GENOME-WIDE GENE EXPRESSION ANALYSIS OF *CORYNEBACTERIUM GLUTAMICUM* DURING PENICILLIN-INDUCED GLUTAMIC ACID PRODUCTION

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Introduction. A coryneform bacterium *Corynebacterium glutamicum* is known as a glutamic acid-producer. Glutamic acid production by this microorganism is induced by biotin limitation, addition of β-lactam antibiotics such as penicillin and addition of fatty ester surfactants such as Tween 40. In our previous report on proteomics (1), de novo protein synthesis after penicillin addition is necessary for penicillin-induced glutamic acid production, and one of such proteins is OdhI which is an inhibitory protein of 2-oxoglutarate dehydrogenase complex (ODHC) and contributes to the decrease in ODHC activity during glutamic acid production (2–4). However, since the comprehension of proteomics analysis is low, other comprehensive analysis is necessary to understand the mechanisms of glutamic acid production by *C. glutamicum*. In this study, genome-wide gene expression analysis of *Corynebacterium glutamicum* during penicillin-induced glutamic acid production was performed.

Methods. The *Corynebacterium glutamicum* wild-type strain ATCC 13032 was used in this study. The *C. glutamicum* cells were cultivated in the synthetic medium and 10 μM penicillin G was added to the culture after cell growth reached the early exponential phase to induce glutamic acid production. Before and after addition of penicillin, cells were harvested and total RNA was isolated. For DNA microarray analysis, custom-designed DNA microarray was used. All the experiments were carried out using a custom-designed DNA microarray an experimental platform of Agilent Technologies Inc. The obtained data were applied to quantile normalization and the ratios of gene expression after penicillin addition to that before addition were calculated. Functional category analysis was performed using functional categories of clusters of orthologous groups of proteins (COGs).

Results. One hour after penicillin addition, expression of 100 genes were upregulated and that of 179 genes were downregulated. Functional category analysis indicates that the category “signal transduction” was overrepresented for the upregulated genes, and the category “amino acid transport and metabolism” was overrepresented for the downregulated genes.

We next examined the expression of the genes encoding the proteins which are known to involve in glutamic acid production by *C. glutamicum*. The expression of the genes related to the glyoxylate shunt was downregulated by penicillin addition, while the expression of *icd* gene encoding isocitrate dehydrogenase, *odhI* gene encoding the inhibitory protein for ODHC and NCgl1221 encoding a mechanosensitive channel protein was upregulated by penicillin addition.

It is known that the treatments inducing glutamic acid production (i.e., biotin limitation, penicillin addition and Tween 40 addition) affect the integrity of cell surface structure and change the cell surface tension. Moreover, one of the two-component signal transduction systems MtrAB senses the change in osmotic pressure of the environment in *C. glutamicum* (5). Therefore, the change in expression of the MtrAB-regulated genes by penicillin addition was analyzed. As a result, 9 genes among 31 MtrAB-regulated genes showed altered expression by penicillin addition and this was statistically significant. Our results suggest that the MtrAB-regulated genes might involve in sensing the change in cell surface tension by penicillin addition.

Conclusions. In this study, genome-wide gene expression analysis of *C. glutamicum* during glutamic acid production was performed using DNA microarray. As expected, we could successfully analyze the change in expression of the genes related to glutamic acid production by this microorganism. Moreover, the two-component signal transduction system MtrAB which involves in sensing the change in osmotic pressure appears to be responsible for sensing the change in cell surface tension by penicillin addition. It was reported that the mechanosensitive channel NCgl1221 protein senses the change in membrane tension and its conformational change is occurred during glutamic acid production (6, 7). Further investigation of the relationship between the MtrAB-regulated genes and NCgl1221 will be required.

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