

Supporting Information

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SI Materials and Methods

Mutant Construction. Mutants in the genes *cj0481*, *cj0483*, *cj0486*, *cj0487*, and *cj0490* were constructed using a mutagenesis and gene-replacement strategy. The target gene was PCR-amplified using the primers listed in Table S3 and the genomic DNA of NCTC 11168 was used as the template. All primers used for these cloning reactions were designed for use with the In-fusion Dry down PCR cloning kit (Clontech). The PCR products were cloned into BamHI-digested pUC19 plasmids using the manufacturer's protocols. Once recombinant plasmids were obtained, they were used as a template for an inverse PCR, so as to delete regions within the gene, and create a site for the insertion of a chloramphenicol resistance cassette (*cat*). The primers used for the inverse PCR are listed in Table S3. The *cat* gene was amplified from pRY111 using Pfx polymerase (Invitrogen) and the primers indicated in Table S3. The *cat* gene was then cloned into the inverse PCR product using the Clontech kit, as above. Constructs were confirmed by sequencing and the recombinant plasmids were naturally transformed into motile *Campylobacter jejuni* NCTC 11168, as described previously (1, 2) by selecting for chloramphenicol resistance on MH agar. The exception to this protocol was the mutation of *cj0486*. The *C. jejuni* *cj0486* mutant was created by blunt insertion of the SmaI-cut 1488 bp kanamycin cassette from pWM2 into the SpeI-cut *C. jejuni* *cj0486* cloned into pBR322 described above. Electroporation of 1 μ g of this plasmid into *C. jejuni* NCTC 11168 was performed, as described previously (3), selecting for kanamycin-resistant *C. jejuni* transformants. Constructs were confirmed by PCR and sequence analysis of the recombinant gene.

***C. jejuni* Mutant Complementation.** The plasmid used to construct CjWM227a was kindly provided by Qijing Zhang (Iowa State University, Ames, IA). This construct contained the amplified region *cj0486-cj0490*, cloned into the pRRK vector (4) as described in by Muraoka et al. (5). This construct was transformed into the Δ *cj0487* mutant to create the *cj0486-cj0490* complemented Δ *cj0487* strain. To construct the Δ *cj0486+* (*cj0486-cj0490*) strain, the pRRKcJ0486-cj0490 plasmid was digested with MfeI-HF to remove the kanamycin-resistance cassette and replace it with a chloramphenicol-resistance cassette. The resultant plasmid pRRC486-490 was transformed into the Δ *cj0486* mutant.

Complementation of an *Escherichia coli* *fucP* Mutant with *Ec-fucP* and *cj0486*. The *fucP* genes from *Escherichia coli* and *C. jejuni* (*cj0486*) were PCR-amplified to contain ribosome binding sites. A 1,354-bp PCR product containing the *E. coli* *fucP* gene was obtained with oligonucleotides EfucP_F, EfucP_R, and chromosomal DNA of *E. coli* K12; a 1,317 bp *C. jejuni-fucP* containing PCR product was obtained using oligonucleotides CfucP_F, CfucP_R, and chromosomal DNA of *C. jejuni* NCTC11168. Full-length *E. coli* *fucP* and *C. jejuni* *cj0486* genes were cloned into BamHI or SalI cut pBR322 within the tetracycline gene. For further complementation analysis of the *E. coli* *fucP* mutant plasmid, pCE111-28 was linearized with EcoRV and ligated with the *fucP* gene containing PCR products. One positive candidate after restriction analyses with PstI (for the insertion and correct orientation of the *E. coli* *fucP* gene) or HindIII (for the insertion and correct orientation of the *C. jejuni* *fucP* gene) was used to complement the *E. coli* *fucP* mutant strain.

Mucin Isolation and Electrospray Ionization Mass Spectrometry Analysis. Intestinal sections (duodenum, jejunum, ileum, and

cecum) recovered from killed piglets, or ceca recovered from broiler chicks, were cut longitudinally to reveal the mucus lining. Luminal contents were removed by washing with PBS buffer and the internal surface was scraped into a microcentrifuge tube using a scalpel. The mucus was emulsified with 15% vol/vol 1 \times PBS buffer and was centrifuged twice at 28,400 \times *g* for 20 min to remove solid material. The crude mucin was washed in 70% ethanol and then dialyzed in MilliQ water and lyophilized overnight. All mucin samples were then treated with NaBH₄ and NaOH overnight at 42 $^{\circ}$ C (β -elimination) to release the *O*-linked glycans (6). Purified mucin *O*-glycans were analyzed using a 4000 Q-Trap (AB/MDS Sciex) equipped with a microspray interface. Precursor ion scans at *m/z* 224, corresponding to the fragment ion of the reducing end, were acquired with a scan time of 2 s at a step size of 0.5 Da. The collision energy was ramped from 40 to 80 V over the scan range of *m/z* 600 to 1,400.

Confirmation of Operonic Structure. The organization of the genes *cj0481* to *cj0490* was confirmed to be in an operonic structure by PCR amplification of regions spanning neighboring genes using cDNA as a template. Total RNA was extracted from cultures grown in medium containing L-fucose, as described earlier. The total RNA was converted to cDNA using SuperScript II reverse transcriptase (Invitrogen), following the manufacturer's protocols. To amplify the regions in between genes, the primers previously used for the inverse PCR step of mutant construction were used (Table S3). PCR products from the cDNA template were compared against products from a control genomic DNA template.

Total RNA Extraction for Real-Time PCR and Microarray Studies. Cultures of *C. jejuni* NCTC 11168 were inoculated into filter-sterilized MEM α medium containing 20 μ M FeSO₄ and 25 mM L-fucose, UV-sterilized MEM α medium containing 2 mg/mL porcine gastric mucin (Sigma) or control medium of MEM α containing 20 mM sodium pyruvate and 20 μ M FeSO₄. Cultures were grown to an OD at 600 nm of 0.2, representing midlog phase, at which point the RNA quality was preserved by one of two methods. A 1/10-volume stop solution of 10% buffer saturated phenol (pH 4.3) and ethanol was added to the fucose-containing culture. However, this stop solution proved insufficient to preserve RNA quality in the mucin-containing culture so a 1/1 volume of RNA-later (Ambion) was used in this case. Total RNA was extracted using a hot-phenol extraction method as described previously (1, 2, 7). The RNA was treated twice with Dnase I (Epicentre) and the absence of genomic DNA was confirmed by PCR. Final RNA quality and quantity was ascertained using BioRad's Experion StdSensRNA system using the manufacturer's protocols.

Microarray Hybridization and Analysis. Microarray slides and labeled cDNA probes were prepared and hybridized as described previously (1, 2, 7). Complementary DNA probes from control cultures grown in MEM α containing pyruvate were labeled with indocarbocyanine [Cy3], and experimental samples, from cultures containing L-fucose or mucin were labeled with indocarbocyanine [Cy5]. Once prepared, the arrays were scanned with a laser-activated confocal scanner (ScanArray Gx; Perkin-Elmer) at a resolution of 10 μ m. The signal intensities of each spot were collected using ScanArray software. Spots exhibiting hybridization anomalies were excluded from the analysis. The raw median fluorescence intensity values were background-subtracted, and spots with background-subtracted values less than three times the SD of the local background in both channels were also excluded from further analysis.

Subsequently, the background-subtracted fluorescent intensity in each wavelength (channel 1 for Cy3 and channel 2 for Cy5) was normalized using the MIDAS software (available from TIGR, <http://www.tigr.org/software/>) and by applying a locally weighted linear regression (Lowess), as previously described (7). Microarray data were collected from three independent biological replicates and three technical replicates for each biological replicate, for a total of nine microarray slides for each condition. Each slide contained triplicates of each probe, yielding a total of 27 replicate spots for analysis. Finally, the ratio of channel 2 to channel 1 was converted to \log_2 , and the data were statistically analyzed using the empirical Bayes method, as previously described (7).

Quantitative RT-PCR. Quantitative RT-PCR was conducted using the Applied Biosystems 7300 DNA analyzer and the Qiagen Quantitect SYBR Green RT-PCR kit using the manufacturer's protocols and as described previously (1). Each sample used total RNA that was extracted from cultures of *C. jejuni* NCTC11168, $\Delta cj0481$, $\Delta cj0486$, $\Delta cj0487$, and the complemented strains: $\Delta cj0486+(cj0486-cj0490)$ and $\Delta cj0487+(cj0486-cj0490)$, grown in MEM α supplemented with 25 mM L-fucose. RNA expression relative to the wild-type control was determined for the genes *cj0480c*, *cj0481*, *cj0486*, and *cj0487*, with *rpsL* acting as an endogenous control.

SI Results

Intestinal Mucin O-glycan Compositions Differ in Chickens and Pigs. Because intestinal mucin glycoproteins are heavily glycosylated with O-linked glycans, we characterized the neutral and acidic O-glycans released from porcine and chicken gastrointestinal mucins to identify differences in glycan composition between these two model systems that may be relevant to *C. jejuni* disease. Although both chicken and pig mucin O-glycans showed similar levels of fucosylation, the fucosylated oligosaccharides from chicken mucin were predominantly sulfated (38% versus 11–14% in the pig samples) and contained relatively low amounts of NeuAc (Fig. S5). In contrast, the pig mucin O-glycans showed very little modification with sulfate, but contained greater amounts of NeuAc and also NeuGc (Fig. S5). The lack of de-

tectable NeuGc in chick mucins is consistent with what has recently been reported (8).

Genes *cj0481* to *cj0490* Exist as a Single Operon. We used PCR of sections spanning neighboring genes and cDNA derived from extracted total RNA to confirm that the genes *cj0481* to *cj0490* are expressed as a single transcript. PCR products were obtained from regions spanning each pair of genes *cj0481-cj0483*, *cj0483-cj0484*, *cj0484-cj0485*, *cj0485-cj0486*, *cj0486-cj0487*, and *cj0487-cj0490*. Each of these products was of equal size to the product obtained from a genomic DNA template. The exception to this was the region in between *cj0480c* and *cj0481*. As these genes are oriented in opposite directions, they should not be transcriptionally linked. A product was obtained from the genomic DNA template, but none from the cDNA template.

Other Genes Transcriptionally Regulated in the Presence of L-Fucose. Cultures grown in the presence of L-fucose also exhibited an up-regulation of metabolic genes, including most of the citric acid cycle, proline metabolism (*putAB*), and genes involved in aspartate/glutamate transport and metabolism (*peb1A*, *cj0919*, *cj0920*, *pebC*, *aspB*). Notable groups of genes exhibiting down-regulation included oxidative stress response genes (*ahpC*, *sodB*, *tpx*), elements of the electron transport chain (*cydAB*, *petABC*), chemotaxis (*cetAB*, *tlp4*), and the outer membrane protein (*omp50*).

Disruption of the L-Fucose Permease Gene *cj0486* Disrupts Expression of Genes both Up and Downstream. Quantitative RT-PCR was conducted on the mutants *cj0481*, *cj0486*, and *cj0487*, as well as mutants complemented with the region *cj0486-cj0490* (Fig. S3). Although the mutant *cj0481* exhibited slightly reduced expression of the downstream genes *cj0486* and *cj0487*, the mutants *cj0486* and *cj0487* saw little to no expression of other genes within the operon (in particular *cj0481* was not expressed in either mutant). Although restoring copies of the genes into an rRNA cluster within the genome partially restored expression of the target genes, this did not restore the expression of other genes within the operon. This finding could indicate that a higher level of transcription of the permease genes is necessary to allow for sufficient uptake of L-fucose to stimulate transcription of the operon.

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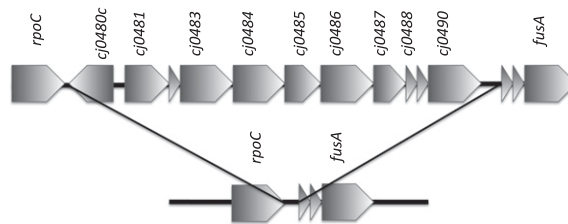


Fig. S1. Genomic region surrounding the putative fucose permease (*fucP*, *cj0486*). The upper strand is the region as it appears in *C. jejuni* strains NCTC 11168, RM1221, CF93-6, 84–25, *C. jejuni* subsp. *doylei* 269.97, and *C. coli* RM2228. The lower strand is the region as it appears in the strains lacking *fucP*: *C. jejuni* 81–176, CG8486, HB93-13, 260.94, and 81116.

<i>EcFucP</i>	MGNTSIQTQSYRAVDKIDAGQSRYSYIIPFALLCSLFFLWAVANNLNILLPQFQQAFTLTN	60
<i>Cj0486</i>	-----MTDSKNIKIAIVLVTSLEFLWGVSYGLIIVMNKFNQNLHHSQ	43
<i>Cj0484</i>	-----MKHANSKLELVCKKISWRILPLIVLMPCLSMLEDRNTISFVKSHIETIDAGTGE	53
<i>EcFucP</i>	FQAGLIQSIFYFYFIPIIPAGILMKKLSYKAGIITGLFLYALGAALFWPAAEIMNYTLF	120
<i>Cj0486</i>	HESGFLQFAYFGAYFIALPAGYIANRFSYKMGIIIFGLALYATGALLIIPATNLASFHLF	103
<i>Cj0484</i>	AAAYLGAGIFFIGYAIPEVPSNLFHLKLGAKIWLRSRIMITWGLVTMAMIFIQGEISFYVLL	113
<i>EcFucP</i>	LVGLFIIAAGLG--CLTAANPFVTVLGPESGHRFLNLAQTFISFGAIIAVVFGQSLIL	178
<i>Cj0486</i>	LFAPFLLACGIG--SLTSANPYMTKLGDEKNASFRINAAQSFVGLGQFVGPPIIGGALFL	161
<i>Cj0484</i>	RFLGLTEAGFSPGIIILYLSYFFPAIYRSKAYGIYQMGVPIAFVFGSLISGFILDYTPNI	173
<i>EcFucP</i>	SNVPHQSQDVLDKMSPEQLSAYKHSLVLSVQTPYMIIVAILLVALLIMLTKFPALQSDN	238
<i>Cj0486</i>	SITKQE-----EGASKEQIQAAALVANMGNVQLVYIGIAVIVILILIAFVANKLP--EGSA	214
<i>Cj0484</i>	YFKNWQWMLIEGGITVLVGFICFLYLDLSDHPKDAKWLDIKEKIDILLKHIEISNTKAKDYS	233
<i>EcFucP</i>	HSDAKQGSFASLSRLARIRHWRWAVLAQFCYVGAQTACWSYLIRYAVEEIPGMTAGFAA	298
<i>Cj0486</i>	VSDDYKQKDDSKPIYVFKRHRFNLGLLAQFLYIANQVAAGAFFINVVVEHNEGLKDAQCA	274
<i>Cj0484</i>	IKDIFKS-----ILVWKFFVVFYFCIQLSVYGLFVLPKIAQILQINVGFEV	280
<i>EvFucP</i>	NYLTGTMVCFPIGRFTGTWLISRFAHPKVLAAAYALIAMALCLISAFAGGHVGLIALTLCS	358
<i>Cj0486</i>	YFYSIALVAFMLGRIVSTPLMKIIEKELKILGFYSLINVLICFSLYFASGFFSIVLLIALF	334
<i>Cj0484</i>	GLLN--AIPWIFVPIALPIFTSLADKHSWNHALFLLLASLMIATSFVFNLAFLFF	338
<i>EcFucP</i>	AFMSIQYPTIFSLGIKNLG-QDTKYGSSFIVMTIIG-----GGIVTPVMGFVSDAAGN-	410
<i>Cj0486</i>	FFMSISFPTIFAVATKNLPLNQVKGSLVMSIVG-----GAIMPIIIGFINDHYGTG	388
<i>Cj0484</i>	ISLAAIGFIVIQPIFWNLPTQVLKGRGAAAATALIGSLGNLGGFVAPTLLKTYIENHFQVE	398
<i>EcFucP</i>	IPTAELIPALCFVAVIFARFRSQTATN--	438
<i>Cj0486</i>	AGYLAMAPFLYVAWYGFVIGSKVRKNAKDF	418
<i>Cj0484</i>	FGLIVLALITAIL-----	410

Fig. S2. The sequence alignment between the *E. coli* FucP, and the *C. jejuni* Cj0486 and Cj0484 proteins. The alignment was done using the online program ClustalW2 from EMBL-EBI, <http://www.ebi.ac.uk/Tools/msa/clustalw2/>. Highlighted in green are the amino acid residues Asp46, Glu135, and Asn146.

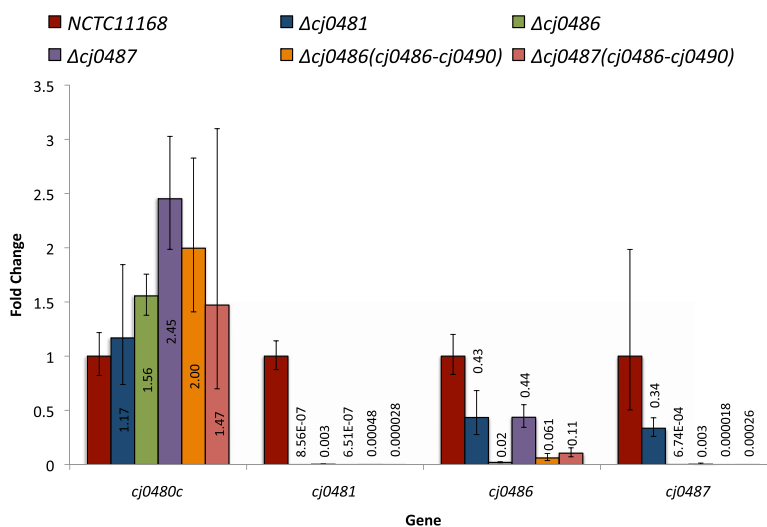


Fig. S3. Expression of the genes *cj0480c*, *cj0481*, *cj0486*, and *cj0487* as determined by real-time PCR from total RNA extracts from *C. jejuni* NCTC11168, $\Delta cj0481$, $\Delta cj0486$, $\Delta cj0487$, and the complemented strains: $\Delta cj0486+(cj0486-cj0490)$ and $\Delta cj0487+(cj0486-cj0490)$. Results are displayed as fold-change relative to the *C. jejuni* NCTC11168 wild-type.

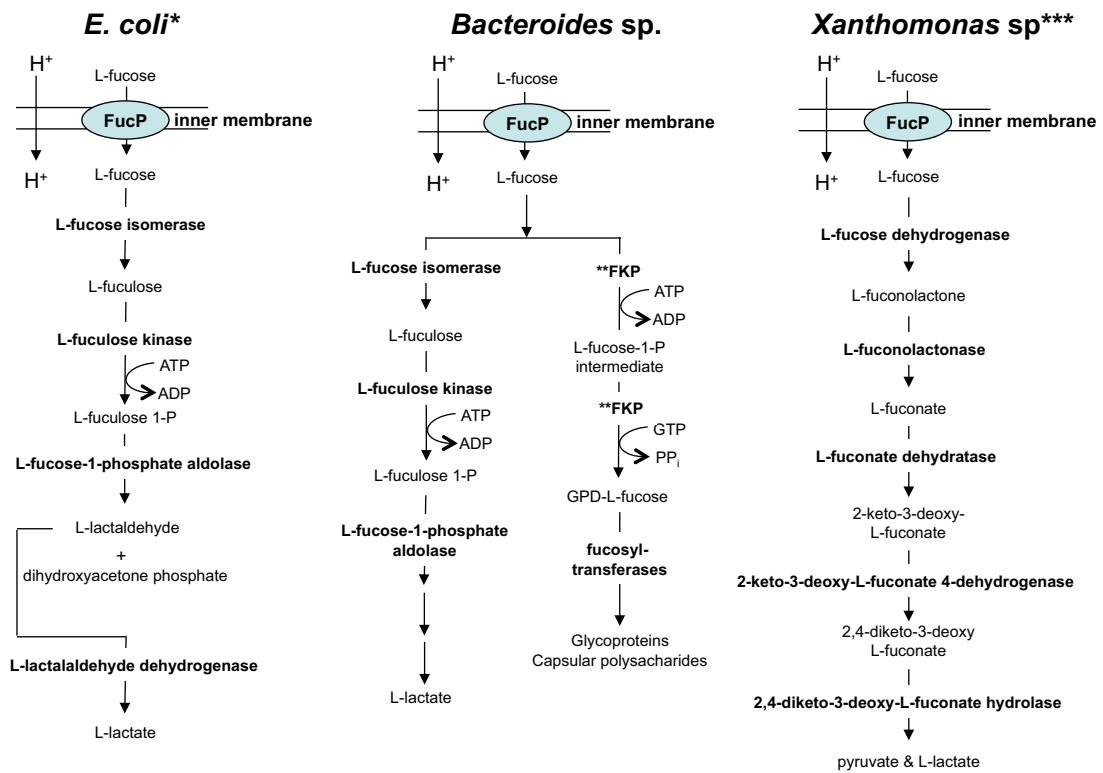


Fig. S4. General pathways for fucose uptake and utilization in *E. coli*, *Bacteroides* sp., and *Xanthomonas* sp. are depicted. FucP, fucose proton symporter of the major facilitator family; FucK, fucose kinase. Biosynthetic proteins are in bold; *adapted from Baldomà and Aguilar (1), **FKP: bifunctional L-fucokinase/ GDP-fucose pyrophosphorylase (2, 3); ***modified from Yew et al. (4)

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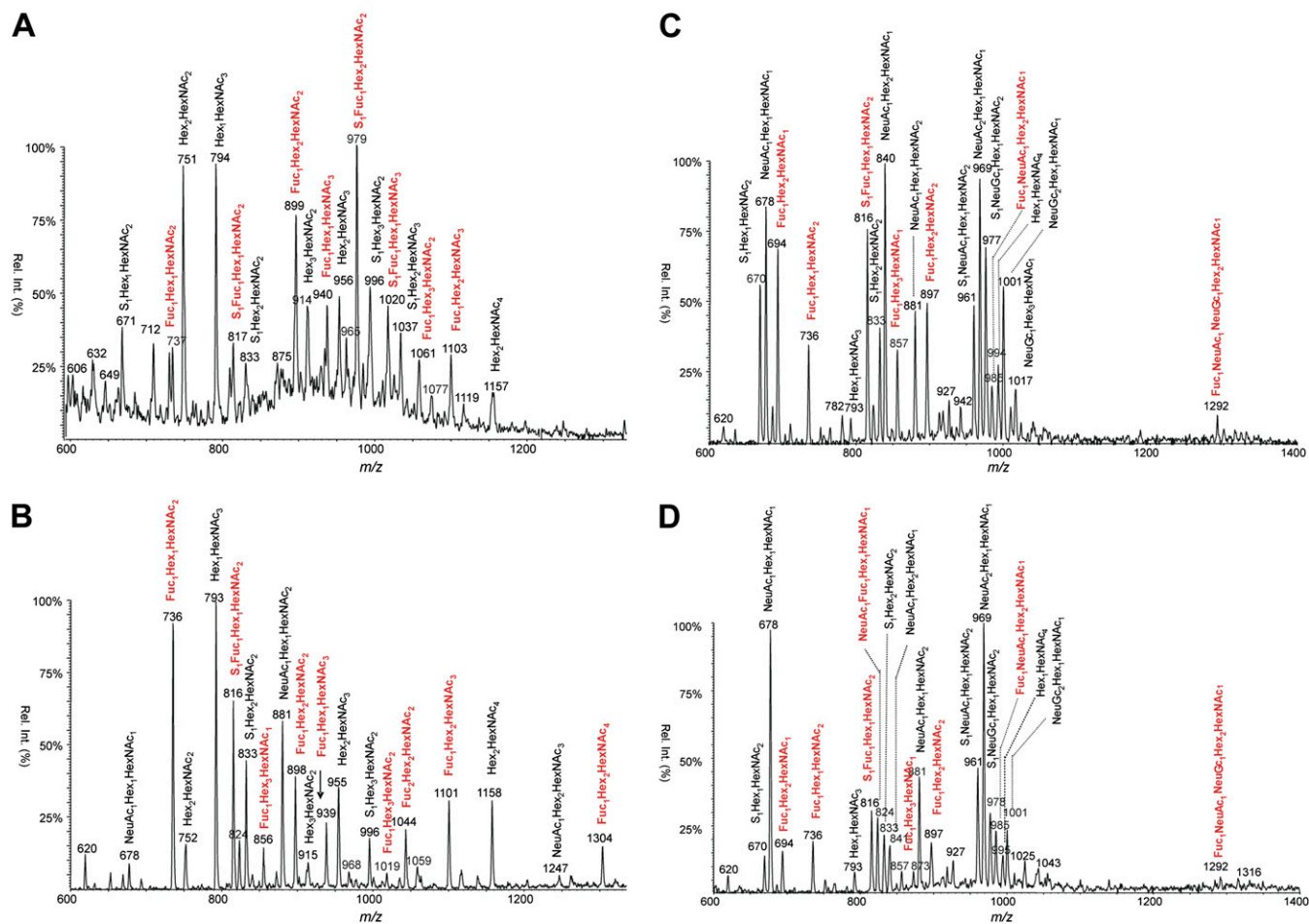


Fig. S5. Precursor ion mass spectra of ion at m/z 224 (positive ion detection mode): (A) O-glycans released from chicken cecal mucin; (B) O-glycans released from pig gastric mucin (purchased from Sigma); (C) O-glycans released from piglet ileum; (D) O-glycans released from piglet cecum.

Table S1. Complete list of genes identified to be differentially expressed in *C. jejuni* NCTC 11168 when grown in minimal media in the presence of 25 μ M L-fucose compared with growth in media lacking L-fucose: Fucose microarray

Gene number	Gene name	Fold-change log ₂	Bayes.p	Genome annotation
Up-regulated				
cj0481	dapA	4.36611	3.05E-11	Putative dihydrodipicolinate synthase
cj0483	uxaA	4.36413792	4.40E-13	Putative altronate hydrolase
cj0484	cj0484	3.74781682	2.32E-11	Putative MFS (Major Facilitator Superfamily) transport protein
cj0486	cj0486	3.69370417	1.97E-11	Putative sugar transporter
cj0485	cj0485	3.38307458	4.27E-11	Putative oxidoreductase
cj0487	cj0487	3.14743458	4.22E-10	Putative amidohydrolase
cj0490	ald	2.99369783	6.14E-10	Putative aldehyde dehydrogenase
cj1503c	putA	2.65269167	2.78E-11	Putative proline dehydrogenase
cj1502c	putP	2.535035	1.03E-10	Putative sodium/proline symporter
cj0917c	cstA	2.41062708	2.50E-09	Putative integral membrane protein
cj1537c	acs	2.31594208	3.43E-10	Acetyl-CoA synthetase
cj0488	cj0488	2.01624375	3.53E-08	Conserved hypothetical protein
cj0482	uxaA	1.91812375	1.82E-09	Putative altronate hydrolase
cj0921c	peb1A	1.87636	4.81E-08	Aspartate/glutamate-binding ABC transporter protein
cj0919c	cj0919c	1.80535083	8.53E-10	Putative ABC-type amino acid transporter permease protein
cj1682c	gltA	1.70775458	1.81E-09	Citrate synthase
cj0922c	pebC	1.56586042	1.42E-08	ABC-type amino acid transporter ATP-binding protein
cj0920c	cj0920c	1.53398875	3.99E-08	Putative ABC-type amino acid transporter permease protein
cj1541	cj1541	1.50247792	3.13E-11	Conserved hypothetical protein
cj0021c	cj0021c	1.43879667	3.69E-09	Putative fumarylacetoacetate (FAA) hydrolase family protein
cj1158c	cj1158c	1.430605	1.42E-05	Small hydrophobic protein
cj0489	ald	1.41285565	4.90E-07	Putative aldehyde dehydrogenase
cj0074c	cj0074c	1.3800625	2.01E-10	Putative iron-sulfur protein
cj0075c	cj0075c	1.3614325	1.57E-09	Putative oxidoreductase iron-sulfur subunit
cj0437	sdhA	1.35764458	1.42E-06	Succinate dehydrogenase flavoprotein subunit
cj0438	sdhB	1.214365	8.08E-06	Putative succinate dehydrogenase iron-sulfur protein
cj1619	kgpP	1.21231	2.21E-05	α -Ketoglutarate permease
cj0073c	cj0073c	1.18393542	8.59E-08	Conserved hypothetical protein
cj0835c	acnB	1.16229458	1.85E-10	Aconitate hydratase
cj0833c	cj0833c	1.11848833	1.83E-09	Putative oxidoreductase
cj0689	ackA	1.09401826	5.11E-09	Acetate kinase
cj1681c	cysQ	1.04634458	8.37E-11	CysQ protein homolog
cj1192	dctA	1.03423458	1.09E-09	Putative C4-dicarboxylate transport protein
cj0533	cj0533	1.0199275	5.59E-09	Putative integral membrane protein
cj0832c	cj0832c	0.977855	4.56E-13	Putative Na ⁺ /H ⁺ antiporter family protein
cj0853c	hemL	0.96895417	3.07E-09	Glutamate-1-semialdehyde 2,1-aminomutase
cj0688	pta	0.96819375	2.66E-08	Putative phosphate acetyltransferase
cj0522	cj0522	0.95287417	1.45E-08	Putative membrane protein
cj1625c	sdaC	0.92356917	3.00E-10	Amino acid transporter
cj0439	sdhC	0.91213167	0.00011757	Putative succinate dehydrogenase subunit C
cj0850c	cj0850c	0.90949087	5.03E-12	Putative MFS (Major Facilitator Superfamily) transport protein
cj1388	cj1388	0.89035625	1.19E-05	Putative endoribonuclease L-PSP
cj0076c	lctP	0.88469348	1.58E-08	L-Lactate permease
cj1542	cj1542	0.87182792	9.77E-08	Putative allophanate hydrolase subunit 1
cj0427	cj0427	0.84059	4.65E-09	Hypothetical protein
cj0203	cj0203	0.82591125	1.92E-12	Putative citrate transporter
cj0903c	cj0903c	0.80671125	1.33E-06	Putative amino acid transport protein
cj0982c	cjaA	0.79958792	1.36E-11	Putative amino acid transporter periplasmic solute-binding protein
cj0762c	aspB	0.78819083	1.35E-06	Aspartate aminotransferase
cj0393c	mgo	0.78807833	4.02E-09	Putative malate:quinone oxidoreductase
cj0834c	cj0834c	0.78551348	5.66E-08	Ankyrin repeat-containing putative periplasmic protein
cj1543	cj1543	0.76831917	3.90E-10	Putative allophanate hydrolase subunit 2
cj1624c	sdaA	0.72731542	1.28E-07	L-Serine dehydratase
cj1389	cj1389	0.71259182	7.42E-07	Pseudogene (putative C4-dicarboxylate anaerobic carrier)
cj0534	sucD	0.70539083	2.65E-11	Succinyl-coA synthetase α chain
cj0554	cj0554	0.69407667	2.00E-06	Hypothetical protein
cj0533	sucC	0.67438458	1.57E-10	Succinyl-coA synthetase β chain
cj1200	cj1200	0.64165539	2.86E-05	Putative NLPFA family lipoprotein
cj0531	icd	0.63176625	4.58E-09	Isocitrate dehydrogenase
cj1719c	leuA	0.62830458	1.63E-09	2-Isopropylmalate synthase
cj0373	cj0373	0.61054458	2.23E-10	Putative D-2-hydroxyacid dehydrogenase

Table S1. Cont.

Gene number	Gene name	Fold-change log ₂	Bayes.p	Genome annotation
<i>cj1008c</i>	<i>aroB</i>	0.60434	1.59E-09	3-Dehydroquinate synthase
<i>cj0555</i>	<i>cj0555</i>	0.60296727	2.30E-09	Putative dicarboxylate carrier protein MatC
<i>cj0069</i>	<i>cj0069</i>	0.5911	7.86E-08	Hypothetical protein
<i>cj0981c</i>	<i>cjaB</i>	0.58550875	2.96E-07	Putative MFS (Major Facilitator Superfamily) transport protein
<i>cj1717c</i>	<i>leuC</i>	0.58027391	1.42E-08	3-Isopropylmalate dehydratase large subunit
<i>cj0374</i>	<i>cj0374</i>	0.5777755	6.82E-07	Conserved hypothetical protein
<i>cj0987c</i>	<i>cj0987c</i>	0.56922083	1.36E-07	Putative MFS (Major Facilitator Superfamily) transport protein
<i>cj0348</i>	<i>trpB</i>	0.56829625	3.04E-06	Tryptophan synthase beta chain
<i>cj0536</i>	<i>oorA</i>	0.55900417	1.46E-11	OORA subunit of 2-oxoglutarate:acceptor oxidoreductase
<i>cj0346</i>	<i>trpD</i>	0.53065333	6.20E-05	Anthranilate synthase component II
<i>cj0374</i>	<i>cj0374</i>	0.52706261	2.98E-08	Conserved hypothetical protein Cj0374
<i>cj0375</i>	<i>cj0375</i>	0.513895	6.32E-10	Putative lipoprotein
<i>cj0532</i>	<i>mdh</i>	0.50484125	5.74E-09	Malate dehydrogenase
Down-regulated				
<i>cj1185c</i>	<i>petB</i>	-0.5030075	2.42E-06	Putative ubiquinol-cytochrome C reductase cytochrome B subunit
<i>cj0912c</i>	<i>cysM</i>	-0.5038296	0.00012051	cCysteine synthase
<i>cj1220</i>	<i>groES</i>	-0.503845	4.72E-05	10 kDa chaperonin (cpn10)
<i>cj0613</i>	<i>pstS</i>	-0.5137573	3.20E-06	Putative periplasmic phosphate binding protein
<i>cj1659</i>	<i>p19</i>	-0.5246338	6.47E-08	Periplasmic protein p19
<i>cj1688c</i>	<i>secY</i>	-0.5303296	4.35E-06	Preprotein translocase subunit
<i>cj1001</i>	<i>rpoD</i>	-0.5452921	9.37E-07	RNA polymerase σ factor (σ -70)
<i>cj0224</i>	<i>argC</i>	-0.54543	4.95E-06	<i>N</i> -acetyl- γ -glutamyl-phosphate reductase
<i>cj0515</i>	<i>cj0515</i>	-0.5500692	2.24E-08	Putative periplasmic protein
<i>cj0118</i>	<i>cj0118</i>	-0.5565643	2.52E-05	Conserved hypothetical protein
<i>cj0012c</i>	<i>rrc</i>	-0.5577417	3.25E-10	Nonhaem iron protein
<i>cj0281</i>	<i>tal</i>	-0.5627229	9.98E-07	Putative transaldolase
<i>cj1511c</i>	<i>fdhA</i>	-0.5698267	8.23E-07	Putative formate dehydrogenase large subunit (Selenocysteine containing)
<i>cj1221</i>	<i>groEL</i>	-0.5822396	7.97E-08	60 kDa chaperonin (cpn60)
<i>cj0082</i>	<i>cydB</i>	-0.5890079	3.96E-05	Cytochrome bd oxidase subunit II
<i>cj0343c</i>	<i>cj0343c</i>	-0.6022113	8.88E-05	Putative integral membrane protein
<i>cj0081</i>	<i>cydA</i>	-0.6309096	4.68E-05	Cytochrome bd oxidase subunit I
<i>cj1190c</i>	<i>cetA</i>	-0.6329646	0.00026076	Bipartate energy taxis response protein
<i>cj0328c</i>	<i>fabH</i>	-0.6329863	4.43E-06	3-Oxoacyl-[acyl-carrier-protein] synthase
<i>cj1438c</i>	<i>cj1438c</i>	-0.6341713	4.58E-07	Putative CPS glycosyltransferase
<i>cj1181c</i>	<i>tsf</i>	-0.6379096	8.15E-08	Elongation factor TS
<i>cj1184c</i>	<i>petC</i>	-0.6417317	8.63E-09	Putative ubiquinol-cytochrome C reductase cytochrome C subunit
<i>cj1180c</i>	<i>cj1180c</i>	-0.6453832	2.54E-09	Putative ABC transporter ATP-binding protein
<i>cj1514c</i>	<i>cj1514c</i>	-0.6476025	4.48E-09	Hypothetical protein
<i>cj0134</i>	<i>thrB</i>	-0.6604627	2.90E-07	Putative homoserine kinase
<i>cj1434c</i>	<i>cj1434c</i>	-0.6673438	1.24E-07	Putative CPS glycosyltransferase
<i>cj0475</i>	<i>rplA</i>	-0.67948	6.65E-10	50S ribosomal protein L1
<i>cj1474c</i>	<i>ctsD</i>	-0.6918808	1.11E-07	Putative type II protein secretion system D protein
<i>cj0117</i>	<i>pfs</i>	-0.6924929	2.35E-08	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
<i>cj1612</i>	<i>prfA</i>	-0.7133457	2.08E-07	Peptide chain release factor 1
<i>cj1596</i>	<i>rplQ</i>	-0.7141754	1.14E-10	50S ribosomal protein L17
<i>cj0343c</i>	<i>cj0343c</i>	-0.7166329	4.91E-05	Putative integral membrane protein
<i>cj1500</i>	<i>cj1500</i>	-0.7177971	3.37E-06	Putative integral membrane protein
<i>cj0025c</i>	<i>cj0025c</i>	-0.7422229	5.74E-06	Putative sodium:dicarboxylate family transmembrane symporter
<i>cj0190</i>	<i>cj0190c</i>	-0.7711905	2.60E-05	Conserved hypothetical protein
<i>cj1189c</i>	<i>cetB</i>	-0.7724913	6.90E-06	Bipartate energy taxis response protein
<i>cj0264c</i>	<i>cj0264c</i>	-0.7761913	4.05E-08	Molybdopterin containing oxidoreductase
<i>cj0265c</i>	<i>cj0265c</i>	-0.8130454	2.87E-09	Putative cytochrome C-type haem-binding periplasmic protein
<i>cj0370</i>	<i>rpsU</i>	-0.8188554	2.71E-12	30S ribosomal protein S21
<i>cj1153</i>	<i>cj1153</i>	-0.8466967	2.36E-11	Putative periplasmic cytochrome C
<i>cj0779</i>	<i>tpx</i>	-0.8684075	1.93E-06	Thiol peroxidase
<i>cj0169</i>	<i>sodB</i>	-0.9068563	6.42E-09	Superoxide dismutase (Fe)
<i>cj0379c</i>	<i>cj0379c</i>	-0.9803817	1.33E-07	Putative molybdenum containing oxidoreductase
<i>cj0262c</i>	<i>cj0262c</i>	-1.0401525	8.48E-11	Putative methyl-accepting chemotaxis signal transduction protein
<i>cj1169c</i>	<i>cj1169c</i>	-1.1581492	1.17E-08	Putative periplasmic protein
<i>cj0239c</i>	<i>cj0239c</i>	-1.3205179	2.18E-07	NifU protein homolog
<i>cj0334</i>	<i>ahpC</i>	-1.3638779	4.18E-13	Alkyl hydroperoxide reductase
<i>cj0415</i>	<i>cj0415</i>	-1.3721392	1.12E-06	Putative GMC oxidoreductase subunit

Table S1. Cont.

Gene number	Gene name	Fold-change log ₂	Bayes.p	Genome annotation
<i>cj0240c</i>	<i>iscS</i>	-1.3789683	4.98E-07	Cysteine desulfurase (NifS protein homolog)
<i>cj0414</i>	<i>cj0414</i>	-1.5476567	1.57E-07	Putative oxidoreductase subunit
<i>cj0453</i>	<i>thiC</i>	-1.6295863	0	Thiamin biosynthesis protein
<i>cj0017c</i>	<i>dsbI</i>	-1.7155813	6.61E-10	Disulphide bond formation protein
<i>cj1170c</i>	<i>omp50</i>	-1.8208058	1.07E-08	50 kDa outer membrane protein precursor

Displayed are the gene numbers, name, fold-change in log₂, *P* value, and annotated function. Boldface indicates genes belonging to the fucose operon.

Table S2. Expression of the genes within the operon *cj0481–cj0490* in *C. jejuni* NCTC 11168 when grown in minimal media containing 2 mg/mL porcine gastric mucin compared with the control: Mucin microarray

Gene Number	Gene name	Fold-change log ₂	Genome annotation
<i>cj0481</i>	<i>dapA</i>	1.862548148	Putative dihydrodipicolinate synthase
<i>cj0483</i>	<i>uxaA</i>	1.39661625	Putative altronate hydrolase
<i>cj0484</i>	<i>cj0484</i>	1.094871923	Putative MFS (Major Facilitator Superfamily) transport protein
<i>cj0485</i>	<i>cj0485</i>	0.98345	Putative oxidoreductase
<i>cj0486</i>	<i>cj0486</i>	1.219888462	Putative sugar transporter
<i>cj0487</i>	<i>cj0487</i>	0.777173333	Putative amidohydrolase
<i>cj0490</i>	<i>ald</i>	0.51669037	Putative aldehyde dehydrogenase

Displayed are the gene numbers, name, fold change in log₂, *P* value, and annotated function.

Table S3. List of primers used in this study

Primer name	Sequence
Gene Cloning	
<i>cj0481L</i>	CGGTACCCGGGGATCCAAAAGGAACTTTACCGGCTTTA
<i>cj0481R</i>	CGACTCTAGAGGATCCTTGCTTCATCAAAGCGAGTG
<i>cj0483L</i>	CGGTACCCGGGGATCCGGAGCAATGCCAATGTTTTT
<i>cj0483R</i>	CGACTCTAGAGGATCCGCTCATCACGCACTTCTTCTT
<i>cj0487L</i>	CGGTACCCGGGGATCCACCTTTGGGATTTGGAAAA
<i>cj0487R</i>	CGACTCTAGAGGATCCCACTGGATAATTGCTCCCAAA
<i>cj0490L</i>	ATGGCAAGAGATGAAAGCACAC
<i>cj0490R</i>	CCATCAGCTCCGCCTATGC
Inverse PCR	
<i>cj04811</i>	GAACTAAAGGGCGCAGCATAGGTGGAGTTTTTCCAG
<i>cj04812</i>	GAACACCGCCGAGCAACTTGCTTCCACGCTCAA
<i>cj04831</i>	GAACTAAAGGGCGCATTATGAATACACCAGGCAATGA
<i>cj04832</i>	GAACACCGCCGAGCATGCAGCACTTCAAGCATT
<i>cj04871</i>	GAACTAAAGGGCGCAGTTTTAAATCATCTAGGAAGTCCA
<i>cj04872</i>	GAACACCGCCGAGCACGCTAAACAAAACAAAAGATTATGAA
<i>cj04901</i>	AGTGTGCAAAGATGCGGATA
<i>cj04902</i>	TAAAGCAGGTGCCATCTTCC
Chloramphenicol cassette	
<i>catSE</i>	TGCTCGGCGGTGTTCTTTCCAAG
<i>catAS</i>	TGCGCCCTTAGTTCCTAAAGGGT
Allelic replacement	
<i>CfucP_F</i>	CGGGATCCTGGGATGAAAATAAGGAGTA
<i>CfucP_R</i>	ACGCGTCGACAAAGGTGTAGATGAGCATCAA
<i>EfucP_F</i>	CGGGATCCTGAACCTAAGAGGATGCTATG
<i>EfucP_R</i>	ACGCGTCGACTTCAGTTAGTTGCCGTTTG