論文 / 著書情報
Article / Book Information

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Title(English)	Effects of EGTA treatment on cell surface structures of Corynebacterium glutamicum
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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 has been widely utilized in industrial biotechnology due to its many beneficial traits compared with other biological systems. In recent years, application of this bacterium as the protein expression system has been gaining an interest due to its low growth requirements, a limited number of secreted host proteins, and low extracellular proteolytic activity. This bacterium harbors dual protein secretion pathway allowing secretion of the recombinant protein into culture media. These features may result in the accumulation of recombinant proteins in culture supernatants with high purity and high quality, which simplify the purification process.

However, the surface of *C. glutamicum* cells is covered with the mycolic acid-containing layer and the para-crystalline protein surface layer (S-layer), which have been considered as a permeability barrier against antibiotics and lytic enzymes. Although extensive studies have established the mechanisms of protein translocation across the plasma membrane, knowledge on how proteins are passed through the upper layers to culture media is still lacking. In spite of the cell wall structure complexity, EGTA (ethylene glycol tetraacetic acid), a calcium chelator, is able to inhibit the growth of *C. glutamicum* at relatively lower concentrations compared with other Gram-positive and Gram-negative bacteria. This dissertation intends to identify the effects of EGTA on cell surface structures of *C. glutamicum* ATCC 13869 and its subsequent implications towards cell wall permeability.

Simultaneous addition of EGTA and lysozyme resulted in cell lysis, whereas addition of these reagents separately had no such effect. This result demonstrated that EGTA increases cell wall permeability of *C. glutamicum*. FE-SEM showed the failure of S-layer assembly and formation of membrane vesicles on the new cell poles. The imaging results further confirmed alteration of cell surface structures in EGTA-treated cells. The protein profile showed that EGTA treatment caused the release of a number of cell surface proteins into the medium, especially the CspB protein, the monomer protein of the S-layer. Hence, this investigation provides evidence that calcium ions are required for the assembly of *C. glutamicum* S-layer. In addition, the defect of the S-layer possibly has a role in the increase of *C. glutamicum* cell wall permeability.

Analysis of cell wall proteins also showed that EGTA treatment resulted in degradation of CspB protein in culture media. Contrasting this result, previous studies have been suggesting that *C. glutamicum* does not show significant extracellular proteolytic activity. I suspected that EGTA treatment consequently causes the release and/or activation of a protease from *C. glutamicum*. I investigated which protease(s) is

responsible for the protein degradation upon EGTA treatment using protease/peptidase mutant library based on YDK010 strain, a strain derived from the wild-type ATCC 13869. From all SDS-PAGE profile of the deletion mutants observed, only a strain with a deletion of ORF 1386 (NCgl1048), which encodes DegQ, a trypsin-like serine protease, lost the ability to degrade CspB. Hence, it was suggested that EGTA treatment caused a release or activation of the DegQ1386 protease.

Although *degQ1386* deletion is able to stop CspB degradation, EGTA treatment on other 4 DegQ paralog deletion mutants of *C. glutamicum* still leads to the production of shortened CspB protein. Deletion of each DegQ paralog also resulted in different vulnerability against lytic enzymes and antibiotics. Furthermore, sequence analysis suggests specific localizations of each DegQ paralog in the cell wall structure of *C. glutamicum*. These results suggest that each DegQ paralog contributes to a specific role in *C. glutamicum* cell wall.

Construction and proteome analysis of *degQ1386* deletion mutant and complemented strain based on ATCC13869 confirmed that DegQ1386 is the key factor for CspB degradation in culture media upon EGTA treatment. While deletion of the *degQ1386* did not affect cell growth under normal growth conditions, an increase in lysozyme sensitivity and lethal synergetic effects of EGTA-lysozyme was observed. Regarding the known role of DegQ as protease/chaperone, it raises the possibility that the DegQ1386 plays a role in protein quality control in the cell wall, which contributes to lysozyme resistance of *C. glutamicum.*

Taken together, the present research explores, for the first time, the importance of calcium ion on the integrity of *C. glutamicum* cell wall structure. It also demonstrated that increase of cell wall permeability by EGTA treatment leads to release and /or activation of an extracellular protease, which subsequently degrades unfolded CspB protein in culture media. DegQ1386 protease, the enzyme responsible for the degradation, plays a role in protein quality control in *C. glutamicum* cell wall.