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Review Article

Carbohydrate esterases involved in deacetylation of food components by the human gut microbiota

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Non-carbohydrate modifications such as acetylations are widespread in food stuffs as well as they play important roles in diverse biological processes. These modifications meet the gut environment and are removed from their carbohydrate substrates by the resident microbiota. Among the most abundant modifications are *O*-acetylations, contributing to polysaccharides physico-chemical properties such as viscosity and gelling ability, as well as reducing accessibility for glycosyl hydrolases, and thus hindering polysaccharide degradation. Of particular note, *O*-acetylations increase the overall complexity of a polymer, thus requiring a more advanced degrading machinery for microbes to utilize it. This minireview describes acetylesterases from the gut microbiota that deacetylate various food polysaccharides, either as natural components of food, ingredients, stabilizers of microbial origin, or as part of microbes for food and beverage preparations. These enzymes include members belonging to at least 8 families in the CAZy database, as well as a large number of biochemically characterized esterases that have not been classified yet. Despite different structural folds, most of these acetylesterases have a common acid–base mechanism and belong to the SGNH hydrolase superfamily. We highlight examples of acetylesterases that are highly specific to one substrate and to the position of the acetyl group on the glycosyl residue of the carbohydrate, while other members that have more broad substrate specificity. Current research aimed at unveiling the functions and regioselectivity of acetylesterases will help providing fundamental mechanistic understanding on how dietary components are utilized in the human gut and will aid developing applications of these enzymes to manufacture novel industrial products.

Introduction

Resident bacterial communities (collectively known as the gut microbiota) inhabiting the distal gastrointestinal tract of humans are responsible for the degradation of undigested food components, such as carbohydrates, which cannot be directly processed by their host [1]. In return, the host receives a wide range of microbial metabolites, including short-chain fatty acids (SCFAs) that contribute to up to 10% of the bodily energy budget and exert a range of health-promoting functions [2]. The most abundant SCFAs are acetate, propionate, and butyrate. These three metabolites represent 90–95% of the SCFAs present in the colon. Other fermentation products such as lactate, succinate and 1,2-propanediol are also generated, but do not accumulate in high concentrations, as they act as substrates for metabolism by other bacteria [1]. The dominating phyla in the gut microbiota of humans are Bacteroidetes and Firmicutes; their composition and abundance vary extensively between individuals and geographically as an effect of variations of diet, lifestyle, and health status [3,4].

To facilitate metabolism of dietary polysaccharides, the genomes of gut bacteria often contain an arsenal of many dozens or hundreds of individual carbohydrate-active enzymes (CAZymes), frequently co-regulated and co-localized in gene clusters known as polysaccharide utilization loci (PULs) [5].

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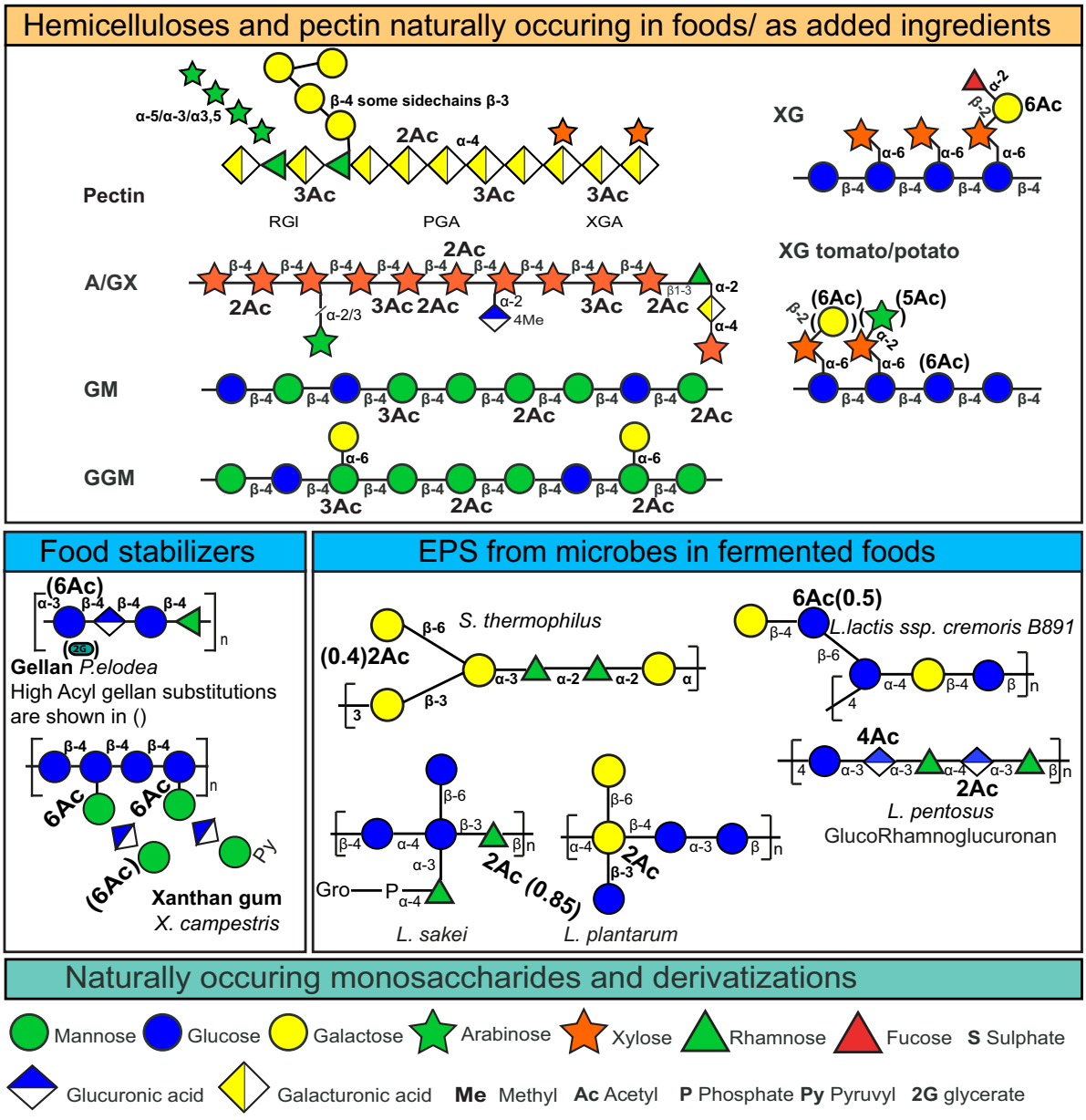

Figure 1. Examples of polysaccharides, found in foods, that contain different extents of acetylations.

XG, xyloglucan; A/GX, arabino/glucuronoxylan; GM, glucomannan; GGM, galactoglucomannan; Gro, glycerol (in L. sakei 0-1's EPS structure). Structures with parenthesis represent variants of the polysaccharide in question, brackets with numbers indicate the degree of acetylation (number of acetylations per monosaccharide annotated). Symbols are drawn according to [12,13].

CAZymes are classified based into families in the CAZy database ([6,7]). The members of a family share the same fold, the same catalytic apparatus and mechanism. PUL-encoded systems from gut Bacteroidetes and Firmicutes have been shown to target a wide range of complex carbohydrates, including common terrestrial and marine plants, microbial exopolysaccharides and host glycans [3].

The non-cellulosic plant cell wall polysaccharides such as hemicellulose and pectin are differentially esterified by *O*-acetyl groups (Figure 1) as a form of defense against hydrolytic enzymes secreted by different fungi and bacterial pathogens. Furthermore, *O*-acetyl groups exist in a large number of food commodities (some examples are sketched in Figure 1). These ornamentations can be present on a wide range of structural motifs including glucose, xylose, galacturonic acid, mannose, rhamnose, as substituents in different positions of the monosaccharides. Specifically, these

modifications are naturally existing in common foods as pectin (including homogalacturonan, xylogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II [8]), xyloglucan [9,10], glucuronoxylan, glucuronoarabinoxylan, glucomannans, and galactoglucomannans. Furthermore, *O*-acetyl groups are present as structural components of bacterial exopolysaccharides that are widely used as food stabilizers (e.g. gellan and xanthan gum). In addition, there are *O*-acetylation on exopolysaccharides produced by bacteria directly used for food and beverage production [11], which in many cases play a crucial role for the texture and sensoric palatability of foodstuffs as in, e.g., yoghurt, kefir, and various cheeses. In nature, cleavage of the ester bonds between carbohydrate residues and acetyl groups is catalyzed primarily by microbial carbohydrate esterases (CEs) that are classified into 20 families according to the CAZy database (Table 1, http://www.cazy.org/Carbohydrate-Esterases.html, as per February 2023).

In this review, we focus on carbohydrate esterases found in the human gut microbiota and describe recent studies that have led to the discovery of new specialized esterase activities from gut bacteria. This knowledge is essential to fully understand the important role of CEs in degradation of both plant polysaccharides and bacterial exopolysaccharides, as the removal of acetylations present on these polysaccharides modifies their physicochemical properties and allows the process of hydrolysis through glycoside hydrolases, and also provides fundamental insight into human digestion.

The SGNH fold

Most of the known *O*-acetylesterases are found among members of the SGNH hydrolase superfamily. This superfamily comprises a variety of carbohydrate-modifying enzymes, including but not limited to the carbohydrate esterase families 2, 3, 6, 12, 17, and 20 [14,15]. Several excellent reviews have recently described the current knowledge for these proteins [14,16]. In short, SGNH hydrolases share a conserved catalytic fold and mechanism. The SGNH domain contains five blocks of the highly conserved catalytic Ser, Gly, Asn, and His in blocks I, II, III, and V, respectively, while block IV does not have a catalytic residue but is structurally important for the overall fold. The mechanism for removal of acetyl sidechains involves the nucleophilic attack of the carbonyl carbon on the ester substrate by the nucleophilic Ser residue. The resulting tetrahedral oxyanion intermediate is stabilized by a positively charged pocket, known as the oxyanion hole, which is formed by the backbone amides of the Block I Ser and Block II Gly residues, and the sidechain amide of the Block III Asn residue. In the final step, a water molecule freely binds the covalent acetyl-enzyme intermediate and is deprotonated by the basic His, initiating a nucleophilic attack of the carbonyl carbon. Catalysis proceeds with the formation of an oxyanion intermediate, stabilized by hydrogen bonding in the oxyanion hole, that finally collapses to liberate the acetyl coproduct and the free enzyme [17].

Deacetylation of pectin and xyloglucan

Pectins are common dietary fibers abundant in fruit and vegetables as well as being widely used as stabilizers and gelling agents [18]. Structurally, pectins consist of four major elements, all characterized by the presence of D-galacturonic acid (GalUA). They include homogalacturonan (HG), that is a linear polymer of 1,4-linked α-D-GalUA residues that can be acetylated and esterified with methanol [19]; xylogalacturonan [20]; type I rhamnogalacturonan (RGI), composed of repeating disaccharide units of -,4-α-D-GalUA-(1,2)-α-L-Rha-1,- to which different arabinan, galactan, and arabinogalactan side chains are attached [21,22] to the L-rhamnose residues; and rhamnogalacturonan II (RGII) that has a backbone of HG with complex sidechains of rare sugars attached to the GalUA residues (Figure 1). RGI and HG are arranged to form the backbone of pectins, with HG being the most abundant component (>65%) [19]. GalUA units of RG-I can be 2-*O* and/or 3-*O*-acetylated [23] which is an acetylation pattern similar to that found in homogalacturonan and xylogalacturonan [17].

Xyloglucan (XyG) is built of repeats of predominantly β-1,4-linked glucose tetramers (general structures are drawn in Figure 1), with various types of sidechain compositions (about 25 different types) [24]. Some parts of the xyloglucan are acetylated [25]. To date only three distinct XyG glycosyl units have been found to be acetylated [24]. *O*-Acetyl substituents are located at *O*-6 of the unbranched (internal) β-D-Glc*p* residue, *O*-6 of the terminal β-D-Gal*p* residue, and/or at *O*-5 of the terminal α-L-Ara*p* residue [26] (Figure 1).

Although esterase activities on pectin and xyloglucan have been known for a long time [27], there are several recent discoveries of novel acetylesterase activities which together take care of the large range of different acetylation in these complex polymers [28], a substrate complexity that is in line with the presence of a range of different acetylesterases in the pectin and xyloglucan PULs of gut microbes.

The fact that both pectin and xyloglucan have acetylations in different structural elements (Figure 1) may explain why several esterase genes are involved in their deacetylation. CE6s and CE12s are the predominant esterases for pectin deacetylation. An example is shown in Figure 2D; despite this is an uncharacterized enzyme, it has a very

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Table 1 Carbohydrate esterase families and corresponding characterized enzyme activities

CE family	B	E	Known activities	EC number	Catalytic residues	3D structure
CE1	●	●	Acetyl xylan esterase	EC 3.1.1.72	SHD	α/β hydrolase fold; central β-sheet flanked by α-helices on both sides
			Cinnamoyl esterase	EC 3.1.1.-		
			Feruloyl esterase	EC 3.1.1.73		
			Carboxylesterase	EC 3.1.1.1		
			S-formylglutathione hydrolase	EC 3.1.2.12		
			Diacylglycerol *O*-acyltransferase	EC 2.3.1.20		
			Trehalose 6-*O*-mycolyltransferase	EC 2.3.1.122		
CE2	●	●	Acetyl xylan esterase	EC 3.1.1.72	SH	C-terminal SGNH domain with repeated α/β/α motifs; N-terminal jellyroll domain with two β-sheets in opposite orientation
			Acetyl β mannan esterase	EC 3.1.1.6		
CE3	●	●	Acetyl xylan esterase	EC 3.1.1.72	SHD	Repeated α/β/α motifs
CE4	●	●	Acetyl xylan esterase	EC 3.1.1.72	DHH	Conserved catalytic core known as NodB domain; distorted $(\alpha/\beta)_7$ barrel or (α/β)8 barrel; dependency of metal ion (Co^{2+}, Zn^{2+}) for enzymatic activity
			Chitin deacetylase	EC 3.5.1.41		
			Chitooligosaccharide deacetylase	EC 3.5.1.-		
			Peptidoglycan GlcNAc deacetylase	EC 3.5.1.-		
			Peptidoglycan N-acetylmuramic acid Deacetylase	EC 3.5.1.-		
CE5	●	●	Acetyl xylan esterase	EC 3.1.1.72	SHD	α/β fold; six-stranded parallel β-sheet surrounded by four/eight α-helices
			Cutinases	EC 3.1.1.74		
CE6	●	●	Acetyl xylan esterase	EC 3.1.1.72	SHE	Repeated α/β/α motifs
CE7	●		Acetyl xylan esterase	EC 3.1.1.72	SHD	α/β hydrolase fold; common hexameric quaternary structure
CE8	●	●	Pectin methylesterases	EC 3.1.1.11	DDR	Right-handed β-helix fold
CE9	●		N-acetylglucosamine 6-phosphate deacetylase	EC 3.5.1.25	D	Distorted $(\beta/\alpha)_8$ barrel; dependency of metal ion (Fe^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}) for enzymatic activity
			N-acetylgalactosamine 6-phosphate deacetylase	EC 3.5.1.80		
CE11	●	●	UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylases	EC 3.5.1.-	E	Two-layer-sandwich with two domains, each containing a five-stranded β-sheet and two α-helices; Zn^{2+}-dependent
CE12	●	●	Pectin acetylesterases	EC3.1.1.-	SHD	Repeated α/β/α motifs
			rhamnogalacturonan acetylesterases	EC 3.1.1.86		
			acetyl xylan esterase	EC 3.1.1.72		
CE13		●	Pectin acetylesterase	EC3.1.1.-	SHD	Unknown
CE14	●	●	*N*-acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-D-glucopyranoside deacetylase	EC 3.5.1.89		α/β hydrolase fold; N-terminal with a Rossmann fold motif; Zn^{2+}-dependent
			Diacetylchitobiose deacetylase	EC3.5.1.-		
			Mycothiol S-conjugate amidase	EC3.5.1.115		
CE15	●	●	4-*O*-methyl-glucuronoyl methylesterase	EC 3.1.1.-	SHE	α/β hydrolase fold; N-terminal with a Rossmann fold motif
CE16	●	●	Acetylesterase	EC 3.1.1.6	Unknown	Unknown
CE17	●		Acetylmannan esterase	EC 3.1.1.-	SHD	α/β hydrolase fold
CE18	●	●	N-acetylgalactosamine deacetylase	EC 3.5.1.-	DHH	$(\beta/\alpha)_7$ barrel; Zn^{2+}-dependent
CE19	●		Pectin methylesterase	EC 3.1.1.11	D	Unknown
CE20	●		Xyloglucan acetylesterase	EC 3.1.1.-	SHD	α/β hydrolase fold

Abbreviations: B, Bacteria; E, Eukaryota.
*CE10s are not included in this Table as they are esterases acting on non-carbohydrate substrates.

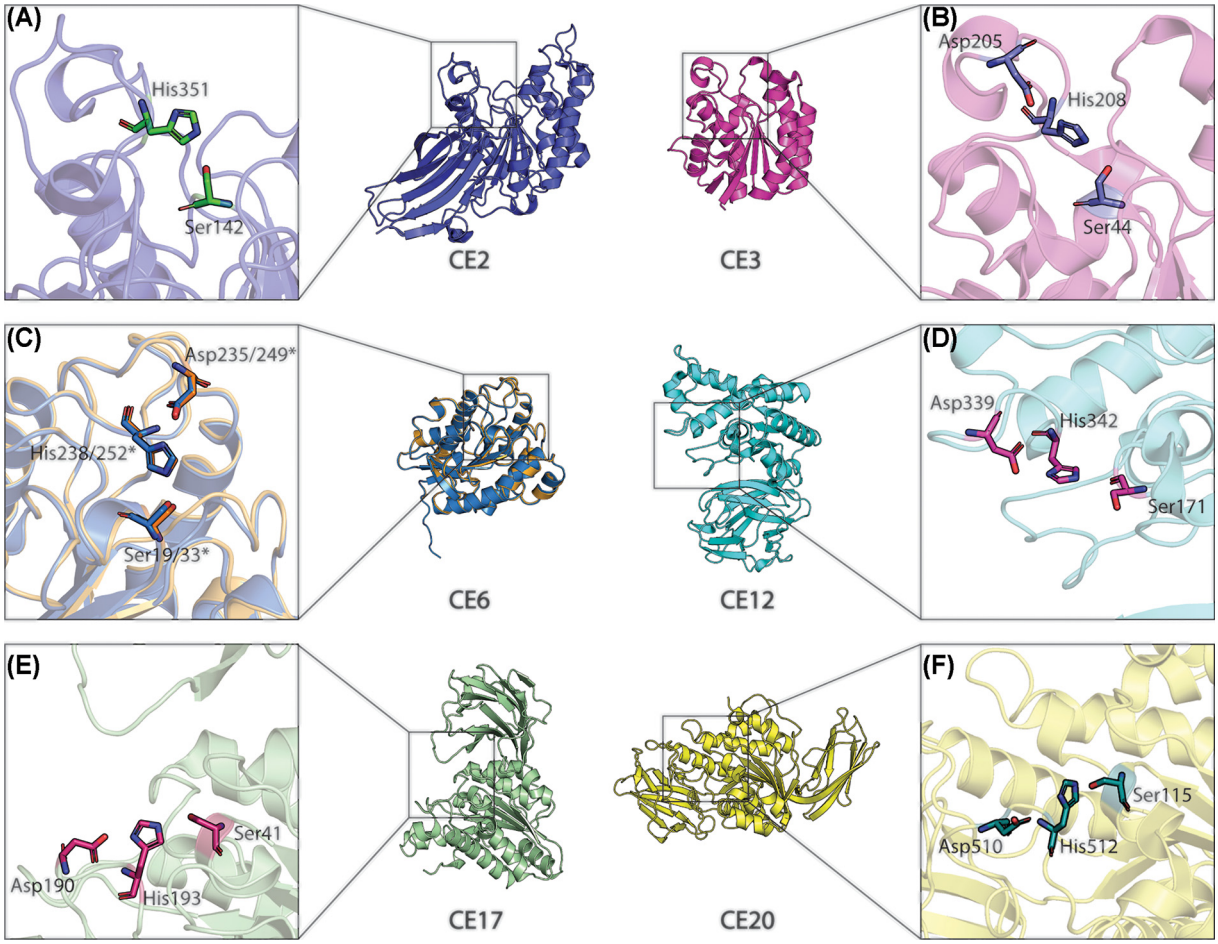

Figure 2. Structures of acetylesterases from the families CE2, 3, 6, 12, 17, and 20 (all being representatives of the SGNH superfamily).

(**A**) An acetyl xylan esterase (AXE) from *Butyrivibrio proteoclasticus* from CE2 (PDB: 3U37). (**B**) AXE from *Clostridium thermocellum* from CE3 (PDB: 2VPT). (**C**) Two superimposed structures of AlphaFold-predicted catalytic domains of AXEs from *Bacteroides intestinalis* (blue) and *Bacteroides ovatus* (orange) from CE6 (* annotates the active site residues for *B. ovatus*) (Protein: BACINT_04211 and BACOVA_03435, respectively). (**D**) Uncharacterized AXE/ pectin-/ rhamnogalacturonan acetylesterase from *Bacillus subtilis* from CE12 (PDB: 2O14). (**E**) An acetyl mannan esterase from *Roseburia intestinalis* from CE17 (PDB: 6HFZ). (**F**) Xyloglucan acetylesterase from *Xanthomonas citri* pv. *citri* from CE20 (PDB: 7KMM). #The CE6 structures from *B. intestinalis* and *B. ovatus* are from AlphaFold [30] and the Ser-His-Asp catalytic triad was predicted from the structure of an AXE CE6 from *Arabidopsis thaliana* (PDB: 2APJ), using superimposition of the structures in PyMOL (RMSD = 0.2 Å). *B. intestinalis* and *B. ovatus* are common members of the gut microbiota and a PUL from each (*B. intestinalis* DSM 17393, PUL 1, protein: BACINT_04211; *B. ovatus* ATCC 8483, PUL 73, protein: BACOVA_03435) with the CE6, flanked by xylanases, was selected for the predicted xylan activity.

low RMSD score in comparison with an RGI-active CE12. CE12s are part of a number of pectin PULs like, e.g., in *Bacteroides cellulosilyticus* [29]. CE20s seem to be the predominant esterases present in xyloglucan-PULs. One example is the recently characterized funding member of the CE20 family that is active on acetylated xyloglucan [15] (Figure 2F). When looking into the CAZy database for other representatives of the CE20 family, these are found in a number of commensal gut microbes and are closely associated with enzyme systems involved in degradation of xyloglucan as well as xylan and pectin PULs.

Enzyme systems for deacetylations of the more heavily ornamented regions of pectins have been discovered more recently in *Bacteroides thetaiotamicron*, although not characterized in detail. RGI-PUL harbors a gene encoding the esterase BT4158 which releases acetyl groups from D-GalUA in the RGI backbone, an important step in the depolymerization of the polymer [31]. Notably, BT4158 is active on RGI pectin as well as on xylan and glucomannan and is typically broad specific; this esterase is not affiliated with any CAZy family yet [31]. Another relatively recently

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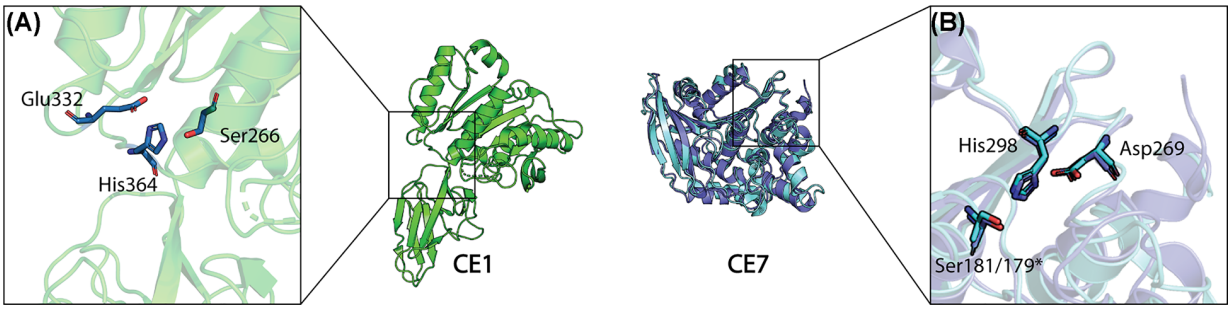

Figure 3. Acetylesterases that are not part of the SGNH superfamily.

(**A**) AXE from *B. intestinalis* from CE1 (PDB: 6NE9). (**B**) AXE/cephalosporin C deacetylase from *B. subtilis* (purple) and *Lactococcus lactis* (cyan) from CE7. The two structures were superimposed in PyMOL with an RMSD of 1.1 Å (* annotates the predicted active site residues for *L. lactis*) (PDB: *L. subtilis*: 1ODS; *L. lactis*: 7CUZ).

identified esterase is Bt0985 in the RGII-PUL of *B. thetaiotamicron*, which is a CE20 active on an aceric acid and a fucose in RGII pectin [32]. Bacteroides sp. 2_2_4 has a galactan PUL with two esterases, one CE12 and one unclassified esterase containing a GDSL like (PF16255) domain (a fold often identified with esterase activities). The presence of esterases in this PUL [33] might be explained by the fact that galactans, even though are not acetylated themselves, are often part of the ramified RGI structure. It is evident that a huge number of pectin PULs consist of proteins with esterase activities or hypothetical proteins with sequence identity to GDSL-like proteins.

Deacetylation of xylan

Xylans are major components of commonly consumed cereal grains [29], fruits, and vegetables, where they can comprise 30–50% of the dry weight. Depending on the plant origin, xylans show substantial heterogeneity and structural variation. A conserved feature in xylans is the β-1,4-linked xylose main chain that can be substituted with L-arabinosyl substitutions linked at xylose 2-*O* and/or 3-*O* (arabinoxylan or AX), D-glucuronic acid and/or 4-*O*-methyl-D-glucuronic acid linked to the 2-*O* position (glucuronoxylan or GX, glucuronarabinoxylan or GAX), or p-coumaric acid and ferulic acid esterified on the 5-*O* of the substituted arabinose. The backbone of AX, GAX and GX is usually acetylated at 2-*O* and/or 3-*O*, with the latter being more frequent. 3-*O* acetylations can also be present on 4-*O*-methylglucuronic acid substituted Xyl*p* residues in GX [34].

Acetylxylan esterases catalyzing the removal of acetyl groups in xylans belong to the families CE1–7 and 16 [34]. The different families seem to act on specific positions, whereby CE4 can deacetylate both 2-*O* or 3-*O* substitutions but only if the xylose residue is mono-acetylated, while CE1 (Figure 3A), 5, 6 (Figure 2C) and 16 can act on double acetylated xylose residues. Removal of the 3-*O*-acetylation of methyl-D-glucuronic acid residues has been reported to be mediated by CE16 [34], but no gut bacteria-derived enzymes are represented in this family. The CE3 family (Figure 2B) has only a few bacterial representatives but none is from the most common gut bacteria. The CE7 family (Figure 3B) is considered multifunctional as its members display activity towards xylooligosaccharides and the antibiotic cephalosporin C [35]. Furthermore, several CE7s seem to be associated to PULs not related to xylan degradation and this family of esterases may have a broader substrate specificity than is known today.

Acetyl esterases have been reported in PULs encoding enzymes for the depolymerization of both simple and complex xylans by Firmicutes and Bacteroidetes. Among the Firmicutes, *Roseburia intestinalis* harbors a PUL with a gene coding for a newly described xylan esterase named *Ri*AXE. The enzyme showed activity toward beechwood glucuronoxylan oligosaccharides with efficient deacetylation of both 2-*O*-acetylated xylose and 3-*O*-acetylated xylose but with a preference for 2-*O* substitutions [36].

In human gut Bacteroidetes, *Bacteroides intestinalis* was shown to possess esterase-enriched PULs (with up to five esterase genes) encoding CE1s or multimodular CE1-CE6 enzymes targeting acetyl groups in arabinoxylan. The 3D structure of the two esterases Bi1039-CE1 and Bi1033-CE1 consist of an N-terminal Ig-like fold of approximately 100 residues followed by the esterase domain. Despite sharing structural homology and a conserved architecture of the active site, Bi1039-CE1 (Figure 3A) was shown to be an acetyl-xylan esterase able to remove acetyl groups from oat spelt xylan, while Bi1033-CE1 is a ferulic acid esterase [37]. Another Bacteroidetes with a strong emphasis on plant-derived xylans abundant in dietary staples like cereal grains is *B. cellulosilyticus* [29] which has two tightly regulated xylan PULs, with one of them harboring genes enconding a CE1 and a CE6.

A CE6 was recently identified and characterized in a PUL targeting xylan in the gut Bacteroidetes *Dysgonomonas mossii*. The enzyme was shown to be a highly efficient esterase on different model substrates, removing both 2-*O* and 3-*O* acetylations, as well as on complex biomass like GAX from corn cob. Two CE1 esterases encoded by the same PUL were also shown to remove feruloyl residues, suggesting that *D. mossii* devotes remarkable resources to promote accessibility of xylan and boost xylanase degradation capabilities of complex substrates [37].

Deacetylation of β-mannans

β-Mannans are hemicellulosic polysaccharides based on a backbone of β-1,4 linked mannose (Figure 1). They are the most abundant form of hemicellulose in the secondary cell wall of softwoods, legume seeds and coffee beans. In softwoods, β-mannans constitute approximately 20% of dry wood mass [38]. In glucomannans, such as konjac (Amorphophallus konjac), the backbone presents non-repeating patterns of β-1,4 linked mannosyl and glucosyl units in varying ratios. In galactomannans, such as carob (Ceratonia siliqua), the backbone is substituted with α-1,6-linked galactose residues [39]. Galactoglucomannans have a main chain which consists of randomly distributed mannose and glucose sugars and branched galactose units linked only to the mannosyl units. Mannans, glucomannans and galactoglucomannans can also be decorated with acetyl groups at the positions 2-*O*, 3-*O*, 4-*O* (only in non-reducing end) or 6-*O* (Figure 1), which enhance their solubility and their ability to form gels. Both glucomannans and galactomannans are widely utilized in the food industry due to their properties such as thickeners, binders, and stabilizers [40]. Galactomannans can act synergistically with other polysaccharides such as agar, xanthan gum and carrageenans resulting in mixed gel formation and increased viscosity. In addition, galactoglucomannan from spruce wood has recently been shown to have prebiotic properties by promoting the selective stimulation of beneficial bacterial populations in the pig gut microbiota [41].

Several CE families such as CE2 and CE17 [42] contain enzymes targeting β-mannans as well as a hitherto unclassified pectin active BT4158 mentioned above [31]. While the CE2 family is classified as acetyl xylan esterases in the CAZy database, several characterized CE2s are seemingly nonspecific and are also active on acetylated konjac glucomannan and galactoglucomannan [42,43] Some CE2s have been reported to have a preference for deacetylating the 3-*O* and 4-*O* position over 2-*O* position on monoacetylated xylopyranosides, whereas on mannopyranosyl and glucopyranosyl units it shows high specificity towards 6-*O* acetylations [44]. A characteristic feature for the CE2s (Figure 2A, this CE2 has an RMSD score of 1.6 Å with the AlphaFold predicted model of *R. intestinalis* CE2) is the two-domain architecture, with a C-terminal catalytic SGNH domain and an N-terminal jelly roll domain. Many of the CE2s differ from other CE families with having a conserved Ser-His catalytic dyad instead of the more usual Ser-His-Asp catalytic triad [43]. CE2s are often found together with CE17s within PUL in Firmicutes members of the gut microbiota. Indeed, *R. intestinalis* and *Faecalibacterium prausnitzii* possess PULs containing a pair of CE2 and CE17 that catalyze the specific deacetylation of β-mannans. While CE2s from both *R. intestinalis* and *F. prausnitzii* remove various acetylations on mannose units, they were shown to be inactive on the axially oriented 2-*O* acetylations [42,45]. On the contrary, CE17s were found to act specifically on 2-*O* acetylations and work in tandem with CE2, where a CE17 is required to first remove the 2-*O* acetylation and give access to CE2 for removal of acyl substitutions in other positions. The characterized CE17 from *R. intestinalis* (*Ri*CE17) (Figure 2E) consists of an N-terminal SGNH hydrolase domain connected to a C-terminal carbohydrate binding module 35 (CBM35) domain. The structure of *Ri*CE17 co-crystallized with a β-mannan showed that the CBM35 is important for substrate recognition and binding cooperatively with the SGNH domain creating a narrow tight 'clamp' that facilitates substrate binding with the C2-OH group on mannose units suggestively acetyl in this position directly attacked by the catalytic Ser-OH group close to the oxyanion hole.

On a peculiar note, in *Lactococcus lactis* the CE7 (Figure 3B) is flanked with a mannosidase (GH113) which implies that *L. lactis* may be part able to utilize simple mannan structures and pinpoints a broader specificity in this CE family. Of note, *Lactococcus lactis* produces acetylated EPS (Figure 1), [46] and the CE7 may be part of housekeeping genes modulating its own EPS formation during growth.

Acetyl orientation and migration in polysaccharides

Compared to other acetylated hemicelluloses, a unique feature of the *O*-acetyl groups linked to the mannose residues of the β-mannans backbone is their orientation; while the 3-*O*-acetylations are in the equatorial plane of the molecule, 2-*O*-acetylations are axial. A number of studies have also reported the presence, although to a lesser amount, of 6-*O*-acetylations in glucomannans and galactoglucomannans [47]. Acetylations in this position are considered to be a result of a process called migration which is hypothesized to be 'clockwise' (meaning from 2-*O* -> 3-*O* -> 4-*O* -> 6-*O*) migration [48] but is also convincingly shown to occur across the glycosidic bond [49] on the mannose sugar

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ring when the substrate is exposed to high temperatures and pH around 7 [48]. Indeed, acetyl group migration is a spontaneous reaction in aqueous medium, and its occurrence increases with pH and temperature [50]. Notably, migration is of more general nature and occurs rapidly on xylan [50] and neuraminic acids [51], and likely also on other polysaccharides mentioned in this review.

Deacetylation of bacterial exopolysaccharides

A large number of microbes used in food industry produce exopolysaccharides that contribute with important rheological properties [52]. Many of these EPS are acetylated in different monosaccharide residues (rhamnose, galactose, glucose, mannose and glucuronic acid) and positions (Figure 1). Several EPS structures [53] with acetylations have been reported (see Figure 1), including xanthan gum in *Xanthomonas campestris* [54], gellan gum (E418) in *Sphingomonas paucimobilis* strain I-886 [55] (formerly known as *Pseudomonas elodea*), *Lactiplantibacillus plantarum* C88 [56], *Lactobacillus sakei* 0-1 [57] *Lactococcus lactis* subsp. *cremoris* [58], *Lactobacillus pentosus* [59], and *Butyrivibrio fibrisolvens* strain H10b [60]. The acetyl ornamentations contribute to the EPS properties. For example, high acyl gellan gum (GG) forms soft and elastic gels, whereas low acyl GG forms hard, non-elastic, and brittle gels [61]. Despite a recent study that shows indications that three acetylesterases are involved in degradation of xanthan gum (further details below), knowledge regarding deacetylation of other EPS remains elusive.

Generally, little is known about how bacteria interact and cross-feed/recycle each other's cell wall material. Only a few studies have provided some information on how bacteria feed on other bacterial cells, but the understanding on this matter remains largely enigmatic. Some examples which demonstrate bacterial growth on EPS are summarized in [62]: gellan gum promotes growth of probiotic strains such as *Lactiplantibacillus rhamnosus* and *Bifidobacterium bifidum* [61]; *B. thetaiotamicron* grows on lactobacilli EPS [63]; Bacteroides grows on EPS structures produced by bifidobacteria [64]; a fructan-type EPS promotes growth of bifidobacteria [65]; bifidobacteria EPS is readily fermentable by gut microbiota [66] and further alters the gut microbiota composition [67,68]. However, when looking at these growth studies, very little focus has been put on the role of non-carbohydrate modifications, like acetyl substitutions, present on the EPS. To the best of our knowledge, the only representative study to date which has some focus on acetylation is on xanthan degradation described in the following section.

Deacetylation of the food additive xanthan gum

While EPS from fermented food as well as hemicellulosic and pectic polysaccharides have been components of the human diet for millennia, food additives such as xanthan gum (see Figure 1.) have only been consumed, through processed foodstuffs, for the last five decades. Despite the recent inclusion, a single gut microbe from the Ruminococcaceae uncultured genus 13 (R. UCG13) was discovered to possess the complete enzymatic apparatus necessary to depolymerize xanthan gum. The model proposed is that R. UCG13 deploys an extracellular GH5 xanthanase to cleave the XG backbone and generates oligosaccharides for uptake. The degradation products can be utilized by the R. UCG13 itself or by a *B. intestinalis* that contains the appropriate degradation system apart from the initial depolymerizing enzyme [69]. Both microbes possess a lyase and a glucuronyl hydrolase to remove the terminal mannose and glucuronic acid from the pentasaccharide, as well as intracellular carbohydrate esterases (RuCE-A, RuCE-B and BiPL-CE) that cleave the 6-*O* acetyl group from the internal mannose [69], and potentially also the 6-*O* acetyl group on the outer mannose unit (which occur in some of the pentasaccharides [70]). Sequence analysis of three esterases shows that they all are part of the SGNH-hydrolase family and have a typical SHD catalytic triad. Once deacetylated, further hydrolysis of the remaining trisaccharide structure is mediated by an additional α-mannosidase (GH38 or GH92) and a GH94 cellobiose phosphorylase in R. UCG13 or a GH3 β-glucosidase in *B. intestinalis*, respectively.

Closing remarks

O-Acetylated dietary fibers have been a component of the human diet for millennia. Acetylation hinders the access of glycoside hydrolases to the substituted polysaccharide, thus preventing its depolymerization [42,58,71]. The hypercompetitive nature of the densely populated human gut environment has driven microorganisms to adapt and evolve their enzymatic toolboxes to target these non-carbohydrate decorations and gain an advantage over less equipped species. Sequencing technology has facilitated the identification of systems deployed by gut bacteria to utilize acetylated food components and in-depth enzymatic characterizations have uncovered a significant number of new *O*-acetylesterases that differ in substrate and positional specificity [15,31,42,69]. Of note, the gut microbiota also experiences variations of naturally occurring polysaccharides, since some modifications are likely to occur during food/ingredient processing. For example, acetyl migration can occur to a large extent at neutral pH and 'benign' temperatures (60°C) [5]. This increase carbohydrate's structural complexity, and positions of acetylations may differ in

processed foods compared with those found in naturally existing polymers. This aspect should be taken into account for the correct functional characterization of acetylesterases.

A considerable number of acetylesterases from members of the abundant phyla Firmicutes and Bacteroidetes have been described to target hemicelluloses and pectins. Still, apart from three esterases involved in the de-acetylation of the food additive xanthan gum [69], esterases targeting acetylations in bacterial exopolysaccharides are under-explored. Given the abundance of bacterial-derived exopolysaccharides (including several structures carrying acetyl ornamentations) in food and beverages, and the fact that the gut microbiota is constantly exposed to these carbohydrates, it is expected that research on gut microbiology will continue to yield more esterases playing key roles in depolymerization of these complex substrates. Furthermore, it is envisaged that newly discovered deacetylases can be applied to modify physico-chemical and rheological properties of acetylated plant- and microbial- polysaccharides to be used as food additives and in material chemistry.

Summary

- Acetylated hemicellulosic and pectic polysaccharide structures as well as exopolysaccharides from food microbes are very common in the human diet as components of foods and beverages.
- Human gut microbiota members use complex degradation systems equipped with carbohydrate esterases to remove acetyl ornamentations and facilitate the activity of other enzymes targeting the glycan part of the polymer.
- Polysaccharide utilization systems include broad specific and highly specific acetyl esterases targeting acetylations in virtually all positions of glycans.
- Continued efforts in the field, using biochemical and structural studies, will help to precisely illuminate the molecular mechanisms behind complex carbohydrate utilization by human gut microbes and advance the understanding of their role in human nutrition.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

CE, carbohydrate esterase; HG, homogalacturonan; GH, glycoside hydrolase; PUL, polysaccharide utilization locus; RGI, rhamnogalacturonan I; RGII, rhamnogalacturonan II; SCFA, short-chain fatty acid; XyG, xyloglucan.

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