TITLE: A GH20 β-(1,6)-N-acetylglucosaminidase from *Cardiobacterium hominis* is able to degrade *Staphylococcus epidermidis* biofilms

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ABSTRACT:
Biofilms are microbial communities encased in an extracellular polymeric substance (EPS) which assures protection from many environmental threats, including antibiotics, and thus leading to the emergence of highly resistant strains. EPS from different bacteria is composed of proteins, polysaccharides, and extracellular DNA. Biofilms can be found in abiotic surfaces, such as prostheses and catheters used in medical treatments, and in biotic surfaces, such as teeth (dental bacterial plaques) and tissues (infected wound, for example). Thus, biofilms present clinical concern and many efforts are designed to search for therapies able to prevent or eradicate pathogenic biofilms. Several works have reported the use of enzymes from glycoside hydrolases (GHs) group to degrade EPS polysaccharides in an attempt to eradicate bacterial biofilms. In the work reported here, biofilms of *S. epidermidis* ATCC 35984 were grown under static conditions in microtiter plates at 37 ºC for 24 h in Brain Heart Infusion broth supplemented with 0.75% glucose. Then, after washing thrice with phosphate buffered saline (PBS), these biofilms were submitted to treatment with *Ch*GH20. The treatment was conducted using the enzyme at 0.5 mg/mL in PBS, for 4 h at 37 ºC. After the established time, the enzyme solution was removed, and the biofilms were washed and quantified through crystal violet assay. It is known that *S. epidermidis* presents a polymer of β-(1,6)-linked N-acetyl-D-glucosamine residues in its EPS. Thus, we selected GH20 enzyme from *Cardiobacterium hominis* due to its activity as a β-(1,6)-N-acetylglucosaminidase. This enzyme was cloned from *C. hominis* gDNA, expressed in *Escherichia coli* Rosetta and purified by affinity chromatography with the aim to test its therapeutic potential against *S. epidermidis* biofilms. Preliminary results show that *Ch*GH20 was able to degrade approximately 64% of *S. epidermidis* biofilm when compared to the control treatment (only buffer, without enzyme). Comparable results were previously reported for dispersin B, another GH20 β-(1,6)-N-acetylglucosaminidase which presents a similar amino acid sequence to *Ch*GH20. Future experiments are still required to test different *Ch*GH20 concentrations and incubation times, besides its effect on the biofilms from other strains. We also aim to use diverse assays to observe enzyme's effect on the biofilms, such as confocal microscopy.

Keywords: biofilm, *S. epidermidis,* glycoside hydrolase
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