Quorum Sensing inhibition to battle infectious diseases

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Discussion

Antibiotic crisis – Back to the start

A famous quote of Charles Darwin reads: "It is not the strongest of the species that survives, not the most intelligent that survives. It is the one that is the most adaptable to change." And for the human mind the concept of evolution might be hard to grasp, because the time frame of adaptation by natural selection is so large that is almost impossible to observe in a human lifetime. However, every living creature is evolving, is adapting to changes of the environment, all the time. This is true for humans as well as bacteria. Bacteria adapt to changes in their environment, also to changes that are man-made. With the discovery of antibiotics physicians found, adapted and refined powerful compounds to efficiently kill bacteria. The overuse of antibiotics will eventually render the same useless, as we can observe already right now. Against all logic and warnings antibiotics are still being used carelessly and are expelled to the environment in large quantities. And as we see right know pathogenic bacteria adapted to the changed environment of the antibiotic aera. It is described as "antibiotic pollution", antibiotics can be found in human made environments such as sewers and wastewater treatment, but antibiotic molecules are also found in natural freshwater environments and can be also detected in the ground water [253]. The sum of the global antibiotics consumption is not solely composed of the usage in human medicine but also many other sectors of commercial activities such as agriculture. In the US alone the Food and Drug Administration estimated in 2010 that 80% of the annual antimicrobial consumption is used for disease control and growth promotion of food animals [1].

The global consumption of antibiotics for livestock is estimated to be more than 65,000 metric tons per year and this trend is rising [254]. If we are looking for a solution for the global antibiotic crisis it might not be enough to just develop more novel antibiotics but this has to go hand in hand with stricter regulations and modern policies for the usage of existing and novel antibiotics. More than that it is extremely important to sensitize and educate the general public over the use of antibiotics and antibiotic treatment in general.

The future of quorum quenching as a treatment

The colonialization of human skin and intestinal tract with "beneficial bacteria" is integral for a healthy human body. We live in a symbiotic coexistence with the microbial ecosystem in our bodies. Needless to say, the disturbance of this ecosystem also is threatening our health. Enzymatic quorum quenching mediated by PvdQ targets only long chain AHLs, this in turn makes it possible to specifically target pathogenic bacteria communicating with this signal.

As shown in **Chapter 2** enzymatic QQ offers a high degree of selectivity in contrast to conventional antibiotics, which can be beneficial to directly target special auto inducers utilized by pathogens and keep the impact on the gut microbiome as low as possible [255]. AHL molecules can differ in their chain length and also modification at their third carbon atom. The resulting differences can be considered as different dialects of the same language family. Enzymatic QQ offers the possibility to only target a special range of AHLs and accordingly target pathogenic bacteria specifically.

A potential impact of QQ on the human host directly is the finding that AHLs are recognized by the immune system and for instance act as chemo-attractants for neutrophils. They act as immune stimuli the same way as for example glycolipids, external DNA and LPS [256]. Furthermore AHLs are also accredited for pro-apoptotic and pro-inflammatory responses in eukaryotic cells [255]. As already described above enzymatic QQ offers a high specificity towards AHLs as substrates. In the case of the acylase PvdQ as described in **Chapter 3** the enzyme is targeting especially long chain AHLs as they are used by Gram-negative pathogens like *P. aeruginosa* and *A. baumannii*. Shorter chain AHLs will be unaffected and still function as auto inducing molecules as well as important cues for the human immune system. The high specificity of PvdQ represents a targeted approach against specific AHLs.

However, looking at the broad range specificity of some AHL lactonases there is a similar problem to consider as with AI-2 interference. Since lactonases cleave the ester bond of the homoserine lactone ring and have few interaction with the acyl side chain, they can be described as "broad range" QQ enzymes [257]. That means these lactonases degrade many different AHLs with various chain lengths and modifications. Accordingly, lactonases potentially are more prone to induce the side effects described above since they potentially can interfere with the QS system of beneficial bacteria. That means that a more specific QQ approach as it is shown for PvdQ would be beneficial in this scenario and reduces potential negative effects of QQ.

For a further evaluation for QS inhibition as a treatment strategy it is necessary to think about the development of resistance. In mixed bacterial populations individual bacteria can benefit from nutrients that are made available from excreted enzymes from other bacteria. This phenomenon is called social cheating. An illustrative example represents the excretion of the siderophore pyoverdine of *P. aeruginosa*. This compound is considered a public good, since it can be taken up by cells even if they are not able to produce the same [255]. Consequently, this means that even if mutations occur which can restore the QS system under those conditions, they would not have a selective advantage due to the social cheating of bacteria in mixed populations. This could imply that resistance might not spread

in the same fashion as it does when the cells are challenged with conventional antibiotics. One factor which is neglected in almost all available publications is the fact that any kind of treatment eventually effects the bacterial evolutionary fitness because the final goal is to clear the infection and reduce the bacterial load in the host system. So far, the only wholesome approach to actually assess the potency of QQ treatments are *in vivo* animal models. Publications attest for the beneficial effect of enzymatic QQ in invertebrate-, mouse- and rat models [35,36]. Against this background QQ seems to be a mild approach to manage infections. Furthermore, in the near future combination treatments utilizing QQ and conventional antibiotics could be a feasible approach to battle infectious diseases.

Acinetobacter baumannii

As discussed in **Chapter 5**, we propose a combination treatment of the QQ acylase PvdQ and gentamicin to battle the bacterial biofilm of *Acinetobacter baumannii*. Firstly, taking into account the specificity of PvdQ we tested if the enzyme discriminates between long chain AHL with an oxo- and a hydroxy modification at the third carbon at the amid bond. This modification seems not to have any impact on the recognition of the autoinducer by the enzyme. The current understanding of the QS system of *A. baumannii* ATCC 17978 connotates the biofilm formation with the QS system. Indeed, we can observe a significant reduction in biofilm formation upon PvdQ treatment. Additionally, we assessed if clinical isolates of *A. baumannii* also are responsive towards the same treatment and found that the addition of the enzyme leads to a biofilm reduction in more than 70% of the isolates. Next, we assessed the biofilm formation of *A. baumannii* ATCC 17978 with and without PvdQ pretreatment and the combination with gentamicin. We could demonstrate that the viable cells within the biofilm were reduced by 50% with the PvdQ/Gentamicin combination in relation to a treatment with the antibiotic alone. Finally, we could confirm that a QQ treatment with PvdQ prolonged the survival of *G. mellonella* in an infection model. Although these are observations in an infection model, the beneficial impact of the QQ treatment is striking.

Immobilized acylases

Also, in the clinical environment QQ strategies can be applied as shown in **Chapter 4**. We immobilized the acylase PvdQ electrostatically on PDMS silicone and evaluated the attachment of *P. aeruginosa* cells. The colonialization of abiotic surfaces by bacteria is omnipresent

in human-made environments and we can find surface attached bacteria everywhere from the international space station to nosocomial environments [258]. Especially in the latter surrounding this is problematic and the medical personal undertakes a huge effort to keep rooms and equipment as sterile as possible. However, despite all the hard work pathogens like *P. aeruginosa* can be isolated in IC units worldwide. Especially, indwelling medical devices are considered an entryway for pathogenic bacteria in the human body. Also sterilized medical items like catheters will eventually be colonized and act as a reservoir for urinary tract infections. The standard procedure in this case is the regular exchange and replacement. But this causes normally a discomfort for the patient and also can cause small injuries which in turn also make the human body vulnerable for bacterial infections. In this work we could show that we indeed can apply PvdQ to the surface of PDMS. FITC labeled PvdQ could be shown to be deposited evenly on the silicone. The enzyme retained its activity due to the mild conditions for immobilization as we could proof by a biosensor AHL activity assay. Finally, a staining of the attached *P. aeruginosa* cells revealed that indeed the initial attachment was significantly reduced. In conclusion we showed that it is possible to create an activated surface, which actively degrades AHLs and consequently reduces the QS mediated attachment of *P. aeruginosa* PAO1. Activated surfaces can also be applied in joint replacements as a preventive measure against prosthetic joint infections (PJI). The bacterial biofilm is an important factor in the pathogenesis of PJIs, which account for approximately 1 to 4% after primary total knee arthroplasty and 1 to 2% after primary total hip arthroplasty [259]. A common management strategy against PJIs is antibiotic-loaded acrylic cement, however a major drawback is the fact that on average only 10% of the total loaded antibiotics will be released and this can lead to low subinhibitory concentrations. In combination with the surface colonialization of the implants this can enhance the bacteria resistance formation [96]. In this scenario immobilized PvdQ could offer a complementary treatment option by inhibiting the initial attachment of bacteria to the surface of the implant and consequently lower the tolerance towards antibiotics of the planktonic cells in contrast to cells organized in a biofilm.

Inhibition of PvdQ

In **chapter 7** we take another approach to attenuate virulence factors of *P. aeruginosa*. As it is discussed in chapter 3, PvdQ plays an integral part in the maturation of the siderophore pyoverdine. The low bioavailability of iron for biological processes in the environment as well as within host organisms creates the necessity for bacteria

for highly efficient iron uptake systems. Logically interfering with the microbial iron homeostasis can be a target against pathogenic bacteria. In this part a library of 81 chromene based inhibitors against PvdQ was screened. The most potent inhibitor showed an IC_{50} = 4,70 ± 0,51µM. The identified inhibitor 4d could quench the production of pyoverdine in a dose dependent manner. Furthermore, the potency of the compound could be evaluated in a *G. mellonella* infection model challenged with PAO1.

Conclusion

Enzymatic QQ has been proven to significantly attenuate the production of virulence factors as well as to serve as a management tool against biofilm formation and surface attachment *in vitro*. Also, animal experiments confirmed the positive effect of QQ enzymes *in vivo*. The potential of QQ enzymes is very much dependent on the medical context e.g., the place of application and as well as the method of administration. Especially in the times of rising numbers of MDR infections alternative treatment options are of big interest. Particularly in the management of chronic infections, which demand long-term medication enzymatic QQ could be an alternative or addition to conventional antibiotics, since QQ puts less selective pressure on pathogens. It is important to highlight in this context that the goal of this treatment is to attenuate virulence factors and hinder the formation of biofilms. The clearance of the infection will be performed by the immune system of the host or with the help of antibacterial compounds. An interesting application of QQ enzymes could be the functionalization of various medical relevant surfaces to mediate anti biofilm properties. We demonstrated in this work that the QQE PvdQ has indeed the potential to be used against *A. baumannii*, and *P. aeruginosa* infections *in vitro* and *in vivo*. The potential of QQEs justifies to put more effort in research and refinement of this technique and eventually QQ treatment approaches could offer powerful tool to fight bacterial infections in the future.