Developing novel strains for butanol production

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The growing global demand for new energy sources combined with environmental concerns had motivated the search for alternative fuels, produced from renewable raw materials. During the last decade, ethanol was considered the next generation of biofuels. But more recently, n-butanol gained attention due to its superior fuel properties when compared with ethanol. Although n-butanol is naturally produced by solventogenic bacteria through ABE fermentation, the low productivities obtained with this bioprocess discouraged its use. Thus, most of n-butanol produced nowadays is chemical synthesized via petrochemical routes and its price is extremely sensitive to crude oil's price. One possible approach to overcome this issue is to express non-native pathways in microbial factories.

Our work focuses on the production of butanol through heterologous pathways, previously generated within our group [1] using a (hyper)graph-based algorithm, in *Escherichia coli*. Using Optflux (http://www.optflux.org), a software platform for metabolic engineering, along with the iJO1366 metabolic model, we simulated and optimized these routes *in silico* and the most promising ones are currently being implemented *in vivo*. First, we evaluated these pathways according to diverse criteria including size of solution, yield, conservation of carbon atoms and novelty. Then, we tested several different environmental conditions and sets of gene knock-outs with the aim of redirecting the cell metabolism to solvent production. The biological meaning of the predicted phenotypes was analyzed to rational design a strain that couples growth and butanol production.

Using biomolecular techniques, a novel strain of *E. coli* – able to produce butanol through the selected pathway in the same conditions simulated *in silico* – is currently being constructed.

[1] F. Liu, P. Vilaça, I. Rocha, and M. Rocha, "Development and application of efficient pathway enumeration algorithms for metabolic engineering applications," *Comput. Methods Programs Biomed.*, Dec. 2014.