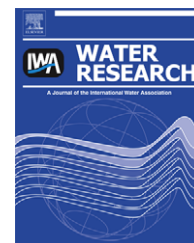


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Adhesion of *Campylobacter jejuni* and *Mycobacterium avium* onto polyethylene terephthalate (PET) used for bottled waters

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ARTICLE INFO

Article history:

Received 6 May 2008

Received in revised form

3 September 2008

Accepted 6 September 2008

Published online 27 September 2008

Keywords:

Campylobacter jejuni

Mycobacterium avium

Polyethylene terephthalate (PET)

Adhesion

Groundwaters

ABSTRACT

Adhesion of the bacteria *Campylobacter jejuni* and *Mycobacterium avium* onto polyethylene terephthalate (PET), a polymer widely used within the bottled water industry was measured in two different groundwater solutions. From this, it was found that whilst the percentage cell adhesion for a given strain did not change between groundwater types, substantial variation was obtained between the two bacterial species tested: *M. avium* (10–30% adhered cells) and *C. jejuni* (1–2%) and no major variations were measured as a function of groundwater composition for a given strain. To explain this, the interfacial electrohydrodynamic properties of the bacteria were investigated by microelectrophoresis, with the resultant data analysed on the basis of electrokinetic theory for soft biocolloidal particles. The results obtained showed that *M. avium* carries a significant volume charge density and that its peripheral layer exhibits limited hydrodynamic flow permeation compared to that of *C. jejuni*. It was also demonstrated that steric hindrance to flow penetration and the degree of hydrophobicity within/of the outer bacterial interface are larger for *M. avium* cells. In line with this, the larger amount of *M. avium* cells deposited onto PET substrates as compared to that of *C. jejuni* can be explained by hydrophobic attraction and chemical binding between hydrophobic PET and outer soft surface layer of the bacteria. Hydrophobicity of PET was addressed by combining contact angle analyses and force spectroscopy using CH₃-terminated AFM tip.

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1. Introduction

Microbial adhesion is the first fundamental step that leads to the formation of biofilm, a form of life for microbes in aqueous environment (Donlan, 2002). Understanding the basic processes governing biofilm formation is of particular interest

within the scope of numerous industrial applications, e.g. in food and beverage industries, since contaminated surfaces may represent a reservoir of microorganisms potentially dangerous for human (Jayasekara et al., 1999; Zottola, 1994). De facto, water quality mandatory survey is only oriented on the detection of microorganisms in bulk water (WHO, 2006).

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doi:10.1016/j.watres.2008.09.009

However, a recurrent issue is the evaluation and understanding of the adhesion affinity of bacteria onto the inner surface of bottles of waters as a function of the physico-chemical composition (nature of ions, concentrations, etc.) of the packaged waters and bottle surface properties.

The very first event leading to bacterial adhesion onto mineral or polymeric material in aquatic environments has been extensively studied and many factors have been attributed to explain bacterial adhesion, e.g. substratum heterogeneity (Chen et al., 2002; Fletcher and Loeb, 1979), chemical, thermal and hydrodynamic features of the suspending media (Chen and Walker, 2007; Fletcher, 1988; Li and Logan, 1999). These factors have been found to affect the interaction forces that govern bacterial adhesion, such as electrostatic forces, hydrophobic interactions or molecular recognition forces. For almost 30 years, researchers have attempted to quantify the hydrophobic characteristics and the electrical properties of bacteria for understanding the key mechanisms governing their adhesion (Boonaert et al., 2002; Bos et al., 1999; Busscher and Weerkamp, 1987; van Loosdrecht et al., 1989). In particular, several macroscopic methods have been widely used to infer cell surface hydrophobicity from water contact angle measurements or microbial adhesion tests to different solvents (e.g. Bellon-Fontaine et al., 1996; Mozes and Rouxhet, 1987; Van der Mei et al., 1998). In addition, these different macroscopic experimental approaches were usually accompanied by analysis based on the extended DLVO thermodynamic model developed by Van Oss et al. (1988) who consider an additional long-range attractive contribution that stems from the hydrophobic character (acid–base Lewis) of the microbial surfaces. Although these methods are still extensively adopted today, several studies have questioned these experimental and theoretical descriptions of the hydrophobic characteristics of microbial surfaces (Gaboriaud et al., 2006, 2008; Poortinga et al., 2002; Salerno et al., 2004). Actually, this physical picture of long-range hydrophobic interactions originates from direct force measurements (mostly obtained with surface force apparatus) between hydrophobic model surfaces with reported interaction ranges between 10 nm and values as large as 300 nm (Israelachvili, 1992). The controversy about the physical origin of hydrophobic interactions has been debated in the literature over the past 70 years for model systems (see the recent review by Meyer et al., 2006). Recent significant progress has been made in understanding hydrophobic interactions with the conclusion that only the short range part of the attraction (<10 nm) represents the true hydrophobic interaction. This result corroborates the arguments proposed by Gaboriaud et al. (2006, 2008) and Salerno et al. (2004) to dismiss the extended DLVO approach and the associated macroscopic methods employed for describing the hydrophobic characteristics of bacterial surfaces. These methods and analysis considerably underevaluate the role of electrostatic interactions in mediating the approach of an organism to a given substrate to be colonized (Gaboriaud et al., 2008).

The emergence of new experimental methods, such as force spectroscopy and advanced soft particle electrokinetic analysis, allow for investigating bacterial organisms in liquid media on the micro- and nanoscales (Dague et al., 2006, 2007; Dufrêne, 2008; Duval et al., 2005a; Duval and Ohshima, 2006;

Gaboriaud et al., 2008; Gaboriaud and Dufrêne, 2007). In particular, progress has been done to quantify the coupled electrostatic and hydrodynamic (permeability) properties of bacterial cells from the recent theoretical concepts describing the electrokinetic behavior of a soft (bio)particle (for a review see Duval, 2007). Such approach is particularly relevant for analyzing rapidly the main component of the interfacial features of bacteria, as demonstrated in a recent study by combining force spectroscopy (a local method) and advanced soft particle electrokinetic analysis (Gaboriaud et al., 2008).

In this study, *Campylobacter jejuni* and *Mycobacterium avium* were selected as bacterial models because they are a matter of serious concern for public health. Indeed, *Campylobacter* infections constitute one of the common causes of water-borne acute gastroenteritis (Allos, 2001; Hänninen et al., 2003; Tauxe, 2002). Its survival in water is depending at least on temperature, biodegradable matter, and strain origin (Guillou et al., 2008; Tatchou-Nyamsi-König et al., 2007). *M. avium* is one of the most important opportunistic pathogen that evolves in a wide variety of environmental reservoirs including natural and municipal waters, soils, aerosols, and protozoa (Primm et al., 2004). Moreover, the structures of the envelopes of these two bacteria are known to be very different. *C. jejuni* has a Gram negative wall surrounded by a polysaccharide capsule (Karlyshev et al., 2001) of which the effect on adhesion to abiotic material is not well documented. *Mycobacterium* are classified as Gram positive bacterium but are unusual in having a thick layer of peptidoglycan linked to a D-arabino-galactan which in turn is esterified by long-chain fatty acids, called mycolic acids. The glycopeptidolipids (GPL), predominant constituents of the cell wall of *Mycobacterium*, are recognized to mediate the initial attachment of *Mycobacterium* to abiotic surfaces (Freeman et al., 2006; Recht and Kolter, 2001). The hydrophobic ends of GPL and mycolic acids may form a hydrophobic monolayer around the bacterial cell (Chatterjee and Khoo, 2001; David et al., 1987) at the origin of the remarkable adhesive properties of *Mycobacterium* (Alsteens et al., 2007; Bendinger et al., 1993; Dague et al., 2007) onto a number of substrates. In the case of *M. avium*, particular GPL structures with oligosaccharide side chains are reported but the expression, the surface density or the three-dimensional structure of the bacterial interfaces should vary significantly within a strain (Chatterjee and Khoo, 2001; David et al., 1987).

As it is recognized that ionic strength (IS) and chemistry composition of the aqueous solution may influence the extent of bacterial adhesion to a given surface, we used two groundwaters (A and B) naturally mineralized and which differed according to both ionic composition and IS ($A = 11 \text{ mmol l}^{-1}$; and $B = 2.76 \text{ mmol l}^{-1}$).

To our knowledge, no study has focused so far on the evaluation of the adhesive properties of *Campylobacter* and *Mycobacterium* with respect to polyethylene terephthalate (PET), a polymer widely used in bottled water industry. The main objectives of our study were both to assess and explain the adhesion behavior of these two bacterial models onto PET surface. To reach this goal, adhesion assays were carried out using PET bottles containing filtered groundwaters A or B, and spiked with bacterial suspension (*C. jejuni* or *M. avium*), the adhered bacteria being then enumerated after few hours of contact. PET surface properties (surface charge, hydrophobic

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