# Control of viability and biofilm formation of foodborne pathogens by amino acids and peptides

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Title ：Control of viability and biofilm formation of foodborne pathogens by amino acids and peptides
(アミノ酸およびペプチドによる食中毒細菌の生存とバイオフィルム形成の制御）

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## Thesis Summary

Illness resulting from the consumption of foods contaminated with pathogens continues to be a public health concern. The prevalence of antibiotic resistance leading to low efficacy of available antibiotics also creates a demand for developing alternative antimicrobial agents. In this thesis, the potential of naturally occurring compounds including L-amino acid, antimicrobial peptides and ε-polylysine has been explored to control foodborne pathogens as well as their biofilm formation.

The effects of various mixture of amino acids, and of specific amino acid deficiency in the medium on *Salmonella* Typhimurium biofilm formation was firstly investigated. Basically, addition of L-amino acid mixture enhanced the biofilm formation, and deprivation of certain single amino acid from the broth including L-Ala, L-Pro and L-Trp was found to greatly increased biofilm mass. The bacterial adhesion to hydrocarbons (BATH) test revealed that the absence of L-Ala, L-Pro and L-Trp significantly increased the cell surface hydrophobicity of *Salmonella* cells. Cultivation of the bacterium in (-) L-Ala, (-) L-Pro broth up regulated the expression of chemotaxis related genes, which were presumed to be a cause of strong biofilm formation.

Secondly, antimicrobial peptides were purified and identified from egg white hydrolysates using multi-step chromatography and mass spectrometry. The amino acid sequences of peptides with promising antibiofilm activity were further engineered using bioinformatics tools, four candidates with higher net charge, hydrophobicity and helicity were designed and synthesized on the basis of the amino acid sequences of peptides derived from egg white hydrolysates. The bioactivity assay indicated that two peptides, P1R3 (KSWKKHVVSGFFLR) and P1C (KSWKKHVVSGFFLRLWVHKK), exhibited potent activity against *S.* Typhimurium but negligible toxicity to Vero cells. Fluorescent microscopy analyses revealed that P1R3 and P1C caused depolarization and increase in permeability of membrane, suggesting damages in membrane integrity. Moreover, P1R3 could interact with genomic DNA, which might also play a role in killing bacteria.

Thirdly, effects of ε-polylysine inhibiting biofilm formation of *S.* Typhimurium were investigated on transcription of the bacterium. The DNA microarray analysis indicated that treatment of *S.* Typhimurium with ε-polylysine down-regulated the expression of genes involved in curli amyloid fibers production, cellulose formation, quorum sensing, and flagella-associated motility, while up-regulated those regulating the synthesis of colanic acid. Overall, these studies not only provided several candidates to control bacterial biofilm infections but also motivated the further development of novel antimicrobial agents.