Research Article

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Microbiological quality and characteristics of probiotic products in China

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Abstract

BACKGROUND: Probiotics are widely used in the food industry and medicine fields in China, but few studies have been conducted to evaluate the actual microbial amounts and species in probiotic products, which may conflict with the labels and mislead consumers to choose inappropriate foods or medicines.

RESULTS: Twenty commercial dairy products and eight commercial 'healthcare' samples were collected from markets in China and tested using culture-dependent and culture-independent methods. The results suggested that the total bacterial counts of most commercial products met the minimum quantitative requirement of the Chinese national standard (6.00 log colony-forming units g^{-1}). However, the bacterial counts of specific species were inconsistent with the labelling. In parallel, denaturing gradient gel electrophoresis analysis indicated that some probiotic-containing products were wrongly labelled; no *Bifidobacterium* species were detected in the products claiming to contain bifidobacteria, and the probiotic characteristics (antimicrobial activity, acid resistance and bile resistance) of some isolates had degraded. Moreover, some contaminating bacteria, e.g. *Enterobacter* sp., *Klebsiella* sp. and *Serratia* sp., were also detected in these products.

CONCLUSION: The combination of culture-dependent and culture-independent methods was proven to quickly and conveniently detect the microbial diversity in probiotic products, and more effort is required to regulate the probiotic market in China.

Keywords: probiotics; PCR-DGGE; viable counts; antimicrobial activities

INTRODUCTION

The development of non-antibiotic and environmentally friendly agents is one of the key factors for human health, and products containing living micro-organisms have been used to restore gut health for a long time.[1] At present, selected strains mainly belonging to the genera *Lactobacillus* and *Bifidobacterium* are increasingly used for probiotics. The definition of probiotics claimed that the strains selected must overcome biological barriers, including acid in the stomach and bile in the intestine, to reach their place of action in order to exert their health-promoting effects, and their safety and efficacy have to be demonstrated for each strain and each product. Moreover, to produce therapeutic benefits, a sufficient number of viable micro-organisms must be present throughout the entire shelf life of the product. However, these organisms often show poor viability in market preparations.[2]

In recent decades, various advanced technologies have been developed to detect the pathogens or hazardous substances contained in foods, but little research has been conducted to evaluate the bacterial biomass and species in commercial probiotic products, not to mention their metabolites. Moreover, the safety assessment of probiotics for human use has long been ignored or considered irrelevant owing to their long history of safe use. At present, the plate count method is still routinely used in the quality control assessment of probiotic products during production; this method usually renders microbial analysis of probiotic products relatively time-consuming, and results may be biased by poor viability or low densities of the target organism. As a powerful tool, the polymerase chain reaction (PCR) denaturing gradient gel electrophoresis (DGGE) method has been widely used to assess microbial communities in fermented soybeans, fermented milk and sourdoughs.[3–8] Thus the combination of viable cell count

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and DGGE method has allowed us to gain an overview of the microbial population structure in 28 probiotic products.

The objective of this study was to determine the microbial biomass and species in 28 commercial products and to evaluate the probiotic characteristics of eight isolates screened from 20 yoghurts, which may contribute to regulate the mislabeling of probiotic products and to establish a much more beneficial regulation for the quality of probiotic products in the near future.

MATERIALS AND METHODS

Commercial products, isolates and pathogens

The commercial products (the most popular brands in China, including 20 yoghurts and eight medicines) were purchased directly from supermarkets and analyzed within 2 days. The 20 yoghurts were named as A–T and the eight medicines were named as A to H. In addition, the probiotic characters of eight isolates (see Table 3) were evaluated.

Eight pathogens, namely *Shigella dysenteriae* 301, *Shigella dysenteriae* 2457, *Staphylococcus* COWan1, *Staphylococcus* CMC, *Enterobacter sakazakii* 45402, *Enterobacter coli* 44102, *Candida albicans* SC5314 and *Enterobacter sakazakii* 45401 (isolated from human feces), were used as control strains. All bacteria used in this study are kept in our labs.

Viable cell counts

Bacterial counts were performed according to a previous report. Plates with 25–250 colonies were replica-plated onto brain/heart infusion (BHI) agar (an enriched non-selective medium for the isolation and cultivation of most anaerobic bacteria and other fastidious micro-organisms, which was used to select the total anaerobic bacteria and total aerobic bacteria in this study), de Man/Rogosa/Sharpe (MRS) agar (an enriched selective medium intended for the isolation and cultivation of *Lactobacillus* found in clinical specimens and dairy and food products, which was used to select the lactobacilli and bifidobacteria in this study) and Luria broth (LB) agar (a rich medium commonly used to culture members of the Enterobacteriaceae as well as for coliphage plaque assays, which was used to selected the common Enterobacteriaceae in this study) and then incubated aerobically or anaerobically at 37 °C for 24–36 h for colony counting.[9,10]

DGGE analysis

DNA was isolated by a bead-beating method.[11] After phenol/chloroform extraction, DNA was precipitated with ethanol and suspended in 50 μL of TE buffer (Tris-hydrochloride buffer, pH 8.0, containing 1.0 mM EDTA). Primers 357f (5′-TACGGGAGGCAGCAG-3′) and 519r (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the total bacterial DNA, a GC clamp in primer was used to create PCR products suitable for separation by DGGE, and PCR was performed with a Taq DNA polymerase kit (Sangon Biotech Co. Ltd, Shanghai, China). Then amplicons of V3 of 16S rDNA were used for sequence separation by DGGE.[7,9,12] DGGE was performed using 40 mmol L^{-1} Tris–HCl (pH 8) as the electrophoresis buffer in a BioRad DGGE system (Bio-Rad, Hercules, CA, USA). Electrophoresis was initiated by pre-running for 5 min at a voltage of 220 V and subsequently run at a fixed voltage of 85 V for 16 h at 60 °C. The gel was stained with $AgNO_3$ and developed after completion of electrophoresis. The gel was then covered with cellophane membrane and dried overnight at 60 °C.

PCR products were subcloned into the pMD18-T vector system I (TaKaRa, Japan) according to the manufacturer's instructions. Cells of *Escherichia coli* DH5a were electrotransformed with recombinant plasmids by a standard method.[13] Selection of transformants was done on LB agar containing 100 mg mL^{-1} ampicillin, and the transformants were randomly picked and sequenced.

Antimicrobial activities of isolates

The agar diffusion assay described in the literature was used to test the antimicrobial activity of isolates, with slight modification.[14] Overnight incubation cultures of the indicator micro-organisms were spread on 10 mL of LB agar in a Petri dish; culture supernatant (200 μL) was added into an Oxford cup (a stainless cylinder of outer diameter 7.8 ± 0.1 mm, inner diameter 6.0 ± 0.1 mm and height 10.0 ± 0.1 mm), which was placed on the surface of the agar. The size of the clear zone around the cup (including that of the Oxford cup, 7.8 mm) was measured. The experiment was performed in duplicate.

Acid and salt tolerance of isolates

Strains were grown in corresponding media at 37 °C overnight, then subcultured into fresh media and incubated for another 24 h. The cultures were centrifuged at 4500 × *g* for 10 min at 4 °C. For acid tolerance, each strain was diluted 1:100 (v/v) in phosphate-buffered saline (PBS) at pH 1.5, 2.5, 3.5, 4.5 and 7 for 4 h; for salt tolerance, freshly prepared cultures were inoculated into corresponding media containing 0.1–0.5% (w/w) bile salts and incubated at 37 °C for another 4 h. Then all bacteria were enumerated using the plate count method.

RESULTS AND DISCUSSION

Although human health improvement by probiotics has gained widespread acceptance in recent years, there is growing concern on the part of consumers and consumer organizations regarding the quality and labeling of commercial probiotic products.[15] Analysis of most probiotics is still based on culture-dependent methods involving the use of specific isolation media and the identification of a limited number of isolates, which makes this approach relatively insensitive, laborious and time-consuming.[16] In this study, the culture-independent DGGE method was compared with a culture-dependent procedure for detection and identification of the strains in probiotic products.

Biomasses of total bacteria, lactobacilli and bifidobacteria, and low-nutritional bacteria

In the USA, probiotics are governed by the Food and Drug Administration (FDA) and must be 'generally recognized as safe' (GRAS) before entering the marketplace. However, there are no specific rules to follow for probiotic fermented dairy products in China, except for the 'Probiotics List for Use in Health Foods' (2001) and the 'Application and Evaluation of Probiotic Health Foods' (2005). The latter states that the viable cell number during the warranty period should not be less than 6.00 log colony-forming units (CFU) g^{-1}, which is below the international standard of 7.00 log CFU g^{-1}, so it is very important to investigate the bacterial diversity in probiotic products in China. In this study, a number of probiotic products were investigated by colony counts of viable bacteria, and the results indicated that the total microbial biomass of 90% of yoghurt samples (except M and Q) met the minimum requirement of the national standard in China (6.00 log CFU g^{-1})

Table 1. Colony counts of viable bacteria in 28 Chinese probiotic products

Product	Micro-organisms declared on label	Declared (log CFU g^{-1})	Viable cell count (log CFU g^{-1})		
			Bifidobacteria and lactobacilli	Spore-forming *Bacillus* species	Total bacteria
Dairy products					
A (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	NM	9.00	ND	9.18
B (liquid)	*L. bulgaricus* *S. thermophilus*	NM	6.46	ND	8.71
C (liquid)	*S. thermophilus* *L. bulgaricus*	NM	ND	ND	8.20
D (liquid)	*S. thermophilus* *L. bulgaricus*	NM	7.30	ND	7.70
E (liquid)	*L. casei*	8.00	8.28	ND	8.45
F (liquid)	LAB	NM	ND	ND	5.54
G (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	7.47	7.00	ND	8.91
H (liquid)	*L. bulgaricus* *S. thermophilus*	NM	8.34	ND	8.40
I (liquid)	*L. bulgaricus* *Bifidobacterium* *L.acidophilus* *S. thermophilus*	NM	8.40	ND	8.78
J (liquid)	*S. thermophilus* *L. bulgaricus* *B. longum* *L. acidophilus*	NM	6.69	ND	8.47
K (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	NM	6.63	ND	8.47
L (liquid)	LAB	NM	8.90	ND	8.95
M (liquid)	*S. thermophilus* *L. bulgaricus*	NM	ND	ND	ND
N (liquid)	*L. bulgaricus* *S. thermophilus* *L. acidophilus* *Bifidobacterium*	NM	7.48	ND	6.92
O (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	7.48	6.78	ND	8.81
P (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	NM	8.9	ND	8.91
Q (liquid)	*S. thermophilus* *L. bulgaricus* *Bifidobacterium* *L. acidophilus*	NM	ND	ND	ND
R (liquid)	LAB	NM	9.30	ND	9.48
S (liquid)	LAB	8.00	ND	ND	8.32
T (liquid)	*L. bulgaricus* *S. thermophilus* *L. acidophilus* *Bifidobacterium*	7.00	8.90	ND	9.48

Table 1. *Continued*

Product	Micro-organisms declared on label	Declared (log CFU g^{-1})	Viable cell count (log CFU g^{-1}) Bifidobacteria and lactobacilli	Spore-forming *Bacillus* species	Total bacteria
'Healthcare' products					
A (capsule)	*E. faecalis*	8.18	9.48	8.81	9.59
	B. subtilis	7.18			
B (tablet)	*B. longum*	7.60	8.93	9.32	9.60
	L. acidophilus				
	E. faecalis				
C (capsule)	*Bifidobacterium*	8.18	10.08	6.38	10.18
D (capsule)	*B. cereus*	8.90	ND	9.60	9.65
E (sachet)	*B. licheniformis*	9.00	ND	10.11	10.18
F (capsule)	*L. acidophilus*	9.78	9.60	ND	9.78
	B. bifidum				
	B. infantis				
G (capsule)	*B. infantis*	5.00	4.5×10^8	7.30	9.04
	L. acidophilus	5.00			
	E. faecalis	5.00			
	B. cereus	5.00			
H (capsule)	*E. faecalis*	8.18	10.66	10.95	11.08
	B. subtilis	8.30			

LAB, lactic acid bacteria; *L.*, *Lactobacillus*; *B.*, *Bifidobacterium*; *S.*, *Streptococcus*; *E.*, *Enterobacter*; NM, no mention; ND, not detected.

Table 2. Sequencing results of typical bands of DGGE patterns from probiotic products

Band no.	Closest relative	Similarity (%)	GeneBank No.
Dairy products			
1, 2, 3, 11	*Enterococcus faecium*	100	HQ641405.1
4	*Streptococcus thermophilus*	99	HM218362.1
5	*Enterobacter* sp.	99	HQ413271.1
6	*Lactococcus lactis*	100	HQ286592.1
7, 8	*Streptococcus thermophilus*	100	HM218518.1
9	*Staphylococcus epidermidis*	100	HM218280.1
10	*Enterobacter cloacae*	100	HQ694184.1
12	*Enterobacter* sp.	99	HQ413274.1
13	*Serratia* sp.	100	HM217122.1
14	*Enterococcus faecium*	99	HQ450696.1
15	Uncultured *Actinomycetales*	100	HM077188.1
'Healthcare' products			
1	*Enterococcus faecium*	100	HQ118100.1
2	*Lactobacillus acidophilus*	100	HQ293112.1
3	*Enterococcus faecalis*	99	HQ641405.1
4	*Enterococcus faecium*	99	HQ450724.1
5	*Bacillus subtilis*	99	CP002453.1
6, 7	*Lactobacillus acidophilus*	99	HQ379177.1
8, 9	*Enterococcus faecium*	100	HQ450730.1
10	*Bacillus cereus*	100	HQ236087.1
11	*Lactococcus lactis*	100	HQ647115.1
12, 13	*Bacillus licheniformis*	100	HQ709382.1
14	*Bacillus licheniformis*	100	HQ684002.1

(Table 1). However, 75% (15/20) of the yogurts did not label the number of viable micro-organisms, and 65% of the products (A, F, G, I, K, L, N, O, P, Q, R, S and T) only labeled the genera *Lactobacillus* and *Bifidobacterium* rather than specific strains. Compared with the total bacterial number counted on BHI agar, the genus *Lactobacillus* accounted for only 1–10% in yoghurts B, G, J, K, O and P, and no *Lactobacillus* species were detected in yoghurts C, F, M, Q and S.

For the probiotic medicines, the microbial biomass was much better. However, product C (only containing *Bifidobacterium*) was found to contain low-nutritional micro-organisms rather than the labeled species. Also, our results indicated that the beneficial genera *Lactobacillus* and *Bifidobacterium* were present only in minor amounts in all products, with low-nutritional bacteria being in the majority owing to their low nutritional requirement and rapid growth rate. Notably, the minimum quantitative requirement of the Chinese national standard (6.00 log CFU g^{-1}) is lower than the currently accepted minimum effective total count for probiotics (9.00 log CFU daily),[17,18] which means that more effort should be made to improve the minimum quantitative requirement of the Chinese national standard.

PCR-DGGE analysis

To detect the bacterial composition in 28 probiotic products and to avoid the limitations of the culture-dependent plate count method, a culture-independent DGGE method was applied. This method does not require the culturing of bacteria, thus avoiding errors during culture progress. Moreover, most bacteria surviving in different environments are unculturable, so the DGGE method provides more realistic results than the culture-dependent method.

From our DGGE results, a minimum similarity of 53% and a maximum similarity of 83% (between yoghurts N and K) were observed among all yoghurt samples (Fig. 1), indicating that

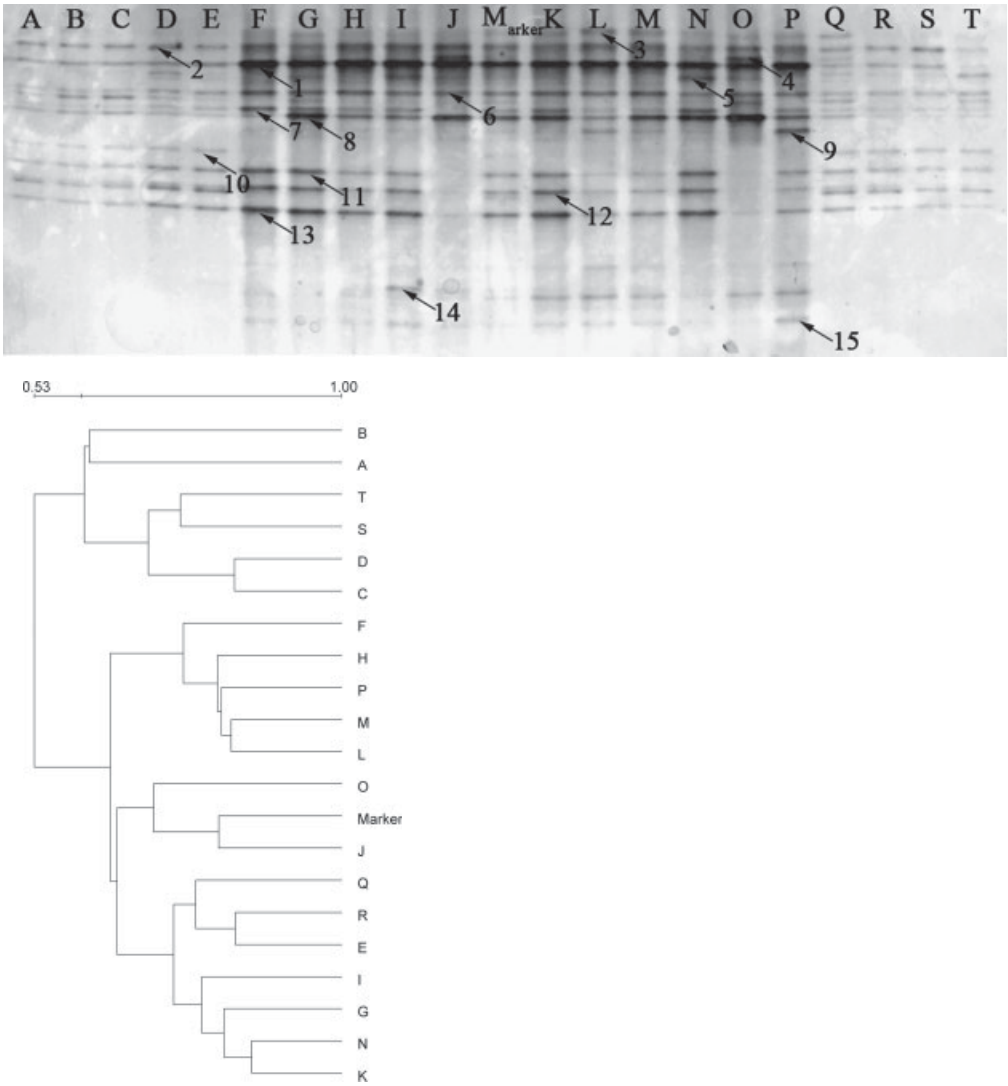

Figure 1. DGGE patterns and similarity index of 20 dairy products: A–T, commercial products purchased from supermarket; Marker, DNA mixture of samples A–T. The characterized bands marked with arrows in the DGGE patterns were sequenced and categorized to different species as shown in Table 2.

Table 3. Sequencing results of isolates from 28 probiotic products

Strain no.	Closest relative	Similarity (%)	GeneBank No.	Source of isolate (see Table 1)
1	*L. bulgaricus* LMG 12168	99	AM28426.1	Dairy product A
2	*B. longum* JCM 1217	99	NC_015067.1	Dairy product A
3	*S. thermophilus* NM62-4	99	HM218518.1	Dairy product A
4	*Bifidobacterium* sp. HGAT10	98	HM245216.1	Dairy product B
5	*B. lactis* Bb 12	99	GQ340905.1	Dairy product E
6	*L. casei* BL23	99	NC_010999.1	Dairy product J
7	*E. faecalis* LW88	100	HQ641405.1	Dairy product Q
8	*L. acidophilus* LC	99	HQ286592.1	Dairy product T

the probiotic species were diverse even in products made by the same company using the same bacteria. Yoghurts O and J, which claimed to contain the same strains *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium* and *Lactobacillus acidophilus*, only possessed a similarity of 71%, while the similarity of yoghurts E (only added *Lactobacillus casei*) and R (only added lactic acid bacteria) was as high as 82%.

When DGGE bands were sequenced (Fig. 1), the dominant bacteria *Enterococcus faecium* (band 1) and *S. thermophilus* (band 8) were detected in all 20 yoghurts, though no products had claimed to contain *E. faecium* (Table 2, Fig. 1). In addition, neither *L. bulgaricus* (appearing on all yoghurt labels) nor *Bifidobacterium* species (appearing on ten yoghurt labels) were detected using the DGGE method. A possible explanation is that *E. faecium* has been

Table 4. Antimicrobial activity of isolates to eight pathogens using Oxford cup method (outer diameter 7.8 ± 0.1 mm, inner diameter 6.0 ± 0.1 mm, height 10.0 ± 0.1 mm)

Isolate	Inhibition zone of probiotic to pathogen (mm)							
	Sh. dysenteriae 301	*Sh. dysenteriae* 2457	*Staphylococcus* COWan1	*Staphylococcus* CMC	*E. sakazakii* 45401	*E. coli* 44102	*C. albicans* SC5314	*E. sakazakii* 45402
L. bulgaricus LMG 12168	13.67 ± 2.89	10.67 ± 0.58	13.00	17.67 ± 1.15	16.67 ± 2.08	10.68 ± 0.58	14.33 ± 0.58	12.33 ± 0.58
B. longum JCM 1217	12.67 ± 0.58	16.67 ± 2.89	14.33 ± 0.58	20.00 ± 1.00	18.00 ± 1.73	14.33 ± 1.15	13.00 ± 1.732	0
S. thermophilus NM62-4	11.33 ± 0.58	0	0	0	0	0	0	0
Bifidobacterium sp. HGAT10	12.67 ± 0.58	0	18.67 ± 1.15	10.66 ± 0.58	11.67 ± 0.58	15.33 ± 0.58	20.33 ± 0.58	11.67 ± 0.58
B. lactis Bb 12	13.33 ± 0.58	14.33 ± 0.58	12.67 ± 0.58	11.67 ± 0.58	15.33 ± 0.58	10.67 ± 0.51	23.00 ± 0.41	15.33 ± 0.32
L. casei BL23	11.00 ± 0.05	0	0	0	10.67 ± 0.51	0	0	0
E. faecalis LW88	10.67 ± 0.27	16.67 ± 0.62	19.67 ± 0.48	17.33 ± 0.53	20.00 ± 1.00	14.67 ± 1.52	14.33 ± 1.12	0
L. acidophilus LC	15.33 ± 0.54	11.00 ± 0.01	14.00 ± 0.12	0	12.33 ± 0.57	11.00 ± 0.11	12.00 ± 0.12	10.00 ± 0.08
E. faecalis PWZ7140[a]	21.67 ± 1.53	20.66 ± 0.58	20.00 ± 0.25	22.67 ± 0.58	16.33 ± 0.51	23.67 ± 0.53	0	15.67 ± 0.54

[a] Isolated from human feces to serve as control group.

Figure 2. DGGE patterns and similarity index of eight 'healthcare' products: A–H, commercial products purchased from supermarket; Marker, DNA mixture of samples A–H. The characterized bands marked with arrows in the DGGE patterns were sequenced and categorized to different species as shown in Table 2.

used as a probiotic for a long time and may be misidentified as other probiotics used in probiotic products. Also, the low nutritional requirement and rapid growth rate of *E. faecium* (band 1) and *S. thermophilus* (band 8) make them the dominant micro-organisms.

Moreover, some contaminating bacteria such as *Enterobacter* sp. (band 5) and *Staphylococcus epidermidis* (band 9) were detected in yoghurts N and P, and *Enterobacter cloacae* (band 10), *Klebsiella* sp. (band 12) and *Serratia* sp. (band 14) were detected in most yoghurts. None of these pathogens was isolated from any of the products using the traditional method, and the low pH (∼4, data not shown) also ensured their elimination. Therefore the only explanation is that these pathogens grew rapidly at the beginning of fermentation but were then killed when the probiotics became dominant, while their DNA content in yoghurts could be identified using the DGGE method.

For the 'healthcare' products, the maximum similarity among the eight medicine samples was only 53% (between A and H), and no *Bifidobacterium* species were detected (Fig. 2). In addition, no contaminating bacteria were detected in the medicine samples, and their microbial biomass ranged from 9.60 to 11.08 log CFU g^{-1} (Table 1).

In a previous study, 58 probiotic products that claimed to contain *Bifidobacterium* strains were analyzed by Masco *et al.*,[15] and only 70.7 and 96.5% of the products were found to contain bifidobacteria by culture-dependent and culture-independent methods respectively. Also, Huys *et al.*[19] studied 26 manufactured probiotic products, and more than 28% of these commercial cultures intended for human and/or animal use were misidentified at the genus or species level. In our study, the culture-dependent method rather than the culture-independent method detected the genus *Bifidobacterium* and *L. bulgaricus*. This is mainly because

Table 5. Acid tolerance of isolates in 28 probiotic products

Isolate	Probiotic biomass at different pH values (log CFU g^{-1})				
	pH 1.5	pH 2.5	pH 3.5	pH 4.5	pH 7
L. bulgaricus LMG 12168	5.43	7.65	8.15	8.41	8.72
B. longum JCM 1217	5.00	7.30	8.08	8.00	7.95
S. thermophilus NM62-4	0	0	5.60	5.78	7.40
Bifidobacterium sp. HGAT10	0	6.85	7.48	7.70	7.60
B. lactis Bb 12	0	7.00	8.08	8.00	7.90
L. casei BL23	0	0	6.65	6.62	7.00
E. faecalis LW88	5.00	6.93	7.30	7.78	8.00
L. acidophilus LC	0	8.61	8.48	8.96	8.93
E. faecalis PWZ7140[a]	6.95	7.32	7.36	7.70	8.85

[a] Isolated from human feces to serve as control group.

Table 6. Bile salt tolerance of isolates in 28 probiotic products

Isolate	Probiotic biomass at different concentrations of bile salt (log CFU g^{-1})			
	0%	0.1%	0.3%	0.5%
L. bulgaricus LMG 12168	8.48	6.11	5.00	4.43
B. longum JCM 1217	8.18	7.30	4.57	3.60
S. thermophilus NM62-4	7.52	6.18	5.00	3.60
Bifidobacterium sp. HGAT10	8.18	7.00	0	0
B. lactis Bb 12	7.00	6.70	0	0
L. casei BL23	7.26	7.00	7.60	3.00
E. faecalis LW88	8.45	3.30	3.00	0
L. acidophilus LC	9.00	6.45	3.00	0
E. faecalis PWZ7140[a]	8.97	5.75	4.67	3.56

[a] Isolated from human feces to served as control group.

our objective was to monitor the total diversity of bacteria in commercial products, so universal bacterial primers rather than specific primers were chosen; thus minor species present at $<1\%$ of the total population might not be detected by DGGE (our species-specific primers could also detect the genus *Lactobacillus*; data not shown). In addition, these primers allowed the detection of *Bifidobacterium* species with minor population, even the residual DNA released by dead *Bifidobacterium* species.

Characteristics of isolates

To further study the characteristics of probiotics contained in commercial probiotic products (mainly for *Lactobacillus* and *Bifidobacterium* species), 64 isolates were selected based on their colony and cell morphologies, and eight probiotics (*Sh. dysenteriae* 301, *Sh. dysenteriae* 2457, *Staphylococcus* COWan1, *Staphylococcus* CMC, *E. sakazakii* 45402, *E. coli* 44102, *C. albicans* SC5314 and *E. sakazakii* 45401) were identified using genomic sequencing (Table 3). Both *L. bulgaricus* LMG 12168 and *Bifidobacterium lactis* Bb 12 showed broad antibacterial spectra and could inhibit all tested pathogens, while *L. casei* BL23 and *S. thermophilus* NM62-4 only exerted antibacterial activity on one or two pathogens (Table 4). Although the antibacterial spectra of other isolates were inferior to those of *L. bulgaricus* LMG 12168 and *B. lactis* Bb 12, they also showed excellent performance in inhibiting pathogens. However, the antimicrobial effect of *Enterobacter faecalis* PWZ7140 (set as a control) was the best.

The acid tolerance test of isolates showed that the amounts of *L. bulgaricus* LMG 12168, *Bifidobacterium longum* JCM 1217 and *E. faecalis* LW88 exceeded 5.00 log CFU g^{-1} at pH 1.5 (Table 5). The bile tolerance test of isolates showed that *L. bulgaricus* LMG 12168, *B. longum* JCM 1217, *S. thermophilus* NM62-4 and *L. casei* BL23 could largely survive at 0.5% bile concentration (Table 6). From Tables 5 and 6 we can conclude that most isolates could survive at low pH (3.5) and high bile concentration (0.3%), suggesting a high survive rate in the stomach and small intestine of humans.[20,21]

CONCLUSION

The probiotic field is an attractive area for industry and scientific research. However, a number of recent reports have clearly highlighted the poor quality of many probiotic products in terms of their contents and label information.[15,16,19,20] On the Chinese market, there are commercial probiotic products that do not comply with what their labels claim. In relation to safety and functionality, it is very important that these products are correctly labeled and contain well-documented probiotic strains.[22]

In this study, culture-dependent and culture-independent methods were used to evaluate the actual microbial biomass and species of 28 commercial products in China. The results will contribute to raising consumer safety awareness and the establishment of relevant laws and regulations on probiotics in China.

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