General aspects of biofilm development and implication in staphylococcal ruminant mastitis: a current literature review

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Biofilm as a survival mechanism

A characteristic of bacteria is the capacity to grow in association with a surface forming complex communities. In the recent decades, these bacterial aggregations have been described as “biofilms”. Biofilms can be defined broadly as a dynamic and well structured microbial community, attached to a solid surface and aggregated by an extracellular matrix. The ability to form biofilm is a widespread feature among prokaryotes (both in the archaeal and bacterial domain) and it has been found in fossil formations dating back 3.2 billion years. From an evolutionary standpoint, the formation of biofilm probably conferred an adaptive advantage by providing homeostasis against extreme conditions and fluctuations of the primitive earth (temperatures, osmotic pressure, pH and exposure to UV radiation). In addition to offering protection from physical and chemical environmental factors, biofilm protects bacteria from being washed or scraped away from aquatic systems, facilitates extracellular catalytic functions (because cells remain close to each other) and promotes the concentration of nutrients on the surface (Hall-Stoodley et al., 2004). Moreover, biofilms are resistant to antimicrobial agents (e.g., antibiotics), what may be due to difficulty in penetration of the antimicrobial agent through the extracellular matrix, to the decreased growth rate of biofilm cells (β-lactam antibiotics are effective in Gram-positive cells that are actively dividing) or the existence of resistant phenotypes among a genetically heterogeneous population.

Biofilm in natural environments and its implication in infections

Biofilm formation is ubiquitous in natural environments. These types of biological structures are found at the bottom of rivers or on the surface of stagnant water; in extreme environments, from hot springs to glaciers in the Antarctic; in showers or bathtubs, favoured by the warm moist environment; inside water ducts or industrial gas and oil pipes; in symbiosis with plants, etc. Moreover, biofilm formation is implicated in the pathogenesis of many human (Hall-Stoodley et al., 2004) and veterinary (Jacques et al., 2010) infections. According to the National Institutes of Health (NIH), biofilm is involved in 80% of bacterial infections (Joo and Otto, 2012). The adhesion of Staphylococcus or Streptococcus to the proteins of the basal membrane of the damaged heart epithelium is a cause of endocarditis. In the case of cystic fibrosis patients, decreased ciliary activity of the respiratory mucosa and mucus hypersecretion promote colonization and biofilm formation by Staphylococcus aureus, Haemophilus influenzae and Pseudomonas aeruginosa. Another well known example of biofilm is the subgingival plaque of Streptococcus mutans. Biofilm formation has also been described in opportunistic strains of Escherichia coli.

In a second step (maturation), adhered bacterial cells multiply and produce an extracellular matrix. The function of the matrix is to provide adhesion between bacterial cells, enabling the accumulation of layers that constitute the biofilm. Adhesive components of the extracellular matrix include exopolysaccharides, proteins and extracellular DNA (released as a consequence of cell lysis). The main constituent of the S. aureus and S. epidermidis extracellular matrix, responsible for the intercellular interactions, is the exopolysaccharide polysaccharide intercellular adhesin (PiA) (Tortorici et al., 2016), synthesized by enzymes encoded in the piaABC operon (Drangsholt et al., 1999). De-acetylation of PiA polysaccharide matrix components (e.g., DNA) or cell wall components (teichoic acids), resulting in a tightly connected matrix network. Likewise, some S. aureus surface proteins have been described that can be involved in intercellular interaction and biofilm formation: biofilm-associated protein (Bap) (Cucarella et al., 2001), protein A (Merino et al., 2009), S. aureus surface proteins C and G (SasC, SasG) (Schroeder et al., 2009) (Corrigan et al., 2007), fibronectin binding proteins (FnBPA, FnBPB) (O’Neill et al., 2008), extracellular matrix protein (Aap) (Conrady et al., 2008). In addition to the intercellular interactions that link bacteria together, biofilm maturation comprises disruptive processes that form channels in the biofilm structure in which nutrients can circulate and reach cells in deeper layers. Enzymatic degradation by proteases and nucleases may have a role in this kind of biofilm structuring. Moreover, a family of peptides with surfactant properties, the phenol-soluble modulins (PSMs), is involved in disruption of noncovalent interactions between biofilm cells and matrix molecules.

Biofilm infection is a complex process, involving the interaction of bacterial and host factors. In biofilm infections, bacteria escape the host’s immune system by forming a biofilm, which protects against host defenses and can lead to chronic infections and antibiotic resistance. The expression of virulence factors is regulated by quorum sensing, a regulatory mechanism that allows bacteria to communicate and coordinate their behavior in response to changes in cell density and environmental conditions. In the case of S. aureus, the agr operon plays a key role in regulating the expression of virulence factors such as PSMs, fibronectin binding proteins, and collagen binding protein Cna, which contribute to biofilm formation and pathogenicity. In addition, biofilm formation is implicated in the pathogenesis of many human and veterinary infections, including infections of medical devices, central venous catheters, orthopedic implants, and urinary tract infections. Moreover, biofilm formation is associated with antibiotic resistance and chronic infections, making the treatment of biofilm infections challenging. The development of new diagnostic tools and therapeutic strategies is essential to combat the increasing prevalence and severity of biofilm infections.
Various studies demonstrate the presence of the icaADBC operon, which encodes the enzymes responsible for the biosynthesis of the PNAQ exopolysaccharide, the main component of the extracellular matrix of the biofilm, in 94.36% (Cucunurca et al., 2004) or 100% (Vásquez et al., 2003) of S. aureus isolated from bovine mastitis. Apart from this genetic propensity, a number of studies have also demonstrated the ability of bovine mastitis isolates to form biofilm in vitro. In this regard, Vázquez et al. (2003) studied 91% of isolates of S. aureus from bovine mastitis that had the ability to form biofilm in vitro by determining the formation of the extracellular matrix of bovine mastitis in vitro as producers of extracellular matrix of biofilm. Dharawade et al. (2010) found that 48.03% of the strains of S. aureus isolated from bovine mastitis had the ability to form biofilm in vitro in the culture test on agar plates with Congo red. Recently, Béault et al. (2013) published that biofilm formation ability was present in all the methicillin-resistant S. aureus (MRSA) isolates analyzed from bovine mastitis in Belgium. Moreover, the biofilm formation capacity of MRSA, encoding mecA or mecC, isolated from bulk tank milk in Great Britain was characterized and we observed that all the strains analyzed were PDR positive for the ica genes and 50% produced biofilm in the microplate assay. This is also the first demonstration of biofilm production by mecC MRSA (Prenafeta et al., 2014). The emergence of MRSA in cattle could imply a reduction of effective antibacterial treatments. Moreover, a high prevalence of biofilm producing MRSA isolates could promote the chronicity of bovine mastitis, with the consequence of persistent bacterial infection and increased shedding and spread from infected animals including potential zoonotic transmission.

While the genetic capacity and in vitro biofilm production in S. aureus isolates from bovine mastitis seems clear, there is some evidence demonstrating the in vivo biofilm formation of S. aureus in the mammary gland. Watson et al. (1998) observed by electron microscopy the production of a polysaccharide matrix called pseudosacculus by the authors in S. aureus cells isolated directly from the milk of sheep and cows with clinical mastitis. Shortly afterwards, Begaña et al. (1993) demonstrated the production of an exopolysaccharide matrix in S. aureus cells by immunocytochemical analysis of mammary gland parenchymal tissue samples from sheep experimentally infected with S. aureus by intramammary route. In the in vivo exopolysaccharide expression has also been shown indirectly by observing the production of specific antibodies against PNAQ and against Some Associated Antigens-Complex (SAAC) in sheep and cows, respectively, experimentally infected with S. aureus by intramammary route (Perez et al., 2009; Prenafeta et al., 2010).

Vaccines against the biofilm of S. aureus to combat mastitis in ruminants

Given that biofilm formation is an important virulence factor of S. aureus in the pathogenesis of mastitis in sheep and cows, the efficacy of different experimental vaccines has been tested, with various levels of protection demonstrated. Watson et al. (1993) and Northcut et al. (1994) used vaccines based on whole S. aureus inactivated cells embedded in their own extracellular matrix called pseudosacculus. The experimental vaccines in a study by Arner and et al. (1994) consisted of a mixture of slime (biofilm exopolysaccharide matrix) in liposomes, formalin and various inactivated S. aureus isolates. More recently, knowing that the PNAQ exopolysaccharide is the major component of the extracellular matrix of the S. aureus biofilm, Prenafeta et al. (2009), conducted an efficacy trial against an intramammary challenge with a virulent S. aureus strain in sheep. When using bacteria (whole and inactivated bacterial cells), crude extract, or purified PNAQ, with different adjuvants as a vaccines. The results of this study showed that bacterins from strong biofilm-producing bacteria induced the highest titres of specific antibodies to PNAQ and conferred the expected protection against an intramammary challenge, compared to vaccines containing bacteria from weak biofilm-producing bacteria, crude extract or purified PNAQ. The study by Prenafeta et al. (2010) clarifies the role of SAAC-associated antigens in protecting the mastitis caused by S. aureus in an experimental infection in cows. SAAC is an isolated cell fraction from S. aureus strains that produce biofilm. The presence of this extracellular component has been determined for all isolates of S. aureus characterized as slime producers in agar plates with Congo red (Figure 2) and its production is directly related to the in vitro biofilm formation (Table 1). The chemical and immunological characterization showed that the SAAC is comprised of deacylated form of PNAQ. It is noteworthy that antibodies to deacylated form of PNAQ are those with the greatest capacity for opsonization (specific antibody binding to antigen) and protection against infection by S. aureus (Cerci et al., 2007).

Figure 2. Analysis by immunoelectrophoresis in agarose gel for bacterial extracts from a strain of S. aureus (red phase) or non-producing strain (blue phase) using a polyclonal antiserum against whole bacterial cells (blue line 2 and 3) and non-producing strain (blue line 1 and 2). The arrow shows the line of immunoprecipitation for the SAAC antigen, which is present only in strain characterized as exopolysaccharide producers in agar plates with Congo-red.