【Grant-in-Aid for Scientific Research (S)】

Broad Section D

Title of Project： High performance microbial cell factories development by model based metabolic design and adaptive laboratory evolution

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Research Project Number：19H05626　Researcher Number：00226250
Keyword：Metabolic Engineering, Bioprocess

【Purpose and Background of the Research】

To establish sustainable society, microbial production of chemicals and fuels from bio-resources has attracted great attention. It is not easy to systematically improve metabolic pathways of microorganisms to optimize productivity of the target product because microorganisms involve many metabolic reactions with complicated interactions in the cells. Development of *in silico* platform to understand metabolic activity at the whole cell level and rational design method of metabolic pathway modification are highly desired.

In this study, we plan to develop the integration method of *in silico* metabolic pathway design of growth coupled production of target chemicals and adaptive laboratory evolution to obtain evolved strains with superior phenotype. Rate limiting steps of the metabolic pathways would be eliminated in the evolved strain. Based on the elucidation information of inherent control mechanism, novel metabolic engineering method to rationally optimize target productivity is established in this research.

【Research Methods】

Novel metabolic engineering principle and methods to understand microbial metabolism and rationally design metabolic pathway modification are established. An industrially important microorganism, *Escherichia coli*, is used as a host cell for bio-production in this study.

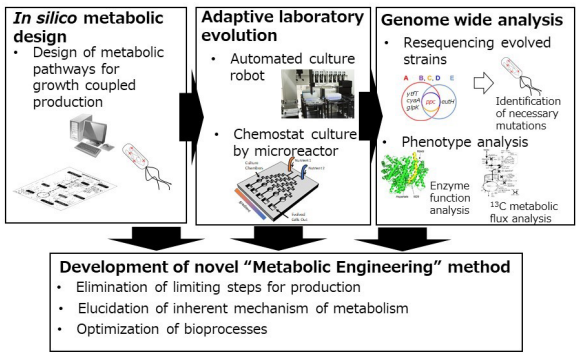

Fig. Plan of Research

To achieve the objectives, we plan to perform this research as following steps. 1) *in silico* design of metabolic pathways as growth coupled production for ten chemicals from different substrates, 2) adaptive laboratory evolution of the chemical producing strains by independent culture series by the automated culture robot, 3) multiple chemostat cultures by microfluidic technology, 4) elucidation of metabolic transition mechanisms by genome and metabolomics analyses, and 5) molecular breeding for superior performance cells with high productivity.

【Expected Research Achievements and Scientific Significance】

In this study, we plan to integrate *in silico* design of metabolic pathways and adaptive laboratory evolution engineering. By comparative genome and metabolic states analyses of the parental and evolved strains, rate limiting steps and inherent control mechanisms in metabolism are revealed. The final goal of the study is establishment of rational modification strategy method to optimize target product. The results provide us a novel understanding of microorganisms and new discipline of construct high performance cell factories in bioprocesses.

【Publications Relevant to the Project】

・Tokuyama, K, Toya, Y, Horinouchi, T, Furusawa, C, Matsuda, F, Shimizu, H. Application of adaptive laboratory evolution to overcome a flux limitation in an *Escherichia coli* production strain, ***Biotechnol Bioeng,*** **115**, 1542-1551 (2018)
・Toya, Y, Shimizu, H. Flux analysis and metabolomics for systematic metabolic engineering of microorganisms, ***Biotechnol Adv***, **31**, 818-826 (2013)

【Term of Project】

FY2019-2023

【Budget Allocation】

144,200 Thousand Yen

【Homepage Address and Other Contact Information】

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