Generation of bacterial strains of production, with a growth-coupled focus to for its application in synthetic biology.

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Abstract

The present project presents a combination of methodologies that manages to turn around the design-construction-test cycle of bacterial strains of Metabolic engineering production. We started with an in silico design generated by the genomic scale model of last generation Escherichia coli (ME-iOL1554). From this, the strains were generated using molecular biology tools. The strains generated were characterized in a simple experimental system but with strict micro aerobic conditions and underwent a process of adaptive evolution in the same experimental system, managing to generate strains with fermentative pathways interrupted but that manage to grow under strict micro aerobic conditions. The strains generated produced L-alanine (although not in titles close to that predicted by the metabolic model at genomic scale), the exo-metabolomic analyzes of one of the strains show that it is igniting latent fermentation pathways not previously described.

This is why this work constitutes a conceptual advance for several reasons. 1) Test the use of computer models as a design tool, a combination of systems biology and synthetic biology is achieved. Both sciences of great importance and relevance today. 2) The concept of growth-coupled (Growth-Coupled), a fundamental quality in a production strain, is experimentally validated. 3) A combination of methodologies was implemented: computational design, molecular biology, fermentations, adaptive evolution and exo-metabolomics by H1-NMR. 4) An advance was achieved in the generation of L-Alanine producing strains, however the most important result of the project was the use of computational models as a design tool and the discovery of latent fermentation pathways (ethylene glycol and methanol) in Escherichia coli, which could reinforce what has been said and proposed by other researchers. At the moment, there are two strains whose characteristics make them candidates for strains ¨Chasis¨.

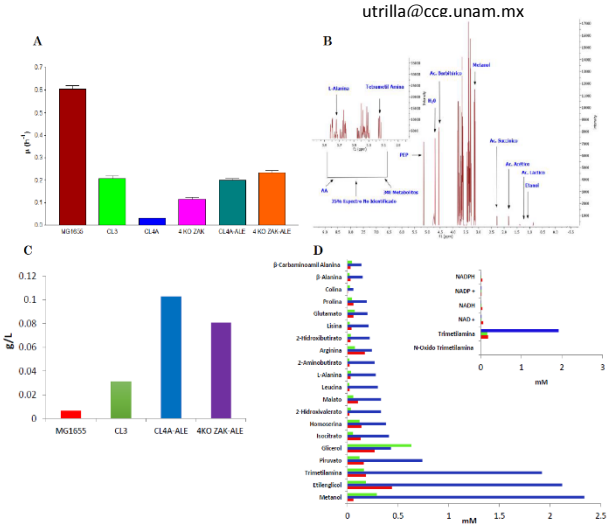


Fig. 1 Growth and production rates of the L-Alanine producing strains.
In subsection **A** the growth rates of the WT, parental and mutant strains are shown. In **B**, the production rates of L-Alanine, in Section **C**, the exometabolome of strain CL4A (mutant strain) analyzed and quantified was shown to be 1 H NMR. In clause **D** the exometabolomes of strain CL4A are shown, the parental strain and the WT strain of *E.coli*, where fermentative routes of production of ethylene glycol and methanol are observed, previously not eroded in *E. coli*.

Recent publications

1. Bolivar, F., Cruz, F., Gosset, G. (2018), ***"Generation and Characterization of Escherichia coli strains production of melanin they express the Tyrosinase enzyme anchored to the outer membrane".*** *(*in review)
2. Cruz, F., Feist, A., Utrilla, J. (2018), ***"Design and generation of Escherichia coli bacterial strains producing L-Alanine through genomic scale metabolic models with a growth-coupled approach"***. *(in construction*).
3. Cruz, F., Lastri, G., Utrilla, J. (2017), ***"Design of biosensors through a cell-free system for its application in synthetic biology´´*** *(in review*).
4. Cruz F, Feist A, Utrilla J. (2016) **XXI Simposio de las Ciencias de la Salud, UAM Iztapalapa: Memorias.** ***¨Generación de cepas bacterianas de producción con un enfoque acoplado al crecimiento para su aplicación en biología sintética¨*** Universidad Autónoma Metropolitana, Unidad Iztapalapa. Departamento de Ciencias de la Salud CBS. Vol. 12: 25-29.
5. Cerezo H., Cruz, F., Utrilla, J. (2015), ***"Introducing synthetic biology in Mexico ´´*** Universidad Autónoma Metropolitana, Unidad Iztapalapa. Departamento de Ciencias de la Salud CBS. Vol. 18: 49-53

Biography



Francisco was born in Mexico City in 1992, is a Biologist from the Metropolitan Autonomous University. In 2014 I made a Research Stay in the Biotechnology Institute (IBT-UNAM) in the lab of Dr. Francisco Bolivar, who in 1979, creates and organizes, based on his pioneering experiments, the first company of Genetic Engineering worldwide (Genentech, Inc.) in 2015 conducts research at the Center of Genomic Sciences in the area of Synthetic Biology and Systems Biology in collaboration with the world leaders in Systems Biology at the University of California, San Diego. He is currently a founder and researcher at the company Biolex.Corp and in September 2017 he started an MRes Synthetic Biology at University College London (UCL) in UK.

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