International Journal of Food Microbiology 220 (2016) 63–72

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Kombucha tea fermentation: Microbial and biochemical dynamics

Somnath Chakravorty [a,1], Semantee Bhattacharya [a,1], Antonis Chatzinotas [b], Writachit Chakraborty [a], Debanjana Bhattacharya [a], Ratan Gachhui [a,*]

[a] Department of Life Science and Biotechnology, Jadavpur University, 188 Raja S. C. Mallick Road, Kolkata 700032, India
[b] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research — UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

ARTICLE INFO

Article history:
Received 28 June 2015
Received in revised form 24 December 2015
Accepted 31 December 2015
Available online 7 January 2016

Keywords:
Kombucha tea
Microbial community structure and dynamics
High throughput sequencing
T-RFLP
Change in fermentation
Radical scavenging

ABSTRACT

Kombucha tea, a non-alcoholic beverage, is acquiring significant interest due to its claimed beneficial properties. The microbial community of Kombucha tea consists of bacteria and yeast which thrive in two mutually non-exclusive compartments: the soup or the beverage and the biofilm floating on it. The microbial community and the biochemical properties of the beverage have so far mostly been described in separate studies. This, however, may prevent understanding the causal links between the microbial communities and the beneficial properties of Kombucha tea. Moreover, an extensive study into the microbial and biochemical dynamics has also been missing. In this study, we thus explored the structure and dynamics of the microbial community along with the biochemical properties of Kombucha tea at different time points up to 21 days of fermentation. We hypothesized that several biochemical properties will change during the course of fermentation along with the shifts in the yeast and bacterial communities. The yeast community of the biofilm did not show much variation over time and was dominated by *Candida* sp. (73.5–83%). The soup however, showed a significant shift in dominance from *Candida* sp. to *Lachancea* sp. on the 7th day of fermentation. This is the first report showing *Candida* as the most dominating yeast genus during Kombucha fermentation. *Komagateibacter* was identified as the single largest bacterial genus present in both the biofilm and the soup (~50%). The bacterial diversity was higher in the soup than in the biofilm with a peak on the seventh day of fermentation. The biochemical properties changed with the progression of the fermentation, i.e., beneficial properties of the beverage such as the radical scavenging ability increased significantly with a maximum increase at day 7. We further observed a significantly higher D-saccharic acid-1,4-lactone content and caffeine degradation property compared to previously described Kombucha tea fermentations. Our data thus indicate that the microbial community structure and dynamics play an important role in the biochemistry of the fermentation of the beverage. We envisage that combined molecular and biochemical analyses like in our study will provide valuable insights for better understanding the role of the microbial community for the beneficial properties of the beverage.

1. Introduction

Kombucha tea is a traditional non-alcoholic fermented beverage originating in the Orient and its inception is shrouded in mystery (Teoh et al., 2004). The beverage has gained substantial popularity especially in the West because of a large number of claims regarding its therapeutic potential against a host of maladies. Some of its beneficial effects have already been demonstrated such as: anti-microbial, anti-oxidant, anti-carcinogenic (Jayabalan et al., 2011), anti-diabetic (Aloulou et al., 2012; Bhattacharya et al., 2013), treatment for gastric ulcers (Banerjee et al., 2010) and high cholesterol (Yang et al., 2009), etc. It has also shown to have impact on immune response (Ram et al., 2000) and liver detoxification (Loncar et al., 2000).

Kombucha tea fermentation is the product of microbial activity by a consortium of both yeast and bacteria (Jarrell et al., 2000). The microorganisms, produce in the course of the fermentation, a thick cellulosic biofilm on the liquid–air interface. In contrast, most of the biofilms that have been studied to date are formed either on liquid–solid or air–solid interface (Nikolaev and Plakunov, 2007).

The beverage is prepared by adding a small portion of the biofilm into sweetened (10% *w*/*v*) black tea. The broth also contains 10–15% of previously fermented Kombucha tea (called hereafter as old soup for convenience). The fermentation is static and the usual fermentation time is 7–12 days at room temperature (Dutta and Gachhui, 2006, 2007). Thus the Kombucha tea microbial community can be divided into two parts; the first one being the cellulosic biofilm and the second one thriving in the underlying liquid or soup. Studies have reported that the entire microbial spectrum of this beverage is dominated by acetic

* Corresponding author at: Department of Life Science and Biotechnology, Jadavpur University, 188 Raja S. C. Mallick Road, Kolkata 700032, India.
E-mail address: ratangachhui@yahoo.com (R. Gachhui).
[1] Equal contributors.

http://dx.doi.org/10.1016/j.ijfoodmicro.2015.12.015
0168-1605/

acid bacteria (AAB) and yeast (Jarrell et al., 2000; Jayabalan et al., 2014; Marsh et al., 2014). Previous explorations however indicated that the microbial community may vary between different Kombucha fermentations across the globe depending upon the source of the inoculum used (Malbaša et al., 2011). The majority of these analyses have been culture-based so far (Dutta and Gachhui, 2006, 2007; Hesseltine, 1965; Liu et al., 1996; Teoh et al., 2004). Only recently, Marsh et al. (2014) has performed sequence-based analysis of the bacterial and yeast populations of Kombucha tea.

The enhanced beneficial activities of Kombucha tea compared to that of the unfermented tea (Banerjee et al., 2010; Bhattacharya et al., 2013; Yang et al., 2009) indicated that some changes have been brought by the microbial community during the fermentation process. Most of the scientific literature available so far either deals with the general microbial community of the system or the beneficial effects of the drink. Extensive exploration of the microbial community dynamics of both the biofilm and the soup along with the dynamics of the fermentation is very scarce. Such an investigation may eventually allow the correlation of the microbial community to the fermentation parameters and thus improve the management and the control of the system.

In this paper we describe concomitantly for the first time the yeast and bacterial community dynamics in both the biofilm and the soup of a Kombucha tea maintained in our laboratory by culture independent methods and also the changes in the fermentation biochemistry and antioxidant property of Kombucha tea during 21 days of fermentation. We hypothesize that several biochemical properties will change during fermentation together with shifts in the microbial communities. High throughput sequencing (HTS) of the yeast internal transcribed spacer 2 (ITS2) and the D1–D2 region of the large sub-unit (LSU) ribosomal RNA (rRNA) gene, as well as of the V3 region of the bacterial 16S rRNA gene was performed for a detailed description of the microbial communities in both compartments. Terminal restriction fragment length polymorphism (T-RFLP) analysis was chosen to monitor bacterial community dynamics during the fermentation process.

2. Materials and methods

2.1. Preparation of Kombucha tea

The Kombucha tea system was maintained in our laboratory as described previously by Bhattacharya et al. (2013). For determining the structure and dynamics of the microbial community, a total of sixteen, 200 mL Kombucha tea batches were maintained. These 16 batches were divided into 4 sets, each set having 4 replicate Kombucha tea systems. Four replicates were prepared so that each replicate was completely harvested after its stipulated period of fermentation. The harvesting was done after 3, 7, 14 and 21 days of fermentation. One set was for the yeast, two sets were for the bacterial T-RFLP and the last set for bacterial next-generation sequencing. The batches were maintained at 28 ± 2 °C.

2.2. Isolation of total DNA

The isolation of DNA for bacteria from both compartments was done using the QIAamp DNA stool mini kit (Qiagen, USA) following the manufacturer's protocol. However for the yeast DNA, a combined protocol using Zymolyase (USBiologicals, USA) and QIAamp DNA stool mini kit was employed, as either of the protocols alone failed to produce quality yeast DNA for this analysis.

DNA isolation from the biofilm required a pre-treatment, which included transfer of the entire biofilm (10 cm diameter) into a beaker and incubation with 750 μL cellulase (Sigma-Aldrich, Germany) and 25 mL of sterile distilled water at room temperature for 30 min. Subsequently the biofilm was manually homogenized. The sample was then centrifuged at 4588 ×*g* for 6 min and the supernatant was discarded. Five mL sorbitol buffer (1 M Sorbitol, 50 mM Tris pH 7.5) supplemented with 30 mM DTT was added to the biofilm and incubated for 10 min at 30 °C. The suspension was centrifuged at 4588 ×*g* for 6 min. Resuspension in 5 mL sorbitol buffer/2 mM DTT was followed by incubation with 400 U of Zymolyase at 30 °C for 45 min. The mixture was then used to isolate the DNA following the manufacturer's protocol. For isolation of total DNA from the soup, 200 mL of the beverage was centrifuged and the pellet was used subsequently using the manufacturer's protocol.

2.3. Next generation sequencing and data analysis

The total yeast DNA from all 4 time points served as templates to amplify the internal transcribed spacer ITS2 and the D1–D2 region of the LSU rRNA genes respectively, using Ion Torrent specific barcoded fusion oligonucleotide primers and PCR protocols as described previously by Tonge et al. (2014). Similarly the bacterial total DNA from the 7 day biofilm and soup were used to amplify the V3 region of the 16S rRNA gene using the primers 341F and 518R as described previously by Mühling et al. (2008). The amplified bacterial products were then attached with dual indices and Illumina sequencing adapters using the Nextera XT Index Kit (Illumina, USA) following manufacturer's protocol. All amplicons were quality checked for fragment size and DNA concentration in a Bioanalyser 2100 (Agilent Technologies). The yeast amplicons were sequenced by the Personal Genome Machine using a 400 bp sequencing kit with a 316v2 chip used for each amplicon in accordance with the manufacturer's standard protocol. The bacterial amplicons were sequenced in an Illumina MiSeq platform (Illumina) using manufacturer's protocol.

Following sequencing the yeast raw data was first filtered within the PGM software to remove low quality and polyclonal sequences. Sequences matching the PGM 3′ adaptor were also automatically trimmed. The reverse primer sequences were trimmed using CutAdapt software. Full length reads were processed using the USEARCH algorithm followed by de-replication of identical reads and individual reads de-noised and chimeric sequences were eliminated using the QIIME pipeline following default parameters (Caporaso et al., 2010). Similarly the bacterial reads were first assembled into candidate 16S rRNA genes from the paired end reads using tools from the UPARSE package and a maximum of two mismatches were allowed in the overlapping region. During this analysis the low quality and polyclonal sequences were filtered out. The reads were then subjected to de-replication using the tool from UPARSE Package in Prefixed De-Replication Mode. Minimum length cut-off of 64 base pairs was applied (Edgar, 2013). De-replicated reads were then de-noised followed by chimera checked using the RDP Gold Database (Cole et al., 2013). The OTU clustering was done at 97% similarities and the clustered OTUs were phylogenetically identified by using the Silva 108 release database (Quast et al., 2013) for the yeast and the bacterial identification was done using the SILVA Incremental Aligner (SINA) tool (Pruesse et al., 2012) against the SILVA and Greengenes databases. In both cases a minimum identity cutoff of 97% was applied for the phylogenetic assignments. The relative abundance was calculated on the basis of the number of sequences assigned to each taxa against the total number of sequences. The raw files were submitted to the SRA database under the BioSample IDs: SAMN02999971, SAMN02595524 and SAMN02602923.

2.4. Terminal restriction fragment length polymorphism (T-RFLP) analysis

T-RFLP analysis with 16S rRNA genes was performed as described previously (Giebler et al., 2013; Giebler et al., 2014). Briefly 16S rRNA genes were amplified using a 6-carboxyfluorescein (6-FAM)-labeled forward primer 27f and 1525r (Lane, 1991; Weisburg et al., 1991). Purified PCR products from each sample were digested overnight at 37 °C with 2 U MspI (NEB, USA). T-RFLP analysis was run on an ABI PRISM 3100 genetic analyzer system using the GeneScanTM 500