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Evaluation of bacterial contamination in raw milk, ultra-high temperature milk and infant formula using single molecule, real-time sequencing technology

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ABSTRACT

The Pacific Biosciences (Menlo Park, CA) single molecule, real-time sequencing technology (SMRT) was reported to have some advantages in analyzing the bacterial profile of environmental samples. In this study, the presence of bacterial contaminants in raw milk, UHT milk, and infant formula was determined by SMRT sequencing of the full length 16S rRNA gene. The bacterial profiles obtained at different taxonomic levels revealed clear differences in bacterial community structure across the 16 analyzed dairy samples. No indicative pathogenic bacteria were found in any of these tested samples. However, some of the detected bacterial species (e.g., *Bacillus cereus*, *Enterococcus casseliflavus*, and *Enterococcus gallinarum*) might potentially relate with product quality defects and bacterial antibiotic gene transfer. Although only a limited number of dairy samples were analyzed here, our data have demonstrated for the first time the feasibility of using the SMRT sequencing platform in detecting bacterial contamination. Our paper also provides interesting reference information for future development of new precautionary strategies for controlling the dairy safety in large-scale industrialized production lines.

Key words: dairy products, single molecule, real-time sequencing technology sequencing, bacterial contamination

INTRODUCTION

Bacterial contamination of dairy products is a great concern throughout the world, as the dairy production and consumption have been expanding extensively during the last decade; intense growth in the dairy industry is anticipated to continue into the future (Fuller et al., 2007). Raw milk, UHT milk, and infant formula are the main types of dairy products consumed by adults and infants. These dairy products are known to contain rich nutrient contents, including carbohydrates, proteins, and minerals, which may promote the growth of microbes as well as some food-borne pathogens (Remenant et al., 2015). The consumption of pathogen-containing products may cause illnesses ranging from upset stomach to more serious symptoms (e.g., diarrhea, fever, vomiting, and so on; Ahmed et al., 2014). The occurrence of other types of bacteria may potentially affect the product nutritional and sensory quality properties and in turn result in significant economic losses (Janštová et al., 2006). Thus, developing effective and accurate methods of assessing microbial food contamination is of the utmost importance.

The traditional way of detecting bacterial contamination in dairy products relies on the use of culture-based methods in combination with biochemical tests (de Boer and Beumer, 1999). The process of sample analysis is time-consuming, not easy to handle, and the results can be ambiguous due to the variable biochemical phenotypes of microbes. These factors have prompted development of reliable and rapid methods for identification of microbes present in dairy products. Several studies have been performed by incorporating various molecular techniques, such as pulsed-field gel electrophoresis, real-time PCR, and microarray, to investigate the prevalence of contaminated bacteria and the level of the contamination in dairy products (Kim et al., 2011; Barancelli et al., 2014; Wareth et al., 2014). Whereas some improvements have been shown using these molecular-based detection techniques, one common limitation shared by these methodologies is the failure in providing an overall microbial profile in the samples, as most of these approaches require the detection of specific target microorganisms. The complete microbial profile offers an objective evaluation on the sample microbial quality and quantifies the contamination level, if any.

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The Pacific Biosciences (**PacBio**; Menlo Park, CA) single molecule, real-time sequencing technology (**SMRT**) was reported to have some advantages in analyzing the bacterial profiles of environmental samples based on the full-length 16S rRNA gene (Amir et al., 2013; Zhang et al., 2015). Thus, the present study aimed to apply such technology in assessing bacterial contamination of dairy products, including raw milk, UHT milk, and infant formula. Our study has demonstrated, for the first time, the feasibility of using the SMRT sequencing system in detecting the bacterial contamination in dairy samples, which would be of interest to the food industry for effective monitoring of microbial food quality and safety during production.

MATERIALS AND METHODS

Sample Collection

A total of 16 samples were collected, including 2 raw milk samples from a local farm, 4 UHT milk samples, and 10 infant formula samples from the supermarkets. Raw milk samples were collected aseptically and all samples were kept in ice boxes during transportation. The sample and sequence information are described in Table 1.

DNA Extraction

The DNA was extracted using the OMEGA DNA isolation kit (D5625–01, Omega, Norcross, GA) following the manufacturer's instructions. The quality of extracted DNA was checked by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260/280 nm ratio). All extracted DNA samples were stored at −20°C for further analysis.

PCR Amplification

The bacterial 16S rRNA was amplified by PCR for barcoded SMRT sequencing. It was amplified using the forward 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and the reverse 1541R (5′- AAGGAGGTGATCCAGCCGCA-3′) primers. These primers contained a set of 16-nucleotide barcodes. The PCR amplifications of the 16S rRNA regions were performed as described previously (Liu et al., 2015). The PCR program was 95°C for 4 min, 30 cycles of 95°C for 60s, 60°C for 45 s, and 72°C for 60 s with a final extension of 72°C for 7 min. The amplicons were sequenced using P6-C4 chemistry on a PacBio RS II instrument (Pacific Biosciences). The quality control for PCR amplifications and sequence preprocessing was performed as described previously (Mosher et al., 2013).

Data Analysis

Raw data processing was carried out using the protocol RS_ReadsOfinsert.1 available in SMRT Portal version 2.7 (PacBio). Filtering parameters, including minimum full passes, minimum predicted accuracy, minimum reads lengths of inserts, and maximum reads lengths, were set up with values 5, 90, 1,400, and 1,800, respectively. The extraction of high-quality sequences was first performed with the QIIME package (Quantitative Insights In to Microbial Ecology; version

Table 1. General information of sequence and diversity

Sample no.	Type	Number of reads	Number of operational taxonomic units	Shannon index	Simpson index	Chao1 index	Observed species index
1	Raw milk	571	387	7.555	0.975	5,222.045	350.816
2	Raw milk	1,366	950	7.854	0.982	5,125.278	379.756
3	UHT milk	1,688	339	5.898	0.968	507.428	146.498
4	UHT milk	1,294	324	5.997	0.949	463.753	177.47
5	UHT milk	1,192	834	7.815	0.979	4,964.68	379.198
6	UHT milk	2,199	1,328	7.513	0.97	3,861.252	350.416
7	Infant formula	872	408	7.025	0.98	1,487.565	263.488
8	Infant formula	916	430	7.14	0.983	1,608.073	266.288
9	Infant formula	1,513	1,045	7.874	0.981	4,648.798	383.314
10	Infant formula	2,029	815	6.945	0.977	1,516.492	259.276
11	Infant formula	3,126	796	6.636	0.962	1,628.741	253.016
12	Infant formula	2,432	815	6.315	0.944	1,191.263	228.288
13	Infant formula	922	630	7.715	0.978	4,061.203	365.938
14	Infant formula	1,192	741	7.728	0.987	3,685.872	345.208
15	Infant formula	3,531	1,280	6.779	0.972	1,622.137	248.942
16	Infant formula	2,133	783	6.732	0.974	1,365.619	239.074