LWT - Food Science and Technology 85 (2017) 151–157

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Microbial diversity of traditional kefir grains and their role on kefir aroma

Enes Dertli [a, *], Ahmet Hilmi Çon [b]

[a] Department of Food Engineering, Faculty of Engineering, Bayburt University, Bayburt, Turkey
[b] Department of Food Engineering, Faculty of Engineering, Ondokuz Mayıs University, Samsun, Turkey

ARTICLE INFO

Article history:
Received 1 June 2017
Received in revised form
7 July 2017
Accepted 11 July 2017
Available online 14 July 2017

Keywords:
Kefir grains
Kefir
Pyrosequencing
Aroma profile

ABSTRACT

Kefir grains consist of rich bacterial and fungal microflora responsible for the production of this traditional fermented milk beverage with unique flavour properties. Here, a pyrosequencing approach was applied for the identification of microbial flora of four kefir grains collected from different regions of Turkey and the volatile compounds in kefir samples produced with these grains were determined. *Lactobacillus kefiranofaciens* presented in all grains at important levels and *Enterobacter*, *Acinetobacter*, *Enterococcus* and *Pseudomonas* spp. were observed in traditional kefir grains. The fungal microflora of kefir grains was dominated by yeast species and *Dipodascaceae* family was dominant and *Saccharomyces cerevisiae* presented in all grains. Other yeast species belonging to *Kazachstania*, *Candida*, *Issatchenkia* and *Rhodotorula* species were also detected in kefir grains. Volatile compounds of kefir samples were also diverse related to the microbial diversity of kefir grains. This study revealed the rich microflora of Turkish kefir grains and their interactions with the aromatic properties of kefir.

1. Introduction

Kefir is a traditional fermented milk beverage thought to be originated from Caucasus and Anatolia regions (Farnworth & Mainville, 2003) that is produced by the biochemical functions of microbial species present in kefir grains as starter cultures (Leite et al., 2012). Kefir grains are small, irregularly shaped granules with a cauliflower-like appearance (Güzel-Seydim, Seydim, Greene, & Bodine, 2000) that contains a diverse microbial community formed by bacterial and yeast species (Leite et al., 2012; Walsh et al., 2016) present in a special exopolysaccharide matrix called kefiran (Riviére, Kooiman, & Schmidt, 1967). Typically, kefir is produced by the inoculation of kefir grains, which contain different microbial species including bacteria and fungi, to the milk followed by the incubation at room temperature for approximately 24 h (Walsh et al., 2016) which results in a unique self-carbonated beverage with exotic sour and yogurt-like taste properties (Güzel-Seydim et al., 2000). The formation of this flavour as well as other characteristics mainly depends on the microbial community of kefir grains (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003) and the type of kefir milk can also be important. So far the microbial community of kefir grains have been investigated by mainly culture dependant methods (Angulo, Lopez, & Lema, 1993; Garrote, Abraham, & De Antoni, 2001; Taş, Ekinci, & Güzel-Seydim, 2012) and in the last decade both culture dependant and independent techniques were used for the identification of microbial community of kefir grains (Walsh et al., 2016; Zhou, Liu, Jiang, & Dong, 2009). The culture independent techniques were mainly based on denaturing gradient gel electrophoresis (DGGE) analysis previously (Kesmen & Kacmaz, 2011; Wang, Li, Jia, Wu, & Guo, 2006; Zhou et al., 2009) but recently next generation sequencing (NGS) became the main technique for the microbial profiling of kefir and kefir grains (Gao et al., 2013; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013; Nalbantoglu et al., 2014; Walsh et al., 2016). This type of analysis is important especially for kefir grains from different parts of world as kefir grains from different regions can have different bacterial and fungal communities (Miguel, Cardoso, de Assis Lago, & Schwan, 2010).

Kefir grains have a diverse bacterial flora and mainly *Lactobacillus* species dominate the grains although presence of *Leuconostoc*, *Lactococcus*, *Streptococcus*, *Acetobacter*, *Pseudomonas*, *Acinetobacter* as well as some other species were shown in different grains (Gao et al., 2013; Leite et al., 2012; Nalbantoglu et al., 2014; Walsh et al., 2016). Similarly, fungal microflora mainly dominated by yeast

* Corresponding author. Department of Food Engineering, Faculty of Engineering, Bayburt University, Bayburt, 69000, Turkey.
E-mail address: enes.dertli@hotmail.com (E. Dertli).

http://dx.doi.org/10.1016/j.lwt.2017.07.017
0023-6438/

species is also diverse and species belonging to *Saccharomyces*, *Kazachstania*, *Kluyveromyces*, *Pichia*, *Issatchenkia* and *Dekkera* were reported to be found in kefir grains (Diosma, Romanin, Rey-Burusco, Londero, & Garrote, 2014; Marsh et al., 2013; Zhou et al., 2009). Therefore, identification of microbial species presented in kefir grains is crucial in order to reveal the technological and therapeutic potentials of these microorganisms. Additionally, these microbial species present in kefir grains are not only important for the production of kefir but also are responsible for the production of volatile-compounds in kefir which result in the formation of the unique flavour properties of this traditional fermented milk product. So, identification of the aroma profile of kefir produced with different kefir grains is important in order to understand the role of different microbial groups on the formation of kefir aroma which can affect its final quality (Beshkova et al., 2003; Walsh et al., 2016).

As the microflora of kefir grains is responsible for the formation of kefir and its aroma characteristics which can be affected by the origin of the kefir grains, in this study, a pyrosequencing approach was applied to elucidate the bacterial and fungal microflora of four kefir grains collected from different regions of Turkey. Additionally, volatile components forming the kefir aroma produced by the biochemical functions of microflora of kefir grains were assessed by GC-MS analysis.

2. Material and methods

2.1. Kefir grains and kefir fermentations

Four traditional kefir grains were obtained from four different geographical regions of Turkey (İstanbul, Kayseri, Balıkesir and İzmir) and kefir grains A-B and C-D were produced in goat and cow milk's, respectively. Kefir grains were activated in laboratory using pasteurised cow milk at 25 °C followed by the removal of the clotted milk by filtering and a final rinsing step with sterile water was applied in order to prepare kefir grains for next step. This activation step was applied for five times and following the sixth propagation, kefir grains were further used for total DNA isolation. Kefir samples in this step were used for the determination of the aroma profile of kefir produced by these four grains.

2.2. Total DNA extraction from kefir grains

Total DNA was extracted from kefir grains. Briefly, in total 250 mg of kefir grain samples were removed from different parts of kefir grains and were then used for the total DNA extraction. A commercial Genematrix food-extract DNA purification kit (Eurx, Poland) was used for DNA extraction using manufacturer's protocol but an additional first step of heating kefir grains at 90 °C for 15 min was added to the protocol in order to eliminate the blocking effect of kefiran. Following the isolation process, total DNA was visualised in agarose gel and further processed.

2.3. Amplicon sequencing

Libraries of 16S rDNA and ITS PCR were generated for each kefir grain samples using locus specific primers targeting V1–V3 hypervariable regions of 16S rRNA gene and ITS region for discrimination of bacterial and fungal communities, respectively. Following the purification of each PCR products, a subsequent PCR, for indexing, was performed using the Nextera XT index kit (FC-131-1001/FC-131-1002), specific to Illumina technology. After amplification of both regions PCR products were treated as described in the Illumina protocol. Samples were then sequenced on Illumina MiSeq platform in IGA Technology Services (Udine, Italia) in accordance with standard Illumina sequencing protocols.

2.4. Bioinformatic analysis

Following the sequencing, data were analysed with Qiime (1.9.0) software (Caporaso et al., 2010) and Usearch (8.1.1756, 32-bit) algorithm (Edgar, 2010) allowed the chimera filtering, grouping of replicate sequences; sorting sequences per decreasing abundance and OTU identification steps. Taxonomy of both regions was assigned with a BLAST search against modified GreenGene database (version 2013_8) and UNITE database for 16S rRNA and ITS regions, respectively. In both pipelines OTUs are constructed at 97% of identity and taxonomy assigned with a Confidence threshold $\geq$0.5. All the biosample raw reads have been deposited at the National Center for Biotechnology Information (NCBI) and are available under the Bioproject ID PRJNA379734.

2.5. Volatile-compound profiling of kefir by GC-MS analysis

The volatile compounds in kefir samples produced by different kefir grains were determined by Headspace-SPME technique using GC-MS (GC-2010; Shimadzu corporation, Kyoto, Japan) and a slightly-modified method of Plessas et al. (2008) was used. For the SPME analysis, 5 mL kefir sample inserted to 20 mL SPME vial with a silicone septum and vial was dipped to the water bath at 55 °C and SPME fiber (2 cm–50/30 mm Supelco, Bellefonte, USA) was exposed to headspace for 50 min. The fiber was retracted and injected into GC inlet and desorbed for 5 min at 250 °C. The samples were analysed in duplicate and a stabilwax column (60 m, 0.32 mm id. 0.25 μm, Restek-Stabilwax, Polyethylene glycol, USA) was used for analysis. The carrier gas was helium and the flow rate was 3 mL/min, at a pressure value of 124.2 kPa. The temperature of the column was set to 40 °C held for 1 min, increased at 7 °C/min to 100 °C and held for 5 min at this temperature followed by the increment of temperature at 4 °C/min to 130 °C and held for 5 min at this temperature, increased by 2 °C/min to 180 °C held for 1 min and then, increased by 15 °C/min to 250 °C and held at that state for 4 min. Total run time was 57.74 min. The interface temperature was 230 °C, the mass range was *m/z* 35–450. Compounds were identified by mass spectrum comparisons to National Institute of Standards and Technology (NIST), Wiley (Wiley Registry of Mass Spectral Data, 7th Edition) and Flavor and Fragrance Natural and Synthetic Compounds (FFNSC) mass spectral libraries.

3. Results and discussion

Kefir is one of the most popular fermented milk products worldwide due to its potential health promoting functions originating from the microbial species that kefir grains have which are also responsible for kefir production. Recent interest in kefir fermentation technology is the identification of the microbial diversity of kefir grains with traditional origin using NGS techniques which allows identification of OTUs at various taxonomic levels including species levels and this approach paved the way to quickly understand formation of microbial floras of fermented food products including kefir and became one of the most preferred methodologies although culture dependent approach is still crucial. From this perspective, this study aimed to unveil the microflora of four kefir grains collected from four different regions of Turkey including bacterial and fungal species and investigate the aromatic profile of kefir samples produced by these kefir grains.

3.1. Kefir grains and bacterial diversity

A high level of diversity was observed in kefir grains and five bacterial phyla that were Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria and Bacteroidetes were identified in kefir