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Sharing a β-Glucan Meal: Transcriptomic Eavesdropping on a *Bacteroides ovatus-Subdoligranulum variabile-Hungatella hathewayi* Consortium

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ABSTRACT Whole-transcriptome analysis was used to investigate the molecular interplay between three bacterial species that are members of the human gut microbiota. *Bacteroides ovatus*, *Subdoligranulum variabile*, and *Hungatella hathewayi* formed associations in cocultures fed barley β-glucan, a constituent of dietary fiber. *B. ovatus* depolymerized β-glucan and released, but did not utilize, 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4). These oligosaccharides provided growth substrates for *S. variabile* and *H. hathewayi* with a preference for DP4 in the case of the latter species. There was increased transcription of a *B. ovatus* mixed-linkage-β-glucan utilization locus, as well as carbohydrate transporters in *S. variabile* and *H. hathewayi* when in batch coculture. Increased transcription of the β-glucan utilization locus did not occur in continuous culture. Evidence for interactions relating to provision of cobalamin, alterations to signaling, and modulation of the "stringent response" (an adaptation to nutrient deprivation) were detected. Overall, we established a bacterial consortium based on barley β-glucan *in vitro*, which can be used to investigate aspects of the functional blueprint of the human gut microbiota.

IMPORTANCE The microbial community, mostly composed of bacterial species, residing in the human gut degrades and ferments polysaccharides derived from plants (dietary fiber) that would not otherwise be digested. In this way, the collective metabolic actions of community members extract additional energy from the human diet. While the variety of bacteria present in the microbial community is well known, the formation of bacterial consortia, and the consequent interactions that result in the digestion of dietary polysaccharides, has not been studied extensively. The importance of our work was the establishment, under laboratory conditions, of a consortium of gut bacteria that formed around a dietary constituent commonly present in cereals. This enabled the metabolic interplay between the bacterial species to be studied. This kind of knowledge is required to construct an interactive, metabolic blueprint of the microbial community that inhabits the human gut.

KEYWORDS RNAseq, bacterial consortium, beta-glucan, gut microbiota, whole-transcriptome analysis

The human gut microbiota is a complex community, in which competitive and cooperative interactions between bacterial species play an important role in its assemblage and maintenance (1–3). Plant polysaccharides (glycans) constitute chemically and structurally diverse sources of carbon and energy for gut bacteria. They are commonly present in the diet but are not degraded by human digestive processes (4, 5). Bacteria in the colon have the ability to depolymerize the glycans and ferment the mono- and oligosaccharides that then become available (6). In at least some cases,

Citation Centanni M, Sims IM, Bell TJ, Biswas A, Tannock GW. 2020. Sharing a β-glucan meal: transcriptomic eavesdropping on a *Bacteroides ovatus-Subdoligranulum variabile-Hungatella hathewayi* consortium. Appl Environ Microbiol 86:e01651-20. https://doi.org/10.1128/AEM.01651-20.

Editor Danilo Ercolini, University of Naples Federico II

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Received 9 July 2020
Accepted 5 August 2020
Accepted manuscript posted online 14 August 2020
Published 1 October 2020

cross-feeding networks form around glycans, in which some species degrade particular substrates that are metabolically inaccessible to others, but leave extracellular hydrolytic products in the habitat that are then used by other species for growth (7–10). Plant polysaccharides can thus form the nucleus for the assembly of combinations of bacterial species (consortia) with special metabolic activities. However, this is a relatively unexplored aspect of gut microbiota research (3).

The purpose of our work was to develop an experimental approach to aid in disentangling the dynamics of the human gut microbiota through synthetic ecology (11). To do this, we built an *in vitro* consortium of gut bacteria based on barley β-glucan, which is a (1→3),(1→4)-β-D-glucan present in plant cell walls, as nutritive substrate (12). Three bacterial species commonly present in the human gut microbiota were selected through experimentation as bacterial constituents of the consortium (13, 14). *Bacteroides ovatus* is a member of one of the dominant phyla present in the human gut microbiota, *Bacteroidetes* (15). Members of this phylum have large genomes that encode many carbohydrate-active enzymes (16). They are considered to be important functionally as "generalist" bacteria in the human gut (5, 17). The metabolic capacity of *Bacteroides* species has been studied for decades, but the molecular systems involved in the degradation of complex glycans have been described more recently (9, 18–29). The remaining members of the bacterial consortium belong to the *Firmicutes*, another dominant phylum in the human gut microbiota. *Subdoligranulum variabile* is a member of the family *Ruminococcaceae* (30), and *Hungatella hathewayi* is a member of the family *Lachnospiraceae* (31, 32). Hence, the consortium contained species that are members of the three predominant families of the human gut microbiota (*Bacteroidaceae*, *Ruminococcaceae*, and *Lachnospiraceae*) (33).

Transcriptomics provides a powerful tool to study consortium interplay (7). It is referred to as a means of eavesdropping on bacterial "conversations" within the community and to identify interactions that sustain the assemblage of bacterial species (7, 34). Therefore, the aim of our study was to determine the transcriptional responses of the different bacteria with regard to their relationships within the consortium.

RESULTS

Choice of bacterial species. *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T were chosen as prospective members of the consortium because of the results of screening experiments using 49 bacterial species (Table 1) commonly present as members of human gut microbiotas (13, 14). Highly significant increases in the amount of growth obtained in β-glucan medium compared to basal medium were evident for five of the species, *Bacteroides cellulosilyticus*, *Bacteroides ovatus*, *Bacteroides uniformis*, *Prevotella copri*, and *Eubacterium rectale*. *B. ovatus* was chosen as the potential β-glucan-degrading member of the consortium because of its ability to grow well in β-glucan medium and because the molecular biology of its degradative abilities is well-described (20, 21). We then tested 19 common members of the gut microbiota for the ability to grow in medium containing β-glucan-derived oligosaccharides produced during the growth of *B. ovatus* (Table 2). Two species (*Hungatella hathewayi* and *Subdoligranulum variabile*) had highly significantly increased growth in oligosaccharide medium compared to basal medium. These species could not grow in β-glucan medium to a greater extent than in basal medium. Therefore, these two species were chosen for further study because they could potentially benefit nutritionally from the hydrolytic activity of *B. ovatus* in the presence of β-glucan. It is noteworthy that some species had less growth in β-glucan medium than in basal medium (such as *Bacteroides pectinophilus* and *Alistipes putredinis*) or less growth in oligosaccharide medium than in basal medium (such as *Bacteroides fragilis*, *Bacteroides massiliensis*, *Bacteroides thetaiotaomicron*, and *Bacteroides vulgatus*). The physiological basis for these inhibitions is not known.

Formation of a bacterial consortium based on the hydrolysis of barley β-glucan. Growth curves of pure cultures of *B. ovatus* in β-glucan medium and of *S. variabile* and *H. hathewayi* in oligosaccharide medium are shown in Fig. 1. Barley

TABLE 1 Bacterial species tested for their ability to grow in barley β-glucan medium[a]

Species	Mean A_{600}	SD	A_{600} ratio	*P* value[b]
Bacteroides cellulosilyticus DSM 14838^T	0.117	0.00173205	1.513	7.6022e-06
Bacteroides ovatus ATCC 8483^T	0.493	0.0057735	2.851	8.8174e-08
Bacteroides uniformis ATCC 8492^T	0.189	0.00503322	1.586	3.8781e-05
Prevotella copri DSM 18205^T	0.143	0.00585947	2.337	5.3198e-05
Eubacterium rectale DSM 17629^T	0.121	0.00251661	3.772	1.9103e-06
Bifidobacterium adolescentis DSM 20083^T	0.134	0.00493288	0.982	
Bifidobacterium breve ATCC 15700^T	0.236	0.00984886	1.354	0.00192629
Bifidobacterium dentium DSM 20436^T	0.203	0.00568624	1.340	0.00040374
Bifidobacterium longum subsp. *longum* ATCC 15707^T	0.133	0.001	0.990	
Bifidobacterium pseudocatenulatum DSM 20438^T	0.217	0.01322876	1.146	0.02529211
Collinsella aerofaciens DSM 3979^T	0.156	0.00650641	0.923	
Bacteroides caccae ATCC 43185^T	0.143	0.00351188	0.953	
Bacteroides dorei DSM 17855^T	0.142	0.01078579	0.806	
Bacteroides eggerthii ATCC 27754^T	0.116	0.00585947	1.600	0.00055184
Bacteroides finegoldii DSM 17565^T	0.250	0.03	2.632	0.00107981
Bacteroides fragilis ATCC 25285^T	0.151	0.0052915	0.998	
Bacteroides intestinalis DSM 17393^T	0.073	0.0034641	1.244	0.00439986
Bacteroides massiliensis DSM 17679^T	0.037	0.00404145	0.836	
Bacteroides pectinophilus ATCC 43243^T	0.003	0.00152753	0.400	
Bacteroides stercoris ATCC 43183^T	0.139	0.00288675	1.146	0.00816623
Bacteroides thetaiotaomicron ATCC 29148^T	0.262	0.01184624	0.913	
Bacteroides vulgatus ATCC 29327	0.056	0.00650641	0.928	
Bacteroides xylanisolvens DSM 18836^T	0.637	0.06110101	3.141	0.00025133
Parabacteroides distasonis DSM 20701^T	0.123	0.002	0.918	
Parabacteroides goldsteinii DSM 19448^T	0.120	0.001	0.832	
Paraprevotella clara DSM 19731^T	0.014	0.00057735	1.433	0.00289001
Alistipes putredinis DSM 17216^T	0.006	0.00152753	0.395	
Lactobacillus salivarius DSM 20555^T	0.236	0.00152753	0.968	
Blautia hansenii DSM 20583^T	0.060	0.03865661	0.767	
Clostridium asparagiforme DSM 15981^T	0.470	0.01464013	0.929	
Hungatella hathewayi DSM 13479^T	0.333	0.0321455	1.020	NS
Clostridium nexile DSM 1787^T	0.129	0.00208167	0.998	
Clostridium scindens DSM 5676^T	0.065	0.00251661	0.920	
Clostridium symbiosum DSM 934^T	0.340	0.03874274	0.899	
Clostridium propionicum DSM 1682^T	0.010	0.00057735	1.526	0.02411011
Coprococcus comes ATCC 27758^T	0.677	0.18175075	0.883	
Dorea formicigenerans DSM 3992^T	0.026	0.00458258	1.300	NS
Dorea longicatena DSM 13814^T	0.135	0.00550757	0.888	
Eubacterium ventriosum DSM 3988^T	0.044	0.00550757	0.771	
Ruminococcus gnavus ATCC 29149^T	0.112	0.00458258	1.222	0.00253049
Ruminococcus torques ATCC 27756^T	0.055	0.003	0.855	
Ruminococcus obeum ATCC 29174^T	0.031	0.00378594	1.022	NS
Clostridium leptum DSM 753^T	0.069	0.0051316	2.286	0.00027607
Faecalibacterium prausnitzii DSM 17677^T	0.018	0.0034448	1.494	0.00631048
Flavonifractor plautii DSM 4000^T	0.176	0.00321455	1.111	0.02816079
Pseudoflavonifractor capillosus DSM 23940^T	0.527	0.1569501	2.476	0.02622434
Subdoligranulum variabile DSM 15176^T	0.012	0.00252982	0.847	
Holdemania filiformis DSM 12042^T	0.062	0.005	0.964	
Bilophila wadsworthia DSM 11045^T	0.073	0.00519615	1.237	0.01855356

[a]Optical density (A_{600}) was measured after 24 h of growth. Bacterial growth in barley β-glucan (0.2% [wt/vol]) medium is reported as mean A_{600} of bacterial cultures ($n = 3$) with standard deviation (SD). The ratio between the mean A_{600} of bacterial cultures grown in barley β-glucan medium and basal medium ($n = 3$) is given. *P* values of <0.05 were considered statistically significant. *P* values are shown only when the A_{600} ratio is >1.
[b]NS, not significant.

β-glucan was hydrolyzed extracellularly (Fig. 2), and 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4) oligosaccharides were released into the medium (Fig. 3A). These oligosaccharides were used by *S. variabile* and *H. hathewayi* for growth (Fig. 3B and C). The three species achieved consistent population levels in replicate batch and continuous (steady-state) cocultures, indicating that the species could reliably form a stable association in which oligosaccharides released as hydrolytic products by *B. ovatus* were used as growth substrates by the other two species (Fig. 4A and B). The *S. variabile* rate of utilization of DP3 was 13.3%/h, slightly faster than for DP4, which was 12.5%/h. In contrast, *H. hathewayi* showed a marked preference for DP4 (10.5%/h) compared to DP3 (2.8%/h). Prioritization of usage of different carbohydrates

TABLE 2 Bacterial species that did not grow in barley β-glucan medium and therefore were tested for their ability to grow on barley β-glucan-derived oligosaccharides[a]

Species	Mean A_{600}	SD	A_{600} ratio	*P* value[b]
Subdoligranulum variabile DSM 15176^T	0.284	0.01635441	23.058	2.293e-12
Hungatella hathewayi DSM 13479^T	0.790	0.04195235	2.685	1.9604e-10
Bifidobacterium adolescentis DSM 20083^T	0.133	0.00519615	1.025	NS
Collinsella aerofaciens DSM 3979^T	0.152	0.00360555	0.896	
Bacteroides dorei DSM 17855^T	0.108	0.00416333	0.806	
Bacteroides fragilis ATCC 25285^T	0.053	0.00288675	0.377	
Bacteroides massiliensis DSM 17679^T	0.011	0.00057735	0.101	
Bacteroides thetaiotaomicron ATCC 29148^T	0.117	0.02083267	0.644	
Bacteroides vulgatus ATCC 29327	0.013	0.00360555	0.140	
Parabacteroides distasonis DSM 20701^T	0.130	0.00602771	0.620	
Parabacteroides goldsteinii DSM 19448^T	0.248	0.04606951	0.996	
Alistipes putredinis DSM 17216^T	0.046	0.0011547	1.631	0.00127325
Lactobacillus salivarius DSM 20555^T	0.287	0.0085049	1.056	NS
Blautia hansenii DSM 20583^T	0.141	0.02129476	1.615	0.00051887
Clostridium nexile DSM 1787^T	0.175	0.07716389	1.314	NS
Clostridium symbiosum DSM 934^T	0.048	0.00173205	0.873	
Dorea longicatena DSM 13814^T	0.152	0.02404163	0.948	
Eubacterium ventriosum DSM 3988^T	0.018	0.00556776	0.628	
Ruminococcus torques ATCC 27756^T	0.046	0.00360555	0.789	

[a]Optical density of cultures (A_{600}) was measured after 48 h of growth. Bacterial growth is reported as the mean A_{600} ($n = 3$) of bacterial cultures grown in medium containing barley β-glucan-derived oligosaccharides (0.05% [wt/vol]), with standard deviation (SD). The ratio between A_{600} of bacterial cultures grown in oligosaccharide medium and basal medium ($n = 3$) (A_{600} ratio) is given. *P* values of <0.05 were considered statistically significant. *P* values are shown only when the A_{600} ratio is >1.
[b]NS, not significant.

for growth may allow potential competitors to cohabit (5, 35), although in this case, *S. variabile* would be predicted to have the overall advantage because it used both oligosaccharides more rapidly than did *H. hathewayi*. However, *H. hathewayi* was numerically dominant over *S. variabile* in both batch and continuous cultures (Fig. 4A and B), indicating that the uptake of fermentable substrates must be complemented by the availability of other growth-limiting nutrients and/or that inhibitory substances are produced by *H. hathewayi*. Experiments would need to be conducted in chemically defined medium to identify the growth-limiting nutrients. This is a difficult and perhaps impossible task in the case of gut commensals that, to date, require rich medium for growth.

Differential transcription of genes. The transcription levels of genes in the genomes of the three species in pure culture were determined and compared using whole-transcriptome analysis of cells collected at different stages of the growth curve. These comparisons were aimed at determining gene transcription during degradation and uptake of nutrients sourced from β-glucan by actively growing cells (for example, *B. ovatus* in pure culture sampled at 16, 20, and 26 h of growth; intraculture comparisons). Transcriptional comparisons of cocultures were made using cells collected after 24 h of growth because all three species were in the same growth phase by then (for example, *S. variabile* in pure culture at 24 h compared to *B. ovatus*/*S. variabile* at 24 h). Although the population levels of the three species were not the same within the coculture at the sampling time, they were similar (no more than 10-fold difference) for a given species between pure culture and coculture, and therefore sufficiently standardized comparisons could be made. *S. variabile* and *H. hathewayi* transcriptomes were compared in pure culture and coculture under batch conditions. Continuous coculture comparisons were not made, due to logistical difficulties in large-scale preparation of oligosaccharide medium required for comparative chemostat experiments. General data relating to RNA sequencing and whole transcriptome analysis are summarized in Table S1 in the supplemental material. Differential transcription of genes in Cluster of Orthologous Genes (COG) categories from the perspective of individual members of cocultures and enrichment of Gene Ontology (GO) terms are summarized in Fig. S1 and Table S2, respectively. The numbers of differentially transcribed genes

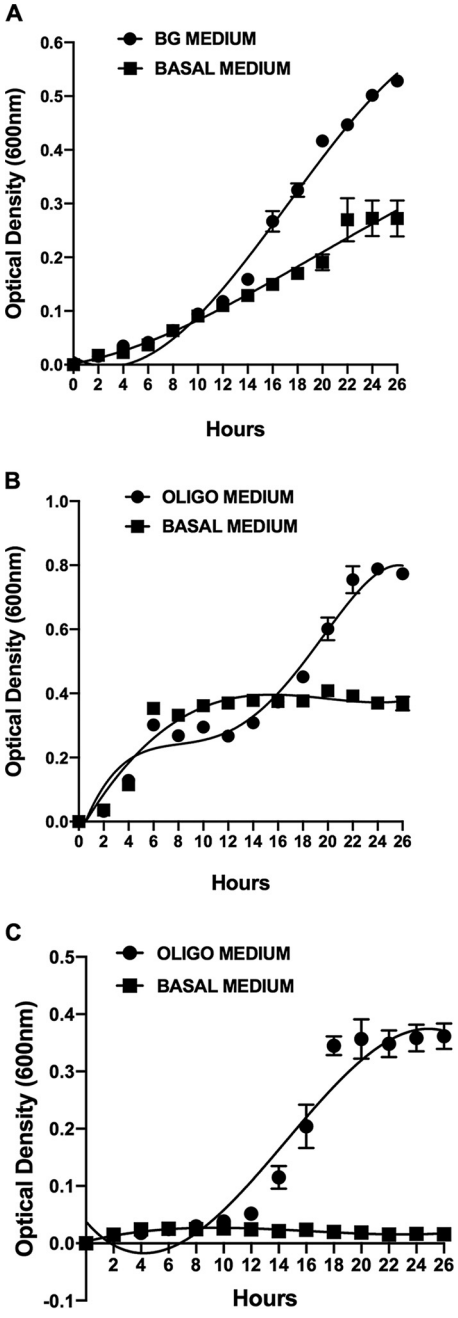

FIG 1 Growth of *B. ovatus* ATCC 8483^T, *H. hathewayi* DSM 13479^T, and *S. variabile* DSM 15176^T in pure batch cultures during anaerobic incubation at 37°C. Mean (standard error of the mean [SEM]) optical densities at A_{600} of triplicate cultures are shown. (A) *B. ovatus* ATCC 8483^T growth in barley β-glucan (BG) medium compared to basal medium. (B) *H. hathewayi* DSM 13479^T growth in oligosaccharide (oligo) medium compared to basal medium. Note the diauxic growth profile in oligosaccharide medium where initial growth relied on constituents in basal medium, followed by utilization of oligosaccharides. (C) *S. variabile* DSM 15176^T growth in oligosaccharide (oligo) medium compared to basal medium.

common or unique to specific combinations of the three bacterial species are summarized in Venn diagrams (Fig. S2).

Particularly noteworthy, transcriptomics analysis revealed the increased transcription of *B. ovatus* genes involved in barley β-glucan degradation (Table 3) during growth in pure culture and coculture under batch conditions. These genes have been recently described as part of a mixed-linkage-β-glucan utilization locus in *B. ovatus* (20, 21). In particular, glycoside hydrolase GH16 is an outer membrane-anchored endo-glucanase

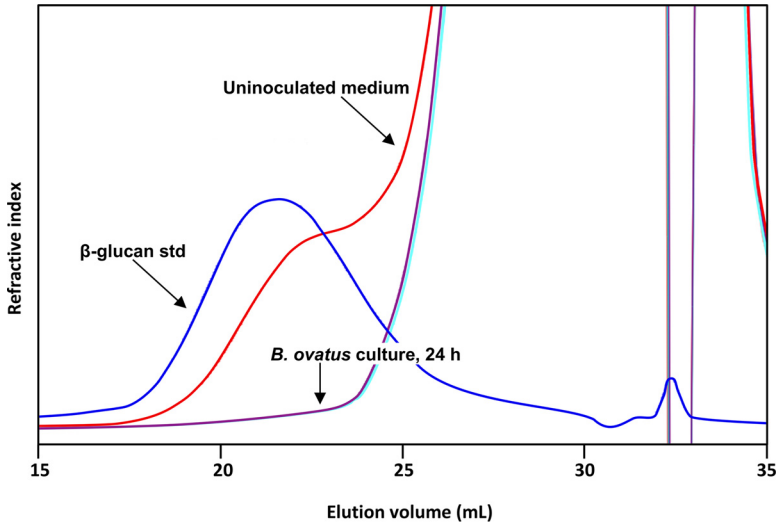

FIG 2 Depolymerization of barley β-glucan by *B. ovatus* ATCC 8483^T in pure batch culture at 37°C. Size exclusion chromatography (SEC) profiles of uninoculated barley β-glucan medium, barley β-glucan standard, and supernatant from *B. ovatus* ATCC 8483^T culture in barley β-glucan medium incubated for 24 h.

responsible for the breakdown of barley β-glucan molecules into oligosaccharides, the smallest released being mixed-linkage tri- and tetrasaccharides (21). Consistent with our results, a recent study showed that cell surface glycan-binding proteins belonging to the *B. ovatus* mixed-linkage-β-glucan utilization locus preferentially bind oligosaccharides with a degree of polymerization >6 and do not bind mixed-linkage tri- and tetrasaccharides (20). The transcription of the β-glucan utilization genes in batch coculture was mainly influenced by the presence of *H. hathaweyi*, because coculture with *S. variabile* did not affect differential transcription of the locus to any extent compared to pure culture. Similarly, transcription of the genes was the same in *B. ovatus* cells obtained from pure culture and coculture under continuous conditions (Table 3). Nevertheless, β-glucan was hydrolyzed in the chemostat, and DP3 and DP4 oligosaccharides were utilized by the coculture as observed for batch conditions (Fig. 3A and 4C).

S. variabile had increased transcription of genes encoding carbohydrate transporters during pure growth but decreased transcription of most of these in coculture, which may be related to competition with *H. hathewayi*, which was numerically dominant (Fig. 4A). *H. hathewayi* had increased transcription of genes encoding carbohydrate receptors and permease transporters in both pure culture and coculture. These gene products are likely to be involved in the uptake of the oligosaccharides (Table 4).

Having obtained evidence of a nutritional relationship based on barley β-glucan oligosaccharides within the coculture of three species, we searched transcriptomics data for indications of other adaptations by the species. Three phenomena of interest were detected, which we report here for future reference. They are, at this stage, observational and thus speculative and require biochemical validation. Cobalamin (vitamin B_{12}) is an essential cofactor for the growth of many bacteria. Species that do not possess the biosynthetic pathway for cobalamin synthesis have mechanisms to acquire it from the environment (36–39). Cobalamin availability may thus represent an important factor in the establishment of microbial networks (36). A search of *S. variabile* and *H. hathewayi* genome sequences (NCBI RefSeq accession assembly numbers GCF_000157955.1 and GCF_000160095.1, respectively) revealed the presence of genes representative of cobalamin biosynthetic pathways. The *B. ovatus* genome sequence (NCBI RefSeq accession assembly number GCF_001314995.1) did not contain evidence of a cobalamin biosynthetic pathway, but a gene encoding a cobalamin-binding protein was present. The transcription of the cobalamin biosynthetic operon of *S.*

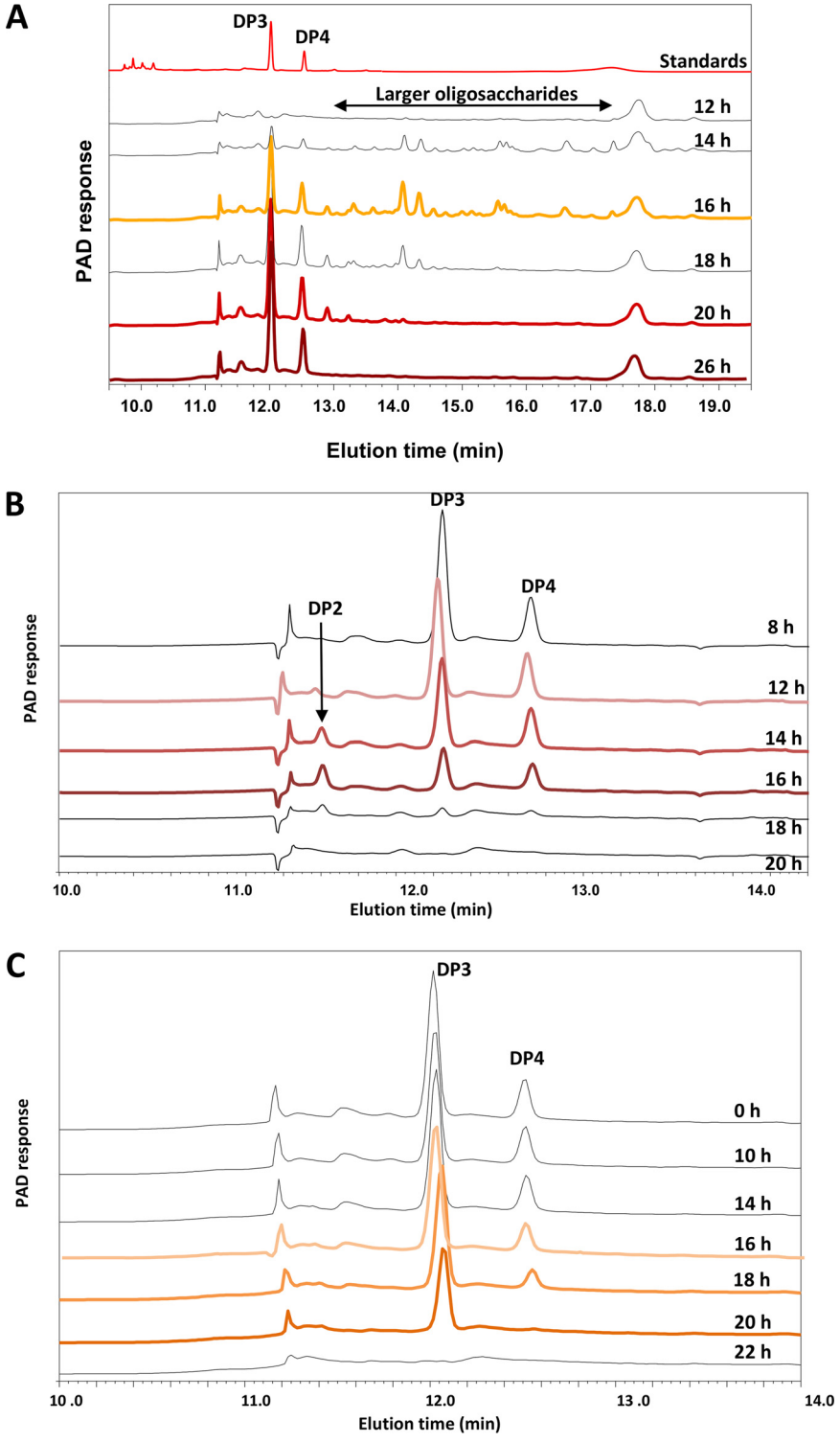

FIG 3 High-performance anion-exchange chromatography (HPAEC) profiles of supernatants collected during the growth of *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T in pure culture during anaerobic incubation at 37°C. Peaks corresponding to DP2 (probably laminaribiose), DP3 (3-*O*-*β*-cellobiosyl-D-glucose), and DP4 (3-*O*-*β*-cellotriosyl-D-glucose) oligosaccharides are indicated by reference to standards. Sampling times are indicated, and chromatographic profiles of samples that were analyzed with whole-transcriptomics analysis (RNA-seq) are colored red/orange. (A) *B. ovatus* ATCC 8483^T supernatants from barley *β*-glucan medium. (B) *H. hathewayi* DSM 13479^T supernatants from oligosaccharide medium. (C) *S. variabile* DSM 15176^T supernatants from oligosaccharide medium.

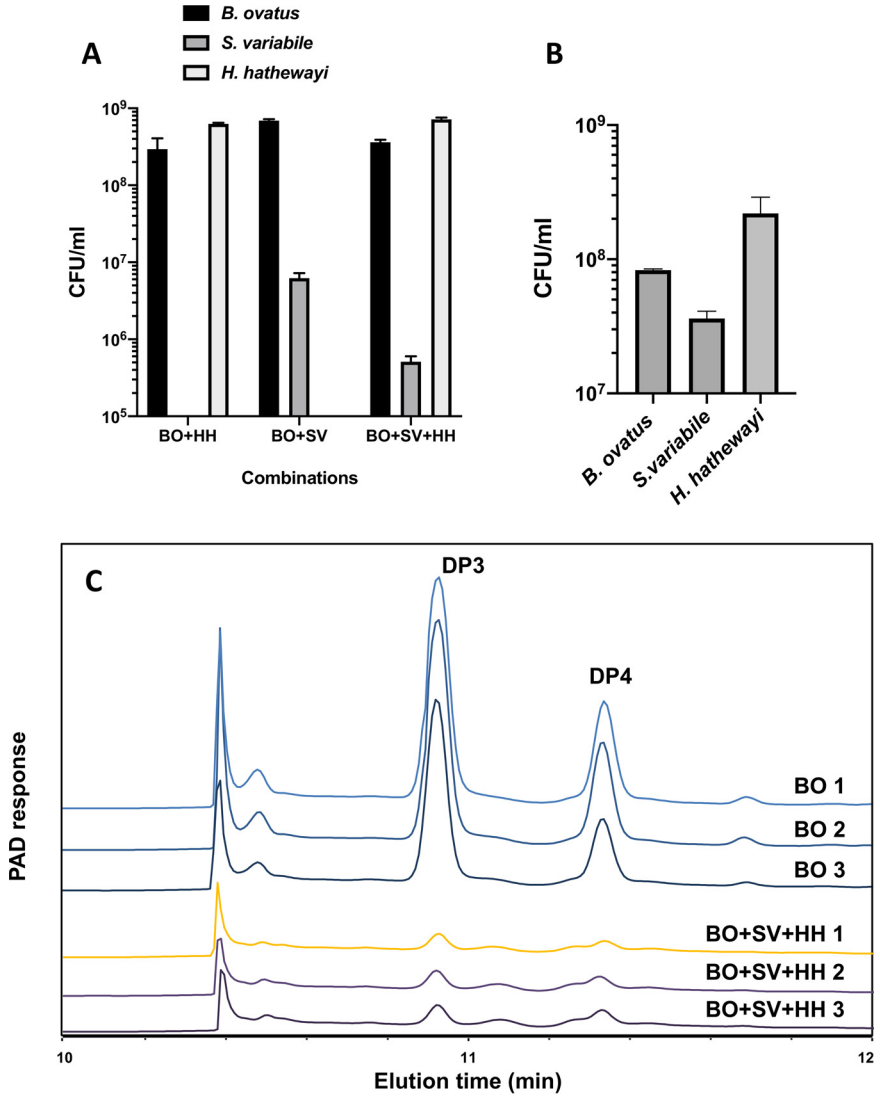

FIG 4 Populations of *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T in batch (24 h of incubation) or continuous coculture and utilization of DP3 and DP4 oligosaccharides in continuous coculture. (A) CFU/ml of *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T in combinations of batch coculture. Means (SEMs) of duplicate cultures are shown. (B) CFU/ml of *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T in continuous coculture. Means (SEMs) of three chemostat runs are shown. (C) High-performance anion-exchange chromatography (HPAEC) profiles of supernatants collected from continuous coculture of *B. ovatus* ATCC 8483^T, *S. variabile* DSM 15176^T, and *H. hathewayi* DSM 13479^T. Profiles from three chemostat runs are shown. BO, *B. ovatus* ATCC 8483T in pure culture; BO+SV+HH, the three species in coculture. Peaks corresponding to DP3 (3-*O*-β-cellobiosyl-D-glucose) and DP4 (3-*O*-β-cellotriosyl-D-glucose) oligosaccharides are indicated.

variabile was unchanged during pure growth or in coculture with *B. ovatus* but had decreased transcription in coculture with *H. hathewayi* (Table 5). This suggests that *H. hathewayi* was the principal source of cobalamin in coculture.

Another possible adaptation detected was in relation to the "stringent response" (40, 41), where the key regulators are the intracellular molecules guanosine-5′-3′-bispyrophosphate (ppGpp) and guanosine pentaphosphate (pppGpp), together denoted by (p)ppGpp (42). In the presence of nutrient starvation conditions, increased levels of (p)ppGpp cause reduced transcription of genes involved in translation machinery and cell growth and division and increased transcription of genes involved in stress response (41, 43). We noted that the bifunctional-(p)ppGpp synthetase/guanosine-3′-5′-bis(diphosphate)-3′-pyrophosphohydrolase (SUBVAR_RS12040) gene

TABLE 3 Differential transcription of genes involved in degradation of barley β-glucan in *B. ovatus* ATCC 8483^T in pure culture and coculture[a]

		Pure culture (batch)[b]			Coculture (batch)[b]				Coculture (continuous)[b]	
Gene name	Product (EC no.)	Log2FC BO16 vs BO20	Log2FC BO16 vs BO26	FDR	Log2FC BOHH24 vs BO24	Log2FC BOSV24 vs BO24	Log2FC BOSVHH24 vs BO24	FDR	Log2FC BOSVHH vs BO	FDR
Bovatus_RS15290	Glycoside hydrolase family 16 (GH16; EC 3.2.1.-)	3.786	7.074	1.55e-04	6.695	–[c]	5.395	1.26e-03	–0.037	0.955
Bovatus_RS15295	SusC/RagA family TonB-linked outer membrane protein	2.819	6.775	3.69e-04	6.413	1.180	5.398	9.49e-04	–0.428	0.358
Bovatus_RS15300	SusC/RagB family nutrient-binding outer membrane lipoprotein	2.734	6.676	2.86e-04	6.113	1.043	5.364	1.52e-03	–0.696	0.083
Bovatus_RS15305	Hypothetical protein	2.507	7.055	1.23e-04	7.252	1.912	6.557	2.73e-04	–0.769	0.053
Bovatus_RS15310	β-Glucosidase (EC 3.2.1.-)	2.542	6.996	5.05e-04	6.862	1.345	6.159	1.12e-03	–0.686	0.080

[a]Gene name, product, Log2FC (fold change) of each comparison, and FDR (false discovery rate) are reported. Genes showing an FDR of <0.05 and absolute value of Log2FC of >1 were considered statistically differentially transcribed.

[b]BO, *B. ovatus*; BOHH, coculture of *B. ovatus* and *H. hathewayi*; BOSV, coculture of *B. ovatus* and *S. variabile*; BOSVHH, coculture of *B. ovatus*, *S. variabile*, and *H. hathewayi*. Comparisons in pure culture are between transcription at 16 h and 20 or 26 h of incubation. Comparisons in coculture are between pure culture and coculture, both at 24 h of incubation.

[c]–, absolute value Log2FC < 1.

TABLE 4 Differential transcription of exemplar genes encoding carbohydrate receptors and transporters in *S. variabile* DSM 15176^T and *H. hathewayi* DSM 13479^T in pure and coculture[a]

Species and gene name	Product	Pure culture[b,c] Log2FC SV12 vs SV14	Log2FC SV12 vs SV16	FDR	Coculture[b] Log2FC BOSV vs SV24	Log2FC BOSVHH vs SV24	FDR
S. variabile							
SUBVAR_RS01140	Carbohydrate ABC transporter permease	1.914	1.805	9.45e-03	–6.402	–3.233	2.98e-32
SUBVAR_RS01150	Carbohydrate ABC transporter substrate-binding protein	1.553	1.271	4.91e-02	–6.100	–5.316	5.21e-58
SUBVAR_RS12285	Carbohydrate ABC transporter permease	1.487	1.109	3.72e-03	–2.595	–2.442	7.40e-32
SUBVAR_RS12505	PTS sugar transporter subunit IIBCA	2.126	2.497	2.23e-03	–7.116	–5.950	2.95e-87
SUBVAR_RS14155	Carbohydrate ABC transporter substrate-binding protein	–	–	NS[d]	1.549	1.911	4.13e-19

		Log2FC HH06 vs HH16	Log2FC HH06 vs HH18	Log2FC HH06 vs HH20	FDR	Log2FC BOHH vs HH24	Log2FC BOSVHH vs HH24	FDR
H. hathewayi								
CLOSTHATH_RS02755	Carbohydrate ABC transporter permease	3.186	4.197	3.932	2.22e-07	2.209	2.352	1.78e-02
CLOSTHATH_RS02760	Sugar ABC transporter permease	3.520	4.275	4.275	8.15e-08	3.949	3.575	4.95e-05
CLOSTHATH_RS02765	Sugar ABC transporter substrate-binding protein	3.820	4.728	4.754	5.50e-06	3.566	3.317	2.13e-04

[a]Gene name, product, and Log2FC (fold change) of each comparison and FDR (false-discovery rate) are reported. Genes showing an FDR of <0.05 and absolute value of Log2FC >1 were considered statistically differentially transcribed.

[b]SV, *S. variabile*; BO, *B. ovatus*; BOSV, coculture of *B. ovatus* and *S. variabile*; BOSVHH, coculture of *B. ovatus*, *S. variabile*, and *H. hathewayi*; HH, *H. hathewayi*; BOHH, *B. ovatus* and *H. hathewayi* in coculture; BOSVHH, *B. ovatus*, *S. variabile*, and *H. hathewayi* in coculture. Comparisons in *S. variabile* pure culture are between transcription at 12 h and 14 or 16 h. Comparisons in *H. hathewayi* pure culture are between transcription at 6 h and 16, 18, or 20 h of incubation. Comparisons in coculture are between pure culture and coculture, both at 24 h of incubation.

[c]–, absolute value Log2FC < 1.

[d]NS, not significant.

TABLE 5 Transcription of genes belonging to the cobalamin biosynthetic operon of *S. variabile* DSM 15176[T][a]

Gene name	Product (EC no.)	Pure culture[b,c]			Coculture[b,c]		
		Log2FC SV12 vs SV14	Log2FC SV12 vs SV16	FDR[d]	Log2FC BOSV vs SV24	Log2FC BOSVHH vs SV24	FDR
SUBVAR_RS06700	Iron ABC transporter permease	–	–	NS[d]	–	−2.055	6.55e-04
SUBVAR_RS06705	Precorrin-8X methylmutase (EC 5.4.99.60)	–	–	NS	–	−1.744	2.62e-03
SUBVAR_RS06720	Cobalamin biosynthesis protein CobD	–	–	NS	–	−1.187	3.60e-03
SUBVAR_RS06735	Adenosylcobinamide-GDP Ribazoletransferase (EC 2.7.8.26)	–	–	NS	–	−1.051	4.98e-02
SUBVAR_RS06750	Cobyrinate a,c-diamide synthase (EC 6.3.5.11)	–	–	NS	–	−1.636	1.30e-02
SUBVAR_RS06775	Precorrin-4 C(11)-methyltransferase (EC 2.1.1.271)	–	–	NS	–	−2.200	1.35e-05
SUBVAR_RS06780	Precorrin-2 C(20)-methyltransferase (EC 2.1.1.151)	–	–	NS	–	−2.206	6.64e-03
SUBVAR_RS06785	Cobalamin biosynthesis protein CbiD	–	–	NS	–	−1.969	4.39e-05
SUBVAR_RS06790	Cobalt chelatase (EC 4.99.1.3)	–	–	NS	–	−2.449	1.97e-06
SUBVAR_RS06805	ABC transporter substrate-binding protein	–	–	NS	–	−2.312	1.09e-06

[a]Gene name, product, and Log2FC (fold change) of each comparison and FDR (false discovery rate) are reported. Genes showing an FDR of <0.05 and absolute value of Log2FC >1 were considered statistically differentially transcribed.
[b]SV, *S. variabile*; BO, *B. ovatus*; BOSV, coculture of *B. ovatus* and *S. variabile*; BOSVHH, coculture of *B. ovatus*, *S. variabile*, and *H. hathewayi*. Comparisons in pure culture are between transcription at 12 h and 14 or 16 h of incubation. Comparisons in coculture are between pure culture and coculture, both at 24 h of incubation.
[c]–, absolute value Log2FC < 1.
[d]NS, not statistically significant.

had decreased transcription when *S. variabile* was in coculture with *B. ovatus* and in coculture with *B. ovatus* and *H. hathewayi* (Table 6). Moreover, ObgE (SUBVAR_RS07640), an essential GTPase that acts as a negative regulator of the stringent response preventing activation in a nutrient-rich environment (40, 44), had increased transcription in *S. variabile* in cocultures (Table 6). Together with increased transcription of ribosomal protein and aminoacyl-tRNA ligase genes in *S. variabile* (Table 6 and Table S2), these observations indicate a suppression of the stringent response when in coculture and hence indicate that the species was not suffering from nutrient starvation. In the case of *H. hathewayi*, however, the bifunctional-(p)ppGpp-synthetase/guanosine-3′-5′-bis(diphosphate)-3′-pyrophosphohydrolase gene (CLOSTHATH_RS21480) had increased transcription in coculture both with *B. ovatus* and with *B. ovatus* and *S. variabile*. The GTPase ObgE (CLOSTHATH_RS30045) gene had increased transcription when *H. hathewayi* was in coculture with *B. ovatus* and *S. variabile*, indicating some nutritional stress (Table 6), but genes encoding ribosomal structural proteins nevertheless had increased transcription in coculture.

Genes belonging to the COG category "signal transduction mechanisms" were differentially transcribed in the three species (Fig. S1). Several of these genes encode sensor-histidine kinases known to be important for sensing and responding to environmental signals (45). Of particular interest was the increased transcription of the gene encoding S-ribosylhomocysteine lyase (SUBVAR_RS06625) of *S. variabile* when in coculture with *B. ovatus* but not with *B. ovatus* and *H. hathewayi* (Table 7). S-ribosylhomocysteine lyase (LuxS) catalyzes the synthesis of autoinducer-2 (AI-2), a molecule considered a universal signal for interspecies communication (46, 47).

DISCUSSION

While there have been major increases in knowledge about the composition of microbial communities during recent decades, details of the functional interactions that occur between bacterial species within communities are much less advanced (3). As a result, Widder and colleagues (11) advocated the development of integrated model systems with which carefully chosen species can be studied *in vitro*. These defined model systems could be used to gain fundamental knowledge of how communities function. Although synthetic communities do not reproduce the complex diversity of natural communities, elucidation of basic principles of the operation of microbial communities is considered to be possible (11). In accordance with these views, we assembled a consortium of bacterial species that are commonly present in the human gut microbiota. Based on the hydrolysis of a common dietary fiber, barley β-glucan, we investigated the functional interplay between members of the consortium using whole-transcriptome sequencing and analysis. Coculture of bacteria produced transcriptional

TABLE 6 Transcription of exemplar genes encoding ribosome structural proteins, aminoacyl-tRNA ligases, and regulators of stringent response in *S. variabile* DSM 15176^T and *H. hathewayi* DSM 13479^T[a]

Species and gene name	Product (EC no.)	Pure culture[b,c] Log2FC SV12 vs SV14	Log2FC SV12 vs SV16		FDR[d]	Coculture[b] Log2FC BOSV vs SV24	Log2FC BOSVHH vs SV24	FDR
S. variabile								
SUBVAR_RS01425	30S ribosomal protein S9	–	–		NS	4.291	3.961	1.89e-54
SUBVAR_RS02945	30S ribosomal protein S18	–	–		NS	3.387	3.033	1.59e-41
SUBVAR_RS04660	50S ribosomal protein L19	–	–		NS	3.291	2.966	1.57e-42
SUBVAR_RS05155	50S ribosomal protein L31	–	–		NS	4.744	4.195	4.26e-60
SUBVAR_RS05835	50S ribosomal protein L10	–	–		NS	4.011	3.423	2.19e-51
SUBVAR_RS07650	50S ribosomal protein L21	–	–		NS	2.640	2.428	1.73e-30
SUBVAR_RS04130	Alanine-tRNA ligase (EC 6.1.1.7)	–	–		NS	2.332	2.945	1.13e-31
SUBVAR_RS04425	Tyrosine-tRNA ligase (EC 6.1.1.1)	–	–		NS	2.301	2.408	3.05e-23
SUBVAR_RS05990	Asparagine-tRNA ligase (EC 6.1.1.22)	–	–		NS	2.185	2.207	3.84e-23
SUBVAR_RS06090	Phenylalanine-tRNA ligase subunit beta (EC 6.1.1.20)	–	–		NS	1.385	1.356	3.14e-11
SUBVAR_RS09185	Lysine-tRNA ligase (EC 6.1.1.6)	–	–		NS	2.172	1.630	1.97e-21
SUBVAR_RS09715	Threonine-tRNA ligase (EC 6.1.1.3)	–	–		NS	1.619	2.114	5.55e-18
SUBVAR_RS12040	Bifunctional (p)ppGpp synthetase/guanosine-3, 5′-bis(diphosphate) 3′-pyrophosphohydrolase (EC 3.1.7.2)	–	–		NS	−1.456	−11.357	1.48e-09
SUBVAR_RS07640	GTPase ObgE	–	–		NS	1.232	1.013	1.91e-08
		Log2FC HH06 vs HH16	**Log2FC HH06 vs HH18**	**Log2FC HH06 vs HH20**	**FDR**	**Log2FC BOHH vs HH24**	**Log2FC BOSVHH vs HH24**	**FDR**
H. hathewayi								
CLOSTHATH_RS11480	50S ribosomal protein L31	−2.080	−2.217	−1.890	6.50e-05	3.550	3.486	8.79e-05
CLOSTHATH_RS18880	30S ribosomal protein S2	−1.550	−1.583	−1.338	5.84e-04	2.598	2.353	2.49e-04
CLOSTHATH_RS18945	30S ribosomal protein S21	−2.511	−2.503	−2.416	4.68e-04	3.912	3.597	4.24e-05
CLOSTHATH_RS20325	50S ribosomal protein L28	−2.543	−2.716	−2.587	1.10e-04	4.578	4.351	2.39e-05
CLOSTHATH_RS28340	50S ribosomal protein L35	−1.908	−1.956	−1.821	1.82e-04	2.545	2.716	2.65e-03
CLOSTHATH_RS30700	30S ribosomal protein S20	−2.869	−2.588	−2.106	2.44e-05	3.198	2.701	1.23e-04
CLOSTHATH_RS07400	Glutamine-tRNA ligase/YqeY domain fusion protein (EC 6.1.1.18)	−1.646	−1.838	−1.653	2.91e-04	2.536	2.572	3.20e-04
CLOSTHATH_RS12900	Glycine-tRNA ligase (EC 6.1.1.14)	−1.202	−1.213	−1.306	2.29e-04	1.131	1.208	1.90e-02
CLOSTHATH_RS21545	Histidine-tRNA ligase (EC 6.1.1.21)	–	−1.031	–	2.32e-03	1.857	2.400	6.22e-04
CLOSTHATH_RS27790	Arginine-tRNA ligase (EC 6.1.1.19)	−1.110	−1.476	−1.347	2.89e-04	1.268	1.598	3.80e-03
CLOSTHATH_RS28080	Lysine-tRNA ligase (EC 6.1.1.6)	−1.161	−1.382	–	1.66e-03	2.201	2.302	3.73e-04
CLOSTHATH_RS21480	Bifunctional (p)ppGpp synthetase/guanosine-3′,5′-bis(diphosphate) 3′-pyrophosphohydrolase (EC 3.1.7.2)	−1.052	−1.239	−1.189	3.80e-03	1.997	1.866	7.53e-04
CLOSTHATH_RS30045	GTPase ObgE	–	–	–	NS	–	1.531	3.28e-02

[a]Gene name, product, and Log2FC (fold change) of each comparison and FDR (false discovery rate) are reported. Genes showing an FDR of <0.05 and absolute value of Log2FC >1 were considered statistically differentially transcribed.

[b]SV, *S. variabile*; BO, *B. ovatus*; BOSV, coculture of *B. ovatus* and *S. variabile*; BOSVHH, coculture of *B. ovatus*, *S. variabile*, and *H. hathewayi*; HH, *H. hathewayi*; BOHH, *B. ovatus* and *H. hathewayi* in coculture; BOSVHH, *B. ovatus*, *S. variabile*, and *H. hathewayi* in coculture. Comparisons in *S. variabile* pure culture are between 12 h and 14 or 16 h of incubation. Comparisons in *H. hathewayi* pure culture are between transcription at 6 h and 16, 18 or 20 h of incubation. Comparisons in coculture are between pure culture and coculture, both at 24 h of incubation.

[c]–, absolute value Log2FC < 1.

[d]NS, not statistically significant.

TABLE 7 Differential transcription of exemplar genes involved in molecular signaling in *S. variabile* DSM 15176^T and *H. hathewayi* DSM 13479^T in pure culture and coculture[a]

Species and gene name	Product (EC no.)	Pure culture[b,c]				Coculture		
		Log2FC SV12 vs SV14	Log2FC SV12 vs SV16		FDR[d]	Log2FC BOSV vs SV24	Log2FC BOSVHH vs SV24	FDR
S. variabile								
SUBVAR_RS00065	HAMP domain-containing histidine kinase (EC 2.7.13.3)	–	–		NS	−2.538	−10.576	4.19e-12
SUBVAR_RS05565	HAMP domain-containing protein	–	–		NS	−2.518	−9.765	5.16e-08
SUBVAR_RS06880	HAMP domain-containing histidine kinase (EC 2.7.13.3)	–	–		NS	−3.041	−11.549	6.32e-22
SUBVAR_RS10870	Sensor histidine kinase (EC 2.7.13.3)	–	–		NS	−1.986	−3.518	8.78e-18
SUBVAR_RS14765	Two-component sensor histidine kinase (EC 2.7.13.3)	–	–		NS	−1.584	−1.732	7.14e-12
SUBVAR_RS15315	HAMP domain-containing histidine kinase (EC 2.7.13.3)	–	–		NS	−3.479	−11.767	1.68e-26
SUBVAR_RS06625	S-ribosylhomocysteine lyase (EC 4.4.1.21)	–	–		NS	1.446	–	1.52e-09
		Log2FC HH06 vs HH16	Log2FC HH06 vs HH18	Log2FC HH06 vs HH20	FDR	Log2FC BOHH vs HH24	Log2FC BOSVHH vs HH24	FDR
H. hathewayi								
CLOSTHATH_RS01390	Sensor histidine kinase (EC 2.7.13.3)	–	–	–	NS	1.815	1.745	1.08e-02
CLOSTHATH_RS14145	Sensor histidine kinase (EC 2.7.13.3)	−1.206	−1.567	−1.550	1.72e-04	1.719	1.214	7.46e-03
CLOSTHATH_RS30795	HAMP domain-containing protein	−1.206	−1.315	−1.390	6.01e-04	1.650	1.582	2.04e-02
CLOSTHATH_RS00055	Diguanylate cyclase (EC 2.7.7.65)	1.070	1.589	1.460	2.99e-04	−1.270	−1.837	6.34e-03
CLOSTHATH_RS07675	Diguanylate cyclase (EC 2.7.7.65)	–	–	–	NS	5.132	–	4.24e-04
CLOSTHATH_RS08820	GGDEF domain-containing protein	−2.119	−1.877	−1.993	7.40e-05	4.138	1.411	2.79e-02
CLOSTHATH_RS17865	Diguanylate cyclase (EC 2.7.7.65)	–	−1.065	−1.089	3.54e-03	1.367	1.308	2.17e-02
CLOSTHATH_RS23095	EAL domain-containing protein	−1.034	−1.008	−1.338	2.58e-02	1.617	1.819	2.23e-02
CLOSTHATH_RS26500	EAL domain-containing protein	–	–	–	NS	1.801	1.686	1.43e-03
CLOSTHATH_RS27510	GGDEF domain-containing protein	–	–	–	NS	−1.461	−1.831	4.49e-02
CLOSTHATH_RS29240	Diguanylate cyclase (EC 2.7.7.65)	–	–	–	NS	−1.040	–	4.91e-02
CLOSTHATH_RS30530	Sensor domain-containing diguanylate cyclase (EC 2.7.7.65)	–	–	–	NS	2.112	2.085	1.08e-02
CLOSTHATH_RS13885	Flagellin	–	1.067	–	3.60e-03	−1.474	−1.415	1.72e-02

[a]Gene name, product, and Log2FC (fold change) of each comparison and FDR (false discovery rate) are reported. Genes showing an FDR of <0.05 and absolute value of Log2FC >1 were considered statistically differentially transcribed.

[b]SV, *S. variabile*; BOSV, coculture of *B. ovatus* and *S. variabile*; HH, *H. hathewayi*; BOHH, *B. ovatus* and *H. hathewayi* in coculture. Comparisons in *B. ovatus* pure culture are between transcription at 16 h and 20 or 26 h of incubation. Comparisons in *S. variabile* pure culture are between 12 h and 14 or 16 h of incubation. Comparisons in *H. hathewayi* pure culture are between transcription at 6 h and 16, 18 or 20 h of incubation Comparisons in coculture are between pure culture and coculture, both at 24 h of incubation.

[c]–, absolute value Log2FC < 1.

[d]NS, not statistically significant.

changes of genes contained in 16 COG categories in all three bacterial species, as well as enrichment or depletion of many GO terms, indicating that the bacteria responded to life in a more complex biological environment. Of particular interest was the increased transcription of genes associated with the hydrolysis of barley β-glucan (*B. ovatus*) and the increased transcription of carbohydrate receptors and transporters when oligosaccharides resulting from β-glucan hydrolysis by *B. ovatus* were available in the medium (*S. variabile* and *H. hathewayi*). The increased transcription of β-glucan utilization genes in *B. ovatus* in batch but not in continuous culture raises the interesting question as to which culture condition is most appropriate in simulating the human gut. Probably, the colon provides conditions that are intermediate between batch and continuous systems. Food travels to the colon in boluses (which could be likened to batches). Movement of digesta into the colon is controlled by the ileo-cecal valve. Thus, the flow of nutrients is semicontinuous, not continuous as is the situation in a chemostat. Technically sophisticated models of the human gut may be required to take into account intermittent addition of digesta to the colon.

β-Glucans are a major component of grains and are therefore common in human foods. The consumption of β-glucans has been reported to reduce postprandial glycemia in type 2 diabetic patients and to reduce hypercholesterolemia and hyperlipidemia (48–52). Although the amount of β-glucan varies in grains according to plant variety and growth conditions, the glycans can be purified to provide products that, when ingested, may therefore support human health. Specific modulation of the gut microbiota through the consumption of β-glucans may also be feasible because, in our screen of 49 common gut commensals, relatively few (mostly *Bacteroides* species) had greatly augmented growth in barley β-glucan medium relative to control medium. The opportunities for syntrophic relationships based on the hydrolysis of barley β-glucan by *B. ovatus* were found to be limited on the basis of the screen of 19 common commensals that could grow in oligosaccharide medium but not in β-glucan medium, of which *H. hathewayi* and *S. variabile* were by far the most competent. *Lactobacillus ruminis* has also been reported to utilize oligosaccharides derived from barley β-glucan hydrolysis (53, 54). This trophic information points to the possibility that highly specific changes to microbiota taxa and associated function could be achieved by dietary intervention using barley β-glucan. Investigation of other plant polysaccharides common in human diets might reveal further clusters of bacterial species that cooperatively degrade particular dietary fibers (2, 3, 55). It would also be interesting to develop this work further by determining the hydrolytic products in culture supernatants that might have been released from barley β-glucan by species other than *B. ovatus* (such as *Bacteroides cellulosilytcus*, *Bacteroides uniformis*, *Prevotella copri*, and *Eubacterium rectale*). Fundamental aspects of microbiota ecology could also be advanced through the development of more complex consortia of gut commensals *in vitro* using trophic information.

The establishment of an *in vitro* consortium of anaerobic bacterial species common in the gut microbiota is consistent with limiting experimental systems to a manageable number of constituent species, making it easier in turn to measure interactions within the coculture (11, 56, 57). For example, augmented propionate production as a proportion of total short-chain fatty acids resulting from the fermentation of a mixture of plant glycans by a synthetic community (*Bacteroides ovatus*, *Bifidobacterium longum* subspecies *longum*, *Megasphaera elsdenii*, *Ruminococcus gnavus*, and *Veillonella parvula*) was recently reported (56). Increased propionate production was due to greater succinate production by *B. ovatus* from galactan fermentation and conversion of succinate to propionate by *V. parvula*. We recognize, nevertheless, that the need to use rich medium for the culture of the fastidious bacterial species in our experiments limits the amount of detailed nutritional information that can be obtained. Missing from our model, too, is a spatial perspective; bacterial cells are associated with plant particulate material in the digesta, and these bacteria doubtless have a role in the hydrolysis and fermentation of plant polysaccharides in the gut (33, 58). Recent work with *Bacteroides* species, including *B. ovatus*, showed that these bacteria have the capacity to bind to beads coated with polysaccharides, characteristic of dietary fiber, in the gut of gnoto-

biotic mice (59). Spatial associations on food particles of hydrolytic bacteria (producers) with other bacterial species that benefit from leakage of hydrolytic products that are potential growth substrates can be envisioned in the development of consortia in the gut ecosystem (2, 3, 7). However, we feel that overall, transcriptomics studies as well as functional studies of consortia, such as we have developed, have the potential to dissect important mechanisms and interactions that take place among bacteria sharing the same meal at the same table (commensalism) (7, 9–11).

MATERIALS AND METHODS

Screening bacterial species for growth on barley β-glucan and β-glucan-derived oligosaccharides. Purified (1→3),(1→4)-β-D-glucan (β-glucan) was prepared from Glucagel (DKSH, Italy) (60). A total of 49 bacterial species (Table 1) commonly present as members of human gut microbiotas (13, 14) were tested for their ability to grow in medium containing barley β-glucan. Individual bacterial strains were cultured under anaerobic conditions for 18 h at 37°C following DSMZ (https://www.dsmz.de/) and ATCC (https://www.atcc.org/) protocols. The screening assay was performed in basal medium (55) containing 2 g/liter of β-glucan. Media were sterilized by autoclaving (121°C for 15 min), prereduced in an anaerobic glove box, and inoculated (1% [vol/vol]) with individual bacterial cultures. Following our previously published procedure (55), optical density (A_{600}) of cultures was measured after 24 h of anaerobic incubation at 37°C, when supernatants from the cultures were collected and stored at −80°C (55). Unpaired *t* test with Welch's correction (GraphPad Prism version 7.0b) was used to compare optical density values of bacterial cultures grown in the presence or absence of β-glucan.

Oligosaccharide medium was prepared from β-glucan medium inoculated (1% [vol/vol]) with *Bacteroides ovatus* ATCC 8483^T culture. After anaerobic incubation at 37°C for 24 h, supernatant was collected by centrifugation at 4,720 × *g* for 20 min at 4°C, pH was adjusted to 6.8 (to match that of basal medium), sterilized by filtration (0.22 μm pore size), and stored at −20°C. The tri- and tetrasaccharides in oligosaccharide medium were present at 0.45 to 0.5 mg/ml, as estimated by comparison of the peak areas of tri- and tetrasaccharides of a standard lichenase digest of barley β-glucan. Bacterial species that did not grow in β-glucan medium were tested for growth after 48 h in oligosaccharide medium following the procedure described above (Table 2).

Pure culture experiments. The temporal pattern of degradation of β-glucan by *B. ovatus* and utilization of β-glucan-derived oligosaccharides by *Subdoligranulum variabile* DSM 15176^T and *Hungatella hathewayi* DSM 13479^T in batch cultures was followed over a 26-h period after inoculation of the medium. *B. ovatus*, *S. variabile*, and *H. hathewayi* cultures were grown for 18 h at 37°C and used to inoculate (1% [vol/vol]) prereduced and prewarmed medium. Sample collection and optical density measurements (A_{600}) were performed every 2 h. Then, 1-ml aliquots of cultures were centrifuged at 14,500 × *g* for 5 min at 4°C to collect bacterial cells and supernatant. Bacterial cell pellets were immediately resuspended in 1 ml of RNAprotect Bacteria Reagent (Qiagen) and stored at −80°C (7, 61, 62), and supernatants were also stored at −80°C. Two technical replicates and three biological replicates were carried out for each time course experiment.

Coculture experiments. Batch cocultures containing combinations of *B. ovatus*, *S. variabile*, and *H. hathewayi* were used to assess the ability of the species to grow together. *B. ovatus*, *S. variabile*, and *H. hathewayi* cultures were grown separately, anaerobically for 18 h at 37°C. The individual bacterial cultures were used to inoculate (1% [vol/vol]; equal proportions of each strain) prereduced and prewarmed (37°C) β-glucan medium in the following combinations: *B. ovatus* and *S. variabile*; *B. ovatus* and *H. hathewayi*; and *B. ovatus*, *S. variabile*, and *H. hathewayi*. The cocultures were sampled over a 26-h period of incubation, and 1-ml aliquots were centrifuged to collect cells and supernatant as described above. Two technical replicates and two biological replicates were carried out for each experiment.

Continuous (steady-state) culture. Continuous culture experiments were carried out in chemostats using cocultures of *B. ovatus*, *H. hathewayi*, and *S. variabile*. β-glucan medium was used in chemostat experiments which were run as described previously (7). This was in order to further demonstrate the persistence of the consortium in the longer term. In brief, the reactor vessel (chemostat) contained 30 ml of medium, and flow rate of 3 ml/h was controlled by peristaltic pumps. The chemostat was inoculated with 200 μl of a mixed culture of *B. ovatus*, *H. hathewayi*, and *S. variabile*. The mixed culture inoculum consisted of a batch culture of the three bacterial species grown together for 18 h at 37°C in anaerobiosis in β-glucan medium. Chemostat cultures were sampled after steady-state was reached (five complete changes of chemostat volume; 7). Chemostat experiments were run three times. Measurement of optical density of cultures and harvest of cells and supernatants was as described above.

Determining CFU/ml. To determine CFU/ml of each bacterial species in cocultures, samples were serially diluted in 10-fold steps to 10^{-6} in prereduced basal medium. Aliquots were plated on brain heart infusion (Difco) agar supplemented with yeast extract (5 g/liter), L-cysteine (1 g/liter), vitamin K (0.5 ml/liter), hemin (5 mg/liter), resazurin (1 mg/liter), and Tween 80 (0.1% [vol/vol]) (BHISA) for *B. ovatus* and *H. hathewayi* enumeration according to their different colony morphologies. BHISA supplemented with tetracycline (16 μg/ml) was used to determine the CFU/ml of *S. variabile*. Plates were incubated for 48 h, or 5 days for BHISA plates supplemented with tetracycline, at 37°C in anaerobiosis before colony enumeration and CFU/ml calculation.

Carbohydrate analysis of culture supernatants. Oligosaccharides that accumulated in culture supernatants were detected by high-performance anion-exchange chromatography (HPAEC) as described previously (55). Identification of DP3 and DP4 oligosaccharides was by reference to standards

as described previously (53). The rate of utilization of DP3 and DP4 was calculated by plotting the amount (%) of oligosaccharide remaining at each time interval, relative to uninoculated medium, in pure cultures of *S. variabile* and *H. hathewayi* and then by determining the slope of the regression curve (percentage/h).

Whole-transcriptomics analysis (RNAseq). Procedures were the same as those used in our previous studies (7, 61, 62). In summary, RNA was extracted from bacterial cells collected from the biological replicates from each culture. Total RNA purification was performed as described previously, standardized by using total RNA at 10 μg/μl from each sample for sequencing (7, 61, 62). RNA samples were sequenced at the Otago Genomics Facility (Dunedin, New Zealand) using the Illumina HiSeq 2500 platform. For RNA-seq analysis, reads were mapped with Rockhopper version 2.0.3 (61, 62) to the reference genomes *B. ovatus* ATCC 8483^T (NCBI RefSeq accession assembly number GCF_001314995.1, complete genome), *S. variabile* DSM 15176^T (NCBI RefSeq accession assembly number GCF_000157955.1, 11 scaffolds), and *H. hathewayi* DSM 13479^T (NCBI RefSeq accession assembly number GCF_000160095.1, 714 scaffolds). Reads from coculture samples were mapped onto the individual reference genomes using a highly stringent cutoff (no mismatches allowed). Reads mapping on rRNA and tRNA were excluded from subsequent analysis to avoid cross-mapping between genomes (63). The number of mapped reads for each replicate is shown in Table S1. Pearson's correlation coefficient was calculated between biological replicates using the R package Hmisc (http://biostat.mc.vanderbilt.edu/Hmisc). EdgeR package version 3.16.5 (64, 65) was used to normalize the raw reads and determine differentially transcribed (DT) genes using the quasi-likelihood F-test. Multiple testing correction was performed using the Benjamini-Hochberg method. Transcripts with counts per million (CPM) less than 0.5 in at least 2 samples were discarded. Genes were considered significantly differentially expressed when the absolute $\log_2$ fold change value in the comparisons was >1 and the false-discovery rate (FDR) value was <0.05. In pure cultures, comparisons were performed between all time points. In coculture, pairwise comparisons between coculture and pure culture at 24 h were carried out.

Gene Ontology (GO) term enrichment analysis for each bacterial species was carried out using GOseq (7, 66). Custom-made category mapping files were prepared to link *B. ovatus*, *S. variabile*, and *H. hathewayi* gene names to the associated GO terms (7). Additional functional enrichment analyses were carried out using eggNOG-mapper (67, 68) based on eggNOG 4.5 orthology data (69, 70). Enzyme nomenclature (EC) numbers were retrieved from the Carbohydrate-Active enZYmes Database (http://www.cazy.org/) or from the ExplorEnz database (https://www.enzyme-database.org/).

Reverse transcription quantitative PCR (RT-qPCR) was performed to validate transcription levels of selected target genes. Primer design and optimization (Table S3), reverse transcriptase reaction, and qPCR were carried out as described previously (7, 61). Two biological replicates and six technical replicates were assessed for each condition. The transcription levels of target genes were calculated by comparative C_T method (71). Transcription levels of selected target genes for each strain were confirmed by RT-qPCR analysis (Table S4).

Data availability. Transcriptomics data are available from NCBI BioProject accession number PRJNA531520.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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