Study on mechanism of action of polyphenols on controlling foodborne pathogens and their toxins

袁, 璐

Name　：袁璐　（YUAN LU）エン　ロ

Title　：Study on mechanism of action of polyphenols on controlling foodborne pathogens and their toxins
（ポリフェノールの食中毒細菌およびその毒素に対する作用機構に関する研究）

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Thesis Summary

Foodborne pathogens are a major cause of foodborne illness and a serious threat to food safety. Traditional physical and chemical control methods may adversely affect the sensory qualities or have the potential risk of leaving chemical residues on the food, which creates a demand for developing safe and effective alternative antimicrobial agents. In this thesis, the effects of polyphenols as natural components from food or plants on foodborne pathogens and their virulence were explored and the underlying mechanism of action was elucidated.

Firstly, nucleotide sequence analysis of a plasmid suggested the presence of 7 different genes related to the emetic toxin (cereulide) production in *Bacillus cereus* BC27 which was isolated from food in 2003. Against the bacterium, epigallocatechin gallate (EGCg), theaflavin-3'- gallate (TF2b), and theaflavin-3, 3'-gallate (TF3) of common tea polyphenols were demonstrated to have strong bactericidal and bacteriostatic activity based on the result of time-kill assays in Luria-Bertani (LB) broth. Among them, the effects were strongest in EGCg. Fluorescence microscope observations suggested that these polyphenols caused damage in the cell envelope, and leakage of cellular material including DNA, resulting in death. After *B. cereus* BC27 spores were incubated in LB with EGCg, TF2b, and TF3, these polyphenols did not inhibit germination but were effective in inhibiting spore outgrowth and killed daughter cells when they were added within 4 h of germination induction. Furthermore, the treatment of *B. cereus* BC27 with EGCg, TF2b, and TF3 reduced transcription of the gene involved in cereulide synthetase and inhibited the production of cereulide. The polyphenols also inhibited the cytotoxicity of cereulide as shown by the HEp-2 cell viability assay.

Secondly, EGCg, butein, isorhapontigenin, hesperetin, morin, luteolin, resveratrol, and rhapontigenin, were demonstrated to have inhibitory effects on the cytotoxicity of Shiga toxin (Stx) 1 and 2 from Stx-producing *Escherichia coli*. Furthermore, Vero cells pretreated with these polyphenols were resistant to the cytotoxicity of Stx. Among them, luteolin showed the most potent Stx inhibitory and cytoprotective effects. Its mechanism of action was investigated using a cell-free protein synthesis assay and qPCR assay to determine RNA-N-glycosylase activity. Luteolin did not inhibit Stxs on cell-free protein synthesis, suggesting that the enzymatic activity of the Stx A subunit was not inhibited by luteolin. The 28S rRNA depurination by Stxs was suppressed in Vero cells treated with Stxs which had been pretreated with luteolin, suggesting that the binding of the Stx to the cell surface receptor Gb3 was inhibited by luteolin. The results obtained in this study suggest that polyphenols are a promising natural antimicrobial and antitoxin agents for controlling foodborne pathogens and their virulence in foods.