

Development of an assay of peptides on HPLC-MS to quantify and to verify the influence of different antibiotics on these peptides

Jennifer DE LEO

Introduction

Staphylococcus aureus is a bacterium responsible for many types of infections, such as nosocomial infections in humans. Plus, it is the second pathogen responsible for this type of infection after Escherichia coli. S. aureus has the ability to secrete, after invasion, adhesion factors, toxins or enzymes. These toxins are capable of forming pores such as PSM. These peptides are produced in large quantities by the community strains, and participate in the success of infection by their ability to lyse neutrophils. PSM of type I called PSM α are those that will be investigated here. The objective is to develop the assay PSM α by HPLC-MS to quantify the expression of PSM α and know later if a particular antibiotic inhibits or otherwise induces the expression of a PSM α Geraldine strain of S. aureus.

Material and method

Analyses were performed on a liquid chromatograph in combination with a mass spectrometer system. The column used is a Strategy US2 C12 (50 mm x 2.1 mm x 2.2 μ m). The flow rate is 0.220 mL/min and the injection volume is 10 μ L.

Time	0	3	3.5	7	20	23	25	30
%A	100	100	60	60	0	0	100	100
%B	0	0	40	40	100	100	0	0

Table 1: Description of the gradient elution (A: 90% acetonitrile, 10% water, 0.1% formic acid ; B: 10% acetonitrile, 90% water, 0.1% formic acid)

About the mass detector conditions, the capillary voltage was at 4 500 volts, the gas temperature at 350 °C, the gas flow at 11 L/min, the nebulizer pressure at 50 psig and the oven temperature at 45 °C.

Results and discussion

In the first instance, the standard range permit to quantify the expression of PSM α in the Geraldine strain of S. aureus (results table 2). Plus, the standard deviation is quite important which means the expression is not stable.

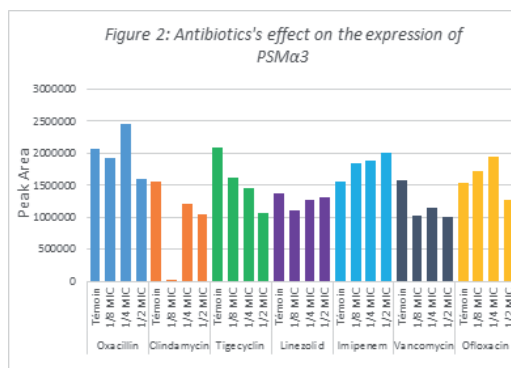
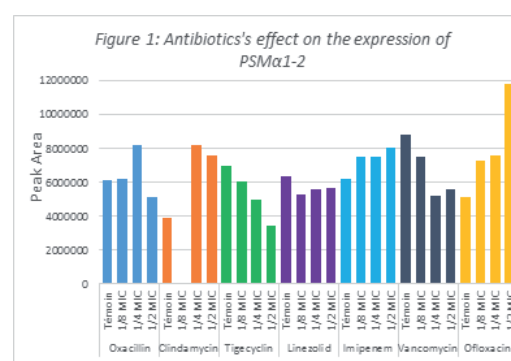
Secondly, antibiotics' effect have been determined. Indeed, Oxacillin and Linezolid have no effect, Clindamycin induces PSM α 1-2 and inhibits PSM α 3, Tigecyclin Vancomycin inhibits and Ofloxacin induces (figure 1 and 2). (MIC = Minimal inhibitory concentration)

Conclusion

To conclude, the results show that PSM α can be quantified with an HPLC-MS but it would be better if some parameters were changed, such as the gradient in order to reduce the analysis time. Moreover some antibiotics can inhibit the PSM α 's expression. It means that, in the future, they will have the opportunity to fight against PSM α .

Table 2: PSM α 's concentration

	Concentration (μ g/mL)	Standard Deviation
PSM α 1-2	530	1400
PSM α 3	520	210



Hôpitaux Civils de Lyon
Service de Bactériologie
Chemin du Grand Revoyet
69495 Pierre-Bénite