



Applying whole genome engineering techniques for the manipulation of *Pseudomonas putida*

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Type of thesis: Experimental

Required competences: Basic microbiology (cell culture, transformation) and molecular biology (PCR design, cloning)

Acquired competences: Advanced molecular biology techniques (TAR, CRISPR/cas, RAGE) , biochemistry and analytic techniques

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Description

Pseudomonas putida has recently emerged as a promising bacterial chassis for the production of interesting compounds [1,2]. This bacterium has many advantages such as a versatile metabolism with high levels of precursors and high amounts of reducing molecules, a high tolerance to xenobiotics and a fairly large set of genetic manipulation tools [3]. In this sense, many genetic engineering tools have been developed in the last years, including recombineering [4,5,6], CRISPR/cas9 [7] and CRISPRi [8]. However, the efficiency of genome editing using the technology available is still low and unreliable, which is especially problematic when targeting multiple loci.

Alternatively to these traditional techniques, some authors has succeeded in manipulating bacterial genomes using yeast cells as a platform for mutagenesis [9,10]. The efficiency of the in-yeast genetic editing tools is high enough to perform a large-scale edition of the bacterial genome [11]. Even, this technology has allowed the chemical synthesis of total bacterial genomes [12,13].

Our objective is to develop a strategy that combines the in-yeast assembly and edition of bacterial genomes or parts of genomes with the technologies available for genetic engineering of *P. putida*. This strategy will allow us to streamline the genome of this bacterium and/or engineering it for specific purposes in a more efficient, reliable way.



References

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