General aspects of biofilm and its implication in ruminant mastitis

### 1. Biofilm as a survival mechanism

Biofilms can be defined broadly as a dynamic structure formed by a mixed microbial community attached to a surface and aggregated by an extracellular polymeric substance (EPS). Biofilms are found in various environments, including natural water systems, soil, the human body, and industrial settings. They provide a protective shield against environmental stresses and can facilitate infections.

### 2. Biofilm in natural environments and its implication in infections

Biofilm formation is ubiquitous in natural environments. The protective effects of biofilms can be manifold. For instance, they can:
- **Protect against host defenses**
- **Reduce the effectiveness of antimicrobial agents**
- **Facilitate infection**
- **Contribute to antibiotic resistance**

Biofilms are often associated with chronic infections, such as those caused by Staphylococcus aureus, which can lead to complications like endocarditis and osteomyelitis. Understanding the biology and mechanics of biofilm formation is crucial for developing strategies to combat these infections.
4. Implication of biofilm in ruminant mastitis caused by *S. aureus*

In bovine and ovine mastitis caused by *Staphylococcus aureus*, bacterial cells adhere to the mammary epithelial cells and grow into the duct system, typically forming a biofilm in the mammary gland. This biofilm formation is a multi-step process, which includes adhesion, proliferation, and colonization of bacterial cells. The biofilm is a complex matrix composed of extracellular polymeric substances (EPS), which are produced by the bacterial cells themselves. 

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

Given that biofilm formation is an important factor in the pathogenesis of mastitis, vaccines that target the biofilm have been developed. Vaccines against biofilm can be designed to target specific components of the biofilm matrix, such as extracellular polymeric substances (EPS). These vaccines can be used to induce an immune response against specific components of the biofilm, thereby preventing bacterial adhesion and colonization. 

### Table 1. Sensitivity to antibiotics of different bacterial genera

<table>
<thead>
<tr>
<th>Bacterial Genus</th>
<th>Sensitivity to Antibiotics</th>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Imipenem 1 (MIC) 1024</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Chloramphenicol 1 (MIC) 32</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Gentamicin 1 (MIC) 2</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Penicillin G 1 (MIC) 0.062</td>
</tr>
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### Table 2. Comparison of biofilm production by *Staphylococcus aureus* isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>OD at 600 nm</th>
<th>CFU/mL</th>
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<tbody>
<tr>
<td>S. aureus 1</td>
<td>0.89</td>
<td>10^7</td>
</tr>
<tr>
<td>S. aureus 2</td>
<td>0.75</td>
<td>10^6</td>
</tr>
<tr>
<td>S. aureus 3</td>
<td>0.63</td>
<td>10^5</td>
</tr>
</tbody>
</table>

### Figure 1. Biofilm formation by *Staphylococcus aureus* in vitro.

- The biofilm matrix is composed of extracellular polymeric substances (EPS), which are produced by the bacterial cells themselves. 
- The biofilm forms a protective layer around the bacterial cells, preventing antibiotic access and immune clearance.

### Figure 2. Detection of biofilm formation by *Staphylococcus aureus* isolates.

- Biofilm formation can be detected by measuring the optical density (OD) at 600 nm of the microplate wells.
- Isolates showing high biofilm production will have a high OD, whereas isolates with low biofilm production will have a low OD.

### Figure 3. Biofilm inhibition by vaccines.

- Vaccines can be used to inhibit biofilm formation by targeting specific components of the biofilm matrix.
- The vaccines can be formulated with adjuvants to enhance the immune response against the biofilm components.

### Figure 4. Biofilm inhibition in experimental trials.

- Clinical trials have shown that biofilm inhibition vaccines can significantly reduce the incidence of clinical mastitis in ruminants.
- Vaccination with biofilm inhibition vaccines can also reduce the severity of clinical symptoms and the duration of the disease.
In bovine and mastitis-related cases, biofilm formation is a well-characterized process that allows the bacteria to resist conventional antibiotics. This resistance is due to the presence of a well-defined barrier, the biofilm matrix, which is composed of extracellular polymeric substances that provide a physical barrier and an environment that is resistant to antibiotics.

3. Development of S. aureus biofilm

Biofilm formation by S. aureus is a well-characterized process that is critical for the pathogenesis of bovine and ovine mastitis. In this context, S. aureus is able to form biofilm structures in the mammary gland, which is a key step in the development of the disease. The biofilm matrix produced by S. aureus is composed of extracellular polymeric substances, which allow the bacteria to resist antibiotics and other antimicrobial agents.

4. Implication of biofilm in ruminant mastitis caused by S. aureus

The implication of biofilm in ruminant mastitis caused by S. aureus has been studied extensively. The biofilm matrix produced by S. aureus in the mammary gland provides a physical barrier that makes the bacteria resistant to antibiotics. This resistance is a key factor in the development of mastitis, as it allows the bacteria to persist in the mammary gland and cause prolonged infections.

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

The development of vaccines against S. aureus biofilm has been a priority in recent years. The rationale behind this approach is that the biofilm matrix produced by S. aureus is a major target for the development of vaccines. The biofilm matrix is composed of extracellular polymeric substances, which are exposed to the host immune system and can be targeted by vaccines.

The main challenge in the development of vaccines against S. aureus biofilm is the need to develop vaccines that are effective against the biofilm matrix. This is a complex problem, as the biofilm matrix is composed of extracellular polymeric substances that are resistant to antibiotics and other antimicrobial agents.

The development of vaccines against S. aureus biofilm has been approached from different angles. One approach is the use of biofilm-specific antibodies, which are capable of inhibiting biofilm formation. This approach has been successful in experimental studies, where the use of biofilm-specific antibodies has been shown to reduce the incidence of mastitis in vaccinated animals.

Another approach is the use of vaccines that target the components of the biofilm matrix. This approach has been successful in preclinical studies, where the use of vaccines that target the components of the biofilm matrix has been shown to reduce the incidence of mastitis in vaccinated animals.

The development of vaccines against S. aureus biofilm is an active area of research, and significant progress has been made in recent years. The development of vaccines that are effective against the biofilm matrix is a priority for the development of vaccines against mastitis in ruminants.

References:
- Dhanawade et al. (2004) demonstrated the ability of bovine mastitis isolates to form biofilm in vitro.
- Bello et al. (2003) demonstrated the production of an exopolysaccharide matrix in microplate (adhesion test). After an incubation period of the isolate in the wells of the microplate, the plate is emptied, the contents of 6.1% of glucosamine and galactosamine were found in the wells washed, and the cells that have adhered fixed, stained and the optical density measured.
- Table 2. Determination of a slime producing phenotype (+/-), biofilm formation capacity in microplate (OD of the biofilm in the test) and production of SAAC (mg SAAC/mg total protein) in isolates of S. aureus. (2009) conducted an efficacy trial against an intramammary challenge, compared to vaccines vacinates based on whole S. aureus, liposomes, toxoid and various inactivated preparations of different experimental vaccines has been tested, showing different levels of protection. Watson et al. (1994) showed an effectiveness of 4.5% in the cows vaccinated with STARTVAC. Nordhaug et al. (1996) demonstrated that antibodies to SAAC are capable of inhibiting biofilm formation in vitro.

The main challenge in the development of vaccines against S. aureus biofilm is the need to develop vaccines that are effective against the biofilm matrix. This is a complex problem, as the biofilm matrix is composed of extracellular polymeric substances that are resistant to antibiotics and other antimicrobial agents.
4. Implication of biofilm in ruminant mastitis caused by S. aureus

In bovine and mammary mastitis caused by *Staphylococcus aureus*, biofilm formation is an important virulence factor that promotes the survival of the bacterium in the mammary gland. Biofilm formation is characterized by the production of a complex extracellular polymeric matrix that protects the bacteria from the host immune response and antibiotics. The biofilm matrix is composed of extracellular DNA, polysaccharides, proteins, and lipids, which together form a protective layer around the bacterial cells. This biofilm matrix serves as a barrier against antimicrobial agents and immune system components. In addition, the biofilm matrix provides a physical barrier that prevents the diffusion of antibiotics into the biofilm, thus making the bacteria more resistant to treatment.

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

Given that biofilm formation is an important factor in the development of mastitis in ruminants, it is crucial to develop vaccines that can target the biofilm formation process. Vaccines that target the biofilm matrix or the production of biofilm enzymes have shown promising results in preventing the establishment of biofilms and the subsequent development of mastitis. In recent studies, vaccines based on whole cell **S. aureus** isolates have been used with success in reducing the incidence of mastitis in ruminants. These vaccines are designed to induce a strong immune response against the biofilm producers, thereby inhibiting the formation of biofilms and reducing the incidence of mastitis. The development of vaccines against the biofilm of **S. aureus** is an active area of research, and further studies are needed to optimize vaccine formulation and delivery systems.
6. Perspectives of biofilm in mastitis

Biofilm formation is a dynamic process characterised by a well-structured, heterogeneous population of bacteria that is embedded in an extracellular matrix. This matrix, known as the extracellular polymeric substance (EPS), provides a protective environment for the bacteria, allowing them to resist host defences and antimicrobial agents.

The EPS matrix consists of various components, including polysaccharides, proteins, and nucleic acids, which are produced by the bacteria in the biofilm. These components help to reinforce the matrix, providing additional protection against environmental stress and antimicrobial agents.

The biofilm is not a static entity but rather an ever-changing structure. Bacteria within the biofilm can rapidly adapt to changing environmental conditions, such as changes in nutrient availability or the presence of antimicrobial agents. This adaptability allows the biofilm to persist and grow, even in the face of antimicrobial pressure.

The biofilm is not only a physical barrier to antimicrobial agents but also provides a platform for the bacteria to colonise and spread. This colonization can lead to the development of drug-resistant strains, which can then be transmitted to other hosts or environments.

The biofilm can also act as a reservoir for bacteria, allowing them to persist in the environment for long periods. This persistence can lead to the intermittent emergence of bacterial infections, as the biofilm can act as a source of bacterial cells that can colonise new environments or hosts.

In conclusion, the biofilm is a complex and dynamic structure that plays a critical role in the development and persistence of bacterial infections. Understanding the biofilm is crucial for the development of effective strategies to prevent and treat these infections.

Bibliographic references


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Bibligraphic references

6. Perspectives of biofilm in mastitis

Biofilm formation is also implicated in ruminant mastitis. The ability to form biofilm is an important virulence factor of S. aureus in the mammary gland by binding to the basal membrane of the damaged heart or to the proteins of the Streptococcus species involved in the development of endotoxins and exotoxins producing inflammation and tissue damage.

Biofilm formation is in general resistance to antimicrobial agents (e.g. antibiotics) may term biofilm as a microcolony at the base of a bacterial cell. Upon formation, biofilms can be defined broadly as a dense and highly structured community attached to a solid surface and aggregated by a polymeric matrix. The ability to form biofilm is a major survival strategy for pathogenic bacteria, allowing them to resist host immune responses and antibiotics. Biofilm formation is characterized by the following properties: adhesion of bacteria to surfaces or other substrates, formation of extracellular polymeric substances (EPS), motility, and quorum sensing. These properties enable biofilms to evade host defenses and antibiotics, leading to chronic infections and treatment failures.

Biofilm formation is also implicated in ruminant mastitis. The ability to form biofilm is an important virulence factor of S. aureus in the mammary gland by binding to the basal membrane of the damaged heart or to the proteins of the Streptococcus species involved in the development of endotoxins and exotoxins producing inflammation and tissue damage.