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Review

Bacteriophages infecting *Bacteroides* as a marker for microbial source tracking

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ABSTRACT

Bacteriophages infecting certain strains of *Bacteroides* are amid the numerous procedures proposed for tracking the source of faecal pollution. These bacteriophages fulfil reasonably well most of the requirements identified as appropriate for a suitable marker of faecal sources. Thus, different host strains are available that detect bacteriophages preferably in water contaminated with faecal wastes corresponding to different animal species. For phages found preferably in human faecal wastes, which are the ones that have been more extensively studied, the amounts of phages found in waters contaminated with human fecal samples is reasonably high; these amounts are invariable through the time; their resistance to natural and anthropogenic stressors is comparable to that of other relatively resistant indicator of faecal pollution such us coliphages; the abundance ratios of somatic coliphages and bacteriophages infecting *Bacteroides thetaiotaomicron* GA17 are unvarying in recent and aged contamination; and standardised detection methods exist. These methods are easy, cost effective and provide data susceptible of numerical analysis. In contrast, there are some uncertainties regarding their geographical stability, and consequently suitable hosts need to be isolated for different geographical areas. However, a feasible method has been described to isolate suitable hosts in a given geographical area. In summary, phages infecting *Bacteroides* are a marker of faecal sources that in our opinion merits being included in the "toolbox" for microbial source tracking. However, further research is still needed in order to make clear some uncertainties regarding some of their characteristics and behaviour, to compare their suitability to the one of emerging methods such us targeting *Bacteroidetes* by qPCR assays; or settling molecular methods for their determination.

Contents

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1. Introduction

Water-borne diseases transmitted by the faecal–oral route make a significant contribution to the burden of diseases worldwide (WHO, 2008). Consequently, faecal pollution of surface and ground waters is a global public health concern. For example, in the USA the pathogen load is one of the main causes of fresh water body impairment (USEPA, 2004). Faecal pollutants can reach water bodies through direct discharge of faecal waste or raw wastewater, secondary effluents from wastewater treatment works, combined sewer and sanitary overflows, animal husbandry activities, the wastewaters from abattoirs and the meat industry, and wildlife (USEPA, 2004). The contribution of each of these faecal sources to the pathogen load varies strongly among watersheds. Determining the source of faecal contamination in aquatic environments is essential for estimating the health risks associated with faecal pollution, facilitating measures to remediate polluted waterways, and resolving legal responsibility for remediation. Microbial source tracking should enable investigators to uncover the sources of faecal pollution in a particular water body (Malakoff, 2002). Different approaches can be used to track the faecal sources. Eukaryotic mitochondrial DNA to differentiate sources in faecally contaminated surface water has been explored (Caldwell et al., 2007). The appropriateness of chemical markers has concentrated on the distribution pattern of chemicals such as caffeine, fragrance substances, fluorescent whitening agents, and faecal sterol isomers (Leeming et al., 1996; Standley et al., 2000; Hagedorn and Weisberg, 2011). As well, many different microorganisms have been tested for source tracking purposes. When using microorganisms, the techniques are then considered microbial source tracking (MST).

2. Microbial source tracking

Candidate microbes, both pathogenic and commensal organisms, determined in many different ways, both by culture-dependent methods and molecular methods, or by library dependent and library independent methods, have been investigated and reviewed as potential tools for the identification of human faecal sources (Scott et al., 2002; Simpson et al., 2002; Field and Samadpour, 2007; Blanch et al., 2008; Hagedorn et al., 2011). However, field studies using most of the numerous chemical and microbiological methods available to track sources of faecal contamination have shown that the existing methods are incomplete and that “tool boxes” of a variable number of different markers are needed, and consequently more research into suitable markers or combinations of markers is still worthwhile (Simpson et al., 2002; Stewart et al., 2003; Blanch et al., 2008).

In our opinion the requirements for an ideal marker are summarized in Table 1. From the many studies performed, all markers investigated so far fail in one or more of the requirements, and this is the reason why a “toolbox” approach is recommended.

3. Bacteriophages infecting *Bacteroides* as a marker for source tracking

Among the numerous tested procedures for microbial source tracking, there are three based on bacteriophages (Jofre et al., 2011) that are library independent methods. The first one is based on the frequencies distribution of the 4 serotypes/genotypes of F-specific RNA bacteriophages (Furuse, 1987; Hsu et al., 1995; Jofre et al., 2011), since serotypes/genotypes II and III predominate in humans and I and IV in non-humans studied so far. The second procedure is based on bacteriophages infecting selected strains of *Bacteroides* ssp. (Tartera and Jofre, 1987; Tartera et al., 1989). *Bacteroides* species are among the most abundant species in the feces of warm blooded animals (Allsop and Stickler, 1985); as well, since the early work of Bernhard and Field (2000) q-PCR assays targeting *Bacteroidetes* associated to certain animal species have become increasingly prominent for MST (Wuertz et al., 2011). And the third is based on bacteriophages infecting certain *Enterococcus* host strains (Bonilla et al., 2010; Purnell et al., 2011; Santiago-Rodriguez et al., 2013). The following text is concerned with bacteriophages, particularly those infecting *Bacteroides* spp.

3.1. *General characteristics of bacteriophages*

Bacteriophages, also called phages, are viruses that infect bacteria. They consist of a nucleic acid molecule (genome)