

APRESENTAÇÃO DE PÔSTER - GENÉTICA DE MICRORGANISMOS

PROSPECTION OF NOVEL ENZYMES FOR MICROBIAL PRODUCTION OF MUCONIC ACID

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Currently, the chemical industry is considered one of the main causes of anthropogenic greenhouse gas emissions, due to its heavy dependence on fossil resources. Thus, the development of an efficient biotechnology industry, based on renewable resources, is essential to reduce environmental impacts and contribute to more sustainable development, meeting the global demand for bioproducts. Among these bioproducts, there is a growing interest in muconic acid (MA), an unsaturated dicarboxylic acid, which can be functionalized into various biopolymers that are indispensable to modern society, such as PET and nylon-6,6. The use of microorganisms as platforms that convert renewable raw materials into MA has been considered a promising alternative for the

development of more sustainable processes. In silico studies conducted by our research group indicated that the metabolic pathway derived from the natural intermediate 3-dehydroshikimate (via DHS-to-MA) presents the highest maximum theoretical yield of MA, using glucose as substrate. This pathway is composed of three heterologous reactions: (1) conversion of DHS into protocatechuate (PCA); (2) conversion of PCA to catechol and (3) conversion of catechol to MA, catalyzed by DHS dehydrogenase, PCA decarboxylase, and catechol 1,2-dioxygenase, respectively. According to the literature, the low activity of PCA decarboxylase limits the yield of this pathway. In this context, this work aimed to prospect new potential genes for PCA decarboxylase and compare their performances against the EcaroY gene from *Enterobacter cloacae*, widely used in the literature. Two novel genes sequences coding (meta1 and meta2) have prospected from metagenomic data set. To test the performance of these potential aroY variants, three strains of *E. coli* were constructed: EcaroY, meta1, and meta2 genes were individually cloned into the pRSM6 plasmid that already contained the other two genes of the heterologous MA production pathway, aroZ from *B. thuringiensis* and catA from *C. glutamicum*. The performance of these three strains was tested in shake flasks experiments for 72 hours using minimal medium and 20 g/L of glucose. The strains containing the EcaroY and meta1 genes were able to produce 0.20 ± 0.02 and 0.11 ± 0.01 g/L of MA, respectively. The same behavior was not observed for meta2 gene. These results indicate that the novel meta1 gene, discovered in this work, was capable of efficiently converting PCA to catechol, enabling the production of MA