Supplemental Materials

Title:
Extracellular vesicles produced by *Bifidobacterium longum* export mucin-binding proteins

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Table S1. Proteomic analysis of the EVs fraction from *B. longum* NCC2705

Band	Protein	MW	Note*	Location**
1	FTR1 family protein	64,804	WP_011068493.1	CM
	Glutamate-tRNA ligase	56,681	WP_007051591.1	C
	Pyruvate kinase	55,268	WP_007055467.1	C
	MHS family MFS transporter	54,634	WP_100986529.1	CM
	Glutamyl-tRNA(Gln) amidotransferase subunit A	54,170	WP_007054751.1	C
	DivIVA domain-containing protein	50,613	WP_007054070.1	U
	Sucrose transport protein	49.455	WP_011067958.1	CM
2	ATP-binding protein	50,192	WP_007051743.1	C
	Dipeptidase	48,282	WP_011068282.1	C
	Serine-tRNA ligase	47,956	WP_007052327.1	C
	Aminotransferase	47,185	WP_007056527.1	C
	Elongation factor Tu	43,936	WP_007051202.1	C
	Hypothetical protein	40,416	WP_007057587.1	C
3	Ketol-acid reductoisomerase	38,552	WP_007051651.1	C
	Glyceraldehyde 3-phosphate dehydrogenase	37,716	WP_007052589.1	C
	putative sulfate exporter family transporter	37,476	WP_007051199.1	CM
4	30S ribosomal protein S3	30,156	WP_007053036.1	C
	vitamin K epoxide reductase family protein	25,926	WP_007054040.1	CM
5	ABC transporter ATP-binding protein	22,932	WP_007051383.1	C
	50S ribosomal protein L25	21,815	WP_007052163.1	C
	Adenine phosphoribosyltransferase	20,251	WP_011068112.1	C
6	Hsp20-family heat shock chaperone	18,766	WP_007055906.1	C
	Inorganic pyrophosphatase	18,310	WP_007054759.1	C
	30S ribosomal protein S9	17,607	WP_003829868.1	C

*Note : Accession in Mascot Search Results

**Location: Protein cellular location was annotated by PSORT II Prediction (https://psort.hgc.jp/form2.html).

C, cytoplasmic; CM, cytoplasmic membrane; U, unknown.

Table S2. Oligonucleotides used in this study

Protein	Gene	Locus tag	Fw 5'-3'	Rv 5'-3'
phosphoketolase	*bl0959*	BL0959	CATATGACGAGTCCTGTTATTGGC	AAGCTTCTCGTTGTCGCCAGCGGTAG
chaperone GroEL	*groEL*	BL0002	CATATGGCAAAGATCATCTCTTATG	AAGCTTGTAGCCCATGTCGGCACCG
elongation factor Tu	*tuf*	BL1097	CATATGGCAAAGGAAAAGTACGAGCG	AAGCTTGGCGAGGATCTTGGTCACACGA
phosphoglycerate kinase	*pgk*	BL0707	CATATGACAAAACACCGCATTCAGTCAGTA	AAGCTTCTCAAGCACCTTCAGGCCAGGGAGCT
transaldolase	*tal*	BL0715	CATATGACTGAAGCAACTCAGCGTAC	AAGCTTCACGCGGTCGATGCCGGACT
Hsp20	*bl0576*	BL0576	CATATGGCAATGTTTCCGGCTTTG	AAGCTTGCCCTCAATCGCGATTTGGTGC

Introduced *Nde*I and *Hind*III restriction site is underlined.

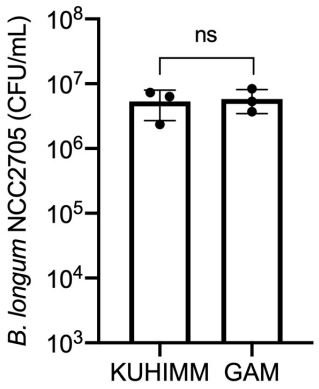


Fig. S1

Viable cell count of *B. longum* NCC2705

B. longum NCC2705 culture broth (after 15 h) was spread on the cell-free KUHIMM (faecal sample code M60) or GAM agar plate, and cultured at 37°C for 30 h under anaerobic conditions. Colonies were scraped and suspended into 1 mL of PBS adjusted to OD_{600} = 0.1. Colony-forming unit (CFU) was determined by serial dilution and spread plating on GAM broth. The error bars indicate the s.d.; data are based on three biological replicates. Statistical significance was determined using from two-tailed, unpaired *t*-tests. “ns”, no significant change in expression ($P > 0.05$).

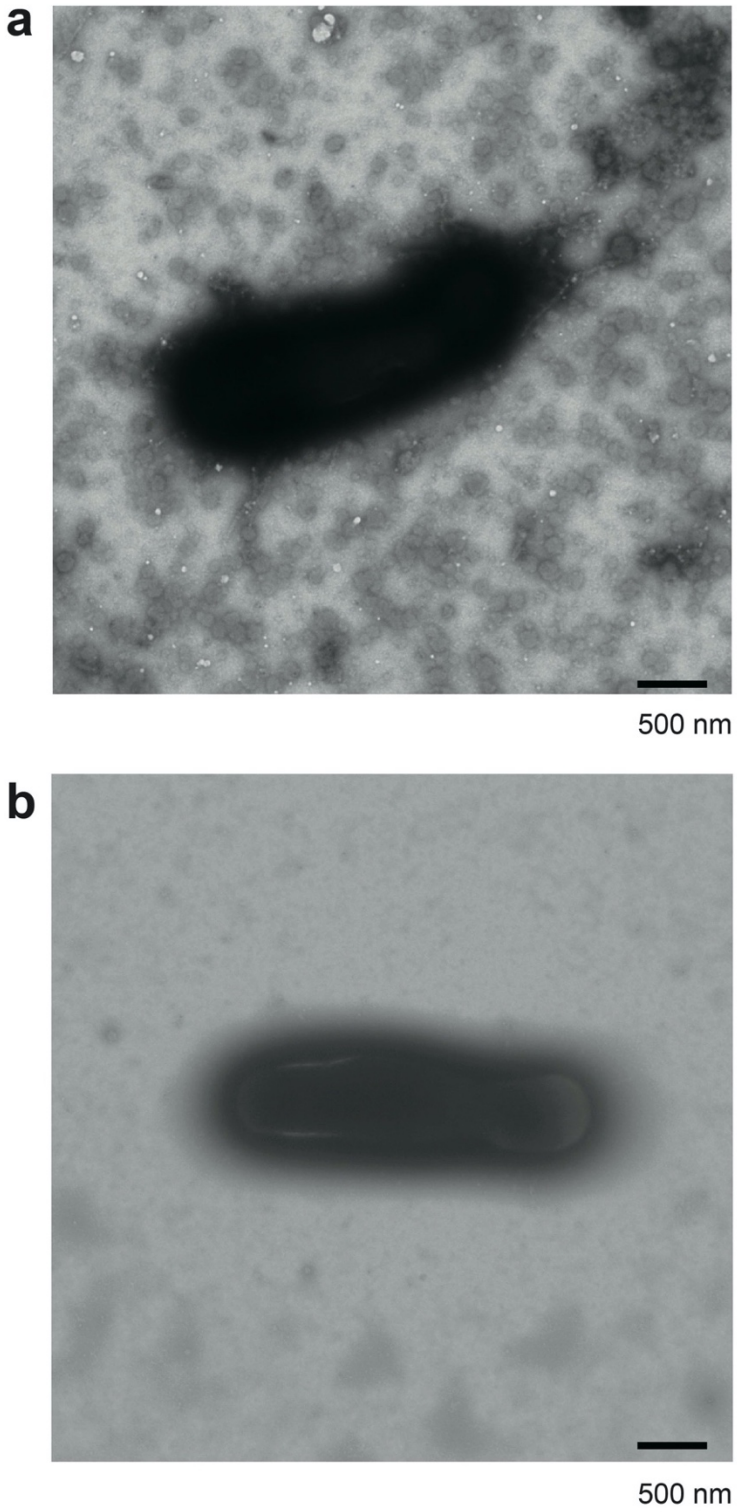
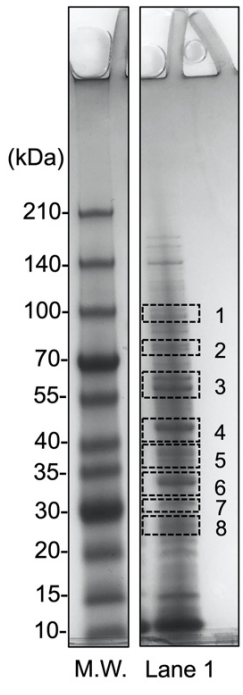


Fig. S2 Electron microscope image of *B. longum* NCC2705

B. longum was cultured with different fecal fermentation HUKIMM broth, which supplemented with individual feces (**a**) sample code F40 and (**b**) sample code M27 [(1)]. The whole bacterial cells were negatively stained with uranyl acetate, and examined by transmission electron microscopy TEM H-7600.

a



Band	Protein	M.W.	Note*
1	beta-galactosidase (LacZ)	114,448	WP_011068233.1
	elongation factor G	78,137	WP_003832662.1
2	DppA2	59,252	WP_011068025.1
	pyruvate kinase	55.267	WP_007055467.1
	ATP synthase	53,309	WP_007051478.1
3	Xaa-Pro aminopeptidase I	58,296	WP_011068002.1
	60 kDa chaperonin, GroEL	56,837	WP_007053188.1
	cell division protein	56,832	WP_011068425.1
	pyruvate kinase	55.267	WP_007055467.1
	type I glutamate--ammonia ligase	53.239	WP_011068284.1
4	phosphopyruvate hydratase (enolase)	46,515	WP_007051126.1
	elongation factor Tu	43,936	WP_007051202.1
	glutamate--cysteine ligase	43,633	WP_011068821.1
5	homoserine dehydrogenase	45,929	WP_011068712.1
	elongation factor Ts	29,979	WP_007052754.1
6	choloylglycine hydrolase	35,125	WP_007052221.1
	phosphoribosylaminoimidazolesuccino carboxamide synthase	28,220	WP_007051209.1
	adenylate kinase	20,500	WP_007053046.1
7	elongation factor P	20,654	WP_007056944.1
8	ABC transporter ATP-binding protein	25,479	WP_007052444.1
	transcription elongation factor GreA	17,135	WP_007055115.1
	peptide deformylase	15,563	WP_007056959.1

*Note: Accession in Mascot Search Results

b

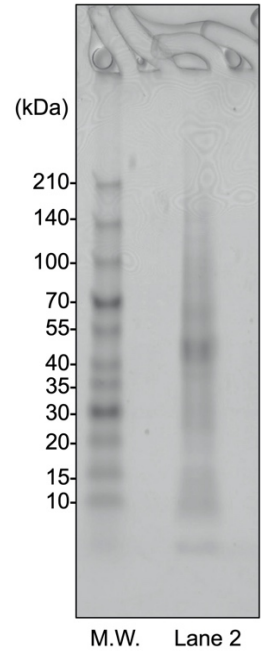


Fig. S3

Protein expression profile of *B. longum* NCC2705

(**a**) The KUHIMM-cultured *B. longum* whole cell lysate (Lane 1, 10 μg protein/lane) or (**b**) KUHIMM-broth (Lane 2, 10 μg protein/lane) was separated by SDS-PAGE and proteins were visualized by Coomassie brilliant blue. (**a**) Eight bands fragments (whole cell lysate) were excised for protein identification by MALDI-ToF MS. Identified proteins and calculated molecular weights are indicated on the right. M.W.: molecular weight marker (Pre-stained XL-Ladder Broad, Integrale, Tokushima, Japan). Masses are indicated in “kDa” to the left of the each gels.

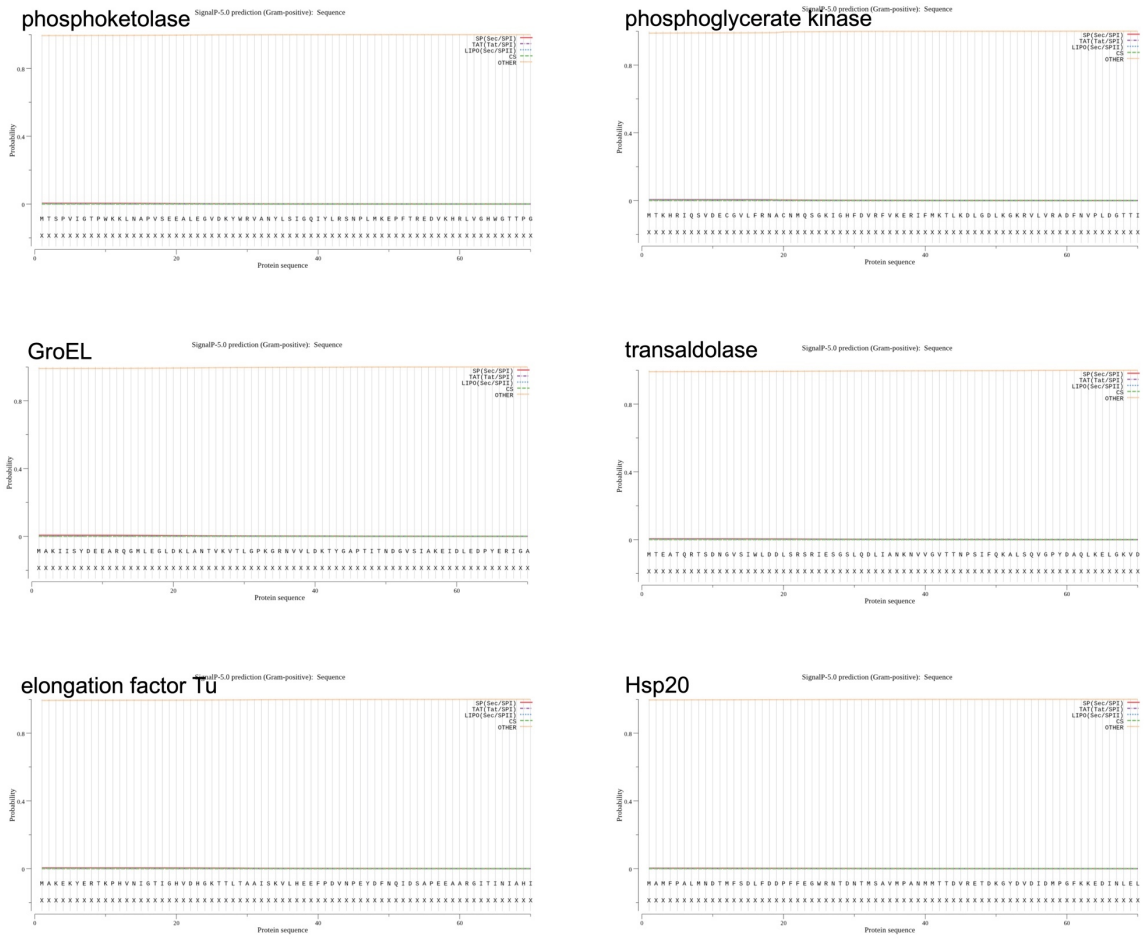
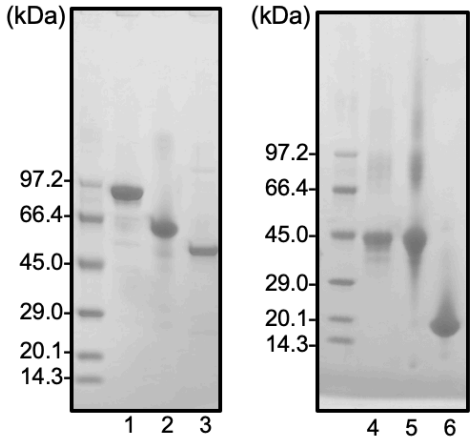


Fig. S4

Prediction of the N-terminal secretory signal

The presence or absence of secretory signal was analyzed by SignalP-5.0 (http://www.cbs.dtu.dk/services/SignalP/).



Lane	Proteins
1	phosphoketolase
2	chaperone GroEL
3	elongation factor Tu
4	phosphoglycerate kinase
5	transaldolase
6	Hsp20/alpha crystallin family protein

Fig. S5

Expression and purification of recombinant proteins from *B. longum* NCC2705

Recombinant His_6-tagged proteins were expressed in *E. coli*. These proteins were verified by SDS-PAGE followed by staining with Coomassie Brilliant Blue.

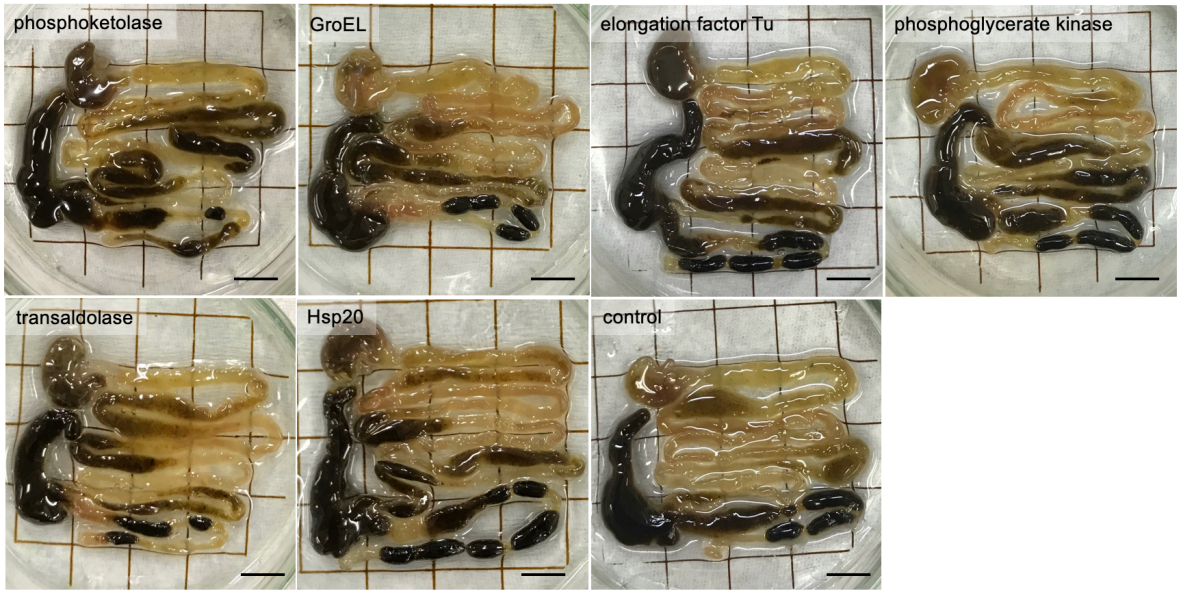


Fig. S6

Visualization of mouse whole gastrointestinal (GI) tract using the tissue clearing method

Recombinant protein immobilized-microbeads were orally administered to mice. After 24 h after administration, whole GI tissues were treated with increasing concentrations of aqueous fructose solutions for tissue clearing. Scale bar, 1 cm.

Reference

(1) Sasaki D, Sasaki K, Ikuta N, Yasuda T, Fukuda I, Kondo A, Osawa R. 2018. Low amounts of dietary fibre increase in vitro production of short-chain fatty acids without changing human colonic microbiota structure. Sci. Rep. 8:435.