A Randomized Study of Microbial Contamination in Ready-to-eat Beef in Shanghai, China

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Abstract: The bacterial contamination of ready-to-eat (RTE) beef products in Shanghai was investigated. Twenty RTE beef samples were randomly collected from five international supermarkets and five street vendors in Shanghai, China. The total number of bacterial colonies was measured using the culture method, and ten colonies from each sample were randomly selected for bacterial classification and identification by 16S rRNA gene sequencing. The average total colony counts of the beef samples collected from the supermarkets and street vendors were 1.08±1.73×10⁸ CFU/g and 2.84±8.49×10⁸ CFU/g, respectively; there was no statistically significant difference (*p*>0.05) between them, but both were higher than the national standard of China (≤8×10⁴ CFU/g). Pathogens that could cause severe food poisoning were not detected in any sample, but several operational taxonomic units (OTUs) that were closely related to food poisoning, urinary tract infections, pneumonia, mastitis, and bacteremia were detected. The results suggest that RTE foods are an important but often overlooked reservoir for pathogenic microorganisms, and we should strengthen our hygienic practices and management of RTE foods to reduce the risk of food-borne disease caused by them.

Key words: ready-to-eat foods; street vendors; supermarkets; reservoir; food borne disease

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中国上海即食牛肉制品微生物污染情况的随机研究

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摘要:本文研究了上海即食牛肉样品中微生物的污染情况。从上海的 5 个国际超市和 5 个街头摊贩随机抽取 20 份即食牛肉样品,采用培养法分析样本的菌落总数,并从每份样品中随机抽取 10 个菌落采用 168 rRNA 基因测序法对细菌进行分类鉴定。超市牛肉样本的菌落总数平均为(1.08±1.73)×10⁸ CFU/g,街头摊贩样本菌落总数平均为(2.84±8.49)×10⁸ CFU/g,二者在统计学上无显著性差异(p>0.05),但均高于国家标准(≤8×10⁴ CFU/g)。所有样本均未检出能引起严重食物中毒的病原体,但检测到种类多样且与食物中毒、尿路感染、肺炎、乳腺炎以及菌血症等疾病相关的多种病原菌操作分类单元(OTU)。结果表明,即食食物是一个经常被忽视的致病微生物的储存库,我国应加强对即食食物的卫生管理,从而降低即食食品引起的食源性疾病传播几率。

关键词: 即食食品; 摊贩; 超市; 存储库; 食源性传染病

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作者简介:吴睿铭(1997-),男,在读研究生,研究方向:食品安全 通讯作者:魏华(1977-),女,博士,讲师,研究方向:食品安全 Ready-to-eat (RTE) foods can be defined as those that have been prepared by manufactures and can be consumed at the point of sale without further cooking or heating. As a result of the increasingly urbanized lifestyle in China, consumption of retail RTE foods has risen significantly in recent years^[1]. Consequently, supermarkets have visibly expanded their RTE sections, and street vendors have established themselves and

dispersed across cities. Contaminated food, an important vehicle for pathogens, can potentially cause many food borne diseases, which remains a global public health issue. Compared to vacuum-packaged meat, RTE foods have greater potential for spoilage and microbial contamination, and therefore pose risks to public health.

There have been endeavors to determine the safety of RTE foods and other fast foods worldwide. In the United Kingdom, cold RTE sliced meats from catering establishments were investigated^[2]. They found that 74% of the 3,494 samples met EU standards, and 15 samples (<1%) were completely unacceptable. A research on RTE foods stored at 18 °C and sold in convenience stores in Taiwan showed that more than 75% of the samples contained coliforms that exceeded the national standard^[3]. Ham had the highest incidence rates of Bacillus cereus (62.5%)Staphylococcus aureus (26.1%)contamination. Among the 480 samples collected at supermarkets and street vendors in Shanghai, Sheng et al. found that only 28.54% of the RTE meats complied with the national standards for total aerobic microbial contamination $\leq 8 \times 10^4$ CFU/g (GB 2726-2005)^[4]. Guan et al. found that the percentage of RTE meats meeting national standards at hotels in Taizhou was 83.33%, 75.00%, 79.17% and 91.67%, in spring, summer, autumn and winter respectively, summer being the most problematic season for microbial contamination^[5]. Fang et al. reported 10.20%~65.00% of RTE meat samples collected from 1998 to 2008 in 8218 samples from 24 different cities and towns across China exceeding Chinese national standards for total aerobic bacteria. These studies demonstrated the potential risk of RTE foods especially for meat products^[6].

However, other than the quantity of total bacteria and some popular bacteria including *coliforms*, *Salmonella*, *Shigella*, and *S. aureus* et al., knowledge of the composition of the contaminating microorganisms is still limited. Therefore, little is known to the public about the risks toward human health and well-being from eating and being exposed to these contaminated RTE meats, despite the huge consumption in China and in many other countries worldwide. Most previous studies have only shown excessive counts of bacteria in such foods, but further exploration, such as the identification of bacterial species and their potential risks, is insufficient. This study

determines and compares the microbiological quality of RTE beef samples randomly collected from supermarkets and street vendors across the city of Shanghai using a standard culture in combination with 16S rRNA gene sequencing.

1 Materials and methods

1.1 Sample collection

RTE beef samples within shelf-life were collected from five international supermarkets and five street vendors. Each sample weighed around 100 g and were packaged with styrofoam base of 24 cm×18 cm and plastic wrapper on top. Two samples were collected from each site on different dates. All samples were collected during afternoons of July 2015. Samples were placed in isothermic bags and transported to the laboratory within 3 hours.

1.2 Bacterial enumeration

25 g of each sample was mixed with 225 mL of normal saline in sterile Ziplocs and placed in a bagmixer for 2 minutes, followed by 10 times serial dilution with normal saline. As a result we had 8 gradients to analyze, from the original mixture down to 10^{-7} of its concentration. 0.1 mL of 10^{-2} to 10^{-7} dilutions of the homogenate was plated in replicate on PCA (plate count agar). The plates were then incubated at 37 °C for 24 h. At the end of the incubation, colonies were counted using an illuminated colony counter (Gallenkamp, England). The count for each plate was expressed as colony forming unit of the suspension (CFU/g). Statistical analysis was done using SPSS 19.0 statistical software package and Student's t-test. p<0.05 was considered as significant.

1.3 16S rRNA gene PCR and sequencing

Ten colonies on each PCA plates with appropriate dilution were randomly selected from each sample. Bacteria DNA were purified using FTA CloneSaver Cards (Whatman® BioScience, USA) according to manufacturer's instructions. The 16S rRNA gene of each colony was PCR-amplified using primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1391R (5'-GACGGGCGGTGWGTRCA-3') and sequenced by

ABI PRISM 3730 XL DNA Analyzer (Life Technologies Corporation, CA). CD-HIT was used for OTUs' picking with similarity cutoff 98%. The partial 16S rRNA sequences (~800 bp) were blasted against type strains in RDP database^[7] to determine their nearest neighbors. Sequences were aligned using Clustal W. Phylogenetic tree was constructed by MEGA6 ^[8] using neighborjoining method. The GenBank accession numbers of 16S rRNA sequences in this study are KX246672 - KX246858.

2 Results and discussion

2.1 Enumeration of total bacterial counts

The total bacterial counts (TBC) of ten RTE samples are summarized in Figure 1. Mean TBC in supermarket samples was $(1.08\pm1.73)\times10^8$ CFU/g (range from 3.8×10^3 to 5.4×10^8), 90% of the samples exceeded the chinese national standards and was 1350-fold higher than standard. Mean TBC in street vendors samples was $2.84\pm8.49\times10^8$ CFU/g (range from 7.0×10^4 to 1.0×10^8), 80% of these samples exceeded the Chinese national standards and was 3550-fold higher than standard. TBC was not significantly different between the groups (p>0.05).

More than 80% of the RTE foods exceeded the national microbiological standards of China, suggesting a relatively high risk of RTE foods in Shanghai, China. The percentage of unqualified RTE foods is higher than the previous reports^[4-6], which is probably due to the sampling time we chose. Summer afternoon is the period with highest microbial contamination risk because of the high temperature and relatively long storage time (generally>8 h, as most of the RTE foods are cooked in the early morning).

2.2 Analysis of 16S rRNA gene sequencing

In comparison to previous studies^[4-6], in this study, we not only enumerated the number of contaminated microbes, but also evaluated their risks by identifying them into species level.

One hundred and eighty-seven of two hundred isolates were successfully sequenced and thirteen colonies from seven samples were sequence failure because of difficulty in purification. Sequences were

grouped into 37 OTUs (Fig.2), 25 OTUs from supermarkets and 23 OTUs from street vendors, and distribution of these OTUs was shown in Table 1. Proteobacteria and Firmicutes were identified as the most abundant phyla in the samples.

68.7% (66/96) of supermarkets derived colonies and 58.2% (53/91) of street vendors derived colonies were non-pathogenic or low risk opportunistic pathogens seldom known to cause human and animal disease, which were closely related to *Bacillus amyloliquefaciens*, *Macrococcus caseolyticus*, *Staphylococcus vitulinus*, *Staphylococcus hominis*, *Carnobacterium divergens*, *Enterococcus sulfurous*, *Enterococcus italicus*, *Enterococcus durans*, *Lactobacillus spp.*, *Weissella spp.*, *Leuconostoc spp.*, *Streptococcus salivarius*, *Citrobacter murliniae*, *Pantoea ananatis* and *Pluralibacter gergoviae*.

Twenty-two colonies belong to coliforms including *Citrobacter spp.*, *Klebsiella spp.* and *Enterobacter spp.* were isolated from all the RTE samples, in which only two colonies were from supermarket samples. It indicated RTE foods from street vendors have poorer hygienic conditions than those from supermarkets.

Escherichia coli, S. aureus, Shigella spp., Bacillus cereus, Salmonella spp. Listeria monocytogenes, Campylobacter spp., Clostridium botulinumand, Yersinia spp., and Clostridium perfringens were not detected in any of these samples. Despite these well-known high risk food-borne pathogenic species, the ones that are forbidden to be in foods, were not observed, sixteen isolates from street vendors and twelve from supermarkets belonging to seven OTUs were closely related to food-borne pathogens Staphylococcus carnosus, Staphylococcus saprophyticus, Enterococcus faecalis, Aeromonas veronii, Cronobacter sakazakii, Klebsiella pneumoniae and Acinetobacter johnsonii. Thirteen isolates from street vendors and twelve from supermarkets belonging to four OTUs were closely related to urethritis pathogens Kurthia gibsonii, K. pneumoniae, E. faecalis and S. saprophyticus. Five isolates belonging to two OTUs which were closely related to pneumonia pathogens K. pneumoniae and A. veroni were only found in sample from street vendors. Seventeen isolates from supermarkets and fifteen from street vendors belonging to eight OTUs were closely related to bacteraemia caused pathogens E. faecalis,

Exiguobacterium acetylicum, Acinetobacter pittii, Acinetobacter soli, Staphylococcus pasteuri, K. pneumoniae, Citrobacter sedlakii and C. sakazakii (Table 1).

OTU6 and OTU9 were identified as S. carnosus and S. saprophyticus, which are food poisoning associated coagulase-negative staphylococci (CNS). They have been isolated from black pudding in southern Brazil and known to produce staphylococcal enterotoxins^[9,10]. S. saprophyticus is a cause of 10~20% of urinary tract infections (UTIs) and in females 17~27 years old, it is the second most common cause of community-acquired UTI after E. coli^[11]. S. saprophyticus was the most frequently detected contaminating species in this study. Since S. saprophyticus is one of the normal flora of human skin, the origin of S. saprophyticus contamination in RTE foods is possibly from handlers' skin. OTU12 was identified as E. faecalis, which is a human opportunistic pathogen and a frequent contaminant on meat^[12, 13]. E. faecalis could also cause non-gonorrheal urethritis[14~16] and human bacteraemia^[17]. OTU26 was identified as A. veronii, which is an important pathogen in aquaculture and has been associated with food borne gastroenteritis. with possible fatal or serious debilitating outcomes^[18] and pneumonia^[19]. OTU30 was identified as *K. pneumonia*. Extended-spectrum β -lactamase (ESBL)-producing K. pneumoniae has been discovered as the reason for a food borne nosocomial outbreak^[20]. K. pneumoniae could also cause bacteremia, an invasive syndrome of liver abscess, meningitis, endophthalmitis, pneumonia urethritis^[21-23]. OTU28 was identified as C. sakazakii, which is an opportunistic food-borne pathogen that has been isolated from various foods including milk, cheese, dried foods, meats, vegetables, rice, bread, tea, herbs, spices, infant formula, water and also restaurant kitchen cutting boards in Seri Kembangan, Malaysia^[24,25]. C. sakazakii can also cause neonatal meningitis, septicemia and enterocolitis^[26]. OTU35 was identified as A. johnsonii which is a very important opportunistic pathogen in humans and has found in polluted food^[27].

Other risk species were also observed, including *K. gibsonii*, *E. acetylicum*, *A. pittii*, *S. pasteuri*, *C. sedlakii*, *A. soli* and *Corynebacterium tuberculostearicum*. OTU3 was identified as *K. gibsonii*, which could cause non-gonorrheal urethritis^[28]. OTU2 and OTU34 were

identified as *E. acetylicum* and *A. pittii*, which could cause human bacteraemia^[29~31]. OTU5 was identified as *S. pasteuri*, which is an agent of nosocomial infections, and could cause human bacteraemia and kestrel infection^[32~34]. OTU32 was identified as *C. sedlakii*, which could cause bacteremia, meningitis and brain abscess in premature infant^[35,36]. OTU36 was identified as *A. soli*, which could cause neonatal bacterial species^[37]. OTU37 was closely related to mastitis pathogens, *C. tuberculostearicum*^[38,39].

The incidence of *E. acetylicum*, *K. gibsonii*, *S. pasteuri*, *S. carnosus*, *A. veronii*, *C. sakazakii*, *K. pneumonia*, and *A. johnsonii* was relatively low and all of them were from street vendors' samples but not from supermarkets, indicating a higher risk of RTE meats from street vendors. The higher occurrence of risk bacterial species in RTE food from street vendors is likely due to its poorer hygienic conditions and worse production practices.

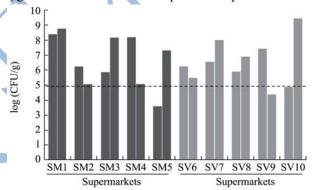


Fig.1 Enumeration of total bacterial counts on PCA plates from ten RTE samples of five international supermarkets and ten RTE samples of five street vendors in Shanghai, China

According to CDC and NHA reports, food-borne diseases are still important health issues in both developed and developing countries. Our study reinforces the possibility of gastrointestinal tract (GIT) infection as well as non-GIT potential serious diseases linked with consumption of RTE contaminated foods. The effective control of food-borne pathogens in RTE foods should be adopted to prevent or limit the risk of not only food-borne transmission of food poisoning, but also UTI, pneumonia, and mastitis pathogen transfer among humans. The contaminating bacteria maybe originated from handlers' hands or other anatomical areas, surrounding environment such as air, knives, spoons, cutting boards, packaging and animal origins, associated with poor hygienic conditions during RTE foods manufacturing.

Wearing disinfected clean cloths, mask and gloves, or adopting correct hand-washing procedures using antibacterial soap or hand sanitizer could reduce contamination risk^[40,41]. Lengthy gaps between preparation and consumption of RTE foods and temperature control for cooking and storage are also

important for preventing microbial contamination especially in warm seasons. In addition to suggesting better hygienic practice to the producers, we also appeal the Chinese regulatory authorities to create a monitoring and management system for better controlling of risk microbes in RTE foods.

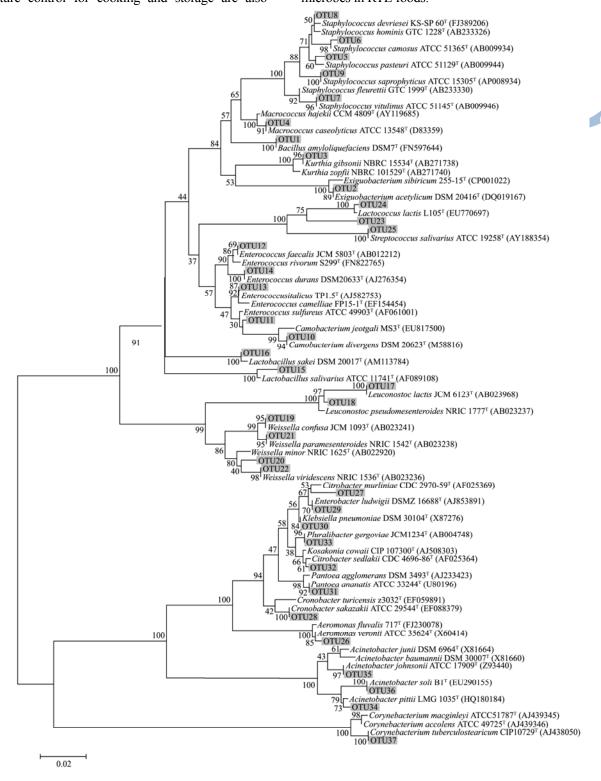


Fig.2 Phylogenetic tree analysis of partial 16S rRNA genes of bacteria isolated from RTE foods. Neighbor-joining dendrograms were generated with bootstrap trials of 100

		Table 1	Distributi	ion of OT	Us among	g samples				
		Supermarkets (SM)			Street Vendors(SV)					
OTU	Nearest species	No. of	No. of		No. of	No of	SM1-1	SM1-2	SM2-1	SM2-2
		samples	isolates		samples	isolates				
OTU1	Bacillus amyloliquefaciens	1	2		4	22	0	0	0	0
OTU2	Exiguobacterium acetylicum	0	0		1	1	0	0	0	0
OTU3	Kurthia gibsonii	0	0		1	4	0	0	0	0
OTU4	Macrococcus caseolyticus	1	1		5	16	0	0	0	0
OTU5	Staphylococcus pasteuri	0	0		1	1	0	0 🦪	0	0
OTU6	Staphylococcus carnosus	0	0		1	3	0	0	0	0
OTU7	Staphylococcus vitulinus	1	2		0	0	2	0	0	0
OTU8	Staphylococcus hominis	1	1		0	0	1	0	0	0
OTU9	Staphylococcus saprophyticus	3	11		2	5	0	0	0	0
OTU10	Carnobacterium divergens	0	0		1	1	0	0	0	0
OTU11	Enterococcus sulfureus	2	2		1	1	0	0	0	0
OTU12	Enterococcus faecalis	1	1		0	0	0	0	0	0
OTU13	Enterococcus italicus	1	1		0	0	0	0	0	0
OTU14	Enterococcus durans	2	17	J	0	0	7	10	0	0
OTU15	Lactobacillus salivarius	1	1		0	0	0	0	1	0
OTU16	Lactobacillus sakei	1	4		0	0	0	0	0	0
OTU17	Leuconostoc lactis	2	2	V	_1	1	0	0	0	0
OTU18	Leuconostoc pseudomesenteroides	1	1		0	0	0	0	0	1
OTU19	Weissella confusa	2	5		4	5	0	0	2	0
OTU20	Weissella minor	0	0		1	1	0	0	0	0
OTU21	Weissella paramesenteroides	1	2		0	0	0	0	2	0
OTU22	Weissella viridescens	3	11		2	2	0	0	0	0
OTU23	Lactococcus lactis*	2	2	1	0	0	0	0	1	0
OTU24	Lactococcus lactis	3	5		1	1	0	0	2	0
OTU25	Streptococcus salivarius	2	2		0	0	0	0	1	0
OTU26	Aeromonas veronii	0	0		1	1	0	0	0	0
OTU27	Citrobacter murliniae	0	0		1	1	0	0	0	0
OTU28	Cronobacter sakazakii	0	0		1	2	0	0	0	0
OTU29_	Enterobacter ludwigii	1	1		5	10	0	0	0	0
OTU30	Klebsiella pneumoniae	0	0		3	4	0	0	0	0
OTU31	Pantoea ananatis	0	0		1	1	0	0	0	0
OTU32	Citrobacter sedlakii	1	1		3	5	0	0	0	0
OTU33	Pluralibacter gergoviae	4	5		1	1	0	0	1	0
OTU34	Acinetobacter pittii	3	12		1	1	0	0	0	7
OTU35	Acinetobacter johnsonii	0	0		1	1	0	0	0	0
OTU36	Acinetobacter soli	2	3		0	0	0	0	0	2
OTU37	Corynebacterium tuberculostearicum	1	1		0	0	0	0	0	0
OTU	Nearest species	SM3-1	SM3-2	SM4-1	SM4-2	SM5-1	SM5-2	SV6-1	SV6-2	
OTU1	Bacillus amyloliquefaciens	0	0	0	0	2	0	0	0	
OTU2	Exiguobacterium acetylicum	0	0	0	0	0	0	0	0	

carried over

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brought fo	orward									
OTU3	Kurthia gibsonii	0	0	0	0	0	0	4	0	
OTU4	Macrococcus caseolyticus	0	0	1	0	0	0	0	3	
OTU5	Staphylococcus pasteuri	0	0	0	0	0	0	0	0	
OTU6	Staphylococcus carnosus	0	0	0	0	0	0	0	0	
OTU7	Staphylococcus vitulinus	0	0	0	0	0	0	0	0	
OTU8	Staphylococcus hominis	0	0	0	0	0	0	0	0	
OTU9	Staphylococcus saprophyticus	7	0	0	0	3	1	0	0	
OTU10	Carnobacterium divergens	0	0	0	0	0	0	0 👞	1	
OTU11	Enterococcus sulfureus	0	0	1	1	0	0	0	1 🛒	
OTU12	Enterococcus faecalis	1	0	0	0	0	0	0	0	
OTU13	Enterococcus italicus	0	0	1	0	0	0	0	0	
OTU14	Enterococcus durans	0	0	0	0	0	0	0	0	
OTU15	Lactobacillus salivarius	0	0	0	0	0	0	0	0	
OTU16	Lactobacillus sakei	0	4	0	0	0	0	0	0	
OTU17	Leuconostoc lactis	0	1	0	1	0	0	0	1	
OTU18	Leuconostoc pseudomesenteroides	0	0	0	0	0	0	0	0	
OTU19	Weissella confusa	0	0	3	0	0	0	0	0	
OTU20	Weissella minor	0	0	0	0	0	0	0	0	
OTU21	Weissella paramesenteroides	0	0	0	0	0	0	0	0	
OTU22	Weissella viridescens	0	1	0	2	0	8	0	0	
OTU23	Lactococcus lactis*	0	1	0	0	0	0	0	0	
OTU24	Lactococcus lactis	0	0	2	1	0	0	0	0	
OTU25	Streptococcus salivarius	0	0	0	1	0	0	0	0	
OTU26	Aeromonas veronii	0	0	0	0	0	0	0	0	
OTU27	Citrobacter murliniae	0	0	0	0	0	0	0	0	
OTU28	Cronobacter sakazakii	0	0	0	0	0	0	0	0	
OTU29	Enterobacter ludwigii	1	0	0	0	0	0	1	1	
OTU30	Klebsiella pneumoniae	0	0	0	0	0	0	0	2	
OTU31	Pantoea ananatis	0	0	0	0	0	0	0	0	
OTU32	Citrobacter sedlakii	1	0	0	0	0	0	0	1	
OTU33	Pluralibacter gergoviae	0	0	1	2	0	1	0	0	
OTU34	Acinetobacter pittii	0	2	0	0	3	0	0	0	
OTU35	Acinetobacter johnsonii	0	0	0	0	0	0	0	0	
OTU36	Acinetobacter soli	0	0	0	1	0	0	0	0	
OTU37	Corynebacterium tuberculostearicum	0	0	1	0	0	0	0	0	
OTU	Nearest species	SV7-1	SV7-2	SV8-1	SV8-2	SV9-1	SV9-2	SV10-1	SV10-2	
OTU1	Bacillus amyloliquefaciens	5	0	8	0	7	0	2	0	
OTU2	Exiguobacterium acetylicum	0	0	0	0	0	0	0	1	
OTU3	Kurthia gibsonii	0	0	0	0	0	0	0	0	
OTU4	Macrococcus caseolyticus	0	9	0	2	0	1	0	1	
OTU5	Staphylococcus pasteuri	1	0	0	0	0	0	0	0	
OTU6	Staphylococcus carnosus	0	0	0	3	0	0	0	0	
OTU7	Staphylococcus vitulinus	0	0	0	0	0	0	0	0	

carried over

现代食品科	Modern Food Science and Technology							2017, Vol.33,	No.8	
brought for	ward									
OTU8	Staphylococcus hominis	0	0	0	0	0	0	0	0	
OTU9	Staphylococcus saprophyticus	3	0	0	2	0	0	0	0	
OTU10	Carnobacterium divergens	0	0	0	0	0	0	0	0	
OTU11	Enterococcus sulfureus	0	0	0	0	0	0	0	0	
OTU12	Enterococcus faecalis	0	0	0	0	0	0	0	0	
OTU13	Enterococcus italicus	0	0	0	0	0	0	0	0	
OTU14	Enterococcus durans	0	0	0	0	0	0	0	0	
OTU15	Lactobacillus salivarius	0	0	0	0	0	0	0	0	
OTU16	Lactobacillus sakei	0	0	0	0	0	0	0	0	
OTU17	Leuconostoc lactis	0	0	0	0	0	0	0	0	•
OTU18	Leuconostoc pseudomesenteroides	0	0	0	0	0	0	0	0	
OTU19	Weissella confusa	0	0	0	1	1 .	1	0	2	
OTU20	Weissella minor	0	0	0	0	0	1	0	0	
OTU21	Weissella paramesenteroides	0	0	0	0	0	0	0	0	
OTU22	Weissella viridescens	0	0	1	1	0	0	0	0	
OTU23	Lactococcus lactis*	0	0	0	0	0	0	0	0	
OTU24	Lactococcus lactis	0	0	0	0	0	1	0	0	
OTU25	Streptococcus salivarius	0	0	0	0	0	0	0	0	
OTU26	Aeromonas veronii	0	1	0	0	0	0	0	0	
OTU27	Citrobacter murliniae	0	0	0	0	0	0	0	1	
OTU28	Cronobacter sakazakii	0	0	0	0	0	0	2	0	
OTU29	Enterobacter ludwigii	0	0	0	0	0	1	5	2	
OTU30	Klebsiella pneumoniae	0	0	0	1	0	0	0	1	
OTU31	Pantoea ananatis	0	0	0	0	0	1	0	0	
OTU32	Citrobacter sedlakii	0	0	0	0	0	3	0	1	
OTU33	Pluralibacter gergoviae	0	0	0	0	0	1	0	0	
OTU34	Acinetobacter pittii	1	0	0	0	0	0	0	0	
OTU35	Acinetobacter johnsonii	0	0	0	0	0	0	0	1	

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Note: * 16S rRNA sequence identity 92%.

Acinetobacter soli

Corynebacterium tuberculostearicum

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OTU36

OTU37

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