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## Effect of the C-Terminal Pro-Domain of Aqualysin I Precursor on Folding for Maturation and Translocation of the Precursor across the Cytoplasmic Membrane in *Escherichia coli*

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To investigate the function of the C-terminal pro-domain in maturation and export pathway of the precursor in *E. coli*, aqualysin I variants were constructed in which deletion mutants of the C-terminal pro-domain lacking its own signal peptide were inserted into pIN-III-ompA3. When *E. coli* cells harboring wild type and mutant plasmids were induced by 0.2 mM IPTG, active aqualysin I was produced by heat treatment and aqualysin I precursors with deletions of more than 5 amino acid residues at the C-terminal end of pro-domain were much more rapidly processed than that of wild type, indicating that the C-terminal pro-domain functions as a inhibitor for processing of aqualysin I precursor. Cellular localization showed that in the case of wild type, most of aqualysin I present in membrane fraction (probably the outer membrane), whereas for the truncated mutants, remain in the cytoplasm, indicating that for deletion mutants, their precursors expressed in cells were not translocated across the cytoplasmic membrane, despite the existence of an N-terminal signal peptide. These results indicate that deletion of the C-terminal pro-domain cause formation of the folded conformation before translocation across the cytoplasmic membrane, resulting in the accumulation of precursor in the cytoplasm and a rapid processing to the mature enzyme.