TRANSCRIPTOMIC ANALYLISIS OF *ESCHERICHIA COLI* (PTS- GLC+) OVERPRODUCING SHIKIMATE STRAIN IN FERMENTER IN ENRICHED MEDIUM USED FOR PRODUCTION

Larisa Cortés Tolalpa[1], Luz María Martínez Mejía [1], Rosa María Gutiérrez Ríos[2], Georgina Hernández[1], Ramón de Anda[1], Francisco Bolívar[1], Adelfo Escalante[1]. Instituto de Biotecnología, UNAM, [1]Departamento de Ingeniería Celular y Biocatálisis, [2] Departamento de Microbiología Molecular, Cuernavaca, Morelos, 6220. larisa_ct@hotmail.com

Key words: Escherichia coli, global transcriptome analysis, shikimate

Introduction. The shikimate (SHK) is used as a synthetic precursor of oseltamivir phosphate (OSF), a potent antiviral against seasonal influenza virus A, B, H5N, A/H1N1. Since 2005 it was shown that the production of OSF is insufficient to satisfy the requirements of the global population, particularly in vulnerable countries like Mexico. The SHK production in engineered strains of *Escherichia coli* from glucose as substrate by biotechnological processes is an attractive alternative compared with the traditional extraction process from Chinese star anise fruits (*Illicium verum*). Applying Metabolic Engineering Pathways (MEP) was generated the E. coli strain PB12.SA22 (PTS-,aroL-,aroK-,aroGfbr,tktA,aroB,aroE) which produces 7.05 g/L of SHK with the highest yield reported so far (1).
In this contribution we report for the first time a global transcriptome analysis (GTA) of a SHK overproducing strain of E. coli in production medium in using the Affymetrix® high density microarrays system.

Methods. Batch cultures of 500 mL in 1 L fermentor using complex production broth were performed as described previously (1). Total high quality RNA was extracted by a modification of the hot phenol-chloroform method in exponential phase (EXP) early stationary (STA) and late stationary (STA2). Global Transcriptome was determined by using the high-density microarray Affimetrix GeneChip® *E. coli* Genome 2.0. Expression data from 4070 geness were mined to identify differentialy expressed genes (upregulated and downregulated) between EXP/STA1, EXP/STA2 and STA1/STA2 stages by the Rank Product method (RP) (2).
Results. For each expression data the RP method calculated a False Discovery Rate (FDR). Those genes with a FDR value = 0 have the highest probability of biological relevance. To identify differentially expressed (upregulated or downregulated) genes among compared growth conditions, a FDR value cutoff = or <0.05 was used to identify those genes differentially expressed (UR or DR). Selected genes were first classified in functional categories according on the gene ontology classes used in the EcoCyc database (ecocyc.org) for *E. coli* MG1655 strain (Table 1). Analysis of EXP/STA1 showed 123 genes differentially expressed genes (43 UR genes and 80 DR genes); EXP/STA2 showed 65 differentially expressed genes (37 UR and 38 DR genes), and analysis of STA1/STA2 showed 97 differentially expressed genes (50 UP and 47 DR genes).
In EXP stage was found the over-expression of several genes related to transport and catabolism of amino acids and carbohydrates controlled by catabolite repression. This suggest that PB12.SA22 strain has an increased cAMP intracellular concentration in response to glucose limitation state, which favors the expression of catabolic enzymes and transporters which allows exploiting several carbon sources in the yeast extract despite the presence of glucose in the medium (3). It was found over-expression of genes that codified the iron citrate ABC transporter, this protein is exclusively expressed when intracellular iron concentrations are low and the iron citrate is present in the medium suggesting that the strain has low intracellular iron concentration probably because its low capability to synthesize enterobactin, the main siderophore produced by *E. coli*. The over-expression of genes related to proline import suggests that the strain uses this amino acid to counteract the effects of the osmolarity of the medium.
In STA stage it observed the over-expression of genes related to amino acid biosynthetic pathways and the sub expression of genes related to transport and catabolism, suggesting that this strain has consumed the vast majority of the compounds in the fermentation broth. Additionally, in STA it was also observed the over-expression of genes involved in several stress response, particularly those involved in decreasing pH increasing acidity, probably related SHK production and nutrient depletion. These results support the proposition that this strain exhibits a more glycolytic than gluconeogenic metabolism (4).

Table 1. Number/functional categories of differentially expresses genes in PB12.SA22 strain during SHK production cultures.

Category[a]	Condition analyzed					
	EXP/STA1		**EXP/STA2**		**STA1/STA2**	
	UE	DR	UR	DR	UR	DR
Adaptation		5	4		13	
Metabolism of sulfur compounds	2		3		2	
Energy metabolism / transport, electron acceptor	4	9	2			
Metabolism, biosynthesis of other compounds		2				1
Metabolism, degradation of macromolecules	1					
Metabolism, carbon utilization, amino acids	8	13	8		13	4
Metabolism, carbon utilization, amino acids/nucleotide		2				
Metabolism, carbon utilization, carbohydrates				1		2
Metabolism, carbon utilization, nucleoside/nucleotide	5	1	1		2	2
Other		1		1		
Cellular processes	2	15	4	13	5	10
Conserved protein with no defined function or prediction	3	18	4	9	2	11
Regulators	2	14		9		2
Transport system	16		10	5	7	11
Total	43	80	37	38	50	47

[a] Base on the orthologous gene classification scheme used in EcoCyc Database (ecocyc.org); UR, upregulated, DR, downregulated.

Conclusions. This is the first work that explores the GTA of SHK overproducing strain in a production system, which imposed the challenge of establishing a RNA extraction methodology to satisfy the RNA requirements of quality and concentration. The results of the application of this omic technique allowed the proposal of a second MEP strategy over the strain PB12.SA22 which takes into account the strain transcriptomic response that triggers the rich medium and the production conditions, unlike other studies using minimum medium. It was also possible to propose a model that explains the particular overproducing strain behavior in the production system.

Acknowledgements. This project was supported by CONACyT FONSEC Salud Proyecto 12679, CONACyT Ciencia Básica 105782 and 106428, DGAPA-PAPIIT IN227409 PAPIIT IN224110, IN205811 and IN206812.

References.

1. Escalante, A., Calderón, R., Valdivia, A., De Anda, R., Hernández, G., Ramírez, O., Gosset, G., Bolívar, F. (2010) *Microb. Cell Fac.*, **9:**21.
2. Breitling R, Herzyk P: Biological master games: using biologists reasoning to guide algorithm development for integrated functional genomics. *OMICS* (2005), **9**: 225-232.
3. Death A, Notley L, Ferenci T: Derepression of LamB protein facilitates outer membrane permeation of carbohydrates into *Escherichia coli* under conditions of nutrient stress. *J. Bacteriol.* (1993), **175:**1475-1483.
4. Hardiman T, Lemuth K, Keller M, Reuss M, Siemann-Herzberg M: Topology of the global regulatory network of carbon limitation in *Escherichia coli*. *Journal of Biotechnology* 2007, **132:**359-374.