Transcript - Innovirology session 7.1 Traditional phage typing

Slide 1:

A traditional biotechnological application of phages is their use to determine the specific strain of isolated bacteria, as well as detect these strains in various samples.

Here, we will discuss the original strategy as well as some adaptations used.

Slide 2:

Microbial typing is the principle of identifying organisms to the strain level, which is important to recognize virulent strains, confirm potential outbreaks, determine the source and routes of infections, and track cross-transmission of pathogens in healthcare. Furthermore it allows the evaluation of control measures to see their effectiveness

Several techniques have been evolved to allow typing and one of these exploits the inherent specificity of phages. Indeed these bacterial viruses only attach to and infect specific host strains, thus by using a phage library a bacterial isolate can be identified at the sub-species level.

Slide 3:

To achieve this, cultured bacteria are plated to create a bacterial lawn on which distinct phages are spotted in specific regions. If infection is successful plaques will be formed, as distinct phages are used, each strain will yield a specific pattern of infection and this allows the determination of the bacterial genus and species.

However a major drawback of this strategy is that it is only able to detect cultivable bacteria and the speed of this diagnostic is furthermore limited by the host bacterial replication rate, a factor that can be problematic for some pathogens such as *mycobacteria*. Another challenge is the labour intensity associated with phage typing as it requires the continued production and storage of large phage stocks.

Slide 4:

To address some of these limitations several adaptations to this strategy have been developed over the years.

A first one is known as the phage amplification assay, which is mainly useful for the detection of slow growing bacteria, such as *mycobacteria*. To circumvent the slow growth rate of this pathogen a second step is introduced using fast growing surrogate cells. The initial sample is still infected with bacteria to detect the presence of specific pathogens, however in the next step virucide is added to destroy all excess bacteriophages. In this way only those phages infecting the bacteria are spared and able to form new phage progeny. Once this infection cycle is finished the new progeny is used to infect fast growing surrogate cells. These cells are plated to allow enumeration of plaques, indicative of bacteriophage replication.

Slide 5:

A second adaption is known as the reporter phage assay. This strategy circumvents the need for plaque formation, allowing a faster detection time. To this end phages are genetically engineered to encode a reporter construct, these constructs can vary from fluorescent proteins to specific enzymes.
5.1: Once the phage genome is inserted in the target cell during infection, the reporter gene will be expressed.
5.2: Then, the signal of this reporter gene can be detected and its signal strength can be correlated to the number of target cells present in the sample.
5.3: One example of a reporter phage system is using luciferase to detect mycobacteria. The infected cell will produce luciferase and by adding luciferin, a light signal will be produced that can be measured.

Slide 6:
A third strategy is the dual phage technology, which enhances the specificity of phage typing even further. In this strategy two phages are used that each encode a distinct resistance gene and are capable of transducing this into their target cell. Furthermore these phages are chemically cross-linked to antibodies targeting different epitopes of the same antigen, which can be derived of specific pathogens.
6.1: Both phages are then mixed with the sample potentially containing the target antigen.
6.2: Next, susceptible bacteria are added to allow infection with the phage-antigen complexes. Here it is important to note that only those phages bound to the same antigen will be able to co-infect the bacteria
6.3: Finally, the infected cells are plated on media containing both selective antibiotics to select for cells that were co-infected. Thus the number of colonies formed on the plate will correlate to the number of antigens present in the original sample.

Slide 7:
The following references offer a good overview of the potential of phages for bacterial detection. This topic will be further discussed in the next section.