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Title(English)	Studies on the Regulatory Mechanism of Glutamate Metabolism in Corynebacterium glutamicum
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種別(和文)	論文要旨
Type(English)	Summary

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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	Bioengineering 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 (Engineering) Doctor of
学籍番号： Student ID Number		指導教員（主）： Academic Advisor(main)	Masaaki Wachi
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Corynebacterium glutamicum is a gram positive, rod-shaped aerobic bacterium used for the fermentative production of L-glutamate and several other amino acids. Wild-type *C. glutamicum* releases more than 80g/L of L-glutamate under appropriate culture conditions. *C. glutamicum* has a unique mechanism of L-glutamate secretion in which special treatments or growth conditions such as biotin-limited conditions as well as addition of fatty acid esters or β-lactam antibiotics is required. It was recently shown that the NCgl1221 gene encoding a protein homolog of the small conductance mechanosensitive channel (MscS) is involved in the mechanism of L-glutamate secretion. Disruption of NCgl1221 greatly diminished L-glutamic acid secretion by biotin limitation. Very recently, it has been demonstrated that the NCgl1221 channel is indeed served as a path for glutamate efflux. Therefore NCgl1221 is necessary for efficient glutamate production. NCgl1221 specific mutation, which results in a truncation of the C-terminal extracytoplasmic domain, induces constitutive L-glutamate production. The mutation of NCgl1221 also caused a 2-fold decrease of ODHC. Since ODHC is located at the branched point between TCA cycle and L-glutamate biosynthesis pathway, a decrease in ODHC activity was suggested to shift the metabolic flux from energy production via TCA cycle to L-glutamate production. Mutation of NCgl1221 gene should effect a reduction of ODHC activity, which may be controlled by a trancriptional regulator. The present research aims to identify the transcriptional regulator(s) in *C. glutamicum*, which connects NCgl1221 with glutamate metabolism.

C. glutamicum wild-type (WT) strain ATCC 13869 and NCgl1221 active mutant strain (BL1) were grown in the glutamate production medium containing excess presence of biotin. WT cannot produce L-glutamate in the presence of biotin, while mutant BL1 can still produce L-glutamate in this condition. Total cellular RNA was isolated for microarray analysis. Genes encoding transcriptional regulators, NCgl1386 and NCgl2814, have been found to be highly down-regulated in the mutant cells. In order to investigate the role of these transcriptional regulators in glutamate metabolism, deletion and overexpression strains of these genes have been constructed.

It was found that glutamate production decreased by overproduction of

NCgl2814 (LldR). LldR is known as a repressor for *ldhA* and *lldD* encoding lactate dehydrogenases. LdhA is responsible for production of lactate, while LldD is for its assimilation. In the wild-type cells, lactate was produced in the first 24 h and it was re-consumed thereafter. On the other hand, in the overproduced cells, lactate was produced like WT, but it was not re-consumed. This means that lactate assimilation, which is catalyzed by LldD, was suppressed by the overproduction of LldR, but lactate production, which is catalyzed by LdhA, was not affected, indicating that LldD functions as a repressor for *lldD* but not for *ldhA* under glutamate-producing conditions. Repression of *lldD* gene was also confirmed by qRT-PCR in the LldR overexpressing strain, while expression of *ldhA* was not affected, indicating that LldR only represses *lldD* during the glutamate production under biotin-limited conditions. From these results, it is suggested that lactate metabolism, which is controlled by LldR, has a buffering function of pyruvate pool for glutamate production.

Furthermore, lactate metabolism during the glutamate production under biotin-limited conditions and penicillin addition conditions were also studied by the *ldhA* disruption compared with WT. Glucose consumption and glutamate production were significantly decreased under biotin-limited conditions in *ldhA* disruptant compared with WT. On the other hand, glucose consumption and glutamate production of *ldhA* disruptant increased to a comparable amount or even higher compared with WT under penicillin addition conditions. Higher L-glutamate was produced under the penicillin addition conditions compared with the biotin-limited conditions in the WT. These results suggest that accumulation of pyruvate may significantly increase under biotin-limited conditions due to the limit of pyruvate carboxylase (PC), which is a biotin-containing enzyme, which consequently leads to a decrease in glycolytic flow. This problem was overcome under penicillin addition conditions where pyruvate accumulation may be abolished due to higher activity of PC compared with that of biotin limitation. Therefore, under penicillin addition conditions, a buffering function of pyruvate pool for glutamate production by lactate metabolism is not needed and *ldhA* disruptant is more efficient for glutamate production under this condition rather than biotin-limited conditions. From these results, it is suggested that the decrease in metabolic flow to lactate formation is not effective for the enhancement of glutamate production under biotin-limited conditions since the produced L-lactate is reused by the cells to produce L-glutamate.

Overproduction of NCgl1386 caused severe growth retardation and a decrease in glutamate production. It is confirmed by RT-PCR that NCgl1386 forms an operon with NCgl1385 (*odhI*), encoding an inhibitor of ODHC, and NCgl1387, which has been found to act as a negative regulator to this operon. Overexpression of NCgl1387 also caused a slight growth inhibition, which is independent from the

inhibition caused by NCgl1386. Therefore, NCgl1386 and NCgl1387 separately function for growth inhibition via the control of some unknown gene(s). NCgl1386 functions as an activator of NCgl1385-1386-1387 and NCgl1387 functions as a negative regulator of NCgl1385-1386-1387. Moreover, NCgl1386 and NCgl1387 control the expression of unknown genes responsible for growth inhibition. However, the regulatory mechanism for growth inhibition by NCgl1386 and NCgl1387 still need to be elucidated.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 2 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 2 copies of 800 Words (English).