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Letter

Safety assessment of probiotic bacteria, *Bacillus coagulans* strain SANK70258, in rats

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ABSTRACT — *Bacillus coagulans* is a lactic acid, spore-forming gram-positive bacteria that has the potential of probiotic benefits. To evaluate the toxicological profiles of *Bacillus coagulans* strain SANK70258 (active element of LACRIS™-S), a 90-day oral gavage dose study was conducted in rats. The microbe was administered by oral gavage to 6-week old Crl:CD(SD) rats (10 males and 10 females/group) for 90 days at dose levels of 0, 500, 1000, and 2000 mg/kg/day. According to the results, no deaths occurred in either males or females, and no treatment-related changes were observed in any clinical sign including a detailed observation with functional observational battery (FOB), functional test, motor activity, body weight, food consumption, ophthalmoscopy, urinalysis, hematology, blood chemistry, organ weight, necropsy or histopathology. In conclusion, *Bacillus coagulans* strain SANK70258 had no subchronic toxicity in rats and the no-observed-adverse-effect-level (NOAEL) was judged to be greater than 2000 mg (about 1×10^{12} CFU)/kg/day. The microbe is harmless and can be used as a probiotic.

Key words: Probiotic, Bacillus coagulans, 90-day subchronic-toxicity study, Rat, NOAEL

INTRODUCTION

Probiotics are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance (Schrezenmeir and De Vrese, 2001). Lactic acid bacteria have a long history of safe use in fermented food production as one of the most beneficial probiotics. The beneficial functions of lactic acid bacteria include: improving digestion and immune function (Rundles *et al.*, 2000); managing lactose intolerance (Granato *et al.*, 2010); degrading cholesterol (Ooi and Liong, 2010); and preventing cancer (Hirayama and Rafter, 2000). However, general lactic acid bacteria are very sensitive to heat, drying, acidity of the stomach, and bile acids. On the contrary, *Bacillus coagulans* stains are the spore-forming gram-positive bacteria and have the ability to overcome these limitations (Keller *et al.*, 2010).

Although traditional Lactobacillus strains have an excellent history of safe use in the formation of dairy products and other foods and some have ‘‘generally recognized as safe’’ (GRAS) status, it is essential to conduct the safety assessment on any new strains with the intent to be added into foods or used as a dietary supplement (Donohue and Salminen, 1996). *Bacillus coagulans* strain SANK70258 was the first successful mass produced by Daiichi-Sankyo Co., Ltd. (Tokyo, Japan). Its beneficial effects have been reported (Ara *et al.*, 2002; Iino *et al.*, 1997a, 1997b; Kajimoto *et al.*, 2005) and this strain has been used for drugs and foods in safety for about 50 years (Mashita *et al.*, 1964; Takaya, 2014); however, reliable data according to recent preclinical guidelines were insufficient for this strain. In this study, we carried out a sub-chronic toxicity study of *Bacillus coagulans* strain SANK70258 when administered daily by oral gavage to rats for 90 days in compliance with Good Laboratory Practice (GLP).

MATERIALS AND METHODS

This study was conducted in accordance with the procedures indicated by Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals “Repeated Dose 90-day Oral Toxicity Study in Rodents” (Test No. 408, Adopted by the Council on 21 September 1998) and carried out by LSI Medience Corporation (Tokyo, Japan) in compliance with OECD Principles of Good Laboratory Practice (as revised in 1997). This experiment was approved by the Institutional

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Animal Care and Use Committee of LSI Medience Corporation accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAAALAC International).

Test article

The test microbe, *Bacillus coagulans* strain SANK70258 (Lot No. 141004AA, 5.1×10^{11} CFU/g), which is an active element of LACRIS™-S, was prepared by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). The microbe is a gram-positive spore-forming rod that is aerobic to microaerophilic in nature. SANK70258 is a pure cell mass consisting solely of *Bacillus coagulans* and light brown powder having a slightly unique odor. The microbe was suspended in water for injection (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to make concentrations of 50, 100, and 200 mg/mL once within 4 days. The stability of the test microbe in water for injection was confirmed for 7 days under refrigeration at 50 and 400 mg/mL.

Animals and husbandry

Male and female Crl:CD Sprague Dawley (SD) rats (4 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Atsugi, Japan). After quarantine and acclimation for 2 weeks, 40 healthy animals (6 weeks of age) of each sex were assigned to each group by the stratified-by-weight randomization method on the basis of the body weights (male: 194.7 g to 234.0 g, female: 147.7 g to 187.7 g). Animals were housed individually in hanging type stainless steel wire cages with enrichments (alumina balls, floor plates and laboratory bedding [Diamond Twists, Harlan Laboratories, Tokyo, Japan]) in an air-conditioned room (temperature: 19.0°C to 25.0°C, relative humidity: 25.0% to 75.0%) with artificial lighting from 7:00 to 19:00 and ventilation providing all fresh air. Food (CR-LPF, radiation sterilized: Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given ad libitum. The initial dosing day was designated as Day 1, and the period from Day 1 to Day 7 was designated as Week 1.

Administration

The dosing suspensions were administered by oral gavage with a disposable syringe attached to a gastric tube for rats. The dose levels were set at 0, 500, 1000, and 2000 mg/kg/day. Each dose group consisted of 10 animals of each sex. The control animals were dosed with the vehicle alone. The dose volume was set at 10 mL/kg, and the individual volume was calculated based on the most recent body weight.

Observation and examinations

Clinical signs were observed twice daily (before and about 1 hr after dosing). Detailed clinical observations were performed by the functional observational battery (FOB) once before initial dosing and once a week (after dosing) during the dosing period. Function tests (approach response, touch response, auditory response, tail pinch response, and aerial righting reaction, grip strength of forelimb and hindlimb using a digital force gauge) and motor activity measurement using a motor activity-measuring device (SUPERMEX, Muromachi Kikai Co., Ltd., Tokyo, Japan) were performed in Week 13. Body weight and food consumption were measured every 7 days. Ophthalmoscopy was performed before the start of dosing and in Week 13. Urinalysis was performed on 5 males and 5 females in each group in Week 13. Fresh urine samples were collected within 3 hr in the morning (before dosing) without food and water supply. Subsequently, 20-hr accumulated urine samples were collected while providing food and water. Paper test (Clinitek 500; Siemens Healthcare Diagnostics K.K, Tokyo, Japan) and sediment test were conducted using fresh urine samples. The volume, specific gravity (Uricon-JE; Atago Co., Ltd., Tokyo, Japan), and electrolytes (sodium [Na], potassium [K], and chlorine [Cl]: TBA-200FR; Toshiba Corporation, Tokyo, Japan) were examined using the 20-hr urine samples. At the end of the dosing period, blood samples were collected from the posterior vena cava under anesthesia (intraperitoneal injection of sodium thiopental) after overnight fasting. A portion of the blood sample was anticoagulated with ethylenediaminetetraacetic acid (EDTA)-2K and analyzed for hematology parameters by an automated hematology analyzer (XT-2000iV; Sysmex Corporation, Hyogo, Japan). A portion of the blood samples was anticoagulated with 3.2 w/v% trisodium citrate solution, and then plasma samples were obtained for examination of prothrombin time (PT) and activated partial thromboplastin time (APTT) by an automated coagulation analyzer (CA-510; Sysmex Corporation, Hyogo, Japan). In addition, serum samples were obtained from the other portion of the blood samples and analyzed for blood chemical parameters by an auto-analyzer (TBA-200FR, Toshiba Corporation, Tokyo, Japan). After the blood samples were collected, animals were necropsied. Organs and tissues were removed and fixed 10 vol% phosphate-buffered formalin. The eyeball with the optic nerve and Harderian gland was fixed with Davidson's solution, and the testes were fixed with Bouin's solution before storage in 10 vol% phosphate-buffered formalin. The main organs were weighed and the relative organ weights (organ to body weight ratio) were calculated. All organs and tissues

were processed by the standard method to prepare hematoxylin and eosin-stained (HE) specimens, and examined microscopically.

Statistical analysis

Numerical data were first analyzed by Bartlett's test ($P < 0.01$). When the group variance was homogeneous, Dunnett's test ($P < 0.05$, two-tailed) was employed to compare with the mean value of the control group. When the group variance was heterogeneous, Bartlett's test was performed again after logarithmic conversion of the data. When the variance was homogeneous, Dunnett's test was applied. When the variance was heterogeneous, Steel test ($P < 0.05$, two-tailed) was applied to compare with the mean value of control group after rank transformation of the data.

RESULTS

Systemic clinical status

There were no deaths or moribund animals or any treatment-related change noted in any animal according to the standard observation or detailed observation by FOB, functional test, or motor activity throughout the experimental period. There were no differences in body weight or food consumption among groups throughout the experimental period (Fig. 1 and Table 1).

Ophthalmoscopy

No treatment-related changes were noted in any animal. Various changes were observed in each group; however, none of them were considered to be treatment-related because they are often observed in normal rats or they were already noted before the start of dosing.

Clinical chemistry

No treatment-related differences were noted in the urinalysis, hematology or blood chemistry tests among groups (Tables 2 and 3). Several statistically significant differences were observed; however, they were within the range of the background data of the test facility or there was no dose-relationship. They were not concluded to be treatment-related.

Pathological examination

No treatment-related changes were noted in organ weights (Table 4), necropsy, or histopathology in any group. A statistically significant increase in thyroid weight was observed in males at 2000 mg/kg; however, it was not of toxicological significance because there were no corresponding abnormalities in the histopathology. Various changes were observed in each group including the control group; however, they were not considered to be treatment-related because similar changes are often observed in normal rats and/or there was no clear dose-dependency.

DISCUSSION

Many *Lactobacillus* stains have been afforded GRAS classification, and are among the most important bacteria in food microbiology and human nutrition due to

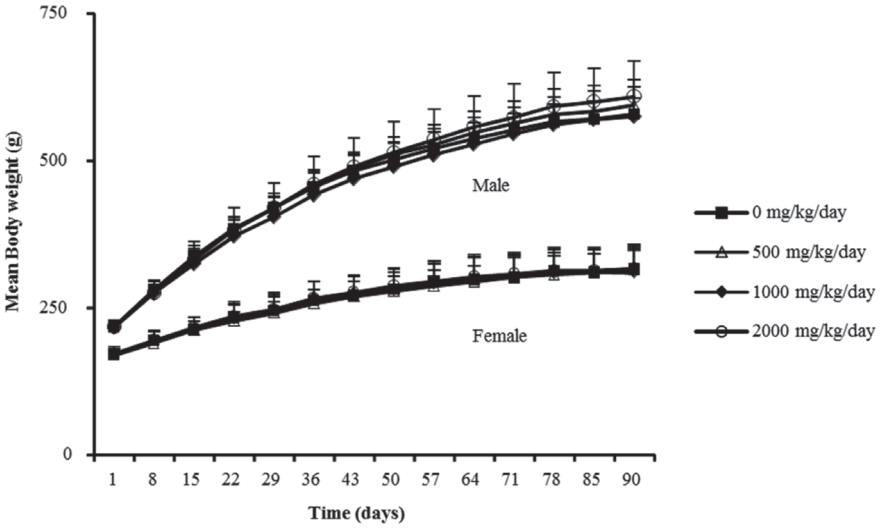

Fig. 1. Body weight changes of male and female rats administered *Bacillus coagulans* strain SANK70258 by gavage for 90 days. The data represents means ± standard deviation for each group (n = 10 animals per group).

Table 1. Mean daily food consumption (g/day) of male and female rats administered *Bacillus coagulans* strain SANK70258 by gavage for 90 days.

Doses (mg/kg/day)	Day	1 to 8	8 to 15	15 to 22	22 to 29	29 to 36	36 to 43	43 to 50	50 to 57	57 to 64	64 to 71	71 to 78	78 to 85	87 to 90
Males														
0	Mean	29.04	31.11	31.45	31.80	32.75	32.17	31.15	31.39	30.76	30.55	30.19	29.83	28.57
	SD	2.26	2.47	2.35	2.68	2.55	1.96	1.85	2.04	1.73	1.77	1.65	1.88	2.09
500	Mean	29.24	31.56	31.56	31.64	32.61	32.07	32.06	31.50	31.57	31.54	30.62	29.88	28.64
	SD	2.31	3.61	4.21	3.74	3.32	3.52	3.35	2.95	3.42	3.62	3.77	3.17	3.51
1000	Mean	28.03	30.06	30.32	30.40	31.17	30.84	30.76	30.63	30.30	29.82	29.40	29.35	28.49
	SD	2.37	2.98	2.85	3.24	3.29	2.91	3.00	2.97	2.89	2.56	2.44	2.79	3.27
2000	Mean	28.24	30.88	31.71	32.30	32.52	32.74	32.44	32.14	32.00	31.39	31.63	30.66	29.42
	SD	2.76	2.39	1.88	1.83	2.80	2.05	2.13	1.82	1.91	1.67	2.13	1.81	2.51
Females														
0	Mean	20.94	21.70	22.54	22.37	22.83	22.91	23.19	23.25	21.98	21.57	21.73	21.75	21.21
	SD	2.01	2.34	2.58	2.16	3.07	2.26	2.54	2.98	2.54	1.75	1.96	1.60	2.11
500	Mean	20.05	21.00	20.87	21.51	21.47	22.26	22.48	21.60	21.64	21.21	20.66	21.11	20.19
	SD	1.89	2.59	2.30	2.78	2.41	2.48	2.29	2.17	2.32	2.03	1.92	2.48	2.45
1000	Mean	20.64	20.94	21.64	22.37	22.81	22.75	22.27	22.35	22.14	21.24	21.15	21.19	19.26
	SD	2.23	2.85	3.19	3.33	3.75	2.70	3.28	3.04	2.78	2.89	2.96	2.48	2.15
2000	Mean	20.85	21.78	22.19	22.95	23.61	23.84	23.25	23.17	22.56	22.40	21.86	22.00	20.98
	SD	2.15	1.98	2.16	2.85	2.64	1.96	2.32	2.49	2.81	1.99	2.06	2.11	2.31

(n = 10 animals per group)

Table 2. Hematology parameters of male (A) and female (B) rats administered *Bacillus coagulans* strain SANK70258 by gavage for 90 days.

(A) Males

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
Red Blood Cell Count	(10^6/μL)	9.023 ± 0.302	8.832 ± 0.443	8.780 ± 0.532	8.892 ± 0.394
Hemoglobin conc.	(g/dL)	15.69 ± 0.65	15.73 ± 0.53	15.11 ± 0.78	15.58 ± 0.77
Hematocrit	(%)	45.43 ± 1.89	45.81 ± 1.37	44.34 ± 2.08	45.52 ± 2.27
MCV	(fL)	50.34 ± 1.17	51.96 ± 2.23	50.54 ± 1.35	51.23 ± 2.41
MCH	(pg)	17.39 ± 0.38	17.83 ± 0.60	17.22 ± 0.40	17.54 ± 0.75
MCHC	(g/dL)	34.53 ± 0.31	34.35 ± 0.41	34.07 ± 0.38*	34.23 ± 0.25
Platelet Count	(10^3/μL)	1113.9 ± 78.3	1119.0 ± 122.7	1110.0 ± 63.6	1159.7 ± 126.3
Reticulocyte	(%)	3.718 ± 0.578	3.223 ± 0.707	3.457 ± 0.624	3.319 ± 0.509
PT	(sec)	15.05 ± 3.25	8.93 ± 0.36**	8.78 ± 0.51**	8.73 ± 0.51**
APTT	(sec)	22.23 ± 2.35	16.11 ± 1.91**	16.58 ± 1.32**	17.03 ± 0.95**
White Blood Cell Count	(10^3/μL)	8.331 ± 1.578	8.776 ± 2.063	8.527 ± 1.166	8.418 ± 2.160
Lymphocyte	(%)	76.59 ± 7.44	77.36 ± 6.36	72.51 ± 6.07	77.15 ± 6.33
Neutrophil	(%)	19.19 ± 6.73	17.20 ± 5.79	22.01 ± 6.09	18.31 ± 5.98
Eosinophil	(%)	1.49 ± 0.47	1.56 ± 0.50	1.52 ± 0.78	1.28 ± 0.26
Basophil	(%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocyte	(%)	2.73 ± 1.00	3.88 ± 0.99*	3.96 ± 1.22*	3.26 ± 0.63
Lymphocyte	(10^3/μL)	6.374 ± 1.362	6.791 ± 1.745	6.215 ± 1.173	6.571 ± 2.040
Neutrophil	(10^3/μL)	1.607 ± 0.661	1.514 ± 0.748	1.852 ± 0.460	1.466 ± 0.349
Eosinophil	(10^3/μL)	0.120 ± 0.028	0.135 ± 0.045	0.126 ± 0.052	0.109 ± 0.042
Basophil	(10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Monocyte	(10^3/μL)	0.230 ± 0.100	0.336 ± 0.094*	0.334 ± 0.092*	0.272 ± 0.084

(n = 10 animals per group), *: Significantly different from control ($P < 0.05$) **: Significantly different from control ($P < 0.01$)

(B) Females

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
Red Blood Cell Count	(10^6/μL)	8.129 ± 0.447	8.108 ± 0.405	8.396 ± 0.426	8.256 ± 0.443
Hemoglobin conc.	(g/dL)	15.03 ± 0.41	15.02 ± 0.52	15.29 ± 0.66	15.21 ± 0.69
Hematocrit	(%)	43.51 ± 1.22	43.45 ± 1.64	44.58 ± 2.05	44.26 ± 1.88
MCV	(fL)	53.63 ± 2.40	53.64 ± 1.27	53.14 ± 2.09	53.65 ± 1.57
MCH	(pg)	18.52 ± 0.74	18.53 ± 0.37	18.23 ± 0.63	18.44 ± 0.56
MCHC	(g/dL)	34.54 ± 0.58	34.58 ± 0.44	34.30 ± 0.22	34.38 ± 0.45
Platelet Count	(10^3/μL)	1110.8 ± 147.5	1160.4 ± 71.4	1145.9 ± 77.8	1161.2 ± 119.5
Reticulocyte	(%)	2.925 ± 0.590	3.375 ± 0.420	2.815 ± 0.577	3.186 ± 0.673
PT	(sec)	7.69 ± 0.36	7.89 ± 0.26	7.71 ± 0.25	7.64 ± 0.24
APTT	(sec)	15.51 ± 1.61	14.99 ± 2.00	14.94 ± 1.28	14.93 ± 1.97
White Blood Cell Count	(10^3/μL)	5.702 ± 3.029	5.133 ± 1.515	6.503 ± 1.434	5.553 ± 2.246
Lymphocyte	(%)	85.12 ± 5.16	84.39 ± 3.63	82.09 ± 7.65	82.11 ± 5.10
Neutrophil	(%)	11.91 ± 5.08	12.09 ± 3.48	13.86 ± 6.91	14.11 ± 4.98
Eosinophil	(%)	1.33 ± 0.47	1.45 ± 0.53	1.40 ± 0.54	1.61 ± 0.67
Basophil	(%)	0.01 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocyte	(%)	1.63 ± 0.73	2.07 ± 0.92	2.65 ± 1.08*	2.17 ± 0.93
Lymphocyte	(10^3/μL)	4.900 ± 2.748	4.335 ± 1.274	5.392 ± 1.531	4.606 ± 2.038
Neutrophil	(10^3/μL)	0.627 ± 0.291	0.614 ± 0.255	0.860 ± 0.374	0.750 ± 0.347
Eosinophil	(10^3/μL)	0.072 ± 0.032	0.077 ± 0.040	0.087 ± 0.026	0.083 ± 0.033
Basophil	(10^3/μL)	0.001 ± 0.003	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Monocyte	(10^3/μL)	0.102 ± 0.093	0.107 ± 0.054	0.164 ± 0.068	0.114 ± 0.061

(n = 10 animals per group), *: Significantly different from control ($P < 0.05$)

their contribution to fermented food production or their use as probiotics (Donohue and Salminen, 1996). Toxicological studies were conducted on several *Bacillus coagulans* species, and demonstrated that there are no safety issues (Endres *et al.*, 2009; Keller *et al.*, 2010; Sudha *et al.*, 2011). The purpose of this study is to assess the potential sub-chronic toxicity of *Bacillus coagulans* strain SANK70258, which is an active element of LACRIS™-S, and to confirm commercial availability as the probiotic strain. *Bacillus coagulans* strain SANK70258 was administered by oral gavage at 0, 500, 1000, and 2000 mg/kg/day to SD rats for 90 days to assess its toxicity according to OECD guideline in compliance with GLP.

According to the results, no deaths occurred in males or females, and no treatment-related changes were observed in any examination. Since the test microbe was administered for a period longer than 90 days in this study, it has no potentials to produce some toxins or induce abnormal fermentation in the intestinal tract causing diarrhea. In the microbe safety assessment, it is important to eval-

Table 3. Blood chemistry parameters of male (A) and female (B) rats administered *Bacillus coagulans* strain SANK70258 by gavage for 90 days.

(A) Males

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
ASAT	(U/L)	76.3 ± 9.2	79.3 ± 14.6	85.9 ± 15.1	94.9 ± 23.7*
ALAT	(U/L)	25.9 ± 5.6	21.3 ± 5.0	21.8 ± 4.9	26.0 ± 6.9
Gamma GT	(U/L)	0.9 ± 0.3	0.8 ± 0.4	1.0 ± 0.0	0.9 ± 0.3
ALP	(U/L)	278.5 ± 30.8	257.7 ± 43.2	247.3 ± 66.6	259.2 ± 60.8
Total Bilirubin	(mg/dL)	0.06 ± 0.05	0.08 ± 0.06	0.11 ± 0.06	0.10 ± 0.00
Total Bile Acid	(μmol/L)	22.67 ± 14.20	16.55 ± 6.67	13.39 ± 5.85	16.70 ± 6.67
Urea Nitrogen	(mg/dL)	13.07 ± 1.40	13.33 ± 1.93	13.57 ± 1.73	13.82 ± 1.30
Creatinine	(mg/dL)	0.28 ± 0.04	0.25 ± 0.05	0.27 ± 0.05	0.29 ± 0.03
Glucose	(mg/dL)	149.1 ± 8.0	146.8 ± 10.8	146.4 ± 11.9	148.1 ± 7.5
Total Cholesterol	(mg/dL)	76.2 ± 15.7	68.9 ± 19.2	75.1 ± 16.7	70.5 ± 18.6
Phospholipid	(mg/dL)	113.2 ± 18.2	108.3 ± 23.7	110.1 ± 19.0	113.9 ± 23.4
Triglyceride	(mg/dL)	47.3 ± 17.6	64.6 ± 22.7	49.5 ± 10.8	68.0 ± 33.9
Total Protein	(g/dL)	7.23 ± 0.38	6.98 ± 0.35	6.79 ± 0.29*	7.18 ± 0.27
A/G Ratio		0.980 ± 0.113	0.986 ± 0.107	0.947 ± 0.067	0.937 ± 0.116
Albumin	(%)	49.37 ± 2.77	49.49 ± 2.68	48.59 ± 1.78	48.22 ± 2.90
Alpha1 Globulin	(%)	20.24 ± 2.80	19.67 ± 2.40	19.46 ± 2.83	19.73 ± 2.35
Alpha2 Globulin	(%)	10.22 ± 1.25	9.73 ± 0.99	10.18 ± 1.39	10.25 ± 1.28
Beta Globulin	(%)	15.21 ± 1.24	15.73 ± 1.04	15.98 ± 0.87	15.75 ± 1.36
Gamma Globulin	(%)	4.96 ± 0.92	5.38 ± 1.23	5.79 ± 1.65	6.05 ± 1.10
Albumin	(g/dL)	3.57 ± 0.21	3.45 ± 0.21	3.30 ± 0.16**	3.46 ± 0.17
Alpha1 Globulin	(g/dL)	1.47 ± 0.24	1.38 ± 0.21	1.33 ± 0.23	1.42 ± 0.19
Alpha2 Globulin	(g/dL)	0.74 ± 0.08	0.68 ± 0.07	0.69 ± 0.07	0.74 ± 0.09
Beta Globulin	(g/dL)	1.10 ± 0.13	1.10 ± 0.09	1.08 ± 0.07	1.13 ± 0.12
Gamma Globulin	(g/dL)	0.36 ± 0.07	0.37 ± 0.08	0.39 ± 0.12	0.44 ± 0.09
Ca	(mg/dL)	10.53 ± 0.47	10.38 ± 0.41	10.31 ± 0.24	10.57 ± 0.28
IP	(mg/dL)	7.61 ± 0.45	7.61 ± 0.39	7.80 ± 0.53	7.44 ± 0.53
Na	(mmol/L)	148.4 ± 1.0	148.1 ± 1.3	147.7 ± 0.8	150.2 ± 4.7
K	(mmol/L)	4.39 ± 0.17	4.40 ± 0.13	4.45 ± 0.23	4.59 ± 0.34
Cl	(mmol/L)	104.3 ± 1.3	105.0 ± 1.1	105.0 ± 1.5	106.7 ± 3.8

(n = 10 animals per group), *: Significantly different from control ($P < 0.05$) **: Significantly different from control ($P < 0.01$)

(B) Females

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
ASAT	(U/L)	94.2 ± 26.7	93.3 ± 12.5	103.7 ± 17.6	97.7 ± 16.1
ALAT	(U/L)	20.6 ± 6.6	21.3 ± 4.4	23.9 ± 3.9	25.4 ± 6.3
Gamma GT	(U/L)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
ALP	(U/L)	141.3 ± 37.1	127.8 ± 25.5	109.8 ± 24.7	115.8 ± 29.4
Total Bilirubin	(mg/dL)	0.09 ± 0.03	0.11 ± 0.06	0.15 ± 0.05*	0.12 ± 0.04
Total Bile Acid	(μmol/L)	28.90 ± 17.26	32.96 ± 22.58	31.83 ± 15.54	26.65 ± 11.34
Urea Nitrogen	(mg/dL)	15.76 ± 2.18	15.87 ± 2.16	17.05 ± 2.33	15.2 ± 1.88
Creatinine	(mg/dL)	0.33 ± 0.05	0.30 ± 0.00	0.32 ± 0.06	0.30 ± 0.0
Glucose	(mg/dL)	140.1 ± 23.4	131.4 ± 15.4	128.7 ± 15.6	138.2 ± 9.9
Total Cholesterol	(mg/dL)	83.2 ± 18.0	78.6 ± 15.7	88.1 ± 13.7	86.9 ± 13.9
Phospholipid	(mg/dL)	146.3 ± 25.9	138.9 ± 25.6	148.8 ± 17.0	144.1 ± 20.3
Triglyceride	(mg/dL)	26.4 ± 11.5	23.3 ± 9.0	23.5 ± 8.3	21.7 ± 9.4
Total Protein	(g/dL)	7.39 ± 0.60	7.41 ± 0.35	7.55 ± 0.30	7.31 ± 0.30
A/G Ratio		1.284 ± 0.155	1.260 ± 0.141	1.275 ± 0.128	1.175 ± 0.178
Albumin	(%)	56.05 ± 2.97	55.59 ± 2.66	55.90 ± 2.52	53.77 ± 3.56
Alpha1 Globulin	(%)	15.47 ± 1.76	15.07 ± 2.21	15.73 ± 2.16	16.52 ± 1.82
Alpha2 Globulin	(%)	7.91 ± 1.42	8.24 ± 1.49	7.86 ± 1.47	7.63 ± 1.50
Beta Globulin	(%)	14.32 ± 1.62	14.23 ± 1.53	13.56 ± 1.15	14.92 ± 1.79
Gamma Globulin	(%)	6.25 ± 1.38	6.87 ± 1.50	6.95 ± 1.35	7.16 ± 1.70
Albumin	(g/dL)	4.15 ± 0.51	4.12 ± 0.32	4.22 ± 0.30	3.94 ± 0.39
Alpha1 Globulin	(g/dL)	1.14 ± 0.14	1.12 ± 0.16	1.19 ± 0.16	1.21 ± 0.14
Alpha2 Globulin	(g/dL)	0.58 ± 0.11	0.61 ± 0.11	0.59 ± 0.11	0.56 ± 0.10
Beta Globulin	(g/dL)	1.06 ± 0.13	1.05 ± 0.13	1.02 ± 0.10	1.09 ± 0.12
Gamma Globulin	(g/dL)	0.46 ± 0.09	0.51 ± 0.11	0.52 ± 0.09	0.52 ± 0.12
Ca	(mg/dL)	10.02 ± 0.33	10.02 ± 0.15	10.13 ± 0.33	10.23 ± 0.37
IP	(mg/dL)	6.51 ± 0.47	6.50 ± 0.57	6.44 ± 0.50	6.58 ± 0.67
Na	(mmol/L)	146.2 ± 1.3	146.2 ± 0.6	145.9 ± 0.7	146.1 ± 0.9
K	(mmol/L)	4.22 ± 0.26	4.29 ± 0.20	4.46 ± 0.25	4.24 ± 0.32
Cl	(mmol/L)	106.4 ± 2.2	107.0 ± 1.1	107.0 ± 0.8	107.1 ± 0.7

(n = 10 animals per group), *: Significantly different from control ($P < 0.05$)

Table 4. Absolute and relative organ weights of male (A) and female (B) rats administered *Bacillus coagulans* strain SANK70258 by gavage for 90 days.

(A) Males

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
Final Body Weight		544.40 ± 30.70	560.23 ± 75.58	543.98 ± 53.28	577.38 ± 32.34
Absolute organ weights					
Brain	(g)	2.178 ± 0.067	2.203 ± 0.086	2.141 ± 0.085	2.242 ± 0.073
Pituitary	(mg)	13.90 ± 1.81	14.95 ± 1.54	14.72 ± 1.71	14.34 ± 1.58
Thyroids	(mg)	31.70 ± 4.31	34.02 ± 4.25	34.00 ± 3.69	38.11 ± 3.96**
Thymus	(mg)	346.0 ± 72.0	379.3 ± 80.8	336.3 ± 115.5	341.9 ± 123.8
Submand.GLs	(g)	0.714 ± 0.054	0.789 ± 0.091	0.767 ± 0.071	0.754 ± 0.124
Lungs	(g)	1.680 ± 0.147	1.715 ± 0.167	1.674 ± 0.128	1.730 ± 0.162
Heart	(g)	1.710 ± 0.155	1.588 ± 0.159	1.578 ± 0.151	1.660 ± 0.163
Liver	(g)	13.396 ± 0.998	13.753 ± 2.485	13.545 ± 1.766	14.454 ± 1.686
Spleen	(g)	0.899 ± 0.112	0.828 ± 0.161	0.853 ± 0.139	0.901 ± 0.200
Kidneys	(g)	3.291 ± 0.120	3.432 ± 0.416	3.420 ± 0.434	3.354 ± 0.232
Adrenals	(mg)	58.40 ± 7.53	65.48 ± 11.56	60.84 ± 7.75	59.87 ± 9.94
Testes	(g)	3.678 ± 0.148	3.781 ± 0.283	3.580 ± 0.229	3.686 ± 0.359
Prostate	(g)	0.741 ± 0.111	0.821 ± 0.167	0.827 ± 0.181	0.717 ± 0.184
Epididymides	(g)	1.435 ± 0.104	1.494 ± 0.159	1.492 ± 0.192	1.475 ± 0.110
Relative organ weights					
Brain	(%)	0.401 ± 0.029	0.398 ± 0.038	0.397 ± 0.035	0.389 ± 0.020
Pituitary	(10^{-3}%)	2.56 ± 0.33	2.68 ± 0.12	2.71 ± 0.20	2.49 ± 0.27
Thyroids	(10^{-3}%)	5.82 ± 0.68	6.12 ± 0.78	6.28 ± 0.66	6.61 ± 0.62*
Thymus	(10^{-3}%)	63.30 ± 12.10	67.73 ± 10.90	61.62 ± 19.81	59.06 ± 20.02
Submand.GLs	(%)	0.131 ± 0.009	0.142 ± 0.014	0.142 ± 0.014	0.131 ± 0.020
Lungs	(%)	0.309 ± 0.020	0.308 ± 0.025	0.309 ± 0.026	0.300 ± 0.026
Heart	(%)	0.315 ± 0.027	0.286 ± 0.028	0.292 ± 0.032	0.287 ± 0.019
Liver	(%)	2.462 ± 0.136	2.446 ± 0.190	2.486 ± 0.142	2.499 ± 0.195
Spleen	(%)	0.165 ± 0.018	0.147 ± 0.013	0.156 ± 0.018	0.156 ± 0.031
Kidneys	(%)	0.606 ± 0.033	0.616 ± 0.058	0.628 ± 0.046	0.582 ± 0.043
Adrenals	(10^{-3}%)	10.79 ± 1.85	11.74 ± 1.75	11.29 ± 1.87	10.40 ± 1.88
Testes	(%)	0.677 ± 0.045	0.685 ± 0.099	0.663 ± 0.065	0.640 ± 0.065
Prostate	(%)	0.137 ± 0.023	0.150 ± 0.039	0.152 ± 0.030	0.124 ± 0.029
Epididymides	(%)	0.264 ± 0.019	0.270 ± 0.038	0.275 ± 0.031	0.256 ± 0.028

(n = 10 animals per group), *: Significantly different from control ($P < 0.05$) **: Significantly different from control ($P < 0.01$)

(B) Females

Items	Unit	Doses (mg/kg/day)			
		0	500	1000	2000
Final Body Weight		297.16 ± 41.06	297.02 ± 34.00	296.16 ± 39.47	300.74 ± 40.01
Absolute organ weights					
Brain	(g)	2.019 ± 0.102	1.960 ± 0.062	1.975 ± 0.088	1.982 ± 0.088
Pituitary	(mg)	18.32 ± 2.76	18.70 ± 2.37	18.50 ± 2.26	19.09 ± 1.74
Thyroids	(mg)	22.84 ± 4.01	23.91 ± 3.13	26.27 ± 4.79	25.04 ± 3.51
Thymus	(mg)	280.2 ± 68.4	267.1 ± 36.6	245.7 ± 44.5	293.2 ± 74.4
Submand.GLs	(g)	0.472 ± 0.046	0.482 ± 0.055	0.452 ± 0.040	0.471 ± 0.069
Lungs	(g)	1.239 ± 0.089	1.259 ± 0.078	1.273 ± 0.123	1.259 ± 0.083
Heart	(g)	0.995 ± 0.099	1.006 ± 0.117	0.975 ± 0.125	1.034 ± 0.092
Liver	(g)	7.537 ± 1.054	7.542 ± 1.043	7.442 ± 0.939	7.906 ± 0.980
Spleen	(g)	0.517 ± 0.068	0.563 ± 0.090	0.550 ± 0.067	0.565 ± 0.071
Kidneys	(g)	1.903 ± 0.226	1.930 ± 0.216	1.908 ± 0.273	1.993 ± 0.126
Adrenals	(mg)	64.57 ± 9.42	66.19 ± 6.80	62.40 ± 5.09	65.94 ± 10.39
Ovaries	(mg)	92.23 ± 16.75	84.41 ± 18.41	91.91 ± 16.98	89.78 ± 13.67
Uterus	(g)	0.823 ± 0.338	0.764 ± 0.214	0.566 ± 0.089	0.782 ± 0.367
Relative organ weights					
Brain	(%)	0.692 ± 0.106	0.667 ± 0.073	0.675 ± 0.069	0.668 ± 0.083
Pituitary	(10^{-3}%)	6.22 ± 0.91	6.31 ± 0.58	6.31 ± 0.91	6.41 ± 0.76
Thyroids	(10^{-3}%)	7.78 ± 1.54	8.11 ± 1.17	9.01 ± 2.19	8.40 ± 1.27
Thymus	(10^{-3}%)	94.78 ± 21.29	90.10 ± 9.79	82.97 ± 10.04	96.86 ± 16.56
Submand.GLs	(%)	0.161 ± 0.021	0.163 ± 0.017	0.154 ± 0.015	0.158 ± 0.022
Lungs	(%)	0.422 ± 0.049	0.427 ± 0.034	0.433 ± 0.034	0.423 ± 0.041
Heart	(%)	0.337 ± 0.027	0.339 ± 0.024	0.330 ± 0.021	0.346 ± 0.021
Liver	(%)	2.542 ± 0.187	2.538 ± 0.178	2.518 ± 0.140	2.633 ± 0.103
Spleen	(%)	0.175 ± 0.013	0.189 ± 0.018	0.188 ± 0.030	0.189 ± 0.024
Kidneys	(%)	0.644 ± 0.059	0.652 ± 0.052	0.645 ± 0.051	0.671 ± 0.078
Adrenals	(10^{-3}%)	22.17 ± 4.76	22.52 ± 3.36	21.39 ± 3.19	22.12 ± 3.55
Ovaries	(10^{-3}%)	31.49 ± 6.78	28.69 ± 6.21	31.14 ± 4.79	30.14 ± 5.01
Uterus	(%)	0.285 ± 0.133	0.259 ± 0.075	0.194 ± 0.038	0.271 ± 0.146

(n = 10 animals per group)

uate whether the microbe has a potential to infect mammals. In this study, there were no increases in white blood cell count (WBC), neutrophil, or eosinophil, or any histopathologic changes indicative of inflammation in any organ/tissue including the digestive tract at the maximum dose level, suggesting that the test microbe did not infect rats.

The no-observed-adverse-effect-level (NOAEL) of *Bacillus coagulans* strain SANK70258 was judged to be greater than 2000 mg/kg/day in males and females. The dose level is equivalent to 1×10^{12} CFU/kg/day. It corresponds to 7×10^{13} CFU/human/day when the body weight in adult is regarded as 70 kg. Considering the expected daily intake of about 10^8 to 10^9 CFU in human, the test microbe is harmless and available as a probiotic.

Conflict of interest---- The authors declare that there is no conflict of interest.

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