博士（栄養学）学位論文要旨

Effects of *Lactobacillus plantarum* No.14 (LP14) on Several Clinical Parameters and Influences of Gastrointestinal Transit on LP14

Lactobacillus plantarum No.14 (LP14) の各臨床項目に対する効果と LP14 が消化管において受ける影響

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Lactobacillus plantarum No.14 (LP14) was isolated from pickled shallots, a traditional Japanese food, and has long been used in food processing. A placebo-controlled, double-blind study was conducted in 2005.[7] It was suggested that LP14 was useful lactic acid bacteria which improved both allergy and overweight. LP14 was the first reported lactic acid bacterium that decreased body fat percentage in humans.

These effects of LP14 were investigated further in this work. The administration studies were conducted on allergy in spring and autumn. The gastrointestinal tolerance of LP14 was evaluated *in vitro*. Body fat percentage was assumed to decrease because LP14 induced thermogenesis, and the effect of LP14 on human body temperature was measured in two administration studies. The effect of intragastric injection of LP14 on sympathetic nerve activity innervating the brown adipose tissue (BAT-SNA) of rats was examined. A novel fermented food including LP14 was developed based on above research: a kind of jelly packing fermented rice powder employing a gelling agent. The effects of the intake of the rice jelly on a body fat, allergy were examined. In addition, the properties of the jelly were observed in artificial digestive juices *in vitro*. The number of cells of LP14 released from the jelly was counted after digestion.

Chapter 1: Improvements in Seasonal Allergic Disease with LP 14

Two randomized, placebo-controlled, double-blind studies were conducted in female students with seasonal allergic diseases in spring and autumn. For subjects who took 8.7×10^8 of living LP14 cultured in Rogosa medium that fructose was used as carbon source, a significant improvement in ocular symptom-medication score was observed in the spring study. In the placebo group, the T helper type 1 (Th1)/T helper type 2 (Th2) ratio tended to decrease after a 6-week intake period, while in the LP14 group, the percentage of Th1 cells significantly increased. Post-intake eosinophil counts significantly increased in comparison to those at intake cessation in the placebo group, but it appeared to be suppressed in the LP14 group. There were no changes in fecal microflora. In the autumn study, there were no significant differences, but the nasal and the ocular symptom score in the LP14 group were lower than those in placebo group.

These studies indicate the clinical effects of LP14 on seasonal allergic diseases. LP14

might behave as an effective counter-regulator of Th2-skewed immunity. Some lactic acid bacteria are thought to be one of the tools for establishing a Th1 predominance through changes in the composition of the host's intestinal microflora by their affects or by their direct action towards gut-associated lymphoid tissue (GALT). No significant change in fecal microflora was observed in the LP14 group. The underlying mechanism might be explained by direct action on the host systemic immune system.

Chapter 2: Gastrointestinal Transit Tolerance of LP14

Transit tolerance was determined at 37°C against simulated gastric juices at pH values of 2.5, 3.0, and 3.5, and against simulated small intestinal juices containing 0%, 0.2%, or 0.4% oxgall. LP14 obtained from the culture with glucose had high gastrointestinal transit tolerance, but that with fructose had low tolerance. Hence the amounts of exopolysaccharide (EPS) from LP14 cultured in various carbon sources were compared. The EPS levels were 146.5 ± 8.1 mg/l (culture) with glucose, and 20.1 ± 17.0 mg/l (culture) with fructose. When EPS was removed by centrifugation, the simulated gastric tolerance of LP14 cultured with glucose decreased markedly, but that with fructose did not decrease.

These results suggested that the simulated gastrointestinal transit tolerance of LP14 varied with the carbon source of fermentation. The gastrointestinal transit tolerance of LP14 related to EPS contents. Because the living LP14 was considered to insufficient tolerance for gastrointestinal transit under the conditions of the sample preparation in Chapter 1, it might affect humans also in the state of being killed.

Chapter 3: Thermogenesis by LP14

The effect of LP14 on human body temperature was measured in 4-week administration study and single-administration study. The 4-week administration study had a randomized, placebo-controlled, double-blind design. The intervention group received one killed LP14 capsule (1.8×10^{10} CFU). The basal body temperature increased significantly only in the LP14 group over the intake period. The average increase in body temperature was 0.019±0.114°C in the placebo group, and 0.036±0.070°C in the LP14 group. The single-administration study had a randomized, double-blind, cross-over design.

The sample capsules were filled with 0.2 g (1.6×10^{11} CFU) of killed LP14. The chest temperature, blood pressure and task performance were significantly higher after LP14 ingestion than after placebo ingestion, and the arousal score tended to be higher. The average increase in chest temperature was 0.10±0.32°C in the placebo group and 0.24±0.32°C in the LP14 group. The effect of LP14 was examined on BAT-SNA in rats. Killed LP14 (9.7×10^{8} CFU) or water was administered through a gastric cannula. LP14 enhanced the BAT-SNA in rats.

These results demonstrated that killed LP14 induced thermogenesis in human. Nagata *et al.* reported that LP14 induced gene expression of an endogenous pyrogen, *IL-1β*.[20] It was considered that LP14 decreased the body fat percentage by a mechanism in which LP14 induces *IL-1β*, activates the sympathetic nerve, and results in thermogenesis.

Chapter 4: Influences of the Intake of a Processed Food Containing LP14 on Several Clinical Parameters

The intervention study was conducted under the design being randomized, placebo-controlled and double-blinded. The intervention group received one LP14 jelly (2.5×10^{11} CFU/50 g), and the placebo group received one placebo jelly every day for 12 weeks. No effects of LP14 were observed on the body composition or allergy, which was considered to be suppressed by the presence of food components or the matrix of gel. The properties of the jelly were observed in artificial digestive juices under a microscope (×400). The amoeba-like gel was recognized to wrap most of LP14 in rice jelly. Amoeba-like gel wrapping LP14 remained after intestinal juice digestion. The number of cells of LP14 released from the jelly was counted after digestion. The jelly after being swallowed was considered to be crushed to a same extent in the particle size with Stomachere processing. In the jelly crushed by Stomachere, 14 % of LP14 was liberated after digestion.

In conclusion, the rice jelly was ineffective to body composition or allergy in spite of increased intake amount of LP14. It was considered that most cells of LP14 in the rice jelly were buried in gel matrix, leading to excretion. LP14 was considered to affect human immune cells of an intestinal tract. Therefore, LP14 buried in gel matrix could not affect humans. The problem became clear about processing LP 14 into foods.