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Genetic relationships among *Enterococcus faecalis* isolates from different sources as revealed by multilocus sequence typing

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ABSTRACT

Enterococcus faecalis is part of the natural gut flora of humans and other mammals; some isolates are also used in food production. So, it is important to evaluate the genetic diversity and phylogenetic relationships among *E. faecalis* isolates from different sources. Multilocus sequence typing protocol was used to compare 39 *E. faecalis* isolates from Chinese traditional food products (including dairy products, acidic gruel) and 4 published *E. faecalis* isolates from other sources including human-derived isolates employing 5 housekeeping genes (*groEL*, *clpX*, *recA*, *rpoB*, and *pepC*). A total of 23 unique sequence types were identified, which were grouped into 5 clonal complexes and 10 singletons. The value of standardized index of association of the alleles ($I_A^S = 0.1465$) and network structure indicated a high frequency of intraspecies recombination across these isolates. *Enterococcus faecalis* lineages also exhibited clearly source-clustered distributions. The isolates from dairy source were clustered together. However, the relationship between isolates from acidic gruel and one isolate from a human source was close. The MLST scheme presented in this study provides a sharable and continuously growing sequence database enabling global comparison of strains from different sources, and will further advance our understanding of the microbial ecology of this important species.
Key words: *Enterococcus faecalis*, multilocus sequence typing, genetic relationship

INTRODUCTION

Enterococcus faecalis is part of the natural gut flora of humans and other mammals and grows under restricted environmental conditions. In humans, they can be considered as opportunistic pathogens and may be present in vegetables, fruits, meat, and milk, as a consequence of fecal contamination. However, current legislation sets no limit for the presence of this species in food, because it is not always associated with fecal contamination (Commission Regulation, 2007). Some isolates are not pathogenic and are added to foods (such as cheeses) to extend their shelf life and improve their sensory properties (Giraffa, 2003). Some isolates of dairy origin also produce bacterocins (enterocins) that inhibit the growth of food spoilage or pathogenic bacteria, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio cholerae*, *Clostridium* spp., and *Bacillus* spp. (Giraffa, 2003; Cocolin et al., 2007).

Enterococcus faecalis is a member of the lactic acid bacteria (**LAB**) that consists of gram-positive cocci or rods that predominantly produce lactic acid as a by-product of carbohydrate fermentation. Unlike most LAB, *E. faecalis* cannot be considered as "generally recognized as safe" because some isolates are potential pathogens, especially in the nosocomial environment, and some of them were considered as a reservoir of genes encoding antibiotic resistance, which could be transferred to other microorganisms in the gut environment (Pesavento et al., 2014). So, understanding the relationship among the isolates from different sources is becoming more important.

In recent years, various genotyping methods were used to identify isolates or to further track their sources, including pulsed field gel electrophoresis, multiple variable number tandem repeat analysis, and multilocus sequence typing (**MLST**). Among them, MLST is a popular one. Nallapareddy et al. (2002) first evaluated the discriminatory power of MLST compared with pulsed field gel electrophoresis for *E. faecalis* and in his pilot study showed that sequence-based typing had potential to differentiate the isolates at the subspecies level and identify outbreak isolates. Subsequent studies confirmed the potential of MLST as an excellent tool for isolate characterization and long-term epidemiologic analysis in the related species *E. faecium* (Homan et al., 2002).

Although some studies have been done on the properties and epidemiological characteristics of *E. faecalis*, few studies are available on the relationship between

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Table 1. Isolates of *Enterococcus faecalis* and their sources

	Source		
Isolate reference number	Origin	Region	Year
IMAU 10057, IMAU10119, IMAU10134	Yogurt	Wulatezhong Banner, Bayan Nur City, Inner Mongolia	2002
IMAU10060, IMAU10063, IMAU10064, IMAU10075, IMAU10078, IMAU10130, IMAU10133	Fermented sheep milk	Wulatezhong Banner, Bayan Nur City, Inner Mongolia	2002
IMAU10087	Cow butter	Wulatezhong Banner, Bayan Nur City, Inner Mongolia	2002
IMAU10091, IMAU10094, IMAU10098, IMAU10099, IMAU10100, IMAU10102, IMAU10103	Fermented sheep milk	Wulatehou Banner, Bayan Nur City, Inner Mongolia	2002
IMAU10095	Sheep-milk cheese	Wulatehou Banner, Bayan Nur City, Inner Mongolia	2002
IMAU10351, IMAU10440, IMAU10483	Yogurt	New Barag Left Banner, Hulunbeier League, Inner Mongolia	2009
IMAU10826, IMAU10861, IMAU10868, IMAU10917	Yogurt	Balinyuo Banner, Chifeng, Inner Mongolia	2009
IMAU10052	Sheep butter	Wulatezhong Banner, Bayan Nur City, Inner Mongolia	2002
IMAU70078, IMAU70121, IMAU70122	Acidic gruel	Togtoh Country, Huhhot, Inner Mongolia	2008
IMAU40025, IMAU40105	Kurut	Gonghe County, Hainan Profecture, Qinghai	2005
IMAU40027	Kurut	Tianjun County, Haibei Profecture, Qinghai	2005
IMAU40046	Yak milk	Gonghe County, Hainan Profecture, Qinghai	2005
IMAU60007	Yogurt	Gyangze County, Shigatse area, Tibet	2007
IMAU60129, IMAU60134, IMAU60135	Yogurt	Danxung County, Lhasa area, Tibet	2007
IMAU60196	Yak milk	Gyangze County, Shigatse area, Tibet	2007
C19315WT	Human pathogens	Broad Institute of MIT and Harvard, Cambridge, MA	2013
T2	Human urine	Sapporo, Japan	2009
RP2S-4	Nonhuman source	Unknown	2007
V583	Blood culture derived from a chronically infected patient	Barnes Hospital, St. Louis, MO	1987

properties of *E. faecalis*, particularly whether they are pathogens, probiotics, or otherwise useful in the food industry, and their original source. Moreover, the related data for *E. faecalis* from food sources were limited. In this study, we tried to compare the genetic profile of the strains from Chinese traditional food products with some human pathogens, and then provide some more data for the MLST analysis of *E. faecalis*.

MATERIALS AND METHODS

Bacterial Isolates and DNA Extraction

A total of 39 *E. faecalis* isolates were obtained from the Collection Centre of Lactic Acid Bacteria of Inner Mongolia Agriculture University, China, and identified by 16S rRNA analysis. These isolates originated from traditional Chinese food products (including yogurt, fermented sheep milk, cow butter, sheep-milk cheese, kurut, yak milk, acidic gruel, and sheep butter) from 10 regions in 3 provinces of China (Inner Mongolia, Qinghai, and Tibet) from 2002 to 2009 (Table 1). Stock cultures were stored in 10% glycerol at −80°C. Working cultures were activated by 2 subcultures in M17 broth (Oxoid, Unipath Ltd., Basingstoke, UK), each at 37°C for 18 to 24 h under anaerobic conditions. The related sequences of another 4 reference isolates of *E. faecalis* (C19315WT, RP2S-4, T2, and V583) were obtained from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/genome/?term=Enterococcus+faecalis). *Enterococcus faecalis* RP2S-4 was isolated from an unknown nonhuman source, but the others were all human pathogens (Table 1).

Each isolate was cultured overnight at 37°C in M17 broth and then the total genomic DNA was extracted using the methods described by Dan et al. (2014).

MLST Analysis

Purified DNA was diluted to a final concentration of 100 ng/μL for evaluation. Primers were designed using Premier 5.0 (Table 2) based on the consensus sequences of 5 housekeeping genes in *E. faecalis* V583 (*pepC*, *clpX*, *recA*, *rpoB*, and *groEL*) and used to amplify fragments from those 5 housekeeping genes. General standards