The BaKillus Concept

Ever increasing microbial resistance to classic antibiotics urges the development of novel pathogen-killing strategies to treat infectious diseases. Exploiting bacterial communication mechanisms such as quorum sensing (QS) is a promising strategy to specifically target certain pathogens. Towards this goal, the synthetic organism BaKillus was designed to specifically target respective QS-dependent pathogenic bacteria. Here, the core element is a pathogen-detection device to detect *Staphylococcus aureus* and *Streptococcus pneumoniae*. By utilizing QS-dependent promoters, BaKillus will activate pathogen-killing devices like the production of antimicrobial peptides or biofilm degrading enzymes in the presence of target pathogens (see Fig. 1 for details). As a safety measure, a delayed suicide-switch guarantees non-persistence of genetically modified *B. subtilis* in the absence of pathogens.

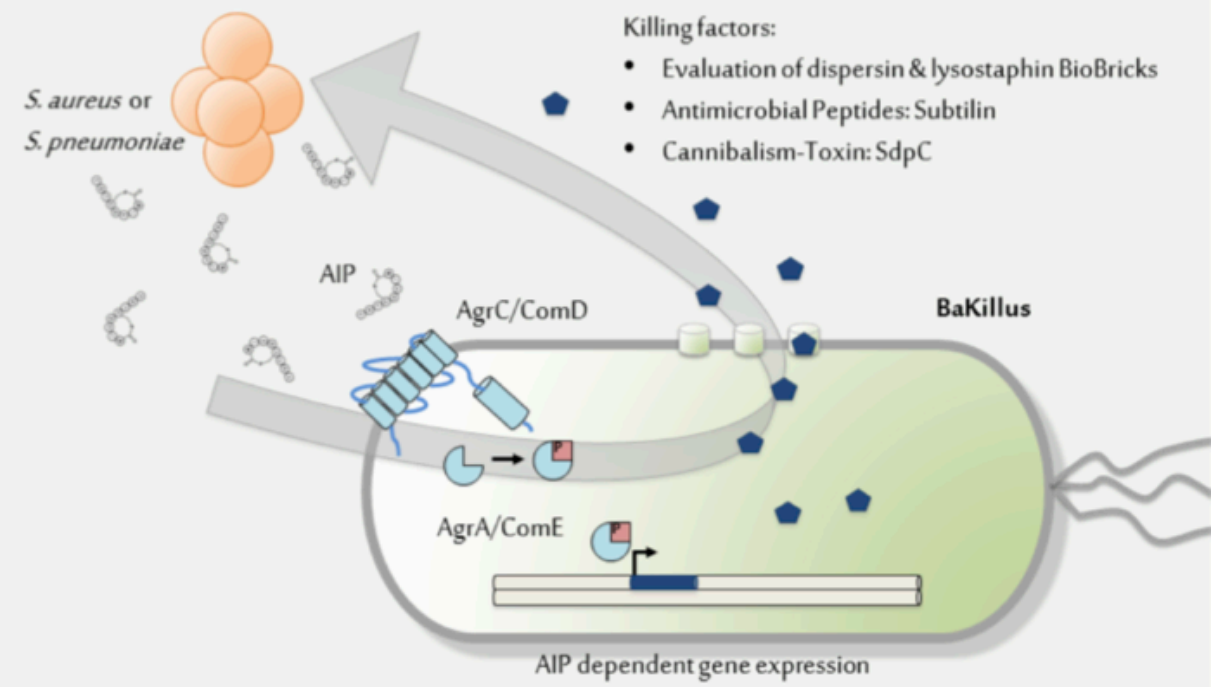

Figure 1: Basic concept of BaKillus: *S. aureus* and *S. pneumoniae* use autoinducer-peptides (AIP) for cell-density dependent regulation of gene expression (quorum sensing), e.g. regulation of pathogenicity. BaKillus with the respective two-component sensing systems AgrAC and ComDE is enabled to activate killing devices in an AIP dependant manner. Uncommon antimicrobial peptides like subtilin or the cannibalism toxin, the peptidase lysostaphin and the hydrolase dispersin will be used to ensure effective killing of the targeted pathogens.

Application

BaKillus could be applied in two independent ways, first as a point-of-care diagnostic tool to identify pathogens and second, as a drug-producing microbe to treat bacterial infections.

Pathogen-identification diagnostic tool:

Problem:

Current tests to identify *S. aureus* are PCR based to detect specific genes. Theoretically, a PCR-result could be obtained within three hours, whereas it takes round seven hours in practice. However, PCR based methods are error-prone and therefore verification by molecular biologic test are recommended. Current biological tests (e.g. coagulase) require a time-consuming (18-24h) culturing step. Based on the test results, rapid and strict hygiene rules, e.g. separation from non-infected persons, seem to be very effective to lower the spread of health-care associated MRSA.

Here, we want to suggest an alternative strategy that can potentially facilitate the current diagnosis procedure.

The BaKillus S. aureus diagnosis tool:

We are planning to develop a ready-to use diagnosis tool for point-of-care testing by the professional staff in hospitals, pharmacies or medical offices (see Fig. 2). A smear of a patient into a BaKillus-reporter culture could be enough to verify the presence of *S. aureus* within 0.5-2 hours (just limited by the time for BaKillus spore germination and reporter gene expression). This culture-independent detection could be precautionary used by patients of certain risk groups. The fast result would enable health-care institutions to start directly a subsequent treatments/hygiene measure just after arrival of the patient and thus contact between infected and non-infected can be lowered.

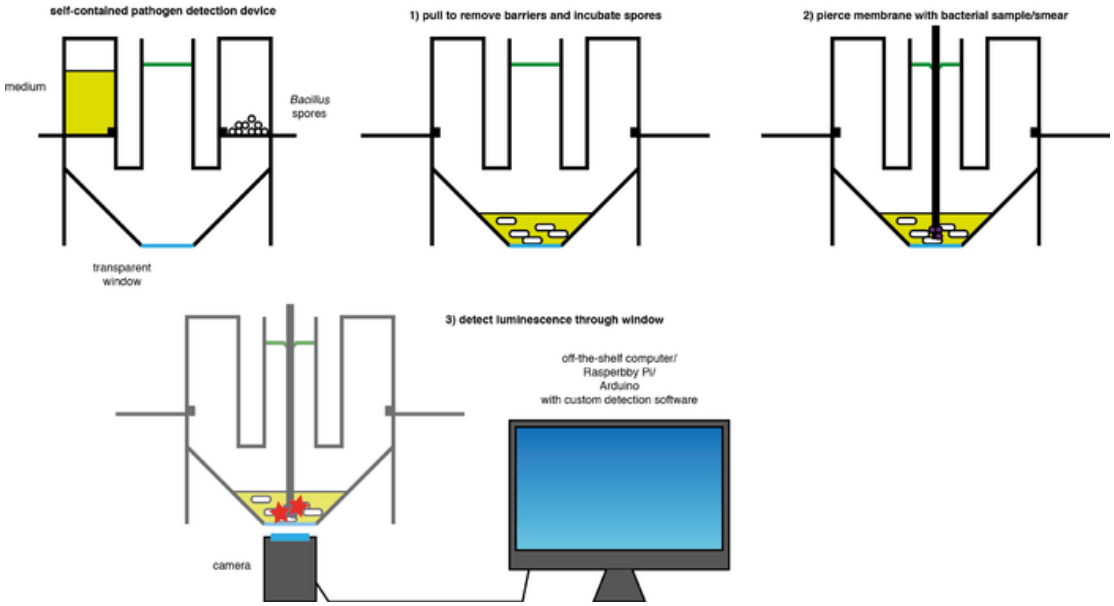

Figure 2: Basic concept of the BaKillus diagnostic tool. BaKillus spores are stored separately in a self-contained device. About one hour before use, spores and medium are mixed and incubated for the germination of the spores. The sample of a patient (e.g. a smear) can be applied into the culture and after about 30 minutes the result can be analysed with a simple camera-computer-station.

Table 1: Discussion of the BaKillus diagnostic tool.

Strengths	Weaknesses
Detection & Identification of AIP dependent pathogens	Application is limited to targets that exhibit QS-dependent pathogenicity.
Pigments (http://2012.igem.org/Team:Groningen/pigmentproduction) would be ideal to use as bio-reporter (the output of the detection device). The change of colour can be observed by eye and thus the product needs no high-end technology or electricity => Highly portable and independent device for usage in outlands and crisis regions	Initial assessment of the sensitivity of the test and tests for reliability of the tool required
Self-contained device prevents release of GMO and thus a product needs only a proposal for release (personal communication Dr. Ulrich Ehlers, Commissioner for GMO-release in Germany, BVL and Dr. Hans Schrubar Commissioner for Bio-Safety in Bavaria)	German laws are unspecific about bacterial GMO. Success and risks are hard to calculate

Pathogen treatment tool:

Problem:

'Inappropriate use of antimicrobials' in humans is considered the 'most important cause' for antimicrobial resistance (AMR) emergence by the WHO. This includes both over and underuse. First, every use of antimicrobials puts the human microbiome under selective pressure, enhancing selectively the proliferation of resistant strains. Second, non-lethal quantities of an antimicrobial drug enables pathogens to develop resistance and prolongs the infection, giving resistant organisms increased possibility to spread

The BaKillus pathogen treatment tool:

Based on the pathogen-detection device, BaKillus will activate its pathogen-killing devices (see Fig. 1) to kill *S. aureus* or *S. pneumoniae*. Both are often associated with surface infections of the human body system and thus BaKillus could be used to cure infected patients. We discussed potential applications with doctors and immunologists and plan an application as a nasal injection, e.g. to treat chronic inflammation of the ENT tract.

The approach's main advantage is that the antibiotic production strictly depends on the QS of the pathogen. Antibiotics are just produced in the presence of an inflammation caused by a pathogen. Once the pathogen is killed, antibiotic production is down-regulated. By this means, the BaKillus strategy increases the efficiency of antimicrobial treatments and strongly reduces the mis- and overuse of antibiotics.

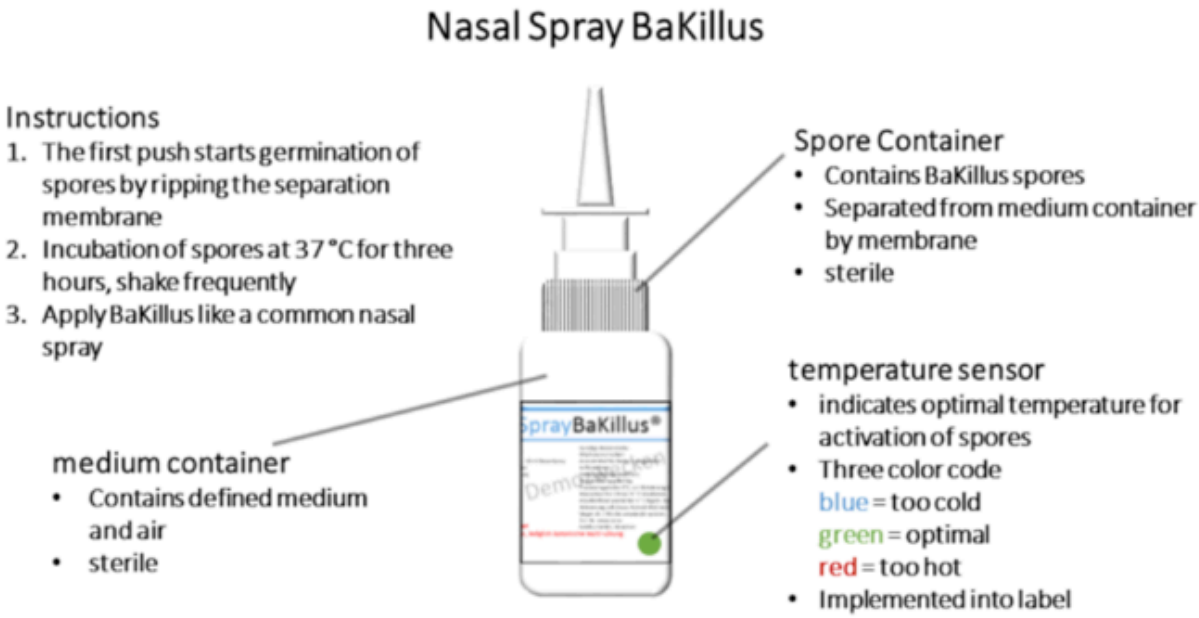

Table 2: Discussion of the BaKillus nasal spray.

Strengths	Weaknesses
Novel antibiotics allow treatment of resistant superbugs like MRSA	Unknown efficiency and interaction of the drugs with the human body system, therefore individual evaluation and approval required for each novel antibiotic.
Local treatment dependent on presence of pathogen => no need for broad spectrum-antibiotics	
Combination of drugs allows effective killing and little survival of resistant subpopulations	Strong legal regulations to use GMOs on humans could make the approval a long endeavour and obstruct the substitution of sensing and killing devices for personalized needs, as individual approval is required.
Low production costs	Immense costs to fulfil legal regulation for approval and clinical studies
Bacillus subtilis is Generally Recognized As Safe (GRAS status, FDA), so BaKillus is probably non-pathogenic. Additionally, the Suicide Switch and the Trytophane auxotrophy add addtional safety layers.	
	Technically, BaKillus is a drug and therefore it needs to prove itself in clinical studies (see Fig. 3 and 4)

Realisation:

In view of the possible BaKillus application process, we first plan to focus on the development of the diagnostic tool inside the university. Funding might be possible by DFG or SynBio- or entrepreneurship-funding organizations. Once our idea is able to leave the lab, we would start a business and develop a prototype with the help of an industry partner (e.g. a small engineering company) and venture capital funds. Alternatively, we would look into the possibility of acquiring the necessary financial means through a crowdfunding campaign. Throughout the development process, we would apply rapid prototyping techniques such as 3D printing to quickly reach a market-ready product. At the same time, intellectual property (e.g. patents) will be built up to protect our invention and provide an income for the growth of our start-up. Through the success of our diagnostic tool, we can convince further investor and finally could be able to afford the approval process of the BaKillus nasal spray. Once we have brought this product to the market, we can push for new legal regulations regarding the approval of SynBio products.

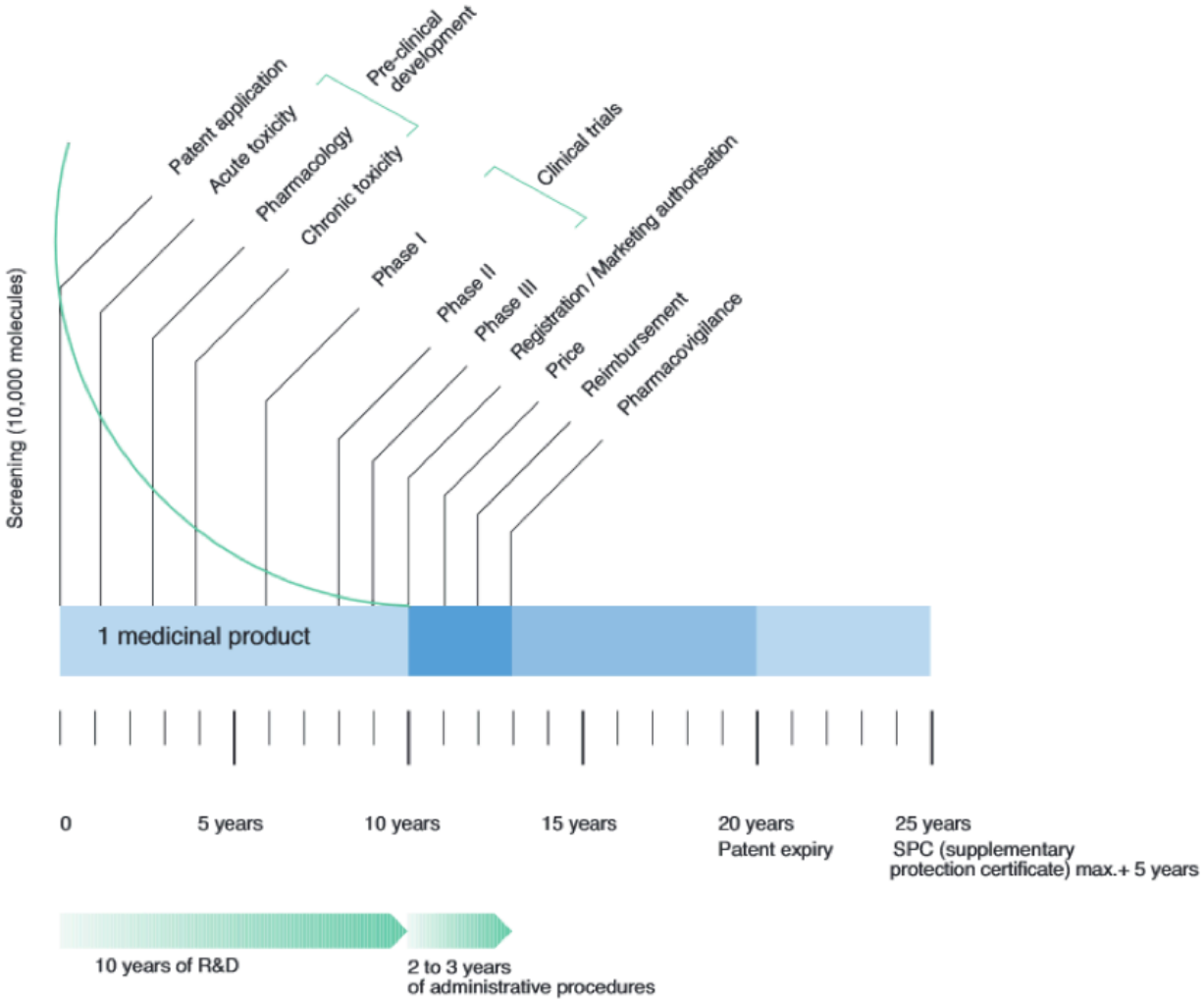

Figure 3: Typical time frame for the development of new drug applications

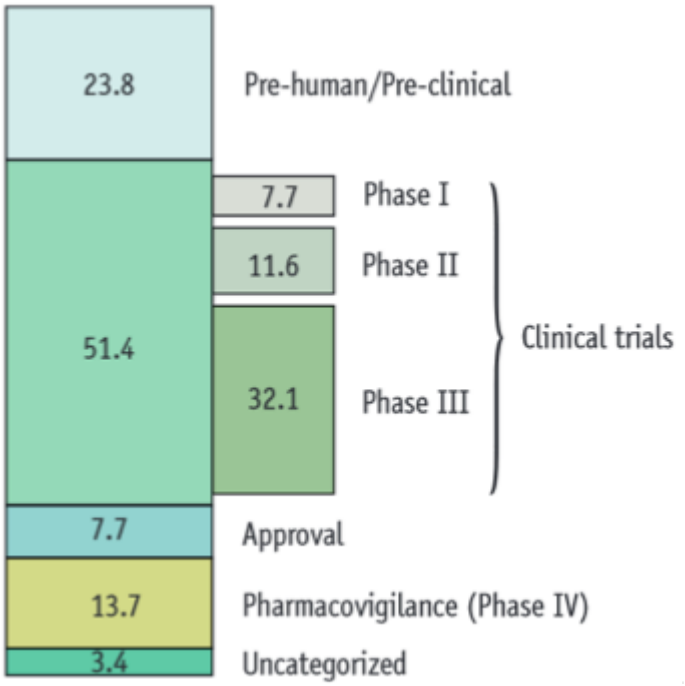

Figure 4: Distribution of costs for development of a new drug application