ANTIBIOFILM ACTIVITY OF BROMELAIN IN *Pseudomonas aeruginosa* AND S*taphylococcus aureus* ISOLATES

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Introduction

Bromelain is a set of proteolytic enzymes from members of the Bromeliaceae family and pineapple [*Ananas comosus* (L.) Merril], is the main representative. Although bromelain has known anti-inflammatory and necrotic tissue debridement properties, little is known about the proteolytic potential of this enzyme against opportunistic pathogens, usually related to the development of biofilms and the worsening of chronic wounds, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Thus, this work aims to investigate bromelain's antibacterial and antibiofilm activity against strains of *P. aeruginosa* and *S. aureus*.

Material and Methods

P. aeruginosa and *S. aureus* come from chronic wounds and blood cultures, belonging to the culture collection of the Microbiological Control Laboratory of the Faculty of Pharmacy at UFF. Strains were activated in Tryptic Soy Broth (TSB) medium and seeded on Tryptic Soy Agar (TSA) and incubated for 20-24 h at 35ºC (± 2ºC). Bromelain solution (Sigma Aldrich®) was prepared according to the manufacturer's instructions. Control strains: ATCC 27853, PAO1, ATCC 29213, and ATCC 25923. The antibacterial activity of bromelain was evaluated using the Mueller-Hinton agar dilution [1]. In this, we used 30 strains of *P. aeruginosa* and 27 strains of *S. aureus*. In the growth curve experiment as in biofilm formation assays, two control and two clinical strains for each species were analyzed. Test tubes containing bacterial inoculum (0.2 ml/ McFarland scale: 0.5), TSB (3.6 ml) and 1% bromelain (0.2 ml) were incubated (20-24 h at 35ºC± 2ºC). Tubes with TSB, TSB+inoculum, and TSB+bromelain were used as control. Readings were performed in a spectrophotometer (Optical density[OD] of 600 nm) every hour of the experiment, for 8h and the last reading in 24h. In the biofilm formation assay, 10µL of the bacterial cultures diluted and adjusted in TSB (1:100/ OD600 0.01) were transferred to the microtiter plate wells previously containing TSB (190 µL). The plate was incubated (35°C ± 2°C, 24 h). After, the crystal violet assay was performed according to previous work [2], except for some modifications: washing with distilled water and using 95% ethanol; optical density was taken at 570nm; strains were classified according to Stepanovic and colleagues [3]. For *S. aureus*, TSB was supplemented with 1% glucose. In biofilm inhibition test, biofilm formation assay was repeated, but now the bacterial inoculum (10µL) was incubated (20-24 h at 35ºC± 2ºC) with TSB (180 µL)+1% bromelain(10 µL) and TSB (150 µL)+4% bromelain (40 µL). While in the destruction biofilm assay, preformed biofilms received TSB+bromelain and were incubated once more (20-24 h at 35ºC± 2ºC). The crystal violet assay was performed, and the antibiofilm activity of bromelain could be detected by comparing the absorbance readings (OD570) [3]. Controls: wells with TSB only.

Results and Discussion

Bromelain did not show antibacterial activity. All the strains of *P. aeruginosa* and *S. aureus* grown at different concentrations of bromelain (0.25%, 0.5%, 1.0%, 2.0% and 4.0%). In the growth curve test with 1% bromelain, this enzyme did not alter the strains' growth profile and generation time. Previous work of our group demonstrated that papain, a proteolytic enzyme similar

to bromelain, did not show antibacterial activity against *P. aeruginosa* [4] and methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* [5] strains. These results raise the hypothesis that the proteolytic enzymes, bromelain, and papain, tend not to show antibacterial activity. Although, here we found for the first time that bromelain can destroy both *P. aeruginosa* and *S. aureus* mature biofilms, being more effective in the last one (Figure 1). Bromelain also could inhibit the biofilm formation of *S. aureus* strains (Figure 1). The formation of the extracellular matrix of *S. aureus* biofilms may be protein-dependent and predominantly proteinaceous, as in methicillin-resistant *S. aureus* strains [7], which would enable the greater performance observed of bromelain in *S. aureus* biofilms. This antibiofilm function of plant proteolytic enzymes was also seen in past studies [4],[5],[6].

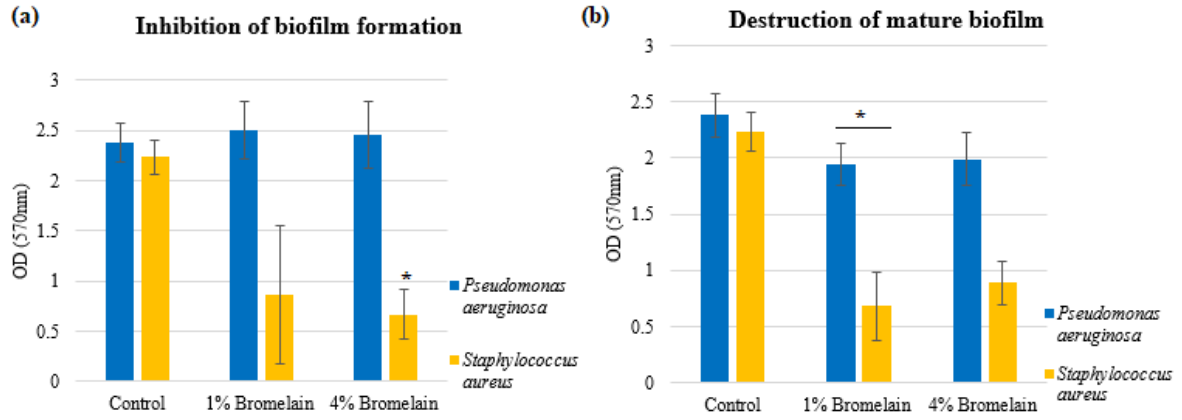

Figure 1- Potential antibiofilm activity of bromelain against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. (a) Inhibition of biofilm formation in the presence of bromelain (1%,4%): The biofilm of *S. aureus* was significantly inhibited in 4% bromelain. (b) Destruction of mature biofilm treated with bromelain (1%, 4%): one percent of bromelain significantly destroyed mature biofilms of the species studied, mainly of *S. aureus* strains.*Statistically significant difference compared to the control group. For *S. aureus*, data are represented as median and interquartile ranges and *P. aeruginosa* as mean and standard deviation. The statistical tests ANOVA oneway and Kruskal-Wallis were applied for analysis of variance ($p < 0.05$). The experiments were carried out in triplicate.

Conclusion

Bromelain is a new potential agent in the treatment of mature biofilms, especially of *S. aureus*, and can be considered a target for developing therapeutic alternatives to combat the presence of these mature biofilms in chronic wounds.

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