SHIKIMATE PRODUCTION IN *ESCHERICHIA COLI* STRAIN PB12.SA22R IN BATCH CULTURE AT 40 °C

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Introduction. Shikimate (SHK) (3, 4, 5-trihydroxy-1-cyclohexene-1-carboxylic acid) is a naturally occurring compound, important intermediate in the biosynthesis of lignin, aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and most alkaloids of plants and microorganisms.SHK and other aromatic pathway intermediates are of industrial importance for the production of various chemicals such as herbicides, anti-bacterial agents. Recently, SHK has emerged as a key molecule for the synthesis of the neuraminidase enzyme inhibitor, Oseltamivir phosphate (OSF) which is used as an oral antiviral for prevention and treatment several influenza infections including and recently the A/H1N1 influenza virus (1). As SHK is extracted traditionally form the fruit of Chinese starry anise (*Illicium verum*), this process results in low yields. Recently, several engineered strains of *Escherichia coli* have been developed to overproduce SHK by fermentative process (2).
In this contribution we report the production of SHK in an engineered strain of *E. coli* in batch cultures evaluating different temperature conditions.

Methods. Strain PB12.SA22 (2) was used as genetic background for the generation of a PB12.SA22R *pykF-* derivative by was obtained by transduction with phage P1 using the PB28 strain as *ΔpykF* donator. Batch culture fermentations were performed by triplicate in bioreactors at 37° and 40 °C (Applikon), nominal volume of 1 L with a working volume of 0.5 L, equipped with a console (ADI 1025, Applikon) and a controller (ADI 1010, Applikon) in order to set parameters such as temperature, pH, agitation and aeration, using the production medium.
Samples were withdrawn and growth, substrate consumption and SHK, and other aromatic intermediates were determined.

Results. Resultant strain PB12.SA22R *pykF-* was cultured at 37 °C, showing a clear decrease in SHK production compared with parental strain PB12SA22 (3.5 and 7.8 g/L SHK, respectively). However, when culture temperature was increased to 40 °C SHK production raised to the same SHK production level observed for the parental strain 7.8 g /L.
The TOPO plasmids are vectors with high copy number due to mutation of regulatory RNA I replication origin (3). The effect of this mutation is manifested when the cell is cultured at 40-42 °C. Resulting in an increase in the number of copies of cloned genes *aroB* and *aroE*, coding for two limiting enzymes of SHK pathway.

Conclusions. The amount of SHK obtained in strain PB12.SA22R *pykF-* at 40 ° C was due to an increment in plasmid copy number at this temperature, resulting in an an increase in the number of genes coding for enzymes involved in the production of SHK, and not necessarily to an increased flow of carbon into the aromatic pathway

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