Staphylococcus epidermidis and *Staphylococcus capitis* quorum-sensing as a strategy to control atopic dermatites

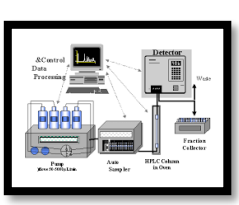
Poster ID 264

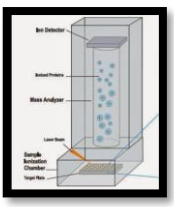
Fuzeti, Deborah Correa; Silva, Júlia Pereira; Segura, Wilson Dias; Andreo, Márcio Adriano; Andreo-Filho, Newton; Leite-Silva, Vânia Rodrigues*; Lopes, Patricia Santos
* Presenting author
Departamento de Ciências Farmacêuticas, Instituto de Pesquisa Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Campus Diadema, São Paulo, Brazil

Introduction

Atopic dermatitis is a common inflammatory skin disease with a complex etiology that affects about 15 to 20% of children and 1 to 3% of adults worldwide [1]. The disease has as main symptoms intense itching, recurrent eczema, dry skin and roughness, especially in sensitive skin regions [1, 2, 7].
The causes of AD are complex and multifactorial [2], and one of the factors that contributes to the worsening of AD symptoms is the dysbiosis that occurs in the skin microbiota of these patients, since studies show that patients with AD have excessive colonization by *Staphylococcus aureus* in their skin (rates ranging from 30 to 100%), which is associated with severity and exacerbation of the disease [3, 4].
S. aureus can also increase AD severity through the secretion of its virulence factors, the best known and most studied are superantigens (Sags) [5].
It is believed that the rebalancing of the microbiota of patients with AD leads to a decrease in the main symptoms of the disease, so the objective of the work is to look for ways to reestablish the quorum-sensing [6] of the other gram-positive bacteria that are in this microbiota, such as *Staphylococcus capitis* and *Staphylococcus epidermidis*, evaluating their activity on inhibiting the growth of *Staphylococcus aureus*.

Materials & Methods:





S. capitis, *S. epidermidis* and *S. aureus* in broth, suspension of 10^8 CFU/mL → Separation of supernatant from the cells, by double centrifugation and filtration using a 0.45 µm filter. Extraction of the supernatant with ethyl acetate (nonpolar) and butanol (polar) analyzed in the HPLC (Agilent, model Infinity 1260 with DAD detector, automatic injector, oven for column

The samples analyzed in the mass spectrometry MALDI-TOF were the same as those used in the HPLC. The device was used Bruker Daltonik, GT 0264, with the flexControl Version 3.4 software and the flexAnalysis Version 3.4 software for sample analysis) with MBT identification method, which has a length from 1,800 to 20,000 m/z, matrix suppression of 700 Da, and the calibrant used was the BTS (Bruker). The samples were placed on the appropriate plate and analyzed in triplicate, after drying and addition of 0.1 µL of matrix.

Results & Discussion

The results showed that both microorganisms chosen, *S. capitis* and *S. epidermidis*, have proteins, which can be potential quorum-sensing molecules. Analyzing the results obtained by HPLC of the samples extracted with ethyl acetate, due to its more nonpolar property [8][9], it was possible to find peaks at the end of the run, at a wavelength of 360 nm, where the largest amount of solvent present in the equipment was methanol, which may indicate a molecule not yet investigated. The samples obtained through extracts from butanol, due to its more polar property [8], showed peaks (fig.2) at the beginning of the run, with a wavelength of 254 nm, where the largest amount of solvent present in the equipment was water. The peaks found for being of polar character, match the results that we obtained in the MALDI-TOF image spectrometry method (fig.1), thus confirming that the molecules found in the extracts from butanol are peptides and proteins, and a probable indication of the same being the molecules that we seek from quorum-sensing.

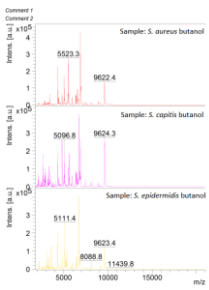


Figure 1. Peak and mass/charge results from the analysis of the three replicates of sample obtained from *S.aureus* butanol extraction (A), *S.capitis* butanol extraction (B) and *S. epidermidis* butanol extraction (C)

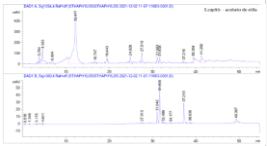


Figure 2. Chromatogram of the *S capitis* Acetate. In the upper part we can observe the wavelength of 254 nm, and in the lower part we can observe the wavelength of 360 nm.

Conclusions:

So far, the research has shown to be promising in identify potential proteins that can be quorum-sensing molecules, through both the MALDI-TOF imaging spectrometry method, with a secondary analysis of HPLC and the presence of lipids through the HPLC method, however our research is ongoing, and more tests will be carried out until your research is completed.

Acknowledgements:

Thanks to the Capes Development Agency for supporting and Scholarship.
Thanks to Gabriel Souza Palhano and Jefferson Modesto Oliveira for their support in collaborating with the tests.

References:

1.Luger T, Amagai M, Dreno B, Dagnelie MA, Liao W, Kabashima K, et al. Atopic dermatitis: Role of the skin barrier, environment, microbiome, and therapeutic agents. J Dermatol Sci [Internet]. 2021;102(3):142–57. Available from: https://doi.org/10.1016/j.jdermsci.2021.04.007
2. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet [Internet]. 2020;396(10247):345–60. Available from: http://dx.doi.org/10.1016/S0140-6736(20)31286-1
3. Edslev SM, Agner T, Andersen PS. Skin microbiome in atopic dermatitis. Acta Derm Venereol. 2020;100(100-year theme Atopic dermatitis):358–66.
4. Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, et al. The microbiome in patients with atopic dermatitis. J Allergy Clin Immunol [Internet]. 2019;143(1):26–35. Available from: https://doi.org/10.1016/j.jaci.2018.11.015
5. Williams MR, Gallo RL. The Role of the Skin Microbiome in Atopic Dermatitis. Curr Allergy Asthma Rep. 2015;15(11).
6. Le KY, Otto M. Quorum-sensing regulation in staphylococci-an overview. Front Microbiol. 2015;6(OCT):1–8.
7. Kim J, Kim BE, Leung DYM. Pathophysiology of atopic dermatitis: Clinical implications. Allergy Asthma Proc. 2019;40(2):84–92.
8. Freed BK, Biesecker J, Middleton WJ. Spectral polarity index: a new method for determining the relative polarity of solvents [1]. J Fluor Chem. 1990;48(1):63–75.
9. Sri Widyawati P, Budianta TDW, Kusuma FA, Wijaya EL. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. Int J Pharmacogn Phytochem Res. 2014;6(4):850–5.
10. https://www.vectorstock.com/royalty-free-vectors/centrifuge-vectors
11. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.ufjf.br/quimica/files/2016/08/AULA-HPLC.pdf
12. http://2.bp.blogspot.com/-elZGtf1bP68/UobDOKpAIQI/AAAAAAAAAkI/PM5JqjpqNf8/s640/maldi-tof.jpg