Transcriptional analysis of the effect of pH condition on the production shikimic acid

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Introduction. The shikimic acid (SA) is a key compound for the synthesis of neuraminidase inhibitor (oseltamivir), which is used to treat influenza. The production methods of SA suggest the use of recombinant bacteria (1) and large scale bioreactors. Although, in bioreactors a common problem is the gradients formation of pH, dissolved oxygen, among others (2). In a previous work, *E. coli* PB12-SA22 strain changed the pH value during the culture, which could affect the production of SA (3). In this study we attempt to understand the interaction between the strain PB12-SA22 at different pH conditions, and the affect on the production of SA. To achieve this, metabolic behavior and the messenger RNA differential expression of some key genes involved in the SA biosynthesis, were evaluated.

Methods. Fermentations were performed using the PB12.22 (strain PTS$^-$ which internalize glucose trough galactose permease) (4), with different pH conditions 6, 7, 8 and without pH control. This in 1L reactor at 37 °C, 30% dissolved oxygen and minimum medium supplemented with yeas extract (30 g/L) and glucose (100 g/L) (5). Samples, to measure metabolites and mRNA transcripts were taken in exponential, early stationary and late stationary phase. The metabolites (glucose, AS and by-products) were quantified by the HPLC. Analysis of 32 genes related with central metabolism and SA synthesis was carried out by RT-PCR, comparing with homologous phase sample collected in a culture without induction at pH 7.

Results. The maximum concentration of SA was found without pH control (15.46 g/L), follow by pH 6 and 7 (14.5 and 14.2 g/L, respectively). In cultures at pH 8 only 66 % of the maximum concentration of SA, was obtained. Nevertheless, the culture at pH 7, without induction produced only 3.5 g/L of SA, and reached around 15 g/L of biomass like all other cultures. Transcriptional analysis showed a similar expression pattern of genes involved in the glycolysis for the condition of pH 6, 7 and without pH control, implying a similar flux of glucose. Particularly, over expression of *galP* in the culture without pH control was observed. In all stationary phase from all cultures, the *glk* and *pgi* were overexpressed, indicating the rapid glucose transformation. The *poxB* respiratory gene was upregulated in stationary phase (20 folds) of culture without pH control, in agreement with 6 g/L of acetate accumulated at the end of the culture. Genes evolved in pentose phosphate pathway like *pgl*, *talA* and *tkatA/B* were overexpressed in cultures at pH7 and without pH control in the stationary phase, suggesting the synthesis increase of ribose-5-P and E4P, important for the SA synthesis. For cultures at pH 6 or 7, no major changes in the TCA gene expression were observed. In the late stationary phase of culture at pH 8 *sucB* and *fumA* were highly upregulated, while all TCA genes were upregulated during the stationary phase in culture without pH control. Related with the chorismate pathway, significant upregulation of the gene *aroG* principally in the cultures at pH 6, 7 and without control was measured. Moreover, in culture without pH control the highest overexpression of genes *aroC*, *aroD*, *aroE* and *aroF*, in stationary phase was observed. Importantly, *shiA* gene coding for SA transporter increased it expression in culture without pH control. Although, in culture pH8 was the highest overexpression.

Conclusions. The maximum SA (15.46 g/L) was produced in the condition without pH controlled, simplifying the bioprocess. Because, it will not require the addition of acid or base, avoiding the possibility of contamination, and being cheap in the high scale. Moreover, the culture without pH control accumulated 2 g/L of dihidroshikimate, and around 6 g/L of acetate. Transcriptional analyses revealed highly upregulation in the culture without pH control in the early and late stationary phase of the glycolysis, TCA, pentose pathway and SA biosynthesis. Suggesting that the carbon was channeling to the formation of aromatic compounds such as SA, DHS and GA. In agreement with the upregulation of the *aroG,* which is responsible to channeling the flux of carbon into this pathway.

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