# 学位論文内容の要旨

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学 位 論 文 題 目

Studies on Histamine Production Properties and Control of *Morganella psychrotolerans*

（*Morganella psychrotolerans* のヒスタミン産生特性と制御に関する研究）

Histamine fish poisoning is a foodborne toxicity caused by ingesting high levels of histamine. The U.S. Food and Drug Administration (FDA) guideline for safe levels of histamine concentration in seafood is 50 mg/kg, while histamine concentration greater than 500 mg/kg is hazardous to humans. Histamine accumulation in food is caused by histamine-producing bacteria that have histidine decarboxylase (HDC). This enzyme decarboxylates free histidine into histamine. Once histamine is formed in food, it cannot be destroyed by common food processing treatments such as heating. *Morganella psychrotolerans* is a novel psychrotolerant histamine-producer, that can grow even at 0 - 2 °C. Previous studies have documented the poisoning incidents caused by *M. psychrotolerans,* and our survey revealed that this bacterium has been commonly distributed in retail seafood in Japan. In this study, histamine production properties of *M. psychrotolerans* were investigated and its control strategies were proposed.

In Chapter 1, a comprehensive investigation has been conducted the histamine production ability of *M. psychrotolerans* (in canned tuna and culture broth), and the activity of HDC extracted and the expression level of the *hdc* gene at different pH, NaCl concentration and temperature conditions. Although the low temperature (4 °C) and high salt concentration (4 %) retarded the growth rate of *M. psychrotolerans*, the high level of histamine (> 2600 mg/kg or 3500 mg/mL) was accumulated by *M. psychrotolerans* under all the conditions (pH 5–8, 0–4 % NaCl, and 20 and 4 °C) tested in canned tuna and culture broth. Expression of histidine decarboxylase gene *hdc* of *M. psychrotolerans* was induced at low pH conditions. The optimal temperature, pH, and NaCl concentration for histamine production with crude HDC extracted from *M. psychrotolerans* cells

were 30 °C, pH 7 and 0 % NaCl, respectively. The activity of the crude HDC at 10 °C retained 45% of the activity at 30 °C. All the results were confirmed that *M. psychrotolerans* have high histamine producing ability even at low temperature. Once the enzyme HDC was produced, it could retain activity even if the bacteria were not active. The inhibitory effect of tea catechins on the activity of HDC was examined. Of 4 major types of tea catechins, epigallocatechin gallate (EGCG) exhibited the highest inhibitory activity on HDC extracted from *M. psychrotolerans*.

Development of a suitable disinfection means is an important for control of the growth and histamine formation of *M. psychrotolerans* in seafood. In Chapter 2, the sanitizing effect of peracetic acid (PAA) against *M. psychrotolerans* was compared to sodium hypochlorite (SH) commonly used in food industry. 20 ppm PAA and 40 ppm SH could reduce the populations of *M. psychrotolerans* below the detection level by the treatment for 5 min *in vitro*. For challenge test on saury surface, the treatment with 80 ppm PAA and 100 ppm SH for 5min showed about 2.23 and 1.99 log CFU/cm$^2$ reduction of *M. psychrotolerans*, respectively. PAA could be proposed to be a better sanitizer for seafood processing to replace SH. The mode of antimicrobial action of PAA was related to damage the cell membrane and not to the DNA.

In addition, concerns for consumer's demand for safer and natural additives have resulted in developing the potential sanitizing strategy. Organic acid treatments have shown clear efficiency against food pathogens. In Chapter 3, antibacterial effect of organic acid and its combination with PAA was evaluated. It was confirmed that lactic acid (LA) significantly showed antimicrobial activity against *M. psychrotolerans in vitro*. The effective antimicrobial activity of LA was caused by depolarizing cell membrane potential and decreasing in intracellular ATP and intracellular pH of *M. psychrotolerans.* Furthermore, the combination of 2 % LA and 10 ppm PAA showed a good efficiency for reducing *M. psychrotolerans* population from saury surface for 5 min, and the efficacy was similar to the treatment with 200 ppm PAA for 5 min.

This study devises strategies for reducing histamine poisoning from the control of *M. psychrotolerans* and its HDC activity. Potential HDC inhibitors such as EGCG and combined sanitizer of PAA and LA would be expected to reduce histamine food poisoning and ensure the safety of seafood during storage and processing.