In vitro and *in vivo* models to study biofilm formation by *Streptococcus dysgalactiae* subsp. *dysgalactiae*

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Streptococcus dysgalactiae subsp. *dysgalactiae* (SDSD) is an important agent of bovine mastitis. This infection causes an inflammatory reaction of the udder tissue, being the most important disease in the dairy industry, due to treatment costs and loss in milk production and quality. Recurrent mastitis is often attributed to biofilm, which allows bacteria to survive and proliferate in hostile environments and resist antimicrobial therapy. Recently, we have described the *in vitro* biofilm formation by SDSD. Imaging and computer methods were used to characterize the *in vitro* formation of biofilms. In this work, we demonstrated the capability of SDSE to develop biofilm *in vivo*, using a murine animal model. For the *in vivo* assays, bovine SDSD was selected based on the ability to form strong (n=2), moderate (n=2) or weak biofilms (n=1). The expression profile of genes associated with biofilm formation was analyzed. Overall, our results showed an increased ability to accumulate biofilm on catheter surfaces implanted in mice compared with *in vitro* biofilm formation. In addition, increased biofilm accumulation on glass surfaces was observed after animal passage. An increase of expression of *brpA-like* (biofilm regulatory protein) and *fbpA* (fibronectin-binding protein A) genes was observed for all the tested SDSD isolates. However, the mRNA expression of *htrA* (serine protease) and *sagA* (Streptolysin S) homologs was more dramatic for SDSD isolates displaying weaker biofilm production. Because the expression of *brpA*-like correlated with *in vitro* and *in vivo* biofilm formation in SDSD, homology modeling was used to predict the 3D structure of BrpA-like protein. Additionally, using high throughput virtual screening and molecular docking, we selected five ligand molecules with strong binding affinity to the hydrophobic cleft of the protein, making them inhibitor candidates of BrpA-like protein from SDSD. These results warrant further investigations for the development of potential novel anti-biofilm drugs for SDSD.