ANALYSIS OF L,D-TRANSPEPTIDASE INVOLVED IN LYSOZYME SENSITIVITY IN *Corynebacterium glutamicum*

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Introduction. *Corynebacterium glutamicum* is a Gram-positive bacterium that belongs to the mycolic acid containing actinomycetes. *C. glutamicum* is widely used for industrial production of L-glutamic acid. Glutamic acid production by *C. glutamicum* is induced by biotin limitation, penicillin treatment or fatty acid ester treatment. All these treatments affect cell surface structures of *C. glutamicum*. However, the reason why these treatments induce L-glutamate production is still unclear. In this study, to clarify the relationship between the cell surface structure and the L-glutamate production of *C. glutamicum*, we analyzed lysozyme sensitive mutants of *C. glutamicum* and identified a new gene responsible for lysozyme sensitivity.

Methods. *C. glutamicum* wild-type strain KY9611 and a lysozyme sensitive mutant strain KY9708 were used in this study. Cells were grown in L medium at 30°C. Growth was monitored by measuring OD_{660}.

Results. *C. glutamicum* KY9708 was isolated as a lysozyme sensitive mutant. We found that KY9708 carries a missense mutation in the *mtrB* gene encoding the sensor kinase of the two component system MtrAB. The *mtrB* mutation in KY9708, named *mtrB9708*, was transferred into a wild-type strain by *sacB* gene mediated successive homologous recombination. The constructed defined *mtrB9708* mutant strain also showed lysozyme sensitivity (Fig. 1A). MtrAB two-component system is known to regulate genes involved in formation of cell surface structures (1). To identify a gene(s) responsible for lysozyme sensitivity under control of MtrAB, microarray analysis was carried out. 16 genes were found, expression of which were decreased in the mutant strain compared to the wild type. We then constructed expression plasmids of these genes. As a result, it was found that plasmid expressing NCgl2388 suppressed lysozyme sensitivity of the *mtrB* mutant strain (Fig. 1B). NCgl2288 gene encodes a homolog of L,D-transpeptidase, which is involved in modification of peptidoglycan.

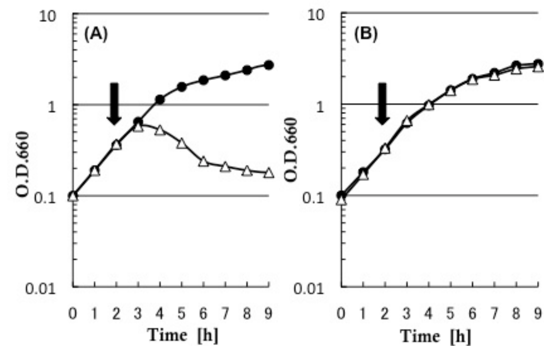

Fig.1 Suppression of lysozyme sensitivity of the *mtrB* mutant by NCgl2388. (A) mtrB9708/pECt (empty vector), (B) mtrB9708/pNCgl2388. Lysozyme (25 μg/mL) was added at 2 h. ●: control, △: + lysozyme.

We then constructed a NCgl2388 deletion mutant strain. As expected, the ΔNCgl2388 mutant strain also showed lysozyme sensitivity (Fig. 2).

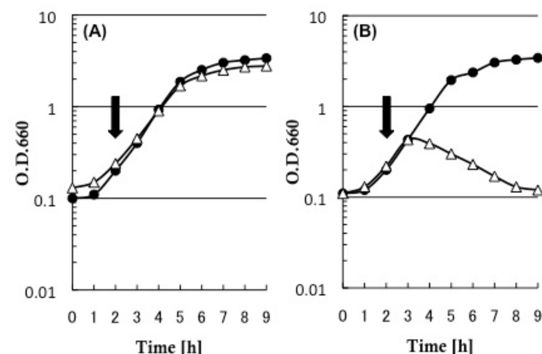

Fig.2 Lysozyme sensitivity of ΔNCgl2388 mutant. (A) wild type, (B) ΔNCgl2388. Lysozyme (25 μg/mL) was added at 2 h. ●: control, △: + lysozyme.

Conclusions. These results indicate that NCgl2388 under control of the MtrAB two-component system is responsible for lysozyme sensitivity. Decreased expression of NCgl2388 may cause a defect in peptidoglycan layer, which renders cells lysozyme sensitive.

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References.

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