Session III (Bioengineering) – Poster 6

The use of antibiotics to improve phage detection and enumeration by the double-layer agar technique.

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The double-layer agar technique is widely and extensive used in labs where phage investigation occurs. This technique is widely used to identify indicator strains, to enumerate and identify phages and to isolate phage mutants and new phages from different sources. The majority of known phages form large and well-defined plaques that are easily observed allowing their identification and enumeration when plated by the double-layer agar technique. However, some phages give rise to small and turbid plaques that are very difficult to detect and enumerate. This has a particular importance in modelling phage-bacteria systems in which it is crucial to have an accurate number of phages. To overcome these problems some authors suggested the use of tetrazolium to improve the contrast between clear plaques and turbid host lawns, facilitating observation. Although, for certain concentrations of tetrazolium salts some phages are suppressed having this dye an opposite effect from that desired.

Based on the Phage-Antibiotic Synergy discovered by Comeau et al. (2007), we used antibiotics to increase plaque size facilitating phage identification and enumeration. In this work several antibiotics with different mechanisms of action, namely antibiotics that affect cell wall, nucleic acids and proteins synthesis, were tested. An increase of the phage plaques of up to 50 times was obtained for some of the antibiotics used in certain concentrations. Furthermore, the use of antibiotic did not suppress the phages tested, what is critical in phage enumeration, and decreased the standard deviations of the PFU readings.

This work presents a modification of the double-layer agar technique that can be used routinely in the lab leading to the isolation and to a more accurate enumeration of phages that other way would be difficult or even impossible to perform.

Notes:

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