153d Determination of the Fractional Contribution of Individual Elementary Modes to the Overall Metabolism of *Escherichia Coli*

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Elementary Mode Analysis (EMA) is a powerful tool that simplifies the complex metabolism of a cell into a set of unique, indivisible elementary modes. The operating space for the metabolic network is defined by a positive, linear combination of these elementary modes that each contribute to the overall metabolism. The degree of contribution can be expressed by a set of unique weighting factors. These weighting factors are experimentally accessible by measuring the production rates of metabolites. Typically a system has many more elementary modes than measurable metabolites resulting in an underdetermined system restricting the identification of the weighting factors. We have implemented several gene knockout mutations, in combination with defined medium and anaerobic bioreactor conditions to reduce the number of available modes. This strategy restricted the system to 40 remaining modes for the transgenic production of Poly-(R)-3-hydroxybutyric acid (PHB) in *Escherichia coli*. After these modifications, the remaining elementary modes were grouped into five families of modes that have the same overall stoichiometry. The resulting model became overdefined, allowing for an optimizing approach that found the best fit of weighing factors to the experimental data. A comparison of weighting factors between a PHB producing metabolism and a non-PHB producing control indicates how the metabolic network shifts when the capability of PHB synthesis is introduced. This approach has guided the design of more efficient PHB yielding strains. Preliminary work has already doubled the combined yield of 3-hydroxybutyric acid (3HB) and PHB on glucose from 0.06 g/g to 0.13 g/g when compared to a control. In addition, the specific synthesis rate of PHB increased from 0.54 g/(g hr) to 0.91 g /(g hr). The data collected from these bioreactors was used to further refine the weighting factors. The improved model provides additional insight for optimizing the PHB yield, which provides another set of data to derive a new set of weighting factors. The strategy developed in this study describes the method for calculating unique weighting factors for elementary modes in a transgenic metabolic network. The information found is useful for the identification of necessary regulation patterns when new pathways are introduced to a metabolic network.