Fermented products – shotgun sequencing methods

DNA extractions

For each sample, 45 mL of a homogenised fermented beverage was centrifuged at 4000 rpm for 20 minutes. Approximately 0.3 g of the resulting pellet was transferred into a PowerBead Tube supplied with the DNeasy PowerSoil Pro DNA Extraction Kit (Qiagen). DNA was then extracted and purified using the DNeasy PowerSoil Pro Kit following the manufacturer's instructions. For each fermented beverage, three biological replicates were processed independently. DNA extraction was perfomed at UAB Sequench Lithuania (Klaipeda) by Aurelija Samuilovienė on 16 June 2025.

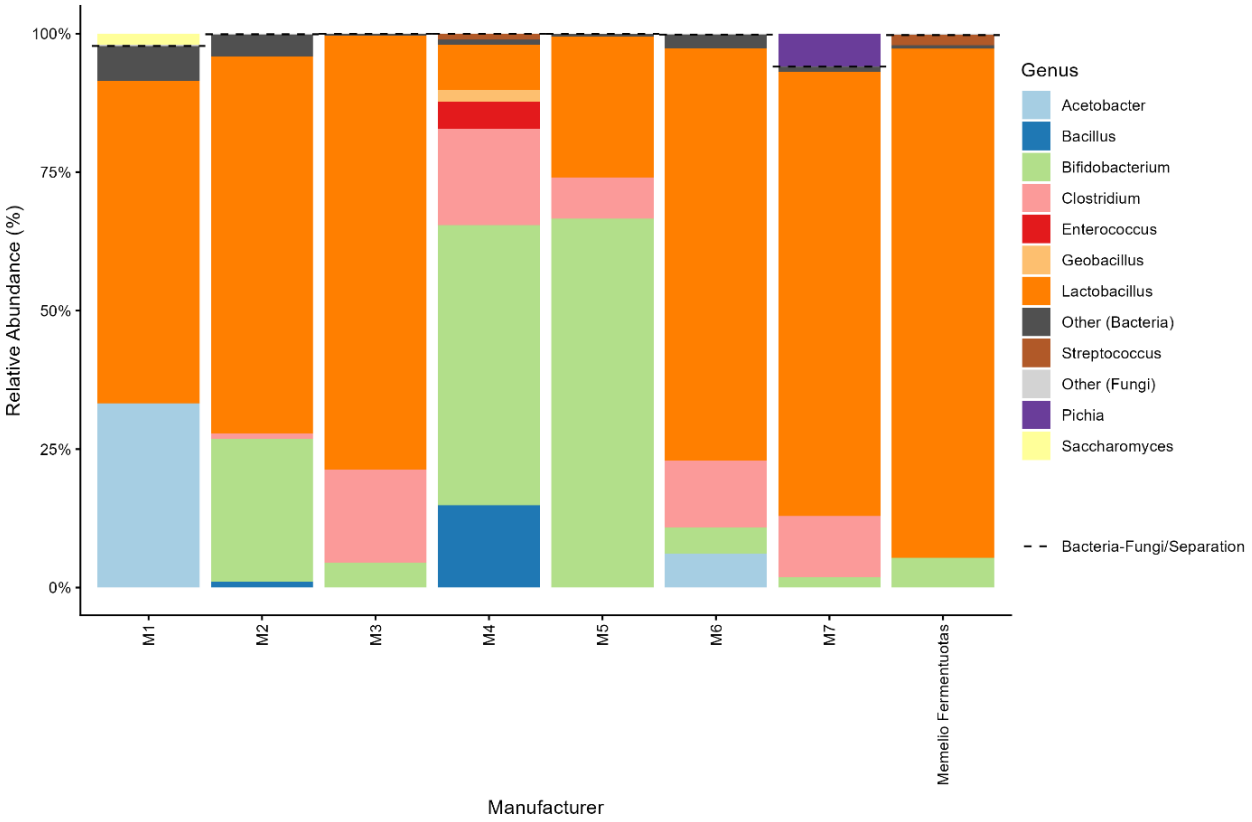
Extracted DNA samples were sent to Sequench Ltd. (Nelson, New Zealand) for further processing.

High-throughput sequencing

The genomic sequencing library was prepared using Illumina's DNA Prep library prep kit, following manufacturer's protocol (https://support-docs.illumina.com/LP/IlluminaDNAPrep/Content/LP/Illumina_DNA/DNA-Prep/Protocol_IDP.htm). Briefly, the extracted genomic DNA was tagmented using Bead-Linked Transposomes (BLT) and cleaned-up. Then the tagmented DNA was amplified using a limited-cycle PCR programme using a T100 Thermal Cycler (Bio-Rad, USA). This step adds Index 1 (i7) adapters, Index 2 (i5) adapters (xGen™ Amplicon UDI Primers, Integrated DNA Technologies, USA), and sequences required for sequencing cluster generations. Then, a double-sided bead purification procedure was performed to purify the amplified and indexed libraries. Libraries were quantified and normalized, the quality of the individual libraries and the pool was assessed on a 2100 Bioanalyzer instrument (Agilent Technology, USA), using a High Sensitivity DNA chip. Library preparation, indexing and quality control was performed on July 1, 2025 by Kate Mathers. The final library was diluted to the starting concentration of 4nM, denatured and diluted to the final concentration of 6 pM, adding a 5% PhiX spike. The sequencing was performed on Illumina's MiSeq platform with v2 Micro 300 cycle kit (2 x 151 bp) (Illumina, USA). Final dilution and loading of the library on the MiSeq was performed on July 16, 2025 by Kate Mathers and Neli Zakio.

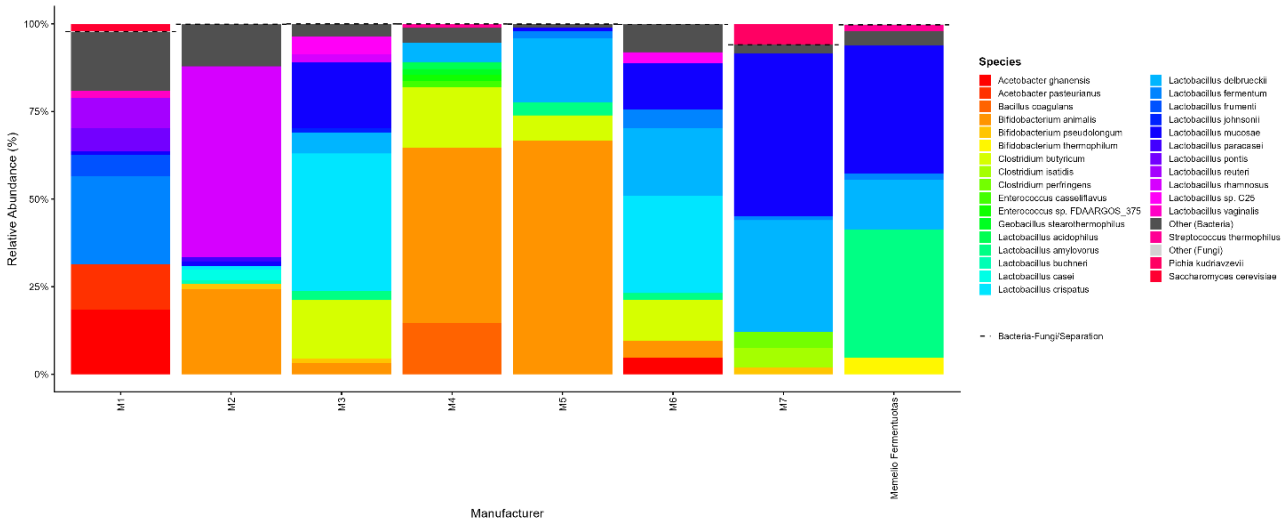
The library preparation room was sterilized using UV sterilization for a minimum of 30 minutes before each use. The reaction set-up was performed in a DNA/RNA UV-cleaner box with a UV cleaner–recirculator (BioSan, Latvia). Aerosol barrier tips (Accumax, Interlab, NZ) were used throughout.

Relative Abundance of Bacterial and Fungal Genera (>1%) by Manufacturer



https://www.sequench.co.nz/

Relative Abundance of Bacterial and Fungal Species (>1%) by Manufacturer



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