# Comparative transcriptomics reveals key differences in the response to milk oligosaccharides of infant gut-associated bifidobacteria.

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## SUPPLEMENTARY INFORMATION

Description: This file contains 19 Supplementary Tables and 2 Supplementary Figures.

**TABLE S1:** List of *Bifidobacterium* strains used in this study.

| Code | Species | Additional strain information[a] | Source |
|---|---|---|---|
| JCM1254 | *B. bifidum* | DSM20082 | Intestine of adult |
| JCM1255 | *B. bifidum* | ATCC29521; DSM20456 | Infant feces |
| JCM1209 | *B. bifidum* | ATCC11863; DSM20082 | Infant feces |
| JCM7002 | *B. bifidum* | | Infant feces |
| JCM7003 | *B. bifidum* | | Infant feces |
| JCM7004 | *B. bifidum* | | Infant feces |
| S28-a | *B. bifidum* | Roy et al. 1996 | Infant feces |
| PRL2010 | *B. bifidum* | Turroni et al. 2010 | Infant feces |
| SC112 | *B. bifidum* | This study | Infant feces |
| SC126 | *B. bifidum* | This study | Infant feces |
| SC555 | *B. bifidum* | This study | Infant feces |
| SC572 | *B. bifidum* | This study | Infant feces |
| SC583 | *B. bifidum* | This study | Infant feces |
| ATCC15697 | *B. longum subsp. infantis* | JCM1222; DSM20088 | Infant feces |
| ATCC25962 | *B. longum subsp. infantis* | JCM1210; DSM20223 | Infant feces |
| ATCC17930 | *B. longum subsp. infantis* | JCM1260; DSM20218 | Infant feces |
| ATCC15702 | *B. longum subsp. infantis* | JCM1272; DSM20090 | Infant feces |
| JCM7007 | *B. longum subsp. infantis* | LMG18901 | Infant feces |
| JCM7009 | *B. longum subsp. infantis* | LMG18902 | Infant feces |
| JCM7011 | *B. longum subsp. infantis* | | Infant feces |
| SC30 | *B. longum subsp. infantis* | This study | Infant feces |
| SC97 | *B. longum subsp. infantis* | This study | Infant feces |
| SC117 | *B. longum subsp. infantis* | This study | Infant feces |
| SC142 | *B. longum subsp. infantis* | This study | Infant feces |
| SC143 | *B. longum subsp. infantis* | This study | Infant feces |
| SC145 | *B. longum subsp. infantis* | This study | Infant feces |
| SC268 | *B. longum subsp. infantis* | This study | Infant feces |
| SC417 | *B. longum subsp. infantis* | This study | Infant feces |
| SC523 | *B. longum subsp. infantis* | This study | Infant feces |
| SC569 | *B. longum subsp. infantis* | This study | Infant feces |
| SC600 | *B. longum subsp. infantis* | This study | Infant feces |
| SC605 | *B. longum subsp. infantis* | This study | Infant feces |
| SC638 | *B. longum subsp. infantis* | This study | Infant feces |
| SC680 | *B. longum subsp. infantis* | This study | Infant feces |
| JCM10602 | *B. animalis subsp. lactis* | DSMZ 10140 | Dairy product |

[a] The original strain numbers are also noted, if known. JCM, Japan Collection of Microorganisms, ATCC, American Type Culture Collection; DSMZ, German Collection of Microorganisms and Cell Culture.

**TABLE S2:** MLST genes and primers.

| Gene | PCR primer (5´-3´)*,a | Expected Amplicon size (bp) | Anneling Temp. (ºC) |
|---|---|---|---|
| *clpC* | GAG TAC CGC AAG TAC ATC GAG | 748 | 63 |
| | CAT CCT CAT CGT CGA ACA GGA AC | | |
| *purF* | CAT TCG AAC TCC GAC ACC GA | 977 | 62 |
| | GTG GGG TAG TCG CCG TTG | | |
| *gyrB* | AGC TGC ACG CBG GCG GCA AGT TCG | 811 | 66 |
| | GTT GCC GAG CTT GGT CTT GGT CTG | | |
| *fusA* | ATC GGC ATC ATG GCY CAC ATY GAT | 784 | 66 |
| | CCA GCA TCG GCT GMA CRC CCT T | | |
| *Iles* | ATC CCG CGY TAC CAG ACS ATG | 789 | 66 |
| | CGG TGT CGA CGT AGT CGG CG | | |
| *rplB* | GGA CAA GGA CGG CRT SCC SGC CAA | 498 | 67 |
| | ACG ACC RCC GTG CGG GTG RTC GAC | | |
| *rpoB* | GGC GAG CTG ATC CAG AAC CA | 1057 | 62 |
| | GCA TCC TCG TAG TTG TAS CC | | |

*= Upper sequence, forward primer; Lower sequence, reverse primer.

[a] In the primer sequence R indicates (A/G), S (C/G), Y (C/T).

**TABLE S3:** Descriptive evolutionary analysis of MLST data.

| Gene | Fragment analyzed(bp)* | G+C (mol%) | polymorphic sites | Allele frequencies | π | k |
|---|---|---|---|---|---|---|
| | | | *Bifidobacterium bifidum* | | | |
| *clpC* | 717 (0,26) | 64,8 | 9 | 6 | 0,00356 | 2,54945 |
| *purF* | 875 (0,57) | 64,4 | 16 | 12 | 0,00325 | 2,84615 |
| *gyrB* | 724 (0,35) | 61,1 | 9 | 7 | 0,00354 | 2,5604 |
| *fusA* | 748(0,35) | 62,9 | 14 | 10 | 0,0048 | 3,5934 |
| *Iles* | 743(0,22) | 63,2 | 17 | 10 | 0,00618 | 4,5934 |
| *rplB* | 422 (0,50) | 66,3 | 2 | 3 | 0,00138 | 0,58242 |
| *rpoB* | 984 (0,27) | 65 | 18 | 11 | 0,00474 | 4,65934 |
| | | | *Bifidobacterium infantis* | | | |
| *clpC* | 721 (0,27) | 62,55 | 30 | 8 | 0,01297 | 9,35238 |
| *purF* | 883 (0,58) | 64,21 | 65 | 9 | 0,02431 | 21,467 |
| *gyrB* | 711 (0,34) | 64,56 | 41 | 7 | 0,01932 | 13,733 |
| *fusA* | 766(0,36) | 62,01 | 42 | 9 | 0,01862 | 14,26667 |
| *Iles* | 757(0,22) | 62,88 | 55 | 8 | 0,02234 | 16,686 |
| *rplB* | 427 (0,51) | 65,81 | 9 | 7 | 0,00567 | 2,419 |
| *rpoB* | 965 (0,27) | 64,46 | 39 | 8 | 0,00754 | 7,276 |

*Percentage of the gene is given in parenthesis.

π = mean pairwise nucleotide difference per site.

k = mean pairwise nucleotide difference per sequence.

**TABLE S4:** Allelic profiles of 13 *B. bifidum* strains analyzed by MLST.

| Strains | ST[a] | Allele | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *clpC* | *purF* | *gyrB* | *fusA* | *Iles* | *rplB* | *rpoB* |
| JCM1254 | 1 | 1 | 2 | 4 | 1 | 10 | 2 | 9 |
| S28-a | 1 | 1 | 2 | 4 | 1 | 10 | 2 | 9 |
| PRL2010 | 2 | 2 | 1 | 6 | 7 | 2 | 3 | 7 |
| JCM1255 | 3 | 6 | 12 | 3 | 2 | 6 | 3 | 2 |
| JCM1209 | 4 | 5 | 6 | 1 | 6 | 8 | 3 | 1 |
| JCM7002 | 5 | 3 | 11 | 7 | 3 | 1 | 3 | 5 |
| JCM7003 | 6 | 4 | 9 | 2 | 8 | 4 | 3 | 4 |
| JCM7004 | 7 | 6 | 8 | 1 | 5 | 10 | 3 | 9 |
| SC112 | 8 | 6 | 3 | 3 | 10 | 3 | 1 | 8 |
| SC126 | 9 | 6 | 10 | 5 | 9 | 9 | 2 | 6 |
| SC555 | 10 | 6 | 5 | 4 | 2 | 5 | 3 | 10 |
| SC572 | 11 | 6 | 4 | 3 | 7 | 7 | 3 | 11 |
| SC583 | 12 | 6 | 7 | 7 | 4 | 5 | 3 | 3 |

[a]ST Indicates specific sequence type.

**TABLE S5:** Allelic profiles of 15 *B. infantis* strains analyzed by MLST.

| Strains | ST[a] | Allele | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *clpC* | *purF* | *gyrB* | *fusA* | *Iles* | *rplB* | *rpoB* |
| ATCC15697 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 8 |
| SC30 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 5 |
| SC97 | 3 | 2 | 8 | 1 | 1 | 2 | 2 | 5 |
| SC117 | 4 | 3 | 9 | 2 | 7 | 3 | 3 | 7 |
| SC142 | 5 | 4 | 8 | 3 | 4 | 4 | 2 | 5 |
| SC143 | 6 | 4 | 2 | 1 | 4 | 4 | 2 | 5 |
| SC145 | 7 | 5 | 7 | 1 | 6 | 5 | 4 | 6 |
| SC268 | 8 | 6 | 5 | 3 | 2 | 6 | 5 | 4 |
| SC417 | 9 | 2 | 6 | 1 | 1 | 2 | 2 | 5 |
| SC523 | 10 | 6 | 5 | 4 | 2 | 6 | 5 | 4 |
| SC569 | 11 | 7 | 4 | 1 | 5 | 7 | 6 | 3 |
| SC600 | 12 | 2 | 3 | 5 | 8 | 2 | 7 | 2 |
| SC605 | 12 | 2 | 3 | 5 | 8 | 2 | 7 | 2 |
| SC638 | 12 | 2 | 3 | 5 | 8 | 2 | 7 | 2 |
| SC680 | 13 | 8 | 3 | 7 | 9 | 8 | 5 | 1 |

[a]ST Indicates specific sequence type.

**Table S6:** Genome statistics of the draft genome of *B. bifidum* SC555

| | Number | % of Total |
|---|---|---|
| DNA, total number of bases | 2237461 | 100.00% |
| DNA coding number of bases | 1933914 | 86.43% |
| DNA G+C number of bases | 1401692 | 62.65% [1] |
| | | |
| DNA scaffolds | 22 | 100.00% |
| Average lenght contigs | 101703 | |
| | | |
| Genes total number | 1894 | 100.00% |
| Protein coding genes | 1835 | 96.88% |
| RNA genes | 59 | 3.12% |
| rRNA genes[a] | 3 | 0.16% |
| 5S rRNA | 1 | 0.05% |
| 16S rRNA | 1 | 0.05% |
| 23S rRNA | 1 | 0.05% |
| tRNA genes | 53 | 2.80% |
| Other RNA genes | 3 | 0.16% |
| Protein coding genes with function prediction | 1363 | 71.96% |
| without function prediction | 472 | 24.92% |
| Protein coding genes with enzymes | 516 | 27.24% |
| w/o enzymes but with candidate KO based enzymes | 29 | 1.53% |
| Protein coding genes connected to Transporter Classification | 212 | 11.19% |
| Protein coding genes connected to KEGG pathways[3] | 537 | 28.35% |
| not connected to KEGG pathways | 1298 | 68.53% |
| Protein coding genes connected to KEGG Orthology (KO) | 907 | 47.89% |
| not connected to KEGG Orthology (KO) | 928 | 49.00% |
| Protein coding genes connected to MetaCyc pathways | 502 | 26.50% |
| not connected to MetaCyc pathways | 1333 | 70.38% |
| Protein coding genes with COGs[3] | 1354 | 71.49% |
| with KOGs[3] | 677 | 35.74% |
| with Pfam[3] | 1447 | 76.40% |
| with TIGRfam[3] | 593 | 31.31% |
| with IMG Terms | 166 | 8.76% |
| with IMG Pathways | 96 | 5.07% |
| with IMG Parts List | 66 | 3.48% |
| in paralog clusters | 427 | 22.54% |

| | | |
|---|---|---|
| in Chromosomal Cassette | 1894 | 100.00% |
| Chromosomal Cassettes | 249 | - |
| Biosynthetic Clusters | 9 | - |
| Genes in Biosynthetic Clusters | 66 | 3.48% |
| Fused Protein coding genes | 3 | 0.16% |
| Protein coding genes coding signal peptides | 108 | 5.70% |
| Protein coding genes coding transmembrane proteins | 459 | 24.23% |
| COG clusters | 982 | 0.00% |
| KOG clusters | 487 | 0.00% |
| Pfam clusters | 1251 | 0.00% |
| TIGRfam clusters | 574 | 0.00% |

[a] Since SC555 is a draft genome, rRNA number is just a reference

**Table S7:** Gene number of putative HMO-genes in the genomes of *B. bifidum* SC555 and *B. bifidum* PRL2010.

| | Solute Binding Protein Family 1 | Sialidase (GH33) | α-Fucosidase (GH29) | α-Fucosidase (GH95) | β-Hexosaminidase (GH20) | Lacto-N-biosidase | β-Galactosidase GH2 (2334) | β-Galactosidase (GH42) |
|---|---|---|---|---|---|---|---|---|
| SC555 | 2 | 2 | 1 | 1 | 3 | 1 | 3 | 2 |
| PRL2010 | 3 | 2 | 1 | 1 | 3 | 1 | 3 | 2 |

**Table S8:** General features of the RNA-seq experiments

| Sample | Number of reads | Counted fragments to genes | Counted fragments to intergenic [a] | Uncounted fragments [b] | % uncounted | % reads aligning to genes | % Intergenic | %16S | %23S | %5S |
|---|---|---|---|---|---|---|---|---|---|---|
| *B. infantis* experiment | | | | | | | | | | |
| LAC-A | 9580821 | 6593523 | 1028625 | 1958673 | 20.44 | 68.82 | 10.74 | 0.38 | 2.57 | 0.01 |
| LAC-B | 8509364 | 5915002 | 895479 | 1698883 | 19.96 | 69.51 | 10.52 | 0.35 | 2.60 | 0.03 |
| HMOearly-A | 7431284 | 6008022 | 628754 | 794508 | 10.69 | 80.85 | 8.46 | 0.26 | 1.81 | 0.01 |
| HMOearly-B | 7869591 | 6394589 | 652910 | 822092 | 10.45 | 81.26 | 8.30 | 0.24 | 1.59 | 0.01 |
| HMOmid-A | 11927818 | 8563335 | 2084420 | 1280063 | 10.73 | 71.79 | 17.48 | 0.05 | 0.17 | 0.01 |
| HMOmid-B | 12697748 | 9421352 | 2021218 | 1255178 | 9.89 | 74.20 | 15.92 | 0.04 | 0.18 | 0.01 |
| HMOlate-A | 10085888 | 7409454 | 1580720 | 1095714 | 10.86 | 73.46 | 15.67 | 0.03 | 0.13 | 0.01 |
| HMOlate-B | 8538230 | 6526725 | 1190321 | 821184 | 9.62 | 76.44 | 13.94 | 0.10 | 0.28 | 0.02 |
| LNT-A | 10348666 | 8226643 | 1037151 | 1084872 | 10.48 | 79.49 | 10.02 | 2.12 | 2.54 | 0.00 |
| LNT-B | 9872022 | 8035279 | 935991 | 900752 | 9.12 | 81.39 | 9.48 | 1.73 | 2.06 | 0.00 |
| LNnT-A | 15999785 | 8613627 | 2890595 | 4495563 | 28.10 | 53.84 | 18.07 | 0.02 | 0.24 | 0.00 |
| LNnT-B | 15999800 | 7889369 | 2742892 | 5367539 | 33.55 | 49.31 | 17.14 | 0.03 | 0.43 | 0.01 |
| 2FL-A | 11834663 | 8320115 | 2235223 | 1279325 | 10.81 | 70.30 | 18.89 | 0.01 | 0.15 | 0.00 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2FL-B | 7999912 | 5625018 | 1510477 | 864417 | 10.81 | 70.31 | 18.88 | 0.02 | 0.34 | 0.01 |
| 3FL-A | 8249294 | 6065228 | 1423663 | 760403 | 9.22 | 73.52 | 17.26 | 0.02 | 0.32 | 0.02 |
| 3FL-B | 12352540 | 9300426 | 1831905 | 1220209 | 9.88 | 75.29 | 14.83 | 0.01 | 0.20 | 0.01 |
| 6SL-A | 9626876 | 6611304 | 1935766 | 1079806 | 11.22 | 68.68 | 20.11 | 0.02 | 0.47 | 0.03 |
| 6SL-B | 8959521 | 5237849 | 2577162 | 1144510 | 12.77 | 58.46 | 28.76 | 0.04 | 1.00 | 0.16 |
| *B.bifidum* experiment | | | | | | | | | | |
| LAC-A | 6832262 | 5063710 | 1155573 | 612979 | 8.97 | 74.11 | 16.91 | 0.02 | 0.38 | 0.01 |
| LAC-B | 9743954 | 7404364 | 1501297 | 838293 | 8.60 | 75.99 | 15.41 | 0.05 | 0.55 | 0.04 |
| HMOearly-A | 7137336 | 5796097 | 492958 | 848281 | 11.89 | 81.21 | 6.91 | 0.10 | 1.65 | 0.07 |
| HMOearly-B | 9440882 | 7717524 | 677384 | 1045974 | 11.08 | 81.75 | 7.18 | 0.06 | 1.12 | 0.03 |
| HMOmid-A | 5700702 | 4524342 | 533781 | 642579 | 11.27 | 79.36 | 9.36 | 0.02 | 1.10 | 0.03 |
| HMOmid-B | 5943078 | 4830773 | 525726 | 586579 | 9.87 | 81.28 | 8.85 | 0.14 | 1.35 | 0.02 |
| HMOlate-A | 8268172 | 6684808 | 807839 | 775525 | 9.38 | 80.85 | 9.77 | 0.09 | 0.92 | 0.02 |
| HMOlate-B | 6142644 | 4973114 | 637948 | 531582 | 8.65 | 80.96 | 10.39 | 0.07 | 1.57 | 0.03 |
| LNT-A | 7525773 | 4944007 | 1708234 | 873532 | 11.61 | 65.69 | 22.70 | 0.01 | 0.29 | 0.01 |
| LNT-B | 3199964 | 2113113 | 707284 | 379567 | 11.86 | 66.04 | 22.10 | 0.08 | 1.57 | 0.09 |
| LNnT-A | 3149953 | 2145609 | 680370 | 323974 | 10.29 | 68.12 | 21.60 | 0.06 | 0.95 | 0.01 |
| LNnT-B | 21902887 | 16216489 | 3132582 | 2553816 | 11.66 | 74.04 | 14.30 | 0.06 | 0.64 | 1.02 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2FL-A | 9647475 | 7141735 | 1687548 | 818192 | 8.48 | 74.03 | 17.49 | 0.02 | 0.40 | 0.02 |
| 2FL-B | 9782588 | 7335764 | 1658551 | 788273 | 8.06 | 74.99 | 16.95 | 0.01 | 0.44 | 0.06 |
| 3FL-A | 9562131 | 7163248 | 1511244 | 887639 | 9.28 | 74.91 | 15.80 | 0.04 | 0.67 | 0.04 |
| 3FL-B | 9335729 | 6902333 | 1569093 | 864303 | 9.26 | 73.93 | 16.81 | 0.03 | 0.54 | 0.01 |
| 6SL-A | 12108746 | 9491459 | 1487133 | 1130154 | 9.33 | 78.39 | 12.28 | 0.02 | 0.25 | 0.02 |
| 6SL-B | 8108806 | 6356291 | 995662 | 756853 | 9.33 | 78.39 | 12.28 | 0.04 | 0.55 | 0.06 |
| MUC-A | 11392726 | 8978246 | 1257843 | 1156637 | 10.15 | 78.81 | 11.04 | 0.03 | 0.47 | 0.21 |
| MUC-B | 12561151 | 10048642 | 1489804 | 1022705 | 8.14 | 80.00 | 11.86 | 0.02 | 0.39 | 0.39 |
| | | | | | | | | | | |
| Summary | | Reads aligning to genes | Reads aligning to IGR | Uncounted reads | % uncounted | % reads align genes | % Intergenic | %16S | %23S | %5S |
| Total reads B.infantis | 187883823 | 130756860 | | | | | | | | |
| Average B. infantis | 10437990 | 7264270 | 1622404 | 1551316 | 13.81 | 70.94 | 15.25 | 0.30 | 0.95 | 0.02 |
| SD | 2572943 | 1309715 | 708267 | 1277312 | 7.04 | 9.04 | 5.16 | 0.60 | 0.95 | 0.03 |
| Total reads B. bifidum | 177486959 | 135831668 | | | | | | | | |
| Average B. bifidum | 8874348 | 6791583 | 1210893 | 871872 | 9.86 | 76.14 | 14.00 | 0.05 | 0.79 | 0.11 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SD | 4002486 | 3049299 | 633710 | 454424 | 1.29 | 4.96 | 4.80 | 0.03 | 0.45 | 0.23 |

[a] Total reads aligning to intergenic regions, identified in both genomes.

[b] Total reads not aligned to any genic or intergenic region.

**Table S9:** Function of genes associated to HMO consumption in *B. infantis* described in Figure 4

| Locus tag | Gene lenght | Annotation | Possible function [a] |
|---|---|---|---|
| Blon_0344 | 978 | Mandelate racemase/muconate lactonizing enzyme, N-terminal domain protein | Fucose metabolism |
| Blon_0247 | 1206 | major facilitator superfamily MFS_1 | HMO import |
| Blon_0248 | 1218 | Alpha-L-fucosidase( EC:3.2.1.51 ) | Fucose metabolism |
| Blon_2177 | 696 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_2016 | 1407 | Beta-galactosidase( EC:3.2.1.23 ) | HMO enzymatic degradation |
| Blon_0732 | 768 | glycoside hydrolase, family 20 | HMO enzymatic degradation |
| Blon_2332 | 1593 | sugar (Glycoside-Pentoside-Hexuronide) transporter | HMO transport |
| Blon_2351 | 1437 | extracellular solute-binding protein, family 1 | ABC transporter for HMO |
| Blon_2350 | 2349 | extracellular solute-binding protein, family 1 | ABC transporter for HMO |
| Blon_2352 | 438 | extracellular solute-binding protein, family 1 | ABC transporter for HMO |
| Blon_2348 | 1356 | Exo-alpha-sialidase( EC:3.2.1.18 ) | HMO enzymatic degradation |
| Blon_2349 | 3072 | dihydrodipicolinate synthetase( EC:4.2.1.52 ) | Fucose metabolism |
| Blon_0538 | 75 | UDP-glucose 4-epimerase( EC:5.1.3.2 ) | Galactose metabolism |
| Blon_2331 | 1317 | sugar (Glycoside-Pentoside-Hexuronide) transporter | HMO transport |
| Blon_2354 | 792 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_2355 | 1278 | glycoside hydrolase, family 20 | HMO enzymatic degradation |
| Blon_0346 | 630 | hypothetical protein | HMO enzymatic degradation |
| Blon_0341 | 2835 | binding-protein-dependent transport systems inner membrane component | ABC transporter for HMO |
| Blon_2204 | 1164 | binding-protein-dependent transport systems inner membrane component | ABC transporter for HMO |
| Blon_0345 | 1410 | major facilitator superfamily MFS_1 | HMO transport |
| Blon_2202 | 1539 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_0343 | 912 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_0342 | 519 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2203 | 1125 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2475 | 450 | ABC transporter related | ATPase for ABC transporters |
| Blon_2063 | 1365 | galactose-1-phosphate uridylyltransferase( EC:2.7.7.12 ) | Galactose metabolism |

| | | | |
|---|---|---|---|
| Blon_2171 | 2061 | UDP-glucose 4-epimerase( EC:5.1.3.2 ) | GlcNAc metabolism |
| Blon_2184 | 1317 | phosphoglucomutase, alpha-D-glucose phosphate-specific( EC:5.4.2.2 ) | Galactose metabolism |
| Blon_2062 | 261 | galactokinase( EC:2.7.1.6 ) | Galactose metabolism |
| Blon_2334 | 933 | glycoside hydrolase family 2, TIM barrel( EC:3.2.1.23 ) | HMO enzymatic degradation |
| Blon_0879 | 813 | ROK family protein | GlcNAc metabolism |
| Blon_2341 | 801 | protein of unknown function DUF624 | HMO transport |
| Blon_2353 | 897 | hypothetical protein | HMO transport |
| Blon_2176 | 1284 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2175 | 1704 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2174 | 690 | hypothetical protein | Galactose metabolism |
| Blon_2172 | 633 | UDP-glucose--hexose-1-phosphate uridylyltransferase( EC:2.7.7.12,EC:2.7.7.10 ) | Galactose metabolism |
| Blon_2173 | 1602 | aminoglycoside phosphotransferase( EC:2.7.1.162 ) | Galactose metabolism |
| Blon_0645 | 945 | N-acylglucosamine-6-phosphate 2-epimerase( EC:5.1.3.9 ) | NeuAc metabolism |
| Blon_0644 | 912 | ROK family protein( EC:2.7.1.2 ) | NeuAc metabolism |
| Blon_0646 | 684 | glycosyl hydrolase, BNR repeat-containing protein( EC:3.2.1.18 ) | HMO enzymatic degradation |
| Blon_0881 | 1338 | glucosamine-6-phosphate isomerase( EC:3.5.99.6 ) | GlcNAc metabolism |
| Blon_0882 | 912 | N-acetylglucosamine-6-phosphate deacetylase( EC:3.5.1.25 ) | GlcNAc metabolism |
| Blon_2347 | 1533 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_2344 | 1200 | extracellular solute-binding protein, family 1 | HMO transport |
| Blon_2342 | 1296 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2345 | 675 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2343 | 2238 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2346 | 1524 | binding-protein-dependent transport systems inner membrane component | HMO transport |
| Blon_2337 | 1347 | RbsD or FucU transport( EC:5.1.3.- ) | Fucose metabolism |
| Blon_2338 | 1116 | dihydrodipicolinate synthetase( EC:4.2.1.52 ) | Fucose metabolism |
| Blon_2335 | 1254 | alpha-1/2-fucosidase | HMO enzymatic degradation |
| Blon_2336 | 1326 | alpha-1,3/4-fucosidase, putative( EC:3.2.1.51 ) | HMO enzymatic degradation |
| Blon_2339 | 690 | short-chain dehydrogenase/reductase SDR( EC:1.1.1.100 ) | Fucose metabolism |

| | | | |
|---|---|---|---|
| Blon_2340 | 1404 | Mandelate racemase/muconate lactonizing enzyme, C-terminal domain protein( EC:5.1.2.2 ) | Fucose metabolism |

[a] Possible function was proposed from previous works referenced throughout the manuscript, as well as gene annotation.

**Table S10:** Function of genes associated to HMO consumption in *B. bifidum* described in Figure 5.

| Locus tag | Gene lenght | Annotation | Possible function |
|---|---:|---|---|
| BBIF_01022 | 1.791 | ABC-type dipeptide transport system, periplasmic component | HMO transport |
| BBIF_00180 | 1.308 | ABC-type sugar transport system, periplasmic component | HMO transport |
| BBIF_00172 | 1.023 | UDP-glucose-4-epimerase( EC:5.1.3.2 ) | Galactose metabolism |
| BBIF_00503 | 5.796 | PEGA domain./Uncharacterised Sugar-binding Domain./F5/8 type C domain. | HMO enzymatic degradation |
| BBIF_01401 | 1.128 | ABC-type sugar transport systems, ATPase components | ATPase for ABC transporters |
| BBIF_00527 | 1.551 | PTS system, N-acetylglucosamine-specific IIBC component | HMO transport |
| BBIF_00528 | 507 | PTS system, glucose subfamily, IIA component( EC:2.7.1.69 ) | HMO transport |
| BBIF_01068 | 813 | glucosamine-6-phosphate isomerase( EC:3.5.99.6 ) | GlcNAc metabolism |
| BBIF_00546 | 3159 | Beta-galactosidase/beta-glucuronidase( EC:3.2.1.23 ) | HMO enzymatic degradation |
| BBIF_00547 | 1476 | sugar (Glycoside-Pentoside-Hexuronide) transporter | HMO transport |
| BBIF_01155 | 5.562 | Beta-galactosidase/beta-glucuronidase( EC:3.2.1.23 ) | HMO enzymatic degradation |
| BBIF_01261 | 5.880 | Bacterial surface proteins containing Ig-like domains | HMO enzymatic degradation |
| BBIF_00173 | 1.515 | Galactose-1-phosphate uridyltransferase( EC:2.7.7.12 ) | Galactose metabolism |
| BBIF_00174 | 1.089 | Putative homoserine kinase type II (protein kinase fold) | Galactose metabolism |
| BBIF_01067 | 1.257 | N-acetylglucosamine-6-phosphate deacetylase( EC:3.5.1.25 ) | GlcNAc metabolism |
| BBIF_00178 | 951 | ABC-type sugar transport system, permease component | HMO transport |
| BBIF_00179 | 972 | ABC-type sugar transport systems, permease components | HMO transport |
| BBIF_00177 | 2.256 | 1,3-beta-galactosyl-N-acetylhexosamine phosphorylase | HMO enzymatic degradation |
| BBIF_00369 | 1251 | galactose-1-phosphate uridylyltransferase, family 1( EC:2.7.7.12 ) | GlcNAc metabolism |
| BBIF_01218 | 456 | Lacto-N-biose phosphorylase. | HMO enzymatic degradation |
| BBIF_00871 | 1677 | phosphoglucomutase, alpha-D-glucose phosphate-specific( EC:5.4.2.2 ) | Galactose metabolism |
| BBIF_01021 | 1.431 | Aminopeptidase C( EC:3.4.22.40 ) | Galactose metabolism |
| BBIF_01217 | 333 | Lacto-N-biose phosphorylase. | HMO enzymatic degradation |
| BBIF_00368 | 1.251 | galactokinase( EC:2.7.1.6 ) | Galactose metabolism |

| | | | |
|---|---|---|---|
| BBIF_00533 | 1.155 | Lacto-N-biose phosphorylase. | HMO enzymatic degradation |
| BBIF_01442 | 3498 | Alpha-L-arabinofuranosidase | HMO enzymatic degradation |
| BBIF_00707 | 2505 | NPCBM-associated, NEW3 domain of alpha-galactosidase./BNR/Asp-box repeat.( EC:3.2.1.18 ) | HMO enzymatic degradation |
| BBIF_00008 | 4.500 | Alpha-L-fucosidase( EC:3.2.1.51 ) | HMO enzymatic degradation |
| BBIF_01050 | 957 | Galactose mutarotase and related enzymes | Galactose metabolism |
| BBIF_00852 | 4884 | N-acetyl-beta-hexosaminidase( EC:3.2.1.52 ) | HMO enzymatic degradation |
| BBIF_00176 | 942 | Transcriptional regulator/sugar kinase( EC:2.7.1.2 ) | GlcNAc metabolism |
| BBIF_00136 | 3183 | N-acetyl-beta-hexosaminidase | HMO enzymatic degradation |
| BBIF_00175 | 1155 | Transcriptional regulator/sugar kinase | GlcNAc metabolism |
| BBIF_01445 | 2337 | N-acetyl-beta-hexosaminidase | HMO enzymatic degradation |
| BBIF_00550 | 1014 | UDP-glucose-4-epimerase( EC:5.1.3.2 ) | Galactose metabolism |
| BBIF_01073 | 1284 | Fucose permease | HMO transport |
| BBIF_00706 | 5388 | GDSL-like Lipase/Acylhydrolase./Laminin G domain./BNR/Asp-box repeat.( EC:3.2.1.18 ) | HMO enzymatic degradation |

[a] Possible function was proposed from previous works referenced throughout the manuscript, as well as gene annotation.

**Table S11:** Enriched annotations, *B. infantis* genes UP-regulated in relative to lactose

| Type | ANNOT_ID | ANNOT_DESC | HMO-E | HMO-M | HMO-L | LNT | LNnT | 2FL | 3FL | 6SL |
|---|---|---|---|---|---|---|---|---|---|---|
| COG | COG1175 | ABC-type sugar transport systems, permease components | NS | NS | NS | NS | 0.059 | 0.019 | NS | 0.09 |
| COG | COG0395 | ABC-type sugar transport system, permease component | 0.041 | NS | NS | 0.027 | NS | 0.08 | 0.015 | 0.024 |
| COG | COG2801 | Transposase and inactivated derivatives | NS | NS | NS | NS | NS | NS | NS | 0.029 |
| COG | COG2197 | Response regulator containing a CheY-like receiver domain and an HTH DNA-binding domain | NS | NS | NS | 0.046 | 0.04 | NS | NS | NS |
| COG | COG1653 | ABC-type sugar transport system, periplasmic component | 0.004 | NS | 0.006 | 0.063 | 0.08 | 0.003 | 0.062 | 0 |
| GO | GO:0005215 | transporter activity | 0 | NS | NS | NS | 0.011 | NS | NS | 0.007 |
| GO | GO:0005975 | carbohydrate metabolic process | NS | NS | 0.008 | NS | NS | NS | NS | NS |
| GO | GO:0043565 | sequence-specific DNA binding | NS | NS | NS | 0 | NS | NS | NS | NS |
| GO | GO:0008643 | carbohydrate transport | 0.003 | NS | NS | 0.01 | 0.01 | 0.001 | 0.001 | 0 |
| SRI | TRNA CHARGING | | NS | 0.005 | NS | NS | NS | NS | NS | NS |

**Table S12:** Enriched annotations, *B. infantis* genes DOWN-regulated in relative to lactose

| Type | ANNOT_ID | ANNOT_DESC | HMO-E | HMO-M | HMO-L | LNT | LNnT | 2FL | 3FL | 6SL |
|---|---|---|---|---|---|---|---|---|---|---|
| GO | GO:0003723 | RNA binding | NS | NS | NS | NS | NS | NS | 0.017 | NS |
| KEGG | MAP00240 | Pyrimidine metabolism | NS | 0.005 | 0.016 | 0.038 | NS | NS | NS | NS |
| KEGG | MAP00230 | Purine metabolism | NS | 0.001 | 0.009 | 0.005 | NS | NS | NS | NS |
| SRI | PEPTIDOGLYCAN BIOSYNTHESIS III MYCOBACTERIA | | NS | NS | NS | NS | NS | NS | 0.041 | NS |
| SRI | TRNA CHARGING | | NS | NS | NS | NS | NS | 0.045 | NS | NS |

**Table S13:** Enriched annotations, *B. bifidum* genes UP-regulated relative to LACTOSE

| Type | ANNOT_ID | ANNOT_DESC | HMO-E | HMO-M | HMO-L | MUC | LNT | LNnT | 2’FL | 3FL | 6’SL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| COG | COG1609 | Transcriptional regulators | NS | NS | NS | NS | NS | 0.041 | NS | 0.029 | NS |
| GO | GO:0007155 | cell adhesion | 0.002 | 0.001 | 0.052 | NS | NS | 0 | NS | NS | NS |
| GO | GO:0005975 | carbohydrate metabolic process | 0.006 | 0.05 | NS | 0.095 | NS | 0.087 | NS | NS | NS |
| GO | GO:0008643 | carbohydrate transport | NS | 0.089 | 0.015 | 0.416 | NS | 0.126 | NS | NS | NS |
| GO | GO:0019843 | rRNA binding | 0 | NS | NS | 0.102 | NS | NS | NS | NS | NS |
| GO | GO:0006412 | translation | 0 | 0.158 | NS | 0.029 | NS | NS | NS | NS | NS |
| GO | GO:0003735 | structural constituent of ribosome | 0 | 0.032 | NS | 0.004 | NS | NS | NS | NS | NS |
| GO | GO:0005840 | ribosome | 0 | 0.163 | NS | 0.008 | NS | NS | NS | NS | NS |
| GO | GO:0005351 | sugar:hydrogen symporter activity | NS | NS | NS | 0.042 | NS | NS | NS | NS | NS |
| GO | GO:0009401 | phosphoenolpyruvate-dependent sugar phosphotransferase system | NS | 0.246 | NS | 0.027 | NS | 0.162 | NS | NS | NS |
| GO | GO:0043169 | cation binding | 0.007 | 0.338 | NS | NS | NS | 0.042 | NS | NS | NS |
| KEGG | MAP00520 | Amino sugar and nucleotide sugar metabolism | NS | 0.094 | NS | 0.022 | NS | NS | NS | NS | NS |
| KEGG | MAP00330 | Arginine and proline metabolism | NS | 0.197 | 0.035 | NS | NS | NS | NS | NS | NS |
| KEGG | MAP00250 | Alanine, aspartate and glutamate metabolism | NS | 0.043 | 0.033 | NS | NS | NS | NS | NS | NS |
| KEGG | MAP00260 | Glycine, serine and threonine metabolism | NS | NS | NS | NS | NS | NS | NS | NS | 0.013 |
| KEGG | MAP03010 | Ribosome | 0 | 0.01 | 0.079 | 0.001 | NS | NS | NS | NS | NS |
| KEGG | MAP00511 | Other glycan degradation | NS | 0.01 | NS | NS | NS | NS | NS | NS | NS |
| SRI | PWY-5686 | uridine-5-phosphate biosynthesis | NS | 0.045 | 0.013 | NS | NS | NS | NS | NS | NS |
| SRI | TRNA-CHARGING-PWY | tRNA charging | NS | NS | NS | NS | 0 | 0 | NS | NS | NS |
| SRI | PWY0-162 | pyrimidine ribonucleotides *de novo* biosynthesis | NS | 0.081 | 0.049 | NS | NS | NS | NS | NS | NS |

**Table S14:** Enriched annotations, *B. bifidum* genes DOWN-regulated relative to LACTOSE

| Type | ANNOT_ID | ANNOT_DESC | HMO-E | HMO-M | HMO-L | MUC | LNT | LNnT | 2’FL | 3FL | 6’SL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GO | GO:0006281 | DNA repair | NS | NS | NS | NS | NS | 0.016 | NS | NS | NS |
| KEGG | MAP03010 | Ribosome | NS | NS | NS | NS | NS | NS | 0.008 | NS | NS |
| KEGG | MAP00190 | Oxidative phosphorylation | NS | NS | NS | 0.012 | NS | NS | NS | NS | NS |
| SRI | TRNA-CHARGING-PWY | tRNA charging | NS | NS | NS | NS | NS | NS | 0 | 0.028 | NS |

**Table S15:** Number of orthologs differentially regulated between *B. bifidum* and *B. infantis* in response to the same substrate

| Substrate | # DE orthologs | # Up in Infantis | # Up in Bifidum |
|---|---|---|---|
| LAC | 1003 | 509 | 494 |
| HMO early | 988 | 504 | 484 |
| HMO mid | 902 | 448 | 454 |
| HMO late | 995 | 491 | 504 |
| LNT | 1113 | 554 | 559 |
| LNnT | 863 | 434 | 429 |
| 2’FL | 1078 | 550 | 528 |
| 3’FL | 1034 | 520 | 514 |
| 6’SL | 772 | 387 | 385 |

* DE: Differentially expressed

**Table 16:** Orthologs highly up-regulated in *B. bifidum* relative to *B. infantis* in response to HMO (early time point)

| Mean Counts B. infantis | MeanCounts B. bifidum | Fold Change | Adjusted p-value | Infantis Ortholog | Bifidum Ortholog | Product Name |
|---|---|---|---|---|---|---|
| 1218.4 | 180768.6 | 148.4 | 0 | Blon_2471 | BBIF_00527 | PTS system, *N*-acetylglucosamine-specific IIBC subunit |
| 214.7 | 29899.2 | 139.3 | 0 | Blon_2470 | BBIF_00528 | PTS system, glucose subfamily, IIA subunit |
| 379.6 | 35529.9 | 93.6 | 0 | Blon_2176 | BBIF_00179 | binding-protein-dependent transport systems inner membrane component |
| 2191.6 | 183539.1 | 83.7 | 0 | Blon_2177 | BBIF_00180 | extracellular solute-binding protein, family 1 |
| 395.4 | 32137.9 | 81.3 | 0 | Blon_2175 | BBIF_00178 | binding-protein-dependent transport systems inner membrane component |
| 747.3 | 44345.8 | 59.3 | 0 | Blon_2174 | BBIF_00177 | hypothetical protein |
| 794.6 | 22423.6 | 28.2 | 5.30E-166 | Blon_1694 | BBIF_01584 | protein of unknown function DUF214 |
| 52.1 | 1227.7 | 23.6 | 1.05E-172 | Blon_1605 | BBIF_01817 | Phosphoglycerate mutase |
| 251.1 | 5653.3 | 22.5 | 0 | Blon_1695 | BBIF_01583 | ABC transporter related |
| 88.8 | 1941.7 | 21.9 | 1.08E-234 | Blon_2046 | BBIF_00354 | hypothetical protein |
| 471.2 | 10236.9 | 21.7 | 0 | Blon_2173 | BBIF_00174 | aminoglycoside phosphotransferase |
| 253.7 | 5132.2 | 20.2 | 2.96E-25 | Blon_0197 | BBIF_00642 | narrowly conserved hypothetical protein |
| 537.1 | 10706.1 | 19.9 | 0 | Blon_2172 | BBIF_00173 | UDP-glucose--hexose-1-phosphate uridylyltransferase |
| 53.9 | 1004.8 | 18.7 | 4.91E-136 | Blon_2042 | BBIF_01215 | ATPase associated with various cellular activities, AAA_3 |
| 179.4 | 3293.2 | 18.4 | 1.45E-142 | Blon_0177 | BBIF_00649 | Phosphotransferase system, |

| Mean Counts B. infantis | MeanCounts B. bifidum | Fold Change | Adjusted p-value | Infantis Ortholog | Bifidum Ortholog | Product Name |
|---|---|---|---|---|---|---|
|  |  |  |  |  |  | phosphocarrier protein HPr |
| 1098.1 | 15143.5 | 13.8 | 0 | Blon_2322 | BBIF_00631 | FAD-dependent pyridine nucleotide-disulphide oxidoreductase |
| 92.5 | 1268.8 | 13.7 | 2.12E-144 | Blon_2044 | BBIF_00352 | transglutaminase domain protein |
| 36.4 | 470.5 | 12.9 | 3.02E-63 | Blon_2043 | BBIF_00351 | protein of unknown function DUF58 |
| 166.8 | 2112.5 | 12.7 | 3.78E-197 | Blon_0509 | BBIF_00169 | integral membrane sensor signal transduction histidine kinase |
| 189.1 | 2310.3 | 12.2 | 1.06E-19 | Blon_2411 | BBIF_01430 | glycoside hydrolase, family 43 |
| 148.9 | 1738.3 | 11.7 | 1.52E-165 | Blon_2047 | BBIF_00355 | FHA domain containing protein |
| 144.3 | 1572.1 | 10.9 | 7.57E-71 | Blon_2003 | BBIF_01861 | Cystathionine gamma-synthase |
| 70.9 | 769.5 | 10.9 | 9.66E-88 | Blon_1376 | BBIF_00234 | VanZ family protein |

**Table 17:** Orthologs highly up-regulated in *B. infantis* relative to *B. bifidum* in response to HMO (early time point)

| Mean Counts Infantis | Mean Counts Bifidum | Fold Change | Adjusted p-value | Infantis Ortholog | Bifidum Ortholog | Product Name |
|---|---|---|---|---|---|---|
| 10420.8 | 79.6 | 130.9 | 0 | Blon_1721 | BBIF_01591 | NLPA lipoprotein |
| 16909.8 | 151.1 | 111.9 | 0 | Blon_2339<br>Blon_2308 | BBIF_01220 | short-chain dehydrogenase/reductase SDR |
| 3830.4 | 76.0 | 50.4 | 0 | Blon_1720 | BBIF_01594 | ABC transporter related |
| 8573.4 | 178.5 | 48 | 0 | Blon_1905 | BBIF_01559 | Beta-glucosidase |
| 1924.1 | 46.2 | 41.6 | 4.43E-264 | Blon_0342<br>Blon_2203 | BBIF_00011 | binding-protein-dependent transport systems inner membrane component |
| 1920.5 | 62.9 | 30.5 | 2.39E-246 | Blon_1719 | BBIF_01595 | binding-protein-dependent transport systems inner membrane component |
| 529.2 | 32.0 | 16.5 | 1.81E-72 | Blon_1053 | BBIF_00193 | hypothetical protein |
| 1194.0 | 90.5 | 13.2 | 1.77E-130 | Blon_0449<br>Blon_1356<br>Blon_2279 | BBIF_01373 | Integrase, catalytic region |
| 6951.8 | 543.5 | 12.8 | 0 | Blon_1232<br>Blon_1233 | BBIF_01490<br>BBIF_00727 | hypothetical protein |
| 1011.5 | 93.1 | 10.9 | 1.77E-103 | Blon_0821 | BBIF_00404 | carbohydrate kinase, FGGY |
| 246.8 | 24.0 | 10.3 | 1.27E-31 | Blon_0300 | BBIF_01716 | hypothetical protein |
| 7981.0 | 785.2 | 10.2 | 1.66E-315 | Blon_1454 | BBIF_00063 | 5,10-methylenetetrahydrofolate reductase |
| 208.5 | 20.7 | 10.1 | 1.77E-25 | Blon_1423 | BBIF_00221 | amidohydrolase-like protein |

**Table S18:** Number of differentially regulated genes in *B. bifidum* relative to lactose

| **Treatment** | **Number DE genes** |
|---|---:|
| 2’FL | 230 |
| 3’FL | 138 |
| 6’FL | 826 |
| MUC | 1129 |
| LNT | 1273 |
| LNnT | 1129 |
| HMO Early | 1170 |
| HMO Mid | 1280 |
| HMO Late | 1271 |

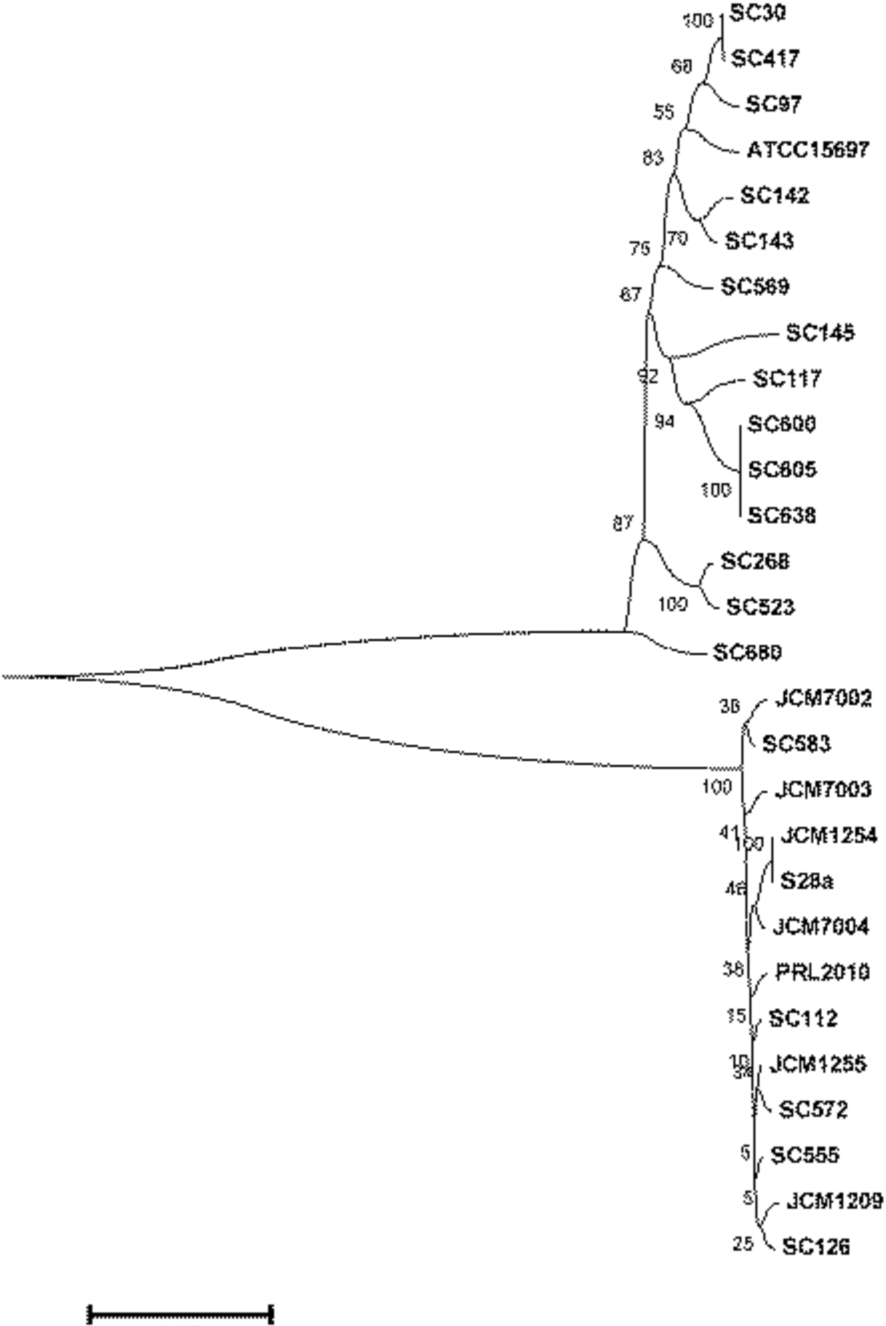
**Table S19:** Number of differentially regulated genes in *B. infantis* relative to lactose

| **Treatment** | **Number DE genes** |
|---|---:|
| 2’FL | 1148 |
| 3’FL | 1277 |
| 6’FL | 1385 |
| LNT | 992 |
| LNnT | 963 |
| HMO Early | 815 |
| HMO Mid | 605 |
| HMO Late | 1014 |

## LEGEND TO SUPPLEMENTARY FIGURES

**FIGURE S1**: Evolutionary relationship of *B. infantis* (upper branch) and *B. bifidum* (lower branch) strains used in the study. The tree is drawn to scale, with branch lengths in the same units (number of base substitutions per site) as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary history was inferred using the Minimum Evolution method, followed by 1000 bootstrap replicates.

**FIGURE S2:** Venn diagram indicating the number of ortholog and unique genes in B. bifidum strains SC555 and PRL2010.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SC600
SC605
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SC638
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SC268
100
SC523
SC680
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SC583
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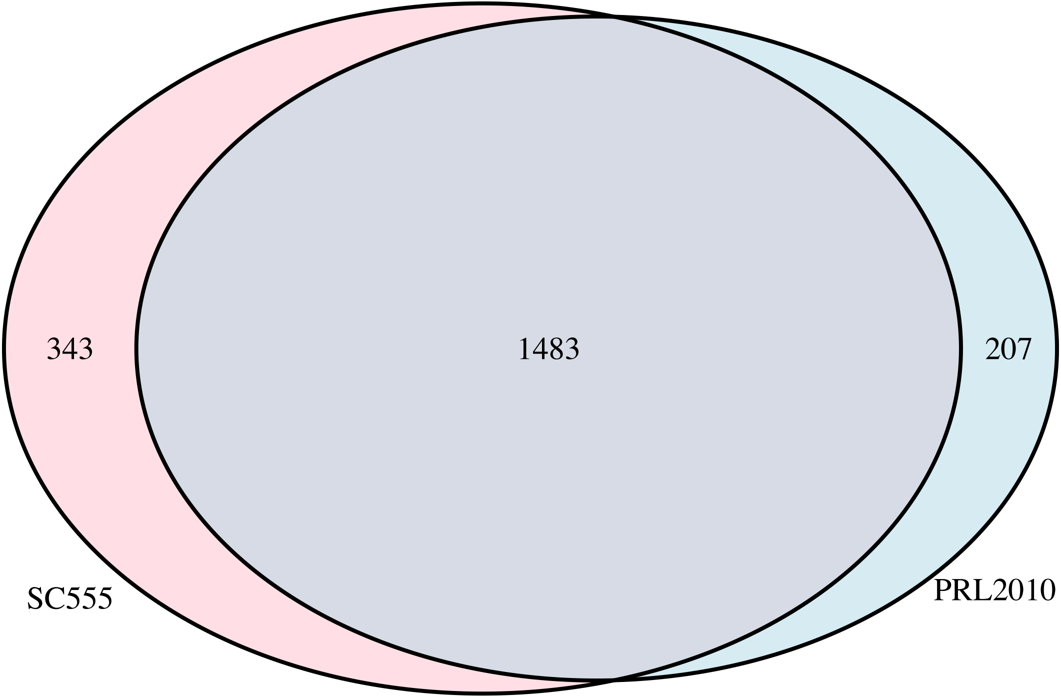
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