Detection of streptomycin resistance and resistance genes in lactic acid bacteria from Sichuan Pickle of China

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ABSTRACT: Objective To detect the streptomycin resistance and resistance genes of lactic acid bacteria (LAB) in Sichuan Pickle of China. **Methods** The streptomycin resistant LABs were isolated by MRS containing 8 μg/ml streptomycin, and were identified by 16S rRNA analysis. The minimum inhibitory concentration (MIC) for streptomycin was determined for each isolate by broth microdilution method. And then susceptibility status was determined by comparing MIC values to breakpoints proposed by European Food Safety Authority (EFSA). In the streptomycin resistant LABs, the candidate streptomycin resistance genes (*str*A, *str*B, *aad*A, *aad*E, *ant*(6), *aac*(6')-*aph*(2'), and *aph*(3')-Ⅲa) were detected by PCR. **Results** Sixty-seven lactic acid bacteria which belonged to *Pediococcus ethanolidurans* (36), *Lactococcus garvieae* (14), *Lactobacillus buchneri* (12), *Lactobacillus acetotolerans* (2), *Lactococcus lactis* (1) and *Staphylococcus*.spp (2) were isolated from Sichuan Pickle of China. In these isolates, all *Lactococcus garvieae*, *Lactobacillus acetotolerans*, *Lactococcus lactis* and *Staphylococcus*.spp strains displayed streptomycin resistance, while only 20 *Pediococcus ethanolidurans* and 7 *Lactobacillus buchneri* isolates were streptomycin resistant. Except *Lactobacillus acetotolerans*, isolates belonged to the other 5 species harbored several or all candidate resistance genes. The *str*A and *aph*(3')-Ⅲa genes were detected in all 5 species isolates with detection rate of 50% - 100% and 21.4% - 100%, respectively. The detection rates of *str*B gene in *Pediococcus ethanolidurans*, *Lactobacillus buchneri*, *Lactococcus garvieae* and *Lactococcus lactis* were 70%, 42.9%, 28.6% and 100%, respectively. The *aac*(6') - *aph*(2') genes were detected only in 3 *Pediococcus ethanolidurans*, one *Lactobacillus buchneri*, one *Lactococcus garvieae* and 2 *Staphylococcus* spp strains. And no amplicon resistance genes such as *aad*A, *aad*E and *ant*(6) were detected in either isolate. In conclusion, the detection rates of *strA*, *strB* and *aph*(3')-Ⅲa genes were more than other genes. **Conclusion** These results indicated that lactic acid bacteria with streptomycin resistance were living in the brine of Sichuan Pickle, and it would be a potential health risk.

KEY WORDS: Sichuan Pickle; lactic acid bacteria; streptomycin resistance; streptomycin resistance genes

四川泡菜中乳酸菌链霉素抗性与抗性基因的检测

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摘　要: 目的　探明四川泡菜中链霉素抗性乳酸菌及抗性基因的种类。**方法**　利用含有 8 μg/ml 链霉素的 MRS 初步分离泡菜液中的抗性乳酸菌，通过 16S rRNA 分析确定抗性乳酸菌的分类地位后，测定同种不同菌株对链霉素的 MIC，并与欧洲食品安全官方机构（EFSA）建议的最低临界值比较确定抗性菌株；通过 PCR 扩增链霉素抗性基因 *str*A、*str*B, *aad*A、*aad*E、*ant*(6)、*aac*(6')-*aph*(2')和 *aph*(3')-Ⅲa，确定抗性菌株的抗性基因。**结果**　分离到 67 株链霉素敏感性或抗性菌株，这些菌株分别属于 *Pediococcus ethanolidurans* (36), *Lactococcus garvieae* (14), *Lactobacillus buchneri* (12), *Lactobacillus acetotolerans* (2), *Lactococcus lactis* (1)和 *Staphylococcus*.spp (2)。其中 *Lactococcus garvieae*、*Lactobacillus acetotolerans*、*Lactococcus lactis* 和 *Staphylococcus*.spp 全部为抗性菌株，*Pediococcus ethanolidurans* 中有抗性菌株 20 株、*Lactobacillus buchneri* 中有 7 株。在抗性基因检测中，除 *Lactobacillus acetotolerans* 抗性菌株没有检测到被检基因外，其他 5 个种的抗性菌株中均检测到部分或全部抗性基因。*str*A 和 *aph*(3')-Ⅲa 基因在除 *Lactobacillus acetotolerans* 外的被检菌株中均有检出，其检出率分别为 50%-100%和 21.4%-100%；*str*B 基因在抗性菌株 *Pediococcus ethanolidurans*, *Lactobacillus buchneri*, *Lactococcus garvieae* 和 *Lactococcus lactis* 检测率分别为 70%、42.9%、28.6%和 100%；*aac*(6')-*aph*(2')基因仅在 3 株 *Pediococcus ethanolidurans*、1 株 *Lactobacillus buchneri*、1 株 *Lactococcus garvieae* 和 *Staphylococcus* spp 中检测到。*aad*A、*aad*E and *ant* (6)在所有抗性菌株中都没有检测到。总体而言，*strA*、*strB* 和 *aph*(3')-Ⅲa 基因在链霉素抗性菌株中的检出率高于其他抗性基因。**结论**　当前的研究结果表明：四川泡菜中存在链霉素乳酸菌抗性菌株，这些抗性菌株对四川泡菜存在潜在安全风险。

关键词: 四川泡菜；乳酸菌；链霉素抗性；链霉素抗性基因

1　Introduction

Lactic acid bacteria (LAB), as probiotics bestowing human health, are generally recognized as safe (GRAC) food grade microorganisms and widely used in food industry [1-3]. In the maintenance of gastrointestinal health, LAB plays an important role. However, in recent years, the overuse and misuse of antibiotics have created a tremendous selective pressure of antibiotics resistant to bacteria in environment. The emergence of antibiotic resistance is a global threat because it reduces the efficiency of the antibiotic therapy [4, 5]. Hence the usage of LAB in food production has raised an important safe question which is the nature of acquiring and distribution of antimicrobial resistance gene in the LAB [6-8]. Different strategies for the resistance to various antibiotics have been found in microorganisms. In general, most of them can be classified into three categories: (1) intrinsic or natural resistance, which is inherent to a bacterial species, (2) acquired resistance caused by the mutation of indigenous genes, and (3) acquired resistance due to acquisition of exogenous resistance genes [9]. And the exogenous acquired resistance would be very harmful due to horizontal transfer of antibiotic resistance genes between bacteria.

In the recent past, most published reports on selection and dissemination of antibiotic resistance genes within the complex bacterial community of the human gut were mainly focused on clinically relevant species [10]. Currently, the role of LAB as a reservoir of antibiotic resistance determinants with transmission potential to pathogenic species is now increasingly acknowledged [11-13], and thus representing a potential health risk in the food chain in dissemination of antimicrobial resistance is also concerned. However, the report of antibiotic resistance about LAB in fermented foods is seldom found at home and abroad.

Streptomycin is extensively used in plant agriculture for bacterial disease control in China, particularly against soft rot of cabbage, angular leaf spot of cucumber, corky scab of hot pepper and so on. Therefore, the resistant strains of environment, such as air, water and soil, are unavoidable gathered at the surface of the vegetable in vegetable growing process. The Sichuan Pickle is traditional fermented vegetables food by natural inoculation, which contains live LAB, and without secondary disinfection. The food chain has been recognized as one of the key routes of transmission of antibiotic resistance between hosts [14]. Owing to there is a no-contact barrier between LAB as normal flora in the intestinal tract, the resistance LAB can easy distribute resistance genes to other bacteria, especially pathogen bacteria. This will cause perishing danger in the treatment of disease. So it is necessary to investigate streptomycin resistance of LAB in pickle. This study would be very useful for safety evaluation of LAB strains in Sichuan Pickle.

2 Materials and methods

2.1 Isolation of streptomycin resistant LAB

Pickle brine samples were collected from family-made Sichuan pickle, and 0.1 mL aliquots of 10- and 100-fold dilution of the culture were spread onto MRS plates containing calcium carbonate and 8 μg/mL streptomycin, which was equal to the lowest breakpoints as suggested in EFSA [15]. After incubation at 37 ℃ for 2~3 d, the colony, which had dissolver zone of calcium carbonate, were randomly selected from streptomycin plate and purified by re-streaking. And these strains were stored at -80 ℃ in MRS broth containing 25% glycerol.

2.2 Identification of streptomycin resistant LAB

Genomic DNA was extracted and purified from cells in the mid-logarithmic growth phase according to the methods described by Xiang *et al* [16]. The polymerase chain reaction (PCR) amplification of 16S rRNA gene of the isolates were performed using the universal primers EU27F (5'-AGA GTT TGA TCC TGG CTC AG C-3') and 1490R (5'-GGT TAC CTT GTT ACG ACT T-3'). The 25 μL PCR reaction mixture contained 2.5 μL 10 × PCR Buffer (with Mg^{2+}), 0.25 μL DNA Polymerase (Bioedlfy Biotech, Nanjing, China), 2 μL dNTP mixture (2.5 mmol/L each), 1 μL chromosome (5-100 ng/μL), 1 μL PCR primer EU27F (20 pp),1 μL PCR primer 1490R (20 pp) and 17.25 μL ultrapure water. Approximate 1500 bp fragments of the 16S rRNA were amplified by PCR, and were sequenced by the Sanger's dideoxy-chain termination method (Shanghai, China). The 16S rRNA sequence was compared with those in GenBank Database by using the BLAST program and the percent similarity was then determined.

2.3 MIC assays

The minimum inhibitory concentration (MIC) for streptomycin was determined for each isolate by broth microdilution method[17]. Briefly, a 96-well plate was inoculated with 198 μL MRS broth containing serial (1 : 2) concentrations of antibiotics (8-1024 μg/mL streptomycin) and 2 μL fresh LAB samples. LAB cultures were first grown in 5 mL MRS for 24 h at 37 ℃ and then subsequently diluted in 0.85% (w:v) physiological saline to the concentration of approximately 1×10^7 cell/mL. Bacteria inoculated in MRS were used as positive control, and a bacteria-free well was used as negative control. Then the plates were incubated at 37 ℃ for 48 h. MIC values of streptomycin of each strain were visually evaluated as the lowest concentrations at which no growth was observed. Susceptibility status was determined by comparing MIC values to proposed breakpoints [15]. All the tests were repeated at least twice. In duplicate experiments, the differences of MIC for independent sample never exceeded one order of dilution.

2.4 PCR detection of streptomycin resistance genes

Chromosomes were isolated from the LAB strains and used as templates for PCR to detect the candidate streptomycin resistance genes (*str*A, *str*B, *aad*A, *aad*E, *ant*(6), *aac*(6')-*aph*(2'), and *aph*(3')-Ⅲa) [6, 18]. The primer sequences, annealing temperature and elongation time were listed in Table 1. PCR products (5 μL) were separated by conventional 1.0 % (*w*:*v*) agarose

Table 1 Candidate primers for detection of streptomycin resistance genes

Resistance genes	Primers (5' to 3')	Annealing temperature (℃)	Reference
*str*A	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	55	[6]
*str*B	F: TCGTCAAGGGATTGAAACC R: GGATCGTAGAACATATTGGC	56	
*aad*A	F: ATCCTTCGGCGCGATTTTG R: CAGCGCAATGACATTCTTG	56	
*aad*E	F: ATGGAATTATTCCCACCTGA R: TCAAAACCCCTATTA AAGCC	50	
ant(6)	F: ACTGGCTTAATCAATTTGGG R: GCCTTTCCGCCACCTCACCG	58	
aac(6')-*aph*(2')	F: CCAAGAGCAATAAGGGCATA R: CACTATCATAACCACTACCG	60	[18]
aph(3')-*III*a	F: GCCGATGTGGATTGCGAAAA R: GCTTGATCCCCAGTAAGT CA	60	

gel electrophoresis in 1× TAE buffer and visualized by ethidium bromide staining. And the amplicons were randomly selected to further confirm by sequencing.

3 Results

3.1 Isolation and identification of streptomycin resistant LAB

In this study, 67 LAB strains were isolated from family-made Sichuan Pickle samples. All strains were identified by 16S rRNA. The 16S rRNA blast suggested that LAB isolates were classified to 6 species, 36 strains of *Pediococcus ethanolidurans*, 14 strains of *Lactococcus garvieae*, 12 strains of *Lactobacillus buchneri*, 2 strains of *Lactobacillus acetotolerans*, one strains of *Lactococcus lactis*, and 2 *Staphylococcus*. These strains shared at least 97% 16S rRNA similarity to that of GenBank Database reference strains, and even some achieved 100% similarity.

3.2 MIC assays of LAB isolates

The susceptibility of 67 LAB strains to streptomycin was assessed by broth microdilution method, and their MIC distributions were shown in Table 2. The unimodal distributions were observed for all strains except for *Lactobacillus buchneri* with different distributions. For *Pediococcus ethanolidurans*, the MIC range was from 16 to 1024 μg/mL, and the MICs of 20 strains were more than EFSA breakpoints 64 μg/mL [15]. All the 14 strains of *Lactococcus garvieae* showed the higher MIC with the range from 256 to 1024 μg/mL and possessed streptomycin resistance. For *Lactobacillus buchneri,* the MICs of 10 strains were between 32 and 64 μg/mL and one strain at 512 μg/mL, one with more than 1024 μg/mL. The MICs of 2 strains *Lactobacillus acetotolerans* were 128 and 256 μg/mL respectively. In *Lactococcus lactis*, only one strain showed streptomycin resistance, with MIC of 64 μg/mL. And the MICs of 2 *Staphylococcus* spp. were 256 μg/mL. In all 67 strains, the total 46 strains showed resistance for streptomycin based on the breakpoints suggested by EFSA [15].

3.3 Detection of streptomycin resistance genes in LAB strains

The 46 LAB strains were tested by PCR for the presence of the candidate streptomycin resistant genes: *str*A, *str*B, *aad*A, *aad*E, *ant*(6), *aac*(6')-*aph*(2'), and *aph*(3')-Ⅲa. The corresponding positive amplicons were presented in Table 3. In the 46 streptomycin resistance LAB strains, the *str*A and *aph*(3')-Ⅲa gene have been detected respectively in 34 and 26 strains containing 5 species except *Lactobacillus acetotolerans*; The *str*B gene has been detected in 25 strains, including *Pediococcus ethanolidurans, Lactobacillus buchneri, Lactococcus garvieae* and *Lactococcus lactis;* Although *aac*(6')-*aph*(2') gene were detected in only 7 strains, it contained *Pediococcus ethanolidurans, Lactobacillus buchneri, Lactococcus garvieae* and *Staphylococcus* spp. And no amplicon corresponding to *aad*A, *aad*E and *ant*(6) genes were detected in all isolates. Moreover, no gene has detected in *Lactobacillus acetotolerans*.

Statistical analysis (showed in Table 4) found that the distribution and occurrence frequency of streptomycin resistance genes were different in various species. In *Pediococcus ethanolidurans*, the detection rate of *aph*(3')-Ⅲ*a* gene was the highest, reaching 75%. In *Lactococcus garvieae* and *Lactobacillus buchneri*, the detection rates of *str*A gene were the highest, with 92.9% and 100% respectively. On the whole, the detection rates of *strA*, *strB* and *aph*(3')-Ⅲa genes were more than other genes. These results indicated that the mechanism and pathway of acquired resistance genes may be different between species, but there are some similarities in all streptomycin resistance strains.

Table 2　MIC distribution of 5 species to streptomycin

Species	Number of strains with MIC (μg/mL)								Total strains
	16	32	64	128	256	512	1024	>1024	
P. ethanolidurans	4 S	12 S	6 R	5 R	4 R	3 R	2 R		36
L. garvieae					6 R	7 R	1 R		14
L. buchneri		5 S	5 R			1 R		1R	12
L. acetotolerans				1 R	1 R				2
L. lactis			1 R						1
Staphylococcus spp					2 R				2

Table 3 LAB strains with streptomycin resistance genes and their MICs

LAB Species	Strain	MIC(μg/mL)	Resistance genes detected by PCR
P. ethanolidurans	A9	256	*aph*(3')-IIIa
	A14	64	*str*A, *str*B, *aph*(3')-IIIa
	A16	128	*str*B, *aph*(3')-IIIa
	A23	64	*str*A, *aph*(3')-IIIa
	A31	256	*str*A, *str*B, *aph*(3')-IIIa
	A33	128	*aph*(3')-IIIa
	J6	64	*str*A, *str*B
	J8	128	*str*A, *str*B
	J17	64	*str*A, *str*B
	J20	128	*str*A, *str*B, *aph*(3')-IIIa
	J28	256	*str*B, *aac*(6')-*aph* (2'),
	J29	512	*str*A, *str*B
	J35	512	*str*B, *aph*(3')-IIIa
	J38	512	*str*A, *str*B, *aph*(3')-IIIa
	J42	128	*str*B, *aph*(3')-IIIa
	S4	≥1024	*str*B, *aph*(3')-IIIa
	S6	64	*str*A, *str*B, *aph*(3')-IIIa
	C20	≥1024	*aac*(6')-*aph*(2'), *aph*(3')-IIIa
	K3	64	*str*A, *str*B, *aph*(3')-IIIa
	K11	256	*str*A, *str*B, *aac*(6')-*aph*(2'), *aph*(3')-IIIa
L. garvieae	L32	1024	*str*A, *aph*(3')-IIIa
	L33	512	*str*A, *str*B, *aph*(3')-IIIa
	L34	512	*str*A
	L35	512	*str*A
	L36	512	*str*A
	L41	256	*str*A, *str*B
	J2	256	*str*A
	J15	512	*str*B, *aac*(6')-*aph*(2'), *aph*(3')-IIIa
	J18	256	*str*A, *str*B
	J21	256	*str*A, *str*B
	J23	256	*str*A
	J24	512	*str*A
	J25	256	*str*A
	J34	512	*str*A
L. buchneri	L2	512	*str*A, *str*B, *aph*(3')-IIIa
	L7	64	*str*A, *aph*(3')-IIIa
	L13	64	*str*A, *str*B, *aph*(3')-IIIa
	L17	64	*str*A, *aph*(3')-IIIa
	L18	64	*str*A, *aph*(3')-IIIa
	A2	64	*str*A, *str*B, *aac*(6')-*aph*(2')
	S1	≥1024	*str*A, *aph*(3')-IIIa
L. acetotolerans	L37	256	-
	L40	128	-
L. lactis	L31	64	*str*A, *str*B, *aph*(3')-IIIa
Staphylococcus spp	L39	256	*aac*(6')-*aph*(2')
	S5	256	*str*A, *aac*(6')-*aph*(2'), *aph*(3')-IIIa

-: not dectected

Table 4 Statistical analysis of the detection rate of streptomycin resistance genes

LAB Species	Total strains	Streptomycin resistant strains	Streptomycin resistant genes							
			*str*A		*str*B		*aac*(6')-*aph*(2')		*aph*(3')-Ⅲa	
			AMT	%	AMT	%	AMT	%	AMT	%
P. ethanolidurans	36	20	12	60%	14	70%	3	15%	15	75%
L. garvieae	14	14	13	92.9%	4	28.6%	1	7.1%	3	21.4%
L. buchneri	12	7	7	100%	3	42.9%	1	14.3%	6	85.7%
L. acetotolerans	2	2	0	0	0	0	0	0	0	0
L.lactis	1	1	1	100%	1	100%	0	0	1	100%
Staphylococcus spp	2	2	1	50%	0	0	2	100%	1	50%
In total	67	46	34	73.9%	23	50%	7	15.2%	26	56.5%

ATM: amount
%: The percentage of the strain detected corresponding streptomycin resistance gene in total resistant strains

4 Discussion

Streptomycin had been spred in the environment when used in plant agriculture for bacterial disease control in the late 1950s, leading to the selection of streptomycin resistant bacteria [19]. These bacteria may live in or on fruits, vegetables. Dissemination of antibiotic resistance by the food chain is an important public health issue.

In this study, the LABs in the family-made Sichuan Pickle, including *Pediococcus ethanolidurans*, *Lactococcus garvieae*, *Lactobacillus buchneri, Lactobacillus acetotolerans*, *Lactococcus lactis and Staphylococcus* spp. The EFSA describes that microbiological breakpoints are set by studying the distribution of MICs of the chosen antimicrobials in bacterial populations belonging to a single taxonomical unit (species or genus) and that the part of the population that clearly deviates from the normal susceptible populations is categorized as resistant [15]. In current works, *Pediococcus ethanolidurans* and *Lactococcus lactis* could be clear deviates from the breakpoints. However, in 46 streptomycin resistant strains, each strain has different MIC. For *Lactococcus garvieae*, *Lactobacillus acetotolerans*, *Lactococcus lactis* and *Staphylococcus* spp, the MICs for streptomycin exceeded the EFSA's breakpoints. However, for *Pediococcus ethanolidurans* and *Lactobacillus buchneri*, the MICs were less or more than the EFSA's breakpoints. And strains *L. buchneri* S1, *P. ethanolidurans* S4, *L. garvieae* L32 and *P. ethanolidurans* C20 showed very high MIC, even more than 1024 μg/mL. These results indicated that various species have different resistances in Sichuan Pickle, and for the same species, the resistances of different isolates were different. These differences possibly can be attributed to resistant gene differences.

The aminoglycoside resistance genes, including *aadA*, *aadE* and *ant*(6) were not detected in the isolates. It has been reported that none of the *strA*, *strB*, *aadA* and *aadE* were found in streptomycin resistance strains of *Lactobacilli*, *Streptococci*, *Lactococci* and *Leuconostoc* species [6]. And in Rojo-Bezareset' report, some streptomycin resistance strains also did not get *ant*(6) gene [18]. In the past, the *aac*(6')-*aph*(2') aminoglycoside resistance gene was found only in *Lactobacilli*, *Pediococci* and *Oenococcus*. Interestingly, it was found in *Pediococcus*, *Lactococcus* and *Staphylococcus* in present study. However, no gene has detected in *Lactobacillus acetotolerans* may suggest a new mechanism of resistance, which can be due either to acquired genes or to the mutation of indigenous genes.

In our investigation, the relationship between the MIC values and resistance genes was not found. The intrinsic or natural resistance genes' mutation in the chromosome might be the reasons why a same species with same streptomycin resistance genes showed the different MIC values. In many cases, resistance to streptomycin at high concentrations has been documented to be caused by mutations in the chromosome rpsL gene [20, 21]. Furthermore, the

‘amount’ of a resistance gene present in all of the bacteria might be an important reason of the MIC values distinction. Anderson *et al* [22] indicated that alteration in the gene copy number provides a simple way to change expression levels and alter the phenotype. Therefore, it requires further the mammoth task to elucidate the complex problem of streptomycin resistance in the Sichuan Pickle.

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参考文献

[1] FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, a joint FAO/WHO expert consultation Cordoba, Argentina. Available, 2001. http://www.who.int/foodsafety/publications/fs_management/probiotics/en/. Accessed 2013 Dec.10.

[2] FAO/WHO. Guidelines for the Evaluation of Probiotics in Food: Joint FAO/WHO Working Group meeting. London Ontario, Canada. Available, 2002. http://www.who.int/foodsafety/publications/fs_management/probiotics2/en/. Accessed 2013 Dec.10.

[3] Vásquez1 A, Eva Forsgren E, Fries I, *et al*. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees [J]. PLOS One, 2012, 7(3): e33188.

[4] Schlegelová J, Babák V, Klímová E, *et al*. Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples [J]. J Vet Med B, 2002, 49: 216-225.

[5] Pan L, Hu XQ, Wang XY. Assessment of antibiotic resistance of lactic acid bacteria in Chinese fermented foods [J]. Food Control, 2011, 22: 1316-1321.

[6] Toomey N, Bolton D, Fanning S. Characterisation and transferability of antibiotic resistance genes from lactic acid bacteria isolated from Irish pork and beef abattoirs [J]. Res Microbiol, 2010, 161: 127-135.

[7] Vankerckhoven V, Huys G, Vancanneyt M, *et al*. Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project [J]. Trends Food Sci Technol, 2008, 19: 102-114.

[8] Ammor MS, Belén Flórez A, Mayo B. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria [J]. Food Microbiol, 2007, 24: 559-570.

[9] Kiwaki M, Sato T. Antimicrobial susceptibility of Bifidobacterium breve strains and genetic analysis of streptomycin resistance of probiotic *B.breve* strain Yakult [J]. Int J Food Microbiol, 2009, 134: 211-215.

[10] Devirgiliis C, Zinno P, Perozzi G. Update on antibiotic resistance in foodborne *Lactobacillus* and *Lactococcus* species [J]. Front Microbiol, 2013, 4.

[11] Van Reenen CA, Dicks LM. Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review [J]. Arch Microbiol, 2011, 193: 157-168.

[12] Marshall BM, Ochieng DJ, Levy SB. Commensals: underappreciated reservoir of antibiotic resistance [J]. Microbe, 2009, 4: 231-238.

[13] Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food [J]. Anton Leeuw Int J G, 1999, 76: 115-137.

[14] Patel AR, Shah NP, Prajapati JB. Antibiotic resistance profile of lactic acid bacteria and their implications in food chain [J]. World J Dairy Food Sci, 2012, 7 (2): 202-211.

[15] EFSA. Technical guidance prepared by the panel on additives and products or s*ubstances use*d in *animal feed* (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (question No. EFSA-Q-2008-004) [J]. The EFSA Journal, 2008, 732, 1-15.

[16] Xiang WL, Liang HZ, Liu S, *et al*. Isolation and performance evaluation of halotolerant phosphate solubilizing bacteria from the rhizospheric soils of historic Dagong Brine Well in China [J]. World J Microbiol Biotechnol, 2011, 27: 2629-2637.

[17] Klare I, Konstabel C, Muller-Bertling S, *et al*. Evaluation of new broth media for microdilution antibiotic susceptibility testing of *Lactobacilli*, *Pediococci*, *Lactococci*, and *Bifidobacteria* [J]. Appl Environ Micro, 2005, 71: 8982-8986.

[18] Rojo-Bezares B, Sáenz Y, Poeta P, *et al*. Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine [J]. Int J Food Microbiol, 2006, 111: 234-240.

[19] Yashiro E, McManus PS. Effect of streptomycin treatment on bacterial community structure in the apple phyllosphere [J]. PLoS One, 2012, 7(5): e37131.

[20] Pestka, S. Inhibitors of ribosome functions [J]. Annu Rev Microbiol, 1971, 25: 487-562.

[21] Spahn CM, Prescott CD. Throwing a spanner in the works: antibiotics and the translation apparatus [J]. J Mol Med, 1996, 74:

423-439.

[22] Anderson TJC, Patel J, Ferdig MT. Gene copy number and malaria biology [J]. Trends Parasitol, 2009, 25(7): 336-343.

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“油脂加工与质量安全”专题征稿函

随着生活水平的日益提高, 消费者对油脂及油脂食品的品质与安全性有了更高的要求。需要更加完善、先进、快捷、准确的检测方法来控制油脂的安全。

鉴于此, 本刊特别策划了“**油脂加工与质量安全**”专题, 由**武汉轻工大学**的**何东平教授**担任专题主编。何教授兼任中国粮油学会常务理事, 中国粮油学会油脂分会常务副会长。全国粮油标准化技术委员会油料及油脂工作组组长, 国家粮食局粮油资源综合开发工程技术研究中心主任, 湖北省(武汉市)微生物学会常务理事。本专题主要围绕**油脂加工工艺, 加工过程中的品质、有害物质、外来物质的检测方法和研究现状, 油脂掺伪鉴别, 油脂检测的新技术**等方面或者您认为在油脂加工与质量安全方面有意义的内容进行论述, 计划在 **2015 年 2、3 月**出版。

鉴于您在该领域的成就, 本刊编辑部及**专题主编何东平教授**特邀请您为本专题撰写稿件, 以期进一步提升该专题的学术质量和影响力。综述、实验报告、研究论文均可, 请通过网站或 E-mail 投稿。我们将快速处理并优先发表。

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