3B10-1 Effect of *fadR* gene knockout on the metabolic regulation of *Escherichia coli* in terms of proteome profiling, enzyme activities and intracellular metabolite concentrations

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A comparative study was conducted between a wild type and its *fadR* mutant *Escherichia coli* based on proteome, enzyme activity and intracellular metabolite concentration analyses to understand the effect of *fadR* knockou on the metabolism of *E. coli*. 2DE and MALDI-TOF-MS were used for proteome profiling. Phenotypes showed that acetate production in the *fadR* mutant was reduced by 12% in the final acetate concentration compared to the wild type, whereas the final cell concentration of the *fadR* mutant rose by about 13%. Besides, specific glucose consumption rate slightly increased in the *fadR* mutant. Enzyme assays revealed that the activities of Icl and MS involved in glyoxylate shunt were significantly induced by 3.7- and 1.9-fold in the *fadR* mutant, respectively. The CS, Acn, Fum and MDH in the TCA cycle were also upregulated to some extent. However, the activities of ICDH decreased by 0.8-fold and α-KGDH by 0.6-fold. Moreover, $NADP^+$-dependent Mez was upregulated but NAD^+-dependent Mez downregulated. Measurements of intracellular metabolites demonstrated that the intracellular PYR and AcCoA concentrations were 0.65- and 0.3- fold lower in the *fadR* mutant, respectively. On the other hand, however, intracellular pool sizes of MAL and OAA were elevated. In addition, *fadR* mutation caused lower ATP/ADP, NADH/NAD and NADPH/NADP ratios.

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Key words *Escherichia coli*, *fadR* mutant, proteome, metabolic regulation

3B10-2 Effects of *lpd*A, *suc*A and *suc*C genes knockout on the metabolic regulation in *Escherichia coli*

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・[Objective] Both alpha-ketoglutarate dehydrogenase complex and succinyl-CoA synthetase play important roles in the TCA cycle. In the present research, the metabolic regulation mechanism was investigated using *lpd*A, *suc*A and *suc*C genes knockout *E.coli* by measuring enzyme activities, intracellular metabolite concentrations, and the metabolic flux distribution obtained by ^{13}C-labeling experiments.・[Methods and Results] *E.coli* strains BW25113 and its *lpd*A, *suc*A and *suc*C mutants were used in the present study. Batch cultivations were carried out at 37 ℃ in 2-liter fermentor under aerobic condition using glucose as a carbon source. The continous cultivation was also carried out in 1-liter fermentor. The result shows that *lpd*A and *suc*A mutants produced high concentration of L-glutamate, while *suc*C mutant produced more acetate than the parent strain. The glyoxylate shunt was activated in the *lpd*A, *suc*A and *suc*C mutant. The oxidative pentose phosphate pathway was activated in the *lpd*A and *suc*A mutants, which may be due to overproduction of NADPH which was consumed for L-glutamate production. The acetate kinase was downregulated in the sucA mutant which might be due to the overproduction of L-glutamate, while the activation of acetate kinase in the sucC mutant might be due to the deficiency of succinyl-coA synthetase. The metabolic flux distribution of lpdA mutant was consistent with the enzyme activity result.

Effects of *lpd*A, *suc*A and *suc*C genes knockout on the metabolic regulation in *Escherichia coli*

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Key words lpdA sucA sucC, E.coli, metabolic regulation

3B10-3 大腸菌の *ace*E 遺伝子破壊が代謝調節に及ぼす影響

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[目的]解糖系とTCA回路を結ぶピルビン酸脱水素酵素(PDH)は、*ace*E,Fおよび *lpd*A によってコードされたサブユニットの複合体である。本研究では、*ace*E遺伝子の破壊が大腸菌効能の代謝調節に及ぼす影響を調べた。
[実験方法と結果]本研究は、大腸菌K12株と*ace*E欠損株である。本研究では、炭素源としてグルコースを含んだLB培地、あるいは最少合成培地を用い、好気条件で回分培養を行った。培養pHは7.0、温度は37℃で実験を行った。また、培養途中でサンプルを採取し、酵素活性及び2次元電気泳動によるタンパク質の発現解析を行った。*ace*E欠損株を、グルコースを炭素源として、好気条件で培養を行った結果、グルコースの消費に伴い、ピルビン酸、乳酸、酢酸、ギ酸が生成され、グルコースがなくなると、この順に消費されることがわかった。また、野性株を培養した結果と比較すると、グルコース消費速度が低く最終菌体量も少ないことがわかった。また、野性株 では殆ど生成されなかった蟻酸や乳酸が著しく生成されており、かなり異なる代謝特性を示すことがわかった。2 次元電気泳動によるタンパク質の発現解析の結果、TCA回路関連のタンパク質の発現量が大腸菌K12株の場合に比べて低くなっており、大腸菌 K12 株では発現していなかった *Ldh*、*Pfl*、及び糖新生経路の FBP が発現していることがわかった。また、合成培地で同様の実験を行った結果、酢酸の生成量が低くはなっているが、蟻酸、ピルビン酸、乳酸が生成されるなど、やはり野性株と著しく異なる特性を示すことがわかった。

Effect of *ace*E gene knockout on the metabolic regulation in *Escherichia coli*

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Key words *ace*E gene knockout, *Escherichia coli*, PDH, enzyme activity

3B10-4 Effects of *cra* and *fnr* genes knockout on the global metabolic regulation of *Escherichia coli*

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[Objective] Among the global regulatory genes, the catabolite repressor/activator gene *cra* is mainly activated under gluconeogenic condition, whereas flavin nitrate reductase gene *fnr* is activated under anaerobic condition to control a wide range of genes from central metabolic pathways to electron transport chains. In the present study, these two gene knockout mutants were cultivated, and the metabolic regulation was investigated based on the enzyme activities and gene expressions as well as intracellular metabolite concentrations.[Results and Discussion]Under glucose abundant batch culture condition, the increased flux was observed through glycolysis for *cra* mutant. On the other hand, *fnr* mutant did not show any significant change in major metabolic pathways. The TCA cycle and glyoxylate shunt enzymes of both *cra* and *fnr* mutants were severely repressed during glucose limited chemostat culture, while, the pentose phosphate pathway was activated in *cra* mutant. During the continuous cultivation under glucose limited condition, the gene expression study indicated two other global regulatory genes such as *arcA* and *crp* were up-regulated in *fnr* mutant. The formic acid production was reduced in *fnr* mutant, whereas the succinate production was increased in *cra* mutant under anaerobic condition.

Effects of *cra* and *fnr* genes knockout on the global metabolic regulation of *Escherichia coli*

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Key words *cra* gene knockout, *fnr* gene knockout, *Escherichia coli*, metabolism