Metabolic Flux Analysis Based on Isotope Distributions Using NMR and GC-MS and Metabolic Control Analysis with Modeling

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The recent progress in biotechnology enables us to improve the quality of our lives and environment, and its research field has been expanded from basic science to engineering such as (1) bioinformatics including functional genomics and proteomics, (2) protein engineering including protein structure - function and structure – activity relationship etc., (3) recombinant techniques including random mutation, DNA shuffling, and phage – display technique, (4) metabolic engineering, and (5) bioprocess engineering.

The primary objective of industrial fermentation is either to increase the rate of a desired product or to reduce the rate of undesired side-products etc. For this, it is important to improve the cellular activities by manipulation of enzymatic transport and regulatory functions of the cell with the use of recombinant DNA technology based on the metabolic systems analysis. Of central importance to this approach is the notion of "cellular metabolism as a network or as a system", and to consider the participating reactions in their "entirety", rather than on an individual basis. From this viewpoint, we are focusing our attention to the research on metabolic systems engineering. This research field is multidisciplinary, drawing on information and techniques from biochemistry, genetics, molecular biology, cell physiology, chemistry, chemical engineering, systems science, and computer science.

From the above viewpoint, we have investigated on the following research topics such as : (1)metabolic signal flow diagram, (2)metabolic flux analysis (MFA)with or without isotopomer distribution, (3)optimization of metabolic flux distribution (MFD), (4)modification of MFD by gene manipulation and by controlling culture environment, (5)identification of unknown pathways by isotopomer distribution, (6)metabolic control analysis (MCA), and dynamics of a mixed culture system. The applications were made for poly-3-hydroxybutyrate (PHB) production using *Ralstonia eutropha* and recombinant *Escherichia coli*, lactate production by recombinant *Saccharomyces cerevisiae*, pyruvate production by vitamin auxotrophic yeast *Toluropsis glabrata*,

lysine production using *Corynebacterium glutamicum*, and cultivation of photosynthetic microorganisms, such as *Chlorella* cell and Cyanobacteria..

In my presentation at the Fall Meeting of the Korean Society for Biotechnology and Bioengineering, I will focus on the following topics:

(1) Metabolic flux analysis of *C.glutamicum* to find the optimal culture condition for the efficient amino acid production.
(2) Modeling of metabolic pathway reactions for lysine synthesis.
(3) Application of metabolic control analysis to find the limiting pathway for lysine synthesis with experimental verification.
(4) Metabolic flux analysis using isotopomer distributions using NMR and GC-MS for cultivation of Cyanobacteria.
(5) Gene and protein expressions using RT-PCR and 2D electrophoresis in relation to metabolic regulations of Cyanobacteria.

Finally, I would like to mention about the 5-year project on cell modeling conducted at the Institute for Advanced Biosciences of Keio University, where we measure the intracellular metabolites concentrations and enzyme activities as many as possible for the development of the quantitative model.