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Bacterial biofilm

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General features

Biofilms are a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (Costerton et al., 1999). This can constitute a protected niche that allows bacteria to grow and survive in a hostile environment, particularly in environments characterized by a continuous flow. When biofilms are formed in low shear environments, they are generally more sensitive to mechanical breakage. In addition to protection against physical and chemical environmental agents, the biofilm promotes extracellular catabolism and the concentration of nutrients on cell surface.

In most natural environments, microorganisms try to adhere to available surfaces. Hence, the free-swimming (planktonic) phase can be viewed as a bacterial dispersal from one surface to colonize another. Thus, the initial phase of biofilm formation involves two stages: the first one consists in attachment of cells to a surface, facilitated by cell wall associated adhesins, which are products of various genes (Mack, 1999). Attachment to native polymeric surfaces is increased in the presence of matrix proteins including fibronectin, and fibrinogen. Following initial attachment of cells to a surface, the primary cell aggregates produce exopolysaccharides to facilitate clumping. The second stage is characterized by cell multiplication and formation of a mature structure consisting of many layers of cells, connected each other by extracellular polysaccharides (Yarwood and Schlievert, 2003). Finally, in the process of maturation, many staphylococci generate a glycocalyx, a slime layer that further protects the biofilm bacteria. The chemical nature of these slime layers is still not entirely elucidated, but evidence suggests that it consists predominantly of hydrated polysaccharides.

The growth potential of any bacterial biofilm is limited by the availability of nutrients to the cells within the biofilm and distinct flow-through channels across the biofilm aim to maintain perfusion (Stoodley et al., 2002). Other factors that are known to control biofilm maturation include internal pH, oxygen perfusion, carbon source and osmolarity (Dunne, 2002). Biofilm lives a a dynamic equilibrium and when it reaches a critical mass the outermost cell layer begins to shed planktonic organisms. These bacteria are free to escape the biofilm and to colonize other surfaces (Dunne, 2002). The formation of biofilms is often involved in the pathogenesis of many human infections caused by various microrganisms such as staphylococci, streptococci, *Ps. areuginosa, Haem. influenzae*, in many urinary infections caused by *E. coli*, as well as in infections in case of use of prostheses and implants (Hall-Stoodley et al., 2004).

Action mechanisms

Biofilm production allows bacteria to resist to antibiotic therapy, ensures infection persistence and the resistance to host immunity.

Resistance to antimicrobial agents (e.g. antibiotics) of bacteria within biofilm seems to be related to several factors: a) increased difficulty of the antibiotic to penetrate through the extracellular matrix, b) a decrease in rate of cell division (β-lactam antibiotics are effective against Gram-positive bacteria in active multiplication), c) the presence of resistant phenotypes in a bacterial population genetically heterogeneous, d) greater resistance to phagocytosis (Costerton et al., 1999). Despite some studies have reported an unimpaired antimicrobial penetration (Anderl et al., 2003), to induce the production of beta-lactamases by bacteria established in the heart of a biofilm is necessary the exposure to a higher concentration of antibiotic than in bacteria in the peripheries of biofilm (Bagge et al., 2004). Biofilm penetration of positively charged aminoglycosides is retarded by binding to negatively charged matrices, such as alginate in *Pseudomonas aeruginosa* biofilms (Walters et al., 2003). Finally, biofilm from coagulase-negative staphylococci reduced the effect of glycopeptide antibiotics, even in planktonic bacterial cultures (König et al., 2001; Souli & Giamarellou, 1998).

Resistance to host immunity contribute to maintain persistent infections. Normally planktonic bacteria are able to stimulate the production of antibodies but these are not effective against bacteria into biofilm deeper layers and may cause immune complex damage to surrounding tissues (Cochrane et al., 1998).Even in non-immunosuppressed individuals, infections caused by biofilm-producing pathogens are rarely resolved by the host defense mechanisms (Khoury et al., 1992).

All these mechanisms allow several human and animal infections to become chronic. The specific mode of growth of biofilm through release of planktonic cells is particularly related to the capability to colonize new sites and perpetuate infections.

Staph. aureus biofilm

Staph. aureus represents a major agent of contagious bovine mastitis and its ability to form biofilm suggests that it is a possible important virulence factor in the establishment of staphylococcal infection (Costerton et al. 1999).

The main constituent of the extracellular matrix, responsible for intercellular

Staph. aureus interactions, is the exopolysaccharides poly-N-acetyl-β-1, 6 glucosamine (PNAG) synthesized by enzymes encoded from icaADBC operon. Some studies have found icaADBC operon, coding for the enzymes responsible for the biosynthesis of PNAG exopolysaccharides, in 94.36% (Cucarella et al., 2004) or in 100% (Vasudevan et al., 2003) strains of *Staph. aureus* isolated from bovine mastitis.

Besides this genetic trait, other studies have also shown a remarkable ability to produce biofilm in vitro by *Staph. aureus* isolated from cases of bovine mastitis (Vadusevan et al., 2003, Olivera et al., 2007).

The in vivo presence of the exopolysaccharides complex was also demonstrated indirectly by observing the production of specific antibodies against PNAG (Pérez et al., 2009) and SAAC (Slime Associated Antigenic Complex; Prenafeta et al., 2010) respectively in ewes and cows with experimentally induced *Staph. aureus* intramammary infections.

Vaccination against *Staph. aureus* intramammary infections

The attention paid to prevent antimicrobial resistance, particularly in meticillin-resistant *Staph. aureus* (MRSA), and a general trend, in the future, to reduce the use of antibiotics in livestock (FDA, 2010), explain the effort to develop new effective vaccines against bacterial infections.

Especially in the regards of *Staph. aureus* intramammary infections, several studies were performed to find an effective vaccine in order to decrease the spread of infection among and within herds. The targets in vaccination against mastitis are to obtain reduced inflammation at the site of injection, high efficiency against disease, a cost-efficient bacterial inoculum and an immunological parameter that could help to predict the success of vaccination (Pérez et al., 2009).

First study about vaccination against whole bacterial cells surrounded by their own biofilm matrix containing PNAG conferred protection against *Staph. aureus* infection and mastitis in a challenge study in sheep. The protection level was related to the features of the immunizing strain (degree of biofilm formation and PNAG production) and consequently to the rate of antibodies to *Staph. aureus* PNAG. Whereas of it was independent of the adjuvant and capsular polysaccharide type of the challenge strain (Pérez et al., 2009).

Further study by Prenafeta et al. in cattle (2010) has point out the active role of specific antibodies against SAAC. The immunogenicity of SAAC was demonstrated when this component was administered associated with the *Staph. aureus* bacterin in dairy heifers. Cows immunized with a greater amount of SAAC associated with the *Staph. aureus* bacterin triggered the highest SAAC-specific antibody levels in serum after vaccination. In conclusion, this study reports the immunogenicity of SAAC in dairy cows when this component is embedded in a *Staph. aureus* bacterin of a strong biofilm-producing strain and candidate it as an effective target for vaccination (Prenafeta et al.2010).

One of the benefit of using PNAG or SAAC, as antigenic component of the vaccine, is that no different serotypes have been highlighted of *Staph. aureus* in relation to the production of the two fractions mentioned above. Therefore, the antibodies induced by vaccination with these antigens give cross-protection against several strains of *Staph. aureus*.

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