Adipic acid is one of the most important dicarboxylic acid for commercial purposes, mainly used as building block for nylon polymers. Around three million tons per year of adipic acid are produced by chemical synthesis using petrochemical resources. The current chemical production process has serious consequences for the environment. Therefore, the implementation of a by a bio-based process using renewable feedstocks would be highly beneficial for the society and the environment.

The study of microbial metabolic pathway to produce adipic acid using L-lysine as precursor is a potential alternative. The first step of the pathway converts L-lysine into 6-aminohex-2-enoic acid (6-AHEA) (Fig. 1), however, no enzymes able to carry out this reaction have been disclosed yet. The main goal of the research is the generation a novel enzyme activity for this conversion.

### STUDY OF HISTIDINE and 3-METHYLSAPRATATE AMMONIA-LYASES

The enzymatic activity necessary to catalyze the required deamination is defined as ammonia lyase and results in the removal of the α-amino group. Histidine ammonia lyase (HAL) and 3-methylaspartate-ammonia lyase (MAL) are known and characterized enzymes acting on histidine and 3-methylaspartate, respectively. They were selected among the top candidates to be engineered to catalyze the deamination of L-lysine.

HAL from Pseudomonas putida and MAL from Clostridium tetanomorphum and Carboxydothermus hydrogenoformans were recombinantly produced in E. coli and purified. The capability of these enzymes to catalyze L-lysine deamination was tested (Figure 2).

None of the native ammonia-lyases tested showed activity towards L-lysine in the tested conditions (Fig. 2 A-C). On the other hand, L-lysine was proven to be a competitive inhibitor of HAL (Fig. 2D). The constant of inhibition ($K_i$) obtained was 5.6 ± 1.1 mM and the relation $K_i / K_m$ was 1.45, which showed that L-lysine is a strong inhibitor of HAL.

### COMPUTATIONAL STUDY AND SITE-DIRECTED MUTAGENESIS ON MAL

Computational structural biology methodologies were combined with protein engineering techniques in order to design MAL mutant enzyme variants potentially active on L-lysine. 

*In-silico* saturation mutagenesis tools were used to model all the possible mutations in the active site or its surroundings that are expected to increase affinity for the L-lysine substrate. Following the results of the structural study and using a rational design, the residues Cys361 and Leu384, located in the binding pocket, were mutated.

The MAL mutant variants were produced and purified, and the activity on the natural substrate (3-methyl aspartate) and L-lysine was tested by monitoring the production of 6-AHEA and the release of ammonia (Fig. 4). The kinetic constants towards 3-methyl aspartate were impaired to different extents and no L-lysine deamination was observed.

### CONCLUSIONS

The studied HAL and MAL did not show the capability to deaminate lysine. On the contrary, L-lysine was showed to be an inhibitor on HAL. Preliminary results obtained in the protein engineering of MAL did not showed positive results. On going studies are being performed.