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Dietary fibre complexity and its influence on functional groups of the human gut microbiota

Petra Louis[1]*, Michael Solvang[1], Sylvia H. Duncan[1], Alan W. Walker[1] and Indrani Mukhopadhya[1,2]

[1]*Gut Health Group, Rowett Institute, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK*
[2]*EnteroBiotix Limited, Aberdeen Blood Transfusion Centre, Foresterhill, Aberdeen, AB25 2ZW, Scotland, UK*

The aim of this review is to provide an overview of the complex interactions between dietary fibre and the resident microbial community in the human gut. The microbiota influences both health maintenance and disease development. In the large intestine, the microbiota plays a crucial role in the degradation of dietary carbohydrates that remain undigested in the upper gut (non-digestible carbohydrates or fibre). Dietary fibre contains a variety of different types of carbohydrates, and its breakdown is facilitated by many different microbial enzymes. Some microbes, termed generalists, are able to degrade a range of different carbohydrates, whereas others are more specialised. Furthermore, the physicochemical characteristics of dietary fibre, such as whether it enters the gut in soluble or insoluble form, also likely influence which microbes can degrade it. A complex nutritional network therefore exists comprising primary degraders able to attack complex fibre and cross feeders that benefit from fibre breakdown intermediates or fermentation products. This leads predominately to the generation of the short-chain fatty acids (SCFA) acetate, propionate and butyrate, which exert various effects on host physiology, including the supply of energy, influencing glucose and lipid metabolism and anti-carcinogenic and anti-inflammatory actions. In order to effectively modulate the gut microbiota through diet, there is a need to better understand the complex competitive and cooperative interactions between gut microbes in dietary fibre breakdown, as well as how gut environmental factors and the physicochemical state of fibre originating from different types of diets influence microbial metabolism and ecology in the gut.

Dietary fibre: Gut microbiota: Anaerobic metabolism: Microbial genetics

Dietary fibre is mainly composed of structural components and storage carbohydrates in dietary plants and fungi that are not broken down in the upper intestinal tract and reach the colon, either because the appropriate host digestive enzymes are lacking to break them down for absorption or because they cannot be accessed by digestive enzymes[1]. In the lower gut, fibre serves as a major energy and carbon source for the resident microbial community, called the intestinal microbiota[2–6]. The activities of this microbiota influence the human host in numerous ways and modulate its health status. Some microbial actions help prevent disease, whereas others can contribute to disease development. Microbial functions associated with health encompass a

Abbreviations: CAZymes, carbohydrate-active enzymes; GH, glycoside hydrolase; RS, resistant starch; SCFA, short-chain fatty acids.
***Corresponding author:** Petra Louis, email p.louis@abdn.ac.uk

wide range of actions, including providing a barrier against incoming pathogens, modulation of the immune system and a plethora of metabolic reactions[7,8]. Microbial metabolism can lead to the modification of compounds entering the gut that can influence their bioavailability or bioactivity[9,10], and the fermentation of dietary fibre leads to the production of fermentation products that affect host health. The major organic end products generated by the microbiota from fibre are the short-chain fatty acids (SCFA) acetate, propionate and butyrate[9]. These SCFA influence gut and systemic health via several mechanistic routes, including by interaction with host receptors, which has been reviewed elsewhere[11]. Crucially, the individual SCFA differ in their actions, for example, butyrate plays a special role as a source of energy for the colonocytes and there is a large body of evidence to indicate that it prevents colorectal cancer[11,12]. Therefore, it is important to understand the microbial fermentation of fibre in order to optimise nutritional strategies to promote gut microbiota compositions that lead to a health-promoting SCFA production profile. Due to the complexity of fibre and the complex microbial interactions for its breakdown, this is not a trivial task. In this review, we will consider how dietary fibre influences different functional microbial groups and their ecological interactions with each other. The microbiota consists of prokaryotes, eukaryotes and viruses, with prokaryotic bacteria likely contributing the bulk of functions related to carbohydrate breakdown. This review will therefore mainly consider the bacterial component of the microbiota.

Dietary fibre: composition and physicochemical properties

In Western diets, grain products are the largest contributor to dietary fibre (about one-third to half of all dietary fibre), followed by vegetables, fruits and potatoes, with legumes contributing the smallest amounts[13]. Plant cell walls and storage carbohydrates contribute to dietary fibre[14].

Plant cell wall carbohydrates

Plant cell walls are complex insoluble structures that contain different types of carbohydrates (Table 1) plus non-carbohydrate constituents (mainly protein and lignin, approximately 10 % of dry weight)[15,16]. Cellulose microfibrils are crosslinked by a range of other carbohydrates collectively designated as hemicellulose (excluding α-galacturonate-rich carbohydrates) or pectin (α-galacturonate-rich carbohydrates)[16]. Pectin also serves as an adhesion layer between adjacent cells, called the middle lamella. As a rough rule of thumb, each of the three major cell wall components accounts for approximately 30 % of dry weight in many dietary plants belonging to dicotyledons (e.g. apple, berries, carrot, legumes, nuts) and monocotyledons (e.g. asparagus, bananas, onions), with their primary cell walls being designated type I cell walls[16,17]. Pectin consists of four different structural domains, homogalacturonan (approximately 15 % of total cell wall dry weight), rhamnogalacturonan I (approximately 10 %), rhamnogalacturonan II (approximately 1–4 %) and xylogalacturonan (usually very low amounts) (Table 1). The exact cell wall composition differs between plants and also depends on other factors, such as plant growth conditions, ripeness and plant storage[18]. Monocotyledon plants belonging to the Poales (including the dietary grains barley, maize, oats, rice, rye and wheat) have type II primary cell walls[16,17]. They have a much lower pectin and xyloglucan content (xyloglucan, a hemicellulosic carbohydrate, constitutes approximately 20–25 % of total dry weight in type I and 4 % in type II cell walls). Xylans (including arabinoxylans and glucuronoarabinoxylans), conversely, constitute approximately 30 % of total dry weight in type II cell walls compared to about 5–8 % in type I. Furthermore, type II cell walls contain approximately 30 % total dry weight of β-glucans, which are absent in type I cell walls[16,17] (Table 1).

Storage carbohydrates

A major plant storage carbohydrate present in cereals, legumes, rhizomes, roots and tubers is starch[19], a polymer consisting of linear (amylose) and branched (amylopectin) α-linked glucose residues (Table 1). Starch is principally digestible in the human upper gut by pancreatic α-amylase, but some starch, termed resistant starch (RS), can escape host digestion due to its physicochemical properties. Starch digestibility depends on several factors, which form the basis for the classification of RS[20,21]. RS1 is physically inaccessible within the food matrix, for example, within intact plant cells; RS2 is inaccessible due to the native starch conformation, for example, high amylose starches that have a more crystalline structure; RS3 is generated during food processing, such as cooking and cooling (retrogradation), which leads to a change in physicochemical properties, such as an increase in its crystallinity; RS4 is chemically modified, for example, by cross-linking or esterification, to reduce its digestibility; RS5 includes amylose-lipid complexes and this category has recently been proposed to be extended to include natural or manufactured self-assembled complexes of starch with other macromolecules[22]. Only a small fraction of the total starch within foods escapes upper gut digestion (typically within the range of 0–20 %), with large differences between plants, food processing and preparation techniques[23].

Other plant storage carbohydrates also contribute to dietary fibre, including inulin-type fructans and raffinose-family oligosaccharides (Table 1). Both contain a terminal sucrose residue, as plants synthesise them starting with sucrose[24], which is extended either with fructose residues in the case of fructans or with galactose residues in the case of raffinose-family oligosaccharides (also called α-galactosides). Raffinose-family oligosaccharides are present in legumes and are mostly comprised of raffinose, stachyose and verbascose, containing 1–3 galactose residues[1]. Different types of fructans are present in plants[24,25], but in dietary fibre, inulin-type fructans are the predominant form, with the main food sources being onions, Jerusalem artichoke, chicory and

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Table 1. Main characteristics of major plant dietary fibre carbohydrate constituents[1,5,16,26]

Carbohydrate (occurrence in plant)*	Backbone residue(s) and linkage type†	Major side chain linkages†	Other side chain monosaccharides†
Cellulose (PCW)	β-(1→4)-glucose	None	None
Xyloglucans (PCW-hemicellulose; storage in some seeds)	β-(1→4)-glucose (±Ac)	α-(1→6)-xylose	β-galactose (±Ac), α-fucose, α-/β-arabinose, β-xylose, α-L-galactose
Xylans, arabinoxylans, glucuronoxylans, glucuronoarabinoxylan (PCW-hemicellulose)	β-(1→4)-xylose (±Ac)	mainly α-(1→2)- (type I PCW) or α-(1→3)- (type II PCW) arabinose, α-(1→2)-glucuronic acid (±Me)	β-xylose, D-/L-galactose
Mannans, galactomannans (PCW-hemicellulose; storage in some seeds)	β-(1→4)-mannose	±α-(1→6)-galactose	None
Glucomannan, galactoglucomannans (PCW-hemicellulose)	β-(1→4)-mannose (±Ac) and β-(1→4)-glucose	±α-(1→6)-galactose	None
β-glucans/mixed linkage glucans (PCW-hemicellulose, type II PCW only)	β-(1→3)- and β-(1,4)-glucose	None	None
Homogalacturonan (PCW-pectin domain)	α-(1→4)-galacturonic acid (±Me/Ac)	None	None
Rhamnogalacturonan-I (PCW-pectin domain; galactans also storage in some seeds)	(α-(1→2)-galacturonic acid (±Ac) – α-(1→4)-rhamnose)$_n$	β-(1→4)-galactose, α-(1→4)-arabinose (bound to rhamnose)	α-fucose, β-xylose, β-glucuronic acid (minor residues)
Rhamnogalacturonan-II (PCW-pectin domain)	α-(1→4)- galacturonic acid	β-(1→2)-apiose, α-(1→3)-Kdo, β-(2→3)-Dha, α-(1→3)-arabinose	α-aceric acid, α-arabinose (incl. pyranose form), β-arabinose, α-fucose (±Me), β-galactose, α-L-galactose, α-/β-galacturonic acid, β-glucuronic acid, α-xylose (+Me), α-/β-rhamnose
Xylogalacturonan (PCW-pectin domain)	α-(1→4)-galacturonic acid (±Me)	β-(1→3)-xylose; α-fucose	β-(1→3)-xylose; α-fucose
Resistant starch (storage)	α-(1→4)-glucose	α-(1→6)-glucose	None
Inulin-type fructans (storage)	(β-(2→1)-fructose)$_n$ – α-glucose	None	None
Raffinose family oligosaccharides/α-galactosides (storage and transport of carbon)	(α-(1→6)-galactose)$_{1–3}$ – α-(1→2)-glucose – β-fructose	None	None

PCW, plant cell wall; Ac, acetyl ester; Me, methyl ester; Kdo, (2-Keto) – 3-deoxy-β-D-manno-octulosonic acid; Dha, (2-Keto) – 3-deoxy-β-D-lyxo-heptulosaric acid.
* Plant exudates and mucilages (including galactans and glucuronomannans)[5,14,16] are not listed separately here as they typically constitute a relatively small fraction of dietary fibre.
† All monosaccharides in D configuration unless specified otherwise.

wheat[1]. They are often designated as non-digestible oligosaccharides, but this only includes molecules of a degree of polymerisation of up to nine units[1]. As inulin-type fructans include molecules of up to degree of polymerisation of 60, small non-digestible carbohydrates are alternatively classified as resistant short-chain carbohydrates, whereas larger polysaccharides that do not contain α-(1→4)-linked glucose are referred to as non-starch polysaccharides (NSP)[1]. Whilst not a major contributor to dietary fibre, it should be noted that some hemicellulosic carbohydrates also take on storage functions in seeds[26] (Table 1).

Biochemical and physicochemical complexity of dietary fibre

Considering the number of different monosaccharides, the presence of non-sugar constituents (such as methyl- and acetyl-groups, phenolic compounds) and the number of different glycosidic linkages present in dietary fibre (Table 1), a multitude of microbial enzymes are required for its degradation. In addition to the biochemical complexity, physicochemical factors also need to be considered when assessing microbial fibre fermentation. A large fraction of fibre arrives in the large intestine in the form of complex insoluble particles, such as intact plant cells, cell wall fragments or granular macromolecular aggregates, especially on diets containing mostly whole plant-based foods with little processed ingredients[13,23], thus limiting access to the individual carbohydrate molecules for microbial degradation. The intrinsic solubility of the different constituents also differs and depends on their specific properties in different plants. For example, the solubility of pectins, which are negatively charged due to the presence of galacturonic acid residues, is affected by pH and by their degree of

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methylation, as the methyl groups render carboxylic acid residues neutral[16]. The solubility of xyloglucans differs depending on the plant source, as type I cell wall xyloglucans are typically highly branched and therefore more soluble than cereal type II xyloglucans[16]. Further structural differences between the two different cell wall types include a lower galactose-, arabinose- and fucose-content in type II cell wall xyloglucans and more extensive oligosaccharide side chains and esterification with acetyl, feruloyl and 4-coumaroyl groups in type II cell wall xylans[16].

The importance of the type of glycosidic linkage in determining physicochemical properties of carbohydrates is exemplified by fibre constituents exclusively composed of glucose monosaccharides, namely cellulose, β-glucans and RS. The β-(1→4)-linkages in cellulose result in linear molecules that tightly align with each other via hydrogen bonds and form highly insoluble microfibrils, which makes cellulose an excellent scaffolding material to provide strength to the plant cell wall[16]. Cereal β-glucans also contain β-(1→4)-linkages, but those are interspersed with β-(1→3)-linkages (which is the basis for their alternative designation as mixed-linkage glucans), which results in more flexible molecules that do not form highly ordered microfibrils and are more soluble, but relatively viscous[16]. The α-(1→4)-glucose linkages in amylose-fractions of starch can adopt different conformations including helical structures, and the α-(1→6)-branchpoints in amylopectin result in very complex structures of the overall starch molecule. Starch granules contain both amorphous and crystalline regions, and the overall starch structure differs between dietary plants[19].

Microbial breakdown of dietary fibre

Collectively, the microbiota provides the plethora of different enzymatic functions required for fibre breakdown. Carbohydrate-active enzymes (CAZymes) belonging to glycoside hydrolases (GH, cleavage of glycosidic bonds within carbohydrates or between a carbohydrate and a non-carbohydrate moiety), polysaccharide lyases (cleavage of uronic acid-containing polysaccharide chains such as present in pectins) and carbohydrate esterases (removal of ester substituents, including methyl- or acetyl-groups and phenolics), plus auxiliary activities such as carbohydrate-binding domains, work together to deconstruct the complex fibre[27]. The carbohydrate-active enzymes database (www.cazy.org[28]) is an excellent resource that describes the different enzyme families by their structural relatedness based on amino acid sequence similarities[29]. Individual species within the diverse microbial ecosystem both compete for the available resources as well as cooperate with each other in fibre breakdown, which is reflected in their carriage of different CAZymes. In order to coexist and not outcompete each other, different species occupy different ecological niches. Some species, called generalists, can use a wide range of different carbohydrates as substrates, whereas specialists have a much narrower substrate range. Examples of generalist and specialist gut microbial species are further discussed in subsequent sections of this review.

Genetics and physiology of fibre breakdown strategies in gut microbes

Much of what is currently known about fibre degradation by individual members of the gut microbiota has been learned from *in vitro* investigations with cultured isolates in the laboratory and *in silico* analyses of their genomes. Fibre breakdown genes and their regulation have been most extensively investigated in *Bacteroides* species belonging to the dominant phylum Bacteroidetes. Members of this phylum contain numerous (often over a hundred) genetic polysaccharide utilization loci, which are operons that encode CAZymes required for the breakdown of specific dietary fibre carbohydrates together with corresponding carbohydrate binding, transport and regulatory functions[5]. This enables the bacteria to sense the presence of many different types of carbohydrates and induce the corresponding functions for their degradation and uptake. Thus, *Bacteroides* species are regarded as generalists that are able to access many different potential growth substrates, although the level of metabolic flexibility differs between species[3,6]. It appears that *Bacteroides* species with overlapping substrate spectra limit competition with each other by prioritising different carbohydrates when grown together on a mix of substrates[30,31]. The initial polysaccharide degradation in Bacteroidetes takes place at the cell surface and oligosaccharides are imported across the outer membrane into the periplasmic space for further degradation and transport into the cytoplasm[6].

Species within the other dominant phylum, the Firmicutes, encode fewer CAZymes on average than Bacteroidetes species[27] and often have smaller genomes overall. However, there is also large variation between the many different species[3,6]. For example, a study of genomes from eleven strains belonging to five Firmicutes species within the *Roseburia* spp./*Eubacterium rectale* group of the *Lachnospiraceae* family showed that most strains harboured between fifty-six and eighty-six GH genes, whereas the three *Roseburia intestinalis* strains contained between 102 and 146[32]. Many CAZymes present in this group of Firmicutes are also organised as operons including regulatory and transport functions, but there are differences to the polysaccharide utilization locus organisation found in Bacteroidetes, reflecting the Gram-positive cell surface architecture of the Firmicutes. Gram-positive cells lack an outer membrane and periplasmic space, leading to differences in the composition and organisation of the carbohydrate-degrading machinery[3]. CAZyme operons found in Firmicutes have therefore been designated Gram-positive polysaccharide utilization loci[32].

Some bacteria within the *Ruminococcaceae* family of Firmicutes employ a number of different CAZymes encoded across several sites of the genome to build multi-enzyme complexes on the bacterial cell surface. This has been extensively studied in *Ruminococcus champanellensis*, the only bacterium from the human gut described

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so far able to degrade crystalline cellulose[33,34]. Multiple enzymes form a protein complex with structural scaffoldin proteins via protein–protein binding between dockerin and cohesin domains, and scaffoldin proteins also tether the complex to the cell surface. In addition, individual proteins often contain complex multi-modular domain structures, which may include several catalytic and carbohydrate-binding domains. The resulting cellulosome complex contains enzymes for the degradation of cellulose as well as hemicellulosic carbohydrates. The close proximity of the different enzymatic functions likely leads to synergism and enables the degradation of highly recalcitrant crystalline cellulose as well as complex particulate plant cell wall matter[33]. Some of the CAZymes present in the *R. champanellensis* cellulosome are strongly upregulated during growth on cellulose compared to cellobiose[34].

Another *Ruminococcus* species, *Ruminococcus bromii*, also makes use of scaffoldins, dockerin and cohesin domains to build multienzyme complexes on its cell surface, but those are amylosomes rather than cellulosomes, as their GH are amylases that target starch rather than cellulose[35]. *R. bromii* is a highly specialised starch-degrading species, as analysis of several strains showed that they contain less than 30 GH in their genomes, the majority of which are involved in starch breakdown[36]. The genes are scattered around the genome and mostly not linked to other GH. Amylase activity was constitutively expressed in *R. bromii* L2-63[35], which further confirms it to be an extreme specialist adapted to starch breakdown. Indeed, *R. bromii* may play a keystone role in RS degradation, as was discovered during human dietary intervention studies involving a dietary period with very high intakes of RS[37,38]. In a trial with fully controlled diets comparing a high NSP to a high RS intake, the relative abundance of *R. bromii* increased in faecal samples of the volunteers within a few days on the high RS diet, and quickly decreased again after its discontinuation[39,40]. Two volunteers who had low or undetectable levels of *R. bromii* excreted a large fraction of the ingested RS in their faeces, whereas faecal starch levels were very low for all other volunteers[39]. *In vitro* incubations of faecal microbiota from one of the two volunteers and addition of individual known starch degraders (*Bacteroides thetaiotaomicron*, *Bifidobacterium adolescentis*, *E. rectale*, *R. bromii*) revealed that only *R. bromii* was able to restore starch degradation to levels seen in healthy volunteers[41]. As the genome of *R. bromii* does not contain an exceptional number of starch-degrading enzymes compared to other starch-degrading bacteria from the human gut, it appears that it is their organisation into amylosomes that provides its enhanced ability to degrade recalcitrant RS[36].

Dockerin-cohesin pairs and other protein domains likely to be involved in the formation of cell surface CAZyme complexes have also been identified in other bacteria, including in the host mucin-degrading opportunistic pathogen *Clostridium perfringens*[42]. The *Ruminococcaceae* pectin-degrading specialist *Monoglobus pectinilyticus* contains some putative dockerin domains in proteins of unknown function, whereas several of its CAZymes contain other domains that may facilitate the assembly of multi-enzyme complexes[43], suggesting that further biochemical variations on the theme of multifunctional enzyme complexes exist in nature.

Within the other Gram-positive phylum that is commonly detected in the human gut, the Actinobacteria, most research has been carried out on *Bifidobacterium* species. There is diversity in which types of fibre are utilised by different species, but many species appear to be adapted to utilise mainly oligosaccharides or monosaccharides rather than complex insoluble fibre, and some species utilise host-derived carbohydrates[6,44,45]. Furthermore, RS-degrading species such as *B. adolescentis* have also been reported[21,41]. Regulators have been found associated with the corresponding genes for substrate breakdown, suggesting that the bacteria can sense and respond to the available substrates and have preference hierarchies for different carbohydrates[45].

Prediction of microbial function from genomic sequence information

Genome sequence information is invaluable in providing hypotheses on the likely physiology and behaviour of different microbes, but function cannot always be deduced from sequence alone. Thus, it can be difficult to establish substrate specificity of CAZymes from their amino acid sequences, as several CAZyme families include enzymes targeting different substrates[28]. The limitations of establishing the ecological niche of a bacterial species from its genome sequence are exemplified by a recent study of *Coprococcus eutactus* within the *Lachnospiraceae* family of the Firmicutes phylum. It was found to contain two GH9 genes, a GH family containing mainly cellulases[46]. They are relatively rare in human gut bacterial genomes and are mostly present in bacteria with confirmed cellulose-degrading ability, especially when more than one GH9 gene is present[47]. Four GH5 genes were also present in *C. eutactus* ART55/1, another GH family containing many cellulases[48], suggesting that this species may be able to degrade cellulose. However, when growth tests were performed on a range of soluble and insoluble substrates, no growth was detected on cellulose[47]. Instead, growth profiles and gene expression analyses suggest that β-glucans are the preferred growth substrate for this species, with lower growth on gluco/galactomannans, galactan and starch. Interestingly, a closely related species, *Coprococcus* sp. L2-50, was more specialised towards β-glucan, showing only limited growth on starch and no growth on mannan, glucomannan, galactomannan or galactan[47]. Thus, phylogenetically closely related bacteria can exhibit major functional differences. This is usually not well captured in studies that analyse microbiota changes based on 16S rRNA gene amplicon sequencing, as this often does not allow for phylogenetic resolution down to species level.

Another limitation of deducing microbial function from sequencing-based microbiota profiling is the fact that many bacteria share the same genus name despite not being phylogenetically closely related, as they were originally misclassified based solely on phenotypic

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characteristics before phylogenetic classification based on genome sequence information was available. For example, several species currently within the genus *Coprococcus* require taxonomic reclassification as they are not sufficiently closely related to *C. eutactus*, which is also reflected in functional differences, such as differences in their growth substrate profiles[47]. Thus, when sequence-based studies find associations between certain bacterial genera (including Firmicutes such as *Clostridium*, *Coprococcus*, *Eubacterium*, etc.) and health outcomes or nutritional factors, it can be difficult to deduce function if it is not clear which specific species, or even phylogenetically related taxa, this actually represents.

The functionality of a given species can also depend on its environmental context at the time, which has to be taken into consideration when assigning function based on presence in microbiota sequence-based profiles. For example, *Coprococcus catus* produces butyrate from fructose, a breakdown product of fructans provided by primary fructan degraders within the microbiota. It can alternatively also grow on the fermentation acid lactate, but produces mainly propionate instead of butyrate on this substrate[49]. Thus, the balance between butyrate and propionate production of this species depends on its ecological context within the complex community, including the abundance of cross-feeders providing the different growth substrates, as well as competitors for those substrates.

Microbial community interactions during dietary fibre fermentation

In vitro human faecal microbiota incubations have been employed to assess which bacterial species or genera are stimulated by different types of dietary fibre within the complex microbial community (Table 2). The results are often in agreement with studies based on pure strain analyses and *in vivo* dietary intervention trials, for example, an increase of *R. bromii* on starch[40,41] or of *Anaerostipes hadrus* on fructans[50,51]. However, microbial community interactions are complex and the ability to degrade a particular carbohydrate in pure culture does not necessarily lead to a stimulation of the species within the complete community and conversely, absence of the necessary CAZymes to degrade a particular carbohydrate does not mean that a species cannot be stimulated indirectly within the community.

Factors affecting microbial competition

Direct competition for dietary fibre substrates between different microbes depends on the substrate specificity of their CAZymes (including the chain length of oligosaccharides and substitution with non-carbohydrate ligands[52]) and also seems to be influenced by their biochemical organisation on the cell surface. Thus, close proximity of different enzymes likely leads to synergism between them to facilitate the breakdown of insoluble complex substrates[33,36]. Differences in the efficiency of substrate binding and transport also need to be considered to understand competitive interactions between gut microbes. For example, it has been hypothesised that the four carbohydrate-binding domains of an *R. intestinalis* xylanase give this species superior ability to compete for insoluble xylans over *Bacteroides* species in co-culture competition assays[52]. Transporter specificities for xylan breakdown products also vary between the different species, likely enabling their co-existence on a pool of xylo-oligosaccharides of varying lengths[52]. Detailed investigation of a mannan utilisation locus in *Bifidobacterium animalis* subsp. *lactis* revealed high affinity transport of manno-oligosaccharides, which enables the bacterium to effectively compete with *Bacteroides ovatus* on carob galactomannan in co-culture. This was found despite the fact that its β-mannanase for extracellular mannan breakdown is secreted rather than cell-attached, which suggests that galactomannan breakdown is likely more physically distant from its cell surface transporters than that of *Bacteroides* species with their cell surface-associated CAZymes and transporters being in close proximity[53].

Other aspects of bacterial physiology should also be considered when examining competitive relationships. The pH in the gut fluctuates with the level of microbial activity due to the formation of acidic fermentation products. It tends to be mildly acidic in the proximal gut, where dietary fibre substrate concentrations are high and acid production exceeds the uptake capacity of the gut wall. It shifts to a more neutral pH in the distal colon, as carbohydrate fermentation slows down due to exhaustion of easily fermentable fibre[54]. Different bacteria vary in their tolerance of acidic pH, as was exemplified in continuous culture studies of human faecal microbiota on different carbohydrates, which showed higher levels of Bacteroidetes at pH 6·5 and of Firmicutes at pH 5·5[54,55]. However, this broad categorisation is somewhat simplistic and there can be large differences in acid tolerance between closely related species. For example, *E. rectale* within the *Lachnospiraceae* family of the Firmicutes exhibited good growth in media with an initial medium pH of as low as 5·1, whereas growth of a relatively closely related species, *Roseburia inulinivorans*, was severely curtailed below pH 5·5 and absent at pH 5·1[56]. This potentially poor competitiveness at lower pH values may partially explain why *R. inulinivorans* was not found to be stimulated within the microbiota by fructans *in vivo*[57] or *in vitro*[58], despite showing good growth on fructans of different chain lengths when grown in pure culture[51]. The requirement for other growth factors (minerals, amino acids, vitamins, etc.) may also disadvantage certain microbes if they are not available in sufficient quantities in the gut environment. For example, a recent study found several vitamin auxotrophies in a range of butyrate-producing Firmicutes from the human gut[59].

Microbial cooperation by metabolic cross-feeding

Microbial cross-feeding plays an important role in providing growth substrates to the wider microbial

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Table 2. Bacterial species enriched after batch or continuous culture using human faecal microbiota *in vitro* incubation with different types of dietary fibre or found to grow on the respective carbohydrate in pure culture

Carbohydrate type	Bacteria enriched	References
Polysaccharides		
α-Glucans		
Potato starch	*Prevotella* spp., *Eubacterium rectale*, *Ruminococcus bromii*, *Bifidobacterium adolescentis*	(41,75)
Pullulan	*Bacteroides thetaiotaomicron*, *Roseburia* spp., *R. bromii*, *Bifidobacterium* spp., *B. adolescentis*	(41,58)
RSII	*E. rectale*, *R. bromii*, *Bifidobacterium* spp.	(41,58,76)
RSIII	*R. bromii*, *Bifidobacterium* spp.	(41,58)
RSIV	*Parabacteroides distasonis*, *B. adolescentis*	(76,77)
β-Glucans		
From oat and barley	*Bacteroides* spp., *Prevotella* spp., *Blautia* spp., *Coprococcus eutactus*, *Roseburia* spp., *Eubacterium ventriosum*, *Lactobacillus* spp., *Bifidobacterium* spp.	(47,58,78,79)
Pectin		
From apple and citrus	*Bacteroides* spp., *Prevotella* spp., *Anaerobutyricum hallii*, *Lachnospira eligens*, *Roseburia* spp., *Faecalibacterium prausnitzii*	(55,58,80–84)
Hemi-cellulose		
Oat spelt xylan	*Bacteroides intestinalis*, *Bacteroides dorei*, *Bacteroides xylanisolvens*, *Roseburia intestinalis*	(85–87)
Arabinoxylan	*Lachnospiraceae*, *Lactobacillus* spp., *Bifidobacterium* spp.	(88–91)
Arabinogalactan from larch	*Bacteroides* spp., *Prevotella* spp., *F. prausnitzii*, *Bifidobacterium* spp.	(92,93)
Guar gum	*Bacteroides* spp., *C. eutactus*, *Roseburia/E. rectale* spp., *Bifidobacterium* spp.	(58,94–96)
Galactomannan	*R. intestinalis*, *Lactobacillus* spp., *Bifidobacterium* spp.	(97)
Resistant short-chain carbohydrates and monosaccharides		
Fructans		
Inulin/oligofructose (DP = 1–9, ≥10 and ≥23)	*Bacteroides uniformis*, *Bacteroides caccae*, *Anaerostipes hadrus*, *C. eutactus*, *Dorea longicatena*, *Roseburia* spp., *R. inulinivorans*, *E. rectale*, *Lactobacillus* spp., *F. prausnitzii*, *R. bromii*, *Bifidobacterium* spp.	(50,51,55,57,58,98–102)
Arabinoxylans		
Arabinoxylan-oligosaccharides	*Prevotella* spp., *Roseburia* spp., *E. rectale*, *Lactobacillus* spp., *Bifidobacterium* spp.	(99,103–105)
Deoxysugars		
Rhamnose	*A. hallii*, *Blautia* spp.	(58)

RS, resistant starch; DP, degree of polymerisation.

community, as only some species, termed primary degraders, are able to degrade the fibre as it arrives in the large intestine (Fig. 1). For example, the previously described keystone role of *R. bromii* in making RS available to other bacteria has been demonstrated *in vivo* and *in vitro*[21,37–41]. The level to which primary degraders share their resource with other gut bacteria varies[6]. *R. bromii* releases extensive amounts of glucose and maltose from RS during *in vitro* growth, which can be utilised by other microbes. As *R. bromii* cannot utilise glucose itself and prefers longer oligosaccharides over maltose, it is a cooperative cross-feeder benefitting other microbes[41]. Nutritional cooperation has also been established for *Bacteroides ovatus* when grown on inulin[60]. Despite the fact that *B. ovatus* takes up intact inulin molecules without extracellular breakdown, it also expresses two extracellular enzymes that make shorter oligosaccharides available to other bacteria. Co-culture and *in vivo* studies suggest that *B. ovatus* receives benefits from the cross-feeding beneficiaries in return, in this case *Bacteroides vulgatus*[60]. Other primary degraders seem to have a much more selfish approach to external degradation of fibre. For example, co-culture studies of *B. thetaiotaomicron* wild type with mutant strains that had a deletion in amylopectin- and levan-targeting extracellular CAZymes showed that there was only limited cross-feeding of carbohydrate degradation intermediates from the wild type to the mutant[60].

Cross-feeding also takes place at the level of fermentation products[61] (Fig. 1). Hydrogen is produced by many fermentative gut bacteria and consumed by three different microbial groups, sulphate-reducing bacteria (which can also convert fermentation acids), acetogens and methanogenic Archaea[62]. Formate cross-feeding was also established between *R. bromii* and the acetogenic bacterium *Blautia hydrogenotrophica* in continuous culture. Transcriptomic analysis revealed further metabolic interactions, including amino acid catabolism and vitamin acquisition, between the two species[63]. Cross-feeding can have considerable benefits for host health. For example, lactate is produced by many different gut microbes, but is known to have a range of potentially deleterious effects on the host, and can have de-stabilising effects on gut microbiota composition by lowering pH and inhibiting the growth of other gut bacteria[64]. Fortunately, lactate can be utilised and converted to either butyrate or propionate by other gut bacteria, although this activity is limited to certain species[49,61,65,66]. These lactate-utilising bacteria therefore play an important role in preventing the build-up of detrimental concentrations of lactate in the colon[64,67].

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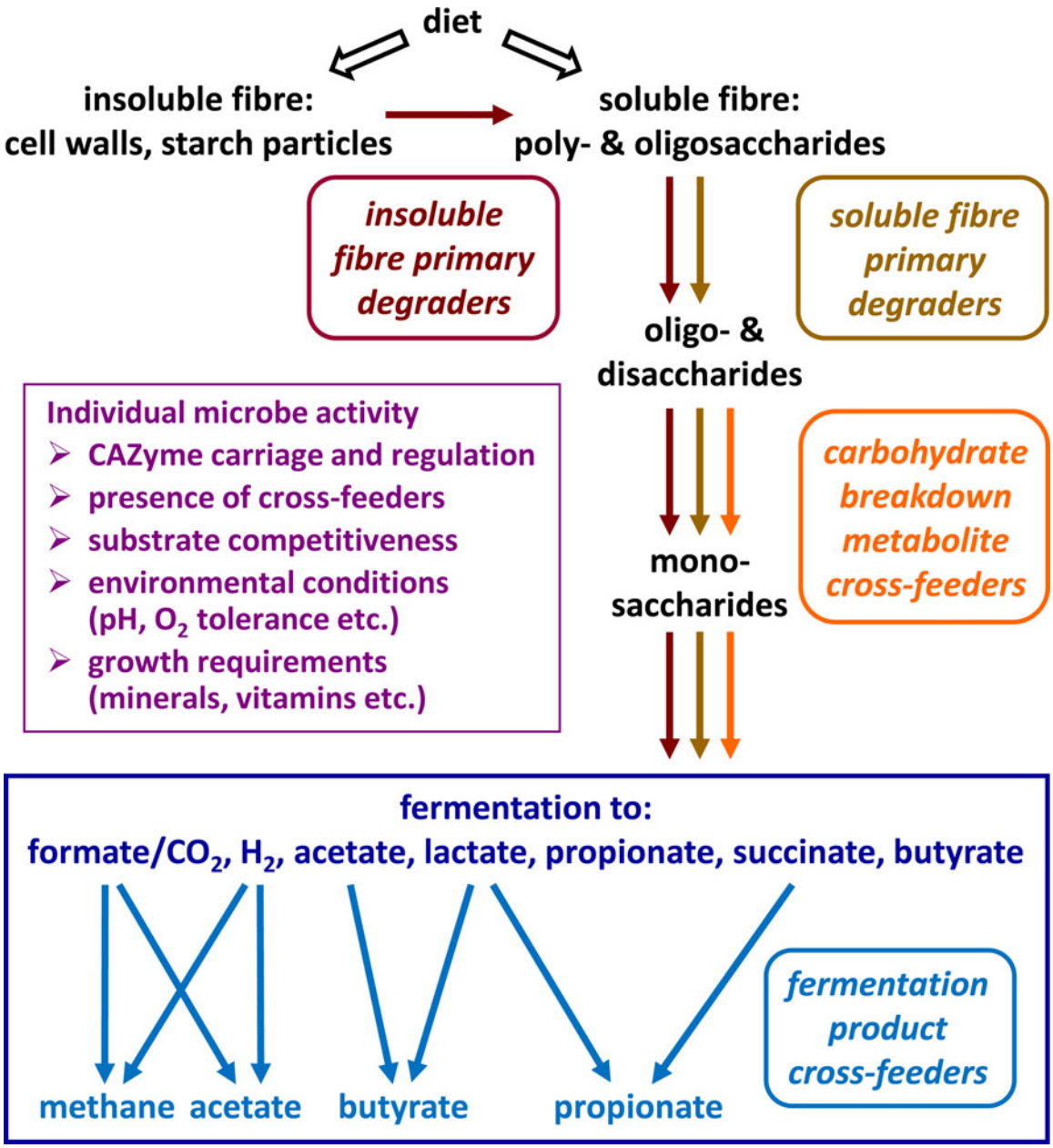

Fig. 1. Main routes of metabolic cross-feeding of dietary fibre by the human gut microbiota and major factors affecting the activity of individual microbes. CAZyme, carbohydrate-active enzyme.

Microbes may also benefit from the production of other compounds such as vitamins by co-inhabitants, based on *in vitro* evidence[59]. Furthermore, metabolic interactions also likely take place during the breakdown of secondary compounds (xenobiotics, phytochemicals). Thus, an *in vitro* study of wheat bran degradation by human faecal microbiota suggested that the release and biotransformation of the abundant phenolic phytochemical, ferulic acid, was due to the action of several different microbial species. The primary wheat bran-degrading bacterial species responsible for breaking down the fibre and releasing ferulic acid only showed very limited further transformation of this compound[68]. Overall plant-derived metabolite pools in the human gut are therefore dependent on both primary degraders of plant material and the wider gut microbiota, which can further biotransform released metabolites.

Conclusions

Microbial functions within the complex gut microbiota are highly dependent on the ecological context of their intestinal environment. The gut ecosystem is highly dynamic and the amount and type of dietary fibre entering the large intestine constantly fluctuates[69,70], which

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influences the complex cooperative and competitive relationships between the individual microbes present. Our understanding of how eukaryotes and viruses influence the actions of the overall community is limited, but it is likely that they contribute to the dynamics within the gut microbiota[71]. For example, the majority of viruses in the gut are comprised of bacteriophages and the host–prey dynamics may alter the composition of the gut bacteria and influence disease[72]. This review has mainly focused on the influence of dietary fibre, but further factors involved in bacterial antagonism and cooperation (e.g. production of antimicrobials such as bacteriocins, quorum sensing interactions) and host factors (bile secretions, immune interactions, etc.) also need to be further studied and considered for a full understanding of gut microbial function. Furthermore, much of our understanding about the metabolism of dietary fibre by gut microbes has been gained from experiments with purified carbohydrates, with fewer studies investigating complex insoluble fibre breakdown[68,73]. Microbial biofilm formation on fibre particles likely plays an important role in their breakdown and creates spatial structures that may allow for the co-existence of different microbes with similar nutritional profiles[69,74]. Insoluble complex dietary fibre–microbiota interactions are more difficult to study than those with soluble fibre, but such studies will be required for a deeper understanding of how diets rich in whole foods influence the microbiota. By better understanding the impact that specific dietary components can have on members of the gut microbiota, this type of research should ultimately lead to more effective nutritional advice to improve human health and will form the basis for the development of novel microbiota-targeted functional food ingredients with health-promoting properties.

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Conflict of Interest

None.

Authorship

The authors had sole responsibility for all aspects of preparation of this paper.

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