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Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods

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ABSTRACT

Attachment of potential spoilage and pathogenic bacteria to food contact surfaces and the subsequent biofilm formation represent serious challenges to the meat industry, since these may lead to cross-contamination of the products, resulting in lowered-shelf life and transmission of diseases. In meat processing environments, microorganisms are sometimes associated to surfaces in complex multispecies communities, while bacterial interactions have been shown to play a key role in cell attachment and detachment from biofilms, as well as in the resistance of biofilm community members against antimicrobial treatments. Disinfection of food contact surfaces in such environments is a challenging task, aggravated by the great antimicrobial resistance of biofilm associated bacteria. In recent years, several alternative novel methods, such as essential oils and bacteriophages, have been successfully tested as an alternative means for the disinfection of microbial-contaminated food contact surfaces. In this review, all these aspects of biofilm formation in meat processing environments are discussed from a microbial meat-quality and safety perspective.

1. Introduction

During the last decades, it has become increasingly clear that bacteria, including foodborne pathogens such as *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*, together with common meat spoilage bacteria, such as *Pseudomonas* spp., *Brochothrix thermosphacta* and *Lactobacillus* spp. grow predominantly as biofilms on surfaces, in most of their habitats, rather than in planktonic mode (Frank, 2001; Lindsay & von Holy, 2006). A biofilm can be broadly defined as a microbially derived sessile community characterized by cells that are attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (Donlan & Costerton, 2002; Lazazzera, 2005).

Interestingly, it has been observed that the resistance of biofilm cells to antimicrobials is significantly increased compared with what is normally seen with the same cells being planktonic (Costerton, Stewart, & Greenberg, 1999; Gilbert, Allison, & McBain, 2002; Mah & O'Toole, 2001). Thus, it is believed that biofilm formation enhances the capacity of foodborne bacteria to survive stresses that are commonly encountered within food processing (e.g. refrigeration, acidity, salinity, disinfection) (Brooks & Flint, 2008; Giaouris, Chorianopoulos, Skandamis, & Nychas, 2012; Kumar & Anand, 1998; Møretrø & Langsrud, 2004). In the meat industry, biofilms formed by pathogenic and spoilage bacteria may create a persistent source of product contamination, leading to serious hygienic problems and also economic losses due to food spoilage (Jessen & Lammert, 2003; Sofos & Geornaras, 2010). While food spoilage and deterioration may result in huge economic losses, food safety is a major priority in today's globalizing market with worldwide transportation and consumption of raw, fresh and minimally processed foods (Shi & Zhu, 2009). However, it should also be noted that in the industry of fermented meat products (e.g. traditional sausages), biofilm formation by some useful (safe technological) bacteria (e.g. staphylococci, lactobacilli) can be desirable, due to the possible enhancement of food fermentation, and more importantly as a mean of protection against the establishment of biofilms formed by undesirable spoilage and/or pathogenic bacteria (Chorianopoulos, Giaouris, Skandamis, Haroutounian, & Nychas, 2008;

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Dagher, Ragout, Siñeriz, & Bruno-Bárcena, 2010; Leriche & Carpentier, 2000; Leriche, Chassaing, & Carpentier, 1999; Leroy et al., 2009; Ndahetuye, Koo, O'Bryan, Ricke, & Crandall, 2012; Zhao et al., 2006).

In real food processing environments, biofilm communities may be inhabited by numerous different species in close proximity (Carpentier & Chassaing, 2004; Habimana, Heir, Langsrud, Asli, & Møretrø, 2010; Pan, Breidt, & Kathariou, 2009; Sanders, Boothe, Frank, & Arnold, 2007). Spatial and metabolic interactions between species contribute to the organization of multispecies biofilms, and the production of a dynamic local environment (Moons, Michiels, & Aertsen, 2009; Nadell, Xavier, & Foster, 2009; Tolker-Nielsen & Molin, 2000). Mixed-species biofilms are usually more stable than mono-species biofilms, while cell-to-cell interactions have been demonstrated to play a key role in biofilm formation, biofilm structure, as well as in the resistance of biofilm community members against antimicrobial treatments (Burmølle et al., 2006; Kostaki, Chorianopoulos, Braxou, Nychas, & Giaouris, 2012; Remis, Costerton, & Auer, 2010; Rieu, Lemaître, Guzzo, & Piveteau, 2008; Uhlich, Rogers, & Mosier, 2010; van der Veen & Abee, 2011).

Various approaches to inhibit biofilm development have been used for many years in the food industry. The focus has mostly been concentrated on the prevention of bacterial contamination by both physical and chemical intervention. However, concerns have been raised over both the effectiveness and safety of these approaches, which has resulted in the search, development and application of novel means for removing and/or inhibiting biofilm formation. Alternative biocides must be safe for the consumers and also harmless to the environment. Such an intriguing case are the essential oils (EOs) extracted from various herbs and spices, together with some EO components (Hyldgaard, Mygind, & Meyer, 2012; Nychas, Tassou, & Skandamis, 2003). Besides the well-established antimicrobial action of these compounds against planktonic microorganisms, in recent years this action was also confirmed against biofilm embedded microorganisms (Chorianopoulos et al., 2008; Desai, Soni, Nannapaneni, Schilling, & Silva, 2012; Jadhav, Shah, Bhave, & Palombo, 2013; Knowles, Roller, Murray, & Naidu, 2005; Kwiecińska, Eickb, & Wójcika, 2009; Laird, Armitage, & Phillips, 2012; Lebert, Leroy, & Talon, 2007; Niu & Gilbert, 2004; Nostro, Scaffaro, et al., 2012; Perez-Conesa, Cao, Chen, McLandsborough, & Weiss, 2011; Schillaci, Arizza, Dayton, Camarda, & Di Stefano, 2008). Apart from EOs, many other novel antimicrobial strategies, which also exhibit antibiofilm properties (e.g. enzymes, quorum sensing inhibitors, bacteriocins, phages, nanoemulsions, surfactants), have also been successfully investigated, in an effort to find effective alternatives for the control of biofilms.

The objective of this article is to provide an overview of the current knowledge related to bacterial attachment and biofilm formation in meat processing environments, to review available scientific data on the influence and impact of bacterial interactions on the establishment of mixed-culture food related biofilm communities, and finally, to provide up-to-date data on the efficient disinfection of biofilm communities using alternative novel methods. Experimental data regarding the detachment of cells from the biofilm structure and the subsequent cross-contamination of food products are also discussed.

2. Common molecular features and advantages of the biofilm phenotype

The molecular mechanisms by which bacteria are able to form biofilms in food processing plants are the subject of increasing interest in recent years and appear more complex than initially assumed (Hall-Stoodley, Costerton, & Stoodley, 2004; Kim & Wei, 2009; Smith, Fratamico, & Uhlich, 2009; Van Houdt & Michiels, 2010). While irreversible bacterial attachment constitutes the first step of biofilm formation, attached bacterial cells do not necessarily proceed into sessile development. From the initial interaction with a substratum to the subsequent sessile growth, significant changes in expression of many genes occur in the bacterial cells. Thus, high-throughput DNA microarray studies have been conducted to study biofilm formation in many model microorganisms and have identified a large number of genes showing differential expression under biofilm conditions (Beloin et al., 2004; Hamilton et al., 2009; Lazazzera, 2005; Shemesh, Tam, & Steinberg, 2007; Whiteley et al., 2001). Compared to planktonic growth, gene expression profile is different in biofilm cells and, most importantly, it not only depends on the temporal stage of biofilm development, but also on the spatial localization of the bacteria within the biofilm (McDougald, Rice, Barraud, Steinberg, & Kjelleberg, 2011; Stewart & Franklin, 2008).

Despite this physiological heterogeneity, some common molecular features have been uncovered for biofilm formation (Lasa, 2006), namely: (i) cell-to-cell communication (including quorum sensing) involving signaling molecules, essentially acyl-homoserine lactone (AHL), oligopeptide and/or furanone autoinducers (AIs) (Renier, Hébraud, & Desvaux, 2011; Schauder & Bassler, 2001; Skandamis & Nychas, 2012; Waters & Bassler, 2005), (ii) nucleotide second messengers, such as the allosteric regulation by c-di-GMP, which metabolism involves proteins with GGDEF/EAL domains, or (p)ppGpp (Cotter & Stibitz, 2007; Kalia et al., 2012; Potrykus & Cashel, 2008; Schirmer & Jenal, 2009), (iii) proteins exposed on the outer bacterial cell surface, namely Bap (biofilm-associated protein) family, and other surface appendages, such as flagella and various types of pili (including fimbriae and curli) (Lasa & Penadés, 2006; Latasa, Solano, Penadés, & Lasa, 2006; Van Houdt & Michiels, 2010), and (iv) exopolymers ranging from exopolysaccharides (e.g. LPS — lipopolysaccharide-, PIA — polysaccharide intercellular adhesin — also called PNAG — poly-β-1,6-linked N-acetylglucosamine-, glucose-rich Pel — pellicle-, or cellulose), alginate, colanic acid, polyglutamate, to extracellular DNA (eDNA) (Candela & Fouet, 2006; Montanaro et al., 2011; Ryder, Byrd, & Wozniak, 2007; Solano et al., 2002).

From a bacterial cell point of view, biofilm formation provides numerous advantages, which in turn can be problematic for the meat industry to maintain food quality and safety. Bacterial biofilm formation could be driven by at least four interconnected teleonomic values: (i) protection from stressful/harmful environmental conditions, by providing a certain degree of shelter and homeostasis, (ii) competition for and appropriation of available nutrients in a delimited area, (iii) benefits of metabolic interactions between microbial species from commensalism, cooperation to mutualism, and (iv) gene transfer enabling acquisition of new adaptative phenotypic traits (Davey & O' Toole, 2000; Molin & Tolker-Nielsen, 2003). Apparently, the most important characteristic of biofilm cells for the meat industry is their increased resistance to to sanitizing treatments (e.g. disinfection procedures), compared to planktonic cells. One might discriminate at least four mechanisms, which can be combined, to explain this increased resistance (Donlan & Costerton, 2002; Gilbert et al., 2002): (i) the exopolymeric matrix forming a physical barrier that limits the diffusion of sanitizers within the biofilm, (ii) the resistance mechanisms (e.g. detoxifying membrane transporters), which can even be encoded on a plasmid and can be horizontally transferred among biofilm cells, (iii) the differentiation of bacterial cells into different physiological states (e.g. dormant cells) less susceptible/receptive to treatments, and (iv) the modification of the microenvironment (e.g. local acidic pH) rending a particular sanitizer less efficient.

3. Attachment and biofilm formation by foodborne bacteria in meat processing environments

The ability of bacteria to attach to abiotic surfaces and form biofilms is a cause of concern for many food industries, including those occupied with meat production and processing (Chmielewski & Frank, 2003). In such environments, inadequately cleaned surfaces promote soil build-up, and, in the presence of water, contribute to the development of bacterial biofilms. The adhesive properties of fimbriae salmonellae