Carbohydrate Polymers 270 (2021) 118377

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Challenges of pectic polysaccharides as a prebiotic from the perspective of fermentation characteristics and anti-colitis activity

Yu Chengxiao [a], Wu Dongmei [a], Zhu Kai [a], Lijuan Hou [a], Hang Xiao [a], Tian Ding [a], Donghong Liu [a], Xingqian Ye [a], Robert J. Linhardt [b], Shiguo Chen [a,*]

[a] College of Biosystems, Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, Ningbo Research Institute, Zhejiang Engineering Laboratory of Food Technology and Equipment, Zhejiang University, Hangzhou, China

[b] Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, USA

ARTICLE INFO

*Keywords:*
Pectic polysaccharides
Prebiotic
Probiotic
IBD

ABSTRACT

Several studies are described that contribute to the systematic exploration of new aspects of digestion, fermentation, and biological activities of pectic polysaccharides (PPS) leading to a better understanding of prebiotics. Inflammatory bowel disease (IBD) is thought to be associated with the dysbacteriosis induced by different environmental agents in genetically susceptible persons. PPS are considered as an indispensable gut-microbiota-accessible carbohydrate that play a dominant role in maintaining gut microbiota balance and show a better effect in ameliorating IBD than some traditional prebiotics. The aim of this review is to summarize the fermentation characteristics of PPS, highlight its role in improving IBD, and propose a view that PPS may be a new and effective prebiotic.

## 1. Introduction

Inflammatory bowel disease (IBD), which includes Ulcerative colitis (UC) and Crohn's disease (CD), is a spontaneous inflammatory bowel disorder (Ishisono et al., 2019). Epidemiological studies show that more than 1.2 million people in North America and 2 million people in Europe suffered from IBD, and its prevalence exceeded 0.3% in North America and Europe (Ng et al., 2017). The prevalence of IBD has increased not only in developed countries, but also in some newly industrialized countries in Africa and South America since 1990 (Ng et al., 2017). Although the precise etiology of IBD is still unclear, it is generally considered that a combination of gut microbiota disorder and susceptible genotype results in an abnormal immune response in the intestinal mucosa (Lennardjones, 1989). The usual clinical treatment for IBD is an anti-inflammatory biologic or painkiller, but this approach has potential side effects such as fever and gastrointestinal irritation (Celiberto et al., 2018). Fecal microbiota transplantation (FMT) has been an effective method for the treatment of IBD, but its safety remains to be evaluated (Vaughn et al., 2019).

Prebiotics like inulin have been considered promising substance for improving colon inflammation, mainly by regulating the dysbiosis of gut microbiota and metabolic butyrate to modulate gut barrier function and anti-inflammatory effect (Ferreira-Lazarte et al., 2019; Kolida et al., 2002). Over 20 years ago, fructan (FOS and inulin) and galactan (GOS) were recognized as common prebiotics for their effects on enrichment of *Lactobacillus* and/or *Bifidobacterium* spp., thus bringing health benefits to the host. Since then, fructan and galactan have dominated the prebiotic category as evidenced by numerous studies (Gibson et al., 2017). However, some common prebiotics which have been widely recognized for their safety have exposed shortcomings in specific cases due to fermentation characteristics (Singh, Zapata, et al., 2018). It has been reported that some soluble fiber can induce dysbiosis of gut microbiota (Tingirikari, 2018), colon inflammation (Singh et al., 2019), and even cancer (Singh, Zapata, et al., 2018). This is mainly because of their rapid fermentation characteristics, which may result in the lack of fermentable carbohydrates for bacteria in the distal colon, and in turn degrade

*Abbreviations:* IBD, Inflammatory bowel disease; UC, Ulcerative colitis; CD, Crohn's disease; FMT, Fecal microbiota transplantation; FOS, Fructooligosaccharide; GOS, galactooligosaccharide; PPS, Pectic polysaccharides; XOS, Xylo-oligosaccharides; SCFA, short-chain fatty acids; HG, Homogalacturonan; RG-I, Rhamnogalacturonan I; RG- II, Rhamnogalacturonan II; HM, High methoxyl; LM, Low methoxyl; DE, Degree of esterification; OTU, operational taxonomic unit; TJ, Tight junction; LPS, Lipopolysaccharides; ZO, Zonula occludins; HDAC, histone deacetylase; COX-2, Cyclooxygenase-2.

* Corresponding author.
*E-mail address:* chenshiguo210@163.com (S. Chen).

https://doi.org/10.1016/j.carbpol.2021.118377
Received 18 March 2021; Received in revised form 22 June 2021; Accepted 23 June 2021
Available online 26 June 2021

proteins, peptides or fats producing a range of harmful metabolites (Moro Cantu-Jungles et al., 2019). Hence, the previously overlooked dynamic fermentation process of prebiotics in the whole intestinal tract has received more attention. Large polymers, like PPS, due to their slow-fermentation resulting from complex structures, have shown some special bioactivities, particularly in colon inflammation, compared with common prebiotics (Moro Cantu-Jungles et al., 2019). Thus, these polymers have been gradually emerging as potential candidates as a new prebiotic. However, due to their long use as excellent thickeners and emulsifiers in the food industry, the potential biological activities, particularly those associated with a large number of active neutral sugars have been overlooked.

Fermentation and benefits are two fundamental indicators for potential prebiotic candidates (Gibson et al., 2017). Compared with common prebiotics, bacteria cannot rapidly utilize PPS and produce short chain fatty acid (SCFA) due to its complex structure. Thus, PPS has not been considered as a suitable fermentable substance for gut microbiota (Moro Cantu-Jungles et al., 2019). However, the fermentation of PPS has received more attention since the recent clarification of different fermentation models, including single and mixed, batch and continuous, and *in vitro* to *in vivo* fermentations (Ferreira-Lazarte et al., 2018; Ferreira-Lazarte et al., 2019; Gómez et al., 2016; Gómez et al., 2019; Larsen, Bussolo de Souza, et al., 2019). SCFA is the main fermentation product of fiber by gut microbiota and is also important substance that brings health benefits to the host. Table 1 summarizes the SCFA-producing bacteria species and the benefits of SCFA. After being utilized by intestinal bacteria, the impact of common prebiotics is restricted due to the uncoordinated proportion and local sudden release of SCFA at cecum and proximal colon (Cho et al., 2015; Singh et al., 2019; Singh, Zapata, et al., 2018), while PPS does not (Chung et al., 2016). These differences from the conventional prebiotics suggest that PPS has a better performance for certain biological activities.

The current review suggests a view that PPS could be a strong prebiotic candidate from the perspective of its fermentation characteristics and its anti-colitis effects compared to conventional prebiotics. We introduce PPS fermentation models developed in recent years and evaluate its fermentation capacity mainly in regulating intestinal flora and SCFA. Research on PPS in the treatment of colitis is summarized and mechanisms responsible have been documented. Finally, we provide a theoretical basis and reference for PPS as a new prebiotic based on its fermentation and anti-colitis effects.

**Table 1**
The SCFA-producing bacteria species and the functions of SCFA.

| SCFA | Gut microbiota | References | Function | References |
|---|---|---|---|---|
| Acetate | *Anaerostipes -caccae* *Roseburia* *Coprococcuscatus* *Eubacterium rectale* *Eubaterium hallii* *Bifidobacterium* | Lordan et al. (2020) | Anti-inflammatory activity | Lordan et al. (2020) |
| | | | Main source of energy for intestinal flora | Xavier-Santos et al. (2020) |
| | | | Involved in cholesterol metabolism and lipogenesis | Frost et al. (2014) |
| | | | Control central appetite regulation | Frost et al. (2014) |
| | | | Modulate the immune response | Zhang, Hu, et al. (2019) |
| | | | Lower the lumen pH and Resistance to bacterial pathogens | (Prandi et al., 2018) |
| Propionate | *Prevotella* *Ruminococcus* *Bacteroides* *F. prausnitzii* *Clostridium* *Roseburia* *Salmonella* *Lactobacillus* | Lordan et al. (2020); Rowland et al. (2018) | Energy source of epithelial cells | De Vadder et al. (2014) |
| | | | Activate liver glycogen heterogenesis | Tazoe et al. (2008) |
| | | | Control of glycemia | Li, Zhang, and Yang (2018) |
| | | | Increase leptin production | Williams et al. (2017) |
| Butyrate | *Firmicutes* *F. prausnitzii* *Roseburia* *Eubacterium-rectale* *Coprococcus* *Prevotella* *Megasphaera-elsdenii* | Lordan et al. (2020); Singh et al. (2019) | Provides energy to the host | den Besten et al. (2013) |
| | | | Protect intestinal epithelial cells | Donohoe et al. (2012) |
| | | | Stimulate T cell growth | (Lutter et al., 2018) |
| | | | Activate intestinal gluconeogenesis | De Vadder et al. (2014) |
| | | | Against colitis and colon cancer | Li, Han, et al. (2020) |

## 2. The structure and source of PPS

The starting biochemical definition of pectin is that it is a group of polysaccharides that are rich in galacturonic acid (Willats et al., 2001), or the most complex polysaccharide existing in the cell wall of all higher plants (Kacurakova et al., 2000). Pectin is not a single structure and comprises of several PPS domains: 1) Homogalacturonan (HG), is a linear polymer of galacturonic acid linked by 1,4-α-glucosidic bonds (Willats et al., 2001); 2) Rhamnogalacturonan-I (RG- I), its backbone is composed of (1 → 2)-α-L-rhamnose-(1 → 4)-α-D-galacturonic acid (Albersheim et al., 1996). The C-4 of rhamnose can be substituted with neutral sugar side chain. Some common structural features about side chains include (1 → 5)-α-L-arabinosyl and (1 → 4)-β-D-galactosyl residue, (1 → 4)-β-linked D-galactan with non-reducing terminal-arabinose (type I arabinogalactan) and (1 → 3)-β-linked D-galactosyl residues (type II arabinogalactan) also occur in RG-I (Carpita & Gibeaut, 1993; Guillon & Thibault, 1989). 3) RG- II has been reported to has a backbone of around 9 GalA linked by (1 → 4)-α and is substituted by 4 heteropolymeric side chains (Oneill et al., 1996). There are more than 10 different sugars like aceric acid, apiose and 2-keto-3-deoxy-D-manno-occtulosonic acid in RG-II (Vidal et al., 2000). RG-II can dimerize by borate ester links through apiosyl residues which is different from HG and RG-I (Ishii et al., 1999), and hence is considered to be the most complex domain in PPS having an undetermined structure (Maric et al., 2018); and 4) xylogalacturonan with a backbone mainly composed of galacturonic acid and pyranxylose successively connected and with the O-3 position of galacturonic replaced by xylose (Gómez et al., 2016). The galacturonic acid residues of the PPS backbone can be partially esterified by methyl or acetyl groups. PPS can be divided into high-methoxyl (HM) PPS (DE > 50%), and low-methoxyl (LM) PPS (DE < 50%) based on the degree of esterification (Oakenfull & Scott, 1984). Fig. 1 shows the possible structure diagram of PPS.

PPS is generally derived as by-product of fruit processing wastes (Singh & Tingirikari, 2021). PPS from different sources can vary widely in structure, thus, these show variable fermentation characteristics and bioactivity. Traditionally, citrus peel and apple pomace are the main sources of commercial PPS, rich in HG domains isolated using hot acid to remove the side chains (Grassino et al., 2016). Some novel sources PPS, such as banana peel (Emaga et al., 2008), tomato (Grassino et al., 2016), potato (Byg et al., 2012), ginseng, mango (Deng et al., 2020), pumpkin (Zhao et al., 2017), carrot (Jonker et al., 2020) have been reported. These PPS can show unique structural characteristics. For example, PPS from pear, berries, cabbage, leek, and onion show low solubility by chelating agent, but they can be extracted easily in alkali condition,

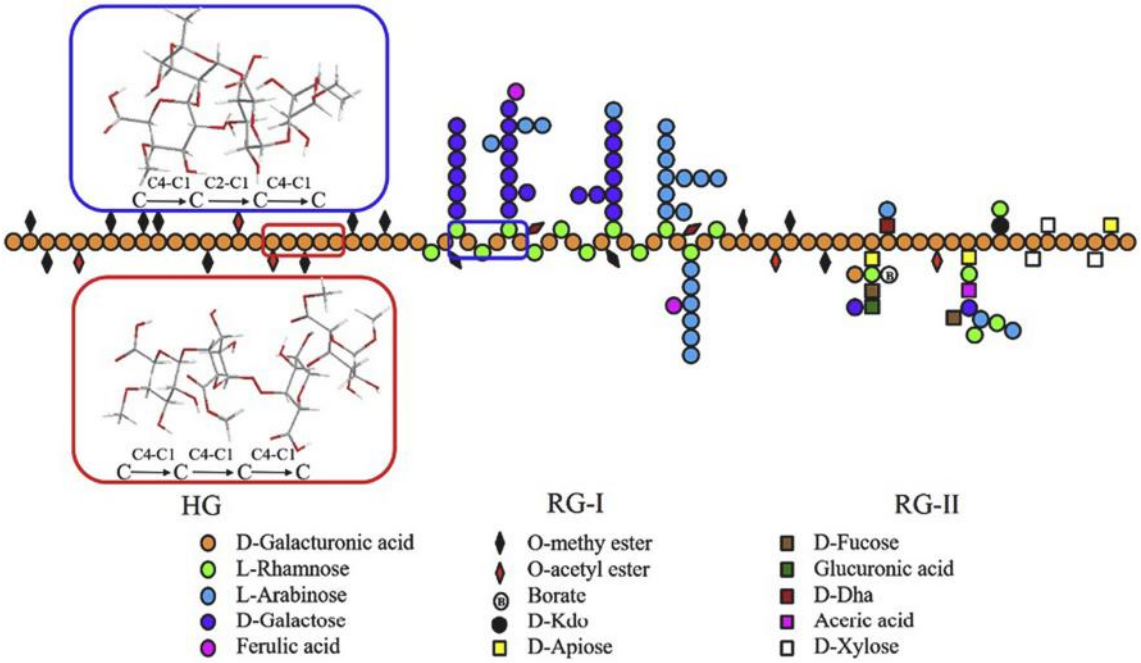


**Fig. 1.** Schematic diagram of different structural domains of PPS. The red box and blue box show the connection of HG backbone and RG-I backbone respectively (Mao et al., 2019).

indicating that these PPS may interact more strongly with cell wall (hemi)cellulose, because chelating agent dissolves PPS by breaking ionic bridge between calcium and non-esterified GalA residues while alkaline condition can break the covalent bond between PPS and (hemi)cellulose (Muller-Maatsch et al., 2016). In contrast, PPS from tomato, rapeseed cake, and grape pomace show opposite properties (Muller-Maatsch et al., 2016). With regard to more detailed structural characteristics, commercial PPS are usually composed of more than 65% galacturonic acid and some neutral sugars (Muller-Maatsch et al., 2016). Commercial PPS mainly consist of HG domains with full of straight chains rather than side chains. However, PPS from Goji (*Lycium barbarum*) (Zhou et al., 2020), carrot (Jonker et al., 2020), sugar beet (Moon et al., 2015), mulberry (Li, Li, et al., 2018), and raspberry (Jakobsdottir et al., 2014) are rich in arabinose and galactose and have a molar ratio of galacturonic acid under 50%, suggesting that they are mainly composed of RG domain. Although these PPS have similar composition of neutral sugars, their structures in the side chains are totally different. Mulberry PPS has the most side chains but the shortest lengths of side chains, while Goji PPS is completely different. In conclusion, the above studies show the structures of PPS is strongly influenced by its source. Since different extraction methods have been used in different studies, this results further studies on structures of different PPS.

## 3. Fermentation of PPS by gut microbiota

In recent years, there has been increasing research on the bioactivity of PPS. Many studies on the structure of PPS include studies on its biological activities, suggesting an increased emphasis in research on bioactivity of PPS (Beukema et al., 2021; Fan et al., 2020; Ishisono et al., 2019; Liu et al., 2020). Traditionally, PPS was thought simply as a non-digestible carbohydrate and the role of its bioactivities was almost entirely based on the gut microbiota (Ferreira-Lazarte et al., 2019).

### 3.1. *In vitro fermentation*

The *in vitro* fermentation model for PPS developed from batch and continuous presentations of specific human fecal bacteria, and from simple tank fermentations used to simulate the complex human colon. Although *in vitro* fermentations cannot simulate the fermentation process as realistically as *in vivo* fermentations, it was more widely used. Table 2 summarizes the *in vitro* fermentation studies of PPS.

#### 3.1.1. *Single or mixed strains culture fermentation*

Fermentation with single or mixed strains was mainly used in the evaluation of PPS regulating the whole intestinal flora. In our gut microbiota, *Bacteroides*, *Prevotella*, *F. prausnitzii*, *Ruminococcus* and some probiotics have the potential to utilize PPS (Ding et al., 2019; Liu et al., 2020). *Bacteroides* is considered the main PPS-degrading bacterial species. One study shows that *B. ovatus* can utilizes glycans including β-glucan, PPS and arabinoxylan (Liu et al., 2020), and *B. thetaiotaomicron* has also been reported to possess a series of PPS-degrading enzymes (Luis et al., 2018). The contribution of *Bacteroides* to degradation of PPS is illustrated by cross-feeding between other bacteria like *Bifidobacterium* (Rogowski et al., 2015), because many bacteria in gut microbiota cannot directly utilize PPS unless it is degraded into oligosaccharide or even monosaccharide (Ferreira-Lazarte et al., 2019). In many cases, this model is mainly used to quickly and simply evaluate the prebiotic effect of PPS. One classic approach is to culture specific probiotics directly with PPS and then determine whether it can stimulate the growth of these probiotics. Generally, *Bifidobacteria* and *Lactobacillus* are the probiotics most widely used for the evaluation of prebiotics. PPS has been shown perfect effect on stimulating the growth of *Bifidobacteria* and *Lactobacillus* (Liu et al., 2020). Other beneficial indicator bacteria like *Bacteroides*, *Blautia* (Bui et al., 2019) and *Coprococcus* (Gamonpilas et al., 2021), some pathogen like *Clostridium perfringens* (Gamonpilas et al., 2021) and *Enterohemorrhagic Escherichia coli* (Beukema et al., 2021) have also been used to evaluate the function of PPS.

In addition to the above advantages, the more important role of the above model is to conveniently evaluate the functions and mechanisms of some specific bacteria species during fermentation. The survival and colonization of probiotics during the passage through gastrointestinal tract is the major issues evaluated by this model. Recently, it has been found that potato fiber (PPS and (hemi)cellulose) can protect *Lactobacillus* strains *in vitro* (Larsen, de Souza, et al., 2019).

Furthermore, there are several researches about the molecular

**Table 2**
*In vitro* fermentation of PPS.

| Substrate source | Treatment method | Molecular weight | Model | Fermentation time | Gut microbiota | SCFA[1] | References |
|---|---|---|---|---|---|---|---|
| Citrus peel | | Sigma-Aldrich (P-9135) | Batch | 48 h | *B. longum B. ovatus V. parvula*↑[2] | Ac ≫ Pr ≈ Bu > Va | Liu et al. (2020) |
| Citrus | | Megazyme Co. | Batch | 48 h | *B. fragilis* ATCC 25285 *B. thetaiotaomicron B. uniformis*↑ | Pr > Ac ≈ La Bu ≈ 0 | Tingirikari (2019) |
| Sugar beet | | | Batch | 6 days | *B. thetaiotaomicron A.rhamnosivorans*↑ | Ac ≫ La ≈ Pr Bu ≈ 0 | Bui et al. (2019) |
| Soy | | | Batch | 6 days | *B. thetaiotaomicron A.rhamnosivorans*↑ | Ac ≫ Bu > Pr La ≈ 0 | |
| Pomelo | Hot acid extraction | 227 ± 50 kDa | Batch | 48 h | *Bifidobacterium Lactobacillus Enterococcus Bacteroides Coprococcus Eubacterium*↑ *Clostridium perfringens*↓ | | Gamonpilas et al. (2021) |
| Goji | Hot water extraction | 1725.5 ± 48.9 kDa | Batch | 24 h | *Bacteroides Bifidobacterium Clostridium* XlVb *Prevotella*↑ | Ac > Pr > Bu > La | Ding et al. (2019) |
| Peach | Hot water extraction | 17 kDa | Batch | 24 h | | Ac ≫ Pr ≈ Bu | Moro Cantu-Jungles et al. (2019) |
| PPS | | 17 kDa, 62 kDa | Batch | 24 h | *Bacteroides B. uniforms F. prausnitzii*↑ *Ruminococcaceae Lachnospiraceae*↓ | Ac ≫ Pr ≈ Bu | Moro Cantu-Jungles et al. (2019) |
| Sunflower | Acid hydrolysis | 12.5 kDa | Batch | 24 h | *Bifidobacterium* 164 *Bacteroides* 303 *Lactobacillus* 158↑ | Ac ≫ Pr > Bu | Ferreira-Lazarte et al. (2018) |
| Artichoke | Acid hydrolysis | 80-300 kDa | Batch | 24 h | *Bifidobacterium* 164 *Bacteroides* 303 *Lactobacillus* 158↑ | Ac ≫ Pr > Bu | Ferreira-Lazarte et al. (2018) |
| Citrus | | 472 kDa, 553 kDa | Continuous fermentation | 34 days | *Bifidobacterium Bacteroides F. prausnitzii*↑ *Lactobacillus Enterococcaceae*↓ | Ac ≫ Bu > Pr > Va ≈ La | Ferreira-Lazarte et al. (2019) |
| Potato | Alkaline extraction | | Continuous fermentation | 56 days | *Bacteroides Bifidobacterium Lactobacillus*↑ *Clostridium leptum*↓ | Ac > Pr > Bu > La | Khodaei et al. (2016) |
| Potato fiber | | Purchased from KMC | Continuous fermentation | 72 h | *Lachnospira B. ovatus*↑ *Ruminococcus*↓ | Ac > Pr > Bu > Va | Larsen, de Souza, et al. (2019) |
| Citrus | Acid and enzymatic extraction | | Continuous fermentation | 72 h | *Bifidobacterium Bacteroides Lactobacillus*↑ *Ruminococcus Sutterella*↓ | Ac > Pr ≈ n-Bu | Larsen, Bussolo de Souza, et al. (2019) |

1 Fo: Formate; Ac: Acetate; Pr: Propionate; Bu: Butyrate; n-Bu: n-Butyrate; Va: Valerate; La: Lactate; Su: Succinat; 2 ↑: It means that the relative abundance of bacteria in front of the arrow has increased; 3 ↓: It means that the relative abundance of bacteria in front of the arrow has decreased.

mechanism or pathway of PPS degrading by single bacterium *in vitro* fermentation. For *Bacteroides thetaiotaomicron*, its glycan-degrading system is encoded by PULs (polysaccharide utilization loci), which includes surface glycan binding proteins, SusC and SusD homologues, outer membrane oligosaccharide transporters and carbohydrate active enzymes (CAZymes) (Lombard et al., 2014). Luis et al. (2018) have characterized the function and genomic organization of pectin PULs in *Bacteroides thetaiotaomicron*. There are 30 glycoside hydrolases and polysaccharide lyases involved in the degradation of major pectin domains. According to Luis et al., the glycoside hydrolases and polysaccharide lyases encoded by BT 4667 to BT 4673 contribute to the degradation of galactan, while BT 0348 to BT 0369, BT 4108 to BT and BT 4145 to BT 4183 contribute to the degradation of arabinan, homogalacturonan and RG-I respectively. For Lactobacillus, Li and Ganzle (2020) have identified the function of an arabinan utilization operon and characterized two putative extracellular arabinanases AbnA and AbnB in *Lactobacillus crispatus* DSM29598, which are homologous to family GH 43 (glycoside hydrolases 43). AbnA can utilize the linear and branched arabinn from pectin to produce lactate and acetate *via* phosphoketolase pathway, while AbnB can only degrade the linear arabinan.

### 3.1.2. Fermentation with human fecal bacteria

Human fecal bacteria inoculation replaced specific bacterial inoculation can better stimulate the fermentation conditions (Khan et al., 2019). The model is based on improvements in bacterial sequencing technology, which allows the quantitative analysis of human gut microbiota. Researchers have been more concerned with the production of SCFA by PPS than the ability of PPS to regulate gut microbiota. Similarly, Moro Cantu-Jungles et al. (2019) conducted a human fecal bacteria fermentation experiment on peach gum PPS. The experiment

focused on the ability of PPS to produce SCFA and found that compared to FOS, peach gum PPS resulted in a lower production of branched chain fatty acids and butyrate (112%), and had a higher production of acetate (16.2%) and propionate (6.2%). The emphasis of polysaccharides fermentation has shifted from regulating flora to stimulating SCFA production. It is noteworthy that the ability of PPS from various sources to produce SCFA in this model appears to be similar, with acetate much greater than propionate and propionate slightly greater than butyrate, valerate and succinate being negligible (Ferreira-Lazarte et al., 2018), but there was no obvious pattern in the first model. The stability of human fecal bacteria in this model has been speculated to be the reason, although there were slight differences in the fecal bacteria used in the studies. Most human fecal bacteria can produce acetate (Rowland et al., 2018), the main propionate producer is *Bacteroidetes*, the main butyrate producer is *Firmicutes*, and the ratio of *Bacteroidetes* to *Firmicutes* is approximately 1:1 (Kramer et al., 2013). The composition of gut microbiota determines the proportion of SCFA produced by fermentation of PPS.

#### 3.1.3. Continuous fermentation

In recent years, with the rise of precision nutrition, there has been a growing focus on the fermentation of PPS in different parts of intestinal tract. There are many aspects that cannot be studied in the traditional culture medium. For example, it has been reported that the fermentation rate of PPS in different parts of the intestine is not the same (Ferreira-Lazarte et al., 2019). Since the distal large intestine lacks fermentable polysaccharides, proteins or peptides can be fermented by bacteria to produce harmful metabolites (Ferreira-Lazarte et al., 2019). The development of a model simulating the complex human intestinal tract was inevitable (Khodaei et al., 2016; Larsen, Bussolo de Souza, et al., 2019).

The first study about the digestion and fermentation of PPS, using complex human intestinal tract simulator, was explored in 2019 (Ferreira-Lazarte et al., 2019). The dynamic gastrointestinal simulator (Simgi*) could simulate processes in the stomach, small intestine, ascending, transverse and descending colon. Thus, it could be used in both digestion and fermentation. The entire cycle of simulated digestion and fermentation is about 34 days, the first 12 days is the model stable period. On the 13th day, PPS is continuously supplied to the instrument for a total of 15 days, and the last 7 days is the wash-out period. In the ascending, transverse and descending colon, the total SCFA content was about 100–300 mM, and the change of six kinds of SCFA was acetate > butyrate> propionate> valerate> formate> lactate.

Similarly, the TIM-1 system and a stirred glass bioreactor (BIOSTAT Qplus) were applied to simulate the digestion and continuous fermentation of potato RG-I PPS (Khodaei et al., 2016), the fermentation conditions are: PPS concentration is 2 g/L, pH is controlled at 6.2, 4 days is a fermentation cycle according to Khodaei. However, it could not simulate the colon as detailed as well as the dynamic gastrointestinal simulator (Simgi*). The biggest highlight of this model was the immobilization of fecal inoculum in gel beads. The fecal bacteria were fixed on the glass beads in a fashion similar to an immobilized enzyme. Although the two studies used advanced fermentation models, they only focused on changes in SCFA.

The TIM-2 model may be more suitable to explore the ability of PPS to beneficially modulate the gut microbiota. Compared to the TIM-1 model, the TIM-2 model is more often used to study the changes of gut microbiota in the colonic fermentation process, which was believed to be a predictive model for clinical trials. The researchers found that gut microbiota in the colon could be directly regulated by different domains of PPS. For example, the RG- I domain is mainly fermented by *Bacteroides* to produce propionate, while the HG domain is mainly utilized by *Ruminococcus* (Larsen, Bussolo de Souza, et al., 2019). The whole fermentation cycle is 3 days, and 7.5 g PPS is fed in every day. Similarly, another study also used TIM-2 model, and found that FiberBind 400 (containing potato PPS) could protect *Lactobacillus* strains from digestive juices throughout the whole digestive tract and selectively regulated the gut microbiota (Larsen, de Souza, et al., 2019).

#### 3.1.4. Impact of the structure of PPS

The impact of PPS structure on fermentation characteristics has been extensively studied. Structure features like the type of monosaccharides, degree of branching and substitution may also determine the access of bacterial enzymes to the polymers thus affecting the fermentation rate in the colon (Holscher, 2017). The above structure features of PPS are more complex than xyloglucan, and hence its fermentation rate is slower than that of xyloglucan (Moro Cantu-Jungles et al., 2019). Meanwhile, some studies suggest that the degree of methylation (DM) greatly impacts the fermentation rate and SCFA production. Furthermore, the lower the DM, the faster the fermentation rate and higher the formation of SCFA. Larsen, Bussolo de Souza, et al. (2019) hypothesized that DM was the most important factor among all the factors affecting PPS fermentation. They found a strong correlation between degree of esterification and bacterial taxa, and they believed that the low surface charge in LM pectin may contribute to strong interactions with bacterial cell wall groups (hydroxyl, carboxyl) negatively charged at neutral pH as strong electrostatic repulsion. However, Alvaro Ferreira-Lazarte et al. (2019) found that the DM value of sunflower and Jerusalem artichoke PPS had little impact on their ability to produce SCFA, which was contrary to the hypothesis of Larsen, Bussolo de Souza, et al. (2019).

For molecular weight, there are several researches using aicd or enzymatic hydrolysis to modify the PPS to explore the effect of molecular weight on fermentation (Ferreira-Lazarte et al., 2018; Gómez et al., 2016; Khodaei et al., 2016; Prandi et al., 2018a). It seems that PPS shows a weaker fermentation ability in SCFA compared to corresponding pectic oligosaccharides (POS), but there is no significant difference in the ability in stimulating probiotics between PPS and POS (Ferreira-Lazarte et al., 2018; Gómez et al., 2016). Interestingly, it may not be that the smaller molecular weight of PPS or POS, the better effect for fermentation. Prandi et al. (2018) found that the 700–1000 Da fraction of sugar beet POS (degraded by nitric acid) showed a stronger ability to stimulate *Lactobacillus* than 400–700 Da fraction, and the 400–700 Da fraction (degraded by enzymes) also showed a stronger ability to stimulate *Lactobacillus* than <400 Da fraction. It is difficult to change the molecular weight while ensuring that other structural information is roughly similar, and there are still a lot of researches to be done.

The structure of PPS and its fermentation characteristics have not been thoroughly studied and the contradictions in reports require additional in-depth researches, and the development of improved fermentation mode.

### 3.2. In vivo fermentation

*In vivo* fermentation is not used as commonly as *in vitro* fermentation due to the disadvantages of difficult operations, time consuming, high cost and poor repeatability. However, as the result of *in vivo* fermentations are more convincing, and it is easier to study the fermentation of PPS in various parts of intestinal tract. Thus, research on PPS based *in vivo* fermentation has gradually increased. There are two main aspects of *in vivo* PPS fermentation. One is to simply evaluate the fermentation characteristics of PPS and the other is to explore biological activities.

Most studies show that PPS can increase diversity in the gut microbiota. The abundance of probiotics, such as *Bifidobacteria* and *Lactobacillus*, reduce the abundance of pathogens (Tian et al., 2016; Tian et al., 2019; Xavier-Santos et al., 2020; Zhu et al., 2020). For some common intestinal flora, the results of *in vivo* fermentation of PPS regulation are similar to those observed in *in vitro* fermentation. In a high-fat diet induced obesity mice model, supplement 100 mg of PPS daily for 8 weeks mainly regulates *Bacteroides*, *Lachnospiraceae*, *Roseburia*, *Ruminococcus* and *Clostridium* cluster IV (Zhu et al., 2020). Interestingly, it seems that different forms of PPS (or POS) may give completely different results. For example, POS can significantly improve the relative

abundance of *Bacteroides*, *Firmicutes*, *Bifidobacterium*, *Lactobacillus* compared to FOS (healthy mice, 2.5 g POS or FOS daily per kg weight for 4 weeks), but the production of SCFA would show no significant difference (Zhang, Hu, et al., 2019). However, the PPS group shows a lower fecal bacterial load, a lower relative abundance of *Bacteroides*, *Bifidobacterium*, *Lactobacillus* and a lower concentration of SCFA than inulin group (healthy rats, about 15.81 g citrus PPS/ 7 days) (Paturi et al., 2017). The reason for this difference is speculated to be that the gut bacteria have a different level of response to PPS having a different degree of polymerization and molecular weight. It should be noted that most *in vitro* fermentation studies use bacteria from human feces, while most of *in vivo* fermentation studies use intestinal flora from mice or rats. The difference of intestinal structures between human and mice may bring uncertainty to results. A study based on rats reported that the fermentation efficiency of PPS is higher than that of xylan (Tian et al., 2019). In contrast, another study, based on human fecal fermentation shows the opposite and even shows that there was almost no change on gut microbiota in the fermentation whole PPS (Moro Cantu-Jungles et al., 2019). The gut microbiota of mice might not truly reflect the human gut microbiota. One approach is to use human flora-associated (HFA) mice or rats to minimize the differences between the models. One study combined 15 kinds of common human intestinal bacteria species and colonized the mice to establish a humanized mouse model (Patnode et al., 2019). Patnode et al. did a *in vivo* screen of the effects of 34 common fibers on members of a defined human gut microbiota, they observed a significant expansion of *B. thetaiotaomicron*, *B. cellulosilyticus*, *B. finegoldii* and a few the *Ruminococcaceae* after 7 days of citrus pectin administration (the concentration of citrus pectin is 10% (*w*/w)). This method may narrow the result differences caused by the differences in the intestinal flora of mice and humans.

## 4. The anti-IBD effect of PPS

### 4.1. Microbiota-dependent ways

#### 4.1.1. The microbiota dysbiosis is a key factor inducing IBD

Inflammatory bowel disease, including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract (Pittayanon et al., 2020). Over the past two decades, the incidence of IBD has increased worldwide and has become a public health challenge (Kaplan, 2015). It has been reported that the prevalence of IBD has exceeds 0.3% in North America, Oceania, and many countries in Europe. The estimated prevalence and incidence for UC is 139.8–286.3 per 100,000 person and 8.8–23.14 per 100,000 person-years in North America, while the data is 90.8–505.0 and 1.7–57.9 in Northern Europe (Ng et al., 2017). Although the exact pathogenesis of IBD remained unknown, the most recognized one is an immune response towards environmental agents and personal genetics (Pittayanon et al., 2020). Many studies suggested that the disorder of the gut microbiome is probably one of the important factors inducing IBD (Ananthakrishnan, 2015).

A striking feature of the gut microbiota of IBD patients is the reduction in α diversity (Halfvarson et al., 2017). α diversity indicates the diversity within sample community species richness (Yang et al., 2020). A comparison of the intestinal flora of UC/CD patients and healthy volunteers shows the α diversity of gut microbiota in CD patients is significantly reduced, while it is almost unchanged in UC patients (Alam et al., 2020). At the phylum level, more than 90% of bacterial species in healthy human belong to four major phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* (Clemente et al., 2012), excess levels of *γ-proteobacteria* can cause impairment of the intestinal barrier and even induce inflammation (Yang et al., 2020), but for other phyla, it is relatively difficult to find out the regularity of changes in gut microbiota in IBD patients, and the results are often confusing. For example, a large scale research of 48 studies comparing the differences in the intestinal flora of IBD patients and healthy volunteers shows none of the above four major phyla appears obvious change law between patients and volunteers (Pittayanon et al., 2020). At a lower taxonomic level, several types of gut bacteria species are closely related to the development of IBD, which is summarized in Table 3. The most widely assessed bacteria species is *Faecalibacterium prausnitzii*. Most studies have reported that the content of *F. prausnitzii* is negatively correlated to the seriousness of IBD, so it is regarded as key bacteria species reflecting IBD (Rowland et al., 2018). The second bacteria species is *Sutterella*, the increase of *Sutterella* is related to the disordered of intestinal flora and intestinal inflammation (Li, Han, et al., 2020). The third bacteria specie is *Akkermansia*. There are two opposing views on the effect of *Akkermansia* on IBD. As *Akkermansia* is a mucin-degrading bacterium, the mainstream belief is that it causes impairment of gut barrier, and, thus, aggravates IBD (V. Singh et al., 2019). Moreover, single *Akkermansia* strain, colonizing germ-free IL-10-deficent mice, can induce colitis (Seregin et al., 2017). In contrast, other researchers have found a decrease of *Akkermansia* in UC patients leading them to speculate that it has an anti-inflammatory effect (Bajer et al., 2017). The fourth is *Escherichia coli.*, which is widely regarded as a potential cause of intestinal infection, bleeding and inflammation (Jimenez et al., 2020). Some adherent-invasive *E. coli* can adhere to and invade intestinal epithelial cells, thus, leading to IBD (Palmela et al., 2018). Interestingly, one study shows that transplanting the intestinal flora of IBD patients into germ-free mice will cause to have symptoms of intestinal inflammation, while transplanting the intestinal flora of healthy volunteers will not cause the disease (Britton et al., 2019), which suggests that the microbiota dysbiosis is one of key factors inducing IBD. The gut bacteria associated with UC and CD is listed in Table 3.

#### 4.1.2. PPS could improve the microbiota dysbiosis

PPS can effectively regulate disorder in gut microbiota, which is one of the mechanisms of an anti-IBD effect. PPS displays a favorable impact on improving gut microbiota diversity and reducing the intestinal bacteria load. In a study for UC patients who had received FMT treatment, the α-diversity of gut microbiota is significantly increased, while that of *Firmicutes* to *Bacteroidetes* when patients treatment with PPS (Wei et al., 2016). In a notable study, Goji polysaccharides increased the abundance of *Actinomycetes*, while other major phylum, such as *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* remain unchanged, but this polysaccharides also show a good anti-colitis effect (Kang et al., 2018). Recently, however, some studies show that PPS can reduce the diversity of gut microbiota. For example, jujube polysaccharides reduce operational taxonomic unit (OUT) by 43.68%, and further studies show a reduction of the relative abundance of *Firmicutes*, *Cyanobacteria*, *Deferribacteres*,

**Table 3**
The gut bacteria associated with ulcerative colitis and Crohn's disease.

| UC[1] | CD[2] | References |
|---|---|---|
| *Faecalibacterium prausnitzii*↓[3] | *Faecalibacterium prausnitzii*↓ | Khan et al. (2019); Pittayanon et al. (2020) |
| *Coriobacteriaceae*↓ | *Coriobacteriaceae*↓ | |
| | *Christensenellaceae*↓ | |
| *Akkermansia*↓ | *Akkermansia*↓ | |
| *Eubacterium rectale*↓ | | |
| *Escherichia coli*↑[4] | *Escherichia coli*↑ | |
| *Veillonella*↑ | *Veillonella*↑ | |
| *Bifidobacteria*↓ | *Bifidobacteria*↓ | |
| *Lactobacillus*↓ | *Lactobacillus*↓ | |
| *Proteobacteria*↑ | *Proteobacteria*↑ | |
| *Bacteroidetes*↓ | *Bacteroidetes*↓ | |
| *Verrucomicrobia*↓ | *Verrucomicrobia*↓ | |
| | *Lachnospiracea*↓ | |
| *Clostridium leptum*↓ | | |
| *Ruminococcus*↓ | *Ruminococcus*↓ | |

1 UC: the change of gut bacteria in UC patients; 2 CD: the change of gut bacteria in CD patients; 3 ↓: the decrease of the relative abundance of bacteria; 4 ↑: the increase of the relative abundance of bacteria.

*Spirochaetes* and *Mucoromycota* (Ji et al., 2020). The diversity decreases as does the abundance of some pathogenic bacteria and this is accompanied by a small increase of some probiotics, leading to the anti-IBD effect of jujube polysaccharides.

PPS improves the relative abundance of *Bifidobacterium*, *Lactobacillus* and other probiotics (Li, Han, et al., 2020). Both *Bifidobacterium* and *Lactobacillus* are known to be the most effective probiotics against colitis, and both can selectively utilize PPS. More than 8% of the genes in the *Bifidobacterium* genome are related to oligosaccharide metabolism and encode for more than 40 glycosyl hydrolases (Schell et al., 2002). Synbiotics, containing *Lactobacillus rhamnosus GG* and *Lactobacillus lactis Bb12*, can effectively inhibit the growth of colon cancer cells. Interestingly, since *Bacteroides* is the main bacteria involved in the degradation of PPS, its high abundance is thought to be helpful, but excess *Bacteroides* has recently been linked to immunosuppression and colon cancer (Yu et al., 2018). Butyrate is generally considered as a substance with anti-inflammatory activity and for improving gut barrier function. PPS can increase the abundance of butyrate-producing bacteria, such as *Clostridium cluster XIVa*, *Lachnospiraceae-Ruminococcaceae* family and *Roseburia* spp., but an abnormal increase in some butyrate-producing bacteria, like *Lachnospiraceae*, may also cause colon tumors (Esposito et al., 2013). Some common pathogens can potentially induce IBD and PPS has been shown to effectively inhibit *E. coli* (Tingirikari, 2018), *Fusobacterium nucleatum*, *γ-proteobacteria*, *Sutterella*.

#### 4.1.3. The effect of butyrate produced by PPS degradation on inflammation

SCFA is the main fermentation product of PPS, and it is considered to be an important mediator between gut microbiota and host. Many studies partially attribute the anti-inflammatory effect of PPS to butyrate (Beukema et al., 2020; Ishisono et al., 2019; Singh et al., 2019; Van den Abbeele et al., 2020). Here, we summarize the effects of butyrate on inflammation.

Butyrate can regulate the differentiation of intestinal epithelial cells and Th cells (L. Chen et al., 2019). The contents of Th1 and Th17 have a negative correlation to improving inflammation within a certain range (Geuking et al., 2011; Moschen et al., 2019). Butyrate stimulates Th1 cell differentiation by promoting IFN-γ and T-bet secretion and suppresses differentiation by inhibiting the secretion of IL-17, Rorα and Rorγ (Britton et al., 2019). Another possible mechanism is that butyrate down-regulates the transcription of HDAC (Zhou et al., 2018). Butyrate, as an HDAC inhibitor, can improve the acetylation of Foxp3 protein and, thus, promote the differentiation of Th1 cells (Furusawa et al., 2013). Additionally, the low expression of HDAC can down-regulate IL-6R and phosphorylation of STAT 3 induced by IL-6, and suppress the expression of IL-17 to inhibit the differentiation of Th17, which suggests HDAC to be a possible target for the clinical treatment of IBD (Glauben et al., 2014).

Butyrate can regulate the growth and function of monocytes and macrophages (Van den Abbeele et al., 2020). In monocytes, butyrate can control the secretion of cytokines and prostaglandin E2 to participate in the inflammatory response. Butyrate also reduces the stimulation of dendritic cells to T cells weakening the immune response throughout the body (Park et al., 2015). In macrophages, butyrate suppresses the expression of IL-6 by inhibiting the TLR2/4 receptor (Ishisono et al., 2019). In addition, butyrate can also suppress the secretion of LPS by RAW264.7 macrophages, which can induce a variety of inflammatory cytokines to mediate systemic inflammation (Bailon et al., 2010). A similar result has also been demonstrated in the bone marrow-derived macrophage, RAW264.7 (Chang et al., 2014). However, other studies have found that excess butyrate inhibits the proliferation of colon stem cells and damages the colonic mucins. Moreover, direct gavage of high concentrations of butyrate in mice can even aggravate intestinal inflammation (Kaiko et al., 2016). The specific mechanism may be that butyrate might activate G protein coupled receptor 41/43, mediated the mitogen-activated protein kinase pathway and trigger inflammatory response (G. J. Chang et al., 2019).

Butyrate can regulate the level of inflammatory cytokines. Studies found that some pro-inflammatory cytokines, induced by LPS, including TNF-α, IL-1β and IL-6 are reduced on treatment with butyrate (Xavier-Santos et al., 2020). Butyrate can also increase the level of IFN-γ and some anti-inflammatory cytokines by Th1 cell, the result of butyrate promoting Th1 cell differentiation. In addition, butyrate can induce T cells to stimulate the expression of Blimp1 protein. When T cells lacked Blimp1 protein, their ability to up-regulate the expression of IL-10, a recognized anti-inflammatory and immunosuppressive factor, will be impaired.

### 4.2. Microbiota-independent ways

#### 4.2.1. Repair the damaged gut barrier

Transgenic animal models demonstrate that the intestinal epithelial barrier injury can cause chronic intestinal inflammation, and the main cause of intestinal epithelial barrier injury is a lack of TJ proteins (Kang et al., 2018). It is worthy to note that both the DSS/TNBS/IL-10-deficient mice models and IBD patients both show significantly higher colon permeability, and the expression and adhesion of TJ proteins are significantly reduced compared with control (Kim et al., 2019). A few studies have reported that PPS can up-regulate claudin2, ZO-1/2, MUC1/3 (Pacheco et al., 2018; Sabater et al., 2019), occludin (Fan et al., 2020) and other genes related to intestinal barrier function. One study shows that orange PPS, and even orange by-products can increase the expression of ZO-1, occludin and MUC-3 (Pacheco et al., 2018). In addition to TJ proteins, PPS can significantly increase the content of IgA in feces (Kang et al., 2018), which suggests an improvement in intestinal mucosal immune response, affording a new perspective of immunology. PPS can also strengthen mucus layer *via* stimulating goblet cells *in vitro* and *in vivo* (Hino et al., 2013). A reasonable explanation is that PPS are negatively charged in most cases, it cannot interact with the same negatively charged mucosa, so it can penetrate the mucosa to reach the epithelial layer, thereby stimulating goblet cells to secrete mucus (Liu et al., 2005). Traditionally, high DE PPS cannot penetrate the mucosal layer, but it can interact with the mucosal layer through the formation of hydrogen bonds to prolong the residence time in the mucosal layer, which acts as a physical barrier to prevent the invasion of some pathogens (Sriamornsak et al., 2010).

#### 4.2.2. Interaction with innate immune cells

The innate immune cells like macrophages respond to the intestinal microbes and their metabolites through pattern recognition receptors, leading to the activation of adaptive immune cells to produce cytokines and chemokines, and developing the process of inflammation when the intestinal barrier function is damaged (Neurath, 2019). So the relative number and status of innate immune cells are closely related to the degree of intestinal inflammation (Neurath, 2019).

In some *in vitro* experiments, several related researches have reported the regulatory effect of PPS structure on macrophages. It seems that only PPS with complete backbone has the ability to activate macrophages, and this ability will disappear when the backbone is destroyed by β elimination (Suh et al., 2013). RG-rich components can significantly increase the IL-6, TNF-α and NO level of macrophages, activate the MAPK and NF-κB pathways to achieve immune enhancement effects (Park et al., 2019). Ho et al. (2015) found that the ability of elderberry PPS to stimulate macrophages gradually weakened after PPS was gradually hydrolyzed with weak acid to remove arabinose and galactose side chains, indicating that the RG side chain of PPS may be the position to bind to the pattern recognition receptors. Interestingly, PPS from *Biophytum petersianum* containing arabinogalactan types I and II, and RG- I and II, it seems that RG- I is more important for stimulating macrophages than RG- II, and the bioactivities of PPS decreased with the decreased neutral sugar side chains (Inngjerdingen et al., 2008).

*4.2.3. Affect some common signal pathways related to inflammation*

NF-κB, enhancing the κ-light chain of B cells activated by nuclear factor, is a protein complex for intracellular nuclear transcription factor involved in the inflammatory and immune response (Rahmani et al., 2020). After NF-κB is activated, it will promote the pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 (Wang et al., 2018) and inhibit anti-inflammatory cytokines like IL-4/10/13, thus, leading to IBD (Granja et al., 2019). Studies on the subunit of NF-κB, p65, show that the transcription of p65 mRNA in the colonic mucus of UC patients is up-regulated and its level is positively correlated with the degree of inflammation, strongly suggesting that p65 protein is involved in the induction and development of inflammation in UC patients, showing that p65 protein can be used as a molecular target of various anti-colitis drugs (Granja et al., 2019). Moreover, since the activation of NF-κB requires phosphorylation of IκB, inhibition of the activity and phosphorylation of IkB is also considered as a molecular target against colitis (Miao et al., 2019). It has been reported that PPS can significantly inhibit the secretion of p65, up-regulate the activity of IκB, inhibit the NF-κB pathway, and reduce the expression of TNF-α and IL-6, thus, ameliorating inflammation (Freysdottir et al., 2016; Miao et al., 2019; Rajagopal et al., 2018). However, the opposite may also be true; PPS may activate the phosphorylation of NF-κB, which leads to internuclear migration of p65 protein and the overexpression of IL-6 and TNF-α (Park et al., 2019). The activation of NF-κB not only promotes the secretion of inflammatory factors, but also promotes the expression of cyclooxygenase-2 (COX-2). Studies have shown that the activity of COX-2 is inhibited in the normal intestinal epithelial cells, while it is highly expressed in inflammatory cells. Some non-steroidal anti-inflammatory drugs can significantly inhibit the activity of COX-2 slowing down the effect of colitis (Kang et al., 2017). This suggests that inhibiting the activity of COX-2 may be a feasible method for the clinical treatment of colitis. Based on this view, a few studies have shown that Goji polysaccharides (Kang et al., 2017), hawthorn PPS (Li et al., 2019) and turmeric PPS (Rajagopal et al., 2018) can significantly inhibit the expression of COX-2, thus, ameliorating colon inflammation.

JAK/STAT, the janus kinase/signal transducer and activator of tranions, is a common pathway for signal transduction of many cytokines related to inflammation, and some essential factors which maintained intestinal cellular immune function, such as IL-2, 6, 10 and 12, are regulated by the JAK/STAT pathway (Salas et al., 2020). It has been demonstrated that STAT 1, 3, 4 are directly involved in the pathological process of intestinal inflammation, and IL-6, 12 in the downstream can enhance the body's susceptibility to colitis (Hedl et al., 2016). IL-6 plays an important role in immune response, cell proliferation, tumor metastasis, and activation of JAK/STAT phosphorylation (Jiang et al., 2020). Therefore, inhibition of the JAK/STAT pathway might be a key target for the chemoprophylaxis and treatment of colitis. PPS may decrease the level of IFN-γ and IL-4 *via* downregulating the expression of pSTAT1 and STAT6, which are the main cytokines produced by Th1 and Th2 in the development of colitis (Ye & Lim, 2010). In addition, PPS can suppress various cytokines by macrophages to inhibit JAK/STAT, so one can speculate that PPS indirectly inhibits the JAK/STAT pathway by reducing the level of inflammatory cytokines (Tan et al., 2018).

MAPK, mitogen-activated protein kinase, is one of the most important pathways in proliferation, differentiation, maturation and immune function of dendritic cells, and is the signal transduction mechanism of LPS-induced dendritic cell maturation. As mentioned above, the MAPK pathway is the up-stream pathway of NF-κB, so the activation of MAPK will also activate its down-stream NF-κB pathway. Dendritic cells play an important role in inflammation, and the inhibition of p38 can inhibit tumor effector T cells and the transformation of regulatory T cells (Miao et al., 2019). Moreover, blocking the MAPK pathway can inhibit pro-inflammatory cytokines and reduce apoptosis of normal colonic epithelial cells. Therefore, inhibition of the MAPK pathway is also an effective method to treat intestinal inflammation. Different PPS have inconsistent effects on the MAPK pathway. For example, PPS from *Rauvolfia verticillata* suppressed the phosphorylation of ERK, p38, and JNK protein and, thus, inhibit the MAPK pathway, as well as its down-stream NF-κB pathway (Miao et al., 2019), while PPS from jujube fruit *(Zizyphus jujuba cv. Junzao)* inhibits phosphorylation of p38 and JNK, not ERK (Zhan et al., 2018). PPS from *Smilax china* L. has a significant anti-inflammatory effect by inhibiting the phosphorylation of ERK1/2 and JNK, but not the phosphorylation of p38 (Zhang, Pan, et al., 2019). However, PPS from Korean citrus does not alter the expression of p38 ERK JNK protein, but significantly induces the phosphorylation of p38 and ERK in RAW264.7 (H. R. Park et al., 2019). The effect of PPS on NF-κB, JAK/STAT and MAPK pathway is summarized in Fig. 2, and the anti-inflammatory effect and possible mechanism of PPS on IBD is listed in Table 4.

## 5. The potential of PPS as prebiotic candidate

PPS show excellent fermentation characteristics and good activity towards colitis making PPS competitive prebiotic candidates. Research on prebiotics is a very active research area, but what were prebiotics? The definition of a prebiotic is directly related to its selection criteria. In 1995, prebiotic was defined as "a non-digestible food ingredient that beneficially impacts the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon." (Gibson & Roberfroid, 1995). In 2002, the defining effect of prebiotics was to stimulate selectively the growth of *Bifidobacteria* and *Lactobacilli* in the gut and, thereby, increased the body's natural resistance to invading pathogens (Cummings & Macfarlane, 2002). In 2004, the definition was altered to "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health." (Gibson et al., 2004). Since 2004, the definition of prebiotic has shifted to the fermentable. In 2008, the definition was again altered to "a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota." (Pineiro et al., 2008). The definition began to emphasize that prebiotics deliver benefits by regulating the microbiota. In 2010, the definition was yet again redefined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit upon host health." (Gibson et al., 2010). Five years later, the definition changed to "a nondigestible compound that, through its metabolite by microorganisms in the gut modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host" (Bindels et al., 2015). The prebiotic-regulated microbiota was still limited to bacteria in the gastrointestinal tract, excluding bacteria on the skin, vagina or other tissues and organs. The most recent definition of prebiotic was proposed in 2017 as "a substrate that is selectively utilized by host microorganisms conferring a health benefit." (Gibson et al., 2017). This definition emphasizes that prebiotics must be selectively used by specific gut bacteria and no longer restricts the way the gut microbiota utilizes them in fermentation.

The development of the definition of a prebiotic clearly reflects the criteria for prebiotic selection. A substance must satisfy four conditions to be a prebiotic: 1) non-digestion; 2) promoting the growth of probiotic; 3) producing benefits on the host; and 4) being selectively by certain gut bacteria. PPS is an indigestible polysaccharide because humans lack PPS-degrading enzymes. Thus, most PPS, usually >80%, remains intact as it passes through the stomach and small intestine (Khodaei et al., 2016). Additionally, PPS can stimulate the growth of beneficial bacteria, such as *Bifidobacterium*, *Bacteroides*, and *Lactobacillus* (Larsen, Bussolo de Souza, et al., 2019), fully meeting the condition of a "stimulating probiotic." Scientists did not understand the mechanism of PPS degradation by bacteria until Gilbert and co-workers (Luis et al., 2018) reported the specific mechanism of pectic glycan degradation by *Bacteroides thetaiotaomicron*. This is precisely one of reasons why PPS can be a prebiotic.

Common prebiotics include inulin, GOS, FOS, chito-

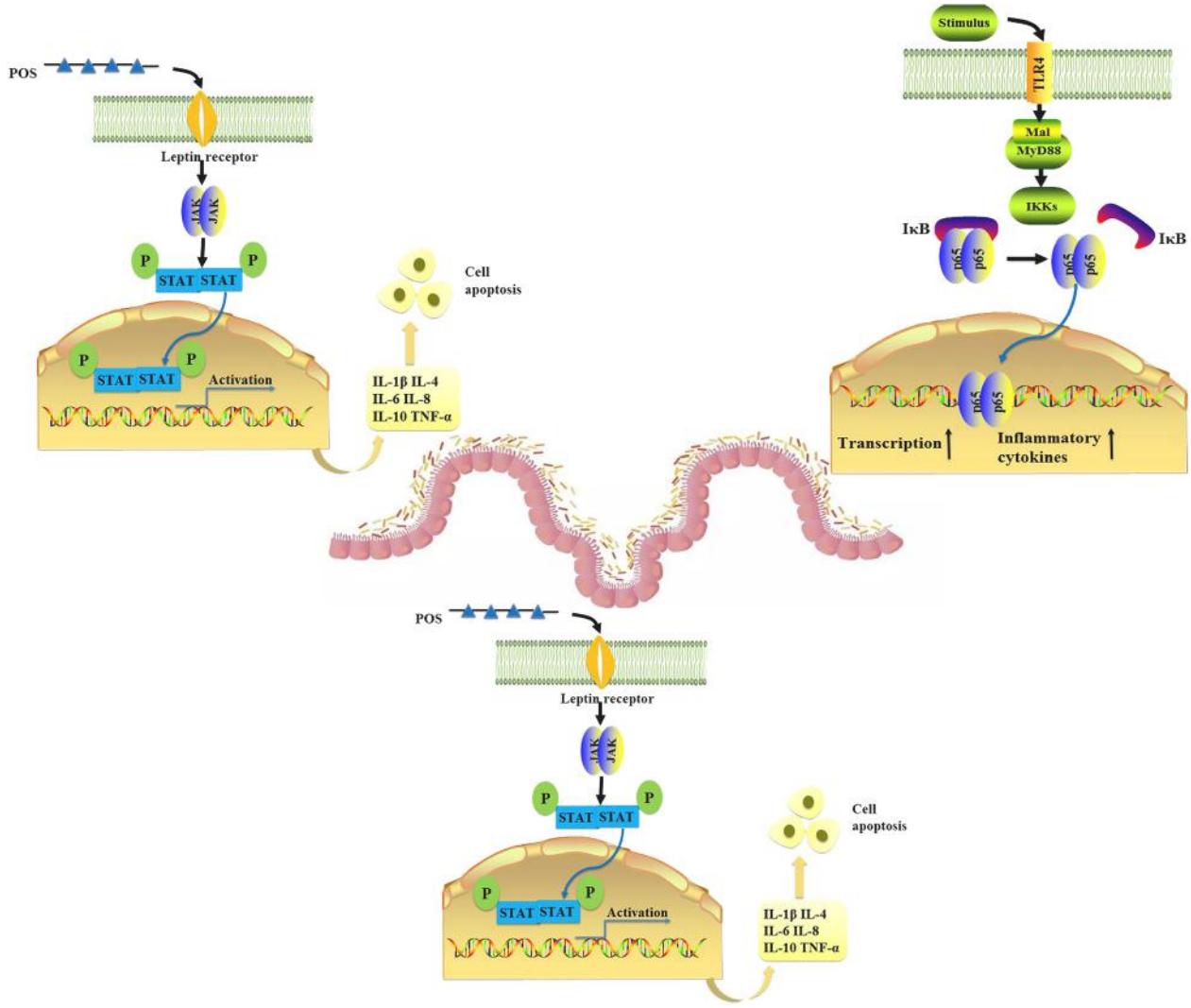


**Fig. 2.** NF-κB, JAK/STAT, MAPK pathway related to gut inflammation.

oligosaccharides, XOS and lactulose (Sanders et al., 2019). The bioactivities of PPS are similar to those of common prebiotics, including:

1) PPS improving host immunity: Some uncommon PPS have shown special immunological competence compared with commercial PPS. For example, sweet cherry PPS significantly induces the expression of NO and some immune proteins such as IL-6 and IL-10 (Cao et al., 2018). Moreover, PPS from silver linden flowers enhance mice immunity by inducing ROS and NO, suppressing iNOS (Georgiev et al., 2017). One study shows that PPS from *Gentiana crassicaulis* may improve host immunity from the perspective of immune complement fixation (Zou et al., 2017).
2) PPS displays anti-cancer activity: PPS had a significant effect on the inhibition of colon cancer, and its possible mechanism is by inhibiting cancer cell division and activated apoptosis by decreasing ICAM1 (Prado et al., 2017). Moreover, researchers also found that PPS could bind and inactivate galectin-3, and the inactivation of galectin-3 promotes apoptosis in cancer cells.
3) PPS can improve chronic metabolic syndrome including hyperlipidemia, hyperglycemia and obesity: Okra PPS, rich in RG domain, can effectively reduce blood sugar levels and improve glucose tolerance in mice (Liu et al., 2018). Another study went a step further, discovering that the RG domain is hypoglycemic primarily through the stimulus of INS-1 cells to secrete insulin (Chen et al., 2018). Additionally, the anti-hyperlipidemic effect of PPS has also been studied, but it is rarely studied in isolation, more focused on anti-obesity activity. It has been speculated that the possible lipid-lowing mechanism of PPS is through inhibiting pancreatic lipase activity, binding bile acid and showing antioxidant activity (Zeng et al., 2016). More importantly, PPS has also been well studied in its most representative activities in chronic metabolic syndrome and obesity.
4) PPS can regulate gastrointestinal inflammation. The effect and mechanism of amelioration of colon inflammation has been discussed in detail in Section 2. In gastric ulcers, PPS prevent gastric ulcer mainly by protecting the gastric mucosa, reducing inflammatory cytokines and apoptosis of gastric epithelial cells (Ortac et al., 2018). The possible mechanism may involve a variety of molecular signaling including MAP kinases, COX-2, PGE2, galectin-3 and MMPs (Manjegowda et al., 2017). Table 5 shows recent researches on bioactivities and functions of PPS.

The possible ways that PPS exert bioactivities were very similar to conventional prebiotics, which could be divided into two aspects: 1) depending on the gut microbiota; and 2) its metabolite SCFA (Ishisono et al., 2019). However, due to the complexity of gut microbiota and the limitations of existing analytical techniques, current research on the molecular mechanism of interaction between PPS and gut microbiota

**Table 4**
Anti-inflammatory effect and possible mechanism of PPS.

| Substrate source | Disease | Model | Gut microbiota | Others | Possible mechanism | References |
|---|---|---|---|---|---|---|
| | IBD | IL-10 deficient mice | *γ-Proteobacteria* *Clostridium cluster XIVa* *Lachnospiraceae* *Ruminococcaceae*↓[1] | Ac Pr Bu↑[2] Ac > Pr > Bu[3] | Slow the enrichment of butyric acid producing bacteria | Singh et al. (2019) |
| Citrus/ Orange | IBD | TNBS[4]mice | | Orange:Ac Pr Bu↑ Ac > Pr > Bu Citrus:Pr Bu↑ Ac > Pr > Bu | The arabinose side chains and galactose side chains of orange pectic polysaccharides can regulate IL-6 | Ishisono et al. (2019) |
| *Myrciaria jaboticaba* | IBD | TNBS mice | *Bifidobacteria* *Lactobacillus* *Enterobacteria*↑ | Bu↓ | Anti-inflammatory and anti-oxidant activity | da Silva-Maia et al. (2019) |
| Goji berry | IBD | IL-10 deficient mice | *Bifidobacterium* *Butyricicoccus* *Clostridium XIVb XVIII* *Pseudoflavonifractor* *Sporobacter*↑ *Clostridium XI*↓ | Ac↓ Bu↑ | Cross-feed butyrate-producing bacteria and Bifidobacteria | Kang et al. (2018) |
| Goji berry | IBD | DSS mice | | Claudin-2 ICM-1 VCAM-1 COX-2 IL-6 MCP-1↓ | Reduce neutrophil infiltration Suppress the expression of CXCL-1, MCP-1 and COX-2 | Kang et al. (2017) |
| *Rauvolfia verticillata* | IBD | DSS mice's dendritic cell | | NF-κB and MAPKs pathways | Suppress the NF-κB and MAPKs pathways | Miao et al. (2019) |
| Citrus | IBD | DSS mice | | Ac Pr n-Bu↑ Ac > Pr > n-Bu | Improve tight junction protein | Kawabata et al. (2018) |
| Artichoke | IBD | DSS mice | | iNOS ICAM-1 TLR-4↓ | The arabinose side chains and galactose side chains of artichoke pectic polysaccharides can regulate IL-6 and IL-1β | Sabater et al. (2019) |
| Orange | IBD | DSS mice | | ICAM1 iNOS↓ MUC protein ZO-1 ↑ | Improve tight junction protein | Pacheco et al. (2018) |
| Apple | IBD | IL-10 deficient mice | | T-bet GATA-3 CD8+ ↓ | Moderate the production of pro-inflammatory cytokines and immunoglobulins | Ye and Lim (2010) |

1 ↓: decrease; 2 ↑:increase; 3 Ac: Acetate; Pr: Propionate; Bu: Butyrate; n-Bu: n-Butyrate; 4 TNBS: Trinitrobenzene sulfonic acid.

are not in-depth, and only structural changes in gut microbiota have been analyzed although the roles and even mechanism of SCFA have been extensively studied, particularly in inflammation, anti-oxidation, and improvement of metabolic syndrome.

## 6. PPS may be healthier than conventional prebiotics in some special cases

In recent years, some conventional prebiotics have shown deficiencies in their fermentation characteristics and bioactivities. Some may even be harmful to humans in certain cases. However, PPS have shown some different fermentation characteristics, which may make up for the shortcomings of traditional prebiotics and even play a more prominent role in some bioactivities (Singh & Tingirikari, 2021).

PPS can travel from cecum, to the proximal colon, to the distal colon. Most conventional prebiotics like inulin and galactan starts to ferment in the cecum and finishes in the transverse colon (Moon et al., 2015). A traditional non-digestible carbohydrate can only benefit the microbiota in the cecum and proximal colon while PPS could benefit almost the whole colon as shown in Fig. 3. There are few studies that compare the fermentation differences between PPS and inulin (FOS), and the results basically conform to this hypothesis (Moro Cantu-Jungles et al., 2019). In addition, the lack of available fermentation substrates for microbiota in distal colon may cause a series of serious consequences, such as flatulence, inflammation and even cancer. When non-digestible polysaccharides is consumed in the proximal colon, the bacteria in distal colon are forced to ferment proteins or peptides for energy, which produce gases like hydrogen, methane, and hydrogen sulfide (Canfora et al., 2019). Besides, other amino acid metabolites like BCFA, phenols and indoles may impair the gut barrier integrity and insulin resistance (Dodd et al., 2017). Some mucus-degrading bacteria can play a dominant role when the colon lacks fiber, which can lead to damage of the gut barrier or even colitis (Desai et al., 2016).

PPS has a more comprehensive ability to regulate gut microbiota. Conventional prebiotics, like inulin, focus too much on the regulation on probiotics such as *Bifidobacterium* or *Lactobacillus*, neglecting the remaining microbiota. Although PPS show a lower ability to promote some specific probiotics, the number of OTU that responds to PPS being more than that of inulin. One study showed that PPS can impact six of the eight most abundant *Bacteroides* in human gut microbiota, while inulin can only impact two (Chung et al., 2016), and the PPS-fed group appears to provide better microbiota diversity than the inulin-fed group. PPS seemed to regulate the microbiota more slowly and gently than inulin (FOS), which means that PPS does not cause drastic changes in the gut microbiota (Chung et al., 2016). The structural differences of fermentation substrates may be one of the reasons for different responses. The differences of metabolites may also be an important reason. Due to the complex monosaccharide composition of PPS, the proportion of SCFA produced by fermentation is more balanced than for inulin, contributing to cross feeding between microbiota and its metabolites (Ferreira-Lazarte et al., 2019).

The SCFA produce by PPS fermentation is more balanced. It is certain that polysaccharides with different structures can produce specific metabolites. A study on monosaccharide and disaccharide fermentation reveals that galactose tends to be fermented into acetate, while rhamnose and arabinose tends to propionate, and galacturonic acid tend to butyrate, suggesting that the metabolites of PPS may be more abundant than for inulin or FOS (Mortensen et al., 1988). In addition, it seemed that the different structural domains of PPS also tend to different metabolites. The HG domain of PPS tend to be fermented into acetate, the AG domain tends to propionate (Moro Cantu-Jungles et al., 2019), and the RG domain tends to propionate and butyrate (Larsen, Bussolo de Souza, et al., 2019). Excessive mono-metabolites, produced by prebiotics, seem to have a negative effect on the host. Inulin can produce a

**Table 5**
Recent researches on bioactivities and functions of PPS.

| Substrate source | Chemical information | Model | Bioactivities | Possible mechanism | References |
|---|---|---|---|---|---|
| *Prunus avium* | High RG-I and arabinose | RAW 264.7 cells | Immune-enhancing | Induces the the release of NO and IL-6 IL-10 TNF-a | Cao et al. (2018) |
| *Gentiana crasskaulis Sambucusnigra* | High RG-I and galactose | sensitized sheep red blood cells (SRBC) | Immune-enhancing | Complements fixation | Zou et al. (2017) |
| Potato | High RG-I | HT29, DLD1, HCT116, LoVo Caco 2 colon cancer cells | Against colon cancer | Reduces ICAM1 gene expression | Maxwell et al. (2015) |
| Tomato | RGI-AG (arabinogalactan) | AGS human gastric carcinoma cells and NIH-3T3 normal mouse embryonic fibroblast cells | Against gastric cancer | Promotes apoptosis of cancer cells | Kapoor and Dharmesh (2017) |
| Papaya | High HG | HCT116 HT29 PC3 colon cancer cells | Against colon cancer | Promotes apoptosis of cancer cells and damage the interaction of cell lines and EMPs | Prado et al. (2017) |
| *Panax ginseng* | High RG-I | Galectin-3 | Antitumor | Inhibits galectin-3 | Cui et al. (2019) |
| *Lonicera japonica* | High RG-I and arabinose | BxPC-3 PANC-1 pancreatic cancer cells | Antipancreatic cancer | Inhibits the growth of cancer cells | Lin et al. (2016) |
| *Fortunella margarita (Lour.)* | High RG-I | | Hypolipidemic effect | Inhibits pancreatic lipase activity and bind bile acid | Zeng et al. (2016) |
| *Pseudostellaria heterophylla* | High HG | Rat insulin one cell line | Hypoglycemic effect | Stimulates insulin secretion of INS-1 cell | Chen et al. (2018) |
| *Tilia tomentosa* | Low-esterified HG/ glucuronidated RGI polymers | Female C3H/HeJ mice | Immune-enhancing | Induces ROS and NO, suppressing iNOS | Georgiev et al. (2017) |
| Okra | High RG-I and galactose | Male C57BL/6 mice | Hypoglycemic effect | Decreases blood glucose level | J. Liu et al. (2018) |
| High-esterified pectic polysaccharides | High-esterified HG | Wistar rats | Anti-obesity | Modulates both brown-adipocyte metabolism and browning | Garcia-Carrizo et al. (2020) |
| *Acmella oleracea* | High RG-I | Female Wistar rats | Anti-gastric ulcer | Protects the gastric mucosa and reduce inflammatory cytokine | Maria-Ferreira et al. (2014) |
| Black cumin | High RG-I | Wistar albino rats | Anti-gastric ulcer | Stimulates molecular signaling cascade including MAP kinases, COX-2, PGE2, galectin-3 and MMPs | Manjegowda et al. (2017) |
| Okra | | Wistar rats | Anti-gastric ulcer | Improves antioxidant defense system | Ortac et al. (2018) |
| Acerola | High HG | Male Swissmice | Anti-fatigue activity | Improves antioxidant defense in the brain | Klosterhoff et al. (2018) |
| Orange | High RG | Male C57BL/6 mice | Anti-colitis | Reduces inflammatory cytokine | Ishisono et al. (2019) |

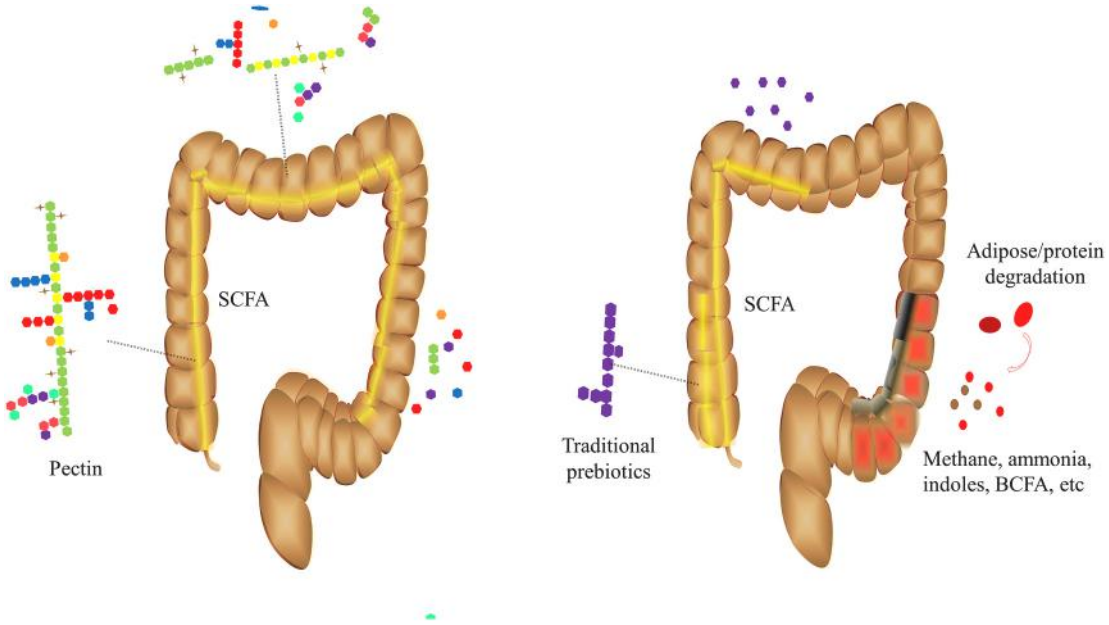


**Fig. 3.** The different fermentation characteristic between PPS and conventional prebiotic.

large amount of butyrate in a short time due to its rapid fermentation in the cecum and the proximal colon, and the accumulation of butyrate can lead to intestinal inflammation for the proliferation of butyrate-producing bacteria phylum, especially *γ-Proteobacteria* (Singh, Zapata, et al., 2018).

## 7. Future directions for PPS as a prebiotic

With the development of prebiotic research, the range of traditional prebiotics urgently needs to be expanded. PPS as a new prebiotic have become more popular, due to good fermentation characteristics and anti-obesity and anti-colitis activities and other positive effects on chronic metabolic syndromes. However, there are also a few shortcomings in the field of PPS bioactivity:

1) The structure-activity relationship of PPS for various biological activities needs to be elucidated. Although the effects of molecular weight, degree of esterification and monosaccharide composition of PPS on its fermentation properties have been reported (Prandi et al., 2018), no clear PPS structure and the influence of structural differences on experimental results have been elucidated. The future goal is to understand the effect of a PPS precise structural differences on its biological activities.
2) PPS are rarely considered solely as a prebiotic. Our daily intake of fruits and vegetables is accompanied with the presence of considerable PPS. We do not need extra PPS hence PPS is considered a functional food ingredient rather than prebiotic. In addition, unlike traditional prebiotics with simple structure, it is difficult to develop specific PPS with unified quality standards and stable efficacy on large scale use due to its variety, complex structure and differential efficacy. There is also a current thinking that prebiotic can be classified as: disaccharides, oligosaccharides and polysaccharides based on the number of monomers bound (Markowiak & Slizewska, 2017).
3) PPS are rarely used as a clinical prebiotic. Few studies have directly used PPS as prebiotics in human clinical studies compared with FOS and GOS. There are two main reasons. One reason is that PPS are the most complex plant-derived polymer which is reflected in its wide range of sources, complicated monosaccharide composition and structure. The complex structure of PPS undoubtedly makes it difficult to control its quality. The other reason is that PPS may not bring benefit and even cause serious consequences in some cases. In a 28-day randomized, double-blind, placebo-controlled, parallel study for 52 healthy young adults and 48 healthy elderly, PPS supplementation did not affect *in vivo* gastroduodenal, small intestinal, colonic and whole gut permeability in young nor in elderly, which is contradictory with the previous role of PPS in improving gut barrier (Wilms et al., 2019). Another surprising study claimed that soluble fibers like PPS, which was previously considered broadly health-promoting, may cause severe icteric hepatocellular carcinoma in T5KO mice (Singh, Zapata, et al., 2018). This suggests that PPS and even the conventional prebiotic inulin, can still have negative effects on the host in some way.

   Therefore, it is necessary to be careful in the clinical application of PPS. It should overcome some crucial barriers for PPS to translation to the clinic. One is that it should provide more safety data in short-term or long-term clinical trials. In addition, it is necessary to provide a high quality and effective commercial products with similar structure. Also, available efficacy data provided by clinicians in an evidence-based manner is indispensable, especially in unbiased systematic review. Finally, safety clarification about PPS are needed to inform patients, such as: is it safe, who will benefit (how and to what extent), and can the labels be trusted (Sanders et al., 2019)?
4) PPS rarely combine with probiotics to form synbiotics. There is no research on the combination of PPS and probiotics resulting in synbiotics, and the metabolic process of PPS-derived synbiotics is still unknown *in vivo*. The interaction between prebiotics and probiotics has always been a research hotspot, particularly the synergistic effect of the two. Therefore, it is necessary to study the synergistic effect of PPS and probiotics in the future.

## Funding

This work was supported by Military Medical Innovation Project (18CXZ044).

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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