Policy Forum

Enumeration, Genetic Characterization and Antimicrobial Susceptibility of Lactobacillus and Streptococcus Isolates from Retail Yoghurt in Beijing, China[*]

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Lactic acid bacteria (LAB) are widely used in food industries. Correct identification and safety evaluation of these bacteria at the species even strain level should take considerations into account. In this study, the LAB were recovered from yoghurt and characterized phenotypically and genetically. Fifty-two isolates of LAB from 31 yoghurt samples were cultured and grouped into 6 species including *Lactobacillus bulgaricus* (24 isolates), *Streptococcus thermophilus* (15 isolates), *L. acidophilus* (7 isolates), *L. paracasei/casei* (3 isolates), *L. delbrueckii* (2 isolates), and *L. fermentum* (1 isolate), based on their Gram-staining, colony morphology and biochemical properties. 16S rRNA gene sequencing identified all isolates as either *Lactobacillus* or *S. thermophilus*, that completely matched with those obtained by phenotyping. PFGE analysis revealed that isolates from yoghurts produced by different manufacturers share the same PFGE profiles. All isolates were susceptible to penicillin and ampicillin. Five isolates were either resistant to vancomycin and gentamicin or resistant to both. One isolate of *S. thermophilus* was resistant to gentamicin, clindamycin and erythromycin. It is necessary for the Chinese government to speed up formulating the integrated regulations for LAB safety evaluation.

Lactobacillus species and *Streptococcus thermophilus* belong to LAB and are extensively used in food industries for many years. Some of them can favorably improve the balance of intestinal flora in humans and animals by increasing the number of beneficial bacteria, inhibiting the growth of various enteric foodborne pathogens, increasing the total amount of volatile fatty acids in the gastrointestinal environment, activating the immune response or anti-mutagenic as well as anti-carcinogenic activities[1-5]. Many of these bacteria have been given the so-called generally regarded as safe (GRAS) status by Food and Drug Administration of the United States, and are considered to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment by the European Food Safety Authority[6-7]. Microorganisms with GRAS or QPS status are food-grade organisms without imposing a health risk for consumers and environment. However, it was reported that antimicrobia-resistant genes are expressed in food-associated LAB[8-11]. The antimicrobia-resistant traits can potentially be transferred to the human or animal commensal flora and to pathogenic bacteria temporarily residing in the hosts, when located on mobile genetic elements such as plasmids transposons. Hence, it is very important to verify whether daily consumed LAB strains are resistant to antibiotics.

It is crucial to identify LAB at the species level correctly and maintain the number of live microorganisms in the end product at the level higher than 10^6 CFU/g (mL) within a shelf-life, according to the Chinese regulatory requirement. Traditional phenotypic identification of LAB based mainly on morphological cell characteristics and biochemical profiles are still widely applied on a routine basis, although it is extremely labor intensive and time consuming. Additionally, as many LAB have similar nutritional and growth requirements, it is often difficult to use conventional microbiological methods to differentiate them correctly even to genus level. Research has focused on the application of molecular biology approaches that allows the visualization of the predominant genetic diversity for the rapid detection and differentiation of these microorganisms. It is the trend that phenotypic properties in combination with the full 16S rRNA gene sequencing which compare the sequences with

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those in databases can unambiguously identify LAB at the species level. On the other hand, strain-specific detection based on pulsed field gel electrophoresis (PFGE) is strongly recommended by the World Health Organization and Food and Agriculture Organization[12]. In this study, the LAB including *Lactobacillus* species and *Streptococcus thermophilus* from retail yoghurt in Beijing were enumerated, the isolates were characterize phenotypically and genetically, and to evaluate the antimicrobial susceptibility profiles of the isolates were assessed in order to provide the scientific base for risk assessment and policy-making.

Viability, Enumeration and Phenotypic Characterization of LAB from Commercialized Yoghurt

Thirty-one yoghurt samples produced by 14 domestic manufacturers were purchased from 3 supermarkets in Beijing, China. Detailed information on the manufacturers and LAB composition labeled on sample packagings was listed in Table 1. A test portion of 25 mL (g) yoghurt was suspended in 225 mL phosphate buffer solution (PBS) and a series of decimal dilutions were prepared. Three appropriate dilutions were inoculated onto De Man, Rogosa, Sharpe (MRS, Becton Dickinson Company, USA) agar plates and incubated for 48 h at 37 °C in anaerobic jars (BioMerieux, Inc. France). The viability of both *Lactobacillus* and *S. thermophilus* was enumerated

Table 1. Information on LAB Composition

Samples	LAB Composition Labeled	Manufacturers
1	*S. thermophilus, L. bulgaricus*	MF1
2	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 2
3	*S. thermophilus, L. bulgaricus*	MF 2
4	*S. thermophilus, L. acidophilus, Bifidobacterium* spp.	MF 3
5	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 4
6	*S. thermophilus, L. bulgaricus*	MF 5
7	*S. thermophilus, L. bulgaricus*	MF 5
8	*S. thermophilus, L. bulgaricus*	MF 5
9	*S. thermophilus, L. bulgaricus*	MF 5
10	*S. thermophilus, L. bulgaricus*	MF 6
11	*S. thermophilus, L. bulgaricus*	MF 6
12	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. casei*	MF 7
13	*S. thermophilus, L. bulgaricus, L. casei*	MF 8
14	*S. thermophilus, L. bulgaricus*	MF 9
15	*S. thermophilus, L. bulgaricus*	MF 9
16	*S. thermophilus, L. bulgaricus*	MF 9
17	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. casei*	MF 10
18	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. casei*	MF 10
19	*S. thermophilus, L. bulgaricus*	MF 3
20	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 3
21	*S. thermophilus, L. bulgaricus*	MF 6
22	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 6
23	lactic acid bacteria	MF 11
24	*L. casei* subsp *casei*	MF 12
25	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 13
26	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 13
27	*S. thermophilus, L. bulgaricus*	MF 6
28	*S. thermophilus, L. bulgaricus*	MF 6
29	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 3
30	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. acidophilus*	MF 14
31	*S. thermophilus, L. bulgaricus, Bifidobacterium* spp., *L. casei*	MF 10

by examining the duplicate MRS plates after a 2 d anaerobic incubation and further identified at the genus level according to their Gram-staining, colony morphology, catalase test and biochemical tests (API System, Biomerieux Company, France). Additionally, reference cultures including *L. casei* subsp *casei* 1.2435, *L. delbrueckii* subsp *bulgaricus* 1.2161, *L. plantanum* 1.2158, *L. acidophilus* 1.2686, and *Bifidobacterium adolescentis* 1.2190 were used. All reference strains were purchased from National Institutes for Food and Drug Control and kept at -80 °C before use.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentration (MIC) of 6 antimicrobials was measured via broth micro-dilution methods according to the interpretive standards of Clinical and Laboratory Standards Institute (CLSI, Table 2)[13]. The antimicrobials including penicillin (0.25-32 mg/L), gentamicin (0.25-128 mg/L), ampicillin (0.03-32 mg/L), vancomycin (0.125-256 mg/L), clindamycin (0.00755-32 mg/L) and erythromycin (0.015-128 mg/L). *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were employed as the quality control in antimicrobial susceptibility test. All antimicrobials and reference cultures were obtained from National Institutes for Food and Drug Control.

16S rRNA Gene Amplification and Sequencing of Lactobacillus Species and S. thermophilus

Frozen isolates were revived from the glycerol stock by inoculating into MRS broth and incubated at 37 °C for 24 h in an anaerobic jar. An aliquot of 1.5 mL broth was centrifuged at 3000 g/min for 10 min and the genomic DNA was extracted from the cell pellets using a bacterial genomic DNA extraction kit (TakaRa Biochemicals Inc., Shiga, Japan) according to its manufacturer's instructions. The concentration of DNA was measured with a Quant-It^{Tm} dsDNA HS assay kit (Invitrogen, Carlsbad, California, USA) and stored at -20 °C before use.

All suspected colonies of *Lactobacillus* and *S. thermophilus* isolated from yoghurt samples were further characterized by 16S rRNA gene sequencing to verify the phenotypic identification. The DNA sample was amplified in 0.5 mL micro-centrifuge tubes with 50 μL reaction mixture: containing 5 μL of 10×PCR buffer, 4 μL of 25 mmol/L $MgCl_2$, 0.3 μL of Taq DNA polymerase (5 U/μL, TaKaRa, Japan), forward primer (20-mer, 5'-AGAGTTTGATCCTGGCT CAG-3'), reverse primer (21-mer, 5'-ACGGCTACCTT GTTACGACTT-3')[14], 2 μL of DNA template for 30 cycles at 95 °C for 3 min, 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, and a final chain elongation for 4 min following the Taq DNA polymerase manufacturer's instructions. The amplicons were separated by gel electrophoresis on 1.0% agarose gels (Xinjingke Biotech Company Limited, Beijing, China) and stained with ethidium bromide (Sigma Co., St. Louis, Mo). The rest of PCR products were purified on EZ-10 spin column (TaKaRa, Japan) and reserved for cloning.

The purified PCR products of *Lactobacillus* species and *S. thermophilus* isolates were cloned into the pUC18-derived T vector (EcoRV digestion and T-tailing with Taq DNA polymerase) with T4 DNA ligase (TaKaRa, Japan) and transformed into *E.coli* JM109 for amplification. The cells were spread onto Muller Hinton agar plates containing ampicillin (100 μg/mL), IPTG (24 mg/mL), X-Gal (20 mg/mL) and incubated in the dark for 24 h at 36±1 °C. White colonies were picked up and spread onto the Muller Hinton agar plates containing ampicillin (100 μg/mL) and incubated for another 24 h at 36±1 °C. Bulk DNA from the recombinant *Lactobacillus* species and *S. thermophilus* was extracted, amplified and sequenced. The sequences for the entire cloned PCR products were analyzed using the BLAST network service of the National Center for Biotechnology Information. Each gene was identified by comparison with those of sequences in the GenBank (http://rdp.cme.msu.edu/).

Table 2. CLSI Criteria for Determination of Antimicrobial Resistance

Antibiotics	*Enterococcus Faecalis* ATCC 29212 (mg/L)	*E. coli* ATCC 25922 (mg/L)	Criteria for MIC (mg/L)		
			S	I	R
Penicillin	2	-	≤8	-	-
Gentamicin	-	1	≤4	8	≥16
Ampicillin	1	-	≤8	-	-
Vancomycin	4	-	≤4	8-16	≥32
Clindamycin	8	-	≤0.5	1-2	≥4
Erythromycin	2	-	≤0.5	1-4	≥8

Pulsed Field Gel Electrophoresis (PFGE)

PFGE was performed on the CHEF-Mapper system (BIO-RAD, USA) in accordance with the standardized protocol to determine the genetic relationships among the isolates of *Lactobacillus* species and *S. thermophilus* identified by both phenotyping and 16S rRNA PCR sequencing. Briefly, all isolates were anaerobically incubated in MRS broth at 37 °C for 16-20 h. The genomic DNA of *Lactobacillus* species and *S. thermophilus* were digested with restriction endonuclease of either Not I or Apa I, respectively and separated on 1% SeaKem gold agarose (Cambrex bio Science Rockland, USA)[15]. The PFGE patterns were interpreted with BioNumerics software (Applied Maths, St-Martens-Latern, Belgium) using the dice similarity coefficient. Dendrograms were constructed on the basis of the un-weighted pair group method of averages, with a position tolerance of 1%. Clusters were defined as DNA patterns sharing a similarity ≥85%. The reference strain employed was *Salmonella* H9812 and the positive controls were *L. casei* subsp. *casei* 1.2435, *L. bulgaricus* 1.2161, *L. plantanum* 1.2158, and *L. acidophilus* 1.2686.

Analysis

A total of 52 isolates were obtained. The microscopy revealed a cellular rod form in 37 strains and a cellular ball form in 15 strains. All isolates were found to be Gram-positive and catalase-negative. Biochemical profile from each isolate was compared with those from the reference strains. The isolates could be classified as *L. bulgaricus* (*n*=24), *L. acidophilus* (*n*=7), *L. paracasei* (*n*=3), *L. delbrueckii* (*n*=2), *L. fermentum* (*n*=1), and *S. thermophilus* (*n*=15). Six yoghurt samples containing *L. fermentum*, *L. bulgaricus*, *L. delbrueckii,* or *L. acidophilus* had no description on the labels of the yoghurt products.

The total viable cells of *Lactobacillus* species and *S. thermophilus* in yoghurt samples varied from 1×10^4 CFU/mL(g) to 1.5×10^8 CFU/mL(g). Of the 31 samples examined, 19 (61.3%) had a viable cell concentration higher than 10^6 CFU/mL(g), a Chinese regulatory minimum viable number of LAB in the final products at the end of the shelf-life, 12 (38.7%) had a viable cell concentration below 10^6 CFU/mL(g) and did not meet the requirement of the Chinese regulation.

The antimicrobial resistance profiles differed among the isolates and antimicrobials. Six different antimicrobial resistance types were detected in the 52 LAB isolates (Table 3). Penicillin and ampicillin exhibited comparable antimicrobial activities against all isolates examined. All except one isolate of *L. bulgaricus* (2.7%, 1/37) and two isolates of *S. thermophilus* (13.3%, 2/15) were susceptible to gentamicin with a MIC ranging from 0.25 mg/L to 16 mg/L for *Lactobacillus* and 1 mg/L to 32 mg/L for *S. thermophilus*. All isolate of *Lactobacillus* species and *S. thermophilus* tested were more susceptible to vancomycin, apart from 3 isolates of *L.paracasei/ casei* (8.1%, 3/37) and 1 isolate of *L. fermentum* (2.7%, 1/37) which exhibited resistance to vancomycin with a MIC higher than 256 mg/L. Additionally, most *Lactobacillus* isolates displayed a MIC at the concentration ≤0.5 mg/L but all 7 *L. acidophilus* isolates displayed intermediate resistance to clindamycin with a MIC of 1-2 mg/L. In contrast, 1 isolate of *S. thermophilus* showed a high resistance to clindamycin with a MIC of 32 mg/L. Only 1 isolate of *S. thermophilus* was more resistant to erythromycin. It should point out that 1 (6.7%, 1/15) isolate of *S. thermophilus* showed multidrug resistant profile to gentamicin, clindamycin and erythromycin with a MIC higher than 32 mg/L, 32 mg/L, and 128 mg/L, respectively.

All isolates were subjected to PCR analysis in order to confirm the phenotypic identification results. Of the 52 isolates, 50 were successfully cloned in PCR, and 7 species were identified, including 24 isolates of *L. bulgaricus*, 12 isolates of *S. thermophilus*, 7 isolates of *L. acidophilus*, 3 isolates of *L. paracasei/casei*, 2 isolates of *L. delbrueckii*, 1 isolate of *L. fermentum*, and 1 isolate of *S. lutetiensis*. All phenotypic identification results matched with those by PCR, except 4 isolates which were initially identified as *S. thermophilus* and *L. paracasei* by their morphological and biochemical properties but were characterized as *S. lutetiensis* and *L. paracasei/casei* by PCR. The relationship of 16S rRNA gene sequences between the representative isolates and related type strains was analyzed by phylogenetic tree analysis. The genetic distances of various *Lactobacillus* species and *S. thermophilus* from yoghurt are shown in Figure 1.

It can be seen in Figure 1 that there are 10 distinct divisions among *Lactobacillus* species and *S. thermophilus*. One isolate of *L. bulgaricus* and 7 isolates of *L. acidophilus* were grouped into cluster I, revealed that this *L. bulgaricus* isolate was closely related to *L. acidophilus*. Three isolates of *L.paracasei/casei* fell into cluster IV. *L. delbrueckii* (2 isolates) and *L. bulgaricus* (12 isolates) were very closely related and belong to cluster VI, because *L.*

delbrueckii subsp. *bulgaricus* was known as *L. bulgaricus* before 1984. No homology was observed in 24 isolates of *L. bulgaricus*, which were grouped into four different clusters including cluster I (1 isolate), cluster VI (12 isolates), cluster IX (1 isolate), and cluster X (10 isolates), respectively. The same results were found in 13 isolates of *S. thermophilus* which were grouped into cluster III, cluster V, cluster VII? and cluster VIII. One isolate was identified as *S. thermophilus* by API system but classified as *S. lutetiensis* by 16S rRNA sequencing, which is consistent with those in a previous study[16].

Table 3. MIC in *Lactobacillus* Species and *S. thermophilus*

Antimicrobials	Species (No. of Isolates tested)	MIC Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Penicillin	*L. bulgaricus* (24)	≤0.25	0.25	0.25
	L. acidophilus (7)	≤0.25	0.25	0.25
	L. paracasei (3)	≤0.25	0.25	0.25
	L. delbrueckii (2)	≤0.25	0.25	0.25
	L. fermentum (1)	≤0.25	0.25	0.25
	S. thermophilus (15)	≤0.25-0.5	0.25	0.25
Gentamicin	*L. bulgaricus* (24)	≤0.25-16	2.25	7
	L. acidophilus (7)	4-8	3.4	4.1
	L. paracasei (3)	8	8	8
	L. delbrueckii (2)	4-8	4	5
	L. fermentum (1)	4	4	4
	S. thermophilus (15)	1-32	2.9	8.5
Ampicillin	*L. bulgaricus* (24)	<0.03-0.125	0.06	0.125
	L. acidophilus (7)	0.25-0.5	0.5	0.5
	L. paracasei (3)	0.5-1	0.5	0.9
	L. delbrueckii (2)	0.06-0.125	0.0925	-
	L. fermentum (1)	0.125	0.125	0.125
	S. thermophilus (15)	0.06-0.5	0.06	0.275
Vancomycin	*L. bulgaricus* (24)	1	1	1
	L. acidophilus (7)	1-2	1	2
	L. paracasei (3)	>256	>256	>256
	L. delbrueckii (2)	1-2	1.5	-
	L. fermentum (1)	>256	>256	>256
	S. thermophilus (15)	1-2	1	2
Clindamycin	*L. bulgaricus* (24)	0.015-0.06	0.045	0.06
	L. acidophilus (7)	1-2	2	2
	L. paracasei (3)	<0.0075-0.03	0.03	0.03
	L. delbrueckii (2)	0.125	0.125	-
	L. fermentum (1)	0.03	0.03	0.03
	S. thermophilus (15)	<0.0075->32	0.03	0.324
Erythromycin	*L. bulgaricus* (24)	0.03-0.06	0.06	0.06
	L. acidophilus (7)	0.125-0.25	0.25	0.25
	L. paracasei (3)	0.03-0.25	0.25	0.25
	L. delbrueckii (2)	0.125-0.25	0.188	-
	L. fermentum (1)	0.125	0.125	0.125
	S. thermophilus (15)	0.06->128	0.125	0.25

Stable and reproducible PFGE profiles for the 52 isolates are shown in Figures 2 and 3. It was found that isolates from the same brand of yoghurts had different PFGE profiles. Twenty-three PFGE clusters or distinct profiles were identified. Of the 52 isolates, 24 isolates of *L. bulgaricus* were identified as clusters A-H, 15 isolates of *S. thermophilus* were identified as clusters N-O and Q-W, 7 isolates of *L. acidophilus* were identified as cluster K-M, 2 isolates of *L. delbrueckii* belonged to 2 different PFGE profiles,

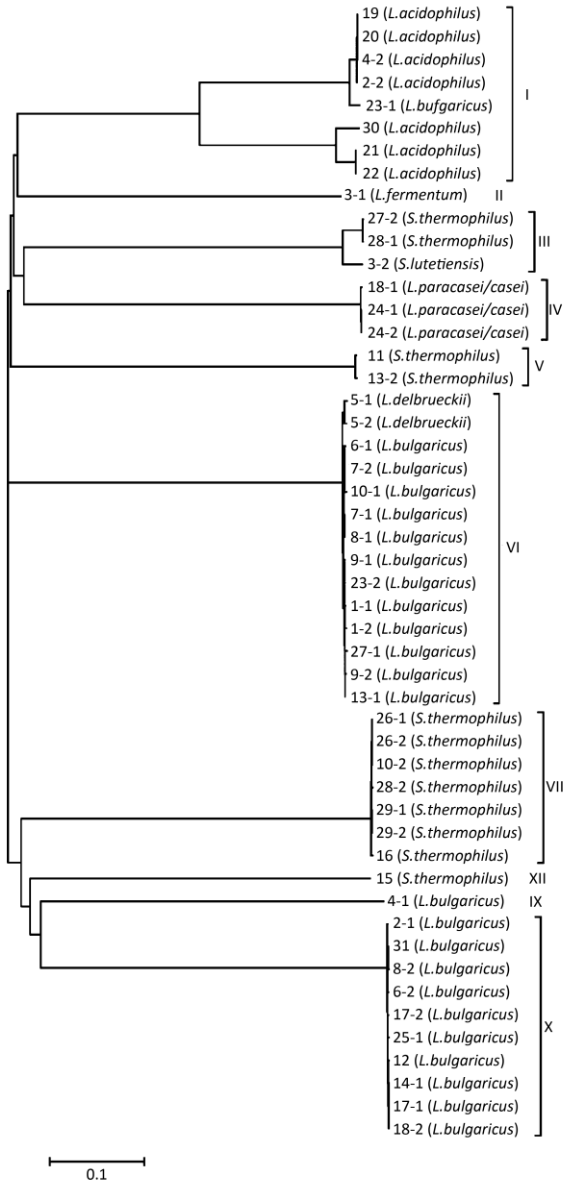

Figure 1. Phylogenetic tree of 16S rRNA gene sequences obtained from 50 isolates of *Lacto-bacillus* and *S. thermophilus* from yoghurt.

and 1 isolates of *L. fermentum* was identified as cluster I, indicating that the characteristics of the isolates used by the same manufacturer may not be the same at the strain level.

On the other hand, different yoghurt manufacturers may use the same species of *Lactobacillus* or *S. thermophilus* at the strain level. For instance, *L. bulgaricus*, *S. thermophilus,* and *L. acidophilus* isolated from yoghurts belong to the same PFGE cluster, suggesting that different yoghurt manufacturers may purchase the strains from the same supplier. Dendrogram patterns of the 52 isolates of *Lactobacillus* and *S. thermophilus* and the antimicrobial susceptibility are shown in Figures 2 and 3.

It was observed that 3 isolates of *L. paracasei/casei* resistant to vancomycin isolated from yoghurt samples produced by 2 different manufactures shared the same PFGE profiles. One *S. thermophilus* isolate resistant to gentamycin, clindamycin and erythromycin was identified as PFGE cluster W, one *L. bulgaricus* isolate resistant to gentamycin was identified as PFGE pattern A, and the unique isolate of *L. fermentum* resistant to gentamycin and vancomycin was identified as PFGE pattern I.

Traditional methodologies for identification and characterization of *Lactobacillus* and *S. thermophilus* mainly rely on their biochemical and physiological properties[17-18] while the identification of their isolates by DNA-DNA hybridization sometimes depends on reference strains as a standard[19]. These methods have limitations since many of the reference strains were characterized by biochemical tests, which may not be reliable. Besides, biochemical, physiological and DNA hybridization can not establish the phylogenetic distances between different *Lactobacillus* and *S. thermophilus*. In the present study, the phenotypes of *Lactobacillus* and *S. thermophilus* isolates were identified and confirmed by genotyping. Identification of *Lactobacillus* and *S. thermophilus* at the species level obtained by phenotypic analysis was highly matched with that by genotyping. Three isolates initially identified as *L. paracasei* based on their morphological and biochemical properties were characterized as *L. paracasei/casei* by PCR. Klein et al.[20] reported that since *L. casei* and *L. paracasei* can not easily be differentiated biochemically, the taxonomy and nomenclatural status of the *L. casei* group species still remain controversial.

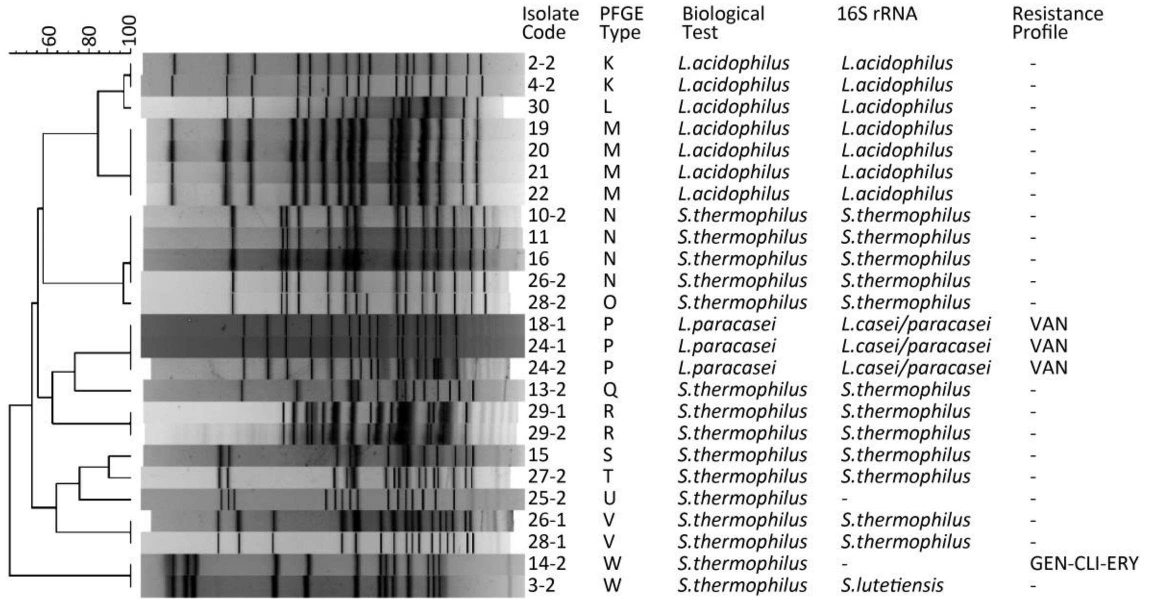

Figure 2. Dendrogram of PFGE patterns of 25 *Lactobacillus* and *S. thermophilus* isolates form yoghurt digested with Apa I restriction endonuclease.

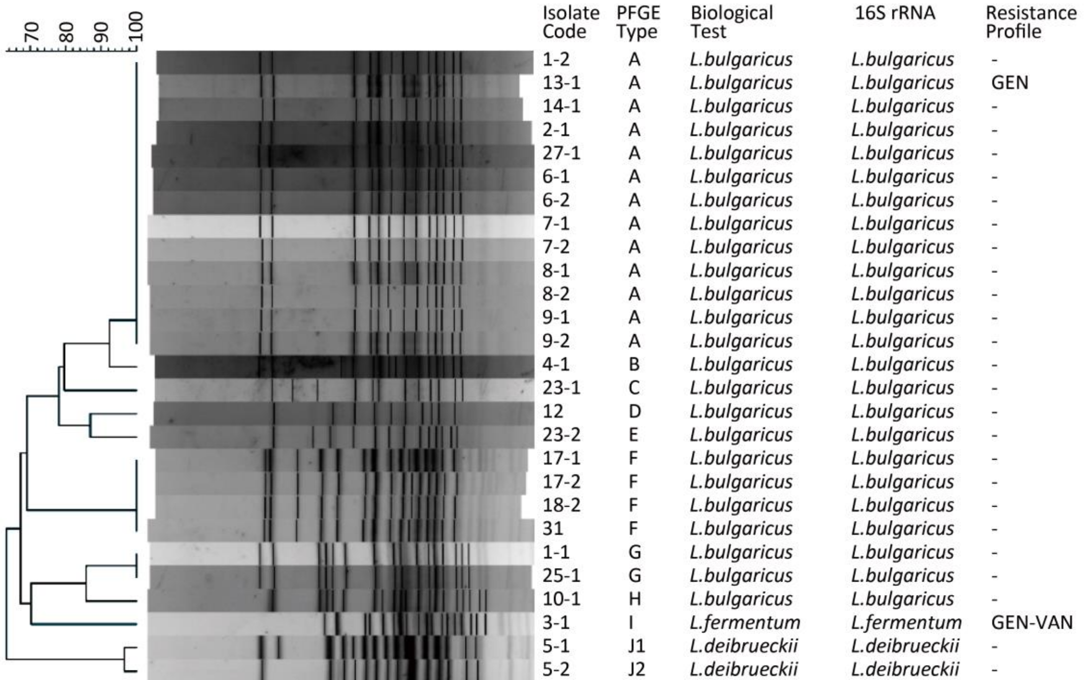

Figure 3. Dendrogram of PFGE patterns of 27 *Lactobacillus* isolates from yoghurt digested with Not I restriction endonuclease.

One iaolate of *L. fermentum* exhibited its resistance to vancomycin, which is consistent with that reported in a previous study[10]. In addition, a species-specific pattern of drug-resistance was detected in 3 *L. paracasei/casei* strains, which were resistant to vancomycin with the a MIC higher than 256 mg/L. It is well known that vancomycin is recommended for the treatment of methicillin-resistant *Staphylococcus aureus* infection in hospitals, especially in specialties such as haematology, transplantation and renal medicine. When the treatment with beta-lactam antibiotics or other anti-bacterial drugs fails, vancomycin is considered to be the last choice in clinical practice[11,21-22]. Resistance to vancomycin reduces the chances for antibiotic treatment where clinical infection is evident, suggesting that government should pay more attention not only to the quality control of LAB employed in yoghurt production but also to the detection of antimicrobial susceptibility of LAB. On the other hand, it is the manufacturers' responsibility to guarantee that any given LAB strain is not a significant risk with regard to transferable antibiotic resistance or other opportunistic virulence properties. The above findings can be used for a thorough strain-specific safety evaluation by the producers.

PFGE is a very powerful method for strain typing and frequently used in epidemiological studies[23]. Most strains of *Lactobacillus* and *S. thermophilus* used for yoghurt production are provided by the designated supplier and rarely developed by the manufacturers themselves. Therefore, some species of *Lactobacillus* and *S. thermophilus* isolated from yoghurt produced by different producers share the same PFGE profiles. However, isolates classified by PFGE are sometimes different from those classified in the same manner by PCR, except for most of the isolates exhibiting unique patterns. Besides, isolates cultured from the same brand yoghurts have different PFGE profiles, which may be due to the repetitious subculture resulting in the occurrence of genetic variation during the long-term use or spurious matches. In theory, a restriction enzyme may cleave two non-homologous genomes to yield similar fragment sizes, thus producing spurious matches. Minor genetic events such as a single-nucleotide mutation can produce the same banding change as a major genetic event.

According to FAO/WHO, the genus, species and strain designation used or supplemented during the food production, minimum viable number of LAB strains at the end of the shelf-life should be described on the label[12]. A couple of yoghurt samples containing *L. fermentum*, *L. bulgaricus*, *L. delbrueckii* and *L. acidophilus* analyzed in the present study were not well described on the label. Since China has no official molecular methods for LAB identification, characterization and safety assessment, the Chinese government should speed up the development of integrated regulations for safety evaluation of LAB in food for the benefit of consumers.

Contribution and Conlict Interest Statement

All authors contributed to the conception, acquisition, analysis and interpretation of data, design and revision of the manuscript and have no conflict of interest to declare.

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