Study on antibacterial mechanism of polyphenols on Staphylococcus aureus

アピサダ, キティシャルニチェン

Name ：Apisada Kitichalermkiat

Title ：Study on antibacterial mechanism of polyphenols on *Staphylococcus aureus* (黄色ブドウ球菌に対するポリフェノールの抗菌作用機構に関する研究)

Category：Kou

Thesis Summary

Increasing demands for safety and quality of food leads to an increase in the important role of food additives in food industries. Catechins, a class of polyphenol abundant in green tea, have several useful properties including, antibacterial activity. Among catechins, epigallocatechin gallate (EGCg) has particularly strong antibacterial activity and is more effective against Gram-positive than Gram-negative bacteria. *Staphylococcus aureus* is one of the major foodborne pathogens that produce a wide variety of extracellular toxins. Naturally occurring antimicrobials, such as EGCg, do not induce acquisition of the resistance to the substance in bacteria compared to antibiotics. Although EGCg is widely studied on their antibacterial properties, the antibacterial mechanism of EGCg against *S. aureus* remains unclear. Clarification of mechanism of antibacterial action of EGCg on *S. aureus* will promote the application of EGCg as a natural food preservative. In this study, the effects of EGCg on viability, cell morphology, and cellular proteins, gene transcription, and Enterotoxin A (SEA) production were investigated in *S. aureus*.

S. aureus cells were recoverable from injury caused by EGCg in the nutrient broth but not in buffer. After 24-h treatment with 500 mg/L EGCg, *S. aureus* cells remained damaged but proliferated very slowly in broth. In buffer, the viable counts of *S. aureus* decreased in time-dependent manner in the presence of 500 mg/L EGCg. The morphological observation by fluorescence microscopy suggested that EGCg bound to the surface of the cells. Abnormal and misplaced division septa were observed in the cells treated with EGCg above 250 mg/L by staining with DAPI. Two-dimensional electrophoresis of proteins prepared from EGCg-treated *S. aureus* cells indicated that some proteins disappeared or showed markedly decreased intensity compared to those from control cells. These proteins were identified to be DnaK, elongation factor G, DNA-directed RNA polymerase, L-lactate dehydrogenase, pyruvate dehydrogenase, and acetate kinase. Moreover, *S. aureus* showed decreased glucose uptake after the treatment with 500 mg/L EGCg.

DNA Microarray analysis revealed that *S. aureus* increased and decreased transcription of genes related to membrane transport and toxin production, respectively, after 1-h treatment with 500 mg/L EGCg. The changes of transcription of all the above genes were confirmed by Real-Time qPCR. The membrane potential of the cells treated with 500 mg/L EGCg largely decreased, indicating the cell membrane was damaged by EGCg. It seems that *S. aureus* increased transcription of the genes for membrane transport to recover the function of the membrane.

EGCg reduced SEA production but not the growth of *S. aureus* after 24-h incubation at 125 mg/L. The transcription of *sea* increased after incubation for 1 h but decreased after incubation for 4 and 24 h with 125 mg/L EGCg. The results suggest that *S. aureus* increased *sea* transcription at 1 h as a result of the general environmental stress response caused by EGCg. It seems that EGCg decreased SEA production by

suppressing the transcription of *sea* in *S. aureus*.

These results suggest that EGCg has the potential to be natural antibacterial agent to control ***S. aureus*** and its enterotoxin production.