Table S1, related to Figures 2, 4-6. Doubling times[1] for bacterial strains used in the study.

	B. theta	***B. caccae***	***B. vulgatus***	***B. uniformis***	***B. fragilis***	***B. ovatus***
Glucose	1.3	4.8	2.8	2.0	1.5	1.4
Fructose	1.2	13.5	3.2	2.1	1.8	1.9
Sucrose	1.6	1.4	2.4	2.1	2.1	1.3
Levan	2.7	-	-	-	-	-
FOS	5.6	2.1	3.0	2.8	2.0	1.4
Inulin	96	2.7	-	3.9	9.9	2.2

	BTΔ1754	**BTΔ1754: BT1754**	**BTΔ1760**	**BTΔ1760: BT1760**	**BTΔ1762**	**BTΔ1762: BT1762**
Glucose	2.6	2.6	2.9	3.4	2.1	ND
Fructose	11.8	2.5	2.6	2.1	1.9	ND
Sucrose	8.3	3.1	3.2	ND	2.5	ND
Levan	-	2.1	-	3.6	19.2	8.9
FOS	-	3.0	5.8	ND	3.8	3.5
Inulin	-	-	-	-	-	-

[1]Doubling times in hours of the Bacteroides species (top table) and the *B. thetaiotaomicron* mutants (bottom table) in minimal media containing a single carbon source. Doubling times were calculated using the KinetiCalc software (F. Breidt, 1994). ND : not determined, - : no growth.

Table S2, related to Figure 4. Kinetic parameters of *Bacteroides thetaiotaomicron* GH32 enzymes against β2-6 and β2-1 fructans and fructooligosaccharides

Enzyme	1759			1760			1765			3082		
	kcat (min^{-1})	K_M (mM)	kcat/ K_M (min^{-1} mM^{-1})	kcat (min^{-1})	K_M (mM)	kcat/ K_M (min^{-1} mM^{-1})	kcat (min^{-1})	K_M (mM)	kcat/ K_M (min^{-1} mM^{-1})	kcat (min^{-1})	K_M (mM)	kcat/ K_M (min^{-1} mM^{-1})
Sucrose	816 ±136	7.8 ±1.2	104	NA[a]	-	-	27087 ±3782	2.2 ±0.7	11286	1421 ±35	2.4 ±0.2	592
Kestose	379 ±75	2.7 ±0.8	140	NA	-	-	3329 ±922	11.8 ±3.0	282	2004 ±204	0.9 ±0.0	2227
Kestotetraose	706 ±125	0.9 ±0.1	784	NA	-	-	3829 ±684	5.1 ±2.1	751	1365 ±159	0.7 ±0.1	1950
Kestopentaose	545 ±45	1.2 ±0.1	454	NA	-	-	4437 ±263	9.0 ±0.0	456	1910 ±412	1.0 ±0.5	1910
Inulin	156 ±63	0.3[b] ±0.1	520	NA	-	-	~1235	>30[b]	-	1013 ±119	0.6[b] ±0.1	1688
Levanbiose	1052 ±209	6.0 ±0.3	175	ND[c]	-	-	9330 ±1509	8.8 ±0.6	1060	2184 ±250	5.3 ±0.5	412
Levantriose	594 ±129	7.3 ±0.6	81	ND	-	-	1857 ±67	9.3 ±0.3	200	1600 ±281	5.6 ±0.8	286
Levantetraose	762 ±175	3.0 ±1.7	254	ND	-	-	2696 ±181	18.8 ±1.5	143	1841 ±192	5.6 ±0.4	329
Levanpentaose	737 ±74	3.0 ±1.6	246	ND	-	-	4043 ±272	34.7 ±0.4	116	1881 ±110	5.4 ±0.4	348
Levan	205 ±47	1.0mg/ml ±0.3	205 (min^{-1} mg/ml^{-1})	32987 ±9383	10.6mg/ml ±1.6	3112 (min^{-1} mg/ml^{-1})	~288	>100mg/ml	-	834 ±95	1.4mg/ml ±0.3	596 (min^{-1} mg/ml^{-1})

Values shown are the mean and SD of at least 3 independent assays for each enzyme/substrate pair.
Kestoligosaccharides are β2-1 linked fructose with a terminal non-reducing glucose. Levanoligosaccharides are β2-6 linked fructose with no terminal glucose moiety.
[a]NA - No activity detected.
[b]mM K_M for inulin based on average MW of ~4000 (Sigma; Chicory, Cat. no. I2255).
[c]ND - Not determined.

Table S3. List of primers used in this study.

Quantitative RT-PCR

BT1754_forward	CGCAATCTGATCGATTCTCA
BT1754_reverse	ACACGGCTTAGTCCCATGTC
BT1757_forward	GTCTTTCAAACGCTGCAACA
BT1757_reverse	GAGCTTCGGGAACAGACTTG
BT1763_forward	ATGCCTGGTCACCTACGAAC
BT1763_reverse	CAAGCGGTCCATTCTCATTT
BT1765_forward	AGACCTGATGCATTGGGAAC
BT1765_reverse	CCATTTACTCTCCGGTGCAT
BT3082_forward	CACCGAACTGACTTTGCGTA
BT3082_reverse	CGTGGCATGAGAGAGTTTGA
BT3305_forward	CGGTGGGGACAAAGTATCAC
BT3305_reverse	AAAGTCTTTCCCGCACTGAA

Quantitative PCR

*B. theta*_forward	GGGGGTATCTTCACCTTCGT
*B. theta*_reverse	ATTCGGTTGAACGCTTGTCT
*B. caccae*_forward	CAGCCGCTACTTTGAAGCTC
*B. caccae*_reverse	TTGACGGAGGCAAAAATAGG
*B. vulgatus*_forward	TAGAGATCCGCCTCGTGTCT
*B. vulgatus*_reverse	TCCAAACGAGGAAGCCATAC

***B. theta* mutant generation**

BTΔ1754

BTΔ1754_1000up	AAAAGGATCCACCGGAAAAGTGGAAAGTGA
BTΔ1754_750up	AAAAGGATCCACTTTTGCTGAAAGCGGAGA
BTΔ1754_700up	AAAAGGATCCTCCATGTCTCATTTGCCAAC
BTΔ1754_sewing_forward	CCTCTTTACGATATCAATGAAATTACATTTCATAGTTCTTTCTGTAATCC
BTΔ1754_1000dwn	AAAATCTAGATGGCATTGTTGCTGTGCTAT
BTΔ1754_750dwn	AAAATCTAGAACACTTCCGTGCACTTCAGA
BTΔ1754_700dwn	AAAATCTAGATCTGCTCTTTTTGGGGAATTT
BTΔ1754_sewing_reverse	GGATTACAGAAAGAACTATGAAATGTAATTTCATTGATATCGTAAAGAGG

BTΔ1754:BT1754

BTΔ1754:BT1754_forward	AAAATCTAGAATCATTCAGTTTTCTGTTGGTTACTT
BTΔ1754:BT1754_reverse	AAAAGGATCCTTAAAATCCGATGTTAAGTCCGAA

BTΔ1760

BTΔ1760_1000up	AAAAGGATCCCTGTAGTCGTTCCGCTGACA
BTΔ1760_750up	AAAAGGATCCTTTGTCGGTACGGCTTCTTC
BTΔ1760_700up	AAAAGGATCCGGATAATGTATCGATGTCCGAAT
BTΔ1760_sewing_forward	CTTGCCGGTGTAGTTTTCATCACATTGTTGCTTATTCTTTTTATTTACA
BTΔ1760_1000dwn	AAAATCTAGACAGCGTTTTGTTGCTTCGTA
BTΔ1760_750dwn	AAAATCTAGACGGTGTTCCCAGTTTACGAG
BTΔ1760_700dwn	AAAATCTAGACTGATAGCGTGTCCCCAGTT

BTΔ1760_sewing_reverse	TGTAAATAAAAAGAATAAGCAACAATGTGATGAAAACTACACCGGCAAG

BTΔ1760:BT1760

BTΔ1760:BT1760_forward	AAAATCTAGACCGGAGTTCTCAGGATTCC
BTΔ1760:BT1760_sewing_reverse	CTATAGGTAAGATCATATTTTTCATCATAGGATATTCAGAGTTATACTAGTG
BTΔ1760:BT1760_sewing_forward	CACTAGTATAACTCTGAATATCCTATGATGAAAAATATGATCTTACCTATAG
BTΔ1760:BT1760_reverse	AAAAGGATCCTCAATAAGTGCTTACCTGAACGT

BTΔ1762

BTΔ1762_1000up	AAAAGGATCCCGGTATCGACTTCAGCCTGTT
BTΔ1762_750up	AAAAGGATCCGAATTGCCGGAAACGGTAGC
BTΔ1762_700up	AAAAGGATCCGAAGAGTGTAGTCGGACATAC
BTΔ1762_sewing_forward	CTTCATCAATAAAACGAATGATCATGTAACTAACCATCTTAAAACAAAGAATA
BTΔ1762_1000dwn	TATTCTTTGTTTTAAGATGGTTAGTTACATGATCATTCGTTTTATTGATGAAG
BTΔ1762_750dwn	AAAATCTAGAAGATATATTCGGACATCGATACATT
BTΔ1762_700dwn	AAAATCTAGAGGGACTCGTCAGAAGACTG
BTΔ1762_sewing_reverse	TATTCTTTGTTTTAAGATGGTTAGTTACATGATCATTCGTTTTATTGATGAAG

BTΔ1762:BT1762

BTΔ1762:BT1762_forward	AAAATCTAGAATCATTCAGTTTTCTGTTGGTTACTT
BTΔ1762:BT1762_sewing_reverse	CGATTGTTGCTATATATATTATCTTTTTCATTAGTTTAATGTTATTAATTTAAAAGTAC
BTΔ1762:BT1762_sewing_forward	CGTACTTTTAAATTAATAACATTAAACTAATGAAAAAGATAATATATATAGCAACAAT
BTΔ1762:BT1762_reverse	AAAAGGATCCTTACCAACCGAAATTCTGTGTAT

BTΔ1763

BTΔ1763_1000up	AAAAGGATCCATAGACCGACTACGGGAGCA
BTΔ1763_750up	AAAAGGATCCGCATTAAAATCCGGCCAATA
BTΔ1763_700up	AAAAGGATCCCGCACCGATTTGAAAATACA
BTΔ1763_sewing_forward	CATTCGTTTTATTGATGAAGTAAGTTACATTAGTTTAATGTTATTAATTTAAAAGTA
BTΔ1763_1000dwn	AAAATCTAGACAGTATCTGCGGAGTGGTCA
BTΔ1763_750dwn	AAAATCTAGACTTCGTTGATTCCGGTAAGG
BTΔ1763_700dwn	AAAATCTAGATTTTTGCAAGGTAAGCAGCA
BTΔ1763_sewing_reverse	TACTTTTAAATTAATAACATTAAACTAATGTAACTTACTTCATCAATAAAACGAATG

BT(In+)

BCexch_BT1763_forward	AAAAGCGGCCGCATCATTCAGTTTTCTGTTGGTTACTT
BC02727_reverse	AAAATCTAGAAATGTTCTGTATTTAGGATAAAAGATTAAA
BC02728-BC02731_forward	AAAATCTAGAATGAAATTGAAATATATCCTTGCGG
BC02728-BC02731_reverse	AAAAGGATCCTTATTGCTTCAACCGGTAGACG
BC_BT1763_sewing_reverse	TTTTGTTCTTCATAATACTGAGCATTAGTTTAATGTTATTAATTTAAAAG
BC_BT1763_sewing_forward	CTTTTAAATTAATAACATTAAACTAATGCTCAGTATTATGAAGAACAAAA

Amplification of fructan PUL genes for expression[1]

BT1754-PD_F	CTC**CCATGG**ATGATACACCCCATTTTCGTATTG
BT1754-PD_R	CCG**CTCGAG**GACCTGTTGTGTAGCTAC
BT1759_F	CTC**CCATGG**ATGCGGATTCTCCTTTGC

BT1759_R	CCG**CTCGAG**TTTCTTCAGTTTGTAAACC
BT1760_F	CTC**CCATGG**ATAGTGACGAGACTGAC
BT1760_R	CCG**CTCGAG**ATAAGTGCTTACCTG
BT1765_F	CTC**CCATGG**ATAAAACAACTCCTTGGATAAAAC
BT1765_R	CCG**CTCGAG**TAAACCTAATCTATACACAC
BT3082_F	CTC**GGATCC**GGAGAAGTATCTTTTAAAATAACCAAGC
BT3082_R	CTC**GAATTC**CTACCAAATGGATTCTACGGAAAAGAC
BT1761_F	CTC**GGATCC**TAGTGATGACTTCAAATCCGGCC
BT1761_R	CTC**GAATTC**CTATTTACACAAGTAGTTGATTGCATTGAGAG
BT1762_F	CTC**GGATCC**GACGATTTTTTGGACCGTCAGGTTCC
BT1762_R	CTC**GAATTC**TTACCAACCGAAATTCTGTGTATAATTTCC

[1]All GH32 enzymes (except BT1765), BT1754-PD, BT1761 and BT1762 were expressed without their predicted endogenous signal peptides (http://www.cbs.dtu.dk/services/SignalP/ and http://www.cbs.dtu.dk/services/LipoP/). BT1754-PD, BT1759, BT1760 and BT1765 were cloned into pET22 or pET28 (Novagen) such that the recombinant protein contained a C-terminal His_6 tag. BT1761, BT1762 and BT3082 were cloned into pRSETA (Invitrogen) encoding an N-terminal His_6 tag. Restriction sites introduced for cloning are highlighted.

Table S4, related to Figure 3. X-ray diffraction data collection and refinement statistics for BT1754-PD.

Data collection	
Space group	P 65
Cell dimensions	a=b=111.84, c=115.171
Resolution Range (Å)	37-2.64 (2.78-2.64)[a]
No. of unique reflections	23919 (3512)
Completeness (%)	99.7 (99.7)
Redundancy	5.9 (6.0)
Rmerge	0.10 (0.35)
Mean I/(s)I	12.4 (5.6)
Refinement	
Resolution	35.7-2.64 (2.70-2.64)
Rwork	18.6 (22.8)
Rfree	23.1 (28.6)
No. non-H atoms	4741
Protein	4596
Ligand	24
Water/ion	121
Mean B, all atoms ($Å^2$)	36.2
Protein	36.1
Ligand	25
Water/ion	39.5
Rmsd B values ($Å^2$) (m.c./s.c.)	0.5/1.1
No. Ramachandran outliers	2
Rmsd bond lengths (Å)	0.010
Rmsd bond angles (°)	1.3

[a] Values in parentheses refer to values in the highest resolution shell

Supplemental Data References

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