USE OF SITE-DIRECTED MUTAGENESIS IN THE UNDERSTANDING OF THE CATALYTIC ACTIVITY OF THE HUMAN SACCHAROPINE DEHYDROGENASE (SDH) ENZYME.
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Abstract
The Saccharopine Pathway is the main degradation pathway of Lysine in plants and animals. Defects in the ALDH7A1 gene causes PDE, a rare epilepsy disease. The project aims to produce mutated SDH domain of the AASS enzyme, the main enzyme responsible for the conversion of Saccharopine in AASA(Aminoadipate Semialdehyde). Being an important enzyme for the Lysine Catabolism, the mutated SDH domain can then be used to verify the important residues via enzyme kinetics.

Key words: Mutagenesis, Lysine, AASS.

Introduction
The Saccharopine pathway is the main degradation pathway of Lysine in plants and animals. The first gene of the pathway, AASS, encodes the bifunctional enzyme Aminoadipate-Semialdehyde Synthase, responsible for the conversion of Lysine in Aminoadipate Semialdehyde (AASA) via the intermediate Saccharopine. AASA is then oxidized to Aminoadipic Acid by AASA Dehydrogenase enzyme (ALDH7A1). Defects in this enzyme causes Piridoxine Dependent Epilepsy, resulted from toxic accumulation of AASA and its cyclic form piperideine-6-carboxylate (P6C). Recently our group have solved the structure of the Saccharopine Dehydrogenase (SDH) domain of human AASS enzyme co-crystallized with NAD+.

Results and Discussion
The selected residues (Y105, Y127, D153, W201 and R275) were mutated to Alanine using Q5® Site-Directed Mutagenesis Kit. Potential mutant clones were screened by Sanger sequencing using the ABI3730 platform. Final contig sequences were obtained using the softwares Phred, Phrap and Consed. Mutations were validated by alignment of the potential mutant and wild type SDH sequences and manual curation of the chromatograms.

Image 1. A Blast alignment of the SDH mutated sample against the SDH without the mutation.

Image 2. Four bacterial plates containing clones with the desired mutation in the specific point. This plating was then sequenced to validate the accuracy of the mutagenesis process.

Conclusions
We expect to see reduced activity in case the mutated residue is important for catalysis. This project will provide basis for the understanding of the activity and catalytic site of Saccharopine Dehydrogenase (SDH).

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