

Figure S1. Number of differentially transcribed (DT) genes grouped according to Cluster of Orthologous Genes (COG) categories. **A.** *B. ovatus* ATCC 8483^T DT genes uniquely expressed in co-cultures. **B.** *S. variabile* DSM 15176^T and **C.** *H. hathewayi* DSM 13479^T DT genes uniquely expressed in co-cultures. Genes that

had increased transcription in co-culture compared to pure culture conditions are indicated in red bars, genes with decreased transcription in light blue bars. COG categories: [A], RNA processing and modification; [B], Chromatin structure and dynamics; [C], Energy production and conversion; [D], Cell cycle control cell division chromosome partitioning; [E], Amino acid transport and metabolism; [F], Nucleotide transport and metabolism; [G], Carbohydrate transport and metabolism; [H], Coenzyme transport and metabolism; [I], Lipid transport and metabolism; [J], Translation ribosomal structure and biogenesis; [K], Transcription; [L], Replication recombination and repair; [M], Cell wall/membrane/envelope biogenesis; [N], Cell motility; [O], Post-translational modification protein turnover and chaperones; [P], Inorganic ion transport and metabolism; [Q], Secondary metabolites biosynthesis transport and catabolism; [R], General function prediction only; [S], Function unknown; [T], Signal transduction mechanisms; [U], Intracellular trafficking secretion and vesicular transport; [V], Defense mechanisms; [W], Extracellular structures; [Y], Nuclear structure; [Z], Cytoskeleton.

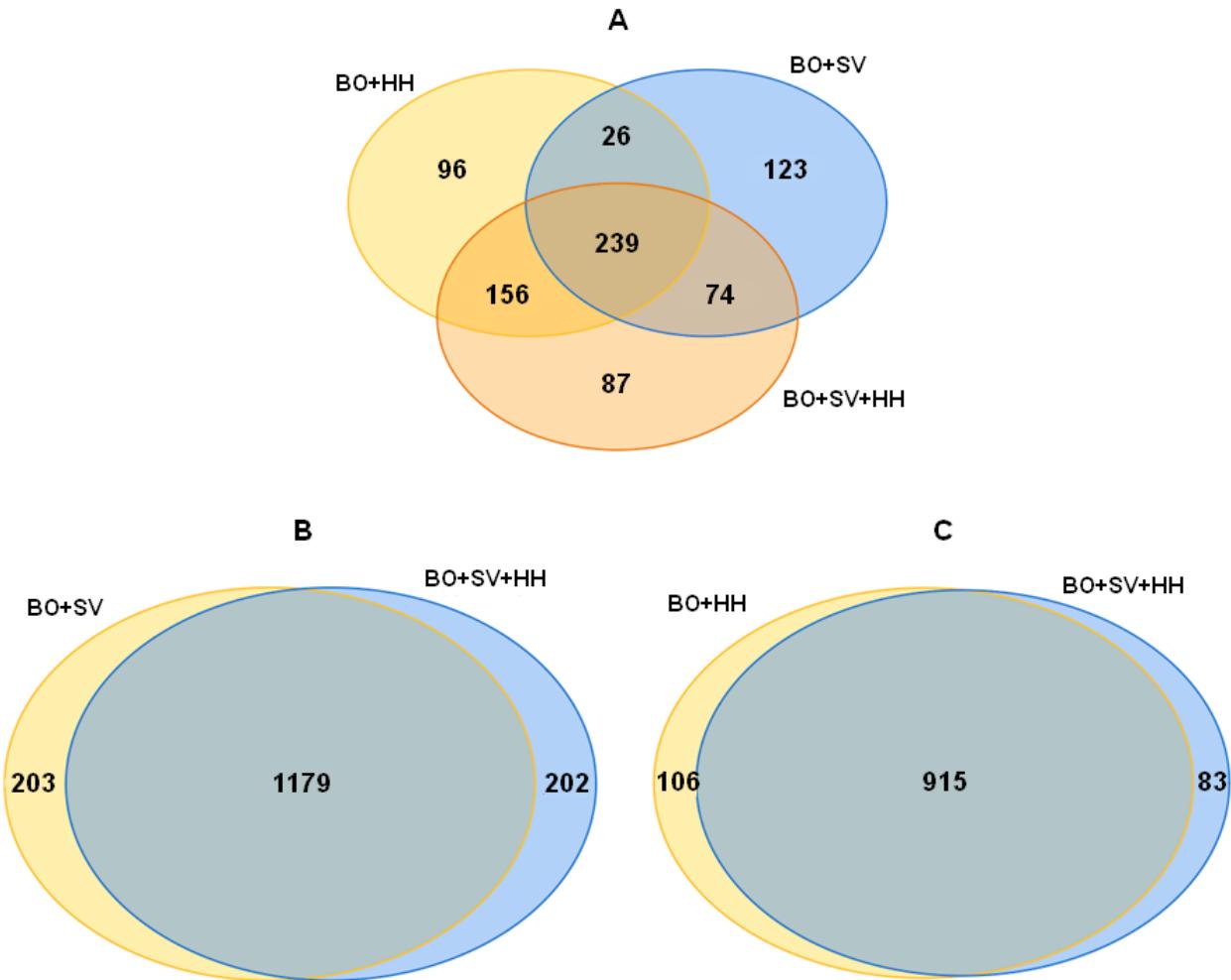


FIG S2. Venn diagrams of the number of common and unique differentially transcribed genes of *B. ovatus* ATCC 8483^T (BO) (**A**), *S. variabile* DSM 15176^T (SV) (**B**) and *H. hathewayi* DSM 13479^T (HH) (**C**) in co-cultures.

Table S1. Summary of RNA-seq data obtained from all pure and co-culture samples.

BO16, BO20, BO24 and BO26 indicate *B. ovatus* ATCC 8483^T pure culture samples at 16, 20, 24 and 26 h, respectively. SV12, SV14, SV16 and SV24 indicate *S. variabile* DSM 15176^T pure culture samples at 12, 14, 16 and 24 h, respectively. HH06, HH16, HH18, HH20 and HH24 indicate *H. hathewayi* DSM 13479^T pure culture samples at 6, 16, 18, 20 and 24 h, respectively. BO+SV, BO+HH and BO+SV+HH indicate co-culture samples of *B. ovatus* and *S. variabile*, *B. ovatus* and *H. hathewayi*, and *B. ovatus*, *S. variabile* and *H. hathewayi*, respectively.

Pure culture

| <i>B. ovatus</i> ATCC 8483 ^T | | | | | | | | | |
|--------------------------------------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|------------------|------------------|-------------------|
| Sample | BO16 | | BO20 | | BO24 | | BO26 | | |
| Biological replicate | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | |
| Adapter- and quality-trimmed reads (pair) | 6275610 | 6171428 | 5672569 | 5780334 | 18119414 | 19225175 | 6134182 | 6146063 | |
| Reads mapped to genome (pair) | 5834141 (93%) | 5640524 (91%) | 5275180 (93%) | 5239690 (91%) | 15315133 (85%) | 16358203 (85%) | 5607694 (91%) | 5738493 (93%) | |
| Reads aligned (sense) to protein-coding genes | 94% | 94% | 94% | 94% | 94% | 94% | 93% | 93% | |
| Reads aligned (antisense) to protein-coding gene | 1% | 1% | 1% | 1% | 1% | 1% | 1% | 1% | |
| Reads aligned (sense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | |
| Reads aligned (antisense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | |
| Reads aligned (sense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | |
| Reads aligned (antisense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | |
| Reads aligned (sense) to miscellaneous RNAs | 1% | 0% | 1% | 1% | 1% | 1% | 1% | 1% | |
| Reads aligned (antisense) to miscellaneous RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | |
| Reads aligned to unannotated regions | 4% | 5% | 5% | 5% | 4% | 5% | 5% | 5% | |
| <i>S. variabile</i> DSM 15176 ^T | | | | | | | | | |
| Sample | SV12 | | SV14 | | SV16 | | SV24 | | |
| Biological replicate | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 |
| Adapter- and quality-trimmed reads (pair) | 5296033 | 4899087 | 5395150 | 5872683 | 4050882 | 5027329 | 5260404 | 4947739 | 16503690 |
| Reads mapped to genome (pair) | 4803047 (91%) | 4740926 (97%) | 4963869 (92%) | 5326185 (91%) | 3412214 (84%) | 4651029 (93%) | 4798418 (91%) | 4750547 (96%) | 13545113 (82%) |
| Reads aligned (sense) to protein-coding genes | 87% | 81% | 90% | 89% | 86% | 88% | 89% | 90% | 64% |
| Reads aligned (antisense) to protein-coding gene | 1% | 2% | 1% | 1% | 1% | 1% | 1% | 1% | 4% |
| Reads aligned (sense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (antisense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (sense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (antisense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (sense) to miscellaneous RNAs | 1% | 2% | 1% | 2% | 2% | 2% | 2% | 1% | 3% |
| Reads aligned (antisense) to miscellaneous RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned to unannotated regions | 10% | 16% | 7% | 8% | 11% | 9% | 8% | 8% | 29% |

| Reference genome | <i>H. hathewayi</i> DSM 13479 ^T | | | | | | | | | | | |
|--------------------------------------------------|--------------------------------------------|------------------|--------------------------------------------|------------------|-----------------------------------------|------------------|--------------------------------------------|------------------|-----------------------------------------|--------------------------------------------|--------------------------------------------|---------------|
| Sample | HH06 | | HH16 | | HH18 | | HH20 | | HH24 | | | |
| Biological replicate | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | | |
| Adapter- and quality-trimmed reads (pair) | 5673589 | 5437765 | 5659507 | 5376647 | 5203979 | 5455634 | 5742994 | 5545527 | 16039801 | 18032570 | | |
| Reads mapped to genome (pair) | 4533453 (80%) | 4371695 (80%) | 4580845 (81%) | 4422405 (82%) | 4407666 (85%) | 4613236 (85%) | 4888885 (85%) | 2748512 (50%) | 11533717 (72%) | 13561227 (75%) | | |
| Reads aligned (sense) to protein-coding genes | 77% | 77% | 76% | 84% | 84% | 83% | 84% | 82% | 48% | 67% | | |
| Reads aligned (antisense) to protein-coding gene | 2% | 3% | 3% | 3% | 3% | 3% | 3% | 3% | 2% | 3% | | |
| Reads aligned (sense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| Reads aligned (antisense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| Reads aligned (sense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| Reads aligned (antisense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| Reads aligned (sense) to miscellaneous RNAs | 1% | 1% | 1% | 1% | 0% | 0% | 0% | 1% | 2% | 1% | | |
| Reads aligned (antisense) to miscellaneous RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| Reads aligned to unannotated regions | 20% | 20% | 20% | 13% | 12% | 13% | 12% | 14% | 48% | 40% | | |
| Co-culture | | | | | | | | | | | | |
| Sample | BO+SV | | | | BO+HH | | | | BO+SV+HH | | | |
| Reference genome | <i>B. ovatus</i> ATCC 8483 ^T | | <i>S. variabile</i> DSM 15176 ^T | | <i>B. ovatus</i> ATCC 8483 ^T | | <i>H. hathewayi</i> DSM 13479 ^T | | <i>B. ovatus</i> ATCC 8483 ^T | <i>S. variabile</i> DSM 15176 ^T | <i>H. hathewayi</i> DSM 13479 ^T | |
| Biological replicate | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Adapter- and quality-trimmed reads (pair) | 5563190 | 7185843 | 5563190 | 7185843 | 5310034 | 5463546 | 5310034 | 5463546 | 5664614 | 5625551 | 5664614 | 5625551 |
| Reads mapped to genome (pair) | 2541504 (46%) | 3477039 (48%) | 959115 (17%) | 1067613 (15%) | 2879519 (54%) | 2988344 (55%) | 541846 (10%) | 507501 (9%) | 2873130 (51%) | 2814112 (50%) | 50819 (1%) | 28326 (1%) |
| Reads aligned (sense) to protein-coding genes | 94% | 94% | 89% | 89% | 94% | 94% | 71% | 67% | 94% | 94% | 84% | 78% |
| Reads aligned (antisense) to protein-coding gene | 1% | 1% | 1% | 1% | 1% | 1% | 3% | 3% | 1% | 1% | 1% | 2% |
| Reads aligned (sense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (antisense) to ribosomal RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (sense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (antisense) to transfer RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned (sense) to miscellaneous RNAs | 1% | 1% | 2% | 2% | 0% | 1% | 1% | 1% | 0% | 0% | 2% | 3% |
| Reads aligned (antisense) to miscellaneous RNAs | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reads aligned to unannotated regions | 5% | 5% | 8% | 8% | 5% | 5% | 25% | 29% | 5% | 5% | 12% | 17% |

Table S2. List of enriched and depleted GO terms and their category number for each individual species in each co-culture condition.

| <i>B. ovatus</i> ATCC 8483 ^T perspective | | | | |
|--------------------------------------------------------|-------------------|-----------------------------------------------------------------------------|-------------------|------------------------------------------------------------------------------------|
| | Enriched GO terms | | Depleted GO terms | |
| Common between BO+SV+HH, BO+SV and BO+HH | - | | GO:0046872 | metal ion binding |
| Common between BO+SV+HH and BO+HH | GO:0020037 | heme binding | GO:0030001 | metal ion transport |
| | GO:0017004 | cytochrome complex assembly | GO:0006568 | tryptophan metabolic process |
| | GO:0006814 | sodium ion transport | GO:0046373 | L-arabinose metabolic process |
| | GO:0008565 | protein transporter activity | GO:0046556 | alpha-L-arabinofuranosidase activity |
| Unique to BO+HH | GO:0016887 | ATPase activity | GO:0005215 | transporter activity |
| | GO:0005622 | intracellular | GO:0016491 | oxidoreductase activity |
| Common between BO+SV+HH and BO+SV | - | - | GO:0015655 | alanine:sodium symporter activity |
| Unique to BO+SV | GO:0016861 | intramolecular oxidoreductase activity, interconverting aldoses and ketoses | GO:0007165 | signal transduction |
| | GO:0016310 | phosphorylation | GO:0006310 | DNA recombination |
| | GO:0030599 | pectinesterase activity | GO:0005515 | protein binding |
| | GO:0042545 | cell wall modification | GO:0004565 | beta-galactosidase activity |
| | GO:0006457 | protein folding | GO:0009341 | beta-galactosidase complex |
| | GO:0004872 | receptor activity | GO:0052855 | ADP-dependent NAD(P)H-hydrate dehydratase activity |
| | GO:0005618 | cell wall | GO:0042279 | nitrite reductase (cytochrome, ammonia-forming) activity |
| | | | GO:0004673 | protein histidine kinase activity |
| | | | GO:0051536 | iron-sulfur cluster binding |
| | | | GO:0006270 | DNA replication initiation |
| | | | GO:0019288 | isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway |
| | | | GO:0050992 | dimethylallyl diphosphate biosynthetic process |
| | | | GO:0051745 | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase activity |
| Unique to BO+SV+HH | GO:0008948 | oxaloacetate decarboxylase activity | GO:0004594 | pantothenate kinase activity |
| | GO:0015081 | sodium ion transmembrane transporter activity | GO:0015074 | DNA integration |
| | GO:0071436 | sodium ion export | GO:0004784 | superoxide dismutase activity |
| | GO:0016829 | lyase activity | GO:0006801 | superoxide metabolic process |
| | GO:0008654 | phospholipid biosynthetic process | | |
| <i>S. variabile</i> DSM 15176 ^T perspective | | | | |
| | Enriched GO terms | | Depleted GO terms | |
| Common between BO+SV+HH and BO+SV | GO:0003735 | structural constituent of ribosome | GO:0043565 | sequence-specific DNA binding |
| | GO:0006412 | translation | GO:0006355 | regulation of transcription, DNA-templated |
| | GO:0005840 | ribosome | GO:0003700 | transcription factor activity, sequence-specific DNA binding |
| | GO:0005622 | intracellular | GO:0005975 | carbohydrate metabolic process |
| | GO:0004812 | aminoacyl-tRNA ligase activity | GO:0055114 | oxidation-reduction process |
| | GO:0042626 | ATPase activity, coupled to transmembrane movement of substances | GO:0016491 | oxidoreductase activity |
| | GO:0016021 | integral component of membrane | GO:0004553 | hydrolase activity, hydrolyzing O-glycosyl compounds |
| | GO:0055085 | transmembrane transport | GO:0006064 | glucuronate catabolic process |
| | GO:0000166 | nucleotide binding | GO:0000160 | phosphorelay signal transduction system |
| | GO:0006418 | tRNA aminoacylation for protein translation | GO:0045454 | cell redox homeostasis |
| | GO:0030246 | carbohydrate binding | GO:0008927 | mannose dehydratase activity |
| Unique to BO+SV+HH | GO:0042173 | regulation of sporulation resulting in formation of a cellular spore | | |
| | GO:0006614 | SRP-dependent cotranslational protein targeting to membrane | GO:0006281 | DNA repair |
| | GO:0009116 | nucleoside metabolic process | | |

| | | | | |
|------------------------|------------|-------------------------------------------------|------------|------------------------------------------|
| | GO:0015986 | ATP synthesis coupled proton transport | | |
| | GO:0015078 | hydrogen ion transmembrane transporter activity | | |
| | GO:0008184 | glycogen phosphorylase activity | | |
| | GO:0006396 | RNA processing | | |
| | GO:0006810 | transport | | |
| | GO:0006520 | cellular amino acid metabolic process | | |
| Unique to BO+SV | GO:0004356 | glutamate-ammonia ligase activity | GO:0050662 | coenzyme binding |
| | GO:0003924 | GTPase activity | GO:0030288 | outer membrane-bounded periplasmic space |
| | GO:0000049 | tRNA binding | | |
| | GO:0009086 | methionine biosynthetic process | | |
| | GO:0019888 | protein phosphatase regulator activity | | |
| | GO:0030289 | protein phosphatase 4 complex | | |
| | GO:0015031 | protein transport | | |

***H. hathewayi* DSM 13479^T perspective**

| | Enriched GO terms | | Depleted GO terms | |
|------------------------------------------|-------------------|-----------------------------------------------------------------|-------------------|--------------------------------------------------------------------|
| Common between BO+SV+HH and BO+HH | GO:0003735 | structural constituent of ribosome | GO:0006810 | transport |
| | GO:0005840 | ribosome | GO:0055114 | oxidation-reduction process |
| | GO:0006412 | translation | GO:0006777 | Mo-molybdopterin cofactor biosynthetic process |
| | GO:0005622 | intracellular | GO:0004134 | 4-alpha-glucanotransferase activity |
| | GO:0005525 | GTP binding | GO:0016491 | oxidoreductase activity |
| | GO:0004812 | aminoacyl-tRNA ligase activity | GO:0045454 | cell redox homeostasis |
| | GO:0015986 | ATP synthesis coupled proton transport | GO:0005506 | iron ion binding |
| | GO:0006418 | tRNA aminoacylation for protein translation | GO:0008452 | RNA ligase activity |
| | GO:0015078 | hydrogen ion transmembrane transporter activity | GO:0009116 | nucleoside metabolic process |
| | GO:0000166 | nucleotide binding | | |
| | GO:0045263 | proton-transporting ATP synthase complex, coupling factor F(o) | | |
| | GO:0004594 | pantothenate kinase activity | | |
| | GO:0016876 | ligase activity, forming aminoacyl-tRNA and related compounds | | |
| | GO:0043039 | tRNA aminoacylation | | |
| | GO:0016021 | integral component of membrane | | |
| | GO:0003924 | GTPase activity | | |
| | GO:0006633 | fatty acid biosynthetic process | | |
| | GO:0009007 | site-specific DNA-methyltransferase (adenine-specific) activity | | |
| | GO:0032775 | DNA methylation on adenine | | |
| | GO:0008033 | tRNA processing | | |
| Unique to BO+SV+HH | GO:0006400 | tRNA modification | GO:0005975 | carbohydrate metabolic process |
| | GO:0015991 | ATP hydrolysis coupled proton transport | | |
| | GO:0003899 | DNA-directed RNA polymerase activity | | |
| | GO:0006351 | transcription, DNA-templated | | |
| | GO:0005328 | neurotransmitter:sodium symporter activity | | |
| | GO:0006836 | neurotransmitter transport | | |
| | GO:0006855 | drug transmembrane transport | | |
| | GO:0015238 | drug transmembrane transporter activity | | |
| | GO:0015297 | antiporter activity | | |
| | GO:0046933 | proton-transporting ATP synthase activity, rotational mechanism | | |
| Unique to BO+HH | GO:0009002 | serine-type D-Ala-D-Ala carboxypeptidase activity | GO:0015558 | p-aminobenzoyl-glutamate uptake transmembrane transporter activity |
| | GO:0006164 | purine nucleotide biosynthetic process | GO:1902604 | p-aminobenzoyl-glutamate transmembrane transport |
| | GO:0015031 | protein transport | | |

Table S3. List of primer sets used for RT-qPCR. All primer sets were designed in this study.

| Name | Sequence | Target ^a | |
|--------------------------------------------------|-----------------------|------------------------------|--------------------------------------------------------|
| | | Gene name | Product |
| <i>B. ovatus</i> ATCC 8483^T | | | |
| Bovatus_RS01400_F | CGTTTCTATCTGCCGTGG | Bovatus_RS01400 | MFS transporter |
| Bovatus_RS01400_R | TGAGGGCGAACAGGAAGAAT | | |
| Bovatus_RS15290_F | AGACTAACGGCGGACTATGG | Bovatus_RS15290 | Glycoside hydrolase family 16 protein |
| Bovatus_RS15290_R | TCCGCTCTGTTCTCCATT | | |
| Bovatus_RS15310_F | CTTGTGGCTTCAGTACCCAT | Bovatus_RS15310 | Beta-glucosidase |
| Bovatus_RS15310_R | CCCCTGTTCCAGTACCCAT | | |
| Bovatus_RS21310_F | GTCTTGGAGCGGCAGTAATG | Bovatus_RS21310 | PTS sugar transporter subunit IIC |
| Bovatus_RS21310_R | GAGCAATGCCGTCACTACTG | | |
| Bovatus_RS06075_F | GGGAGCTAACAAAGGCGG | Bovatus_RS06075 | ABC transporter ATP binding protein |
| Bovatus_RS06075_R | GGGCAATCGTTTTCCCT | | |
| Bovatus_RS09210_F | AGAGTCTTATCCTCCCTGC | Bovatus_RS09210 | Molecular chaperone GroES |
| Bovatus_RS09210_R | CCGTGACCTATTGCCACAAC | | |
| Bovatus_RS03700_F | CGAGGGGACAAAGTTGGTG | Bovatus_RS03700 ^b | DNA polymerase III subunit delta |
| Bovatus_RS03700_R | TCTGTTCAGGAGTTACGCGT | | |
| <i>S. variabile</i> DSM 15176^T | | | |
| SUBVAR_RS12285_F | CAGCAGAACATCATCACCGG | SUBVAR_RS12285 | Carbohydrate ABC transporter permease |
| SUBVAR_RS12285_R | CCCGGAATGACGTATAACCA | | |
| SUBVAR_RS12505_F | CTCCGGCTCCATCTATGTGT | SUBVAR_RS12505 | PTS beta-glucoside transporter subunit IIBCA |
| SUBVAR_RS12505_R | GAGGTTGGGGTGGATCATCA | | |
| SUBVAR_RS14140_F | CAACGACAATGTGCTACCCC | SUBVAR_RS14140 | Beta-glucosidase |
| SUBVAR_RS14140_R | CAATCTCGCTGGTCAGGTG | | |
| SUBVAR_RS14155_F | CTCCCGCATTATCAGGACT | SUBVAR_RS14155 | Carbohydrate ABC transporter substrate binding protein |
| SUBVAR_RS14155_R | CCCTGGAAGTAGTCGCTGAA | | |
| SUBVAR_RS14170_F | TTTGAGACCAGCCGCATCTA | SUBVAR_RS14170 | Cellobiose phosphorylase |
| SUBVAR_RS14170_R | TTCATCAAATGTTGGCGG | | |
| SUBVAR_RS02860_F | TTCAATTGCCCCGATCGTG | SUBVAR_RS02860 | CidA/LrgA family protein |
| SUBVAR_RS02860_R | ACCTGTTCCAGTCTGAGCAA | | |
| SUBVAR_RS02935_F | ACCATCCCCACCAAGAAGAG | SUBVAR_RS02935 | Molecular chaperone DnaK |
| SUBVAR_RS02935_R | TTGCTCATGTTGGTGGAGC | | |
| SUBVAR_RS15530_F | CCTACTTGCAGGATCTGGC | SUBVAR_RS15530 ^b | Class D sortase |
| SUBVAR_RS15530_R | CTCCCCAGCGAACCTTATCA | | |
| <i>H. hathewayi</i> DSM 13479^T | | | |
| CLOSTHATH_RS01805_F | CCAAGTTTGCCTACCCCC | CLOSTHATH_RS01805 | Glucan binding protein |
| CLOSTHATH_RS01805_R | CAGCCGGTAACCAAATAGCC | | |
| CLOSTHATH_RS19270_F | CGACGACACAGATTTCAGCAG | CLOSTHATH_RS19270 | Carbohydrate ABC transporter permease |
| CLOSTHATH_RS19270_R | GGCCGTCATTGTGGAAAAGT | | |
| CLOSTHATH_RS02765_F | GGCGGAAATAAGGAAGCAGG | CLOSTHATH_RS02765 | Sugar ABC transporter substrate binding protein |
| CLOSTHATH_RS02765_R | GTCTCCTCGTAAATCGGC | | |
| CLOSTHATH_RS07435_F | CCGCTGGTATTCTGTACGC | CLOSTHATH_RS07435 | Iron ABC transporter permease |
| CLOSTHATH_RS07435_R | CCTTCGCCGATTAACATGGG | | |
| CLOSTHATH_RS09305_F | CTCTGGCGTGGATCATGTG | CLOSTHATH_RS09305 | Stage III sporulation protein J |

| | | | |
|---------------------|----------------------|--------------------------------|---------------------------------------------|
| CLOSTHATH_RS09305_R | TTCCACGATCGTCCCTGAA | | |
| CLOSTHATH_RS16585_F | ATGCAGTCCATCAGCTCAGT | CLOSTHATH_RS16585 | MATE family efflux transporter |
| CLOSTHATH_RS16585_R | GATACAAAAGCTGCTCCGGG | | |
| CLOSTHATH_RS09845_F | GGCGATGCTGGTGAAAATCT | CLOSTHATH_RS09845 ^b | Sigma 70 family RNA polymerase sigma factor |
| CLOSTHATH_RS09845_R | CGGAATGGTTCTGCTCGA | | |

^a *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T and *H. hathewayi* DSM 13479^T reference genome locus tags, respectively.

^b Reference genes used for gene expression level normalization.

Table S4. Transcriptional level of selected gene targets for *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T and *H. hathewayi* DSM 13479^T, measured by RT-qPCR and RNA-seq. Expression levels of DT genes are indicated as log₂ fold change (Log2FC) for comparison between condition 1 and 2, as indicated in the Table. BO16, BO20, BO24 and BO26 indicate *B. ovatus* pure culture samples at 16, 20, 24 and 26 h, respectively. SV12, SV14, SV16 and SV24 indicate *S. variabile* pure culture samples at 12, 14, 16 and 24 h, respectively. HH06, HH16, HH18, HH20 and HH24 indicate *H. hathewayi* pure culture samples at 6, 16, 18, 20 and 24 h, respectively. BO+SV, BO+HH and BO+SV+HH indicate co-culture samples of *B. ovatus* and *S. variabile*, *B. ovatus* and *H. hathewayi*, and *B. ovatus*, *S. variabile* and *H. hathewayi*, respectively. RT-qPCR values are means of all the biological replicates, and for each biological replicate three technical replicates were measured, ± SEM (standard error of the mean).

| <i>Bacteroides ovatus</i> ATCC 8483 ^T | | Pure culture | | | | | | | | Co-culture | | | | | | | | | | | |
|--------------------------------------------------|-----------------|---------------|-------|-----------------|--------|---------------|--------|-----------------|-------|----------------|-------|-----------------|-------|----------------|-------|-----------------|-------|-------------------|-------|-----------------|--|
| | | BO16 vs. BO20 | | | | BO16 vs. BO26 | | | | BO+HH vs. BO24 | | | | BO+SV vs. BO24 | | | | BO+SV+HH vs. BO24 | | | |
| | | RT-qPCR | | RNA-seq results | | RT-qPCR | | RNA-seq results | | RT-qPCR | | RNA-seq results | | RT-qPCR | | RNA-seq results | | RT-qPCR | | RNA-seq results | |
| | | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | Mean Log2FC | SEM | | |
| MFS transporter | Bovatus_RS01400 | -1.065 | 0.093 | -1.668 | -1.373 | 0.081 | -1.659 | | | 0.998 | 0.241 | 1.049 | | | | | | | | | |
| Glycoside hydrolase family 16 protein | Bovatus_RS15290 | 4.080 | 0.327 | 3.786 | 7.016 | 0.600 | 7.074 | 7.562 | 0.807 | 6.695 | | | | | | | 6.363 | 0.221 | 5.395 | | |
| Beta glucosidase | Bovatus_RS15310 | 2.911 | 0.382 | 2.542 | 7.491 | 0.841 | 6.996 | 7.742 | 0.677 | 6.862 | 1.995 | 1.203 | 1.345 | 7.025 | 0.606 | 6.159 | | | | | |
| PTS sugar transporter subunit IIC | Bovatus_RS21310 | 1.424 | 0.145 | 1.203 | | | | 1.099 | 0.318 | 1.069 | 1.937 | 0.302 | 2.006 | 1.541 | 0.071 | 1.886 | | | | | |
| ABC transporter ATP binding protein | Bovatus_RS06075 | | | | | | | 1.688 | 0.050 | 4.486 | | | | | | 2.020 | 0.139 | 4.892 | | | |
| Molecular chaperone GroES | Bovatus_RS09210 | | | | | | | | | 1.749 | 0.512 | 1.919 | 1.061 | 0.423 | 1.389 | | | | | | |

| | | | | | | | | | | | | | |
|--------------------------------------------------------|----------------|-------|-------|-------|-------|-------|-------|--------|-------|--------|--------|-------|--------|
| Carbohydrate ABC transporter permease | SUBVAR_RS12285 | 1.728 | 0.184 | 1.487 | 1.561 | 0.076 | 1.109 | -2.169 | 1.116 | -2.595 | -1.853 | 0.384 | -2.442 |
| PTS beta glucosidase transporter subunit IIBCA | SUBVAR_RS12505 | 2.215 | 0.138 | 2.126 | 2.659 | 0.020 | 2.497 | -6.686 | 1.059 | -7.116 | -6.166 | 0.031 | -5.950 |
| Beta glucosidase | SUBVAR_RS14140 | | | | | | | 2.158 | 0.954 | 1.712 | 2.079 | 0.295 | 1.804 |
| Carbohydrate ABC transporter substrate binding protein | SUBVAR_RS14155 | | | | | | | 2.283 | 0.321 | 1.549 | 2.771 | 0.268 | 1.911 |
| Cellobiose phosphorylase | SUBVAR_RS14170 | | | | | | | 3.220 | 0.135 | 2.020 | 3.590 | 0.147 | 2.785 |
| CidA/LrgA family protein | SUBVAR_RS02860 | | | | | | | 4.123 | 0.002 | 4.617 | 4.260 | 0.174 | 3.864 |
| Molecular chaperone DnaK | SUBVAR_RS02935 | | | | | | | -3.367 | 0.216 | -3.404 | -2.655 | 0.109 | -3.737 |

| <i>Hungatella hathewayi</i> DSM 13479 ^T | Pure culture | | | | | | | | | | | | Co-culture | | | | | | | | |
|----------------------------------------------------|-------------------|--------|--------|-----------------|--------|--------|---------------|--------|--------|-----------------|--------|--------|---------------|--------|--------|-----------------|-----|--------|----------------|-----|--------|
| | HH06 vs. HH16 | | | | | | HH06 vs. HH18 | | | | | | HH06 vs. HH20 | | | | | | BO+HH vs. HH24 | | |
| | RT-qPCR | | | RNA-seq results | | | RT-qPCR | | | RNA-seq results | | | RT-qPCR | | | RNA-seq results | | | RT-qPCR | | |
| | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC | Mean Log2FC | SEM | Log2FC |
| Glucan binding protein | CLOSTHATH_RS01805 | 1.647 | 0.243 | 1.643 | 2.141 | 0.261 | 1.805 | 2.557 | 0.235 | 2.099 | | | | | | | | | | | |
| Carbohydrate ABC transporter permease | CLOSTHATH_RS19270 | -5.370 | 0.406 | -7.013 | -5.611 | 0.006 | -7.289 | -4.785 | 0.121 | -6.755 | | | | | | | | | | | |
| Sugar ABC transporter substrate binding protein | CLOSTHATH_RS02765 | 4.447 | 0.503 | 3.820 | 5.502 | 0.271 | 4.728 | 5.807 | 0.550 | 4.754 | 3.832 | 0.876 | 3.566 | 3.686 | 1.340 | 3.317 | | | | | |
| Iron ABC transporter permease | CLOSTHATH_RS07435 | 4.729 | 0.123 | 4.872 | 6.537 | 0.214 | 7.646 | 5.683 | 0.705 | 6.126 | -2.157 | 0.156 | -4.349 | -1.319 | 0.039 | -1.642 | | | | | |
| Stage III sporulation protein J | CLOSTHATH_RS09305 | -1.497 | 0.029 | -1.472 | -1.534 | 0.325 | -1.687 | -1.227 | 0.152 | -1.583 | 2.431 | 0.346 | 2.339 | 2.725 | 0.207 | 2.469 | | | | | |
| MATE family efflux transporter | CLOSTHATH_RS16585 | -1.051 | 0.040 | -1.405 | -1.098 | 0.123 | -1.911 | -0.879 | 0.087 | -1.868 | 1.534 | 0.987 | 2.291 | 1.276 | 0.698 | 2.084 | | | | | |