Study on antibacterial and antitoxin activities of plant polyphenols

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Thesis Summary

Enterohaemorrhagic *Escherichia coli* (EHEC) is an important foodborne pathogen. It may be transferred to humans by the consumption of contaminated food such as undercooked meat products, contaminated fresh vegetables and fruits. EHEC produces Shiga toxin (Stx), can give rise to hemorrhagic colitis and hemolytic uremic syndrome. Plant polyphenols have various properties including antibacterial, antioxidant and antitoxin activities. They have been reported to inhibit the growth of various bacteria and the activity of toxins produced by the bacteria. In this study, the antibacterial and antitoxin activities of plant polyphenols were investigated..

The first objective of this study, the combined effects of Teavigo (epigallocatechin gallate (EGCg) formulation including 95% EGCg) and TF40 (theaflavin formulation including 40% theaflavins) and 5 food additives on the growth of *E. coli* O157:H7 were evaluated by determining minimum inhibitory concentration (MIC) and the fractional inhibitory concentrations (FIC) index. The 2 tea polyphenol formulations showed stronger antibacterial effects against *E. coli* O157:H7 in combination with 5 food additives than those of the single use. The combination of Teavigo with EDTA or Na citrate, TF40 with EDTA showed the synergistic effect. The partial synergy effect was observed in the combination of Teavigo with ethanol, NaCl or Na acetate, and the combination of TF40 with ethanol or Na citrate. There was no combined effect of TF40 and Na acetate or NaCl in this study. The results of time-kill assay confirmed the combined effects of the tea polyphenol formulations and food additives against *E. coli* O157:H7. It is possible to take advantage of the different antibacterial activity of these compounds and the application of combined use of plant polyphenols and additives in food preservation is feasible.

Secondly, the effects of Teavigo and heat treatment against of *E. coli* O157:H7 were investigated. Heat treatment at 55 ºC for 10, 15, and 20 min significantly decreased the viable count of *E. coli* in the presence of Teavigo compared to those in the absence of it. After heating at 55ºC for 20 min, there was the recovery of injured cells after heating both in the absence and the presence of Teavigo at 125 and 250 mg/L. However, at higher concentration of Teavigo (500 and 1000 mg/L), the injured *E. coli* O157:H7 did not recover. In addition, after the heat treatment of *E. coli* at 55 ºC for 20 min, both protein and nucleic acid leakages increased with increasing heating time and Teavigo concentration. These results suggested that the mild heat treatment in the presence of EGCg at the concentrations without growth inhibition in the single use caused the significant damage in the membrane leading to the leakages of cellular protein and nucleic acid related substances and subsequent death in *E. coli* cells.

Lastly, effects on cytotoxicity of both Stx1 and Stx2 were investigate on 7 compounds selected by *in silico* screening of the natural compound database according to the structural properties of EGCg which inhibited cytotoxicity of both Stx1. Among them, baicalein inhibited the cytotoxicities of both Stx1 and Stx2

against Vero cells, after preincubation at 0.13 mmol/L. Baicalein also reduced the susceptibility of Vero cells against both Stx1 and Stx2. Real-time qPCR showed that baicalein increased transcription of *stx1* but not of *stx2*. However, baicalein had no effects on both the intracellular Stxs production and the secretion of Stxs. Docking simulation suggested that baicalein inhibited cytotoxicity of both Stx1 and Stx2 due to the formation of stable complex at the pocket of the Stx1B and Stx2B pentamers.

The findings in this study showed the antibacterial and antitoxin effects of plant polyphenols for controlling pathogenic EHEC. The combination of polyphenols and food additives or heating was more effective than the single use against EHEC. Polyphenols is also one of the potential candidates for inhibiting Stxs from EHEC. Natural polyphenols are highly safe as they have experience in eating and are expected to be one of the means to contribute to the reduction of health risks derived from foods. For this purpose, it is necessary to further verify the combined effect with various additives and naturally derived antibacterial substances, elucidate its mechanism, and systematically elucidate the inhibitory effect against various bacterial toxins.