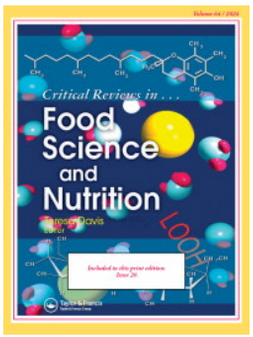

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REVIEW ARTICLE

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The pectin metabolizing capacity of the human gut microbiota

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ABSTRACT

The human gastrointestinal microbiota, densely populated with a diverse array of microorganisms primarily from the bacterial phyla Bacteroidota, Bacillota, and Actinomycetota, is crucial for maintaining health and physiological functions. Dietary fibers, particularly pectin, significantly influence the composition and metabolic activity of the gut microbiome. Pectin is fermented by gut bacteria using carbohydrate-active enzymes (CAZymes), resulting in the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which provide various health benefits. The gastrointestinal microbiota has evolved to produce CAZymes that target different pectin components, facilitating cross-feeding within the microbial community. This review explores the fermentation of pectin by various gut bacteria, focusing on the involved transport systems, CAZyme families, SCFA synthesis capacity, and effects on microbial ecology in the gut. It addresses the complexities of the gut microbiome's response to pectin and highlights the importance of microbial cross-feeding in maintaining a balanced and diverse gut ecosystem. Through a systematic analysis of pectinolytic CAZyme production, this review provides insights into the enzymatic mechanisms underlying pectin degradation and their broader implications for human health, paving the way for more targeted and personalized dietary strategies.

KEYWORDS

Gastrointestinal microbiota; dietary fibers; pectin fermentation; short-chain fatty acids (SCFAs); carbohydrate-active enzymes (CAZymes); microbial cross-feeding

1. Introduction

The human gastrointestinal microbiota is densely populated with diverse microbial communities, fulfilling distinct roles in human health and physiology (Holscher 2017; Wardman et al. 2022). These microbial communities consist of approximately 1500 different species and comprise an estimated 10^{11}–10^{12} bacterial cells per mL (Johnson and Klaenhammer 2014; Rivera-Piza and Lee 2020; Ye et al. 2022). Several factors regulate the gut microbiome, including host physiology, genetic makeup, use of medication, and environmental factors such as living conditions and dietary habits. Diet, especially dietary fiber, is considered a crucial factor that can alter the composition of the gut microbiome and mediate its role in metabolic functions (Johnson and Klaenhammer 2014; Rivera-Piza and Lee 2020). The impact of dietary fiber on the human gastrointestinal microbiota is an intricate phenomenon that is essential for promoting homeostasis and influencing overall health and disease susceptibility (Ye et al. 2022).

Within this context, dietary fibers, particularly pectin, have emerged as vital components, offering a fascinating and multifaceted contribution to human health. Although pectin cannot be digested by humans, it is fermented by beneficial bacteria in the large intestine via carbohydrate-active enzymes (CAZymes) (Blanco-Pérez et al. 2021; Kaoutari et al. 2013; Tan and Nie 2020). The production of CAZymes by gut bacteria is an adaptive response to dietary pectin. CAZymes are responsible for breaking down glycosidic bonds within both carbohydrate and non-carbohydrate structures. Several classes of CAZymes includes glycoside hydrolases (GHs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), Glycosyl transferases (GTs), carbohydrate binding modules (CBMs), and auxiliary activities (AAs) (Drula et al. 2022; Ye et al. 2022). All these enzymes play a fundamental role in pectin degradation and are systematically cataloged in the CAZY database (www.cazy.org, version: 2021). In recent years, breakdown products of pectin, also known as pectin oligosaccharides (POSs), have gained significant attention as prebiotics because of their potential to alter microbiome composition (Tingirikari 2018). The beneficial properties extend from gastrointestinal regulation to potential roles in allergy and inflammatory disease prevention, as well as in cancer therapy (Blanco-Pérez et al. 2021; Liu et al. 2016; Palko-Łabuz et al. 2021). This surge in interest has led to extensive research efforts exploring the interplay between pectin and the gut microbiota.

As non-starch polysaccharides are abundant in the cell walls of fruits, vegetables, and other plant sources, pectins contribute to the firmness and texture of plant tissues while

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also serving as a soluble dietary fiber with numerous physiological benefits (Chen et al. 2015; Voragen et al. 2009; Zandleven et al. 2007). Pectins are complex polysaccharides composed of up to 29 different monosaccharides connected by over 20 different glycosidic bonds (Scheller et al. 2006; Voragen et al. 2009). Structurally, pectin comprises various domains, including homogalacturonan (HG), xylogalacturonan (XGA) rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II), each with distinct properties and functionalities (Liu, Willför, and Xu 2015). The side chains of RG-I exhibit significant diversity owing to the presence of four types of branched oligosaccharides, which vary in length. These oligosaccharides are generally made up of linear and branched arabinan, galactan, and arabinogalactan I (AG-I), and arabinogalactan II (AG-II) chains (Mikshina, Petrova, and Gorshkova 2015). The specific composition of these chains depends on the plant source (Ye et al. 2022). Furthermore, the structure of pectin can be classified based on its degree of esterification (DE), which includes its degree of methylation (DM) and acetylation (DAc) (Thibault and Rinaudo 1986). Owing to this intricate structure, predicting the specific effects of pectin on individual microbiota is challenging.

Specific groups of gut bacteria have developed the genetic capacity to produce a diverse array of CAZymes that target different components of pectin. Fermentation of pectin results in the production of SCFAs including acetate (C_2), propionate (C_3), and butyrate (C_4) (Wardman et al. 2022; Ye et al. 2022). SCFAs confer various beneficial effects on the host gastrointestinal tract, including serving as an energy source for colonocytes, mitigating inflammation, and preserving intestinal barrier function (Nicholson et al. 2012; Wardman et al. 2022; Ye et al. 2022). Moreover, SCFAs have been associated with numerous health benefits throughout the body and modulate various physiological pathways, such as the immune, endocrine, vagal, and humoral pathways (Clarke et al. 2014). Additionally, SCFAs have been suggested to play a vital role in communication along the microbiota-gut-brain axis (Stilling et al. 2016). However, our understanding of the growth and metabolism of gut bacteria in the presence of pectin remains limited. Owing to the inter-individual diversity of the microbiota and its susceptibility to various factors, including diet, it is challenging to study changes in individuals, as not everyone reacts in the same way when fibers are introduced (Cantu-Jungles and Hamaker 2023). This symbiotic relationship between the gut microbiota and the host highlights the intricate interplay between dietary fibers, gut bacteria, and pectin metabolism within the human gut ecosystem.

In addition to its complexity, the gut microbiome provides metabolites that are nutrients to other microbiome species; this is called cross-feeding. This can occur through a variety of mechanisms, including the secretion of CAZymes, resulting in metabolized intermediates, such as oligosaccharides or other compounds, which the receiving species cannot utilize on their own (Ye et al. 2022). Cross-feeding is thought to play a key role in maintaining a diverse and balanced gut microbiome. By providing nutrients to other species, microbes can support the growth and survival of their community members, which can help to maintain a stable and functional microbiome (Payling et al. 2020). The quality of the gut microbiome is influenced by its diversity and stability, with greater diversity and stability indicating a healthier microbiome status. The stability of a microbiome reflects its capacity to resist changes caused by external factors such as shifts in diet, antibiotic use, and environmental conditions. A stable microbiome can preserve its diversity and function over time despite external stressors, indicating a better capacity to adapt to changing conditions and maintain healthy gut function (McClements 2019).

This review aims to comprehensively examine the fermentation of pectin by the human gut microbiota after degradation of pectin through CAZyme production. We focused on elucidating the role of CEs, GHs, and PLs, in the intricate interplay between dietary fibers and gut microbiota. A list of these CAZymes was compiled previously (Yüksel, Kort, and Voragen 2024). A systematic manual approach was used to map the production capacity of these pectinolytic CAZyme families of important microbiota colonizing the colon. Additionally, the impact of pectin degradation on the microbial ecology of the gastrointestinal tract, including the production of SCFAs, is discussed. Furthermore, this review addresses the missing links and complexities in understanding the gut microbiome composition in the context of pectin degradation. By examining the repertoire of CAZyme families involved in pectin degradation, this review provides a comprehensive understanding of the enzymatic machinery utilized by the gastrointestinal microbiota in processing pectin dietary fibers, and provides insights into the process of cross-feeding and nutrient exchange within the microbial community.

2. Pectinolytic human gut microbes

The human gastrointestinal tract harbors diverse microbial communities encompassing various phyla. Among these phyla, four were consistently recognized as the most predominant, collectively accounting for approximately 98% of the total microbial population. These four phyla are Bacillota, Bacteroidota, Actinomycetota, and Pseudomonadota. Bacillota, with a prevalence ranging from 58% to 88%, is the most abundant phylum in the human gut. Bacteroidota follow closely behind, typically comprising 8.5%–28% of the microbial population. Pseudomonadota, although less abundant, still plays a significant role, representing 0.1%–8% of the total population. Actinomycetota, with a prevalence of 2.5%–5%, completes the quartet of dominant phyla in the human gut (Donaldson, Lee, and Mazmanian 2016; Wu, Bushmanc, and Lewis 2013).

Pectinolytic organisms were identified in two ways: by isolating them from human feces and cultivating them on a specialized growth medium with pectin as the primary carbon source, or by testing previously isolated strains for their ability to degrade pectin (Bayliss and Houston 1984; Jensen and Canale-Parola 1985). Moreover, the identification of these pectin-degrading organisms has been facilitated not only by culture-based methods, but also by genomic analyses. By identifying CAZyme genes, researchers have been

able to predict the ability of different gastrointestinal tract isolates to break down pectin (Centanni et al. 2019; Ndeh et al. 2017). These studies have helped to broaden our understanding of the role of pectinolytic organisms in the gut microbiota. In this manner, pectin degradation was found to impact bacterial species associated with human health, including key species such as *Faecalibacterium prausnitzii*, *Prevotella copri*, and multiple *Bifidobacterium*, and *Bacteroides* species, depending on the pectin source (Larsen, Bussolo de Souza, et al. 2019).

To bridge the genomic-based data found in the CAZy database with experimental findings, we developed a CAZyme family profile, elucidating the relationships between gut microbes and the production of CAZyme families through a manually performed systematic categorization approach, as shown in Table 1. Members of the gut microbiota were selected based on previous studies by Elshahed et al. (2021), Gullón et al. (2013), Pascale et al. (2022), Tan and Nie (2020) and Ye et al. (2022). These studies compiled and summarized the specific gut bacteria that demonstrated growth in response to pectin supplementation, as shown in Table S1. The CAZY database was used to identify specific CAZyme families associated with multiple strains of these gut bacteria (Drula et al. 2022). These findings were compared with relevant experimental literature to validate and support the observed correlations between gut microbes and their CAZyme family profiles. During our investigation, we did not discern any Pseudomonadota species that demonstrated enhanced growth as a result of pectin degradation. Therefore, this phylum was excluded from the analysis. Moreover, genes of the CAZyme family have not been reported for *Bacteroides finegoldii*, *Bacteroides salyersae*, *Bacteroides galacturonicus*, *Bacteroides massiliensis*, and *Bifidobacterium adolescentis*.

2.1. Bacteroidota

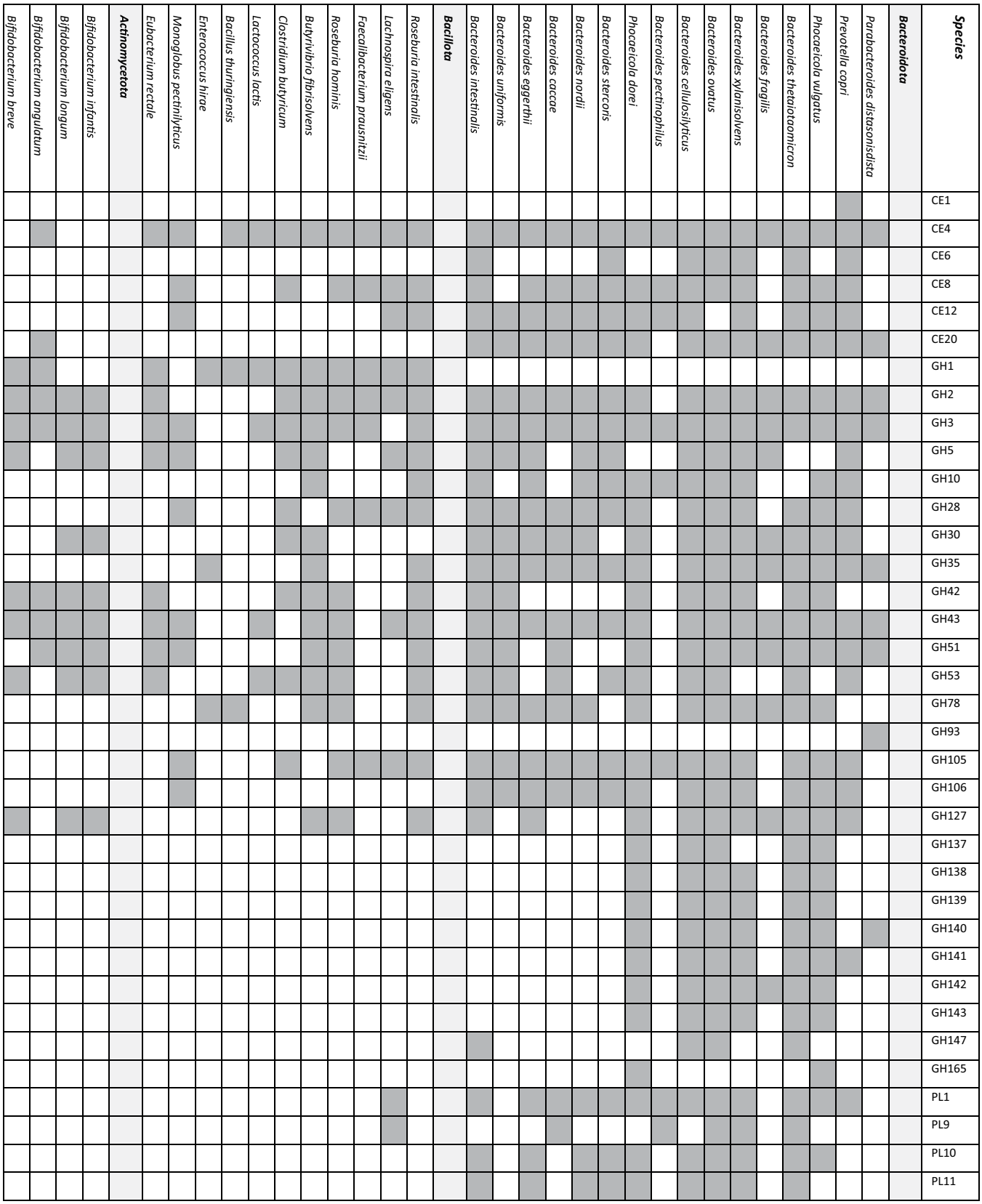
The phylum Bacteroidota is well known for its dominant role in polysaccharide degradation in the human gastrointestinal tract (El Kaoutari et al. 2013). Within the phylum Bacteroidota, the genus *Bacteroides* is notable for encoding a significant number of CAZymes, ranging from 100 to over 300. This abundance allows them to efficiently utilize a diverse array of dietary fibers (Ye et al. 2022). Additionally, *Bacteroides* species, known for their high degree of pectin and other pectic substrate utilization, exhibit a rapid and specific response to the presence of pectin in their surroundings (Chung et al. 2016). This is attributed to the possession of advanced systems that compete for carbohydrate substrates (Lopez-Siles et al. 2012). This ability is relatively widespread in *Bacteroides* (Martens et al. 2011). Studies have shown that populations of *Bacteroides* increase during *in vitro* fermentation with various pectin sources, such as citrus pectin, sugar beet pectin, and RG-I from carrots (Cui et al. 2020; Holck, Hjernø, et al. 2011; Van den Abbeele et al. 2020). Therefore, these species are recognized as the main pectin-degrading bacteria in the gut (Martens et al. 2011).

Two of the most prevalent microorganisms in the gastrointestinal tract are *Bacteroides thetaiotaomicron* and *Bacteroides ovatus* (Arumugam et al. 2011; Martens et al. 2011). These bacteria have been extensively studied using enzymatic and genomic analyses, which have revealed a diverse repertoire of genes and enzymes involved in the degradation of various pectic compounds. For instance, genomic analysis of *Ba. thetaiotaomicron* revealed the presence of several CAZymes from multiple gene families, such as GH28, as well as other CAZymes including several CE, GH and PL CAZyme families, associated with the degradation of the entire pectin backbone chain (Chung et al. 2017; Martens et al. 2011). Moreover, genetic data in the CAZY database revealed that *Ba. thetaiotaomicron* possesses more CAZyme families, which play a role in the breakdown of galactan, arabinan, AG-I, and AG-II chains, as shown in Table 1 (Drula et al. 2022). The presence of these diverse enzymes expands an organism's capacity to efficiently degrade a wide range of pectic compounds (Bayliss and Houston 1984; Centanni et al. 2019; Martens et al. 2011; Ndeh et al. 2017). Various other species within the *Bacteroides* genus are also well equipped with enzymes to degrade pectin, according to Table 1, such as *Bacteroides xylanisolvens*, *Phocaeicola vulgatus* (formerly known as *Bacteroides vulgatus*), *Bacteroides dorei*, *Bacteroides caccae*, and *Bacteroides cellulosilyticus*.

For an extended duration, it was unclear which organism(s) were responsible for the deconstruction of RG-II in nature and whether it is mediated by a single organism or microbial consortia in a regionally selective and temporally sequential manner (Ndeh et al. 2017). Owing to the extensive branching and unique glycosidic linkages in RG-II, research on RG-II depolymerizing human gut microbes has lagged behind that of HG and RG-I. Nonetheless, recent advancements in enzymatic characterization and genetic studies have shed light on the microbes involved in RG-II degradation. Recent studies have demonstrated that both *Ba. thetaiotaomicron* and *Ba. ovatus* can utilize RG-II, which is mainly found in apples and red wine, as their sole carbon source (Centanni et al. 2019; Martens et al. 2011). Enzymatic activities and the corresponding genes necessary for the breakdown of 20 out of 21 glycosidic bonds were identified in RG-II using *Ba. thetaiotaomicron* as a model organism. Genomic analysis of these bacteria has identified a range of CAZymes, including GH137 to GH143, which are involved in RG-II depolymerization (Ndeh et al. 2017). As shown in Table 1, this range of enzymes was found in more *Bacteroides* species, including *Ba. caccae*, *Ba. cellulosilyticus*, *Ba. dorei*, *Ba. xylanisolvens*, *Ba. finegoldii*, *Ba. salyersae*, and *Bacteroides uniformis* (Centanni et al. 2019; Ndeh et al. 2017).

The same study also reported that *Bacteroides eggerthii*, *Bacteroides stercoris*, *Bacteroides nordii*, and *Bacteroides intestinalis* have the ability to degrade RG-II (Centanni et al. 2019; Ndeh et al. 2017). Notably, their ability to degrade RG-II could not be attributed to their known CAZyme family potential, as illustrated in Table 1. This finding suggests that these bacteria employ either a distinct set of enzymes to degrade RG-II or that the relevant database has not yet been fully established. For instance, Table 1 shows that *Ba. stercoris* has the potential to degrade RG-II using members of CE20 and GH2 enzyme families. CE20 and GH2 have been shown to employ new

Table 1. Pectin degrading microbes and CAZyme production.



Compilation of data from Elshahed et al. (2021), Gullón et al. (2013), Pascale et al. (2022), Tan and Nie (2020), Ye et al. (2022), and the CAZY database.
†*Ba. massiliensis*, *Ba. salyersae*, *Ba. finegoldii*, *Ba. galacturonicus* and *Bi. adolescentis* are not described in the CAZY database.
‡CE13, GH54, GH59, GH62, PL2, PL3 and PL4 were not found to be associated within the selected gut microbes in the CAZY database.

activities in the degradation of RG-II. Furthermore, *Ba. intestinalis* possesses GH127, which is also implicated in RG-II degradation (Drula et al. 2022; Ndeh et al. 2017). In addition, these bacteria could participate in RG-II degradation after deconstruction of the ramified side chains of RG-II. According to Table 1 it could be suggested that

P. vulgatus may also have the potential to degrade RG-II, but this degradation has not been mentioned in literature studies.

Another example of contrasting findings came from the analysis of *Bacteroides pectinophilus*. As the name suggests, it has been found to degrade pectin structures containing high percentages of HG and RG-I side chains (Centanni et al. 2019). This ability may be attributed to the presence of GH105 and several CEs and PLs encoded by CAZymes in the genome, suggesting its ability to target the HG and RG-I backbone (Drula et al. 2022). However, *Ba. pectinophilus* lacks the CAZymes required for the degradation of arabinan, galactan, and AG-I and AG-II structures. These findings indicate that *Ba. pectinophilus* has the potential to degrade the RG-I backbone but lacks the necessary CAZymes to degrade the RG-I side chains.

Parabacteroides distasonis is a species within the *Parabacteroides* genus, belonging to the phylum Bacteroidota, which is included in the CAZyme family profile. Previous research has suggested that treatment with apple pectin results in an increase in *Parabacteroides* (Nie et al. 2021). Analysis of the CAZyme family profile revealed that this bacterium lacks the enzymes involved in the depolymerization of simple pectin molecules. For instance, *P. distasonis* does not contain enzymes from GH28 or any of the PL families that are known to play important roles in degrading the HG domain. Instead, the genomic data presented in the database suggest that it is more likely to act on the RG-I domain with CAZymes, such as GH43, GH51, and GH106. This suggests that *P. distasonis* may have limited ability to degrade simple pectin structures. However, experimental data suggest that *P. distasonis* can degrade pectin from citrus fruits that contain a high composition of HG (Bayliss and Houston 1984).

The *Prevotella* genus, belonging to the phylum Bacteroidota, plays a significant role in the gut microbiome owing to its ability to utilize various complex carbohydrates. Notably, the presence of arabinan side chains in pectic substrates positively correlates with higher counts of *P. copri*. (Larsen, Bussolo de Souza, et al. 2019). *P. copri* possesses a wide range of CAZymes, including: GH43, GH51, GH53, GH105, GH106, targeting the RG-I side chains by CAZymes. It is also likely that *P. copri* may be capable of degrading the pectin backbone, given its possession of the GH28 CAZyme family. However, it is important to note that most studies have investigated *Prevotella* in conjunction with other bacterial genera, rather than in isolation. This makes it challenging to conclusively determine the specific effect of pectic substrates on *P. copri* growth (Pascale et al. 2022).

2.2. Bacillota

Although Bacteroidota species generally have larger genomes and encode a higher number of GH and PL enzymes than Bacillota, some members of the phylum Bacillota are also capable of utilizing pectin. Candidates stimulated by pectin include *Butyrivibrio fibrisolvens*, *Clostridium butyricum*, *Lactococcus lactis*, *Bacillus thuringiensis*, *Enterococcus hirae, and Eubacterium rectale* (Gullón et al. 2013; Tan and Nie 2020). Bacillota possesses a unique organization of extracellular enzymes, sometimes forming large complexes, which enable them to effectively attack robust materials with limited surface area accessibility and robust hydrogen-bonding networks (Flint, Duncan, and Louis 2017; Williams et al. 2017; Ze et al. 2013). However, the mechanism by which Bacillota degrades pectin in the human gut is not well understood. Currently, our knowledge of pectin-degrading Bacillota is limited because only a few species have been identified to possess this capability. The scarcity of known pectin-degrading Bacillota species highlights the need for further research to identify additional species within this phylum that contribute to pectin degradation in the human gut.

Research has shown that, within the phylum Bacillota, the *Ruminococcaceae* family is favored in the fermentation of native pectin (Tan and Nie 2020). The *Ruminococcaceae* family represents 10%–20% of the gut microbiome in healthy humans (Pryde et al. 2002). The genus *Faecalibacterium*, which belongs to this family, has commonly been observed to increase during fermentation with pectic substrates. Specifically, *F. prausnitzii* has been shown to be promoted by citrus pectin rich in RG-I and RG-II, as well as oligosaccharides from sugar beets (Cui et al. 2020; Leijdekkers et al. 2014; Reichardt et al. 2018). Studies have also revealed that the distribution of linear and branched structural regions in pectin can affect fermentation by the human gut microbiota. This suggests that different microorganisms may have specific preferences for certain substrates. This is the case with *F. prausnitzii*, which was shown to have a preference for utilizing the HG backbone compared to the RG-I backbone, but still contains the enzymatic capabilities to degrade both, as shown in Table 1. This phenomenon has also been reported in previous studies, where the enzymatic ability of *F. prausnitzii* was found to degrade both the RG-I and HG backbones (Heinken et al. 2014; Ndeh and Gilbert 2018). This variation in substrate utilization was strain-dependent (Lopez-Siles et al. 2012).

The *Lachnospiraceae* family is another family of bacteria that possesses the enzymatic ability to degrade pectic substrates, and is significantly increased in the gut microbiota of healthy subjects with HG- and RG-I-rich pectin sources. Within this family, *Lachnospira eligens* (previously known as *Eubacterium eligens*), *Roseburia hominis* and *Roseburia intestinalis* have been shown to possess various enzymes capable of degrading pectin (Cantu-Jungles et al. 2021; Cantu-Jungles et al. 2019; Chung et al. 2017; Larsen, Bussolo de Souza, et al. 2019; Larsen, de Souza, et al. 2019). *R. intestinalis* and *R. hominis* contain a similar CAZyme potential, in which *R. intestinalis*, for instance, contains various enzyme families such as GH35, GH42, GH43, GH51, and GH53, which could potentially degrade the RG-I side chains. *L. eligens*, on the other hand, possesses lyases, including PL1 and PL9, as well as other enzymes capable of degrading easily accessible pectic structures, according to Table 1. Within experimental data, PL9 was constitutively produced in *L. eligens* upon exposure to HG in pectin. Other GHs and PLs predicted to act on pectin were absent among the proteins exhibiting pectin-induced expression (Chung et al. 2017). However, when compared to *R. intestinalis* and *R. hominis*, *L. eligens* exhibits less capability to degrade the side chains of RG-I. The variation in

enzymatic capabilities among members of the *Lachnospiraceae* family may explain why the pectin-degrading capacity of this family varies among studies. The stimulation of *L. eligens* is unique to pectic substrates and is not observed in fructans or resistant starch (Cantu-Jungles et al. 2021; Larsen, Bussolo de Souza, et al. 2019).

Another gut bacterium was recently discovered to be capable of breaking down pectin and named *Monoglobus pectinilyticus*. It belongs to the family *Oscillospiraceae* and is classified as a species within the Order Eubacteriales and Class Clostridia. The genetic makeup of *M. pectinilyticus* includes 48 enzymes that are potentially involved in the breakdown of various pectin sources (Centanni et al. 2019; Kim et al. 2017; Kim et al. 2019). The CAZY database suggests that the CAZyme family includes GH43, and GH51, which may have the ability to break down arabinan within the RG-I chain. In addition, *M. pectinilyticus* contains GH105 and GH106, which act on the RG-I backbone. Table 1 also lists GH28, which may target the backbone of the pectin molecules.

2.3. Actinomycetota

Bifidobacterium exhibits selective fermentation of specific carbohydrate substrates with a preference for shorter chain molecules, as most bifidobacteria exhibit increased growth on oligosaccharides compared to pectins in experimental studies (Olano-Martin, Gibson, and Rastell 2002; Rastall et al. 2022). Table 1 shows that the selected *Bifidobacterium* species generally lack depolymerization enzymes, such as PLs and GHs, including GH28. For instance, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Bifidobacterium angulatum*, *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium infantis* have been suggested to have limited capacity to degrade the pectin backbone compared to *Bacteroides* (Chung et al. 2017; Pascale et al. 2022).

Several studies have shown that *Bifidobacterium* species efficiently ferment arabino-oligosaccharides, which can also arise from the depolymerization of RG-I arabinan side chains, indicating their ability to degrade specific pectin components (Holck et al. 2011; Moon et al. 2015; Onumpai et al. 2011; Sulek et al. 2014). Enzyme studies have shown the presence of CAZymes, such as GH43, GH51, and GH127, in *Bifidobacterium* species, which are potentially involved in the breakdown of arabinan-rich chains (Chung et al. 2017). These CAZymes were also identified in the database (Table 1). Furthermore, *Bifidobacterium* has been reported to have the ability to break down galactan side chains in RG-I (Van Laere et al. 2000). CAZymes associated with the selected bifidobacteria include GH42 and GH53, which target galactan, as shown in Table 1. These findings suggest that *Bifidobacterium* is more adapted to degrade specific side chains, such as arabinan and galactan, within pectin structures. However, inconsistent results were also reported in a previous study that used different pectic substrates, as previous research also pointed out that HG may contribute to the selective stimulation of *Bifidobacterium* as well (Onumpai et al. 2011; Thomassen et al. 2011).

3. Transporting pectin oligosaccharides

After the depolymerization of pectin, several types of oligosaccharides known as POSs remain, such as oligogalacturonides with and without unsaturated C4–C5 bond at the non-reducing end, galacto-oligosaccharides, arabino-oligosaccharides, rhamnogalacturon-oligosaccharides, xylo-oligogalacturonides, and arabinogalacto-oligosaccharides, are formed. These POSs can serve as a nutrient source for the gut microbiota as they are capable of being taken up by transport systems and utilized by these microorganisms for growth and further metabolism (Gullón et al. 2013). However, the specific transport systems involved in the uptake of POSs by gut microbes, particularly those derived from pectin, are not yet fully understood. Figure 1 illustrates the different transport systems associated with dietary fiber uptake by Bacteroidota and Bacillota. However, their role in transporting pectin-derived oligosaccharides in the human gut microbiota has not been extensively studied. Further research is needed to unravel the specific mechanisms by which gut microbes take up and utilize pectin-derived POSs, including identification and characterization of transport systems involved in their uptake.

3.1. Transport systems in Bacteroidota

Bacteroidota utilize a unique mechanism for carbohydrate uptake through gene clusters called polysaccharide utilization loci (PUL). Each PUL targets a specific carbohydrate structure and encodes proteins for substrate binding and transport, signal transduction systems, and CAZyme production (Lapébie et al. 2019). Unlike other bacteria, Bacteroidota lack classical carbohydrate uptake systems like ATP-binding cassette (ABC) transporters, relying instead on specific PULs and their corresponding enzymes for the uptake and utilization of polysaccharides (Brigham and Malamy 2005). Activation of a specific PUL ensures the organism has all components for efficient substrate degradation (Lapébie et al. 2019).

The starch utilization system (Sus) of *Ba. thetaiotaomicron*, a well-studied PUL, encompasses eight genes (*susRABCDEFG*) responsible for starch uptake and utilization (Foley, Martens, and Koropatkin 2018). It also facilitates the import of various plant polysaccharides, including pectin (Collins et al. 1994). The Sus system includes a membrane protein for saccharide transport to the periplasm (Salyers et al. 1977). SusR protein detects polysaccharides and activates *sus* genes. SusD facilitates polysaccharide binding to the cell surface, while SusE and SusF are responsible for binding. SusG hydrolyzes polysaccharides into oligosaccharides and smaller glycans, and SusC, a TonB-dependent transporter, moves them into the periplasm. In the periplasm, SusA and SusB enzymes depolymerize glycosidic linkages, producing monomers for cytoplasmic transport (Flint et al. 2012).

The *sus* locus is organized into two transcriptional units controlled by SusR: one with *susA* and the other with *susB* to *susG*, enabling co-regulation of PUL (Anderson and Salyers 1989; D'Elia and Salyers 1996; Reeves, Wang, and Salyers 1997). This characterization led to identifying several PULs in Bacteroidota, with frequent tandem *susC*- and

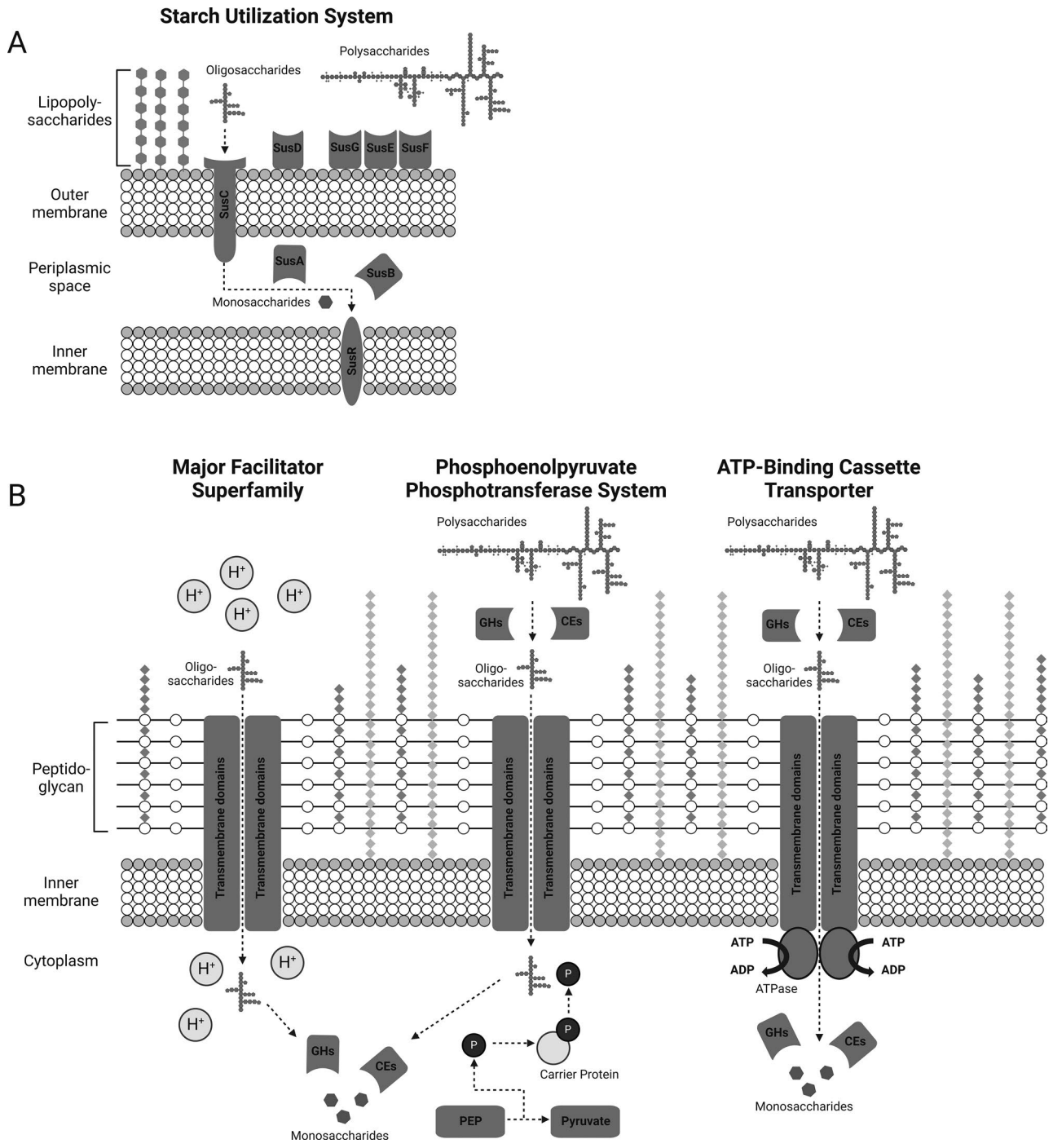


Figure 1. Substrate uptake mechanisms in Bacteroidota and Bacillota. (A) Illustration of the starch utilization system (Sus) in *Ba. thetaiotaomicron*. The Sus, represented by eight genes (susRABCDEFG), is responsible for the uptake and utilization of pectin by *Ba. thetaiotaomicron*. (B) Illustration of substrate uptake mechanisms in Bacillota. Bacillota employ different transport systems for pectin substrate uptake, including the major facilitator superfamily (MFS), the phosphoenolpyruvate phosphotransferase system (PTS), and the ATP-binding cassette (ABC) transporter. Composite figure created with BioRender.com, adapted for clarity and conciseness from Cockburn and Koropatkin (2016) and Hou et al. (2021).

susD-like genes as central units. *Ba. thetaiotaomicron* contains 107 *susC* paralogs, with 101 paired with a *susD*-like gene (Xu et al. 2003). PULs account for about 18% of its genome (Martens, Chiang, and Gordon 2008). Closely related species like *Ba. fragilis*, *P. vulgatus*, and *P. distasonis* also have numerous PULs (Kuwahara et al. 2004; Xu et al. 2007). These PULs likely give Bacteroidota an advantage in the gut environment, explaining their evolution as symbionts. SusD homologs are exclusive to Bacteroidota, suggesting that PULs containing a *susD*-like gene in ancestral Bacteroidota may have driven the emergence and specialization of the phylum in carbohydrate degradation (Thomas et al. 2011).

3.2. Transport systems in Bacillota

Bacillota use various strategies for saccharide transport, including the major facilitator superfamily (MFS), the phosphoenolpyruvate phosphotransferase system (PTS), and

ABC transporters. The ABC transporter in Bacillota includes an extracellular substrate-binding protein that specifically recognizes certain sugars, coupling sugar import with ATP hydrolysis. MFS proteins utilize a cation gradient (H^+ or Na^+) to transport saccharides across the cell membrane, while PTS allows the uptake of small oligosaccharides or sugars with low polymerization, phosphorylating them to prevent diffusion out of the cell and facilitating metabolic processing (Cockburn and Koropatkin 2016).

Recent studies have shown Bacillota using cell wall-anchored, multimodular enzymes combining carbohydrate-binding and enzymatic functions within a single polypeptide, known as cellulosomes, potentially involved in pectin degradation. Cellulosomes consist of scaffoldin, organizing enzymes, cohesin-dockerin interactions for structural integrity, and a carbohydrate-binding module (CBM) for substrate attachment. These insights largely come from studies on *Clostridium thermocellum* (Bayer et al. 2004; Bayer et al. 2008; Doi and Kosugi 2004; Fontes and Gilbert 2010). This phenomenon is observed across various Bacillota species, highlighting its importance in fiber uptake (Lammerts van Bueren et al. 2011; Ramsay et al. 2006). Unlike Bacteroidota's Sus-like complexes, which distribute these functions among different proteins, *Ruminococcus champanellensis* in the human gastrointestinal tract is known to possess a cellulosome, although it cannot degrade pectin (Ben David et al. 2015; Chassard et al. 2012). Such fragments are associated with genera like *Faecalibacterium*, *Eubacterium*, and *Ruminococcus*, with *Faecalibacterium* and *Eubacterium* including documented pectin-degrading species (Elshahed et al. 2021).

The distinct organization of carbohydrate-degrading enzymes in Bacillota may reflect an adaptation to the competitive environment of the large intestine. By retaining these enzymes on the bacterial surface, Bacillota species ensure proximity between the hydrolysis site and the transport systems importing hydrolysis products, enhancing nutrient acquisition and utilization efficiency. Further research is needed to clarify the roles of these enzyme domains and their impact on substrate binding and catalytic activity (Cockburn and Koropatkin 2016).

3.3. Transport systems in Bifidobacterium (Actinomycetota)

Bifidobacteria have various carbohydrate transport systems for utilizing dietary carbohydrates, including ABC transporters, MFS, permeases, and proton symporters (Kelly, Munoz-Munoz, and van Sinderen 2021). However, it is not fully understood if these systems can specifically import POSs, and the mechanism and efficiency of pectin uptake remain under study. ABC transporters in bifidobacteria are notable for their selectivity for oligosaccharides. They consist of two transmembrane domains forming a translocation pore, two cytoplasmic nucleotide-binding domains providing energy for transport, and an extracellular substrate-binding protein (SBP) capturing specific ligands (ter Beek, Guskov, and Slotboom 2014). This selectivity allows bifidobacteria to recognize and internalize specific oligosaccharides. Over time, bifidobacteria have evolved ABC transporters that can bind specific types of oligosaccharides, like α-1,6-galactosides (Theilmann et al. 2019), suggesting they may take up specific substrates after pectin degradation (Arzamasov, van Sinderen, and Rodionov 2018; Fushinobu and Abou Hachem 2021).

4. SCFA production

Fatty acids are organic compounds characterized by the presence of a carboxyl group at one end and a long hydrocarbon chain at the other. Saturated and unsaturated fatty acids are classified based on the presence or absence of double bonds in their hydrocarbon chains (Moss, Smith, and Tavernier 1995). Fatty acids can be further categorized based on the length of their hydrocarbon chains into short-, medium-, or long-chain fatty acids. The diverse properties of SCFAs make them important molecules in various biological processes. They serve as a source of energy for cells, regulate gene expression, participate in signaling pathways, and contribute to host-microbe interactions. SCFAs include formate (CH_2O_2), acetate ($C_2H_4O_2$), propionate ($C_3H_6O_2$), butyrate ($C_4H_8O_2$), and valerate ($C_5H_1O_2$), and their physical and chemical properties vary depending on the number of carbon atoms present (Layden et al. 2013; Nicholson et al. 2012; Wardman et al. 2022; Ye et al. 2022).

The specific composition and abundance of the gut microbiota influence the levels and proportions of SCFAs produced (Cummings et al. 1987). This fermentation process also generates gases, such as hydrogen, methane, and carbon dioxide (Henningsson, Björck, and Nyman 2002). The concentration of SCFAs is relatively low in the ileum, at approximately 13 mM. However, SCFA levels increase in the cecum, which has a more acidic pH than the sigmoid colon and rectum, where the pH is slightly higher. SCFA production significantly increases in the colon, ranging from approximately 130 mM in the cecum to 80 mM in the descending colon. Approximately 500–600 mmol of SCFAs can be produced by fermenting 50–60 g of carbohydrates daily, with acetate, propionate, and butyrate being the most abundant. Collectively, these three SCFAs constitute approximately 80% of the total SCFAs produced in the gut. The relative proportions of acetate, propionate, and butyrate in the colon and feces are approximately 3:1:1, depending on the composition of the gut microbiota (Bergman 1990; Cummings et al. 1987). The gut and its resident microbiota absorb or utilize between 90%–99% of the SCFAs produced (Perry et al. 2016).

In the intricate ecosystem of the human gut, SCFA production by gut microbes plays a vital role in promoting intestinal health and overall wellbeing. For instance, they play a significant role in maintaining the integrity of the intestinal barrier, modulating immune responses, and influencing the composition of gut microbiota. When assessing the effects of pectin fermentation in the gut, the consideration of SCFAs as significant parameters is warranted. Therefore, the intricate relationship between SCFA production and pectin degradation is explored in this section, shedding light on the multifaceted factors that influence this essential process. Additionally, this section discusses the

diverse pathways through which gut microbes synthesize SCFAs, including acetate, propionate, butyrate, and the common by-product lactate.

4.1. Pectin fermentation and SCFA distribution in the gastrointestinal tract

Pectin is primarily fermented in the colon and is commonly recognized for its resistance to digestion in the small intestine (Pascale et al. 2022; Ye et al. 2022). The ileum has a thin mucus layer, a rapid transit time, and relatively low microbial diversity and density. In contrast, the distal colon has a thick mucus layer, slow transit, and high microbial diversity and density (Koropatkin, Cameron, and Martens 2012). Nevertheless, findings from a prior *in vitro* study revealed a significant recovery of citrus pectin, with approximately 90% being successfully reclaimed from the terminal ileum (Ferreira-Lazarte et al. 2019). This notable loss of pectin is likely linked to the activity of gut bacteria, with additional chemical effects, such as the interaction of pectin with bile acids, contributing to the overall process (Cao et al. 2023). Moreover, it is important to note that some degree of de-esterification may occur during transit through the small intestine due to alkaline conditions. De-esterification plays a crucial role in modifying the structure of pectin during its transit through the small intestine. A study investigating pectin degradation in the gastrointestinal tract of weaning pigs indicated that pectin with lower levels of methyl esterification was more readily fermented by the gut microbiota than highly methyl-esterified pectin. Conversely, highly methyl-esterified pectin is predominantly fermented by microbiota in the proximal colon (Tian et al. 2017).

The colon, characterized by varying physiological conditions along its length, provides a nutrient-rich environment for anaerobic bacteria. These microorganisms utilize complex indigestible carbohydrates, including pectin, through a process known as saccharolytic fermentation, ultimately yielding SCFAs (Richards et al. 2016). Upon entering the colon, slightly alkaline conditions may favor lyase activity to act on the pectin molecules. The physical state in which pectin occurs affects its nature, speed, and degree of breakdown, in addition to the pH and degree of substitution (Ye et al. 2022). Despite numerous research efforts aimed at unraveling the fermentative behavior of pectin by the gut microbiome, the intricate structural complexity inherent to pectin continues to impede a precise understanding of its fermentation processes within the human gastrointestinal tract.

However, available scientific literature has confirmed that the fermentation of pectic substrates in the large intestine of humans is slow and complete, as evidenced by their non-detectable presence in adult feces during intervention studies, even at daily doses of up to 30–40 g (Cummings et al. 1979; Holloway, Tasman-Jones, and Maher 1983). Slow fermentation is advantageous because it facilitates sustained production of SCFAs throughout the colon, spanning from the proximal to distal regions. This continuous SCFA production not only contributes to metabolic processes in the colon but also provides protective effects by preventing the growth of harmful bacteria and the accumulation of toxins (Pascale et al. 2022). Additionally, the composition and structure of pectin, including chain length and branching, can significantly affect its interaction with specific bacteria involved in SCFA production (Larsen, de Souza, et al. 2019). Previous studies have found that pectin substrates stimulate higher levels of acetate production by the gut microbiota in healthy adults, regardless of the structure or source of pectin (Min et al. 2015). Moreover, clinical studies have shown that supplementing with 20–25 g citrus fiber rich in pectin results in increased levels of acetate in fecal and blood samples (Fechner, Kiehntopf, and Jahreis 2014; Pomare, Branch, and Cummings 1985).

Other studies have attempted to establish the ratios of acetate, butyrate, and propionate produced during intestinal fermentation of pectin; values that were found were approximately 80%–83%, 7%–10%, and 4%–6%, respectively, highlighting the dominance of acetate and butyrate as the main metabolites. Specifically fermentation of galacturonic acid, mainly found in the HG domain of pectin, has been identified as a significant contributor to acetate production, as observed in a study by Zhao et al. (2021). However, these findings are in contrast to those reported in other studies, which demonstrated the highest concentration of 63%–77% acetate, followed by 17%–26% propionate, and 6%–11% butyrate (Cui et al. 2019). It has also been suggested that pectinolytic microorganisms depolymerize the pectin backbone and utilize saccharides from XGA, such as xylose, as well as from RG-I, including rhamnose, arabinose, and galactose. The pectin structure and monosaccharides included in pectin has been previously discussed in detail (Yüksel, Kort, and Voragen 2024). Fermentation of xylose and galacturonic acid resulted in the production of acetate. Additionally, the fermentation of galacturonic acid and galactose in pectin resulted in butyrate production. The fermentation of rhamnose and arabinose mainly results in propionate production (Zhao et al. 2021). Figure 2 illustrates the relationship between pectinolytic microorganisms and SCFA production during pectin fermentation. However, limited information is available regarding the specific relationship between RG-II degradation and SCFA production by human gut microbes. Most research on SCFA production has focused on the degradation of other pectin components such as HG and RG-I, which constitute a major portion of pectin. However, it is plausible that certain microbial species with RG-II-specialized CAZymes can degrade RG-II and potentially produce SCFAs as byproducts. Further research is needed to elucidate whether RG-II degradation results in SCFA production.

In addition, the pH of the colon is a critical factor that influences the degradation and fermentation of dietary components, including pectin, by the gut microbiota. The pH of the colon typically ranges from 5.5 and 7.5, and this acidic environment has a profound effect on the production of specific SCFAs, such as butyrate. Studies have shown that variations in pH can lead to shifts in microbial populations and subsequent changes in the fermentation products (Louis and Flint 2009; Nugent et al. 2001). In a previous study using fecal matter, researchers observed distinct pH-dependent effects on the abundance of key butyrate-producing bacteria such as *E. rectale* and *F. prausnitzii*. Specifically, these bacteria thrived in an acidic environment with a pH of 5.5, but their

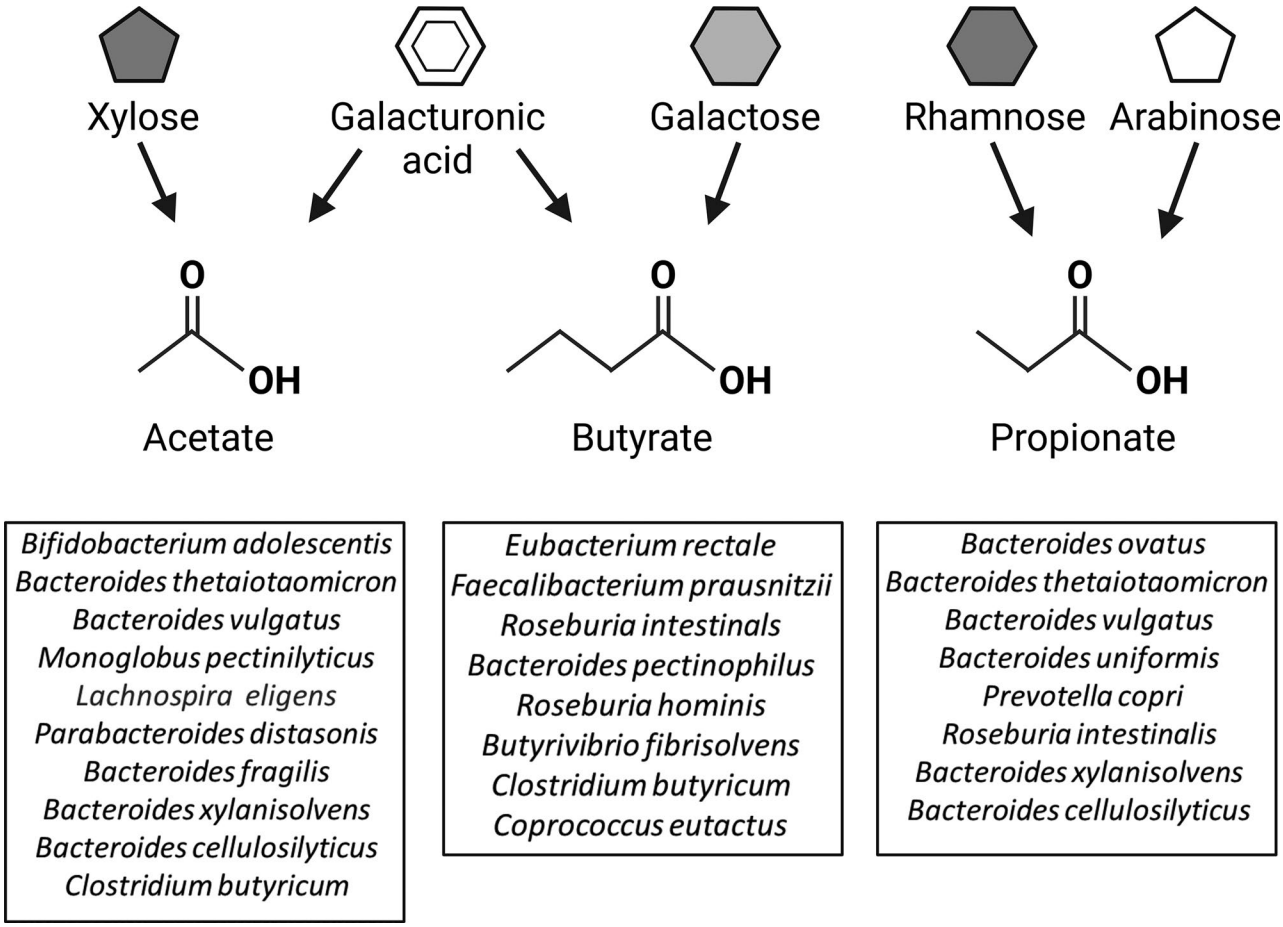


Figure 2. An overview of the pectin structures and their role in SCFA production by the human gut microbiota during intestinal fermentation. Pectinolytic microorganisms employ extracellular CAZymes to degrade the pectin backbone and utilize its side chains, such as rhamnose, arabinose, xylose, galacturonic acid, and galactose. The fermentation of xylose and galacturonic acid primarily leads to the production of acetate, while the fermentation of galactose and galacturonic acid in pectin results in butyrate production. Additionally, the fermentation of rhamnose and arabinose is mainly associated with propionate production. Composite figure created with BioRender.com, adapted and merged for clarity and conciseness from Gullón et al. (2013) and Zhao et al. (2021).

numbers sharply declined when the pH increased to 6.5, with *Bacteroides* species being dominant. This shift in microbial population was accompanied by a corresponding change in the predominant fermentation products, with butyrate being the primary SCFA at pH 5.5, whereas acetate and propionate were the most abundant at pH 6.5. These findings were further supported by the ability of various cultured strains to thrive under low pH conditions, reinforcing the importance of pH in shaping the microbial community and their metabolic activity in the colon (Louis and Flint 2009).

4.2. SCFA production pathways

Chemoheterotrophic anaerobes in the colon can be classified into two main categories: those that have a limited ability to carry out anaerobic respiration and rely solely on substrate-level phosphorylation for ATP production via fermentation and those that are capable of anaerobic respiration. Substrate-level phosphorylation does not involve respiratory chains that utilize terminal electron acceptors such as oxygen or inorganic ions. Instead, it involves organic compounds derived from the original fermentation substrate. Therefore, fermentation reactions must be self-regulating in terms of the generation and utilization of reducing power, with the redox difference between substrates and end products determining the amount of energy that can be generated. Thus, fermentation reactions that produce substantial amounts of acetate generally yield relatively high ATP levels. Phosphorylation reactions that occur via substrate-level phosphorylation are less efficient in terms of energy production than oxidative metabolism, resulting in relatively low ATP yields. Therefore, fermentative microorganisms require significant amounts of substrate for growth, leading to the formation of substantial levels of metabolic end products (Macfarlane and Macfarlane 2003).

The hexose (C_6) and pentose (C_5) saccharides, derived from POSs uptake, are partly directed toward the central glycolytic Embden-Meyerhoff-Parnas (EMP) and pentose phosphate (PP) pathways, respectively (Elshahed et al. 2021). The PP pathway plays a crucial role in the metabolism of many gut bacteria, serving not only to break down pentoses but also to generate essential components such as NADPH for biosynthesis. The breakdown of hexoses involves the oxidative decarboxylation of glucose-6-phosphate into ribulose-5-phosphate, followed by a series of non-oxidative interconversions catalyzed by trans-ketolases and trans-aldolase. These reactions generate triose and hexose

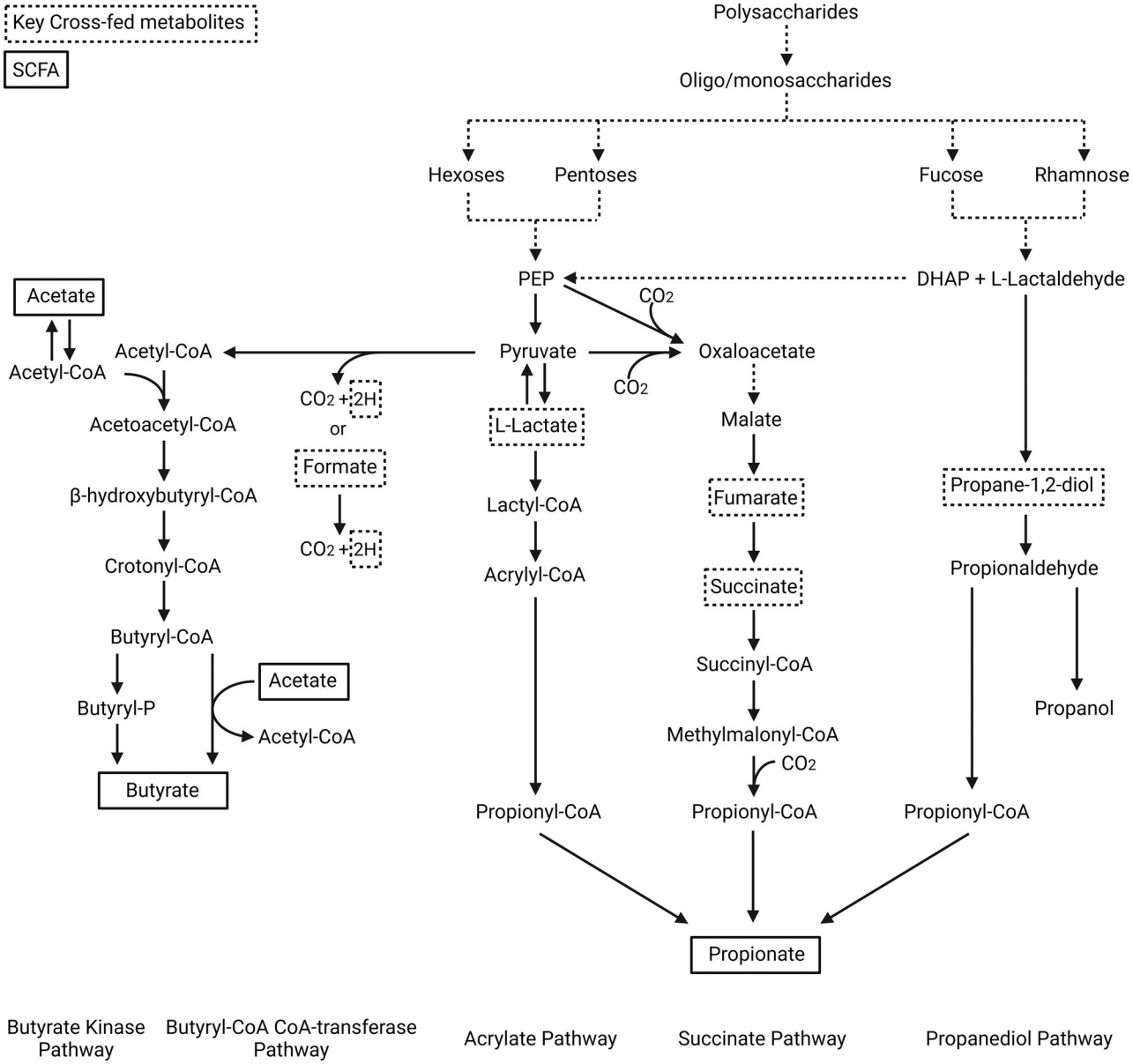


Figure 3. Metabolic pathways involved in the synthesis of acetate, propionate, and butyrate in the gut. Hexoses and pentose saccharides derived from POS uptake are directed toward the Central glycolytic Embden-Meyerhoff-parnas (EMP) pathway and the pentose phosphate (PP) pathway, respectively. Acetate can be produced via two pathways: the decarboxylation of pyruvate to acetyl-CoA, followed by hydrolysis to acetate, and the reduction of carbon dioxide to carbon monoxide, leading to the production of acetyl-CoA. Propionate synthesis occurs through three distinct pathways: the succinate, acrylate, and propanediol pathways. Butyrate is formed by condensing two acetyl-CoA molecules to acetoacetyl-CoA, which is subsequently reduced to butyryl-CoA. These pathways highlight the diverse metabolic capabilities of gut microbes in utilizing POSs and provide insights into the complex interplay between microbial processes and the host gut environment. DHAP refers to dihydroxyacetone phosphate and PEP stands for phosphoenolpyruvate. Composite figure created with BioRender.com, adapted and merged for clarity and conciseness from Nogal, Valdes, and Menni (2021) and Culp and Goodman (2023).

phosphates that play crucial roles in various metabolic pathways. During the dissimilatory metabolism of pentoses, these intermediates are utilized at different levels in the EMP pathway, ultimately resulting in pyruvate synthesis. Hence, the PP pathway is a vital metabolic pathway in gut bacteria that enables them to efficiently utilize diverse carbon sources and generate metabolic intermediates necessary for biosynthesis (Macfarlane and Macfarlane 2003).

Pyruvate serves as an electron acceptor and undergoes reduction to form key intermediates such as butyrate, propionate, acetate, lactate, ethanol, and succinate. However, rhamnose and fucose monosaccharides in the RG-I domain are exceptions, and are metabolized via the propane 1,2-diol pathway (Elshahed et al. 2021). This pathway involves the production of lactaldehyde and dihydroxyacetone phosphate, which are eventually fermented to propanol and propionate, respectively (Petit et al. 2013). Furthermore, metagenomic analyses have facilitated the identification and classification of the main bacteria responsible for SCFAs production (Morrison and Preston 2016). The major pathways involved in the synthesis of acetate, propionate, and butyrate are shown in Figure 3.

4.2.1. Acetate production

Acetate is produced through two main pathways. In one pathway, pyruvate is decarboxylated to acetyl-CoA by pyruvate decarboxylases, and then acetyl-CoA is hydrolyzed to form acetate by acetyl-CoA hydrolase (Miller and Wolin 1996). The Wood-Ljungdahl pathway is an alternative, where acetyl-CoA is synthesized from CO_2 through a series of enzymatic reactions. Acetyl-CoA is a central metabolite involved in various metabolic pathways and serves as a precursor for acetate production (Ragsdale and Pierce 2008). Acetate is absorbed and transported to the liver for further metabolism, limiting its availability in the colon. It can also be a substrate for butyrate production (Cook and Sellin 1998; Diez-Gonzalez et al. 1999). *F. prausnitzii* can produce and consume acetate, which is influenced by acetate production from GalA (Flint et al. 2015). High acetate levels from pectin fermentation align with increased *Lachnospiraceae* abundance, as some species produce acetate (Dusková and Marounek 2001). For example, *L. eligens* produces formate, acetate, and lactate (Lopez-Siles et al. 2012). In addition, *M. pectinilyticus* produces acetate, formate, hydrogen, and CO_2, with minor levels of lactate as the end products (Kim et al. 2017). Other bacteria associated with acetate production are *Bi. adolescentis*, *Ba. thetaiotaomicron*, *P. vulgatus*, *P. distasonis*, *Ba. fragilis*, *Ba. xylanisolvens*, *Ba. cellulosilyticus*, and *Cl. butyricum* (Araki et al. 2002; Chassard et al. 2008; Macfarlane and Macfarlane 2003; Sakamoto and Benno 2006; Zhao et al. 2021).

Increasing the concentration of acetate in the colon may provide additional benefits. One of the effects of increased acetate concentration is a decrease in the pH. This decrease in pH can create an environment that is less favorable for the proliferation of harmful species and the growth of pathogenic bacteria. Pathogenic bacteria typically prefer a higher pH; therefore, a decrease in pH can help regulate their growth and promote a healthier gut microbiota composition (Flint et al. 2015).

4.2.2. Propionate production

Propionate production in the gut occurs mainly through three biochemical pathways: succinate, acrylate, and propanediol pathways (Reichardt et al. 2014). First, the succinate pathway involves phosphoenolpyruvate carboxylation to oxaloacetate, which converts to malate and fumarate, promoting NADH dehydrogenase activity and succinate generation via fumarate reductase. Succinate is then transformed into propionate and CO_2 (Flint et al. 2015; Macy, Ljungdahl, and Gottschalk 1978). Second, the acrylate pathway reduces lactate, a carbohydrate fermentation by-product, to propionate. Third, the propanediol pathway involves converting 1,2-propanediol, derived from other carbohydrate fermentation products, to propionate (Miller and Wolin 1996).

Specific bacterial genera utilize these pathways. The succinate pathway is found in many Bacteroidota and some Bacillota species, including *Bacteroides* species, which produce succinate, propionate, and lactate (Adamberg et al. 2014; Salyers, 1984). Key propionate producers in the gut include *Ba. thetaiotaomicron*, *Ba. uniformis*, *Ba. ovatus*, *P. vul*gatus, and *P. copri* (Flint et al. 2015; Reichardt et al. 2014). The propanediol pathway is mainly observed in *Ruminococcus* species, while the acrylate pathway, present in some Bacillota species, uses lactate as a precursor for propionate synthesis (Bik et al. 2018).

Scientific studies have provided evidence supporting the beneficial effects of propionate on various aspects of health. Propionate inhibits the growth of colon cancer cells, suggesting its potential as a protective factor against colon cancer development (Jan et al. 2002). This finding highlights the significance of propionate in cancer prevention and its potential therapeutic application. Moreover, propionate has been associated with the regulation of feeding behavior and the maintenance of a healthy weight. Studies have demonstrated that propionate can influence appetite control and energy homeostasis, leading to reduced food intake and increased satiety. These effects can contribute to the prevention of excessive weight gain and the management of body weight (Ruijschop, Boelrijk, and Te Giffel 2008).

4.2.3. Butyrate production

Butyrate is synthesized through a series of biochemical reactions in the gut. Initially, two acetyl-CoA molecules condense to form acetoacetyl-CoA, which is subsequently reduced to butyryl-CoA. Butyrate production involves two distinct pathways. The classical pathway relies on lactate fermentation by phosphotransbutyrylase and butyrate kinase. In this pathway, lactate is converted into butyrate. The second pathway involves utilization of acetate as a substrate, which is facilitated by the action of butyryl-CoA: acetate CoA-transferases. Through these mechanisms, butyrate is generated by the combination of two acetyl-CoA molecules to form acetoacetyl-CoA, which is subsequently reduced to butyryl-CoA (Duncan, Barcenilla, et al. 2002; Louis and Flint 2009).

All currently well-known butyrate-producing microorganisms belong to Bacillota, including *E. rectale*, *R. intestinalis*, *F. prausnitzii,* and *Co. eutactus* (Louis and Flint 2009; Notting et al. 2023). *R. intestinalis* and *F. prausnitzii* convert butyrate using acetate (Duncan, Hold, et al. 2002; Khan et al. 2012; Rios-Covian et al. 2015). *Ba. pectinophilus* is also associated with butyrate production (Serino 2019). Previous studies have indicated that the levels of butyrate increase due to the activity of bacteria, such as *Lachnospiraceae* and *Faecalibacterium*, which are also known to produce acetate (Rios-Covian et al. 2015).

Colonic epithelial cells, which form the lining of the colon, rely uniquely on butyrate as their primary energy source. Butyrate serves as a crucial fuel for colonic epithelial cells and plays a vital role in maintaining proper function and overall colonic health (Ramakrishna and Roediger 1990). The precise mechanisms by which butyrate exerts its protective effects in the colon are still under investigation. In addition to its energy-providing role, butyrate also has several beneficial effects on colon health. Studies have suggested that an appropriate supply of butyrate to the colon may prevent the initial stages of tumor formation (Sengupta, Muir, and Gibson 2006). Furthermore, butyrate is believed to influence gene expression and inflammation within colonic tissues, ultimately promoting a healthy colon environment (Furusawa et al. 2013; Smith, Yokoyama, and German 1998).

4.2.4. Lactate production

Lactate is a common end product of bacterial fermentation and is produced by lactic acid bacteria. However, lactate production is not limited to lactic acid bacteria. Many other dominant members of the gut microbiota are also capable of producing lactate along with other fermentation acids (Flint et al. 2015). Observations during pectin fermentation revealed a small quantity of lactate, which corresponded to a minor increase in *Bifidobacterium* and *Lactobacillus* (Scott, Duncan, and Flint 2008). Bifidobacteria, for instance, utilize the bifid shunt pathway, which generates acetate and lactate in a molar ratio of 3:2 (Flint et al. 2015).

Lactate can serve as a substrate for further metabolic pathways and interconversions within the gut microbiota. For example, several bacterial species can utilize lactate for SCFA formation (Gómez et al. 2014; Nogal, Valdes, and Menni 2021). However, acetate formation from lactate is considered to be less energetically favorable than other fermentation pathways. Therefore, these bacterial groups are likely to compete with lactate as a substrate (Thauer, Jungermann, and Decker 1977). In a recent study, it was observed that lactate utilization contributed to approximately 20% of the butyrate pool. This indicates the significant involvement of lactate-utilizing bacteria, including those that produce butyrate, in shaping the colonic metabolome (Scott, Duncan, and Flint 2008).

Lactate-producing bacteria have potential health benefits including improved nutrition, control of intestinal infections, enhanced lactose digestion, cancer prevention, and modulation of cholesterol levels. However, the alleviation of lactose intolerance is one of the few widely accepted health benefits linked to the consumption of lactic acid bacteria and lactic acid-fermented foods (Marco et al. 2017).

5. Cross-feeding behaviour of gut microbes

Cross-feeding in gut microbes is a phenomenon in which one species or strain of bacteria produces and releases certain metabolites or nutrients that can be utilized by other species or strains, resulting in a mutualistic exchange of resources within the gut ecosystem (Culp and Goodman 2023). Commonly used definitions are listed in Textbox 1. Cross-feeding adds to the complexity of the gut microbiome as it involves intricate interactions between different bacterial species. To better understand the role of cross-feeding in the gut microbiome during pectin degradation, it is crucial to explore how interactions between distinct species in the microbial communities, including the gut microbiome, can influence their growth and fitness. These interactions can be classified on the basis of the fitness outcomes of the members involved. Microbial interactions can occur within species (intraspecific) or between species (interspecific), although cross-feeding is typically considered an interspecific interaction (Culp and Goodman 2023).

In vitro intestinal models have been extensively employed to investigate the influence of diet on gut microbes as they provide a means to understand the fermentation mechanisms modulated by the gut microbial community. The complexity and diversity of pectin contribute to its potential for a wide range of interactions and stimulations among pectinolytic organisms in the gut microbiome. The structural variability of pectin provides a rich substrate landscape for microbial degradation (Aguirre et al. 2014). When pectin is introduced into the gut through dietary sources, it serves as a selective pressure that favors the growth and proliferation of pectinolytic organisms capable of efficiently utilizing this complex polysaccharide, leading to the production of specific ranges of SCFAs (Kaur et al. 2018; Martens et al. 2014). This selective pressure can result in the enrichment of specific pectinolytic species or strains that possess the necessary genetic machinery and enzymatic repertoire to effectively degrade pectin, further stimulating cross-feeding behavior. Changes in the composition of the gut microbiota induced by pectin may be more pronounced than those observed in other types of fibers, such as fructo-oligosaccharides or type-2 resistant starch (Chung et al. 2016; Ndeh and Gilbert 2018). These alterations in the gut microbiota composition may be attributed to cross-feeding, a critical process in the gut microbiome that establishes trophic levels.

Nonetheless, cross-feeding dynamics associated with pectic substrates remain a relatively unexplored field of research. This section aims to elucidate the intricate interactions and ecological relationships that occur within the gut microbiome, focusing on the role of cross-feeding in pectin degradation. By conducting a thorough examination and analysis, we can enhance our understanding of how cross-feeding influences microbial communities and their metabolic activity during pectin fermentation.

Textbox 1. Cross-feeding definitions (Culp and Goodman 2023).

Cross-feeding:
Process where different species or strains of microorganisms share and exchange metabolites, which serve as a source of energy and nutrients.
Public goods:
Shared resources or compounds that are produced by one or a few individuals in a community and can be utilized by other members of the community.
Mutualism:
Interaction where both species involved benefit from the relationship.
Commensalism:
Interaction where one species benefits from the relationship without harming or benefiting the other species.
Exploitation:
Interaction where one species benefits at the expense of another species.
Competition:
Interaction where multiple species compete for limited resources or metabolites.
Primary degraders:
First trophic level that breaks down complex polysaccharides into smaller sugars, serving as nutrients for other species in the community.
Primary fermenters:
Second trophic level that has the ability to either directly release the sugars obtained from the primary degraders or acquire them from other microbes in the community.
Secondary fermenters:
Third trophic level that is able to use by-products from primary fermenters in their metabolic pathways to produce SCFAs through fermentative or respiratory pathways.
H_2 consumers:
Fourth trophic level in which microorganisms utilize H_2 as an energy source in their metabolic pathway.

5.1. Primary degraders

Primary degraders at the first trophic level possess specialized enzymatic machinery to break down complex polysaccharides into simpler sugars, which serve as a nutrient source for other species within the microbial community. Primary degraders in the gut microbiome play a crucial role in the carbon food chain, and are dominated by three genera: *Bacteroides*, *Parabacteroides*, and *Prevotella*. The presence of pectin PULs, which encode a specific set of surface-exposed GHs and PLs with N-terminal signal peptidase II sequences for lipoprotein attachment, enables a species to adhere to pectic substrates and release poly- or oligosaccharides, which are considered public goods (Culp and Goodman 2023).

Primary degraders can operate through either selfish or unselfish mechanisms, further influencing cross-feeding dynamics. Certain *Bacteroides* species employ a mechanism characterized by self-interest that restricts the sharing of carbohydrate resources and minimizes the degradation of large polymers at their cell surface. This type of interaction is known as competitive interaction (Culp and Goodman 2023; Payling et al. 2020). Competitive interactions can promote the stability of microbial communities by reducing the strength of mutualism and preventing any single species from dominating the community (Coyte, Schluter, and Foster 2015). Other bacterial species act through unselfish mechanisms, encouraging the sharing and cross-feeding of carbohydrates, such as packaging GHs and PLs into outer membrane vesicles (OMVs). This type of cross-feeding has been observed in various human gut *Bacteroides* but is not universal for all species or CAZymes. Recent studies have suggested that GHs with a more acidic pKa are preferentially packaged into OMVs, although the mechanism involved remains unclear (Payling et al. 2020; Valguarnera et al. 2018).

The secretion of CAZymes to produce public goods is a type of cooperative interaction. This means that compounds and their functions are released into the extracellular environment for the benefit of the community. However, this type of interaction is also prone to cheaters consuming shared resources, without contributing to their production. This could lead to the collapse of cooperative behavior, known as exploitative interaction (Feng et al. 2022; Smith and Schuster 2019). For instance, an experiment was conducted using mono- or co-colonized mice with *Ba. thetaiotaomicron* and *E. rectale*, which are representative members of each phylum. The presence of *Ba. thetaiotaomicron* caused *E. rectale* to downregulate many of its GHs and instead upregulate oligosaccharide transporters, indicating that it utilized the products of glycan degradation from *Ba. thetaiotaomicron*. On the other hand, when *E. rectale* was present, *Ba. thetaiotaomicron* upregulated many of its PUL, including those necessary for host glycan utilization, to avoid competition for mono- and oligosaccharides (Mahowald et al. 2009).

The balance and composition of microbial communities in the human colon are influenced by variations in the expression of CAZyme genes within a species (Payling et al. 2020). Glycosidic linkages determine CAZyme specificity; not every bacterium can produce specific CAZymes that can cleave these linkages. For instance, the cleavage of both AG-I and AG-II requires the action of specific enzymes. A previous study investigated the degradation of these structures and discovered that 14 different *Bacteroides* species were able to cleave the AG-I chain containing a β-1,4 linked galactan chain (Luis et al. 2018). In contrast, AG-II contains a β-1,3 linked galactan chain and is only utilized by three *Bacteroides* species (Cartmell et al. 2018). *Ba. thetaiotaomicron*, *Ba. cellulosilyticus*, and *Ba. caccae* have been experimentally verified to cleave AG-II by exo-β-1,3-galactanases. However, it should be noted that although many pectinolytic bacteria possess enzymes of the GH43 family, many *Bacteroides* species lack the ability to produce the enzyme required for cleaving the β-1,3-galactan backbone of AG-II, as shown in Table 1. In addition, *Ba. thetaiotaomicron* and *Ba. ovatus* demonstrate a preference for products released by *Ba. cellulosilyticus* and *Ba. caccae*, respectively, providing possible examples of discrete AG structure cross-feeding niches provided by primary degraders. Therefore, degradation of this structure is limited to a few important species that possess the necessary enzymatic activity, making them crucial for microbial cross-feeding (Cartmell et al. 2018).

The enzymatic capabilities of pectinolytic organisms allow them to specialize in the degradation of specific pectin structures, contributing to the genetic and functional diversity within the microbial community (Payling et al. 2020). These diverse pectinolytic organisms interact with and stimulate each other, thereby shaping the dynamics of the microbial community. The specific degrading capacities observed in certain species involve the concept of keystone organisms, which are highly ecologically relevant. Keystone organisms are present in relatively low abundance but exert a significant stabilizing influence on their communities. These species possess unique traits that enable them to perform functions that are not easily replicated by other community members. Keystone species are vulnerable components of an ecosystem and can be easily lost (Cockburn and Koropatkin 2016). Another example of this is the presence of PULs. For instance, studies have shown that mutations in the PULs of *Ba. uniformis* can lead to significantly reduced growth of cross-feeding Bacillota species that produce butyrate, resulting in a lower overall butyrate production (Feng et al. 2022). This highlights the intricate interplay between PULs and community dynamics within the gut microbiome, further emphasizing the importance of these gene clusters in modulating metabolism and composition of the gut microbial community (Feng et al. 2022).

Primary degraders are classified as either specialists or generalists, based on their metabolic capabilities and substrate utilization patterns. Specialists, which are microorganisms with a narrower glycan degradation capacity, are mostly responsible for degrading complex carbohydrates, and tend to have smaller genomes than generalists. Generalists are microorganisms that have broad metabolic capabilities and can utilize a wide range of substrates for growth and energy production (Koropatkin, Cameron, and Martens 2012). This difference in genome size reflects their specific adaptations and streamlined metabolic capabilities (Cockburn and

Koropatkin 2016). For instance, the average genome size of Bacillota members, which are often specialists, is approximately 3.4 Mb. In contrast, members of the phylum Bacteroidota, which encompasses more generalists, have larger genomes, with an average size of 7.1 Mb. Primary degraders, such as *Eubacterium*, *Roseburia*, *Ruminococcus*, *Clostridium*, and *Bifidobacterium*, play a critical role in the cross-feeding network by initiating the degradation of complex carbohydrates, which are all Bacillota (Koropatkin, Cameron, and Martens 2012; Leitch et al. 2007).

5.2. Primary fermenters

Once the primary degraders depolymerize pectin molecules, they release various by-products, including polysaccharides, which have undergone partial degradation (Culp and Goodman 2023; Koropatkin, Cameron, and Martens 2012). These partially degraded polysaccharides could serve as nutrients for other species within the microbial community. Bacteria that utilize these released sugars or acquire them from other microbes in the community are considered primary fermenters (also called secondary degraders or cross-feeders in some studies) that belong to the second trophic level (Fan et al. 2023; Ye et al. 2022). Although the majority of saccharolytic bacteria found in the human gut are generally considered generalists, it is important to note that there is a mixture of both generalists and specialists among the primary fermenters. The primary fermenters further metabolize these substrates, contributing to the breakdown of complex carbohydrates and producing valuable end products as a result of their metabolic activities (Coyte, Schluter, and Foster 2015; Payling et al. 2020; Ye et al. 2022). Their involvement in the degradation process enhances the overall efficiency of nutrient utilization and production of beneficial compounds within the gut ecosystem.

The liberated saccharides are used in one of three upper pathways, namely the Entner-Doudoroff pathway, the EMP pathway, or the PP pathway, to produce phosphoenolpyruvate (PEP), eventually resulting in the production of SCFAs and gases such as carbon dioxide and hydrogen (Krautkramer, Fan, and Bäckhed 2021). Both types of degradation, primary degraders and fermenters, contribute to the generation of metabolic end products, including organic acids and gases. Three principal SCFAs are among the predominant organic acids. Additionally, intermediary organic acids, such as lactate and formate, and low concentrations of succinate along with acetate, are cross-fed within the microbiota. Individual bacteria typically do not ferment saccharides until the production of SCFAs but instead secrete intermediates as a result of overflow metabolism (Ye et al. 2022). The specific intermediates that are released depend on factors that optimize energy harvest and growth rate, including partial pressures of CO_2 and H_2 and redox balance (NAD+/NADH) in the cell (Coyte, Schluter, and Foster 2015; Fischbach and Sonnenburg 2011).

Cross-feeding among *Bifidobacterium* strains appears to be relatively common, as several species grow to higher cell densities, accompanied by upregulation of their respective saccharolytic pathways when grown in co-culture as opposed to their growth in monoculture. *Bifidobacterium* has been shown to exhibit a preference for short-chain molecules, indicating their specialization in the utilization of already processed carbohydrate molecules. This suggests that synergy can be achieved even among strains that target the same substrates, perhaps by specializing in the degradation of different motifs within the molecule (Culp and Goodman 2023; Rastall et al. 2022).

5.3. Secondary fermenters

By-products produced by primary fermenters in various fermentative or respiratory pathways can be utilized by secondary fermenters at the third trophic level. Secondary fermenters may be less specific to pectinolytic microbes. Metabolic intermediates are imported by secondary fermenters, leading to the production of SCFAs that accumulate at peak concentrations in the colon. Acetate is produced by many bacteria, whereas propionate and butyrate production are less widespread. In contrast, other fermentation intermediates, such as fumarate, lactate, succinate, and 1,2-propanediol, do not accumulate at high concentrations in the gut (Culp and Goodman 2023).

Acetate cross-feeding is particularly important in the context of pectin degradation, as pectin fermentation predominantly produces acetate as a metabolic by-product (Green et al. 2017; Min et al. 2015). Acetate is the most abundant SCFA in the gut and serves as a crucial precursor for the production of butyrate by butyryl-CoA:acetate CoA-transferases, as the growth of most butyrogenic bacteria relies on the availability of acetate, further emphasizing its significance in microbial metabolism, particularly in the context of pectin utilization (Canani et al. 2011; Duncan, Barcenilla, et al. 2002). Moreover, there are various bacterial species that have the capability to utilize lactate as a substrate for the production of fermentation products, including propionate and butyrate (Bourriaud et al. 2005; Morrison et al. 2006).

5.4. H_2 consumers

Finally, the molecular hydrogen produced by primary and secondary fermenters serves as an electron donor for hydrogen consumers, making up the fourth trophic level (Culp and Goodman 2023). These microorganisms utilize H_2 as an energy source in their metabolic pathways and play a crucial role in the gut microbiome metabolism (Smith et al. 2019). Downstream consumers convert H_2 to acetate, CH_4, or H_2S depending on the microorganism type. A well-known example of mutualistic interactions among gut microorganisms involves H_2-producing organisms. Many bacteria in the gut generate H_2 as an outcome of their carbohydrate fermentation process, which can then be utilized by other microorganisms, such as methanogens for the conversion of CO_2 to methane, acetogens for the production of acetate, or sulfate-reducing bacteria for the production of H_2S (Culp and Goodman 2023).

The utilization of hydrogen by these organisms not only supports their growth and metabolic activities but also has

additional benefits for hydrogen producers (Culp and Goodman 2023). The accumulation of hydrogen in the environment can have negative consequences as it inhibits the regeneration of NAD + from NADH. This inhibition disrupts the fermentation process and hinders the growth of the carbohydrate-fermenting microbes. Therefore, the removal of hydrogen through its utilization by specific functional groups alleviates the inhibitory effect on fermentation and allows for continued growth of the microbial community. This demonstrates the interconnectedness and interdependencies within the microbial ecosystem, where the utilization of metabolic byproducts, such as hydrogen, contributes to maintaining a balanced and functional community (Smith et al. 2019).

6. Discussion

This comprehensive review examines the degradation of pectin dietary fibers by the gut microbiota, specifically focusing on the role of CAZymes in this process. The correlation between specific gut microbes and their potential to produce pectinolytic CAZyme families involved in pectin degradation was investigated in our accompanying review "Structure and degradation dynamics of dietary pectin". By cross-referencing the CAZY database, we created CAZyme family profiles for the most common pectin-degrading microbes, which were then compared with experimental data from scientific literature. A CAZyme family profile helps to predict whether microbes may act on a specific dietary fiber such as pectin by detailing the presence and activity of specific CAZymes that are essential for breaking down pectin (Yüksel, Kort, and Voragen 2024). Moreover, the impact of pectin degradation on microbial ecology and SCFA production highlights existing knowledge gaps in predicting the effectiveness of pectin supplementation. By assessing an individual's gut microbiota composition and their capacity to produce pectinolytic CAZymes, we could produce CAZyme family profiles and predict their ability to degrade pectin as well as other types of dietary fibers. This personalized approach allows for tailored dietary fiber interventions and supplementation strategies, enhancing their effectiveness and optimizing health outcomes. Interestingly, the CAZyme profiles revealed gaps in the potential of pectinolytic CAZyme families. For example, only *Bacteroides* species and *L. eligens* (Bacillota) are linked with pectate lyases (PLs). *Bifidobacterium* species show CAZyme families associated with side chain degradation but lack those targeting the pectin backbone (Chung et al. 2017; Holck et al. 2011; Onumpai et al. 2011; Sulek et al. 2014). Discrepancies between experimental degradation profiles and CAZyme family profiles suggest either novel CAZyme functions or gaps in the database (Ndeh et al. 2017).

The significance of import systems and pectin oligosaccharide recognition cannot be overstated as they play pivotal roles in shaping the dynamics of cross-feeding interactions within microbial communities. Since a cross-feeding model is not only reliant on degrading enzymes, but also on the import of mono- and oligosaccharides, recipient species must possess the necessary genetic and transporters tailored to the utilization of sugar components derived from pectin (Culp and Goodman 2023). These processes are crucial for determining which species function as primary degraders or primary fermenters of pectin. The interplay between import systems and pectin oligosaccharide recognition ultimately determines the ecological roles of different microbial species within the community, influencing their ability to degrade or ferment pectin and contributing to the overall community metabolism. The current understanding of pectin uptake, particularly the recognition and internalization of pectin oligosaccharides, remains limited. Future research could address the technical challenges associated with studying complex carbohydrate-protein interactions. Pectin oligosaccharides are heterogeneous in structure and require sophisticated analytical techniques and specialized tools to accurately characterize and quantify their interactions with microbial receptors or transporters. Furthermore, the diversity of microorganisms present in the gut adds another layer of complexity, as various species or strains may possess unique recognition systems for pectin oligosaccharides.

Another factor to consider when constructing a cross-feeding model is that the strategies used by primary degraders to break down pectin influence the composition and availability of the resulting breakdown products, which in turn affects their suitability as cross-fed substrates for other members of the microbial community (Culp and Goodman 2023). Despite the efficient breakdown of pectin by specialized microorganisms in the gut, a significant portion of the resulting monomers and oligomers is often released into the environment. Moreover, *Bacteroides* species that encode the same PULs may release different types, amounts, and proportions of mono- or oligosaccharides during pectin degradation (Rakoff-Nahoum, Coyne, and Comstock 2014). The released pectin serves as a potential substrate for other microorganisms within the gut community that do not have pectin-degrading capabilities. Other factors that influence the proportions of different fermentation end products are the growth conditions of microorganisms, including whether they are grown in pure culture or co-culture. The rate of microbial growth, levels of CO2 in the environment, and availability of amino acids also influence the fermentation process (Adamberg et al. 2014). These factors can affect the metabolic pathways utilized and the efficiency of energy conversion, ultimately affecting the balance of the end-products produced. Variations in the released products can affect the dynamics and efficiency of cross-feeding interactions, influencing the overall metabolic capabilities and ecological roles of different microbial species within the community. Consequently, pectin plays an indirect role as a substrate for a broad range of microorganisms, thereby contributing to the complexity of the microbial trophic chain in the gut. It is crucial to consider the involvement of non-pectin-degrading microorganisms in pectin metabolism as they have the potential to impact the overall dynamics and functioning of the gut microbiota. Currently, there is limited knowledge regarding the specific cross-feeding behavior of pectin degradation.

Notably, the presence of a specific CAZyme family in a pectinolytic bacterium using our approach does not guarantee

the production of a specific CAZyme capable of degrading pectin in the gut. Despite this limitation, the approach used in this study offers insight into the enzymatic potential of pectinolytic bacteria in the gut microbiota. Moreover, we did not utilize computational techniques to analyze and interpret the data. For computational degradation patterns to be effective, we need a comprehensive list of CAZymes present in the human gut environment, along with the bacteria that produce them. This would allow us to link these CAZymes to the structures found not only in pectin, but also in a broad range of dietary fibers. Computational techniques highlight the potential application of degradation patterns to dietary fiber-rich foods in a broader range of microorganisms, together with the integration of diverse data types, such as metagenomics, metatranscriptomics, and metabolomics. Metatranscriptomics, in particular, holds promising potential for achieving these results, as it enables us to identify individual CAZymes present in the human gut environment and understand which groups of microorganisms respond to pectin or other fiber supplementation. Comparing the microbial composition, diversity, and functional potential across different fibers can provide a comprehensive understanding of how different fiber types influence the gut microbiome. Using this approach, researchers can identify key microbial taxa that are responsive to specific fiber types and metabolic pathways involved in fiber fermentation. By examining the changes in microbial community structure and function in response to different fiber interventions, a better understanding of the specific effects of various fibers on the gut microbiome can be obtained.

The TNO Intestinal Model (TIM), an advanced *in vitro* simulation model of the human gut, is recommended for studying digestion and fermentation processes (Larsen, Bussolo de Souza, et al. 2019). TIM consists of compartments that mimic different regions of the gastrointestinal tract, facilitating the simulation of specific conditions and processes. Previous studies using TIM have examined the relationship between pectin structural properties, gut microbiome composition, and SCFA production using different pectin sources (Larsen, Bussolo de Souza, et al. 2019). However, it would also be interesting to use the TIM method to design experiments to analyze the metagenome and explore the microbial shift after pectin supplementation. The effectiveness of the fermentation process can be assessed by measuring the release of metabolites such as SCFAs. Moreover, the model allows researchers to introduce specific components into colon compartments to monitor degradation processes at colon-specific locations. This approach provides insights into the spatial distribution of pectin-degrading microbes and their activity in the colon.

Another promising method could involve the further utilization of genomic analysis to identify genes encoding CAZymes by analyzing PULs. This method was used in previous investigations and resulted in newly discovered CAZymes (Chung et al. 2017). This approach can provide valuable insights into the genetic potential of microorganisms for pectin degradation. By examining the presence and diversity of PULs in the microbial genomes, researchers can predict the ability of various isolates from the gastrointestinal tract to degrade pectin. Researchers have already explored the pectin-degrading abilities of specific isolated bacterial strains using pectin components as substrates in their experiments (Ndeh et al. 2017). However, food contains a variety of fibers with distinct structures such as cellulose, hemicellulose, pectin, and resistant starch (Ye et al. 2022). Consuming a diverse range of fiber types promotes a more diverse and robust gut microbiome, leading to the production of a wider spectrum of beneficial metabolites. Future studies could explore the use of co-culture systems to investigate the degradation dynamics of these structures (Bayliss and Houston 1984). By co-cultivating pectinolytic organisms from human feces in the presence of pectin as the main carbon source, researchers can simulate realistic conditions that mimic microbial interactions occurring in the gastrointestinal tract. Co-culture studies can reveal the synergistic or competitive interactions between different microbial species and shed light on the dynamics of pectin degradation within a complex microbial community.

Construction of pectinolytic capacity profiles for human gut bacteria represents a significant early phase in personalized nutrition, highlighting its potential implications in various scientific domains. Understanding this capacity could drive advancements in probiotic and prebiotic development by enabling the design of targeted interventions to improve digestion and the gut microbial balance. Additionally, knowledge of pectin degradation capabilities can guide personalized dietary recommendations to optimize the utilization of dietary fiber. Manipulating the pectinolytic capacity of the gut microbiota can influence the microbial composition and metabolic activity, leading to desirable shifts in the microbial community. Overall, understanding the pectinolytic capacity of human gut bacteria will contribute to personalized interventions, improved dietary strategies, disease management, modulation of the gut microbiota, and biotechnological applications, leading to advancements in gut health and overall well-being.

7. Conclusion

Our comprehensive review extensively explores the intricate domain of pectin degradation by the gut microbiota, emphasizing its profound potential for promoting health. We established a robust correlation between specific gut microbes and their intrinsic capacity to produce pectinolytic enzymes, bolstering our findings by rigorous cross-referencing with the CAZY database.

Emphasizing the imperative need to delve into cross-feeding interactions and harness advanced computational techniques for data analysis, we also shed light on the indirect role of pectin as a substrate for non-pectin-degrading microbes and advocate standardized extraction methods and efficient extraction processes. In conclusion, the comprehension of pectinolytic capacity has far-reaching implications, such as personalized dietary interventions to improve gut health and overall well-being. Future studies should investigate the recognition and transport mechanisms of pectin

oligosaccharides within gut microbes, and formulate theoretical profiles of bacteria attainable through fiber supplementation. Moreover, incorporating computational methods with metatranscriptomics could help to identify active CAZymes and responsive microorganisms, enabling personalized dietary recommendations based on pectinolytic capacity.

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