If You Eat It or Secrete It, They Will Grow: the Expanding List of Nutrients Utilized by Human Gut Bacteria

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ABSTRACT In order to persist, successful bacterial inhabitants of the human gut need to adapt to changing nutrient conditions, which are influenced by host diet and a variety of other factors. For members of the *Bacteroidetes* and several other phyla, this has resulted in the diversification of a variety of enzyme-based systems that equip them to sense and utilize carbohydrate-based nutrients from host, diet, and bacterial origins. In this review, we focus first on human gut *Bacteroides* and describe recent findings regarding polysaccharide utilization loci (PULs) and the mechanisms of the multiprotein systems that they encode, including their regulation and the expanding diversity of substrates that they target. Next, we highlight previously understudied substrates such as monosaccharides, nucleosides, and Maillard reaction products that can also affect the gut microbiota by feeding symbionts that possess specific systems for their metabolism. Since some pathogens preferentially utilize these nutrients, they may represent nutrient niches competed for by commensals and pathogens. Finally, we address recent work to describe nutrient acquisition mechanisms in other important gut species such as those belonging to the Gram-positive anaerobic phyla *Actinobacteria* and *Firmicutes* as well as the *Proteobacteria*. Because gut bacteria contribute to many aspects of health and disease, we showcase advances in the field of synthetic biology, which seeks to engineer novel, diet-controlled nutrient utilization pathways within gut symbionts to create rationally designed live therapeutics.

KEYWORDS *Bacteroides*, microbiome, glycans, polysaccharides

The importance of symbiotic gut bacteria to human health has been appreciated for decades. Advances in sequencing, culture, and other technologies have invigorated research into the relationships between the human gut microbiota (HGM) and various diseases, with new connections emerging rapidly over the last decade. As the mechanistic relationships between various altered HGM states and diseases become better understood (1–4), there is a desire to design rational interventions to restore or suppress certain microbes or even implant engineered bacteria that can uniquely exploit rare nutrients (5, 6). A key feature underlying the persistence of many gut bacteria is their ability to utilize fiber and some other carbohydrate-based nutrients consumed by the host, which exert a heavy impact on the lower gut ecosystem because they are not degraded or absorbed in the small intestine. Underscoring the importance of dietary fiber polysaccharides to gut microbial ecology and health, when these nutrients are absent or reduced, the microbiota shifts to foraging on mucosal glycans (7), which increases susceptibility to pathogens (1), changes the spatial organization of gut microbes (8), and leads to exacerbated disease in models of inflammatory bowel disease (9). Significant research has focused on the numerically abundant members of the *Bacteroidetes* phylum and their abilities to access carbon from the diverse array of polysaccharides presented through diet, host secretions, and microbial exopolysaccharides (EPSs) and capsules (10–17). This ability in *Bacteroidetes* is accomplished via

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the expression of polysaccharide utilization loci (PULs), genomic clusters of coexpressed genes that encode products that sense, cleave, transport, and metabolize polysaccharides and other nutrients. As research has extended into the often more abundant but also more fastidious members of the *Firmicutes* phylum, similar clusters of nutrient utilization genes have been observed (18–20), although their enzymes and other products are adapted to the Gram-positive cell surface.

The ability of the *Bacteroidetes* and members of other phyla to utilize complex carbohydrates has been reviewed in the past few years (19, 21, 22). However, a number of recent studies have expanded the known repertoire of polysaccharides and other nutrients that are targeted by gut bacteria, along with increased knowledge of the enzymes and local and global regulatory mechanisms involved. Some nutrients, such as Maillard reaction products (MRPs), formed between reducing sugars and amino acids during cooking (23), or commonly ingested food additives like trehalose are unexpectedly metabolized by some members of the gut microbiome using specifically adapted systems (24). Here, we review recent findings regarding the enzymatic and regulatory strategies through which gut bacteria degrade carbohydrates, with a focus on newly identified substrates and the regulation of these attributes within bacterial cells that are often equipped to utilize many different substrates. We approach this review with the idea that complex carbohydrates are of ubiquitous importance in shaping the ecology and physiology of the HGM and therefore present a convenient lever to intentionally manipulate these communities.

MECHANISMS OF POLYSACCHARIDE UTILIZATION BY HUMAN GUT BACTEROIDETES

Polysaccharides are arguably the most diverse category of biological molecules because of the number of different sugars that they may incorporate, the two different glycosidic linkage types (α or β) that connect them, the ability of some sugars to form 5 (furanose) or 6 (pyranose) atom rings, and the ability of polysaccharides to contain linkages to multiple carbons on constituent sugars. Bacteria that degrade these molecules as nutrients must possess enzymes that are specific for cleaving each of the unique linkages that they contain, although partial degradation may be possible by some bacteria. The degradative carbohydrate-active enzymes (CAZymes) that conduct these reactions are grouped into numbered families that reflect common structure and homology (25) and may also indicate activity. As an introduction to polysaccharide structure and bacterial degradation, Fig. 1 shows an example structure of a plant cell fiber, the hemicellulose galactomannan composed of mannose and galactose, a relatively simple bacterial glycoside hydrolase (GH) pathway for its degradation that has been determined in the gut symbiont *Bacteroides ovatus*, and a schematic of the genes involved (26, 27). A key aspect of this degradative pathway is the sequential action of multiple enzymes, some of which act in an endo fashion (i.e., cleaving within polymers) and others of which work in an exo mode (i.e., cleaving at the ends of polymers). A more detailed catalog of the sugars and linkages that are present in plant, host, and microbial polysaccharides and that impact gut bacteria, as well as a review of the bacterial degradative systems involved, is provided by Porter and Martens (21).

The major Gram-negative and Gram-positive bacterial phyla in the human gut have evolved a variety of different systems to access nutrients, including carbohydrates, and these systems often incorporate various endo- and exo-acting CAZymes at the cell surface or in the periplasm and cytoplasm (Fig. 2). Members of the Gram-negative *Bacteroidetes* usually comprise a substantial proportion of the bacteria present in the HGM, with the genus *Bacteroides* typically being the most abundant in industrialized populations (28). Since several of these species are amenable to genetic manipulation, significant effort has focused on understanding the polysaccharide-degrading mechanisms of certain *Bacteroides* species, which sometimes devote up to 20% of their genomes to PULs (29–32). The first PUL to be discovered encodes the *Bacteroides thetaiotaomicron* starch utilization system (Sus), which has served as an archetype for understanding other PUL-encoded systems and is among the mechanistically best

FIG 1 Structural schematic of the plant polysaccharide galactomannan and the corresponding requirement for sugar- and linkage-specific enzymes for its degradation. (A) Schematic of the two sugars galactose (yellow) and mannose (green) that compose galactomannan as well as the α and β linkages that connect them at positional carbons (carbons that are involved in forming these glycosidic linkages are numbered in red). (B) Symbol schematic of a longer region of galactomannan with the region in panel A delineated in the dashed box and the sequence of enzymatic steps for its degradation. Like proteins and nucleic acids, polysaccharides have a directional orientation that includes a reducing end (right) and one or more nonreducing ends (the arrow at the left, which indicates further elongation of the polymer for which the final residue in the chain is the nonreducing end as well as the galactose branches). Polysaccharide-degrading enzymes, such as glycoside hydrolases (GHs), are sugar and linkage specific, and therefore, gut bacteria that degrade diverse dietary fiber structures often possess hundreds of linkage-specific enzymes in their genomes. GHs and other carbohydrate-active enzymes (CAZymes) are classified into numbered families based on structural homology (25), which may provide some indication of their function, although individual families often contain multiple activities. These enzymes may act on their substrate in an “endo” fashion (i.e., cleaving within a chain to release products longer than two sugars) or may be exo-acting (i.e., mostly cleaving at the nonreducing end to release individual sugars or disaccharides). Endo-acting enzymes generally work early in degradation to release smaller fragments. The example shown is from recent studies on the gut symbiont *Bacteroides ovatus* (26, 27). (C) A series of four enzymes that are coexpressed within a single polysaccharide utilization locus (PUL) are shown as a gene schematic. The sequence and sites at which the encoded enzymes cleave galactomannan are shown in panel B. The initial degrading enzyme is a cell surface GH26 endo- β -mannosidase that cleaves within the β 1,4-linked mannose backbone (green circles) to produce a fragment with a new, nonreducing end. Cleaved fragments are then debranched by a GH36 family α -galactosidase that prefers removing these branches when they are present within chains (26). Finally, the remaining β 1,4 manno oligosaccharide chains are broken down by specific exo-acting activities such as GH26 and GH130 family enzymes. As polysaccharide substrates become more complex and incorporate more or different sugars, the number and activities of the required enzymes and their sequence of action also change and may increase substantially. (D) Schematic of a recently described gpPUL from *Roseburia intestinalis* that is also involved in the degradation of galactomannan and other β -mannans. Note that several GH enzymes are common between the two PULs shown in panels C and D, but the *Roseburia* system relies on an ABC transporter instead of the TonB-dependent transport mechanism that is common in *Bacteroidetes*. (See reference 20 for a complete description of the *Roseburia* degradative pathway.)

FIG 2 Mechanisms of nutrient utilization for Gram-negative (Gram[−]) and Gram-positive (Gram⁺) bacteria. (A) Generic polysaccharide utilization locus (PUL)-encoded multiprotein system with major proteins displayed, including a glycoside hydrolase (GH) and SusC-, SusD-, and SusE/F-like outer membrane proteins. Different mechanisms of either sequestering sugars during degradation ("selfish") or releasing some sugars ("sharing") are illustrated. Outer membrane vesicles (OMVs) that contain acidic hydrolytic enzymes with signal peptidase II lipidation motifs (72) may help facilitate the sharing of polysaccharides or their degradation farther away from the producing cell. (B) Prominent carbohydrate utilization systems for some members of the Gram-positive *Firmicutes* and *Bifidobacterium* phyla. The ABC and other sugar/oligosaccharide transport systems encoded by Gram-positive PULs (gpPULs) are often coexpressed with degradative enzymes (GHs and carbohydrate esterases [CE]) and carbohydrate binding modules (CBMs), the latter sometimes encoded within the same polypeptide as the catalytic domain of glycoside hydrolases or other enzymes. (C) Some Gram-positive *Ruminococcus* species, and other related bacteria, produce complex multiprotein assemblies termed cellulosomes or amylosomes to degrade cellulose or resistant starch, respectively. In addition to the extracellular-facing enzymes and binding proteins, these strains often rely on ABC transporters to import the cleaved products. (D) Many proteobacteria rely on mono- and disaccharides for growth and therefore may rely on the sharing of these resources by primary degraders by the use of cytoplasmic membrane importers such as ABC transporters, major facilitator superfamily (MFS) proteins, and PTSs (blue). Once in the cytoplasm, kinases and isomerases further contribute to sugar metabolism. At least one strain of *E. coli* has been observed to steal or pirate cytoplasmic nutrients from host cells using a type III secretion system/injectisome. The bacterium delivers effector proteins or toxins into host cells, forcing nutrients into an adjacent tube that shuttles carbohydrates or amino acids back to the bacterium.

understood (33–35). This system includes a maltooligosaccharide-specific outer membrane TonB-dependent transporter (SusC) that partners with a starch binding protein (SusD) (36) (Fig. 2A). This transporter/binding protein pair works with a cell surface endo-acting amylase (SusG), two additional cell surface starch binding proteins (SusE and -F), and two periplasmic α -glucosidases (SusA and -B) to bind, transport, and saccharify starch into its component sugar glucose. Upon determining the *B. thetaio-taomicron* genome sequence (30), many Sus variants (that each target different polysaccharides) were identified in this species as well as many other gut *Bacteroidetes* and their environmental relatives. The original definition of a PUL arbitrarily included at least one set of *susC-susD* homologs (37), although they typically encode several CAZymes (38), carbohydrate binding proteins, regulators, and other enzymes (kinases, proteases, and sulfatases, etc.). Each PUL-specific functional suite tailors the system to target a particular substrate (39, 40). However, variant or degenerate forms of these gene clusters exist, and not all meet the original definition. Examples of variant PULs for bacteria or substrates discussed below include the presence of genes that are required for a single complex substrate spread across multiple distinct PULs, as is the case for *B. thetaio-taomicron* rhamnogalacturonan II degradation (14), and uncoupling of essential functions, including *susC-susD*-like genes, from the gene cluster that encodes the enzymes that they work with, as observed for *Zobellia galactanivorans* carrageenan utilization (41).

PUL-encoded systems from HGM *Bacteroidetes* or their environmental relatives have been demonstrated to target a remarkably diverse array of complex carbohydrates, spanning essentially all of the common terrestrial plant and host glycans. While we do not provide a comprehensive catalog of PULs and their corresponding nutrient targets, we provide an overview and highlight several PUL-substrate pairs that have been discovered in recent years. In addition to starch, the plant-derived substrates that gut *Bacteroides* PULs have been shown to target include individual substrates within three

more diverse families of storage and cell wall polysaccharides: fructans, pectins, and hemicelluloses (27, 31, 42–46). Different *Bacteroides* PULs equip some species to target the host-derived O-linked glycans attached to mucus as well as N-linked glycans and glycosaminoglycans in other host glycoconjugates (7, 12, 29, 47, 48). The expression of some of the PULs that target sulfated host glycans, probably those in colonic mucus, has been associated with the ability of *B. thetaiotaomicron* to cause colitis in a sulfate-dependent manner (49). Additional *Bacteroides* PULs have been shown to target human milk oligosaccharides (50), some bacterial exopolysaccharides (15, 51), the α -mannan (10, 52) and β -glucan (11) in fungal cell walls, and the seaweed-derived polysaccharides agarose (17), porphyran (5, 16), laminarin (53), and alginate (54).

PULs contained in non-HGM *Bacteroidetes*, such as those from the soil, rumen, marine, and insect gut ecosystems, extend the list of target substrates even further, having been linked to the degradation of chitin (55), cellulose (56), and additional seaweed polysaccharides (41, 57, 58). Underscoring the adaptability of this system to diverse substrates, this list has continued to expand in recent years, with a landmark study describing the enzymatic mechanism through which *B. thetaiotaomicron* degrades rhamnogalacturonan II—thought to be among the most complex polysaccharides known—through a process that requires 3 separate PULs and 26 different enzymes (14). Related studies on the also complex rhamnogalacturonan I and its attached side chains, which vary between botanical sources, revealed the requirement for at least 4 different *B. thetaiotaomicron* PULs encoding 30 enzymes (13). Extending the range of host glycans targeted via *B. thetaiotaomicron* PULs, another recent study investigated this species' ability to access high-mannose N-glycans (59, 60), including those attached to mucosal immunoglobulin A through the action of several separate PULs (59).

Owing to the structure of their Gram-negative cell envelope, *Bacteroidetes* are able to transport and sequester oligosaccharides into the periplasm prior to full depolymerization (61–63). Efficient utilization of polysaccharides may be enhanced through the action of outer membrane-bound carbohydrate binding proteins such as SusE and SusF and related carbohydrate binding module (CBM)-containing proteins that could prevent diffusion from the cell surface (63, 64). *Bacteroides* species frequently digest polysaccharides using this “selfish” mechanism that is characterized by little release of free oligosaccharide or sugar into the extracellular milieu (Fig. 2A), which has been shown for α -mannan and some xylans (7, 52). However, other studies have implied that when certain substrates are degraded by different *Bacteroides* species, significant amounts of oligosaccharides can be released to be used by other community members (both related *Bacteroides* as well as those from non-*Bacteroidetes* phyla) and thought of as “common goods” (65–67). While released degradation products may become available to competitors, the broad existence of interbacterial antagonism strategies (e.g., type VI secretions systems plus others) may provide protection for the primary degrader, especially from closely related species/strains with similar abilities to access the same resources (68–70). Of particular significance are observations that invading pathogens such as *Salmonella* and *Citrobacter rodentium* are more readily able to colonize the host when they gain access to nutrients liberated from dietary fiber or host mucosal glycans by resident *Bacteroides*.

In some cases, nutrient “sharing” between bacteria may promote species-specific cooperation instead of competition. Highlighting how substrate and strain specificities play deciding roles, *B. thetaiotaomicron* was shown to degrade amylopectin and the fructan levan in a mostly selfish fashion, although some amount of product appeared to be released to cross-feed a non-enzyme-producing mutant of the same strain (66). In contrast, fragments of a different fructan, inulin, were produced in greater abundance by *B. ovatus* during degradation through the secretion of an extracellular enzyme, which was not required by *B. ovatus* itself, and the corresponding products were accessible to a strain of *Bacteroides vulgatus* but not *Bacteroides fragilis* (66). Interestingly, several *Bacteroides* species appear to preferentially pack hydrolytic

enzymes into outer membrane vesicles (OMVs) (71, 72), which as these hydrolytic particles diffuse away from the producing cell have the potential to degrade carbohydrates and generate common-goods resources for other bacteria with the ability to use them (65) (Fig. 2A). The utilization of the oligosaccharide products generated by these enzymes could still be specific to strains that possess enzymes required to finish their degradation but lack the catalytic ability to degrade full-length polysaccharides. Such a phenomenon was recently observed in strains of *B. vulgatus* that can utilize xylooligosaccharides (XOSs) up to 4 sugars long but are unable to degrade larger xylans (73).

While it has been perhaps the best-characterized system, the prototypic *B. thetaiotaomicron* Sus continues to provide new mechanistic insights. All four of the outer membrane lipoproteins in this system have had crystal structures determined with a ligand, revealing 8 binding sites spread across 4 proteins, plus 1 additional active site in SusG, all for a substrate with low sugar/linkage complexity (36, 61, 74). Previous work demonstrated that a binding site mutation in SusD results in the loss of starch binding as well as the loss of the ability to activate growth on starch unless a small amount of maltose is provided (62). However, this *B. thetaiotaomicron* SusD mutant was still able to grow on maltooligosaccharides, suggesting compensatory roles for one or both of the related outer membrane proteins SusE and SusF. A recent study revealed that despite sharing significant sequence and structural homology, SusE exclusively confers this ability to grow on maltooligosaccharides (63). Additional work using live-cell imaging has revealed substantial variability in the surface mobility of Sus proteins. In live anaerobic cells, SusE remains stationary (75), while the hydrolytic enzyme SusG moves dynamically around the cell surface, spending more time in the locality of bound starch molecules (76). These studies provide important insight into the assembly and dynamic function of PUL-encoded machinery and serve as a reference for additional detailed studies that have begun to emerge for other Sus-like systems, including xyloglucan and cereal-, yeast-, and seaweed-derived β -glucans in other *Bacteroides* species (44, 53, 64, 77).

MARINE BACTEROIDETES USE PULs DURING UTILIZATION OF ALGAL POLYSACCHARIDES AND HAVE TRANSFERRED SOME OF THESE ABILITIES TO HUMAN GUT BACTEROIDES

Interestingly, several studies have shown that some human gut *Bacteroides* have the ability to degrade seaweed-derived polysaccharides such as porphyran, agarose, alginate, laminarin, and carrageenan (16, 17, 41, 54, 57, 58, 78, 79), which have been introduced into the human diet through cuisine or as food additives. In at least some cases, the PULs enabling these abilities appear to have been transferred into gut *Bacteroides* via mobile DNA elements, and the genes often have close relatives in marine *Bacteroidetes*, suggesting transfer between these environments (16). Additional PULs that target other algal polysaccharides have been identified in marine *Bacteroidetes* themselves, and these PULs often contain more genes, encode a more expanded enzyme repertoire than those present in gut *Bacteroidetes*, and include genes for adhesion proteins that may promote anchoring to the nutrient source (80). These expanded enzyme repertoires may reflect the complexity of the polysaccharides present in the cell walls of red, green, and brown algae as well as those from cyanobacteria and the variable EPS structures that they produce (81–84). An important role for marine *Bacteroidetes* that possess these PULs is to release fixed carbon during and after algal blooms, and the transcripts and corresponding proteins from these systems have become some of the most highly observed products in marine metatranscriptomic and corresponding metaproteomic samples (85, 86). Growth analysis of cultured organisms has been used to measure the activities of the bacterial members present in such blooms, leading to insight into the diverse polysaccharides being utilized as well as which species establish niches around these complex substrates (80, 87–91).

Similar to studies in gut *Bacteroidetes*, full or partial degradation mechanisms for the algal polysaccharides laminarin (91–95), alginate (54), agarose (96, 97), ulvan (98),

and carrageenan (41) have been determined in some of their marine relatives. Some marine *Bacteroidetes* also appear to utilize α - and β -mannans (99), and the *Bacteroidetes* in this environment tend to utilize substrates more selfishly, perhaps because of the rapid loss due to dilution that might occur with extracellular degradation in the ocean (100, 101). The genes responsible for the utilization of ulvan, a sulfated heteropolymer of rhamnose and uronic acids, have been characterized in *Formosa agariphila*, with an impressive armament of 39 PUL genes and 20 additional non-PUL genes involved in processing this polysaccharide (102). While substrate-specific PULs exist in marine *Bacteroidetes* to target ulvan, fucellaran, and others (103, 104), they have not yet been detected in human gut *Bacteroidetes*, possibly because many algae have not become a routine part of the human diet and provided the corresponding selective pressure for environment-to-gut transfer of the genes involved. Although PULs specific to these additional algal polysaccharides may not have yet been transferred to the human gut, horizontal gene transfer (HGT) of PULs and other genomic islands is widespread among *Bacteroides* and gut bacteria in general, and the acquisition of these systems plays a role in niche partitioning (105). As discussed below, PULs involved in degrading seaweed polysaccharides could be utilized to engineer new “orthogonal niches” into recombinant human gut bacteria to create biotherapeutics that are coupled to the ingestion of polysaccharides that are rare in the human diet (5).

GRAM-POSITIVE BACTERIA AND SOME ENTEROBACTERIACEAE ACCESS POLYSACCHARIDES VIA ALTERNATIVE MULTIPROTEIN SYSTEMS

In contrast to the TonB-dependent transporter systems that are dominant in *Bacteroidetes*, polysaccharide degradation in the Gram-positive *Firmicutes* and *Actinobacteria* is accomplished through different cellular mechanisms that are often built around enzymes with catalytic domains similar to those of *Bacteroidetes* (106). Gram-positive gut symbionts often encode polysaccharide-degrading functions within genetic loci termed Gram-positive PULs (gpPULs) (18), which may encode ATP binding cassette (ABC), major facilitator superfamily (MFS), or phosphoenolpyruvate-phosphotransferase system (PTS) transporters that are coexpressed with associated degradative enzymes (22, 107–109) (Fig. 1D and Fig. 2B). Several examples have been investigated at a mechanistic level, including in *Eubacterium rectale*, which is able to grow on starch and its component oligosaccharides through functions encoded in five separate genomic loci that include three membrane-bound amylases, one of which has five appended carbohydrate binding modules (CBMs) (110, 111). Recently, two separate multienzyme loci were characterized in the butyrate producer *Roseburia intestinalis* that mediate the degradation of β -mannans (20). Similar to *E. rectale*, *R. intestinalis* employs membrane-bound enzymes that harbor carbohydrate binding modules (Fig. 2B) in the same polypeptide, which may fill roles analogous to those of the SusE/F binding domains in *B. thetaiotaomicron* Sus, which are encoded separately. After initial degradation at the membrane or extracellularly, *R. intestinalis* imports partially broken-down oligosaccharides into the cytoplasm using ABC transporters, and the oligosaccharides are further broken down by the action of additional esterases and glycoside hydrolases. *R. intestinalis* also uses dietary xylan by cleaving it into xylooligosaccharides (XOSs), mainly xylotetraose, at the cell surface and then imports these products through an ABC transporter into the cell for further intracellular processing and catabolism, similar to the strategy that it uses for β -mannan degradation, with the addition of an intracellular epimerase that helps shunt foraged sugar products into central metabolism (Fig. 2B) (112). Other species within the genera *Bifidobacterium* and *Lactobacillus* have the ability to utilize xylan, XOSs, or arabinoxyloligosaccharides (AXOSs) (113, 114) through more elaborate mechanisms, including the secretion of non-cell-attached, extracellular enzymes to break down insoluble or complex structures prior to binding and import into the cytoplasm (115). Like some *Bacteroides* noted above that can use only oligosaccharides derived from larger polymers (73, 116), some

bifidobacteria can engage in interphylum cooperation with *Bacteroides*, with the former profiting from the breakdown of xylans from certain botanical sources into accessible xylooligosaccharides by species like *B. ovatus* (117).

Some gut Gram-positive bacteria have adapted another multienzyme system to target resistant starch (RS), cellulose, and possibly other substrates. The “cellulosome” is a mechanistically different strategy to organize polysaccharide-degrading enzymes and associated CBMs (118). Cellulosomes have been well characterized in environmental bacteria (118–120) and have more recently been identified in some human gut bacteria. As their name implies, these systems were originally discovered for their role in degrading cellulose (121) and consist of large, membrane-bound, multienzyme complexes (Fig. 2C). The most widely studied of these complexes was first described in the 1980s in the thermophilic soil bacterium *Clostridium thermocellum* (119, 120). Analogous cellulosomes exist in the gut symbiont *Ruminococcus champanellensis* that target cellulose (122). Interestingly, the same protein organization that is a hallmark of cellulosomes has been adapted by the gut species *Ruminococcus bromii* to equip it to degrade RS using amylases that presumably have gained added catalytic capacity toward this insoluble substrate (123). These so-called “amylosomes” contain component proteins similar to cellulosomes termed dockerins, cohesins, and scaffoldins adapted to display these RS-specific enzymes (123, 124). Although the amylosome of *R. bromii* is the best studied, metagenomics analysis suggests that more human *Ruminococcus* strains use this strategy for the breakdown of RS, cellulose, and perhaps other substrates (125). Given the taxonomic diversity of gut *Firmicutes*, additional polysaccharide degradation strategies employed by these Gram-positive bacteria will likely be uncovered as further investigations are performed on these often fastidious and therefore understudied species, a research horizon that will undoubtedly be propelled by resurgent culturing efforts (126–130).

In contrast to the polysaccharide-dominant nutrient utilization strategies of the more abundant *Bacteroidetes* and *Firmicutes*, most of the common gut *Enterobacteriaceae* such as *Escherichia coli* are predominantly confined to the utilization of more simple mono- and disaccharides. However, a few strains of *Enterobacteriaceae* have evolved strategies for degrading complex carbohydrates. Notably, the pathogen *Yersinia enterocolitica* is able to bind and degrade the plant cell wall pectin polygalacturonic acid via a series of periplasmic carbohydrate binding modules and outer membrane-bound and extracellular polysaccharide lyases (PLs), carbohydrate esterases (CEs), and GHs (131).

MONOSACCHARIDES AND OTHER OVERLOOKED NUTRIENTS

The importance of monosaccharides has been given less attention in studies of HGM species, perhaps because they are often broadly utilized by these bacteria, and many are assumed to be absorbed by the host. However, it is likely that relevant amounts of simple sugars or oligosaccharides may be transiently present, perhaps through the action of nonselfish bacterial catabolism, which might enable some bacteria to rely on consuming these simpler products. For *E. coli*, competition for nutrient niches has been primarily defined by the importance of various monosaccharides to support colonization by different pathogenic and commensal strains. While many species broadly utilize simple sugars, they sometimes vary in their preference for metabolizing these sugars in complex mixtures, leading to alterations in the importance of certain sugars to different strains (132). Studies that examined the ability of invading pathogenic bacteria to access nutrients during infection demonstrated that both enterohemorrhagic *E. coli* (EHEC) and *Salmonella* upregulate genes for the utilization of ribose and other simple sugars during infection of cows, chickens, and mice (133–135). EHEC prefers ribose to some other sugars such as fucose, arabinose, and maltose (132), while the related compounds deoxyribose and DNA can also be utilized during invasion by pathogenic *E. coli* (136, 137). Several pathogenic bacteria such as *Clostridium difficile*, *Salmonella enterica* serovar Typhimurium, *Citrobacter rodentium*, and *E. coli* have an additional strategy that involves scavenging simple sugars such as fucose,

sialic acid, and galacturonic acid, respectively, that have been liberated from larger polysaccharides by the action of commensal *Bacteroides* (138–141).

Often, the loci involved in monosaccharide catabolism by *Proteobacteria* include a sugar kinase, a transporter (e.g., ABC transporter or MFS transporter), a regulator, and additional genes specific for the metabolism of that sugar (e.g., aldolase or isomerase). These systems are generally less complex than PUL-encoded mechanisms in *Bacteroides* (Fig. 2D). However, *B. thetaiotaomicron* has related gene clusters for the catabolism of the monosaccharides L-fucose, L-rhamnose, D/L-arabinose, D-ribose, and D-fructose. For fucose and rhamnose, the corresponding *B. thetaiotaomicron* loci look very similar to those found in *Proteobacteria*, without any additional PUL machinery such as Sus homologs or CAZymes (142–144). Curiously, the machinery for arabinose metabolism is similar to that found in *Proteobacteria* but is embedded within a larger PUL for the degradation of arabinan, a polysaccharide that also contains arabinose. Despite this genetic arrangement, the genes involved in depolymerizing arabinan are activated in response to α 1,5-linked arabinooctaose oligosaccharides (31), while the genes for arabinose utilization are turned on by the free monosaccharide, which may also be liberated from other polymers (145). This observation suggests that some *Bacteroides* PULs may have evolved from simpler systems that enable sugar utilization but with the addition of enzymatic machinery to target larger polysaccharides that are sources of the cognate sugar. Indeed, two other *B. thetaiotaomicron* PULs support this idea. A PUL involved in degrading the β 2,6-linked fructan levan contains substrate-specific CAZymes assembled around a core system encoding fructokinase, fructose permease, and a fructose-activated regulator (43). Interestingly, fructose-activated variants of this PUL exist in other *Bacteroides* species, including strains of *B. thetaiotaomicron* (146), that contain different enzymes for releasing fructose from the β 2,1-linked polymer inulin (43). A PUL involved in the catabolism of ribose, nucleosides (NSs), and RNA (147) also contains two ribose kinases, a permease, and a predicted ribose-responsive regulator. Interestingly, the ability to degrade RNA and NS is dependent on the actions of the ribokinases and a non-PUL-encoded nucleoside phosphorylase (BT4554), despite other CAZymes and SusC/D homologs being present. Partially homologous variants of this ribose PUL are found throughout the phylum in human gut, marine, and soil isolates, with many different CAZyme and genetic architectures. While all of these retain the core regulator, kinase, and permease genes, the accessory genes within these PULs are often highly variable, suggesting that individual species have evolved these variant PULs to access ribose from diverse sources.

Some plants synthesize and store a number of additional carbohydrate-derived molecules such as glucosinolates, flavonoids, and sulfonoacid sugars, and several studies have highlighted the abilities of gut bacteria to metabolize these nutrients, in some cases activating bioactive compounds that then exert effects on the gut. One of these studies demonstrated that *B. thetaiotaomicron* and other *Bacteroides* species possess genes that enable them to cleave glucosinolates, a group of thioglucosides that are abundant in cruciferous plants like broccoli (148). This releases glucose that becomes available to bacteria but also releases an active isothiocyanate molecule, which is variable based on the particular glucosinolate cleaved and with some being associated with protective effects against intestinal cancer and other diseases (149). Another family of plant compounds that have been associated with protective health effects are flavonoids, which occur in free flavonol forms (e.g., quercetin) as well as flavonols with one or more attached mono- or disaccharide sugars (e.g., glucose or rutinose). Some bacteria such as *Eubacterium ramulus* and *Flavonifractor plautii* have developed the ability to ferment these molecules as a growth substrate, although the genes involved have not been identified (150, 151). Finally, strains of *Eubacterium rectale* and *Clostridium* are capable of growing on the plant-derived sugar sulfoquinovose, a sulfonoacid derivative of glucose that is abundant in plants and is a precursor to sulfolipid biosynthesis (152). While these abundant plant compounds can serve as direct nutrients for gut bacteria, the fact that several of them are associated with positive

health effects points to the presence of the proper activating bacteria as a critical factor in unlocking their effects.

Finally, pathogens have also evolved strategies to access nutrients beyond carbohydrates during infection. When the intestine is inflamed, L-serine that is commonly present in the diet provides *Enterobacteriaceae* a competitive edge over resident bacteria through the actions of dedicated L-serine transporters and dehydratases that are up-regulated and provide an inflammation-specific advantage (153). Enteropathogenic *E. coli* (EPEC) was recently shown to employ its type III secretion system (T3SS) injectisome, an apparatus long associated with the injection of effector proteins into the host cell, to steal or “pirate” amino acids from host cells and gain an advantage over commensal bacteria (154) (Fig. 2D). Taken together, it is clear from these studies that both gut pathogens and commensals have forged new nutrient niches by adapting strategies to scavenge or access both monosaccharides and amino acids.

TO COOK OR NOT TO COOK, LET'S ASK THE MICROBIOTA: COOKING AND FOOD PRESERVATION ALTER NUTRIENTS FOR THE GUT MICROBIOTA

Advanced glycation end products (AGEs) or Maillard reaction products (MRPs) are generated from reactions between sugars and proteins during cooking and can also influence the gut microbiota (23, 155, 156). The MRP fructose-asparagine (FA) is metabolized almost exclusively by *Salmonella enterica* during infections because *S. enterica* encodes a high-affinity transporter for FA. Due to *S. enterica* causing inflammation, other microbiota members such as *Clostridia*, which also possess high-affinity FA systems, are excluded from this nutrient niche (157). Another recent study revealed that commensal *Collinsella intestinalis* and *Collinsella aerofaciens* utilize the MRP fructose-lysine (FL) to various degrees based on the presence of other, repressive nutrients such as glucose. In the presence of glucose, *C. intestinalis* does not repress FL utilization genes, while *C. aerofaciens* does. These strains accomplish FL metabolism through the actions of a phosphotransferase system and a deglycase that converts FL into the central metabolite glucose-6-phosphate (158). Finally, the AGE N-ε-carboxymethyllysine is degraded by as-yet-uncultured members of the *Oscillibacter* genus, as inferred based on enrichment culturing experiments, and by a cultured *Cloacibacillus evryensis* strain through an unknown mechanism (159). These recent studies complement earlier reports of the effects that cooking food has on the microbiota (160, 161) through the formation of MRPs and AGEs and indicate that this area requires deeper investigation.

MRPs and AGEs are not the only substrates created or modified by cooking. Starch is perhaps the most important nutritional carbohydrate in the human diet, and its digestibility is dramatically impacted by cooking. While the starch in a raw potato may be 50 to 70% resistant to digestion by human amylases (162) (so-called “resistant starch” that reaches the colon and its resident HGM), cooking a potato increases starch digestibility by both human and bacterial enzymes through a process called gelatinization. Resistant starch (RS) is categorized into 5 types that vary based on whether it is derived from raw foods, foods that have been cooked and cooled to allow starch to retrograde into crystalline RS, or food containing starches that are chemically modified or have physical coatings that prevent enzymatic access (163). Some bifidobacteria and *R. bromii* strains degrade naturally occurring type 2 RS found in foods such as raw potatoes and other uncooked foods (124, 164). Chemical modifications through the process of esterification, cross-linking, transglycosylation, or acid hydrolysis of type 4 RS are thought to make the starch less accessible to bacterial degradation. However, studies have observed that dietary supplementation with this type of RS often changes the composition of the HGM, skewing toward more *Bacteroidetes* and *Actinobacteria* and fewer *Firmicutes* and specifically increasing *Parabacteroides distasonis* and *Bifidobacterium adolescentis* (165, 166). These observations suggest that some species may be able to access this nutrient through an as-yet-unknown mechanism. Other types of RS, such as type 1 (physically inaccessible granules due to being surrounded by a protein matrix or cell wall material) and type 3 (retrograded starch due to heating

and subsequent refrigeration), have also been shown to alter the composition of *in vitro* synthetic communities derived from porcine microbiota. However, studies into direct mechanisms are still needed to determine which species are responsible for these effects (167).

The introduction of other food additives, such as the stabilizer trehalose, has implications for the HGM. Specific *C. difficile* ribotypes are able to sense very low concentrations of trehalose and have acquired a PTS for the high-affinity transport of this disaccharide sugar, which can also lead to greater upregulation of toxin B genes (24). One possible reason for increased *C. difficile* outbreaks, specifically caused by the ribotypes able to use trehalose, is the expanded use of trehalose in the diet since the early 2000s when it became inexpensive to produce, providing a selective pressure for these ribotypes to dominate globally (24). Collectively, these and other studies highlight that additional food modifiers such as emulsifiers (168) and artificial sweeteners (169) should also be considered for their potential to modulate the gut microbiota either because they are directly degraded or because of other indirect mechanisms.

"WATCHING" WHAT YOU EAT: HOW *BACTEROIDES* SPECIES REGULATE THEIR CARBOHYDRATE INTAKE

Given their abundance of carbohydrate-degrading systems, *Bacteroides* species have correspondingly developed a series of finely tuned local and global regulatory networks that enable surveillance for available nutrients without the costly expression of enzymes and other functions in the absence of their substrate. Most PULs are regulated by locally encoded regulators that respond to a polysaccharide's presence and are usually transcription activators since eliminating them blocks PUL activation (12, 170, 171). For these systems to be capable of responding to their specific substrate, there must be a sensory state in which small amounts of critical PUL enzymes and other functions are expressed, an idea that is supported by immunofluorescence imaging of a small number of *B. thetaiotaomicron* Sus proteins on the cell surface during growth under noninducing conditions such as minimal medium supplemented with glucose (62). Upon exposure to starch, the primed Sus system initiates the degradation of starch, yielding the inducing molecule maltose, which initiates transcription activation through the dedicated inner membrane-spanning regulator SusR. The response to starch exposure occurs incredibly quickly, with transcript and surface protein levels reaching their respective maxima in 5 to 10 min and 30 min after exposure (172). This "priming effect" is likely to occur for most PULs, although the details of the promoters and potential signals that mediate or modify basal expression have not been investigated deeply and, as discussed below, could be a critical point at which global regulation intersects with individual PULs to impose catabolite repression-like effects.

SusR is predicted to be an inner membrane-spanning transcription activator and is one of several different types of PUL regulators that share the theme of sensing saccharide cues in the periplasm or outer membrane, a feature that may enable them to both sense these cues quickly and incorporate additional sugar and linkage information before oligosaccharides are degraded into "less informative" single sugars. Only two PULs with SusR-type regulators have been characterized and both target glucose-based polysaccharides, starch or dextran, and this is a minor regulator type (171). A more abundant mode of regulation that *Bacteroides* species commonly employ is a variant of the more typical two-component regulator system in which all of the domains of the sensor histidine kinase and DNA binding response regulator are combined into a single protein termed a hybrid two-component system (HTCS). HTCS regulators are found in PULs involved in degrading a variety of polysaccharides, including most PULs associated with dietary fiber degradation and others that are specific for fungal cell wall mannans, chondroitin and heparin sulfates, seaweed-derived polysaccharides, and mucin O-glycans (10, 16, 43, 173, 174) (Fig. 3A). A third type of regulation is governed by extracytoplasmic function (ECF)- σ /anti- σ regulator pairs (Fig. 3B), which so far have

PUL Regulatory Mechanisms

FIG 3 Local and global regulatory mechanisms involved in *Bacteroides* carbohydrate utilization. (A) Hybrid two-component system (HTCS) regulators are frequently associated with PULs involved in the utilization of dietary fiber polysaccharides, although they are also associated with other substrates. Unlike classical two-component systems composed of separate response regulator and histidine kinase domains, these functions are fused into one multidomain-containing protein, with an oligosaccharide signal being bound and sensed directly by the HTCS, triggering the upregulation of the adjacent PUL genes (31, 201). Highlighted is one additional regulatory strategy in which the polysaccharide being degraded, chondroitin sulfate, yields unsaturated disaccharides that are bound by the HTCS regulator but also further degraded by a periplasmic GH. The GH that cleaves the inducing disaccharide but not the HTCS is upregulated, serving as a brake on the positive-feedback loop initiated by chondroitin sulfate degradation. (B) ECF- σ /anti- σ factor regulators are commonly found in PULs responsible for degrading host polysaccharides. The anti- σ factor spans the inner membrane and directly interacts in the periplasm with a specialized N-terminal domain of SusC-like transporters (170) to transduce the signal generated by substrate transport, ultimately resulting in the activation of the bound ECF- σ factor. In a subset of these systems found in multiple species, antisense small RNAs are expressed from promoters that overlap the start codon of the *susC*-like gene and act to repress transcription by an unknown mechanism (180). RNA Pol, RNA polymerase. (C) Global mechanisms likely act to exert carbon catabolite repression-like effects in *Bacteroides*, although the complete mechanism has not yet been determined. These effects may explain the nutrient utilization hierarchies of some *Bacteroides* strains, which are illustrated in a simple schematic for the utilization of two different polysaccharides in the top right inset. While both polysaccharides initially trigger PUL transcriptional responses, we hypothesize that after sugars are released from the high-priority polysaccharide (black curve), they enter the cell and repress the expression of the low-priority PUL transcript (blue), possibly through the mechanisms described in the text. Additional global mechanisms that have not been fully resolved involve the CRP homolog (BT4338), which directly controls the transcription of some non-PUL sugar utilization genes and also appears to exert indirect effects on several PULs. For simplicity, several additional outer membrane and periplasmic proteins, such as additional CAZymes and surface glycan binding proteins, are not shown in each panel but are required for polysaccharide degradation. UTR, untranslated region.

been almost exclusively associated with systems responsible for utilizing host-derived mucin O- and N-linked glycans (10, 12, 59, 170). These regulators function similarly to the *E. coli* FecI/FecR sigma/anti-sigma factor pair (175, 176) by physically coupling a periplasmic domain of the anti-sigma factor to an N-terminal domain of a corresponding TonB-dependent receptor and activating transcription when the substrate is transported across the outer membrane (170, 177, 178). Additional regulators found in PULs include OmpR/streptomyces antibiotic regulatory protein (SARP) family regulators that are also predicted to span the inner membrane (12, 170) and AraC, GntR, NrtR, and LacI-type regulators, which may function by engaging their regulatory cues in the cytoplasm, although few have been investigated in depth (39, 142–144). Given the similarities in PUL architectures between gut and environmental *Bacteroidetes*, emerging studies are revealing the mechanisms of some of these additional regulator types, including a GntR family member in marine *Zobellia galactanivorans* that acts as a repressor in the absence of the substrate alginate for its associated PUL (179).

While PUL-associated regulators are at the front lines of activating specific responses to polysaccharides, additional layers of local regulation exist. Antisense small RNAs (sRNAs) were recently described in some but not all *B. fragilis* PULs that are regulated by ECF- σ /anti- σ pairs, occur between the cotranscribed anti-sigma and *susC*-like

genes, and have been found in other species (180) (Fig. 3B). These sRNAs repress PUL transcription since eliminating them by mutating the antisense promoter results in increased PUL transcription both at a basal state and after induction by the cognate polysaccharide. Although the mechanisms through which they work and whether or not these sRNAs themselves integrate additional regulatory cues are unknown, the elimination of one *B. fragilis* sRNA in a PUL that is repressed by glucose partially alleviated this effect (180). Interestingly, an intergenic region (IGR) that lies between an anti-sigma-*susC*-like gene pair (the same region in which sRNAs are found) is important for *B. thetaiotaomicron* to exert fructose-based catabolite repression on the PUL in which the IGR is contained. This effect appeared to be localized to just the PUL containing the deleted IGR, since similar loss of repression was not observed at other PULs (47). This observation further suggests that these sRNA-containing regions integrate global sugar availability information but exert their effect locally at their own PUL.

Additional regulatory mechanisms have emerged from the biochemical function of PUL-associated sensors and enzymes. A *B. thetaiotaomicron* HTCS required to activate a PUL involved in chondroitin sulfate utilization (12) was found to compete for its activating signal (a 4-5 unsaturated disaccharide generated by the action of chondroitin lyase) with a periplasmic glycoside hydrolase that cleaves this signal into its component sugars. Since PUL activation via the HTCS increases the expression of the gene encoding the cleaving glycoside hydrolase but not the HTCS itself, this is thought to exert a brake on the positive-feedback loop that is initiated by substrate recognition and balance PUL activation with the rate of substrate processing (Fig. 3A) (181, 182).

Finally, posttranscriptional regulatory effects have recently been discovered in PUL regulation and are likely another point at which global nutrient status is integrated locally at the PUL level. In one *B. thetaiotaomicron* PUL involved in host glycan degradation, a single adjacent HTCS (BT3172) is present to presumably activate expression in response to this substrate (12). Both glucose and fructose reduce the amount of BT3172 protein but not the corresponding transcript, an effect that is mediated by the presence of a 5' leader on the BT3172 message (183). Deletion of this leader alleviates BT3172 repression, and its effect can also be transferred to other HTCS-coding genes to cause them to be repressed by the same sugars.

Several of the observations described above suggest that connections exist between local PUL expression and the global nutrient status of the *Bacteroides* cell. Within several *Bacteroides* species, there are clear nutrient utilization hierarchies, within which PULs that target different polysaccharides are expressed in a defined order, even when all of their respective substrates are present (172, 184, 185). Studies looking for carbon catabolite repression mechanisms similar to those in *E. coli* and other bacteria have failed to detect the presence of cyclic AMP (cAMP) in *Bacteroides* (186, 187), and adding exogenous cAMP to *B. thetaiotaomicron* cultures grown in a mixture of high- and low-priority glycans failed to relieve the repression of PULs involved in degrading lower-priority substrates (47). However, recent studies have determined that a regulator in the same family as the *E. coli* cAMP receptor protein (CRP), originally designated MalR because it plays a role in maltose metabolism (34), indeed plays a role in the utilization of several polysaccharides by *B. thetaiotaomicron*. Deletion of *malR* (BT4338) from *B. thetaiotaomicron* resulted in substantially diminished growth on several mono- and polysaccharides. Moreover, the utilization of some substrates, like polymeric arabinan and its component arabinose, was almost completely eliminated (145). A follow-up study showed that BT4338/MalR acts as a transcription factor by binding to promoter sequences upstream of several loci responsible for the catabolism of monosaccharides (fucose, arabinose, and xylose), for which a BT4338 mutant also exhibits growth defects (188). Reductions in mRNA abundance were observed for several PULs when BT4338 mutant cells were shifted to carbon starvation conditions and compared to wild-type cells subjected to the same treatment. However, direct binding sites for BT4338/MalR near these PULs were not detected, therefore suggesting an indirect mechanism. One of the strongest responses to carbon starvation was the

activation of *fusA2*, a gene encoding a translation elongation factor whose transcription is dependent on BT4338. Comparison of growth defects on multiple carbohydrates shows that a *fusA2* mutant does not exhibit growth defects for the same carbohydrates that the *BT4338* mutant does. This decoupled response suggests that PUL expression upon carbon starvation is not directly mediated by FusA2 but is likely mediated by another signaling pathway involving BT4338, while FusA2 is likely important because it provides a distinct protein synthesis pathway during changes in nutrient pools.

While the complete mechanism(s) for orchestrating carbohydrate utilization by *Bacteroides* remains to be determined, it appears that global signals such as the amount and identity of simple sugars generated by polysaccharide catabolism influence local PUL expression through factors such as BT4338 and perhaps other unknown factors that connect global sugar metabolism to local PUL regulation through transcriptional and posttranscriptional mechanisms. Previous work has revealed that PULs involved in utilizing lower-priority polysaccharides in *B. thetaiotaomicron* are activated early in a mixture containing multiple substrates (172). However, this response quickly fades, and the transcription of lower-priority PULs is fully or partially repressed until later in growth, presumably after repressing substrates have been used up. This suggests a model in which each PUL is activated by its associated sensor/regulator, an event that occurs quickly upon exposure and often in the periplasm. Then, as different sugars are liberated from the polysaccharides in a mixture, certain dominant sugars enter the cell, where they repress the transcription of low-priority PULs (Fig. 3C, inset). These effects could be exerted at a number of points in the PUL expression process described above but are known to be dynamically initiated by introducing a repressing substrate(s) into a culture that is processing lower-priority polysaccharides, resulting in the rapid loss of transcripts for low-priority PULs (47, 172).

Interestingly, a recent study that directly monitored both *B. thetaiotaomicron* PUL transcription and carbohydrate depletion over time in a polysaccharide mixture revealed that PULs involved in degrading high-priority substrates remain activated even after their substrate is largely depleted (172). This suggests that PULs targeting high-priority substrates may lack signals to be silenced during the utilization of other substrates such that they remain activated in the presence of a very small amount of their substrate. Retained expression of these high-priority PULs may equip *B. thetaiotaomicron* to rapidly reengage the degradation of high-priority substrates because the enzymes and other protein apparatus are already present at higher levels on the cell surface. Finally, since there is no general theme connecting the source or component sugar composition of high- and low-priority polysaccharides, it remains to be determined what the biological consequences of this global regulation are for these bacteria in the HGM. Since at least two studies have revealed variations in priority between different species (47, 185), and repressive signals can be recombinantly moved between PULs (183), an intriguing possibility is that polysaccharides are not prioritized due to any intrinsic value differences of their sugars. Instead, these responses could be programmed within each strain or species based on a given set of PULs and their corresponding hierarchical organization. If individual species and strains are differentially programmed with nonoverlapping priorities despite being able to target some of the same substrates, such a phenomenon might decrease competition among gut bacteria and promote the persistence of more diverse HGM communities.

SYNTHETIC BIOLOGY TO ENGINEER GUT BACTERIA

Given the metabolic importance of the HGM and the vast degradative capabilities encoded within its members, there has been increasing interest in using synthetic biology to engineer gut bacteria to perform particular functions. Ethical, regulatory, and consumer considerations aside, a major barrier to approaching this goal is the realization that the majority of species within the HGM lack tools for genetic manipulation. In several foundational experiments, genetically tractable *B. ovatus* was engineered to

couple PUL-mediated transcriptional responses to the ingestion of dietary xylan (189) and induce the production of human cytokines and proteins to treat inflammation, including transforming growth factor β (TGF- β), human intestinal trefoil factor 3, and keratinocyte growth factor 2 (190–192). While it involved only *in vitro* experiments with engineered bacteria, an elegant example of a recent synthetic biology advance was the optimization of 12 independent, small-molecule-inducible sensors that respond to substrates like arabinose, choline, cuminic acid, and naringenin, among others, into a single strain of *E. coli* (193). This system serves as an example of how common HGM strains could be engineered to respond to different nutrients present in the gut and couple these signals to the production of different therapeutics or the expression of compound pathways.

Even members of the genus *Bacteroides*, for which genetic systems have been fairly well developed, have benefited from new tools in recent years that are critical for intentional engineering. These include multichannel fluorescence imaging to distinguish species and strains *in vivo* (194), tunable expression of genes (195), and the ability to respond to endogenous signals via CRISPR-Cas9-mediated gene expression and record exposures to nutrients via a chromosomally encoded integrase (196). One impediment to the manipulation of a broad set of *Bacteroides* strains and species is the availability of broadly applicable selectable and counterselectable markers, which has recently been addressed by the introduction of a minimal PUL for inulin utilization into non-inulin-utilizing strains as a means of positive selection and the corresponding use of a type VI secretion system toxin/antitoxin pair for counterselection (197). Perhaps one of the most interesting approaches is the transfer and manipulation of large *Bacteroidetes* PULs, particularly those that permit the utilization of rare or unique nutrients, into other human gut *Bacteroides* strains. This strategy can be used to design (via diet or supplements) and fill (via engineered bacteria that use those supplements) “orthogonal niches” around specific nutrients like the seaweed polysaccharides discussed above. Strains that are able to utilize the introduced polysaccharide, sometimes almost exclusively, will gain a substantial colonization advantage, permitting them to be engrafted and supported via diet as new strains in an existing HGM ecosystem (6). This idea is not new and has roots in Rolf Freter’s nutrient niche hypothesis (198) but with modern goals of designing and filling a new niche rather than allowing natural competition for nutrients and adaptation to take place.

As a proof of concept for this idea, a strain of *Bacteroides plebeius* that was derived from a Japanese adult (199) and contains a PUL for degrading the seaweed polysaccharide porphyran was transferred into mice fed a diet containing the seaweed *Porphyra yezoensis*, from which it is derived, leading to long-term engraftment of the new strain (6). A similar PUL for porphyran degradation in a *B. ovatus* strain conferred similar engraftment into conventional specific-pathogen-free (SPF) mice or ex-germfree mice colonized with human gut microbiota (5). This PUL was also transferred into *B. thetaiotaomicron* and *B. stercoris*, followed by the successful engraftment of these recombinant strains into conventional SPF mice or ex-germfree mice colonized with complex human microbiomes (5). These effects were tunable based on the amount of purified porphyran added to the diet, and the introduction of this orthogonal niche allowed *B. thetaiotaomicron* to be supported long enough that it colonized colonic crypts and replaced another previously present and otherwise isogenic strain of *B. thetaiotaomicron*, which typically blocks the engraftment of incoming strains (194). Taken together, these emerging technologies that allow for the creation of niches and the prolonged introduction of beneficial strains have the promise to help treat a variety of inflammatory and metabolic disorders both within and outside the gut. A key aspect of the design, construction, and deployment of these engineered microbes is the inclusion of appropriate and often redundant biocontainment systems, such as engineered auxotrophies, that ensure that the modified organisms cannot survive and compete when they are naturally released into the environment through feces (200).

PROSPECTUS

Given the central importance of complex carbohydrates and related nutrients to human gut bacteria, in-depth mechanistic studies are continuing to uncover the intricate metabolism of these nutrients by our gut symbionts. Within the HGM, there are likely to be additional carbohydrate nutrients that can serve as nutrients for some of its members. One of the most chemically diverse groups of polysaccharides in the gut is likely to be those that are produced by endogenous bacteria themselves as capsular and exopolysaccharides, and these might be important nutrients for some HGM members. While few of these polysaccharides have been studied in chemical detail, they are likely to contain different linkages relative to those in common plant and host polysaccharides and even novel sugars. Support for the idea that gut bacteria could degrade some of these microbial polysaccharides could lie in the presence of numerous PULs in otherwise well-studied *Bacteroides* species that have not been connected to known plant-, fungus-, or host-derived polysaccharides. It is probable that the direct production of at least the most abundant and prevalent microbial polysaccharides in the distal gut has provided selective pressure for other gut bacteria to assemble the enzymes and other functions required to utilize them. If the polysaccharides produced endogenously by gut bacteria are capable of feeding others, there could be an unseen and perhaps personalized food web within each of us that sustains and provides resilience to our own HGM.

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REFERENCES

- Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Nunez G, Martens EC. 2016. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167:1339–1353.e21. <https://doi.org/10.1016/j.cell.2016.10.043>.
- Ramakrishna C, Kujawski M, Chu H, Li L, Mazmanian SK, Cantin EM. 2019. *Bacteroides fragilis* polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat Commun* 10:2153. <https://doi.org/10.1038/s41467-019-09884-6>.
- Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472:57–63. <https://doi.org/10.1038/nature09922>.
- Zegarar-Ruiz DF, El Beidaq A, Iniguez AJ, Lubrano Di Ricco M, Manfredo Vieira S, Ruff WE, Mubiru D, Fine RL, Sterpka J, Greiling TM, Dehner C, Kriegel MA. 2019. A diet-sensitive commensal *Lactobacillus* strain mediates TLR7-dependent systemic autoimmunity. *Cell Host Microbe* 25:113–127.e6. <https://doi.org/10.1016/j.chom.2018.11.009>.
- Shepherd ES, DeLoache WC, Pruss KM, Whitaker WR, Sonnenburg JL. 2018. An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature* 557:434–438. <https://doi.org/10.1038/s41586-018-0092-4>.
- Kearney SM, Gibbons SM, Erdman SE, Alm EJ. 2018. Orthogonal dietary niche enables reversible engraftment of a gut bacterial commensal. *Cell Rep* 24:1842–1851. <https://doi.org/10.1016/j.celrep.2018.07.032>.
- Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, Weatherford J, Buhler JD, Gordon JI. 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science* 307:1955–1959. <https://doi.org/10.1126/science.1109051>.
- Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, Amieva MR, Huang KC, Sonnenburg JL. 2015. Quantitative imaging of gut microbiota spatial organization. *Cell Host Microbe* 18:478–488. <https://doi.org/10.1016/j.chom.2015.09.002>.
- Khan S, Waliullah S, Godfrey V, Khan MAW, Ramachandran RA, Cantarel BL, Behrendt C, Peng L, Hooper LV, Zaki H. 2020. Dietary simple sugars alter microbial ecology in the gut and promote colitis in mice. *Sci Transl Med* 12:eay6218. <https://doi.org/10.1126/scitranslmed.aay6218>.
- Cuskin F, Lowe EC, Temple MJ, Zhu Y, Cameron E, Pudlo NA, Porter NT, Urs K, Thompson AJ, Cartmell A, Rogowski A, Hamilton BS, Chen R, Tolbert TJ, Piens K, Bracke D, Vervecken W, Hakki Z, Speciale G, Munoz-Munoz JL, Day A, Peña MJ, McLean R, Suits MD, Boraston AB, Atherly T, Ziemer CJ, Williams SJ, Davies GJ, Abbott DW, Martens EC, Gilbert HJ. 2015. Human gut *Bacteroidetes* can utilize yeast mannan through a selfish mechanism. *Nature* 517:165–169. <https://doi.org/10.1038/nature13995>.
- Temple MJ, Cuskin F, Basle A, Hickey N, Speciale G, Williams SJ, Gilbert HJ, Lowe EC. 2017. A *Bacteroidetes* locus dedicated to fungal 1,6-beta-glucan degradation: unique substrate conformation drives specificity of the key endo-1,6-beta-glucanase. *J Biol Chem* 292:10639–10650. <https://doi.org/10.1074/jbc.M117.787606>.
- Martens EC, Chiang HC, Gordon JI. 2008. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host Microbe* 4:447–457. <https://doi.org/10.1016/j.chom.2008.09.007>.
- Luis AS, Briggs J, Zhang X, Farnell B, Ndeh D, Labourel A, Basle A, Cartmell A, Terrapon N, Stott K, Lowe EC, McLean R, Shearer K, Schuckel J, Venditto I, Ralet MC, Henrissat B, Martens EC, Mosimann SC, Abbott DW, Gilbert HJ. 2018. Dietary pectic glycans are degraded by coordinated enzyme pathways in human colonic *Bacteroides*. *Nat Microbiol* 3:210–219. <https://doi.org/10.1038/s41564-017-0079-1>.
- Ndeh D, Rogowski A, Cartmell A, Luis AS, Basle A, Gray J, Venditto I, Briggs J, Zhang X, Labourel A, Terrapon N, Buffetto F, Nepogodiev S, Xiao Y, Field RA, Zhu Y, O'Neil MA, Urbanowicz BR, York WS, Davies GJ, Abbott DW, Ralet MC, Martens EC, Henrissat B, Gilbert HJ. 2017. Complex pectin metabolism by gut bacteria reveals novel catalytic functions. *Nature* 544:65–70. <https://doi.org/10.1038/nature21725>.
- Lammerts van Bueren A, Saraf A, Martens EC, Dijkhuizen L. 2015. Differential metabolism of exopolysaccharides from probiotic lactobacilli by the human gut symbiont *Bacteroides thetaiotaomicron*. *Appl Environ Microbiol* 81:3973–3983. <https://doi.org/10.1128/AEM.00149-15>.
- Hehemann J-K, Kelly AG, Pudlo NA, Martens EC, Boraston AB. 2012. Bacteria of the human gut microbiome catabolize red seaweed glycans with

- carbohydrate-active enzyme updates from extrinsic microbes. *Proc Natl Acad Sci U S A* 109:19786–19791. <https://doi.org/10.1073/pnas.1211002109>.
17. Pluvinaige B, Grondin JM, Amundsen C, Klassen L, Moote PE, Xiao Y, Thomas D, Pudlo NA, Anele A, Martens EC, Inglis GD, Uwiera RER, Boraston AB, Abbott DW. 2018. Molecular basis of an agarose metabolic pathway acquired by a human intestinal symbiont. *Nat Commun* 9:1043. <https://doi.org/10.1038/s41467-018-03366-x>.
 18. Sheridan PO, Martin JC, Lawley TD, Browne HP, Harris HMB, Bernalier-Donadille A, Duncan SH, O'Toole PW, Scott KP, Flint HJ. 2016. Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrate-producing human colonic Firmicutes. *Microb Genom* 2:e000043. <https://doi.org/10.1099/mgen.0.000043>.
 19. Grondin JM, Tamura K, Dejean G, Abbott DW, Brumer H. 2017. Polysaccharide utilization loci: fueling microbial communities. *J Bacteriol* 199:e00860-17. <https://doi.org/10.1128/JB.00860-16>.
 20. La Rosa SL, Leth ML, Michalak L, Hansen ME, Pudlo NA, Glowacki R, Pereira G, Workman CT, Arntzen MO, Pope PB, Martens EC, Hachem MA, Westereng B. 2019. The human gut firmicute *Roseburia intestinalis* is a primary degrader of dietary beta-mannans. *Nat Commun* 10:905. <https://doi.org/10.1038/s41467-019-08812-y>.
 21. Porter NT, Martens EC. 2017. The critical roles of polysaccharides in gut microbial ecology and physiology. *Annu Rev Microbiol* 71:349–369. <https://doi.org/10.1146/annurev-micro-102215-095316>.
 22. Cockburn DW, Koropatkin NM. 2016. Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease. *J Mol Biol* 428:3230–3252. <https://doi.org/10.1016/j.jmb.2016.06.021>.
 23. Aljadhali N, Carbonero F. 2019. Impact of Maillard reaction products on nutrition and health: current knowledge and need to understand their fate in the human digestive system. *Crit Rev Food Sci Nutr* 59:474–487. <https://doi.org/10.1080/10408398.2017.1378865>.
 24. Collins J, Robinson C, Danhof H, Knettsch CW, van Leeuwen HC, Lawley TD, Auchtung JM, Britton RA. 2018. Dietary trehalose enhances virulence of epidemic *Clostridium difficile*. *Nature* 553:291–294. <https://doi.org/10.1038/nature25178>.
 25. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* 42:D490–D495. <https://doi.org/10.1093/nar/gkt1178>.
 26. Reddy SK, Bagenholm V, Pudlo NA, Bouraoui H, Koropatkin NM, Martens EC, Stalbrand H. 2016. A beta-mannan utilization locus in *Bacteroides ovatus* involves a GH36 alpha-galactosidase active on galactomannans. *FEBS Lett* 590:2106–2118. <https://doi.org/10.1002/1873-3468.12250>.
 27. Bagenholm V, Reddy SK, Bouraoui H, Morrill J, Kulcinskaja E, Bahr CM, Aurelius O, Rogers T, Xiao Y, Logan DT, Martens EC, Koropatkin NM, Stalbrand H. 2017. Galactomannan catabolism conferred by a polysaccharide utilization locus of *Bacteroides ovatus*: enzyme synergy and crystal structure of a beta-mannanase. *J Biol Chem* 292:229–243. <https://doi.org/10.1074/jbc.M116.746438>.
 28. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. *Science* 308:1635–1638. <https://doi.org/10.1126/science.11110591>.
 29. Bjursell MK, Martens EC, Gordon JI. 2006. Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, *Bacteroides thetaiotaomicron*, to the suckling period. *J Biol Chem* 281:36269–36279. <https://doi.org/10.1074/jbc.M606509200>.
 30. Xu J, Bjursell MK, Himrod J, Deng S, Carmichael HC, Chiang H, Hooper LV, Gordon JI. 2003. A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science* 299:2074–2076. <https://doi.org/10.1126/science.1080029>.
 31. Martens EC, Lowe EC, Chiang H, Pudlo NA, Wu M, McNulty NP, Abbott DW, Henrissat B, Gilbert HJ, Bolam DN, Gordon JI. 2011. Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biol* 9:e1001221. <https://doi.org/10.1371/journal.pbio.1001221>.
 32. McNulty NP, Wu M, Erickson AR, Pan C, Erickson BK, Martens EC, Pudlo NA, Muegge BD, Henrissat B, Hettich RL, Gordon JI. 2013. Effects of diet on resource utilization by a model human gut microbiota containing *Bacteroides cellulosilyticus* WH2, a symbiont with an extensive glyco-biome. *PLoS Biol* 11:e1001637. <https://doi.org/10.1371/journal.pbio.1001637>.
 33. Anderson KL, Salyers AA. 1989. Genetic evidence that outer membrane binding of starch is required for starch utilization by *Bacteroides thetaiotaomicron*. *J Bacteriol* 171:3199–3204. <https://doi.org/10.1128/JB.171.6.3199-3204.1989>.
 34. Cho KH, Cho D, Wang GR, Salyers AA. 2001. New regulatory gene that contributes to control of *Bacteroides thetaiotaomicron* starch utilization genes. *J Bacteriol* 183:7198–7205. <https://doi.org/10.1128/JB.183.24.7198-7205.2001>.
 35. Foley MH, Cockburn DW, Koropatkin NM. 2016. The *Sus* operon: a model system for starch uptake by the human gut *Bacteroidetes*. *Cell Mol Life Sci* 73:2603–2617. <https://doi.org/10.1007/s00018-016-2242-x>.
 36. Koropatkin NM, Martens EC, Gordon JI, Smith TJ. 2008. Starch catabolism by a prominent human gut symbiont is directed by the recognition of amylose helices. *Structure* 16:1105–1115. <https://doi.org/10.1016/j.str.2008.03.017>.
 37. Martens EC, Koropatkin NM, Smith TJ, Gordon JI. 2009. Complex glycan catabolism by the human gut microbiota: the *Bacteroidetes* *Sus*-like paradigm. *J Biol Chem* 284:24673–24677. <https://doi.org/10.1074/jbc.R109.022848>.
 38. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. 2013. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol* 11:497–504. <https://doi.org/10.1038/nrmicro3050>.
 39. Ravcheev DA, Godzik A, Osterman AL, Rodionov DA. 2013. Polysaccharides utilization in *Bacteroides thetaiotaomicron*: comparative genomics reconstruction of metabolic and regulatory networks. *BMC Genomics* 14:873. <https://doi.org/10.1186/1471-2164-14-873>.
 40. Terrapon N, Lombard V, Drula E, Lapebie P, Al-Masaudi S, Gilbert HJ, Henrissat B. 2018. PULDB: the expanded database of polysaccharide utilization loci. *Nucleic Acids Res* 46:D677–D683. <https://doi.org/10.1093/nar/gkx1022>.
 41. Ficko-Blean E, Prechoux A, Thomas F, Rochat T, Larocque R, Zhu Y, Stam M, Genicot S, Jam M, Calteau A, Viart B, Ropartz D, Perez-Pascual D, Correc G, Matard-Mann M, Stubbs KA, Rogniaux H, Jeudy A, Barbeyron T, Medigue C, Czjzek M, Vallenet D, McBride MJ, Duchaud E, Michel G. 2017. Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria. *Nat Commun* 8:1685. <https://doi.org/10.1038/s41467-017-01832-6>.
 42. Mardo K, Visnapuu T, Vija H, Aasamets A, Viigand K, Alamae T. 2017. A highly active endo-levanase BT1760 of a dominant mammalian gut commensal *Bacteroides thetaiotaomicron* cleaves not only various bacterial levans, but also levan of timothy grass. *PLoS One* 12:e0169989. <https://doi.org/10.1371/journal.pone.0169989>.
 43. Sonnenburg ED, Zheng H, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN, Sonnenburg JL. 2010. Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. *Cell* 141:1241–1252. <https://doi.org/10.1016/j.cell.2010.05.005>.
 44. Tamura K, Hemsworth GR, Dejean G, Rogers TE, Pudlo NA, Urs K, Jain N, Davies GJ, Martens EC, Brumer H. 2017. Molecular mechanism by which prominent human gut *Bacteroidetes* utilize mixed-linkage beta-glucans, major health-promoting cereal polysaccharides. *Cell Rep* 21:417–430. <https://doi.org/10.1016/j.celrep.2017.09.049>.
 45. Tamura K, Foley MH, Gardill BR, Dejean G, Schnitzlein M, Bahr CME, Creagh AL, van Petegem F, Koropatkin NM, Brumer H. 2019. Surface glycan-binding proteins are essential for cereal beta-glucan utilization by the human gut symbiont *Bacteroides ovatus*. *Cell Mol Life Sci* 76:4319–4340. <https://doi.org/10.1007/s00018-019-03115-3>.
 46. Larsbrink J, Rogers TE, Hemsworth GR, McKee LS, Tauzin AS, Spadiut O, Klinger S, Pudlo NA, Urs K, Koropatkin NM, Creagh AL, Haynes CA, Kelly AG, Cederholm SN, Davies GJ, Martens EC, Brumer H. 2014. A discrete genetic locus confers xyloglucan metabolism in select human gut *Bacteroidetes*. *Nature* 506:498–502. <https://doi.org/10.1038/nature12907>.
 47. Pudlo NA, Urs K, Kumar SS, German JB, Mills DA, Martens EC. 2015. Symbiotic human gut bacteria with variable metabolic priorities for host mucosal glycans. *mBio* 6:e01282-15. <https://doi.org/10.1128/mBio.01282-15>.
 48. Ulmer JE, Vilen EM, Namburi RB, Benjdia A, Beneteau J, Malleron A, Bonnafant D, Driguez PA, Descroix K, Lassalle G, Le Narvor C, Sandstrom C, Spillmann D, Berteau O. 2014. Characterization of glycosaminoglycan (GAG) sulfatases from the human gut symbiont *Bacteroides thetaiotaomicron* reveals the first GAG-specific bacterial endosulfatase. *J Biol Chem* 289:24289–24303. <https://doi.org/10.1074/jbc.M114.573303>.
 49. Hickey CA, Kuhn KA, Donermeyer DL, Porter NT, Jin C, Cameron EA, Jung H, Kaiko GE, Wegorzewska M, Malvin NP, Glowacki RW, Hansson GC, Allen PM, Martens EC, Stappenbeck TS. 2015. Colitogenic *Bacteroides thetaiotaomicron* antigens access host immune cells in a

- sulfatase-dependent manner via outer membrane vesicles. *Cell Host Microbe* 17:672–680. <https://doi.org/10.1016/j.chom.2015.04.002>.
50. Marcobal A, Barboza M, Sonnenburg ED, Pudlo N, Martens EC, Desai P, Lebrilla CB, Weimer BC, Mills DA, German JB, Sonnenburg JL. 2011. Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. *Cell Host Microbe* 10:507–514. <https://doi.org/10.1016/j.chom.2011.10.007>.
 51. Rios-Covian D, Cuesta I, Alvarez-Buylla JR, Ruas-Madiedo P, Gueimonde M, de Los Reyes-Gavilan CG. 2016. Bacteroides fragilis metabolises exopolysaccharides produced by bifidobacteria. *BMC Microbiol* 16:150. <https://doi.org/10.1186/s12866-016-0773-9>.
 52. Jones DR, Xing X, Tingley JP, Klassen L, King ML, Alexander TW, Abbott DW. 2020. Analysis of active site architecture and reaction product linkage chemistry reveals a conserved cleavage substrate for an endo- α -mannanase within diverse yeast mannans. *J Mol Biol* 432:1083–1097. <https://doi.org/10.1016/j.jmb.2019.12.048>.
 53. Dejean G, Tamura K, Cabrera A, Jain N, Pudlo NA, Pereira G, Viborg AH, Van Petegem F, Martens EC, Brumer H. 2020. Synergy between cell surface glycosidases and glycan-binding proteins dictates the utilization of specific beta(1,3)-glucans by human gut Bacteroides. *mBio* 11:e00095-20. <https://doi.org/10.1128/mBio.00095-20>.
 54. Thomas F, Barbeyron T, Tonon T, Genicot S, Czjzek M, Michel G. 2012. Characterization of the first alginate operons in a marine bacterium: from their emergence in marine Flavobacteriia to their independent transfers to marine Proteobacteria and human gut Bacteroides. *Environ Microbiol* 14:2379–2394. <https://doi.org/10.1111/j.1462-2920.2012.02751.x>.
 55. Larsbrink J, Zhu Y, Kharade SS, Kwiatkowski KJ, Eijssink VG, Koropatkin NM, McBride MJ, Pope PB. 2016. A polysaccharide utilization locus from Flavobacterium johnsoniae enables conversion of recalcitrant chitin. *Biotechnol Biofuels* 9:260. <https://doi.org/10.1186/s13068-016-0674-z>.
 56. Mackenzie AK, Naas AE, Kracun SK, Schuckel J, Fangel JU, Agger JW, Willats WG, Eijssink VG, Pope PB. 2015. A polysaccharide utilization locus from an uncultured Bacteroidetes phylotype suggests ecological adaptation and substrate versatility. *Appl Environ Microbiol* 81:187–195. <https://doi.org/10.1128/AEM.02858-14>.
 57. Foran E, Buravenkov V, Kopel M, Mizrahi N, Shoshani S, Helbert W, Banin E. 2017. Functional characterization of a novel “ulvan utilization loci” found in Alteromonas sp. LOR genome. *Algal Res* 25:39–46. <https://doi.org/10.1016/j.algal.2017.04.036>.
 58. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. 2010. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464:908–912. <https://doi.org/10.1038/nature08937>.
 59. Briliüté J, Urbanowicz PA, Luis AS, Baslé A, Paterson N, Rebello O, Hendel J, Ndeh DA, Lowe EC, Martens EC, Spencer DIR, Bolam DN, Crouch LI. 2019. Complex N-glycan breakdown by gut Bacteroides involves an extensive enzymatic apparatus encoded by multiple co-regulated genetic loci. *Nat Microbiol* 4:1571–1581. <https://doi.org/10.1038/s41564-019-0466-x>.
 60. Trastoy B, Du JJ, Klontz EH, Li C, Cifuentes JO, Wang LX, Sundberg EJ, Guerin ME. 2020. Structural basis of mammalian high-mannose N-glycan processing by human gut Bacteroides. *Nat Commun* 11:899. <https://doi.org/10.1038/s41467-020-14754-7>.
 61. Cameron EA, Maynard MA, Smith CJ, Smith TJ, Koropatkin NM, Martens EC. 2012. Multidomain carbohydrate-binding proteins involved in Bacteroides thetaiotaomicron starch metabolism. *J Biol Chem* 287:34614–34625. <https://doi.org/10.1074/jbc.M112.397380>.
 62. Cameron EA, Kwiatkowski KJ, Lee BH, Hamaker BR, Koropatkin NM, Martens EC. 2014. Multifunctional nutrient-binding proteins adapt human symbiotic bacteria for glycan competition in the gut by separately promoting enhanced sensing and catalysis. *mBio* 5:e01441-14. <https://doi.org/10.1128/mBio.01441-14>.
 63. Foley MH, Martens EC, Koropatkin NM. 2018. SusE facilitates starch uptake independent of starch binding in B. thetaiotaomicron. *Mol Microbiol* 108:551–566. <https://doi.org/10.1111/mmi.13949>.
 64. Foley MH, Dejean G, Hemsworth GR, Davies GJ, Brumer H, Koropatkin NM. 2019. A cell-surface GH9 endo-glucanase coordinates with surface glycan-binding proteins to mediate xyloglucan uptake in the gut symbiont Bacteroides ovatus. *J Mol Biol* 431:981–995. <https://doi.org/10.1016/j.jmb.2019.01.008>.
 65. Rakoff-Nahoum S, Coyne MJ, Comstock LE. 2014. An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr Biol* 24:40–49. <https://doi.org/10.1016/j.cub.2013.10.077>.
 66. Rakoff-Nahoum S, Foster KR, Comstock LE. 2016. The evolution of cooperation within the gut microbiota. *Nature* 533:255–259. <https://doi.org/10.1038/nature17626>.
 67. Rogowski A, Briggs JA, Mortimer JC, Tryfona T, Terrapon N, Lowe EC, Basle A, Morland C, Day AM, Zheng H, Rogers TE, Thompson P, Hawkins AR, Yadav MP, Henrissat B, Martens EC, Dupree P, Gilbert HJ, Bolam DN. 2015. Glycan complexity dictates microbial resource allocation in the large intestine. *Nat Commun* 6:7481. <https://doi.org/10.1038/ncomms8481>.
 68. Coyne MJ, Comstock LE. 2019. Type VI secretion systems and the gut microbiota. *Microbiol Spectr* 7:PSIB-0009-2018. <https://doi.org/10.1128/microbiolspec.PSIB-0009-2018>.
 69. Wexler AG, Bao Y, Whitney JC, Bobay LM, Xavier JB, Schofield WB, Barry NA, Russell AB, Tran BQ, Goo YA, Goodlett DR, Ochman H, Mougous JD, Goodman AL. 2016. Human symbionts inject and neutralize antibacterial toxins to persist in the gut. *Proc Natl Acad Sci U S A* 113:3639–3644. <https://doi.org/10.1073/pnas.1525637113>.
 70. Coyne MJ, Bechon N, Matano LM, McEneaney VL, Chatzidakis-Livanis M, Comstock LE. 2019. A family of anti-Bacteroidales peptide toxins widespread in the human gut microbiota. *Nat Commun* 10:3460. <https://doi.org/10.1038/s41467-019-11494-1>.
 71. Valguarnera E, Scott NE, Azimzadeh P, Feldman MF. 2018. Surface exposure and packing of lipoproteins into outer membrane vesicles are coupled processes in Bacteroides. *mSphere* 3:e00559-18. <https://doi.org/10.1128/mSphere.00559-18>.
 72. Elhenawy W, Debely MO, Feldman MF. 2014. Preferential packing of acidic glycosidases and proteases into Bacteroides outer membrane vesicles. *mBio* 5:e00909-14. <https://doi.org/10.1128/mBio.00909-14>.
 73. Tazuin AS, Kwiatkowski KJ, Orlovsky NI, Smith CJ, Creagh AL, Haynes CA, Wawrzak Z, Brumer H, Koropatkin NM. 2016. Molecular dissection of xyloglucan recognition in a prominent human gut symbiont. *mBio* 7:e02134-15. <https://doi.org/10.1128/mBio.02134-15>.
 74. Koropatkin NM, Smith TJ. 2010. SusG: a unique cell-membrane-associated alpha-amylase from a prominent human gut symbiont targets complex starch molecules. *Structure* 18:200–215. <https://doi.org/10.1016/j.str.2009.12.010>.
 75. Tuson HH, Foley MH, Koropatkin NM, Biteen JS. 2018. The starch utilization system assembles around stationary starch-binding proteins. *Biophys J* 115:242–250. <https://doi.org/10.1016/j.bpj.2017.12.015>.
 76. Karunatilaka KS, Cameron EA, Martens EC, Koropatkin NM, Biteen JS. 2014. Superresolution imaging captures carbohydrate utilization dynamics in human gut symbionts. *mBio* 5:e02172-14. <https://doi.org/10.1128/mBio.02172-14>.
 77. Hemsworth GR, Thompson AJ, Stepper J, Sobala LF, Coyle T, Larsbrink J, Spadiut O, Goddard-Borger ED, Stubbs KA, Brumer H, Davies GJ. 2016. Structural dissection of a complex Bacteroides ovatus gene locus conferring xyloglucan metabolism in the human gut. *Open Biol* 6:160142. <https://doi.org/10.1098/rsob.160142>.
 78. Li M, Shang Q, Li G, Wang X, Yu G. 2017. Degradation of marine algae-derived carbohydrates by Bacteroidetes isolated from human gut microbiota. *Mar Drugs* 15:92. <https://doi.org/10.3390/md15040092>.
 79. Mathieu S, Tuvrey-Loiodice M, Poulet L, Drouillard S, Vincentelli R, Henrissat B, Skjåk-Bræk G, Helbert W. 2018. Ancient acquisition of “alginate utilization loci” by human gut microbiota. *Sci Rep* 8:8075. <https://doi.org/10.1038/s41598-018-26104-1>.
 80. Fernandez-Gomez B, Richter M, Schuler M, Pinhasi J, Acinas SG, Gonzalez JM, Pedros-Alio C. 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. *ISME J* 7:1026–1037. <https://doi.org/10.1038/ismej.2012.169>.
 81. Poli A, Anzelmo G, Nicolaus B. 2010. Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. *Mar Drugs* 8:1779–1802. <https://doi.org/10.3390/md8061779>.
 82. Muhlenbruch M, Grossart HP, Eigemann F, Voss M. 2018. Mini-review: phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. *Environ Microbiol* 20:2671–2685. <https://doi.org/10.1111/1462-2920.14302>.
 83. Decho AW, Gutierrez T. 2017. Microbial extracellular polymeric substances (EPSs) in ocean systems. *Front Microbiol* 8:922. <https://doi.org/10.3389/fmicb.2017.00922>.
 84. Popper ZA, Michel G, Herve C, Domozych DS, Willats WG, Tuohy MG, Kloareg B, Stengel DB. 2011. Evolution and diversity of plant cell walls: from algae to flowering plants. *Annu Rev Plant Biol* 62:567–590. <https://doi.org/10.1146/annurev-arplant-042110-103809>.
 85. Mann AJ, Hahnke RL, Huang S, Werner J, Xing P, Barbeyron T, Huettel B, Stuber K, Reinhardt R, Harder J, Glockner FO, Amann RL, Teeling H. 2013.

- The genome of the alga-associated marine flavobacterium *Formosa agariphila* KMM 3901T reveals a broad potential for degradation of algal polysaccharides. *Appl Environ Microbiol* 79:6813–6822. <https://doi.org/10.1128/AEM.01937-13>.
86. Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM, Kassabgy M, Huang S, Mann AJ, Waldmann J, Weber M, Klindworth A, Otto A, Lange J, Bernhardt J, Reinsch C, Hecker M, Peplies J, Bockelmann FD, Callies U, Gerdts G, Wichels A, Wiltshire KH, Glockner FO, Schweder T, Amann R. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336:608–611. <https://doi.org/10.1126/science.1218344>.
 87. Gobet A, Barbeyron T, Matard-Mann M, Magdelenat G, Vallenet D, Duchaud E, Michel G. 2018. Evolutionary evidence of algal polysaccharide degradation acquisition by *Pseudoalteromonas carrageenovora* 9(T) to adapt to macroalgal niches. *Front Microbiol* 9:2740. <https://doi.org/10.3389/fmicb.2018.02740>.
 88. Unfried F, Becker S, Robb CS, Hehemann JH, Markert S, Heiden SE, Hinzke T, Becher D, Reintjes G, Kruger K, Avci B, Kappellmann L, Hahnke RL, Fischer T, Harder J, Teeling H, Fuchs B, Barbeyron T, Amann R, Schweder T. 2018. Adaptive mechanisms that provide competitive advantages to marine Bacteroidetes during microalgal blooms. *ISME J* 12:2894–2906. <https://doi.org/10.1038/s41396-018-0243-5>.
 89. Kappellmann L, Kruger K, Hehemann JH, Harder J, Markert S, Unfried F, Becher D, Shapiro N, Schweder T, Amann R, Teeling H. 2019. Polysaccharide utilization loci of North Sea Flavobacteriia as basis for using SusC/D-protein expression for predicting major phytoplankton glycans. *ISME J* 13:76–91. <https://doi.org/10.1038/s41396-018-0242-6>.
 90. Tang K, Lin Y, Han Y, Jiao N. 2017. Characterization of potential polysaccharide utilization systems in the marine Bacteroidetes *Gramella flava* JLT2011 using a multi-omics approach. *Front Microbiol* 8:220. <https://doi.org/10.3389/fmicb.2017.00220>.
 91. Xing P, Hahnke RL, Unfried F, Markert S, Huang S, Barbeyron T, Harder J, Becher D, Schweder T, Glockner FO, Amann R, Teeling H. 2015. Niches of two polysaccharide-degrading *Polaribacter* isolates from the North Sea during a spring diatom bloom. *ISME J* 9:1410–1422. <https://doi.org/10.1038/ismej.2014.225>.
 92. Alderkamp AC, van Rijssel M, Bolhuis H. 2007. Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. *FEMS Microbiol Ecol* 59:108–117. <https://doi.org/10.1111/j.1574-6941.2006.00219.x>.
 93. Labourel A, Jam M, Jeudy A, Hehemann JH, Czjzek M, Michel G. 2014. The beta-glucanase ZgLamA from *Zobellia galactanivorans* evolved a bent active site adapted for efficient degradation of algal laminarin. *J Biol Chem* 289:2027–2042. <https://doi.org/10.1074/jbc.M113.538843>.
 94. Labourel A, Jam M, Legentil L, Sylla B, Hehemann JH, Ferrieres V, Czjzek M, Michel G. 2015. Structural and biochemical characterization of the laminarinase ZgLamCGH16 from *Zobellia galactanivorans* suggests preferred recognition of branched laminarin. *Acta Crystallogr D Biol Crystallogr* 71:173–184. <https://doi.org/10.1107/S139900471402450X>.
 95. Mystkowska AA, Robb C, Vidal-Melgosa S, Vanni C, Fernandez-Guerra A, Hohne M, Hehemann JH. 2018. Molecular recognition of the beta-glucans laminarin and pustulan by a SusD-like glycan-binding protein of a marine Bacteroidetes. *FEBS J* 285:4465–4481. <https://doi.org/10.1111/febs.14674>.
 96. Jam M, Flament D, Allouch J, Potin P, Thion L, Kloareg B, Czjzek M, Helbert W, Michel G, Barbeyron T. 2005. The endo-beta-agarases AgaA and AgaB from the marine bacterium *Zobellia galactanivorans*: two paralogue enzymes with different molecular organizations and catalytic behaviours. *Biochem J* 385:703–713. <https://doi.org/10.1042/BJ20041044>.
 97. Hehemann JH, Correc G, Thomas F, Bernard T, Barbeyron T, Jam M, Helbert W, Michel G, Czjzek M. 2012. Biochemical and structural characterization of the complex agarolytic enzyme system from the marine bacterium *Zobellia galactanivorans*. *J Biol Chem* 287:30571–30584. <https://doi.org/10.1074/jbc.M112.377184>.
 98. Konasani VR, Jin C, Karlsson NG, Albers E. 2018. A novel ulvan lyase family with broad-spectrum activity from the ulvan utilisation loci of *Formosa agariphila* KMM 3901. *Sci Rep* 8:14713. <https://doi.org/10.1038/s41598-018-32922-0>.
 99. Chen J, Robb CS, Unfried F, Kappellmann L, Markert S, Song T, Harder J, Avci B, Becher D, Xie P, Amann R, Hehemann JH, Schweder T, Teeling H. 2018. Alpha- and beta-mannan utilization by marine Bacteroidetes. *Environ Microbiol* 20:4127–4140. <https://doi.org/10.1111/1462-2920.14414>.
 100. Reintjes G, Arnosti C, Fuchs B, Amann R. 2019. Selfish, sharing and scavenging bacteria in the Atlantic Ocean: a biogeographical study of bacterial substrate utilisation. *ISME J* 13:1119–1132. <https://doi.org/10.1038/s41396-018-0326-3>.
 101. Reintjes G, Arnosti C, Fuchs BM, Amann R. 2017. An alternative polysaccharide uptake mechanism of marine bacteria. *ISME J* 11:1640–1650. <https://doi.org/10.1038/ismej.2017.26>.
 102. Reisky L, Prechoux A, Zuhlke MK, Baumgen M, Robb CS, Gerlach N, Roret T, Stanetty C, Larocque R, Michel G, Song T, Markert S, Unfried F, Mihovilovic MD, Trautwein-Schult A, Becher D, Schweder T, Bornscheuer UT, Hehemann JH. 2019. A marine bacterial enzymatic cascade degrades the algal polysaccharide ulvan. *Nat Chem Biol* 15:803–812. <https://doi.org/10.1038/s41589-019-0311-9>.
 103. Schultz-Johansen M, Bech PK, Hennessy RC, Glaring MA, Barbeyron T, Czjzek M, Stougaard P. 2018. A novel enzyme portfolio for red algal polysaccharide degradation in the marine bacterium *Paraglacicola hydrolytica* S66(T) encoded in a sizeable polysaccharide utilization locus. *Front Microbiol* 9:839. <https://doi.org/10.3389/fmicb.2018.00839>.
 104. Koch H, Freese HM, Hahnke RL, Simon M, Wietz M. 2019. Adaptations of *Alteromonas* sp. 76-1 to polysaccharide degradation: a CAZyme plasmid for ulvan degradation and two alginate systems. *Front Microbiol* 10:504. <https://doi.org/10.3389/fmicb.2019.00504>.
 105. Jiang X, Hall AB, Xavier RJ, Alm EJ. 2019. Comprehensive analysis of chromosomal mobile genetic elements in the gut microbiome reveals phylum-level niche-adaptive gene pools. *PLoS One* 14:e0223680. <https://doi.org/10.1371/journal.pone.0223680>.
 106. Ndeh D, Gilbert HJ. 2018. Biochemistry of complex glycan depolymerisation by the human gut microbiota. *FEMS Microbiol Rev* 42:146–164. <https://doi.org/10.1093/femsre/fuy002>.
 107. Chen J. 2013. Molecular mechanism of the *Escherichia coli* maltose transporter. *Curr Opin Struct Biol* 23:492–498. <https://doi.org/10.1016/j.sbi.2013.03.011>.
 108. Deutscher J, Ake FM, Derkaoui M, Zebre AC, Cao TN, Bouraoui H, Kentache T, Mokhtari A, Milohanic E, Joyet P. 2014. The bacterial phosphoenolpyruvate:carbohydrate phosphotransferase system: regulation by protein phosphorylation and phosphorylation-dependent protein-protein interactions. *Microbiol Mol Biol Rev* 78:231–256. <https://doi.org/10.1128/MMBR.00001-14>.
 109. Yan N. 2015. Structural biology of the major facilitator superfamily transporters. *Annu Rev Biophys* 44:257–283. <https://doi.org/10.1146/annurev-biophys-060414-033901>.
 110. Cockburn DW, Orlovsky NI, Foley MH, Kwiatkowski KJ, Bahr CM, Maynard M, Demeler B, Koropatkin NM. 2015. Molecular details of a starch utilization pathway in the human gut symbiont *Eubacterium rectale*. *Mol Microbiol* 95:209–230. <https://doi.org/10.1111/mmi.12859>.
 111. Cockburn DW, Suh C, Medina KP, Duvall RM, Wawrzak Z, Henrissat B, Koropatkin NM. 2018. Novel carbohydrate binding modules in the surface anchored alpha-amylase of *Eubacterium rectale* provide a molecular rationale for the range of starches used by this organism in the human gut. *Mol Microbiol* 107:249–264. <https://doi.org/10.1111/mmi.13881>.
 112. Leth ML, Ejby M, Workman C, Ewald DA, Pedersen SS, Sternberg C, Bahl MI, Licht TR, Aachmann FL, Westereng B, Abou Hachem M. 2018. Differential bacterial capture and transport preferences facilitate co-growth on dietary xylan in the human gut. *Nat Microbiol* 3:570–580. <https://doi.org/10.1038/s41564-018-0132-8>.
 113. Erlandson KA, Delamarre SC, Batt CA. 2001. Genetic evidence for a defective xylan degradation pathway in *Lactococcus lactis*. *Appl Environ Microbiol* 67:1445–1452. <https://doi.org/10.1128/AEM.67.4.1445-1452.2001>.
 114. Michlmayr H, Hell J, Lorenz C, Bohmdorfer S, Rosenau T, Kneifel W. 2013. Arabinoxylan oligosaccharide hydrolysis by family 43 and 51 glycosidases from *Lactobacillus brevis* DSM 20054. *Appl Environ Microbiol* 79:6747–6754. <https://doi.org/10.1128/AEM.02130-13>.
 115. Riviere A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L. 2014. The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. *Appl Environ Microbiol* 80:204–217. <https://doi.org/10.1128/AEM.02853-13>.
 116. Mendis M, Martens EC, Simsek S. 2018. How fine structural differences of xyloligosaccharides and arabinoxyloligosaccharides regulate differential growth of bacteroides species. *J Agric Food Chem* 66:8398–8405. <https://doi.org/10.1021/acs.jafc.8b01263>.
 117. Zeybek N, Rastall RA, Buyukkileci AO. 2020. Utilization of xylan-type polysaccharides in co-culture fermentations of *Bifidobacterium* and *Bacteroides* species. *Carbohydr Polym* 236:116076. <https://doi.org/10.1016/j.carbpol.2020.116076>.

118. Lamed R, Kenig R, Setter E, Bayer EA. 1985. Major characteristics of the cellulolytic system of *Clostridium thermocellum* coincide with those of the purified cellulosome. *Enzyme Microb Technol* 7:37–41. [https://doi.org/10.1016/0141-0229\(85\)90008-0](https://doi.org/10.1016/0141-0229(85)90008-0).
119. Lamed R, Setter E, Bayer EA. 1983. Characterization of a cellulose-binding, cellulase-containing complex in *Clostridium thermocellum*. *J Bacteriol* 156:828–836. <https://doi.org/10.1128/JB.156.2.828-836.1983>.
120. Bayer EA, Kenig R, Lamed R. 1983. Adherence of *Clostridium thermocellum* to cellulose. *J Bacteriol* 156:818–827. <https://doi.org/10.1128/JB.156.2.818-827.1983>.
121. Artzi L, Bayer EA, Morais S. 2017. Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides. *Nat Rev Microbiol* 15:83–95. <https://doi.org/10.1038/nrmicro.2016.164>.
122. Morais S, Ben David Y, Bensoussan L, Duncan SH, Koropatkin NM, Martens EC, Flint HJ, Bayer EA. 2016. Enzymatic profiling of cellulosomal enzymes from the human gut bacterium, *Ruminococcus champanellensis*, reveals a fine-tuned system for cohesin-dockerin recognition. *Environ Microbiol* 18:542–556. <https://doi.org/10.1111/1462-2920.13047>.
123. Ze X, Ben David Y, Laverde-Gomez JA, Dassa B, Sheridan PO, Duncan SH, Louis P, Henrissat B, Juge N, Koropatkin NM, Bayer EA, Flint HJ. 2015. Unique organization of extracellular amylases into amylosomes in the resistant starch-utilizing human colonic Firmicutes bacterium *Ruminococcus bromii*. *mBio* 6:e01058-15. <https://doi.org/10.1128/mBio.01058-15>.
124. Ze X, Duncan SH, Louis P, Flint HJ. 2012. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J* 6:1535–1543. <https://doi.org/10.1038/ismej.2012.4>.
125. Mukhopadhyay I, Morais S, Laverde-Gomez J, Sheridan PO, Walker AW, Kelly W, Klieve AV, Ouwerkerk D, Duncan SH, Louis P, Koropatkin N, Cockburn D, Kibler R, Cooper PJ, Sandoval C, Crost E, Juge N, Bayer EA, Flint HJ. 2018. Sporulation capability and amylosome conservation among diverse human colonic and rumen isolates of the keystone starch-degrader *Ruminococcus bromii*. *Environ Microbiol* 20:324–336. <https://doi.org/10.1111/1462-2920.14000>.
126. Lagkouvardos I, Pukall R, Abt B, Foesel BU, Meier-Kolthoff JP, Kumar N, Bresciani A, Martinez I, Just S, Ziegler C, Brugiroux S, Garzetti D, Wenning M, Bui TP, Wang J, Hugenholtz F, Plugge CM, Peterson DA, Horneff MW, Baines JF, Smidt H, Walter J, Kristiansen K, Nielsen HB, Haller D, Overmann J, Stecher B, Clavel T. 2016. The Mouse Intestinal Bacterial Collection (mIBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nat Microbiol* 1:16131. <https://doi.org/10.1038/nmicrobiol.2016.219>.
127. Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, Goulding D, Lawley TD. 2016. Culturing of ‘unculturable’ human microbiota reveals novel taxa and extensive sporulation. *Nature* 533:543–546. <https://doi.org/10.1038/nature17645>.
128. Lagier J-C, Khelaifa S, Alou MT, Ndongo S, Dione N, Hugon P, Caputo A, Cadoret F, Traore SI, Seck EH, Dubourg G, Durand G, Mourembou G, Guilhot E, Togo A, Bellali S, Bachar D, Cassir N, Bittar F, Delerue J, Mailhe M, Ricaboni D, Bilen M, Dangui Niekro NPM, Dia Badiane NM, Valles C, Mouelhi D, Diop K, Million M, Musso D, Abrahao J, Azhar El, Bibi F, Yasir M, Diallo A, Sokhna C, Djossou F, Vitton V, Robert C, Rolain JM, La Scola B, Fournier P-E, Levasseur A, Raoult D. 2016. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol* 1:16203. <https://doi.org/10.1038/nmicrobiol.2016.203>.
129. Surana NK, Kasper DL. 2017. Moving beyond microbiome-wide associations to causal microbe identification. *Nature* 552:244–247. <https://doi.org/10.1038/nature25019>.
130. Forster SC, Kumar N, Anonye BO, Almeida A, Viciani E, Stares MD, Dunn M, Mkandawire TT, Zhu A, Shao Y, Pike LJ, Louie T, Browne HP, Mitchell AL, Neville BA, Finn RD, Lawley TD. 2019. A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat Biotechnol* 37:186–192. <https://doi.org/10.1038/s41587-018-0009-7>.
131. Abbott DW, Hrynuk S, Boraston AB. 2007. Identification and characterization of a novel periplasmic polygalacturonic acid binding protein from *Yersinia enterocolitica* [sic]. *J Mol Biol* 367:1023–1033. <https://doi.org/10.1016/j.jmb.2007.01.030>.
132. Fabich AJ, Jones SA, Chowdhury FZ, Cernosek A, Anderson A, Smalley D, McHargue JW, Hightower GA, Smith JT, Autieri SM, Leatham MP, Lins JJ, Allen RL, Laux DC, Cohen PS, Conway T. 2008. Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. *Infect Immun* 76:1143–1152. <https://doi.org/10.1128/IAI.01386-07>.
133. Segura A, Bertoni M, Auffret P, Klopp C, Bouchez O, Genthon C, Durand A, Bertin Y, Forano E. 2018. Transcriptomic analysis reveals specific metabolic pathways of enterohemorrhagic *Escherichia coli* O157:H7 in bovine digestive contents. *BMC Genomics* 19:766. <https://doi.org/10.1186/s12864-018-5167-y>.
134. Harvey PC, Watson M, Hulme S, Jones MA, Lovell M, Berchieri A, Jr, Young J, Bumstead N, Barrow P. 2011. *Salmonella enterica* serovar Typhimurium colonizing the lumen of the chicken intestine grows slowly and upregulates a unique set of virulence and metabolism genes. *Infect Immun* 79:4105–4121. <https://doi.org/10.1128/IAI.01390-10>.
135. Rollenhagen C, Bumann D. 2006. *Salmonella enterica* highly expressed genes are disease specific. *Infect Immun* 74:1649–1660. <https://doi.org/10.1128/IAI.74.3.1649-1660.2006>.
136. Bernier-Fébreau C, du Merle L, Turle E, Labas V, Ordonez J, Gilles A-M, Le Bouguéne C. 2004. Use of deoxyribose by intestinal and extraintestinal pathogenic *Escherichia coli* strains: a metabolic adaptation involved in competitiveness. *Infect Immun* 72:6151–6156. <https://doi.org/10.1128/IAI.72.10.6151-6156.2004>.
137. Palchevskiy V, Finkel SE. 2006. *Escherichia coli* competence gene homologs are essential for competitive fitness and the use of DNA as a nutrient. *J Bacteriol* 188:3902–3910. <https://doi.org/10.1128/JB.01974-05>.
138. Ng KM, Freyre JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL. 2013. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 502:96–99. <https://doi.org/10.1038/nature12503>.
139. Jimenez AG, Ellermann M, Abbott V, Sperandio V. 2020. Diet-derived galacturonic acid regulates virulence and intestinal colonization in enterohemorrhagic *Escherichia coli* and *Citrobacter rodentium*. *Nat Microbiol* 5:368–378. <https://doi.org/10.1038/s41564-019-0641-0>.
140. Pacheco AR, Curtis MM, Ritchie JM, Munera D, Waldor MK, Moreira CG, Sperandio V. 2012. Fucose sensing regulates bacterial intestinal colonization. *Nature* 492:113–117. <https://doi.org/10.1038/nature11623>.
141. Curtis MM, Hu Z, Klimko C, Narayanan S, Deberardinis R, Sperandio V. 2014. The gut commensal *Bacteroides thetaiotaomicron* exacerbates enteric infection through modification of the metabolic landscape. *Cell Host Microbe* 16:759–769. <https://doi.org/10.1016/j.chom.2014.11.005>.
142. Patel EH, Paul LV, Patrick S, Abratt VR. 2008. Rhamnose catabolism in *Bacteroides thetaiotaomicron* is controlled by the positive transcriptional regulator RhaR. *Res Microbiol* 159:678–684. <https://doi.org/10.1016/j.resmic.2008.09.002>.
143. Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JL. 1999. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. *Proc Natl Acad Sci U S A* 96:9833–9838. <https://doi.org/10.1073/pnas.96.17.9833>.
144. Chang C, Tesar C, Li X, Kim Y, Rodionov DA, Joachimiak A. 2015. A novel transcriptional regulator of L-arabinose utilization in human gut bacteria. *Nucleic Acids Res* 43:10546–10549. <https://doi.org/10.1093/nar/gkv1005>.
145. Schwalm ND, III, Townsend GE, II, Groisman EA. 2016. Multiple signals govern utilization of a polysaccharide in the gut bacterium *Bacteroides thetaiotaomicron*. *mBio* 7:e01342-16. <https://doi.org/10.1128/mBio.01342-16>.
146. Joglekar P, Sonnenburg ED, Higginbottom SK, Earle KA, Morland C, Shapiro-Ward S, Bolam DN, Sonnenburg JL. 2018. Genetic variation of the SusC/SusD homologs from a polysaccharide utilization locus underlies divergent fructan specificities and functional adaptation in *Bacteroides thetaiotaomicron* strains. *mSphere* 3:e00185-18. <https://doi.org/10.1128/mSphereDirect.00185-18>.
147. Glowacki RWP, Pudlo NA, Tuncil Y, Luis AS, Sajjakulnukit P, Terekhov AI, Lyssiotis CA, Hamaker BR, Martens EC. 2020. A ribose-scavenging system confers colonization fitness on the human gut symbiont *Bacteroides thetaiotaomicron* in a diet-specific manner. *Cell Host Microbe* 27:79–92. <https://doi.org/10.1016/j.chom.2019.11.009>.
148. Liou CS, Sirk SJ, Diaz CAC, Klein AP, Fischer CR, Higginbottom SK, Erez A, Donia MS, Sonnenburg JL, Sattely ES. 2020. A metabolic pathway for activation of dietary glucosinolates by a human gut symbiont. *Cell* 180:717–728.e19. <https://doi.org/10.1016/j.cell.2020.01.023>.
149. Herr I, Buchler MW. 2010. Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. *Cancer Treat Rev* 36:377–383. <https://doi.org/10.1016/j.ctrv.2010.01.002>.
150. Simmering R, Pforte H, Jacobasch G, Blaut M. 2002. The growth of the flavonoid-degrading intestinal bacterium, *Eubacterium ramulus*, is stimulated by dietary flavonoids in vivo. *FEMS Microbiol Ecol* 40:243–248. <https://doi.org/10.1111/j.1574-6941.2002.tb00957.x>.
151. Ulbrich K, Reichardt N, Braune A, Kroh LW, Blaut M, Rohn S. 2015. The microbial degradation of onion flavonol glucosides and their roasting

- products by the human gut bacteria *Eubacterium ramulus* and *Flavonifractor plautii*. *Food Res Int* 67:349–355. <https://doi.org/10.1016/j.foodres.2014.11.051>.
152. Frommeyer B, Fiedler AW, Oehler SR, Hanson BT, Loy A, Franchini P, Spittler D, Schleheck D. 2020. Environmental and intestinal phylum Firmicutes bacteria metabolize the plant sugar sulfoquinovose via a 6-deoxy-6-sulfofructose transaldolase pathway. *iScience* 23:101510. <https://doi.org/10.1016/j.isci.2020.101510>.
 153. Kitamoto S, Alteri CJ, Rodrigues M, Nagao-Kitamoto H, Sugihara K, Himpel SD, Bazzi M, Miyoshi M, Nishioka T, Hayashi A, Morhardt TL, Kuffa P, Grasberger H, El-Zaatari M, Bishu S, Ishii C, Hirayama A, Eaton KA, Dogan B, Simpson KW, Inohara N, Mobley HLT, Kao JY, Fukuda S, Barnich N, Kamada N. 2020. Dietary L-serine confers a competitive fitness advantage to Enterobacteriaceae in the inflamed gut. *Nat Microbiol* 5:116–125. <https://doi.org/10.1038/s41564-019-0591-6>.
 154. Pal RR, Baidya AK, Mamou G, Bhattacharya S, Socol Y, Kobi S, Katsowich N, Ben-Yehuda S, Rosenshine I. 2019. Pathogenic *E. coli* extracts nutrients from infected host cells utilizing injectisome components. *Cell* 177:683–696.e18. <https://doi.org/10.1016/j.cell.2019.02.022>.
 155. Snellson M, Coughlan MT. 2019. Dietary advanced glycation end products: digestion, metabolism and modulation of gut microbial ecology. *Nutrients* 11:215. <https://doi.org/10.3390/nu11020215>.
 156. Carmody RN, Bisanz JE, Bowen BP, Maurice CF, Lyalina S, Louie KB, Treen D, Chadaideh KS, Maini Rekdal V, Bess EN, Spanogiannopoulos P, Ang QY, Bauer KC, Balon TW, Pollard KS, Northern TR, Turnbaugh PJ. 2019. Cooking shapes the structure and function of the gut microbiome. *Nat Microbiol* 4:2052–2063. <https://doi.org/10.1038/s41564-019-0569-4>.
 157. Wu J, Sabag-Daigle A, Borton MA, Kop LFM, Szkoda BE, Deatherage Kaiser BL, Lindemann SR, Renslow RS, Wei S, Nicora CD, Weitz KK, Kim YM, Adkins JN, Metz TO, Boyaka P, Gopalan V, Wrighton KC, Wysocki VH, Ahmer BMM. 2018. Salmonella-mediated inflammation eliminates competitors for fructose-asparagine in the gut. *Infect Immun* 86:e00945-17. <https://doi.org/10.1128/IAI.00945-17>.
 158. Wolf AR, Wesener DA, Cheng J, Houston-Ludlam AN, Beller ZW, Hibberd MC, Giannone RJ, Peters SL, Hettich RL, Leyn SA, Rodionov DA, Osterman AL, Gordon JL. 2019. Bioremediation of a common product of food processing by a human gut bacterium. *Cell Host Microbe* 26:463–477.e8. <https://doi.org/10.1016/j.chom.2019.09.001>.
 159. Bui TPN, Troise AD, Fogliano V, de Vos WM. 2019. Anaerobic degradation of N-epsilon-carboxymethyllysine, a major glycation end-product, by human intestinal bacteria. *J Agric Food Chem* 67:6594–6602. <https://doi.org/10.1021/acs.jafc.9b02208>.
 160. Helou C, Marier D, Jacolot P, Abdennebi-Najar L, Niquet-Leridon C, Tessier FJ, Gadonna-Widehem P. 2014. Microorganisms and Maillard reaction products: a review of the literature and recent findings. *Amino Acids* 46:267–277. <https://doi.org/10.1007/s00726-013-1496-y>.
 161. Deppe VM, Klatte S, Bongaerts J, Maurer KH, O'Connell T, Meinhardt F. 2011. Genetic control of Amadori product degradation in *Bacillus subtilis* via regulation of *friBONMD* expression by *FriR*. *Appl Environ Microbiol* 77:2839–2846. <https://doi.org/10.1128/AEM.02515-10>.
 162. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. 2019. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio* 10:e02566-18. <https://doi.org/10.1128/mBio.02566-18>.
 163. Birt DF, Boylston T, Hendrich S, Jane JL, Hollis J, Li L, McClelland J, Moore S, Phillips GJ, Rowling M, Schalinske K, Scott MP, Whitley EM. 2013. Resistant starch: promise for improving human health. *Adv Nutr* 4:587–601. <https://doi.org/10.3945/an.113.004325>.
 164. Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, Flint HJ. 2006. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol* 72:3593–3599. <https://doi.org/10.1128/AEM.72.5.3593-3599.2006>.
 165. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J. 2010. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One* 5:e15046. <https://doi.org/10.1371/journal.pone.0015046>.
 166. Upadhyaya B, McCormack L, Fardin-Kia AR, Juenemann R, Nichenametta S, Clapper J, Specker B, Dey M. 2016. Impact of dietary resistant starch type 4 on human gut microbiota and immunometabolic functions. *Sci Rep* 6:28797. <https://doi.org/10.1038/srep28797>.
 167. Maier TV, Lucio M, Lee LH, VerBerkmoes NC, Brislawn CJ, Bernhardt J, Lamendella R, McDermott JE, Bergeron N, Heinzmann SS, Morton JT, Gonzalez A, Ackermann G, Knight R, Riedel K, Krauss RM, Schmitt-
 - Kopplin P, Jansson JK. 2017. Impact of dietary resistant starch on the human gut microbiome, metaproteome, and metabolome. *mBio* 8:e01343-17. <https://doi.org/10.1128/mBio.01343-17>.
 168. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, Gewirtz AT. 2015. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 519:92–96. <https://doi.org/10.1038/nature14232>.
 169. Wang QP, Browman D, Herzog H, Neely GG. 2018. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. *PLoS One* 13:e0199080. <https://doi.org/10.1371/journal.pone.0199080>.
 170. Martens EC, Roth R, Heuser JE, Gordon JL. 2009. Coordinate regulation of glycan degradation and polysaccharide capsule biosynthesis by a prominent human gut symbiont. *J Biol Chem* 284:18445–18457. <https://doi.org/10.1074/jbc.M109.008094>.
 171. D'Elia JN, Salys AA. 1996. Effect of regulatory protein levels on utilization of starch by *Bacteroides thetaiotaomicron*. *J Bacteriol* 178:7180–7186. <https://doi.org/10.1128/JB.178.24.7180-7186.1996>.
 172. Rogers TE, Pudlo NA, Koropatkin NM, Bell JS, Moya Balasch M, Jasker K, Martens EC. 2013. Dynamic responses of *Bacteroides thetaiotaomicron* during growth on glycan mixtures. *Mol Microbiol* 88:876–890. <https://doi.org/10.1111/mmi.12228>.
 173. Cartmell A, Lowe EC, Basle A, Firbank SJ, Ndeh DA, Murray H, Terrapon N, Lombard V, Henrissat B, Turnbull JE, Czjzek M, Gilbert HJ, Bolam DN. 2017. How members of the human gut microbiota overcome the sulfation problem posed by glycosaminoglycans. *Proc Natl Acad Sci U S A* 114:7037–7042. <https://doi.org/10.1073/pnas.1704367114>.
 174. Ndeh D, Basle A, Strahl H, Yates EA, McClurg UL, Henrissat B, Terrapon N, Cartmell A. 2020. Metabolism of multiple glycosaminoglycans by *Bacteroides thetaiotaomicron* is orchestrated by a versatile core genetic locus. *Nat Commun* 11:646. <https://doi.org/10.1038/s41467-020-18097-1>.
 175. Angerer A, Enz S, Ochs M, Braun V. 1995. Transcriptional regulation of ferric citrate transport in *Escherichia coli* K-12. *FecI* belongs to a new subfamily of sigma 70-type factors that respond to extracytoplasmic stimuli. *Mol Microbiol* 18:163–174. <https://doi.org/10.1111/j.1365-2958.1995.mmi.18010163.x>.
 176. Stiefel A, Mahren S, Ochs M, Schindler PT, Enz S, Braun V. 2001. Control of the ferric citrate transport system of *Escherichia coli*: mutations in region 2.1 of the *FecI* extracytoplasmic-function sigma factor suppress mutations in the *FecR* transmembrane regulatory protein. *J Bacteriol* 183:162–170. <https://doi.org/10.1128/JB.183.1.162-170.2001>.
 177. Braun V, Mahren S, Ogierman M. 2003. Regulation of the *FecI*-type ECF sigma factor by transmembrane signalling. *Curr Opin Microbiol* 6:173–180. [https://doi.org/10.1016/S1369-5274\(03\)00022-5](https://doi.org/10.1016/S1369-5274(03)00022-5).
 178. Schalk IJ, Yue WW, Buchanan SK. 2004. Recognition of iron-free siderophores by TonB-dependent iron transporters. *Mol Microbiol* 54:14–22. <https://doi.org/10.1111/j.1365-2958.2004.04241.x>.
 179. Dudek M, Dieudonne A, Jouanneau D, Rochat T, Michel G, Sarels B, Thomas F. 2020. Regulation of alginate catabolism involves a GntR family repressor in the marine flavobacterium *Zobellia galactanivorans* DsjT. *Nucleic Acids Res* 48:7786–7800. <https://doi.org/10.1093/nar/gkaa533>.
 180. Cao Y, Forstner KU, Vogel J, Smith CJ. 2016. cis-encoded small RNAs, a conserved mechanism for repression of polysaccharide utilization in *Bacteroides*. *J Bacteriol* 198:2410–2418. <https://doi.org/10.1128/JB.00381-16>.
 181. Raghavan V, Groisman EA. 2015. Species-specific dynamic responses of gut bacteria to a mammalian glycan. *J Bacteriol* 197:1538–1548. <https://doi.org/10.1128/JB.00010-15>.
 182. Raghavan V, Lowe EC, Townsend GE, II, Bolam DN, Groisman EA. 2014. Tuning transcription of nutrient utilization genes to catabolic rate promotes growth in a gut bacterium. *Mol Microbiol* 93:1010–1025. <https://doi.org/10.1111/mmi.12714>.
 183. Townsend GE, II, Han W, Schwalm ND, III, Raghavan V, Barry NA, Goodman AL, Groisman EA. 2019. Dietary sugar silences a colonization factor in a mammalian gut symbiont. *Proc Natl Acad Sci U S A* 116:233–238. <https://doi.org/10.1073/pnas.1813780115>.
 184. Schwalm ND, III, Townsend GE, II, Groisman EA. 2017. Prioritization of polysaccharide utilization and control of regulator activation in *Bacteroides thetaiotaomicron*. *Mol Microbiol* 104:32–45. <https://doi.org/10.1111/mmi.13609>.
 185. Tuncil YE, Xiao Y, Porter NT, Reuhs BL, Martens EC, Hamaker BR. 2017. Reciprocal prioritization to dietary glycans by gut bacteria in a competitive environment promotes stable coexistence. *mBio* 8:e01068-17. <https://doi.org/10.1128/mBio.01068-17>.

186. Siegel LS, Hylemon PB, Phibbs PV, Jr. 1977. Cyclic adenosine 3',5'-monophosphate levels and activities of adenylate cyclase and cyclic adenosine 3',5'-monophosphate phosphodiesterase in *Pseudomonas* and *Bacteroides*. *J Bacteriol* 129:87–96. <https://doi.org/10.1128/JB.129.1.87-96.1977>.
187. Cotta MA, Wheeler MB, Whitehead TR. 1994. Cyclic AMP in ruminal and other anaerobic bacteria. *FEMS Microbiol Lett* 124:355–359. <https://doi.org/10.1111/j.1574-6968.1994.tb07308.x>.
188. Townsend GE, II, Han W, Schwalm ND, III, Hong X, Bencivenga-Barry NA, Goodman AL, Groisman EA. 2020. A master regulator of *Bacteroides thetaiotaomicron* gut colonization controls carbohydrate utilization and an alternative protein synthesis factor. *mBio* 11:e00301-20. <https://doi.org/10.1128/mBio.00301-20>.
189. Farrar MD, Whitehead TR, Lan J, Dilger P, Thorpe R, Holland KT, Carding SR. 2005. Engineering of the gut commensal bacterium *Bacteroides ovatus* to produce and secrete biologically active murine interleukin-2 in response to xylan. *J Appl Microbiol* 98:1191–1197. <https://doi.org/10.1111/j.1365-2672.2005.02565.x>.
190. Hamady ZZR, Farrar MD, Whitehead TR, Holland KT, Lodge JPA, Carding SR. 2008. Identification and use of the putative *Bacteroides ovatus* xylanase promoter for the inducible production of recombinant human proteins. *Microbiology (Reading)* 154:3165–3174. <https://doi.org/10.1099/mic.0.2008/019109-0>.
191. Hamady ZZ, Scott N, Farrar MD, Lodge JP, Holland KT, Whitehead T, Carding SR. 2010. Xylan-regulated delivery of human keratinocyte growth factor-2 to the inflamed colon by the human anaerobic commensal bacterium *Bacteroides ovatus*. *Gut* 59:461–469. <https://doi.org/10.1136/gut.2008.176131>.
192. Hamady ZZ, Scott N, Farrar MD, Wadhwa M, Dilger P, Whitehead TR, Thorpe R, Holland KT, Lodge JP, Carding SR. 2011. Treatment of colitis with a commensal gut bacterium engineered to secrete human TGF-beta1 under the control of dietary xylan 1. *Inflamm Bowel Dis* 17:1925–1935. <https://doi.org/10.1002/ibd.21565>.
193. Meyer AJ, Segall-Shapiro TH, Glassey E, Zhang J, Voigt CA. 2019. *Escherichia coli* “marionette” strains with 12 highly optimized small-molecule sensors. *Nat Chem Biol* 15:196–204. <https://doi.org/10.1038/s41589-018-0168-3>.
194. Whitaker WR, Shepherd ES, Sonnenburg JL. 2017. Tunable expression tools enable single-cell strain distinction in the gut microbiome. *Cell* 169:538–546.e12. <https://doi.org/10.1016/j.cell.2017.03.041>.
195. Lim B, Zimmermann M, Barry NA, Goodman AL. 2017. Engineered regulatory systems modulate gene expression of human commensals in the gut. *Cell* 169:547–558.e15. <https://doi.org/10.1016/j.cell.2017.03.045>.
196. Mimee M, Tucker AC, Voigt CA, Lu TK. 2015. Programming a human commensal bacterium, *Bacteroides thetaiotaomicron*, to sense and respond to stimuli in the murine gut microbiota. *Cell Syst* 1:62–71. <https://doi.org/10.1016/j.cels.2015.06.001>.
197. Garcia-Bayona L, Comstock LE. 2019. Streamlined genetic manipulation of diverse *Bacteroides* and *Parabacteroides* isolates from the human gut microbiota. *mBio* 10:e01762-19. <https://doi.org/10.1128/mBio.01762-19>.
198. Freter R, Brickner H, Botney M, Cleven D, Aranki A. 1983. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. *Infect Immun* 39:676–685. <https://doi.org/10.1128/IAI.39.2.676-685.1983>.
199. Kitahara M, Sakamoto M, Ike M, Sakata S, Benno Y. 2005. *Bacteroides plebeius* sp. nov. and *Bacteroides coprocola* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 55:2143–2147. <https://doi.org/10.1099/ijs.0.63788-0>.
200. Lee JW, Chan CTY, Slomovic S, Collins JJ. 2018. Next-generation biocontainment systems for engineered organisms. *Nat Chem Biol* 14:530–537. <https://doi.org/10.1038/s41589-018-0056-x>.
201. Lowe EC, Basle A, Czjzek M, Firbank SJ, Bolam DN. 2012. A scissor blade-like closing mechanism implicated in transmembrane signaling in a *Bacteroides* hybrid two-component system. *Proc Natl Acad Sci U S A* 109:7298–7303. <https://doi.org/10.1073/pnas.1200479109>.