METABOLIC ENGINEERING FOR THE PRODUCTION OF SHIKIMIC ACID IN AN *Escherichia coli* W3110 STRAIN LACKING THE PHOSPHOENOLPYRUVATE: CARBOHYDRATE PHOSPHOTRANSFERASE SYSTEM

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Introduction. *Escherichia coli* is one of the most widely used organism for fermentation processes and glucose is commonly utilized as a carbon source. The phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS) is responsible for the proper transport and phosphorylation of this sugar at the cost of one molecule of phosphoenolpyruvate (PEP), this could be inconvenient for the production of certain metabolites such as aromatic compounds. *E. coli* strains lacking PTS have the potential to accumulate high amounts of PEP that can be then used as a precursor for the synthesis of commercially valuable compounds. Our group previously reported the development of an *E. coli* strain lacking PTS that can growth on glucose, PB12 (PTS^- Glc^+), by adaptive evolution [1] and further characterization of this strain revealed the genetics changes occurring during its evolution process that allowed recovering a higher growth rate on glucose [2]. However, PB12 contains numerous mutations making it difficult to predict its response to certain industrial processes.

In this work we generated PTS^- Glc^+ strains with a more controlled genotype by incorporating a limited number of the mutations found on PB12 genome, and subsequently used them as a more robust organism for industrial applications such as shikimic acid (SA) production.

Methods. From an *E. coli* W3110 PTS^- strain (WPTS) the mutants WPTS Δ*galR* and WPTS Δ*rppH* Δ*galR* were generated by one-step inactivation of chromosomal genes technique using PCR products as previously reported [3]. These strains were tested for growth on glucose as a sole carbon source. Shake flask cultures were performed in 250 mL baffled flasks with 50 mL of M9 medium supplemented with 2.5 g/L of glucose, at 37°C, 300 rpm. Once was found that these mutants were able to consume glucose, shake flask cultures were performed in 250 mL baffled flask with 50 mL of fermentation medium, whose composition has been previously reported for the production of SA, and grown as described above. This medium contained 25 g/L of glucose and 15 g/L of yeast extract [4]**.** Metabolites concentrations were determined by HPLC using a Waters system equipped with an Aminex HPX-87H column.

Results. By inactivating *galR* and *rppH* we were able to recover up to 50% of the parental strain's growth rate in minimal medium and also increase glucose consumption rate. For the growth on a complex medium we found out that the behavior of the WPTS Δ*galR* and WPTS Δ*rppH* Δ*galR* strains emulates the displayed by the wild type strain W3110, which indicates that these mutants are totally capable of consuming glucose under the conditions used to produce SA in our laboratory. Due to the high glucose consumption rate, these strains present acetic acid accumulation, which was overcome after transforming the strains with the production plasmid pJLB*aroG*^{fbr}*tktA* accumulating up to 4g/L of SA (data not shown).

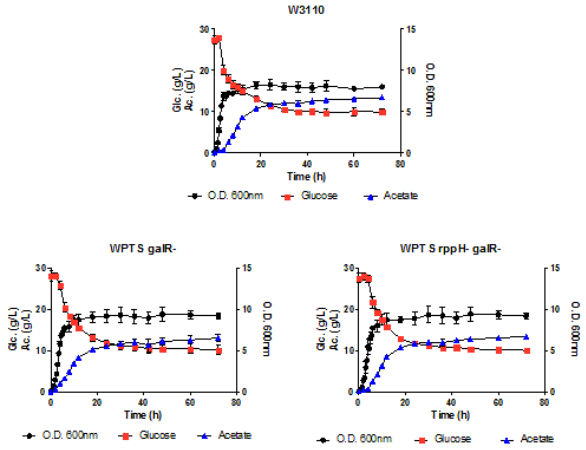

Fig.1 Biomass, glucose and acetate concentrations in W3110 and PTS derivatives growing in fermentation medium (Glc 25 g/L, YE 15 g/L).

Conclusions. This first approach to generate W3110 PTS^- Glc^+ strains from the mutations found on a laboratory evolved strain proved to be robust while strains were growing either minimal or complex mediums at rates comparable to those of the wild type strain. The PEP accumulation by PTS inactivation and the high glucose consumption rates make these mutants suitable for fermentation proposes especially for SA production.

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References.

1. Flores N, Xiao J, Berry A, Bolívar F, Valle F. (1996). *Nat Biotechnol.* 14(5):620-623.
2. Aguilar C, Escalante A, Flores N, de Anda R, Riveros-McKay F, Gosset G, Morett E, Bolívar F. (2012). *BMC Genomics* 13:385
3. Datsenko K, Wanner B. (2000). *Proc Natl Acad Sci USA*. 97(12):6640-6645.
4. Chandran S, Yi J, Draths K, von Daeniken R, Weber W, Frost J. (2003). *Biotechnol Prog.*, 19(3):808-804.