PAPER

ANTIBIOTIC SUSCEPTIBILITY OF POTENTIALLY PROBIOTIC *LACTOBACILLUS* STRAINS

JUNHUA HAN[a], DAHUAN CHEN[a], SHANSHAN LI[c], XINGFENG LI[a], WEN-WEN ZHOU[d], BOLIN ZHANG[b,*], YINGMIN JIA[a,*]

[a]College of Biological Science and Engineering, Hebei University of Science and Technology, Shijiazhuang, Heibei, 050018, China

[b]School of Biological Science and Biotechnology, Beijing Forestry University, Beijing, 100083, China

[c] Dongcheng District Center For Disease Control And Prevention, Beijing, 100009, China

[d]School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou Zhejiang, 310058, China

*Corresponding authors:

Yingmin Jia, Tel. +86 311 81668010, jjyymm0311@163.com

Bolin Zhang, Tel. +86 10 62338221, zhangbolin888@163.com

ABSTRACT

Susceptibility of 29 *Lactobacilli* to 13 antibiotics was assayed by paper disc diffusion method. Plasmids and gastrointestinal tolerance were detected. The relationship between plasmids and antibiotic resistance was discussed. The results showed that all of the strains were resistant to bacitracin, polymyxin B, kanamycin, and nalidixic acid. Many strains were relatively sensitive to chloramphenicol and tetracycline. Six strains contained plasmids and showed good gastrointestinal tolerance. β-lactam resistance gene *blr* was found in the plasmid of *L. plantarum* CICC 23180 by PCR. The study will be helpful to promote the safety evaluation and development of potentially probiotic lactic acid bacteria.

- Keywords: antibiotic resistance; *Lactobacillus*; gastrointestinal tolerance; plasmid; probiotic -

1. INTRODUCTION

Due to the claimed benefits, *Lactobacillus* bacteria are widely used in food, feed, medical and health related fields. Many lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*, have been used safely for a long history. They are agreed to be secure and do not have the possibility of pathogenic. Currently, new beneficial bacteria are being developed continuously and will enter the market. However, the security of these new strains has caused great concern. Evaluation of antibiotic sensitivity is an important part of safety assessment.

Now, overuse of antibiotics has become a serious social problem. This led to the emergence of a large number of antibiotic-resistant strains. Once the resistance-related factors are tranferred to other microorganisms, especially pathogens via food carrier, it will cause tremendous problems. The evolution of antibiotic-resistant foodborne pathogens has been widely reported (THRELFALL *et al.*, 2000; WALSH *et al.*, 2008; WHITE *et al.*, 2002). Moreover, the resistance and resistance-related genes of *Bifidobacterium*, *Lactobacillus* and *Pediococcus* strains to different antibiotics were studied systematically (HUMMEL *et al.*, 2007; HUYS *et al.*, 2004; MARIA *et al.*, 2007). The *tetM* gene transfer of tetracycline resistance in *Lactobacillus plantarum* among strains was reported by NIAMH *et al.* (2010).

In this study, 29 *Lactobacillus* strains isolated from the food environment with potentially probiotic effects (JIN *et al.*, 2009; LI *et al.*, 2009; LIU *et al.*, 2011; SUN *et al.*, 2009; ZHAO *et al.*, 2013) were used. These strains were assayed for susceptibility to 13 antibiotics by agar disc diffusion method. Furthermore, some strains with higher resistance were analysed for the presence of plasmids. Then, the tolerance of the plasmid-containing strains under simulated gastrointestinal conditions was investigated. By plasmid elimination and PCR, the relationship between the plasmid profiles and resistance patterns of the strains was explored. This will provide a reference for the safety evaluation method and also will be helpful to improve the evaluation system of probiotics.

2. MATERIALS AND METHODS

2.1 Bacterial strains and cultivation

29 *Lactobacillus* strains used in the study were listed in Table 1. *Lactobacillus* strains were cultured in MRS (De Man, Rogosa, and Sharpe) medium at 37°C for 18h under anaerobic condition.

Quality control strain recommended by Clinical and Laboratory Standards Institute (CLSI) in the antibiotic sensitivity test was *E. coli* ATCC25922 purchased from the Institute of Microbiology, Chinese Academy of Sciences. The *E. coli* ATCC25922 was activated and cultivated in LB medium (yeast extract 5 g/L, tryptone 10 g/L, NaCl 10 g/L) at 37°C.

2.2 Testing of antibiotic susceptibility

13 kinds of antibiotics paper discs were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Table 2), each piece with a diameter of 6.5 mm. The quality was fully complied with the WHO criteria.

Antibiotic susceptibility was semi-quantitatively determined with K-B method by antibiotic paper disc diffusion referring to the CLSI as described by CHARTERIS *et al.* (1998a).

Briefly, 1.0 mL *Lactobacillus* suspension (approximately 1.5×10^8 CFU/mL) was added to sterile petri dish with diameter of 90 mm, and then mixed with a 15 mL MH (Muller Hinton, MH) agar (beef extract powder 6g/L, casein ac-

Table 1 - Source of the tested strains for antibiotic susceptibility test.

Species	Source (original number)
Lactobacillus plantarum	CICC[a] 23124 (L11), CICC 23131 (B31), CICC 23135 (B37), CICC 22195 (C35), CICC 23166 (ZJ1), CICC 23138 (C8-1), CICC 23180 (CH8)
Lactobacillus rhamnosus	CICC 23119 (1132), CICC 22175 (LL), ATCC[b] 7469, CICC 22151 (LK-Mt), CICC 22173 (R11)
Lactobacillus salivarius	CICC 23182 (CH-10)
Lactobacillus acidophilus	CICC 22162 (CH-2)
Lactobacillus casei	CICC 23184 (Y5-2b)
Lactobacillus helveticus	CICC 22154 (LLB)
Lactobacillus pentosus	CICC 23116 (SN23), CICC 22161 (Lp-4), CICC 22160 (Lp-5), CICC 22159 (Lp-B), CICC 22156 (Ind-3), CICC 22157 (Lp-A)
Lactobacillus paralimentarius	CICC 22148 (412), CICC 22149 (413)
Lactobacillus delbrueckii	CICC 22153 (LB), CICC 22163 (LC)
Lactobacillus paracasei	CICC 22165 (5M1), CICC 22167 (5M7), CICC 23183 (D-400)

[a]CICC, China center of industrial culture collection. [b]ATCC, American type culture collection.

Table 2 - The content of antibiotic paper discs and criterion for judgement.

Antibiotics	Content/disc	inhibition zone diameter (mm)		
		R[c]	I	S
Vancomycin	30 μg	≤9	10-11	≥12
penicillin G	10 U	≤14	15-17	≥18
ampicillin	10 μg	≤21	22-28	≥29
Bacitracin	0.04 U	≤10	10-12	≥12
cephalothin	30 μg	≤14	15-17	≥18
streptomycin	10 μg	≤11	12-14	≥15
kanamycin	30 μg	≤13	14-17	≥18
tetracycline	30 μg	≤11	12-14	≥15
chloramphenicol	30 μg	≤12	13-17	≥18
gentamicin	10 μg	≤12	13-14	≥15
nalidixic acid	30 μg	≤13	14-18	≥19
multi-polymyxin B	300 μg	≤11	12-14	≥15
rifampicin	5 μg	≤16	17-19	≥20

[c]Note: R-Resistant; S-Susceptible; I-Intermediate.

ids hydrolysate 17.5 g/L, soluble starch, 1.5 g/L, agar 17 g/L, pH 7.3±0.1) until the medium solidified. The antibiotic paper discs were pasted closely onto the solidified medium with sterile tweezers after 5min at room temperature. Three discs were pasted in each dish. The distance was more than 24 mm of each disc center and more than 15 mm from disc edge to the inner edge of dish. Next, the dishes were placed at room temperature for 1.5 h and then incubated at 37°C. After 24 h, the inhibition zone diameter was measured around the antibiotic disc with vernier caliper and recorded. For one tested strain, each antibiotic disc was done 3 times. The inhibition zone diameter was averaged

Standard sensitive strain of *E. coli* ATCC25922 was used as the control. The operation was the same as the above.

The antibiotic susceptibility of the tested strains was evaluated according to the CLSI criteria (Table 2).

2.3 Plasmid DNA extraction

10 mL of *Lactobacillus* suspension cultured overnight was centrifugated at 10000 rpm for 5 min. Then the precipitation was suspended with 500 μL of lysozyme solution (10 mg/mL). The mixture was placed in a water bath for 45 min at 37°C. Then plasmid DNA of *Lactobacilli* strains was extracted and purified with DNA extraction and purification kit of Tiangen Biotech (Beijing) Co., LTD. Plasmid DNA was observed by agarose gel electrophoresis.

Antibiotic susceptibility and plasmid stability were tested after cultivated 30 generations at 37°C in MRS medium according to the above methods.

2.4 Gastrointestinal tolerability test

In order to explore the application safety in human, the gastrointestinal tolerability of those lactic acid bacteria containing the plasmids were tested.

For acid tolerance test, Lactobacillus cells were harvested by centrifugation at 6000 rpm for 15 min, washed twice with 0.01 mol/L PBS, pH 7.2 after cultured for 18 h at 37°C in MRS broth, and then suspended in 20 mL sterile saline (0.85%, w/v) adjusted to pH 2.5 with sterile hydrochloric acid.

For bile tolerance test, the modified method of LEE *et al.* (1999) was referred to test bile tolerance. The *Lactobacillus* cells were centrifuged (6000 rpm, 15 min) after cultivated for 18 h at 37°C in MRS broth and suspended in 20 mL sterile saline (0.85 %, w/v) supplemented with 0.3% (w/v) bile salts (taurocholate, Sigma) at pH 6.8.

For pepsin and trypsin tolerance test, *Lactobacillus* cells were centrifuged (6000 rpm, 15 min) after cultivated for 18 h at 37°C in MRS broth, then suspended in 20 mL sterile simulated gastric and pancretic juices. Fresh simulated gastric and pancreatic juices were prepared daily according to Charteris *et al* (1998b). Pepsin (Sigma) was added into the simulated gastric juice with a final concentration of 5 mg/mL. Then the pH was adjusted to 2.5 with sterile hydrochloric acid. Trypsin (Sigma) was added into the simulated pancreatic juices with a final concentration of 10 mg/mL. Then pH was adjusted to 8.0 with 0.1 M NaOH.

All of the tolerability detection, the initial bacterial counts were adjusted to about 10^8 CFU/mL and were checked by viable count determination on MRS agar. For the tolerance assay, the bacterial suspensions were incubated and counted at 37°C for 0,1,2,3,4,5,6 h, respectively.

All tests were repeated three times to estimate the standard error.

2.5 Detection of antibiotic resistance genes

Part of the antibiotic-resistant genes of those lactic acid bacteria containing both plasmids and high tolerance were investigated. The β-lactam resistance-related gene sequence of *blr*, *ECP-1569*, *nps-1* and the chloromycetin resistance-related gene sequence of *cmlA*, *cat*, *cmlA1* in plasmids were found in National Center for Biotechnology Information (NCBI). The primers were designed and synthesized by Beijing Sunbiotech Co., LTD (Table 3).

The PCR programmes were performed with the plasmid template of the tested strains according to the following procedures: initial heating at 94°C for 4 min was followed by 34 cycles of the following sequence: 94°C for 30 s, 72°C for 1 min, and 72°C for 1 min. Final extension took place at 72°C for 7 min.

The amplification products were separated

Table 3 - The primers of the resistance genes in the experiment.

Gene	Sequence of the primer	Annealing temperature	Fragment size
blr--up *blr*--down	5'-CGTCTTATTGAATTAACAGGTTGG -3' 5'-CACGAAGCCATGTTGTGTTC -3'	53°C	125 bp
ECP-1569--up *ECP-1569*--down	5'-CAATCAACAGAGATGTGGGCTG-3' 5'-GTACCGTAGTACTCTGTTCAGGTGG-3'	57°C	155 bp
nps-1--up *nps-1*--down	5'-TCATTCTTCTGGCCTGTAGC-3' 5'-GGCGATACCGCTCAGTTAC-3'	54°C	782 bp
cmlA--up *cmlA*--down	5'-CAAGGAGATGGTTTCGTGCG-3' 5'-CATGCCCAAACCTAGAAACGC-3'	56°C	551 bp
cat--up *cat*--down	5'-GGCATTTCAGTCAGTTGCTC-3' 5'-TGGAAGCCATCACAAACG-3'	55°C	530 bp
cmlA1--up *cmlA1*--down	5'-GCTGAAGCCAAGCTGAGAC-3' 5'-CTACGTTGTGGCGTCAATG-3'	56°C	492 bp

by conventional 1.0% (w/v) agarose gel electrophoresis (100V, 4°C) in TAE (tris-acetate-EDTA) buffer and visualised by ethidium bromide staining. The target fragment was recovered and sequenced by TaKaRa Biotechnology (Dalian, China) Co., Ltd. The resistance-related gene of plasmid was determined by comparison with the known fragment.

3. RESULTS AND DISCUSSION

3.1 Antibiotic susceptibility

Antibiotic susceptibility of the tested strains was evaluated according to the anti-microbial drug sensitivity standard of CLSI criteria. The sensitivity of the tested *Lactobacillus* to 13 kinds of antibiotics was shown in Table 4. The tested 29 strains were generally resisitant to multi-polymyxin B, bacitracin, kanamycin, nalidixic acid, and were mostly sensitive to chloramphenicol and tetracycline. The same species of *Lactobacillus* generally had similar resistance patterns. But there was species specificity such as the different antibiotic sensitivity in *L. plantarum*, *L. rhamnosus*, and *L. pentosus*. Moreover, the antibiotic-resistant level of different strains is also different.

Antibiotic resistance of the foodborne lactic acid bacteria had heen reported in the 1980s. The researchers generally believed that the resistance was a result of the long evolution and it was generally endogenous resistance and obtained resistance (Zeng *et al.*, 2004). So, the resistant lactic acid bacteria of natural or isolated from human intestinal can indirectly reflect the habitat of used antibiotic.

It can be seen from Table 5, the 29 strains showed different patterns of resistance to 13 kinds of antibiotics. To bacitracin, polymyxin B, kanamycin and nalidixic acid, the resistance rate of the 29 tested strains was 100%. To β-lactam and aminoglycosides, the resistance percentage was 20.7%-37.9% and 86.2%-100%, respectively. All of the 29 strains were mostly sensitive to chloramphenicol and tetracycline.

Among of the tested antibiotics, the nalidixic acid and polymyxin B can inhibite DNA synthesis and interfer cell membrane formation, respectively. The resistance of lactobacillus to these kinds of antibiotics may be due to the thicker cell wall of Gram-positive bacteria. While the tested strains showed different sensitivity to the antibiotics, such as streptomycin, kanamycin, tetracycline, chloramphenicol, gentamicin with protein synthesis inhibitition effect. Most lactobacillus strains showed resistance to those antibiotics against gram-negative bacteria, for example, streptomycin, gentamicin, kanamycin. This was consistent with report of Zhang *et al* (2007).

3.2 Plasmid DNA extraction of antibiotics-resistant lactobacillus strains

16 CICC strains with relatively strong antibiotic resistance were screened for plasmid extraction. As can be seen from Fig. 1, among these strains, only CICC 23180, 22161, 22175, 22157, 23124, and 22154 contained plasmids.

L. plantarum CICC 23180 showed 6 plasmid DNA bands and there is one band greater than 23 kb. *L. pentosus* CICC 22157 showed two plasmid DNA bands of 10 kb and 5 kb, respectively. *L. rhamnosus* CICC 22175 and *L. plantarum* CICC 23124 contained respectively 2 and 4 of plasmid DNA bands and both of the two strains contained a 10 kb plasmid. *L. helveticus* CICC

Table 4 - The sensitivity results of 29 *Lactobacillus* strains to 13 antibiotics.

	L plantarum							*L pentosus*							*L rhamnosus*				*L salivarius*	*L acidophilus*	*L casei*	*L.helveticus*	*L paracasei*			*L delbrueckii*		*L paralimentarius*	
	CICC 23166	CICC 23131	CICC 23180	CICC 23135	CICC 23124	CICC 23138	CICC 22195	CICC 22161	CICC 22157	CICC 22160	CICC 22159	CICC 22156	CICC 23116	CICC 22175	CICC 23119	ATCC 7469	CICC 22151	CICC 22173	CICC 23182	CICC 22162	CICC 23184	CICC 22154	CICC 22165	CICC 22167	CICC 23183	CICC 22153	CICC 22163	CICC 22148	CICC 22149
Vancomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	R	R
penicillin G	R	R	S	R	R	R	R	S	R	—	R	S	S	—	R	R	—	—	S	S	S	S	S	S	S	S	S	S	R
cephalothin	R	R	R	R	S	S	S	—	—	—	—	S	S	R	S	—	—	—	—	—	S	S	S	S	S	S	S	—	—
Bacitracin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ampicillin	R	—	—	—	—	—	—	—	—	R	—	R	—	R	—	—	R	—	R	—	—	R	—	—	R	—	—	R	R
Multipolymysin B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
streptomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	—	R	R
kanamycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
tetracycline	R	—	S	R	S	—	—	—	S	S	S	S	—	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
chloramphenicol	—	S	R	S	S	S	S	R	S	—	—	—	—	R	S	—	—	—	—	S	—	—	S	S	S	S	S	—	S
gentamicin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	—	R	R
nalidixic acid	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
rifampicin	R	R	R	—	R	R	S	R	—	R	—	S	S	—	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R

Table 5 - The percentage of the antibiotic resistance of 29 *Lactobacillus* strains.

Antibiotics	Quantity of resistant strains	Percentage of resistance (%)
vancomycin	26	89.7
penicillin G	11	37.9
cephalothin	6	20.7
bacitracin	29	100
ampicillin	10	34.5
multi-polymyxin B	29	100
streptomycin	27	93.1
kanamycin	29	100
tetracycline	3	10.3
chloramphenicol	3	10.3
gentamicin	25	86.2
nalidixic acid	29	100
rifampicin	10	34.5

22154 showed only one plasmid DNA band of about 10 kb.

Lactic acid bacteria generally contain plasmids. The plasmid size was usually 1.9 kb-84.8 kb. Most of the plasmid was less than 20 kb (WANG and LEE, 1997). In the culture process from generation to generation, many plasmids might disappear from the bacterial cell, but most of the plasmids were stable. In the study, the plasmids of the above six strains and the antibiotic susceptivity showed no changes after cultivated 30 generations.

3.3 Gastrointestinal tolerability

Resistance to gastrointestinal stress is very important for one strain to play the potential probiotic function (GUGLIELMOTTI *et al.*, 2007). If the strains have a high tolerance to gastrointestinal stress, it will have the chance to survive and play the probiotic effects in the gastrointestinal environment.

The tolerance of the selected six strains to low

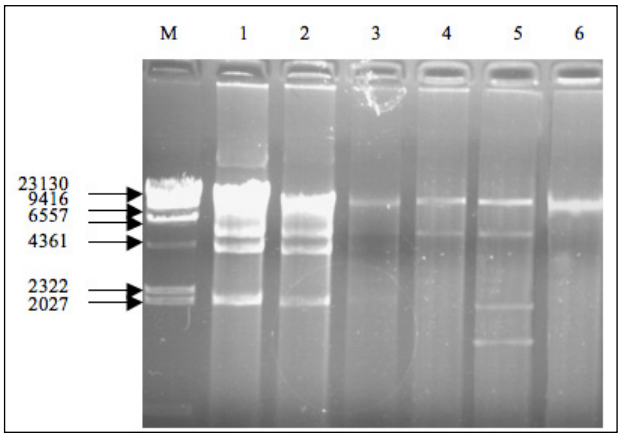

Fig. 1 - The plasmids in *Lactobacillus* (1.CICC 23180, 2.CICC 22161, 3.CICC 22175, 4.CICC 22157, 5.CICC 23124, 6.CICC 22154. M. λHindIII marker).

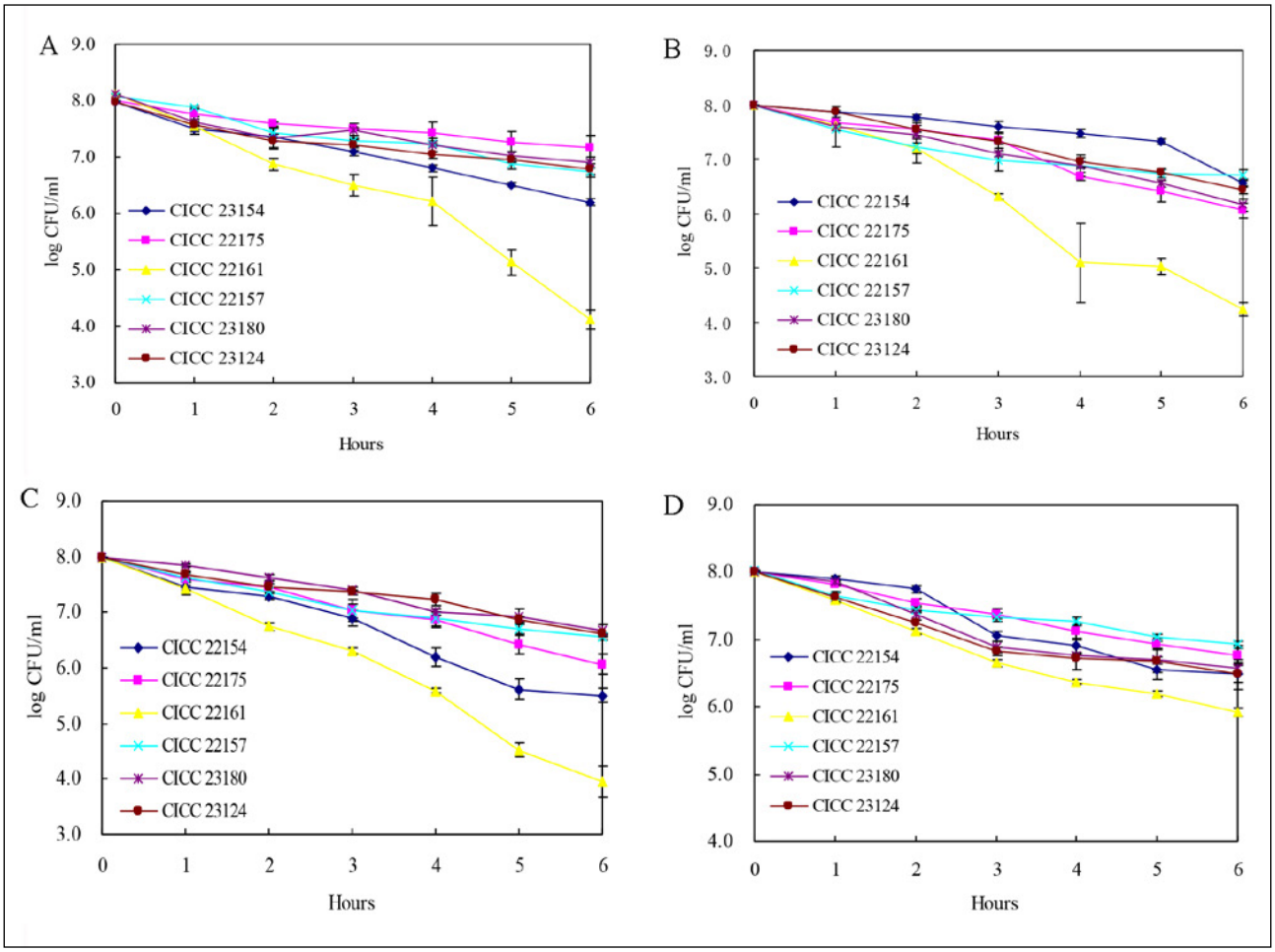

Fig. 2 - The viable counts of strains [C]CICC 22154, 22175, 22161, 22157, 23180 and 23124 in the gastrointestinal environment after 6 hs at 37°C.
A: pH 2.5; B: 3 mg/mL bile; C: 5mg/mL pepsin; D: 10 mg/mL trypsin.

pH, bile salt, pepsin and trypsin is presented in Fig. 2. As shown in Fig. 2A, the viable counts of *L. pentosus* CICC 22161 strain reduced to below 10^6 CFU/mL after 4 h and 1.32×10^4 CFU/mL after 6 h. However, the viable numbers of *L. helveticus* CICC 22154, *L. pentosus* CICC 22157, *L. plantarum* CICC 23124, 23180 and *L. rhamnosus* CICC 22175 were still more than 10^6 CFU/mL after 6 h in the gastric acid of pH 2.5. Thus, these five strains showed higher tolerance in acid environment.

For bile tolerance, except the *L. pentosus* CICC 22161, the viable counts of the other five strains were still more than 10^6 CFU/mL after 6 h in the medium containing bile salt (Fig. 2B). However, the viable cells of *L. pentosus* CICC 22161 had decreased to 2.0×10^6 CFU/mL within 3 h. And it declined to only 1.8×10^4 after 6 h.

For pepsin tolerance, among of six strains, the viable cells of *L. pentosus* CICC 22161 and *L. helveticus* CICC 22154 decreased significantly in 6 h and it is less than 10^4 CFU/mL and 10^6 CFU/mL after 6 h exposure to 5 mg/mL pepsin solution (pH 2.5), respectively (Fig. 2C).

For trypsin tolerance, as can be seen in Fig. 2D, the viable counts of the tested six strains still remained at 10^6 CFU/mL or more after 6 h exposure to 10 mg/mL trypsin solution (pH 8.0).

3.4 Detection of Resistance genes

According to the above results, except strain *L. pentosus* CICC 22161 and *L. helveticus* CICC 22154, the tested strains may be able to survive in the simulated gastrointestinal environment. However, if the above strains contain antibiotics-resistant plasmids, there is the possibility of resistance transfer to other bacteria, especially pathogenic bacteria. It will be a potential hazard to human health and be a serious social problem. So, the plasmid-determined resistant gene should be checked firstly before subsequent utilization.

After 0.02% SDS combined with heat treatment of the four strains (CICC 22175, 22157, 23124, 23180), only the plasmids of *L. plantarum* CICC 23180 were removed and the resistance to cephalothin and chloromycetin disappeared simultaneously (unpublished results). So, the primers of β-lactam resistance-relat-

ed genes including *blr*, *ECP-1569* and *nps-1* as well as chloromycetin resistance-related genes including *cmlA*, *cat* and *cmlA1* were designed. The plasmid-determined resistant genes of *L. plantarum* CICC 23180 were detected by PCR. As shown in Fig. 3, the plasmid of *L. plantarum* CICC 23180 contained β - lactam resistance gene *blr*, excluding other resistant genes. *blr* gene encodes beta-lactamase, which can hydrolyze β-lactam ring and then make the β-lactam antibiotic inactivation. This is probably the main reason of the bacteria resistant to β-lactam antibiotics. In the present study, the successful amplification of *blr* gene in *L. plantarum* CICC 23180 indicated that its cefalotin

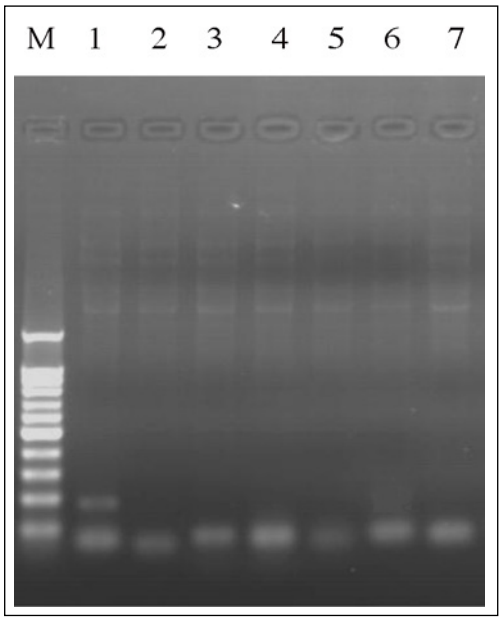

Fig. 3 - The PCR result in the genome and plasmid of CICC 23180.
M. Marker; 1. blr; 2. ECP-1569; 3. nps; 4. cmlA; 5. cat; 6. cmlA1; 7.control.

resistance may be due to the effect of the beta-lactamase to β-lactam antibiotics.

While in the study, the genes of *cat*, *cmlA* and *cmlA1* were not detected in the plasmids of *L. plantarum* CICC 23180. However, *L. plantarum* CICC 23180 strain was resistant to chloramphenicol. At the same time, plasmid elimination and *Escherichia coli* transformant test showed that chloramphenicol resistance-related genes should be present in plasmid DNA of *L. plantarum* CICC 23180 (unpublished results). Therefore, the plasmid of the CICC 23180 strain may contain other genes encoding chloramphenicol resistance.

In recent years, more studies have been done on antibiotic resistance of probiotics. It was shown that the antibiotic resistance was variable, species-dependent and related to the product types. And studies have shown that more genes associated with antibiotic resistance are located in plasmids and transposons (DOUCET *et al.*, 1992; MAYYA *et al.*, 2011). But unlike the chromosome DNA, both plasmids and transposons can provide the possibility of transferability for resistance genes between bacteria. PIER *et al.* (2003) proved the high transferability of plasmid pCF10 that encodes tetracycline resistance from *Enterococcus faecalis* OG1rf to *Enterococcus faecalis* BF3098c during cheese and sausage fermentation. JOANNA *et al.* (2008) reported the transferability of erythromycin resistant plasmid (pAMβ1) from *Lactococcus lactis* SH4174 to *Lactococcus lactis* Bu2-60. A similar study also indicated that the transferability of tetracycline resistance in *E. italicus* LMG 22195 from fermented milk (MIRIAM *et al.*, 2010).

So, the assessment of antibiotic resistant of potentially probiotic lactic acid bacteria used in food industry, especially the resistance-related genes and the transferability are very necessary. We can also say that, exploring the probiotic property and safety of lactic acid bacteria are equally important.

4. CONCLUSIONS

The tested 29 strains of potential probiotic *lactobacillus* showed different resistance to antibiotics. Those resistant strains containing both plasmids and high tolerance to gastrointestinal condition may cause food safety problems. So these strains need to be re-assessed carefully. The study found that the plasmid of *L. plantarum* CICC 23180 exactly carried the cephalothin-related gene *blr*. However, the transferibility of the resistance-related gene remains to be further studied. This study provides a reference in investigating the relationships between antibiotic resistance spectrum and the plasmids and evaluating the safety of probiotics.

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