Supplementary Materials

Lactate Cross-Feeding between *Bifidobacterium* species and *Megasphaera indica* Contributes to Butyrate Formation in the Human Colonic Environment

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Supplementary Tables

Table S1. Pairwise average nucleotide identity (ANI) values among *Bifidobacterium* and *Megasphaera* strains.

Bacterial isolate	Type strain	ANI value (%)
OTU10_Megasphaera_unidentified_BWX	Megasphaera sp. NH10--GCA_000417505.1	99.09[1]
OTU10_Megasphaera_unidentified_BWX	Megasphaera sp. BL7--GCA_000417525.1	99.03[1]
OTU10_Megasphaera_unidentified_BWX	Megasphaera_elsdenii--GCF_003010495.1	90.89[2]
OTU10_Megasphaera_unidentified_BWX	Megasphaera_massiliensis--GCF_000455225.1	82.43[2]
OTU10_Megasphaera_unidentified_BWX	Megasphaera_cerevisiae--GCF_001045675.1	78.19[2]
OTU10_Megasphaera_unidentified_BWX	Megasphaera_paucivorans--GCF_900103535.1	77.35[2]
OTU10_Megasphaera_unidentified_BWX	Megasphaera_micronuciformis--GCF_000165735.1	77.34[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_pseudocatenulatum--GCF_001025215.1 (Bifidobacterium pseudocatenulatum DSM 20438)	98.17[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_kashiwanohense--GCF_000800455.1	92.03[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_catenulatum--GCF_001025195.1	91.59[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_adolescentis--GCF_000010425.1	84.47[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_ruminantium--GCF_000770925.1	83.03[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_dentium--GCF_001042595.1	82.57[2]
OTU2_Bifidobacterium_pseudocatenulatum_BWX	Bifidobacterium_moukalabense--GCF_000522505.1	82.56[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_longum--GCF_000196555.1 (Bifidobacterium longum subsp. longum JCM 1217)	98.83[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_breve--GCF_001025175.1	86.84[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_reuteri--GCF_000741695.1	83.25[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_saguini--GCF_000741715.1	82.66[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_scardovii--GCF_001042635.1	82.19[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_aesculapii--GCF_001417815.1	82.18[2]
OTU13_Bifidobacterium_longum_BWX	Bifidobacterium_stellenboschense--GCF_000741785.1	82.14[2]

[1] Values were obtained through comparing genome sequences using the OrthoANI tool (https://www.ezbiocloud.net/tools/ani).

[2] Values were generated from the Protologger webserver (www.protologger.de).

Table S2. Composition of FBVA medium.

Component	Composition (per liter)
NaCl	0.47 g
HNa_2PO_4	3.46 g
H_2NaPO_4	1.87 g
Na_2SO_4	0.1 g
KCl	0.45 g
Urea	0.20 g
NH_4Cl	0.36 g
resazurin	1 mg
cysteine-HCl	0.25 g
$CaCl_2 \cdot 2H_2O$	72.8 mg
$MgCl_2 \cdot 6H2O$	100 mg
P1 metal stock solution[1]	1 mL
ATCC vitamin stock (ATCC MD-VS, Hampton, NH)	10 mL
Amino acid mixture[2]	10 mL

[1] P1 metal stock solution (per liter) is composed of 34.26 g H_3BO_3, 4.32 g $MnCl_2 \cdot 4H2O$, 315 mg $ZnCl_2$, 44 mg $Na_2MoO_4 \cdot 2H_2O$, 3 mg $CuSO_4 \cdot 5H2O$, 12.15 mg $CoCl_2 \cdot 6H_2O$, 259 mg $NiCl_2$, 0.28 mL EDTA (10 mM), and 1 mL $FeCl_3 \cdot 6H_2O$ (3.89 mg/mL in 0.1 M HCl).

[2] The amino acid mixture consisted of all 20 amino acids (1 mM each).

Supplementary Figures

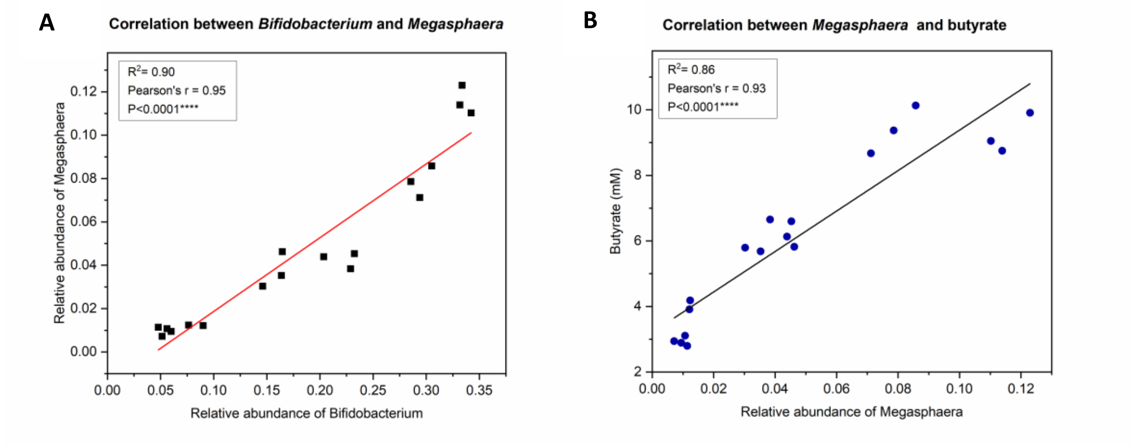
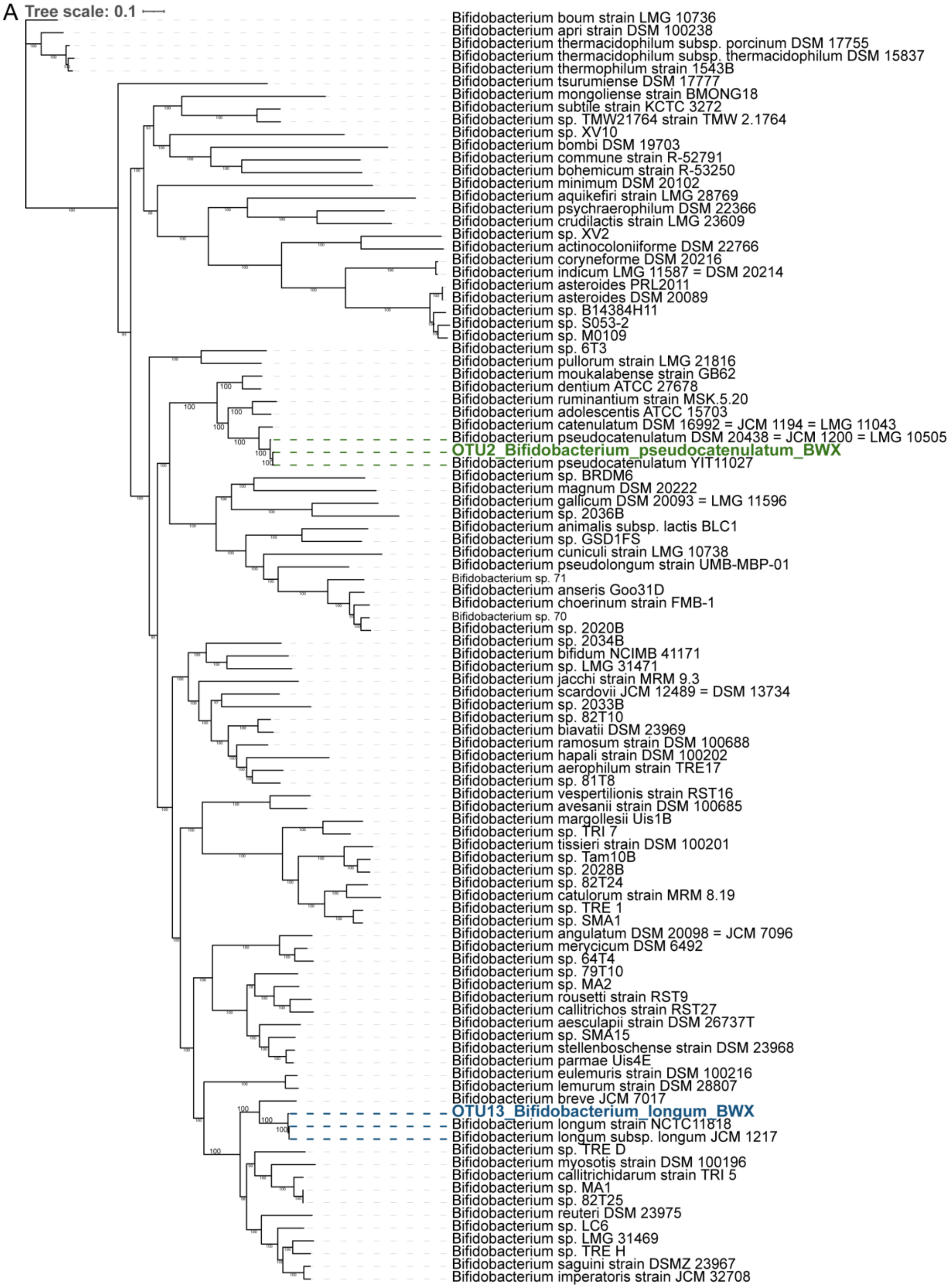


Fig. S1. Correlations between the relative abundance of the genera *Bifidobacterium* and *Megasphaera* in BWX30-based fecal fermentation cultures (A). Correlations between the relative abundance of *Megasphaera* and butyrate yields.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Bifidobacterium apri strain DSM 100238
Bifidobacterium thermacidophilum subsp. porcinum DSM 17755
Bifidobacterium thermacidophilum subsp. thermacidophilum DSM 15837
Bifidobacterium thermophilum strain 1543B
Bifidobacterium tsurumiense DSM 17777
Bifidobacterium mongoliense strain BMONG18
Bifidobacterium subtile strain KCTC 3272
Bifidobacterium sp. TMW21764 strain TMW 2.1764
Bifidobacterium sp. XV10
Bifidobacterium bombi DSM 19703
Bifidobacterium commune strain R-52791
Bifidobacterium bohemicum strain R-53250
Bifidobacterium minimum DSM 20102
Bifidobacterium aquikefiri strain LMG 28769
Bifidobacterium psychraerophilum DSM 22366
Bifidobacterium crudilactis strain LMG 23609
Bifidobacterium sp. XV2
Bifidobacterium actinocoloniiforme DSM 22766
Bifidobacterium coryneforme DSM 20216
Bifidobacterium indicum LMG 11587 = DSM 20214
Bifidobacterium asteroides PRL2011
Bifidobacterium asteroides DSM 20089
Bifidobacterium sp. B14384H11
Bifidobacterium sp. S053-2
Bifidobacterium sp. M0109
Bifidobacterium sp. 6T3
Bifidobacterium pullorum strain LMG 21816
Bifidobacterium moukalabense strain GB62
Bifidobacterium dentium ATCC 27678
Bifidobacterium ruminantium strain MSK.5.20
Bifidobacterium adolescentis ATCC 15703
Bifidobacterium catenulatum DSM 16992 = JCM 1194 = LMG 11043
Bifidobacterium pseudocatenulatum DSM 20438 = JCM 1200 = LMG 10505
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Bifidobacterium sp. BRDM6
Bifidobacterium magnum DSM 20222
Bifidobacterium gallicum DSM 20093 = LMG 11596
Bifidobacterium sp. 2036B
Bifidobacterium animalis subsp. lactis BLC1
Bifidobacterium sp. GSD1FS
Bifidobacterium cuniculi strain LMG 10738
Bifidobacterium pseudolongum strain UMB-MBP-01
Bifidobacterium sp. 71
Bifidobacterium anseris Goo31D
Bifidobacterium choerinum strain FMB-1
Bifidobacterium sp. 70
Bifidobacterium sp. 2020B
Bifidobacterium sp. 2034B
Bifidobacterium bifidum NCIMB 41171
Bifidobacterium sp. LMG 31471
Bifidobacterium jacchi strain MRM 9.3
Bifidobacterium scardovii JCM 12489 = DSM 13734
Bifidobacterium sp. 2033B
Bifidobacterium sp. 82T10
Bifidobacterium biavatii DSM 23969
Bifidobacterium ramosum strain DSM 100688
Bifidobacterium hapali strain DSM 100202
Bifidobacterium aerophilum strain TRE17
Bifidobacterium sp. 81T8
Bifidobacterium vespertilionis strain RST16
Bifidobacterium avesanii strain DSM 100685
Bifidobacterium margollesii Uis1B
Bifidobacterium sp. TRI 7
Bifidobacterium tissieri strain DSM 100201
Bifidobacterium sp. Tam10B
Bifidobacterium sp. 2028B
Bifidobacterium sp. 82T24
Bifidobacterium catulorum strain MRM 8.19
Bifidobacterium sp. TRE 1
Bifidobacterium sp. SMA1
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Bifidobacterium merycicum DSM 6492
Bifidobacterium sp. 64T4
Bifidobacterium sp. 79T10
Bifidobacterium sp. MA2
Bifidobacterium rousetti strain RST9
Bifidobacterium callitrichos strain RST27
Bifidobacterium aesculapii strain DSM 26737T
Bifidobacterium sp. SMA15
Bifidobacterium stellenboschense strain DSM 23968
Bifidobacterium parmae Uis4E
Bifidobacterium eulemuris strain DSM 100216
Bifidobacterium lemurum strain DSM 28807
Bifidobacterium breve JCM 7017
OTU13_Bifidobacterium_longum_BWX
Bifidobacterium longum strain NCTC11818
Bifidobacterium longum subsp. longum JCM 1217
Bifidobacterium sp. TRE D
Bifidobacterium myosotis strain DSM 100196
Bifidobacterium callitrichidarum strain TRI 5
Bifidobacterium sp. MA1
Bifidobacterium sp. 82T25
Bifidobacterium reuteri DSM 23975
Bifidobacterium sp. LC6
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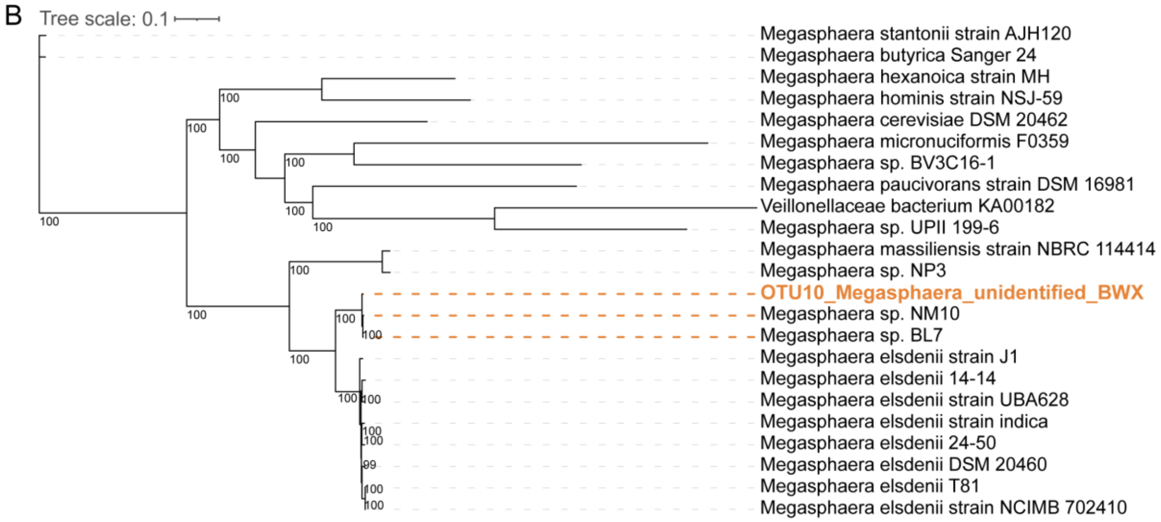
Bifidobacterium sp. TRE H
Bifidobacterium saguini strain DSMZ 23967
Bifidobacterium imperatoris strain JCM 32708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Megasphaera butyrica Sanger 24
Megasphaera hexanoica strain MH
Megasphaera hominis strain NSJ-59
Megasphaera cerevisiae DSM 20462
Megasphaera micronuciformis F0359
Megasphaera sp. BV3C16-1
Megasphaera paucivorans strain DSM 16981
Veillonellaceae bacterium KA00182
Megasphaera sp. UPII 199-6
Megasphaera massiliensis strain NBRC 114414
Megasphaera sp. NP3
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Megasphaera sp. BL7
Megasphaera elsdenii strain J1
Megasphaera elsdenii 14-14
Megasphaera elsdenii strain UBA628
Megasphaera elsdenii strain indica
Megasphaera elsdenii 24-50
Megasphaera elsdenii DSM 20460
Megasphaera elsdenii T81
Megasphaera elsdenii strain NCIMB 702410
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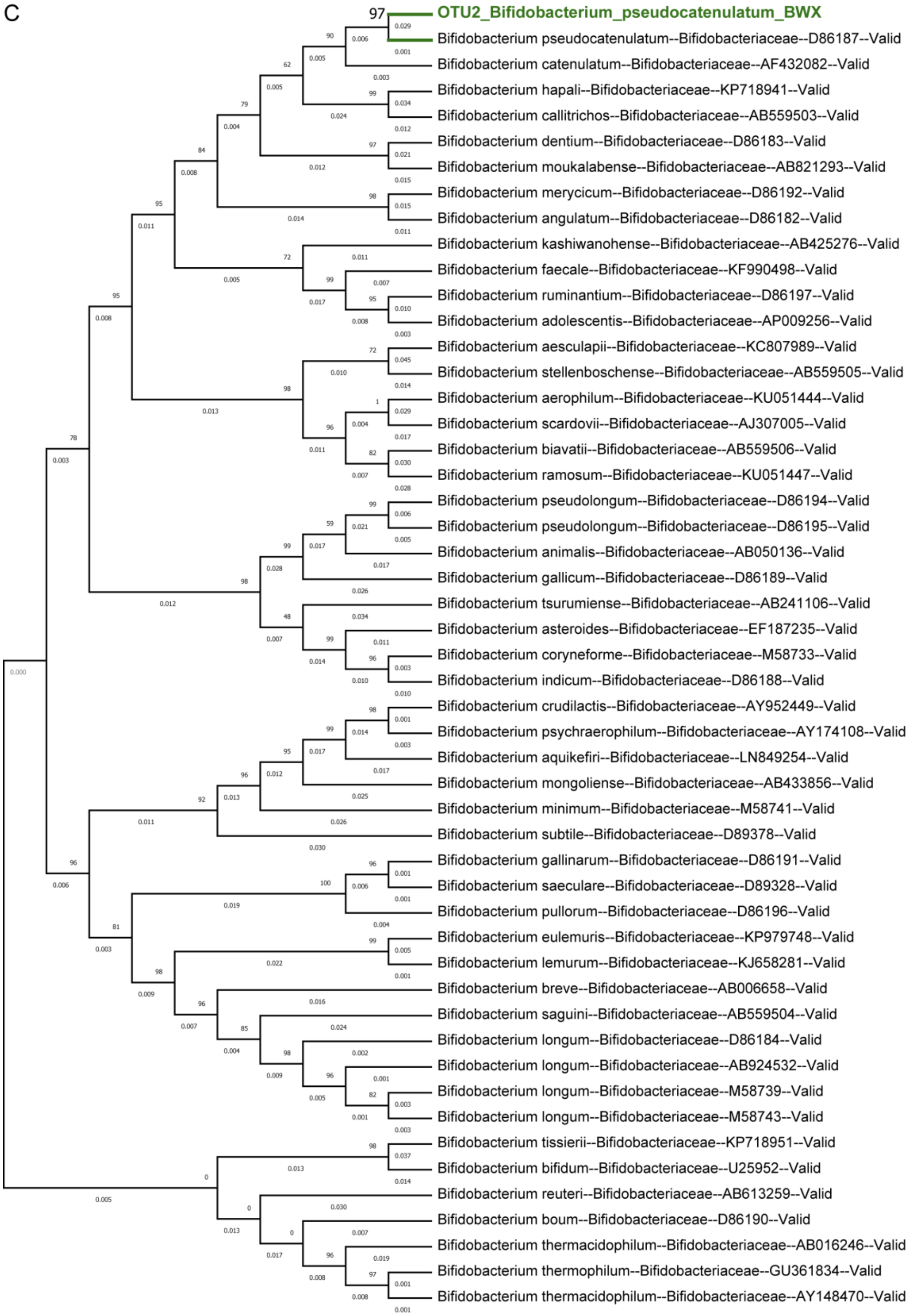
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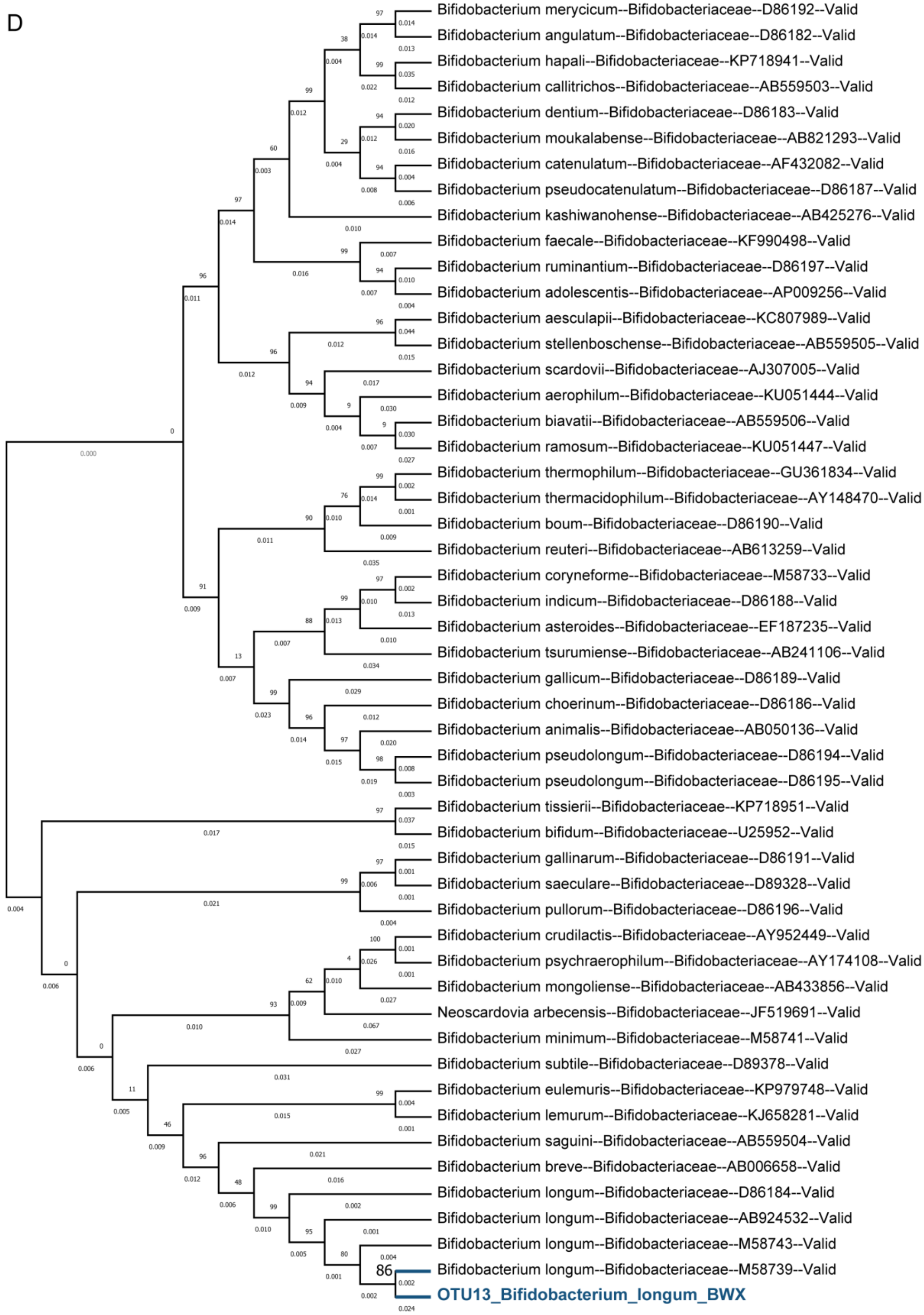
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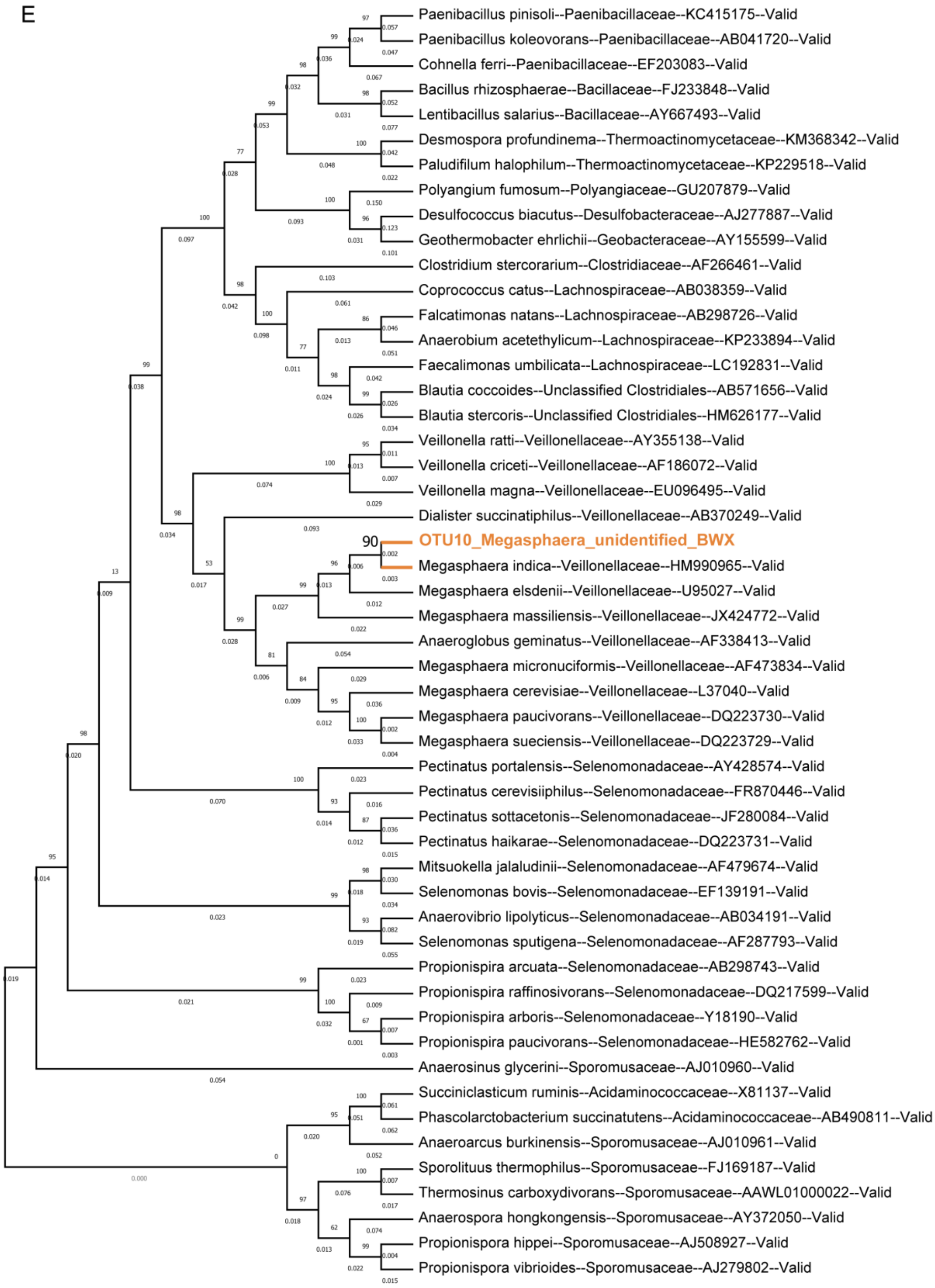
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Fig. S2. Phylogenetic trees depicting the evolutionary relationships among species. (A) Codon tree of genus *Bifidobacterium* based on 160 core genes. (B) Codon tree of genus *Megasphaera* based on 375 core genes. Diagrams A & B were constructed using the BV-BRC server (https://www.bv-brc.org/app/PhylogeneticTree). (C) Phylogenetic tree of OTU2_*Bifidobacterium_pseudocatenulatum*_BWX. (D) Phylogenetic tree of OTU13_*Bifidobacterium_longum*_BWX. (E) Phylogenetic tree of OTU10_*Megasphaera*_unidentified_BWX. Diagrams C, D, & E were constructed based on the 16S rRNA gene sequence using Protologger (http://protologger.de/).

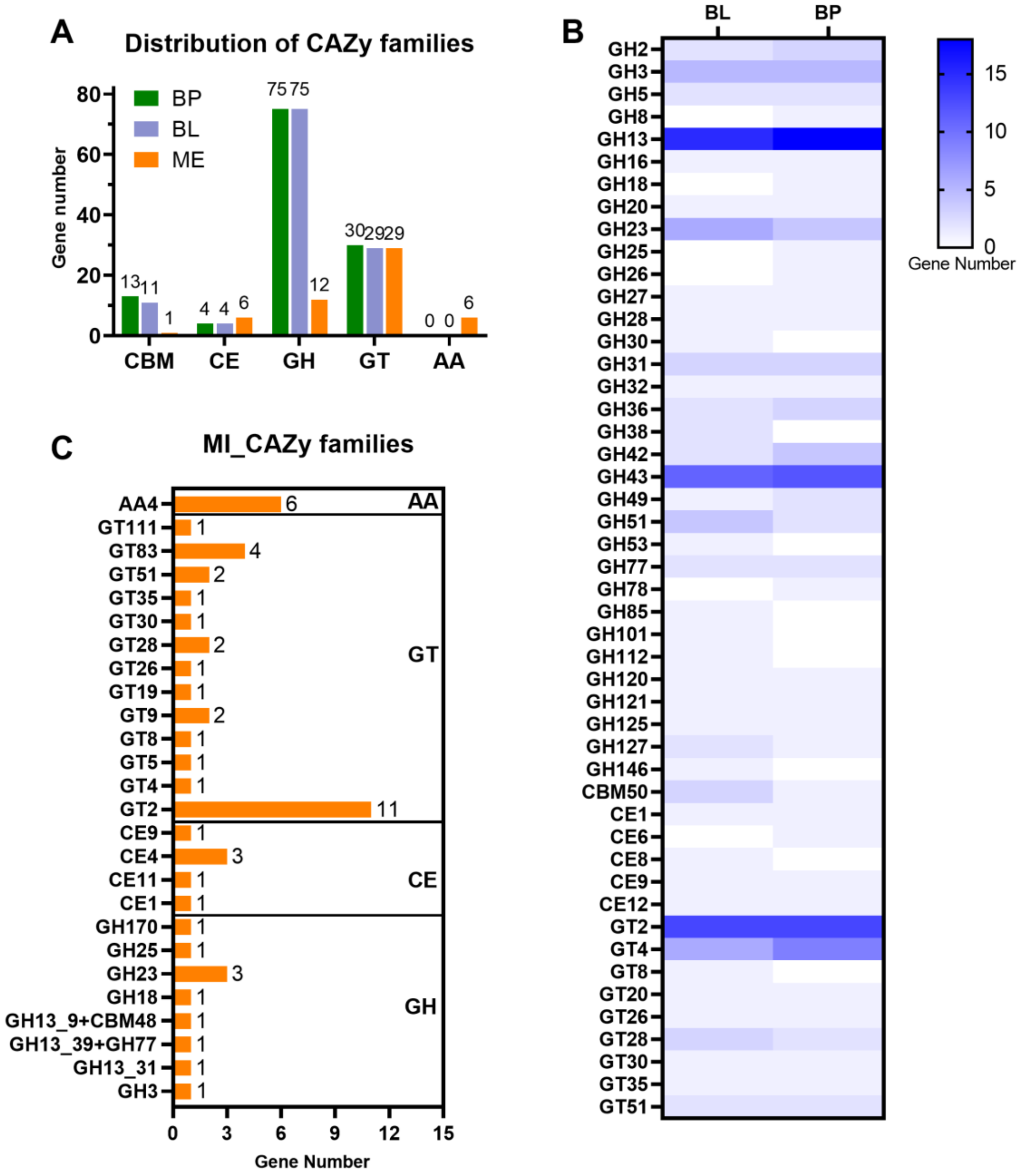


Fig. S3. Distribution of the CAZymes (Carbohydrate active enzymes) in the three bacterial genomes. CBM, carbohydrate binding module; CE, carbohydrate esters; GH, glycoside hydrolase; GT, glycoside transferase; AA, auxiliary activities family.

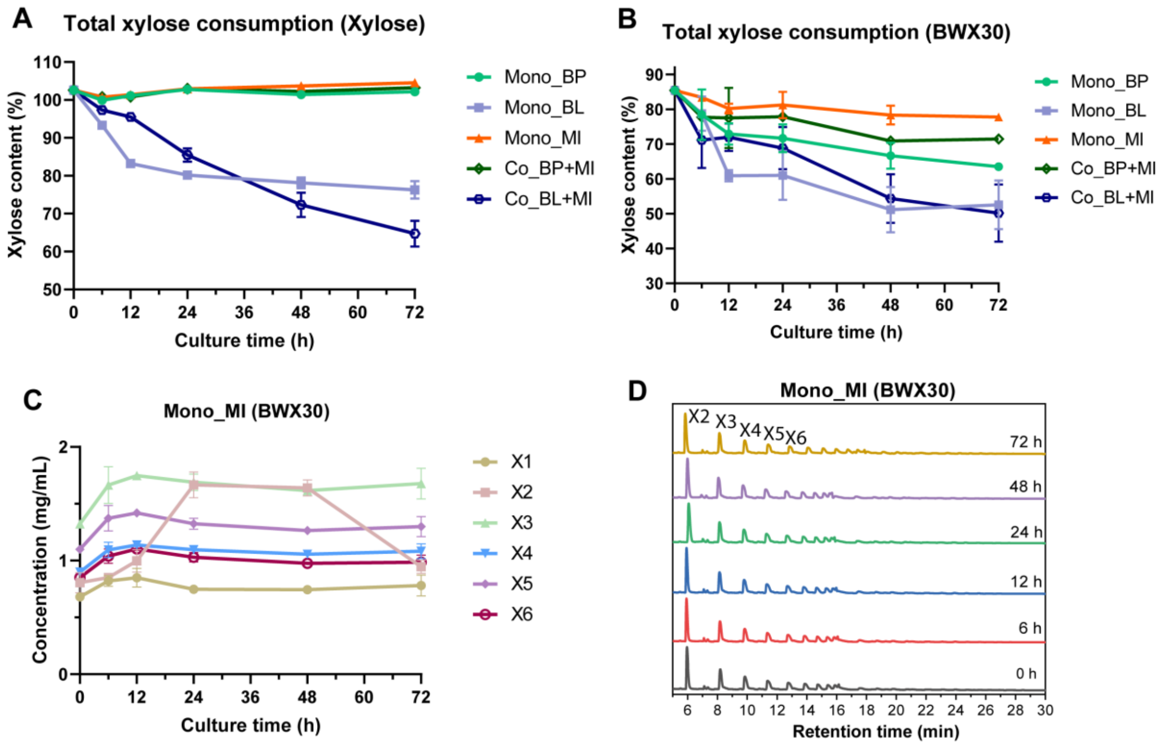


Fig. S4. Substrate consumption during monoculture and coculture fermentation. Total xylose concentration in the medium with xylose (A) or BWX30 (B) as the carbohydrate source. (C) Concentration of xylose and xylooligosaccharides in the MI fermented BWX30 medium. (D) Profile of the oligomers in medium with BWX30 as the carbohydrate source. Mono, monoculture; Co, coculture. BP, *Bifidobacterium pseudocatenulatum_BWX*; BL, *Bifidobacterium longum_BWX*; MI, *Megasphaera indica_BWX*; X1, xylose; X2, xylobiose; X3, xylotriose, X4, xylotetraose; X5, xylopentaose and X6, xylohexaose. The range bars in line charts denote the standard deviation of three biological replicates.

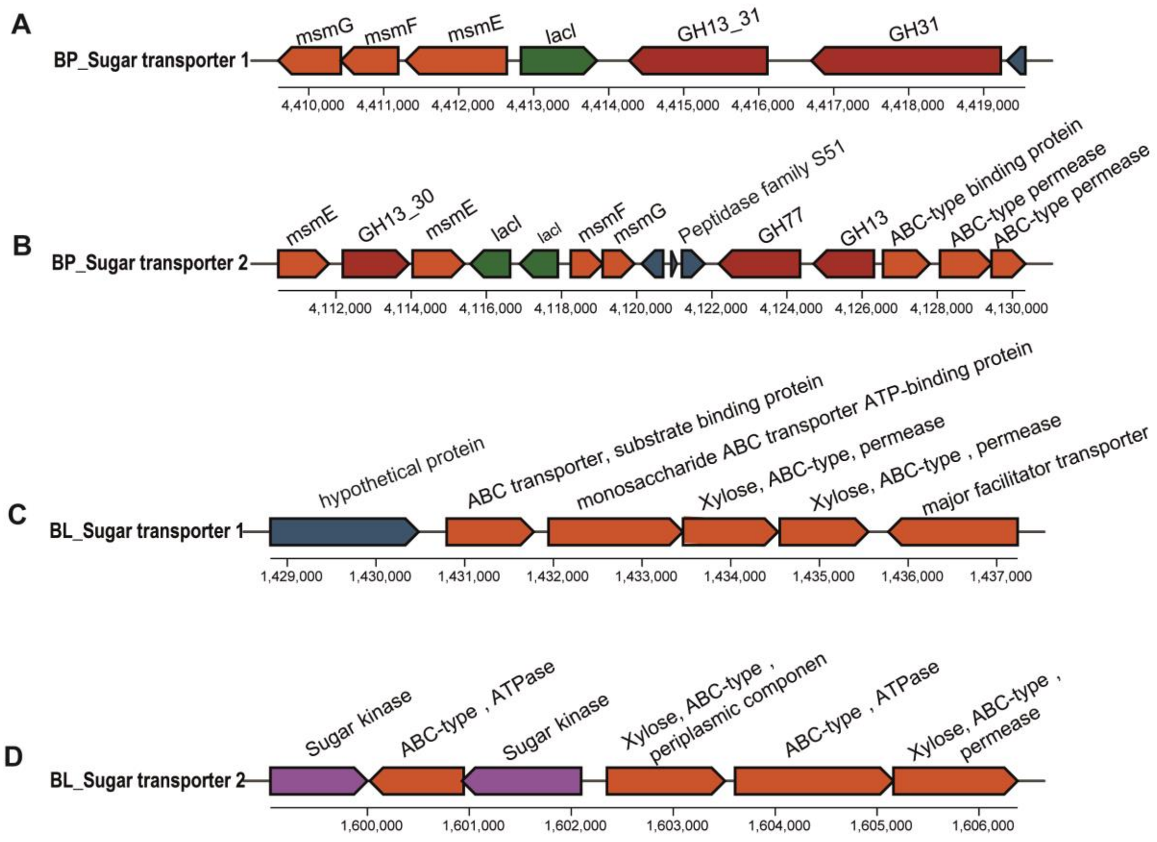
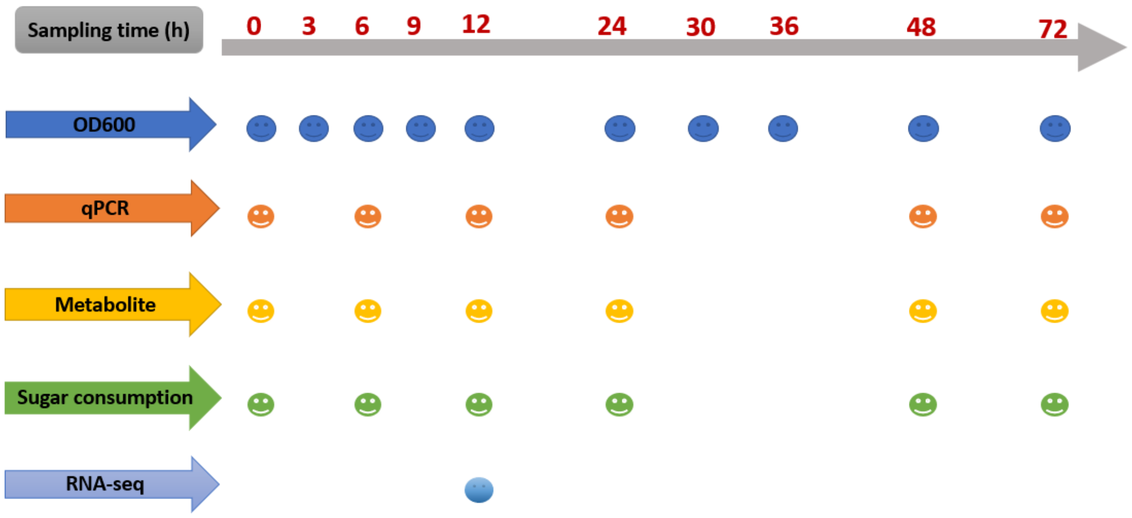


Fig. S5. Schematic representation of sugar transport system in the genome of (A-B) *Bifidobacterium pseudocatenulatum*_BWX (BP), and (C-D) *Bifidobacterium longum*_BWX (BL).



Sampling time (h)	0	3	6	9	12	24	30	36	48	72
OD600	●	●	●	●	●	●	●	●	●	●
qPCR	●		●		●	●			●	●
Metabolite	●		●		●	●			●	●
Sugar consumption	●		●		●	●			●	●
RNA-seq					●					

Fig. S6. Sampling time points for each analysis during the monoculture and coculture fermentations.