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Bile acid-lowering properties of *Lactobacillus plantarum* Sanriku–SU3 isolated from Japanese surfperch fish

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ABSTRACT

In vitro bile acid-lowering capacity of lactic acid bacteria (LAB) strains (ten *Lactobacillus plantarum*, four *Lactococcus lactis*, and two *Leuconostoc mesenteroides*) isolated from fermented fish and fish intestine were compared with those of type strains. Among the isolated strains, *Lb. plantarum* Sanriku–SU3, *Lc. lactis* Suzuki 8, and *Ln. mesenteroides* BF6, isolated from Japanese surfperch, Japanese seabass, and Korean rockfish, respectively, clearly showed bile acid-lowering capacity. Although *Lc. lactis* Suzuki 8 and *Ln. mesenteroides* BF6 showed activity on deoxycholic and taurocholic acid media, respectively, rather than glycocholic acid, *Lb. plantarum* Sanriku–SU3 showed the lowering capacity with all of the three cholic acids. These capacities were higher with heat-killed cells than live cells. Plasma cholesterol in mice fed heat-killed cells of *Lb. plantarum* Sanriku–SU3 tended to be low, while liver cholesterol and plasma glucose were lowered in mice fed live Sanriku–SU3 cells. These results suggest that live as well as heat-killed *Lb. plantarum* Sanriku–SU3 could ameliorate metabolic syndrome.

1. Introduction

Hypercholesterolemia is a major human health concern, which is implicated in cardiovascular disease (Yamamoto & Rakugi, 2015) as well as various other diseases such as diabetes (Ramadan & Kabbara, 2015), osteoporosis (Pelton et al. 2012), Alzheimer's disease (El-Sayyad, 2015), immune response disorders (Han, Leka, Lichtenstein, Ausman, & Meydani, 2003) and cancers (Pelton et al., 2014). There are many reports about the lowering of low-density lipoprotein (LDL)-cholesterol, cholesterol and bile acid-binding and excretion, and the properties of food materials such as various dietary fibers (Niijar, Burke, Bloesch, & Rader, 2010).

In the normal human body, 70–80% of cholesterol is biosynthesized. Some probiotics, such as *Lactobacillus fermentum, Lactobacillus casei,* and *Bifidobacterium bifidum*, show cholesterol lowering as a result of bile acid hydrolase activity (Guo & Li, 2014; Kim, Miyamoto, Meighen, & Lee, 2004; Pereira, McCartney, & Gibson, 2003). In addition, the cholesterol lowering effect is attributed to cholesterol binding by the putative probiotic cells (Choi & Chang, 2015; Kuda, Yazaki, Ono, Takahashi, & Kimura, 2013; Zeng, Pan, & Guo, 2010). The latter property might be attributed to the membrane structure; even the heat-killed cells of these probiotics can lower cholesterol *in vitro* and have an effect on the plasma cholesterol level *in vivo*.

The Ministry of the Environment, Government of Japan, has defined *Satoumi* as a coastal area where biological productivity and biodiversity are increased owing to human interaction (Berque & Matsuda 2013). This laboratory has reported the testing of lactic acid bacteria from fermented fish and intestines of fish from *Satoumi* regions for probiotic properties such as acid, bile acid, and salt tolerances, and also determined the anti-oxidant and/or anti-inflammation capabilities of the selected strains (Kawahara et al., 2015; Kuda & Kawahara, 2014; Kuda & Noguchi, 2014). Compared to the type strains, their bile and salt-tolerances are higher (Kuda et al., 2013; Kuda, Kyoi, Takahashi, Obama, & Kimura, 2011).

In this study, probiotic LAB showing cholesterol-lowering effects were identified for use as probiotics and/or starter strains by determining the bile acid-lowering capacities of heat-killed cells of 10 *Lactobacillus plantarum*, 4 *Lactococcus lactis,* and two *Leuconostoc mesenteroides* strains isolated from fish intestine, algal beach casts, and fermented fish products obtained from the *Satoumi* regions, and compared them with the type strains. The properties of live and heat-killed cells of selected strains were then determined in mice.

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2. Material and methods

2.1. Lactic acid bacteria (LAB) cultures

A total of 19 lactic acid bacteria (LAB) strains (Table 1) were used in this study. Among them, 10 *Lactobacillus plantarum*, 4 *Lactococcus lactic* subsp. *lactis*, and two *Leuconostoc mesenteroides* subsp. *mesenteroides* were isolated from fish intestine and fermented fish products obtained from *Satoumi* areas of Sanriku (Kuda & Kawahara, 2014), Kyusyu (Kuda et al., 2011), Noto (Kuda et al., 2013) and Rausu (Kuda & Noguchi, 2014), Japan. These 16 *Satoumi* LAB strains and their type strains (*Lb. plantarum* NBRC15891^T, *Lc. lactis* NBRC100933^T, *Ln. mesenteroides* NBRC10496^T) were pre-incubated in 4 ml of de Man, Rogosa and Sharpe (MRS) broth (Oxoid, Basingstoke, UK) at 37 °C for 48 h.

2.2. In vitro screening of total bile acid-lowering capability of Satoumi LAB strains

Bile acid-lowering capacity of heat-killed LAB cells was determined as previously reported (Kuda et al., 2013), with some modifications. Pre-cultures of the strains (0.2 ml) were inoculated into 30 ml of MRS broth and incubated at 37 °C for 48 h. The cultured broths were boiled for 20 min and centrifuged (2200*g* for 10 min at 4 °C; Model 3740, Kubota, Tokyo, Japan). Then, cell turbidity at OD 660 nm was measured using a spectrophotometer (U0080D, Hitachi High-Technologies, Tokyo, Japan) and adjusted to OD 660 nm = 10 (about 10 log CFU/ml) with phosphate buffered saline (PBS, Nissui Pharmaceutical, Tokyo, Japan) containing 0.25 mg/ml bile (Gall, powder, Wako Pure Chemical, Osaka, Japan), and was then centrifuged without incubation. After the centrifugation, the remaining bile acid content in the supernatant was determined using a commercial kit (Total Bile Acid Test Wako, Wako Pure Chemical) according to the manufacturer's instructions.

2.3. Effect of heat inactivation on bile acid-lowering capacity of selected Satoumi LAB strains

The type strain and one *Satoumi* LAB of each species were used in this experiment. The bacterial suspension were adjusted to OD 660 nm = 10 without heating and were then split between two test tubes. One of the tubes was boiled for 20 min and the bile acid-lowering capacity was then determined as above. The bile acid lowering capacity of the other live cell suspension was measured before and after incubation at 37 °C for 30 min.

2.4. Cholic acid-lowering properties of selected Satoumi LAB strains

The three selected *Satoumi* LAB strains and their type strains were also used in this experiment. The cell suspensions were prepared as above with the PBS containing 0.1 mg of cholic acid (sodium glycocholate, sodium taurocholate, or deoxycholic acid, Wako Pure Chemical) instead of gall powder. Then, the cholic acid-lowering capacities were determined as above. In this experiment, the capacities of live cells were determined after incubation at 37 °C for 30 min.

2.5. Effect of live and heat-killed cells of selected LAB strains on plasma and liver lipid levels

From the results of the above experiments, a *Satoumi* LAB strain which showed the clear all three cholic acid-lowering capacities was selected for the animal experiment. The live and heat-killed bacterial cells were prepared as above and suspended in distilled water; OD 660 nm was adjusted to 10. The cell suspension was stored at 4 °C and used within 3 days. Then, the suspension was diluted with 4 volumes of distilled water as drinking water. The animal experiment was done in compliance with the fundamental guidelines for the proper conduct of animal experiments and related activities in academic research institutions, under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and approved by the animal experiment committee of the Tokyo University of Marine Science and Technology (Approval no. H26-3).

Twenty-one 5-week-old male Kwl:ddY mice were obtained from Tokyo Laboratory Animal Science (Tokyo, Japan). The mice were acclimated in a negative pressure rack maintained at 20–24 °C and were fed a CE2 diet (CLEA Japan, Tokyo, Japan) and distilled water. The CE2 is a standard diet for rodent containing 24.9 g/100 g of crude protein, 4.6 g/100 g crude fat, 51 g/100 g of nitrogen free extract, 4.1 g/100 g of crude fiber, and 345 kcal/100 g of energy. After 7 days, the mice were divided into 3 groups ($n = 7$) and the diet was changed to Quick Fat (high-fat diet, CLEA Japan) containing 24.8 g/100 g of crude protein, 14.4 g/100 g crude fat, 45.7 g/100 g of nitrogen free extract, 2.45 g/100 g of crude fiber, and 415 kcal/100 g of energy. The main fat source was beef tallow. The control group was administered distilled water (DW) and the other two groups were administered live or heat-killed cells in drinking water. The food and drinking water were changed every day. After 14 days of Quick Fat administration, whole blood was collected from the abdominal aorta while the mice were under anesthesia with diethyl ether. Following the lethal exsanguination, the liver, kidneys, spleen, cecum, and epididymal fat pads were excised and weighed.

To measure triacylglyceride (TG), phospholipid (PL), and total

Table 1
Lactic acid bacteria strains used in this study.

Name of speicies	Strain no.	Source	Areas	Reference
Lactobacillus plantarum	NBRC15891^T	Type strain		
	Sanriku–SU1	Salted SQUID	Sanriku	Kuda and Kawahara (2014)
	Sanriku–SU3	Japanese surfperch		
	Sanriku–SU4	Korean rockfish		
	Sanriku–SU5	Blue mackerel		
	AN1, 2, 3, 6, 7, 13	Fermented fish	Noto	Kuda et al. (2013)
		Horse mackerel (*aji-narezushi*)		
Lactococcus lactis subsp. *lactis*	NBRC100933^T	Type strain		
	BF3	Chum salmon	Rausu	Kuda and Noguchi (2014)
	Suzuki 7, 8, 13	Japanese seabass	Kyusyu	Kuda et al. (2011)
Leuconostoc mesenteroides	NBRC10496^T	Type strain		
	BF6, 7	Chum salmon	Rausu	Kuda and Noguchi (2014)