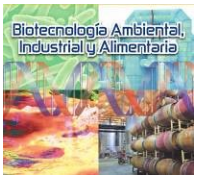
Talk



Characterization of the antimicrobial activity of a clone with metagenomic DNA from a refinery

Andreo-Andreu, Luis (1), Flores Díaz, Amando (1), Alias-Villegas, Cynthia (1), Acosta-Jurado, Sebastián (1), Díaz-Moscoso, Alejandro (2), Camacho Fernández,

Eva María*(1)

(1)Dpto. de Biología Molecular e Ingeniería Bioquímica. Centro Andaluz de Biología del Desarrollo. Universidad Pablo de Olavide, Ctra. Utrera, km 1, 41013 Sevilla, España.

(2) Plataforma de Proteómica y Bioquímica. Centro Andaluz de Biología del Desarrollo. Universidad Pablo de Olavide, Ctra. Utrera, km 1, 41013 Sevilla, España.

Tutor académico: Flores Díaz, Amando

Keywords: antimicrobial compounds; functional metagenomics; Methicillin-resistant *Staphylococcus aureus*

ABSTRACT

Motivation: The increase in the number of antibiotic resistance mechanisms in microorganisms added to the low rate of new antimicrobial (AM) development supposes a threat for public health that should be urgently approached. Bacteria are one of the biggest sources of AM but only a small fraction of them can be cultured in vitro. To overcome this limitation our lab uses a functional metagenomic approach and had previously developed a heterologous expression system that allows the study of all the gene pool from a specific environmen[1,2]. Using this strategy we found different clones whose metagenomic DNA expresssion produces AM activity against *Micrococcus luteus* and also against the Methicillin-resistant *Staphylococcus aureus* (MRSA), one of the World Health Organization's main priorities regarding resistant bacteria. In this work we study the properties and the activity of the AM produced by three different subclones with homologous genes related to phenol metabolism that were found in two different metagenomic libraries and we further characterize one of these subclones, pMPO1718, which proceeds from a refinery metagenomic library.

Methods: The AM is produced in liquid cultures, the antimicrobial production is increased with arabinose for 6-7h and then the supernatant is filtered and lyophilized. The AM activity is tested on LB soft agar plates inoculated with the target strains. The AM is separated in different fractions by chromatography to study in which of them the activity is present.

Results: We have defined a protocol to produce the AM in minimum media complemented with tryptophan and we have observed AM activity against M.luteus in the filtered culture and even higher activity in the total culture extracted with acetone 50%. The last one was analyzed by high performance liquid chromatography and mass spectrometry by Fundación MEDINA and the comparison with databases showed that it may be an AM not previously described. Some AM properties are characterized in this work such as the minimum inhibitory concentration, the minimum bactericidal concentration, its thermostability, its activity in different solvents or against different bacteria.

Conclusions: The filtered supernatant of the three different subclones has AM activity against M.luteus and MRSA produced by phenol hydroxylase related genes. This AM is thermostable until more than 100ºC, has bactericidal activity and can be produced either in LB medium or in M9 medium complemented with tryptophan.

REFERENCES

1. Terrón-González L, Medina C, Limón-Mortés MC, Santero E. Heterologous viral expression systems in fosmid vectors increase the functional analysis potential of metagenomic libraries. *Sci Rep*. 2013;3(1):1107. doi:10.1038/srep01107
2. Terrón-González L, Martín-Cabello G, Ferrer M, Santero E. Functional Metagenomics of a Biostimulated Petroleum-Contaminated Soil Reveals an Extraordinary Diversity of Extradiol Dioxygenases. Parales RE, ed. *Appl Environ Microbiol*. 2016;82(8):2467-2478. doi:10.1128/AEM.03811-15