Mutual cross-feeding interactions between *Bifidobacterium longum* NCC2705 and *Eubacterium rectale* ATCC 33656 explain the bifidogenic and butyrogenic effects of arabinoxylan-oligosaccharides

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Supplemental Material:

Materials and methods M1, Figures S1-S6, and Tables S1-S4

Supplemental materials and methods

M1: Identification of glycoside hydrolases possibly involved in (A)XOS degradation in *Eubacterium rectale* ATCC 33656

A more detailed functional prediction of genes possibly involved in (A)XOS degradation in the genome of *Eubacterium rectale* ATCC 33656 was performed according to Lagaert (2013). Glycoside hydrolase (GH) families GH1, GH3, GH5, GH8, GH30, GH31, GH33, GH42, GH43, GH51, GH52, GH54, GH62, GH120, and GH116 contain GHs with (A)XOS-degrading activity (CAZy database; 1). The genome of *E. rectale* ATCC 33656 encodes nine GHs (locus tags EUBREC_0616, EUBREC_1040, EUBREC_1042, EUBREC_1044, EUBREC_2454, EUBREC_2578, EUBREC_2820, EUBREC_2879, and EUBREC_3687) belonging to six of these GH families (GH1, GH3, GH5, GH8, GH43, and GH51) (CAZy database; Taxonomy ID 515619). In accordance with Lagaert (2), multiple protein sequence alignments were performed for each of these GH families using the protein sequences corresponding to the GHs of interest with experimentally characterized GHs from the CAZy database (1). Hereto, ClustalW was used within the Geneious Pro R6 software (Biomatters, San Francisco, CA), followed by the construction of bootstrap neighbor-joining trees. In this way, phylogenetic trees were obtained for each GH family. Function prediction of the GHs of *E. rectale* ATCC 33656 was done by comparing their positions with the positions of the experimentally characterized GHs. Signal peptides were predicted in the translated protein sequences using SignalP (3).

Results

Comparing the positions of the GHs of *E. rectale* ATCC 33656 with the positions of the experimentally characterized GHs in each GH tree indicated that EUBREC_3687 [belonging to GH1; clustered together with proteins with 6-phospho-*β*-glucosidase activity (EC 3.2.1.86)], EUBREC_2879 [belonging to GH3; clustered together with proteins with *β*-glucosidase activity (EC 3.2.1.21)], EUBREC_2820 (belonging to GH3; clustered together with proteins with *β*-glucosidase activity (EC 3.2.1.21)], and EUBREC_0616 [belonging to GH5; clustered together with proteins with endo-*β*-1,4-glucanase/cellulase activity (EC 3.2.1.4)] does not possess the proper functionality needed for (A)XOS degradation (Figures S1, S2, and S3). Although the genome annotation of *E. rectale* ATCC 33656 (NCBI accession number NC_012781) suggested that EUBREC_1044, belonging to GH3, displayed *β*-glucosidase activity, the current *in silico* analysis showed that EUBREC_1044 clustered together with experimentally characterized *β*-xylosidases (EC 3.2.1.37) instead of *β*-glucosidases (EC 3.2.1.21) (Figure S2). This was also the case for other proteins, such as the protein encoded by the gene *EUBREC_1040* (belonging to GH8), which was annotated as a xylanase Y (NCBI accession number NC_012781), that clustered together with exo-oligoxylanases (EC 3.2.1.156; Figure S4); the protein encoded by the gene *EUBREC_1042* (belonging to GH43), which was annotated as an *α*-arabinofuranosidase (NCBI accession number NC_012781), that clustered together with a *β*-xylosidase/*α*-arabinofuranosidase with dual function (EC 3.2.1.37/55) (Figure S5); and the protein encoded by the gene *EUBREC_2578* (belonging to GH43), which was annotated as a polyfunctional esterase/*β*-

xylosidase/*α*-arabinofuranosidase (NCBI accession number NC_012781), that clustered together with *α*-arabinofuranosidases (EC 3.2.1.55) (Figure S5). For the protein encoded by the gene *EUBREC_2454* (belonging to GH51), which was annotated as an *α*-arabinofuranosidase (NCBI accession number NC_012781), the same function (EC 3.2.1.55) was found during the present study (Figure S6). All these predicted (A)XOS-degrading enzymes are most likely located intracellularly in *E. rectale* ATCC 33656, as no signal peptides were found in their protein sequences.

Supplemental Figures

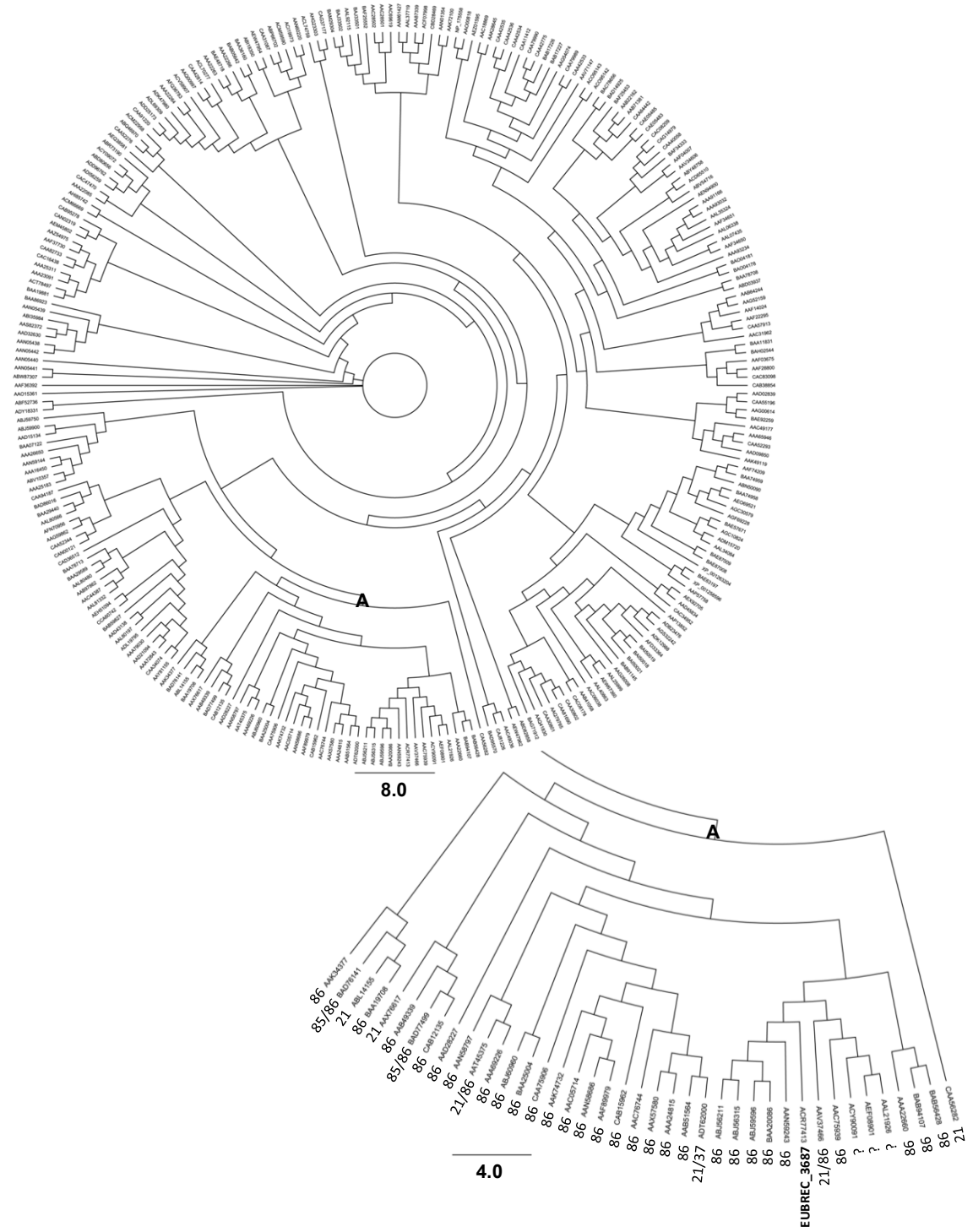

Figure S1: Phylogenetic tree based on protein sequences of GH1 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_3687, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 21, *β*-glucosidase, 37, *β*-xylosidase, 85, 6-phospho-*β*-galactosidase, 86, 6-phospho-*β*-glucosidase, ?, not known). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

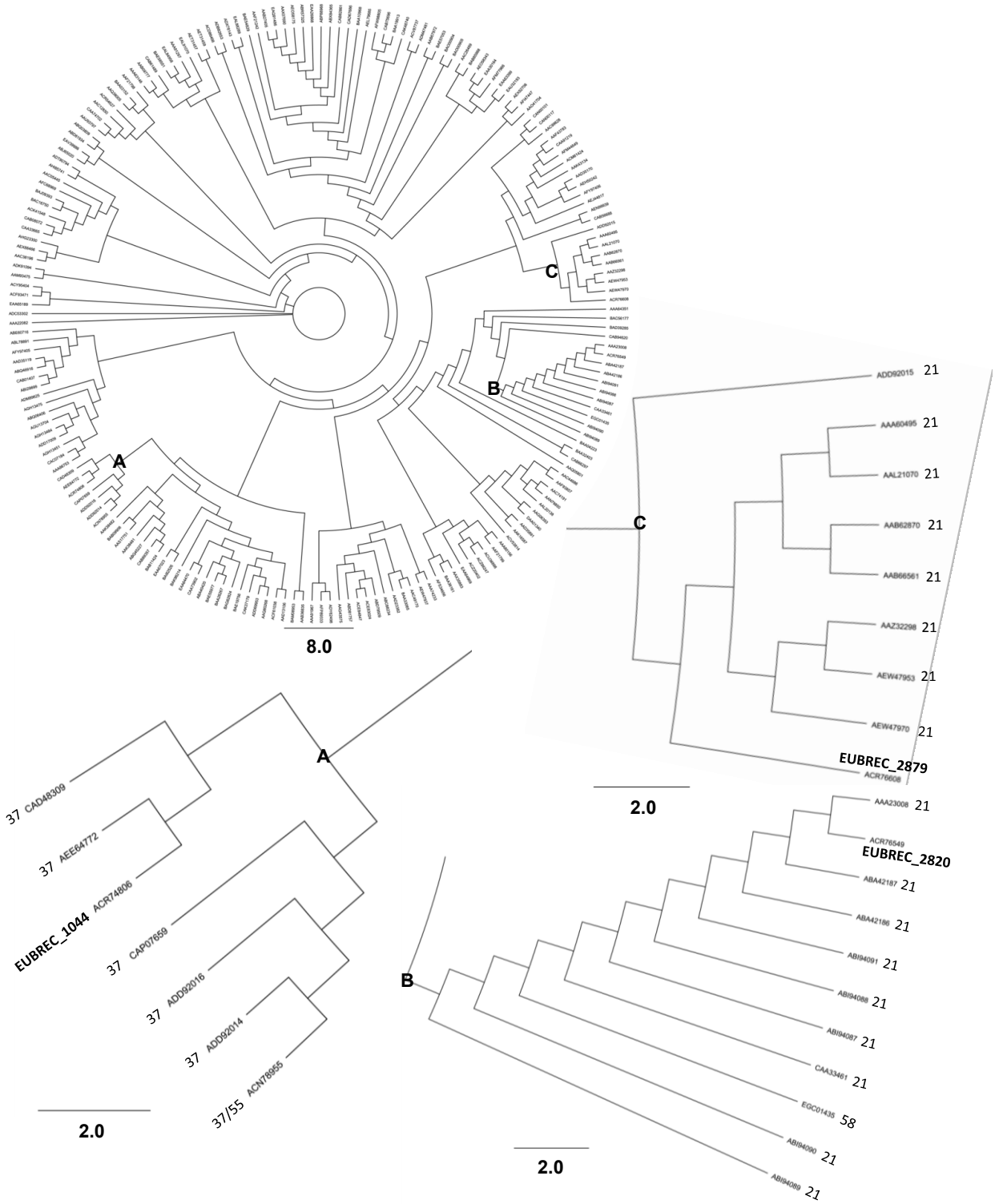

Figure S2: Phylogenetic tree based on protein sequences of GH3 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_1044 EUBREC_2820, and EUBREC_2879, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 21, *β*-glucosidase, 37, *β*-xylosidase, 55, *α*-arabinofuranosidase, 58, glucan 1,3-*β*-glucosidase). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

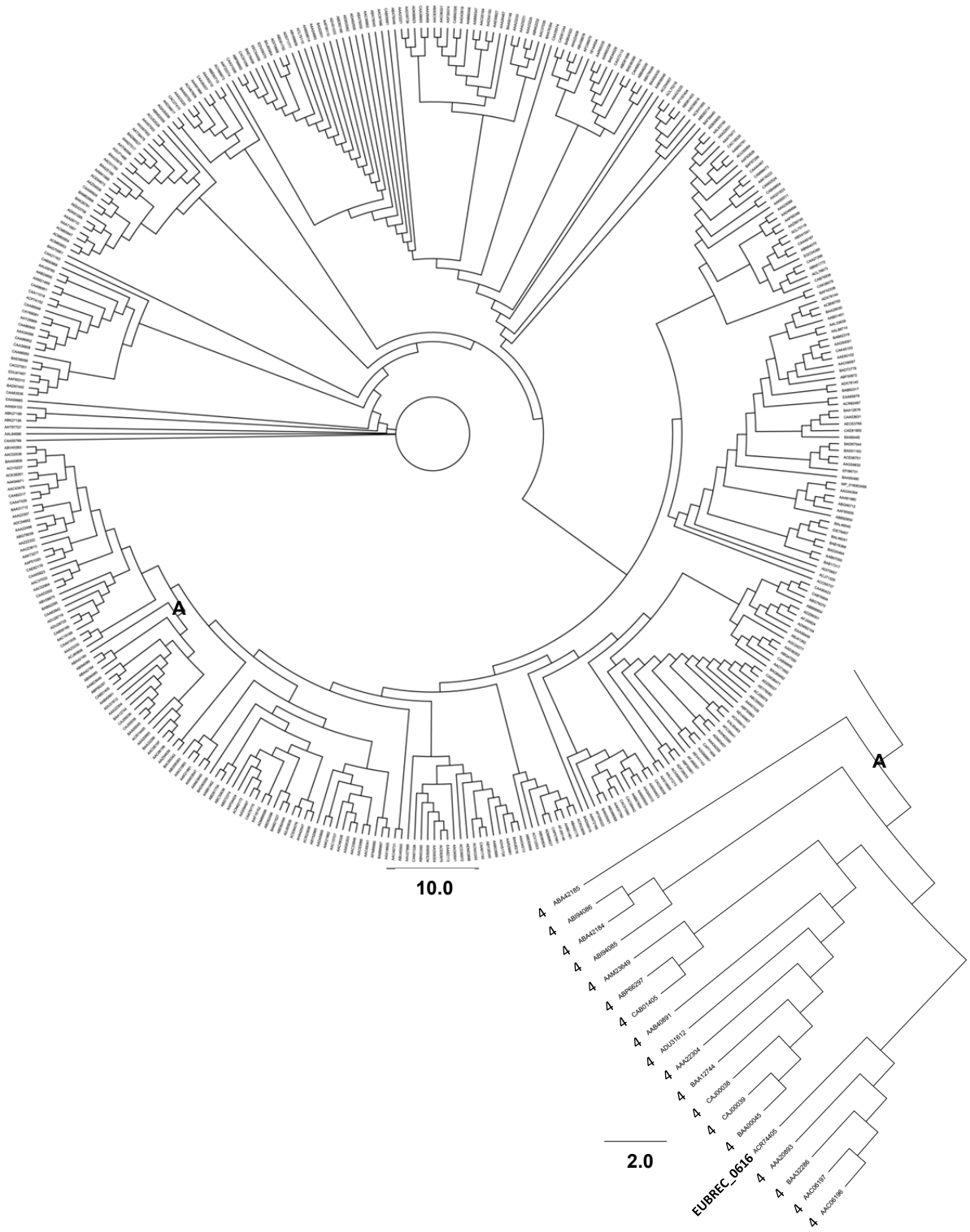

Figure S3: Phylogenetic tree based on protein sequences of GH5 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_0616, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 4, endo-β-1,4-glucanase/cellulase). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

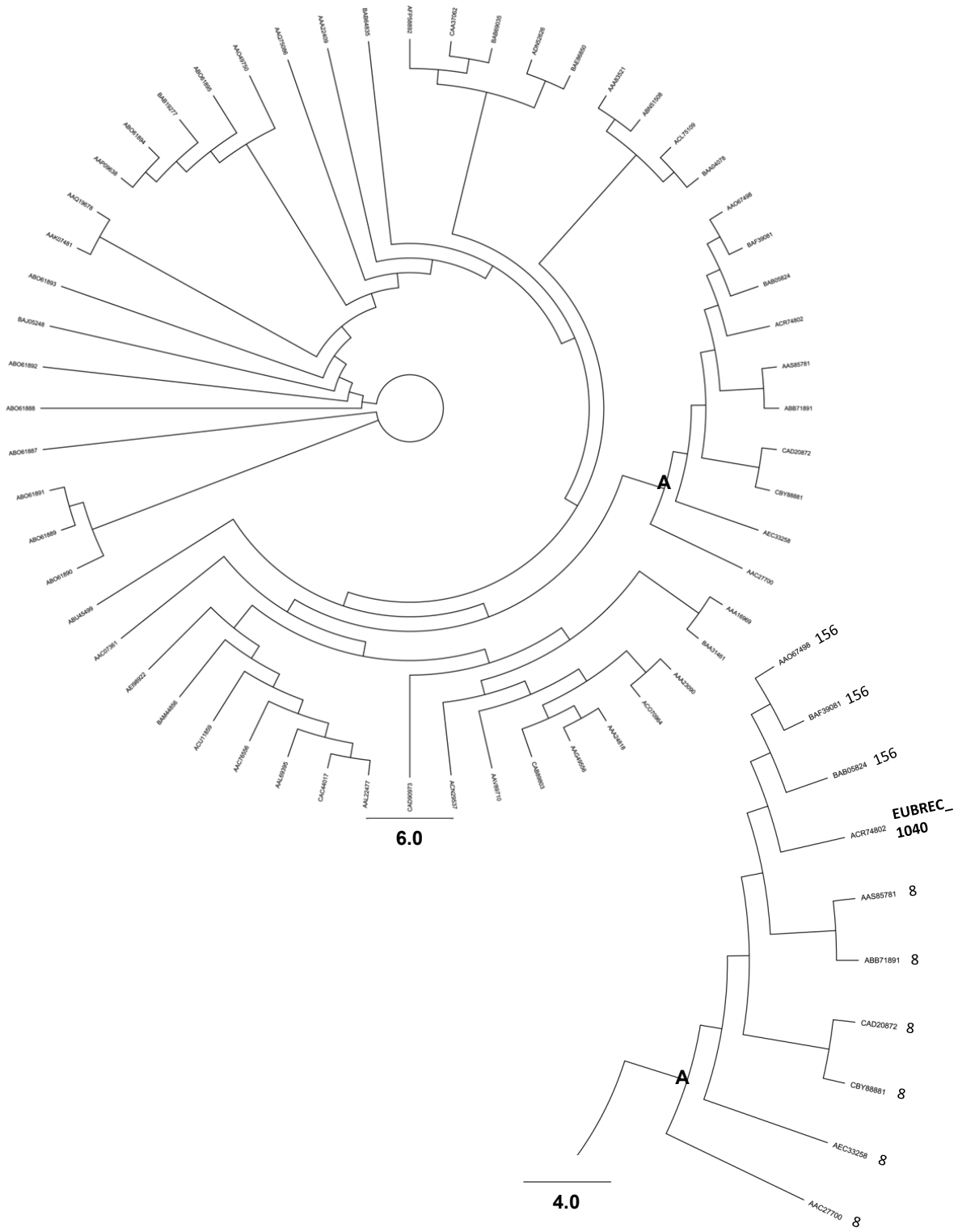

Figure S4: Phylogenetic tree based on protein sequences of GH8 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_1040, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 8, endo-1,4-*β*-xylanase, 156, exo-oligoxylanase). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

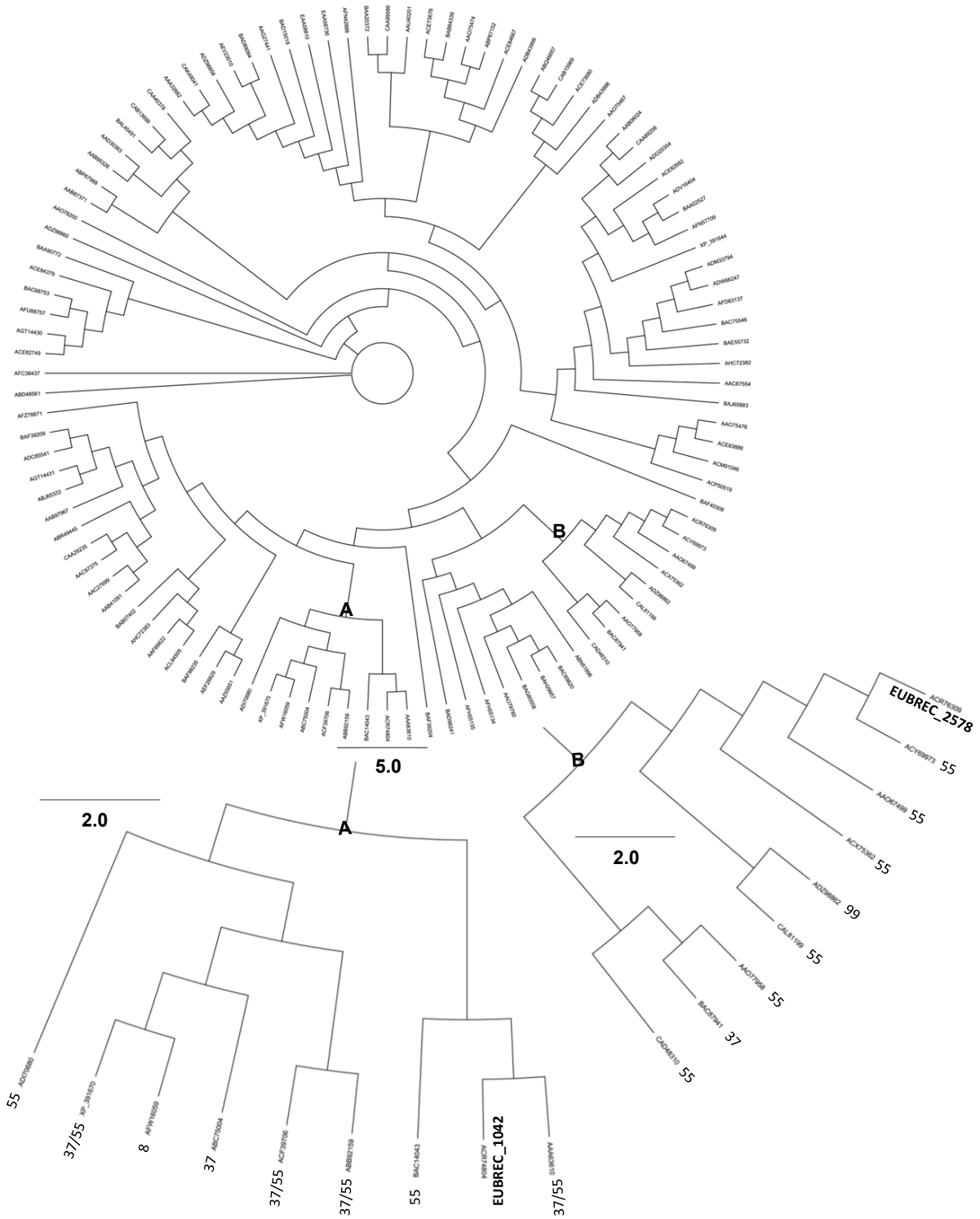

Figure S5: Phylogenetic tree based on protein sequences of GH43 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_1042 and EUBREC_2578, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 8, endo-1,4-*β*-xylanase, 37, *β*-xylosidase, 55, *α*-arabinofuranosidase, 99, arabinanase). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

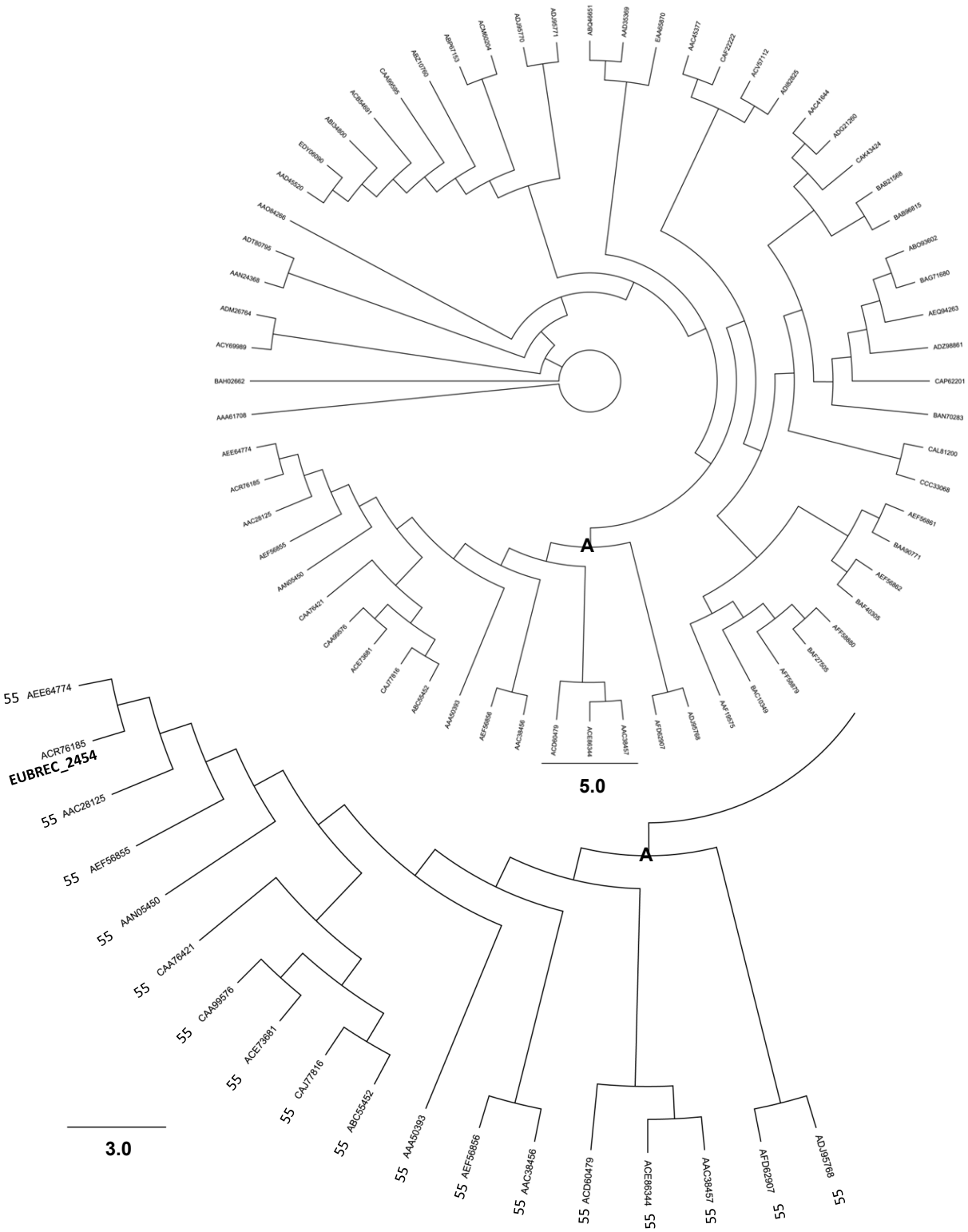

Figure S6: Phylogenetic tree based on protein sequences of GH51 glycoside hydrolases obtained from the CAZy database and the protein sequence derived from EUBREC_2454, indicated by their GenBank accession numbers. Numbers represent the glycoside hydrolase activity (EC number, excluding 3.2.1.; 55, *α*-arabinofuranosidase). The scale bars indicate the length of the branches of the tree (expressed in units of substitutions per site of the sequence alignment).

Supplemental tables

Table S1. Primer sets used for quantitative PCR (qPCR) and propidium monoazide-qPCR (PMA-qPCR) analyses.

Target organism	Locus tag	Forward primer (5' → 3')	Reverse primer (5' → 3')	Amplicon length (bp)	Optimized primer concentration (forward/reverse) (μM)	C_t value with genomic DNA of *B. longum*	C_t value with genomic DNA of *E. rectale*
Bifidobacterium longum NCC2705	BL1097[a]	AAGGTGCTGC ACGAGGAGTT	GAATCGATCTG GTTGAAGTCGTACT	62	2.5/6.25	19	30
Eubacterium rectale ATCC 33656	EUBREC_0383[b]	ACAGGTCGTG GTACTGTTGC	TCGTCGTTGAG GTGAAGTGT	62	2.5/2.5	16	31

[a] Accession number whole-genome sequence = NC_004307 (NCBI, NCBI Resource Coordinators, 2014; accessed in January 2014).
[b] Accession number whole-genome sequence = NC_012781 (NCBI, NCBI Resource Coordinators, 2014; accessed in January 2014).

Table S2. Primer sets used to generate[a] PCR amplicons containing qPCR primer-binding sites (qPCR standards).

Target organism	Forward primer (5' → 3')	Reverse primer (5' → 3')	Amplicon length (bp)	Molecular mass (g mol^{-1})
Bifidobacterium longum NCC2705	TGGGCGATGTTGATGGTGATAC	TAAGACTACCCTGACTGCCG	135	83,305
Eubacterium rectale ATCC 33656	AGGACGTATTCACAATCACAGGT	ACACCAAGGATCTCTAACTCGTC	97	59,811

[a] Each 50-μL PCR assay mixture contained 5 μL of 10× *Taq* buffer (Feldan, Québec, QC, Canada), 0.25 μL (1.25 U) of *Taq* DNA polymerase (Feldan), 1 μL of deoxynucleotide mix (10 μM; Invitrogen, Burlington, ON, Canada), 1 μL of genomic DNA, 2 μL of each primer (10 μM), and 38.75 μL of sterile nuclease-free water (Qiagen, Mississauga, ON, Canada). The PCR amplification program consisted of an initial denaturation step at 95.0°C for 2 min; followed by 30 three-step cycles at 95.0°C for 20 s, 61.8°C for 40 s, and 72.0°C for 30 s; and a final extension step at 72.0°C for 5 min using a TProfessional Basic Gradient thermocycler (Biometra, Montreal, QC, Canada).

Table S3. TaqMan gene expression assays (primers and probes) used for RT-qPCR analyses in *Bifidobacterium longum* NCC2705 and their assessment of specificity towards *B. longum* NCC2705 and *Eubacterium rectale* ATCC 33656.

Metabolic pathway	Gene[a]	Locus tag	Primer sequences			Amplicon length (bp)	Efficiency (E)	C_t value with genomic DNA of *B. longum*	C_t value with genomic DNA of *E. rectale*
			Probe	Forward primer	Reverse primer				
Bifid shunt and metabolite production	*BL0274*, ribulokinase	BL0274	AGACGTGCTCT TCCTTGTTGA	GCGAGTGTGG TGAAGTAAGC	TGGTCCATCGC TCACCTGTA	80	1.92	21	34
	xylB, xylulokinase	BL1709	CGTCCAGAAT GCGCGAAC	TCGGCCAGTTC GTCGTAGT	CTCGCCTGCAC AATCAACG	80	1.87	20	33
	BL0959, bifunctional xylulose-5-P/fructose-6-P phosphoketolase	BL0959	CTGGCCGATG GAAAGGTAGT	CCTTCATCAGC GGGTTGGAA	AGGCGTTGAC AAGTACTGGC	80	1.94	21	35
	ldh1, lactate dehydrogenase	BL0710	TCGAAGAGAA GACCCGCAC	CGACAACCAA GACCGTTTCG	CGACGATCTCA TTGCCACGG	80	1.87	21	34
	ldh2, lactate dehydrogenase	BL1308	CGACATCGCTA CCCACGTG	TACATGCTCAT CACCAACCCG	AAGATCTGGTT CTCGGGCAG	80	1.93	21	Nd[c]
	ackA, acetate kinase	BL0969	CGCTGAACAA GGAAATCGCC	TCCTTCTTCTT CCAGCTGCC	CGGCGGATGT GGTACTGATC	80	1.84	19	35
	adh2, bifunctional acetaldehyde-CoA/alcohol dehydrogenase	BL1575	CCGAACGCCA TCAAGTACCT	AAGATCCCGG CCAAGACCTA	ATGACGGCCTT CTCGATGC	80	1.81	20	34
(A)XOS degradation	*BL1543*, extracellular *α*-arabinofuranosidase (GH43, EC 3.2.1.55)[b]	BL1543	CGAGTACGCC GATTACACG	TTTCGATTCCT GCCGGTGAT	CACATGATGC ACCCCTGTCA	80	1.89	20	33
	BL1544, extracellular *α*-arabinofuranosidase (GH43, EC 3.2.1.55)	BL1544	TGACTTGAGCG ACGTGTTGA	CCAAGGAGAA TACGGGTGGTT	CACAGCTTTGC CGTCAGAAG	80	1.89	20	34
	BL0181, intracellular *α*-arabinofuranosidase (GH51, EC 3.2.1.55)	BL0181	CAAGTAGTGC GCGAAGGC	TTCGCTTGGCC GTGGATT	CCATTGAAGTT TCCGTGCCG	80	1.86	20	Nd
	abfA1, intracellular *α*-arabinofuranosidase (GH51, EC 3.2.1.55)	BL0544	CAGGAAGCTG GCCATGTCG	TTGATGAAGCC GTCCATATCCA	CAGCCGTTCTT CAAGGAGGA	80	1.85	20	32
	abfA2, intracellular *α*-arabinofuranosidase (GH51, EC 3.2.1.55)	BL1166	ATGGGGCGTA TGGTATTCGG	CCAACAACGG CACCAAAGAC	TTCCTGCTCGT TCCACTTGT	80	1.91	20	Nd
	abfA3, intracellular *α*-arabinofuranosidase (GH51, EC 3.2.1.55)	BL1611	GAAGACCAGA ACCGAGTCGTT	CTGCACGAGG ATGATCTGCT	GTGATGGTGGT GTTGGGGT	80	1.88	20	34
	BL1138, intracellular *α*-arabinofuranosidase (GH51, EC 3.2.1.55)	BL1138	CGTATGCCGTG TCCATCGAG	CTGTTTTCCAC GCATCTGGG	TACAGATGTTT GGCGGGGC	80	1.95	21	Nd

Stress and cellular responses	*groEL*, chaperonin GroEL	BL0002	GCCTGGACCTC GACTTCAC	CGTTGTGACCG TTGAAGACA	TGTAGCCCTTG TCAAAACGC	80	1.85	19	34
	dnaK, chaperone DnaK	BL0520	CCGACAGCCA CAACCTCAT	CTTACGGTCGC CCTTGATGA	CGAACCAGTC CGTGAACCC	80	1.79	22	Nd
	BL0618, Dps family protein	BL0618	ACATCATCCAG GACCACGTG	CTGGACGAGC TGGATGTGAT	CGCATGAACC ACTGGAACTT	80	1.84	21	34
	ftsZ, cell division protein	BL0118	ATTGCTGCTGG TTTTGACCC	ATGGCGATGA AGTGCGTGTC	CGTGCTCTGAG TGTCGTCAT	80	1.88	20	35
	atpA, F_oF_1-ATP synthase, subunit α	BL0359	AAGGTTGAAC GCCAGGCC	GATCACCACG CCGATCTCA	CCAATGAGCT GCTGACGTTC	80	1.85	20	Nd
	tuf, elongation factor Tu	BL1097	GACGTCGGTG GTGCGGAA	TGAACCATCTC GACGCCTTC	AACTACCGTCC GCAGTTCTAC	80	2.02	20	Nd
Reference genes	*rpoB*, DNA-directed RNA polymerase, subunit β	BL1205	TCGTAGTTGTA GCCGTTCCAC	AGGCGCTGGG AGATGATCA	CTGGGCAAGA ACCTGCTCAT	80	1.89	19	36
	atpD, F_oF_1-ATP synthase, subunit β	BL0357	CGGGTTGCGGT GGATAGG	CTCCAGCTGAT CGAAGGCC	ACAAGAAGCC GGATGAGACC	80	1.88	19	34
	recA, recombinase A	BL1415	TGTTCTTCGGC AACCCTGAA	CTTCATCAACC AGCTGCGTG	ACTTCAGAGCT TTACCACCGG	80	1.92	21	35
	ileS, isoleucyl-tRNA synthetase	BL1777	TTGGGTGGACT TCGAGCAC	CGAATGGCAG AACTACGTGC	TGTACGGGAT GTTCAGGGTC	80	1.86	20	35
	pcrA, ATP-dependent DNA helicase PcrA	BL0890	TCGTAGAACTT GGTGCCGC	CCAGCGCATCC TTGATTTCG	CTGATCAACAC GAATCAGCCG	79	1.92	20	35

[a] Putative gene functions were obtained based on information from the RefSeq database, Lagaert (2013), Schell *et al.* (2002), and the pathway databases BioCyc and the Kyoto Encyclopedia of Genes and Genomes (KEGG).
[b] Glycoside hydrolase family (GH) according to the CAZy database; EC numbers as determined by Lagaert (2013) according to the numerical classification of the enzyme commission (EC).
[c] Nd = not determined by RT-qPCR.

Table S4. TaqMan gene expression assays (primers and probes) used for RT-qPCR analyses in *Eubacterium rectale* ATCC 33656 and their assessment of specificity towards *Bifidobacterium longum* NCC2705 and *E. rectale* ATCC 33656.

Metabolic pathway	Gene[a]	Locus tag	Primer sequences			Amplicon length (bp)	Efficiency (E)	C_t value with genomic DNA of *B. longum*	C_t value with genomic DNA of *E. rectale*
			Probe	Forward primer	Reverse primer				
Pentose phosphate pathway and metabolite production	*EUBREC_0668*, ribulokinase	EUBREC_0668	ATGGTGGTGCTTGAAAAGGC	CGGAAATGTATCAGCGGGCA	CAAGCTCCTCATGAACTGCC	80	1.91	36	17
	EUBREC_2559, xylulokinase	EUBREC_2559	AGCGCTCCTGTCAAAATCTT	ATGCATCTGACCTCCGAAGC	CCGTGGCTGGGCTTATAGAG	80	1.92	36	19
	EUBREC_2460, transketolase domain protein	EUBREC_2460	CCAGACCTGTCCCTCCTGAA	CAAGTCTTCTGAAGCCCGCA	ATATACACTTCTCGGCGATGG	80	1.86	35	18
	EUBREC_1724, lactate dehydrogenase	EUBREC_1724	TCTGGTTTTTGCATGCGAGG	TATGGCTGTGTCTCCGATGC	CCTGTCCTGAATGCAACGG	80	1.88	36	18
	EUBREC_2331, lactate dehydrogenase	EUBREC_2331	TTGAGAGAACCACACCGTCG	TCCACTCCGTTTGCTCCAAC	TCACACATGATGCACGGACA	80	1.82	36	18
	EUBREC_ 1472, pyruvate:ferredoxin oxidoreductase	EUBREC_ 1472	TCACACTTACATCAAAGGCCG	CATTCAACCCTGCACTCAAGG	CTCCTGGTAAGATCCGTCGC	80	1.92	35	19
	EUBREC_0735, butyryl-CoA dehydrogenase belonging to the butyryl-CoA dehydrogenase/electron transferring flavoprotein (Bcd/Etf) complex	EUBREC_0735	ATGTTCCTGACCTTGCTTCA	TGACATTCGGTACAGAGGAGC	AAGACCGAAAGCACCGATCT	80	1.92	35	19
	EUBREC_3075, butyryl-CoA:acetate CoA-transferase	EUBREC_3075	CGTTATCGTCAGGCTTACGG	CCGGAAAAATCACAGGAGCA	CATCTTCTTGGTTCCGGCAC	80	1.88	35	18
	EUBREC_2390, ferredoxin hydrogenase	EUBREC_2390	TCTGTGGGTCGATATGTGAGC	AGCGTCCACACTCCTTACAC	TGCATGTAAGTTCGGAGCAA	80	1.80	35	19
	EUBREC_1646, ferredoxin belonging to the membrane-bound ferredoxin oxidoreductase (Rnf) complex	EUBREC_1646	TCATGTAATTATGGTTGCCTTGGC	TGCAATGTCATTTGTACCGGG	ATGCCTTCACACAGCTTCCA	80	1.86	36	19
(A)XOS degradation	*EUBREC_1040*, xylanase Y (GH8, EC 3.2.1.156, exo-oligoxylanase)[a]	EUBREC_1040	GAAAGGCTTCAGAAGTTTTATTGTG	TGCAGATCCGTGGCAGATTA	ACCCCATCCCAATTGTCCTTC	81	1.80	35	18
	EUBREC_1042, *α*-arabinofuranosidase (GH43, EC 3.2.1.37/55, bifunctional *β*-xylosidase/α-arabinofuranosidase)	EUBREC_1042	ATGGGAAAACGAATGGCCGG	AGCAGTATGGGCAGGGAAAC	TGTCCTATCCCGGGTGCTATT	80	1.81	35	19
	EUBREC_1044, *β*-glucosidase (GH3, EC 3.2.1.37, *β*-xylosidase)	EUBREC_1044	TATGCAAGCGGTGACAAGCT	AAGGTGAAGAGGGGGATGCC	TTCCTCCTGAAGTCCCGGAA	80	1.81	36	18
	EUBREC_2454, *α*-arabinofuranosidase (GH51, EC 3.2.1.55, *α*-arabinofuranosidase)	EUBREC_2454	CCGCTTCCTAAATTTCCGTTCA	CCACTCACTCATCTCGCGTA	AGCTTTGCAGACAGTTAGGC	80	1.86	36	19
	EUBREC_2578, polyfunctional esterase/*β*-xylosidase/*α*-	EUBREC_2578	CCACTATTCCTCCCTGTGCC	CCTGAAACAGCATTGCATACCA	TGGATATTGTGGACAGGGCG	80	1.85	35	18

	arabinofuranosidase (GH43, EC 3.2.1.55, *α*-arabinofuranosidase)								
Stress and cellular responses	*EUBREC_2270*, chaperonin GroEL	EUBREC_2270	ACTGTGTTCTCCTTCTGAACCT	CTTGTCTCCGGCACCATCAA	CAATCGATATGCTTGGCCGTG	80	1.83	35	19
	EUBREC_0020, chaperone DnaK	EUBREC_0020	TGAGGCTGATACGCTTGTGC	ACAGACGGACAGAGAAAGGC	GCCATGACATTTTCCACCTGG	80	1.82	36	19
	ftsZ, cell division protein	EUBREC_2247	AGCTGTCTGATTCGGATATACC	TGTGTTCCCATGCCTGAAACA	AGGAATGGCAGGCGGTATG	80	1.84	36	19
	atpA, F_oF_1-ATP synthase, α subunit	EUBREC_0122	ATCGCACCATACTCAGGCTG	TCAGAGCTTGCACCGTTACA	TCCTGTCCGTTTTCCATCCA	80	1.95	36	18
	EUBREC_0383, elongation factor Tu	EUBREC_0383	TGGTGATAACATCGGAGCACT	AGCAGTTAGACGAGGCTCAG	TCCACGAACGATCTGGTCAC	80	1.86	36	19
Reference genes	*EUBREC_0379*, DNA-directed RNA polymerase, subunit β	EUBREC_0379	ACCTCATTCCTTACGGCTCAC	AAACAACGAGACAGGCGAGA	GCTCCTGTCCGTCTACAACC	77	1.85	35	19
	EUBREC_0124, F_oF_1-ATP synthase, subunit β	EUBREC_0124	AGCTTGGTATTTATCCGGCTGT	TGCCACGACAGTACTTGAGC	TACGCGATGTAGATGCCAGC	80	1.82	35	19
	EUBREC_2512, preprotein translocase, subunit SecY	EUBREC_2512	CTGTGGCAGCATAAATACCGC	CCGGGTGCAGATAATATAAGCC	TGCAATGCCGCTTATGTTCA	80	1.84	35	19
	EUBREC_1905, DnaJ protein	EUBREC_1905	CTGTGCCATCAAATGCTGCA	CCATGCCTGAGAAATCAAAGCC	ACGCCAGTATGATCAGTTCGG	80	1.85	35	18
	EUBREC_0328, DNA polymerase III, subunits γ and τ	EUBREC_0328	TGATGATGAGAATGCGTATGAGT	TCGCTGGGCAATTCATCTGA	CCTGCACGGTTTCAGAGAG	80	1.81	36	19

[a] Putative gene functions were obtained based on information from the RefSeq database, Mahowald *et al.* (2009), and the pathway databases BioCyc and the Kyoto Encyclopedia of Genes and Genomes (KEGG).
[b] Glycoside hydrolase family (GH) according to the CAZy database; EC numbers were determined according to Lagaert (2013) (Supplemental materials and methods M1) according to the numerical classification of the enzyme commission (EC).

References

1. **Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B.** 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res **42:**D490–D495.

2. **Lagaert S.** 2013. Study of the arabinoxylan and arabinoxylan oligosaccharide-degrading enzymes of *Bifidobacterium adolescentis* and *Bifidobacterium longum*. Ph.D. thesis. KU Leuven, Leuven, Belgium.

3. **Petersen TN, Brunak S, von Heijne G, Nielsen H.** 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods **8:**785–786.

4. **NCBI Resource Coordinators.** 2014. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res **42:**D7–D17. doi: 10.1093/nar/gku1130.

5. **Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore RD, Arigoni F.** 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. Proc Natl Acad Sci U S A **99:**14422–14427.

6. **Mahowald MA, Rey FE, Seedorf H, Turnbaugh PJ, Fulton RS, Wollam A, Shah N, Wang C, Magrini V, Wilson RK, Cantarel BL, Coutinho PM, Henrissat B, Crock LW, Russell A, Verberkmoes NC, Hettich RL, Gordon JI.** 2009. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A **106:**5859–5864.