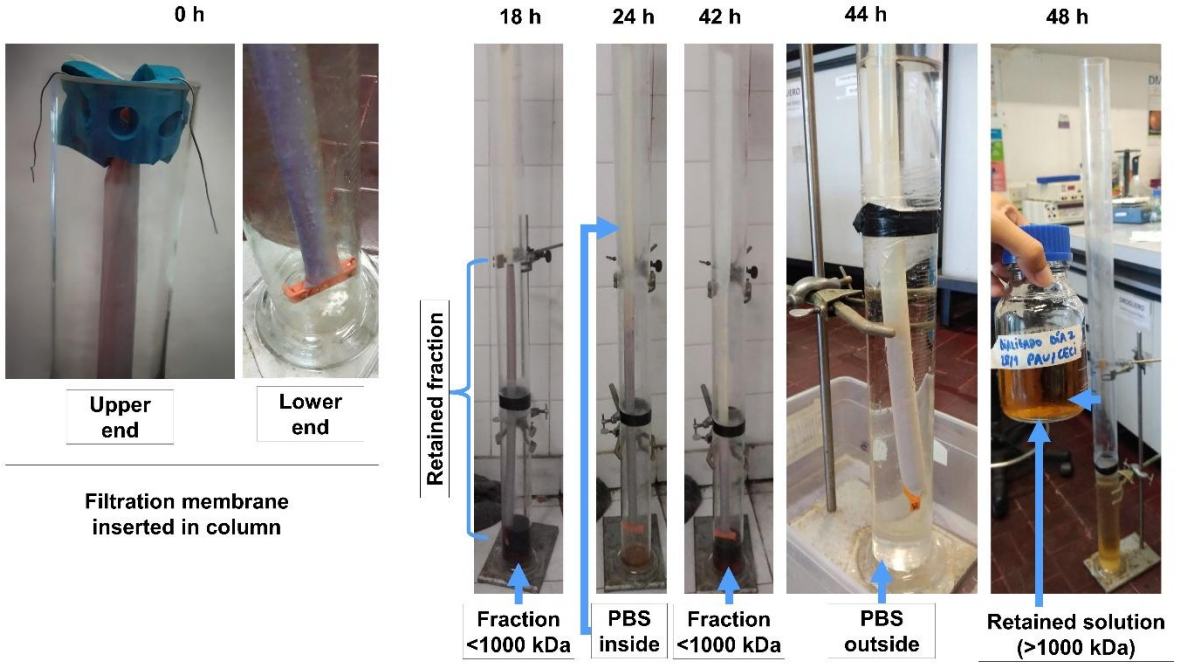
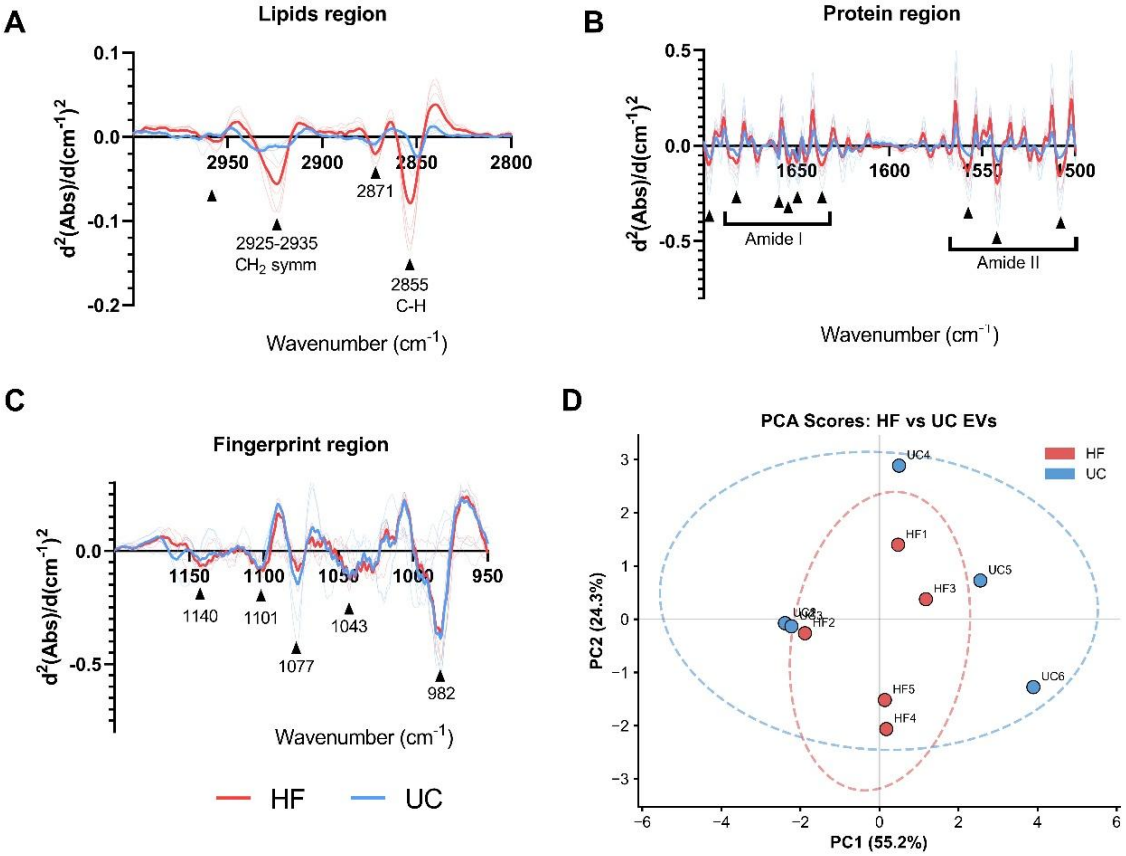
Supplementary materials



Supplementary Fig. 1 Hydrostatic filtration process using a dialysis membrane inserted into a column. Initially, the membrane was filled with cell-free supernatant from a *Lacticaseibacillus casei* BL23 culture. After 24 h, PBS was added inside the membrane to perform an inside-out wash. At 44 h, PBS was added to the column (externally to the membrane) to perform a 4-hour outside-in wash (dialysis). The final retained solution contains the EVs.



Supplementary Fig. 2 Second derivative spectra of EV preparations isolated by hydrostatic filtration (HF) (red) and ultracentrifugation (UC) (blue) across three spectral regions. Individual biological replicates are shown as thin lines and group means as bold lines. (**A**) Lipid region (2800–3000 cm^{-1}). (**B**) Protein region (1500–1700 cm^{-1}), with resolved components within the amide I (1700–1600 cm^{-1}) and amide II (1580–1500 cm^{-1}) envelopes reflecting contributions from α-helices, β-sheets, β-turns, and random coil structures. (**C**) Fingerprint region (900–1200 cm^{-1}), showing peaks at 1140 cm^{-1} (carbohydrates), 1101 cm^{-1} (nucleic acid sugar-phosphate backbone), 1077 cm^{-1} (symmetric PO_2^- stretching), 1043 cm^{-1} (RNA ribose C-O stretching and polysaccharides), and 982 cm^{-1} (ribose-phosphate backbone). Peak positions were determined from second derivative minima of mean spectra and are indicated by arrowheads. (**D**) PCA scores plot of second derivative spectra. Each point represents one biological preparation; dashed ellipses indicate 95% confidence regions for each group. PC1 and PC2 account for 55.2% and 24.3% of total spectral variance, respectively. PERMANOVA revealed no statistically significant difference between groups ($F = 0.65$, $p = 0.573$). HF: n=5; UC: n=6 independent biological preparations.

Supplementary Table 1. Proteins identified and quantified in the supermere-like EPs from *Lacticaseibacillus casei* BL23 by nano-LC-MS/MS (Sequest HT) and ranked by emPAI-derived relative molar abundance. emPAI% = relative molar abundance. ID Confidence: High = ≥ 5 unique peptides or ≥ 10 PSMs; Confident = ≥ 3 unique peptides or 2 UP + ≥ 5 PSMs; Acceptable = 2 unique peptides, < 5 PSMs.

Rank (emPAI%)	Accession (BL23 RefSeq)	Protein Description	Functional Category	MW (kDa)	pI	# AAs	Coverage (%)	# Peptides	# Unique Peptides	# PSMs	emPAI	emPAI (%)	Relative Abundance	ID Confidence
1	WP_012490875.1	Cell wall hydrolase P75	Cell wall modulation / adhesion	49.7	5.06	494	14.0%	5	5	11	2.162	22.18%	High	High
2	WP_003564265.1	Type I type I glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Moonlighter /metabolism (glycolysis)	36.7	6.04	340	11.8%	6	6	13	1.581	16.22%	High	High
3	WP_003576121.1	RlpA-like double-psi β-barrel protein	Cell wall modulation	35.3	6.80	344	17.4%	3	3	8	1.512	15.51%	High	Confident
4	WP_003572828.1	Cell wall hydrolase P40	Cell wall modulation / adhesion	42.4	7.52	411	10.2%	5	5	11	1.154	11.84%	Moderate	High
5	WP_012490867.1	Cell wall hydrolase (small isoform, 23 kDa)	Cell wall modulation	23.0	4.97	228	14.9%	2	2	5	0.995	10.21%	Moderate	Confident
6	WP_012491782.1	C40 family peptidase	Cell wall modulation	41.5	8.78	405	12.8%	5	5	8	0.833	8.55%	Moderate	High
7	WP_012491163.1	ABC transporter substrate-binding protein	Transport	34.1	9.70	322	17.7%	4	4	5	0.833	8.55%	Moderate	Confident
8	WP_014566708.1	Leucine-rich repeat (LRR) protein	Adhesion / surface	134.6	4.60	1305	4.1%	5	5	7	0.417	4.28%	Low	High
9	WP_012492167.1	Hypothetical protein (flagellar-associated)	Unknown / flagellar	41.3	8.98	390	8.2%	2	2	2	0.259	2.66%	Low	Acceptable

Abbreviations: emPAI, exponentially modified protein abundance index; MW, molecular weight; pI, isoelectric point; AAs, amino acids; PSMs, peptide spectrum matches; RefSeq, NCBI Reference Sequence Database.