Study of food additives on inhibition of biofilm formation by pathogenic bacteria on food and food contact surfaces

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（食中毒細菌のバイオフィルム形成阻害に関する研究）

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論　文　内　容　の　要　旨

The consumption of fresh produce has been increasing over the last few years due to a demand for healthier diets. Increased consumption of minimally processed fruits and vegetables has led to an increase in the number of outbreaks of foodborne illness to these products in the world. The fresh produce can be contaminated with pathogenic bacteria at any point from farm to fork through incidental contact with the organism and the sources may include soil, feces, organic manure, irrigation or wash water, insects, wild and domestic animals, handling by workers, and contact surfaces. In this study, to reduce the risks of foodborne illness associated with fresh produce, effective method for the adhesion inhibition of foodborne pathogen on the surface of vegetables by combined use of food additives was developed. The molecular mechanism for adhesion inhibition by the safer food additives was investigated.

The adhesion inhibition effects on pathogenic bacteria by the combined use of some selected food additives such as sucrose fatty acid ester (SE) C18, gardenia yellow (GY), monascus pigment (MP), protamine (PT), ε-polylysine (PL) and milk serum protein (MSP) was investigated. The adhesion of those pathogenic bacteria was reduced by the combination of MSP and PL or PT. The combination of 0.001% PL and 0.25% MSP was effective to reduce the viable counts of secondary-contaminated *Salmonella* Enteritidis and *Escherichia coli* O157 by around 2.5 log on the cabbage leaves by washing with water compared to that of control. However, the pretreatment was not effective against *Listeria monocytogenes*. The pretreatment of lettuce leaves with the same combination significantly reduced ($P < 0.05$) the secondary-contaminated *S*. Enteritidis by around 1.5 log and 1.2 log by washing with water, and combination of washing and disinfection with low concentration of 0.02% NaOCl for 5 min, respectively compared to that of control. In case of sprouts, the pretreatment of the contaminated seeds by 0.01% PL facilitated the reduction of *S.* Enteritidis by 1 log and 1.6 log after washing and combination of washing and disinfection, respectively, on sprouts cultivated from the contaminated seeds. This combination of food additives did not affect the quality attributes of the vegetables, including the vitamin C and chlorophyll contents and appearance.

To clarify the molecular mechanism for adhesion inhibition by food additives, changes of gene expression of *S.* Typhimurium after the treatment with food additives were examined by using DNA microarray. Among the regulated genes (≥2-fold expression), 337, 70, 94, and 50 genes were upregulated, whereas 299, 29, 31, and 58 genes were downregulated in the samples treated with 0.001% PL, 0.25% MSP, 0.05% SE, and the combination of 0.001%PL and 0.25% MSP, respectively. Among these genes, the downregulation of curli production, cellulose formation, chemotaxis and quorum sensing related genes along with *adrA* (diguanylate cyclase AdrA) genes seemed to be the main factors in the inhibition of initial attachments of *S.* Typhimurium on plastic surfaces by using PL. The flagellar assembly, *cpx* envelope stress response and quorum sensing related genes may have the principle role in the inhibition of attachment of *S.* Typhimurium on plastic surface by MSP and SE.

In conclusion, the successful application of these food additives for reduction of secondary-contaminated pathogenic bacteria and decontamination of vegetable may reduce the use of chemicals having any adverse effects on human health for commercial decontamination of vegetables. In addition, the successful completion of the further study would provide a suitable model to predict the mechanism for inhibition of interaction of *S.* Typhimurium cells with food and food contact surfaces by using food additives. This knowledge would be helpful to take the novel strategy to overcome the problem associated with biofilm formation on food and food contact surfaces in the food industry.