Poster

Identification and characterization of resistance genes to hospital antibiotics and new antimicrobial compounds in environmental samples.

Gómez Tostado, José Francisco; Acosta Jurado, Sebastián; Flores Díaz, Amando

Área de Microbiología. Centro Andaluz de Biología del Desarrollo

Universidad Pablo de Olavide, Ctra. Utrera, km 1, 41013 Sevilla.

Tutor académico: Flores Díaz, Amando

Keywords: multiresistance; antimicrobials; functional metagenomics.

ABSTRACT

Motivation: The emergence and spread of drug-resistant pathogens, endowed with novel resistance mechanisms, continue to compromise our ability to deal with common infections. Of particular concern is the rapid global spread of multidrug-resistant and pan-resistant bacteria, commonly referred to as "superbacteria" which pose a serious threat to public health (1). Therefore, there is an urgent need to develop new antimicrobials and antifungal compounds and to understand the mechanisms of resistance against antibiotics restricted to hospitals. Given that most antimicrobials are produced by microorganisms and that resistance genes originate from environmental bacteria, functional metagenomic analysis is a promising approach to address this problem.

Methods: In our laboratory, we have developed a metagenomic library (2) with DNA from soils of Los Alcornocales Natural Park, a pristine site with a high biological diversity. It is being screened to identify clones that produce novel antimicrobials or carry resistance genes for last-resort or hospital-restricted antibiotics. Recently, fosmids with genes that produce resistance to Beta-lactams (3) have been obtained in our laboratory but, although they have been sequenced, the genes responsible for the resistance are not yet known.
In the context of this approach, the following work has been carried out:
1. Screening of the metagenomic library to select clones that produce antifungal compounds against Candida albicans.
2. Assays of Beta-lactamase activity in droplets of clones with fosmids previously selected in the laboratory that bear unknown resistance mechanism to identify those that produce these enzymes.
3. Experimental evolution of fosmids to select clones with a higher level of resistance to Beta-lactam antibiotics.

Results and conclusions:
1. Despite having screened 403.280 clones, no antifungal-producing fosmids have been found yet, so we consider that, under our screening conditions, these compounds are not present in the metagenomic library or cannot be detected.
2. Among the 9 sequenced fosmids with unknown resistance genes, 3 allow the growth of satellite colonies around them, so they probably encode mechanisms similar to Beta-lactams.
3. From these fosmids with unknown Beta-lactam resistance genes, derivatives with increased antibiotic resistance have been obtained and will soon be sequenced to identify the mutations that lead to this increased resistance.

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