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

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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 metabolomics 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, was experimentally validated, (3) a combination of methodologies was implemented: Computational design, molecular biology, fermentations, adaptive evolution and exo-metabolomics by H1-NMR and (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¨.

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