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Microbial biogeography of the transnational fermented milk matsoni

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ABSTRACT

The fermented milk matsoni is a traditional, national food product of both Georgia and Armenia. Little is known about the effects of biogeography and milk type on the microbial biodiversity of matsoni or the fungal composition of matsoni fermentations. High-throughput marker-gene sequencing was used to survey the bacterial and fungal communities of matsoni from different milk types and regions throughout Armenia and Georgia. Results demonstrate that both production region and milk type influence matsoni microbiota, suggesting that the traditional production methods preserve the transfer of unique regional microbiota from batch to batch. Bacterial profiles were dominated by *Lactobacillus* and *Streptococcus* species. Yeast profiles varied dramatically, with *Kluyveromyces marxianus*, *Candida famata, Saccharomyces cerevisiae, Lodderomyces elongisporus,* and *Kluyveromyces lactis* being the most important species distinguishing production regions and milk types. This survey will enable more detailed capture and characterization of specific microbiota detected within these fermentations.

1. Introduction

Matsoni (*syn.* mazun, matsoon) is a traditional Caucasian fermented milk product largely used from ancient times. It is considered an analog of yogurt and prepared from milk of cow, sheep, goat, buffalo, or a mixture thereof. Matsoni is made from heated or pasteurized milk, cooled to 35–42 °C, and inoculated with a portion (approximately 1% wt/wt) of finished matsoni, thereby maintaining an ongoing culture consortium of lactic acid bacteria and yeasts. It is fermented at 35–42 °C overnight and the finished product has a characteristic pleasant, cultured milk taste and aroma. It is a national food throughout the Caucasus, where it is widely considered to have beneficial health effects, particularly for intestinal disorders, and to increase longevity.

In contrast to yogurt, which is fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, matsoni microbiota comprise more abundant and different microbial species. Essentially important is that the microbial composition of matsoni apparently differs from various Caucasian areas but is very stable and characteristic for the region of origin (Afrikyan, 2009, 2012). Several studies have described the microbiota of matsoni fermentations, variously reporting *Lactobacillus delbruekii* subsp. *lactis, L. delbrueckii* subsp. *bulgaricus, L. acidophilus*, *S. thermophilus, Lactococcus lactis, L. lactis* subsp. *cremoris, Geotrichum candidum, Saccharomyces, Candida,* and other species of yeasts (Saroukhanian, 1960; Yerzinkian, 1965, 1971; Ter-Ghazarian, 1993; Afrikian, 2009, 2012; Quero et al., 2014; Uchida et al., 2007; Reddy et al., 1986; Merabishvili and Chanishvili, 2001). Other bacteria, including *Lactobacillus helveticus*, *Lactobacillus paracasei*, and *Leuconostoc lactis* are also involved in some fermentations (Quero et al., 2014). This inconsistent bacterial composition has led some to conclude that the bacterial diversity of matsoni fermentations may be

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influenced by region of production (Uchida et al., 2007; Afrikian, 2009, 2012). Such biogeographical patterns have been described previously in other food fermentations (Bokulich and Mills, 2013a; Bokulich et al., 2014b; Yu et al., 2011) and may explain regional differences in matsoni product qualities, but a comprehensive survey of regional matsoni fermentations has yet to be described. The effect of milk type on microbial composition is similarly uncharacterized in matsoni or similar dairy products.

The goal of this study was to better characterize how production region and milk type shape the bacterial and fungal constitution of matsonis in Armenia and Georgia. We used high-throughput amplicon sequencing as a culture-independent surveillance tool to characterize regional and milk-derived patterns in the bacterial and yeast populations of matsoni. Results indicate that regional and substrate-driven conditions shape the bacterial and fungal consortia of matsoni fermentations, and indicate that matsonis are a diverse source of microbial cultures for dairy fermentations.

2. Methods

2.1. Sample collection

Samples were collected aseptically on-site at the farms or production facilities producing matsoni, or at local markets, and transported on ice to the laboratory for immediate processing. A total of 194 samples were collected from different sites within 18 distinct regions in Georgia, Armenia, and Nagorno Karabakh (Fig. 1, Table 1). Matsoni fermentations typically last 24 h and all samples were collected from finished fermentations. DNA was extracted using the ZR-96 Fecal DNA MiniPrep Kit (Zymo Research, Irvine, CA), with bead beating in a FastPrep-24 bead beater (MP Bio, Solon, OH), and stored at −20 °C until further processing.

2.2. Marker-gene sequencing library construction

Amplification and sequencing was performed as described previously for bacterial (Bokulich et al., 2012) and yeast communities (Bokulich and Mills, 2013b). Briefly, the V4 domain of bacterial 16S rRNA genes was amplified using primers F515 (5′−*NNNNNNNN***GT**GTGCCAGCMGCCGCGGTAA−3′) and R806 (5′−GGACTACHVGGGTWTCTAAT−3′) (Caporaso et al., 2011), with the forward primer modified to contain a unique 8 nt barcode (italicized poly-N section of primer above) and 2 nt linker sequence (bold, underlined portion) at the 5′ terminus. PCR

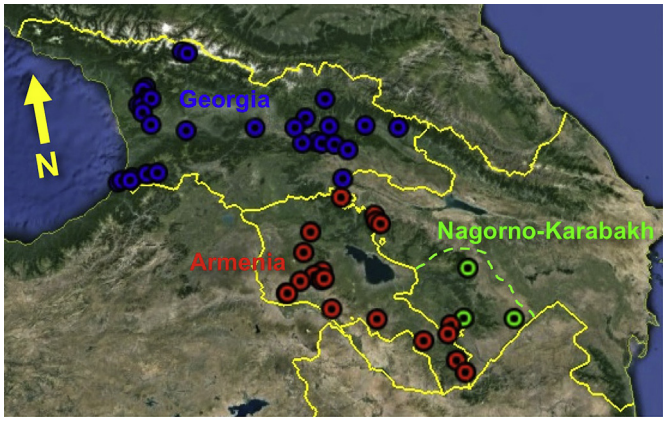

Fig. 1. Matsoni sample collection map. Each point represents the coordinates of a single sampling site within Armenia (red), Georgia (blue), or the Nagorno Karabakh region (green, bordered by dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Description of matsoni samples collected.

	Cow	Buffalo	Goat	Sheep	Total
Armenia	21	22	46	32	121
Georgia	10	10	16	8	44
Nagorno Karabakh	4	11	14	0	29
Total	35	43	76	40	194

reactions contained 5–100 ng DNA template, 1X GoTaq Green Master Mix (Promega, Madison, WI), 1 mM $MgCl_2$, and 2 pmol of each primer. Reaction conditions consisted of an initial 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension of 72 °C for 10 min. Fungal internal transcribed spacer (ITS) 1 loci were amplified with primers BITS (5′−*NNNNNNNN***CT**ACCTGCGGARGGATCA−3′) and B58S3 (5′−GAGATCCRTTGYTRAAAGTT−3′) (Bokulich and Mills, 2013b), with a unique 8 nt barcode and linker sequence incorporated in each forward primer. PCR reactions contained 5–100 ng DNA template, 1X GoTaq Green Master Mix (Promega, Madison, WI), 1 mM $MgCl_2$, and 2 pmol of each primer. Reaction conditions consisted of an initial 95 °C for 2 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, and a final extension of 72 °C for 5 min. Amplicons were combined into two separate pooled samples (keeping bacterial and fungal amplicons separate) at roughly equal amplification intensity ratios, purified using the Qiaquick spin kit (Qiagen, Valencia, CA), and submitted to the UC Davis Genome Center DNA Technologies Core for Illumina paired-end library preparation, cluster generation, and 250-bp paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

2.3. Data analysis

Raw fastq files were demultiplexed, quality-filtered, and analyzed using QIIME v.1.8.0 (Caporaso et al., 2010b). The 250-bp reads were truncated at any site of more than three sequential bases receiving a quality score <Q10, and any read containing ambiguous base calls or barcode/primer errors were discarded, as were reads with <75% (of total read length) consecutive high-quality base calls (Bokulich et al., 2013b). Reverse primer sequences were trimmed from the ends of ITS sequences following demultiplexing. Operational taxonomic units (OTUs) were clustered at 97% identity using the QIIME subsampled reference OTU-picking pipeline using UCLUST-ref (Edgar, 2010) against either the Greengenes 16S rRNA gene database (May 2013 release) (McDonald et al., 2012) or the UNITE fungal ITS database (Abarenkov et al., 2010; Koljalg et al., 2005), modified as described previously (Bokulich and Mills, 2013b). OTUs were classified taxonomically against these same databases using RDP classifier (Wang et al., 2007). Any OTU comprising less than 0.01% of total sequences for each run were removed prior to further analysis (Bokulich et al., 2013b). Bacterial 16S rRNA gene sequences were aligned using PyNAST (Caporaso et al., 2010a) against a reference alignment of the Greengenes core set (McDonald et al., 2012). From this alignment, chimeric sequences were identified and removed using ChimeraSlayer (Haas et al., 2011) and a phylogenic tree was generated from the filtered alignment using FastTree (Price et al., 2010). Sequences failing alignment or identified as chimera were removed prior to downstream analysis.

Beta-diversity (between-sample community dissimilarity) estimates were calculated within Phyloseq (McMurdie and Holmes, 2013) using weighted UniFrac (Lozupone and Knight, 2005) distance between samples for bacterial 16S rRNA reads (evenly sampled at 1500 sequences per sample) and Bray–Curtis