EFECT OF THE *ydiB* GENE IN THE PRODUCTION OF SHIKIMIC ACID IN AN EVOLVED STRAIN OF *Escherichia coli* PTS$^-$

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Introduction. Cultures of engineered strains of *Escherichia coli* for shikimic acid (SA) production results in quinic acid (QA) and dehydroshikimic acid (DHS) byproducts formation, reducing significantly SA yield and impair downstream processes (1). QA synthesis has been proposed to be driven by equilibration process, which turns on the backflow of normal SA producing pathway to SA→DHS→DHQ→QA, where probably the enzyme quinate/shikimate dehydrogenase coding by *ydiB* is responsible of the synthesis of QA in cultures of engineered *E. coli* strains for SA production (2).
This work evaluated the inactivation and upregulation of *ydiB* gene in *E. coli* strain PB12.SA22 (PTS$^-$ glc$^+$ *aroG*^{fbr} *tktA aroB aroE aroK*$^-$ *aroL*$^-$) on SA and byproducts in 50h batch fermentation and transcriptional profile analysis of selected genes during exponential (EXP) and early stationary (STA) stages.

Methods. Inactivation of *ydiB* gene was by the procedure described previously in *E. coli* JM101 (3), then it was transducted to PB12.SA22 using P1*vir* phage, resulting in PB12.SA22*ydiB*$^-$ (*ydiB*$^-$) strain. For the upregulation, *ydiB* gene was cloned in pTOPO*aroEaroB* to obtain PB12.SA22-OE*ydiB* (OE*ydiB*) strain. Batch cultures using mineral medium supplemented with 25 g/L glucose and 15 g/L of yeast extract, and analytical methods were performed for the three strains as it was described before (4). The RNA extraction, cDNA synthesis, RT-qPCR and data analysis were performed as reported previously (5).

Results. Transcriptional expression analysis of selected genes showed differences between EXP and early STA stages, suggesting that during EXP stage, the yeast extract have an important role in support growth, meanwhile glucose consumption is mainly associated with aromatic product synthesis during early STA stage. Also, during this stage, *ydiB*$^-$ strain decreased 70% QA concentration and OE*ydiB* strain resulted in an increase of 6.4 times the QA concentration, resulting in overproduction strain for this compound.
During late STA stage (glucose depleted) only PB12.SA22 strain showed the exchange of aromatic compounds, with the increasing 45% the QA concentration at the end of the culture. In order to show the change in QA and DHS respect to SA, the molar byproducts ratios were calculated (Table 1). The different values obtained to QA showed that deletion of *ydiB* gene is a good strategy for reduce QA concentration during SA production, or the upregulation is a good strategy for the production of QA. On the other hand, DHS concentration maintains the same relation to SHK in the three strains, probably because the feedback inhibition of SA to the enzyme coding by *aroE* (1).

Table 1. Molar byproducts ratio of QA and DHS respect to SA at 50 h.

Strain	Molar byproducts ratio (mol/mol SA)	
	QA	DHS
PB12.SA22	0.14 ± 0.01	0.09 ± 0.008
PB12.SA22*ydiB*$^-$	0.04 ± 0.01	0.10 ± 0.01
PB12.SA22OE*ydiB*	1.14 ± 0.10	0.11 ± 0.01

Conclusions. The enzyme coding for *ydiB* gene has an important role in the synthesis of AQ, and in the exchange of byproducts when glucose is depleted. Even when the inactivation of this gene does not increase the SA concentration, reduces QA and as consequence potentially streamlines purification processes of SA.
DHS concentrations do not have a relation with the *ydiB* gene, this metabolite is related to SA concentration.

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