Universidad de Navarra
Facultad de Ciencias

MÁSTER EN INVESTIGACIÓN BIOMÉDICA
Research Project Proposal
Academic year 2024-2025

Project Nº 47

Title: Breaking Bacterial Silence: Activating Dormant BGCs in *Xenorhabdus nematophila* for Antimicrobial Innovation

Department/ Laboratory *Laboratory where the project will be carried out indicating Department, Area, Faculty, CUN, CIMA etc.*
Department/ Laboratory: Department of Microbiology and Parasitology-Edificio de Investigación

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Summary *Short summary of the project with a **maximum extension of 250 words**, including the goals and the methodology that will be used.*

The discovery of novel antibiotics is critical to combat the rising threat of antibiotic-resistant bacteria. This project aims to uncover novel antibiotic compounds by activating underexpressed biosynthetic gene clusters (BGCs) in *Xenorhabdus nematophila* ATCC 19061, a bacterium known for its rich secondary metabolite profile. The methodology involves random promoter insertion to activate these silent BGCs.

First, a transposon (Tn) mutant library of *X. nematophila* ATCC 19061 is generated using the pBT20 vector via conjugation. Successful transconjugants are selected on gentamicin (Gent20) plates, harvested, and stored as frozen stocks. Next, the Tn library is thawed and cultured in liquid medium (LB-Gent20+IPTG) for four days to induce the expression of underexpressed BGCs.

To ensure compatibility with target strains, it is crucial that the target strains to be killed (i.e. *Acinetobacter baumannii* and *Klebsiella pneumoniae*) are resistant to Gent20. Supernatants from both the Tn library and wild-type *X. nematophila* ATCC 19061 cultures are collected, and overlay assays are performed at 1X and 10X concentrations to measure inhibition zones against the target strain, to confirm antibiotic activity.

If significant inhibition zones are observed, a screening assay is conducted to identify individual antibiotic-producing colonies. A new Tn library is generated, cultured on IPTG-Gent20 plates for four days, and overlaid with the target strain to identify clones with inhibition halos.

This approach aims to unlock the hidden potential of *X. nematophila* by activating silent BGCs, facilitating the discovery of novel antibiotics crucial in the fight against resistant pathogens.

yes	
no	**X**

Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?