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Distribution and Stress Resistance of Resident Lactobacilli in Mouse Intestinal Tract

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In this paper, we studied the spatial distribution and stress responses of resident *Lactobacillus* in various intestinal regions in order to clarify the ecological and functional properties of the microbes in mouse normal microflora. *Lactobacillus reuteri* and *Lactobacillus intestinalis* were identified as resident species by 16S rDNA analysis. Both lactobacilli were present in all regions of the intestinal tract (duodenum, jejunum+ileum, cecum and colon) at almost the same ratios based on colony shapes on LBS agar and the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) profiles using a genus-specific primer pair. Survivability of *L. reuteri* against heat shock, acid and bile salt treatments was markedly higher than that of *L. intestinalis*. Furthermore, almost all isolates of *L. reuteri* obtained from all intestinal regions grew in MRS broth with 0.5% bile salts, while almost all of the isolates of *L. intestinalis* failed to grow in the broth with 0.1% bile salts. These results suggest that resident lactobacilli, *L. reuteri* and *L. intestinalis*, in mice are able to colonize all intestinal regions with different environmental conditions, regardless of their stress response potentials.

Key words: resident lactobacilli; mouse; intestine; stress response

INTRODUCTION

The gastrointestinal tracts of animals harbor a complex and diverse microbial community comprising hundreds of bacterial species *(6, 21)*. Some of these bacteria are able to grow and colonize the gut, resulting in a characteristic intestinal microflora for each host species. These microflora provide a first line of defense against pathological microbe colonization. Much attention has recently been focused on the cross-talk between intestinal microflora and the host, e.g. development and regulation of the host immune system *(8, 12)*.

Lactobacilli are commonly present in the intestines of humans and animals. Particularly in the gastrointestinal tracts of mice and rats, lactobacilli are present in large numbers in all regions *(16, 20)*. This means that some lactobacilli in these animals are able to adhere to the epithelial cells throughout the intestine. Itoh et al. reported that lactobacilli played an important role in experimental mice in gnotobiotic studies *(9–11)*. Furthermore, it is known that lactobacilli inhabiting the gastrointestinal tract have a major influence on intestinal biochemistry *(20)*. Tannock et al. demonstrated that the bile salt hydrolase activity of intestinal contents was mainly due to the presence of lactobacilli *(22)*, and unconjugated bile acids increased in intestinal contents with intentional lactobacilli colonization *(23)* in a lactobacillus-free mouse model.

Mitsuoka reported that *Lactobacillus murinus*, *Lactobacillus intestinalis* and *Lactobacillus reuteri* were representative inhabitants of the mouse intestine *(15)*. Park and Itoh *(16)*, and Salzman et al. *(17)* also identified several *Lactobacillus* strains isolated from mouse feces and the gastrointestinal tract: *L. intestinalis*, *L. johnsonii*, *L. murinus*, *L. vaginalis* and *L. reuteri*.

Microorganisms in the gastrointestinal tract must endure many environmental stresses. It is known that some *Lactobacillus* strains have health benefits and are utilized as probiotics for production of fermented foods. Thus, the stress responses of probiotic *Lactobacillus* strains have been widely investigated from physiological and molecular biological perspectives *(3, 26)*.

It is important to know the ecological and functional characteristics of the intestinal resident lactobacilli, because intestinal microflora are closely related to the physiological and immunological responses of the host. There are some reports on the traits of mouse resident lactobacilli using a lactobacillus-free mouse model *(22, 23)* and gnotobiotic SCID-mice colonized with defined flora *(18)*, but few on the distribution of lactobacilli in the intestine and the stress resistance of lactobacilli in normal mice. Therefore, in this study, we examined the species distribution of resident lactobacilli in different

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intestinal regions and the stress response potentials of these lactobacilli in normal mice.

MATERIALS AND METHODS

Animals

Six-week-old female BALB/c mice obtained from Charles River Japan (Yokohama, Japan) were maintained at 22–24°C and around 50% relative humidity under a 12-hr light-dark cycle for more than two weeks before use. Commercial diet (CE-2, Clea Japan, Tokyo, Japan) and water were available *ad libitum*. The experimental protocol was approved by the Animal Experiment Committee of Rakuno Gakuen University and the animals were managed in accordance with the same institution's Guidelines for the Care and Use of Laboratory Animals.

Bacterial strains

L. reuteri JCM 1081, *L. murinus* JCM 1717^T, and *L. intestinalis* JCM 7548^T were purchased from the Japan Collection of Microorganisms. All strains were grown in MRS broth at 37°C for DNA extraction.

Isolation of Lactobacillus from intestinal tissues

After collecting feces, mice were sacrificed by cutting the carotid artery under ether anesthesia. The duodenum, jejunum+ileum, cecum, and colon were removed and gently rinsed with sterilized saline in order to wash out the contents. Feces and tissues were then homogenized with 99 times their volumes of saline (10^{-2} dilution). The homogenate was serially diluted to 10^{-8} and 0.1-ml aliquots were spread onto LBS agar (Becton Dickinson, Sparks, MD, U.S.A) plates. The plates were incubated at 37°C under anaerobic conditions with AnaeroPack (Mitsubishi Gas Chemical Co. Inc., Tokyo) for 48 hr. After counting colony forming units (CFU), all colonies on the plate were wiped out with a sterilized cotton swab and suspended in MRS broth. The suspension was stored at –80°C until use.

DNA extraction from bacteria

Bacterial cells were collected by centrifugation at 13,400×g and washed twice with saline. The cells were resuspended in 180 μl of TE buffer (20 mM tris, 2 mM EDTA, pH 8.0) containing 1.2% Triton X-100 and 20 mg/ml lysozyme (Merck KGaA, Darmstadt, Germany), and were incubated at 37°C for 60 min. DNA was purified from the cell suspension using the DNeasy tissue extraction protocol (Qiagen K.K., Tokyo, Japan).

PCR amplification and sequencing of 16S rDNA for identification of isolated Lactobacillus species

Twenty-four colonies with visibly different shape were isolated from the feces on LBS agar plates. These colonies were grouped by the results of microscopic and biochemical analysis (catalase activity, gas production from glucose and optical rotation of lactic acid), and sugar fermentation ability (glucose, cellobiose, mannose, melibiose, rhamnose, sorbitol, trehalose, xylose, mannitol, salicin and arabinose). Then, two colonies in each group were identified by 16S rDNA sequencing. PCR amplification was carried out using an MJ minicycler (Bio-Rad, Hercules, CA, USA) and the primer pair 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-GGMTACCTTGTTACGACTT) *(14)*. The reaction mixture (100 μl) contained reaction buffer (final concentrations, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, pH9.0), 1.5 mM $MgCl_2$, 62.5 μM deoxyribonucleotide triphosphates, 0.6 μM primers, 75 ng of bacterial template DNA and 2.5 U of Taq DNA polymerase (Promega, Madison, WI, USA). PCR conditions were 94°C for 1 min, 30 cycles of 94°C for 30 sec, 49°C for 30 sec and 72°C for 2 min, followed by 72°C for 10 min. PCR products were purified with the QIAquick PCR purification kit (Qiagen K.K.). Purified PCR products were sequenced with the Thermo sequenase cycle sequencing kit (USB Co. Cleveland, OH, USA), the LI-COR system (Lincoln, NE, USA), and IRD 800-labeled 27f primer. To determine the closest relatives of the partial 16S rDNA sequence, the retrieved sequences were compared with those in the DDBJ nucleic acid databases using the BLAST algorithm *(1)*.

Analysis of Lactobacillus species by PCR-DGGE

Total DNA was extracted from the stored colony suspension, as described above. PCR was carried out with a group-specific primer pair of *Lactobacillus* species, Lac1 (5'-AGCAGTAGGGAATCTTCCA-3') and a 40-bp GC clamp (5'-CGCCCGGGGCGCGCCCC GGGCGGCCCGGGGGCACCGGGGG-3') attached to Lac2 (5'-CATGTGTAGCGGTGRAAT-3') *(28)*. The reaction mixture composition was as described above. The amplification program and DGGE conditions followed the method of Walter et al. *(29, 30)*.

Assay for survival against heat shock, NaCl, acid and bile salt treatments

The methods of Kim et al. *(13)* were employed to study survival assessment against heat shock, NaCl and bile salt treatments, with some modification. Briefly, each of three isolated colonies of *L. intestinalis* and *L.*

Table 1. Total number of lactobacilli and the ratio of colony type

Mouse no.	Log no. of lactobacilli / g of tissue (% ratio of smooth colony)									
	Duodenum		Jejunum + Ileum		Cecum		Colon		Feces	
	Log CFU / g	%	Log CFU / g	%	Log CFU / g	%	Log CFU / g	%	Log CFU / g	%
1	5.11	(66.3)	5.36	(65.8)	7.88	(60.3)	7.60	(67.5)	9.15	(58.3)
2	4.82	(78.8)	5.04	(78.4)	6.88	(74.5)	5.98	(75.3)	8.40	(78.8)
3	6.53	(67.6)	6.81	(56.3)	7.50	(47.6)	6.45	(50.9)	9.32	(52.8)
4	4.93	(70.8)	5.90	(67.8)	6.42	(60.4)	7.28	(39.5)	9.26	(66.4)
5	5.57	(64.9)	6.07	(35.1)	6.36	(65.2)	6.70	(50.0)	9.26	(61.1)
Average	5.39	(69.7)	5.84	(60.6)	7.01	(61.4)	6.80	(56.5)	9.08	(63.5)
SD	0.70	5.55	0.68	16.31	0.67	9.73	0.65	14.47	0.38	9.87

Each value is the $\log_{10}$ numbers of lactobacilli per gram of wet tissues or feces. The numbers in parentheses are the percentages of smooth colonies (%).

reuteri from feces were cultured in MRS broth at 37°C for 20 hr. An aliquot (0.5 ml) of culture broth was resuspended in 4.5 ml of MRS broth. To examine heat-shock stress tolerance, cultures were incubated at 37°C, 45°C, 50°C, 55°C or 60°C for 30 min. For assessment of NaCl tolerance, cell culture broth was resuspended in MRS broth containing 0 to 10% NaCl and incubated at 37°C for 2 hr. To assess acid stress tolerance, cell culture broth was resuspended in MRS broth adjusted to pH 2.0, and incubated at 37°C for 4 hr. For assessment of bile stress tolerance, cell culture broth was resuspended in MRS broth containing 0 to 0.5% bile extracts (SIGMA, St. Louis, MO, USA), and incubated at 37°C for 3 hr. After these stress treatments, residual viable cells were enumerated on MRS agar plates.

Assay for acid and bile resistance

Single colonies of *Lactobacillus* isolated from intestinal tissues were cultured in MRS broth at 37°C for 20 hr. For the acid tolerance assay, an aliquot of the broth was inoculated into wells of a microplate containing MRS broth adjusted to pH 2 or pH 6.4, and the microplates were incubated at 37°C for 4 hr. Following incubation, an aliquot of cell culture was resuspended in the wells of another microplate filled with freshly prepared MRS broth (pH 6.4). For bile tolerance, an aliquot of cell culture broth was inoculated into the wells of a microplate containing MRS broth with (0.1–0.5%) or without bile extracts.

After covering the surface of the wells with mineral oil the microplates were incubated at 37°C for 72 hr and cell growth was monitored by optical density (OD) at 655 nm using a microplate reader (model 550, Bio-Rad).

RESULTS

Identification of resident Lactobacillus species

From mouse fecal samples, two types of colonies were recognized and differentiated by their morphologies on LBS agar plates. Colonies of the one type (type A) were milky white, and smooth and round in shape. Colonies of the other type (type B) were semi-transparent, and rough and irregular in shape. Each colony type was constituted of one group of *Lactobacillus* species judging from the biochemical analysis and sugar fermentation ability. The 16S rDNA of four strains of both types were sequenced and related strains were identified using BLASTN. The most closely related species to type A and type B were *L. reuteri* and *L. intestinalis*, respectively, with more than 98% similarity. The 16S rDNA sequences of the lactobacilli determined in this study have been deposited in the DDBJ/EMBL/GenBank database with the accession numbers AB260941 for *L. reuteri* R-13 and AB 280764 for *L. intestinalis* LF-01.

Spatial distribution of Lactobacillus species in intestinal regions of mice

In order to determine whether the spatial distribution of resident *Lactobacillus* species differs in each region of the mouse intestinal tract, we examined the cell numbers, ratios, and PCR-DGGE profiles of lactobacilli.

The numbers of lactobacilli in the cecum and colon were higher than in the small intestine (Table 1). The ratio of the two types did not show marked differences among the intestinal regions of the same individuals, and intestinal regions with only one of the two types were not seen (Table 1).

We also examined the spatial distribution of the resident *Lactobacillus* species by PCR-DGGE. It was reported that the Lac1 and Lac2 primers were useful for

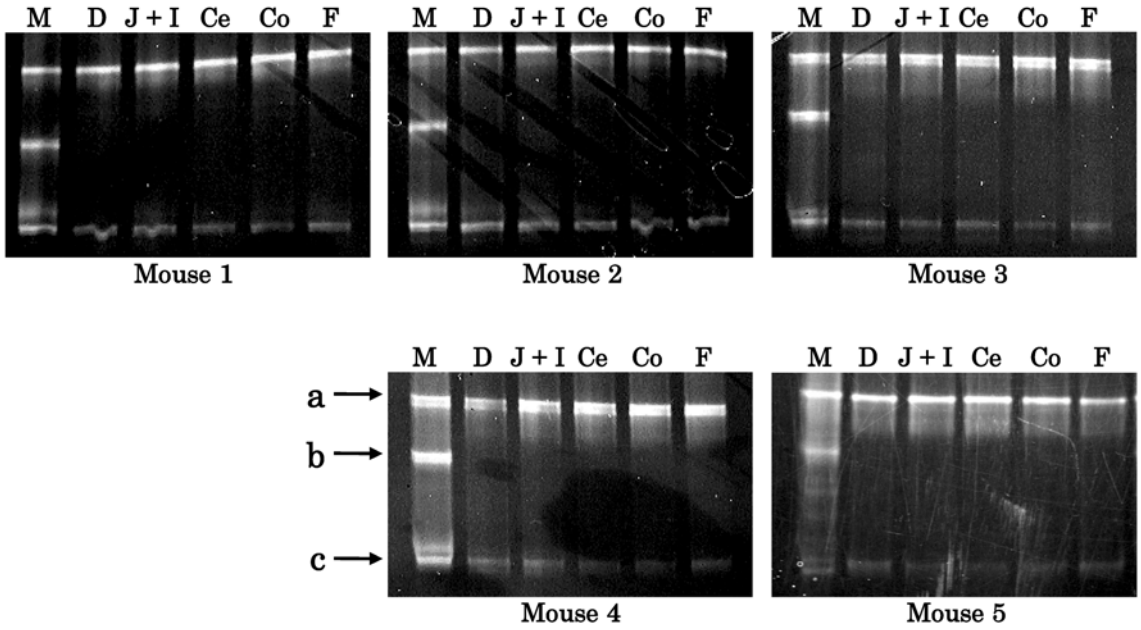

Fig. 1. DGGE profiles of 16S rDNA fragments obtained from lactobacilli in the intestinal tract. M: Mixture of *Lactobacillus intestinalis* JCM7548^T (a), *Lactobacillus murinus* JCM1717^T (b) and *Lactobacillus reuteri* JCM 1081 (c); D: duodenum; J+I: jejunum and ileum; Ce: cecum; Co: colon; F: feces.

studying Lactobacillus populations in fecal samples from human subjects *(29)*. As shown in Fig. 1, the Lac1 and Lac2-GC primers were also able to distinguish between the PCR fragments generated from *L. intestinalis*, *L. murinus* and *L. reuteri*. The DGGE profiles showed that fragments other than *L. intestinalis* and *L. reuteri* were not seen in the intestinal tracts of the mice used in this study, and that the relative intensities of the two fragments were almost the same for all intestinal regions.

Survivability of resident lactobacilli against heat shock, NaCl, acid and bile salt treatments

We compared the survivability of isolates of *L. intestinalis* and *L. reuteri* against heat shock, NaCl, acid and bile salt treatments. As shown in Table 2, *L. reuteri* was more resistant to heat shock than *L. intestinalis*. The CFU count of *L. reuteri* in MRS broth was only slightly decreased, even with treatment at 60°C. To investigate the lactobacilli response to an increase in osmolarity of the environment, the survivability of the lactobacilli in increasing NaCl concentrations was evaluated. The viable cell ratios of both bacteria were not influenced by the addition of up to 10% sodium chloride.

L. reuteri showed stronger resistance to acid and bile salt treatment when compared to *L. intestinalis*. These treatments reduced viable cells by about 0.4 to 0.6 log CFU/ml for *L. reuteri*, and by about 2 log CFU/ml for *L. intestinalis*.

Acid and bile salt resistance of resident lactobacilli

In order to further characterize the stress response of resident lactobacilli, each of several colonies of *L. reuteri* and *L. intestinalis* were isolated from different intestinal regions and their growth ability after acid treatment, and in the presence of bile salts was investigated. As shown in Table 3, growth of *L. reuteri* inhabiting all intestinal regions was markedly enhanced by acid treatment. Over 90% of isolated *L. reuteri* grew better with acid treatment than without acid treatment. This promotion of growth was also observed in isolates of *L. intestinalis* obtained from all intestinal regions, but the ratio of growth-promoted isolates was 15% and 40–50% from the duodenum and other regions, respectively.

Next, we examined bile salt resistance of isolated lactobacilli from several intestinal regions. Table 4 summarizes the number of isolated colonies in MRS broth containing the indicated bile salt concentration showing 20% more OD at 655 nm than in the bile salt-free broth. *L. reuteri* showed potent growth in the presence of bile salts. All isolates obtained from the intestinal tracts were able to grow in MRS broth containing 0.5% bile salts. However, there were fewer

Table 2. Change of viable cell numbers after various stress treatments

	L. reuteri		*L. intestinalis*	
	Log CFU / ml	SD	Log CFU / ml	SD
Initial	8.01	0.10	5.83	0.32
Temperature (°C)				
37	7.98	0.10	5.97	0.32
45	7.91	0.10	5.99	0.33
50	8.03	0.01	5.56	0.45
55	7.80	0.11	4.79	0.16
60	7.00	0.37	3.89	0.18
NaCl (%)				
0	8.00	0.03	6.20	0.12
2.5	8.02	0.06	6.16	0.24
5	7.89	0.08	5.95	0.05
10	7.91	0.06	5.72	0.20
pH				
6.5	8.46	0.08	7.66	0.11
2.0	7.88	0.11	5.53	0.61
Bile extract (%)				
0	8.34	0.03	6.22	0.09
0.1	7.99	0.08	6.18	0.09
0.3	7.87	0.21	4.55	0.10
0.5	7.91	0.03	4.33	0.15

Each value is the average of three isolates and standard deviation (SD).

isolates of *L. intestinalis* showing potent resistance to bile salts. The ratios of isolates which were able to grow in the presence of 0.5% bile salts were 10–15% and less than 5% in the small and large intestines, respectively.

Bile salt resistance of subcultured strains of L. reuteri

The effects of subculture without bile salts on the tolerance of *L. reuteri* were determined. As shown in Fig. 2, colonies of *L. reuteri* freshly isolated from feces were able to grow in the presence of 0.5% bile salts, while *L. reuteri* R-13, which was isolated from mouse feces and subcultured several times in MRS broth, did not grow, even in the presence of 0.3% bile salts.

DISCUSSION

We identified two predominant species of resident *Lactobacillus* isolated from mouse feces: *L. reuteri* and *L. intestinalis*. Both species have already been isolated from the mouse intestine. Other than these two *Lactobacillus* species, *L. johnsonii*, *L. murinus* and *L. vaginalis* have previously been isolated from mouse feces by Park and Itoh *(16)*. The predominant *Lactobacillus* species probably varies with mouse strain, and breeding and feeding conditions *(5, 9)*.

It is known that lactobacilli are widely present throughout the intestinal tract. There are numerous reports analyzing intestinal lactobacilli using fecal samples, but few on the microbes inhabiting intestinal tissues. *L. reuteri* and *L. intestinalis*, which were identified as the resident species based on feces, were found to inhabit all regions of the intestinal tract (duodenum, jejunum+ileum, cecum and colon).

There was no region-specific inhabitance seen among the present lactobacilli. Furthermore, the population ratio of the two lactobacilli was almost the same throughout the intestine, based on culture method and DGGE profiles of PCR products amplified with a group-specific primer pair. Sarma-Rupavtarm et al. *(18)* reported that *L. murinus*, a resident *Lactobacillus* species, was able to colonize the entire intestine in a complex defined-flora model mouse with altered Schadler flora. Watanabe et al. *(31)* also reported that *L. murinus* was the predominant population of *Lactobacillus* in the lower part of the small intestine of conventional rats and in all parts of the gastrointestinal tracts of gnotobiotic rats, except for the wall of the non-glandular part of the stomach. Although Charles River use altered Schadller flora (including *L. murinus*) to establish a barrier sustained mouse colony, in this experiment *L. murinus* was not obtained from the

Table 3. Cell growth of resident lactobacilli isolated from the mouse intestinal tract after acid treatment

GI (%)	Duodenum		Jejunum + Ileum		Cecum		Colon		Feces	
	No.	%*	No.	%*	No.	%*	No.	%*	No.	%*
Lactobacillus reuteri										
0–50	0	0.0 (-)	0	0.0 (-)	0	0.0 (-)	0	0.0 (-)	0	0.0 (-)
50–100	3	6.3 (96.8)	2	4.2 (95.4)	1	2.1 (64.3)	0	0.0 (-)	0	0.0 (-)
> 100	45	93.8 (110.2)	46	95.8 (115.8)	47	97.9 (116.4)	48	100 (119.6)	48	100 (118.3)
Total	48	100	48	100	48	100	48	100	48	100
Lactobacillus intestinalis										
0–50	0	0.0 (-)	0	0.0 (-)	5	10.4 (37.3)	3	6.3 (27.7)	6	15.0 (38.9)
50–100	17	85.0 (82.5)	10	55.6 (78.5)	18	37.5 (73.7)	25	52.0 (76.8)	17	42.5 (83.0)
> 100	3	15.0 (116.0)	8	44.4 (128.2)	25	52.1 (109.5)	20	41.7 (114.3)	17	42.5 (112.2)
Total	20	100	18	100	48	100	48	100	40	100

Growth index (GI) was calculated by the following formula: $GI\ (\%) = \frac{\text{OD 655 nm at 72hr (pH 2.0 treat.)}}{\text{OD 655 nm at 72hr (pH 6.5 treat.)}} \times 100$. Isolated colonies of lactobacilli were cultured in MRS broth at 37°C under anaerobic condition after acid treatment (pH 2.0, 4hr). *Values in parentheses are the averages of GI.

Table 4. Bile salt tolerance of resident lactobacilli isolated from the mouse intestinal tract

Conc. of bile salts* (%)	Duodenum		Jejunum + Ileum		Cecum		Colon		Feces	
	No.	%	No.	%	No.	%	No.	%	No.	%
Lactobacillus reuteri										
0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0.5	48	100	48	100	48	100	48	100	48	100
Total	48	100	48	100	48	100	48	100	48	100
Lactobacillus intestinalis										
0	34	83.0	24	75.0	42	87.5	45	93.8	40	83.3
0.1	1	2.4	4	12.5	4	8.3	2	4.2	1	2.1
0.5	6	14.6	4	12.5	2	4.2	1	2.1	7	14.6
Total	41	100	32	100	48	100	48	100	48	100

Isolated colonies of lactobacilli were cultured in MRS broth with bile salts. *Maximum concentration of bile salts at which the isolates could grow.

colonies on LBS agar plates, so we could not elucidate the distribution of *L. murinus* in the mouse intestinal tracts. It is possible that the number of *L. murinus* in the mice used in this study was much lower than that of *L. intestinalis* and *L. reuteri*, or that the LBS agar plate was not suitable for the selection of *L. murinus*. Other isolation methods are needed for further ecological observation of lactobacilli in mice intestinal tracts.

Based on our results and several ecological observations of *L. murinus (18, 31)*, mouse resident lactobacilli have the capacity to colonize epithelial cells of all intestinal regions, even under different environmental conditions.

Resident microbes colonize and multiply in the intestinal tract despite various environmental stresses. It is known that lactobacilli are present in large numbers in the mouse stomach and intestinal tract. Therefore, gastric contents are inoculated with lactobacilli inhabiting the epithelia of the stomach, ensuring that large numbers of these bacteria are present throughout the intestinal tract. Thus, investigating both the survivability and growth potential after acid treatment and in the presence of bile salts is useful for understanding the stress response potential of resident lactobacilli in the intestinal tract.

Some investigators have reported that *L. reuteri* exhibits higher bile salt resistance than *L. plantarum* and *L. rossiae (4)*, and hypocholesterolemic effects *(25)*. It is

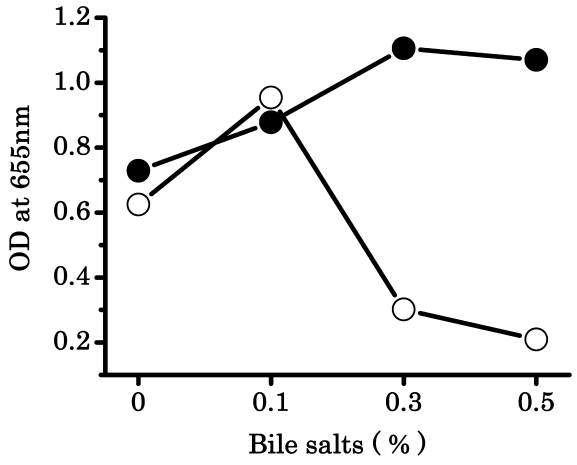

Fig. 2. Cell growth of fecal isolates of *Lactobacillus reuteri* (●) and *Lactobacillus reuteri* R-13 (○) subcultured without bile salts. Microbes were cultured at 37°C in MRS broth containing bile salts for 72 hr under anaerobic conditions. The values for the fecal isolates are the means of 48 isolates.

known that the action mode of stress responses in lactic acid bacteria varies between species and is dependent on the type of stress *(2, 26)*. Flahaut et al. *(7)* and Kim et al. *(13)* reported that bile adaptation increased the resistance not only to bile salts but also to heat shock in *Enterococcus faecalis* and *L. acidophilus*. In the present study, the survivability of *L. reuteri* was clearly higher than that of *L. intestinalis* when the microbes were treated with not only bile salts but also heat shock. As intestinal resident microbes are constantly exposed to bile stress under constant temperature conditions, *L. reuteri* inhabiting the mouse intestine may have a cross-protection pathway for bile salts and heat shock, although the mechanism remains unclear. Most of the isolates of *L. intestinalis* didn't have strong resistance to bile salt, but some of them showed considerable growth even in MRS broth containing 0.5% bile salt. Since we examined the resistance of isolates from colonies isolated independently from the intestinal region, the resident *L. intestinalis* appears to be composed of several strains with different bile salt tolerances.

An appropriate bile concentration is necessary for the resident *L. reuteri* to maintain high bile salt tolerance, as subculturing without bile salts reduced this tolerance. Several pathways, such as deconjugation by bile salt hydrolase and the multidrug resistance transporter system are thought to be involved in bile salt resistance in lactic acid bacteria *(26)*. Recently, Taranto et al. reported that bile salts and cholesterol induce changes in the lipid profile of the cell membrane and play key roles in the response of *L. reuteri* to environmental stress *(24)*. It is worth investigating whether the reduced bile resistance of *L. reuteri* is restored by culturing with bile salts. Such findings would help better understanding of the stress response mechanism of *L. reuteri*.

Bile salt resistance is a major criterion for the selection and development of probiotics, as microbes with higher bile resistance are better able to colonize and survive in the intestinal tract. However *L. intestinalis*, which is markedly less tolerant to bile salts, compared to *L. reuteri*, colonized not only the colon but also the duodenum. Thus, bile salt resistance may not be crucial for colonizing the intestinal tract.

The results obtained from the *in vitro* bile salt tolerance examinations are limited for several reasons: (1) bile salt concentration in the intestine is not static, but changes with time and region; (2) numerous microbes are present in the intestinal tract and may interact with one another in bile salt degradation; and (3) bile salts form micelles with phospholipids and have lower antibacterial activity than artificial bile salt solution *(19)*.

Genes from *L. reuteri* specifically induced in the mouse gastrointestinal tract have recently been identified, and were found to have a close relationship with colonization potential *(27, 28)*. Such analyses are also needed to understand colonization and growth mechanisms in other lactobacilli.

Acid stress reduced viability of the resident lactobacilli, but growth promotion was also seen in many of the surviving cells of both *L. intestinalis* and *L. reuteri* obtained from all intestinal regions, particularly *L. reuteri*. The acid response of lactic acid bacteria is an intricate process involving the synthesis of various proteins *(26)*. Factors affecting cell growth promotion might be involved in newly synthesized proteins after acid treatment.

L. intestinalis and *L. reuteri* are common inhabitants of mice and rats *(15)*. There is, however, little knowledge on the functional properties of *L. intestinalis* compared with *L. reuteri*. Thus, the present results are the first observations of the stress response potential of *L. intestinalis*. Although the precise mechanisms explaining the differences in colonization and stress response potentials between *Lactobacillus* species are unknown, the present findings will assist in elucidating them.

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