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Screening of *Lactobacillus* from breast milk and infant feces and evaluation of their bile salt tolerance

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RESEARCH ARTICLE

Abstract

The intestinal bile salt concentration of infants is lower than that of adults, and the necessary bile salt tolerance of probiotics for infants remains unclear. In this study, *Lactobacillus* strains were isolated from breast milk and infant feces. The strains with better bile salt tolerance were screened, and their bile salt tolerance was compared with *Lactobacillus* strains from the adult intestine. The results showed that the bile salt concentration had a better distinguishing effect when it was 0.075 and 0.1%. Among the 28 strains of *Lactobacillus* with better bile salt tolerance, there were 16 strains of *Lactobacillus plantarum*, among which eight strains had greater than 70% survival under 0.075% bile salt and two strains had more than 50% survival under 0.1% bile salt. *L. plantarum* strains isolated from breast milk and infant feces had a significantly lower survival rate than those isolated from the adult intestine ($P < 0.05$) under 0.1% bile salt, while there was no significant difference in the survival rate under 0.075% bile salt ($P > 0.05$). Therefore, the demands for bile salt tolerance of probiotics for infants might be lower than that for adults. It is suggested that the standard for the screening of probiotics from breast milk and infant feces should differ from adults.

Keywords: bile salt tolerance; breast milk; infant feces; *L. plantarum*; probiotics

Introduction

According to the FAO/WHO (2006), probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. The most used probiotics belong to the genera of *Lactobacillus* and *Bifidobacterium* (Garcia *et al.*, 2014). *Lactobacillus* strains, as one of the most important flora in the human intestine, can colonize competitively in the intestine, promote the digestion and absorption of the body, regulate and maintain the balance of the intestinal flora, and reduce the incidence of diseases and enhance the body's immune function (Rodriguez *et al.*, 2015). The establishment of a healthy intestinal flora in the first 1000 days of life plays an important role in the development and maturation of the intestinal mucosal immune system and the systemic immune system (Martin *et al.*, 2010).

At present, it is generally believed that healthy human intestines, traditional fermented dairy products, and pickled products such as kimchi are valuable resources for screening probiotics. However, the influence of probiotics on the host and flora is strain-specific and host-specific. In recent years, the infant health industry has been experiencing rapid development, and researchers have also turned their attention to infant feces and breast milk to obtain probiotics suitable for infants after considering the difference between infant intestines and adult intestines.

In the past, breast milk has always been considered sterile. In recent years, studies have found that breast milk is an important source of oral (Ruiz *et al.*, 2019) and gastrointestinal bacteria in breastfed infants and this has an important impact on the early intestinal flora and the health of infants (McGuire and McGuire, 2015). With the maturity of strain isolation technology and culture conditions, a large number of probiotics, such as *Lactobacillus rhamnosus* (Li *et al.*, 2020), *L. plantarum*, *Bifidobacterium breve* (Choi *et al.*, 2021), and *Bifidobacterium longum* (Hikaru *et al.*, 2020) have been isolated from breast milk and infant feces.

We are generally strict in probiotics used for infants due to safety concerns, so it is especially important to study the properties of strains isolated from breast milk and infant feces. Probiotics colonize and exert probiotic effects that require a certain degree of bile salt resistance when they enter the human body. Healthy adults have a baseline bile salt concentration of 2.6 mM–3.0 mM. Secretions from the gallbladder during meal digestion increase the bile salt concentration in the duodenum to 12.0 mM–16.0 mM (Poquet and Wooster, 2016). Term infants have a baseline bile salt concentration of 3.0 mM. However, bile salt concentration decreases dramatically to 1.0 mM after feeding (Glasgow *et al.*, 1980). Bile salt concentration is even lower in preterm infants (Järvenpää *et al.*, 1983). The intestinal bile salt concentration of infants is low, and it is unclear whether the bile salt concentrations used for screening probiotics for adults are applicable to probiotics for infants. In this study, a total of 114 acid-producing and Gram-positive strains were isolated from breast milk and infant feces. We investigated their bile salt tolerance properties, strains with better bile salt tolerance were screened, and their bile salt tolerance was compared with that of *Lactobacillus* strains from the adult intestine, to provide a reference of bile salt tolerance level for the development and research of probiotics for infants.

Materials and Methods

Bacterial strains

Ten *L. plantarum* strains from the adult intestine were used in this study, including grx-L4, grx-L11, grx-L39, grx-L54, grx-L58, grx-L67, grx-L97, grx-L114, grx-L117, and grx-L128, isolated from the intestines of long-lived elderly people in Bama, Guangxi, China and archived at the Key Laboratory of Dairy Biotechnology and Safety Control of Jiangsu Province, China. Probiotics from breast milk and infant feces were all isolated from the samples collected from Hunan Province, China.

Sample collection

Forty mothers and their respective infants were recruited for this study as a source of breast milk and infant feces. Breast milk samples were obtained by manual expression and collected in sterile containers (Liu *et al.*, 2020). The volunteers wore disposable sterile gloves while collecting the samples, and washed the nipple and surrounding skin with sterile water. After the first 1 mL of breast milk was discarded, approximately 5 mL of breast milk was collected and placed in a 10 mL sterile centrifuge tube with a 30% volume fraction of glycerol. Infant fecal samples were obtained from their diapers (Peirotén *et al.*, 2018). All samples were quickly placed in a portable ice box with an anaerobic gas-generating bag and immediately sent to the laboratory for separation as soon as possible.

Separation and purification of lactic acid bacteria

Breast milk or infant feces were diluted by the appropriate amount (breast milk: 10^{-2}, 10^{-3}, 10^{-4} ; infant feces: 10^{-5}, 10^{-6}, 10^{-7}) and 200 μL of each sample was coated on triplicate sets of MRS agar with 0.3% bromocresol purple (Kemio Chemical Reagent Co., Ltd., Tianjin, China), and incubated anaerobically (80% N_2, 10% H_2, and 10% CO_2) at 37°C for 72 h (Gu *et al.*, 2008). The colony morphology, size, color, transparency, and surface roughness were recorded. Three to four different single colonies with typical lactic acid bacteria (LAB) morphology and bromocresol purple discoloration reactions were selected from each plate. The colonies were purified on separation agar medium and incubated at 37°C for 72 h. Single colonies were subjected to Gram staining, and the cell morphology was observed under a microscope. Ultimately, Gram-positive bacteria with typical *Lactobacillus* morphology were obtained. The selected bacteria were frozen in MRS broth with 30% glycerol at −80°C. Before the characterization experiments, the bacteria were recovered in MRS broth culture medium at 37°C for 24 h under anaerobic conditions.

Optimization of the test conditions for bile salt tolerance

A total of 1.0 mL of LAB suspension was inoculated into 9.0 mL of MRS broth containing the corresponding concentration of bile salt, and it was cultured anaerobically at 37°C for 3 h.

To prepare the bile salt culture medium, MRS broth containing 0.03%, 0.05%, 0.07%, 0.08%, 0.09%, or 0.1% (w/v) bile salt (Solab Technology Co., Ltd., Beijing, China) was prepared, the pH level was adjusted to 6.5 (Aguilar-Uscanga *et al.*, 2013; De Man *et al.*, 1960; Gao *et al.*, 2009), and then sterilized at 121°C for 15 min.

The survival rate was evaluated via plate count on MRS agar (Zhang *et al.*, 2016). The mean of three independent experiments was calculated:

$$\text{Survival rate (\%)} = N_1/N_0 \times 100$$

where N_0 is the number of viable bacteria at 0 h (CFU/mL) and N_1 is the number of viable bacteria in bile salt culture medium at 3 h (CFU/mL).

Identification of LAB

A rapid bacterial genomic DNA extraction kit (Shenggong Bioengineering Co., Ltd., Shanghai, China) was used to extract the DNA from the LAB. The purified DNA was used as a PCR template for amplification in a polymerase chain reaction instrument (Nexus+x2eco type, Eppendorf, Germany). The primers were universal primers (Shenggong Bioengineering Co., Ltd., Shanghai, China) (27F: AGAGTTTGATCCTGGCTCAG; 1492R: GGTTACCTTGTTACGACTT), and the fragments were amplified under the following conditions: 94°C, 30 cycles of 94°C for 45 s, 55°C for 30 s, and finally 72°C for 10 min (Riaz Rajoka *et al.*, 2017). The PCR products were detected by 1% agarose gel electrophoresis and then sequenced (Shenggong Bioengineering Co., Ltd., Shanghai, China). The sequence results were logged into the NCBI database for BLAST homology search and comparison analysis.

MEGA-X software was used for multiple sequence comparison analysis, and neighbor-joining (Saitou and Nei, 1987) was used to construct a phylogenetic tree. *L. plantarum* ATCC 14917, *Lactobacillus fermentum* ATCC 14931, *L. rhamnosus* ATCC 7469, *Lactobacillus casei* ATCC 393, and *Lactobacillus paracasei* ATCC 25302 were used as the standard strains.

Comparison of the bile salt tolerance of L. plantarum isolated from different sources

Ten strains of *L. plantarum* isolated from the intestines of long-lived elderly people were used as a comparison, and the differences in bile salt tolerance of *L. plantarum* from different sources were analyzed by one-way ANOVA.

Statistical analysis

The results were expressed as the mean ± standard deviation. The differences between the means of the test were evaluated by Duncan's test, and $P < 0.05$ was considered significantly different by one-way ANOVA.

Accession number

The sequences of 16S rDNA of the strains had been submitted to GenBank.

Results and Discussion

Isolation of LAB

After Gram staining and microscopic observation (Figure 1), 114 strains of acid-producing and Gram-positive strains were initially screened from breast milk and infant feces, among which 96 strains were isolated from infant feces and 18 strains were isolated from breast milk.

Optimization of the conditions for the bile salt tolerance test

Bile helps the enzymes of the human body break down fats into free fatty acids and facilitates fat molecule digestion in the small intestine through emulsion formation. All bacteria have high lipid content in their cell walls, which could be degraded by bile salt, leading to bacterial content leakage and cell death (Saboktakin *et al.*, 2021).

Figure 1. Microscopic pictures of partial strains.

Gram-positive bacteria are more sensitive to bile salt, and some LAB and bifidobacteria have a low bile salt tolerance (Margolles *et al.*, 2003). Moreover, the bile salt tolerance of different strains has significant specificity. Because of the low concentration of bile salt in the intestine of infants, most strains cannot survive under 0.3% bile salt, and the survival rate under 0.1% bile salt is extremely low. Therefore, it is necessary to explore a more suitable bile salt concentration to screen probiotics from breast milk and infant feces. The mass concentration of bile salt in the human small intestine fluctuates between 0.03 and 0.3% (Kashket, 1987), and the bile salt concentration in the intestinal tract of infants is even lower (He *et al.*, 2020), so the bile salt concentration should be set to 0.03 to 0.1% to further explore the optimal screening conditions.

Sixteen strains of *Lactobacillus* were selected to test the distinguishing ability of 0.03–0.1% bile salt. As shown in Table 1, the survival rates of 16 strains treated with 0.03% bile salt were significantly different, but the overall survival rate was relatively high. Among them, the survival rates of 14 strains were greater than 100% under 0.03% bile salt, and the survival rates of M704 and M705 were greater than 80%. Obviously, it was impossible to distinguish strains with poor bile salt tolerance. Under 0.1% bile salt, only the survival rate of M716 was greater than 1%, and for the remaining 15 strains it was less than 1%. There was no significant difference in the survival rates of these 15 strains treated with 0.1% bile salt, so 0.1% bile salt had no screening ability. According to our further study, 0.05% bile salt still had no screening ability (Table 1). However, under 0.07 and 0.08% bile salt, the survival rates of different strains were significantly different ($P < 0.05$), and 0.075% was finally selected as the bile salt tolerance concentration for screening. Considering that 0.1% bile salt can screen strains with higher bile salt tolerance, the subsequent bile salt test concentration was set as 0.075 and 0.1%. The optimized bile salt concentrations of 0.075 and 0.1% were used to test the bile salt tolerance of the isolates to screen strains with bile salt tolerance.

Bile salt tolerance test and strain identification of Lactobacillus

After 0.075 and 0.1% bile salt tolerance tests, a total of 36 strains whose survival rates were greater than 10% under 0.075% bile salt were obtained, and then 28 strains were identified as *Lactobacillus*. Based on the 16S rDNA sequence, a phylogenetic tree was constructed via neighbor-joining (Figure 2). Among the 28 strains of *Lactobacillus*, there were 16 strains of *L. plantarum*, three strains of *L. fermentum*, three strains of *L. paracasei*, two strains of *L. casei*, two strains of *L. rhamnosus*, and two strains of *Lactobacillus pentosus* (Table 2).

Table 1. Survival rate of strains under 0.03–0.1% bile salt.

Isolates	Survival rate (%)					
	0.03%	0.05%	0.07%	0.08%	0.09%	0.1%
M701	109.98 ± 7.08^{f}	–	–	–	–	<0.001^{b}
M703	106.38 ± 9.38^{f}	–	–	–	–	<0.001^{b}
M705	94.16 ± 6.49^{g}	–	–	–	–	<0.001^{b}
M706	108.40 ± 6.21^{f}	–	–	–	–	<0.001^{b}
M707	123.24 ± 2.70^{e}	–	–	–	–	<0.001^{b}
M708	114.72 ± 3.75^{ef}	–	–	–	–	<0.001^{b}
M709	110.75 ± 3.80^{f}	–	–	–	–	<0.001^{b}
M712	141.03 ± 5.88^{d}	–	–	–	–	<0.001^{b}
M715	126.92 ± 5.77^{e}	–	–	–	–	<0.001^{b}
M702	116.90 ± 6.38^{ef}	115.56 ± 0.96^{b}	–	–	–	<0.001^{b}
M718	180.09 ± 4.74^{b}	115.95 ± 7.44^{b}	–	–	–	0.21 ± 0.02^{b}
M731	114.94 ± 0.90^{ef}	47.62 ± 4.12^{d}	–	–	–	0.024 ± 0.02^{b}
M716	219.43 ± 4.51^{a}	132.61 ± 8.96^{a}	78.38 ± 2.70^{a}	45.95 ± 2.70^{a}	28.83 ± 1.56^{a}	1.86 ± 0.26^{a}
M704	87.50 ± 4.33^{g}	103.23 ± 6.45^{c}	19.00 ± 1.21^{b}	9.25 ± 0.94^{b}	1.08 ± 0.11^{b}	<0.001^{b}
M710	126.86 ± 4.48^{e}	101.94 ± 6.80^{c}	17.22 ± 0.66^{b}	4.53 ± 0.19^{c}	0.26 ± 0.03^{b}	<0.001^{b}
M713	152.27 ± 7.41^{c}	106.25 ± 2.08^{bc}	7.96 ± 0.26^{c}	0.74 ± 0.03^{d}	0.02 ± 0.03^{b}	<0.001^{b}

Values with different superscripts in the same column differ significantly ($P < 0.05$).

Table 2. Identification of strains screened by bile salt tolerance.

Isolates	Latin name	Accession No.*	Homology/%
M498	*L. paracasei*	SUB10562625	99.33
M511	*L. paracasei*	SUB10573239	99.52
M547	*L. plantarum*	SUB10573250	100.00
M555	*L. pentosus*	SUB10573257	99.27
M561	*L. plantarum*	SUB10573255	99.13
M589	*L. plantarum*	SUB10575755	99.61
M595	*L. casei*	SUB10575760	99.54
M596	*L. casei*	SUB10575765	99.39
M598	*L. paracasei*	SUB10575779	99.15
M600	*L. fermentum*	SUB10583278	98.98
M612	*L. fermentum*	SUB10583285	98.65
M621	*L. plantarum*	SUB10583289	99.15
M658	*L. plantarum*	SUB10583311	98.71
M660	*L. plantarum*	SUB10583314	98.79
M661	*L. plantarum*	SUB10583322	99.34
M662	*L. plantarum*	SUB10585957	99.04
M701	*L. fermentum*	SUB10585983	99.14
M703	*L. plantarum*	SUB10585987	99.14
M706	*L. rhamnosus*	SUB10585991	98.94
M707	*L. rhamnosus*	SUB10586000	99.89
M731	*L. plantarum*	SUB10586013	98.76
M747	*L. plantarum*	SUB10586019	99.58
M748	*L. plantarum*	SUB10586026	99.25
M750	*L. pentosus*	SUB10586031	98.70
M751	*L. plantarum*	SUB10586034	98.73
M781	*L. plantarum*	SUB10586038	98.80
M782	*L. plantarum*	SUB10586044	99.25
M783	*L. plantarum*	SUB10586045	98.73

*GenBank Accession No. on NCBI website.

Comparison of the bile salt tolerance of *L. plantarum* from different sources

Most strains isolated from breast milk and infant feces with high bile salt tolerance were *L. plantarum*. Therefore, *L. plantarum* strains from the adult intestinal tract were selected as a reference to analyze the bile salt tolerance characteristics of LAB from breast milk and infant feces. As shown in Table 3, among the strains isolated from the feces of long-lived elderly individuals (randomly isolated, without being screened by bile salt tolerance), the bile salt tolerance of the five isolates grx-L114, grx-L54, grx-L117, grx-L97, and grx-L128 was significantly higher than that of the others ($P < 0.05$), and their survival rate was greater than 70% under 0.075% bile salt and greater than 60% under 0.1% bile salt. The bile salt tolerance of isolates grx-L67, grx-L4, and grx-L39 was weaker, and their survival rate was greater than 40% under 0.075% bile salt and greater than 10% under 0.1% bile salt, while grx-L11 and grx-L58 had the weakest bile salt tolerance.

Among the strains isolated from infant feces, M547, M589, M621, M703, M747, and M748 had a survival rate greater than 70% under 0.075% bile salt; only M589 and M621 had survival greater than 50% under 0.1% bile salt, and the rest were between 10 and 22%. M751 had a survival rate of approximately 50% under 0.075% bile salt and a survival rate of approximately 17% under 0.1% bile salt. M561 and M662 had the weakest bile salt tolerance, with a survival rate of approximately 20% under 0.075% bile salt and less than 1% under 0.1% bile salt.

Specifically, among the strains isolated from breast milk, the bile salt tolerance of M731 and M783 was significantly higher than that of the other isolates under 0.075% bile salt, with a survival greater than 70%. M781 and M782 followed with survival rates greater than 40%, and the rest were between 20 and 30%. However, their survival rates were all between 0 and 24% under 0.1% bile salt.

Although all of these strains belonged to *L. plantarum*, the survival rates of the different strains under 0.075 and 0.1% bile salt were significantly different. The survival rate of *L. plantarum* E680 was as high as 80.79 ± 0.94% under 0.3% bile salt (Zheng *et al.*, 2020). A strain of *L. plantarum* WLPL04 was isolated from human breast milk, and its survival decreased slightly under 0.15% bile salt and was reduced by 0.83 and 1.17 log CFU/mL under 0.30 and 0.45% bile salt, respectively, for 6 h (Jiang *et al.*, 2016). A recent study indicated that there was a significant difference in the tolerance to bile among the growth stages, and the *L. plantarum* YM-4–3 possessed better bile tolerance during the early exponential phase points than in the middle stationary phase (Jiang *et al.*, 2021). In this study, the bile salt tolerance of probiotics isolated from breast milk and infant feces reflected the strain specificity of *L. plantarum*.

Under 0.075% bile salt, there was no significant difference in the survival rate of *L. plantarum* isolated from the feces of long-lived elderly people and infants, and breast milk ($P > 0.05$) (Figure 3). The 0.075% bile salt tolerance of *L. plantarum* isolated from breast milk and infant feces could reach the level of that isolated from the adult intestine after being screened by the bile salt tolerance test. However, under 0.1% bile salt, the survival rate of *L. plantarum* isolated from the feces of long-lived elderly individuals was significantly higher than that isolated from infant feces and breast milk ($P < 0.05$). After screening for bile salt tolerance, the bile salt tolerance of *L. plantarum* isolated from breast milk and infant feces was still far less than that of strains from the adult intestine without bile

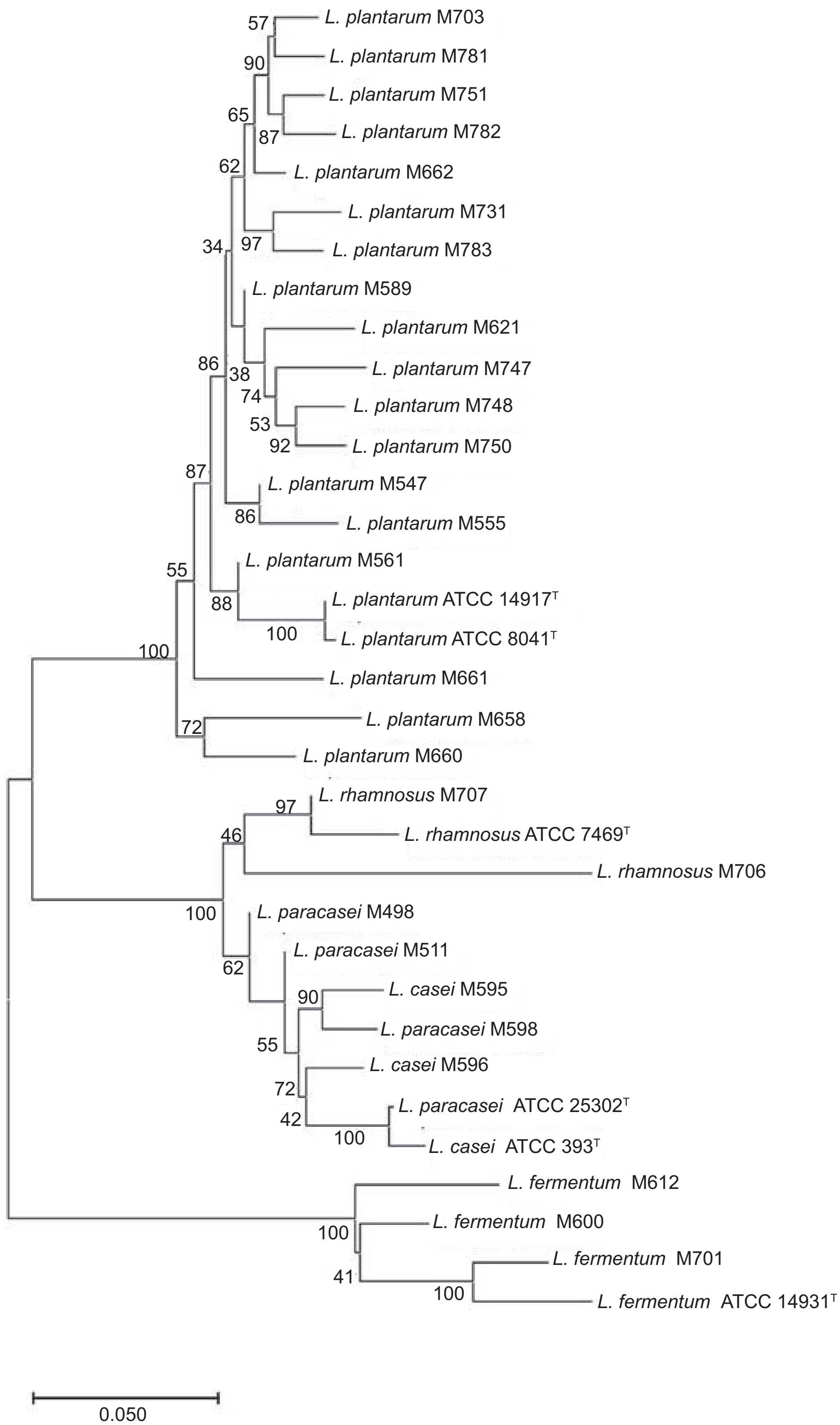

Figure 2. Phylogenetic tree based on the 16S rDNA gene sequences of the isolates and the related standard strains.

Table 3. Survival rate under 0.075 and 0.1% bile salt of *L. plantarum* from different sources.

Isolates	Source	Survival rate of 0.075% bile salt (%)	Survival rate of 0.1% bile salt (%)
grx-L4	Stool of long-lived people	45.54 ± 1.46^{f}	12.74 ± 1.10^{f}
grx-L11		20.63 ± 0.58^{g}	5.99 ± 1.00^{g}
grx-L39		46.64 ± 2.65^{f}	23.70 ± 1.32^{e}
grx-L54		88.89 ± 2.96^{b}	70.62 ± 1.71^{a}
grx-L58		13.72 ± 0.55^{h}	0.89 ± 0.11^{h}
grx-L67		52.22 ± 1.92^{e}	41.11 ± 1.92^{d}
grx-L97		80.08 ± 1.16^{c}	66.15 ± 2.32^{bc}
grx-L114		93.87 ± 2.20^{a}	67.65 ± 3.19^{b}
grx-L117		86.97 ± 2.73^{b}	66.29 ± 0.85^{bc}
grx-L128		71.29 ± 1.74^{d}	64.26 ± 1.74^{c}
M547	Infant feces	108.07 ± 6.45^{A}	11.53 ± 1.11^{E}
M561		21.87 ± 2.61^{D}	<0.001^{F}
M589		76.22 ± 3.58^{B}	56.16 ± 2.63^{B}
M621		76.76 ± 0.88^{B}	64.71 ± 3.67^{A}
M662		20.00 ± 2.35^{D}	0.71 ± 0.12^{F}
M703		109.68 ± 2.11^{A}	10.86 ± 0.12^{E}
M747		75.07 ± 1.94^{B}	21.74 ± 0.70^{C}
M748		76.03 ± 3.31^{B}	19.83 ± 1.51^{CD}
M751		51.75 ± 3.04^{C}	17.37 ± 1.47^{D}
M658	Breast milk	20.12 ± 2.05	0.47 ± 0.00
M660		30.50 ± 1.37	1.07 ± 0.12
M661		22.74 ± 1.26	0.51 ± 0.13
M731		78.45 ± 0.86	0.19 ± 0.01
M781		52.70 ± 1.10	14.73 ± 0.58
M782		44.72 ± 1.41	23.93 ± 2.96
M783		76.56 ± 1.35	17.19 ± 1.35

The values of different superscripts in the same column in each group are significantly different ($P < 0.05$).

salt resistance screening under 0.1% bile salt. The poor tolerance of *L. plantarum* from infants under a high concentration of bile salt might be due to their adaptation to a low bile salt concentration environment. On the other hand, the tolerance of *L. plantarum* from infants was comparable to that of strains from adults under a low bile salt concentration, indicating that probiotics for infants should also have a certain level of bile salt tolerance.

It is proposed that the deconjugation of bile salt by bile salt hydrolase (BSH) may be a detoxification mechanism and might increase bile tolerance and survival of microbes in the gastrointestinal tract (Bi *et al.*, 2016). *Lactobacillus* strains possessing BSH activity hydrolyze conjugated bile acid into free bile salt (Miremadi *et al.*, 2014). Bile salt is important for helping to solubilize the liberated fatty acid. Thus, strains possessing high BSH activity may bring some risks to the neonate, given that bile salt is significantly lower in them. However, the microbiota has been shown to contribute to the diurnal variation in lipid absorption, as well as other nutrients (Kuang *et al.*, 2019). Therefore, it is necessary to screen suitable and safe strains for infants.

The survival rates of the strains isolated from infant feces and breast milk were both very different ($P < 0.05$) and the distribution structure was similar under 0.075 and 0.1% bile salt, indicating that the bacterial flora distribution in breast milk and infant feces might be similar. At present, the specific process of the vertical transmission of bacteria from mother to baby is not completely understood, but the *Bifidobacterium* strains shared between the baby and the mother have been isolated from breast milk (Makino *et al.*, 2015). Another study also showed that the microbiomes in breast milk and baby feces were related to each other (Kimberly *et al.*, 2020).

Conclusion

In this study, a total of 114 acid-producing and Gram-positive strains were isolated from breast milk and infant feces. Twenty-eight strains of *Lactobacillus* with relatively better bile salt tolerance were screened and identified, including 16 strains of *L. plantarum*. Under 0.1% bile salt, the bile salt tolerance of *L. plantarum* isolated from breast milk and infant feces was far less than that of strains isolated from adult intestines that were not screened for bile salt tolerance. Therefore, the demand for bile salt tolerance of probiotics for infants might be lower than that for adults. The bile salt tolerance of strains for infants should be in line with their actual situation out of safety concerns; hence, the screening standards for the in vitro bile salt tolerance of probiotics should be reduced during the development of special LAB for infants. Further studies are required to examine the dynamic changes of bile salt tolerance of strains from different sources during subculture, and whether the difference of bile salt tolerance will be maintained needs to be explored. Furthermore, the differences of gene sites related to bile salt tolerance of strains during subculture should be considered.

Authors' contributions

Ruixia Gu and Chenchen Zhang conceived and designed the experiments; Junjuan Yu, Yujun Huang, and Haodong Yan completed the experiments; Yujun Huang, Wenli Kang, and Lina Pan processed statistics and proofread the text; Jiaqi Wang and Zhiyong Dai wrote the manuscript. All authors checked and approved the final manuscript.

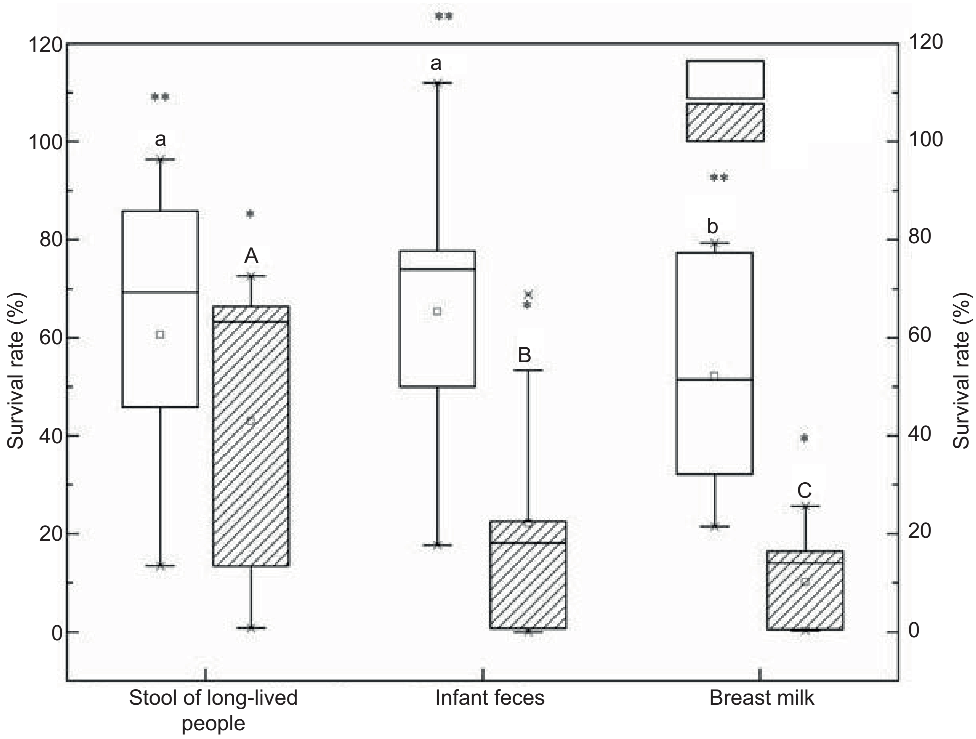

Figure 3. Comparison of bile salt tolerance of *L. plantarum* from different sources. Lowercase letters indicate that the survival rate of strains between different groups under 0.075% bile salt was significantly different at the *P* < 0.05 level. Capital letters indicate that the survival rate of strains between different groups under 0.1% bile salt was significantly different at the *P* < 0.05 level. "*" indicates that the survival rate of strains under the two concentrations of bile salt in each group was significantly different at the *P* < 0.05 level.

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflicts of interest with the current work or its publication.

Ethical approval

All mothers that participated in the study gave written informed consent to the protocol, which had been previously approved by the Ethical Committee of Xiangya School of Public Health Central South University (China). The approval number was XYGW-2020-37. The study was carried out in accordance with the Declaration of Helsinki.

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