

Microbial degradation of complex carbohydrates in the gut

Harry J. Flint,^{1,*} Karen P. Scott,¹ Sylvia H. Duncan,¹ Petra Louis¹ and Evelyne Forano²

¹Rowett Institute of Nutrition and Health; University of Aberdeen; Bucksburn, Aberdeen UK; ²INRA; UR454 Microbiologie; Saint-Genès Champanelle, France

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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 Verrucomicrobium 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 fermentors) 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 rumen 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.⁹⁻¹¹ 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.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

*Correspondence to: Harry J. Flint; Email: h.flint@abdn.ac.uk
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Table 1. Predicted CAZymes encoded by the genomes of selected fibrolytic gut bacteria

Ecosystem	Phylum (family)	Bacterium	Total CAZymes	GH	GT	PL	CE	Total CBMs
Human colon	Bacteroidetes	<i>Bacteroides thetaioaomicron</i> VPI-5482	386	263	87	16	20	31
		<i>B. xylanisolvens</i> XB1A*	349	224	81	22	22	26
		<i>B. vulgatus</i> ATCC-8482	279	177	78	7	17	18
		<i>B. fragilis</i> 638R	223	138	78	1	6	26
	Firmicutes: (Lachnospiraceae) (Ruminococcaceae)	<i>Roseburia intestinalis</i> XB6B4*	175	115	46	0	14	11
		<i>Butyrivibrio fibrisolvens</i> 16/4*	115	75	37	0	3	31
		<i>Ruminococcus champanellensis</i> 18P13*	87	54	12	9	12	34
	Actinobacteria	<i>Bifidobacterium adolescentis</i> ATCC15703	94	54	37	0	3	6
Rumen	Fibrobacteres/ Acidobacteria	<i>Fibrobacter succinogenes</i> S85	183	100	54	12	17	73
	Bacteroidetes	<i>Prevotella ruminicola</i> 23	215	133	60	3	19	16
		<i>P. bryantii</i>	203	107	53	14	19	un
	Firmicutes: (Ruminococcaceae)	<i>Ruminococcus albus</i> 7	145	96	24	7	18	128
<i>R. flavefaciens</i> FD1		140+	101	un	13	26	68	

(GH, glycoside hydrolases; GT, glycosyl transferases; PL, polysaccharide lyases; CE, carbohydrate esterases; CBM, carbohydrate binding modules)

*For these strains, data were provided by the Pathogen Genomics group at the Wellcome Trust Sanger Institute and can be obtained from <http://www.sanger.ac.uk/resources/downloads/bacteria/metahit/>; The information presented is available from the CAZY website, except in the case of *R. flavefaciens* FD1^{27,30} and *P. bryantii*.⁴⁴ Un, information not available.

degradation, whereas amorphous forms are more accessible. Xylan is a heterogenous polymer of $\beta(1,4)$ -linked xylose residues substituted with acetyl, arabinosyl and 4-O-methyl-glucuronol residues; ester cross linkages can also occur between arabinosyl substituents and ferulic acid present in lignin. Pectins are a family of complex polysaccharides that contain $\alpha(1,4)$ -linked D-galacturonic 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.⁶ 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 cellulosome-type enzyme complex, where the assembly of protein subunits depends on specific interactions between dockerin and cohesin modules found in the protein subunits.²⁴ 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 *sca* gene cluster.²⁵⁻²⁷ 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.²⁷ The cellulosomal xylanases in this species display remarkably complex structures^{28,29} 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.^{27,30} The whole complex is anchored to a small protein that is bound to the bacterial cell surface by a sortase-mediated linkage.³¹ Dockerins, 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^{8,32} however and both of these species of ruminococci produce prominent GH48 enzymes³³ that are assumed to play a key role in cellulose hydrolysis, as related enzymes function as exo-acting cellobiohydrolases in *Clostridium* spp.²⁴ Adhesion to the insoluble plant cell wall substrate involves multiple CBMs within enzyme subunits, together with cellulose binding pili in *R. albus*³⁴ and a specific attachment protein in *R. flavefaciens*.³⁵ 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 fulfil the same role.^{12,36} The organization of fibrolytic enzymes in this species, which achieves highly efficient degradation of crystalline cellulose, remains unclear.

Prevotella 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. bryantii* (formerly *P. ruminicola*) B₁4 grows well on water-soluble, but not on water-insoluble, xylans.³⁷ Two gene clusters are now known to play an important role in xylan-utilizing *Prevotella* spp One includes a GH10 xylanase and GH43 β -xylosidase that contribute most of the assayable xylanase activity in cell extracts and whose expression is induced in response to xylo-oligosaccharides by a linked hybrid two component regulator (HTCS).³⁷⁻³⁹ Subsequent transcriptomic studies have revealed more than 50 genes whose expression is significantly higher during growth on xylans as compared with xylose and arabinose in *P. bryantii* B₁4.⁴⁰ The most highly induced genes belonged to a second cluster (*xus*) that includes two *susC* and two *susD* paralogs (discussed further below) in tandem, and an endoxylanase gene (*xyn10C*). *Xyn10C* is unusual in carrying CBM sequences within the catalytic domain^{41,42} and is thought to be responsible for cleavage of xylan molecules at the cell surface. The related *P. ruminicola* 23 has been shown to encode at least 16 esterases that are involved in de-acetylation and de-methylation of xylans and pectins, as well as removing ester-linked phenolic acids.^{43,44}

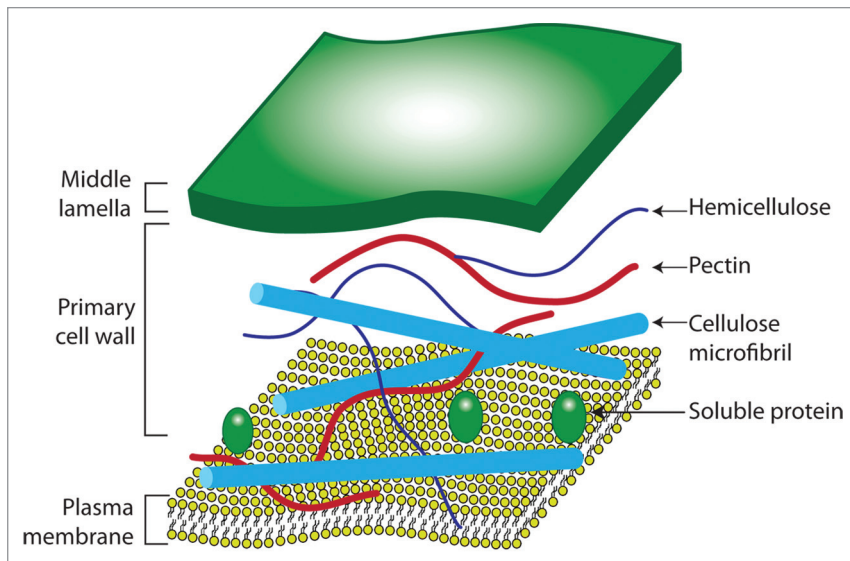


Figure 2. Plant cell wall structure. Diagrammatic representation of the major structural polysaccharide components of a “typical” primary plant cell wall.

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.²² were related to GH2 or GH3 glycosidases). It is not clear whether this primarily reflects the difficulty of recovering DNA from tightly adherent cellulolytic 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.⁴⁵ This emphasizes the important and distinctive contribution that is made by 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^{4,46} together with metagenomic surveys⁴⁷ 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 cultures⁴ (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 carbohydrate intake in the short-term⁴ and apparently also in the longer term.^{5,48} De Filippo et al.⁵ ascribed the differences they observed in fecal microbiota composition between two groups of children to different intakes of fiber and starch, with Bacteroidetes, especially *Prevotella* spp, being favored in a group of rural African

children and Firmicutes in Italian children. An association between a *Prevotella*-dominated microbiota and fiber intake, and between a *Bacteroides*-dominated microbiota and protein intake, has also been noted in adults.⁴⁸ Major inter-individual variation in microbiota composition is also evident however and this can strongly affect individual responses to dietary carbohydrate.^{4,49} It has recently been proposed that inter-individual variation in the microbiota can be classified into three discrete enterotypes across the healthy human population.⁵⁰

There is limited information on the spatial distribution of bacteria in the human intestine, with most information being available from fecal samples that are assumed mainly to reflect events in the distal colon. In human fecal material, Firmicutes, in particular certain Ruminococcaceae, have been shown to be enriched in the particulate fraction, with *Bacteroides* more prevalent in the liquid phase.¹⁰ This is likely to reflect different ecological niches and roles in substrate breakdown. Relatively little is known about the small intestinal microbiota in humans, but passage rates are more rapid and microbial concentrations lower than in the large intestine, making it unlikely 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,⁵¹ but the major energy sources appear to be simple carbohydrates.⁵²

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 glycans.^{53,54} 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 (75%, 51% and 44%, respectively).⁵⁵

Degradation of diet-derived carbohydrates. It is estimated that around 20–60 g of dietary carbohydrates reach the colon each day^{56,57} having escaped digestion by host enzymes. The main categories are resistant starches, plant cell wall polysaccharides and non-digestible oligosaccharides, although some di- and mono-saccharides (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.^{56–58} 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.⁵⁹ 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

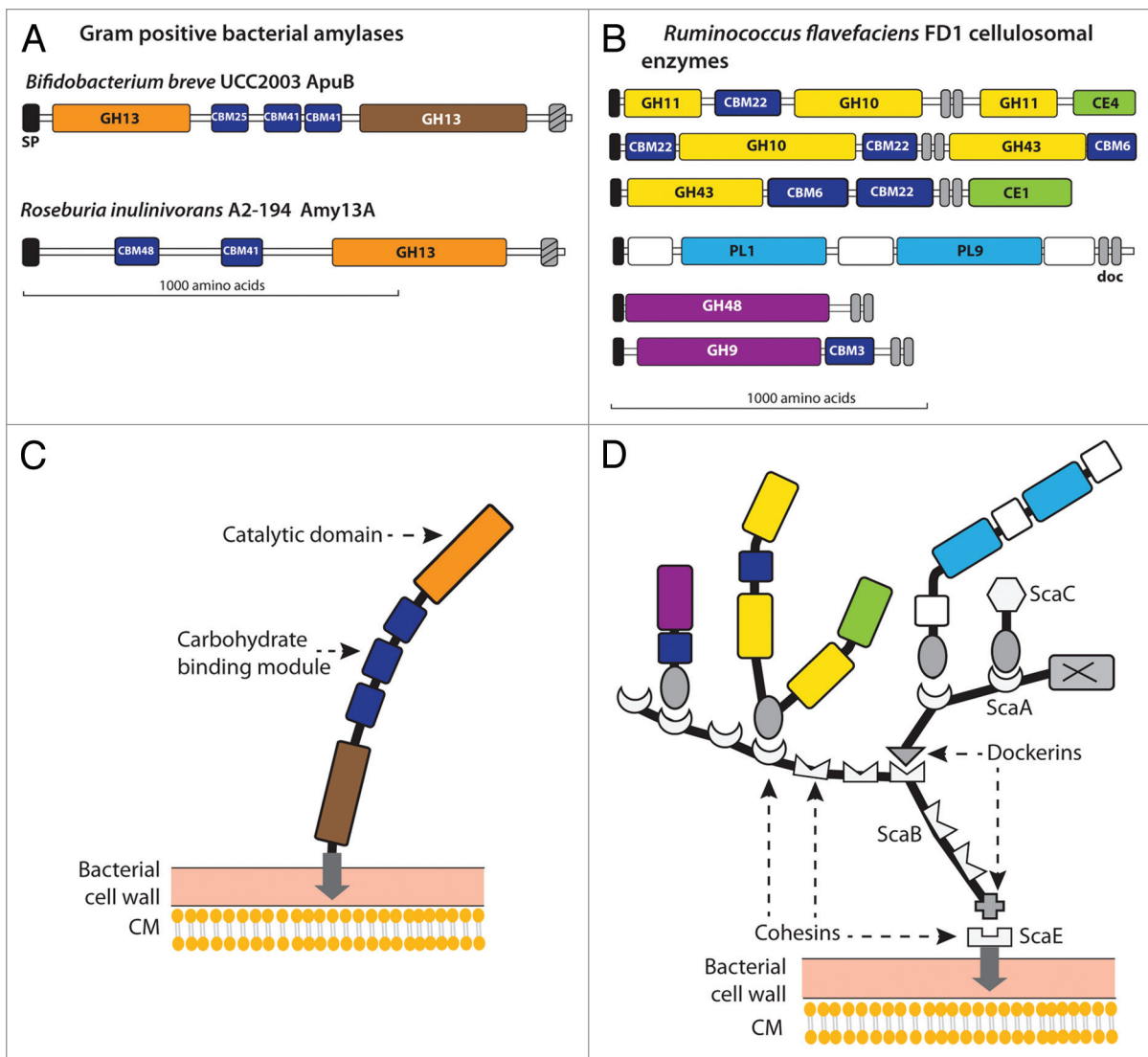


Figure 3. Examples of cell surface organization of carbohydrate-degrading enzymes in anaerobic Gram-positive gut bacteria. (A and C) show the domain structures and organization of two major cell-surface anchored amylases from two human intestinal anaerobes (Numbering refers to the enzyme family (as in 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; scaE, 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).

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.⁶⁰ 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 α 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

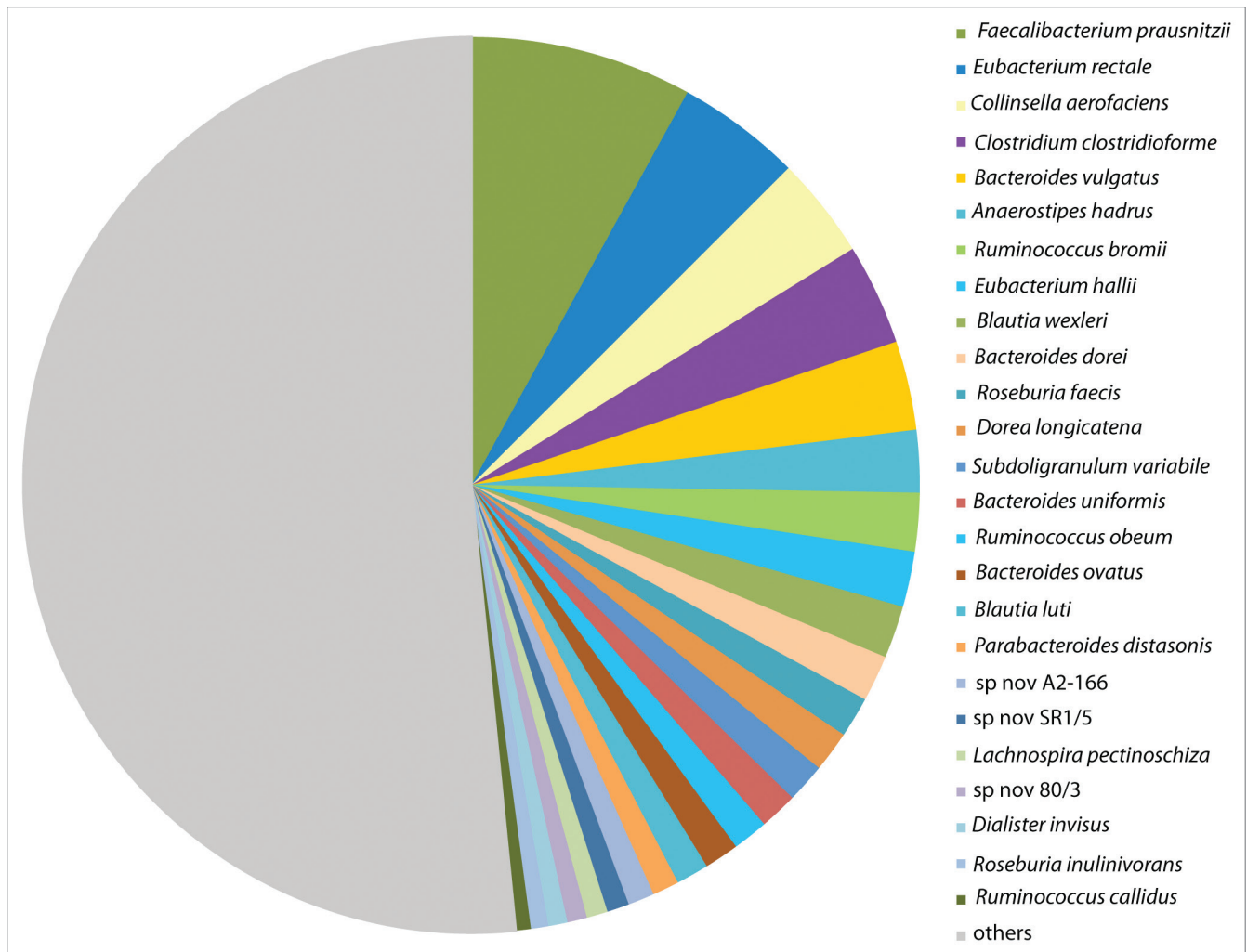


Figure 4. Dominant bacterial species identified by analysis of 16S rRNA sequences in fecal samples from six individuals. Data are from Walker et al. (2011),⁴ and represent the mean of 26 fecal samples from six obese male volunteers (4, or in one case 6, samples per person) taken during a 12 week controlled dietary study. Phylotypes 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 phylotypes (both cultured and uncultured organisms) that were detected.

community of the human large intestine remains somewhat limited. The digestibility of cellulose and hemicellulose in a group of seven women on a standardised diet was estimated at 70% and 72% respectively,⁶² 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.⁶² 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.⁶³⁻⁶⁵ Cellulolytic strains isolated from human feces have been classified as *Ruminococcus* sp, *Clostridium* sp, *Eubacterium* sp and *Bacteroides* sp.⁶³⁻⁶⁶ 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.⁶⁵ It was hypothesized that these differences might be linked to H₂ transfer between H₂-producing cellulolytic bacteria (the ruminococci) and methanogenic archaea⁶⁵ although gut transit also tends to be slower in methanogenic individuals.⁶⁷

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”.^{68,69} Currently used prebiotics are mainly low digestible carbohydrates that are found naturally in foods. These include xylo-oligosaccharides (XOS), galacto-oligosaccharides (GOS) and fructans, including inulin and fructo-oligosaccharides (FOS).^{70,71} Any dietary substrate that remains undigested in the upper GIT, and that may have

beneficial effects, is however a potential prebiotic. The health benefits attributed to various prebiotics, 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 $\beta(2,1)$ -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 $\beta(2,1)$ bonds in sucrose, FOS and inulin.⁷² Galactooligosaccharides (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 ($\beta(1,2)$; $\beta(1,3)$; $\beta(1,4)$) 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 the introduced bifidobacteria, and increased numbers of native bifidobacterial populations in some volunteers.⁷³ Whereas earlier work to investigate the effects of prebiotics on the gut microbiota tended to focus entirely on the intended target organisms (normally *Bifidobacterium* spp), it is now possible, and certainly desirable, to monitor the response of the whole community so as to assess the selectivity of different prebiotics. Utilization of prebiotic carbohydrates is proving to be more widespread among phylogenetically diverse bacteria than was originally considered.⁷⁴

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 L-fucose, D-glucose or D-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.⁷⁵ In total, human milk contains around 200 different oligosaccharides, with as many as 130 in milk from a single mother.⁷⁶ 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 bacterium *Akkermansia muciniphila*, a member of the Verrucomicrobium phylum, which can comprise as much as 3% of gut bacteria detected in feces of adults.⁷⁷ 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.⁷⁸ 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.⁷⁸ 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 Colonic Bacteroides

Early work showed that human *Bacteroides* species were able to degrade diverse plant polysaccharides, including pectin, galactomannan, arabinogalactan, alginate, laminarin and xylans,^{53,79,80} while more recent work has extended this to include xyloglucan, rhamnogalacturonans I and II, β -glucans and glucomannan.⁸¹ *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.^{4,46,47} The xylanolytic microbiota was recently re-investigated, yielding new isolates belonging to *B. intestinalis*, *B. ovatus*, *B. dorei*, *B. cellulolyticus* 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.⁸³ The main cellulose-degrading bacteria isolated recently from non-methane-excreting subjects belonged to the new species *B. cellulolyticus*, which is the only cellulolytic *Bacteroides* described to date.^{65,84} 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. ovatus*.^{85,86} Polygalacturonases from *B. thetaiotaomicron* were also characterized.⁷⁹ All of the enzymes or activities identified in these *Bacteroides* species were found cell-associated rather than extracellular, and their production appeared highly regulated by the substrate. For most of them, the cellular location was either in the outer membrane or in the periplasm.^{79,85} Xylan utilization has been studied more recently in *B. xylanisolvens*, which has proved to be the most active of several newly described xylanolytic *Bacteroides* species.^{87,88}

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 amylose, amylopectin and pullulan as well as the corresponding malto-oligosaccharides.⁵³ 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.⁸⁹ The starch binding activity and the degradative enzymes are maltose inducible, with these functions encoded by an operon of eight genes (*susRABCDEFGF*).⁹⁰ 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).

SusCDEFG are localized at the cell surface and bind, degrade and import soluble starch molecules.⁹²⁻⁹⁴ SusDEFG are lipoproteins anchored at the outer membrane of the cell. SusD is

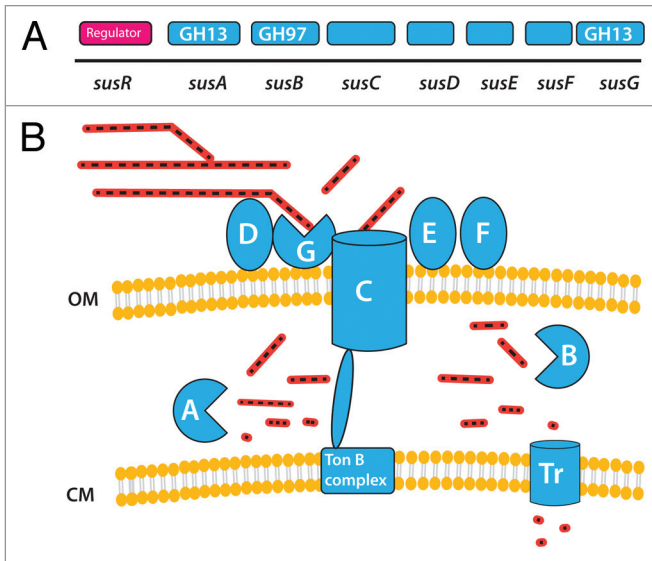


Figure 5. *Bacteroides thetaiotaomicron* *sus* system. (A) shows the order of genes in the *sus* cluster that is responsible for starch utilization in this species. (B) shows the inferred organization of gene products on or near the bacterial cell surface (OM outer membrane, CM cytoplasmic membrane). 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.⁹⁵ SusE and SusF are also likely to be involved in starch binding.^{91,92} SusC 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.⁹⁶ Unlike the other TBDT characterized to date, SusC cannot bind the ligand alone and requires the starch-binding protein SusD for starch import. Therefore, SusD 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,^{92,93} 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:⁹¹ 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 GH 97 α -glucosidase, respectively.⁹⁷ 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, amylose, amylopectin and pullulan but is

also controlled by the regulatory protein MalR.⁹⁸ The Sus system thus appears to be a very efficient and well controlled selfish 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.^{16,99,100} 35 of these have been reported to degrade mucin or other host-derived glycans and include enzymes that target glycan decorations such as sulfatases and acetyl-esterases.^{99,100} The other PULs are probably involved in the degradation of plant polysaccharides, ten of them being dedicated to pectins.⁸¹ PULs have also been identified for the utilization of FOS and levans.¹⁰¹ 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.⁹⁹ 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.¹⁰² All these mechanisms of adaptation are based on the regulation of the expression of the different PULs, which very efficiently sense the substrates available.

More generally, Sus-like complexes appear as a paradigm for glycan uptake in bacteria belonging to the phylum Bacteroidetes, and have been identified in other human gut *Bacteroides* as well as in ruminal *Prevotella* and in environmental *Bacteroidetes*.^{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 *xus* cluster) and galactomannans.^{41,103,104} 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.¹⁰⁵

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 *sus* 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.^{15,99,103} 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 environmental Bacteroidetes.

Regulation. PULs also include regulators belonging to the 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.¹⁰⁶ In *B. thetaiotaomicron*, at least 18 of the 88 *susC/susD* clusters contain ECF-sigma factors and adjacent anti-sigma factors.¹⁰³ Analysis of the TBDDT sequences in *B. fragilis* genome indicated that most of them were associated to CAZymes and possessed an N-terminal extension identifying them as putative transducers.¹⁰⁶ Protein contacts are made in the periplasm (TBDDT/anti- σ interaction) and then at the cytoplasmic face of the inner membrane (anti- σ /ECF- σ interaction). The signal is transduced across the entire bacterial cell envelope and results in activation of the ECF- σ transcription factor which activates the PULs. The protein interactions were experimentally demonstrated for some of the *B. thetaiotaomicron* PULs, in particular mucin O-glycan signaling.¹⁰⁴ *B. thetaiotaomicron* also contains an expanded collection of 32 hybrid two-component systems (HTCS), of which 17 are adjacent to *susC/susD* genes.¹⁰⁷ HTCS are proteins that incorporate all domains found in classical two-component environmental sensors into one polypeptide. Twenty eight of the 32 *B. thetaiotaomicron* HTCS reside within loci that are induced transcriptionally in response to modified polysaccharide content of the host mice diet, suggesting the contribution of these systems to glycan sensing.⁹⁹ The various HTCS and anti- σ /ECF- σ factors might sense specifically the glycan ligand released in the cytoplasm by their associated TBDDTs allowing the induction of the corresponding genes or operons. This allows a sophisticated integrated control of the carbohydrate hydrolytic and metabolic machinery of the cell in response to the availability of nutrients in the gut environment.

Evidence for horizontal transfer of polysaccharide utilization genes. Horizontal transfer within the gut microbiota, but also from microbes living outside the gut and ingested with the food, has probably played an important role in the diversification of the degrading-activity of each species.^{21,108} The plasticity of Bacteroidetes genomes appears to be driven by frequent genetic rearrangements, gene duplications and horizontal gene transfers (HGT) between species. Using a phylogenetic approach, around 5.5% of the genes in gut Bacteroidetes genomes were inferred to be laterally acquired from non gut-associated bacteria, among which glycosyltransferases (GT) were significantly over-represented.¹⁰⁵ In addition, the convergence of GT and GH repertoires in gut Bacteroidetes was due mainly to massive HGT rather than gene duplications.¹⁰⁹ Recently, a porphyrin/agar degradation locus was discovered and characterized in a member of the marine Bacteroidetes.¹⁰⁸ Surprisingly, homologs were found in the human gut bacterium *B. plebeius* that was isolated from Japanese individuals who are used to eat seaweeds.¹⁰⁸ Comparative gut metagenome analysis show that porphyrinases and agarases are frequent in the Japanese population while they are absent in metagenomic data from North Americans.¹⁰⁸ The authors concluded that gut bacteria were able to acquire new functions via transfer of a complete degradation pathway from

food-associated environmental bacteria.¹⁰⁸ Thus, acquisition of selectively advantageous genes by successive HGT events could explain how gut symbionts acquired CAZymes involved in the degradation of plant polysaccharides.

Actinobacteria

This phylum of high percentage G + C Gram-positive bacteria includes the highly abundant *Collinsella aerofaciens* and *Atopobium* spp, but by far the greatest amount of work has been focused on *Bifidobacterium* spp. Many bifidobacterial genes are conserved between species, creating a “core genome” and of the conserved genes, 6.5% are concerned with carbohydrate metabolism.¹¹⁰ Approximately 8% of the genome of *B. longum* subsp *longum* is dedicated to carbohydrate metabolism, with many genes organized into clusters containing a LacI-type of repressor protein.¹¹¹ These sugar-responsive regulators, which carry sugar-binding motifs, presumably permit a rapid response to changes in the availability of different substrates.¹¹¹ *B. longum* subsp *infantis* contains more unique genes than other sequenced *Bifidobacterium* spp,¹¹² many of which are located in a large cluster of carbohydrate utilization genes with both enzymatic and transport activities. Many of these genes were found to be specific for mammalian derived carbohydrates and were absent in bifidobacterial species normally associated with adults.¹¹³

Starch utilization. *Bifidobacterium* spp have been reported to be particularly effective degraders of high amylose starches⁵⁸ and some strains are known to be able to attach to starch particles.^{55,114} Degradation of RS and of pullulan appears to be associated with particular strains and species, especially *B. breve* and *B. adolescentis*.¹¹⁵ Detailed work on *B. breve* has identified a major cell surface anchored enzyme that comprises distinct $\alpha(1,4)$ amylase and type 1 pullulanase domains together with multiple CBMs (Fig. 3). Deletion of this one gene abolished 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.¹¹⁷ 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).¹¹⁸ Two types of β -fructofuranosidase have been identified in *Bifidobacterium* spp: those that are more active against the $\beta(2,1)$ glucose-fructose bonds, releasing only the terminal glucose residue for growth¹¹⁹ and those that are more active against $\beta(2,1)$ fructose-fructose links.¹²⁰ Both types of β -fructofuranosidase however had only low activities against long-chain inulin molecules.¹¹⁹⁻¹²¹ Only eight out of 55 *Bifidobacterium* strains tested, from five different species, were able to grow on long chain inulin, although all grew well on FOS.¹²² It appears that bifidobacteria 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 scFOS and short chain inulin.¹²³ Fructan-utilizing ability was not species-specific, with strains of *B. longum* for instance falling into different clusters.¹²³ The chain length of the substrate is therefore likely to be critical to its

subsequent effect on the composition of the microbial community. None of the *Bifidobacterium* sp tested were active against inulin chains longer than 20 units long.¹²³ 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.¹²⁴ 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 acidic fermentation products.¹⁴

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.¹²⁵ 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.¹²⁶ 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.¹²⁷

It has also been shown that substrate responses occur at a species-specific level. *B. adolescentis* was elevated in response to FOS/inulin in humans⁷⁴ and in humanised rats.¹²⁸ *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.¹²⁹ Supplementation with the novel prebiotic long-chain arabinoxylan significantly increased numbers of bifidobacteria in humanised rats, particularly boosting *B. longum*.¹²⁸

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 *Bifidobacterium* species having variable abilities.¹³⁰ *B. longum* subsp *infantis* expresses specific genes in direct response to the composition of the milk; with fucosidases only detected during growth on HMOs.¹³¹ The *B. longum* subsp *infantis* genome is also enriched in family 1 solute binding proteins (F1SBPs) that are particularly associated with oligosaccharide uptake. Different classes of F1SBPs were induced specifically during growth on different substrates.¹³² In a separate study *B. breve* was found to be prevalent in breast-fed babies and not in those fed on formula milk.¹³³ 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.¹³⁴

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 human adults based on 16S rRNA analyses. These include some highly oxygen-sensitive organisms and are seriously underrepresented by available cultured isolates, but they are responsible for some of the key metabolic conversions within the intestinal community.¹³ They include for example the major butyrate-producing species,^{49,135,136} as well as species that convert lactate to butyrate or propionate¹³⁷ and species that perform reductive acetogenesis.^{138,139} 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.^{4,140,141} This group was also prominent among fecal bacteria shown by stable isotope probing to utilize ¹³C labeled starch in vitro.¹⁴² Walker et al.⁴ saw a mean increase of > 4-fold (from 3.8% to 17%) in the overall proportion of cluster IV *Ruminococcus*-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 phylotypes 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.⁴ Additional evidence has now been obtained that supports the view that *R. bromii*-related organisms may indeed play a 'keystone' role in the initial stages of breakdown of particulate resistant starch.⁶¹ *R. bromii* showed a much greater ability to degrade raw or boiled RS2 and RS3 starches than *B. thetaiotaomicron*, and non-growing *R. bromii* cells were found to greatly enhance the utilization of these starches by three other prominent human amylolytic species, *B. thetaiotaomicron*, *E. rectale* or *B. adolescentis*.⁶¹ 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.¹⁴³ The enzyme systems that allow *R. bromii* to efficiently utilize particulate starch have not yet been fully investigated. In contrast to other abundant amylolytic bacteria found in the human colon *R. bromii* fails to grow on glucose, and grows more rapidly on malto-oligosaccharides than on maltose.⁶¹

Among the *Lachnospiraceae*, the ability to utilize starch has been reported for most members of the *Roseburia/Eubacterium rectale* group of butyrate-producing bacteria.¹⁴⁴ Furthermore the population of this group in fecal samples has been found to increase on average in human volunteers on RS-enriched diets⁴ and to decrease on diets low in total carbohydrate.¹⁴⁵ *Roseburia* spp produce a major, high molecular weight (> 180 kDa) amylase that is detectable by zymogram analysis. The enzyme from *R. inulinivorans*, Amy13A, includes a GH13 amylase and two or more CBMs and is able to cleave α (1,4) linkages in amylose, amylopectin and pullulan.¹⁴⁶ 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. 3). *R. inulinivorans*

Amy13A 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.¹⁴⁷ Genome sequences indicate 9 to 13 GH13 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 *R. bromii*.⁶¹ Although GH13 genes are present in most sequenced representatives of the *Lachnospiraceae*, the contribution of other species to starch degradation in the colon is currently unknown.

Plant cell wall polysaccharides. Salyers et al.^{53,54} 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. flavefaciens*, is the only human colonic bacterium so far reported to degrade microcrystalline cellulose.¹⁴⁸ Human colonic strains related to *R. albus* were reported to utilize galactomannan.⁵⁴ 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.¹⁰ Among the *Lachnospiraceae*, cellulolytic activity was reported in the acetogenic bacterium *Bryantella formatexigens* on first isolation, but apparently proved unstable.¹⁴⁹ Xylan-utilization has been reported for *Roseburia intestinalis*,^{82,150} and also for the human *B. fibrisolvens* strain 16/4, which was isolated from a wheat bran enrichment.¹⁵¹ 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.¹⁴³ The only other pectin-utilizing Firmicutes species identified so far from the human colon are *Eubacterium eligens*⁵⁴ and *Lachnospira pectinoschiza*.

Prebiotics. Relatively little attention has been paid to the utilization 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^{4,49} that include strains able to grow on inulin and FOS in pure culture. *R. inulinivorans* encodes a β -fructofuranosidase that acts against short and long chain length molecules.¹⁴⁷ The genes for the major β -fructofuranosidase and a linked ABC sugar transport system in *R. inulinivorans* are upregulated during growth on inulin compared with starch.¹⁴⁷

In a human study where a FOS/inulin mixture was supplemented to the diet, numbers of both *Bifidobacterium* spp and *Faecalibacterium prausnitzii* were significantly increased.⁷⁴ GOS consumption has also been reported to increase *F. prausnitzii*.¹²⁶ In human flora-associated rats, *Lachnospiraceae* numbers increased following dietary supplementation with either an inulin/FOS mixture or inulin alone, but no effect was observed with FOS alone.¹⁵² Increases have also been reported in the numbers of *Lachnospiraceae* upon in vitro fermentation of both pectic

oligosaccharides and FOS¹⁵³ while inulin enhanced the survival of *R. inulinivorans* against a background of total fecal bacteria in a fermentor system designed to simulate the proximal colon.¹⁵⁴

Increased production of butyric acid has been noted with FOS supplementation, although the intended targets, bifidobacteria, do not produce butyrate.¹⁵³ This may be explained in part by reduced pH favoring butyrogenic bacteria^{155,156} and in part by the ability of butyrate-producing bacteria such as *R. inulinivorans* and *F. prausnitzii* to metabolise fructans including long chain inulins.^{74,143,147,154} Humanised rats fed with a FOS/inulin mixture had increased cecal concentrations of butyrate, which correlated with a higher incidence of butyrate producing bacteria in the *Roseburia/E. rectale* group.¹²⁸ In addition, bifidobacteria form acetate and lactate as major end products and these products can be co-metabolised by cross-feeders including *Anaerostipes* spp and *Eubacterium hallii* to form butyrate.¹³⁷

Host-derived carbohydrates. Certain *Lachnospiraceae*, notably *R. torques*, have been identified as mucin-degraders.¹⁵⁷ Strains of *F. prausnitzii* were recently shown to utilize N-acetyl glucosamine for growth, although none was able to utilize mucin.¹⁴³ The sugar fucose is found extensively in host glycoconjugates and can be utilized by *R. inulinivorans* as a growth substrate.¹⁵⁸ Interestingly *B. thetaiotaomicron* can only partially utilize fucose for growth,¹⁵⁹ whereas *R. inulinivorans* can convert the propane-1,2-diol intermediate into propionate and propanol via the toxic propionaldehyde intermediate.¹⁵⁸ A similar metabolic route for fucose metabolism has been described in the gut pathogen *Salmonella* serovar Typhimurium LT2. The fucose utilization genes in *R. inulinivorans* A2-194 are strongly upregulated during growth on fucose.¹⁵⁸ Genome searching indicates that other species of *Lachnospiraceae* normally found in the human colon, *R. obeum* and *R. gnauvus*, 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 microbiota 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.^{155,160} 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.^{145,161,162} 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.¹⁶² Separately De Preter et al.¹⁶³ demonstrated in vitro studies that there was a dose dependent stimulation of saccharolytic 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 certain minerals such as calcium, and enhance absorption and expression of calcium-binding proteins.¹⁶⁴ 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.¹⁶⁵ High fiber diets increase fecal bulking, short chain fatty acid production and transit rates along the large intestine.^{166,167} 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 studies¹⁶⁸ and the presence of methanogens is also associated with slower gut transit in humans.⁶⁷ 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 cancer¹⁶⁹ 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 diet³) 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.¹⁷⁰ 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 proving far more complex than was first proposed. Variation in microbiota composition has the potential to influence “energy harvest” from fiber¹⁷¹ if it affects key groups involved in energy release and recovery, but factors such as gut transit and absorption seem likely to be more important¹⁷² (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.^{173,174} 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 obesity¹⁷⁵ has recently been balanced by a study reporting the opposite effect.¹⁷⁶ 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.¹⁷⁶ Potential links between the gut microbiota and metabolic disease have also been under intense investigation in recent years.¹⁷⁷⁻¹⁷⁹ Serum levels of lipopolysaccharide (LPS), derived from Gram-negative bacteria, are reported to increase in obese, diabetic or high-fat fed subjects and reproduction of similar LPS levels by chronic injection lead to a loss of insulin sensitivity in animals.¹⁷⁸ 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-2.¹⁷⁸

Physiological impact of SCFA. Besides supplying energy to the host, SCFA, along with other microbial metabolic products, have wider effects on host physiology. The gut microbiota may influence the expression of host peptides and hormones by production of short-chain fatty acids via their interaction with free fatty acid receptors FFA2 and FFA3, thus influencing host energy metabolism and appetite regulation.¹⁸⁰ Propionate has been shown to increase satiety and improve glucose homeostasis also when taken orally.¹⁸¹ The effect of butyrate on the host has received much attention due to its anti-inflammatory and anti-carcinogenic effects, but it also appears to be involved in the regulation of other host functions.¹⁸² A recent study found that oral administration of butyrate to mice fed a high-fat diet prevented development of insulin resistance and obesity. This effect was not due to a reduced food intake, but to increased energy expenditure.¹⁸³ Butyrate concentrations in plasma were only increased by approximately 1.6-fold compared with control animals, therefore manipulation of the microbiota to increase systemic butyrate levels via the colonic route could possibly be achieved, despite the fact that most bacterially produced butyrate is consumed in the colonic wall.

Another potential route linking microbial activity with the host is via the gut-brain axis, a bi-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.¹⁸⁴ Recent rodent studies indicated that changes in the microbiota composition led to behavioral changes and altered levels of brain-derived neurotrophic factor (BDNF) in different brain regions.¹⁸⁵ 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.¹⁸⁵

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.¹⁸⁶

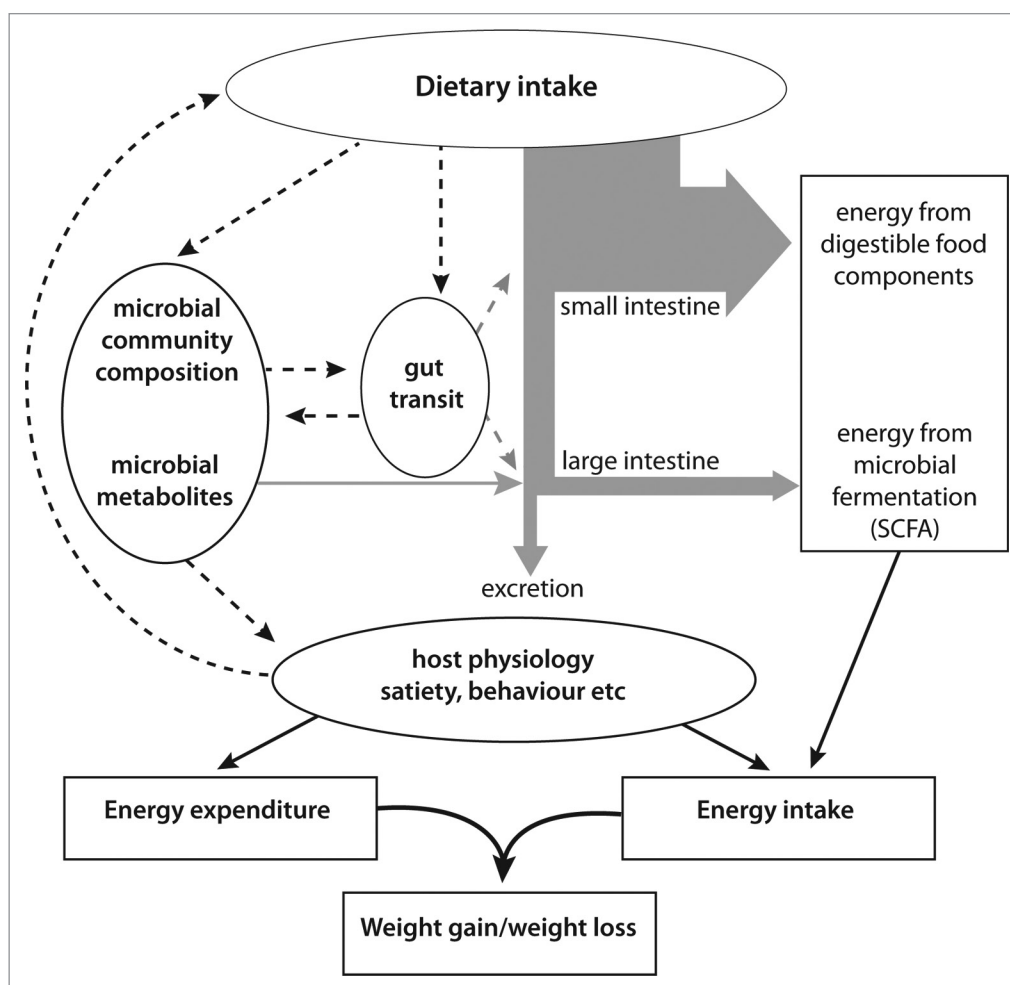


Figure 6. Energy intake and expenditure. The diagram summarizes the potential of gut microorganisms to influence energy gain and expenditure in a mono-gastric animal such as man.¹⁶⁸ The energy arising from microbial fermentation via the absorption of short chain fatty acids may be influenced by microbiota composition, but more especially by gut transit, affecting the efficiency of substrate breakdown, digestion and absorption. Other potentially important, but little understood, influences however include possible effects of microbial products on satiety and energy expenditure.

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 approaches¹⁸⁷ to integrate and model the many complex interactions between functional groups.

Acknowledgments

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