Assessment of the microbial diversity of Chinese Tianshan tibicos by single molecule, real-time sequencing technology

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Received: 19 April 2018 / Revised: 20 July 2018 / Accepted: 29 August 2018 / Published online: 3 September 2018
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Abstract Chinese Tianshan tibico grains were collected from the rural area of Tianshan in Xinjiang province, China. Typical tibico grains are known to consist of polysaccharide matrix that embeds a variety of bacteria and yeasts. These grains are widely used in some rural regions to produce a beneficial sugary beverage that is slightly acidic and contains low level of alcohol. This work aimed to characterize the microbiota composition of Chinese Tianshan tibicos using the single molecule, real-time sequencing technology, which is advantageous in generating long reads. Our results revealed that the microbiota mainly comprised of the bacterial species of *Lactobacillus hilgardii*, *Lactococcus raffinolactis*, *Leuconostoc mesenteroides*, *Zymomonas mobilis*, together with a *Guehomyces pullulans*-dominating fungal community. The data generated in this work helps identify beneficial microbes in Chinese Tianshan tibico grains.

Keywords Chinese Tianshan tibicos · Microbial diversity · Single molecule · Real-time sequencing technology · *Leuconostoc mesenteroides* · *Guehomyces pullulans*

Introduction

Tibicos, also known as water kefir or sugary kefir, are very similar to milk kefir grains both in terms of structure and microbiological constitution (da Miguel et al., 2011). The composition of tibico grains is mainly polysaccharide, primarily dextran, which is not associated with milk fermentation. A variety of microorganisms is residing within the exopolysaccharide biofilm matrix structure, which forms a complex stable symbiotic microbial consortium, including *Lactobacillus*, *Acetobacter*, yeasts, and many other members (Gulitz et al., 2011). These small, translucent, and crystal-like microbe-embedding grains can produce a sugary beverage by utilizing and converting sugars present in the environment to produce low concentrations of alcohol, lactic acid, and carbon dioxide. The fermented beverage is straw-colored, carbonated, slightly acidic, and has a low level of alcohol. Tibicos are found worldwide; however, their compositions greatly varied between geographical locations due to climatic and regional differences.

In China, the tibicos are spontaneously fermented in brown sugary solutions. After incubation for 24 h, the tibico grains are removed by sieving and are reused in newly prepared sugary solutions. With each subsequent incubation, the size of the tibico grains increases slightly. The tibico fermented beverage is believed to exert health-promoting effects, such as strengthening immunity, inhibiting tumors, reducing blood sugar and cholesterol, and improving intestinal microbiota. However, the microbiota composition of Chinese Tianshan tibicos and their beneficial effects have not been scientifically defined and confirmed.

Traditionally, the microbial diversity of tibico grains has been assessed by classical culturing methods using

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selective growth media (Vardjan et al., 2013). However, due to the presence of a high proportion of uncultivable microorganisms, the resultant microbial profile determined based on culturing methods could be incomplete, especially for such complex ecosystem (Jany and Barbier, 2008). Recently, culture independent methods, such as quantitative real-time PCR, ribosomal gene targeted 454-pyrosequencing, and single-cell amplification technique, have been proven to be powerful tools for describing the microbial compositions of fermented foods (Liu et al., 2015; Yao et al., 2017).

Zero-mode waveguide (ZMW) technology is the heart of PacBio's DNA sequencing instrument. The ZMWs are 70 nm in diameter (100 nm in depth nanoholes) that are affixed with polymerase enzyme at the bottom. Thus, sequencing results can be acquired based on monitoring the DNA template-directed synthesis process by uninterruptedly incorporation of different fluorescently labelled nucleotides by the DNA polymerase (Eid et al., 2009). Such technology is advantageous in generating long reads and thus allows rapid and accurate identification of microbial taxa in a complex microbial ecosystem, including species that are difficult to culture or are present in low abundance.

The aim of our study was to assess the biodiversity within the complex microbial consortium of Chinese Tianshan tibicos by single-molecule, real-time (SMRT) sequencing technology. This is the first report studying the fungal and bacterial microbiota of Chinese Tianshan tibicos using SMRT sequencing technology approach; our data provide insights into the microbial composition of these natural products.

Materials and methods

Tibico grain samples

Chinese Tianshan tibicos used in this study were collected from rural area of Tianshan in Xinjiang province, China. The sample belonged to a local family that cultivated the tibico grains in private household for self-consumption. As soon as the sample was collected, it was stored at 4 °C until further processing.

Total DNA extraction

Before DNA extraction, the tibico grains were washed twice with sterile water. The total DNA was extracted from samples using a modified CTAB method with glass bead to disrupt the cell walls (Lick et al., 1995). The yield and quality of DNA were checked through agarose gel electrophoresis (1%) which was stained with nucleic acid dye

(0.1%); and the OD_{260/280} ratio was measured by spectrophotometry. All DNA samples were stored at − 20 °C for further analysis.

PCR amplification and SMRT sequencing

For the bacterial community analysis, the full-length 16S rRNA genes of the samples were amplified by polymerase chain reaction (PCR) for SMRT sequencing. The primer pair was 27F (5'-AGAGTTTGATCGTGGCTCA-3') and 1492R (5'-ACCTTGTTACGACTT-3') (Mosher et al., 2013). For the fungal community analysis, the 18S rDNA and ITS region genes were amplified by the primer pair, NS1F (5'-GTAGTCATATGCTTGTCTC-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (Chen et al., 2012).

Thermocycling was conducted in a 2720 Thermal Cycler (AB Applied Biosystems, USA) under the following conditions: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 2 min; and a final extension step at 72 °C for 10 min. The quality of the amplified PCR products was checked by electrophoresis in 1% agarose gel. Products were quantified using an Agilent DNA 1000 Kit and an Agilent 2100 Bioanalyser (Agilent Technologies) according to the manufacturer's instructions. The quantified DNA products were sequenced using P6-C4 chemistry on a PacBio RS II instrument.

Data analysis

Raw data acquired from the PacBio RS SMRT chip were processed through Pacific Biosciences SMRT Portal (version 2.7) for length filtering and quality control. The parameters set to filter the sequences were performed as described previously (Hou et al., 2015). The bacterial taxonomy of each OTU representative sequence was assigned using the Ribosomal Database Project II database that classified at a minimum bootstrap threshold of 80% (Cole et al., 2007). The fungal taxonomy used the UNITE database following Kõljalg et al. (2013) (<https://unite.ut.ee/repository.php>). The Shannon–Wiener index, Simpson's diversity, Chao1, and rarefaction estimators were calculated to evaluate the alpha diversity.

Nucleotide sequence accession numbers

The sequence data reported in this study have been deposited in the MG-RAST database (accession no.: 4709272.3–4709273.3).

Results and discussion

A total of 2293 bacterial and 814 fungal high-quality reads were generated from the sample. All sequences were clustered with representative sequences, and a total of 291 bacterial and 67 fungal OTUs were obtained with a 97% sequence identity cut-off. For both bacteria and fungi, the Shannon curve but not Rarefaction curve reached the saturation phase, suggesting that although new phylotypes would be expected with an increase in sequencing depth, the majority of microbial phylotypes present in the sample had already been captured (Figs. 1, 2). Sample OTU richness and diversity are listed in Table 1.

Using the Ribosomal Database Project classifier, Firmicutes and Proteobacteria constituted the two most dominant bacterial phyla (contributing to 53.01% and 46.88% of the total sequences, respectively), whereas Bacteroidetes only comprised a low proportion (0.11%). At

Fig. 1 Rarefaction (A) and Shannon diversity index (B) curves of the bacterial community in the sample

Fig. 2 Rarefaction (A) and Shannon diversity index (B) curves of the fungal community in the sample

the genus level, the relative abundances of *Gluconobacter*, *Lactobacillus*, *Lactococcus*, *Serratia*, *Leuconostoc*, *Ochrobactrum*, *Pseudomonas*, and *Zymomonas* were found to be larger than 1% (Fig. 3A). In particular, the genus *Serratia* of the Proteobacteria phylum was the most abundant genus (contributing to 36.99% of the total sequences). The second-most dominant bacterial genus was *Leuconostoc* (30.11%). In addition, a low proportion (all about 0.11%) of *Alistipes*, *Gluconacetobacter*, and *Kluyvera* was detected in the present study.

A total of 15 bacterial species were identified in the sample. Reads belonging to the species *Lactobacillus hilgardii*, *Lactococcus raffinolactis*, *Leuconostoc mesenteroides*, *Ochrobactrum lupini*, *Pseudomonas fragi*, and *Zymomonas mobilis* were detected (each comprised > 1%) (Fig. 3B). Among these detected bacteria, *Leuconostoc mesenteroides* was the most dominant member. This species is likely responsible for the production of dextran that

Table 1 Information on sequence analysis and diversity indices of the sample

Classification	Number of reads	Number of OTUs	Shannon index	Simpson index	Chao 1 index	Number of observed species
Bacteria	2293	219	0.939	0.174	224.043	38.95
Fungi	814	67	0.687	0.166	29.456	11.506

Fig. 3 Relative abundances of bacteria identified in the tibicos sample at the genus (A) and species (B) levels

forms the grains; when it grows in sucrose solution, it converts the sugar to dextrans (Rodina et al., 2015). Furthermore, *Leuconostoc mesenteroides* strains have a wide spectrum of antifungal activity against *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium roqueforti*, and some other common spoilage fungi. Thus, *Leuconostoc mesenteroides* has the potential to become a biopreservative in food industry (Lee and Chang, 2016; Muhialdin et al., 2015). Lee et al. (2016) also isolated a new phenyl lactic acid derivative and some known metabolites from the culture medium of *Leuconostoc mesenteroides* that showed high radical-scavenging activities, suggesting that this species could produce antioxidative compounds.

Lactococcus raffinolactis was the second dominant bacterial species. This food-associated bacterium is present in a wide range of environments such as fish, plant, and many other materials (Hagi and Hoshino, 2009; Ikeda et al., 2005). In the dairy environment, this species has not only been found in raw cow milk and camel milk, but also in natural dairy starter cultures, especially in different kinds of cheeses (Meslier et al., 2012; Terzic-Vidojevic et al., 2014). *Lactococcus raffinolactis* does not only ferment α -galactosides such as melibiose and raffinose, but also has a high ability of degrading cholesterol (Boucher et al., 2003; Li et al., 2015).

The other two minor bacterial species were *Lactobacillus hilgardii* and *Zymomonas mobilis*. Among them, *Lactobacillus hilgardii* is believed to be the primary

exopolysaccharide producer (Davidovic et al., 2015). The *Zymomonas mobilis* species is likely related to ethanol production; it showed a high performance in response to external stresses such as high ethanol concentration, high temperature, and harsh acidic fermentation conditions (Wang et al., 2016; Zhang et al., 2016). In addition, *Zymomonas mobilis* has anti-leukemic activity, antitumor effects, and prebiotic activity (Einsfeldt et al., 2016; Lorenzetti et al., 2015; Yoon et al., 2004). No *Acetobacter* was detected in this study, despite representatives of this genus have previously been isolated from other tibicos (Gulitz et al., 2011; Marsh et al., 2013).

The dominant fungal sequence reads were distributed across the Basidiomycota and Ascomycota phyla. Basidiomycota was the predominating phylum, constituting 95.88% of the fungal population of the sample. Ascomycota (3.60%) and uncultured fungi (0.51%) were the minor fungal phyla. At the genus level, six fungal genera were identified, namely *Guehomyces*, *Saccharomyces*, *Cladosporium*, *Mrakia*, *Pichia*, and *Rhodospiridium* (Fig. 4A). The most abundant genus was *Guehomyces* (67.57%), followed by *Saccharomyces* (3.09%), while the other four genera were present in low amounts (all about 0.13%). Unidentified fungi constituted 28.83% of all clean reads.

A total of six fungal species were identified (Fig. 4B). An interest finding of the current work is the observation of the major fungal species, *Guehomyces pullulans*; its

Fig. 4 Relative abundances of fungi identified in the tibico sample at the genus (A) and species (B) levels

presence in tibico samples is firstly reported by this study. This result is contrasting to some previous studies (Leite et al., 2012; Marsh et al., 2013). The discrepancy may due to the regional difference between samples. The current sample was collected from a local household of Tianshan region, where the temperature is rather low annually. Such climatic difference may contribute to shaping the microbiota structure of fermented products. The psychrotolerant yeast *Guehomyces pullulans* has the potential to produce β -galactosidase at low temperature. Beta-galactosidase is an enzyme commonly used in both the food and chemical industries. It hydrolyzes lactose to glucose and galactose; thus, its presence may improve the suitability of the fermented products to lactose intolerant individuals (Nakagawa et al., 2006).

Saccharomyces cerevisiae was a minor fungal species within the tibicos microbiota. This species has previously been reported to be present in the tibico grains (da Miguel et al., 2011; Leite et al., 2012). Beta-1,3-glucan is a major cell wall component of *Saccharomyces cerevisiae* that has a significant immune stimulatory effect (Shokri and Asadi, 2008). The beta-glucan originated from *Saccharomyces cerevisiae* has shown potent antitumor properties (Ning et al., 2016). The remaining 4 species together with the unclassified sequences made up < 1% and 29.2% of the total fungal sequences, respectively.

The species *Gluconacetobacter liquefaciens*, *Gluconobacter oxydans*, *Lactobacillus hilgardii*, *Lactobacillus paracasei*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Zymomonas mobilis*, and *Saccharomyces cerevisiae* found in this study were also identified in tibico grains from other countries (da Miguel et al., 2011; Gultiz et al., 2011; 2013). Theoretically, the difference in the microbiota composition of the tibico grains should not be too obvious even though their difference in geographical origin as long as a similar growth environment is provided. However, over time and under different environmental

conditions, particularly different ways of domestic handling and cultivation, the microbiota within the grains might have evolved divergently. Such differences might further influence the fermentation properties and organoleptic characteristics of products. On the other hand, the differences in the microbial composition of tibico grains can also be a result of the different detection and identification techniques used (Gultiz et al., 2013; Leite et al., 2012).

Traditional fermented foods are obtained of natural fermentations of complex microbial communities. However, the natural fermentation approach may risk the presence of pathogens. Thus, it is important to characterize the microbial consortium in the tibico beverages to ensure safe human consumption. Our results did not detect sequences from major food pathogens. Instead, many of the detected microorganisms are potentially beneficial to human health and confer desirable product features.

In conclusion, we have described the microbial profile of a Chinese Tianshan tibicos sample by SMRT sequencing technology. The dominant population was composed mainly of the species *Leuconostoc mesenteroides*, *Lactococcus raffinolactis*, and *Guehomyces pullulans*. Our work here is only a first step in characterizing the microbial members present in such kinds of samples; further studies are needed to connect the role of individual microbial members with the organoleptic characteristics, fermentation properties, and beneficial effects of this popular beverage.

Acknowledgements The authors wish to thank those who provided the tibico samples for use in this study and the National Natural Science Foundation of China for financial support of this research (Grant No. 31571815).

Compliance with ethics standards

Conflict of interest The authors declare no conflict of interest.

Human and animal rights This paper does not contain any studies with human or animal subject.

References

- Boucher I, Vadeboncoeur C, Moineau S. Characterization of genes involved in the metabolism of alpha-galactosides by *Lactococcus raffinolactis*. Appl. Environ. Microb. 69: 4049–4056 (2003)
- Chen X, Li J, Zhang L, Xu X, Wang A, Yang Y. Control of postharvest radish decay using a *Cryptococcus albidus* yeast coating formulation. Crop Prot. 41: 88–95 (2012)
- Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrity GM, Tiedje JM. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Res. 35: D169–D172 (2007)
- da Miguel MG, Cardoso PG, Magalhães KT, Schwan RF. Profile of microbial communities present in tibico (sugary kefir) grains from different Brazilian States. World J. Microbiol. Biotechnol. 27: 1875–1884 (2011)
- Davidovic S, Miljkovic M, Antonovic D, Rajilic-Stojanovic M, Dimitrijevic-Brankovic S. Water Kefir grain as a source of potent dextran producing lactic acid bacteria. Hem. Ind. 69: 595–604 (2015)
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. Real-time DNA sequencing from single polymerase molecules. Science. 323: 133–138 (2009)
- Einsfeldt K, Baptista IC, Pereira JC, Costa-Amaral IC, Costa ES, Ribeiro MC, Land MG, Alves TL, Larentis AL, Almeida RV. Recombinant L-Asparaginase from *Zymomonas mobilis*: A potential new antileukemic agent produced in *Escherichia coli*. PLoS ONE 11: e0156692 (2016)
- Gulitz A, Stadie J, Ehrmann MA, Ludwig W, Vogel RF. Comparative phylobiomic analysis of the bacterial community of water kefir by 16S rRNA gene amplicon sequencing and ARDRA analysis. J. Appl. Microbiol. 114: 1082–1091 (2013)
- Gulitz A, Stadie J, Wenning M, Ehrmann MA, Vogel RF. The microbial diversity of water kefir. Int. J. Food Microbiol. 151: 284–288 (2011)
- Hagi T, Hoshino T. Screening and characterization of potential probiotic lactic acid bacteria from cultured common carp intestine. Biosci. Biotech. Bioch. 73: 1479–1483 (2009)
- Hou Q, Xu H, Zheng Y, Xi X, Kwok LY, Sun Z, Zhang H, Zhang W. Evaluation of bacterial contamination in raw milk, ultra-high temperature milk and infant formula using single molecule, real-time sequencing technology. J. Dairy Sci. 98: 8464–8472 (2015)
- Ikeda S, Fujimura T, Ytow N. Potential application of ribosomal intergenic spacer analysis to the microbial community analysis of agronomic products. J. Agric. Food Chem. 53: 5604–5611 (2005)
- Jany JL, Barbier G. Culture-independent methods for identifying microbial communities in cheese. Food Microbiol. 25: 839–848 (2008)
- Köljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22: 5271–5277 (2013)
- Lee SH, Chang HC. Isolation of antifungal activity of *Leuconostoc mesenteroides* TA from kimchi and characterization of its antifungal compounds. Food Sci. Biotechnol. 25: 213–219 (2016)
- Lee YG, Cho J-Y, Moon J-H. Isolation and antioxidative activity of amino acid derivatives produced by *Leuconostoc mesenteroides*. Food Sci. Biotechnol. 25: 329–334 (2016)
- Leite AM, Mayo B, Rachid CT, Peixoto RS, Silva JT, Paschoalin VM, Delgado S. Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiol. 31: 215–221 (2012)
- Li X, Li J, Li M, Meng X. Screening and functional properties of cholesterol-degrading lactic acid bacteria from Jiangshui. Wei Sheng Wu Xue Bao = Acta microbiologica Sinica. 55: 1001–1009 (2015)
- Lick S, Keller M, Bockelmann W, Heller KJ. Optimized DNA extraction method for starter cultures from yoghurt. Milchwissenschaft. 51: 183–186 (1995)
- Liu W, Zheng Y, Kwok LY, Sun Z, Zhang J, Guo Z, Hou Q, Menhe B, Zhang H. High-throughput sequencing for the detection of the bacterial and fungal diversity in Mongolian naturally fermented cow's milk in Russia. BMC Microbiol. 15: 45 (2015)
- Lorenzetti MFS, Moro MR, García-Cruz CH. Alginate/PVA beads for levan production by *Zymomonas mobilis*. J. Food Process Eng. 38: 31–36 (2015)
- Marsh AJ, O'Sullivan O, Hill C, Ross RP, Cotter PD. Sequence-based analysis of the microbial composition of water kefir from multiple sources. FEMS Microbiol. Lett. 348: 79–85 (2013)
- Meslier V, Loux V, Renault P. Genome sequence of *Lactococcus raffinolactis* strain 4877, isolated from natural dairy starter culture. J. Bacteriol. 194: 6364 (2012)
- Mosher JJ, Bernberg EL, Shevchenko O, Kan J, Kaplan LA. Efficacy of a 3rd generation high-throughput sequencing platform for analyses of 16S rRNA genes from environmental samples. J. Microbiol. Meth. 95: 175–181 (2013)
- Muhialdin BJ, Hassan Z, Abu Bakar F, Alghoory HL, Saari N. Novel antifungal peptides produced by *Leuconostoc mesenteroides* DU15 effectively inhibit growth of *Aspergillus niger*. J. Food Sci. 80: M1026–M1030 (2015)
- Nakagawa T, Ikehata R, Uchino M, Miyaji T, Takano K, Tomizuka N. Cold-active acid beta-galactosidase activity of isolated psychrophilic-basidiomycetous yeast *Guehomyces pullulans*. Microbiol. Res. 161: 75–79 (2006)
- Ning Y, Xu D, Zhang X, Bai Y, Ding J, Feng T, Wang S, Xu N, Qian K, Wang Y, Qi C. Beta-glucan restores tumor-educated dendritic cell maturation to enhance antitumor immune responses. Int. J. Cancer. 138: 2713–2723 (2016)
- Rodina NN, Shutova VV, Revin VV. Effect of sucrose on the growth and dextransucrase activity of *Leuconostoc mesenteroides*. J. Biotechnol. 208: S117 (2015)
- Shokri H, Asadi F, Khosravi AR. Isolation of β -glucan from the cell wall of *Saccharomyces cerevisiae*. Nat. Prod. Res. 22: 414–421 (2008)
- Terzic-Vidojevic A, Mihajlovic S, Uzelac G, Veljovic K, Tolinnacki M, Nikolic M, Topisirovic L, Kojic M. Characterization of lactic acid bacteria isolated from artisanal Travnik young cheeses, sweet creams and sweet kajmaks over four seasons. Food Microbiol. 39: 27–38 (2014)
- Vardjan T, Mohar Lorbeg P, Rogelj I, Canzek Majhenic A. Characterization and stability of lactobacilli and yeast microbiota in kefir grains. J. Dairy Sci. 96: 2729–2736 (2013)
- Wang H, Cao S, Wang WT, Wang KT, Jia X. Very high gravity ethanol and fatty acid production of *Zymomonas mobilis* without

- amino acid and vitamin. *J. Ind. Microbiol. Biot.* 43: 861–871 (2016)
- Yao G, Yu J, Hou Q, Hui W, Liu W, Kwok LY, Menghe B, Sun T, Zhang H, Zhang W. A perspective study of koumiss microbiome by metagenomics analysis based on single-cell amplification technique. *Front. Microbiol.* 8: 165 (2017)
- Yoon EJ, Yoo SH, Cha J, Lee HG. Effect of levan's branching structure on antitumor activity. *Int. J. Biol. Macromol.* 34: 191–194 (2004)
- Zhang A, Guo E, Qian L, Tang NY, Watt RM, Bartlam M. Purification, crystallization and X-ray crystallographic analysis of a putative exopolyphosphatase from *Zymomonas mobilis*. *Acta Crystallogr.* 72: 172–178 (2016)