Biofilm formation is a key virulence strategy of *Proteus mirabilis* during catheter-associated urinary tract infection

Mario Maierl[1], Michael Jörger[1], David Siebenhofer[1], Ellen Zechner[2] and Andreas Reisner[1*]

[1] University of Applied Sciences, Biomedical Science, 8020 Graz, Austria

[2] University of Graz, Institute of Molecular Biosciences, 8010 Graz, Austria

Abstract

Objective: Catheter-associated urinary tract infections (CAUTI) are the most frequent infections in hospitals and other health care facilities. *Proteus mirabilis* is the most important pathogen that colonizes the bladder during long-term catheterization. Colonization of the bladder is associated with formation of a crystalline biofilm leading to catheter blockage, stone formation and severe complications for the patient. The relevance of *Proteus mirabilis* biofilm formation on urethral catheters for CAUTI pathogenicity as well as the mechanisms underlying efficient biofilm formation of *Proteus mirabilis* are largely unknown. We aimed to identify genetic factors crucial for biofilm formation on urethral catheters.

Methods: To identify genetic factors crucial for biofilm formation on urethral catheters, a random mutagenesis approach was applied. 5500 individual Tn5 mutants of *Proteus mirabilis* HI4320 were evaluated for loss of biofilm formation in a static biofilm model system. In parallel, signature-tagged mutagenesis was used to identify novel virulence determinants of *P. mirabilis* crucial for colonization of urethral catheters during CAUTI in a dynamic catheterized bladder model in vitro. Attenuated mutants of both libraries were co-challenged with the parent strain in the dynamic catheterized bladder model. Mutants that were significantly outcompeted by the parent strain were identified by sequencing the transposon insertion sites and further tested for growth deficiencies and phenotypes like swarming, hemagglutination, and urease activity. Expression of affected genes during CAUTI infection in vitro was evaluated.

Results: Of 7300 mutants, we identified 28 mutants that were significantly outcompeted by the parent strain in the catheterized bladder model, 16 of which were out-competed more than 100-fold for colonization of bladder and catheter, and 10 of which were also attenuated for biofilm formation in a static biofilm model. Swarming was not found to be necessary for catheter colonization. We identified genes affecting MR/P pili synthesis, urease activity, aerobic respiration control, osmolarity control, extracytoplasmic stress, and key metabolic pathways as requirements for *P. mirabilis* colonization.

Discussion: Our results suggest that biofilm formation is a key virulence strategy of *Proteus mirabilis* and indicate molecular pathways that support *Proteus mirabilis* biofilm formation, providing therapeutic targets for combating CAUTIs.

Aims

- Identify genes relevant for *P. mirabilis* biofilm formation
- Identify *P. mirabilis* genes relevant for bladder and catheter colonization during CAUTI in vitro
- Evaluate relevance of biofilm formation in CAUTI pathogenicity

Methods

Tn*5*-based mutagenesis of *P. mirabilis* HI4320[1]

Screen for loss of biofilm formation in static biofilm model

- 96-well microdish (PS)
- Biofilm quantification based on Crystal Violet staining after 24 hours of static growth in LB medium

Characterization of mutants attenuated in biofilm formation

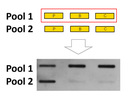
- Growth characterization in LB medium compared to wildtype strain
- Identification of transposon insertions via plasposon rescue and sequencing

Signature tagged mutagenesis of *P. mirabilis* HI4320

Competition of pools of 24 signature-tagged mutants in an in vitro model of CAUTI

- Harvest of Preculture and Bladder/Catheter after 24 hours
- Tag amplification of samples from Preculture / Bladder / Catheter

Immobilization on membrane and hybridization with Tag-specific probe



- Immobilization of samples from Preculture (P) /Bladder (B) / Catheter (C)
- Hybridization with 24 different probes
- Visual identification of attenuated mutants

Analysis of mutant colonization in an in vitro model of CAUTI[2]

- Inoculation with 1x10^8 cfu of mutant in competition with wild type strain in catheterized bladder
- Controlled continuous urine feed (artificial or human) mimicking physiological conditions (~700 ml /d)
- Quantification of bladder and catheter colonization after 24-48 hours

Growth rate comparison of outcompeted mutants in artificial urine compared to wildtype

Results

Tn5 Based Mutagenesis

17 of 5500 tested *P. mirabilis* Tn*5* mutants show significantly reduced biofilm formation and no growth deficiency

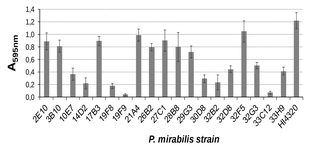


Fig.1 : Biofilm formation of 19 attenuated mutants compared to HI4320 wild type

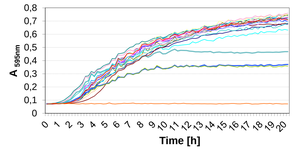


Fig.2 : Growth curve of 19 attenuated mutants compared to HI4320 wild type

Signature Tagged Mutagenesis

1st screening round

- 39 of 1920 tested *P. mirabilis* signature tagged mutants show significantly reduced attenuation in both bladder and catheter and no growth deficiency
- 202 potential catheter-attenuated mutants are pooled in 10 pools for secondary STM screen

2nd screening round

- 28 mutants with same attenuation as in 1st screen

Competition of 17 biofilm and 67 STM mutants with *P. mirabilis* HI4320 wild type strain in catheter model

28 mutants are outcompeted by *P. mirabilis* HI4320 wild type strain during competitive colonization of bladder and catheter in vitro

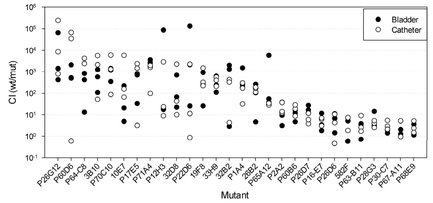


Fig.3 : Competitive indices (CI) of attenuated mutants in comparison to wild type strain. CI indicate ratio of wildtype cells to mutant cells isolated from inoculated bladders and catheter, respectively, relative to the onset of the experiment.

Growth rates of attenuated mutants in artificial urine

Only 6 mutants have significantly reduced growth in artificial urine

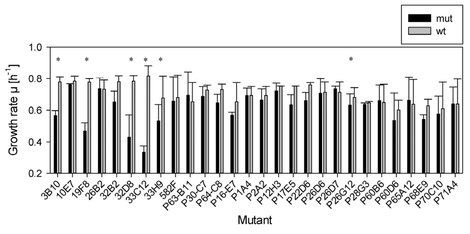


Fig.4 : Growth rates of mutants significantly outcompeted by HI4320 wildtype in bladder and catheter (mut) in artificial urine compared to wildtype (wt). Asterisk (*) indicates significant difference.

Transposon insertions of mutants affected in biofilm formation and / or colonization of CAUTI model

Mutants defective in several functional categories were identified via sequencing

Mutants affected in pili synthesis	*mrpB*	subunit of MR/P fimbriae	
	dsbA	disulfide-bond forming enzyme	
Mutants affected in metabolic pathways	*ureR, ureD*	urease	
	PMI0229	ABC transporter (Fe-transport)	
	sufS, sufC	cysteine desulfurase	
	sdhB, sdhC	succinate dehydrogenase	
Mutants affected in regulatory pathways	*arcA*	aerobic respiration control protein	
	arcB	aerobic respiration control protein	
	cpxA	two-component system sensor kinase	
	ompR	osmolarity transcriptional regulator	

Conclusions

- Mutants that are attenuated in static biofilm formation and / or CAUTI model survival could be isolated
- Of 28 mutants all except one are attenuated in bladder AND catheter colonization
- Biofilm formation is an important virulence strategy for CAUTI
- Stress response and anaerobic growth are required for bladder and catheter colonization in vitro

Outlook

- Determine biofilm formation of selected mutants in flow chamber biofilms grown on silicone-coated surfaces
- Analyze expression of MR/P fimbriae during CAUTI colonization
- Determine impact of MR/P and ATF fimbriae mutation on CAUTI colonization
- Reveal expression patterns of *arcA/B*, *ompR* and *cpx* regulon during CAUTI colonization

References:

1. Pearson MM, et al. *J Bacteriol. 2008;190(11):4027-4037.*
2. Stickler DJ, et al. *Methods Enzymol. 1999;310:494-501.*

*Contact: **andreas.reisner@fh-joanneum.at**

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