THE ANTIBIOFILM ACTIVITY OF WOUND MICROBIOTA SUPERNATANTS IN STRAINS OF *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]. The human microbiota is a source of molecules with antivirulence activity, including against multidrug-resistant pathogens [4]. The aim of the study is to evaluate *in vitro* the influence of metabolites produced by *Morganella morganii* and *Corynebacterium striatum* on biofilm formation in *S. aureus* strains, and to evaluate the chemical composition of the bioactive molecules studied.

Material and Methods

Strains of *M. morganii* and *C. striatum* obtained from patients with chronic wounds were selected to obtain metabolites from its growth in Tryptic Soy Broth, in addition to 12 strains of *S. aureus*, originating from different clinical sources as blood culture and wound swabs from patients treated at University Hospital Antônio Pedro, to verify the action of the metabolites produced by the selected microorganisms on the biofilm formation of these strains. Biofilm formation tests were carried out in 96-well polystyrene plates, with spectrophotometer reading, regarding a control group, test group with usual supernatant usage. To test the stability of the bioactive molecule, the supernatant was treated by heating (50 ºC), boiling (100 ºC) and proteinase K, and then a new biofilm formation test was performed. Statistical analysis was performed using the ANOVA test using Graphpad Prism v:8.2 software.

Results and Discussion

The supernatants of *M. morganii* and *C. striatum*, grown aerobically and anaerobically, showed the ability to inhibit biofilm formation in 12 different strains of *S. aureus*, including standard strain ATCC 29213 and clinical strains of Methicillin-resistent *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA). The supernatants obtained from anaerobic cultures showed greater inhibition activity when compared to the supernatants from aerobic cultures ($P < 0.0001$) in different concentrations (0,5x and 0,25x) to strain ATCC 29213 and clinic strain MRSA CM-5. Figure 1 shows the influence of *M. morganii* monoculture supernatant and a coculture supernatant involving *M. morganii* and *C. striatum*. Statistical analysis shows significance with $P < 0,0001$.

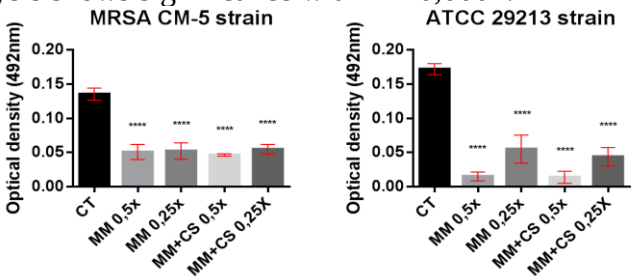

Figure 1 – Inhibition of biofilm formation comparing the control group (CT) with the use of different supernatans in different concentrations (0,5X or 0,25X), grown in anaerobic conditions, such as *M. morganii* monoculture (MM) and a coculture involving *M. morganii* and *C. striatum* (MM+CS), against strains ATCC 29213 and CM-5. **** P < 0,0001.

Beyond MRSA CM-5 and ATCC 29213, other 10 clinical strains of *S. aureus* were also tested, to evaluate if the antibiofilm molecules are strain- or species-specific. Statistical significance shows an inhibition of the culture supernatant on the biofilm formation compared to the control group in all of the strains. This data is shown to be reproducible, as the production of metabolites by *M. morganii* and *C. striatum* was performed in duplicate.

To test the stability of the bioactive molecule, the supernatant was treated, and no statistical significance was observed between the antibiofilm activity of the Untreated Supernatant (US) compared to the heated (50), boiled (100) and Proteinase K (PK) treated supernatants of *M. morganii* (MM) and *C. striatum* (CS). For the *M. morganii* and *C. striatum* co culture supernatant (MM+CS), there was an induction of biofilm formation that was statistically significant (** P < 0,01) as shown in Figure 2.

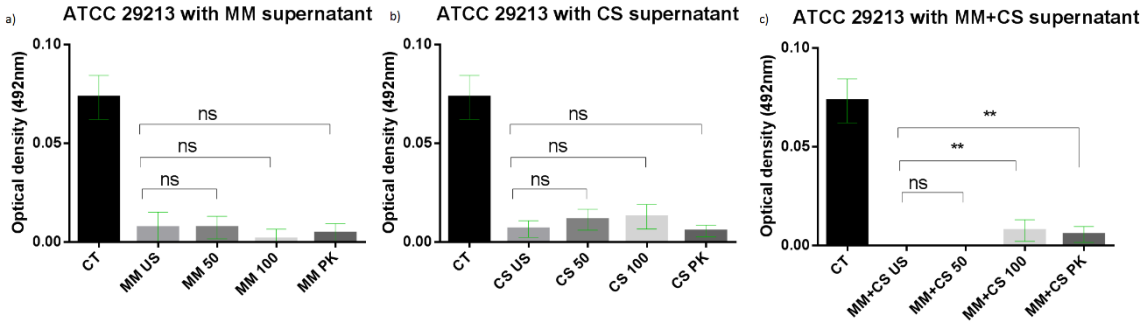

Figure 2 – Inhibition of biofilm formation comparing the control group (CT) and the untreated supernatants (US) grown in anaerobic conditions with the use of treated supernatans by heating (50), boiling (100) and Proteinase K (PK) against strain ATCC 29213. ns = no statistical significance; ** P < 0,01.

Conclusion

The studied supernatants exerted antibiofilm activity in strains of *S. aureus*. This activity is exerted by non-proteic molecules.

Acknowledgments

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