

ENGINEERING OF ARTIFICIAL ENZYMES TOWARD THE CONSTRUCTION OF A NEW METABOLIC PATHWAY FOR 2,4-DIHYDROXYBUTYRIC ACID PRODUCTION

Romain Irague¹⁻⁴, Yannick Malbert¹⁻⁴, Thomas Walther¹⁻⁴, Christopher M. Topham¹⁻⁴, Clément Auriol¹⁻⁴, Clémentine Dressaire¹⁻⁴, Audrey Baylac¹⁻⁴, Marion Stodel¹⁻³, Manon Carmona¹⁻³, Hélène Cordier¹⁻⁴, Isabelle André¹⁻⁴, Marc Maestracci⁵, Robert Huet⁵, Magali Remaud-Simeon¹⁻⁴, Jean Marie François¹⁻⁴

¹Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Ranguueil, 31077 Toulouse, France; ²INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France; ³CNRS, UMR5504, Toulouse, France; ⁴TWB, 3 rue des Satellites, Canal Biotech Building 2, 31400 Toulouse, France; ⁵Adisseo SA, Antony Parc II, 10 place General de Gaulle, 92160 Antony, France.

Key Words: 2,4-Dihydroxybutyric acid, engineered enzymes, semi-rational design, computer-based engineering, artificial metabolic pathway, screening

Methionine is an essential amino acid that is required in animal diet and is the first limiting amino acid in poultry. Its world market is huge and keeps growing. Today, most of the commercialized methionine is chemically synthesized and leads to mixtures of D- and L-enantiomers. To develop alternative and bio-based production routes, the past ten years have seen the development of novel modes of production of the pure enantiomer L-methionine by fermentation of engineered microorganisms. Besides, the chemical synthon 2,4-Dihydroxybutyric acid (DHB) is a promising molecule as it may serve as a precursor of methionine analogue (2-hydroxy-4-(methylthio)butyrate). However, petrochemical synthesis of DHB is not economically viable. In addition, no natural metabolic pathways exist for the biochemical production of this compound.

In this work, the focus was placed on the design of a new and artificial metabolic pathway dedicated to DHB production. The idea was to change the homoserine pathway that starts with aspartate to a DHB pathway starting from malate. To this end, three catalysts had to be engineered, which include one malate kinase, one malate semi-aldehyde dehydrogenase and one malate semi-aldehyde reductase activities (Figure1). Candidate enzymes acting on sterically cognate substrates were first identified using data mining. Following a computer-aided design strategy, small libraries of mutants were generated for each enzyme and screened with medium-throughput microtiter plate assays. Mutants displaying the desired substrate specificities were sorted out and used to produce DHB.

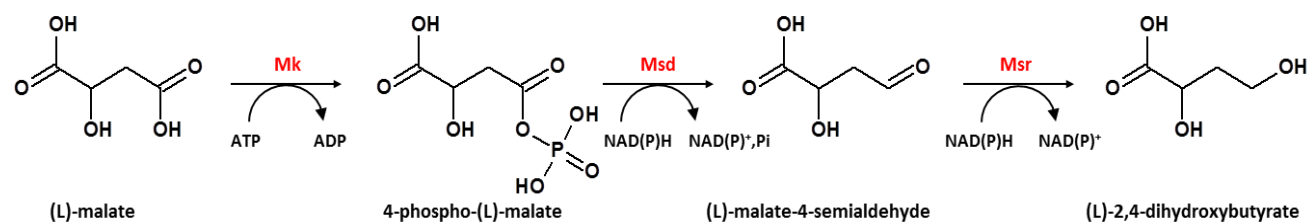


Figure 1. Synthetic pathway for the production of 2,4-Dihydroxybutyric acid. The three artificial catalysts depicted in red correspond to a malate kinase (Mk), a malate semialdehyde dehydrogenase (Msd) and a malate semialdehyde reductase (Msr) enzyme.