



MÁSTER EN INVESTIGACIÓN BIOMÉDICA

Research Project Proposal

Academic year 2022-2023

Project Nº 23 ASIGNADO

Title: Identification of Novel Factors of *Klebsiella pneumoniae* Required for Macrophage Survival Using Transposon Sequencing (Tn-seq) Analysis

Department/ Laboratory: Department of Microbiology and Parasitology-Edificio de Investigación

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Introduction. *Klebsiella pneumoniae* is an important cause of multidrug-resistant infections worldwide. Understanding the virulence mechanisms of *K. pneumoniae* is a priority and timely to design new therapeutics.

Goal. Although it is well established that *K. pneumoniae* survives intracellularly in macrophages the mechanism behind remains to be clearly understood. This project will aim to fill this knowledge gap, performing a genome-wide screening of *K. pneumoniae* to search for novel factors required for this bacterium survival within human macrophages.

Methodology. Transposon sequencing (Tn-seq) is a method that combines large-scale Tn mutagenesis with next-generation sequencing to estimate the fitness contribution of each gene in a bacterial genome under defined selective conditions.

In this project, a *K. pneumoniae* library composed of 12,000 mutants arrayed in 96 well plates will be pooled and used to infect human macrophages (Thp-1 cells). After 2h, samples will be treated with gentamicin to kill extracellular cells and incubated an additional 24h. Macrophages will then be lysed and surviving Tn mutants retrieved plating lysates on tryptic soy plates. Sequencing libraries will be prepared from DNA extracted from the input library used for infection and output mutants grown in macrophages and high-throughput sequencing analysis performed on these libraries to identify Tn mutants whose abundance is significant decreased in output versus input samples, presumably encoding factors critical for *K. pneumoniae* intramacrophage growth.

Follow-up validation of previously identified factor will then be performed in competition studies infecting macrophages with wild type and selected Tn mutant strains retrieved from our arrayed Tn library.

yes	
no	X

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