Polysaccharide fractions from *Fortunella margarita* affect proliferation of *Bifidobacterium adolescentis* ATCC 15703 and undergo structural changes following fermentation

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**A B S T R A C T**

In this study, the relationships between the proliferation effect of polysaccharide fractions from the citrus shrub *Fortunella margarita* on *Bifidobacterium adolescentis* ATCC 15703 and their resulting structural changes were investigated. Four polysaccharide fractions, FP20, FP40, FP60, and FP80, were obtained by graded precipitation at ethanol concentrations of 20%, 40%, 60% and 80%, respectively. The results showed that polysaccharide fractions, especially FP20, FP40, and FP60, enhanced the proliferation of *B. adolescentis* ATCC 15703 and their effects were better than those of FP80, inulin or glucose. Moreover, acetic acid was mainly produced during fermentation. After fermentation, the molecular weight of polysaccharide fractions decreased and the tightly structural chain conformations of FP20, FP40, and FP60 changed to highly branched structures. The prebiotic effect of these polysaccharide fractions might be related to their molecular weight and chain conformation. Thus, these polysaccharides could be used as potential prebiotics.

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1. Introduction

The balance of intestinal flora in the human body has an important role in maintaining human health [1]. Therefore, how to promote the growth of beneficial bacteria, such as *Bifidobacterium*, in the gut has become a significant focus of research [2]. *Bifidobacterium* is an anaerobic gram-positive bacterium [3] that grows at pH 4.5–8.5 [4], and is known to be beneficial for human health. It can prevent the growth of harmful bacteria by reducing the intestinal pH value [5]. *Bifidobacterium* also secretes active substances involved in carbohydrate metabolism, including glycosyl hydrolases (GHs) and carbohydrate-binding proteins (CBPs) [6]. Members of the GH43, GH3, and GH13 families of GHs in *Bifidobacterium* are crucial for the degradation of plant polysaccharides, with the latter family containing mainly α-amylases [7,8]. *Bifidobacterium* can also secrete exopolysaccharides, and the average molecular weight of exopolysaccharides produced by *Bifidobacterium* exceeds $2.0 \times 10^8$ g/mol [9,10]. *Bifidobacterium adolescentis* is one of the most abundant *Bifidobacterium* spp. in the healthy human intestinal flora [11], and the complete gene sequence of *B. adolescentis* ATCC 15703 is now available (https://www.ncbi.nlm.nih.gov/genome/?term=ATCC+15703). The genome of *B. adolescentis* ATCC 15703 encodes several carbohydrate transport systems and enzymes that are involved in carbohydrate metabolism [12]. *Bifidobacterium* utilizes not only monosaccharides, but also some polysaccharides, such as those from citrus [13], herbs [14], and mushrooms [15]. Short-chain fatty acids (SCFAs) are organic fatty acids of 1–6 carbon atoms and are the main anions produced by bacterial fermentation of polysaccharides, resistant starch, dietary fiber, oligosaccharides, and glycoproteins [16,17]. SCFAs, as an acidic metabolite, can promote the growth of beneficial bacteria and inhibit the growth of harmful bacteria [18].

The citrus shrub *Fortunella margarita* (Lour.) Swingle, which originates in southeastern China, is cultivated for its health benefits in many parts of the world, including Europe, Japan, USA, Brazil, Australia, and India [19]. It is rich in nutrients and bioactive compounds, such as polysaccharides, limonoids, essential oils, flavonoids, phenolic acids, vitamins, dietary fiber, and amino acids, with carbohydrates accounting for 15.9% (g/g) of the dry weight of the plant [20]. In addition, polysaccharides are one of several important biologically active compounds in *F. margarita*. Previous studies showed *F. margarita* polysaccharides comprised four components [21,22]. Zeng et al. [21] reported that the hypolipidemic activity of *F. margarita* polysaccharide fractions was affected by their monosaccharide composition, glycosidic linkage,
molecular weight, and chain conformation in aqueous solution. Additional studies showed that polysaccharides could promote the proliferation of bifidobacteria, such as rapeseed polysaccharides [23], arabinoxylan [24], konjac glucomannan [25], xylo-polysaccharides [26]. The relationship between polysaccharide structure and the proliferation of bifidobacteria were studied usually, but the changes of polysaccharide structure after fermentation were rarely investigated.

Thus, in the current study, the effects of polysaccharide fractions extracted from *F. margarita* on the proliferation of *B. adolescentis* ATCC15703 and any preliminary structural changes to the fractions as a result of fermentation were investigated. Growth curves and pH change curves were measured and SCFAs were determined by high performance liquid chromatography (HPLC). Molecular structural characteristics of *F. margarita* polysaccharides after fermentation were determined by size-exclusion chromatography (SEC), multi-angle laser light-scattering (MALLS), and refractive index (RI).

2. Materials and methods

2.1. Extraction of *F. margarita* polysaccharides

*F. margarita* was provided by the Youxi Agricultural Bureau, Sanming City, Fujian Province, China. The method used to prepare the polysaccharide fractions is shown in Fig. 1. Briefly, after cleaning and removing seeds, fresh *F. margarita* was added to 10 volumes of water and crushed in a juicer. The cloudy juice was poured into a beaker and kept for 2.5 h at 80 °C in a constant-temperature water bath (HH-6, Ronghua, Jiangsu, China). The extracted solution was centrifuged at 4000 rpm for 20 min [27] using a centrifuge (H1850R, Xiang Yi Laboratory Instrument Development Co., Ltd., Hunan, China) to remove the precipitate. The supernatant was deproteinized by Sevag solution (chloroform: butyl alcohol = 4: 1) for 20 min and centrifuged at 4000 rpm for 20 min to remove the precipitate. After removing the protein, absolute ethanol was added to the *F. margarita* polysaccharide solution at a final ethanol concentration of 20% (v/v). After sedimentation for 20 h, the mixture was centrifuged at 4000 rpm for 20 min and the polysaccharide precipitate was labeled ‘FP20’. According to the above methods, FP40, FP60 and FP80 were also obtained by 40%, 60% and 80% ethanol concentration, respectively.

2.2. Fermentation of polysaccharides

2.2.1. Activation of media

*B. adolescentis* ATCC 15703 was purchased from the Guangdong Microbial Culture Center. The activation methods were as follows: the activation medium comprised 5.00 g/L soya peptone, 5.00 g/L tryptone,
2.3. Determination of SCFAs, lactic acid and pH

The basal medium was prepared as described previously [28] with a slight modification. It comprised 5.00 g/l beef extract, 5.00 g/l peptone, 3.00 g/l yeast extract, 5.00 g/l NaCl, 0.50 g/l L-cysteine-HCl, 3.00 g/l sodium acetate, and 0.02 g/l polymyxin. The pH of the basal culture medium was adjusted to 6.80 ± 0.02. All medias were sterilized by autoclaving (MJ-54A; Stik Co., Ltd., Shanghai, China) at 121 °C for 15 min before use. The basal medium was adjusted to pH 6.80 ± 0.02. All medias were sterilized by autoclaving (MJ-54A; Stik Co., Ltd., Shanghai, China) at 121 °C for 15 min before use. The activated B. adolescentis was incubated for 18 h in a basal culture medium supplemented with 5.00 g/l glucose as the bacterial mother liquor.

The sources of carbon in the basal culture medium were supplemented with 10.00 g/l FP20, FP40, FP60, FP80, inulin (Aladdin, Shanghai, China), and glucose (Aladdin), respectively. The bacterial mother liquor (5.00%) was transferred to the basal culture medium with FP20, FP40, FP60, inulin, and glucose. Media were incubated in an anaerobic incubator at 37 °C for 4, 8, 14, 20, 32, or 48 h, respectively.

2.2.2. Fermentation

The basal medium was prepared as described previously [28] with a slight modification. It comprised 5.00 g/l beef extract, 5.00 g/l peptone, 3.00 g/l yeast extract, 5.00 g/l NaCl, 0.50 g/l L-cysteine-HCl, 3.00 g/l sodium acetate, and 0.02 g/l polymyxin. The pH of the basal culture medium was adjusted to 6.80 ± 0.02. All medias were sterilized by autoclaving (MJ-54A; Stik Co., Ltd., Shanghai, China) at 121 °C for 15 min before use. The activated B. adolescentis was incubated for 18 h in a basal culture medium supplemented with 5.00 g/l glucose as the bacterial mother liquor.

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2.2.3. Determination of bacterial concentration

Cell suspensions were centrifuged at 4000 rpm for 15 min and the precipitate was resuspended with distilled water. Aseptic deionized water was used as a blank control. The optical density at 600 nm (OD600 nm) (UV-1100; Mapada Instrument Co., Ltd., Shanghai, China) was measured to determine the number of cells, as described previously [29,30]. At the same time, the dry weight method was used to measure the concentration of bacteria suspension, and a standard curve was obtained. The relationship between the absorbance value of the bacteria suspension (y) and the concentration of bacteria (x) was drawn according to the relationship between the absorbance value of the bacteria suspension (y) and the concentration of bacteria (x). The standard curve equation was as following equation:

\[
y = 0.3602x - 0.0188 \quad (R^2 = 0.9813)
\]

2.3. Determination of SCFAs, lactic acid and pH

The method was carried out as described by De Baere et al. [31] with a slight modification. Ultrapure water was used to prepare a solution of acetic acid, propionic acid, butyric acid, and lactic acid to a concentration (mL) at 37 °C and passed twice.

\[
Y = 3.92 \times 10^7x + 7.11 \times 10^4 \quad \text{(lactic acid, } R^2 = 0.9998) \quad (5)
\]

using SCFAs and lactic acid with a concentration (x) as the abscissa, and peak area (y) as the ordinate. The SCFAs and lactic acid contents of the samples filtered through a 0.45 μm filter were determined using GLC analysis. The relationship between the absorbance value of the bacteria suspension (y) and the concentration of bacteria (x) was drawn according to the relationship between the absorbance value of the bacteria suspension (y) and the concentration of bacteria (x). The standard curve equation was as following equation:

\[
y = 0.3602x - 0.0188 \quad (R^2 = 0.9813)
\]

2.4. Determination by SEC-MALLS-RI

Before the experiment, the mobile phase was prepared with a 0.10 mol/L NaCl solution. The molecular weight was measured as previously described [32,33], with some modification. The molecular weights of FP20, FP40, and FP80 were measured using a SB-806 M HQ column (Shodex, Tokyo, Japan), whereas the molecular weights of FP20, FP40, FP60, and FP80 after 20 h and 48 h were measured using a SB-806M HQ column connected to a SB-803 HQ column (Shodex). The mobile phase operated at a flow rate of 0.50 mL/min and a column temperature of 25 °C. The instrument system was normalized with dextran [34]. The data obtained from this SEC-MALLS-RI system were analyzed by ASTRA 6.1 software (Wyatt Technology, CA, USA). Bovine serum albumin was used to determine the delay volume between MALLS and RI. The refractive index (dn/dc) was set as 0.145 mL/g in accordance with the literature [35].

2.5. Statistical analysis

All the experiments were repeated in triplicate. All results are shown as the mean ± standard deviation. One-way ANOVA and Duncan tests were used to analyze repeated measures data in SPSS (version 13.0, Chicago, IL, USA). All the figures were drawn using Origin 8.0 (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. B. adolescentis proliferation

The growth curves of B. adolescentis in media containing different carbon sources are shown in Fig. 2A. After 4 h fermentation, B. adolescentis in the media containing FP20, FP40, and FP80 was in the logarithmic growth phase, peaking at a constant amount of cells during the stationary phase after 20 h for FP20 and FP40, and 14 h for FP80. With the increase of incubation time, not only the number of B. adolescentis, but also its proliferation rate increased. However, after 20 h, the number of cells and the rate of proliferation increased only slowly in media containing FP20, FP40, and FP60. This might be because, after 20 h fermentation, FP20, FP40, and FP60 had been broken down and only a few molecules remained. After 14 h, B. adolescentis was in a stable state of growth in media containing FP80, inulin, and glucose. The concentration of B. adolescentis at 48 h is shown in Fig. 2B. The concentration of B. adolescentis in media containing FP20, FP40, and FP60 was significantly higher (p < 0.05) than those in media containing FP80, inulin and glucose, suggesting that FP20, FP40, and FP60 better promote the proliferation of B. adolescentis compared with FP80, inulin, and glucose.

Bacteria exhibit four phases of growth: lag phase, logarithmic growth phase, stationary phase, and death phase. The factors affecting the growth of microorganisms include acidity, carbon sources, carbon structure (e.g., glycosidic linkages and degree of molecule branching), and the relationship between bacteria, the substrate, and metabolites (i.e., SCFAs) [4,36,37]. High-molecular-weight polysaccharides are fermented more slowly than small-molecular-weight polysaccharides, with a longer period required for them to be utilized [38]. In this experiment, the growth time of B. adolescentis media supplemented with FP20, FP40, and FP60 were not only longer than that of media supplemented with FP80, inulin, and glucose.
supplemented with FP80, inulin, and glucose but there were also more cells in the medias with FP20, FP40 and FP60 after 48 h fermentation. This could be explained by the higher molecular weights of FP20, FP40, and FP60 compared with FP80, inulin, and glucose. These results also suggested that the molecular weight of F. margarita polysaccharides decreased with an increase in the ethanol concentration, which was consistent with the results reported by Ma et al. [39]. The molecular weight of Dioscorea opposita polysaccharides decreased with the increasing concentration of alcohol solutions. In addition, it might be that FP20, FP40 and FP60 provided more accessible nutrients to the bacteria, with the nutrient levels in the media containing FP80, inulin, and glucose being depleted more quickly than in the media containing FP20, FP40, and FP60.

3.2. SCFAs, lactic acid production, and pH value

The metabolic pathways of sugar metabolism in Bifidobacterium differed from those in other microorganisms [40]. In this pathway, 1.0 mol of glucose produces 2.5 mol of ATP, 3.0 mol of acetic acid and 2.0 mol of lactic acid, which is either released to the media or used for de novo fatty acid synthesis [41]. In this study, the amounts of SCFAs and lactic acid produced by B. adolescentis ATCC 15703 were measured after fermentation in media containing different carbon sources and only acetic acid and lactic acid were detected (Table 1), which was consistent with a previous report that mainly acetic acid and lactic acid were produced by B. infantis ATCC 15697 after growth on different prebiotics [42]. In the current study, only acetic acid was detected in the medium with FP80, which might be related to the specificity of the medium substrate [42]. Acetate acid was the primary fermentation product. With the extension of fermentation time, the acetic acid concentration gradually increased. Before 4 h, the generation rate of acetate acid in the media containing FP20 and FP40 was slow, whereas after 20 h fermentation, the concentration of acetate acid was stable. After 14 h, the concentration of acetate acid in the media containing FP80 was in a steady state. At 4 h, there was a substantial concentration of acetate acid in the media containing FP60, inulin, and glucose. The production of acetate acid was in accordance with the growth curves of B. adolescentis. With the increase in fermentation time, the amount of lactic acid initially increased, then decreased in the media containing FP40, FP60, and inulin. This might reflect the stationary phase of bacterial growth, in which lactic acid was used a carbon source by B. adolescentis [43]. At 20 h, a substantial concentration of lactic acid had been generated in the media containing FP40 and inulin.

The medium pH value can reflect the growth of B. adolescentis [44], although the changes in pH value are related not only to the production curves, but also to the type and concentration of acid metabolites. The pH changes in the media containing carbon sources are shown in Fig. 3A. At 4 h, the pH value in the medium containing FP60 was the lowest among the different treatments. It might be that, at 4 h, the rate of cell proliferation was the fastest (Fig. 2A) among the treatments, resulting in more acid metabolites being produced. At 20 h, the pH values of the media containing FP20, FP40, and FP60 was lower than

Table 1
The SCFAs concentration in fermentation media at different time.

| Time/h | FP20 (g/mL) | FP40 (g/mL) | FP60 (g/mL) | FP80 (g/mL) | Inulin (g/mL) | Glucose (g/mL) |
|--------|-------------|-------------|-------------|-------------|---------------|----------------|
| Acetic acid concentration | | | | | | |
| 4 | 0.3933 ± 0.1138Ea | 0.6443 ± 0.1055Ec | 1.2750 ± 0.1001Eb | 1.1060 ± 0.0026Da | 1.9200 ± 0.1364Ed | 2.7450 ± 0.7128Da |
| 8 | 2.7360 ± 0.1455Ca | 2.7353 ± 0.6641Cc | 1.9200 ± 0.1508Cb | 3.4260 ± 0.6327Ca | 2.6773 ± 0.3247Da | 2.7340 ± 0.4779Ca |
| 14 | 3.5053 ± 0.2312Ch | 2.9887 ± 0.1382Cf | 2.4923 ± 0.1365Ce | 7.0587 ± 0.3449Dc | 3.6733 ± 0.0516Dc | 3.2807 ± 0.2394Db |
| 20 | 6.0000 ± 0.1755Ca | 4.7537 ± 0.1326Bd | 5.4937 ± 0.2169Dc | 7.0570 ± 0.0630Dc | 4.0373 ± 0.0263Dc | 3.8777 ± 0.1439G |
| 32 | 6.2067 ± 0.0457Ca | 4.6737 ± 0.0527Bc | 5.5437 ± 0.0251Dc | 7.8330 ± 0.0660Dc | 4.4507 ± 0.0899Ba | 4.5007 ± 0.0703Ba |
| 48 | 6.8067 ± 0.0213Cg | 5.4883 ± 0.0661Ac | 6.0163 ± 0.1450Ec | 8.7473 ± 0.0052Ac | 4.5657 ± 0.0680Ac | 4.5817 ± 0.0380Ba |
| Lactic acid concentration | | | | | | |
| 4 | 0.1303 ± 0.0165Ec | 0.0933 ± 0.0042Ab | 0.0107 ± 0.0133Fb | – | 0.1810 ± 0.0343Cr | 0.1730 ± 0.0340Ge |
| 8 | 0.1560 ± 0.0136Ec | 0.2477 ± 0.0044Ec | – | 0.2373 ± 0.0110Ab | 0.4427 ± 0.0014Fd | 0.3733 ± 0.1348Dc |
| 14 | 0.3783 ± 0.0407Ec | 0.4400 ± 0.0021Ec | 0.4007 ± 0.0046Ec | – | 1.3620 ± 0.1841Db | 0.9293 ± 0.1572Ee |
| 20 | 1.0473 ± 0.0027Ec | 2.7440 ± 0.1850Ec | 1.7900 ± 0.1198Ff | – | 3.5680 ± 0.1610Hf | 1.5187 ± 0.1261Hf |
| 32 | 1.4203 ± 0.0624Ed | 4.6990 ± 0.0546Ad | 2.8130 ± 0.1501Db | – | 2.6760 ± 0.4575Ba | 2.2503 ± 0.2178Bc |
| 48 | 2.2637 ± 0.1611Eb | 3.3600 ± 0.0182Ec | 2.4763 ± 0.0991Ec | – | 1.6747 ± 0.0153Cf | 2.2097 ± 0.0210Ec |

Different lowercase letters in the same row represent significant differences between different treatments (p < 0.05). Different capital letters in the same column represent significant differences between different treatment time (p < 0.05).
Fig. 4. Chromatograms of the molar mass distribution of *F. margarita* polysaccharides fermented by *B. adolescentis*: (A1): FP20 (0 h); (A2): FP20 (20 h); (A3): FP20 (48 h); (B1): FP40 (0 h); (B2): FP40 (20 h); (B3): FP40 (48 h); (C1): FP60 (0 h); (C2): FP60 (20 h); (C3): FP60 (48 h); (D1): FP80 (0 h); (D2): FP80 (20 h); (D3): FP80 (48 h).

Fig. 3. pH changing curves (A) and pH value at 48 h (B) in the presence of FP20, FP40, FP60, FP80, inulin and glucose. Different letters mean statistical differences in the category (*p* < 0.05).
those of the media containing inulin and glucose, in accordance with the growth curves of B. adolescentis. These results might be related to the higher levels of acid metabolites produced in media with FP20, FP40 and FP60 fermentation compared with the media with inulin and glucose. Kong et al. [45] reported that fermented media contained more SCFAs, resulting in a lower pH value. The total acid metabolite content was not recordable in media containing FP20, FP40, FP60, and FP80, whereas the pH values of the media containing FP20, FP40 and FP60 carbon sources were lower compared to the media containing FP80. It might be related to the type of acid metabolites present. The amount of acid production was not consistent with the growth curve, in accordance with previous studies. Zhang et al. [44] reported that the content of lactic acid showed a decrease after 24.5 h fermentation with lotus seed resistant starch while the amount of bacteria increased.

The pH values at 48 h in the presence of FP20, FP40, FP60, FP80, inulin, and glucose are shown in Fig. 3B. The pH values in the presence of FP20, FP40, and FP60 were significantly lower (p < 0.05) than those in the presence of FP80, inulin, and glucose. The pH value of the medium containing FP80 was significant higher (p < 0.05) than those in the presence of FP20, FP40, FP60, inulin and glucose, which might be because only acetic acid was produced in the media containing FP80 [45].

3.3. Molecular weight changes of polysaccharides

The molar mass distributions of the F. margarita polysaccharides at different fermentation time are shown in Fig. 4. The MALDI and RI signals of F. margarita polysaccharides only had one peak (Fig. 4A1, B1, C1, and D1). Furthermore, the polydispersity of FP20, FP40, FP60, and FP80 was 1.253, 1.233, 1.180, and 1.127, respectively, indicating that graded alcohol precipitation was suitable for separating F. margarita polysaccharides with different molecular mass [46,47]. With the increase in fermentation time, the time of the peaks was delayed, demonstrating that the molecular weight of FP20, FP40, FP60, and FP80 had decreased. The molecular weights of F. margarita polysaccharides at different fermentation time are shown in Table 2. The absolute molecular weights of FP20, FP40, FP60 and FP80 were 1.341 × 10^6, 6.881 × 10^5, 6.000 × 10^5 and 1.941 × 10^5 g/mol, respectively, indicating that high ethanol concentrations resulted in the precipitation of the lower-molecular-weight F. margarita polysaccharides, whereas low ethanol concentrations resulted in the precipitation of relatively macromolecular F. margarita polysaccharides. This might be because the higher the molecular weight of the polysaccharides, the lower its polarity, resulting in it being precipitated by ethanol with a lower volume fraction. Low-molecular-weight polysaccharides had better solubility compared with high-molecular-weight polysaccharides, which required a higher volume of ethanol to precipitate [48]. After 20 h fermentation, the molecular weights of FP20, FP40, FP60, and FP80 had decreased to 1.065 × 10^5, 1.258 × 10^5, 1.743 × 10^4, and 1.086 × 10^4 g/mol, respectively. After 48 h of fermentation, their absolute molecular weights were 7.636 × 10^3, 5.834 × 10^3, 1.017 × 10^3 and 9.667 × 10^2 g/mol, respectively. These results demonstrated that F. margarita polysaccharide fractions could be utilized by B. adolescentis to promote its growth.

The cumulative weight fractions of the F. margarita polysaccharide fractions fermented by B. adolescentis at different time are shown in Fig. 5. After fermentation, there was no molecular weight distribution of FP20, FP40, FP60, and FP80 above 2.0 × 10^5, indicating that B. adolescentis could not secrete extracellular polysaccharides in the media containing F. margarita polysaccharides. Before fermentation, the main molecular weight of FP20 was ~1.0 × 10^6. However, there was a larger molecular weight distribution with <1.0 × 10^5 of FP20 after 48 h fermentation compared with after 20 h. Before fermentation, the molecular weight of FP40, FP60 and FP80 ranged from 1.0 × 10^5 to 1.0 × 10^6. After 20 h fermentation, the main molecular weight distribution of FP40 and FP60 was 1.0 × 10^5–1.0 × 10^6, whereas, after 48 h, it was <1.0 × 10^5. By contrast, after 20 h fermentation, the molecular weight of 1.0 × 10^5–1.0 × 10^6 of FP80 accounted for 47.52% of total polysaccharide molecules, whereas after 48 h fermentation it accounted for 25.85%. The results indicated that these four polysaccharide fractions could be utilized by B. adolescentis ATCC 15703.

![Fig. 5. Mass fractions of F. margarita polysaccharides fermented by B. adolescentis of different molar mass ranges.](image-url)
3.4. Chain conformation of polysaccharides

The SEC-MALLS-RI system provides not only molecular weight data, but also the chain conformation of a macromolecule. The chain conformation is obtained by determining the slope of molar mass against molecular radius [49]. The chain conformations of the *F. margarita* polysaccharide fractions are shown in Fig. 6. Before fermentation, the slope value of the FP20 plot was $0.24 \pm 0.00$ (Fig. 6A1), indicating that it had a tight, uniform, spherical conformation. The slope value of the FP40 plot was $0.53 \pm 0.00$ (Fig. 6B1) and the FP60 plot was $0.64 \pm 0.01$ (Fig. 6C1), suggesting that these both had a random coil conformation. However, the FP80 plot revealed a rod-like conformation (Fig. 6D1). By contrast, after 20 h and 48 h fermentation, FP20 (Fig. 6A2 and A3), FP40 (Fig. 6B2 and B3), and FP60 (Fig. 6C2 and C3) displayed U-shaped curves, suggesting a typical highly branched structure. In addition, after 20 h and 48 h fermentation, the slope value of the FP80 plot was $>1.00$, indicating that it had a rod-like conformation (Fig. 6D2).

*F. margarita* polysaccharides comprise galactose, glucose, galacturonic acid, rhamnose, mannose, and arabinose [21]. The GH protein family of *B. adolescentis* ATCC 15703 includes $\alpha$-amylases, $\beta$-glucosidase, $\alpha$-L-arabinofuranosidase, arabinofuranohydrolase, $\alpha$-glucosidase, $\beta$-galactosidase, $\alpha$-galactosidase, and $\alpha$-mannosidase ([https://www.ncbi.nlm.nih.gov/genome/?term=ATCC+15703](https://www.ncbi.nlm.nih.gov/genome/?term=ATCC+15703)). These hydrolytic enzymes can partially hydrolyze *F. margarita* polysaccharides, resulting in the decrease in the molecular weight of the latter. The resulting hydrolysates are the unutilized by *B. adolescentis* ATCC 15703, promoting its proliferation. Given the specificity of the enzymes, *F. margarita* polysaccharides were partially hydrolyzed, resulting in the changes to their chain conformation.

3.5. *F. margarita* polysaccharides metabolism by *B. adolescentis* ATCC 15703

According to the growth curves and the structural changes above, the potential metabolism of *F. margarita* polysaccharides used by *B. adolescentis* is summarized in Fig. 7. Firstly, *F. margarita* polysaccharides were degraded into low-molecular-weight polysaccharides by GHs (such as $\alpha$-amylases, $\beta$-glucosidase, $\alpha$-L-arabinofuranosidase, arabinofuranohydrolase, $\alpha$-glucosidase, $\beta$-galactosidase, $\alpha$-galactosidase, and $\alpha$-mannosidase), and then into monosaccharides and oligosaccharides. In Table 2, the molecule weight of *F. margarita* polysaccharide fractions decreased after 20 h fermentation. Secondly, expression increased of the core genes of *B. adolescentis* ATCC 15703 encoding gluconate transporters and the major facilitator super family transporters [12], resulting in proteins that transported hydrolysates (monosaccharides and oligosaccharides) into the bacterial cells. It can be seen in Fig. 2A, with the increase of fermentation time, the number of *B. adolescentis* increased, indicating that the *F. margarita* polysaccharide fractions were used by *B. adolescentis*. Finally, the hydrolysates were then fermented in bacterial cells, resulting in the generation of acetic acid and lactic acid. From Table 1 and Fig. 3A, the content of acetic acid and lactic acid increased, and pH value of media decreased.

![Fig. 6. Chromatograms of chain conformation in aqueous of *F. margarita* polysaccharides fermented by *B. adolescentis*: (A1): FP20 (0 h); (A2): FP20 (20 h); (A3): FP20 (48 h); (B1): FP40 (0 h); (B2): FP40 (20 h); (B3): FP40 (48 h); (C1): FP60 (0 h); (C2): FP60 (20 h); (C3): FP60 (48 h); (D1): FP80 (0 h); (D2): FP80 (20 h); (D3): FP80 (48 h).](image)
4. Conclusions

In this study, the effects of *F. margarita* polysaccharide fractions on the proliferation of *B. adolescentis* ATCC 15703, with a focus on the relationships between bacterial proliferation and polysaccharide structural changes were investigated. Polysaccharide fractions, especially FP20, FP40, and FP60, enhanced the proliferation of *B. adolescentis* ATCC 15703, suggesting these fractions as potential prebiotics. A large amount of acetic acid was produced by *B. adolescentis* ATCC 15703, suggesting these fractions as potential prebiotics. A large amount of acetic acid was produced by *B. adolescentis* ATCC 15703 after polysaccharides fermentation. The molecular weights of FP20, FP40, FP60 and FP80 were reduced after fermentation, and the chain conformations of FP20, FP40 and FP60 also changed. These prebiotic effects might be related to the preliminary structural properties of the polysaccharides, such as molecular weight and chain conformation, or even to the degree of utilization by *B. adolescentis*.

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