6. Phage-inspired antibacterials

6.2 Phages to combat biofilms

Slide nr. and content:

1) How can phages help us to combat biofilms? Before we try to solve this question, we will introduce you to the world of biofilms. Next, we will discover how phages and their enzymes can help us to overcome the biofilm threat.

2) So, what is a biofilm? Biofilms are structured groups of bacterial cells, either formed by one group of bacteria or by a combination of several bacterial species. The bacteria are embedded in a structured matrix of self-produced extracellular polymeric substances, also called EPS. This EPS matrix is often composed of polysaccharides, but can also comprise proteins and even DNA.

   To illustrate this biofilm composition, we will quickly go over its actual formation. First, bacterial cells attach themselves to the surface. Secondly, the production of EPS starts, leading to irreversible surface attachment of the cells. In a third phase, the cells are moving and forming clusters, ending in a mature biofilm. At this stage, the EPS matrix is a structured complex with channels to allow nutrient and oxygen transport, resulting in distinct cell types throughout the biofilm. Finally, cells can detach again and migrate to start a new biofilm.

   As you can notice, the EPS matrix is one of the main characteristics of a biofilm, but is also responsible for the troubles concerning biofilm treatment. The EPS doesn't only protect the cells against environmental stresses like oxidation, but also shields them from antibiotics and the host immune responses, making biofilms harder to treat than free-living bacteria.

3) So, how can phages help to combat these biofilms? Phage cocktails are able to reduce the biofilm amount. Experiments showed that a cocktail of three phages could eradicate a biofilm composed of only 1 bacterial species. But, most biofilms are build up by multiple types of bacteria. In this case, the cocktails failed to even eliminate 1 bacterial species out of the biofilm, let along to erase the entire structure.

   There are some limitations when using phages and phage cocktails. As indicated in the figure, you will need very complex and diverse phage cocktails to reduce multispecies biofilms. In this type of biofilms, the target bacteria are surrounded by others, who are non-susceptible to the used phage, thereby protecting the target bacteria. At last, there is still the biological drawback of bacteria becoming resistant to phages.

Phages alone are not enough to combat biofilms, but phages found some way to infect bacteria in a biofilm, by using specific enzymes. We are now going to focus on one type of enzyme, namely the EPS or polysaccharide depolymerase.

4) Different groups of phages produce polysaccharide depolymerases. These depolymerases can be attached to the phage itself, especially on the tail or capsid, or can be secreted into the environment. The enzymes dissolve the bond between polysaccharides. They target the lipopolysaccharides, also known as LPS, so the phage can attach onto the bacterial envelope, but they can also degrade the complex EPS matrix structure.

   And this demonstrates how these enzymes can help us to combat biofilms. The depolymerases degrade the EPS matrix, thereby disrupting the protective structure around the bacterial cells, making the bacteria available for antibiotic treatment. The combination of depolymerases and antibiotics can lead to complete biofilm removal.

5) Next, we are going to use some research examples to have a closer look into the potential of these depolymerases.

   The first case is 'Depolymerases and Cystic Fibrosis'. CF patients are confronted with very dense and viscid mucus in their lungs, giving them difficulties to breathe, but the mucus is an excellent spot for biofilm formation of pathogens like *Pseudomonas aeruginosa*. To combat these *P. aeruginosa* biofilms, the search started for phages that can degrade the EPS matrix. Four phages were found, able to degrade polysaccharides originating from the EPS *P. aeruginosa* matrix (Figure: How higher the absorbance, the more polysaccharides were degraded). This demonstrates the ability to identify phages producing depolymerases to combat specific biofilm diseases.

   Now, other researchers went a step further, when they investigated phage depolymerases against *Staphylococcus aureus*, a frequent cause of hospital-acquired infections. An EPS depolymerase was discovered, able to remove up to 90% of the biofilm cells. But the greatest achievement was that the protein could work independently from the phage. This opens the way for combination treatment of depolymerases and antibiotics to eliminate biofilms.

   To conclude, I will give you a final tip on depolymerase selection. As the actual composition of the EPS depends on the bacteria inside the biofilm, you will find the most therapeutically useful depolymerases in phages that have co-evolved with the target bacteria.

6) To conclude on this topic, we are showing an example of how phage engineering can be used to combine the bacterial killing properties of lytic phages with the EPS degrading capacities of enzymes.

If you want to perform this strategy, you first select a bacteriophage that is specific for a well-represented bacteria in the biofilm. Then, you let the bacteria express a strong broad spectrum depolymerase, that can be secreted into the environment. This study used Dispersin B, an enzyme targeting multiple species.

The engineered phage infects the biofilm. After infection, the phage is replicated inside the bacteria, while Dispersin B gets expressed. Upon cell lysis, both the phage as the enzyme are released, leading to a continued infection of the phage, and so the killing of the bacteria, while the enzyme strongly degrades the EPS, allowing the phage to reach all bacteria. This strategy resulted in a 99% removal of biofilm cells.