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Effects of EGTA treatment on cell surface structures of *Corynebacterium glutamicum*

(*Corynebacterium glutamicum* の細胞表層構造に対する EGTA 処理の影響)

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 pathways 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. This investigation suggests 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.

Taken together, the present research explores, for the first time, the importance of calcium ion on the integrity of *C. glutamicum* cell wall structure.