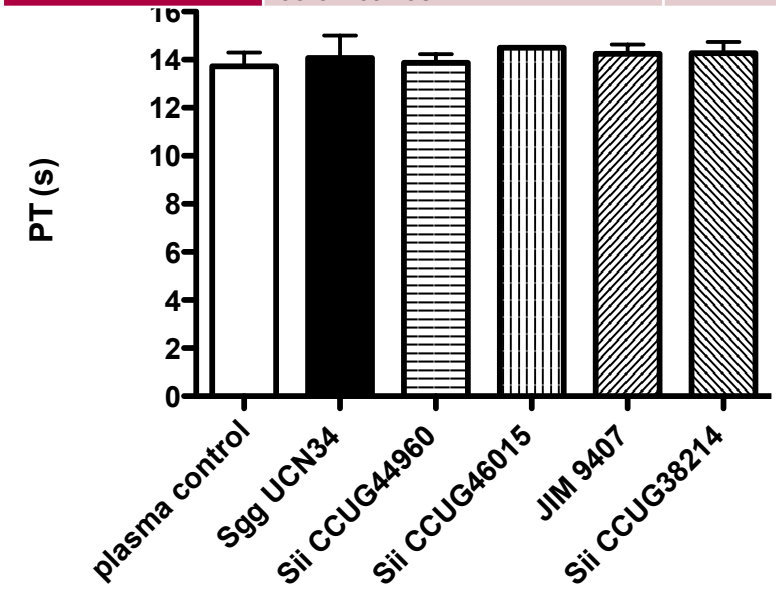
(Corredoira et al., 2008) (Schlegel et al., 2003)
(Rusniok et al., 2010)

SBSEC interfere with aPTT clotting time



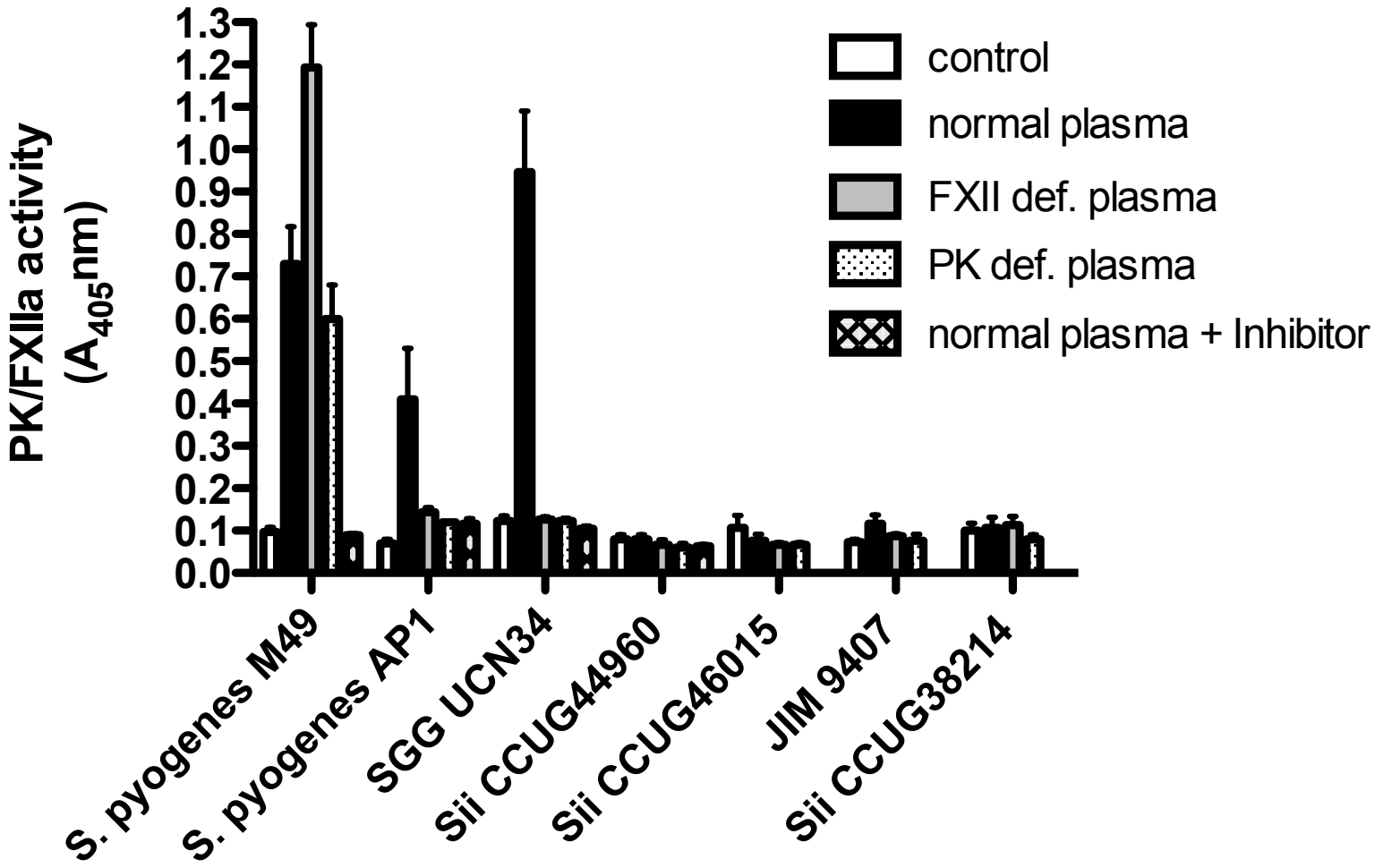
Strain	Source	Origin
Sii JIM 9407	Human bacteremia	Spain
Sii CCUG38214	Human blood	Sweden
Sii CCUG44960	Human blood	Sweden
Sii CCUG46015	Human	Sweden
Sgg UCN34	Infectious endocarditis and colon cancer	France





➢ **Interference of *Sii* and *Sgg* with intrinsic pathway of coagulation**

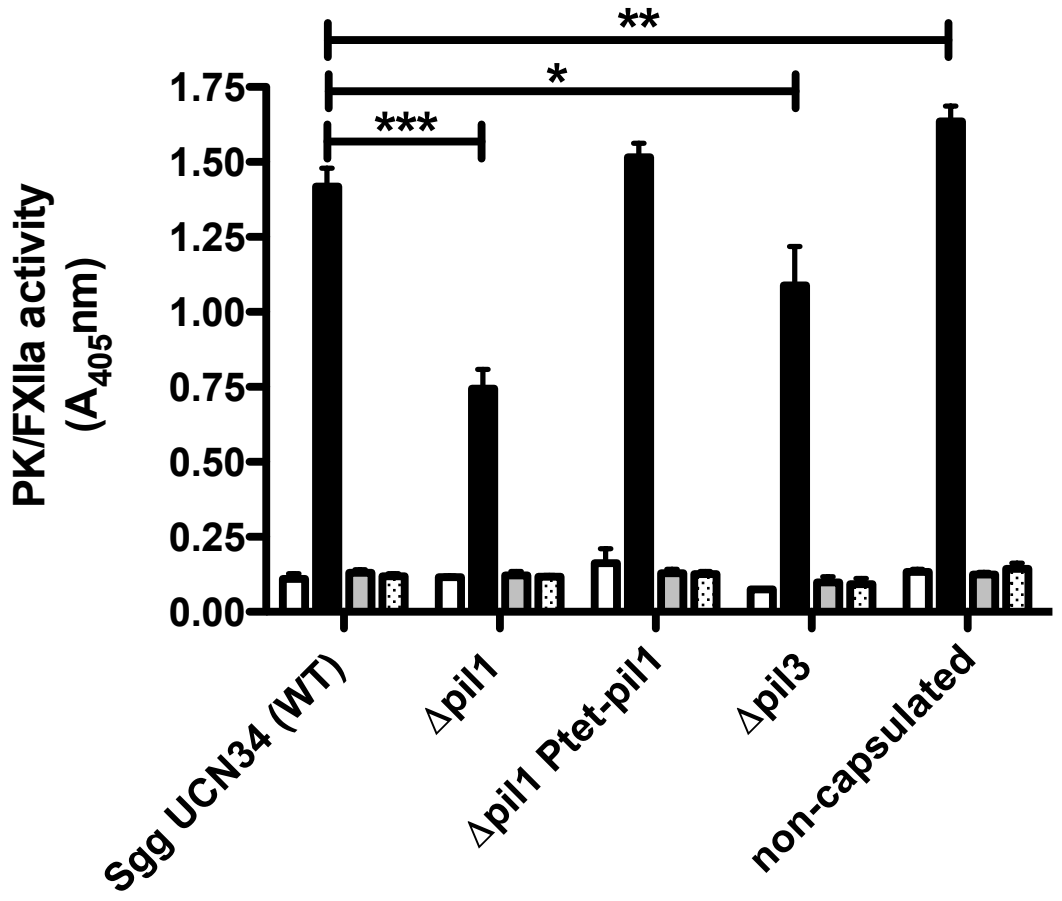
Sgg UCN34 activates contact factors



- ***Sii*** **strains bind contact factors, but do not activate the system!**
- **Binding <u>and</u> activation of contact factors by *Sgg* UCN340**

Isenring et.al,Virulence (2017)

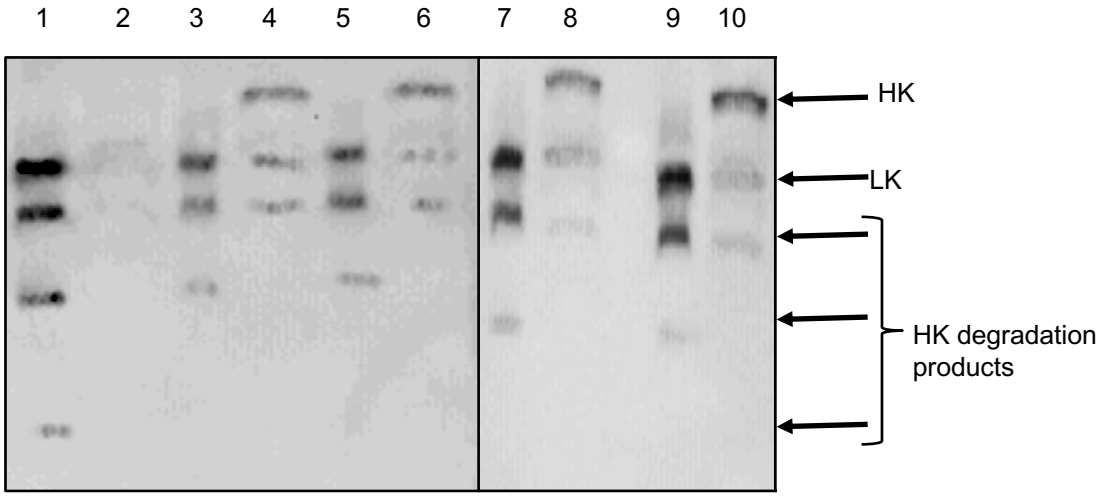
Pili and capsule are involved with contact system activation at the bacterial surface

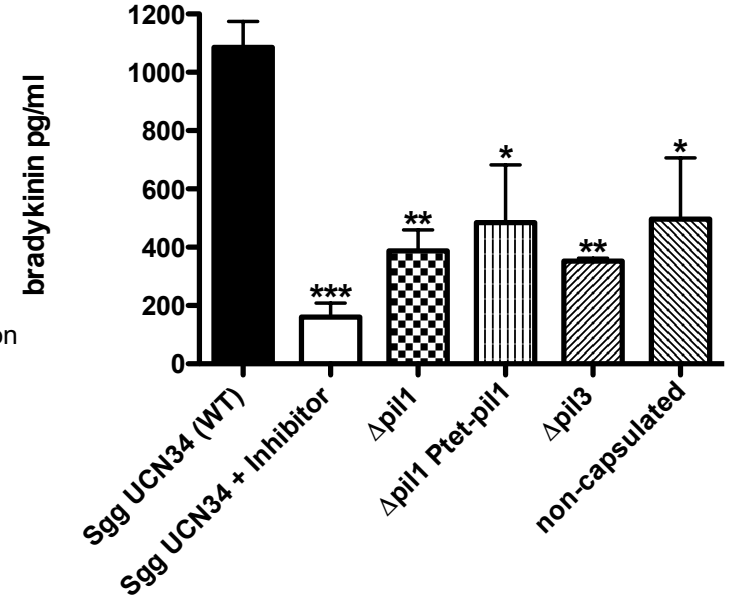


Mutant	Description
SGG UCN 34	*S. gallolyticus* wildtype
Δpil1 mutant	lack the *pil1* operon
Δpil1 Ptet-pil1 mutant	Complementation of pilus 1
Δpil3 mutant	lack the *pil3* operon
Non- capsulated mutant	lack the capsule

- **Decreased activation by Pil1 and Pil3 mutants**
- **Increased activation by non-capsulated mutant**

Kininogen binding and release of bradykinin from the bacterial surface of *Sgg*

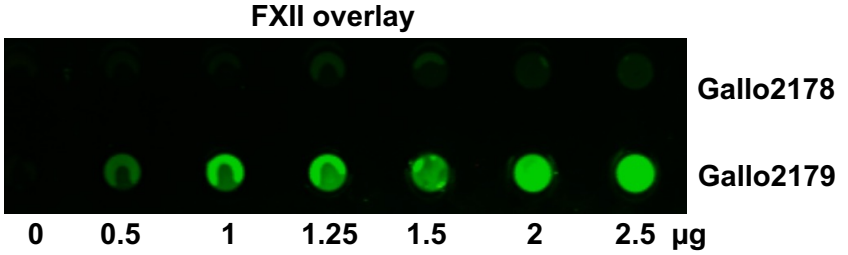


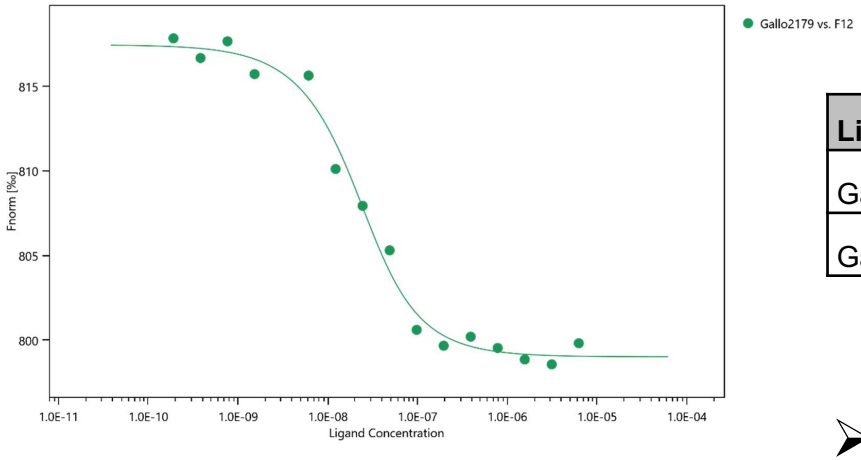


1 *Sgg* UCN34 eluate
2. *Sgg* UCN34 plasma supernatant
3 Δpil1 eluate
4 Δpil1 plasma supernatant
5 Δpil1 Ptet-pil1 eluate
6 Δpil1 Ptet-pil1 plasma supernatant
7 Δpil3 eluate
8 Δpil3 plasma supernatant
9 non-capsulated eluate
10 non-capsulated plasma supernatant

FXII binds to Pil1 protein (Gallo2179)

pil1 operon in *Sgg* UCN34





Surface Plasmon Resonance

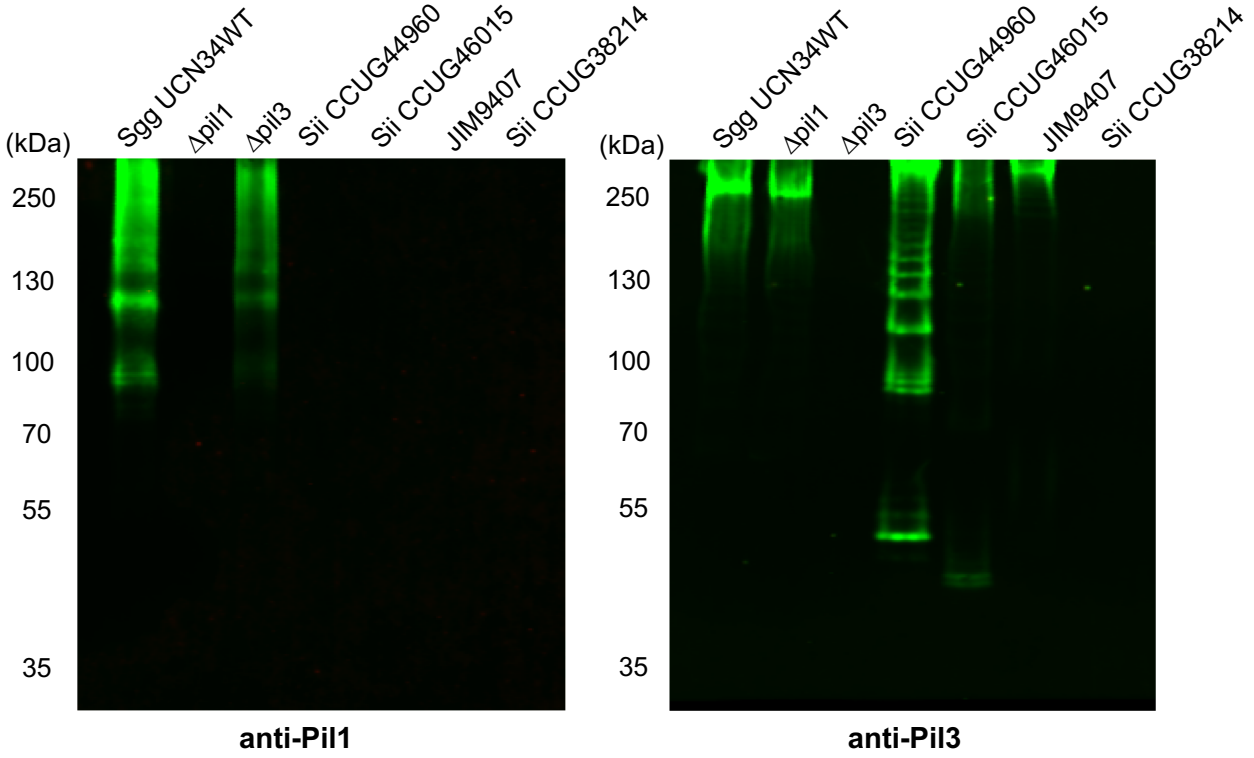
Ligand	Analyte	ka (1/Ms)	kd (1/s)	KA(1/M)	KD (M)
Gallo2179	Coll. I	5.68×10^{5}	2.89×10^{-3}	1.97×10^{8}	**5.08×10^{-9}**
Gallo2179	FXII	0.9×10^{3}	1.27×10^{-5}	7.09×10^{7}	**14.1×10^{-9}**

➢ **specific interaction of Pil1 with FXII**

Isenring et.al,Virulence (2017)

Danne et. al, JID, 2011

Biogenesis of Pil1 and Pil3 in SBSEC



- as previously indicated [2] , *Sii* express Pil3 but not Pil1
- pil1 is important for binding and activation of the contact system

Isenring et.al,Virulence (2017)

[2] Papadimitriou K et. al, BMC Genomics, 2014

Conclusion

- ***Sgg* binds FXII and kininogen on its surface**
- **FXII is bound by Pil1 adhesin**
- **binding of contact factors causes activation**
- **bradykinin will be released from the bacterial surface**
- **Pil1 is produced by *Sgg* but not *Sii* strains**

➢ **contact activation by *Sgg* might contribute to the pathogenesis of infective endocarditis**

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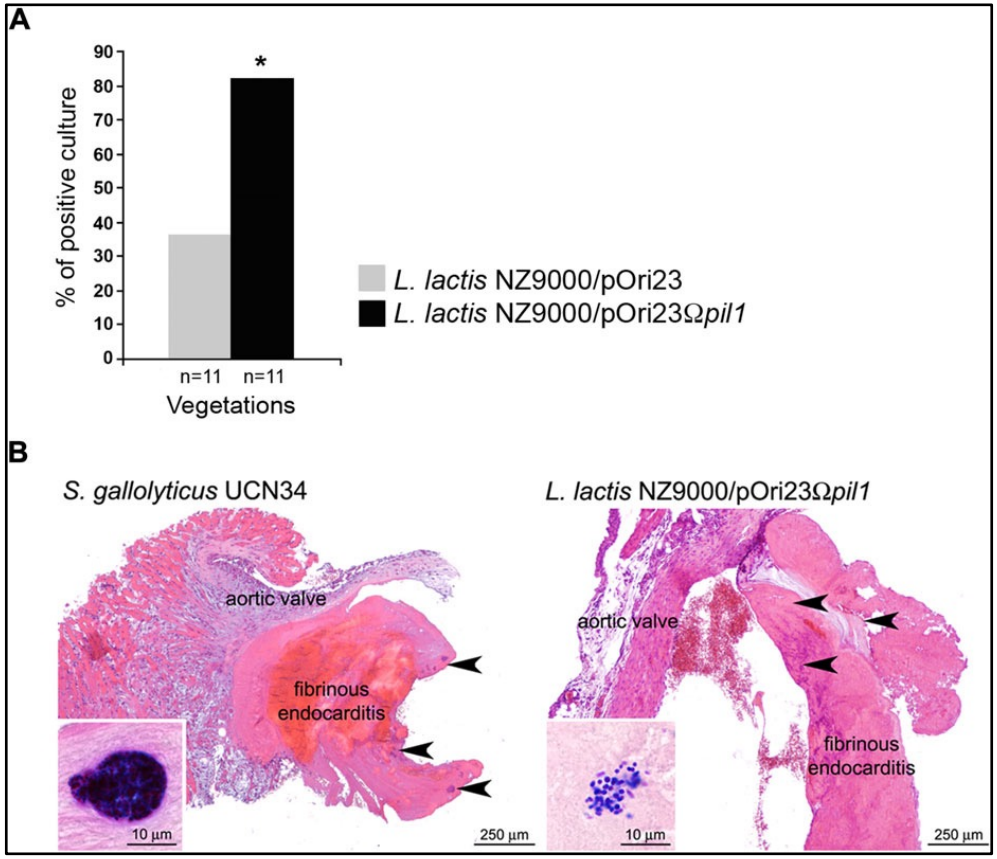
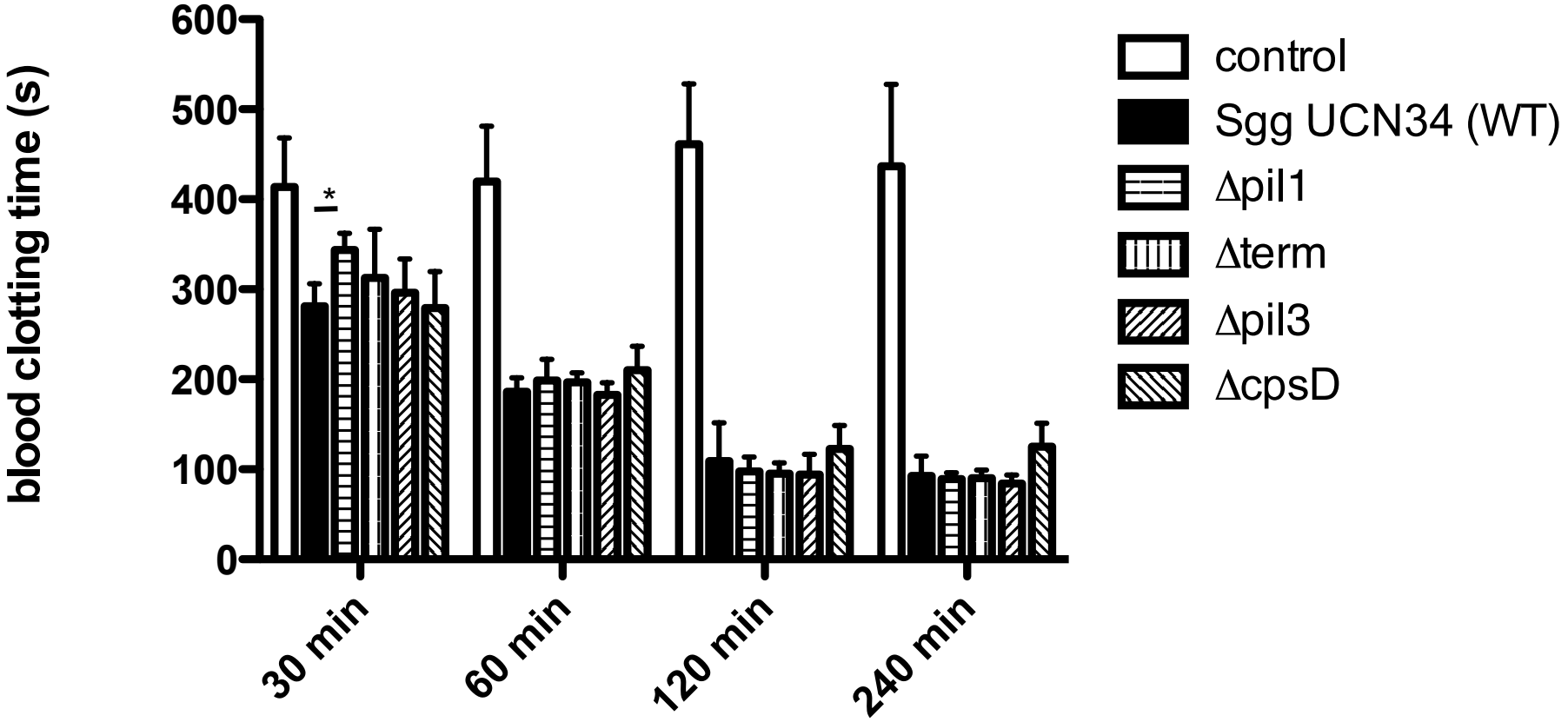
Pilus 1 of *Sgg* is involved in experimental infective endocarditis



Danne, C., Entenza, J. M., Mallet, A., Briandet, R., Débarbouillé, M., Nato, F., et al. (2011). Molecular characterization of a Streptococcus gallolyticus genomic island encoding a pilus involved in endocarditis. *The Journal of Infectious Diseases*, *204*(12), 1960–1970. http://doi.org/10.1093/infdis/jir666

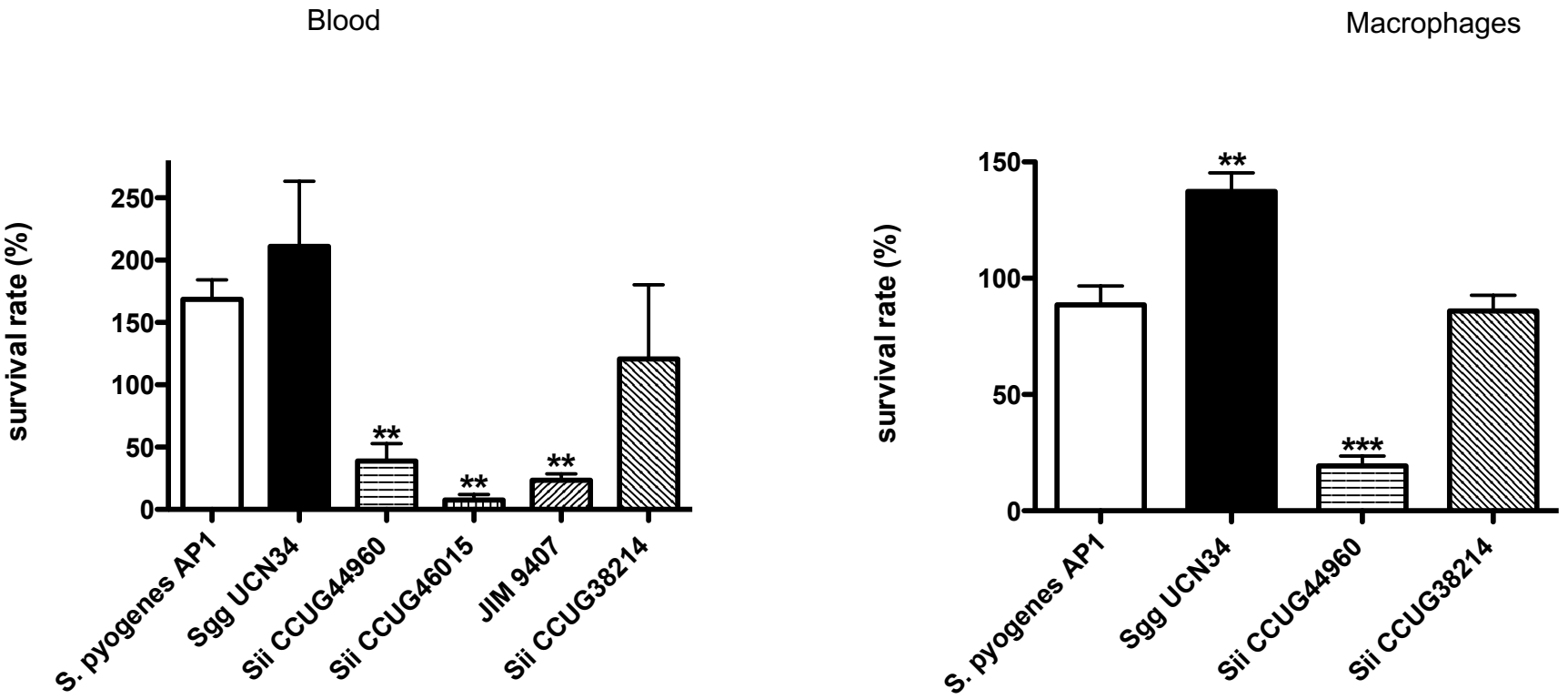
Activation of coagulation in whole blood by *Sgg*



- Coagulation is activated after incubation of bacteria in whole blood
- pilus knockout strain activates coagulation slowly

Isenring et.al,Virulence (2017)

Survival of selected *SBSEC* strains in blood and in the presence of macrophages



- **Low/no survival of *Sii* strains**
- **Growth of *Sgg* UCN34 in blood**