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Pectin in diet: Interactions with the human microbiome, role in gut homeostasis, and nutrient-drug interactions

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ABSTRACT

Pectins are a part of daily diet as well as food additives that are indigestible polysaccharides by human enzymes, however, they can be easily degraded by gut bacteria with the production of short chain fatty acids (SCFAs). Knowledge of pectin gut homeostasis and further how pectin affect gut bacterial communities is insufficient and limited. This review focuses on providing the whole story of how pectin functions as prebiotics in the gut. Understanding the interplay between functional and immunological responses inside animal or human gut as influenced by pectin in diets is provided. The interaction between pectin and gut microbiota is presented from both sides, in terms of how pectin affects gut microbiome and or the fermentation products produced in response by gut bacteria. This knowledge can be used to define preferred dietary pectins, targeting beneficial bacteria, and favoring balanced microbiota communities in the gut to maximize pectins' health benefits.

1. Introduction

Pectins are naturally occurring anionic heteropolysaccharide polymers that are rich in polygalacturonic acid. Pectins are especially abundant in fruits *e.g.* apples, plums, pears and citrus from which it is extracted and used as a gelling agent (Renard, Crépeau, & Thibault, 1995). In addition to vascular plants, pectin is also abundant in the cell wall of Streptophyta and green algae (Chlorophyta), members of which have a global distribution in aquatic freshwater and marine habitats (Domozych, 2001).

Structurally, pectin is one of the most complex naturally occurring plant polysaccharides (Mohnen, 2008). Homogalacturonan (HG) is the most abundant form of pectin, representing up to 60–65 % of pectin (Mohnen, 2008). HG is a polymer of α-1,4-linked D-galacturonic acid units. The galacturonic acid backbone in HG could be methyl esterified at *O*-6 and at *O*-2, or *O*-3 positions. Rhamnogalacturonan I is the second most abundant type of pectin, constituting 20–35 % of pectin (Mohnen, 2008). The backbone in Rhamnogalacturonan I (RG-1) are alternating alpha linked D-galacturonic acid and L-rhamnose. Side chains of arabinan, galactan, or arabinogalactan occur in approximately half the L-rhamnose subunits in RG-I; these units are substituted at the *C-4* position with side-chains. Finally, the most complex type of pectin is Rhamnogalacturonan II (RG-II), which constitutes approximately10 % of pectin. Its structure is largely conserved across plant species and consists of an oligo-HG decorated with side branches of 13 different types of sugars in 21 different linkages (Ndeh et al., 2017). HG backbone is often heavily substituted giving rise to additional pectin motifs such as xylogalacturonan, apiogalacturonan-1, galactogalacturonan or arabinogalacturonan.

Pectin is a non-digestible carbohydrate, and its degradation in the human GIT is mediated by a fraction of the microbial community residing in the human intestine (Fig. 1). The prevailing anaerobic conditions in the human GIT tract promotes incomplete fermentative degradation processes, leading to the production of fermentation end products *e.g.* short chain fatty acids (SCFA). As such, the pectin molecule as well as its partial and complete degradation products interact with human GIT cells and mediate a wide range of physiological and immunological functions. Indeed, pectin is increasingly recognized to play an important role in gut inflammation, immunomodulation, and drug/nutrient interactions and have been promoted as a diet supplement for improving cholesterol levels, lowing blood pressure, and promoting overall gut health.

As an indigestible substrate, pectin represents an excellent model to assess human-microbiome interactions and how the human cells and the

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human microbiome act synergistically as a single holobiome unit for the host's benefit. Prior microbiome-oriented studies have assessed mechanisms of pectin degradation in the GIT and the impact of pectin ingestion on the microbial community. Similarly, physiology/human/pharmacology-oriented studies have examined how pectin modulates human heath at the cellular levels. However comprehensive efforts to link microbial metabolism of pectin in the human GIT, with pectin (and its products) impact on the physioloigical/pharmacological levels have been sparse.

Here, we aim to provide a holistic overview of pectin's fate and impact on the human GIT. We follow the fate of pectin post ingestion and evaluate the microbial communities mediating its degradation in the human GIT. Subsequently, we examine how pectin and its products, generated by microbial degradation, impact gut inflammation, immunology, nutrient and drug absorption. The review hence aims to bridge a gap between microbiome research and human physiology and highlight the interconnectivity between dietary intake, microbiome community structure and activity, and human health effects.

2. Microbial degradation of pectin

2.1. Overview of pectin turnover in nature

Pectin turnover represents a crucial process in global carbon cycling not only in the human GIT tract, but also in a wide range of terrestrial, plant-rich habitats *e.g.* forests (Yeager et al., 2017), as well as marine and aquatic environments where green algae survives and blooms (Hallegraeff, 1993; Hoshaw & McCourt, 1988). Within the microbial world, an arsenal for pectin degradation enzymes have been identified and characterized from a wide range of pectinolytic organisms (Ndeh & Gilbert, 2018). As well, studies on genetic regulation of pectin degradation and epigenetic, post-transcriptional and translational regulation have also received considerable attention in the last few years (Benoit et al., 2012; Kabisch et al., 2014). However, it is important to note that such studies have been conducted on a relatively small number of model pectinolytic organisms *e.g.* the commensal bacterium *Bacteroidetes thetaiotaomicron* (Phylum Bacteroidetes) from the human GIT (Dongowski, Lorenz, & Anger, 2000; Xu et al., 2003), the phytopathogenic *Erwinia chrysanthemi* (Phylum Proteobacteria) from soil and plant surfaces (Abbott & Boraston, 2008), and members of the fungal genus *Aspergillus* from terrestrial habitats (de Vries & Visser, 2001; Yadav et al., 2017). Due to its structural complexity, pectin degradation by microorganisms requires the production of multiple distinct carbohydrate active enzymes (CAzymes) for backbone depolymerization and side chain removal during pectin degradation. Indeed, a significant body of knowledge on the various biochemical aspects of pectin degradation by various classes of pectinolytic enzymes (*e.g.* substrates range, physiological preferences, induction patterns, kinetic properties, catalytic mechanisms) has been accumulating (Ndeh et al., 2017; Pickersgill, Smith, Worboys, & Jenkins, 1998).

2.2. Pectin degradation in the human GIT tract

Pectin is an integral component of the human diet. However, as a non-digestible plant polysaccharide, human digestive enzymes are incapable of breaking down the absolute majority of glycosidic linkage within pectin molecules (Fu, Liu, Zhu, Mou, & Kong, 2019). As such, pectin degradation is achieved *via* the diverse microbial community residing in the human intestine (Fig. 1). Arriving almost intact to the large intestine, the ingested pectin is attacked by the relatively small fraction of the ≈500–1000 distinct species typically residing in the intestine that possess the enzymatic machinery necessary for pectin degradation. The large MW of plant polysaccharides, including pectin, precludes the direct uptake of intact molecules for intracellular degradation. Rather, pectin degradation process is initiated by the secretion of extracellular cell-free and cell-bound enzymes for the depolymerization of pectin backbone, as well as the removal of accessory side chains, resulting in the production of mono/oligosaccharides for subsequent uptake and intracellular catabolism.

The human intestinal microbiome represents the largest mass and concentration of microorganisms in the entire body, with estimates of 10^{14} cells, 0.2 Kg total mass, and 500–1000 distinct microbial species

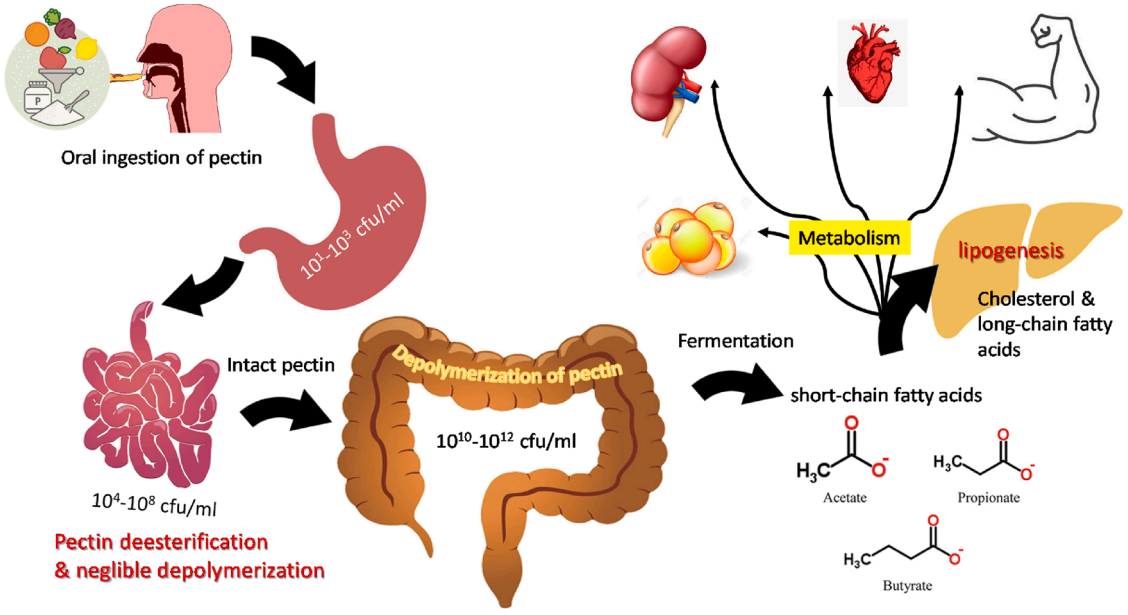

Fig. 1. Overview of Pectin metabolism. Pectin (ingested as part of the human diet or as a dietery supplement) passes through the stomach, small, then large intestine. The stomach and small intestine microbial community do not degrade or modify the ingested pectin to any extent except for some deesterification, reaching almost intact to the large intestine. Pectin depolymerization to sugar monomers, and subsequent fermentation of sugars occurs in the large intestine, in which short chain fatty acids (SCFAs) are generated that are subsequently utilized by the liver for lipogenesis as a substrate for the synthesis of cholesterol and long-chain fatty acids and as a cosubstrate for glutamine and glutamate synthesis. Other tissues including heart, adipose tissue, kidney, and muscle metabolize the remainder of SCFAs.

per individual (Huttenhower et al., 2012; Sender, Fuchs, & Milo, 2016; Qin et al., 2010). Representatives of 12 different phyla have been identified in humans GIT tract, with members of two phyla (Bacteroidetess & Firmicutes) usually representing 50–70 % of the microbial community; and four phyla Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria typically representing >95 % of the microbial community (Arumugam et al., 2011; Lopetuso, Scaldaferri, Petito, & Gasbarrini, 2013). Due to the anoxic conditions and dearth of alternate electron acceptors in the human intestine, fermentative -rather than respiratory- processes predominate in the human gut, with strict anaerobic bacteria outnumber aerobic and facultative anaerobic bacteria by 100 to 1,000-fold (Sommer & Bäckhed, 2013). Fermentative breakdown of non-digestible carbohydrates (including pectin) leads to the formation of a wide range fermentation end products, mainly short-chain fatty acids (SCFAs) that are absorbed by the host cells as a source of energy, as well as mediate a wide range of physiological effects to be discussed in the next sections (Fig. 2).

3. Pectinolytic organisms in the human GIT tract

The involvement of microorganisms in the degradation of dietary pectin as well as the localization of the process in the human colon has been observed as early as 1941 (Werch & Ivy, 1940). Subsequent studies have obtained pure cultures of pectinolytic organisms from human feces either by enriching and isolation on growth media containing pectin as the sole/main carbon source (Baylisss & Houston, 1984; Jensen & Canale-Parola, 1985) or testing strains previously isolated general media for their capacity for pectin degradation (Benítez-Páez, Gómez del Pulgar, & Sanz, 2017; Centanni et al., 2019; Ndeh et al., 2017; Salyers, West, Vercellotti, & Wilkins, 1977). Recently genomic analysis has been used to identify carbohydrate active enzymes (CAZymes) genes and predict capability to degrade pectin in multiple GIT isolates (Chung et al., 2017). So far, the capacity of pectin degradation has convincingly been demonstrated on members of the phyla Bacteroidetes and Firmicutes (Table 1).

3.1. Pectin uptake by phylum Bacteroidetes

Bacteroidetes species shown to degrade pectin (Table 1) overwhelmingly belong to the genus Bacteroides, one of the most abundant bacterial genera in the human GIT tract (Arumugam et al., 2011). Members of the genus Bacteroides are known to play a central role in the degradation of a wide range of plant polymers in the GIT and to possess a wide range of CAZymes in their genomes (Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013).

A wide range of organisms belonging to the Bacteroidetes possess a demonstrated capacity for pectin degradation (Table 1). B. *thetaiotaomicron* is one of the most abundant microorganisms in the human GIT (Arumugam et al., 2011), and has long been utilized as a model species for understanding the physiological preferences, biochemical capacities, and ecological role of the GIT Bacetorides (Xu et al., 2003). Experimental studies have shown its ability to grow on a wide range of polygalacturonas and rhamnogalacturonans, as well as pectin preparations extracted from various sources *e.g.* citrus pectin preparations with different degrees of methylation (Chung et al., 2017; Dongowski et al., 2000; Martens et al., 2011; Ndeh et al., 2017; van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000). Enzymatic studies and genomic analysis have uncovered its possession of wide range of genes and enzymes mediating for the degradation of various pectic entities *e.g.* GH28 polygalacturonase, GH78 α-L-rhamnosidase, GH105 unsaturated rhamnogalacturonyl hydrolase, GH106 α-L-rhamnosidase, PL1 pectin lyase, PL9 Pectate lyase, PL10 pectate lyase, PL11 Ramnogalacturonan lyase, and carbohydrate esterases (CE) families CE8 pectin methylesterase, and CE12 pectin/rhamnogalacturonan acetylesterase. In addition to PG and

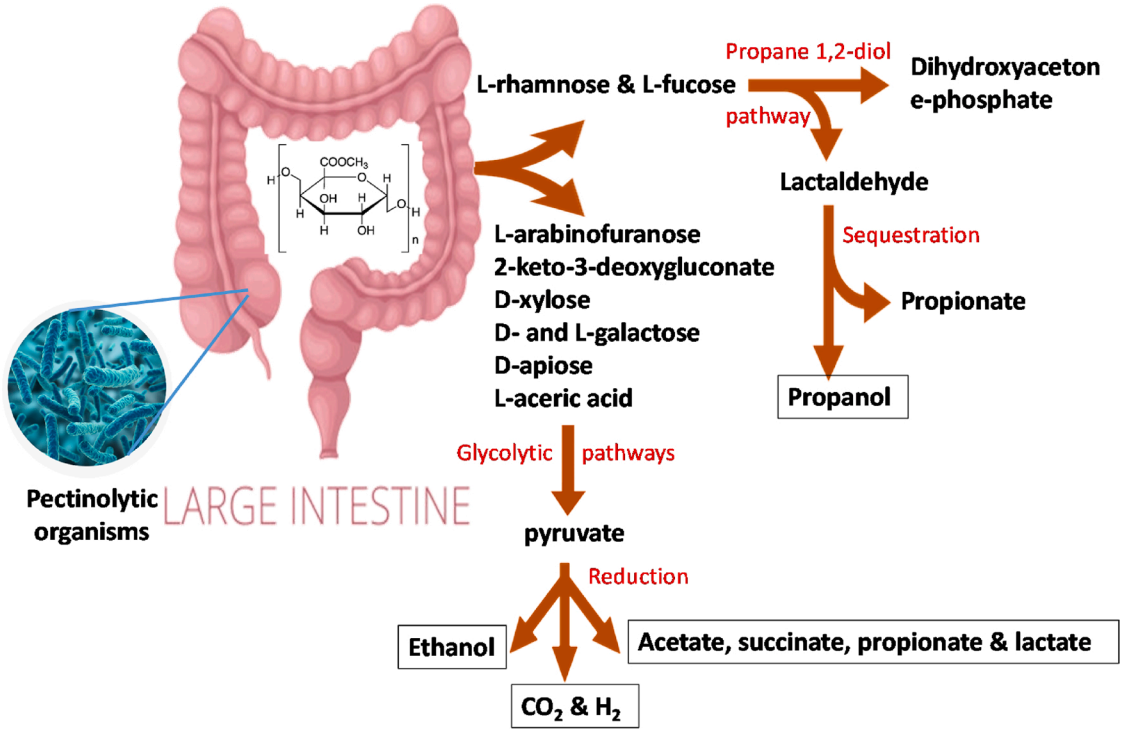

Fig. 2. Outline of the pectin degradation processes by the human intestinal microbiota. Pectinolytic microorganisms belonging to the phyla Firmicutes and Bacteroidetes (Table 1, Sections 3.1 and 3.2) utilize extracellular cell-free and cell-bound enzymes for the depolymerization of pectin backbone, as well as the removal of accessory side chains. The produced sugars and sugar acids (L-rhamnose & L-fucose, L-arabinofuranose, 2-keto-3-deoxygluconate, D-xylose, D- and L-galactose, D-apiose, L-aceric acid) serve as a carbon and energy source not only to pectinolytic members of the community, but also by the wider cohort of secondary degraders within the intestinal microbiome. Fermentative degradation pathways are the predominant mechanism for sugars-degradation in the human GIT, resulting in the production of SCFAs, ethanol, CO_2, and H_2 as final end products highlighted in black boxes.

Table 1
List of pectinolytic microorganisms in isolated from the human GIT tract.

Organism	Pectin type (Reference)
Phylum Bacteroidetes	
Bacteroides pectinophilus	- Polygalacturonic acid (Jensen & Canale-Parola, 1985; Centanni et al., 2019) - Pectin*, (Jensen & Canale-Parola, 1985, 1986) - Pectin from spinach and Karaka berries (Centanni et al., 2019)
Bacteroides galacturonicus	- Pectin* (Jensen & Canale-Parola, 1985; Jensen & Canale-Parola., 1986) - Polygalacturonate (Jensen & Canale-Parola, 1985)
*Parabacteroides distasonis***	Pectin from citrus fruit (Baylisss & Houston, 1984)
Bacteroides ovatus	- Pectin from citrus fruit (Baylisss & Houston, 1984) - Pectin from spinach and Karaka berries (Centanni et al., 2019) - Polygalacturonate(Centanni et al., 2019; van Laere et al., 2000; Martens et al., 2011) - RhamnogalacturonanI(Martens et al., 2011) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides thetaiotaomicron	- Citrus pectin (Gerhard Dongowski et al., 2000) - Homogalacturonan (Martens et al., 2011) - RhamnogalacturonanI (Martens et al., 2011) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides xylanisolvens	- Polygalacturonan, rhamnogalacturonanI and pectin* (Chung et al., 2017)*** - Pectin and galacturonic acid (Chassard, Delmas, Lawson, & Bernalier-Donadille, 2008) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides dorei	- Polygalacturonan, rhamnogalacturonanI and pectin* (Chung et al., 2017)*** - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides vulgatus	Polygalacturonan, rhamnogalacturonanI and pectin* (Chung et al., 2017)***
Bacteroides cellulosilyticus	- Pectin from citrus (Robert, Chassard, Lawson, & Bernalier-Donadille, 2007) - Pectin from spinach and Karaka berries (Centanni et al., 2019) - Polygalacturonate (Centanni et al., 2019) - -Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides Finegoldii	- Pectin from spinach and Karaka berries (Centanni et al., 2019) - Polygalacturonate (Centanni et al., 2019) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides intestinalis	- Pectin from spinach and Karaka berries (Centanni et al., 2019) - Polygalacturonate (Centanni et al., 2019) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides stercoris	- Pectin from spinach and Karaka berries (Centanni et al., 2019) - Polygalacturonate (Centanni et al., 2019) - Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Bacteroides salyersae, B. nordii, B. caccae, B. eggerthii. B. massiliensis, B. uniformis	Rhamnogalacturonan II (red wine and apple) (Ndeh et al., 2017)
Phylum Firmicutes	
Eubacterium eligans	- Pectin*, polygalacturonate (Salyers et al., 1977) - Polygalacturonan, rhamnogalacturonanI and pectin* (Chung et al., 2017)***
Faecalibacterium prausnitzii	Apple pectin (Lopez-Siles et al., 2012)
Monoglobus pectinilyticus	Kiwifruit pectin (Kim et al., 2017)

* Identity, origin, composition of pectin not specified in publications.
** Originally named Bacteroides distasonis, and subsequently reclassified *Parabacteroides distasonis*.
*** Genomic based prediction, no experimental evidence.

RG1, a remarkable capacity was recently uncovered in the *Bacteroides* regarding the ability of multiple Bacteroides spp. to metabolize RGII in pure culture. RGII is widely spread in nature, being a component of the primary cell wall of a wide range of angiosperms, gymnosperms. Structurally, RGII is the most complex plant polysaccharide in nature containing 13 different sugars and 21 different glycosidic linkages in six different side chains (Ndeh et al., 2017). Uncertainty about the identity of organism(s) mediating RGII turnover in nature has long prevailed, specifically whether the process of RGII deconstruction is mediated by a single organisms or microbial consortia in a regionally selective and temporally sequential manner. Work by Martens et al. clearly demonstrated the capacity of the human intestinal organisms *B. thetaiotaomicron* and *B. ovatus* to grow on RGII as the sole carbon source, suggesting their capacity to utilize at least components of RGII for its growth (Martens et al., 2011). Subsequently, Ndeh et al. (Ndeh et al., 2017) demonstrated that multiple Bacteroides strains could grow on RGII (Table 1). Using *B. thetaiotaomicron* as a model organism, the authors proceeded to identify enzymatic activities, and corresponding genes organized in three genomic loci, necessary for the breakdown of 20 out 21 glycosidic bonds in this molecule and in the process, identify and characterize seven new glycoside hydrolase families (GH137-GH143).

3.2. Pectin uptake by Bacteroidetes spp

Large polysaccharides, including pectin could not be directly imported into the microbial cell without prior breakdown to its sugar monomers/oligomers. Therefore, beyond mere possession of genes encoding pectinolytic activities, distinct strategies for pectin foraging, binding, extracellular breakdown of the polymer, and transport of degradation product into the intracellular space should be employed to maximize efficiency. The Bacteroides utilize sus-like systems for plant polysaccharide acquisition and degradation (Tancula, Feldhaus, Bedzyk, & Salyers, 1992). The system, originally identified as part of *B. thetaiotaomicron* starch degradation machinery, encodes a membrane protein mediating substrate attachment to the outer membrane, binding of extracellular and periplasmic enzymes to the substrate, and sugar transport of sugar products across the outer membrane to the periplasm (Salyers et al., 1977). Subsequent research has demonstrated that *sus* systems are not only employed for starch degradation, but rather for a wide range of plant polysaccharides including pectin (Collins et al., 1994). Within bacterial genomes, *sus* genes are present as part of polysaccharide utilization loci (PUL); clusters of polysaccharide catabolizing genes that are prevalent in Bacteroidetes genomes (Lapébie, Lombard, Drula, Terrapo, & Henrissat, 2019). In *B. thetaiotaomicron*, 88 polysaccharide utilization loci were identified, comprising 18 % of all genes in the genome. In addition to the *sus* genes for substrate binding and transport, these loci contain two-component histidine kinase signal transduction systems, sigma and anti-sigma factors, and genes encoding various carbohydrate-binding enzymes for plant polysaccharide deconstruction (Collins et al., 1994; Xu et al., 2003). The activation of entire machinery encompassed within a specific PUL ensures the organism's timely possession of all enzymatic and structural components necessary for efficient degradation of a sensed substrate with minimal loss/leakage to other members within the community. Transcriptional studies have been utilized to identify the involvement of specific PUL in specific substrates degradation, and 3–4 loci have been implicated in pectin degradation in *B. thetaiotaomicron* (Ndeh et al., 2017; Xu et al., 2003).

3.3. Pectin uptake by Phylum Firmicutes

Bacteroidetes are generally regarded as the more dominant plant polysaccharides degraders in the human GIT. In addition to experimental evidence, Bacteroidetes genomes are larger in size, compared to members of the phylum Firmicutes, and encode on average a higher number of GH and PL enzymes (137.1 per genome) when compared to Firmicutes (39.6 per genome) (Kaoutari et al., 2013). Nevertheless, several members of the phylum Firmicutes have also been shown to utilize pectin (Table 1). The relative contribution of Bacteroidetes *versus* Firmicutes for the *in-situ* pectin degradation and how different dietary, life-style, and health impact such balance between pectinolytic members of both phyla is yet unclear. The first member of the Firmicutes isolated from human feces shown to metabolize pectin was *Eubacterium eligens* (Salyers et al., 1977) (family Eubacteraceae, order Eubacterales, Class Clostridia), a member of the Clostridium group XIV or *Clostridium coccoides* group (as per the widely proposed classification outlined in Collins et al. (Collins et al., 1994) (Table 1). Such capacity has been further confirmed by genomic analysis (Chung et al., 2017). Subsequently, the ability of *Faecalibacterium prausnitzii* (Originally described as *Fusobacterium prausnitzii)* to grow on apple pectin has also been reported (Lopez-Siles et al., 2012). Members of the genus *Faecalibacterium* (Family *Oscillospiraceae,* Order *Eubacteriales,* Class *Clostridia)* are members of Clostridium group IV, also known as *clostridium leptum* group (as per (Collins et al., 1994)) (Table 1). The genus is one of the most abundant microbial genera identified in the human feces (Arumugam et al., 2011). Recently, a novel pectinolytic microorganism was isolated from human feces (Kim et al., 2017), and phylogenetic analysis supports its accommodation into a new genus and species *Monoglobus pectinilyticus* gen. nov., sp. nov. within the family Oscillospiraceae, Order Eubacteriales, Class Clostridia (Table 1). A subsequent study has identified the occurrence of *M. pectinilyticus* in fecal samples from 10 out of 44 random healthy subjects in New Zealand, and quantitative PCR analysis estimated at a mean relative abundance of 0.3 % (Kim et al., 2019).

3.4. Pectin uptake by Firmicutes spp

Uptake and foraging of glycans by the gram positive Firmicutes have been extensively investigated, driven by the growing interest in utilizing various cellulolytic and hemicellulolytic clostridia in biofuel production from crops and lignocellulosic biomass. Surprisingly, little research has been conducted on pectin or glycan foraging by specific Firmicutes strains residing in the human GIT. A recent study investigated the pectinolytic machinery in *M. pectinilyticus* provided important novel insights into the process (Kim et al., 2019). The genome of *M. pectinilyticus* encodes 48 different enzymes putatively implicated in pectin degradation with products listed in Table 1. Interestingly, most polysaccharide lyases (PLs) as well as carbohydrate esterases (CEs) CE8 and CE12 genes possess a signal peptide, suggesting their extracellular localization and involvement of initial attack on pectin molecules; while most poly/-rhamnogalacturonyl hydrolases (GH28 and GH105) appear to be located intracellularly, suggesting their involvement in oligomers degradation post uptake. 42 Genes contained S-layer homology (SLH) domains, 8 of which were associated with pectin-degrading enzymes. SLH proteins have previously been shown to mediate cell surface attachment of CAZymes to the cell surface in a wide range of Firmicutes (Conway et al., 2016; Fuchs, Zverlov, Velikodvorskaya, Lottspeich, & Schwarz, 2003) and the involvement of implicated CAZymes, including those harboring SLH domains have further been confirmed by comparative proteomic analysis. In general, such strategy of sequestering extracellular enzymes involved in polymer degradation by attachment to surface layer proteins, and sugar uptake primarily *via* dedicated ABC transporters is widely employed by anaerobic plant polymer degrading organisms in nature *e.g.*, members of the order Clostriales (Lynd, Weimer, van Zyl, & Pretorius, 2002), and this study confirms the importance of such strategy in pectin degradation by a model human GIT Firmicutes.

Sugar monomers and oligomers generated extracellularly are imported mainly *via* ABC (ATP-binding cassette) transport systems in Firmicutes. The genome of *M. pectinilyticus* (Kim et al., 2019) and multiple additional Firmicutes (Kaoutari et al., 2013; Cerisy et al., 2019) encodes a large number of ABC transporters that are co-localized with CAZyme genes in the genome. Indeed, the role of many of these ABCs for specific sugars uptake has been confirmed by transcriptomic analysis and mutation studies (Cerisy et al., 2019). This attests to the importance of ABC transport systems for THE uptake of pectin products, as opposed to the alternate sympott/antiport or phosphotransferase (PTS) uptake mechanisms.

3.5. Could cellulosome-harboring microorgansisms be involved in pectin degradation in the human GIT?

Another tantalizing, yet-unproven, possibility is the involvement of cellulosomes in pectin degradation in human GIT strains. Cellulosomes are extracellular structures that harbor multiple extracellular enzymes bound to scaffoldins (Lamed, Setter, & Bayer, 1983). These efficient cell surface-attached structures are produced by some members of the Firmicutes as well as members of the anaerobic gut fungi in the Phylum Neocallimastigomycota (Lamed et al., 1983; Youssef et al., 2013). Many key organisms involved in plant biomass production in the bovine rumen and other environment relies on their cellulosomes as a unit to bind, degrade, and uptake substrates (Bensoussan et al., 2017). Within the human GIT, *Ruminococcus champanellensis* is the only organism, to our knowledge, that possesses a cellulosome. *R. Champanellensis* is capable of cellulose degradation, but not pectin (Chassard, Delmas, Robert, Lawson, & Bernalier-Donadille, 2011; David et al., 2015). However, it is interesting to note that metagenomic and metatranscriptomic studies of human fecal samples often yield genomic fragments harboring genes encoding cohesion and dockerin domains, the hallmark structural features within a cellulosome (Turnbaugh et al., 2010; Collins et al., 1994). Remarkably, these fragments are often affiliated with the genera *Faecalibacterium, Eubacterium* and *Ruminococcus*, the first two of which include known pectin-degrading members as described earlier. Direct analysis of the pectin lysis activity of *Ruminococcus champanellensis* likewise to its cellulosome activity has yet to be reported using either *in vitro* or *in vivo* assays to be conclusive. Nevertheless, such epigenetics proof can help in characterizing organisms of unknown metabolic functions.

3.6. Are there novel pectinolytic organisms residing in the human GIT tract that has yet to be discovered?

The variation in microbiome structure due to food intake variation between individuals and across cultures, the highly dynamic nature of the human microbiome, and the relatively limited number of studies attempting to isolate pectinolytic organisms from the human GIT strongly suggest that new genera (and certainly new species) are yet indeed to be isolated and characterized from the human GIT. Further, it is important to note that isolation only captures a fraction of microbial communities within an ecosystem. While the proportion of cultured to uncultured taxa appear to be higher in the human microbiome as compared to other ecosystems *e.g.* soils, marine sediments (Lloyd et al., 2018 mSystems.00055-18), there still remains a wide range of yet-uncultured taxa within the human GIT (Fodor et al., 2012). Alternatively, mining metagenomic datasets for fragments containing pectinases could be used to identify putatively novel organisms mediating pectin degradation in the human GIT.

4. Fermentation of pectin sugar monomers and the production of SCFA in the human GIT

Pectin degradation, mediated by pectinolytic organisms (discussed in the previous section) results in the formation of a wide range of sugar monomers are generated from pectin breakdown to include 2-keto-3-deoxygluconate, (from glucoronic and galacturonic acids), L-rhamnose, L-fucose, L-arabinofuranose, D-xylose, D- and L-galactose, D-apiose, and L-aceric acid (Fig. 2). The majority of these sugars are channeled to central glycolytic pathways (Embden-Meyerhoff-Parnas) pathway for hexoses, and pentose phosphate pathway for the pentoses xylose and arabinose for the production of pyruvate (Fig. 2). However, an important exception is L-rhamnose and L-fucose, the metabolism of which proceeds *via* a distinct pathway (propane 1,2-diol pathway) that involves the formation of lactaldehyde and dihydroxyacetone-phosphate, with the subsequent sequestration of lactaldehyde into an intracellular micro compartment and formation of propanol and propionate as a final end product. Such unique pathway and spatial sequestration is dictated by the formation of toxic propionaldehyde as an intermediate (Havemann & Bobik, 2003; Petit et al., 2013). Pyruvate, acting as an electron acceptor, is further reduced to a wide range of fermentation end products *e.g.* butyrate, propionate, acetate, lactate, ethanol, and succinate, hydrogen, and carbon dioxide (Fig. 2). Some of these products are further metabolized by additional trophic members within the community *e.g.* CO_2 and H_2 could be converted to methane by hydrogenotrophic methanogens in the GIT (*Methanobrevibacter smithii*), and to acetate by acetogens in the human GIT (*e.g. Blautia hydrogenotrophica and Eubacterium* maltosivorans.

Within pectinolytic microorganisms, end products generated from sugar fermentation have been well characterized within the Bacteroides, with acetate, succinate, propionate (Salyers, 1984), and lactate (Adamberg et al., 2014) as the main final products identified in *B. thetaiotaomicron*. The relative proportion of products is though dependent on many factors *e.g.*, growth in pure *versus* co-culture, growth rate, CO_2 levels in the media and amino acids availability (Adamberg et al., 2014; Salyers, 1984). In contrast, within the Firmicutes, *Eubacterium eligens* produced formate, acetate, and lactate as fermentation end products (Lopez-Siles et al., 2012). *Faecalibacterium prausnitzii is* known to produce butyrate, formate and lactate, but not hydrogen as fermentation products (Duncan, Hold, Harmsen, Stewart, & Flint, 2002; Lopez-Siles et al., 2012). Finally *M. pectinilyticus* produced acetate formate, hydrogen, and CO_2, and minor levels of lactate as final end products (Kim et al., 2017).

However, as described above, it is not only pectinolytic microorganisms that benefit from the introduction of pectin into the GIT tract. Regardless of the pectin foraging and sequestration mechanism employed, a fraction of monomers and oligomers generated by the breakdown of pectin is often released for consumption by the wider secondary degraders within the GIT community. As such, pectin could serve indirectly as a substrate for a wider cohort of microorganisms within the complex food web of the human GIT, and an appreciation of non-pectin degrading microorganisms contributing to its metabolism is warranted. A detailed discussion of microbial community involved in sugar degradation in the human GIT tract is clearly beyond the scope of this review. Broadly, the most important and physiologically relevant products generated from pectin and non-digestible carbohydrates degradation are the SCFAs propionate and butyrate. Acetate, propionate, and butyrate are present in the human intestine in a broad ratio of 3:1:1 (Louis, Hold, & Flint, 2014), and provide substantial health benefits and energy to the human intestinal cells. Recent research and reviews have provided a detailed analysis of the identity of microorganisms involved in SCFA production in the human intestinal tract, as well as the predominant metabolic pathways involved. Briefly, the major propionate producers in the human GIT are members of the Phylum Bacteroidetes (*Bacteroides uniformis* and *B. vulgatus*, *Prevotella copri*, and *Alistipes putredinis*), the Firmicutes (*Roseburia intestinalis*, *Eubacterium hallii*, *Blautia obeum, Coprococcus catus*. *Dialister invisus*, and *Phascolarctobacterium succinatutens*), and the Verrucomicrobia *Akkermansia muciniphila* (Louis & Flint, 2009; Reichardt et al., 2014). Butyrate is produced in the human GIT from the combination of two molecules of acetyl-CoA to form acetoacetyl-CoA, followed by stepwise reduction to butyryl-CoA. All currently recognized butyregenic microorganisms are members of the Firmicutes *e.g.*, *Eubacterium rectale, Roseburia inulinivorans, R. intestinalis, Eubacterium hallii, Anaerostipes hadrus, Coprococcus eutactus, C. catu, Faecalibacterium prausnitzii, Subdoligranulum variabile, and Holdemanella biformis* (Louis & Flint, 2009, 2017; Vital et al., 2013).

5. Culture-independent approaches to examine the impact of pectin intake on the microbial community in the human intestine

The introduction of pectin to the human GIT clearly impacts the microbial community structure and diversity beyond merely stimulating pectinolytic organisms. Study of microbial community structure, diversity, and dynamics, as well as correlating such information to specific measurable parameters is best achieved using culture-independent, amplicon-based 16S rRNA gene diversity surveys. A plethora of human microbiome 16S rRNA gene studies have been conducted to provide a baseline of the human intestinal GIT community across countries, cultures, and diets, and to examine the impact of nutritional manipulations, diseases, age, and on the community. A small fraction of these studies have focused on understanding the impact of pectin administration on human intestinal microbiome (Table 2). Such studies have utilized a range of experimental designs (batch cultures, fermenters, clinical trials), pectin types, pectin concentrations, and sampling regiments. Most studies concurrently monitored SCFA production as an important measure of pectin benefits on the gut microflora. 16S Data analysis has focused on identifying taxa enriched by pectin, as well as patterns of overall change in diversity and community structure associated with pectin intake. Keeping in mind the wide range of experimental variables described above, we attempt to summarize salient studies below.

5.1. *In vitro Batch studies*

Multiple studies have investigated the impact of pectin amendments into a single container anaerobic enrichment containing fecal matters as an inoculum. The focus of such studies was to provide a baseline understanding of how pectin impacts the GIT community. Reinhard et al. (Reichardt et al., 2018) tested the effect of apple pectin and rhamnogalacturonic acid from potato on fecal microbial community in an *in vitro* batch culture seeded with fecal inoculum from four healthy subjects using a combination of 16S diversity surveys and group specific qPCR (Table 2). 16S rRNA Diversity survey data provides information on the relative abundance of a specific taxon within a sample (*e.g.* % the phylum Firmicutes makes of the overall community in a sample), while qPCR provides information on the relative enrichment of a specific taxon between samples (*e.g.* fold increase in the number of Firmicutes in time final sample, compared to that in time zero sample). Since pectin degradation causes acidification. The experiments were conducted at two pHs (5.5 and 6.5) to test the impact of colon acidification *via* SCFA production on the community. After 24 h incubation, the total number of microorganisms (16S rRNA gene copies in qPCR data) increased in all incubations, including substrate unamended controls. Higher net SCFA production was observed at pH 6.5 compared to 5.5, and a significant increase in propionate levels was observed in Rhamnogalacturonan at pH5.5 and 6.5 compared to unamended controls at both pHs. 16S rRNA diversity analysis identified an increase in the relative abundance of *Faecalibacterium prausnitzii* in apple pectin at both pHs, and *Lactobacillus rogosae* and *Clostridium bolteae* on RG at pH 5.5, and *Oscallibacter ruminatum* at pH 6.5 for RG. Collectively these results show that pH levels greatly influence microbial community stimulated by pectin in fecal

Table 2
Summary of salient 16S rRNA gene-based diversity surveys that examined how pectin ingestion effects the microbial community in the human GIT tract. Studies are divided into Batch studies, studies conducted in fermenters, and in-viv (clinical studies). The main question(s) and findings of each study are summarized.

Study	Inoculum and general setting	Pectin type	Concentration	Sampling time	Question	Findings	Reference
Batch studies							
Reichardt et al. (2018)	Fecal samples from three donors. *in vitro* batch incubations at pH 5.5 and 6.5, 37 °C	Rhamongalacturonan from potato, apple pectin	0.2 %	0, 6, 24 h,	Effect of pectin on fecal microbial community structure and numbers at different pH levels relevant *in vivo*	1 Increase in total number of microorganisms in all incubations. 2 Higher net SCFA production at pH 6.5 compared to 5.5 3 Increase in propionate levels at both test pH in RG incubations. 4 Increase in the relative abundance of *Faecalibacterium prausnitzii* in apple pectin at both pHs, and *Lactobacillus rogosae* and *Clostridium bolteae* on RG at pH 5.5, and *Oscallibacter ruminatum* at pH 6.5 for RG.	(Reichardt et al., 2018)
Yang et al. (2013)	Fecal samples from 15 healthy donors *in vitro* batch incubation at 37 °C	Unknown,	1%	12h	Effect of pectin on microbial community structure	1 Decrease in relative abundance of pectinolytic Bacteroidetes and Firmicutes 2 Increase in relative abundance of non-pectinolytic SCFA producer genus *Bifidobacterium*.	(Yang et al., 2013)
Bang et al. (2018)	Fecal samples from 3 healthy male donors. *in vitro* batch incubation in organic rich media amended by pectin.	Citrus peel pectin	1%	0, 6, 12, 18, 24, 36, and 48h	Effect of pectin and on microbial community structure	1 Decrease decrease in the relative abundance of pectinolytic Bacteroidetes 2 Increase in relative abundance of the non-pectinolytic SCFA producer genera *Lachnospira, Dorea,Clostridium* and *Sutterella*	(Bang et al., 2018)
Fermenter studies							
Chung et al. (2016)	Fecal samples from 3 healthy donors. Anaerobic continuous single stage fermenter	Apple pectin	0.5 %	0, 3, 6, and 9d	Microbial community dynamics associated with pH drop due to SCFA production during pectin degradation	1 At pH 6.9: increase in relative abundance of the pectinolytic organisms B. vulgatus/dorei, B. stercoris, B. eggerthii, B. cellulosilyticus/intestinalis, B. ovatus, and B. thetaiotaomicron and Eubacterium eligens 2 At pH 5.5: increase in relative abundance of the pectinolytic Faecalibacterium prausnitzii.	(Chung et al., 2016)
Bianchi et al. (2018)	Fecal sample from three obese adults. Incubation in a simulator of the Human Intestinal Microbial Ecosystem (SHIME®)	1. Lemon pectin. *2. B. longum* + Lemon pectin 3. *B. longum only*	Pectin: 2% (w/v). *B. longum :* 10^8 CFU ml^{-1}	1 week	Impact of pectin and the SCFA producer *B. longum* administration on modulating the microbial community structure in obese individuals	1 Pectin treatment resulted in increase in relative abundance of the genus *Succinivibrio* and an unclassified genus within the Ruminococcaceae. 2 Pectin + B. longum treatment yielded a similar microbial community to that observed in pectin only treatment. 3 Pectin, rather than *B. longum* had the predominant effect in modulating the microbial community.	(Bianchi et al., 2018)
Aguirre (2012)	Pooled fecal samples from 4 Obese subjects and pooled samples from 4 lean subjects. Proximal colon: TIM-2 system a proximal colon laboratory simulator	Sugar beet pectin	7.5 g/d	3 days	Impact of pectin on microbial community in lean *versus* obese subjects	1 Higher Firmicutes and lower Bacteroidetes in obese *versus* lean subjects at time zero. 2 Differential response to pectin in lean *versus* obese subjects: a Higher SCFA production in lean subjects. b Clostridium group XIV, *Faecalibacterium*, *Bacteroides*, *Bifidobacterium*, and *Parasutterella* were enriched in lean subjects, while *Anaerostipes, Caprococcus, Dorea*, *Lactobacillus* and *Lachnospiraceae* were	(Aguirre et al., 2014)

(*continued on next page*)

Table 2 (*continued*)

Study	Inoculum and general setting	Pectin type	Concentration	Sampling time	Question	Findings	Reference
						enriched in obese individuals.	
Larsen et al. (2019)	Pooled fecal samples from 8 healthy Caucasian adults TIM-2 proximal colon model	Rhamnogalacturonic acid, pectins from Orange, lemon, sugar beet, and lime	7.5 g pectin per day	0, 24, 48, 56, and 72 h	Would structural variations between various types of pectin would elicit and enrich distinct microbial communities?	1 Shifts in microbial community were correlated to salient structural variations in pectin preparations. 2 Substrate specific signals in microbial community shifts were identified, with degree of esterification, HG/RGI ratio, degree of branching, and presence of amide groups significantly impacting microbial community development.	(Larsen et al., 2019)
Clinical trials							
An et al. (2019)	Randomized double blind, placebo controlled human trial 100 subjects: 52 young adults, 48 elderly adults	Sugar beet pectin, maltodextrin as a control.	15 g/ d, 7.5 g twice daily	4 weeks	Effect of pectin on fecal microbiota composition, (SCFAs), and exhaled volatile organic compounds in young *versus* elderly subject.	No change in pre and post diversity measurements in both young and older groups.	(An et al., 2019)
Wei et al. (2016)	20 ulcerative colitis patients who underwent fecal transplantation by colonoscopy. Effect of pectin in microbial community stability.	Apple pectin	20 g/day	Five days	Can pectin promote stability of transplanted microbial community and prolonging the effects of transplantation?	1 Clinical outcome (Mayo Score) was better in the pectin-supplied *versus* control group. 2 Microbial community analysis showed a slightly higher level of diversity in pectin-supplied *versus* control group.	(Wei et al., 2016)

samples. Pure culture representatives of many of the stimulated taxa *e.g. Faecalibacterium prausnitzii* and *Eubacterium eligens* are known for their capability to degrade pectin (*i.e.* primary pectinolytic organisms), while others (*e.g. Bifidobacterium bifidum*) are not and as such, primarily benefit from the sugar released by pectinolytic members of the community.

Another *in-vitro* Batch study was conducted by Yang et al. conducted an in-vitro study to directly assess the impact of pectin on microbial community in in-vitro batch cultures (Yang, Martínez, Walter, Keshavarzian, & Rose, 2013) (Table 2). Batch incubations using fecal samples from healthy donors were amended with commercial pectin preparation. Surprisingly, pectin amendments resulted in the increase in relative abundance of the phylum Actinobacteria, mostly due to increase in the relative abundance of the SCFA (acetate, lactate) producer genus *Bifidobacterium*. Members of this genus are not known to degrade pectins, but are rather known sugar fermenters in the human GIT tract. Surprisingly, a decrease in the relative abundance of both phyla Bacteroidetes and Firmicutes harboring primary pectinolytic organisms, and a wide range of secondary metabolizing sugar fermenters was not observed. The reason and significance of this unexpected failure to enrich pectinolytic taxa is unclear.

Finally, Bang et al. conducted an *in-vitro* batch study to examine the impact of pectin on intestinal GIT community (Bang et al., 2018). The authors observed an increase of members of clostridium group XIV genera *Lachnospira, Dorea,* and *Clostridium* and the β-Proteobacterium *Sutterella* in pectin-amended cultures. Pure culture representatives of these above mentioned taxa are incapable of pectin degradation, and hence the observed enrichment is probably associated with cross feeding following pectin degradation by pectinolytic taxa. A surprising decrease in Bacteroidetes was observed, several members of which are known to degrade pectin as described above. However, this could be a reflection of the fast degradation of pectin observed in the experimental setting employed (pectin was degraded within the first 18 h), after which secondary metabolizers dominate the ecosystem. Further, it is important to note that the enrichment procedure was conducted in a rich medium containing high levels of rumen fluid, yeast extract, and peptone.

5.2. *In vitro studies in fermenters*

Several studies employed a fermenter setting to examine pectin degradation by the human GIT microbiota. Utilization of fermenters allows for continuous monitoring and adjustment of various parameters (*e.g.* pH, nutrients composition), as well as controlled shifts to measure to impact of a specific parameter on the microbial community. While some studies have utilized a single container fermenter under anaerobic conditions (Chung et al., 2016), others have employed more sophisticated systems that could better simulate the human GIT (*e.g.* SHIME® GIT model, dynamic gastrointestinal simulator simgi® model, and TIM-2 proximal column model) (Aguirre, Jonkers, Troost, Roeselers, & Venema, 2014; Barroso, Cueva, Peláez, Martínez-Cuesta, & Requena, 2015; Bianchi et al., 2018; Larsen et al., 2019).

Chung et al. (Chung et al., 2016) examined pectin degradation in an anaerobic continuous single stage fermenter system using apple pectin and seeded using fecal samples from three healthy donors (Table 2). The experimental design, employing controlled pH levels in a fermenter setting aimed to identify direct changes associated with pectin degradation at the initial in-situ column pH (6.9), rather than those mediated by general acidification of the colon following SCFA formation from pectin (pH 5.5). In general, 6 different operational taxaonomic units (OTUs), roughly corresponding to *B. vulgatus/dorei*, *B. stercoris*, *B. eggerthii*, *B. cellulosilyticus/intestinalis*, *B. ovatus,* and *B. thetaiotaomicron* and one Firmicutes (*Eubacterium eligens*) were stimulated in pectin-supplemented fermenters. Remarkably, pure culture representatives of all these species have been shown to metabolize pectin, attesting to the prevalence of primary degraders in this pH controlled environment. In addition, lower pH has been shown to increase the relative proportion of *Faecalibacterium prausnitzii* compared to various Bacteroides species at lower pH levels (5.5 *versus* 6.9).

Collectively, this study shows the importance of pH levels in shaping intestinal communities mediating pectin degradation and implies for a dynamic, pH-dependent changes in the gut community associated with various stages of pectin degradation.

As a non-digestible carbohydrate, the use of pectin as a probiotic to combat obesity has been reported. Bianchi et al. (Bianchi et al., 2018) explored the impact of pectin administration on modulating the microbial community structure in obese individuals (Table 2). Fecal samples from three obese individuals were used to seed the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), a dynamic model of the complete gastrointestinal tract developed to simulate and investigate GIT microbial community in a controlled *in vitro* setting by controlling pH, residence time, and temperature in an *in vitro* setting. The study utilized harsh pectin extract from lemon as a substrate and examined the microbial community after 1 week of incubation. Pectin treatment resulted in an increase of the family Ruminococcaceae, mainly due to the increase in relative abundance of the genus *Succinivibrio* and an unclassified genus within the Ruminococcaceae. Surprisingly, in a parallel treatment where pectin administration was combined with the addition of the SCFA producer *Bifidobacterium longum* (10^8 cells per treatment/mLs) as a probiotic, the changes in microbial community almost paralleled that observed in pectin only treatment, suggesting that in such setting, pectin had the predominant effect in modulating the microbial community.

Similarly, Aguirre et al. have examined how multiple dietary probiotics would impact the microbial community in lean *versus* obese subjects (Aguirre et al., 2014). The study was conducted in a TIM-2 proximal column model that mimics *in vivo* conditions by maintaining anaerobiosis, peristaltic movements, and metabolites removal, with sugar beet pectin (7.5 g/day for three days) being one of the probiotics examined (Table 2). The models were seeded with pooled fecal samples from 4 lean or 4 obese individuals, and shifts in microbial community (expressed as fold change between treatment and control was examined after 72 h). Initial microbial communities in lean *versus* obese individuals varied, with higher proportion of Firmicutes (92 ± 3.3 % *versus* 74 ± 1) and a lower proportion of Bacteroidetes (3.6 ± 1.9 % *versus* 22 ± 0.9 %) in obese *versus* lean individual. Total SCFA production in sugar beet pectin amended substrates was significantly higher in lean *versus* obsess subjects. Finally, representatives of the Clostridium group XIV, *Faecalibacterium*, *Bacteroides*, *Bifidobacterium*, and *Parasutterella* were significantly enriched in apple pectin amended samples from lean individuals, while *Anaerostipes, Caprococcus, Dorea*, *Lactobacillus* and *Lachnospiraceae* were significantly enriched in apple pectin amended samples from obese individuals. Interestingly, patterns of SCFA production and taxa stimulated within lean and obese individuals were drastically different with other substrates examined in the same study (Lactulose and apple fiber). The significant differences between substrates and lean/obese individuals renders the study at best preliminary and the degree of stochasticity within and between different studies precludes the formulation of any generalized principles regarding how pectin impacts microbial community and SCFA production in obese *versus* lean subjects (Aguirre et al., 2014).

An interesting, yet-relatively unexplored question is whether structural variations between various types of pectin would elicit and enrich distinct microbial communities. Larsen et al. (Larsen et al., 2019) attempted to address such issue by utilizing multiple pectin preparations from multiple sources (orange, lemon, sugar beet, lime), with various extraction procedures (mild, harsh) to prepare pectins with various degrees of esterification (Table 2). These pectin preparations, in addition to RG-I was introduced into a TIM-2 proximal column model that mimics *in vivo* conditions by maintaining anaerobiosis, peristaltic movements, and metabolites removal. The system was seeded by pooled fecal samples from eight healthy volunteers, and microbial community was followed at 0−72 hours. Shifts were correlated to salient structural variations within pectins preparations. Substrate specific signals in microbial community shifts were identified, with degree of esterification, HG/RGI ratio, degree of branching, and presence of amide groups significantly impacting microbial community development.

These results provide a proof of principle for the feasibility of substrate-specific microbiome engineering using various pectin preparations to elicit the development of a microbial taxa/community of interest. Replication of the observed results and extrapolation of such studies into human (rather than simulated column) settings presents the next logical step in such efforts.

5.3. Clinical in vivo trials

In general, few general trials examining the effects of pectin on human GIT health are available. One trial investigated the differences elicited by pectin intake on microbial community in young adults *versus* elderly subjects. It has been postulated that dietary fibers, including pectin could contribute to healthy aging by beneficially impacting gut microbiota and metabolite profiles. To test this hypothesis, a randomized double blind, placebo controlled human trial was conducted by An et al. to investigate the effect of pectin supplementation on fecal microbiota composition, short chain fatty acids (SCFAs), and exhaled volatile organic compounds (VOCs) in young adults *versus* elderly subjects (An et al., 2019). All subjects (52 young adults, 48 elderly adults) consumed 15 g/day sugar beet pectin or maltodextrin as a control for four weeks. Microbial community analysis, fecal VFA profiles, and exhaled VFA (acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate) profiles were measured (Table 2). The authors report no change in pre and post diversity measurements in both young and older groups.

Finally, Wei et al. evaluated using pectin in a fairly novel role: as a prebiotic for patients undergoing fecal microbiota transplants (FMT). The rationale is that pectin administration could promote stability of the desirable transplanted microbial community for longer periods hence prolonging the beneficial effects of fecal transplantation. A small clinical trial was conducted were a cohort received pectin (20 g/day) for five consecutive d post FMR *versus* a control group (Table 2). Various biochemical measurements C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), and inflammatory bowel disease questionnaire (IBDQ) criteria, and Clinical outcome (Mayo score), and microbial diversity and community structure were examined. Although all biochemical tests did not show significant differences between both cohorts, clinical outcome (Mayo Score) was better (significantly lower) in the pectin-supplied *versus* control group. Microbial community analysis showed a slightly higher level of diversity in pectin-supplied *versus* control group. Based on the modest improvement in clinical outcome and modest increase in microbial community diversity, the authors concluded that pectin treatment have provided beneficial clinical effect by preserving the diversity of the gut flora following FMT in ulcerative colitis patients. The results are at best modest and the correlation between diversity and clinical outcome does not elevate to the level of causation. More work is needed to confirm the observed relatively subjective clinical effect, replicate the observation of higher diversity in pectin amendments, and provide a plausible path to examine whether better clinical outcomes are truly due to microbial community changes or any other factors.

6. Pectin and gut inflammation

Pectin protects against intestinal inflammation by both gut microbiota-dependent and -independent pathways (Ishisono, Mano, Yabe, & Kitaguchi, 2019). Such anti-inflammatory activity is significantly influenced by the presence of neutral sugar side chains and degree of methyl esterification (DM) in pectin. A simplified pectin structure depicting the main chemical features which influence the anti-inflammatory, tumor preventive and immunomodulating effects is illustrated in Fig. 3.

As shown in Fig. 4, a high neutral sugar content, a high degree of

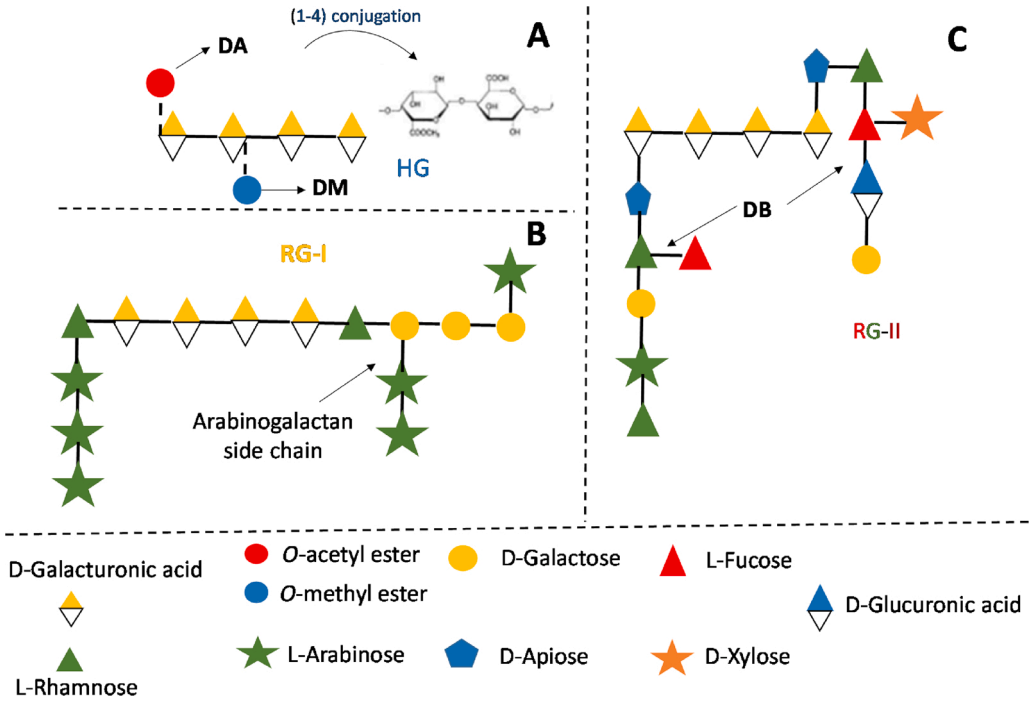

Fig. 3. A diagrammatic sketch representing pectin structure and SAR discussed in Figs. 4 and 6. The recognition between pectin chemical features and SAR, where each section represents: (A) = degree of acetylation (DA) and degree of methyl esterification (DM) of homogalacturonan (HG); (B) = arabinogalacton side chain of rhamnogalacturonan I (RG-I) and (C) = degree of branching (DB) of rhamnogalacturonan II (RG-II).

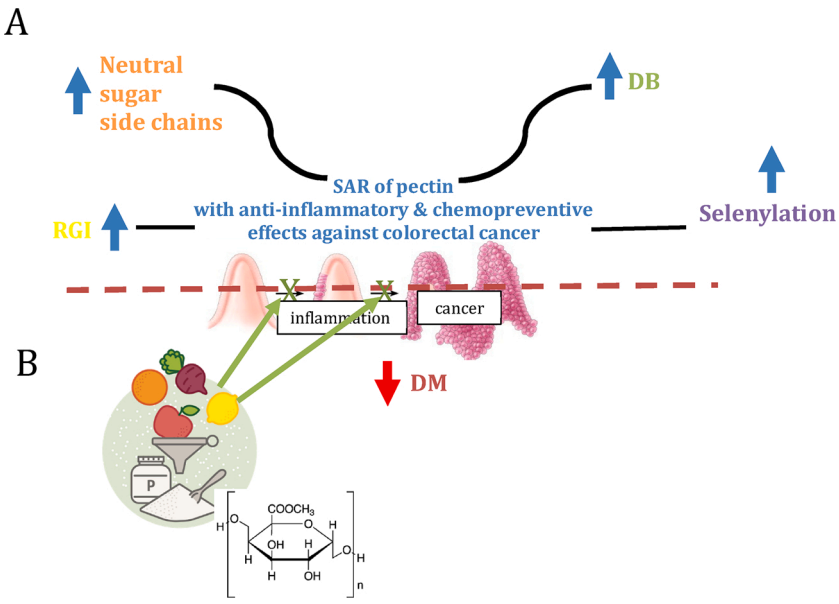

Fig. 4. Major structural features positively (A) and negatively (B) influencing intestinal anti-inflammatory activity and protective effects against colorectal cancer of pectin. Pectin's capacity to protect against intestinal inflammation and colorectal cancer is strongly affected by structural features. The presence of neutral sugar side chains and rhamnogalacturonan I type structure (homogalacturonan segments and neutral sugar side chains), a high degree of branching, a low degree of methyl-esterification are essential for highly potent anti-inflammatory and tumor preventive effects. Structural modifications such as selenylation improve the anti-inflammatory potential of pectin. SAR – structure-activity relationship, DB – degree of branching, DM – degree of methyl esterification, RGI – rhamnogalacturonan I, ↑ - direct SAR, ↓ - inverse SAR.

branching and a low DM potentiate the anti-inflammatory activity (do Nascimento, Winnischofer, Ramirez, Iacomini, & Cordeiro, 2017; Popov et al., 2013; Sabater et al., 2019; Sahasrabudhe et al., 2018), suggesting that these structural motifs can be prioritized for designing more active pectin or synthetic or semisynthetic derivatives to be used as anti-inflammatory agents in the gut.

Inflammation as a process is orchestrated by several mediators and signalling cascades including cytokines, pro-inflammatory (IL-1β, -6, -8, -12, TNF-α, IFN-γ) and anti-inflammatory (IL-4, -10, -11), enzymes (inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, high-mobility group box 1 (HMGB1), NADPH oxidase (NOX)), reactive oxygen and nitrogen species. The inflammatory response is regulated by multiple pathways, the most common ones being the nuclear factor-kB (NF-kB), mitogen-activated protein kinase (MAPK) and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways (Chen et al., 2018). The former is a transcription factor inducing the expression of numerous inflammatory genes, whereas MAPK pathway (ERK1/2, JNK and p38) is involved in the production of inflammatory mediators (Zhang, Pan, Ran, & Wang, 2019).

Intestinal mucosa hosts the largest pool of macrophages in the body playing important roles in managing gut inflammation and maintaining immunological homeostasis (Wang, Ye, Zeng, & Qiao, 2019). In the gut, pectin can directly interact with intestinal macrophages modulating their activity as revealed using *in vitro* studies. For example, pectins from

various sources (*Smilax china* rhizome, *Sedum dendroideum* leaf) were reported to reduce the release of inflammatory mediators (NO, TNF-α, IL-1β, IL-6) in macrophages in response to lipopolysaccharide (LPS) stimulation (de Oliveira, do Nascimento, Iacomini, Côrtes Cordeiro, & Cipriani, 2017; Zhang et al., 2019). In case of *Smilax china* pectin, such effect was mediated by the suppression of NF-kB, ERK1/2 and JNK pathways (Zhang et al., 2019). A pectin isolated from fresh green sweet pepper fruits (*Capsicum annuum* cv Magali) reduced the pro-inflammatory TNF-α and IL-1β and increased the anti-inflammatory IL-10 release by LPS-stimulated macrophages. Its product of partial acid hydrolysis, devoid of side chains, was found more active in reducing TNF-α and less active in enhancing IL-10 than the native pectin, whereas both pectins exhibited similar effects on IL-1β secretion (do Nascimento et al., 2017). Such results suggest that the anti-inflammatory action is differentially regulated by different parts in pectin structure (Fig. 4).

The anti-inflammatory effects of pectin have also been investigated in different murine chemical induced colitis models. Acetic acid- and dextran sulfate sodium (DSS)-induced colitis are widely used experimental models of inflammatory bowel disease. Pectin, given orally before induction and/or during colitis, improved the disease activity index (DAI) scores (bleeding, stool consistency, weight loss), food intake (indicator of the discomfort caused by the intestinal inflammation), colon weight/length ratio (indicator of inflammation and cell infiltration) and tissue damage (inflammatory cell infiltration, crypt loss) (Ishisono et al., 2019; Pacheco et al., 2018; Silveira et al., 2017). Overall, pectin attenuated colon inflammation by modulating various mediators and inflammatory pathways.

The destruction of the mucus layer followed by mucosal and submucosal necrosis, infiltration of neutrophils and production of ROS in the colonic mucosa, epithelial erosions and ulcerations are common features of colonic inflammation evoked in murine acetic acid-induced colitis (Popov, Markov et al., 2006; Popov, Ovodova, Markov, Nikitina, & Ovodov, 2006; Randhawa, Singh, Singh, & Jaggi, 2014). Oxycoccusan, comaruman and crude and purified rauvolfian, pectins isolated from *Vaccinium oxycoccos* (common cranberry) fruits, *Comarum palustre* (marsh cinquefoil) aerial parts and *Rauwolfia serpentina* dried callus, respectively, reduced colonic myeloperoxidase activity (marker of neutrophil infiltration) concurrent with an increase in the production of colonic mucosal layer. Further, oxycoccusan also attenuated the oxidative stress (lipid peroxidation) in the intestinal wall (Popov, Markov et al., 2006; Popov et al., 2007). In DSS-induced acute colitis in mice, citrus pectin (10 % in the diet) significantly protected against the increase in neutrophil and eosinophil recruitment if supplied prior to the induction of colitis. Citrus pectin decreased colonic CXCL1 and CCL11 (chemokines controlling neutrophil and eosinophil recruitment), but also myeloperoxidase and eosinophil peroxidase activities (markers of neutrophil and eosinophil infiltration, respectively). In contrast to acute colitis models, in chronic DSS-induced colitis, citrus pectin (10 % in diet) showed protection when given prior to colitis induction, whereas less protection (decrease in eosinophil infiltration according to eosinophil peroxidase activity) was evidenced if supplied after the disease progression. Overall, citrus pectin seems to be more efficient as preventive rather than therapeutic agent in acute and chronic colitis (Silveira et al., 2017). Citrus pectin also lowered DSS-induced expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), intercellular adhesion molecule (ICAM)-1 (involved in neutrophil adhesion to intestinal mucosa) and iNOS in the colonic tissue (Pacheco et al., 2018; Sumagin et al., 2016).

The epithelial barrier integrity is significantly compromised in bowel inflammation. Reduced mucus production (reduction of mucus-secreting goblet cells), colonic mucus weakening (decrease in mucin glycosylation), altered expression or functional abnormalities of tight junctions, adherens junctions and desmosomes, impairment of gap junctional intercellular communication, decrease in intestinal alkaline phosphatase (IAP) activity are hallmarks of bowel inflammation allowing mucosal penetration of bacterial fragments and leading to not only local but also systemic inflammatory responses (Bilski et al., 2017; Chelakkot, Ghim, & Ryu, 2018; Dorofeyev, Vasilenko, Rassokhina, & Kondratiuk, 2013; Ey, Eyking, Gerken, Podolsky, & Cario, 2009). Citrus pectin attenuated DSS-induced reduction in the expression of the membrane-associated mucin (MUC)-3 and two tight junction proteins, namely occludin and zonula occludens (ZO)-1 (Dorofeyev et al., 2013; Pacheco et al., 2018). A pectin from noni (*Morinda citrifolia*) fruits increased the expression of tight junction proteins (ZO-1, occludin), mucus secretion by goblet cells and number of goblet cells/crypt and reduced the crypt damage in the colon of DSS-treated mice (Jin et al., 2019). Full characterization of such pectin has yet to be reported to determine active sites mediating for these effects.

A major mechanism contributing to the anti-inflammatory activity of pectin lies in the interaction with Toll-like receptors (TLRs), a family of pattern recognition receptors (PRRs) expressed in the intestine (Sahasrabudhe et al., 2018). Pectin usually interacts with TLR2 and TLR4 receptors. Upon activation, TLR2 dimerizes with either TLR1 or TLR6. TLR2-TLR1 heterodimers induce an anti-inflammatory response *via* IL-10 expression whereas TLR2-TLR6 heterodimers are pro-inflammatory *via* IL-6 and TNF-α expression (Ey et al., 2009). In contrast to TLR2, TLR4 forms homodimers upon activation with induction of a pro-inflammatory response (MYD88-dependent pathway) *via* IL-1, IL-6 and TNF-α expression (Hug, Hasan Mohajeri, & La Fata, 2018). Supplementation with apple pectin (5%) attenuated the alteration of gut barrier function (increase in TLR4 expression, decrease in IAP and claudin expression) and ileal inflammation (increase in TNF-α and IL-6, decrease in IL-10) induced by a high fat diet in rats (Jiang et al., 2016). Another pectin to target TLRs is artichoke pectin which reduces colonic TLR4 expression and also other DSS-induced inflammatory markers such as iNOS, TNF-α, IL-1β, IL-6 and ICAM-1. Further release of arabinose and galactose side chains by enzymatic hydrolysis of pectin led to arabinose-free and galactose-free modified artichoke pectins, respectively, having different anti-inflammatory profiles as compared to parent pectin. Thus, removal of arabinose and galactose resulted in the loss of activity. In contrast to the artichoke pectin, modified pectins did not reduce IL-1β and IL-6 levels. Galactose-free modified pectin also lacked the ability to reduce TNF-α and ICAM-1 (Sabater et al., 2019), suggestive for the crucial role of galactose side chain in mitigating against bowel inflammation. Whether such role is also preserved towards other effects in pectin *i.e.*, immunomodulation has yet to be reported.

It is though obvious that the neutral sugar side chains are essential for the anti-inflammatory activity of pectin. Orange pectin, containing more neutral sugar side chains than citrus pectin (isolated from lemon and lime peels), but having comparable average molecular weights, DM and lengths of side chains, as expectedly, showed more protection against TNBS-induced colitis. In contrast to citrus pectin, pre-feeding with orange pectin (5% in diet) significantly downregulated IL-1β, IL-6 and TNF-α in the colon tissue, IL-6 suppression being mediated by blockade of TLR2 and TLR4 signaling. Moreover, orange pectin decreased IL-1β, IL-6 and TNF-α in antibiotic-treated mice suggesting for a microbiota-independent mechanism in ameliorating TNBS-induced colonic inflammation (Ishisono et al., 2019).

In general, pectins with low DM (DM $<$ 50 %) were found more effective in protecting against intestinal inflammation than those with high DM (DM $>$ 50 %) (Popov et al., 2013; Sahasrabudhe et al., 2018). A lemon pectin with low DM (DM7) efficiently ameliorated doxorubicin-induced ileitis in mice. Doxorubicin induces a TLR2-dependent inflammatory response with cell death in intestinal crypts and neutrophil influx in the peritoneum. Both effects were strongly inhibited by pectin DM7 with a significant reduction of TNF-α, IL-6, monocyte chemoattractant protein-1 (MCP-1, CCL2) and growth-regulated oncogene (GRO)-α (CXCL1) in peritoneal fluid (Deshmane, Kremlev, Amini, & Sawaya, 2009; Egesten et al., 2007; Sahasrabudhe et al., 2018). The mechanism underlying the anti-inflammatory activity of DM7 involves direct binding to intestinal

TLR2 with subsequent inhibition of heterodimerization with TLR1 and production of pro-inflammatory cytokines. The TLR2-TLR1 inhibition was DM- and concentration-dependent. At low concentrations, low-DM pectin efficiently suppressed TLR2-TLR1 whereas, at high levels, higher-DM pectin (lemon pectin DM22, DM45, DM60 and DM75) were also inhibitory. This DM- and concentration-dependent activity is likely attributed to the interaction between pectin and TLR2 ligand binding site. Low-DM pectin encompasses high levels of unesterified galacturonic acid units of highly negative charge interacting by electrostatic forces with positively charged amino acids surrounding TLR2 ligand binding site. In case of high-DM pectin, such interactions are possible only at high concentrations due to a higher level of negatively charged unesterified regions (Sahasrabudhe et al., 2018). High-DM pectins also form gel networks with mucosal glycoproteins which reduce their ability to penetrate the mucus layer (Liu, Fishman, Hicks, & Kende, 2005; Popov et al., 2013). In murine acetic acid-induced colitis, both low-DM and high-DM citrus pectin (CU701, 36 % DM and CU201, 72 % DM, respectively) reduced the macroscopic damage score, total square of injury and colonic myeloperoxidase activity, with CU701 found more active than CU201, confirming that low-DM pectin is more active regarding bowel inflammation than high-DM. Such hypothesis was also confirmed by artichoke pectin (19.5 % DM) found more effective than citrus pectin (71 % DM) in reducing colonic pro-inflammatory markers (TNF-α, IL-1β, IL-6, iNOS, ICAM-1, TLR-4) in murine DSS-induced colitis (Sabater et al., 2019).

DM also affects pectin ability to protect the gut barrier integrity. Lemon pectin of different DM (DM30, DM56 and DM74) protected T84 intestinal epithelial cells against phorbol 12-myristate 13-acetate-induced increase in membrane permeability *via* activation of TLR2 in a DM-dependent manner (Vogt et al., 2016).

Anti-inflammatory activity of pectin can also be further improved by selenylation. Thus, selenylation conferred to *Ulmus pumila* root pectin the capacity to inhibit NO production in LPS-stimulated macrophages by downregulation of iNOS protein expression, the effect being selenium content-dependent (Lee et al., 2018).

Chronic inflammation is a major risk factor for the development of colorectal cancer (Monteleone, Pallone, & Stolfi, 2012), a more aggrevated disease form. Galectin-3 (gal-3) and MUC-1 are important targets in controlling chronic inflammation and protection against colorectal cancer (Li et al., 2012; Sun et al., 2018), both found up-regulated in colorectal cancer and other types of cancer. Their overexpression is associated with the development of colorectal cancer metastasis; MUC-1 expression increases as colitis progresses to colon cancer. In addition, MUC-1 competes with β-catenin to bind to cadherin and thereby, it destroys the intercellular junctions and enables metastasis (Sun et al., 2018). In a mouse model of colitis-associated colon cancer (exposure to colon carcinogen 1, 2-dimethyl-hydrazyne (DMH) and colitis-inducing agent DSS), modified *Malus domestica* (apple) pectins (smaller linear water soluble fractions generated by partial hydrolysis) reduced intestinal toxicity of DMH/DSS (piloerection, bowing wast, anal prolapse), tumor formation (up to 5%), inflammation, hyperplasia and MUC-1 expression in the colonic mucosa (Li et al., 2012; Sun et al., 2018). Modified apple pectin also increased apoptotic index in colonic epithelial cells, with galactose units most likely acting as ligands for gal-3, thus inhibiting its binding to receptors (Li et al., 2012), and further confirming the role of residual galactose in pectin function.

Rhamnogalacturonan I type structure (RGI) (including homogalacturonan segments and neutral sugar side chains) was reported to be essential for reducing ICAM-1 expression in DLD1 colon cancer cells (Maxwell et al., 2015).

To conclude, some structural features (high neutral sugar content, high degree of branching, low DM, RGI) and structural modifications (selenylation, partial hydrolysis) significantly improve the ability of pectin to reduce intestinal inflammation and/or protect against colorectal cancer. Such aspects on structure-activity relationship (Fig. 4) can be used for the rational design of pectins having more potent intestinal anti-inflammatory and tumor preventive effects.

Asides from pectin structural parts to finction as anti-inflammatory agent in the gut, their fermentation products *i.e.*, short chain fatty acids (SCFAs) are also responsible, at least in part, for the biological effects of pectins. Such effects point to the value of *in vivo* assays to assess pectin effects in the gut. The anti-inflammatory effects of SCFAs are mediated *via* the inhibition of colonic histone deacetylases (HDACs) and activation of G-coupled protein receptors (GPRs). HDACs inhibition results in positive effects on intestinal mucosa such as suppression of NF-kB and decreased production of inflammatory cytokines (IL-6, IL-8, TNF-α) and other inflammatory mediators (Burger-van Paassen et al., 2009; van der Beek, Dejong, Troost, Masclee, & Lenaerts, 2017). In addition, SCFAs control intestinal inflammation through the activation of GPRs on intestinal endothelial and immune cells. For example, butyrate, a major SCFA, predominantly activates GPR109a resulting in suppression of NK-kB signaling pathway. SCFAs binding to GPR43 induces activation of NLRP3 inflammasome which plays a key role in intestinal homeostasis and epithelial integrity (Macia et al., 2015). Increase in SCFAs production *via* stimulation of SCFAs-producing bacteria is obviously an important approach to control intestinal inflammation.

7. Pectin and gut immune homeostasis

Pectin exhibits beneficial effects on intestinal immune system as outlined in Fig. 5. Upon oral administration, pectin interacts with gut-associated lymphoreticular tissue consisting of lamina propria aggregates, Peyer's patches and mesenteric lymph nodes hosting a wide variety of immune cells (B and T lymphocytes, macrophages, dendritic cells, M cells) (Bain & Schridde, 2018; Suh et al., 2013) and initiates immunomodulating effects through PRRs, mainly TLRs (Prado et al., 2020). TLRs play a major role in pathogens recognition and activation of innate immunity as well as in the maintenance of healthy intestinal epithelial barrier. Pectin was reported to activate macrophages to produce and release various cytokines (TNF-α, IL-1β, IL-10). It has yet to be concluded whether in the presence of pro-inflammatory stimuli (LPS), same pectins can mitigate against the inflammatory response, namely to reduce pro-inflammatory and increase anti-inflammatory cytokine production (TNF-α, IL-1β and IL-10, respectively) (de Oliveira et al., 2017; do Nascimento et al., 2017). The abilities of pectin to bind TLRs are dependent on their structural features as previously stated. Pectin from *Carica papaya* fruits with a structure of homogalacturonan backbone and low-DM inhibited TLR3 and TLR9, albeit activated TLR2 and TLR4 while the derivatives with a reduced homogalacturonan region and medium-DM expressed a favorable profile for the activation of TLRs signaling (TLR2, TLR3, TLR4, TLR5 and TLR9) (Prado et al., 2020). The immunomodulating activity of pectin is strongly influenced by both DM and molecular weight. Lemon pectin (DM30, DM56 and DM74) stimulated THP1 MD2-CD14 cells (express all TLRs) *via* TLR signaling with further NF-kB/AP-1 activation in a DM-dependent manner. The intensity of activation decreased as follows: DM74 > DM56 > DM30. A higher DM is therefore essential for TLR-mediated NF-kB/AP-1 activation. Moreover, enzymatic hydrolysis to lower the degree of polymerization generated pectin oligomers having weaker TLR activating properties (Vogt et al., 2016). The content of galacturonic acid as well as structure of branched region in pectin also influence the immunomodulatory effects. It appears that pectin containing less than 75 % galacturonic acid were more immune effective, while a developed ramified or branched structure favors the immune stimulatory activity by increasing phagocytosis and antibodies production (Popov & Ovodov, 2013), and all to suggest that different pectin components trigger different immune reactions and warranting that structural modification of pectin could yield even more effective analogues.

Structural modifications indeed affect the immunomodulatory potential of pectins. For example, the linear low methyl-esterified homogalacturonan, generated by partial acid hydrolysis of fresh green sweet pepper fruit pectin, exhibited different cytokine secretion effects in

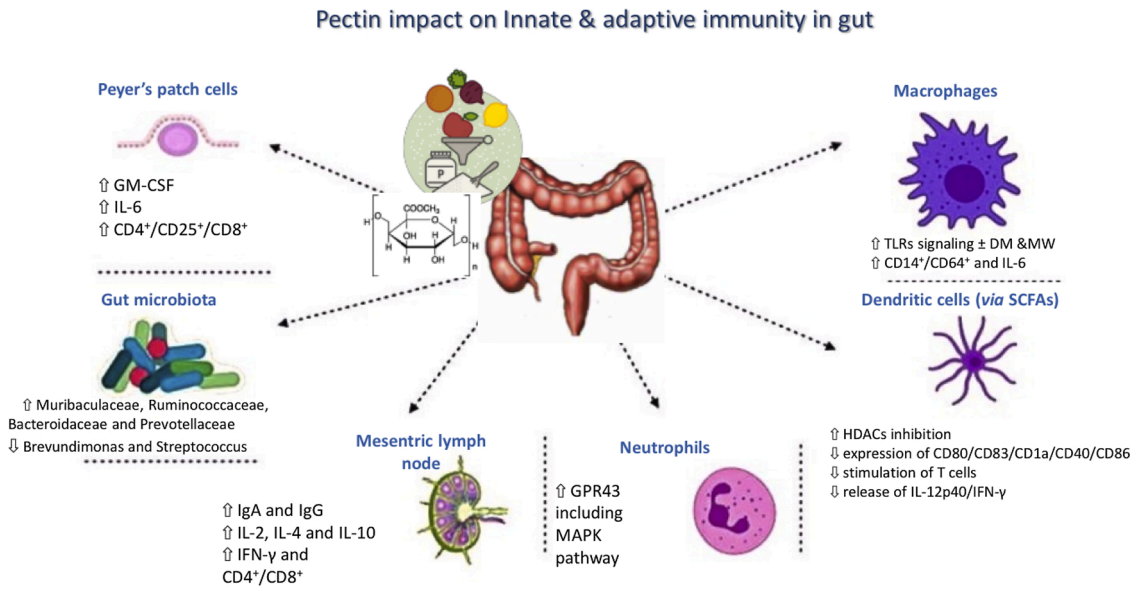

Fig. 5. Outline of the immunomodulation effects of pectin and its SCFAs. Pectin exerts pleiotropic immunoregulatory effects influencing both innate and adaptative immunity. They activate Peyer's patches cells to secrete immunomodulating cytokines (GM-CSF, IL-6), enhance $CD4^+/CD25^+$, $CD8^+/CD25^+$ regulatory T lymphocytes, promote macrophage activation *via* Toll-like receptors (TLRs) signaling pathway, increase the levels of some cytokines with pleiotropic immunologic functions (IgA, IgG, IL-2, IFN-γ), and they increase the beneficial bacterial populations that contribute to the gut and systemic immune homeostasis. Short-chain fatty acids (SCFAs) activate mitogen-activated protein kinases pathway (MAPK) *via* stimulation of G-protein-coupled receptor 43 (GPR43) supporting neutrophils function, suppress development of dendritic cells through histone deacetylases (HDACs) inhibition and modulate the proliferation and differentiation of T lymphocytes.

comparison with the native pectin (do Nascimento et al., 2017). Partial deacetylation and de-esterification of a pectin isolated from cacao (*Theobroma cacao*) pod husks enhanced the immunomodulating activity. The modified pectin was more active than the native form in activating murine peritoneal macrophages to produce NO, TNF-α, IL-12 and IL-10 (Amorim, Vriesmann, Petkowicz, Martinez, & Noleto, 2016). A low degree of esterification allows a high penetration of the mucin intestinal layer by pectin and promotes the interaction with intestinal epithelium and immune intestinal structures (Popov & Ovodov, 2013).

A purified linden pectin, that is acetylated and highly glucuronidated, induced neutrophil and macrophage activation concurrent with the suppression of various tumor cells. The highest macrophage-stimulating activity was observed for a linden pectin fraction that is low-esterified and has a predominant homogalacturonan region and RGI structure with highly branched arabinogalactan II units (AGII). It has been suggested that RGI and AG side chains contribute essentially to the macrophage-activating properties. Besides, pectin presented high complement fixation activity to play an important role in phagocytosis, antibody function and inflammation (Georgiev et al., 2017; Minzanova et al., 2018). Such activity was also described for *Brassica oleracea* (cabbage) varieties and lavender pectins. The high complement-fixing activity of cabbage pectin is positively associated with large neutral side chains carrying high amounts of (1→6) and (1→3,6)-linked galactose (Samuelsen et al., 2007). In addition to the type of glycosidic bonds in the side chains, the type of linkage in the branched residues appeared to play a key role for immunomodulatory potential (Popov & Ovodov, 2013). Also, the AGII and arabinan moieties appeared to be important for the complement-fixing activity. The removal of arabinose in the furanose form units by acid hydrolysis led to a decrease of complement activation (Westereng, Yousif, Michaelsen, Knutsen, & Samuelsen, 2006) confirming the role of galactose residues in pectin functions as previously described (Sabater et al., 2019). Purified low-acetylated and high-methoxylated lavender pectin triggered innate and adaptive immune responses. They showed complement fixation activity *via* classical and alternative pathways concurrent with induced macrophage and neutrophil activation, the latter being associated with the increase of CD18 expression and reduction of CD62 L expression. Such results confirm that both native and derivatized pectins exhibit similar action regarding activation of intestinal Peyer's patches.

Dietary apple pectin (5%, 2 weeks) increased IgA, IL-2, IFN-γ levels in mesenteric lymph node lymphocytes from mice with DSS-induced colitis. In addition, pectin intake lowered IL-4 and IL-10 production. Also, pectin-fed rats (5%, 2 weeks) showed high levels of IgA and IgG as well as a high $CD4^+/CD8^+$ ratio in the mesenteric node lymphocytes (Lim et al., 1997). These findings suggest the intervention of pectin in the differentiation of T-cells toward Th1-dominant immunity (Lim, Lee, Park, & Choue, 2003).

The immunoregulatory activity of pectin is also expressed by the mitigation of immunosuppresive drugs effect. Oral gavage of hydrolyzed citrus pectin (300, 600, 100 mg/kg b.w., 21 days) modulated gut microbiota and alleviated CYP-induced dysbiosis. It increased beneficial *Muribaculaceae*, *Ruminococcaceae*, *Bacteroidaceae* and *Prevotellaceae* bacteria and decreased pathogenic *Brevundimonas* and *Streptococcus* bacteria in the gut, with medium and high doses found more effective. The decrease of molecular weight and increase of galacturonic acid content in pectin appeared to improve its immunomodulatory properties (Chen et al., 2020).

It is obvious that some structural features (DM, degree of polymerization, branching, galacturonic acid content) or structural modifications (deacetylation, de-esterification) significantly potentiate the immunomodulatory effects of pectin. A summary of all these structure-activity relationships are presented in Fig. 6.

The microbial fermentation metabolites of pectins, SCFAs, are also major players in the regulation of the gut immune system. SCFAs affect both innate and adaptive immunity. They activate signalling cascades that are involved in the intestinal immune responses *via* cell-surface GPRs (GPR41 = FFAR3, GPR43 = FFAR2) agonism. SCFAs-induced GPR43-dependent signalling, including MAPK pathway activation, supports neutrophil recruitment. Also, SCFAs can modify the effector function of neutrophils (TNF-α production, NF-kB activation, CXCL8 production) being dependent on the stimuli and state of activation of the cells. SCFAs suppressed the development of dendritic cells through HDACs inhibition and subsequent modulation of some transcription factors expression (PU.1, Rel B). PU.1 is involved in the differentiation/

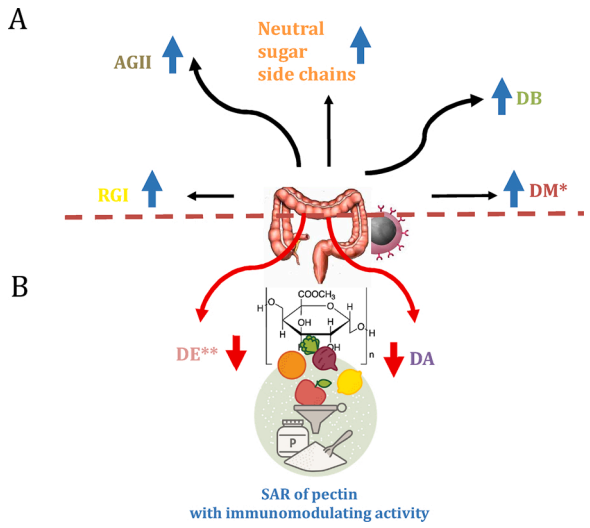

Fig. 6. Major structural features positively (A) and negatively (B) influencing intestinal immunomodulating activity of pectin. Pectin modulates intestinal immune system in relation to their structural features. The presence of neutral sugar side chains, rhamnogalacturonan I and arabinogalactan II type structures, degree of branching are determinant for immunostimulating activity. The degree of esterification differently modulates various immune mechanisms. A high degree of methyl-esterification is essential for TLR-mediated NF-kB/AP-1 activation whereas a low esterification and acetylation results in activation of macrophages to produce NO, TNF-α, IL-12 and IL-10. SAR – structure-activity relationship, DA – degree of acetylation, DB – degree of branching, DE – degree of esterification, DM – degree of methyl esterification, AGII – arabinogalactan II, RGI – rhamnogalacturonan I, ↑ - direct SAR, ↓ - inverse SAR, *TLR-mediated NF-kB/AP-1 activation, **NO, TNF-α, IL-12 and IL-10 production by macrophages.

activation of macrophages or B-cells and Rel B, in NF-kB signalling (Bhatt & Ghosh, 2014). Among SCFAs, butyrate induced a delay in human dendritic cell maturation that was associated with a reduction in some surface markers expression (CD80, CD83, CD1a, CD40, CD86). Besides, butyrate decreased the capacity of dendritic cells to stimulate T cells and to release pro-inflammatory cytokines (IL-12p40, IFN-γ). As regard to the adaptative immunity, SCFAs modulated the proliferation and differentiation of T lymphocytes by inducing Treg production and affecting Th1, Th2, Th17 cells differentiation and activation (Corrêa-Oliveira, Luís Fachi, Vieira, Sato, & Vinolo, 2016; Yang et al., 2018). Apart from the effects on the gut T cells, SCFAs promoted B cell differentiation and boosted intestinal and systemic antibody production. They increased the expression of *Xbp1* and *Aicda* genes and cellular metabolism in mouse and human B cells to aid and support the antibody production (Kim, Qie, Park, & Chang, 2016). Structure-activity relationship studies on the different SCFAs, with most reports on butyrate and less on other acids, is an area that needs to be further explored.

8. Nutrient and drug-pectin interactions

Due to structural features and gelling properties, pectin can influence the absorption of nutrients and drugs in the intestinal tract. Among macronutrients, proteins interact significantly with pectin, leading to impaired digestibility of proteins, reducing the proteolysis *via* formation of electrostatic complexes and/or inhibition of digestive enzymes. *In vitro*, low-methylated pectin (DE<50) decreased more strongly peptic digestibility of β-lactoglobulin even at low concentrations (1%) than high-methylated pectin. High charge density of low-methylated pectin allows a good electrostatic interaction with proteins. In addition, high pectin levels reduced the enzymatic activity of pepsin due to viscosity or direct interaction between pectin and enzyme (Nacer, Sanchez, Villaume, Mejean, & Mouecoucou, 2004). Supplementation of diets with highly esterified apple pectin (40 and 80 g/kg diet) decreased ileal digestibility of protein and amino acids in pigs. The reduction in ileal protein digestibility is related to many factors including, among others, the increase of digesta viscosity, formation of complexes with digestive enzymes, increase of endogenous nitrogen losses and digesta passage rate (Święch, Tuśnio, Taciak, Boryczka, & Buraczewska, 2012). Pectin also impairs starch digestion rate (Sasaki & Kohyama, 2012; Sasaki, Sotome, & Okadome, 2015). A recent *in vitro* digestibility study reported that citrus peel pectin slows starch digestion rate by interacting with amyloglucosidase (non-specific interaction *via* electrostatic complexation and/or hydrogen bonding) and increasing medium viscosity thus limiting enzyme access to starch granules (Bai et al., 2017). Pectins with high molecular weight and DM or extensive branching (high methoxylated citrus pectin, peach water-soluble pectin) efficiently inhibited lipid digestion under simulated gastrointestinal conditions mainly due to their rheological properties (increase in gastrointestinal medium viscosity with restricted diffusion of both lipolytic enzymes to lipid droplets and lipid digestion products to micelles) (Cervantes-Paz et al., 2017; Espinal-Ruiz, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2016; Zhou, Bi, Chen, Wang, & Richel, 2021). In addition, low-DM pectins have high calcium ions binding capacity which improves their ability to form gels through dimerization and cross-linking. Pectin gelification reduces lipid digestion by impairing lipase activity on oil droplets. An impaired lipid digestion results in an altered bioavailability of dietary carotenoids as lipid digestion products (free fatty acids, mono- and diglycerides, lysophospholipids) form micelles having an essential role in carotenoid transport and absorption. In addition, bile salts are essential for lipid emulsification and micellarization. Pectin binds bile salts through hydrophobic interactions thus reducing their availability for micelle formation; the effect varies with pectin concentration (high binding capacity at low pectin concentration) and DM (high binding capacity at high DM) (Cervantes-Paz et al., 2017). Also, the ingestion of pectin can impair absorption of minerals such as magnesium and iron (Greger, 1999). The absorption of nutrients can also be influenced by pectin due to their slowing gut transit properties. Pectin/SCFAs-induced secretion of peptide YY contributes to the delayed gastro-intestinal transit altering the nutritional profile. Moreover, peptide YY and glucagon-like peptide-1 (GLP-1) are involved in the activation of so-called “ileal brake” that reduces food intake and increase satiety levels (Maljaars, Peters, Mela, & Masclee, 2008) contributing to pectin slimming effect.

With regards to pectin-drugs interaction, *in vitro* permeation model system revealed that the permeation of drugs (propranolol) across lipid membranes was significantly increased by pectin with a high DE (more than 70 %). On the other hand, blockwise distribution of free carboxyl groups and the high DA of pectin were associated with a strong decrease of drugs absorption. Besides, a high molecular weight and a great viscosity of pectin may decrease the transport of drugs across the membranes (Dongowski, Neubert, Haase, & Schnorrenberger, 1996; Dongowski, Schnorrenberger, Plätzer, Schwarz, & Neubert, 1997). Pectin can also modify the effects of bile salts on drug absorption. The addition of pectin to the bile salts above critical micellar concentrations slightly increased the permeation of drugs such as propranolol due to the pectin-induced disruption of micelles and reduction in the interaction drug-bile salts (Dongowski et al., 1996). Such effects can be exploited for the production of sustained drug release systems using pectin.

Development of pectin hydrogels or cross-linked pectin films as matrices for drug controlled release and/or target delivery is another interesting picture of the drug-pectin interactions. Formulation of delivery systems based on pectin cross-linked with acrylic acid-co-acrylamide or 2-acrylamido-methyl propane sulfonic acid matrices sustained the release of orally administered ibuprofen. The encapsulation of indomethacin in pearls of modified citrus pectin hydrogels as well as of doxorubicin in esterified calcium pectinate hydrogels showed a high loading efficiency. Pectin-ethyl cellulose coated beads were found to be a favorable matrix for colon-targeted delivery of the antitumor

drug fluorouracil. Aminated pectin hydrogels were proposed for transdermal delivery of insulin, whereas citrus pectin aerogels provided a good loading capacity and release for theophylline (Naqash et al., 2017; Tiwary & Rana, 2016).

9. Summary and conclusions

Pectin degradation in the human GIT is entirely mediated by microorganisms. The initial debranching and depolymerization of the complex pectin structure is carried out by a relatively small fraction of the overall community in the GIT. Pectinolytic organisms belong to the genera *Bacteroides* (Phylum Bacteroidetes), *Clostridium*, *Eubacterium*, *Faecalibacterium*, and *Monoglobus* (Phylum Firmicutes). Genes and enzymes mediating pectin degradation and uptake has been thoroughly characterized in few model microorganisms (*e.g. Bacteroides thetaiotaomicron*). While similar pectin degradation mechanisms *via* pectin/pectate lyases and polygalacturonic acid hydrolases are widespread in all pectinolytic organisms; distinct mechanisms for uptake have been observed. Bacteroidetes rely on *sus* systems, while Firmicutes employ ABC transporters for sugar products uptake. Discovery of novel pectinolytic taxa within the human microbiome is entirely possible, as well as the possibility of pectin degradation by cellulosome-harboring microorganisms. Sugars generated from pectin degradation is fermented by both pectinolytic and non-pectinolytic organisms *via* cross feeding. SCFA-produced are mainly acetate, propionate, and butyrate, known to provide substantial health benefits to the human intestinal cells. Culture independent 16S rRNA gene-based analysis has been utilized to examine the impact of pectin ingestion on the microbial community in the human intestine using various settings (batch cultures, fermenters, and clinical trials). The collective results of these studies indicate that pectin degradation stimulates a wide range of organisms that could be directly or indirectly involved in pectin degradation, or benefiting from the altered physiological conditions brought about by pectin ingestions.

Pectin, by itself or by its microbial derived metabolites such as SCFAs, is an important modulator of gut homeostasis *via* anti-inflammatory and immunomodulatory effects demonstrated in numerous *in vitro* and in *vivo* studies. Depending on plant source and certain structural features, pectin reduces intestinal inflammation, protects against tumor development and modulates gut immunity. The results of up-to-date studies clearly indicate structural particularities needed for certain anti-inflammatory and immunomodulatory effects enabling targeted therapeutic applications which are to be confirmed by further investigations in humans. A high neutral sugar content, a high degree of branching and the RGI type structure are important for mitigation of intestinal inflammation, prevention of tumor development and modulation of gut immunity whereas the AGII type structure and a high DM potentiate some immunomodulatory effects. Moreover, chemical modifications of native pectin is a promising approach to produce more potent analogues than native pectin. Selenylation, partial hydrolysis and de-esterification (deacetylation) of pectin improve the gut anti-inflammatory, tumor preventive and immunomodulatory effects, respectively. Human studies are highly needed to confirm higher potency shown *in vitro*. Besides, an increase in SCFAs production, particularly butyrate, *via* supplementation with prebiotics and/or probiotics, plays a key role in maintaining gut homeostasis. Detailed understanding of the regulation of SCFAs production inside the human gut is still though unexplored area and, with the advancements in omics tehcnologies, particularly metabolomics that can monitor a large chemical pool, questions as such are expected to be answered. Further, omics studies ought to be considered in the future by analyzing stool metabolism in rats harboring human gut microbiota consortium exposed to pectin from different dietary sources, which would provide a true translation of the several *in vitro* yet fundamental assay results to future research in the field of food pectin – gut microbiota interaction.

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