
Poster

A metagenomic approach to search for new antibiotic resistance genes



María Teresa Álvarez (1), Amando Flores (1), Laura Terrón (1)

(1) Área de Microbiología. Departamento de Biología Molecular e Ingeniería Bioquímica. Universidad Pablo de Olavide

Keywords: antibiotic resistance; metagenomic library; functional screening

ABSTRACT

Motivation: Microorganisms produce antibiotics as a form to interact with other microorganisms and both, the producers and target microorganisms, develop resistance mechanisms to thrive in the presence of natural concentrations of these antibiotics, which are usually low (Aminov, 2009). However, for many years, high concentrations of antibiotics have been used to treat bacterial infections or to improve production of food or animal products on the farm, which have affected the natural resistance mode generating bacteria more resistant to antibiotics. This increase in the resistance level is due to the accumulation of mutations in the genes that encode these resistances. The problem is exacerbated when mutations and new resistances are transferred between different bacteria, both related and not phylogenetically.

For this reason, it is necessary to study resistance genes in human associated bacteria and environment, which will help to find new antimicrobial compounds that could be used as therapeutic agents. Since most microorganisms have not yet been cultivated in laboratory, functional metagenomics is an alternative approach to search for genetic determinants coding for new antimicrobial resistance genes.

Methods: In this work, we are focusing on the construction of a metagenomic library of 22 strains which have been provided by Virgen Macarena University Hospital and that are resistant to colistin and ceftriaxone, two antibiotics used in hospital as a last resort against bacterial infections. This library will contain the chromosomal DNA of all of these bacteria cloned into the fosmid vector pMPO1670, which allows processive transcription of long stretches of environmental DNA by using two viral transcriptional machineries (Terrón-González et al., 2013). One is based on the phage T7 RNA-polymerase, which is insensitive to many of the bacterial transcription terminators. The second system involves the use of the lambda N-anti-termination protein coupled to a salicylate inducible promoter. After its construction, metagenoteca will be transferred by triparental conjugation to a specialized receptor strain to select fosmids that confer resistance to one of the two antibiotics. The fosmids obtained will be studied and metagenomic DNA will be sequenced to characterize molecular mechanisms of resistance.

REFERENCES

- Aminov, R. I. (2009). Minireview The role of antibiotics and antibiotic resistance in nature, 11, 2970–2988. <http://doi.org/10.1111/j.1462-2920.2009.01972.x>
- Terrón-González L., Medina, C., Limón-Mortés, M. C. & Santero, E. (2013). Heterologous viral expression systems in fosmid vectors increase the functional analysis potential of metagenomic libraries. <http://doi.org/10.1038/srep01107>

