METABOLIC ENGINEERING AND EVOLUTION OF *Escherichia coli* FOR THE PRODUCTION OF LACTATE OR ETHANOL FROM XYLOSE

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Introduction. Several issues remain to be solved for the production of biocommodities from lignocellulosic hydrolysates. Also, several lignocellulosic biomasses need to be accounted for a sustainable production of biofuels and biochemical compounds. Sustainable production of these molecules, including fuel ethanol and lactate, requires the use of agricultural residues as feedstocks. Thermochemical and enzymatic hydrolysis of lignocellulosic material generates a mixture of sugars, comprising: hexoses (mainly glucose, mannose and galactose), pentoses (xylose and arabinose) and disaccharides (cellobiose), being glucose and xylose by far the most abundant.
The objective of this work was to metabolic engineering efficient homo-fermentation strains of *Escherichia coli*, which besides glucose can efficiently metabolize xylose and sugar mixtures to lactate or ethanol.

Methods. The wild type *Escherichia coli* strain MG1655 was metabolic engineering and evolved with the ability to produce Lactate or Ethanol as the sole fermentation products from xylose, glucose and other sugars. The strategies used for the development of such biocatalysts include: interruption of the competing fermentation pathways that uses pyruvate; chromosomal integration of genes that code for the desired pathway; identification of a new xylose transporter; and metabolic evolution to contend with the acetic acid present in hydrolysates from biomass. These strains had been evaluated using pure sugars and hydrolysates from different agricultural residues from Mexico.

Results. *E. coli* MG1655 (wild type) was metabolic engineered with the genotype *ΔpflB, ΔadhE, ΔfrdA*, to produce D-Lactate as the only fermentation product. This strain produces D-Lactate with a volumetric productivity of 1.3 g/L/h (1), but this parameter was very low when xylose was used as the only carbon source (2). The slow growth of homolactic *E. coli* strains in xylose has been attributed to the low ATP yield in this carbon source (2). By deleting the ATP dependent xylose transport *xylFGH* and using adaptive evolution a new strain (JU15) was isolated, which displayed a high productivity of lactate in xylose mineral media. Genome sequence of JU15 revealed several changes, including a point mutation in the *gatC* gene (2). The mutation is a change of a serine for a leucine in the 184 amino acid of the GatC protein. The mutation is located in the PTSIIC domain in a predicted transmembranal region. Previous analyses have indicated that Gat IIC proteins could have promiscuous functions and may act as secondary carriers. By knocking out the *gatC* gene and introducing the mutation in a non-evolved strain the participation of *gatCS184L* as a xylose transporter was confirmed. Its function as a xylose transporter was previously unknown. *E. coli* JU 15 shows a D-Lactate volumetric productivity of 0.8 g/L/h when xylose is used as the only carbon source.
Further, JU15 was engineered to obtain the homoethanologenic strain MS04 (3): *E. coli* MG1655: *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, Δreg 27.3 kb, gatC S184L, ΔmidarpA, ΔldhA, PpflB::pdcZm-adhBZm*. Main results revealed an increase in the specific growth rate, cell mass formation, and ethanol volumetric productivity at moderate concentrations of sodium acetate (2–10 g/l), in addition to a high tolerance to acetate because MS04 (as well as JU15) was able to grow and produce a high yield of ethanol in the presence of up to 40 g/l of sodium acetate. Genomic analysis of the strains JU15 and MS04 allow identifying that a chromosomal deletion of 27.3 kb generates the improved growth and acetate tolerance in these strains. This deletion comprises genes related to the respiration of nitrate, repair of alkylated DNA and synthesis of the *ompC* gene coding for porin C. *E. coli* MS04 shows an ethanol volumetric productivity close to 0.8 g/L/h when glucose, xylose, mixtures of xylose-glucose or hydrolysates from different agricultural residues from Mexico were used as carbon sources.

Conclusions. These results suggest that xylose transport is the limiting step in xylose fermentative metabolism in *E. coli*. In addition to the well characterized xylose transporters, xylose is transported by GatC and by GatCS184L in the metabolic engineered-evolved strain JU15 and ethanologenic derivatives (MS04). The newly found transporter can be used to engineer strains for converting ligno-cellulosic feedstocks into valuable chemicals. MS04 is advantageous for the production of ethanol from hemicellulosic hydrolysates that contain acetate. This work illustrates the power of metabolic engineering and genomics to reveal ambiguous industrial phenotypes, a common industrial paradigm in strains obtained through random mutagenesis.

References.

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