Supporting Information

Scott et al. 10.1073/pnas.1000091107

SI Materials and Methods

Microarray Hybridization Analysis. The fluorescence of each spot was measured in two channels using a GeneTAC LS IV (Genomic Solutions) using GTLS software. The microarray data were log2 transformed and normalized by Loess normalization to remove intensity-dependent dye effects. Because each clone was represented by three spots, we calculated the median of the three log2 (Cy5/Cy3) values, a robust measure of relative gene expression. In total, the fluorescence of 12 hybridizing spots was compared for each clone. *P* values were calculated by applying a one-sample *t* test to the two log ratios from the two replicate experiments, whereas the two dye-swapped arrays within each experiment were combined by averaging. Because these *P* values were based on only two biological replicates, they were not used as the main selection criterion. Instead, a threshold of at least 5-fold induction was used to select clones for further analysis. The whole experiment conformed to the Minimum Information about a Microarray Experiment (MIAME) criteria for the documentation of microarray experiments.

Reverse Transcription–Quantitative PCR. Primers were designed and checked using Netprimer to specifically amplify fragments of 80–200 bp from selected clones. The optimal annealing temperature for each primer pair was determined in a standard PCR amplification, using the appropriate clone DNA as a template, in a gradient PCR machine (Bio-Rad) capable of running eight different annealing temperatures simultaneously. The same RNA purification (10 ng) used to hybridize to the array slides was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). Aliquots of the resulting cDNA (2 μL) were used in quantitative PCR (Q-PCR) amplifications using the Bio-Rad iCycler iQ and incorporating specific primers in the iQ SYBR Green Supermix (Bio-Rad), according to the manufacturer's recommendations. Control samples lacking the reverse-transcriptase enzyme were included to assess DNA contamination of the RNA sample, and triplicate samples of a 5-fold dilution series were amplified in each case. Amplification conditions were 1 cycle of 95 °C for 3 min followed by 40 cycles of 95 °C for 30 s and the specific annealing temperature for 30 s, with a final melt curve-analysis step (heating the PCR mixture from 52 °C to 95 °C by 1 °C every 10 s) to confirm specificity of amplification and lack of primer dimers.

The value used for comparison was the threshold cycle (Ct), the cycle number at which the fluorescence is above the baseline level (set at 200). The Ct values for RNA purified from each substrate were compared with the values for glucose to determine the fold difference, or gene induction, relative to growth on glucose. The Ct values were first converted from a logarithmic to a linear scale using the formula $x = 2^{-Ct}$, and the larger of these mean values was divided by the smaller to obtain the fold difference (1). The log-transformed data were analyzed using residual maximum likelihood (REML) with substrate, gene, and their interaction as fixed effects followed by a posthoc *t* test to compare substrate means.

TLC. Samples and standards were separated on TLC plates (K5 silica gel plates) from Whatman 4850–820 using a butanol:acetic acid: water solvent in a ratio of 5:4:1. Samples and standards were applied to the plate, 1 cm from the bottom at 1-cm intervals, applying a total of 1 μL in 0.5-μL aliquots, allowing to dry before subsequent applications. The standard ladder was a mix of 1% substrates comprised of glucose, fructose, sucrose, GF4 (1-fructofuranosylnystose), GF3 (nystose), and GF2 (kestose). Separation was carried out until the solvent front was 1 cm from the top of the plate; the plate was allowed to dry and then, it was rerun in the same way and allowed to dry. Spots were visualized by spraying the plate with 0.2% orcinol in methanol:sulphuric acid (90:10) and heating at 105 °C for 10 min or until spots became visible.

FISH. Samples (0.5 mL) were removed from triplicate bacterial cocultures on Synergy1 after 0, 4, 8, and 24 h and after 0, 6, 10, and 24 h from triplicate bacterial cocultures on inulin, maintaining anaerobic conditions. Samples were fixed by mixing in a 1:3 ratio in 4% (wt/vol) paraformaldehyde at 4 °C for 16 h and 0.5-mL aliquots stored at −20 °C. FISH analysis was performed as described by Walker et al. (2).

Hybridized cells were counted automatically using an Olympus BX61 upright fluorescence microscope with a digital imaging system; 30 microscopic fields were counted per experimental sample. The samples were hybridized to the Erec482 probe to specifically detect *Roseburia inulinivorans* and the Bif164 probe to enumerate *Bifidobacterium longum.* Total bacterial numbers were estimated using the universal probe Eub338. Details of these probes are contained within ref. 2.

Total Protein Extraction. Bacterial cultures (7.5 mL) were centrifuged (5,000 × *g*, 10 min, 4 °C), and the pellet was washed three times with 1 mL of ice-cold washing Buffer (10 mM Tris·HCl, pH 7.4, 100 mM sucrose). Final pellets were resuspended in 300 μL of freshly prepared Extraction buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% BioRad Biolyte, pH 3–10) and sonicated (8 × 30-s bursts, with cooling on ice, using a Soniprep 150). Alternatively, the pellets were stored frozen at −80 °C. After sonication, the samples were centrifuged (13,000 × *g*, 15 min, 12 °C), and the supernatant was divided into 60-μL aliquots and frozen until further use. Total protein concentration was assayed by Bradford method (3) using BSA (Sigma Aldrich) as a standard.

2D Gel Electrophoresis. Sonicated protein extracts were thawed at room temperature. A total of 150 μg protein was loaded for each gel, and the sample volume was adjusted to 340 μL by adding the Extraction buffer and 15 μL 30% DTT. The samples were disrupted for 5 min in a water-bath Decon F5100b sonicator (RT; Decon Laboratories Ltd) and centrifuged (13,000 × *g*, 5 min, 20 °C), and the supernatant was applied to 17-cm Immobilized pH gradient (IPG) strips (pH 4–7) (BioRad). Strip rehydration and 1D isoelectrofocusing was carried out in Proteon IsoElectric Focusing Cells (BioRad) using the following parameters: passive rehydration: 1 h, 20 °C; active rehydration: 50 V, 18 h, 20 °C; isoelectrofocusing: step 1, 250 V, 15 min; isoelectrofocusing: step 2, stepwise increase in voltage to 10,000 V over 3 h; isoelectrofocusing: step 3, 10,000 V until a total of 60,000 Vh had been applied. Afterward, the strips were equilibrated for 15 min in Reduction buffer (6 M urea, 2% SDS, 375 mM Tris·HCl, pH 8.8, 20% glycerol, 130 mM DTT) followed by a 15-min incubation in Alkylation buffer (6 M urea, 2% SDS, 375 mM Tris·HCl, pH 8.8, 20% glycerol, 135 mM iodoacetamide) using freshly prepared buffers; 2D slab electrophoresis was performed on 8–16% gradient polyacrylamide gels using the Hoefer ISO-DALT deca-gel System (200 V, 9 h, 4 °C), and gels were then stained with colloidal Coomassie blue (0.01%) as previously described (4). To assess reproducibility, samples were purified from five independently growing bacterial cultures, and each sample was run in triplicate, yielding a total of 15 gels for each growth condition.

Gels were scanned and digitized and then, they were analyzed using PDQuest version 7.0.1 software (BioRad). For qualitative comparisons, total spot-volume normalization was performed (i.e., density value of a given spot was calculated as a percentage of the sum of volumes of all spots detected and present on all gels) (5). The apparent abundance of any protein spot was considered conserved when the overall gel-to-gel variation did not exceed a factor of 2. Pairwise comparisons between resolved proteomes of cultures grown in a medium supplemented with the appropriate growth substrate were made using at least six gels (from three biological replicates) for each of the compared growth conditions.

Protein spots displaying the greatest increases in their apparent intracellular abundance in the proteomes of *R. inulinivorans* A2-194 cells grown in the presence of either inulin or starch were chosen for subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

LC-MS/MS and Protein Identification. In-gel digestion with trypsin (Promega) and extraction of proteins from gel slices were conducted using a Mass*Prep* station (MicroMass), according to the manufacturer's instructions. MS data from tryptic peptide mixtures were obtained using an Ultimate pump, Famos autosampler, and Switchos microcolumn switching device (LC Packings) interfaced with the nanoflow electrospray ionization (ESI) source of a hybrid quadrupole linear ion trap (Q-Trap) mass spectrometer (Applied Biosystems). The LC-MS/MS analyses were performed at the Rowett Research Institute. For protein identification, the acquired LC-MS/MS spectra were searched using Mascot (http://www.matrixscience.com) (6) and the NCBInr and UniProt KB/TrEMBL databases (http://www.ebi.ac.uk/uniprot) followed by individual inspection of the matched peptides.

1. Hancock LE, Shepard BD, Gilmore MS (2003) Molecular analysis of the *Enterococcus faecalis* serotype 2 polysaccharide determinant. *J Bacteriol* 185:4393–4401.
2. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ (2005) pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 71: 3692–3700.
3. Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of proteins utilising the principle of protein-dye binding. *Anal Biochem* 72: 248–254.
4. Anderson NL, et al. (1994) *Two Dimensional Gel Electrophoresis: Operation of the ISO-DALT System* (Large Scale Biology Press, Rockville, MD), 2nd Ed.
5. Choe LH, Lee KH (2003) Quantitative and qualitative measure of intralaboratory two-dimensional protein gel reproducibility and the effects of sample preparation, sample load, and image analysis. *Electrophoresis* 24:3500–3507.
6. Perkins DN, Pappin DJ, Creasy DM, Cottrell JS (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. 20: 3551–3567.

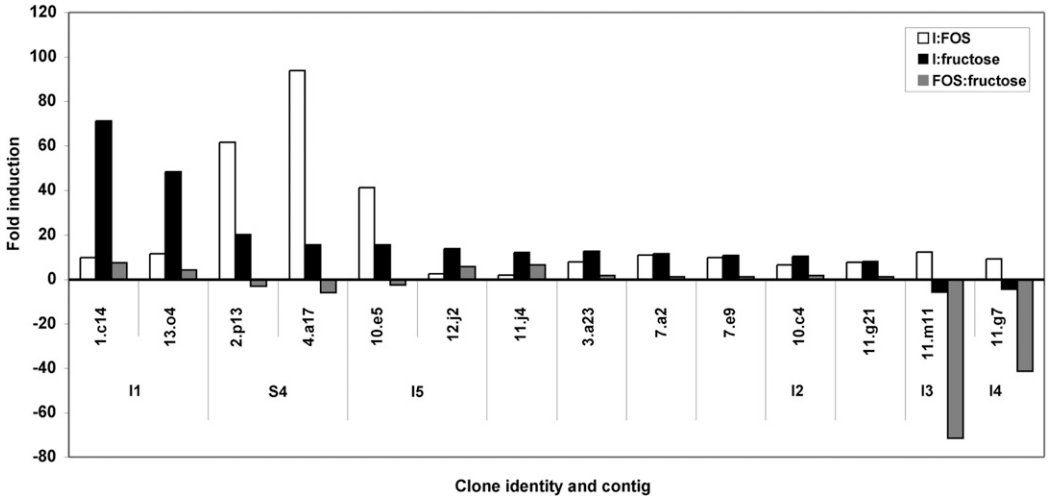

Fig. S1. Relative induction of selected genes (clones) on inulin, fructo-oligosaccharides (FOS), and fructose in pair-wise comparisons after microarray analysis. I:FOS, induction on inulin compared with FOS; I:fructose, induction on inulin compared with fructose; FOS:fructose, induction on FOS compared with fructose. Negative values indicate induction on the substrate shown second. Clone identities and related contigs are indicated as appropriate.

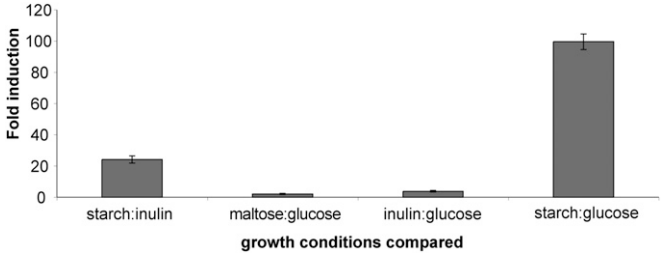

Fig. S2. Results of reverse transcription–Q-PCR (RT-Q-PCR) amplification of the flagellin gene *Fla*A. Primer pairs were used to amplify mRNA extracted from cells during exponential growth [optical density $(OD)_{650}$ = 0.4] on glucose, maltose, starch, and inulin (Dahlia inulin; Sigma). The fold induction of the data converted from logarithmic to linear scale ($x = 2^{-Ct}$) was calculated relative to the glucose standard. The expression of *Fla*A on starch compared with inulin was compared directly in a separate experiment. Results are the means of nine independent RT-Q-PCR amplifications.

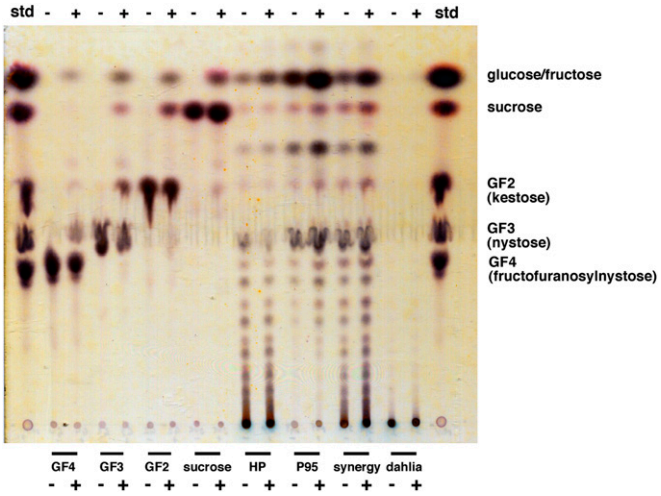

Fig. S3. TLC illustrating the separation of sugar substrates before (–) and after (+) incubation for 24 h with the purified cloned β-fructofuranosidase enzyme. Defined oligosaccharides G4 (G-F4), G3 (GF3), and G2 (GF2) were obtained from Wako Chemicals (GmbH). Comparison with the five standard sugars, shown at each side of the plate (std), enables identification of the oligosaccharide-degradation products.

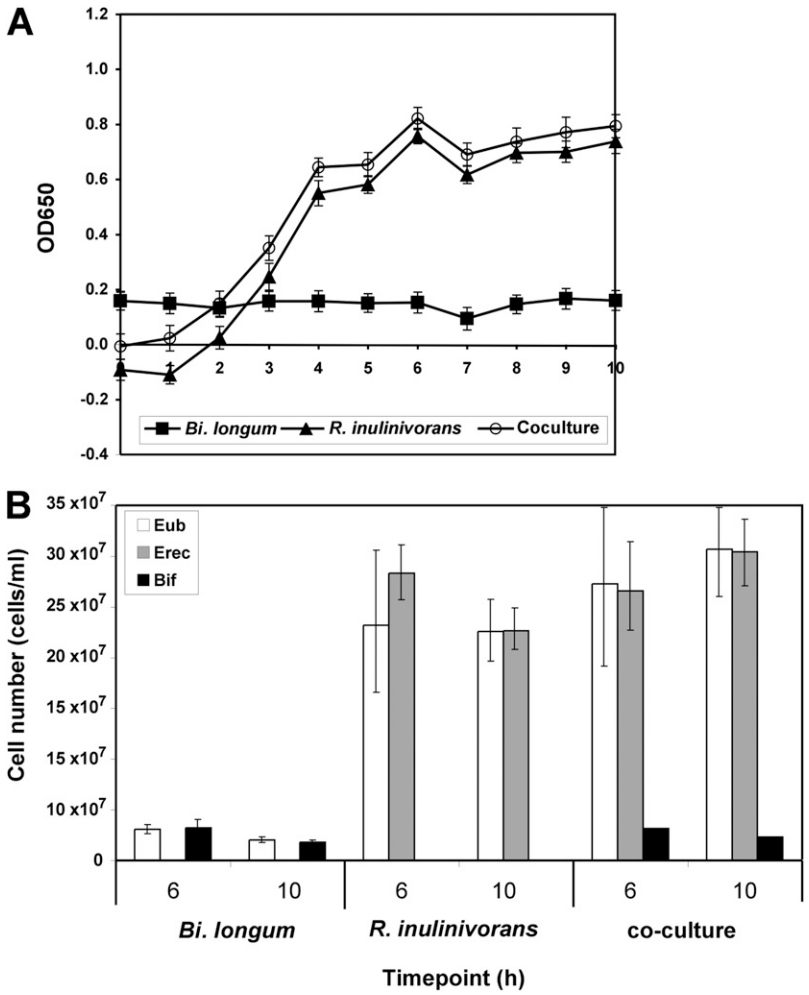

Fig. S4. Data from the coculture experiment after growth of *R. inulinivorans* A2-194 and *Bi. longum* 20219 on inulin. (*A*) Growth curve showing the increasing OD_{650} at hourly intervals of *Bi. longum* (■), *R. inulinivorans* (▲), and coculture (○). (*B*) Number of bacterial cells at two time points enumerated by FISH using the eubacterial probe (Eub338) and probes to specifically detect *R. inulinivorans* (Erec482) and *Bi. longum* (Bif164).

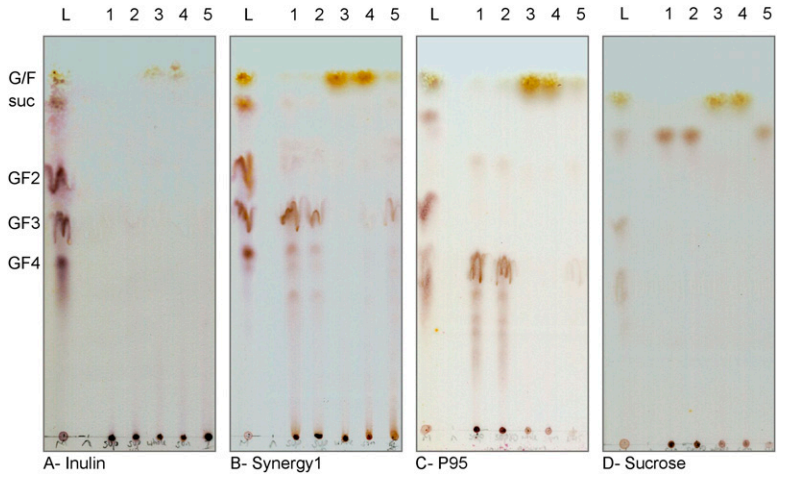

Fig. S5. Activity of fractionated cell extracts of overnight cultures of *R. inulinivorans* grown on YCFA-inulin against (*A*) inulin, (*B*) Synergy1, (*C*) P95, and (*D*) sucrose. Cell extracts (10 μL) were incubated with specific substrates (0.2 mg) for 67 h at 37 °C and the products (1 μL) then separated by TLC. Lanes: L, ladder [1:1 ratio of glucose/fucose (G/F), sucrose (suc), GF2, GF3, and GF4]; 1, concentrated supernatant (15-mL culture supernatant concentrated to 200 μL); 2, heat-treated concentrated supernatant (15-mL culture supernatant heated at 80 °C for 10 min and concentrated to 200 μL); 3, whole cells (cell pellet collected by centrifugation at 5,000 × g, washed two times in 2 mL 50-mM sodium phosphate buffer, pH 6.5, resuspended in 1/30 volume, and stored frozen); 4, sonicated cells (frozen cell pellets, thawed and sonicated for three 20-s bursts before storing frozen); 5, substrate control.

Table S1. Summary of the ORFs and main contigs identified

	Strain A2-194 protein			Related protein					
Contig or clone	ORF	Predicted function	Identity (%)	Name of closest relative	Closest species	e value	Accession number	No. of clones	Fold induction*
Clones induced >5-fold on starch compared with inulin									
Contig S1 (3,077 nt)	1 (510)	4-α glucanotransferase	45	4-α glucanotransferase (GH family 77)	*C. perfringens*	6e-131	GU591781	6	1b4–19.39
	2 (429)	Binding protein	48	Extracellular sugar binding protein (ABC transporter)	*C. diptheriae*	1e-97			7i4–14.81
									11e12–8.98
									2j10–8.3
									1a12–7.22
									12m6–5.73
Contig S2 (3,607 nt)	1 (98)	Regulator	87	Two-component response regulator	*C. phytofermentans*	4e-41	GU591774	4	14e23–10.32
	3 (142)	Regulator	>80	Transcriptional regulatory protein	various	1e-40			12l18–8.94
	4 (94)	Regulator	48	CsrA global regulatory protein family	various	0.006			10a5–7.75
	5 (392)	Flagellin (FlaA)	80	Flagellin protein Fla1 or FlaA	*E. rectale*	2e-53			14h23–7.59
	7 (180 tr)	Regulator	48	XRE family transcriptional regulator	*C. phytofermentans*	1e-27			
Contig S3		Amylopullulanase	100	alpha-amylase accession no. AM055811	*R. inulinivorans*	e0.0	AM055811	3	2b20–5.29
									4b7–4.93
									7e22–4.65
Contig S4 (1,538 nt)	1 (437)	4-α glucanotransferase	52	4-α glucanotransferase (GH family 77)	*C. perfringens*	2e-133	GU591776	2	4a17–4.47
									2p13–4.41
Single clones									
10.e6	1 (110 tr); 2 (311)	Membrane protein	32–50	Hypothetical proteins, possible membrane proteins	various	1e-20	GU591782	1	10.e6–13.08
6.o9 (4,493 nt)	1 (354 tr)	Arginosuccinate lyase	70	Arginosuccinate lyase	*C. phytofermentans*	4e-60	GU591783	1	6.o9–10.01
	3 (736)	Glycogen phosphorylase	71	Glycogen phosphorylase	*C. phytofermentans*	e0.0			
	4 (260)	Transcriptional regulator	32	AraC transcriptional regulator	*S. sanguinis*	4e-29			
12.g10	(371 tr)	alpha-amylase	50	alpha-amylase catalytic region 22% identity to AM055811	*C. phytofermentans*	2e-117	GU591784	1	12.g10–9.27
7.b10	(189 tr)	tRNA synthetase	76	Alanyl-tRNA synthetase	*C. phytofermentans*	1e-75	GU591780	1	7.b10–6.10
2.j15	(275 tr)	Transposase	48	Transposase like protein	various	2e-64	GU591779	1	2.j15–5.98
8.c12	(306 tr)	Membrane protein	84	Protein-export membrane protein secD	*R. intestinalis*	9e-147	GU591778	1	8.c12–5.5
10.f5	(232 tr)	DNA topoisomerase	54	DNA topoisomerase	*Alkaliphilus metalliredigenes*	7e-63	GU591777	1	10.f5–5.06

Table S1. Cont.

Contig or clone	Strain A2-194 protein: ORF	Strain A2-194 protein: Predicted function	Strain A2-194 protein: Identity (%)	Related protein: Name of closest relative	Related protein: Closest species	Related protein: e value	Accession number	No. of clones	Fold induction*
Clones induced >5-fold on inulin compared with starch									
I1 B-fructosidase cluster	5 (318)	Phosphofructokinase	55	6-phosphofructokinase (pfkB)	*Akkermansia muciniphila*	6e-94	GU591787	4	1c14–22.92
(7,858 nt)	6 (505)	beta-fructofuranosidase	48	Sucrose 6P hydrolase	*C. beijerinckii*	2e-145			13o4–14.23
	7 (556)	Sugar-transport system	58	Extracellular solute binding protein	*C. beijerinckii*	e0.0			6m13– 9.33
	8 (298)	Sugar-transport system	71	Sugar-transport protein	*C. beijerinckii*	4e-119			14e7–7.7
	9 (312)	Sugar-transport system	67	Sugar-transport protein	*C. beijerinckii*	1e-114			
	10 (341)	Repressor protein	50	Ribose operon repressor	*C. perfringens*	1e-98			
I2 ferredoxin (428 nt)	1 (143 tr)	Oxidoreductase	76	Aldo/keto oxidoreductase (ironsulphur binding)	*C. phytofermentans*	5e-62	GU591772	2	10c4–12.34
									4a5–8.24
Contig I3 (2,211 nt)	1 (539)	PTS transport	70	PTS I, E1 component	*C. phytofermentans*	e0.0	GU591775	1	11g7–4.96
Contig I4 (1,490 nt)	1 (309)	1-phosphofructokinase	60	1-phosphofructokinase	*C. botulinum*	3e-102	GU591773	2†	2n22–5.97
	2 (174 tr)	PTSII	48	PTSII fructose specific	*C. botulinum*	6e-39			11m11†
Contig I5 (1,364 nt)	1 (68 tr)	Transcription regulator	40	N-terminal transcription regulator	various	7e-6	GU591786	2†	12j2,† 11j4†

Identification and assembly into functional clusters of those clones induced >5-fold during growth on inulin or starch. The database accession numbers of each DNA sequence are given. The match of each ORF within a contig to the closest database homolog is shown (% identity and e value). Tr , truncated ORF.

*Relative induction of each clone within the contig sequence is given for the two-way starch–inulin comparison.

†Clones that were identified in the three-way inulin. FOS, fructose comparison.

Clones induced at least 8-fold on inulin in three-way comparison between inulin, FOS, and fructose

Clone	Fold change: Inulin:fructose	Fold change: FOS:inulin	Fold change: FOS:fructose	Related contig identified in other analyses	Protein identification (from database identity)
1.c14	71.23	−9.73	7.32	I1	Sucrose 6P hydrolase *C. bejerinckii*
13.o4	48.27	−11.54	4.18	I1	6-phosphofructokinase
1.n24	33.09	−12.49	2.65		Pyridine nucleotide-disulphide oxidoreductase, *B. thetaiotaomicron*
2.p13	20.03	−61.58	−3.07	S4	4-α glucanotransferase (different to others)
4.a17	15.58	−93.85	−6.02	S4	53% 4-α glucanotransferase
10.e5	15.56	−41.22	−2.65		65% (6e-69) hypothetical protein *R. intestinalis* L1-82 (01379)
12.j2	13.78	−2.40	5.74	I5	40% N terminus of transcriptional regulators/DNA binding proteins
3.a23	12.54	−7.83	1.60		33% hypothetical protein
11.j4	12.02	−1.89	6.37	I5	Overlaps 12.j2
7.a2	11.49	−10.80	1.06		48% metallophosphoesterase, // 44% AbrBfamily transcription regulator
7.e9	10.73	−9.66	1.11		44% (2e-58) putative integrase/recombinase
10.c4	10.24	−6.47	1.58	I2	Aldoketo 4Fe4S ferredoxin
11.g21	8.03	−7.53	1.07	Up-regulated in fucose microarray (1)	Glycerol-3P-dehydrogenase (NAD dependent dehydrogenase)
Clones significantly induced on fructose					
11.m11	−5.86	−12.22	−71.55	I4	61% PTS system II bc component *Clostridium spp.*
11.g7	−4.48	−9.24	−41.39	I3	PTS I *C. phytofermentans*

1. Scott KP, Martin JC, Campbell G, Mayer CD, Flint HJ (2006) Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "*Roseburia inulinivorans*." *J Bacteriol* 188:4340–4349.

Table S2. Identity of the two main up-regulated (>5-fold) spots (4802S and 4606I) identified in the *R. inulinivorans* proteome, which were excised and the peptide sequences were determined

Spot identity	Up-regulated on	Matching peptides*†	Microarray identity (clone)
4802S	Starch	LLSNNLINLG**I**Y**E**DVKK NLTLFLYPDDSD**DK**GR GI**L**MDEAIDIVSK TCAYTNHTILAEALEK QQMNALYVIHK AAPAYVIAK VVMVENYNVTLAEK	Glycogen phosphorylase (6.o9)
4606I	Inulin	FSG**S**AIEADGK HVLVYTGVTRIKQPDGSE ENDTYYMIVGNK NQIGQVVLCSSK FETILASNESGK ETTLCGIEGR ISNPEGLKK	β-fructosidase (I1 – 1.c14)

The peptides matching the translated sequence of the clones detected on the microarray are shown; mismatching amino acids are shown in bold and underlined.

*Peptide sequences were determined as described in *SI Materials and Methods*.

†Underlined bold face type shows the mismatching amino acids or amino acids whose identity was not unequivocally determined by LC-MS/MS.

Table S3. Sequences producing significant alignments using Blast P, searching with A2-194 β-fructofuranosidase sequence (July 20, 2010)

Accession no.	Description	Max score	*E* value	Identity (%)
ZP_03755395.1	Hypothetical protein ROSEINA2194_03834 [Roseburia inulinivorans DSM 16841]	1068	0	100
ZP_03801019.1	Hypothetical protein COPCOM_03306 [Coprococcus comes ATCC 27758]	840	0	79
YP_002936195.1	Sucrose-6-phosphate hydrolase [Eubacterium rectale ATCC 33656]	780	0	73
CBK92563.1	Sucrose-6-phosphate hydrolase [Eubacterium rectale M104/1]	780	0	72
CBK92067.1	Sucrose-6-phosphate hydrolase [Eubacterium rectale DSM 17629]	780	0	73
CBL20803.1	Sucrose-6-phosphate hydrolase [Ruminococcus sp. SR1/5]	683	0	63
ZP_02040442.1	Hypothetical protein RUMGNA_01206 [Ruminococcus gnavus ATCC 29149]	677	0	63
ZP_01994901.1	Hypothetical protein DORLON_00890 [Dorea longicatena DSM 13814]	594	1e-167	57
CBK82500.1	Beta-fructosidases (levanase/invertase) [Coprococcus sp. ART55/1]	558	4e-157	52
CBK83251.1	Beta-fructosidases (levanase/invertase) [Coprococcus sp. ART55/1]	532	4e-149	53
ZP_02205360.1	Hypothetical protein COPEUT_00119 [Coprococcus eutactus ATCC 27759]	529	4e-148	52
YP_001310947.1	Sucrose-6-phosphate hydrolase [Clostridium beijerinckii NCIMB 8052]	518	8e-145	48
ZP_06597629.1	Sucrose-6-phosphate hydrolase [Oribacterium sp. oral taxon 078 str. F0262]	516	3e-144	47
ZP_02075141.1	Hypothetical protein CLOL250_01917 [Clostridium sp. L2-50]	511	9e-143	47
ZP_05853370.1	Sucrose-6-phosphate hydrolase [Blautia hansenii DSM 20583]	509	2e-142	46
CBL25413.1	Beta-fructosidases (levanase/invertase) [Ruminococcus torques L2-14]	495	7e-138	47
ZP_01963924.1	Hypothetical protein RUMOBE_01648 [Ruminococcus obeum ATCC 29174]	491	9e-137	46
ZP_04857545.1	Sucrose-6-phosphate hydrolase [Ruminococcus sp. 5_1_39B_FAA]	480	2e-133	46
ZP_05615941.1	Sucrose-6-phosphate hydrolase [Faecalibacterium prausnitzii A2-165]	470	2e-130	47
CBK98452.1	Beta-fructosidases (levanase/invertase) [Faecalibacterium prausnitzii L2-6]	468	7e-130	48
CBK75003.1	Beta-fructosidases (levanase/invertase) [Butyrivibrio fibrisolvens 16/4]	455	8e-126	43
ZP_05981823.1	Sucrose-6-phosphate hydrolase [Subdoligranulum variabile DSM 15176]	379	5e-103	39
YP_003477892.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter italicus Ab9]	367	2e-99	40
ZP_06598787.1	Sucrose-6-phosphate hydrolase [Oribacterium sp. oral taxon 078 str. F0262]	362	6e-98	40
ZP_05492959.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter ethanolicus CCSD1]	361	2e-97	39
YP_003677389.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter mathranii subsp. mathranii str. A3]	356	5e-96	39
YP_003477499.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter italicus Ab9]	355	6e-96	39
YP_001662752.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter sp. X514]	354	1e-95	39
YP_003598941.1	Sucrose-6-phosphate hydrolase [Bacillus megaterium DSM319]	353	2e-95	40
ZP_04788153.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter brockii subsp. finnii Ako-1]	352	7e-95	39
ACN59531.1	Invertase [uncultured bacterium]	352	7e-95	38
ZP_05336575.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacterium thermosaccharolyticum DSM 571]	347	2e-93	37
YP_001907823.1	Sucrose-6-phosphate hydrolase [Erwinia tasmaniensis Et1/99]	343	3e-92	38
ZP_02693416.1	Sucrose-6-phosphate hydrolase [Epulopiscium sp. 'N.t. morphotype B']	343	3e-92	37
YP_001664628.1	Sucrose-6-phosphate hydrolase [Thermoanaerobacter pseudethanolicus ATCC 33223]	343	3e-92	39
ZP_05657431.1	Beta-fructofuranosidase/sucrose 6 phosphate hydrolase [Enterococcus casseliflavus EC20]	342	6e-92	39
ZP_05654203.1	Beta-fructofuranosidase/sucrose 6 phosphate hydrolase [Enterococcus casseliflavus EC10]	341	2e-91	37
ZP_05647830.1	Beta-fructofuranosidase/sucrose 6 phosphate hydrolase [Enterococcus casseliflavus EC30]	338	1e-90	38
YP_003472710.1	Sucrose-6-phosphate hydrolase [Staphylococcus lugdunensis HKU09-01]	329	6e-88	38
ZP_03992025.1	Possible beta-fructofuranosidase [Oribacterium sinus F0268]	328	1e-87	38
YP_081250.1	Glycoside hydrolase family protein [Bacillus licheniformis ATCC 14580]	326	5e-87	38
YP_001422743.1	Hypothetical protein RBAM_031820 [Bacillus amyloliquefaciens FZB42]	324	2e-86	38
YP_176610.1	Sucrose-6-phosphate hydrolase [Bacillus clausii KSM-K16]	323	4e-86	40
ZP_05660310.1	Glycosylhydrolase [Enterococcus faecium 1,230,933]	323	5e-86	38
YP_003562050.1	Sucrose-6-phosphate hydrolase [Bacillus megaterium QM B1551]	322	6e-86	38
YP_003596774.1	Sucrose-6-phosphate hydrolase [Bacillus megaterium DSM319]	322	6e-86	38
AAM19071.1	Beta-fructosidase FruA [Bacillus megaterium]	322	1e-85	38
ZP_05674260.1	Beta-fructofuranosidase/sucrose 6 phosphate hydrolase	321	2e-85	37
ZP_04783183.1	Beta-fructofuranosidase [Weissella paramesenteroides ATCC 33313]	321	2e-85	36
ZP_05404140.1	Sucrose-6-phosphate hydrolase [Mitsuokella multacida DSM 20544]	318	1e-84	36
ZP_05737371.1	Sucrose-6-phosphate hydrolase [Granulicatella adiacens ATCC 49175]	317	2e-84	38
ZP_03958139.1	Beta-fructofuranosidase [Lactobacillus ruminis ATCC 25644]	315	1e-83	38
ZP_03624709.1	Glycosyl hydrolase family 32 domain protein [Streptococcus suis 89/1591]	314	2e-83	38
ZP_06189775.1	Sucrose-6-phosphate hydrolase [Serratia odorifera 4Rx13]	312	6e-83	36
YP_003620449.1	Hypothetical protein LKI_10506 [Leuconostoc kimchii IMSNU 11154]	312	7e-83	35
YP_002249945.1	Raffinose invertase [Dictyoglomus thermophilum H-6-12]	311	2e-82	36
YP_001200910.1	Beta-fructosidases (levanase/invertase) [Streptococcus suis 98HAH33]	310	2e-82	37
XP_623540.2	PREDICTED: hypothetical protein [Apis mellifera]	310	4e-82	37
YP_002741065.1	Sucrose-6-phosphate hydrolase [Streptococcus pneumoniae 70585]	310	4e-82	37
ZP_06900985.1	Sucrose-6-phosphate hydrolase [Streptococcus parasanguinis ATCC 15912]	309	6e-82	43
YP_002736736.1	Sucrose-6-phosphate hydrolase [Streptococcus pneumoniae JJA]	308	9e-82	37
NP_359209.1	Sucrose-6-phosphate hydrolase, putative [Streptococcus pneumoniae R6]	308	1e-81	37

BlastP output of sequences producing significant alignments (>35% identity) to the A2-194 β-fructofuranosidase in a BlastP database search performed on July 20, 2010.