

Microbial Interactions in Drinking Water Biofilms

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Drinking water distribution networks may be viewed as a large reactor where a number of chemical and microbiological processes are taking place. Control of microbial growth in drinking water distribution systems (DWDS) often achieved through the addition of disinfectants, is essential to limit the spread of waterborne pathogens. However, microorganisms can resist disinfection through protection within biofilms and resistant host cells. Recent studies into the microbial ecology of DWDS have found that microbial resistance to disinfectants is affected by microbial community diversity and interspecies relationships. The dynamics of the microbial growth and multispecies biofilm formation in drinking water networks is very complex, as a large number of interacting processes are involved. Coaggregation/coadhesion of microorganisms and other interspecies relationships are processes that are believed to play a significant role in the formation of single and multispecies biofilms in drinking water distribution systems, but remain poorly understood. Coaggregation is a process by which genetically distinct microorganisms become attached to one another via specific molecules and cumulative evidences suggest that such cell-cell adhesion influences the development of complex multispecies biofilms since aggregation conveys advantages to microorganisms. The purpose of this review is to gain deeper insights into the fundamental mechanisms of biofilm formation and population dynamics in DWDS.

Biofilms in Drinking Water Distribution Systems

Many problems in drinking water distribution systems (DWDS) are microbial in nature, including biofilm growth, nitrification, microbially mediated corrosion, and the occurrence and persistence of pathogens (Regan *et al.* 2003; Beech and Sunner 2004; Camper 2004; Emtiazi *et al.* 2004). Biofilms are suspected to be the primary source of microorganisms in DWDS that are fed with treated water and have no pipeline breaches, and are of particular concern in older DWDS (LeChevalier *et al.* 1987). By adopting this sessile mode of life, biofilm-embedded microorganisms enjoy a number of advantages over their planktonic counterparts. One advantage is the ability of the extracellular polymeric matrix, they excrete, to capture and concentrate a number of environmental nutrients, such as carbon, nitrogen and phosphate (Simões *et al.* 2006). Another advantage to the biofilm mode of growth is that it enables resistance to a number of

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removal strategies, such as antimicrobial and antifouling agent removal and shear stress (Simões *et al.* 2005a, b). DWDS disinfection with chlorine dioxide and chlorine, for example, can reduce the concentration of planktonic bacteria, but have little to no effect on the concentration of biofilm bacteria (Gagnon *et al.* 2005). This inherent resistance to antimicrobial factors is mediated through very low metabolic levels and drastically downregulated rates of cell division of the deeply embedded microorganisms. Furthermore, biofilms act as a reaction:diffusion barrier, slowing down the penetration, to some antimicrobial agents (Simões *et al.* 2007b). The last advantage to the biofilm mode of growth is the potential for dispersion *via* detachment. Under the direction of fluid flow, detached microorganisms travel to other regions to attach and promote biofilm formation on clean areas (Codony *et al.* 2005). Therefore, this advantage allows a persistent bacterial source population that is resistant to antimicrobial agents, while at the same time enabling continuous shedding to promote bacterial spread.

The current knowledge of the structure and activities in biofilm communities still is limited, because analysis of microbial physiology and genetics have been largely confined to studies of microorganisms from few lineages for which cultivation conditions have been determined and for some process conditions, not mimicking real environments. The dynamics of the microbial growth in drinking water networks is very complex, as a large number of interacting processes are involved. Drinking water pipe inner-surfaces are invariably colonized by biofilm, regardless of the presence of a disinfectant residual. In addition to the possibility of causing corrosion, taste and odour problems, biofilms control the microbiological contents of the distributed water and are a potential source of pathogens (Percival and Walker 1999; Szewzyk *et al.* 2000). The interaction of pathogens with biofilms has predominantly been a concern in man made water systems, particularly drinking water distribution systems. In fact, biofilms formed within potable-water systems contain bacterial pathogens such as *Legionella pneumophila* and coliforms of intestinal and nonintestinal origin (World Health Organization 1993). Furthermore, protozoa are commonly found within water distribution systems and have been associated with the persistence and invasiveness of pathogens (Tyndall and Domingue 1982). Such findings implicate the importance of maintaining a continuous disinfectant residual in DWDS.

Parameters Affecting Biofilm Formation

There exist a number of mechanisms by which numbers of species of microorganisms are able to come into closer contact with a surface, attach firmly to it, promote cell-cell interactions and grow as a complex structure. The attachment of microorganisms to surfaces is a very complex process, with many variables affecting the process. In general, attachment will occur most readily on surfaces that are rougher, more hydrophobic, and coated by surface condi-

tioning films (Donlan 2002, Simões *et al.* 2007a). An increase in flow velocity, water temperature, or nutrient concentration may also encourage attachment, if these factors do not exceed critical levels (Vieira *et al.* 1993; Simões *et al.* 2007b). Properties of the cell surface, especially the presence of extracellular appendages, the interactions involved in cell-to-cell communication and the production by the microorganisms of extracellular polymeric substances, are important and may possibly provide a competitive advantage for one microorganism where a mixed community is involved (Donlan 2002). Cells within biofilms are surrounded by extracellular polymeric substances (EPS), protecting the cells from predation, disinfectants and other stress factors (Stoodley *et al.* 2002). A biofilm will be greatly influenced by the chemical composition, structure and physical properties of the extracellular polymers, in addition to their role as receptors to adhesins, EPS produced by one species may alter the substratum properties and indirectly affect the adhesion of another species (Donlan 2002). Furthermore, microbial surface properties and structure, coaggregation/coadhesion of microorganisms and other interspecies relationships are processes that are believed to play a significant role in the formation of single and multispecies biofilms in DWDS, remaining poorly understood. Table I summarizes the main variables involved in cell attachment and biofilm formation.

Table I Variables important in cell attachment, biofilm formation and development (based on Donlan, 2002)

Adhesion surface	Bulk fluid	Cell
	Flow velocity	
	pH	Cell surface hydrophobicity
Texture or roughness	Temperature	Extracellular appendages
Hydrophobicity	Cations	Extracellular polymeric substances
Conditioning film	Presence of residual disinfectants	Signalling molecules
	Nutrient availability	

Microbial Interactions in Biofilms

Under natural conditions, true monospecies biofilms are rare and in most natural and industrial environments, biofilms are complex communities. Diversity in microbial communities leads to a variety of complex relationships involving interspecies and intraspecies interactions. Interactions among bacterial species may have a profound influence on the initial stages of biofilm formation and development. The conventional analyses of microorganisms in drinking

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water systems include plate counting, microbial biomass determination, and crude microbial metabolic measurement. Unfortunately, those traditional techniques do not give information on the microbial ecology of DWDS (Keinänen *et al.* 2004) and are unlikely to provide further evidence that can contribute to the development of effective biofilm control strategies. The ecology of a biofilm is a complex function of prevailing growth conditions, hydrodynamic forces, presence of microbial metabolites and molecules (cell-to-cell signalling communications) excreted by the microorganisms and dominant microbial inhabitants in the biofilm (Bryers and Ratner 2004).

Surfaces provide a niche that promotes the evolution of complex interactions between bacterial cells. Once cells are firmly bound, the activity of the community is dependent on the metabolism and growth of each member species under local surface conditions. Such metabolic activities can include substrate consumption, cellular growth and replication, and synthesis of extracellular polymeric substances (Bryers and Ratner 2004). The biological complexity of a system is defined by intra as well as interpopulation cell behaviour. The metabolic activities of those microorganisms that become associated with a surface cause these interfacial chemical gradients to evolve over time and space, creating conditions not normally encountered in the bulk aqueous phase (Geesey 2001).

The microbial heterogeneity found in drinking water and the existence of interspecies relationships can provide improved strategies for microbial growth control (Rasmussen *et al.* 2005). Competition for substrate is considered to be one of the major evolutionary driving forces in the microbial world, and experimental data obtained in laboratory conditions showed how different microorganisms may effectively outcompete others because of better utilization of a given energy source (Møller *et al.* 1998; Christensen *et al.* 2002). Central to the structure, composition and function of any community is a complex set of interactions (Hansen *et al.* 2007). For instance, Hansen *et al.* (2007) found that spatial structure was the key environmental factor for *Pseudomonas putida* KT2440 and *Acinetobacter* sp. strain C6 to establish a structured community for interspecies interactions. Previously, Møller *et al.* (1998) showed the metabolic synergy between *P. putida* and *Acinetobacter* sp. community members when biodegrading toluene and related aromatic compounds. There is evidence that biofilm community diversity can affect disinfection efficacy and pathogen survival within biofilms (Burmølle *et al.* 2006). Most research into interspecies interactions within biofilms has focused on the beneficial aspects of these relationships. However, not all interactions will be beneficial, since antagonistic interactions may play an important role in the development of microbial communities. The production of antimicrobial compounds, including toxins, bacteriolytic enzymes, bacteriophages, antibiotics and bacteriocins seems to be a generic phenomenon for most bacteria (Riley

1998; Tait and Sutherland 2002). Table 2 shows relevant interactions found for several multispecies biofilms from diverse environments.

Table 2 Relevant interspecies interactions in biofilm communities

Interspecies interactions	Strains	Reference
Antagonism	Marine epiphytic bacteria	Burgess <i>et al.</i> (1999)
	Enteric bacteria	Tait and Sutherland (2002)
Commensalism	<i>Acinetobacter</i> sp./ <i>Pseudomonas putida</i>	Christensen <i>et al.</i> (2002)
	<i>Lactococcus lactis</i> ssp. <i>cremoris</i> / <i>Pseudomonas fluorescens</i>	Kives <i>et al.</i> (2005)
Competition	<i>Acinetobacter</i> sp./ <i>Pseudomonas putida</i>	Christensen <i>et al.</i> (2002)
	<i>Klebsiella oxytoca</i> / <i>Burkholderia cepacia</i>	Komlos <i>et al.</i> (2005)
Mutualism (protooperation and symbiose)	Soil bacteria	Wolfaardt <i>et al.</i> (1994)
	Oral bacteria	Palmer <i>et al.</i> (2001)
	Marine epiphytic bacteria	Burmølle <i>et al.</i> (2006)
Neutralism	<i>Pseudomonas</i> sp.; <i>Corynebacterium</i> sp.; <i>Candida</i> sp.; <i>Schizosaccharomyces</i> sp.; <i>Saccharomyces</i> sp.; <i>Schizosaccharomyces</i> sp.	Yu <i>et al.</i> (2002)

Coaggregation

Coaggregation, the specific recognition and adherence of genetically distinct bacteria to one another, occurs in a variety of ecosystems (Kolenbrander 2000;

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Malik *et al.* 2003; Rickard *et al.* 2003a) and was first demonstrated for bacteria from dental plaque (Gibbons and Nygaard 1970), where both intergeneric and intrageneric coaggregation occurs (Kolenbrander *et al.* 1999). However, coaggregation is a widespread phenomenon has now been observed amongst bacteria from other biofilm communities in several diverse habitats. More recently, a few reports on the coaggregation abilities of freshwater biofilm bacteria have also been published (Buswell *et al.* 1997; Rickard *et al.* 2000; 2002; 2003a; 2004) and it has been suggested that coaggregation may also mediate in the sequential integration of species of bacteria into freshwater biofilms (Handley *et al.* 2001; Rickard *et al.* 1999). This mechanism of adhesion is highly specific and is thought to have a role in the development of multispecies biofilms in many different environments (Kolenbrander and London 1993; Kolenbrander *et al.* 1999; Rickard *et al.* 2003b) and now recognized as a mechanism for allowing specific association between collaborating bacterial species. Figure 1 shows scanning electron microscopy (SEM) photomicrographs of intergeneric coaggregation between *Acinetobacter calcoaceticus*-*Burkholderia cepacia* (Fig. 1a) and *Acinetobacter calcoaceticus*-*Staphylococcus* sp. (Fig. 1b) three bacteria isolated from a DWDS.

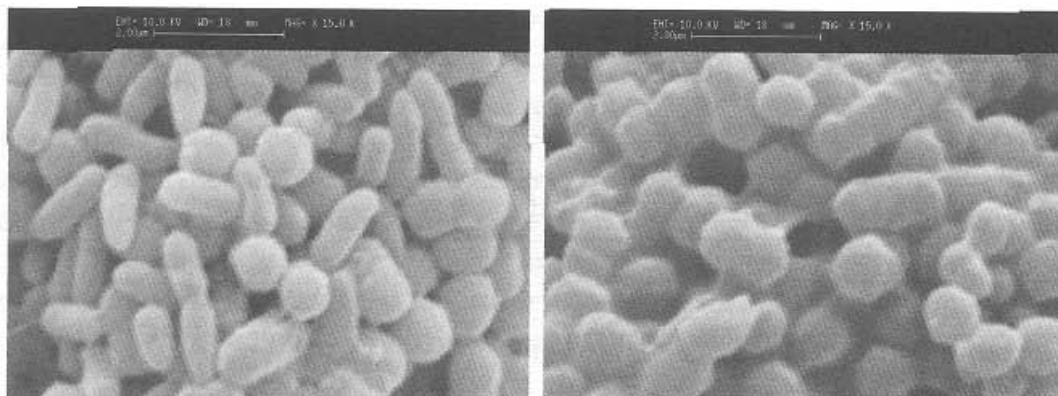


Figure 1 SEM microphotographs of *Acinetobacter calcoaceticus*-*Burkholderia cepacia* (a) and *Acinetobacter calcoaceticus*- *Staphylococcus* sp. (b). X 15000 magnification, bar = 2 μ m.

Aggregation conveys advantages to microorganisms. These include transfer of chemical signals, exchange of genetic information, protection from adverse environmental conditions, metabolic cooperation between different species, as well as cell differentiation in some populations.

Coaggregation interactions contribute to the development of biofilms by two routes. The first route is by single cells in suspension specifically recognizing and adhering to genetically distinct cells in the developing biofilm. The second is by the prior coaggregation in suspension of secondary colonizers followed by the subsequent adhesion of this coaggregate to the developing biofilm (Rickard *et al.* 2003b). In both cases, bacterial cells in suspension specifically adhere to biofilm cells in a process known as coadhesion (Bos *et al.* 1994; Busscher *et al.*

1995).

The coaggregation between pairs of freshwater bacteria is typically mediated by a protein “adhesin” on one cell type and a complementary saccharide “receptor” on the other. These protein-saccharide interactions could be blocked by the addition of simple sugars. Thus, the mechanism mediating adhesion between coaggregating pairs in freshwater biofilm bacteria is very similar to the one verified by oral bacteria.

The coaggregation between freshwater bacteria is growth-phase-dependent and depends on cells being in the optimum physiological state for coaggregation, being maximum when both partner bacteria are in stationary phase. Maximum expression of coaggregation generates clearly visible flocs of cells in mixtures of the two cell types (Rickard *et al.* 1999) and is maintained for up to 48 h into stationary phase, depending on the coaggregating pair. The ability to coaggregate then decreases and eventually is lost completely (Rickard *et al.* 2000). The optimum coaggregation between a pair might be dependent upon a change in coaggregation ability of one or both partner bacteria. As the adhesion on one bacterium and the receptor on the other partner bacteria may not be expressed simultaneously in batch culture.

Studies on freshwater biofilm bacteria have also demonstrated that coaggregation often occurs between bacteria that are taxonomically distant (intergeneric coaggregation) and occasionally between strains belonging to the same species (intraspecies coaggregation) (Buswell *et al.* 1997; Rickard *et al.* 2002). Intergeneric coaggregation is common between oral bacteria (Kolenbrander and London 1993), but intraspecies coaggregation has not yet been described between oral plaque bacteria. Thus, intraspecies coaggregation may well be a characteristic that is unique to freshwater biofilm bacteria. Moreover, and as suggested by Malik *et al.* (2003), the bacterial cell surface properties, namely the hydrophobicity, are other factors thought to play an important role in coaggregation, as well as in cell-substratum interactions. In conclusion, bacteria are affected by the environment they live in and the variety of other species present. The development of a multipopulation model of drinking water biofilms that take into account the effects of disinfectants on microbial ecology will help to determine optimal operational parameters and lead to knowledgeable decisions regarding the management of drinking water supply. Coaggregation can take the form of intra, inter or multigeneric interactions, a combination of which contributes to the overall structure and diversity of the bacterial community in the freshwater biofilms. The specific mechanism for this remains unknown, but a more complete picture of microbial community diversity and interspecies relationships should facilitate a better understanding of disinfection resistance phenomena and will provide new data to design innovative and effective control strategies that will guarantee microbio-

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logically safe and high quality drinking water.

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