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SUPPLEMENTARY MATERIAL OPEN ACCESS

Unravelling the Probiotic and Safety Profile of *Lactiplantibacillus plantarum* 022AE: A Multi-Omics Approach Integrating Genomics and Phenotypic Data

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Supplementary Data

Materials and methods

Whole genome sequencing

Genomic DNA from L. plantarum strain 022AE was used for preparation of Illumina and Nanopore Whole Genome Sequencing (WGS) libraries (Table **A**). Illumina WGS library was prepared as per the workflow described in Fig. (**SA**). with Illumina-compatible SureSelect^{QXT} whole genome library prep kit (Agilent, Santa Clara, CA, U.S.A.).

A total of 25 ng DNA was fragmented and adapter-tagged using Sure Select QXT enzyme. Fragmented and adapter-tagged DNA was purified with High Prep PCR beads, amplified and indexed in a 6 cycle PCR reaction (68°C for 2 min, denaturation at 98˚C for 30 sec, cycling (98˚C for 30 sec, 56˚C for 30 sec and 72˚C for 60 sec). The final PCR product (sequencing library) was purified with High Prep beads (MAGBIO, MD, USA), followed by library quality control check. The Illumina-compatible sequencing library was quantified by Qubit Fluorometer (Thermo Fisher Scientific, MA, USA) and its fragment size distribution was analysed on Agilent Tape Station (Fig. **SB**).

The tape station profile for Illumina library indicates fragment size ranging from 232 to 1315 bp, however, larger proportion of the Illumina-compatible sequencing library had fragment size range between 200 bp to 700 bp. Considering the combined adapter size which was approximately 120 bp, the effective user-defined insert size was 80 bp to 580 bp which was obtained with optimal concentration. A total of 2,941,678 (R1 + R2) reads were generated on Illumina MiSeq platform for *L. plantarum* strain 022AE.

Long read library for Nanopore sequencing was performed using 1 µg of genomic DNA from *L. plantarum* strain 022AE. Genomic DNA was end-repaired (NEBnext ultra II end repair kit, New England Biolabs, MA, USA) and cleaned up with 1x AmPure beads (Beckmann Coulter, USA). Native barcode ligation was performed with NEB blunt/ TA ligase (New England Biolabs, MA, USA) using NBD103 (ONT) and cleaned with 0.5x AmPure beads. Barcode sequences are shown in the Table **B**.

Table A. Description of the library.

Sample ID	Qubit (ng/ul)	Vol (ul)	Yield (ng)	Index1	Index 1 Sequence	Index2	Index 2 Sequence
GT_SO_7892_*L. plantarum* strain 022AE	15.1	10	151	P7i4	TCCTGAGC	P7i18	ACTGCATA

Table B. Barcodes used for sequencing.

Sample ID	Barcode name	Sequence
GT_SO_7892_*L. plantarum* strain 022AE	NB01	CACAAAGACACCGACAACTTTCTT

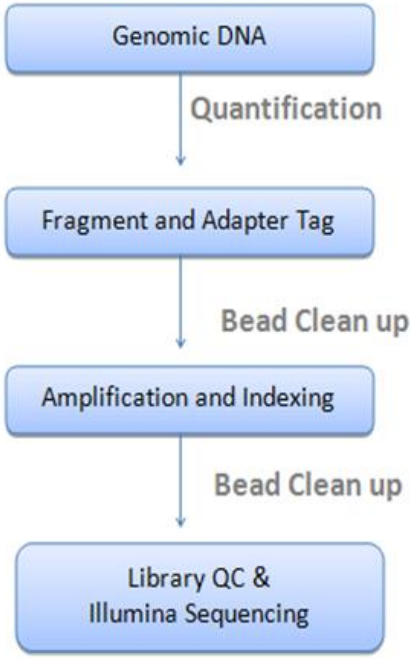
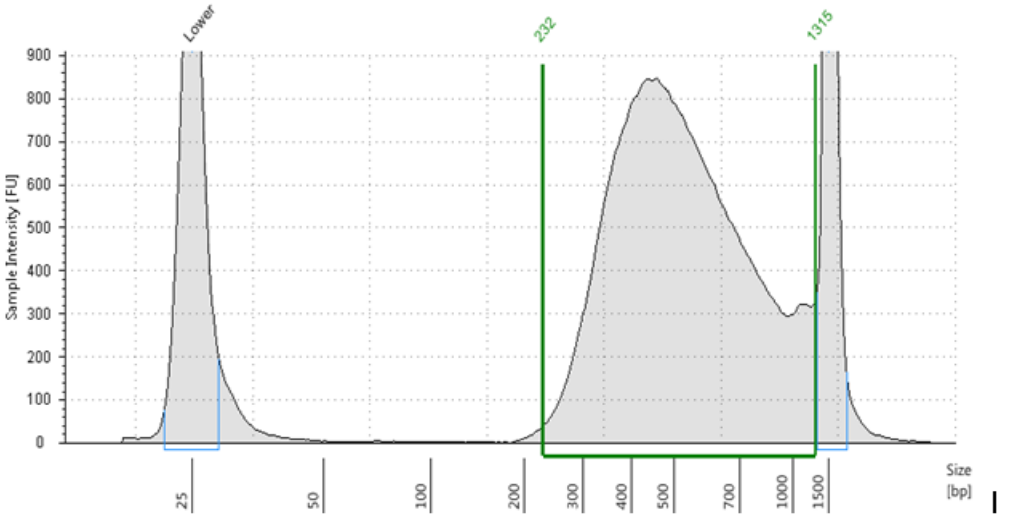


Fig. (SA). Illumina Sure SelectQXT Library preparation workflow (Version D0, November 2015)



From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nm	% of Total
232	1024	530	15.5	49.9	90.22

Fig. (SB). - Tape Station profile of the Illumina library (ePCR1)

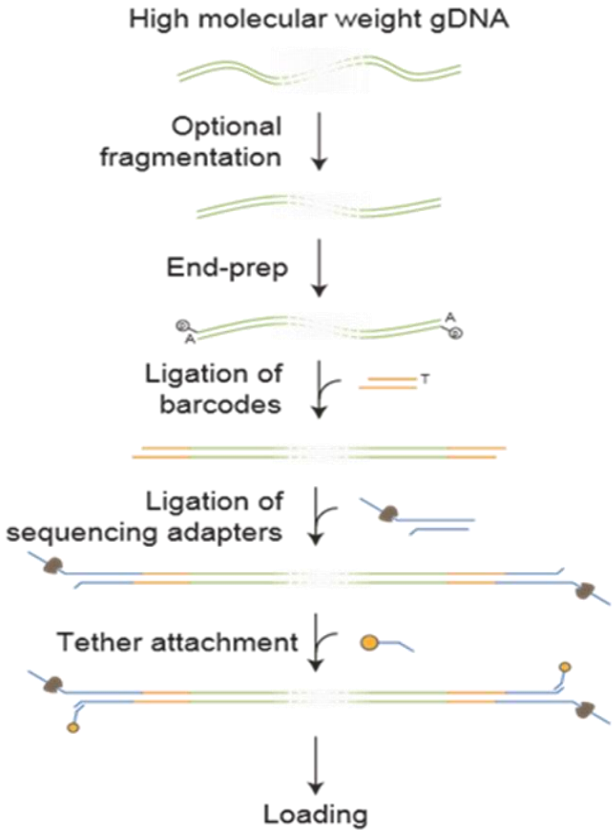


Fig. (SC). Overview of Native barcoding library preparation

Qubit quantified barcode ligated DNA samples were pooled at equimolar concentration to attain 1 µg pooled sample. Adapter ligation (BAM) was performed for 20 minutes using NEBnext Quick Ligation Module (New England Biolabs, MA, USA). Library mix was cleaned up using 0.4X AmPure beads (Beckmann Coulter, USA) and finally sequencing library was eluted in 15 µl of elution buffer and used for sequencing. Native barcoding kit (NBD103) as described in Fig. (**SC**). was used for library preparation.

Statistical data for phenotypic characteristics

Namrata Bhingardeve et al. studied the phenotypic characteristics of L. plantarum 022AE and each experiment was carried out in triplicate, and results are expressed as mean ± standard deviation (SD) in Log10 CFU/g or mL. Statistical tests and graph preparation were performed using Graph Pad Prism (v8.0.2; Graph Pad Software Inc., USA; https://www.graphpad.com/scientifc-sofware/prism/). Differences between groups were assessed using two-way ANOVA, followed by either Tukey's HSD or Dunnett's multiple comparison test, with significance considered at $p < 0.05$.

L. plantarum 022AE remained stable at pH 3.5–7.0 for 5 h (9.137 vs. 9.300 Log10CFU/mL; P = 0.1668) and at pH 2.5 up to 3 h (P = 0.0998), but declined significantly after 4–5 h (≈8.69 Log10CFU/mL; P < 0.05). At pH 1.5, viability dropped rapidly within 1 h (P = 0.0063) and to 6.480 Log10CFU/mL after 5 h (P = 0.0001). In bile, growth was unaffected at 0.01–0.7% for 5 h (P = 0.0997) and at 1% up to 3 h (P = 0.0856), but reduced significantly after 4–5 h (≈8.5 Log10CFU/mL; P < 0.01). L. plantarum 022AE free cells survived all phases of the static gut model, with no significant loss in the gastric phase (P = 0.7929) and 9.010 Log10CFU/mL recovered after the intestinal phase (P = 0.7748). In food matrices, viability was stable in milk (9.253 Log10CFU/mL) and baby food (9.117 Log10CFU/mL), while SAD and SED significantly improved survival in the intestinal phase (9.470, P = 0.0205; 9.407, P = 0.0052, respectively). L. plantarum 022AE exhibited solvent-dependent adhesion (highest with ethyl acetate, lowest with toluene), moderate autoaggregation, variable co-aggregation with pathogens, and significantly higher adherence to mucin compared to control (P = 0.034). L. plantarum 022AE remained stable at 4–25°C for 6 h (P = 0.0578), at 40°C up to 5 h (P = 0.1903) and 50 ^{0}C till 2h (P-value = 0.1011) At 4°C, L. plantarum 022AE maintained high viability in Mcilvaine buffer (97.8%, P = 0.0527) but declined in DW (66.8%, P = 0.0005), with significant losses in oil and buffer–glycerol after 6 months (P ≤ 0.0001) and complete loss in aqueous glycerol by 3

months. At 25°C, survival was highest in buffer (89.5%, P = 0.0012), moderate in aqueous matrix (57.6%, P < 0.0001), and lowest in oil emulsion (21.5%) and buffer–glycerol (4.9%), with no viability in aqueous glycerol and oil beyond 3 months.