PARTITION BETWEEN GLYCOLYSIS AND TCA CYCLE IN *E. coli* PTS⁻ STRAINS ENABLES PEP TO BE REDIRECTED TOWARDS AROMATIC PRODUCTION WHEN COUTILIZING GLUCOSE AND ACETATE

Andrea Sabido[1], Juan C. Sigala[2], Noemí Flores[1], Guillermo Gosset[1], Francisco Bolívar[1]. [1] Instituto de Biotecnología-UNAM. Departamento de Ingeniería Celular y Biocatálisis, Cuernavaca, Morelos 62210; [2] Universidad Autónoma Metropolitana, Unidad Cuajimalpa. Departamento de Procesos y Tecnología, México, D.F. 01120; asabido@ibt.unam.mx

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Introduction. Phosphoenolpyruvate (PEP) is one of the precursors involved in aromatic biosynthesis in *Escherichia coli*. The PEP:: carbohydrate phosphotransferase system (PTS) is the largest consumer of this metabolite. To increase intracellular PEP availability, *E. coli* JM101 strains devoid of PTS have been generated previously (1). Considering that PTS⁻ strains, lack glucose catabolite repression, we explored the possibility of separating glycolysis from the tricarboxylic acid cycle (TCA cycle) by deleting some genes of the PEP node and growing these strains in a mixture of glucose and acetate. Finally, we are evaluating the redirection of most of the PEP derived from glucose catabolism to the aromatic production in these PTS⁻ strains.

Methods. Mutant strains were constructed by deleting *pykA*, *pykF*, and *ppsA* genes using an established protocol (2). Cultures were done in baffled flasks on M9 minimal medium with 2g/L glucose plus 3 g/L acetate. RNA extraction and cDNA synthesis for RT-qPCR analysis were performed as reported elsewhere (3). The quantification technique used to analyze data was the $2^{-\Delta\Delta Cq}$ method (4). Transcription levels of the measured genes for the PB11 and PB12 strains respectively, were used as control to normalize data.

Results. PB11*pykAF⁻ppsA⁻* and PB12*pykAF⁻ppsA⁻* strains were able to reestablish their growth on glucose plus acetate compared to the JM101 derivative (Table 1).

Table 1. Specific growth rate ($\mu=h^{-1}$) on minimal medium with glucose plus acetate. ND: Not detected.

Strain	$\mu_{Glc+Ace}$	Strain	$\mu_{Glc+Ace}$
JM101	0.71	JM101 *pykAF⁻ppsA⁻*	ND
PB11	0.27	PB11 *pykAF⁻ppsA⁻*	0.16
PB12	0.41	PB12 *pykAF⁻ppsA⁻*	0.33

Under the same conditions, expression profile showed that *ppc* and *pckA* genes, which could connect glycolysis and TCA cycle in PB11*pykAF⁻ppsA⁻* are downregulated as compared to PB11 (Fig. 1a). In contrast, they maintained the same expression levels in PB12*pykAF⁻ppsA⁻* strain as PB12, while most of the central carbon metabolism genes are overexpressed in this mutant (Fig. 1b).

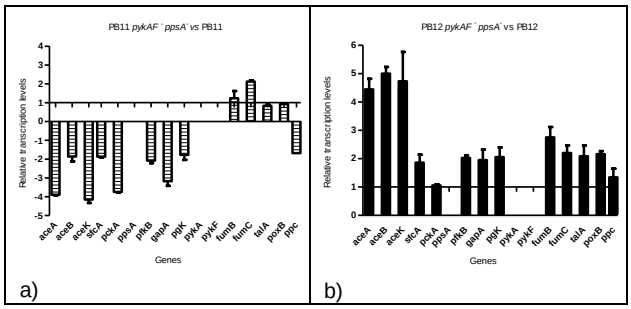

Fig.1 RT-qPCR values of some upregulated genes (1.6 fold or higher) or downregulated genes (-1.6 fold or lower) from PTS⁻ *pykAF⁻ppsA⁻* strains grown in glucose plus acetate. The PB11 and PB12 value for the measured genes was set as one.

Conclusions. Glycolytic and gluconeogenic metabolisms can apparently coexist as separated pathways on strain PB11*pykAF⁻ppsA⁻* when growing on glucose plus acetate. This proposition is also in agreement with results obtained on single substrates where no growth was detected in this mutant (data not shown). On the other hand, partial separation is achieved in PB12*pykAF⁻ppsA⁻* strain. Under this scenario, in PTS⁻*pykAF⁻ppsA⁻* strains most of the PEP derived from glycolysis could be redirected for the synthesis of aromatic compounds while acetate catabolism may supply biosynthetic intermediates and redox power from the TCA cycle.

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