

## Introduction:

Growing bacterial resistance to antibiotics is a major threat to modern society and responsible for thousands of illnesses and deaths annually. This necessitates the development of new strategies in diagnostics, as well as finding new targets to kill pathogens. Our project's major aims are to specifically detect and target certain pathogens using quorum sensing (QS) and specific killing strategies, respectively. As a safety measure a delayed suicide-switch guarantees non-persistence of genetically modified *Bacillus subtilis* W168 in the absence of pathogens. We envision the use of BaKillus as a smart, cheap and simple-to-use medical device for diagnostics and targeted treatment of multiresistant superbugs.

## Results:

- Sensing: The two-component system ComDE from *Streptococcus pneumoniae*, introduced into *B. subtilis*, can sense the QS peptide CSP and activate the corresponding promoters  $P_{comC}$  and  $P_{comAB}$ .
- Adhesion: Three peptides were shown to bind to *S. pneumoniae* when fused to a maltose binding protein. Anchors to the *B. subtilis* cell wall are still in work.
- Killing: Subtilin, a lantibiotic produced by *B. subtilis* ACC633, is able to lyse *Staphylococcus aureus* (kindly evaluated by Groningen) and *S. pneumoniae*. The resistance module *spaIFEG*, once introduced as BioBricks into *B. s.* W168, confers resistance against *B. s.* ACC633.
- Suicide switch: A delayed expression can be achieved by wiring the ecf  $\sigma$  factor  $\sigma_{ecf41}$  and its target promoter  $P_{ydfG}$  between the input promoter and the output. Furthermore, the resistance factor Sdpl leads to higher resistance against the antimicrobial peptide SdpC, enabling it to survive until the suicide switch kicks in.
- Application: we developed a nasal spray for the application of BaKillus against sinusitis.

## Discussion:

- We could obtain results in all major parts of our projects!
- The optimal assay conditions for several parts still have to be found or created, e.g. a resistance-free *B. subtilis* strain that lacks extracellular proteases.
- The modules have to be combined and prove their efficacy against *S. pneumoniae* and *S. aureus*.

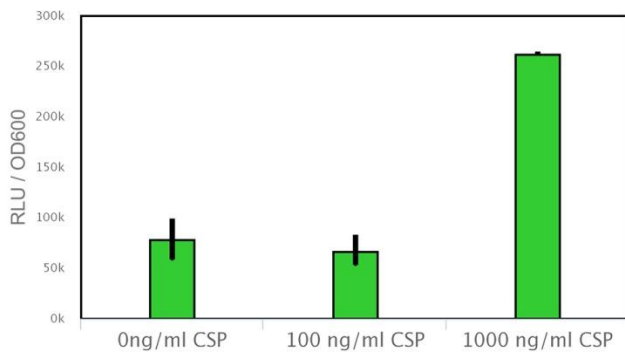
## Conclusions & Significance:

We aim to fight two major human pathogens by equipping *B. subtilis* with appropriate genetic modules. In our sensing module we could show that ComDE can not only be expressed heterologously in *B. subtilis*, but also sense CSP and activate two target promoters.

Our wide-spread correspondence with experts showed us which steps we have to take to use BaKillus as a cheap medical device in the fight against multi-resistant superbugs.

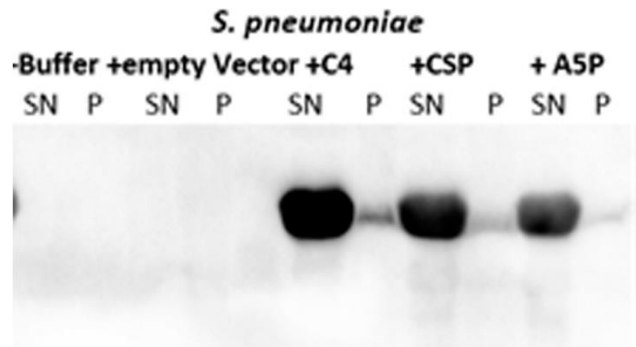
## Sensing of CSP leads to activation of PcomC

*Pxyl - comDE / PcomC - lux*

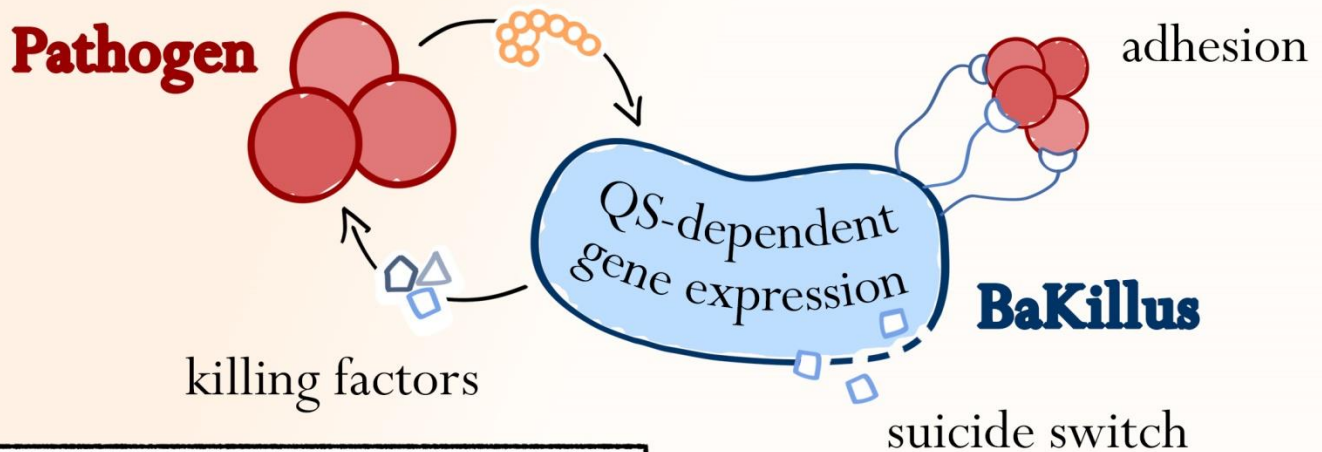


BioBricks: BBa\_K1351039, 16, 24

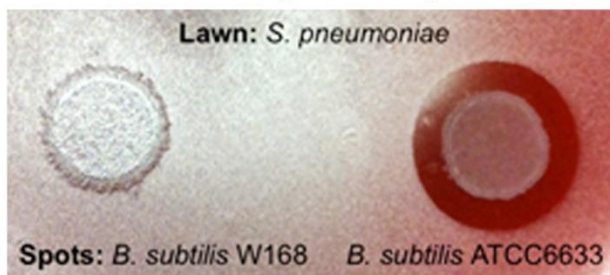
## Peptides C4P, CSP and ASP bind to *S. pneumoniae*



## sensing of QS molecules

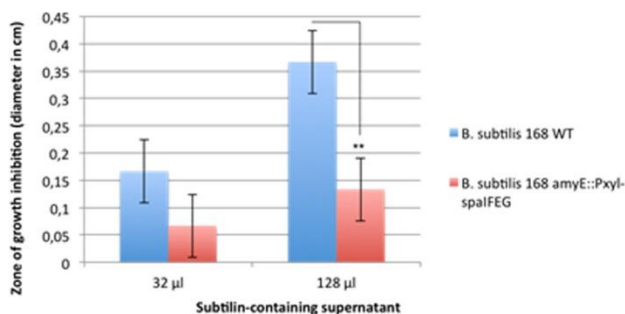


## Subtilin leads to lysis of *S. pneumoniae*



## SpaIFEG confers resistance against subtilin

Supernatant-in-lawn assay



BioBricks: BBa\_K1351014

## Using the cascade $\sigma_{ecf41}$ and PydfG, transcription is delayed

