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Phage Typing of Bacterial Plant Pathogen

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Abstract

Bacteriophages, also known as phages, are viruses that attack bacteria. Depending on the method of replication phages can be broadly classified as virulent phages (replicate via lytic pathway) and temperate phage (replicate via both lytic and lysogenic pathway). In phage typing, a panel of lytic phages is inoculated on a lawn inoculum of the bacteria under investigation. Phages which are able to set up a lytic infection in that isolate produce a clear zone. As the ability to be infected (and lysed) by different phages varies between different strains of bacteria, the pattern of lysis forms the basis of phage typing.

Keywords – Bacteriophage, lytic and lysogenic pathway

Introduction

Microbiologists are using phage typing for several decades to determine the relatedness of species and also for various epidemiological purposes (surveillance, outbreak investigations etc.) This phenotypical method is being replaced by various molecular typing methods Phage-typing methods are gradually being superseded by genotypic techniques such as clustered regularly interspaced short palindromic repeats (CRISPR) typing, whole-genome sequencing etc.

Principle:

Bacterial strains are grown on a suitable culture medium and then subjected to attack by a series of different known phages. Some phages will kill the bacteria and lyse their colony, which can be visualized and measured but others won't be able to kill a given bacteria.

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Depending on which groups of phages can lyse or fail to lyse bacterial strain, the bacteria are given a number, also called phage-type.

Phage typing has been used for decades for subtyping of *Salmonella* Typhimurium to determine the epidemiological relation among isolates. The system distinguishes more than 300 definitive phage types (DT) of *Salmonella* Typhimurium based on their patterns of lysis to a unique collection of Salmonella phages e.g., *S.* Typhimurium DT104. Phage typing is also done for other species of Salmonella e.g., *Salmonella* Enteritidis PT4. Similarly, Staphylococci are typed to determine whether the isolates belonged to the more virulent phage types so that the appropriate infection control method could be instituted.

Procedure

1. Label each plate with the name/number of test bacterium.
2. Place a sterile cotton swab in the bacterial suspension and remove the excess fluid by pressing and rotating the cotton against the inside of the tube above the fluid level.
3. Streak the swab in three directions over the surface of the agar medium to obtain uniform growth. A final sweep is made around the rim of the agar. This is done to make a lawn culture of bacterium.
4. Allow the plates to dry for five minutes.
5. Divide the plate in four quarters (using a pencil, by drawing a line in the backside of the plate) and name each quarter with the name of the bacteriophage which you are going to inoculate in that region.
6. One the agar media dried completely, spot-inoculate 10 µl phages (according to the labelling) by dropping just a tiny amount of the phage suspension from the pipette tip.
7. Repeat the above procedure with a fresh pipette tip and spot-inoculate this phage on its specifically labelled region.
8. Allow the phage inocula to dry completely.
9. Incubate at 37°C for 1-2 days (or 30°C if incubation is more than 2 days).

Limitations

1. Phage typing requires different phages so phage typing is beyond the scope of local diagnostic laboratories. It is generally performed only at reference laboratories.
2. Phage typing requires substantial technical expertise to perform. Careful control of environmental conditions and other variables is technically demanding.

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3. Maintenance of typing phages by the reference laboratory is time consuming and expensive approach.
4. phage-types can change following lysogenic conversion, loss of prophages, or gain or loss of R plasmids, and this variability is coupled with the continuous need to maintain the typing set of bacteriophages in a viable state by regular serial passage.

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