Microbial degradation of complex carbohydrates in the gut

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Bacteria that colonize the mammalian intestine collectively possess a far larger repertoire of degradative enzymes and metabolic capabilities than their hosts. Microbial fermentation of complex non-digestible dietary carbohydrates and host-derived glycans in the human intestine has important consequences for health. Certain dominant species, notably among the Bacteroidetes, are known to possess very large numbers of genes that encode carbohydrate active enzymes and can switch readily between different energy sources in the gut depending on availability. Nevertheless, more nutritionally specialized bacteria appear to play critical roles in the community by initiating the degradation of complex substrates such as plant cell walls, starch particles and mucin. Examples are emerging from the Firmicutes, Actinobacteria and Verrucomicrobiidum phyla, but more information is needed on these little studied groups. The impact of dietary carbohydrates, including prebiotics, on human health requires understanding of the complex relationship between diet composition, the gut microbiota and metabolic outputs.

Introduction

Mammalian genomes do not encode most of the enzymes needed to degrade the structural polysaccharides present in plant material. Instead a complex mutual dependence has developed between the mammalian host and symbiotic gut microorganisms that do possess the ability to access this abundant source of energy. Herbivorous mammals rely on resident gut microorganisms to gain energy from their main food sources, and this has entailed major changes in digestive anatomy and physiology that allow efficient microbial fermentation to take place alongside the recovery of dietary energy by the host.1 Ruminants (foregut fermenters) benefit from microbial protein as well as the absorption of energy that is released by anaerobic microorganisms in the form of fermentation acids. Other herbivores and omnivores derive varying amounts of energy from microbial fermentation in the hind gut of those carbohydrates that are not digested in the upper gut. Interestingly, molecular profiles for the gut microbiota have been shown to group together for animal species that share similar nutrition and digestive anatomy.2 While humans derive a relatively small fraction (perhaps 10%) of their dietary energy through the activities of intestinal microorganisms,3 the microbial communities of the human intestine have important consequences for health and their composition and activities are known to be strongly influenced by the carbohydrate content of the diet.4,5

Most of the plant-derived polysaccharides that enter the rumen and large intestine are in the form of insoluble structures, in particular plant cell wall fragments and starch particles. Early work on the rumen established that only a small subset of rumen microorganisms, that include cellulolytic bacteria, fungi and protozoa, have the capacity to initiate degradation of plant cell walls.6 The most numerous groups of rumin microorganisms however are non-cellulolytic bacteria, many of which possess the ability to grow on soluble polysaccharides that are released by the primary degraders.7,8 Stratification of particle-associated microbial communities is evident from microscopic and fractionation studies both in the rumen and in human colon.9,10 It is reasonable to assume that the most closely adherent organisms will include the primary degraders, but also that more loosely adherent organisms within the consortium will contribute to polysaccharide degradation and utilization. Some primary colonizers are known to be nutritionally highly specialized; many rumen cellulolytic bacteria for example utilize breakdown products of cellulose, but fail to utilize products of xylan breakdown despite possessing a battery of hemicellulases and pectinases that are presumably required to degrade the plant cell wall matrix surrounding the cellulose fibrils.11,12 Solubilisation of the matrix polysaccharides therefore results in cross-feeding to other groups of bacteria. Metabolic cross-feeding is a central feature in anaerobic microbial communities that involves products of fermentation such as hydrogen and lactate as well as partial substrate degradation products.13,14 On the other hand, many other dominant gut bacteria show remarkable nutritional flexibility. The human intestinal species Bacteroides thetaiotaomicron for example encodes a huge repertoire of carbohydrate degrading activities15 and has the ability to switch between diet- and host-derived carbohydrates.16 The expression of genes involved in the degradation of complex carbohydrates by many gut bacteria is tightly regulated not only in response to the availability of specific substrates, but also in response to the host and other bacteria within the gut community.17

This review will focus mainly on the microbial ecology of carbohydrate degradation in the human large intestine, but for comparison also considers the degradation of plant structural polysaccharides in the rumen where this process is both more...
In total 130 families of glycoside hydrolases (GH), 22 of polysaccharide lyases (PL) and 16 of carbohydrate esterases (CE) have now been described from all life forms and a high proportion of these are found to be encoded in microbial genomes (www.cazy.org). These include catalytic domains that degrade plant structural polysaccharides (cellulose, β-glucan, xylan, mannan and pectin) and storage carbohydrates (Fig. 1) and a wide variety of host-derived glycans. In addition there are currently 64 families of carbohydrate binding modules (CBMs) that are frequently found to be associated with the catalytic domains of extracellular degradative enzymes. Draft genomes are now available for several rumen bacteria and for 50–100 species of commensal human intestinal bacteria (with more projected) and these provide important information on the potential polysaccharide-degrading enzyme repertoire of each strain (Table 1). Metagenomic approaches have the potential to identify novel enzymes and enzyme families involved in carbohydrate breakdown through functional screening as well as cataloguing the abundance of known genes via high-throughput sequencing. Metagenomic sequencing applied to the human gut microbiota has detected a large panel of carbohydrate active enzymes (CAZymes). However, the great majority of these potential enzymes remain to be characterized and their regulation studied. It is important to keep in mind also that organisms depend on complex interacting systems of degradative enzymes, transport functions and regulatory circuits in order to utilize complex carbohydrate substrates. For this reason the following sections will concentrate on examining function-based information that has so far been obtained mainly from cultured anaerobic gut bacteria.

### Plant Cell Wall Degradation by Rumen Bacteria

Plant cell walls consist of cellulose fibrils embedded in a matrix of hemicellulose (xylan, mannan, xyloglucan and β-glucan) and pectin, with lignin also present in secondary walls (Fig. 2). Cellulose consists of linear chains of β(1,4)-linked glucose units that form microfibrils through hydrogen bonding. Highly crystalline cellulose is particularly recalcitrant to enzymatic degradation and efficient and better studied. We also consider briefly some of the consequences of carbohydrate fermentation for human health.
degradation, whereas amorphous forms are more accessible. Xylan is a heterogeneous polymer of β(1→4)-linked xylose residues substituted with acetyl, arabinoxy and 4-O-methylglucuronyl residues; ext cross linkages can also occur between arabinoxyl substituents and furalic acid present in lignin. Pectins are a family of complex polysaccharides that contain α(1→4)-linked β-galactosuronic acid or rhamnogalacturonan backbones. Plant cell wall composition and structure, and consequently its digestibility and fermentability in the gut, however varies considerably between plant species, varieties and tissues.

Only actively cellulolytic rumen species have been found to cause extensive solubilisation of plant cell wall material in pure culture. The two main groups of cellulose-degrading bacteria that have been isolated, Gram-positive ruminococci and Gram-negative Fibrobacter spp, possess contrasting fibrolytic enzyme systems. Ruminococcus flavefaciens is the only gut bacterium so far shown to produce a cellulose-degrading enzyme complex, where the assembly of protein subunits depends on specific interactions between dockerin and cohesin modules found in the protein subunits. The genome of R. flavefaciens FD1 encodes 220 dockerin-containing proteins that are potential cellulosome subunits together with multiple cohesin-containing scaffolding proteins, four of which are encoded by the sce genes.25,26 The dockerin-containing proteins include diverse GH, CE and PL domains as well as CBMs and peptidases, but the functions of around 30% of the associated domains remain unknown.37 The cellulosomal xylanases in this species display remarkably complex structures38,39 with as many as five distinct catalytic domains and CBMs (Fig. 3), and include some that are upregulated more than 50-fold by growth on cellulose.37,38 The whole complex is anchored to a small protein that is bound to the bacterial cell surface by a sortase-mediated linkage.41 Dockers, but not cohesins, have been found in the related R. albus, leaving it unclear how enzymes are organized in that species. Multidomain organization is also seen for non-cellulosomal xylanases of R. albus.42 However, both of these species of ruminococci produce prominent GH48 enzymes43 that are assumed to play a key role in cellulose hydrolysis, as related enzymes function as exo-acting cellubiohydrolases in Clostridium spp.44 Adhesion to the insoluble plant cell wall substrate involves multiple CBMs within enzyme subunits, together with cellulose binding pili in R. albus and a specific attachment protein in R. flavefaciens.45 In contrast, F. succinogenes is highly unusual among anaerobic cellulolytic bacteria in lacking dockerin sequences and in lacking a GH48 exo-cellulase, although processive GH9 cellulases may fulfill the same role.46,47 The organization of fibrolytic enzymes in this species, which achieves highly efficient degradation of crystalline cellulose, remains unclear.

Pevrotella spp are among the most abundant bacteria within the rumen community; while none is cellulolytic, other plant cell wall polysaccharides can be utilized by many species. P. brentii (formerly P. ruminicola) B4 grows well on water-soluble, but not on water-insoluble, xylans.48 Two gene clusters are now known to play an important role in xylan-utilizing Pevrotella spp. One includes a GH10 xylanase and GH43 β-xylanosidase that contribute most of the assayable xylanase activity in cell extracts and whose expression is induced in response to xyl-o-oligosaccharides by a linked hybrid two component regulator (HTCS).49,50 Subsequent transcription studies have revealed more than 50 genes whose expression is significantly higher during growth on xylans as compared with xylose and arabinoxy in P. brentii B4.48 The most highly induced genes belonged to a second cluster (xux) that includes two xuc and two xud paralogues (discussed further below) in tandem, and an endoxylanase gene (xyn10C). Xyn10C is unusual in carrying CBM sequences within the catalytic domain51,52 and is thought to be responsible for cleavage of xylan molecules at the cell surface. The related R. amudosus F50 has been shown to encode at least 16 esterases that are involved in de-acylating and de-methylating xylans and pectins, as well as removing ester-linked phenolic acids.53,54
Metagenome surveys of rumen contents have tended to detect a low number of CBMs and a high % of GH domains that are typically associated with the utilization of soluble polysaccharides (e.g., 32% of glycoside hydrolases detected in rumen fiber fractions by Brulc et al. were related to GH2 or GH3 glycosidases). It is not clear whether this primarily reflects the difficulty of recovering DNA from tightly adherent cellulosytic species or low populations of those bacteria that have so far been identified as fiber-degraders. A recent metatranscriptome analysis of the muskox rumen that targeted mRNA of eukaryotic origin, however, yielded very high numbers of glycosyl hydrolase genes. This emphasizes the important and distinctive contribution that anaerobic eukaryotic microorganisms, fungi and protozoa, to fiber degradation in the rumen.

Degradation of Complex Carbohydrates by the Human Intestinal Microbiota

Microbial diversity in the human colon. Recent analyses of directly amplified 16S rRNA genes together with metagenomic surveys have helped to define those phylotypes (species defined by sequencing) that are most abundant within the human fecal microbiota. Perhaps not surprisingly, many of the dominant phylotypes correspond to cultured species, whereas only around 30% of the less abundant phylotypes are represented by cultures (Fig. 4). The dominant bacterial phyla in healthy subjects are the Bacteroidetes, Firmicutes and Actinobacteria, together with Verrucomicrobia and Proteobacteria. The composition of the human fecal microbiota responds to dietary carbohydrates. Recent metatranscriptome analysis of the human colon is making it likely that this is a significant site for microbial fiber degradation. Some reports indicate that the distal ileum harbors a community somewhat similar in composition to that of the proximal colon, but the major energy sources appear to be simple carbohydrates.

Early phenotypic surveys revealed that members of the Bacteroides genus harbor very broad saccharolytic potential, with some strains able to target dozens of different complex carbohydrates. Gram-positive bacteria (especially the Firmicutes) have received far less attention and their importance in polysaccharide breakdown is only now beginning to emerge. 16S rRNA sequences from human colonic bacteria attaching to wheat bran, resistant starch and mucin in a fermentor system were shown to include high proportions of Firmicutes (79%, 51% and 44%, respectively).

Degradation of diet-derived carbohydrates. It is estimated that around 20–60 g of dietary carbohydrates reach the colon each day having escaped digestion by host enzymes. The main categories are resistant starches, plant cell wall polysaccharides and non-digestible oligosaccharides, although some dis- and monosaccharides (e.g., sugar alcohols) also show limited digestion and/or absorption. Resistant starch. While the majority of ingested dietary starch is completely digested in the small intestine, a variable fraction survives to reach the large intestine. This fraction is referred to as "resistant starch" and for most diets it is estimated to provide the single largest source of diet-derived energy for colonic bacteria. Dietary starch can be resistant because of protection from plant cell wall polymers (type 1), granular structure (type 2), retrogradation (resulting from heating and cooling) (type 3) or chemical cross-linking (type 4). It is also likely however that...
polymer comprising $\alpha(1,6)$-linked maltotriose residues, provides a useful test substrate for enzymatic activity. Catalytic domains that hydrolyze $\alpha(1,4)$ linkages (mainly $\alpha$-amylases) and $\alpha(1,6)$-linkages (e.g., type 1 pullulanases) in starches are mostly found within GH family 13, while binding domains belonging to several different families can be responsible for binding starch molecules. It is important to note that the preparation of starches both in cooking and in laboratory experimentation strongly influences their fermentability, as well as digestibility, with autoclaved starches generally being more fermentable by amylolytic human gut bacteria than boiled or raw, starches.61

Plant cell wall polysaccharides. By comparison with the rumen, discussed above, understanding of the fibrolytic microbial system is important. More rapid oro-cecal transit, and perhaps meals that provide a particularly high starch intake, may result in more digestible starch reaching the large intestine. The fraction of dietary starch that is resistant will therefore vary with diet composition and intake, cooking methods and even between individuals. Resistant starch has been suggested to confer a number of human health benefits that may result from its fermentation and stimulation of microbial growth in the colon.59 Dietary starch typically comprises a mixture of amylose (linear chains of $\alpha(1,4)$-linked glucose residues) and amylopectin (amylose chains connected by $\alpha(1,6)$-linked side branches) (Fig. 1). Cereal starches that have a higher content of amylose often show greater resistance to host amylases than those with more amylopectin.59 Pullulan, a repeat polymer comprising $\alpha(1,6)$-linked maltotriose residues, provides a useful test substrate for enzymatic activity. Catalytic domains that hydrolyze $\alpha(1,4)$ linkages (mainly $\alpha$-amylases) and $\alpha(1,6)$-linkages (e.g., type 1 pullulanases) in starches are mostly found within GH family 13, while binding domains belonging to several different families can be responsible for binding starch molecules. It is important to note that the preparation of starches both in cooking and in laboratory experimentation strongly influences their fermentability, as well as digestibility, with autoclaved starches generally being more fermentable by amylolytic human gut bacteria than boiled or raw, starches.60

Figure 3. Examples of cell surface organization of carbohydrate-degrading enzymes in anaerobic Gram-positive gut bacteria. (A and C) show the domain structures and organization of two major cell-surface anchored amylases from two human intestinal anaerobes (Numbering refers to the enzyme family as in Fig. 1 or carbohydrate binding module [CBM] family). (B) shows the domain structures of six examples of cellulosomal polysaccharidases from the rumen bacterium Ruminococcus flavefaciens FD1. (D) shows the likely organization of the cellulosome in R. flavefaciens FD1; scaB, scaA and scaC are structural proteins encoded by the sca gene cluster that interact with each other and with the cellulosomal enzyme subunits via a series of specific, non-covalent dockerin-cohesin pairings (shown, in gray). The arrows in (C and D) indicate sortase-mediated anchoring to the bacterial cell wall (also indicated by cross-hatching in A).
community of the human large intestine remains somewhat limited. The digestibility of cellulose and hemicellulose in a group of seven women on a standardized diet was estimated at 70% and 72% respectively, showing that there is extensive degradation of these polysaccharides in dietary plant cell wall material during passage through the human intestine. The type of cellulose appears to be critical, however, since in the same study only 8% of an added refined cellulose (Solka Floc) was digested. Whereas bacteria able to grow on sources of hydrated, amorphous cellulose, such as spinach cell walls, can apparently be isolated from most individuals, bacteria able to degrade largely crystalline cellulose substrates, such as milled filter paper, are not always recoverable. Cellulolytic strains isolated from human feces have been classified as Ruminococcus sp, Clostridium sp, Eubacterium sp and Bacteroides sp. Interestingly, it has been suggested that the structure and activity of the cellulose-degrading community varies according to the methanogenic-status of the individual; thus among cellulolytic isolates, Ruminococcus sp were predominant in methane excretors and Bacteroides in the non-methane excretors. It was hypothesized that these differences might be linked to H₂ transfer between H₂-producing cellulolytic bacteria (the ruminococci) and methanogenic archaea although gut transit also tends to be slower in methanogenic individuals. Inulin, oligosaccharides and prebiotics. There is a strong interest in optimising the colonic microbiota through dietary manipulation. A prebiotic has been defined as “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” Currently used prebiotics are mainly low digestible carbohydrates that are found naturally in foods. These include xylol-oligosaccharides (XOS), galacto-oligosaccharides (GOS) and fructans, including inulin and fructo-oligosaccharides (POS). Any dietary substrate that remains undigested in the upper GIT, and that may have

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Figure 4. Dominant bacterial species identified by analysis of 16S rRNA sequences in fecal samples from six individuals. Data are from Walker et al. (2011), and represent the mean of 26 fecal samples from six obese male volunteers (4, or in one case 6, samples per person) taken during a 12 week controlled dietary study. Phyotypes corresponding to the 25 most abundant cultured bacterial species, that accounted for almost 50% of all sequences, are shown in descending order of abundance on the right hand side. The gray area on the left represents the 295 additional phyotypes (both cultured and uncultured organisms) that were detected.

- Faecalibacterium prausnitzii
- Eubacterium rectale
- Collinsella aerofaciens
- Clostridium clostridioforme
- Bacteroides vulgatus
- Anaerostipes hadrus
- Ruminococcus bromii
- Eubacterium hallii
- Blautia wexleri
- Bacteroides direc
- Roseburia faecis
- Dorea longicatena
- Subdoligranulum variabile
- Bacteroides uniformis
- Ruminococcus obeum
- Bacteroides ovatus
- Blautia luti
- Parabacteroides distasonis
- sp nov A2-166
- sp nov SR1/5
- Lachnospira pectinovorans
- sp nov 80/3
- Dialister inovius
- Roseburia inulinivorans
- Ruminococcus colitidis
- others
beneficial effects, it is however a potential probiotic. The health benefits attributed to various probiotics, including FOS and GOS, have been extensively reviewed in references 70 and 71.

Most of the available information on prebiotics has focused on fructans which were the first carbohydrates to be used to increase the abundance of bifidobacteria in the human colon. The inulin type fructans are present in foods such as onions, garlic and bananas that are linear polymers of β-(2,1)-linked fructose residues, with terminal glucose residues (Fig. 1). Oligofructose has a DP (degree of polymerisation) of between two and eight units and inulin has a DP of up to 65. Bacterial utilization of fructans is dependent on the presence of β-fructofuranosidases. Different bacterial β-fructofuranosidases vary in their ability to cleave the (β2,1) bonds in sucrose, FOS and inulin.72 Galacto-oligosaccharides (GOS) are chains of galactose residues (DP 3–10) with a terminal glucose residue. GOS can be formed by treating lactose with β-galactosidase, and the final GOS product has a range of linkages (β(1,2), β(1,3), β(1,4)) depending on the production conditions. One of the most abundant natural sources of GOS is human milk, and this has led to the development of GOS-enriched formula milk.

Prebiotics are also used in conjunction with probiotics, the so-called “synbiotic” approach. In a group of elderly patients given a double probiotic mixture of Bifidobacterium bifidum and B. lactis, the inclusion of an inulin/FOS prebiotic enhanced the survival of GOS-enriched formula milk.76

Utilization of host-derived glycans. From birth most infants are exposed to oligosaccharides, present at concentrations of around 10 g/L in human breast milk, that consist mainly of t-fucose, t-glucose or t-galactose residues. Bifidobacterium spp usually dominate in the feces of breast fed babies and this is thought to be due to their abilities to utilize oligosaccharides in breast milk.73 In total, human milk contains around 200 different oligosaccharides, with as many as 130 in milk from a single mother.74 Since none of these can be metabolised by infant digestive enzymes, the reason for their production is assumed to be the selective stimulation of particular bacteria.

The major host-derived source of glycans entering the gut throughout life is mucin, a group of glycoproteins that are produced continuously in large amounts by the gut epithelium. A limited number of microbial species appear able to digest mucin; these include the recently described bacteria Akkermansia muciniphila, a member of the Verrucomicrobia phylum, which can comprise as much as 3% of gut bacteria detected in feces of adults.75,76 Comparing the genome sequence of A. muciniphila with those of other Verrucomicrobia reveals the presence of relatively more genes involved in carbohydrate transport and metabolism.79 Signal peptides were detected in 26% of the predicted proteome from A. muciniphila, indicating a high proportion of exported products that include many with a likely role in mucin degradation.75 Sugars are also present on gut epithelial surface glycoconjugates. Bacterial cells able to use endogenously derived substrates as an energy source are likely to have a competitive advantage during periods of reduced dietary intake.

Human Colon Bacteriodes

Early work showed that human Bacteroides species were able to degrade diverse plant polysaccharides, including pectin, galactomannan, arabinogalactan, alginate, laminarin and xylan, while more recent work has extended this to include xylolanghamnogalacturonans I and II, β-glucans and glucomannan. Bacteroides ovatus, B. thetaiotaomicron and B. uniformis ferment a particularly wide range of polysaccharides, and this versatility may help to explain their prevalence as dominant species in the colon.79,80 The xylanolytic microbe was recently re-investigated, yielding new isolates belonging to B. intestinalis, B. ventosus, B. dentis, B. cellulosolvens and B. xylanisolvens.82 Furthermore, the direct cloning of xylanase genes has suggested that other as yet uncultivated xylanolytic Bacteroides and Prevotella exist in the human intestine.7 The main cellulose-degrading bacteria isolated recently from non-methane-excreting subjects belonged to the new species B. celulosilyticus, which is the only cellulolytic Bacteroides described to date.83,84 Cellulases have not yet been characterized however from any human Bacteroides strain.

Among hemicellulose-degrading activities, enzymes involved in the hydrolysis of xylans, mannans and galactomannans were characterized in B. thetaiotaomicron and B. xylanisolvens.85 Furthermore, the direct cloning of xylanase genes has suggested that other as yet uncultivated xylanolytic Bacteroides and Prevotella exist in the human intestine.7 The main cellulose-degrading bacteria isolated recently from non-methane-excreting subjects belonged to the new species B. celulosilyticus, which is the only cellulolytic Bacteroides described to date.83,84 Cellulases have not yet been characterized however from any human Bacteroides strain.

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Starch utilization and the Sus paradigm. The organization of starch-degrading enzymes was first studied in B. thetaiotaomicron, which can utilize various forms of starch, including amylase, amylpectin and pullulan as well as the corresponding malto-oligosaccharides.86 Salyer’s group showed that the starch degradation enzymes are cell-associated and that the binding of the polysaccharide to the cell surface is the first step in the degradation process.87 The starch binding activity and the degradation enzymes are maltose inducible, with these functions encoded by an operon of eight genes (susABCDEF).88 Gene disruption analysis enabled roles to be assigned to the proteins encoded by the different genes. Salyers proposed the original model for the highly efficient ‘Starch Utilization System’ of B. thetaiotaomicron, that has recently been reviewed in reference 91 (Fig. 5).

SusABCDEF are localized at the cell surface and bind, degrade and import soluble starch molecules.86 SusD is a lipoprotein anchored at the outer membrane of the cell. SusD is
Figure 5. Bacteroides thetaiotaomicron sus system. (A) shows the order of genes in the sus cluster that is responsible for starch utilization in this species. (B) shows the inferred organization of gene products on or near the bacterial cell surface (OM outer membrane, CM cytoplasmic membrane). Starch molecules are shown as sugar chains, at various stages of hydrolysis.

responsible for the binding of starch to the cell surface, and this binding appears to be driven by recognition of the overall three-dimensional shape of the starch molecule. SusE and SusF are also likely to be involved in starch binding. SusG is a pivotal protein in the system: it is a TonB-dependent transporter (TBDT), a group of outer-membrane-spanning β-barrel proteins that sense and transport various molecules in Gram negative bacteria. Unlike the other TBDTs characterized to date, SusC cannot bind the ligand alone and requires the starch-binding protein SusD for starch import. Therefore, SusG likely plays a critical role in targeting polymeric starch to the Sus complex and may facilitate movement of linear oligosaccharides to SusC. SusG is a GH13 α-amylase that may have evolved to work as part of a carbohydrate-processing/import complex rather than just as an outer-membrane amylase. susG deletion mutants could still bind starch at the cell surface, but could not grow on starch, suggesting that SusG is essential for the metabolism of starch, despite the presence of four other predicted amylases in B. thetaiotaomicron genome. The proposed mechanism is as follows: starch molecules are held on the surface of the bacteria through multiple interactions with SusD proteins. This anchors the polysaccharide in close proximity to SusG, and enables the enzyme to hydrolyze the starch. The cleaved maltooligosaccharides still bound to SusD are then presented to the entrance of the SusC porin (Fig. 5). The maltooligosaccharides are translocated by SusC and released in the periplasm where they are broken down by SusA and SusB, a periplasmic GH13 α-amylase and a GH97 α-glucosidase, respectively. The small saccharides produced can then be transported into the cytoplasm (Fig. 4). The sus cluster is regulated by the transcriptional regulator SusR in response to maltooligosaccharides, amylase, amylopentin and pullulan but is also controlled by the regulatory protein MalR. The Sus system thus appears to be a very efficient and well controlled system for the capture, sequestration and degradation of starch, giving B. thetaiotaomicron an ecological advantage in a very competitive ecosystem.

Polysaccharide utilization loci. Since the discovery of the Sus complex, 88 similar Sus-like Polysaccharide Utilization Loci (PULs) have been identified, representing 18% of the B. thetaiotaomicron genome. 82-103 Most of these have been reported to degrade mucins or other host-derived glycans and include enzymes that target glycan decorations such as sulfatases and acetyl-esters.104-106 The other PULs are probably involved in the degradation of plant polysaccharides, ten of them being dedicated to pectins.104 PULs have also been identified for the utilization of FOS and levans.107 Recent studies using B. thetaiotaomicron-associated gnotobiotic mice and bacterial genome transcriptional profiling have shown that this species has evolved mechanisms to adapt glycan utilization to nutrient availability within the ecosystem. When dietary polysaccharides were supplied to the mice, B. thetaiotaomicron expanded its niche from host derived glycans to accommodate the additional diet-derived nutrients.107 When the dietary polysaccharides became less available, the bacterium turned to the utilization of the host mucins. In addition, in the gut of suckling mice, B. thetaiotaomicron relied on host-derived mucosal polysaccharides in addition to mono and oligosaccharides present in mother’s milk, but after weaning, the bacterium expanded its metabolism to exploit abundant, plant-derived dietary polysaccharides.41,103,104 More than 50 Bacteroides genomes are currently available in the NCBI database, and these confirm that gut-associated Bacteroidetes possess an extensive repertoire of genes predicted to encode CAZymes. PULs have been identified that target pectins and hemicelluloses such as xylans (the sus cluster) and galactomannans.103,105,106 Although it was first thought that PULs were adapted only to soluble or well-hydrated polysaccharides, PULs have also been found associated with genes coding CAZymes targeting insoluble polysaccharides.96

Although different PULs encode different repertoires of proteins involved in the utilization of specific polysaccharides, they are all organized in a manner similar to the sux operon of B. thetaiotaomicron (Fig. 5), and comprise SusC-like TBDT and SusD paralogs, as well as CAZymes adapted to the substrate, located at the cell surface and in the periplasm. The susC-like and susD-like genes are the central units of substrate-specific PULs. For example, B. thetaiotaomicron possesses 108 paralogs of susC, of which 101 are paired to a susD-like gene, and 88 of these pairs are associated with CAZyme genes.95,106 Pairs of susC/susD-like genes often appear in tandem, possibly as a result of gene duplication. In conclusion, the PUL system appears to be a generic
feature of carbohydrate nutrient acquisition by gut and environ-
mental bacteria genes. This hybrid two-component histidine kinase response regulators and Extra Cytoplasmic Function (ECF-type) sigma factors and anti-
sigma factors, which participate in trans-envelope signaling.104

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Extracytoplasmic Function (ECF) signal transduction systems in the gut environment.111 Starch utilization. Bifidobacterium spp have been reported to be partic-
ularly effective degraders of high amylopectin117 starch and some strains are known to be able to attach to starch particles.118 Degradation of RS and of pullulan appears to be associated with particular strains and species, especially B. breve and B. adoles-
centis.115 Detailed work on B. breve has identified a major cell surface anchored enzyme that comprises distinct α (1,4) amylase and type 1 pullulanase domains together with multiple CBMs (Fig. 3). Deletion of one these starch abolishes growth on starch, pullulan and glycogen116 although multiple GH13 genes are found in the genomes of B. adolescentis and B. breve.

Other plant polysaccharides and prebiotics. Tannock et al.107 reported that, in addition to Bifidobacterium spp, C. aerofaciens increased in fecal samples from volunteers consuming FOS, and another human study found that numbers of Bifidobacterium and Atopobium were increased by long chain inulin (average Dp >55).109 Two types of β-fructofuranosidase have been identified in Bifidobacterium spp: those that are more active against the β(2,1) glucose-fructose bonds, releasing only the terminal glucose residue for growth110 and those that are more active against β(2,1) fructose-fructose links.111 Both types of β-fructofuranosidase however had only low activities against long-chain inulin mol-
ecules.111-112 Only eight out of 55 Bifidobacterium strains tested, from five different species, were able to grow on long chain inu-
il, although all grew well on FOS.113 It appears that bifidobac-
teria can be split into clusters: those unable to use any fructan (B. bifidum and B. breve); those able to use only short chain FOS (7 species); and those able to use iFOS and short chain inulins. Fructan-utilizing ability was not species-specific, with strains of B. longum for instance falling into different clusters.114 The chain length of the substrate is therefore likely to be critical to its

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subsequent effect on the composition of the microbial community. Next, the bifidobacteria up-regulated were active against insulin chains longer than 20 units long. Complex cross-feeding interactions have been demonstrated for co-cultures between human colonic B. thetaiotaomicron and Bifidobacterium spp that have different abilities to utilize fructan molecules of different chain length. Meanwhile other bifidobacteria can be involved in cross-feeding with butyrate-producing bacteria either by releasing oligo- and mono-saccharides from complex substrates, or via the utilization of acetic fermentation products.

Studies in adults consuming GOS (5 g per day) revealed that there was more than a 100-fold increase in abundance of Bifidobacterium populations in fecal samples. Only 50% of the subjects were responders however, thereby revealing a considerable degree of inter-individual variation. Doses below 5 g GOS per day were not sufficient to induce a response while 10 g per day gave an even greater increase in bifidobacteria in some volunteers suggesting a dose-response effect. Analysis of the pyrosequencing data revealed that GOS enriched for particular Bifidobacterium-related OTUs. B. bifidum possesses four distinct β-galactosidases, which seem to act in complementary ways on different substrate bonds, thus contributing to efficient substrate degradation. It has also been shown that substrate responses occur at a species-specific level. B. adolescentis was elevated in response to FOS/ inulin in humans and in humanized rats. Bifidobacterium spp are also reported to have some ability to utilize arabinoxylans and arabinogalactan, but benefit from initial substrate breakdown of the complex polymers by other bacteria. Supplementation with the novel prebiotic long-chain arabinosylan significantly increased numbers of bifidobacteria in humanized rats, particularly boosting B. longum.

Host-derived carbohydrates. The plethora of genes specific for degradation of mammalian derived carbohydrates in B. longum subsp infantis presumably reflects the adaptation of this species to use human milk oligosaccharides (HMOs) for growth. Only B. bifidum and B. longum subsp infantis were able to grow well on HMOs, with other Bifidobacterial species having variable abilities. B. longum subsp infantis expresses specific genes in direct response to the composition of the milk, with fucoisidases only detected during growth on HMOs. The B. longum subsp infantis genome is also enriched in family 1 solute binding proteins (FISBPs) that are particularly associated with oligosaccharide uptake. Different classes of FISBPs were induced specifically during growth on different substrates. In a separate study R. inulinivorans was found to be prevalent in breast-fed babies and not in those fed on formula milk. The genome sequence of B. bifidum also contains many genes involved in the degradation of host-derived glycans, in particular the O-linked glycans attached to mucin, which appear to be co-regulated.

Firmicutes

Two families of Firmicutes, Lachnospiraceae and the Ruminococcaceae, are particularly abundant in the human large intestine, typically accounting for 50–70% of bacteria in fecal samples from healthy adult humans based on 16S rRNA analysis. These include some highly oxygen-sensitive organisms and are seriously underestimated by available cultured isolates, but they are responsible for some of the key metabolic conversions within the intestinal community. They include for example the major butyrate-producing species R. bromii, as well as species that convert lactate to butyrate or propionate and species that perform reductive acetogenesis. Emerging evidence suggests that these and other Firmicutes play key roles in polysaccharide degradation.

Starch utilization. Three recent studies have reported an increase in Ruminococcus bromii-related bacteria in volunteers consuming diets enriched with RS. This group was also prominent among fecal bacteria shown by stable isotope probing to utilize C-labelled starch in vitro. Walker et al. saw a mean increase of > 4-fold (from 3.8% to 17%) in the overall proportion of cluster IV Ruminococcaceae-related 16S rRNA sequences detected by qPCR in 14 obese volunteers when consuming a diet containing 26 g/day of type 3 RS compared with a low RS, wheat bran-enriched diet. The fractional increase was greater for sequences >98% related to R. bromii (0.4% to 5%). qPCR analysis indicated that further uncultured phyotypes among the Ruminococcaceae also responded to the RS diet. Remarkably, two of the 14 individuals showed no detectable ruminococci in their fecal samples and these were the only two individuals to give low estimates for starch fermentation. Additional evidence has now been obtained that supports the view that R. bromi-related organisms may indeed play a ‘keystone’ role in the initial stages of breakdown of particulate resistant starch. R. bromii showed a much greater ability to degrade raw or boiled RS2 and RS3 starches than B. thetaiotaomicron, and non-growing R. bro-mi cells were found to greatly enhance the utilization of these starches by three other prominent human amyloytic species, B. thetaiotaomicron, E. rectale or B. adolescentis. By contrast, a second, highly abundant group of Ruminococcaceae-related to Faecalibacterium prausnitzii apparently does not utilize starch, based on the cultured strains currently available. The enzyme systems that allow R. bromii to efficiently utilize particulate starch have not yet been fully investigated. In contrast to other abundant amyloytic bacteria found in the human colon R. bro-mii fails to grow on glucose, and grows more rapidly on maltosaccharides than on maltose.

Among the Lachnospiraceae, the ability to utilize starch has been reported for many members of the Roseburia/Eubacterium rectale group of butyrate-producing bacteria. Furthermore the population of this group in fecal samples has been found to increase on average in human volunteers on RS-enriched diets and to decrease on diets low in total carbohydrate. Roseburia spp produce a major, high molecular weight (> 180 kDa) amylose that is detectable by zymogram analysis. The enzyme from R. inulinivorans, Amyl3A, includes a GH1 amylase and two more CBMs and is able to cleave α(1,4) linkages in amylose, amylopectin and pullulan. The pre-protein carries an N-terminal signal peptide and a C-terminal sortase-mediated anchoring sequence indicating that it becomes anchored to the cell wall but extrudes into the extracellular matrix (Fig. S). R. inulinivorans...
Amylase is induced by growth on starch, along with expression of Flagella that are characteristic of this group of bacteria and that may perhaps help cells to migrate toward particulate substrates. 

Genome sequences indicate 9 to 13 GH3 genes in Roseburia spp and in the related species E. rectale, but the roles of the different gene products have not been elucidated. E. rectale was less active against boiled or raw RS than different gene products have not been elucidated. E. rectale was less active against boiled or raw RS than the other GH13 genes are present in most sequenced representatives of the Lachnocloaceae, the contribution of other species to starch degradation in the human colon is currently unknown. Plant cell wall polysaccharides, Salyers et al. reported finding a lower frequency of plant polysaccharide utilizers among Gram-positive anaerobes than among the Bacteroides spp tested. Subsequent evidence, both from molecular studies and new isolations, has however suggested that Firmicutes play a significant role in the degradation of complex plant carbohydrates. In particular, Ruminococcus champanellensis, a new species related to R. flavifaciens, is the only human colon bacillus so far reported to degrade microcrystalline cellulose. Human colonic strains related to R. albus were reported to utilize galactomannan. A shortage of cultured organisms from the Ruminococcaceae means that information on this group remains limited, especially for the human gut, but many appear to be closely associated with particulate material. Among the Lachnocloaceae, cellulolytic activity was reported in the aceticogenic bacterium Bryantella formexigen on first isolation, but apparently proved unstable. Xylan-utilisation has been reported for Roseburia intestinalis and also for the human R. flavefaciens strain 16k, which was isolated from a wheat brain enrichment. The distribution of the two main families of endoxylanases (GH10 and GH11) appears limited among other human intestinal Firmicutes for which draft genome sequences are available. The highly abundant species F. prausnitzii is now known to include strains able to utilize apple pectin for growth. The only other pectin-utilizing Firmicutes species identified so far from the human colon are Eubacterium eligens and Lachnocloaceae pectinicola.

Prebiotics. Relatively little attention has been paid to the utilisation of prebiotic oligosaccharides by Firmicutes. It is clear however that many species can utilize FOS, while some utilize long chain inulin. F. prausnitzii, E. rectale and R. inulinivorans for example are abundant butyrate-producing species. Among the Lachnocloaceae, cellulolytic activity was reported in the acetogenic bacterium Bryantella formexigen on first isolation, but apparently proved unstable. Xylan-utilisation has been reported for Roseburia intestinalis and also for the human R. flavifaciens strain 16k, which was isolated from a wheat brain enrichment. The distribution of the two main families of endoxylanases (GH10 and GH11) appears limited among other human intestinal Firmicutes for which draft genome sequences are available. The highly abundant species F. prausnitzii is now known to include strains able to utilize apple pectin for growth. The only other pectin-utilizing Firmicutes species identified so far from the human colon are Eubacterium eligens and Lachnocloaceae pectinicola.

A similar metabolic route for fucose metabolism has been described in the gut pathogen Salmonella enterica Typhimurium LT2. The fucose utilization genes in R. inulinivorans A2–194 are strongly upregulated during growth on fucose. Genome searching indicates that other species of Lachnocloaceae normally found in the human colon, R. obeum and R. gnavus, also possess homologs of the key R. inulinivorans fucose utilization genes, including those involved in the synthesis of a polyhedral body required for propane-1,2-diol metabolism. This indicates that these bacteria may employ a similar pathway for fucose utilization.

Metabolic Consequences of Carbohydrate Fermentation in the Human Colon

Impact on the gut environment. Addition of any non-digestible but fermentable carbohydrate to the diet will increase fermentative activity, especially in the proximal colon, resulting in increased acid production. This tends to decrease luminal pH, with important consequences for the composition of the microflora and the balance of microbial metabolites. In vitro studies indicate that Bacteroides populations are likely to be curtailed, while butyrate-producing Firmicutes are favored, within the community at mildly acidic pH. Reduced overall intake of complex dietary carbohydrates by obese subjects on weight loss diets was found to decrease short chain fatty acid formation, with a disproportionate decrease in fecal butyrate. Interestingly, the major butyrate-producing bacteria detected on high carbohydrate diets were the starch-utilizing Roseburia spp and E. rectale and the decrease in fecal butyrate on diets very low in carbohydrates was associated with a major decrease in this.
group, with *F. prausnitzii* becoming the main butyrate producer. Dietary complex carbohydrates also decrease the levels of potentially harmful metabolites that arise from proteolytic activity in the colon. Separately De Preter et al. demonstrated in in vitro studies that there was a dose dependent stimulation of sacccharolytic fermentation when fructans were included in their growth medium concomitant with a decrease in toxic peptide fermentation metabolites.

Increased SCFA concentrations may also increase the solubility of cations such as calcium, and enhance absorption and expression of calcium-binding proteins. Changes in intestinal microbial metabolism following the consumption of inulin fructans have also been shown to benefit bone health by increasing calcium absorption while β-glucans may lower total cholesterol levels. High fiber diets increase fecal bulking, short chain fatty acid production and transit rates along the large intestine. Slow transit rates will encourage growth of the slower growing microorganisms such as some of the hydrogen-utilizers including methanogens, that are present in approximately 50% of the population. Methane has been shown to slow gut transit in animal studies and the presence of methanogens is also associated with slower gut transit in humans. Digestion of plant fiber also results in the release of phenolic compounds. Epidemiological studies suggest that there is an inverse association between the intake of polyphenol-rich diets and the incidence of cardiovascular disease, diabetes and cancer but it is unclear at present what proportion of absorbed bioactive phenolic compounds can be ascribed to microbial activity.

**Recovery of energy from dietary carbohydrates: consequences for obesity, weight loss and metabolic health.** A high proportion of the SCFA produced by microbial fermentation of indigestible carbohydrates in the large intestine is absorbed by the host. Thus microbial activity contributes energy to the host (estimated to be around 10% of calories obtained from the diet) that would otherwise be lost through excretion of undegraded substrate in the feces. On the other hand, the calories that are obtained from a sugar via fermentation, followed by absorption and metabolism of SCFA, are estimated to be less than half the amount that would be gained by direct absorption of the same amount of sugar in the small intestine. The net effect of replacing consumption of a digestible carbohydrate in the diet with consumption of the same amount of fermentable, non-digestible carbohydrate is therefore to reduce the calories acquired from the diet.

The possible involvement of the gut microbiota in the development of obesity is growing far more complex than was first proposed. Variation in microbiota composition has the potential to influence “energy harvest” from fiber if it affects key groups involved in energy release and recovery, but factors such as gut transit and absorption seem likely to be more important (Fig. 6). Phylum level differences in the gut microbiota in obese vs. lean individuals have been reported in some studies, but not in others, and it appears that differences in dietary intake are mainly responsible for microbiota changes. Interestingly, however, microbiota composition also has the potential to influence satiety (and thus dietary intake) and energy expenditure. The finding that germ-free animals were apparently protected from developing diet-induced obesity has recently been balanced by a study reporting the opposite effect. These effects were thus shown to be highly dependent on the type of high-fat diet fed to germ-free mice, and were also found to be linked to differences in energy expenditure. Potential links between the gut microbiota and metabolic disease have also been under intense investigation in recent years. Serum levels of lipopolysaccharide (LPS), derived from Gram-negative bacteria, are reported to increase in obese and diabetic subjects and reproduction of similar LPS levels by chronic injection lead to a loss of insulin sensitivity in animals. The increased LPS levels may result from a decrease in the gut barrier function. It was shown that the administration of prebiotics (FOS) improved gut barrier function, which was strongly correlated with reduced portal plasma LPS levels. The effect seems to be mediated by the gut hormone glucagon-like peptide-2. Another potential route linking microbial activity with the host is via the gut-brain axis, a b-directional communication system based on neural, endocrine and immunological mechanisms. There is increasing evidence that there may indeed be a link between the gut microbiota and the brain. Recent rodent studies indicated that changes in the microbiota composition led to behavioral changes and altered levels of brain-derived neurotropic factor (BDNF) in different brain regions. These changes did not appear to be mediated by gut inflammation, specific enteric neurotransmitters or the autonomic nervous system, and it was hypothesized that microbial products acting on the central nervous system are likely to be involved, with butyrate being one potential candidate. The immune system is influenced by microbial metabolic products, but can also recognize a diverse range of microbial cell components. This leads to complex interactions between the species composition of the microbiota and the host’s innate and adaptive immune systems that are thought to underlie many probiotic effects.
Conclusions

Bacteria that colonize the mammalian intestine collectively possess a far wider diversity of genes and a larger repertoire of degradative enzymes and metabolic capabilities than their hosts. Fermentation of complex carbohydrates in the intestine involves interactions between community members that include both nutritionally specialized and widely adapted species. Certain dominant species, notably among the Bacteroidetes, possess very large numbers of genes encoding carbohydrate active enzymes (CAZymes). This allows them to switch readily between different energy sources in the gut depending on availability, using sophisticated sensing and regulatory mechanisms to control gene expression. Other groups encode fewer CAZymes and are clearly more specialized, but some of these organisms appear to play critical roles in the community by initiating the degradation of complex substrates such as plant cell walls, starch particles and mucin. Identification of these ‘keystone’ groups and their roles, particularly among members of the under-investigated Firmicutes phylum, should be a priority for future research. Finally, the impact of dietary carbohydrates, including prebiotics, on health in man requires further progress in understanding of the relationship between diet composition, gut microbiota and metabolic outputs. This demands, in addition to mechanistic understanding, systems-based approaches to integrate and model the many complex interactions between functional groups.

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