DEVELOPMENT OF A METHODOLOGY FOR THE ANALYSIS OF THE DISTRIBUTION ON CARBON FLUXES IN A *Escherichia coli* STRAIN PRODUCING SHIKIMIC ACID.

Susy Carmona, Francisco Bolivar, Adelfo Escalante. Instituto de Biotecnología. UNAM. Departamento de Ingeniería Celular y Biocatálisis. Av. Universidad 2001, Col. Chamilpa Cuernavaca, Mor.; susybcc@ibt.unam.mx.

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Introduction. Omic tools help to elucidate the metabolic state of the cell. Metabolic flux analysis (MFA) provides important information about the metabolism functioning of an organism[1]. In the MFA with isotopic labeling, the biological system is fed with a labeled substrate and its atoms are distributed among the metabolites of the biochemical network until steady-state conditions, the isotopic enrichment of the metabolites can be determined by mass spectrometry (figure 1).

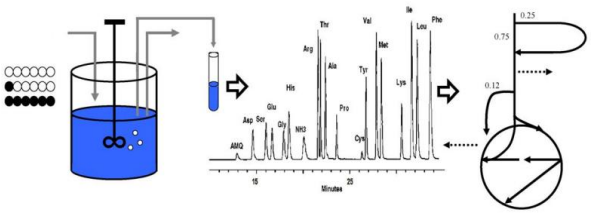

Fig.1 MFA Methodology. 1.-Cultivation with labeled substrate, 2.- measurement of isotopic enrichment and other extracellular flows and 3.- integration of experimental data in a mathematical model.

Shikimic acid (SA), an intermediate in the biosynthetic pathway of aromatic compounds is used as precursor in the chemistry, food and pharmaceutical industries[2]. Different SA producing strains were obtained with the object to have an available source of SA[3]. The objective of this work is to optimize the steps of sample preparation for the GC-MS analysis in order to obtain the necessary data required by a mathematical model for MFA in a SA producing strain of *Escherichia coli* and its parental strain.

Methods. Batch cultures with 1-^{13}C Glc, 6-^{13}C Glc and natural labelling glucose as substrate in minimal media were performed for both strains. Kinetic and production parameters were determined. Aliquots of different volumes of culture were harvested, the pellets were dried, hydrolyzed, neutralized, once filtered samples were lyophilized[4] and derivatized. For derivatization reactions N,O-bis (trimethylsilyl)acetamide (BSA) and N -Methyl- N -(tert –butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) and several solvents were employed. Isotopic enrichment of the proteinogenic aa in the sample was determined by GC-MS.

Results. Acetonitrile and chloroform were the best solvents, however acenonitrile allowed identify higher amount of aa than chloroform; the derivatization reagent BSA only allowed identify 7 aa, while with MTBSTFA were detected 15 of the 20 aa (figure 2).

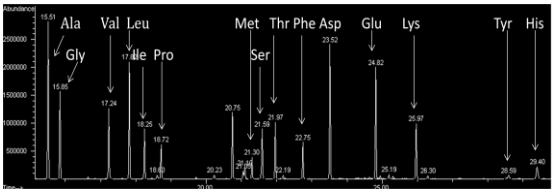

Fig.2 GC-MS analysis of tert-butyl dimethylsilyl (TBDMS) derivatives of aa.

Fragmentation pattern for each aa was obtained and from the relative abundances for the different peaks the isotope mass distribution (IMD) (figure 3 and table 1) was calculated.

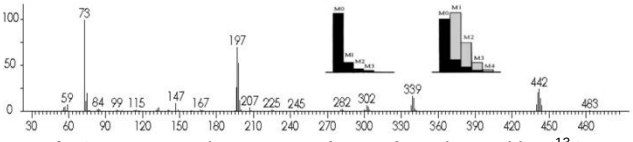

Fig.3 Fragmentation pattern of Hys of a culture with 1-^{13}C glucose of PB12.SA12 producing strain

Table 1. IMD for the fragment M-57 for some proteinogenic aa of a culture with 1-^{13}C glucose of PB12.SA12 producing strain.

				IMD (%)	
Compound	Considered fragment	m (m/z)	m+x (m/z)	m (m/z)	m+x (m/z)
Ala-(TBDMS)2	M-57	260	261	40.45	40.31
Gly-(TBDMS)2	M-57	246	247	69.89	20.50
Met-(TBDMS)2	M-57	320	322	14.71	29.88
His-(TBDMS)2	M-57	440	443	10.61	29.10

Conclusions. Derivatization reagent and solvent play a role important in the appropiate identification of proteinogenic aa. It was possible calculate the IMD corresponding to M-57, M-159 and M-15 fragments for each aa for both strains. This data will be incorporated in a mathematical model for MFA in a SA producing strain of *E. coli* and its parental strain.

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References. 1.- Iwatani S *et al.* (2008). *Biotechnol. Lett.* 30:791–799. 2.- Krämer M, *et al.* (2003) Met. Eng. 5.4:277-283. 3.- Escalante A, *et al.* (2010). *Microb. Cell Fact.* 9.21:1-12. 4.- Wittmann C. (2007). *Microb. Cell Fact.* 6.6:1-17