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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

論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Corynebacterium glutamicum is a well-known bacterium, which has a unique cell wall organization composed of peptidoglycan, arabinogalactan, and mycolic acid. *C. glutamicum* possesses an amidase-like protein encoded by NCgl2986 which is the homologue of Rv3915 of *M. tuberculosis* with 52% homology. The role of amidases has already been characterized in several bacteria. However, the role of NCgl2986 in *C. glutamicum* has not yet been understood. Screening of genes (Matsuda et al., 2014) to improve the production of antibody fragment Fab using *C. glutamicum* YDK010 as a host was conducted by overexpressing hydrolase enzymes. It was found that cell growth was promoted by NCgl2986 overexpression even though Fab production was decreased. It is expected that to uncover the role of NCgl2986 gene in cell growth of *C. glutamicum* will be useful for protein production in industry. Therefore, the objective of this study is to uncover the role of NCgl2986 gene encoding a protein homologous to amidases in the growth of *C. glutamicum*.

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 even though glucose consumption was almost the same compared to the control. Further analysis using a scanning electron microscopy SEM showed that this growth promotion resulted from the increase in cell length (16%) and cell number (31%).

To determine the activity of NCgl2986 protein, *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 these literature study and bioinformatic studies, it is suggested that probably NCgl2986 protein has another function rather than hydrolytic enzyme. Furthermore, MurA overexpression mimicked the NCgl2986 overexpression. From these results, it is suggested that NCgl2986 has a role as an activator of MurA.

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%.

Growth promotion was observed by NCgl2986 overexpression in a small- and a large-scale culture. What still puzzled here is even though cell biomass was increased, glucose consumption rate was not different compared to control. One possible hypothesis is, CO_2 released is less in the overproducer.

The purified NCgl2986 protein could not hydrolyze the cell wall of *M. luteus*. This is probably due to its lack of Zn^{2+} residues in the amidase catalytic domain when compared to other bacterial amidases. Moreover, NCgl2986 and its homologue Rv3915 have no signal peptide. Therefore, possibility of interaction between NCgl2986 and MurA was tested by overexpressing *murA* gene. Results showed that *murA* overexpression mimicked NCgl2986 overexpression. Hence, it is speculated that in *C. glutamicum*, NCgl2986 likely interacts with MurA to initiate peptidoglycan precursor synthesis. Further, NCgl2986 activity is possibly regulated by the phosphorylation involving protein kinase although this hypothesis needs further investigation.

Repression of NCgl2986 by overexpressing antisense RNA resulted in increased glutamic acid production. Controlling the NCgl2986 expression could be a new technique to improve the productivity of bacterial fermentation.