CONSTITUTIVE EXPRESSION OF SELECTED GENES FROM THE PENTOSE PHOSPHATE AND AROMATIC PATHWAYS INCREASES THE SHIKIMIC ACID YIELD IN HIGH-GLUCOSE BATCH CULTURES OF AN *Escherichia coli* STRAIN CARRYING SEVERAL CHROMOSOMAL INACTIVATIONS

Alberto Rodriguez, Juan A. Martínez, José L. Báez, Noemí Flores, Georgina Hernández-Chávez, Octavio T. Ramírez, Guillermo Gosset, Francisco Bolivar. Departamento de Ingeniería Celular y Biocatálisis. Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), A.P. 510-3, Cuernavaca, Morelos, 62250, México. e-mail: rrja@ibt.unam.mx

Keywords: shikimic acid; synthetic operon; aromatic compounds

Introduction. Shikimic acid (SA) is an intermediate compound in the aromatic amino acid biosynthetic pathway in plants and bacteria, utilized as starting material in the chemical synthesis of the anti-influenza drug Tamiflu®. To date, engineered *E. coli* strains can accumulate up to 85g/L of SA using fed-batch processes (1); however, feeding high amounts of glucose (Glc) has invariably rendered low conversion yields (below 33% of the theoretical maximum). This effect can be partially attributed to heterogeneities on the intensity and temporality of the required gene expression, imposed by non-optimal production systems. An alternative platform to overproduce SA is presented in this work, based on plasmid-driven constitutive expression of a six-gene synthetic operon in a laboratory-evolved *E. coli* strain carrying several chromosomal inactivations.

Methods. A synthetic operon was built by sequential cloning of six coding sequences (*aroB, tktA, aroG*^{fbr}*, aroE, aroD* and *zwf)* with consensus Shine-Dalgarno sites, controlled by a single constitutive Trc promoter and inserted into a high-copy plasmid containing a region that confers segregational stability (2). The constructed plasmid, pTrcAro6, was transformed into a modified PB12 strain (3) with inactive *ptsHIcrr, aroK, aroL, pykF,* and *lacI* genes. The resulting production strain, AR36, was evaluated in batch fermentors using mineral media containing three different concentrations of Glc and yeast extract (YE).

Results. SA accumulation was observed from the beginning of the cultures reaching a maximum titer of 43g/L in 30h. In all cases, the yields on Glc of SA and of total aromatic compounds were about 50% and 60% of the theoretical maximum, respectively. Despite the consumption of 100g/L of Glc by strain AR36 in batch-mode fermentors, relatively low titers of acetate and aromatic byproducts were detected, with SA accounting for 80% of the produced aromatic compounds.

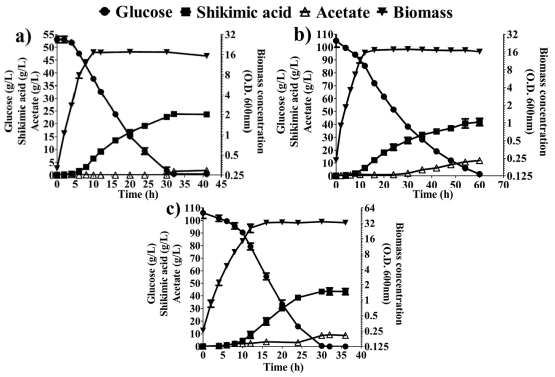

Fig. 1 Fermentation profiles of strain AR36 grown in 1L bioreactors with three different substrate concentrations. a) 50g/L of Glc and 15g/L of YE; b) 100g/L of Glc and 15g/L of YE; c) 100g/L of Glc and 30g/L of YE.

Substrate concentration	50g/L Glc + 15g/L YE	100g/L Glc + 15g/L YE	100g/L Glc + 30g/L YE
SA titer (g/L)	23.80 ± 0.00	41.80 ± 2.83	43.30 ± 0.57
Glc consumed (g/L)	52.65 ± 1.20	103.70 ± 6.79	105.55 ± 4.45
Duration of culture (h)	32	60	30
Acetate titer (g/L)	1.45 ± 0.00	11.90 ± 0.14	8.65 ± 0.92
$Y_{SA/Glc}$ relative to Y_{max} (%)	54	49	49
$Y_{TAC/Glc}$ relative to Y_{max} (%)	67	61	63
SA : TAC (%)	81.4	79.8	80.3

Table 1. Comparison of titers and yields determined from the fermentations depicted in Fig.1. $Y_{SA/Glc}$ = yield of SA from Glc; $Y_{TAC/Glc}$ = yield of total aromatic compounds from Glc; Y_{max} = maximum theoretical yield; SA:TAC = molar percentage of SA to total aromatic compounds.

Conclusions. The obtained SA titers and yields represent the highest reported values for a high-substrate batch process, making this strategy an interesting alternative to the traditionally employed fed-batch processes for SA production.

Acknowledgements. Work supported by grants 105782 (CONACyT) and IN205811 (PAPIIT-UNAM).

References.

1. Chandran, S., Yi, J., Draths, K., von Daeniken, R., Weber, W., Frost, J. (2003). *Biotechnol prog*, vol.19:808-814.
2. Zurita, M., Bolivar, F., Soberón, X. (1984). *Gene*, vol.28:119-122.
3. Aguilar, C., Escalante, A., Flores, N., de Anda, R., Riveros-McKay, F., Gosset, G., Bolivar, F. (2012). *BMC Genomics*, vol.13:385.