Microbial liberation of \(N\)-methylserotonin from orange fiber in gnotobiotic mice and humans

Highlights

- Host-inaccessible compounds in discarded plant fibers are released by gut microbes
- Liberation of orange-fiber \(N\)-methylserotonin shows species/strain-level specificity
- \(N\)-methylserotonin affects adiposity, metabolism, and gut motility in gnotobiotic mice
- \(N\)-methylserotonin and bacterial CAZymes are correlated in orange-fiber-fed humans

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In brief

Potential value from food waste: gut microbes can release host-inaccessible, bioactive compounds from discarded plant fibers.
Microbial liberation of \(N\)-methylserotonin from orange fiber in gnotobiotic mice and humans

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SUMMARY

Plant fibers in byproduct streams produced by non-harsh food processing methods represent bio-repositories of diverse, naturally occurring, and physiologically active biomolecules. To demonstrate one approach for their characterization, mass spectrometry of intestinal contents from gnotobiotic mice, plus \textit{in vitro} studies, revealed liberation of \(N\)-methylserotonin from orange fibers by human gut microbiota members including \textit{Bacteroides ovatus}. Functional genomic analyses of \textit{B. ovatus} strains grown under permissive and non-permissive \(N\)-methylserotonin “mining” conditions revealed polysaccharide utilization loci that target pectins whose expression correlate with strain-specific liberation of this compound. \(N\)-methylserotonin, orally administered to germ-free mice, reduced adiposity, altered liver glycogenesis, shortened gut transit time, and changed expression of genes that regulate circadian rhythm in the liver and colon. In human studies, dose-dependent, orange-fiber-specific fecal accumulation of \(N\)-methylserotonin positively correlated with levels of microbiome genes encoding enzymes that digest pectic glycans. Identifying this type of microbial mining activity has potential therapeutic implications.

INTRODUCTION

Identifying the products of metabolism of dietary components by members of human gut communities and determining how these products mediate microbe-microbe and microbe-host interactions holds the promise of generating new approaches for modulating host biology in ways that improve health status (e.g., Wang et al., 2011; Everard et al., 2013; Cohen et al., 2017; Guo et al., 2017; Chen et al., 2019). Dietary fibers exemplify this point. Fibers are chemically complex; they include but are not limited to structurally diverse polysaccharide components, proteins, and lipids (Macagnan et al., 2016; Capuano, 2017). The association between increased consumption of dietary fiber and improved health status is widely recognized (Kendall et al., 2010; Brownlee, 2011; Turner and Lupton, 2011; Dinggra et al., 2012; Taylor et al., 2021). Some of the underlying mediators and mechanisms are well known. For example, short-chain fatty acids produced by microbial metabolism of otherwise indigestible plant polysaccharides have been linked to beneficial health outcomes (den Besten et al., 2013; Rios-Covia et al., 2016; Dalile et al., 2019). The gut microbiota affects the bioavailability of (poly)phenolic compounds contained in dietary fiber by metabolizing them to smaller bioactive products (Parkar et al., 2013; Juárez et al., 2017; Williamson and Clifford, 2017; Gil-Sánchez et al., 2018).

Population growth, the existential threat posed by climate change, and associated challenges to environmental sustainability have focused attention on food production—this includes management of the massive amount of inorganic as well as organic “waste” generated during food manufacture. Fibers are well represented in many of these manufacturing streams, for example, in the peels, rinds, and seeds discarded from...
different fruits and vegetables. The composition of the fibers present in these byproducts reflects their differing sources as well as the various mechanical, physical, and chemical steps applied during food processing. In addition to their polysaccharide components, fibers represent a potentially enormous biorepository of unknown or largely uncharacterized natural molecular entities that could have health-promoting effects. Defining these molecular species would address a major gap in our understanding of fiber. Moreover, these streams could represent a sustainable and scalable source for such compounds when they are identified. For example, ~140 million tons of citrus were produced worldwide in 2020 (FAO, 2021). Almost half of the total weight of industrially processed fruits are used in the production of juices, yielding tens of millions of tons of citrus waste annually (Leporini et al., 2021; Russo et al., 2021). Disposal of this waste is challenging—low pH and high concentrations of organic compounds make it difficult to manage biologically, with improper disposal leading to the destruction of soil or aquatic ecosystems (Calabrò et al., 2016; Sharma et al., 2017; Zema et al., 2018) while its high content of water makes incineration inefficient (Satari and Karimi, 2018; Wei et al., 2017). One strategy for enhancing the value of citrus waste is through the extraction of commercially important compounds including essential oils, flavonoids, pectins, and dietary fibers (Mahato et al., 2018; Zema et al., 2018).

The current study illustrates an approach for harnessing gut microbes to identify chemical entities naturally contained within fiber preparations emanating from such manufacturing streams, and then defines the effects of one such entity on host physiology, characterizing mechanisms underlying its “mining” by gut bacteria, and determining whether results obtained from preclinical models translate to humans. We previously used gnotobiotic mice colonized with defined consortia of cultured human gut bacterial strains to characterize the effects of adding different dietary fiber preparations to a high saturated fat, low fruit, and vegetable diet (HiSF-LoFV) formulated based on the National Health and Nutrition Examination Survey (NHANES) database of diet consumption patterns by people living in the United States (Rидaura et al., 2013). These mice were used to characterize mechanisms by which gut bacteria compete or cooperate in utilizing specific glycan structures present in these fiber preparations (Pathode et al., 2019; Wesener et al., 2021). We now use gnotobiotic mice colonized with defined consortia of human gut bacterial taxa that were fed this HiSF-LoFV diet, with or without an orange fiber (OF) byproduct of juice manufacture. The results revealed microbe-dependent release (mining) of a compound from the fiber preparation that we identified as N-methylserotonin. The effects of N-methylserotonin on host metabolism, and gene expression in the intestine and liver, were characterized by adding it to drinking water consumed by germ-free animals. Mechanisms underlying N-methylserotonin release were delineated in vitro, initially with 49 phylogenetically diverse human gut bacterial strains, and then by performing functional genomic analyses under different media conditions using 12 different strains of Bacteroides ovatus, a prominent miner in vivo. Finding that B. ovatus mining activity could be regulated by the addition or subtraction of a single component (hemin) from one of the media tested led to the discovery that strain-specific expression of genes involved in the metabolism of pectic glycans in the fiber preparation correlated with the liberation of N-methylserotonin. The administration of an OF-containing snack food prototype, and separately, a control pea fiber-containing snack to adult female dizygotic twins in two open-label, single-group assignment studies demonstrated a dose-dependent, fiber-specific relationship between levels of N-methylserotonin in feces and changes in the representation of bacterial genes encoding glycoside hydrolases (GHs) and polysaccharide lyases (PLs) that break down pectic glycans. Although our study focused on one chemical entity, N-methylserotonin, the approach we describe may be generally useful for identifying components of fibers whose liberation under normal physiological conditions requires microbial activity but whose biological/pharmacological activities are not dependent on further microbial biotransformation.

RESULTS

A gnotobiotic mouse model reveals human gut bacterial liberation of N-methylserotonin from OF

As a starting point for characterizing the liberation of fiber-associated bioactive constituents by gut bacterial taxa, we selected a commercial, food-grade source of OF (see STAR Methods) derived from the byproducts of the juicing process—these by-products include pulp cells, juice vesicles, segment membranes, rag/core, and peel that are mechanically processed (washed with water, heated, dewatered, and sheared) prior to drying. Importantly for the purpose of our experiments, the preparation had not been subject to chemical treatment or extraction; therefore, any proteins, lipids, and small molecules that are not removed by washing with water are retained in the preparation (see Tables S1A and S1B for composition and glycosidic linkage analysis of constituent polysaccharides).

Two groups of adult C57BL/6J germ-free mice were colonized with a 14-member consortium of sequenced human gut bacterial strains and monotonously fed the HiSF-LoFV diet ad libitum, with or without supplementation with 10% (w/w) OF, for 21 days (i.e., the supplemented formulation was composed of the base HiSF-LoFV diet [90% by weight] plus OF [10% by weight]). Two other groups of mice were maintained as germ-free—mice in one of these groups were fed the unsupplemented HiSF-LoFV diet, while those in the other group consumed the 10% OF-supplemented diet (n = 5 animals/treatment group).

Untargeted liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Qtof-MS) of cecal contents harvested at the time of euthanasia revealed 116 features (m/z) that were increased at least 3-fold in the cecal contents of colonized mice consuming the OF-supplemented diet compared with the other three experimental groups (Table S2). A prominent feature with an m/z of 191.1186 was present at high abundance only in colonized mice fed the OF-supplemented diet (Figure 1A). This feature was tentatively identified as methylserotonin, with its major fragment (m/z 160.0760) consistent with methylation of its alkyl amine. Subsequent LC-Qtof-MS/MS co-characterization with a known standard confirmed that this compound was N-methylserotonin (Figure 1B). N-methylserotonin has been previously identified in several plants, including black cohosh (Powell et al., 2008; Nikolić et al., 2014), Japanese pepper (Yanase et al., 2010), and citrus...
fruits (Servillo et al., 2015). There is very limited information on whether this compound has beneficial or potentially detrimental physiologic effects (e.g., Takeda, 1994; Zhang et al., 2018).

Assaying N-methylserotonin content in major global food staples and citrus fibers

Reasoning that the microbial disruption of complex polysaccharides in fibers might be needed to liberate sequestered N-methylserotonin, we performed a set of experiments where 50 mg of OF was incubated separately with 13 commercially available GH preparations (see STAR Methods). The greatest amount of N-methylserotonin was recovered when OF was incubated with a preparation containing a mixture of cellulases from Trichoderma reesei whose broad cellulolytic activity encompassed many of the cellulases and hemicellulases tested individually (total yield; 2,728 ± 26 ng/50 mg OF; Table S1C). By comparison, when OF (50 mg) was subjected to repeated rounds of extraction with methanol, only small quantities of N-methylserotonin were released after each round (31 ng after two rounds and 171 ng in total after 15 rounds; Table S1D). Similarly, low yields were obtained in separate experiments using acetonitrile or acetone (30–31 ng after two rounds of extraction). When 2-methyserotonin, a compound structurally similar to N-methylserotonin was “spiked” into the OF preparation, 95% of it was removed in the first cycle of extraction with methanol, and all of the remaining was removed by the third cycle (Table S1D). These latter observations provide additional support for the notion that N-methylserotonin is “trapped” within the OF.

Figure 1. Colonization and orange-fiber-dependent accumulation of N-methylserotonin in the intestines of gnotobiotic mice

(A) Cecal contents were harvested from germ-free or colonized mice fed either an unsupplemented high saturated fat/low fruits and vegetable (HiSF-LoFV) diet or the HiSF-LoFV diet supplemented with 10% OF and were analyzed by LC-Qtof-MS. The analyte with an m/z of 191.1180 was only found in colonized animals consuming the orange-fiber-supplemented diet. Chromatograms representative of five biological replicates for each treatment group are shown.

(B) Collision-induced dissociation mass spectra of an N-methylserotonin standard (upper portion of the panel) and cecal extracts (lower portion of the panel) obtained by LC-Qtof-MS/MS.

(C) Levels of N-methylserotonin released after a 72 h incubation of each of 14 bacterial strains with OF in TYG medium. Mean values ± SD per 50 mg of OF are shown. See also Tables S1, S2, and S4.
We subsequently added the T. reesei-derived cellulase mixture to 133 different samples of edible plants (each sample assayed in triplicate) (Table S1E). These included major global food staples such as corn, wheat, rice, and cassava (FAO, 2021) as well as commonly consumed fruits and vegetables in the United States (Davis and Lucier, 2021; Kramer et al., 2022). N-methylserotonin was only detected in samples from three sources, all of which are peppers belonging to Zanthoxylum, a genus in the Rutaceae family that includes citrus fruits. These sources consisted of two types of the Japanese mountain pepper (Z. piperitum), which was previously reported to contain N-methylserotonin (Yanase et al., 2010), and the Chinese Sichuan pepper (Z. bungeanum). It was not detected in various types of hot chili peppers, bell peppers (members of Solanaceae), or in common black pepper (a member of Piperaceae).

We performed a follow-up screen of 23 commercially available citrus fibers. The in vitro enzymatic liberation assay revealed that N-methylserotonin was present in all 23 preparations, albeit at widely varying levels (Table S1F). The OF preparation used in our initial gnotobiotic mouse experiment yielded the largest quantity of N-methylserotonin. The method used to manufacture the OF preparation had a marked effect on N-methylserotonin yields—the amount of N-methylserotonin released was 85% ± 1% (mean ± SD) lower in a finely processed counterpart of the same OF (422.8 ± 33.8 ng; p < 0.0001 compared with coarse fiber; unpaired t test).

Based on these results, we repeated the in vivo experiment but now compared different groups of mice colonized with the 14-member consortium and fed the HiSF-LoFV diet supplemented with 10% OF or with 10% pea fiber. The latter was a natural food-grade commercial preparation consisting of insoluble and soluble fibers as well as resistant starch (see STAR Methods and Table S1A for composition). Given that (1) the in vitro enzyme liberation assay had disclosed that levels of N-methylserotonin in the pea fiber preparation were below the limits of detection (Table S1F), and (2) this preparation was available in sufficient quantities for manufacturing diets, we used it as a “control” fiber source for studies of the specificity of microbial N-methylserotonin mining. Targeted liquid chromatography-triple quadrupole mass spectrometry (LC-QqQ-MS) revealed that N-methylserotonin was present at markedly higher levels in cecal and colonic contents and tissues of mice consuming the OF-supplemented diet compared with levels in their small intestine, liver, gastrocnemius muscle, and kidney (173 ± 14 ng/g [mean ± SD] [cecal contents]; 130 ± 13 ng/g [cecal tissue]; 139 ± 13 ng/g [colonic contents]; 164 ± 25 ng/g [colonic tissue]; <1 ng/g [small intestine, liver and kidney]); and 1.22 ± 0.76 ng/mL [plasma]). N-methylserotonin was below the limits of detection (0.5 ng/g) in cecal or colonic contents, nor in any of these intestinal and extra-intestinal tissues harvested from mice consuming the pea fiber-supplemented diet. These findings led us to determine what effects N-methylserotonin might have on host physiology.

**Host effects of N-methylserotonin**

To explore the host effects of N-methylserotonin, we first examined whether it was metabolized in mice colonized with the 14-member bacterial consortium and fed the OF-supplemented HiSF-LoFV diet or a control unsupplemented diet. Non-targeted LC-Qtof-MS and targeted LC-QqQ-MS analysis revealed no statistically significant differences (p > 0.05; unpaired t test) in the levels of serotonin, dimethylserotonin, trimethylserotonin, 5-hydroxyindoleacetic acid, tryptamine, N-methyltryptamine, N,N-dimethyltryptamine, tryptophan, bufotenin or melatonin in small intestinal, cecal and colonic tissue, or liver obtained from animals consuming the unsupplemented versus supplemented HiSF-LoFV diets. These results indicated that the mice were unable to metabolize N-methylserotonin, at least to these products in the tissues examined.

In follow-up experiments, we fed 12-week-old germ-free C57BL/6J mice the unsupplemented HiSF-LoFV diet and administered N-methylserotonin via their drinking water at doses of 1 mg/kg/day or 50 mg/kg/day for 21 days (Figure 2A). The rationale for this design was to examine the effects of N-methylserotonin independent of any potential contributions from other OF components. The 1-mg/kg/day dose was selected because we found that this dose produced fecal N-methylserotonin levels that were equivalent to those documented in mice colonized with the 14-member community consuming the OF-supplemented HiSF-LoFV diet (133.5 ± 15 ng/g versus 131 ± 19-ng/g feces, respectively; p = 0.92, unpaired t test). The 50-mg/kg/day dose was selected because it was equivalent to the estimated total amount of N-methylserotonin consumed each day in the 10% OF-containing diet (based on the yield obtained after in vitro enzymatic digestion of the fiber with T. reesei cellulase). A control group of germ-free animals did not receive any N-methylserotonin. Food and water intake were measured daily and remained similar throughout the experiment among all three groups of mice.

Although a statistically significant decrease in weight gain was observed with N-methylserotonin treatment, interpreting this result is confounded by the abnormally large contribution of the cecum to body weight in germ-free mice (Figure 2B). However, compared with untreated controls, oral administration of N-methylserotonin resulted in a statistically significant reduction in epididymal fat mass at the higher but not the lower dose (Figure 2C).

The higher but not the lower dose of N-methylserotonin also produced a statistically significant increase in liver glycogen (Figure 2D), statistically significant decreases in its metabolic precursors, uridine, and uridine monophosphate (Figures 2E and 2F), and a statistically significant decrease in liver glucose-6-phosphate (Figure 2G)—the latter is a key metabolic intermediate, formed from either glycogenolysis or gluconeogenesis, that is known to directly impact levels of glycogen in the liver (Van Schaftingen and Gerin, 2002). Based on these results, we used untreated control animals and those that received 50 mg/kg/d of N-methylserotonin to characterize gene expression in the liver and colon.

A total of 716 genes exhibited statistically significant differences in their expression in the livers of N-methylserotonin-treated compared with untreated germ-free mice (false discovery rate [FDR]-adjusted p value < 0.05; see STAR Methods and Tables S3A and S3B). Gene set enrichment analysis and over-representation analysis (STAR Methods) of all statistically significant differentially expressed genes revealed that GO Biological Pathway terms pertaining to circadian rhythm and fatty acid...
metabolism were the most significantly enriched (Figure S1). Effects of N-methylserotonin on circadian rhythm-related genes included significantly decreased expression of Arntl and Clock (Rudic et al., 2004; Dang et al., 2016) and lipogenesis (Zhang et al., 2018). Consistent with this observation, there were significant increases in the expression of their regulators Per2, Per3, and Nr1d2. Per2 can promote glycogen synthesis (Zani et al., 2013). Moreover, genetically engineered disruption of Per3 is associated with resistance to leptin with resulting weight gain (Kettner et al., 2015), while its deletion directly leads to increased adipogenesis (Costa et al., 2011; Aggarwal et al., 2017). Nr1d2 acts as a repressor of Nfil3 (Yu et al., 2013), and its statistically significantly increased expression with N-methylserotonin treatment is associated with statistically significantly decreased hepatic levels of Nfil3 mRNA. Nfil3 serves as an important link between the gut microbiota, intestinal epithelial lipid metabolism, and body composition. Nfil3 expression exhibits microbiota-modulated diurnal oscillation in epithelial cells via group 3 innate lymphoid cells, Stat3, and epithelial clock components, with accompanying changes in epithelial lipid absorption and export. Moreover, genetic ablation of Nfil3 attenuates high fat diet-induced obesity in mice (Wang et al., 2017).

Seven hundred forty-eight genes exhibited statistically significant differences in their expression in the colonic tissue of N-methylserotonin-treated versus untreated mice (FDR-adjusted p value < 0.05; Tables S3C and S3D). They include Nr1d2, Per3, Per2, Arntl, and Clock. In vitro studies have indicated that N-methylserotonin binds to various serotonin (5-hydroxytryptamine) G protein-coupled receptors, including 5-Htr7 (Powell et al., 2008) and 5-Htr2A (Hajduch et al., 1999). RNA-seq analysis of the colon did not reveal any statistically significant effects of N-methylserotonin administration on the colonic expression of its known (Htr7 and Htr2A) or related (Htr3, Htr4, Htr5, and Htr6) receptors.

Glutamate levels were significantly higher in colonic tissue harvested from germ-free mice receiving 50 mg/kg/day of N-methylserotonin compared with untreated controls (21 ± 1.4 versus 11 ± 0.9-ng/mg tissue, respectively; p < 0.01, unpaired t test). Glutamate is known to promote the degradation of Arntl when directly applied to tissue slices (Tamaru et al., 2000). Knockout of the Per3 homolog Per2 reduces the expression of the glutamate transporter (Eaat1 and Slc1A3) and uptake of glutamate in the brain (Spanagel et al., 2005). These observations raise the possibility that one way that N-methylserotonin might influence colonic circadian regulators is through its effects on tissue glutamate levels.

Circadian rhythm-related genes are known to be expressed in the myenteric plexus, which coordinates colonic motility (Hoo- genwerf et al., 2010). We used orally administered carmine red to determine the gastrointestinal transit time on day 17 of the

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**Figure 2. Effects of orally administered N-methylserotonin in germ-free mice**

(A) Experimental design. Groups of adult germ-free mice consumed the HiSF-LoFV diet ad libitum. Animals received one of two doses (1 and 50 mg/kg/day) of N-methylserotonin in their drinking water for 21 days.

(B) Percent change in body weight between experimental days 1 and 21.

(C) Epidydimal fat pad weight at the time of euthanasia.

(D–G) Metabolites related to glycogen biosynthesis measured in the liver at euthanasia.

(H) Transit time through the gastrointestinal tract of germ-free mice measured on day 17. Mean values ± SD are shown in (B–H). **p < 0.01; ***p < 0.001; ****p < 0.0001 (one-way ANOVA). The colors used to denote the treatment groups in (B–H) are keyed to the colors employed in (A). Each dot represents results from a single animal. An open or closed circle indicates membership in one or the other of two independent gnotobiotic mouse experiments. n = 4–5 mice/treatment group/experiment.

See also Figure S1 and Table S3.
21-day experiment in germ-free mice whose drinking water was supplemented with 1 mg/kg/day or with 50 mg/kg/day of N-methylserotonin. The assay revealed that both doses of N-methylserotonin produced comparable, statistically significant reductions in transit time (i.e., increased motility) compared to the untreated control group (p < 0.0001, one-way ANOVA; Figure 2H). We repeated the experiment, administering unsupplemented drinking water or water containing the 50 mg/kg/day dose (n = 5 animals/group) and found that changes in host physiologic and metabolic features were comparable with those documented in the first experiment (Figures 2B–2H). These results encouraged us to investigate whether it would be possible to manipulate levels of N-methylserotonin in vivo by changing the composition of the defined community.

**Bacterial strains capable of mining N-methylserotonin from OF**

**In vitro assays of N-methylserotonin liberation by individual members of the defined consortium**

Given that the detection of N-methylserotonin was dependent on colonization with the bacterial consortium and consumption of OF, we investigated which community members were responsible for its appearance. Each of the 14 community members was grown in monoculture to stationary phase in TYG medium, and 10^6 cells of each organism were incubated in 10 mL of a 5-mg/mL suspension of OF for 8, 24, 48, 72, and 168 h. The N-methylserotonin released into the growth medium was quantified using targeted LC-QqQ-MS. N-methylserotonin rose from levels that were not significantly above background at the 8 h time point (background determined by measurements of control incubations containing sterile TYG medium) to levels that reached a maximum at the 72 h time point. At this time point, _Bacteroides ovatus_ strain TSDC 17.2 and a strain of _Parabacteroides distasonis_ yielded the highest quantities (29 ± 1 and 24 ± 2 ng N-methylserotonin/10^6 cells, respectively). By contrast, the 12 other strains yielded ≤1.5 ng/10^6 cells—amounts that were not appreciably higher than background levels (triplicate incubations/organism; Figure 1C). Cultures grown in Wilkins-Chalgren anaerobe broth yielded similar results to those obtained with TYG medium (Table S4A), whereas testing each of these 14 organisms in another nutrient-rich medium (MEGA medium 2.0; Romano et al., 2015) resulted in N-methylserotonin levels ranging from 5 to 25 ng/10^6 cells for _P. distasonis_, _Bacteroides finegoldii_, and _Collinsella aerofaciens_ (Table S4A). Given its capacity to support the growth of a number of cultured anaerobic gut bacterial taxa, we screened 24 other phylogenetically diverse human gut bacterial strains in Wilkins-Chalgren anaerobe broth containing 5 mg/mL of OF, and none yielded amounts of N-methylserotonin significantly above background (≤0.5 ng/10^6 cells) (Table S4B).

Several other experiments were performed to characterize _in vitro_ N-methylserotonin liberation by members of the 14-strain consortium. Adding (1) 10^6 cells of either _B. ovatus_ TSDC 17.2 or the _P. distasonis_ strain that had been grown to stationary phase in TYG medium and then heat-killed, or (2) lysates prepared by bead-beating of 10^6 cells harvested from monocultures of each organism in TYG, or (3) conditioned medium harvested from stationary-phase TYG monocultures of each organism to fresh TYG with OF for 72 h failed to yield levels of N-methylserotonin above background (Table S4C). These data indicate that mining requires intact, viable cells. When N-methylserotonin was added to monocultures of the 14 strains that had been grown to stationary phase in TYG medium without OF, no appreciable degradation was observed over a 72 h period (97% ± 2% of input N-methylserotonin remaining intact/unmodified; Table S4D). We also obtained evidence that _B. ovatus_ TSDC 17.2 was not capable of synthesizing N-methylserotonin. A homology-based search of the bacterial genome failed to reveal gene candidates involved in serotonin biosynthesis and metabolism. Moreover, when we cultured the organism in TYG medium supplemented with either tryptophan, tryptamine, serotonin, dimethylserotonin, trimethylserotonin, methyltryptamine, or S-adenosyl methionine, N-methylserotonin was not detected above background after either 24 or 72 h incubations. Incubating this strain in TYG medium supplemented with either pea fiber or two other commercial dietary fiber preparations (apple pectin or oat beta glucan) also did not yield detectable levels of N-methylserotonin.

**In vivo assays of N-methylserotonin liberation by all or subsets of the bacterial consortium**

Based on these _in vitro_ findings, we proceeded to colonize three groups of adult C57BL/6J germ-free mice with either (1) a 4-member consortium of strains with _in vitro_ mining activity (_B. ovatus_, _P. distasonis_, _B. finegoldii_, and _C. aerofaciens_), (2) the full 14-member consortium, or (3) the 10 “non-mining” strains from the 14-member consortium (n = 5 animals/group). 3 days after gavage, animals were switched from the unsupplemented HiSF-LoFV diet to a HiSF-LoFV diet supplemented with 10% (w/w) OF. This diet was then administered _ad libitum_ for 21 days (Figures 3A and 3B). Short-read shotgun sequencing of DNA isolated from fecal samples collected at the time of euthanasia revealed that all strains in each consortium were able to colonize recipient animals (see Table S5 for their absolute abundances). The total biomass (bacterial genome equivalents/g feces) in mice harboring the 4-member consortium was 2-fold lower than in animals colonized with the 14-member consortium (p < 0.01; one-way ANOVA), whereas there was no significant difference in bacterial load between animals hosting the 10- and 14-member communities (p = 0.81, Figure 3C). Targeted LC-QqQ-MS analysis of fecal samples obtained at the time of euthanasia revealed that levels of N-methylserotonin in mice colonized with the 4-strain consortium were equivalent to those in animals harboring the full 14-member community and significantly higher than in mice colonized with the 10-member consortium (p = 0.002; one-way ANOVA; Figure 3D). Animals colonized with the 4-member consortium had a significantly lower weight gain and lower epididymal fat pad mass compared with mice colonized with the 10-member consortium (p = 0.007 and p = 0.001, respectively; one-way ANOVA). No significant differences in adiposity were noted between mice harboring the 4- and 14-member communities (Figures 3E and 3F). Mice colonized with the 4-member consortium also had a statistically significant reduction in gut transit time compared with animals containing the 10-member community (184 ± 32 min [mean ± SD] versus 319 ± 8 min, respectively; p < 0.0001, one-way ANOVA). The 14-member community was associated with
transit times (278 ± 14 min) that were also significantly shorter compared with mice with the 10-member community (p = 0.025) but still significantly longer than in mice harboring the 4-member community (p < 0.0001) (Figure 3G).

Identifying bacterial genes involved in the release of N-methylserotonin from OF

We employed comparative genomics and functional genomics approaches to characterize the mechanisms underlying the release of N-methylserotonin from the OF preparation. We first compared the N-methylserotonin mining activity of B. ovatus TSDC 17.2 with 11 other human gut-derived strains of B. ovatus. All strains were grown on TYG medium and the protocol described above for assaying N-methylserotonin release from the OF was followed (i.e., 10^8 input bacterial cells/incubation containing 5-mg/mL OF; 72 h incubation; 3 replicate assays/strain). Compared with the control OF incubations lacking B. ovatus where the yield of N-methylserotonin was 1.5 ± 0.2 ng (mean ± SD), we detected N-methylserotonin in the growth medium of all strains. The levels varied between strains but each had mining activity that was significantly lower than that of TSDC 17.2 (one-way ANOVA, all p values < 0.0001). B. ovatus 115 had the lowest activity (2.8 ± 0.1 ng [mean ± SD] N-methylserotonin released/10^6 cells compared with 33.3 ± 2.8 ng/10^6 cells for TSDC 17.2 [Figure 4A; Table S4E]).

As noted above, several of the bacterial taxa tested exhibited mining activity that was dependent upon the growth medium used. We tested whether hemin, a component of TYG and known regulator of gene expression in Bacteroides species (Andrews et al., 2003), was essential for N-methylserotonin release. Although all strains grew to comparable densities in TYG with or without hemin, no N-methylserotonin release occurred when these cells were added to reactions containing fresh hemin-deficient TYG plus OF (controls; incubations containing TYG with or without hemin but lacking OF; Figure 4B; Table S4F).

To identify the genes involved in N-methylserotonin release, we sequenced the genomes of all 12 B. ovatus strains and annotated all of their known or predicted encoded proteins. We performed microbial RNA-seq analysis of gene expression in B. ovatus TSDC 17.2 and B. ovatus 115 grown under conditions identical to those used previously (72 h incubation with or without OF in TYG medium with or without hemin, or in MEGA medium).
We then compared the expression of genes in strain TSDC 17.2 under conditions that were either permissive for N-methylserotonin release (TYG medium with OF) or non-permissive (TYG containing OF but without hemin, MEGA medium with OF, and all media conditions without OF). We identified 133 genes that (1) exhibited a statistically significant \( \log_2 \) fold increase in expression under permissive conditions (Benjamini and Hochberg FDR-adjusted Wald test \( p \) value < 0.05) and (2) were either not significantly differentially expressed (FDR-adjusted \( p \) value > 0.1) or were significantly downregulated (FDR-adjusted \( p \) value < 0.05) under non-permissive conditions (see Table S6A for a list of these 133 genes plus Figure 4C).

Natural products are entrapped in dietary fiber through a variety of chemical and physical interactions (Palafox-Carlos et al., 2011; Quirós-Sauceda et al., 2014). As noted above, the OF preparation contained nearly 60% (w/w) uronic acid, with a prominent representation of homogalacturonan, rhamnogalacturonan, xylan, and arabinan structures (Table S1B). Bac- teroides species possess multiple polysaccharide utilization loci (PULs). These PULs encode proteins (SusC and SusD homologs) involved in the binding and import of various glycan structures as well as carbohydrate-active enzymes (CAZymes) that catalyze their degradation (GHs and PLs). Therefore, we used previously described methods (Terrapon et al., 2015, 2018) to identify PULs and CAZyme gene clusters present in B. ovatus strains TSDC 17.2 and 115. The results revealed that PUL conservation and synteny between the two strains are very high (Table S7).
Among the 133 genes with statistically significant differential expression in *B. ovatus* TSDC 17.2 under permissive conditions, those that manifested the most prominent induction were concentrated in PUL27, PUL28, and PUL29 and to a lesser extent in several other PULs (e.g., PUL4 and PUL13). Proteins encoded by these PULs exhibit >95% amino acid sequence identity with those in *B. ovatus* 115 and share orthologs in the other *B. ovatus* strains tested (Tables S6A and S7). Functional assignments for these proteins were made by identifying their best scoring alignments with the sequences of experimentally characterized CAZymes in the CAZY database (www.cazy.org) (Table S6B). The results disclosed members of CAZyme families with reported activities against the backbones of homogalacturonan (GH family 105 [unsaturated rhamnogalacturonyl hydrolase/unsaturated glucuronyl hydrolase]) or rhamnogalacturonan (PL9 and PL11 [rhamnogalacturonan lyase] and GH28 [RGI-specific α-galacturonidase]) ([Ndeh et al., 2017; Luis et al., 2018]). These structures are prominently represented in pectin and in our OF preparation (>50% of glycosyl linkages, Table S1B). The PULs also included CAZymes with predicted activities directed at oligosaccharides linked to these backbone structures (arabinofuranosidase [GH43_18], galactosidase [GH36], and aposi-dase [GH1140]).

Despite the high degree of PUL conservation between *B. ovatus* TSDC 17.2 and *B. ovatus* 115, almost none of their component genes were expressed in the latter strain under mining-permissive conditions (Table S7). In an attempt to define the origin of the observed differences in expression of these PUL genes, and by extrapolation, the discordant N-methylserotonin mining activities of strains TSDC 17.2 and 115, we reconstructed their potential transcriptional regulators using comparative genomics (Figure S2). PUL27, PUL28, and PUL29 form a large chromosomal cluster of 60–70 genes that encode 28 CAZymes, six SusC/SusD transport systems, and three paralogs of a previously characterized rhamnogalacturonan-specific regulator in *Bacteroides thetaiotaomicron*, Hybrid two-component system (HTCS). Rgu-2 ([Ravcheev et al., 2013]). HTCSs are single polypeptide chains comprised of a transmembrane sensor histidine kinase, a DNA-binding response regulator, and a carbohydrate sensing domain. The reconstructed HTCS_Rgu-2 regulon in *B. ovatus* strains includes 42 genes from PUL27, PUL28, PUL29, and PUL30, of which 30 were significantly upregulated (FDR-adjusted p value < 0.05) in the presence of OF (Table S7). However, all identified HTCS_Rgu-2 binding sites are highly conserved between the 17.2 and 115 strains, and the orthologous pairs of HTCS regulators are 98%–99% identical to each other, suggesting (1) conservation of this feature of the regulation of rhamnogalacturonan-I utilization loci between the two strains of *B. ovatus* and (2) that the observed difference in regulon expression is likely not ascribable to this HTCS alone.

**N-methylserotonin levels and microbiome CAZyme gene abundances in humans consuming fiber snack prototypes**

To assess the translatability of results obtained from these *in vitro* analyses and mouse models to humans, we analyzed fecal samples that had been obtained from participants in two 10-week open-label, single-group assignment studies. The studies involved OF- and pea fiber-supplemented snack food prototypes (the latter as an *N*-methylserotonin-deficient fiber control) and dizygotic twins 36.6 ± 2.9 years old (mean ± SD) recruited from the Missouri Adolescent Female Twin Study (MOAFTS) cohort ([Buchoz et al., 2000; Delannoy-Bruno et al., 2022]). The two studies shared the same design (see STAR Methods) with participants supplementing their normal, unrestricted diets with one or other snack food prototypes. In brief, consumption of the fiber snack prototypes escalated from none consumed during the first 2 weeks, to one snack per day during the third week, then to two servings a day during week 4, and finally, beginning in week 5, three snacks at which time the maximum daily dose of ~25–30 g per day of either pea fiber (study 1; n = 18 participants) or OF (study 2; n = 24 participants, including all 18 from study 1) was achieved. This dose level was then maintained for 4 weeks (see Table S8A for the composition of the snack prototypes and Table S8B for participant characteristics). Importantly, the orange and pea fiber preparations used for these human studies were obtained from the same commercial sources as those used in the preclinical studies. Therefore, analysis of fecal samples collected during these two studies, including from subjects who had participated in both, provided an opportunity to examine the relationship between features of their microbiomes and fecal levels of *N*-methylserotonin as a function of fiber consumption. Specifically, this study enabled us to assess whether mining was robust to different background diets, exhibited specificity for OF, and was dependent upon the amount of OF consumed.

*N*-methylserotonin was present in 98% of the 48 fecal samples obtained from participants consuming the OF snack prototype (Figure 5A; Table S8B) and its levels were significantly correlated with the number of OF snacks consumed per day (Pearson’s r = 0.72; p < 0.0001). Levels documented at the maximal dose were 72.5 ± 38.4 μM (mean ± SD) (based on converting fecal wet weight to volume and assuming 1 g equals 1 mL). To put this concentration in context, the reported binding affinity of *N*-methylserotonin to serotonin receptor subtype 5-HT1A is ~2 nM ([Powell et al., 2008]). Fecal serotonin levels were 0%–8.6% of those of *N*-methylserotonin (7 ± 5.7 μM; mean ± SD) and did not vary significantly with the dose of OF (Pearson’s r = −0.105, p = 0.381; one-way ANOVA p = 0.62) (Table S8B). In contrast, *N*-methylserotonin was undetectable (<0.26 nM) in 87% of the 36 fecal samples collected from individuals consuming the pea fiber snack prototype during the supplementation period (at week 3, when one snack per day was being consumed, and at the end of week 5, when the maximum dose was being administered) (see the legend to Table S8B regarding the four donors who had positive samples).

Neither the relative abundance of *B. ovatus* nor of any of the bacterial taxa (amplicon sequence variants [ASVs]) that exhibited statistically significant changes in their relative abundances in the fecal microbiota after OF and/or pea fiber snack consumption had statistically significant correlations with *N*-methylserotonin levels at the end of week 5 (Spearman correlation r < 0.30) (A statistically significant log2-fold change in the relative abundance of a taxon at week 5 compared with the pre-intervention period was defined by a q value < 0.1 [linear mixed effect model] [see Table S8C and Delannoy-Bruno et al., 2022]). Using shotgun sequencing datasets generated from fecal DNA samples collected at the end of weeks 1 and 5 of the OF study
we performed a Spearman correlation between (1) the abundances of 213 annotated CAZyme genes (GHs and PLs) that were present in at least one study participant at these time points and (2) the fecal levels of N-methylserotonin prior to fiber supplementation and at the end of week 5 (Table S8 D). CAZyme genes whose log2-fold changes in abundance were significantly correlated with levels of N-methylserotonin (q value < 0.1) are shown in Figure 5B. The strongest positive correlation was with PL9 (rhamnogalacturonan lyase; Spearman rho = 0.51, q value = 0.025) (Figure 5B; Table S8E)—a CAZyme whose expression was significantly upregulated in vitro under conditions permissive for mining (log2-fold change 1.2, FDR-adjusted p value [q] = 2.2 \times 10^{-4}, Figure 4C; Tables S6A and S7). The CAZyme gene with the second most positive correlation with levels of N-methylserotonin was GH5_37 (Spearman rho = 0.438, q = 0.08), which has reported specificity for \( \beta \)-glucan/cellulose (Aspeborg et al., 2012). It is notable that our in vitro experiments revealed that an enzyme preparation composed of cellulases and hemicellulases exhibited a high level of N-methylserotonin mining activity (Table S1 C). Other CAZymes that were significantly correlated...
with fecal N-methylserotonin levels included GH30_5 (Spearman rho = 0.44, q = 0.08) and GH59 (Spearman rho = 0.52, q = 0.02), which possess homogalacturonan/rhamnogalacturan processing functions or target pectin components such as galactans and arabinogalactans (Fujita et al., 2014; Kumar et al., 2019; Figure 5B). As was the case with PL9 and GH5_37, the abundances of genes encoding GH30_5 and GH59 increased significantly in the microbiomes of participants consuming the OF snack (a statistically significant log₂-fold change for a CAZyme gene was defined by a q value < 0.1 [linear mixed-effects model] and, using higher-order singular value decomposition, by its positioning at the tails [α < 0.1] of the distribution of CAZyme genes along tensor component 1) (Delannoy-Bruno et al., 2022). Taken together, the results of these human studies revealed an OF-specific, dose-dependent accumulation of N-methylserotonin in feces, where its concentration was positively correlated with the abundance of microbiome genes encoding CAZymes targeting pectic glycans.

**DISCUSSION**

Given the increasing quantities of byproducts generated during food manufacturing, it is important to consider how these waste streams can be better utilized. We have used gnotobiotic mice colonized with defined collections of human gut microbes, together with *in vitro* assays, to show that N-methylserotonin is present in certain preparations of OF generated during food manufacturing and that it can be released and thus rendered bioavailable to the host by specific members of the gut community. In germ-free mice, N-methylserotonin delivered in the drinking water, at a dose equivalent to that which was consumed daily in the form of our OF-supplemented, low fiber high saturated fat “Western” diet, produced effects on body composition and colonic motility. To provide evidence for the clinical translation of these findings, a pilot human study in adult dizygotic twins was performed—it revealed dose-dependent, OF-specific accumulation of N-methylserotonin in feces of study participants. In one sense, the OF preparation and its releasable N-methylserotonin may be viewed as a naturally occurring analog of polysaccharide-based drug delivery systems (Gopinath et al., 2018; Miao et al., 2018).

Many natural products are embedded in dietary fiber through various chemical and physical interactions, including hydrophobic interactions, hydrogen as well as covalent bonds, and/or physical entrapment (Palafax-Carlos et al., 2011; Qurós-Sauceda et al., 2014; Pereira et al., 2021). We propose the term “celobiotic” (from the Latin “conceal or disguise”) to describe a bioactive compound that is liberated from fibers through the actions of one or more microbial enzymes (rather than being synthesized) and whose biological/pharmacologic activities are not dependent upon additional microbial biotransformation. Our findings highlight the possibility that a given fiber type can release various small molecules along the intestine in an environmental and microbe-dependent manner—depending upon the molecule, such release could have beneficial or unwanted effects.

Several key results allowed us to decipher how N-methylserotonin could be liberated from the OF. We discovered a switch for turning mining activity on and off: *Bacteroides ovatus* TSDC 17.2, a prominent miner in our preclinical gnotobiotic mouse model, exhibited hemin-dependent release of N-methylserotonin *in vitro*. Pronounced *B. ovatus* strain-specific differences in N-methylserotonin release were observed under mining- permissive conditions; however, release by all strains was hemin dependent. Taking advantage of this hemin dependency and strain specificity, a comparison of gene expression between a strong versus a weak *B. ovatus* mining strain revealed a set of GH and PL genes associated with release. The known/predicted substrate specificities of enzymes encoded by these genes are consistent with the prominent representation of pectic polysaccharides present in the OF preparation. Furthermore, in the human study, fecal levels of N-methylserotonin correlated most significantly with the abundance of fecal microbiome PL and GH genes that are involved in the processing of glycan structures in pectins. Although the specific means of small molecule entrapment differ, it is noteworthy that several members of Bacteroidetes have recently been shown to possess a polysaccharide utilization locus that encodes esterases capable of extracting ferulic acid, a component of multiple cereal grains (Pereira et al., 2021).

There have been a limited number of reports describing the biological effects of N-methylserotonin, and most of these studies have been conducted *in vitro* (Powell et al., 2008; Nikolic et al., 2014). Similar to serotonin, N-methylserotonin is able to increase glucose uptake in rat muscle via its agonist activity on the 5-HT2A receptor (Hajduch et al., 1999). A maleated form of methylserotonin enhanced insulin secretion in human and mouse beta cells via activation of the 5-HT2B receptor (Bennet et al., 2016). The closely related compound, alpha-methylserotonin, by means of its engagement of 5-HT1 and 5-HT2A, is able to increase glycogen synthesis in rat hepatocytes via a direct increase in glycogen synthase activity as well as cAMP-dependent inactivation of glycogen phosphorylase (Tudhope et al., 2012). Interestingly, the binding of serotonin to 5-HT2B/C receptors has an opposing effect and decreases glycogen synthesis.

We found that oral administration of N-methylserotonin to germ-free mice consuming a high saturated fat, low fiber representative United States diet produced a number of phenotypic changes including reduced adiposity and alterations in hepatic glucose metabolism. Intriguingly, N-methylserotonin affected the expression of regulators of circadian rhythm in both liver and colon, including *Arntl*, *Clock*, *Per2*, *Per3*, plus *Nfil3* and its repressor, *Nrd1d2*. Emerging literature has linked the gut microbiota, microbiota-regulated diurnal oscillation of epithelial expression of clock components, and the effects of these components (e.g., *Nfil3*) on lipid absorption and export (Wang et al., 2017). RNA-seq did not reveal significant effects of N-methylserotonin on intestinal or liver levels of mRNAs encoding its known (Htr7 and Htr2A) or related (Htr3, Htr4, Htr5, and Htr6) receptors. However, the absence of changes in receptor expression does not preclude effects on their signal transduction pathways, or the possibility that N-methylserotonin exerts its effects on circadian regulators through other metabolites, such as glutamate, whose colonic levels increased after N-methylserotonin administration in germ-free animals.

The ability to manipulate luminal levels of N-methylserotonin in gnotobiotic mice fed OF by including or excluding N-methylserotonin-releasing bacterial species in their gut community illustrates one strategy for designing future symbiotics where fibers
containing concealed celobiotics could be administered together with probiotic miners to enhance/expand the biological effects of fibers to the benefit of the host. For example, our finding that administration of free, unbound N-methylserotonin to germ-free mice produced a dose-dependent increase in gastrointestinal transit time suggests that a symbiotic composed of OF plus an N-methylserotonin miner such as B. ovatus could represent an approach for the treatment of certain forms of irritable bowel syndrome (IBS-C, Ford et al., 2020). Moreover, the effects we observed on glycogen metabolism in mice exposed to N-methylserotonin suggest the potential for additional beneficial pharmacological properties.

Our findings underscore how the beneficial effects of dietary fibers should be considered not only from the perspective of their glycan constituents but also from the other natural products that they may harbor, including those that we define here as celobiotics. Celobiotics provide analytic opportunities that should be valuable to both food and microbiome scientists. The liberation of celobiotics from fiber preparations during in vitro incubations of intact uncultured (fecal) microbiota samples, defined consortia of cultured microbes, or single microbial strains could provide a way to operationally define the compositional “equivalence” of different lots of fiber preparation and/or a comparative assessment of the impact of different food processing methods. Knowledge of whether a consumer of a fiber preparation harbors a gut microbiota with miners of a specific celobiotic could also help explain the origins of interpersonal variations in responses to that fiber in longer duration clinical studies. A corollary is that this knowledge could help enable more personalized dietary recommendations about the types of fiber preparations that might provide specific health benefits.

Limitations of the study
Additional work is needed to directly characterize the physical-chemical interactions between N-methylserotonin and glycans in OF. Our pilot human studies had several limitations. They involved ostensibly healthy participants (albeit overweight or obese) and were not designed to evaluate metabolic or disease endpoints. A series of preclinical studies are needed to evaluate parameters that might affect the design and interpretation of larger, randomized controlled human trials designed to test hypotheses about the physiologic effects, as well as the safety of a symbiotic formulation composed of OF and B. ovatus. These parameters include, for example, the effects of gender, age, dose, and duration of treatment and stratification based on host physiologic and metabolic phenotypes. One approach to obtaining a better understanding of the extent to which N-methylserotonin release from a snack fiber could be personalized would be to document, in sufficiently powered human studies, the effect of consuming the snack, or a suitable control formulation, on the expression of CAZyme genes in metagenome-assembled genomes (MAGs) present in the gut microbiomes of study participants. In addition, deciphering the extent to which the range of potential pharmacologic properties of N-methylserotonin is similar to, or distinct from, those of serotonin requires further work. Finally, this report was focused on N-methylserotonin as an illustrative celobiotic, and it was not designed to comprehensively explore the repertoire of potential bioactive small molecules that may be sequestered within the OF preparation tested and releasable by members of the human gut microbiota. A more exhaustive analysis of these compounds could be performed with analytic tools such as atmospheric pressure chemical ionization, normal phase chromatography, gas-chromatography mass spectrometry, and nuclear magnetic resonance. Moreover, networking the resulting fragmentation profiles could help determine if enzymatic cleavage or compound release results in structural changes that will be missed by using the intact compound alone. It is possible compounds are transformed when released, and if this is the case, their structures could be deduced using platforms such as the Global Natural Product Social Molecular Networking site (GNPS; Wang et al., 2016).

STAR METHODS
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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.cell.2022.06.004.

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AUTHOR CONTRIBUTIONS

N.D.H., J.C., and J.I.G. designed the gnotobiotic mouse experiments and N.D.H. conducted them. N.D.H. and J.C. performed the mass spectrometric analyses of the capacity of human gut bacterial strains to release N-methylserotonin from the OF preparation. N.D.H. conducted them. N.D.H. and J.C. performed the mass spectrometric analyses of the capacity of human gut bacterial strains to release N-methylserotonin from the OF preparation in vitro and in vivo, as well as the effects of N-methylserotonin on metabolic activities in germ-free mice. N.D.H. performed the RNA-seq analyses of the liver and colonic gene expression in germ-free mice treated with N-methylserotonin and RNA-seq analyses of B. ovatus cultures grown in differing media conditions. D.W. generated and assembled the genomes of the various B. ovatus strains used in this study. N.T. and B.H. characterized the PULs represented in B. ovatus strains. D.A.R., A.A.A., and A.L.O. conducted the region analysis. A.C.H., M.J.B., O.D.-B., and J.I.G. designed the human studies. D.K.H., A.M., and S.V. oversaw the design, manufacture, and quality control analyses of fibres snack prototypes. A.C.H. was responsible for supervising the execution of the human study. N.D.H., J.C., and J.I.G. wrote the paper with the valuable input from co-authors.

DECLARATION OF INTERESTS

A.O. and D.R. are co-founders of Phenobiome Inc., a company pursuing the development of computational tools for predictive phenotype profiling of microbial communities. A provisional patent application has been filed that covers aspects of this work.

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### STAR METHODS

#### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and Virus Strains** | | |
| Agrobacterium radiobacter DSM 30147 | DSMZ, Stämmier et al., 2016 | Cat#30147 |
| Alicyclobacillus acidophilus DSM 14558 | DSMZ, Stämmier et al., 2016 | Cat#14558 |
| Anaerococcus vaginalis TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Anaerofustis stercorihominis TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides caccae TSDC 17.2-1.2 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides caccae TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides finegoldii TSDC 17.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides fragilis TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides intestinalis TSDC 17.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides massiliensis TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides ovatus 115 | Gehrig et al., 2019 | N/A |
| Bacteroides ovatus TSDC 17.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides ovatus VPI-435 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus VPI-B4-11 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus VPI-C1-45 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus VPI-C16-22 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus VPI-C2-26 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus WH208 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus WH214 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus WH514 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus WH604 | Shoemaker et al., 2001 | N/A |
| Bacteroides ovatus WH711 | Shoemaker et al., 2001 | N/A |
| Bacteroides thetaiotaomicron TSDC 17.2-2.2 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides thetaiotaomicron TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides uniformis TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides uniformis TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bacteroides vulgatus TSDC 17.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bifidobacterium longum TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Bifidobacterium longum TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Clostridiales TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Clostridium bolteae TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Clostridium hylemonae TSDC 20.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Clostridium scindens TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Collinsella aerofaciens TSDC 17.2-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Dialister invisus TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |
| Dorea longicatena TSDC 20.1-1.1 | Faith et al., 2013; Faith et al., 2014; N/A |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| *Eggerthella lenta* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Escherichia coli* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Escherichia coli* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Finegoldia magna* | Faith et al., 2013; Faith et al., 2014; N/A | N/A |
| *Odoribacter splanchnicus* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Parabacteroides distasonis* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Ruminococcus albus* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Ruminococcus gnavus* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Subdoligranulum variabile* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Subdoligranulum variabile* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Veillonella parva* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Veillonella parva* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |
| *Veillonella parva* | Faith et al., 2013; Faith et al., 2014; Ridaura et al., 2013 | N/A |

**Chemicals, Peptides, and Recombinant Proteins**

| Name | Source | Cat# |
|------|--------|------|
| N-methylserotonin | Santa Cruz Biotech | sc-391509 |
| Tryptophan | Sigma | 93659 |
| Tryptamine | Sigma | 193747 |
| Serotonin | Sigma | 14927 |
| Dimethylserotonin | Sigma | B-022 |
| Trimethylserotonin | Sigma | H-133 |
| Methyltryptamine | Santa Cruz Biotech | sc-391685 |
| S-adenosyl methionine | Santa Cruz Biotech | sc-278677 |
| Cellulase | Sigma | C2730 |
| Hemicellulase | Megazyme | E-GERF |
| Xylanase | Megazyme | E-XYAN4 |
| exo-Inulinase | Megazyme | E-EXOIAN |
| endo-Inulinase | Megazyme | E-ENDOIAN |
| beta-Xylanase | Megazyme | E-XYNBS |
| endo-Polygalacturonanase (M2) | Megazyme | E-PGALUSP |
| alpha-Amylase | Megazyme | E-ANAAM |
| Lichenase | Megazyme | E-LUCHN |
| Alginase Lyase | Megazyme | E-ALGLS |
| beta-Mannanase | Megazyme | E-BMANN |
| endo 1, 4 beta D-galactanase | Megazyme | E-GALCJ |
| endo 1, 5 alpha L-arabinanase | Megazyme | E-EARAB |

**Critical Commercial Assays**

| Name | Source | Cat# |
|------|--------|------|
| Glycogen Assay kit | Sigma | MAK016 |
| Takara Nucleospin RNA Plus kit | Takara | 740984 |
| Agilent RNA 6000 Pico kit | Agilent | 5067-1513 |
| Illumina TruSeq Stranded Total RNA | Illumina | 20040529 |
| SMRTBell Express Template Prep Kit 2.0 | Pacific Biosciences | 101-685-400 |
| SMRTbell Enzyme Clean up Kit | Pacific Biosciences | 101-746-400 |

(Continued on next page)
### RESOURCE AVAILABILITY

#### Lead contact

Requests for further information should be directed to and will be fulfilled by the lead contact, Jeffrey I. Gordon (jgordon@wustl.edu)

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Ampure PB beads     | Pacific Biosciences | Cat# 100-265-900 |
| Barcoded Overhang Adapter Kit- 8A, 8B | Pacific Biosciences | Cat# 101-628-400 |
| QIAquick 96 PCR Purification column | Qiagen | Cat# 28181 |
| Illumina Tagment DNA Enzyme and Buffer | Illumina | Cat# 20034211 |
| **Continued**        |        |            |
| **Ampure PB beads**  | Pacific Biosciences | Cat# 100-265-900 |
| **Barcoded Overhang Adapter Kit- 8A, 8B** | Pacific Biosciences | Cat# 101-628-400 |
| **QIAquick 96 PCR Purification column** | Qiagen | Cat# 28181 |
| **Illumina Tagment DNA Enzyme and Buffer** | Illumina | Cat# 20034211 |
| **Continued**        |        |            |
| **Ampure PB beads**  | Pacific Biosciences | Cat# 100-265-900 |
| **Barcoded Overhang Adapter Kit- 8A, 8B** | Pacific Biosciences | Cat# 101-628-400 |
| **QIAquick 96 PCR Purification column** | Qiagen | Cat# 28181 |
| **Illumina Tagment DNA Enzyme and Buffer** | Illumina | Cat# 20034211 |

#### Deposited Data

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| COPRO-Seq shotgun sequences | This study | PRJEB40461 |
| Tissue RNA-seq sequences | This study | PRJEB40461 |
| Microbial RNA-seq sequences | This study | PRJEB40461 |

#### Experimental Models: Organism/Strains

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| C57BL/6J mice | The Jackson Laboratory | Cat# 000664 |

#### Software and Algorithms

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Agilent software | Agilent | N/A |
| QiIME v1.9.2 | Caporaso et al., 2010 | http://qiime.org |
| COPRO-Seq pipeline | Hibberd et al., 2017 | https://github.com/nmcnulty/COPRO-Seq |
| R | The R foundation | https://www.r-project.org/ |
| Prism v9.2 | Graphpad | https://www.graphpad.com |
| STAR | Dobin et al., 2013 | https://github.com/alexdobin/STAR |
| FeatureCounts | Liao et al., 2013 | http://subread.sourceforge.net/ |
| Deseq2 | Love et al., 2014 | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |
| ClusterProfiler | Wu et al., 2021 | https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html |
| Prokka | Seemann 2014 | https://github.com/tseemann/prokka |
| RAST | Aziz et al., 2008 | https://rast.nmpdr.org/ |
| SMRT Link v9.0 | Pacific Biosciences | https://www.pacb.com |
| Cromwell | Voss et al., 2017 | https://cromwell.readthedocs.io/en/develop/ |
| Flye v2.8.1 | Kolmogorov et al., 2019 | https://github.com/fenderglass/Flye |
| RASTTk | Brettin et al., 2015 | https://rast.nmpdr.org/rast.cgi |

#### Other

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| HiSF-LoFV mouse diet | Ridaura et al., 2013 | N/A |
| Pea fiber | Rettenmaier | Cat# Pea Fiber EF 100 |
| Orange fiber | Fiber Star | Cat# CitriFri 100 |
| Carmine red dye | Sigma-Aldrich | Cat# C1022 |
| Lysing Matrix F | MP Bio | Cat# 116915050-CF |
| Reinforced Tubes | Thermo-Fischer | Cat# NC0444131 |
| 96-well, round bottom, deep well plate | Axygen | Cat# P-DW-11-HC |
| ART 200G, Filtered, Sterile pipette tips | Thermo Scientific | Cat# ART 2069G |
| Genomic DNA ScreenTape | Agilent | Cat# 5067-5365 |
| Genomic DNA Reagents | Agilent | Cat# 5067-5366 |
| 4200 TapeStation | Agilent | N/A |
| Quant-IT dsDNA broad range kit | Invitrogen | Cat# Q33130 |
| Sequel System | Pacific Biosciences | N/A |
| Procedure - SMRTBell Express Template Prep Kit 2.0 | Pacific Biosciences | Cat# 101-730-400 |
Materials availability
This study did not generate new unique reagents. All bacterial strains can be obtained from ATCC or as described in the key resources table.

Data and code availability
Annotated B. ovatus genomes, microbial RNA-seq, and COPRO-seq, liver and colonic RNA-seq datasets from gnotobiotic mice have been deposited at the European Nucleotide Archive (ENA: PRJEB40461). Metabolomics data are available in the EMBL-EBI MetaboLights database (MetaboLights: MTBLS2331). Shotgun and 16S rDNA amplicon sequencing datasets generated from human fecal DNAs are available in ENA (ENA: PRJEB44020).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Gnotobiotic mice
Experiments involving gnotobiotic mice were performed using protocols approved by Washington University Animal Studies Committee. Ten-week-old male germ-free C57BL/6J animals were housed in plastic flexible film gnotobiotic isolators (Class Biologically Clean) at 23 °C under a strict 12-hour light cycle (lights on a 0600h, off at 1800h).

Germ-free animals were weaned onto an autoclaved, low-fat, plant polysaccharide-rich chow (catalog number 2018S, Envigo) administered ad libitum. Four days prior to colonization, mice were switched to a diet formulation containing ingredients that in aggregate represented the upper tertile of saturated fat consumption and the lower tertile of fruits and vegetable consumption in the USA population as reported in the National Health and Nutrition Examination Survey (NHANES) database (see Ridaura et al., 2013). Pelleted unsupplemented HiSF-LoFV diet and the diets supplemented with 10% (w/w) OF (CitriFi 100; Fiber Star) or 10% (w/w) pea fiber (EF 100; Rettemmaiers) were vacuum packed in plastic bags and subsequently sterilized by gamma irradiation (20–50 kGy, Steris, Mentor, OH). Sterility was confirmed by culturing the material under aerobic and anaerobic (atmosphere, 75% N2, 20% CO2, 5% H2) conditions at 37 °C in TYG medium.

The bacterial strains used to colonize mice had been cultured from a fecal sample obtained from a lean co-twin in an obesity-discordant twin pair (TSDC 17 in Ridaura et al., 2013; Patnode et al., 2019). Equivalent numbers of bacterial cells (based on OD600 measurements) in monocultures (grown in TYG medium under anaerobic conditions to stationary phase) were pooled to create gavage mixtures. A total of 200 μL of each pool, consisting of all 14 strains, the four strains identified as capable of releasing N-methylserotonin from OF in vitro (B. ovatus, P. distasonis, C. aerofaciens, B. finegoldii), or a mixture of the other 10 strains, were introduced into mice using a plastic-tipped oral gavage needle (Fisher).

Animals were maintained in separate gnotobiotic isolators each dedicated to mice colonized with the same bacterial consortium (n=5 animals/cage). Cages contained autoclaved paper ‘shepherd shacks’ to facilitate their natural nesting behaviors and to provide environmental enrichment. Pre-colonization fecal samples were collected to verify the germ-free status of the mice using both culture and culture-independent assays.

For experiments involving administration of N-methylserotonin to germ-free mice, a stock solution of the compound (100 mg/mL, Santa Cruz Biotechnologies) was prepared in sterile water and filter-sterilized (0.2 μm pore size; Nalgene). The outer surface of tubes containing the stock solution was sterilized with Cidox (Pharmacal) and the tubes were introduced into gnotobiotic isolators using standard procedures. The stock solution was then diluted in darkened glass water bottles (Ancare) in order to administer doses of 1 mg/kg/day or 50 mg/kg/day (based on an experimentally determined average consumption of 5 mL of water/day/mouse). Every four days, bottles were replaced with new ones containing fresh N-methylserotonin. Each arm of the experiment, including the control arm where unsupplemented drinking water was provided, consisted of 5 mice with equivalent average starting body weights per cage. Two independent experiments were performed. One animal in the 50 mg/kg/day treatment group in the first in the two independent experiments died within the first week without any preceding behavioral changes or signs of illness, or decipherable underlying cause.

Fecal samples and body weights were collected weekly, while food and water intake were monitored daily by comparing food pellet mass in the food hopper and the volume of water in water bottles at the beginning and end of a 24h period and dividing these values by the number of mice per cage. All animals were euthanized between 0830h and 0930h without prior fasting. Luminal contents from the proximal and distal halves of the small intestine, the cecum and the colon, host tissues (liver, epididymal fat pads, gastrocnemius muscle, the distal quarter of the small intestine [ileum], cecum, the entire colon) plus serum were collected, flash frozen in liquid nitrogen and stored at -80 °C prior to analyses.

Human studies with pea and OF snack prototypes
Two separate open-label, single group assignment studies were performed involving members of the Missouri Adolescent Female Twin Study (MOAFTS) cohort (Bucholz et al., 2000) who were aged 31–45 years at the time of enrollment. The first study with the pea fiber snack was performed between April and August 2017, while the second study with the OF snack was conducted between August and December 2017. The design of the two studies were identical except for the fiber snack supplement used and the number of participants in each study. All participants provided written informed consent and the studies were approved by the Washington University Institutional Review Board (IRB ID#201611122). (ClinicalTrials.gov NCT03078283).
Details of the human studies are described elsewhere (Delannoy-Bruno et al., 2022). In brief, study 1 involved 9 twin pairs, four of whom were concordant for obesity (BMI ≥ 30 kg/m²) while five pairs were discordant with one member being obese and the other non-obese (n=18 participants; 36.6±2.9 years (mean ± SD); Table S8B). Study 2 involved 24 participants: 12 dizygotic twin pairs (37±2.9 years (mean ± SD)), nine of which had participated in the pea fiber study. For these nine pairs, the interval between cessation of pea fiber snack consumption and initiation of OF consumption ranged from 50 to 106 days [84±26 days (mean ± SD)]. Participants consumed their normal, unrestricted diet for the first two weeks of the study (pre-intervention phase). At the beginning of week three, they supplemented their diets with one 35g fiber snack serving a day for one week, then two 35g snack servings a day the following week, and thereafter, three 35g snacks per day for four weeks (weeks 5-8) at breakfast, lunch and dinner. No attempt was made to adjust the diets of participants other than supplementation with the fiber snack. Snack prototypes were manufactured by Mondeléz International, LLC (see Table S8A for their composition). Participants received their snacks in weekly shipments from the study center. The pea fiber snacks were in the form of rotary biscuits (6.7g total fiber/35g snack) or extruded bars (8.1g fiber/35g snack) with participants having the option to alternate between them. (Note that due to the different food processing techniques used for the biscuits and bars, it was not possible to perfectly match their fiber content). The OF snacks were all in the form of extruded bars (10.2g total fiber/35g snack). Compliance was monitored throughout by the study coordinator through weekly phone calls. The primary outcomes for each study were the effects of the respective snack prototypes on gut microbial community structure and function (see Delannoy-Bruno et al., 2022 for the results of an additional analysis of study participants).

Fecal samples were collected by participants and frozen immediately at -20°C in dedicated freezers provided to participants at the beginning of the study. All samples were shipped, via overnight delivery, in an insulated container containing frozen gel packs, to a biospecimen repository located in Washington University in St. Louis that was overseen by one of the authors (A.C.H.). Once received, samples were stored at -80°C until processing for LC-QqQ-MS analysis of N-methylserotonin levels and culture-independent characterization of ASVs and CAZyme gene abundances.

**Measurement of fecal N-methylserotonin levels**

Each fecal sample was homogenized with a porcelain mortar (4 L) and pestle while submerged in liquid nitrogen; multiple 500 mg aliquots of the pulverized frozen material were stored at -80°C from study participants. Sequencing libraries were generated from each purified fecal DNA sample and sequenced [Illumina International, LLC (see Table S8A for their composition)]. Participants received their snacks in weekly shipments from the study center. The pea fiber snacks were in the form of rotary biscuits (6.7g total fiber/35g snack) or extruded bars (8.1g fiber/35g snack) with participants having the option to alternate between them. (Note that due to the different food processing techniques used for the biscuits and bars, it was not possible to perfectly match their fiber content). The OF snacks were all in the form of extruded bars (10.2g total fiber/35g snack). Compliance was monitored throughout by the study coordinator through weekly phone calls. The primary outcomes for each study were the effects of the respective snack prototypes on gut microbial community structure and function (see Delannoy-Bruno et al., 2022 for the results of an additional analysis of study participants).

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**Shotgun sequencing of fecal DNA and quantification of CAZyme gene abundances**

DNA was purified from fecal samples (Delannoy-Bruno et al., 2021) that had been collected at the 1 week and 5-week time points from study participants. Sequencing libraries were generated from each purified fecal DNA sample and sequenced [Illumina NextSeq 550 and HiSeq 3000 instruments; 10.7 ± 0.6 x 10⁶ (mean ± SD) and 6.9 ± 1.1 x 10⁶ (mean ± SD) 150 nt paired-end reads/sample]. Microbial reads were assembled and annotated using prokka (Seemann, 2014). Counts for each open reading frame (ORF) were generated by mapping paired-end reads from each sample to its assembled DNA contigs. Alignments were processed to generate count data (featureCounts; Subread v. 1.5.3 package; Liao et al., 2014) for each ORF in each sample and normalized (TPM) (Delannoy-Bruno et al., 2021).

ORFs identified in each fecal sample were used as the starting point for CAZyme annotation using a procedure described in Delannoy-Bruno et al. (2021). Aggregating abundance data for each sample allowed us to generate CAZyme gene family/subfamily abundance tables. (The abundances of GH and PL genes annotated with multiple CAZyme families/ subfamilies were propagated to each individual family/subfamily member, and abundances were then summed across all corresponding CAZyme families within each fecal sample; see Delannoy-Bruno et al., 2021).

**16S rDNA amplicon sequencing and identification of ASVs**

PCR was performed using purified fecal DNA and barcoded primers directed against variable region 4 of the bacterial 16S rDNA gene (Caporaso et al., 2010). PCR amplification was performed as described in a previous publication (Gehrig et al., 2019); amplicons with sample-specific barcodes were quantified, pooled and sequenced (Illumina MiSeq instrument, paired-end 250 nucleotide reads). Paired-end reads were demultiplexed, trimmed to 200 nucleotides, merged, and chimeras were removed (version 1.13.0 of the DADA2 pipeline; Callahan et al., 2016). Amplicon sequence variants (ASVs) were aligned against GreenGenes 2016 (v. 13.8) to 97% sequence identity, followed by taxonomic and species assignment [RDP 16 (release 11.5) and SILVA (v. 128)]. The resulting ASV table was filtered to only include those ASVs with ≥ 0.1% relative abundance in at least five fecal samples, and then rarefied to 15,000 reads/sample.

**METHOD DETAILS**

**Enzymatic measurement of N-methylserotonin in plant fiber**

All enzymatic assays were carried out in a 10 mL reaction mixture containing water, 700 active units of enzymes listed in the key resources table and the following quantities of sample: (i) 50 mg of OF for the initial enzyme panel screen as described in Table S1C; (ii) either 1g or 50 mg for the broad fiber screen in Table S1E (1 g of dried materials such as various grains or herbs were ground to a fine powder with mortar and pestle; 1g of wet materials such as raw fruits and vegetables were chopped to a fine paste; and 50 mg of samples marked as either “Fiber” or “Pre-Ground” that were derived from commercially processed sources and required no additional pre-processing); (iii) 50 mg of commercially available fibers for the citrus fiber screen in Table S1F, where samples described as
either “Granule” or “Whole” were first pulverized into a fine powder with mortar and pestle, Samples were mixed via manual pipetting and subsequently incubated for 72h at room temperature (~23 °C) with intermittent shaking. All assays were carried out in triplicate.

**Measurement of gastrointestinal transit times using non-absorbable red carmine dye**

This protocol was adapted from a previously published study (Dey et al., 2015). Carmine red (Sigma-Aldrich) was prepared as a 6% (w/v) solution in 0.5% methylcellulose (Sigma-Aldrich) and autoclaved prior to import into gnotobiotic isolators. Seventeen days after initiation of N-methylserotonin treatment, 200 μL of the carmine red solution were gavaged into each germ-free mouse between 0800h and 0815h. Feces were collected every 15 minutes and streaked across a sterile white napkin to assay for the presence of the carmine red dye. The time from oral gavage to initial appearance of carmine red in the feces was recorded as the total intestinal transit time for that animal.

**Absolute abundances of community members**

Short-read community profiling by sequencing (COPRO-Seq; McNulty et al., 2013) was used to define the absolute abundances of bacterial taxa in fecal samples from colonized mice. For absolute abundance determination, 22.1x10^6 million *Agrobacterium radiobacter* DSM 30147 cells and 6.6x10^8 *Alicyclobacillus acidophilus* DSM 14558 cells were added to each frozen fecal pellet (Stämmler et al., 2016). DNA was isolated from the pellets by adding 500 μL of extraction buffer [200mM Tris (pH 8), 200 mM NaCl, 20 mM EDTA], 210 mL of 20% SDS, and 500 mL of 0.1 mm diameter zirconia beads, followed by treatment with a BioSpec bead beater for 4 minutes, addition of 500 μL phenol:chloroform:isoamyl alcohol (25:24:1), and precipitation of nucleic acids with isopropanol. Libraries were prepared using the Nextera DNA Library Prep Kit (Illumina) and combinations of custom barcoded primers (Adey et al., 2010). Multiplex sequencing of the libraries was performed using an Illumina Hi-Seq instrument (paired-end 75 nt reads; 2.65 x 10^6 ± 1.5 x 10^5 reads/sample). Reads were mapped onto the sequenced genomes of consortium members using an analytic pipeline described in previous publication (Hibberd et al., 2017). Absolute abundances, expressed as genome equivalents per gram of material, were calculated for each community member by multiplying the normalized counts of that member with the abundances of the spike-in (number of cells per normalized count) and dividing by the measured weight of the fecal sample (Stämmler et al., 2016).

**RNA-seq of liver and colonic tissue**

Frozen tissue was broken into small pieces and ground into a very fine powder under liquid nitrogen using a mortar and pestle. A 25 mg aliquot of powdered tissue was then aliquoted into shearing matrix F (MP Bio) pre-chilled in liquid nitrogen; 0.5 mL of buffer LBP (Takara) was added immediately, and the mixture was placed on a 4 °C cold block. Samples were then disrupted (Biospec bead beater; 2 minutes). The remaining steps in the RNA isolation procedure were performed using a Takara Nucleospin RNA Plus kit. After verifying that all purified RNAs had an RNA integrity number (RIN) greater than 8.5 (Agilent RNA Pico), a 10ng aliquot of each sample was used to generate a cDNA library (Illumina TruSeq Stranded Total RNA). Libraries were sequenced using an Illumina Hi-Seq instrument (paired-end 75 nucleotide reads; 1.43 x 10^7 ± 3.74 x 10^6 reads/liver sample, and 3.27 x 10^7 ± 1.23 x 10^6 reads/colon sample). Reads were aligned to the *Mus musculus* GRCm39 genome assembly with STAR version 2.7.0d. Gene count data were generated from the number of uniquely aligned reads (featureCounts Subread version 1.6.2a). The R package DESEQ2 (Love et al., 2014) was used to perform differential gene expression analysis; results were filtered based on an adjusted Benjamini and Hochberg FDR p-value <0.05. Gene set enrichment analysis was carried out using ClusterProfiler (Wu et al., 2021) with an adjusted p-value cut-off of <0.05 and minimum gene-set size of 3; over-representation was carried out using a log2 fold-change cut-off of ≥ 1.

**In vitro screening of bacterial strains for N-methylserotonin releasing activity**

A given bacterial strain was grown in monoculture at 37 °C in TYG medium in an anaerobic chamber (atmosphere; 75% N₂, 20% CO₂ and 5% H₂) to stationary phase. An aliquot was then added to 10 mL of fresh TYG medium with or without 50 mg of OF that had been sterilized by gamma irradiation (30-50 kGy); the mixture was incubated under anaerobic conditions without shaking for 72 hours. A 200 μL aliquot was then removed for targeted LC-QqQ-MS measurement of N-methylserotonin levels; another aliquot was used to define the number of colony-forming units so that levels of the analyte could be expressed per 10^6 cells. An identical protocol was used to compare the amount of N-methylserotonin released when two other rich media, MEGA medium 2.0 (Romano et al., 2015) and Wilkins-Chalgren anaerobe broth (Thermo-Fisher), were used in lieu of TYG. All incubations were performed in triplicate for each condition.

Experiments seeking to determine whether N-methylserotonin can be synthesized de novo by *B. ovatus* were carried out in 10 mL TYG with or without supplementation with tryptophan, tryptamine, serotonin, dimethylserotonin, trimethylserotonin, methyltryptamine, or S-adenosyl methionine (final concentrations; 5 mg/mL; all from Sigma). Experiments seeking to test the capacity of all 14 bacterial strains that were studied in mice to degrade N-methylserotonin in vitro were carried out using 10 mL TYG and 50 ng N-methylserotonin, with samples collected every 24 hours. Assays were performed in triplicate for each condition, using the protocol described above.

Experiments seeking to test the necessity of having live bacteria to extract N-methylserotonin were carried out by first incubating monocultures of *B. ovatus, B. finegoldii, P. distasonis* and *C. aerofaciens* in 10 mL TYG medium at 37 °C under anaerobic conditions to stationary phase. The stationary phase culture was then treated at 70 °C for 1 hour. Cells were recovered by centrifugation (6,000 x g for 15 minutes at 4 °C) and the pellet was added to 10 mL of TYG medium containing 5mg/mL of OF.
Experiments using conditioned media were carried out by taking monocultures of *B. ovatus, B. finegoldii, P. distasonis,* and *C. aerofaciens* that had been grown to stationary phase in TYG under anaerobic conditions, centrifuging the culture for 15 minutes at 6,000 x g at 4 °C to remove bacterial cells and adding 10 mL of the conditioned medium to 50 mg OF.

Experiments using bacterial lysates were carried out by bead-beating of bacterial cells, collected by centrifugation from 10 mL stationary phase TYG cultures for 4 minutes at room temperature; 500 μL of the resulting lysate was added to 10 mL of a solution containing 5 mg OF/mL TYG medium. To ensure sterility in these experiments, aliquots of the heat-treated cells, centrifuged conditioned media, or bacterial lysate were cultured in TYG medium for 7 days and subsequently plated on TYG-agar; the results confirmed the absence of colony forming units. Assays were performed in triplicate for each experimental condition.

For screening the 24 additional non-*B. ovatus* strains, 3 mg of OF was seeded into a deep 96-well plate; a liquid handling robot (Precision XS, Biotek) added 0.6 mL of Wilkins-Chalgren anaerobe broth to each well (yielding a final concentration of 5 mg OF/mL). Each well was subsequently inoculated with 50 μL of a stationary phase culture of the bacterial strain targeted for screening and sealed with foil. The screen was performed in triplicate and carried out under identical conditions as the 14-strain experiment.

**Genomic DNA extraction and purification**

Bacterial isolates were inoculated into TYG medium and were grown at 37 °C in an anaerobic chamber with an atmosphere of 75% N₂, 20% CO₂ and 5% H₂ until reaching stationary phase. A 10 μL aliquot was transferred into 10 mL of fresh TYG medium and was incubated for 72 hours under anaerobic conditions without shaking. A fraction of the culture was removed for full-length 16S rRNA amplicon sequencing to confirm the identity of culture isolates. The remaining culture was centrifuged at 3,000 x g for 5 minutes, yielding a 10-50 mg of cell pellet, which was transferred to a 2 mL cryo-tube for DNA extraction. A 3.97 mm steel ball and 250 μL of 0.1 mm zirconia/silica beads were added to the tube along with 500 μL of a mixture of phenol:chloroform:isoamyl alcohol (25:24:1, pH 7.8–8.2), 210 μL of 20% SDS, and 500 μL of 2X buffer A (200 mM NaCl, 200 mM Trizma base, 20 mM EDTA). Samples were subjected to bead-beating for 1 minute in a Biospec Minibeadbeater-96 and were then centrifuged at 3220 x g for 4 minutes. Following centrifugation, 420 μL of the aqueous phase was transferred to a deep 96-well plate for subsequent DNA isolation. DNA was isolated using a QIAquick 96-well PCR purification kit (Qiagen) with liquid handling performed using a Biomek FX robot. DNA was eluted from the column in 70 μL Tris-EDTA (TE) buffer and was quantified with a Quant-IT dsDNA broad range kit (Invitrogen).

**Long-read library preparation and sequencing**

Approximately 1 μg of genomic DNA from each isolate was transferred into a 96-well, 0.8 mL, deep-well plate and was prepared for long-read sequencing using a SMRTbell Express Template Prep Kit 2.0 from Pacific Biosciences (PacBio) according to the manufacturer’s guidelines for preparing HiFi Libraries from low DNA input, with adaptations for 96-well plate format. Purified DNA was of appropriate quality (DNA range: 6.8-7.9) and size (range of median peak size: 14.1-23.8 kb) for HiFi library preparation; therefore, no DNA shearing or size selection was performed prior to template preparation. All DNA handling and transfer steps were performed with ART wide-bore, genomic DNA pipette tips. Initial steps were performed as described in the PacBio protocol, including removal of single stranded overhands, DNA damage repair, end repair, and A-tailing. Barcoded adapters were ligated to A-tailed DNA fragments by overnight incubation at 20 °C followed by treatment with the SMRTbell Enzyme Cleanup Kit to remove damaged or partial SMRTbell templates. Ligated templates were purified, and size selected with 0.45x AMPure PB beads (45:100, AMPure beads/sample). Size-selected libraries were pooled to yield equal genome coverage (3-6 libraries/pool). A second round of size selection with 0.45x AMPure PB beads was performed after pooling, and DNA was eluted in 12 μL of PacBio elution buffer. Pooled libraries were quantified by Qubit, and the size distribution was evaluated on an Agilent TapeStation using Genomic DNA ScreenTape (Agilent). The median fragment size for the 4 library pools ranged from 14.5 kb to 16.9 kb. Each library was sequenced on a Sequel System from Pacific Biosciences using a Sequel Binding Kit 3.0 and Sequencing Primer v4 with 24 hours of data collection.

**Genome assembly and annotation**

Samples were demultiplexed and Q20 circular consensus sequencing (CCS) reads were generated using a Cromwell workflow configured in SMRT Link. Genomes were assembled using Flye v2.8.1 with hifi-error set to 0.003, min-overlap set at 2000, and other options set to default. Genome quality was evaluated using checkm and annotated using the RASTtk pipeline (Aziz et al., 2008; Brettin et al., 2015; Overbeek et al., 2014).

**Microbial RNA-seq**

Samples were prepared for microbial RNA-seq as described in the section above, except under the following conditions: (i) *Bacteroides ovatus* TSDC 17.2 was grown in TYG, TYG without hemin, and MEGA media and (ii) *Bacteroides ovatus* 115 was grown in TYG with or without 5 mg/mL OF (n=4 separate monocultures of each organism/condition). A volume of 10 mL of 72-hour growth was centrifuged to yield 10-50 mg of pelletated bacteria. A 3.97 mm steel ball and 250 μL of 0.1 mm zirconia/silica beads were added to each sample tube along with a 500 μL mixture of 25:24:1 parts phenol:chloroform:isoamyl alcohol (pH 7.8–8.2), 210 μL of 20% SDS, and 500 μL of 2X buffer A (200 mM NaCl, 200 mM Trizma base, 20 mM EDTA). Samples were then subjected to bead-beating for 1 minute in a Biospec Minibeadbeater-96 followed by centrifugation at 3220 x g for 4 minutes. A 100 μL fraction of the aqueous phase was transferred to a deep 96-well plate along with 70 μL isopropanol and 10 μL 3M NaOAc, pH 5.5 and was mixed by pipetting 10-times. The crude DNA/RNA mixture was chilled at -20 °C for approximately 1 hour and then centrifuging at 3220 x g at 4 °C for
15 minutes before removing 210 μL of the supernatant to yield nucleotide-rich pellets. A Biomek FX robot was used to add 300 μL Qiagen Buffer RLT to the pellets and resuspend the RNA/DNA by pipetting up and down 50-times. A 400 μL volume was transferred to an AllPrep 96 DNA plate and was centrifuged at 3220 x g for 1 min at room temperature. The RNA flow-through was purified as described in the AllPrep 96 protocol; DNA was then eluted from the column and retained.

Libraries were prepared from extracted RNA using the Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus and were sequenced on an Illumina Next-Seq instrument using single end 75-nucleotide reads (1.33 x 10^7 ± 1.06 x 10^7 reads/microbial sample). Reads were aligned to assembled genomes using Bowtie. The resulting counts table was passed onto the R package DESEQ2 for differential gene expression analysis, where results were filtered as described in Figure 4C. Sequence-based comparisons between genes expressed and/or present in B. ovatus strain TSDC 17.2 with B. ovatus strain 115, as well as the other B. ovatus strains were subsequently performed using the SEED system (Overbeek et al., 2014), where a bidirectional BLAST search was carried out setting B. ovatus strain TSDC 17.2 as the reference genome for comparison. Annotation of PULs and regulon analysis were carried out as described (Ravcheev et al., 2013; Terrapon et al., 2015; Terrapon et al., 2018).

Sample extraction for mass spectrometric analyses
All samples were maintained on liquid nitrogen throughout the extraction process. Frozen tissue was broken into small pieces and ground into a fine powder using a mortar and pestle. The powder was aliquoted into open-capped tubes (Reinforced, Thermo) pre-chilled in liquid nitrogen. Each sample was added to a 20 times weight volume of methanol along with 3-5 stainless steel beads (2.8 mm, Biospec) in a reinforced tube (Benchmark Scientific, catalog number D1031-RF) and placed on a pre-chilled block (-20 °C). For intestinal contents, feces and in vitro screening samples, tubes were shaken using a Biospec bead beater for 4 minutes. For host tissues, tubes were shaken using a Biospec bead beater for two cycles of 4 minutes each, switching to a new chilled block each time that the bead beater was activated. For each plasma sample, a 40 μL aliquot was added to 4 mL of extraction solution (40% methanol in water) followed by addition of 20 μL of 100 mM tricarboxylic acid. After a 10-minute incubation at room temperature, samples were briefly vortexed and then centrifuged at 12,000 x g for 10 minutes at 4 °C; 200 μL of the resulting supernatant was transferred to a 2 mL glass tube (Agilent) and dried in a speed vacuum at room temperature for two hours. The dried extract was reconstituted in 100 μL of 90% water/10% acetonitrile and stored at -4 °C prior to injection into a mass spectrometer.

Untargeted LC-QToF-MS
Untargeted metabolomics was performed using an Agilent 1290 LC system coupled to an agilent model 6545 QToF mass spectrometer (Santa Clara, CA). Five μL of each sample extract was injected onto a BEH C18 column (2.1 x 150 mm, 1.7 μm, Waters Corp., Milford, MA) that was heated to 35 °C. For analyses carried out in the positive ESI mode, the mobile phase consisted of 0.1% formic in water (A) and 0.1% formic acid in acetonitrile (B). For analyses in the negative ESI mode, the mobile phase consisted of 5mM ammonium bicarbonate formic in water (A) and 5mM ammonium bicarbonate in acetonitrile/water (95:5 v/v) (B). A flow rate of 0.3 mL/minute was applied (gradient program: from 0 to 14 minutes, mobile phase B eluted from 5% to 100%, followed by 3 minutes at 100% of B). An equilibration time of 3 minutes was used. Data were collected in the range from m/z 50 to 1000, and m/z 150 to 650 for MS full-scan analysis and MS/MS analysis, respectively. The key parameters of QToF were set as follows nozzle voltage, 1000 V for positive and 1500V for negative; capillary voltage, 3000 V for positive and 3500 v for negative; collision gas, high purity N2; drying gas, N2; vaporizer/sheath gas temperature, 325 °C; sheath gas flow rate, 12 L/min. To ensure accurate mass measurements, reference masses m/z 121.0509 and 922.0098 were automatically delivered using a dual ESI source during analyses. The mass accuracy of the LC-MS system was generally better than 4 ppm.

The resulting raw data sets were deconvoluted using MassHunter Profinder B.08.00 software (Agilent Technologies) which generated a list of molecular features. These features were subsequently filtered using in-house scripts to identify those that were only present in all samples obtained from mice that were colonized and fed the OF supplemented HiSF-LoFV diet. Initial characterization of the resulting subset of features was performed by monoisotopic mass search in METLIN (www.metlin.scripps.edu) and HMDB (www.hmdb.ca). These features were fragmented by targeted MS/MS with collision energy from 0 to 40 V. Final metabolite identification was performed by co-characterization with standards.

Targeted LC-QqQ-MS
N-methylserotonin
Five microliters of sample extract were injected into a 1290 Infinity II UHPLC system coupled to a Model 6470 Triple Quadrupole LC/MS system with a Jet Stream electrospray ionization source (Agilent Technologies). Chromatographic separation was performed on a ZORBAX Extend-C18, 2.1 x 50 mm, 1.8 μm column (Agilent Technologies) using the following gradient conditions: 5-95% solvent B (methanol/0.1% formic acid); 0-3 minutes at a flow rate of 0.2 mL/minute. Mass spectra were acquired in positive mode and quantification transitions for N-methylserotonin at 191 → 160.
Other metabolites
Tissue (at least 10 mg) was placed in a reinforced 2 mL tube (Biospec). A 20 times weight volume of extraction solvent was added (40% acetonitrile, 40% methanol, 20% water) and the tissue was disrupted as described above. Samples were centrifuged at 12,000 x g for 10 minutes at 4 °C. A 200 µL aliquot of the resulting supernatant was transferred to a 2 mL glass tube and dried in a speed vacuum at room temperature (25 °C) for two hours. The dry extract was reconstituted in 100 µL of 90% water/10% acetonitrile and stored at -4 °C prior to injection; a 5 µL aliquot was injected into a 1290 Infinity II UHPLC system coupled to a 6470 Triple Quadrupole LC/MS system equipped with a Jet Stream electrospray ionization source (Agilent Technologies). Chromatographic separation was performed on an Agilent ZORBAX Extend C18, 2.1 x 150 m, 1.8 µm column, using the following gradient conditions: mobile phase A, 10mM tributylamine and 15mM acetic acid in 3% methanol (v/v); mobile phase B, 10mM tributylamine and 15mM acetic acid in 100% methanol; 0% solvent B (0-2 minutes); 0-20% solvent B (2-7.5 minutes); 20-45% solvent B (7.5-13 minutes); 45-99% solvent B (13-20 minutes); 99-0% solvent B (20-22 minutes) at a flow rate of 0.25 mL/minute. Mass spectra were acquired in negative mode using the following conditions: capillary voltage set at 2000V; nitrogen as the nebulizer gas (45 psi); drying gas flow rate and temperature of 13 L/minute and 250 °C, respectively; sheath gas flow rate and temperature of 12 L/minute and 325 °C. Transitions were taken from the Agilent Metabolomics dMRM Database.

QUANTIFICATION AND STATISTICAL ANALYSIS
Details regarding statistical tests used, replicates and means and standard deviations are provided in the text, Figure legends and Tables.
Figure S1. Over-representation analysis of GO biological process terms in the set of genes differentially expressed in the livers of germ-free mice in response to orally administered N-methylserotonin, related to Figure 2 and Table S3A. GO terms are ranked by a gene ratio with a p value cutoff of 0.05.
Figure S2. HTCS_Rgu-2 regulon analysis in three *B. ovatus* strains, related to Figure 4 and Table S7

Predicted HTCS_Rgu-2 binding sites (PUL number and the ID of their component genes are based on *B. ovatus* TSDC 17.2 and described in Table S7). Predicted members of the HTCS_Rgu-2 regulon are indicated by the solid line on top. The sequence logo shows the consensus for identified HTCS_Rgu-2 binding sites.