SCREENING, CHARACTERIZATION AND CLASSIFICATION OF 3-DEOXY-D-*ARABINO*-HEPTULOSONATE 7-PHOSPHATE (DAHP) SYNTHASE ENZYMES FROM A SOIL METAGENOMIC LIBRARY

Uriel Gutiérrez-Gómez, Francisco Bolívar, Adelfo Escalante. Universidad Nacional Autónoma de México, Instituto de Biotecnología, Cuernavaca, Morelos 62210, México
E-mail: uriel@ibt.unam.mx

Keywords: Escherichia coli; DAHP; metagenomics

Introduction. The shikimate pathway (SP) is a set of seven reactions involved in the synthesis of aromatic compounds. The first reaction of the SP comprises the aldolic condensation of D-erythrose 4-phosphate (E4P) and phospho*enol*pyruvate (PEP) to produce 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP); this reaction is catalyzed by the enzyme DAHP synthase (DAHPS). In biotechnology, the DAHPS is a key enzyme in order to increase carbon flux from central carbon metabolism to SP in engineered strain for aromatic metabolite production purposes. A powerful approach in functional metagenomics is heterologous expression; this approach has the potential to identify new classes of genes or operons for new or know functions, and it does not require a previous sequence analysis.
The objective of this work is to detect and characterize DAHP synthases from a soil metagenomic library, in order to identify DAHPS with potencial biotechnological utility and contribute with new information about DAHPS diversity.

Methods. The functional screening of DAHPS was realized by transforming an *E. coli* strain, lacking of DAHPS activity Δ*aroF* Δ*aroG::kan* Δ*aroH* (DAHPS$^-$) [1], with an aliquot of DNA extracted from soil metagenomic library constructed using a plasmid as a vector cloning [3]. Transformed *E. coli* strain was growth in M9S minimal medium; the heterologous complementation was observed by restitution of bacterial growth. The sequences of DNA inserts obtained (hypothetical DAHPS) were analyzed in order to find homology with current DAHPS sequences; furthermore, we analyzed some of representative DAHPS sequences and its genomic context in order to update the current classification of DAHPS.

Results. Growth of *E. coli* DAHPS$^-$ mutant in M9S medium implies the restitution of DAHPS activity and synthesis of final products of SP; we obtained 138 clones. The plasmid DNA of each clone was digested with *Pvu* II and *Bve* I enzymes, obtaining 19 unique restriction patterns. These 19 clones will be considered for DAHPS specific activity assays and characterization of bacterial growth in M9S medium. Therefore, we sequenced the DNA inserts of these 19 clones and were analyzed by nucleotide and protein BLAST (homology search with DAHPS sequences). Considering two sequences of representative DAHPS (*E. coli* and *B. subtilis*) we did a phylogenetic analysis in two perspectives: 1) genomic context [2] of microbial DAHPS, AroA$_{I\alpha}$ (*E. coli*-like) and AroA$_{I\beta}$ (*B. subtilis*-like); and 2) analysis of DNA inserts and current classes of DAHPS: microbial DAHPS, representative DAHPS of higher plants (AroA$_{II}$), aminoDAHPS, and DAHPS involved in phenazine biosynthesis (PhzC).

Conclusions.
- We obtained 138 clones which recovered DAHPS activity and growth. From these, we identified 19 unique clones.
- Among these 19 inserts, DNA size is variable (from 100 bp to 3 kb); the average GC content is about 73%.
- Nucleotide BLAST or protein BLAST showed no homology with our 19 DNA inserts, which suggests the existence of new sequences of DAHPS.

Acknowledgement. This work was supported by CONACyT project 106428.

References.

1. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H (2006) *Mol Syst Biol*. 2, 2006.0008.
2. Ciria R, Abreu-Goodger C, Morett E, Merino E (2004) *Bioinformatics*. 20(14):2307-2308.
3. Sirena-Sánchez N (2009) Tesis de Maestría, UNAM. México.