TITLE: ANTIBIOFILM ACTIVITY OF *Cutibacterium acnes* CELL-FREE CONDITIONED MEDIA AGAINST *Staphylococcus* spp.

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Species of *Staphylococcus* are often isolated from human and animal infections, both from hospitals and the community. The rapid spread of antimicrobial resistance among these species indicates the importance of discovering new therapeutic alternatives for the treatment of infections caused by these pathogens. In addition, these species can form biofilms, which make the effective treatment of such infections even more difficult, highlighting the importance of compounds capable of acting on these structures. The microbiota has been widely studied in recent years as a source for new compounds with activity against pathogens and therapeutic potential, and *Cutibacterium acnes*, important colonizer of the human skin, has been described as a skin protector against pathogens. Here, we tested the activity of molecules secreted by *C. acnes* against *Staphylococcus* spp. biofilm formation. To obtain a cell-free supernatant, we grew *C. acnes* for 72 h at 37 ^{o}C under anaerobiosis, collected, filtered and concentrated the supernatant. We then tested its impact on *Staphylococcus* spp. aerobic biofilm formation using a microplate assay. Given the inhibitory activity on biofilm formation, we initiated the characterization of the active molecules present in the supernatant by submitting it to heat, boiling, and treatments with proteinase K, trypsin and sodium metaperiodate. We then tested the activity of the supernatants after each treatment on *Staphylococcus* biofilm formation. Our results showed that among the staphylococci species tested, *S. lugdunensis* and *S. hominis* displayed significant reduction in biofilm formation in the presence of the *C. acnes* supernatant. This effect was lost only when the supernatant was treated with sodium metaperiodate, indicating a polysaccharidic nature of the active molecule against *S. lugdunensis*. None of the treatments inhibited the activity against *S. hominis* biofilm. The supernatant was also able to disperse the mature biofilm of *S. aureus*, *S. epidermidis* and *S. pseudintermedius*. Future experiments will focus on futher characterizing the bioactive molecules, which could have therapeutical potential. Understanding the interactions between different microorganisms could shed light on new compounds with possible applications to help treating and preventing bacterial infections.

Keywords: antibiofilm, *Cutibacterium acnes*, *Staphylococcus*, microbial interactions.

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