PRODUCTION OF COUMARIC AND CINNAMIC ACIDS IN *ESCHERICHIA COLI* W3110 USING PAL/TAL FROM R. *GLUTINIS* AND *A. THALIANA*

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Introduction. Flavonoids are plant-secondary metabolites with beneficial properties on human health (1). Biosynthesis of flavonoids in plants is a continuation from the biosynthetic pathway of aromatic amino acids, where cinnamic acid (CA) and coumaric acid (pHCA) are the starting precursors. CA and pHCA are produced by the deamination of phenylalanine and tyrosine by action of phenylalanine ammonia lyase (PAL). Enzymes from PAL-family also have tyrosine ammonia lyase (TAL) activity (2). In this work the production of CA and pHCA in *E. coli* was evaluated using PAL/TAL enzymes from *Rhodotorula glutinis* and *Arabidopsis thaliana* as model enzymes.

Methods. PAL/TAL genes from *R. glutinis* and *A. thaliana* were cloned in plasmid pTrc99A, to generate plasmids pTrcPALRg and pTrcPALAt, these were individually co-transformed with plasmid pJLBaroG^{fbr}tktA (2) in *E. coli* W3110 strain. Derivative strains WPJPALRg and WPJPALAt were grown in mineral medium supplemented with glucose at 37°C and 300 rpm. IPTG 0.01 mM was used as inducer. Final concentrations of glucose, pHCA, CA and acetic acid were HPLC-determined.

Results. WPJRg produced both CA and pHCA acids; however, final accumulated levels were low (Fig 1, Table1). On the other hand strain WPJPALAt only produced CA with a final yield 6300-times higher than that from strain WPJPALRg. This high yield might be explained by its low rate of glucose consumption (q_S), allowing carbon flow redirection trough the recombinant CA synthesis.

Table 1. Growth, kinetic and production parameters from cultures of E. coli WPJRg and WPJAt strains.

Strain	μ (h-1)	q_S (mmolC/g_{cel}h)	$Y_{P/S}$ (μmol$_{pHCA}$/mmolC)	$Y_{P/S}$ (μmol$_{CA}$/mmolC)
WPJRg	0.39	62.0	0.047	0.075
WPJAt	0.28	35.1	-----	4.756

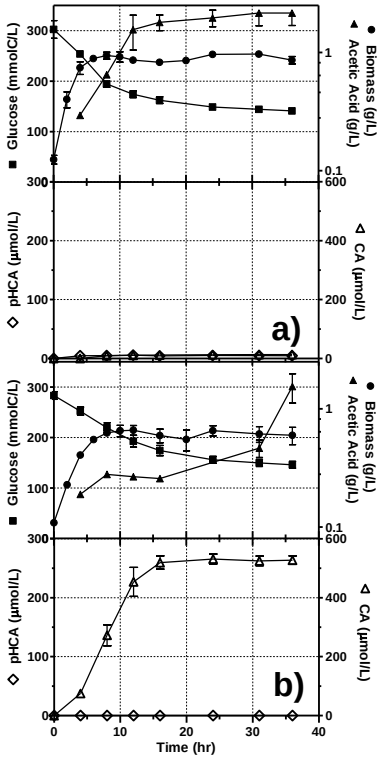

Fig.1. Growth and production kinetics from cultures of a) WPJRg and b) WPJAt strains.

Conclusions. *E. Coli* strain expressing PAL/TAL from *R. glutinis* produced CA and pHCA. Expression of PAL/TAL from *A. thaliana* in *E. coli* resulted in high yield production of CA, possibly due to a low q_S.

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