MOI Project 8

First aid response of C. difficile against antimicrobial peptides

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Clostridioides difficile is the leading cause of antibiotic-associated diarrhea but can also result in more serious, life-threatening conditions. *C. difficile* is an anaerobic, spore-forming bacterium that is one of the most ubiquitous nosocomial pathogens. One group of antimicrobial peptides called lantibiotics are currently in focus to become novel antibiotics against these human pathogenic bacteria. The paradigm of lantibiotics is nisin produced by *L. lactis* which already in 1953 has been commercialized to inhibit the outgrowth of inhibit the outgrowth of *Clostridium tyrobutyricum.* Due to its high bactericidal activity in combination with low toxicity in humans, nisin has been applied for decades in the dairy and food industries to prevent growth of pathogenic bacteria.

Recently our group determined, the adaptation of *C. difficile* to treatment with a sublethal concentration of the lantibiotic nisin in a whole proteomic analysis, mimicking the first aid response of *C. difficile* against lantibiotics (1). This revealed two distinct operons which are upregulated. The cationic antimicrobial peptide resistance cprABC gene cluster, and adjacent a lipoprotein CprI is present, which displays a basal expression level and ensures the first line of defense (2).

Secondly, the vancomycin resistance B operon is upregulated. Here, a special feature is the VanW protein, found only in Clostridia genus.

We have biochemically and structurally characterized both membrane anchored proteins CprI and VanW *in vitro* where both display high affinity to lantibiotics and vancomycin, respectively.

Although our *in vitro* knowledge of VanW and CprI has been increasing it is still lacking the exact function of these proteins on a *in vivo* level which would explain why these gene are upregulated after the first contact of *C. difficile* with lantibiotics.

Within this Project a suitable candidate is searched for to

- Functionally characterize VanW and CprI
- Structural biology on the VanW and CprI protein in complex with the antimicrobial peptid
- *In vivo* determination of the function via microbiology techniques

Using the following techniques:

- Protein expression and purification
- Structural biology (X-ray crystallography and SAXS)
- Biophysical method (SPR and ITC)
- *In vivo* assays (bacterial cell growth, fluorescenz microscopy, inhbitions assays

Literature:

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