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ORIGINAL ARTICLE

PFGE genotyping and antibiotic resistance of *Lactobacillus* distributed strains in the fermented dairy products

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Abstract The aim of this study was to analyze the antibiotic resistance and pulsed-field gel electrophoresis (PFGE) genotypes of *Lactobacillus* strains present in fermented dairy products available on the Chinese market and to analyze the correlation between these. A total of 33 *Lactobacillus* strains identified in fermented dairy products were tested for resistance to 16 antibiotics using the broth microdilution method and also analyzed by PFGE. Almost all of the strains were multidrug resistant and showed high resistance rates to fosfomycin (97.0%), chloramphenicol (87.9%), vancomycin (87.9%), ceftriaxone (81.8%), imipenem (66.7%), trimethoprim/sulfamethoxazone (54.6%), tetracycline (33.3%), cefotaxime (21.2%), gentamicin (18.2%), erythromycin (12.1%), gatifloxacin (12.1%), amoxicillin/clavulanic acid (12.1%), rifampin (9.1%), and clindamycin (9.1%). Based on the PFGE results, the 33 strains were subtyped into 17 pulsotypes (PFPs, pulsed-field profiles), of which six were represented by more than one strain each. In conclusion, the *Lactobacillus* strains identified here in fermented dairy products available on the Chinese market showed a high rate of antibiotic resistance. *Lactobacillus* strains were genotyped well by PFGE; the resistance patterns of the strains among the same PFP were not necessarily similar to each other.

Keywords Antibiotic resistance · PFGE · Fermented dairy products · *Lactobacillus* strains

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Introduction

The term probiotic, meaning "for life," was derived from the Greek language. It was first used by Lilly and Stillwell to describe substances produced by one microorganism which stimulate the growth of another (Lilly and Stillwell 1965). Fuller then redefined the word as a live microbial feed supplement which beneficially affects host animals by improving its intestinal balance (Fuller 1989), while Schaafsma (1996) later defined the word as live microorganisms, which when consumed in adequate amounts, confers a health effect on the host. The Joint FAO/WHO Expert Consultation in 2001 redefined probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host (Food and Agriculture Organization/World Health Organization 2001); this has become the most widely accepted definition.

Most of the probiotic microorganisms identified to date belong to the genera *Lactobacillus* and *Bifidobacterium* (Prasad et al. 1998). However, species which belong to the genera *Enterococcus*, *Lactococcus*, *Saccharomyces*, and *Propionibacterium* are also considered to be probiotic

bacteria (Grant and Salminen 1998; Sanders and Veld 1999).

Many lactic acid bacteria produce antibacterial substances, such as organic acids, hydrogen peroxide, carbon dioxide, and bacteriocins (Klaenhammer 1988). The production of these molecules makes the application of probiotics more valuable.

Antibiotic resistance among probiotic strains is common, so there are always potential health risks associated with using probiotic strains in foods. It is both inevitable and necessary to strengthen the work being carried out on antibiotic resistance in probiotic strains.

Lactobacillus and *Bifidobacterium* are widely used in foods, such as yoghurt and cheese, health foods, dietary supplements, such as edible priobiotic powder, and drugs that are available on the Chinese market. Although traditional probiotics has a long history of safe use, the continual introduction of new strains into food products is highlighting the question of the safety of probiotics, both domestically and internationally, and making it the focus of scientific research.

Genotypic methods used for strain typing are typically PCR methods [e.g., randomly amplified polymorphic DNA (RAPD) analysis] or variations of restriction enzyme analysis [e.g., pulsed-field gel electrophoresis (PFGE) and ribotyping]. Of these methods, PFGE has been shown to be highly effective in epidemiological studies (Hudson et al. 2001; Murase et al. 1995; Seo et al. 2006). A number of researchers have suggested that this genotyping method could provide a reproducible subdivision of *Lactobacillus* and that it is in fact the most discriminatory method (Björkroth et al. 1996; Tynkkynen et al. 1999; Vandamme et al. 1996).

In the study reported here, the strains were genotyped by PFGE to determine their electrophoretic karyotype. The antibiotic resistance testing of the *Lactobacillus* strains identified in fermented dairy products on the Chinese market was performed using the broth microdilution method; then the relationship between the resistance and genomic DNA were analyzed.

Materials and methods

Microorganisms

A total of 33 *Lactobacillus* strains distributed throughout commercially available dairy products were isolated by the Laboratory of Foodborne Disease Surveillance, Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. Strain identification was facilitated using API 50 CHL identification kits (bioMérieux SA, Marcy-l'Etoile, France) (Table 1). Resistance QC (Quality Control) strains were ATCC 49619 and ATCC 25922, which were also obtained from the Chinese Center of Disease Control.

Antibiotic resistance test

The resistance test was performed using the broth microdilution method with Cation-adjusted Mueller–Hinton broth (MH) supplemented with lysed horse blood (2.5–5%, v/v) after incubation at 37°C for 48 h, according to the guidelines of the CLSI (Clinical and Laboratory Standards Institute 2007) and the instruction to the 96-well microplate. The results were determined by reference to NCCLS (National Committee for Clinical Laboratory Standards 2007) and classified as susceptible (S), intermediate susceptible (I), and resistant (R). Multidrug resistance (MDR) was defined as resistance to three or more groups of antimicrobial agents (Clinical and Laboratory Standards Institute 2006). A total of 16 antibiotics were tested (Table 1).

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis was performed as described previously by the Pulse-Net protocol (PulseNet protocol, section 5.3 2009). DNA was digested with 30 U of restriction enzyme *Asc*I (New England Biolabs, Ipswich, MA) at 37°C. The restriction fragments were separated by electrophoresis in 0.5× TBE buffer for 22 h at 14°C in a CHEF Mapper system (Bio-Rad, Hercules, CA) using pulsed times of 1–15 s. *Xba*I-digested *Salmonella* Braenderup H9812 was used as the DNA size marker. PFGE data were analyzed using GelCompar software (ver. 4.0; Applied Maths, Sint-Martens-Latem, Belgium). The extent of variability was determined by the Dice coefficient F, as previously described (Thong et al. 2003). Clustering was based on the unweighted pair group average method (UPGMA) with a position tolerance of 0.10.

Results

Antibiograms

The antibiotic resistance patterns of the different antibiotics tested on the *Lactobacillus* strains are shown in Tables 1 and Table 2.

Lactobacillus strains were almost resistant to a total of 16 antimicrobial agents to varying degrees, and they were resistant to two and more antibiotics. Of the 33 strains tested, 31 were multiple antibiotic resistance strains. All of the strains were resistant to fosfomycin, with the exception of strain 31. The resistance rates which were over 50% were: fosfomycin (97.0%), chloramphenicol (87.9%), van-

Table 1 Antimicrobial resistance of the 33 isolated *Lactobacillus* strains to 16 antibiotics

Strain no.	Strain identity	Antibiotics[a]															
		AMP (0.03–32)	PEN (0.03–32l)	IPM (0.002–2l)	GEN (0.06–64)	VAN (0.25–256)	ERY (0.03–32)	CLI (0.015–16)	SXT (0.0–32)	AMC (0.03–32)	GAT (0.015–16)	CHL (0.015–16)	TET (0.06–64)	FOS (0.2–256)	CRO (0.125–128)	CTX (0.125–128)	RIF (0.015–16)
02	*Lactobacillus rhamnosus*	S	S	I	S	R	R	S	I	S	S	R	R	R	R	S	S
03	*L. rhamnosus*	S	S	R	S	R	S	I	R	S	S	S	S	R	R	I	S
04	*L. rhamnosus*	S	S	R	S	R	S	S	R	R	R	R	S	R	R	S	S
07	*L. rhamnosus*	S	S	R	S	S	S	S	R	S	S	R	S	R	R	S	S
09	*L. rhamnosus*	S	S	R	S	R	S	I	R	R	S	R	S	R	R	R	S
11	*L. rhamnosus*	S	S	R	R	R	S	I	R	S	S	R	R	R	R	R	S
13	*L. rhamnosus*	S	S	R	S	R	R	R	R	S	S	R	S	R	R	I	S
16	*L. rhamnosus*	S	S	R	I	R	S	R	S	S	S	R	S	R	R	R	S
17	*L. rhamnosus*	S	S	R	S	R	S	S	R	R	R	R	R	R	R	S	S
18	*L. rhamnosus*	S	S	R	R	R	I	I	R	S	S	R	S	R	R	I	S
20	*L. rhamnosus*	S	S	R	S	R	S	I	I	S	S	R	R	R	R	I	S
22	*L. rhamnosus*	S	S	S	R	R	I	I	R	I	S	R	R	R	R	R	S
26	*L. rhamnosus*	S	S	R	I	R	I	I	R	S	S	R	S	R	R	I	S
27	*L. rhamnosus*	S	S	S	I	R	S	I	S	I	S	R	R	R	R	R	S
05	*L. plantarum*	S	S	S	S	S	S	I	S	S	S	S	S	R	R	S	S
30	*L. plantarum*	S	S	S	S	R	S	S	S	S	R	R	R	R	S	S	R
32	*L. plantarum*	S	S	S	S	R	S	S	R	R	R	R	R	R	S	S	R
33	*L. plantarum*	S	S	S	S	R	S	S	S	S	I	R	R	R	S	S	R
15	*L. paracasei* ssp. *paracasei*	S	S	S	R	R	I	S	I	S	S	S	I	R	R	I	S
21	*L. paracasei* ssp. *paracasei*	S	S	R	I	R	I	S	R	S	S	R	S	R	R	I	S
28	*L. paracasei* ssp. *paracasei*	S	S	R	S	R	S	S	S	S	S	R	S	R	I	S	S
01	*L. paracasei* ssp. *paracasei*	S	S	R	S	R	I	S	S	S	S	R	R	R	R	S	S
06	*L. paracasei* ssp. *paracasei*	S	S	R	S	R	S	I	S	S	S	R	S	R	R	S	S
10	*L. paracasei* ssp. *paracasei*	S	S	R	S	R	S	I	R	S	S	R	S	R	R	I	S
14	*L. paracasei* ssp. *paracasei*	S	S	R	S	R	S	S	R	S	S	R	S	R	R	S	S
19	*L. paracasei* ssp. *paracasei*	S	S	R	S	S	S	I	R	S	S	R	S	R	R	R	S
24	*L. paracasei* ssp. *paracasei*	S	S	R	I	R	I	S	R	S	S	R	I	R	R	I	S
25	*L. paracasei* ssp. *paracasei*	S	S	R	I	R	I	I	R	S	S	R	S	R	R	S	S
29	*L. paracasei* ssp. *paracasei*	S	S	R	R	R	R	R	S	S	S	R	S	R	R	S	S
12	*L. fermentum*	S	S	I	S	S	S	S	S	S	S	R	R	R	R	R	S
31	*L. delbrueckii* ssp. *lactis*	S	S	S	R	R	R	S	I	S	S	S	S	I	R	I	S
23	*L. curvatus*	S	S	S	S	R	I	S	S	S	S	R	S	R	S	S	S
08	*L. acidophilus*	S	S	R	S	R	S	S	R	S	S	R	S	R	I	S	S
	R (%)	0	0	66.7	18.2	87.9	12.1	9.1	54.6	12.1	12.1	87.9	33.3	97	81.8	21.2	9.1
	I (%)	0	0	6.1	18.2	0	27.3	39.4	12.1	6.1	3	0	6.1	3	6.1	30.3	0
	S (%)	100	100	27.2	63.6	12.1	60.6	51.5	33.3	81.8	84.9	12.1	60.6	0	12.1	48.5	90.9

Table 1 (continued)

Strain no.	Strain identity	Antibiotics[a]															
		AMP (0.03–32)	PEN (0.03–32)	IPM (0.002–2)	GEN (0.06–64)	VAN (0.25–256)	ERY (0.03–32)	CLI (0.015–16)	SXT (0.0–32)	AMC (0.03–32)	GAT (0.015–16)	CHL (0.015–16)	TET (0.06–64)	FOS (0.2–256)	CRO (0.125–128)	CTX (0.125–128)	RIF (0.015–16)
Resistance quality control strains		0.015–2	0.03–4	0.008–1	0.06–8	0.015–2	0.015–2	0.015–2	0.03–4	0.015–2	0.03–4	0.25–32	0.06–8	2–256	0.015–2	0.015–2	0.008–1
ATCC 49619	*Streptococcus pneumoniae*	S	S	S	\	S	S	S	S	S	S	S	S	S	S	S	S
ATCC 25922	*Escherichia coli*	\	\	\	S	\	\	\	\	\	\	\	\	\	\	\	\

R, Resistant; S, Susceptible; I, Intermediate susceptible; \, No Detection

[a] AMP, Ampicillin; PEN, penicillin; IPM, imipenem; GEN, gentamicin; VAN, vancomycin; ERY, erythromycin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazone; AMC, amoxicillin/clavulanic acid; GAT, gatifloxacin; CHL, chloramphenicol; TET, tetracycline; FOS, fosfomycin; CRO, ceftriaxone; CTX, cefotaxime; RIF, rifampin. The value expressed in parenthesis is the range of the minimum inhibitory concentration (MIC; in μg/ml)) of the antimicrobial compound that was tested

comycin (87.9%), ceftriaxone (81.8%), imipenem (66.7%), and trimethoprim/sulfamethoxazone (54.6%). The others were: tetracycline (33.3%), cefotaxime (21.2%), gentamicin (18.2%), erythromycin (12.1%), gatifloxacin (12.1%), amoxicillin/clavulanic acid (12.1%), rifampin (9.1%), and clindamycin (9.1%). There were 11 antibiotics which were intermediate susceptible. All of the strains were susceptible to ampicillin and penicillin, with sensitivity rates to both of 100%, followed by rifampin, with a sensitive rate of 90.9%. On the whole, all of the strains were resistant to six antibiotics, namely, fosfomycin, chloramphenicol, vancomycin, ceftriaxone, imipenem, and trimethoprim/sulfamethoxazone (Table 1).

Genotyping

The PFGE of *Asc*I-digested chromosomal DNA subtyped all 33 *Lactobacillus* strains into 17 pulsotypes (pulsed-field profiles, PFPs), with DNA fragments ranging from 10–20 kb in size, designated A to Q with similarity 100%. Among these 17 different pulsotypes, two (PFP A, PFP C) were represented by two strains each, and they only contained *L. paracasei* ssp. *paracasei*; two (PFP K, PFP M) were represented by three strains each, and they respectively contained one species. PFP K contained *L. rhamnosus*, and PFP M contained *L. plantarum*. One (PFP F) was represented by four strains, and it contained two species, namely, *L. rhamnosus* and *L. paracasei* ssp. *paracasei*. One (PFP J) was represented by eight strains, and it contained four species, namely, *L. acidophilus*, *L. fermentum*, *L. paracasei* ssp. *paracasei*, and *L. rhamnosus.* The remaining PFGE profiles were unique: PFP D, G, H, and I contained one species, *L. rhamnosus*; PFP E, L, O, and P contained one species, *L. paracasei* ssp. *paracasei.*; PFP B contained *L. curvatus*; PFP N contained *L. plantarum*; PFP Q contained *L. delbrueckii* ssp. *lactis* (Fig. 1; Table 2).

Comparison between antibiotic resistance and PFGE profile

PFP A (strains 01, 21) had similar resistance patterns, and the two strains were susceptible to ampicillin, penicillin, clindamycin, amoxicillin/clavulanic acid, gatifloxacin, and rifampin, resistant to imipenem, vancomycin, chloramphenicol, fosfomycin, and ceftriaxone, and intermediate susceptible to erythromycin. PFP C (strains 24, 25) also had similar resistance patterns, and the two strains were susceptible to ampicillin, penicillin, amoxicillin/clavulanic acid, gatifloxacin, and rifampin, resistant to imipenem, vancomycin, trimethoprim/sulfamethoxazone, chloramphenicol, fosfomycin, and ceftriaxone, and intermediate susceptible to gentamicin and erythromycin. Strains among PFP K (strains 13, 22, 27) were all susceptible to ampicillin, penicillin, gatifloxacin, and rifampin and all resistant to vancomycin, chloramphen-

Table 2 Resistance spectrums and PFGE profiles of the 33 *Lactobacillus* strains tested

Strain no./strain identity	Resistance pattern[a]	PFGE profiles[b]
02 *Lactobacillus rhamnosus*	VAN-ERY-CHL-TET-FOS-CRO	F
06 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-CHL-FOS-CRO	F
03 *Lactobacillus rhamnosus*	IPM-VAN-SXT-FOS-CRO	F
04 *Lactobacillus rhamnosus*	IPM-VAN-SXT-AMC-GAT-CHL-FOS-CRO	F
20 *Lactobacillus rhamnosus*	IPM-VAN-CHL-TET-FOS-CRO	G
01 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-CHL-TET-FOS-CRO	A
21 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-SXT-CHL-FOS-CRO	A
26 *Lactobacillus rhamnosus*	IPM-VAN-SXT-CHL-FOS-CRO	I
24 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-SXT-CHL-FOS-CRO	C
25 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-SXT-CHL-FOS-CRO	C
10 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-SXT-CHL-FOS-CRO	J
14 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-SXT-CHL-FOS-CRO	J
08 *Lactobacillus acidophilus*	IPM-VAN-SXT-CHL-FOS	J
09 *Lactobacillus rhamnosus*	IPM-VAN-SXT-AMC-CHL-FOS-CRO-CTX	J
17 *Lactobacillus rhamnosus*	IPM-VAN-SXT-AMC-GAT-CHL-TET-FOS-CRO	J
11 *Lactobacillus rhamnosus*	IPM-GEN-VAN-SXT-CHL-TET-FOS-CRO-CTX	J
07 *Lactobacillus rhamnosus*	IPM-SXT-CHL-FOS-CRO	J
12 *Lactobacillus fermentum*	CHL-TET-FOS-CRO-CTX	J
19 *Lactobacillus paracasei* ssp. *paracasei*	IPM-SXT-CHL-FOS-CRO-CTX	L
18 *Lactobacillus rhamnosus*	IPM-GEN-VAN-SXT-CHL-FOS-CRO	H
29 *Lactobacillus paracasei* ssp. *paracasei*	IPM-GEN-VAN-ERY-CLI-CHL-FOS-CRO	E
16 *Lactobacillus rhamnosus*	IPM-VAN-CLI-CHL-FOS-CRO-CTX	D
28 *Lactobacillus paracasei* ssp. *paracasei*	IPM-VAN-CHL-FOS	P
13 *Lactobacillus rhamnosus*	IPM-VAN-ERY-CLI-SXT-CHL-FOS-CRO	K
27 *Lactobacillus rhamnosus*	VAN-CHL-TET-FOS-CRO-CTX	K
22 *Lactobacillus rhamnosus*	GEN-VAN-SXT-CHL-TET-FOS-CRO-CTX	K
15 *Lactobacillus paracasei* ssp. *paracasei*	GEN-VAN-FOS-CRO	O
31 *Lactobacillus delbrueckii* ssp. *lactis*	GEN-VAN-ERY-CRO	Q
05 *Lactobacillus plantarum*	FOS-CRO	M
33 *Lactobacillus plantarum*	VAN-CHL-TET-FOS-RIF	M
32 *Lactobacillus plantarum*	VAN-SXT-AMC-GAT-CHL-TET-FOS-RIF	M
30 *Lactobacillus plantarum*	VAN-GAT-CHL-TET-FOS-RIF	N
23 *Lactobacillus curvatus*	VAN-CHL-FOS	B

PFGE, Pulsed field gel electrophoresis

[a] For abbreviations of antibiotics tested, see footnote of Table 1

[b] Pulsotype (pulsed-field profile) code

icol, fosfomycin, and ceftriaxone. Of these, different strains were intermediate susceptible to different antibiotics, and the two strains (22, 27) had very similar resistance patterns. Strains among PFP M (05, 32, 33) were all susceptible to ampicillin, penicillin, imipenem, gentamicin, erythromycin, and cefotaxime and resistant to fosfomycin. Of these, different strains were intermediate susceptible to different antibiotics, and strains 32 and 33 had very similar resistance patterns. Strains among PFP F (02, 03, 04, 06) were all susceptible to ampicillin, penicillin, gentamicin, and rifampin and resistant to vancomycin, fosfomycin, and ceftriaxone. Of these, different strains were intermediate susceptible to different antibiotics, and the three strains (03, 04, 06) had very similar resistance patterns each other. Strains among PFP J (07, 08, 09, 10, 11, 12, 14, 17) were all susceptible to ampicillin, penicillin, and rifampin and resistant to chloramphenicol and fosfomycin. Of these, different strains were intermediate susceptible to different antibiotics, and these strains had very similar resistance patterns to each other. The strains among the other PFPs had different resistance patterns, and they were susceptible to ampicillin, penicillin, amoxicillin/clavulanic acid, gatifloxacin, and rifampin. On the whole, PFP G and one

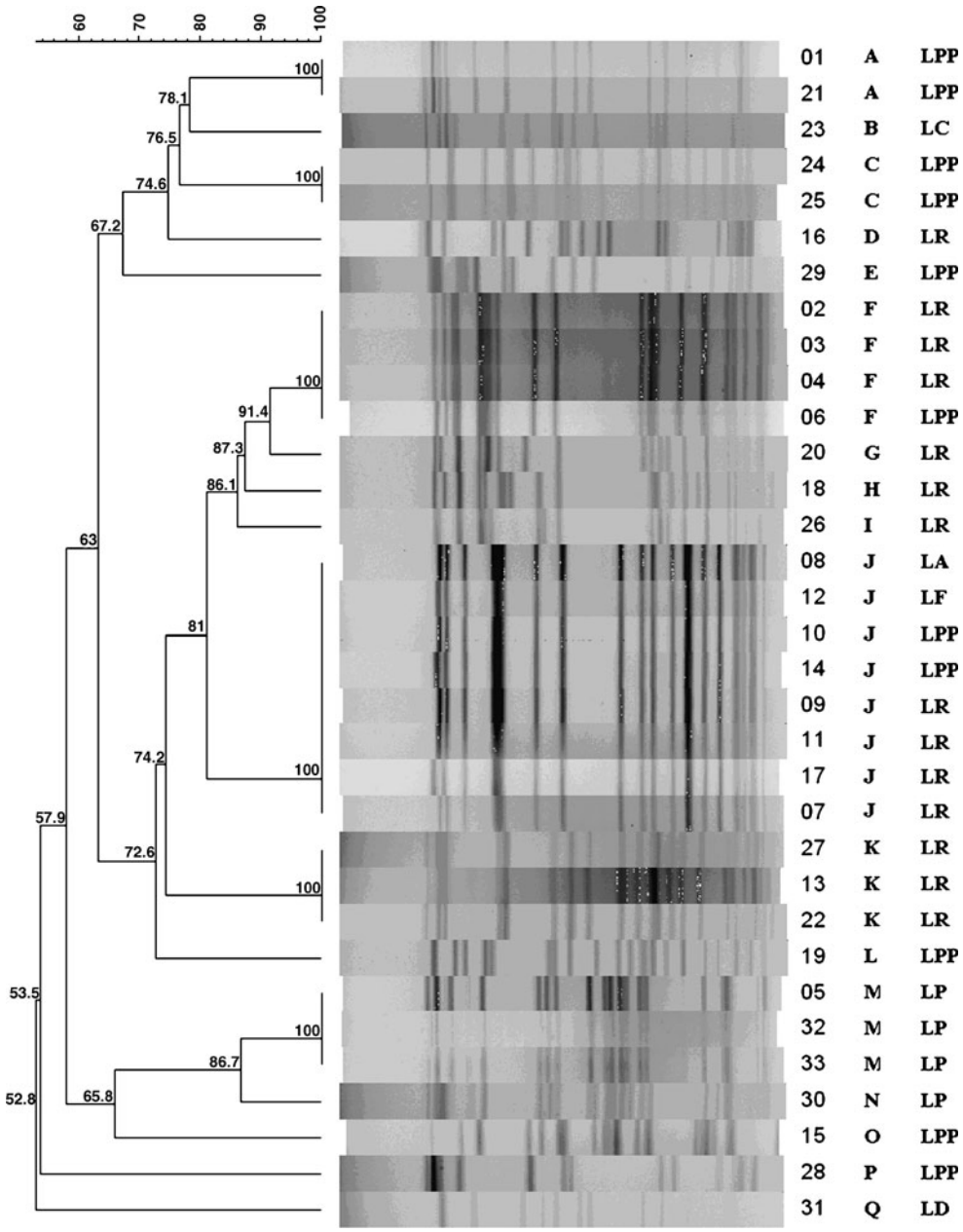

Fig. 1 Dendrogram of cluster analysis of 33 *Lactobacillus* strains generated by GelCompar software using the unweighted pair group arithmetic means. *Numbers* (*01–33*) Strain code, *uppercase letters* (*A–Q*) pulsed field gel electrophoresis. *LA*, *LC*, *LD*, *LF*, *LP*, *LR*, *LPP L. acidophilus*, *L. curvatus*, *L. delbrueckii* ssp. *lactis*, *L. fermentum*, *L. plantarum*, *L. rhamnosus*, *L. paracasei* ssp. *paracasei*, respectively

strain of PFP A (01) displayed the same drug resistance spectrums: IPM-VAN-CHL-TET-FOS-CRO. One strain of PFP A (21), PFP I, PFP C, and two strains of PFP J (10, 14) displayed the same drug resistance spectrums: IPM-VAN-SXT-CHL-FOS-CRO. Similarities can be observed among the drug resistance spectrums of the stains of the other PFPs, while these resistance spectrums were different from each other (Tables 1, 2; Fig. 1).

Discussion

With the addition of probiotics to the fermented foods, an increasing number of problems associated with antibiotic resistance have arisen. The study reported here is the first to compare antibiotic resistance patterns and genotypes of *Lactobacillus* strains found in fermented dairy products in China.

The European Qualified Presumption of Safety (QPS) suggests determining the strain's antibiotic resistance then verifying it at the genetic level, and finally determining whether it can be transferred to intestinal microflora. The Federal Drug Administration (FDA) considers that probiotic fermented food can be considered GRAS (generally recognized as safe) (Saarela et al. 2000). The guidelines of the FAO/WHO (2001) emphasize that experiments on probiotic strains should be performed in both animals and humans on antibiotic resistance patterns.

Most of the *Lactobacillus* strains tested in this study showed a multiple antibiotic resistance pattern. However, the susceptibility and resistance of various *Lactobacillus* strains to many antibiotics are variable and dependent on the species (D' Aimmo et al. 2007; Danielsen and Wind 2003; Mathur and Singh 2005).

Published data demonstrate that *Lactobacillus* is resistant to multiple antibiotics to varying degrees. Temmerman et al. (2003) identified 187 *Lactobacillus* strains as resistant to vancomycin, tetracycline, penicillin, erythromycin, and chloramphenicol, with resistance rates of 65, 26, 23, 16, and 11%, respectively, and reported that 68.4% of the strains these strains were multiple antibiotic resistant strains. The resistance rates of these antibiotics in the present study were 87.9, 33.3, 0, 12.1, and 87.9%, respectively, and more than 90% of the strains were multiple antibiotic resistance strains. Thus, the results of these two studies differ considerably, possibly due to the experimental methodology and the determination standards.

The antibiotic susceptibility profiles of the *Lactobacillus* strains in the present study are similar to those reported by Zhou et al. (2005), who showed that *Lactobacillus* sp. and *Bifidobacterium* sp. are sensitive to erythromycin, tetracycline, and ampicillin. They are also similar to the results of Comunian et al. (2010), who reported that among the 121 isolates of *Lactobacillus paracasei* from Italian fermented products, 77.7% were susceptible to tetracycline and 94.2% were susceptible to erythromycin, 60.6% were susceptible to both tetracycline and erythromycin, and 100% were susceptible to ampicillin. It would therefore appear that the antibiotic resistance of *Lactobacillus* strains is present in different strains, which represents a threat to food safety. Although long-term application of *Lactobacillus* in fermented dairy products has been demonstrated to be safe, and infection caused by such bacteria or pathogenicity has seldom been reported, the safe use of *Lactobacillus* in health products and food should be guided by established criteria, guidelines, and regulations (Liu et al. 2009).

We genotyped the *Lactobacillus* strains by PFGE according to the standardized protocol of PulseNet USA section 5.3. All of the strains showed bands ranging from 10–20 kb. The 33 strains were subtyped into 17 pulsotypes (PFPs), and the PFGE approach was shown to be highly effective. The strains classified to one cluster were basically the same bacteria, except for PFP F and J: PFP F included *L. paracasei* and *L. rhamnosus*, and PFP J included *L. acidophilus*, *L. fermentum*, *L. paracasei*, and *L. rhamnosus.* Strains belonging to different species displayed the same PFGE patterns, which suggests that these bacteria likely descended from a common ancestor. In the course of evolution, different variations were introduced, with these bacteria eventually expressing different phenotypic characteristics and belonging to different species. In several *Lactobacillus* studies, PFGE has been shown to be the most powerful method for strain typing (Ferrero et al. 1996; Roussel et al. 1993; Tynkkynen et al. 1999), which is consistent with our results.

There was no evident correlation between the antibiotic resistance pattern and the PFGE profile. The entire antibiotic resistance pattern of every strain among PFPs A, C, F, J, K, and M was similar to each other, but there was no exact same pattern. Some strains with different PFPs displayed the same drug resistance spectrums. The strains among the same PFP had different antibiotic resistance patterns, likely due to mutations of resistance genes, but the mutation site was not in the position of the restriction enzyme site; consequently, the status of antibiotic resistance of *Lactobacillus* strains is not shown by PFGE genotyping (Ong et al. 2007; Yagüe et al. 2001).

In conclusion, the antibiotic resistance and PFGE genotype were compared among 33 *Lactobacillus* strains isolated from fermented dairy products. High rates of resistance were found among these strains, and MDR patterns were observed. This can raise concerns about their use in foods as their antibiotic resistance genes can be transferred. The strains with same PFPs did not necessarily display the same drug resistance spectrum.

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