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

doi: 10.3967/bes2014.109

*This study was supported by National Health and Family Planning Commission of PRC.

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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