Transcript - Innovirology session 7.2 Emerging methods in phage-based bacterial diagnostics

Slide 1:

The use of phage-based diagnostics goes beyond mere phage typing and some techniques already have industrial applications. In this presentation, we will go into detail into some phage-based technologies already used to detect clinical and foodborne pathogens.

Slide 2:

This table summarizes the commercially available phage-based diagnostics, which target four pathogens (*Mycobacterium tuberculosis*, *Yersinia pestis*, *Bacillus anthracis* and *Staphylococcus aureus*). Roughly these tools use five main strategies to assess if phages could successfully infect the host: 1. Phage amplification, 2. Reporter gene production, 3. Quantification of phage genomic DNA amplification, 4. MS based detection of phage structural protein production, and 5. Membrane dot blot assay.

As listed in the table, each tool has its own distinct sample matrix, detection time and sensitivity characteristics. The matrix ranges from actual blood samples to cultured cells, the detection time can still be long (around 48 hours), however several diagnostics have much shorter detection times down to 2 hours. The sensitivity finally can go down to 100 CFU/ml.

These parameters show the superior speed and sensitivity that can be achieved with phage-based diagnostics, compared to traditional methods used so far. For more details about these tools we refer to the article of Schofield et al.

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Another application area of phage-based diagnostics is the detection of pathogens in the food industry. This industry is subjected to strict bacterial quality regulations to prevent food poisoning, for which the risk is on the rise due to the increase in consumption of ready-to-eat foods and the worldwide distribution thanks to globalization.

Nevertheless the conventional methods to assess the bacterial quality of food products are often slow and inefficient, for example an aerobic count to reveal pathogens can take around 72 hours. Such slow diagnostic methods result in delayed release of the finished food products and ingredients, requiring more storage capacity, as well as slower response to potential outbreaks. Furthermore these techniques are often labour intensive, operator-dependent and require many consumables.

Phage-based diagnostics could offer the tools to circumvent some of these drawbacks and design fast, robust and reliable procedures to detect important foodborne pathogens as you have *E. coli*, *Salmonella*, *Campylobacter* and *Listeria monocytogenes*.

One such procedure is the VIDAS-technology, which has been used to design an automated detection system using recombinant phage protein for various pathogens.

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The technology uses a fixed recombinant phage protein that specifically recognizes the targeted pathogen. This allows the entrapment of the specific pathogen, in a second step another antibody conjugated with an enzyme binds to other antigens of the target bacterium. In a final step this bound enzyme can produce a detectable reaction product of which the intensity can be determined to enable the quantification of the number of target bacteria present in the sample.

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This strategy is incorporated in a single strip as depicted on the top left, in the middle a schematic representation is shown. After an initial enrichment step of around 18-24 hours the sample is loaded in the strip, after which a tip containing the phage-based antibodies is used to collect potential target cells. The consequent slots are used to wash unbinding cells, add the secondary antibody and allow the enzymatic reaction to take place. Finally the output is measured and this entire process takes 48 minutes, as such this technique is faster then other procedures, less labour intensive and requires lees consumables.

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Aside from this technique various other technologies are under development that can depend on phage amplification or reporter phages, these two principles were already discussed in the previous topic. Other options are the measurement of the release of bacterial markers linked to infection (like the release of ATP following cell lysis) or the use of phages as affinity component in biosensor devices. For a more elaborate view on these options we refer to the article of Schmelcher and Loessner.

Slide 7:

One phage component that can be used to detect and recognize specific gram positive bacteria are the cell wall binding domains of endolysins. The paper discussed here shows the optimization of a library consisting of fusion proteins between fluorescent reporters and the cell wall binding domains of different classes of endolysins targeting *Listeria*. Once established this library can detect and differentiate specific *Listeria* strains, to this end the binding affinity of these fusion proteins to different strains was determined. Then those fusion proteins achieving a high affinity and specificity can be mixed with heterogeneous cultures to visualize the different strains present.

Slide 8:

For those who want to learn more about phage-based diagnostics we refer to the following papers. The link displays a movie concerning the use of the VIDAS technology.