METABOLIC RESPONSES TO PHOSPHOTRANSFERASE SYSTEM AND PYRUVATE KINASE KNOCKOUTS IN *Escherichia coli*

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Introduction. The glycolytic intermediate phosphoenolpyruvate (PEP) is a precursor in several metabolic pathways leading to the synthesis of valuable compounds. In *Escherichia coli*, the PEP:sugar phosphotransferase system (PTS) and the pyruvate kinase isozymes (PykA and PykF) are the main PEP consuming reactions when this bacterium grows on glucose(1). Therefore, these activities have been the target of genetic modifications with the aim of increasing PEP availability in *E. coli* production strains (2). However, the effects of such modifications on the central metabolic network are not known in detail.
The objective of this work was to determine carbon flux distribution and aromatics production capacity in *E. coli* strains lacking PTS and pyruvate kinase activities.

Methods. Strain VH33 is a PTS$^-$ glucose$^+$ derivative of *E. coli* W3110. Derivatives of VH33 were generated, lacking PTS and PykA (VH34) or PTS and PykF (VH35). Carbon flux analysis was performed employing [1-^{13}C] labeled glucose in cultures with minimal salts medium (3).

Results. Inactivation of PTS and PykA or PykF caused a reduction in the rates of growth and glucose consumption, as well as the elimination of acetate secretion (Table 1). These results suggested a rearrangement of carbon flux distribution as consequence of the genetic modifications.

Table 1. Growth kinetic parameters in minimal medium with glucose.

Strain	μ (h^{-1})	q_s (mmol/g/ h)	q_{ac} (mmol/g/ h)
W3110	0.69	16.1	4.31
VH33	0.45	6.9	0.23
VH34	0.44	4.2	0
VH35	0.36	4.2	0

To ascertain the effects of PTS and pyruvate kinase isozyme inactivation on central metabolism, flux analysis was performed. It revealed a redirection of carbon flux towards biomass formation pathways as consequence of PTS or PTS plus pyruvate kinase isozyme inactivation. This effect was more pronounced in the acetyl coenzyme A node, resulting in a severe reduction or elimination of overflow metabolism. A metabolic cycle involving PEP carboxylase (Ppc) and PEP carboxykinase (Pck) was detected in all strains, with a net flux value correlating inversely with the specific rate of glucose consumption. Inactivation of gene *pck* in the four studied strains caused a reduction in μ and q_s (Figure 1).

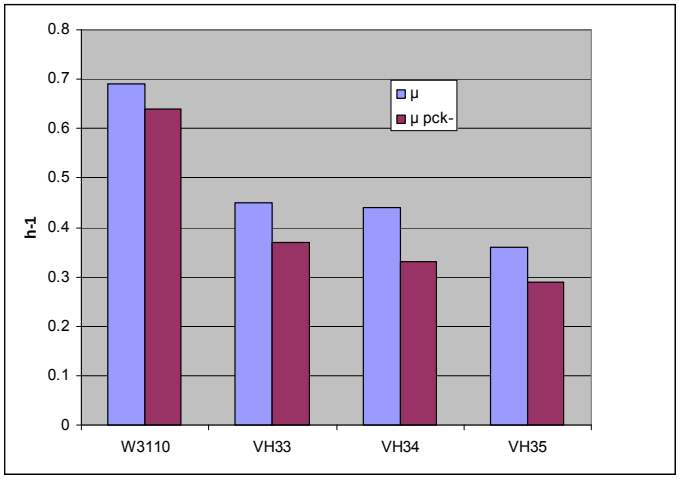

Fig.1 Growth kinetic parameters of *pck*$^-$ derivatives of strains W3110, VH33, VH34 and VH35.

Cultures were performed with derivative strains W3110A, VH33A, VH34A and VH35A that were modified for L-phenylalanine production. As consequence of PTS, PTS PykA and PTS PykF inactivation, 10, 4 and 7-fold higher aromatics yield from glucose were observed, respectively.

Conclusions. Inactivation of PTS and pyruvate kinase isozymes in *E. coli* causes a carbon limitation physiological state resulting in metabolic flux redirection towards biomass formation and carbon atom conservation. The metabolic cycle involving Ppc and Pck was found to have a positive effect on growth capacity in wild type and mutant strains. Aromatics production capacity did not increase as a result of PykA or pykF inactivation, since mutant strains are under a severe carbon limitation state.

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References.

1. Postma PW, Lengeler JW, Jacobson GR. (1993). Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol Rev.* 57:543-594.
2. Gosset G. (2005). Improvement of *Escherichia coli* production strains by modification of the phosphoenolpyruvate:sugar phosphotransferase system. *Microb Cell Fact.* 4:14.
3. Meza E, Becker J, Bolivar F, Gosset G, Wittmann C. (2012). Consequences of phosphoenolpyruvate:sugar phosphotranferase system and pyruvate kinase isozymes inactivation in central carbon metabolism flux distribution in *Escherichia coli*. *Microb Cell Fact*. 11:127.