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Decay of host-associated *Bacteroidales* cells and DNA in continuous-flow freshwater and seawater microcosms of identical experimental design and temperature as measured by PMA-qPCR and qPCR

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ABSTRACT

It is difficult to compare decay kinetics for genetic markers in an environmental context when they have been determined at different ambient temperatures. Therefore, we investigated the persistence of the host-associated genetic markers BacHum, BacCow and BacCan as well as the general *Bacteroidales* marker BacUni in both intact *Bacteroidales* cells and as total intracellular and extracellular marker DNA in controlled batch experiments at two temperatures using PMA-qPCR. Fecal *Bacteroidales* cells and DNA persisted longer at the lower temperature. Using the modified Arrhenius function to calculate decay constants for the same temperature, we then compared the decay of host-associated *Bacteroidales* cells and their DNA at 14°C in field-based flow-through microcosms containing human, cow, and dog feces suspended in freshwater or seawater and previously operated with an identical experimental design. The time for a 2-log reduction (T_{99}) was used to characterize host-associated *Bacteroidales* decay. Host-associated genetic markers as determined by qPCR had similar T_{99} values in freshwater and seawater at 14°C when compared under both sunlight and dark conditions. In contrast, intact *Bacteroidales* cells measured by PMA-qPCR had shorter T_{99} values in seawater than in freshwater. The decay constants of *Bacteroidales* cells were a function of physical (temperature) and chemical (salinity) parameters, suggesting that environmental parameters are key input variables for *Bacteroidales* survival in a predictive water quality model. Molecular markers targeting total *Bacteroidales* DNA were less susceptible to the variance of temperature, salinity and sunlight, implying that measurement of markers in both intact cells and DNA could enhance the predictive

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power of identifying fecal pollution across all aquatic environments. Monitoring *Bacteroidales* by qPCR alone rather than by PMA-qPCR does not always identify the contribution of recent fecal contamination because a signal may be detected that does not reflect a recent fecal event.

1. Introduction

The threat to human health posed by fecal contamination of surface waters is usually estimated by measuring fecal indicator bacteria (FIB) such as total and fecal coliforms, *Escherichia coli* and *Enterococcus*. However, traditional fecal indicators to monitor recreational water quality are often not indicative of the source of fecal pollution and not associated with health risks in ambient water where non-point fecal sources are prevalent (Field and Samadpour, 2007; Santo Domingo et al., 2007). Members of the order *Bacteroidales* have been proposed both as an alternative to FIB and as source identifier because of their abundance in the gastrointestinal tract and the geographic stability of 16S rRNA genetic markers with host-specific distribution (Dick et al., 2010; Dorai-Raj et al., 2012; Drozd et al., 2013; Keity et al., 2012; Kreader, 1998; Layton et al., 2006; Liang et al., 2012; Liu et al., 2010; Reischer et al., 2013; Seurinck et al., 2005). Modification of qPCR methods with propidium monoazide (PMA) (Bae and Wuertz, 2009a; Nocker et al., 2006) can reduce the PCR signal from DNA originating from dead bacterial cells in the environment when determining the decay rates of uncultivable *Bacteroidales* cells in marine and fresh water (Bae and Wuertz, 2009b, 2012). Because anaerobic *Bacteroidales* cells decay much more rapidly in water than does DNA, PMA-qPCR has been proposed as a way to identify only recent fecal pollution (Bae and Wuertz, 2012).

A number of biotic and abiotic factors have been shown to affect survival and persistence of enteric bacteria. Thus biological conditions such as grazing by eukaryotic cells, cell death and biochemical breakdown of the DNA may also influence the decay of *Bacteroidales* in water (Bae and Wuertz, 2009b, 2012; Bell et al., 2009; Dick et al., 2010; Gilpin et al., 2013; Okabe and Shimazu, 2007; Schulz and Childers, 2011; Seurinck et al., 2005; Tambalo et al., 2012; Walters and Field, 2009). Previous research into the persistence of *Bacteroidales* using field microcosms reported that *Bacteroidales* DNA could persist longer at lower temperatures and higher salinities (Bae and Wuertz, 2009b, 2012; Liang et al., 2012; Walters and Field, 2009). However, a direct comparison of decay kinetics in field experiments using freshwater and seawater has not been carried out. Effects of multiple environmental factors on the survival of enteric bacteria can lead to a biphasic decay model consisting of a first period with a relatively high apparent first-order rate constant and a second period with a lower constant (Bae and Wuertz, 2009b, 2012; Schulz and Childers, 2011). Accordingly, the decay pattern of *Bacteroidales* DNA could not be described as single exponential decay (Bae and Wuertz, 2009b, 2012). In particular, the survival of host-associated *Bacteroidales* cells affected by a complex array of physical, chemical, and biological factors was not discerned in earlier studies.

The objectives of this study were (i) to evaluate the influence of temperatures on the persistence of *Bacteroidales* cells and DNA in laboratory-based batch experiments using PMA-qPCR, (ii) to examine the decay models associated with such variances, and (iii) to compare the decay of host-associated *Bacteroidales* in previously run identical field experiments in seawater and freshwater with an identical study design (Bae and Wuertz, 2009b, 2012) after adjustment of decay constants for the same temperature with the modified Arrhenius equation. The results are relevant for microbial source tracking studies where the origin and approximate age of a *Bacteroidales* genetic marker in a watershed are of key importance.

2. Materials and methods

2.1. Fecal sample collection

Fresh human feces was collected within 24 h from six healthy adults and animal fecal samples (cow and dog) were obtained in the early morning of the same day of inoculation from a cattle farm (8 samples; Winters, CA) and a dog park (12 samples; Davis, CA). Individual fecal samples were collected with a sterile utensil placed in a sterile 50-mL tube and kept on ice/dark until used as inoculum. Each fecal sample was immediately transported on ice to the laboratory. To confirm the specificity of each host-associated *Bacteroidales* assay for the corresponding host fecal samples, each feces was serially diluted with 1X PBS and then analyzed once by qPCR without DNA extraction before spiking fecal samples into freshwater. The lab batch experiment was started on the same day when fresh fecal samples were collected.

2.2. Laboratory batch experiment setup

Four grams of each fresh feces was mixed with 2.5 ℓ groundwater obtained from the Aqua Toxicology Laboratory, UC Davis. To confirm the presence or absence of any host-associated *Bacteroidales* prior to adding feces to the microcosm experiments, DNA was extracted from a 100-mℓ freshwater sample using the Ultraclean Water DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA) and qPCR was performed as described below. Hundred-milliliter samples of groundwater amended with fresh human, cow and dog feces were placed in sterile 250-ml Erlenmeyer flasks. To examine the effect of temperature on decay rates, triplicate flasks were