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Article / Book Information

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Title(English)	Growth promotion in Corynebacterium glutamicum by overexpression of NCgl2986 gene encoding a protein homologous to amidase-like proteins
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（博士課程）
Doctoral Program

THESIS OUTLINE

系・コース： Department of, Graduate major in	Life Science and Technology	系 コース	
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申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Engineering)
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Thesis Outline

Chapter 1. Introduction

Glutamic acid is a non-essential amino acid, which is naturally produced in human body in L form. It is odorless and has umami taste. The identification of L-glutamic acid as a substance responsible for umami taste was carried out by Kikunae Ikeda in 1908. Later in the next year (1909), the first commercial umami seasoning was produced by Ajinomoto company and its industrial technology has been advancing until now. Researchers at Kyowa Hakko Kogyo company, Kinoshita, Udaka, and Shimono in 1957, performed a screening of microorganisms isolated from soil samples to find a new candidate for amino acid producer. The new species *Corynebacterium glutamicum* was determined as the highest glutamate producer (Kinoshita, Udaka, & Shimono, 1957).

Several host strains of *C. glutamicum* which showed improved ability of protein secretion were developed by conventional genetic breeding by Ajinomoto. Using YDK010 as a host, Matsuda et al. (2014) succeeded the production of antibody fragment Fab into the medium. They also performed the screening of genes in order to improve the productivity of Fab when overexpressed or deleted. During screening, it was found that cell growth of the Fab producer strain YDK010/pPKStrastFabHL was significantly promoted when NCgl2986 gene encoding a protein homologous to N-acetylmuramoyl-L-alanine amidase was overproduced even though the Fab production was decreased (Matsuda, 2014). The role of NCgl2986 in *C. glutamicum* has not been understood yet. It is expected that to uncover the role of NCgl2986 gene in cell growth of *C. glutamicum* will be useful for fermentation improvement as well as protein production in industry. Hence, the objective of this study is to reveal the role of NCgl2986 gene encoding protein homologous to N-acetylmuramoyl-L-alanine amidase in the growth of *C. glutamicum*.

Chapter 2. NCgl2986 overexpression promoted the cell growth in *C. glutamicum*

- NCgl2986 is active in MMTG medium with high concentration of glucose and $CaCO_3$ addition

Growth promotion effect was also observed in wild-type cells when it was grown in MMTG medium containing high concentration of glucose and neutralizing agent $CaCO_3$ but not in conventional L medium. Growth assay was also carried out using a jar fermenter with total MMTG medium 400 mL. Not only turbidity but also dry cell weight was increased by NCgl2986 overexpression.

- NCgl2986 overexpression increased cell biomass production, cell number and cell volume

SEM analysis showed that the growth promotion effect by NCgl2986 overexpression resulted from the increase in cell length (16%) and cell number (31%).

Chapter 3. NCgl2986 is likely interacts with MurA protein

- NCgl2986 has another function rather than hydrolytic enzyme

In vitro peptidoglycan hydrolysis assay was carried out using purified NCgl2986 protein and *M. luteus* whole cells as substrate. NCgl2986 protein was successfully purified near homogenously but it did not show hydrolase activity. A recent study by Boutte et al. (2016) showed that Rv3915, an amidase-like protein in *Mycobacterium tuberculosis*, interacts with MurA protein which is involved in the first step of peptidoglycan precursor synthesis. Based on this literature and bioinformatic studies, it is suggested that probably NCgl2986 protein has another function rather than hydrolytic enzyme.

- *murA* overexpression mimics NCgl2986 overexpression

MurA overexpression mimicked the NCgl2986 overexpression; it promoted the cell growth and caused cells more susceptible to ampicillin. From these results, it is suggested that NCgl2986 has a role as an activator of MurA.

Chapter 4. Repression of NCgl2986 protein might increase amino acid production

Effects of overexpression or repression of NCgl2986 on glutamic acid production were examined. Repression of NCgl2986 by overexpressing antisense RNA caused increased in the glutamic acid production by 6%.

Chapter 5. General discussion

According to the result explained, it is speculated that the growth promotion effect by NCgl2986 overproduction is a result of its interaction with MurA protein. NCgl2986 in this proposed mechanism functions as a regulator of MurA protein. This interaction leads to the initiation of peptidoglycan precursor synthesis. Furthermore, repression of the NCgl2986 is expected could improve the production of desired amino acid.