THE INFLUENCE OF SUPERNATANTS ON GENE EXPRESSION OF OPERONS RELATED TO BIOFILM FORMATION BY *Staphylococcus aureus*

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Introduction

Staphylococcus aureus is a Gram-positive bacteria that is commensal and pathogenic for humans [1,2]. Infections in chronic wounds, mediated by virulence factors, stand out. Due to its ability to produce biofilm, and high rates of antimicrobial resistance, therapeutic alternatives are limited [3]. Depending on the composition of the biofilm matrix, biofilms of *Staphylococcus spp.* can generally be classified as ica-dependent or -independent. The first ica-dependent mechanism described is mediated by polysaccharide (PIA) or poly-N-acetyl-glucosamine (PNAG) intercellular adhesion (PIA/PNAG), which is synthesized by the *icaADBC* operon [4]. Genes *icaA* and *icaD* play a primary role in the property of exopolysaccharides. Another mechanism associated with biofilm formation by *S. aureus* is the two-component system YycFG. YycG is a protein sensor encoded by the *yycG* gene that detects environmental changes, having the role of phosphorylating the YycF protein, encoded by the *yycF* gene, responsible for modulating the transcription of target genes and being capable to bind to the promoter region of the *icaA* gene and regulate its expression, indicating that biofilm formation by *S. aureus* is also related to this two-component system [5]. The human microbiota is a source of molecules with antivirulence activity, including against multidrug-resistant pathogens [6]. The aim of the study is to evaluate *in vitro* the influence of metabolites produced by *Morganella morganii* and *Corynebacterium striatum* obtained from wound infections on the expression of genes *icaA, icaD, yycF* and *yycG* in *S. aureus*.

Material and Methods

ATCC 29213 strain of *S. aureus* was tested in the presence and absence of cell-free supernatant (CFS) from *M. morganii* and *C. striatum* cultivated in anaerobiosis, which previously showed activity against biofilm formation on a variety of strains of *S. aureus* in phenotypic tests. The strain seeded in TSA was incubated at 35 ºC (± 2 ºC) for 24 hours, and then transferred to: 5.0 mL of TSB without any CFS and incubated at 35 ºC (± 2 ºC) for 12 h; or 5.0 mL of TSB containing CFS from *M. morganii* or *C. striatum* at a concentration of 0.5x, and incubated at 35 ºC (± 2 ºC) for 12 h. After the incubation period, total RNA from bacterial strains (culture in the presence and absence of CFS), in logarithmic growth phase (OD600 = 0.2 to 0.4) was extracted using the PureLink™ RNA Mini Kit (Ambion), according to the manufacturer's instructions. Residual DNA was removed after treatment with RQ1 RNase-Free DNase (Promega). Reverse transcription of 500 ng of mRNA into cDNA was performed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems) according to the manufacturer's protocol. Quantitative PCR (qPCR) was performed using the GoTaq® qPCR Master Mix (Promega), according to the manufacturer's manual, using the StepOnePlus® thermocycler (Applied Biosystems).

The relative expression of each gene was calculated using the 2-ΔΔCT method [7]; where CT (cycle threshold) corresponds to the number of cycles necessary for the fluorescence released by the reaction to reach a detection threshold. Therefore, the result of 2-ΔΔCT expression determines how many times a target gene is expressed in the strain in the presence of CFS, in relation to the strain in the absence of CFS. All tests were performed in triplicate.

Results and Discussion

Analysis of the relative expression of the *icaA* and *icaD* genes revealed that, for the *S. aureus* strain ATCC 29213, the CFS of *M. morganii* (MM) generated an inhibition of the expression of the genes *icaA* (-3.59x) and *icaD* (-6.15x), relative to the biofilm formation pathway dependent on the *icaADBC* operon (Figure 1a). CFS from *C. striatum* (CS) was able to inhibit the expression of the *icaD* gene (-1.39x), but with an increase in the expression of the *icaA* gene (0.61x). A decrease in the expression of *yycF* (-6.91x) and *yycG* (-5.90x) was observed by the CFS of *M. morganii* (MM) in the ATCC 29213 strain of *S. aureus*, these genes being related to the *yyc* operon. The CFS of *C. striatum* (CS) generated little significant inhibition of the *yycF* (-0.68x) and *yycG* (-0.10x) genes (Figure 1b).

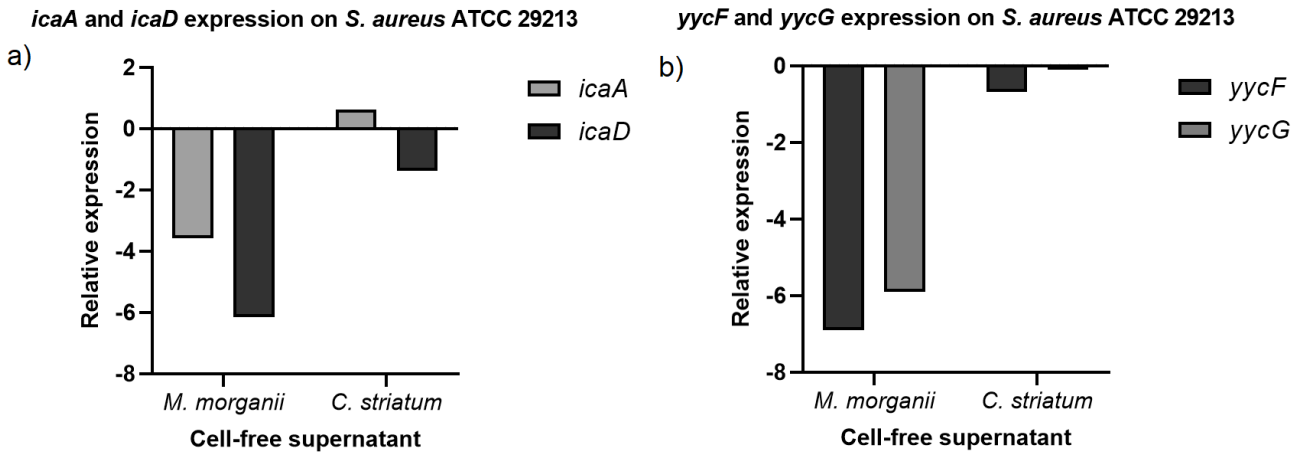

Figure 1 – Evaluation of the inhibition of the expression of the *icaA* and *icaD* genes (a) and *yycF* and *yycG* genes (b) by *M. morganii* and *C. striatum* CFS on the *S. aureus* strain ATCC 29213.

Conclusion

The *M. morganii* CFS was able to promote a mild repression in the gene expression of *icaA* and *icaD*. On the other hand, the CFS of *C. striatum* did not show the expected results, being discrepant in relation to the CFS of *M. morganii*, which was capable of promoting the expected gene repression. Further analysis is required to address the mechanism of biofilm repression induced by the *C. striatum* CFS.

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