



HETEROLOGOUS EXPRESSION OF ANTHRANILATE SYNTHASES OF Streptomyces coelicolor A(3) IN Escherichia coli.

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Introduction. Anthranilate (o-aminobenzoate ANT) it's a highly valuable industrial compound. Current production methods start from nonrenewable sources such as oil derivates. A Escherichia coli (E. coli) strain that produces ANT through the reaction catalyzed by the enzyme anthranilate synthase (AS, EC 4.1.3.27) have been reported (1). Allosteric control of the AS is a main problem for the overproduction of ANT (1). Streptomyces coelicolor A(3) (S. coelicolor) has clusters of genes that code for enzymes of secondary metabolism pathways that are homologous to some enzymes from central metabolism. These enzymes might not be feed-back regulated by the end products of primary metabolism since their pathways synthesize different products.

The aim of this work was to characterize possible AS, by studing their activity in an heterologous system.

Methods. The genes SCO2117 and SCO3214-SCO3213 from the wild strain *S. coelicolor* A(3) where cloned in the expression vector pTrc99a (2). Plasmids pSC2117 and pSC3214-3213 where transformed in strain BW25113*trp*E- (3) for functional complementation assays. These assays where carried out in minimal medium M9 with 10 g/L of glucose as carbon source and supplemented with 0.1 % of casamino acids. Cell growth was measured in a colorimeter KlettScienceware®.

Results. Plasmid pSC2117 complemented the auxotrophy of the mutant strain and this is independent of the level of induction. Plasmid pSC3214-3213 partially complemented the auxotrophy of the strain and this effect is dependent on the level of induction.

Conclusions. Genes SCO2117 and SCO3214-3213 code the biological function of AS and they are expressed correctly in *E*. coli.

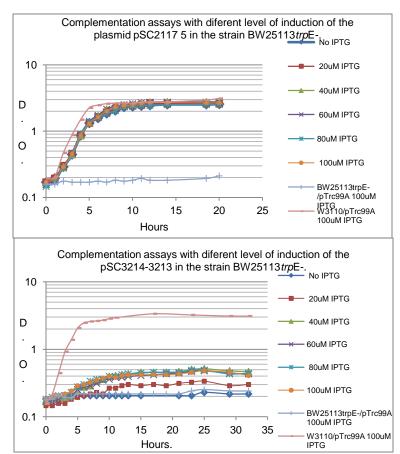


Fig.1. Assay of functional complementation. A IPTG gradient where performed for each one of the strains.

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References.

1. Balderas-Hernández, V. E., Sabido-Ramos, A., Silva, P., Cabrera-Valladares, N., Hernández-Chávez, G., Báez-Viveros, J., L., Martínez, A., Bolivar, F., and Gosset, G. (2009). Microbial Cell Factories 8-19.

2. Amann E., Ochs B. Abel K.-J. (1988). Gene vol. 69, 301-315.

3. Baba T., Ara T., Hasegawa M., Takai Y., Okumura Y., Baba M., Datsenko K. A., Tomita M., Wanner B. L. and Mori H. (2006). *Mol. Sys. Biol.*