Bacteriophages: Update on application as models for viruses in water

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Abstract

Phages are valuable models or surrogates for enteric viruses because they share many fundamental properties and features. Among these are structure, composition, morphology, size and site of replication. Even though they use different host cells, coliphages and *Bacteroides fragilis* phages predominantly replicate in the gastro-intestinal tract of humans and warm-blooded animals where enteric viruses also replicate. A major advantage of phages is that, compared to viruses, they are detectable by simple, inexpensive and rapid techniques. In view of these features, phages are particularly useful as models to assess the behaviour and survival of enteric viruses in the environment, and as surrogates to assess the resistance of human viruses to water treatment and disinfection processes. Since there is no direct correlation between numbers of phages and viruses, phages cannot to a meaningful extent be used to indicate numbers of viruses in polluted water. The presence of phages typically associated with human and animal excreta indicates the potential presence of enteric viruses. However, the absence of these phages from water environments is generally a meaningful indication of the absence of enteric viruses. This is because phages such as somatic coliphages, F-RNA coliphages and *B. fragilis* phages generally outnumber enteric viruses in water environments, and they are at least as resistant to unfavourable conditions including those in water treatment and disinfection processes. However, using highly sensitive molecular techniques viruses have been detected in drinking water supplies which yielded negative results in conventional tests for phages. Initially, data on phages were rather confusing because a wide variety of techniques was used. However, techniques for the detection of phages are being standardised internationally. This applies in particular to somatic and F-RNA coliphages, and *B. fragilis* phages, which are most commonly used in water quality assessment. Reliable and practical techniques now available include direct quantitative plaque assays on samples of water up to 100 mℓ, and qualitative tests on 500 mℓ or more using highly sensitive enrichment procedures.

Introduction

Bacteriophages (phages) are viruses which infect bacteria. They were discovered independently by Frederick W Twort in England in 1915 and by Felix d'Herelle at the Pasteur Institute in Paris in 1917 (Pelczar et al., 1988). Phages were the last of the three major classes of viruses to be discovered during World War I. The other two classes were the plant viruses and animal viruses. It was then hoped that their ability to kill bacteria could be used for the prevention and treatment of bacterial disease, but this did not prove successful due to the rapid selection of resistant bacteria (Goyal et al., 1987). However, phages eventually turned out to have major other benefits, notably as models or surrogates for human viruses in basic genetic research as well as water quality assessment.

Phages proved to be most valuable tools in research on viruses because compared with the human, animal, plant and even insect hosts of other viruses, phages are easily and rapidly cultivated in laboratories which are not particularly demanding with regard to space, facilities, and equipment (Pelczar et al., 1988). Research on the basic genetic properties of phages led to the development of an entirely new science - that of molecular biology - which allowed unprecedented advancements in all the biological and medical sciences. In addition, the way all viruses reproduce was first indicated by work with phages (Ackermann, 1969).

Structure and morphology of phages

Phages basically consist of a nucleic acid molecule (genome) surrounded by a protein coat (capsid). The capsid is made up of morphological subunits called capsomeres. The capsomeres consist of a number of protein subunits or molecules called protomers. Some phages also contain lipid and additional structures such as tails and spikes. These features imply that in terms of composition, structure and morphology, phages share many fundamental properties with human viruses. For instance, F-RNA coliphages (Family Leviviridae) and enteroviruses such as polio viruses (Family Picornaviridae) both have an icosahedral capsid with a diameter of about 25 nm and a single strand (ss)-RNA genome. Under the electron microscope F-RNA coliphages and enteroviruses are hardly distinguishable (Fig. 1). In addition, F-RNA coliphages and enteroviruses are both excreted by humans. For these reasons coliphages are valuable models or surrogates for human enteric viruses. As a result of these similarities, the behaviour of F-RNA coliphages as well as other phages, resembles that of enteric viruses much closer than faecal bacteria such as coliforms commonly used as indicators of faecal pollution. The same applies to properties such as removal by water treatment processes and resistance to disinfection processes. However, there are differences which limit the indicator value of phages. For instance, electrostatic charges on phages may differ from those on enteric viruses, which affect important properties such as adsorption to solid surfaces. This has implications for features like behaviour in the environment, and the efficiency of recovery by techniques based on adsorption-elution principles.

Phage replication

Phages and enteric viruses can replicate only inside host cells, which in the case of phages are susceptible bacteria, and in the case of enteric viruses are susceptible mammalian cells. Phages use the ribosomes, protein-synthesising factors, amino acids, and energy-

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