Poster

Identification and characterization of last-resort antibiotic resistance genes and new antimicrobial compounds in environmental samples.

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ABSTRACT

Motivation: The misuse of antibiotics has led to a global health crisis, with antimicrobial resistance projected to surpass cancer-related deaths by 2050 and potentially reaching ten million deaths annually. This resistance originates in environmental bacteria, which transmit resistance genes to pathogens via horizontal gene transfer. Additionally, most antimicrobials are natural products, primarily produced by bacteria. However, studying these mechanisms in environmental bacteria is limited, as fewer than 1% of them can be cultured. To overcome this, functional metagenomics and massive sequencing are key tools for identifying these genes in microbial communities(1). Previously, in our laboratory, metagenomic libraries from Los Alcornocales Natural Park soils were constructed and screened, isolating clones conferring resistance to last-resort antibiotics or producing novel antimicrobials.

Methods: This work has two objectives:

1.Evaluating MPO223 for NRPS (non-ribosomic peptide synthetase) expression: This new strain produces a PPTase (phosphopantethenil transferase) required for NRP synthesis(2). Its ability to generate NRP compounds is being compared with the control strain MPO554.

2. Resistance to last-resort antibiotics: On the one hand, the capacity of the fosmids that confer resistance to ciprofloxacin in Pseudomonas to confer resistance in Escherichia coli was evaluated by conjugation and determination of the minimum inhibitory concentration (MIC). On the other hand, we have checked whether a specific region of the MerA25 fosmid is associated with resistance to meropenem, through its deletion.

Results: Pseudomonas fosmids confer ciprofloxacin resistance in Escherichia coli, suggesting that resistance genes can be transferred between different bacterial genera. MIC analysis with and without inducers revealed that one of the fosmids carries a salicylate-independent promoter, indicating that heterologous expression is not required for resistance gene expression(3). For the MerA25 fosmid, deletion of a specific region resulted in loss of resistance, indicating that this fragment is essential for that function. For the evaluation of strain MPO223, we are currently constructing plasmids with the bpsA reporter gene to measure the PPTase activity.

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