## 1H15-1 Physiological, enzymatic, and metabolic studies of response of *E.coli* to genetic and environmental modulations

○ Jiao Zhao[1], Tomoya Baba[1], Hirotada Mori[1], Kazuyuki Shimizu[1,2]
([1]Inst. Adv. Biosci., Keio Univ., [2]Dept. of Biochem. Eng. Sci., Kyushu Inst. Technol.)

Construction of gene knockout strains and then study of their characteristics and capabilities is an example of the rapidly growing field of systematic bioscience.In this study, we have determined and compared the response of *E.coli* to genetic and environmental modulations by combining physiological, enzymatic and metabolic approaches. *E.coli* with gene deletion in pentose phosphate pathway (PPP) was surveyed for its growth and metabolic phenotypes as well as enzyme activities that are located at key branch points and involved in the NADPH formation.

It was found that mutations in *gnd* or *zwf* alone led to no significantly different growth phenotypes from those of wild type cells. However, enzyme activity analysis suggested that gene deletion caused aberrant expression of enzymes in different central metabolic pathways and activated enzymes that were not necessarily required in wild type cells. Further metabolic flux analysis confirmed these changes in central metabolic pathways, and the flux distribution values allowed these changes to be quantified. Our results provide evidence that considerable flexibility in central metabolism permits the metabolic network to coordinate itself with genetic and environmental modulations.

### Physiological, enzymatic, and metabolic studies of response of *E.coli* to genetic and environmental modulations

○ Jiao Zhao[1], Tomoya Baba[1], Hirotada Mori[1], Kazuyuki Shimizu[1,2]
([1]Inst. Adv. Biosci., Keio Univ., [2]Dept. of Biochem. Eng. Sci., Kyushu Inst. Technol.)

**Key words** gene knockout, metabolic response, pentose phosphate pathway, central metabolism, metabolic flux, growth phenotype

## 1H15-2 D-Lactate production by *pfl*-gene knockout *Escherichia coli*

○ Jiangfeng Zhu, Kazuyuki Shimizu
(Kyushu Inst. Tech.)

【Objective】 In the present study, several *pfl* knockout *E.coli* strains were cultivated, and the intracellular regulation mechanism for lactate production was investigated by measuring intracellular metabolite concentrations and enzyme activities.

【Methods and Results】 *E. coli* BW25113 and its *pfl* knockout mutants, JW0885 and JW0886 were cultivated using several carbon sources. Some intracellular metabolite concentrations and enzyme activities were measured based on the production or utilization of NADH or NADPH. The result shows that the *pfl*A and *pfl*B mutant strains can readily ferment glucose and fructose as well as glycerol to lactate. The lactate dehydrogenase and glyceraldehyde dehydrogenase activity were significantly higher in *pfl* mutants. Those are consistent with the larger amount of intracellular fructose 1, 6-bisphosphate and pyruvate pool sizes in *pfl*A- and *pfl*B- strains. The intracellular NADH/NAD ratio was also found to be high in the mutants, which promote the lactate production from redox balance point of view.

### D-Lactate production by *pfl*-gene knockout *Escherichia coli*

○ Jiangfeng Zhu, Kazuyuki Shimizu
(Kyushu Inst. Tech.)

**Key words** D-lactate, pfl knockout, metabolism

## 1H15-3 Metabolic analysis of *lpd*A knockout *Escherichia coli*

○ Mai Li, Kazuyuki Shimizu
(Kyushu Inst. Tech.)

【Objective】 Pyruvate dehydrogenase complex plays key roles in the metabolic network of *Escherichia coli*. In the present work, the metabolic regulation mechanism was studied using *lpd*A knockout *E.coli* by measuring enzyme activities and intracellular metabolite concentrations, as well as two-dimensional electrophoresis.

【Methods and Results】 *E.coli* strains BW25113 and its *lpd*A mutant derivative were used in this study. Batch cultivations were carried out at 37℃ in 5-Liter fermentor under aerobic condition using D-glucose , pyruvate and acetate as carbon sources, respectively. The result shows that the *lpd*A mutant produced high concentration of pyruvate using glucose as carbon source. The pool of acetyl-CoA is supplemented by the reactions through pyruvate oxidase, acetate kinase-phospho acetyltransferase, acetyl CoA synthase. The high concentration of pyruvate formation may be due to the deformity of the PDHc, which is the main enzyme catalysing the reaction from pyruvate to TCA cycle.

### Metabolic analysis of *lpd*A knockout *Escherichia coli*

○ Mai Li, Kazuyuki Shimizu
(Kyushu Inst. Tech.)

**Key words** lpd knockout, E.coli, Metabolism

## 1H15-4 Metabolic Response to *ppc* Gene Knockout in *Escherichia coli* Based on Enzyme Activity Assay

○ Lifeng Peng, Kazuyuki Shimizu
(Kyushu Inst.Tech.)

Fermentation characteristics and enzyme regulation patterns were investigated for *ppc* gene knockout in *E.coli*. Aerobic batch cultivations with different carbon sources such as glucose, pyruvate and acetate were carried out for both *ppc*- mutant and its parent strain. Microaerobic fermentation was also conducted with glucose as a carbon. It was found from the aerobic batch culture using glucose as a carbon source that the cell yield was improved by about 14 % in *ppc*- mutant as compared with the parent strain due to the reduced production of acetate and carbon dioxide. Similarly, significant enhancement of cell yield and reduction of acetate production were observed in *ppc*- mutant grown on pyruvate. However, little difference between the two strains was observed when using acetate as a carbon source. Under microaerobic condition, *ppc*- mutant promoted lactate production by more than 2 fold while succinate formation decreased significantly as compared with those in its parent strain. 24 different enzyme activities were assayed. The results showed that Pyk was upregulated in *ppc*- mutant grown on glucose under aerobic condition as compared with its parent strain. Concomitantly, 6PGDH activity in PP pathway significantly decreased. FBA and 6PGDH may be the major enzymes that limits the aerobic glucose utilization in *ppc*- mutant. Surprisingly, it was found that the cells replenish OAA by activating glyoxylate cycle in *ppc*- mutant.

### Metabolic Response to *ppc* Gene Knockout in *Escherichia coli* Based on Enzyme Activity Assay

○ Lifeng Peng, Kazuyuki Shimizu
(Kyushu Inst.Tech.)

**Key words** ppc knockout, E.coli, Enzyme activity, metabolic analysis