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The structure and prebiotic activity of arabinogalactan from *Ferula Kuhistanica*

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ABSTRACT

Acidic arabinogalactan with molecular weight 36 kDa was isolated from the aerial part of *Ferula kuhistanica,* the monosaccharide composition of which is represented by galactose and arabinose in a ratio of 3.6:1. The chemical and spectral methods revealed a main polymer chain consisting of (1 → 6) *β*-galactopyranose residues, where in position of C-3 were the residues of α-arabinofuranose and its 1,5-linked oligomers, as well as *β*-Glc*p*A-4-OMe-(1 → 6)-*β*-Gal*p*-(1→ fragments. In addition, a small part of the main chain carries monosaccharide residues of *β*-Gal*p*-(1→ in position C-2. The effect of arabinogalactan on the growth of an associative culture of bifidobacteria and some mono-strains of lactobacilli was studied and it was shown that it exhibits more effective prebiotic activity.

1. Introduction

Plant polysaccharides are an important class of biopolymers and have long attracted researchers for their high technical and nutritional value. Polysaccharides perform specific functions in many vital processes and are distinguished by high biological activity. To date, there is no information on the chemical composition and beneficial properties of carbohydrates in 80–90% of plants growing on the territory of Uzbekistan. It becomes relevant to obtain information on the distribution of various polysaccharides (galactomannanans, arabinogalactans, glucans, pectin and hemicelluloses) among promising plants in the local region. The raw materials for these compounds are plants of the family *Fabaceae*, *Apiaceae*, *Lamiaceae* and secondary waste of medicinal plants growing in Uzbekistan, which can be considered as sources of new drugs and pharmacologically active substances [1–3]. In this respect, arabinogalactans from plants of the genus *Ferula* (family *Apiacea*) are of great interest. Arabinogalactans are a unique natural polysaccharide characterized by a complex of valuable properties, the most important of which are high biological activity and low toxicity [4–6].

There are about 150 species of plants of the *Ferula* genus in the world, of them about 45 species grow on the territory of the Republic of Uzbekistan. Many *Ferula* species have high feeding qualities and are honey plants, the roots of some species are used by the local population as a source of starch [7]. In chemical terms, the secondary metabolites of the plant are deeply studied, i.e. low molecular weight compounds-flavonoids, esters, essential oils and lipids [8–10]. On their basis, at the Institute of the Chemistry of Plant Substances were created preparations: "Kufesterol" (*F. kuhistanica*), "Tefesterol" and "Ferulen" (*F. tenuisecta*). It should be noted that carbohydrates have not been studied enough. Therefore, obtaining information on the content of various polysaccharides in a given plant genus, studying the structure of polysaccharides and, if possible, the fine structure of their macromolecules is relevant.

The aim of this work is to establish the structure of arabinogalactan *Ferula kuhistanica* by chemical and spectral methods, as well as to reveal its biological activity.

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2. Results and discussion

2.1. Isolation of polysaccharide

Earlier, we reported on the isolation of various groups of polysaccharides from the aerial part of *F. kuhistanica*. The presence of water-soluble polysaccharides (8.0%) was established, the monosaccharide composition of which is presented by neutral monosaccharides and uronic acids [11]. In the IR spectrum of WSPS, broad absorption bands were observed in the region of 3298, 2929 cm^{-1}, characteristic of the OH group, 1414 cm^{-1} shows the presence of CO groups, 1107 cm^{-1} - COC vibrations of glycosidic bonds, 907, 879, 841 cm^{-1} characterize the type of glycosidic bonds (Fig. 1) [12]. An intense absorption band at 1591 cm^{-1} indicates the presence of protein components that were removed by the Sevaga method [13]. WSPS was fractionated with alcohol and obtained a fraction of acidic arabinogalactan –AGF with a yield of 30.7% and a ratio of arabinose and galactose was 8.2:4.3. Arabinogalactan AGF was dissolved in water and loaded onto a DEAE cellulose (OH form) column to separate neutral and acidic polysaccharides. The column was washed with water, a small amount of a neutral fraction was obtained, with the main monosaccharide composition consisting of galactose, glucose, mannose in a ratio of 20.7:4.0:2.7. The acidic fractions were eluted with 0.1 N NaOH solution, followed by purification on a Sephadex G-50 column, and acid arabinogalactan AGF-1 was obtained with a yield of 27.5%.

Arabinogalactan AGF-1 was an amorphous powder of light brown color, completely soluble in water and, according to high-performance size exclusion chromatography, has a molecular weight of 36 kDa. The monosaccharide composition of AGF-1 is represented by galactose and arabinose in a ratio of 3.6:1.

2.2. Smith degradation results

In the final results of the degradation according to Smith of AGF-1 were identified glycerin and free monosaccharide. The detection of glycerin indicates the branching of the polymer and the presence of 1,6-types of bonds between monosaccharide residues. The identification of a free monosaccharide speaks in favor of the 1,3-type of bond in the AGF-1 chain.

2.3. Methylation and GC-MS analysis

Methylation of AGF-1 was carried out as described [14]. The completeness of methylation was monitored by IR spectroscopy by the absence of an absorption band at 3400–3200 cm^{-1} (OH group). The permethylate was subjected to methanolysis, the methanolysis product was acetylated to obtain trimethylsilyl derivatives, which were studied by GC-MS [15]. Gas chromatography-mass spectrometry analysis data showed that AGF-1 contains T-Ara*f* (12.9%), 1,5-Ara*f* (9.4%), galactose residues consist of terminal T-Gal*p* (19.9%), 1,6-linked Gal*p* (25.4%), 1, 3-linked Gal*p* (14.0%), 1,2-linked Gal*p* (2.7%) and 1,3,6-linked Gal*p* (14.2%) (Table 1).

2.4. NMR spectra analysis

The primary structure of AGF-1 was elucidated using NMR spectroscopy. Both ^{1}H (Fig. 2) and ^{13}C (Fig. 3) NMR spectra of the arabinogalactan were resolved enough to apply 2D spectroscopy for the assignment of resonances in the 1D spectra. COSY, TOCSY (not shown) and ROESY (Fig. 5) spectra revealed the presence of β-galactopyranose (β-Gal*p*), α-arbinofuranose (α-Ara*f*) and β-glucopyranoside uronic acid (β-Glc*p*A) residues as sugar component of the polysaccharide (Table 2).

Analysis of the HSQC spectrum (Fig. 4) displayed substitution of the β-Gal*p* at position C-6 (residues ***A*** and ***E***, Table 2), positions C-2 (residue ***D***), positions C-3,6 (residues ***B*** and ***C***) and positions C-2,6 (residue ***D'***), a part of the β-Gal*p* residues was found non-substituted (terminal, ***I***). Two type of α-Ara*f* residues were observed in the polymer, that are terminal (***G*** and ***G'***) and 5-substituted (***H***) ones. The β-Glc*p*A residues (***F***) proved to be substituted at position C-4. All conclusions about substitution in the residues were made on the basis of comparison ^{13}C NMR sub-spectra of the residues with that of parent sugars taking into account the positive α-effects of the substitution [16].

Sequence of the residues was found on the basis of ROESY (Fig. 5) and HMBC (Fig. 6) spectra. ROESY spectra showed that the main chain built up alternating residues type of ***A*** and ***B*** (inter-residue correlation peaks 1***A***/6***A***, 1***A***/6***B*** and 1***B***/6***A*** at δ_H/δ_H 4.46/4.05, 3.92 and 4.42/4.05, 3.92). The inter-residue correlation peaks 1***A***/6***A***, 1***A***/6***B*** and 1***B***/6***A*** at δ_H/δ_C 4.46/70.8 and 4.42/70.8 in the HMBC spectrum confirmed this structural peculiarity of the main chain.

The presence of the peak designated as 1D'/1I and 1D/1A′ in the ROESY spectrum deserves a detailed discussion. The appearance of inter-residue peak for two anomeric protons characteristic of 1,2-linkage between corresponding residues. The homonuclear 2D spectra revealed

Table 1
GC-MS analysis data of hydrolysates of arabinogalactan permethylates of AGF-1.

Methylated product	Content of methyl fragments, %	Linkage pattern
2,3,5-tri-*O*-Me-Ara*f*	15.0	Ara*f*-(1→
2,3-di-*O*-Me-Ara*f*	11.0	→5)-Ara*f*-(1→
2,3,4,6-tetra-*O*-Me-Gal*p*	23.2	Gal*p*-(1→
2,3,4-tri-*O*-Me-Gal*p*	29.6	→6)-Gal*p*-(1→
2,4-di-*O*-Me-Gal*p*	16.6	→3,6)-Gal*p*-
3,4-di-*O*-Me-Gal*p*	3.1	→2)-Gal*p*-(1→

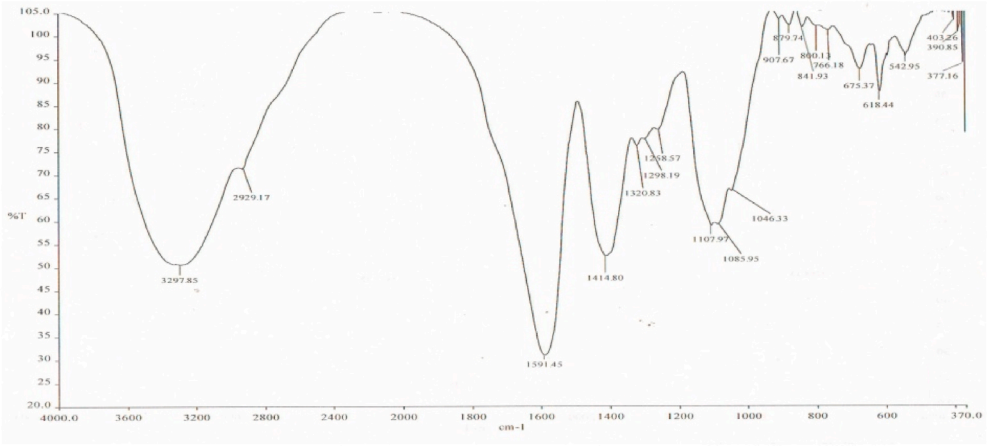

Fig. 1. IR spectrum of WSPS from *Ferula kuhistanica*.

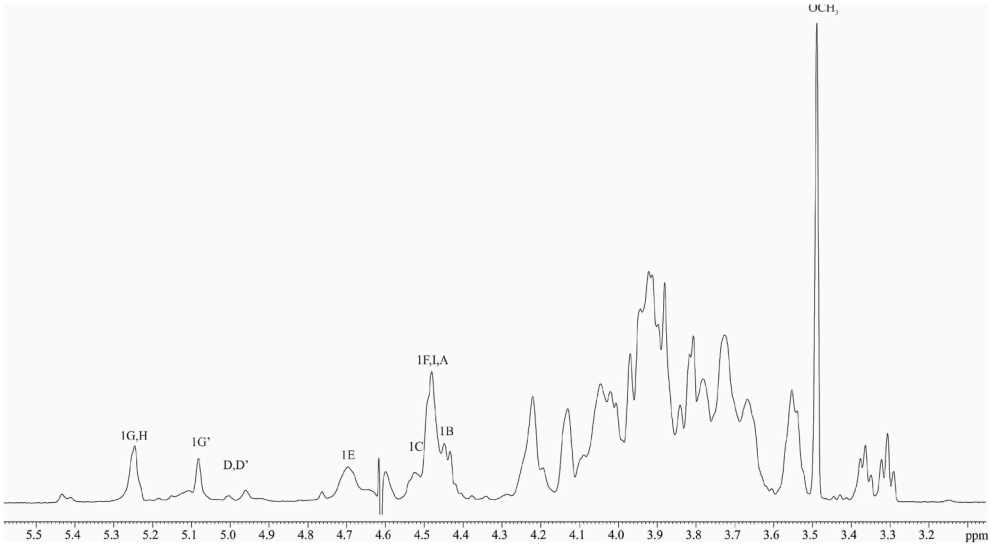

Fig. 2. ^{1}H NMR spectrum of arabinogalactan AGF-1.

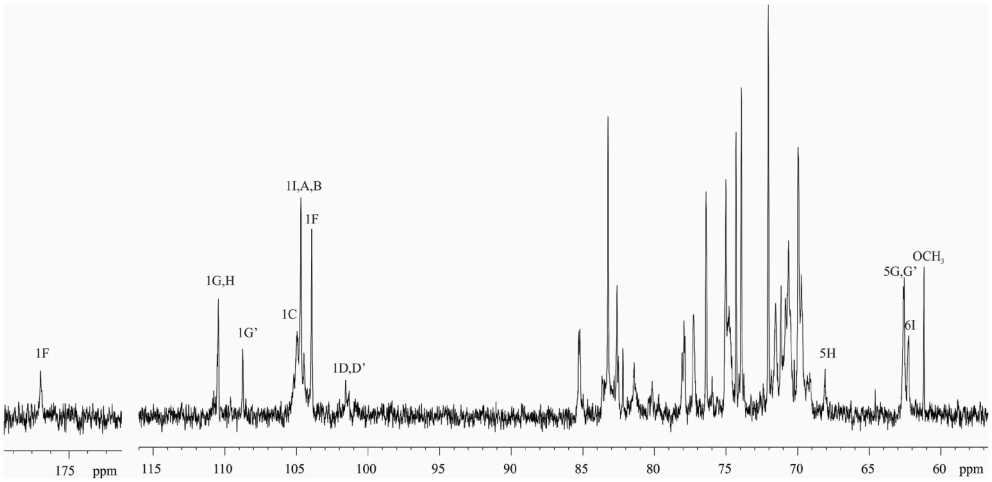

Fig. 3. ^{13}C NMR spectrum of arabinogalactan AGF-1.

2- and 2,6-substituted β-Gal*p* residues (Table 2, ***D*** and ***D'***) as minor components. These NMR data are in complete agreement with the results of methylation analysis, in which a 2-substituted β-Gal*p* residue was found with a content of 3.1% (Table 1). The presence of 2-substituted β-Gal*p* residues suggests their localization in the polymer backbone along with the predominant 1,6-linked residues (ratio about 1:16 according to the methylation analysis).

The ROESY spectrum discovered location Ara*f* residues ***G*** and ***H*** at position C-3 of the residues ***B*** (inter-residue correlation peak 1***G***,***H***/3***B*** at δ_H/δ_H 5.24/3.71), and substitution of the residue ***H*** by terminal residue ***G***′ at position C-5 (peak 1***G'***/5***H*** at δ_H/δ_H 5.08/3.79). The corresponding inter-residue correlation peaks 1***G***,***H***/3***B*** at δ_H/δ_C 5.24/81.5 and 1***G'***/5***H*** at δ_H/δ_C 5.08/68.2 were observed in the HMBC spectrum. The location of the OMe group at C-4 of the uronic acid residue ***F*** confirmed by presence of HMBC correlation peak at δ_H/δ_C 3.49/83.2 (***OMe***/C-4 ***F***, not shown in Fig. 6). The residue F was found linked with C-6 of ***E*** (ROESY correlation peaks 1***F***/6***E*** and 1***F***/6′***E***, Fig. 5). Location of the disaccharide β-Glc*p*A4-OMe-(1 → 6)-β-Gal*p*-(1→ at position C-3 of a 3,6-substituted Gal*p* was confirmed by presence of the correlation peak 1***E***/3***C*** in the ROESY spectrum. The 1)-β-D-Gal*p*-(6 → 1)-Glc*p*-A4-*O*Me fragment was also found in arabinogalactan of *Silybum marianum* [17].

Based on the results obtained, the main structural fragment of arabinogalactan AGF-1 can be represented as follows:

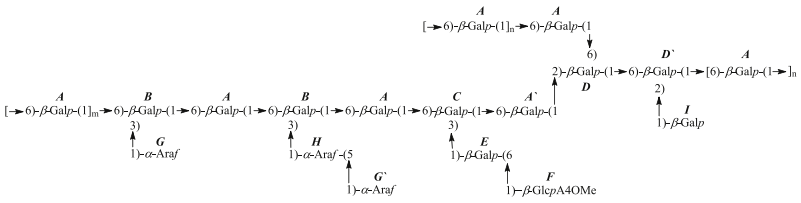

Table 2
^{1}H and ^{13}C NMR chemical shifts of the main fragments of arabinogalactan AGF-1.

Sugar residue		C1 *H1*	C2 *H2*	C3 *H3*	C4 *H4*	C5 *H5,5'*	C6 *H6, H6'*
→6)-*β*-Gal*p*-(1→	***A***	104.7 *4.46*	72.1 *3.54*	74.0 *3.65*	70.8 *3.93*	74.8 *3.91*	70.8[a] *4.05, 3.92*
→6)-*β*-Gal*p*-(1→ 3)	***B***	104.8 *4.42*	71.2 *3.65*	81.5 *3.71*	69.8[a] *4.12*	74.8 *3.91*	70.8[a] *4.05, 3.92*
↑ *α*-Ara*f* -(1	***G***	110.6 *5.24*	82.7 *4.21*	77.9 *3.94*	85.3 *4.13*	62.7 *3.83, 3.71*	
→ 5)-*α*-Ara*f*-(1→3	***H***	110.6 *5.24*	82.7 *4.21*	77.9 *3.94*	83.6 *4.26*	68.2 *3.88, 3.79*	
α-Ara*f*-(1→5	***G'***	108.8 *5.08*	82.2 *4.12*	78.1 *4.00*	85.3 *4.08*	62.7 *3.83, 3.71*	
→6)-*β*-Gal*p*-(1→ 3)	***C***	104.5 *4.53*	71.5 *3.78*	83.2 *3.87*	69.8[a] *4.22*	74.8 *3.91*	70.8[a] *4.05, 3.92*
↑ 1)-*β*-Gal*p* 6) ↑	***E***	105.1 *4.69*	71.2 *3.65*	74.0 *3.65*	69.8[a] *4.12*	75.0 *3.88*	70.7[a] *4.04, 3.92*
1)-*β*-Glc*p*A4OMe	***F***	104.0 *4.49*	74.3 *3.36*	76.5 *3.53*	83.2[b] *3.31*	73.9 *3.73*	176.6
→6)-*β*-Gal*p*-(1→ 2)	***D,D`***	101.6 *4.96*	78.2 *4.00*	74.0 *3.70*	69.8[a] *4.22*	74.8 *3.91*	70.7[a] *4.02, 3.88*
↑ 1)-*β*-Gal*p*	***I***	104.7 *4.45*	73.1 *3.71*	74.0 *3.65*	70.8 *3.93*	76.0 *3.72*	62.4 *3.78, 3.75*

[a] Assignments in the column may be inter-changed.
[b] OMe at δ_C 61.1 и δ_H *3.49*.

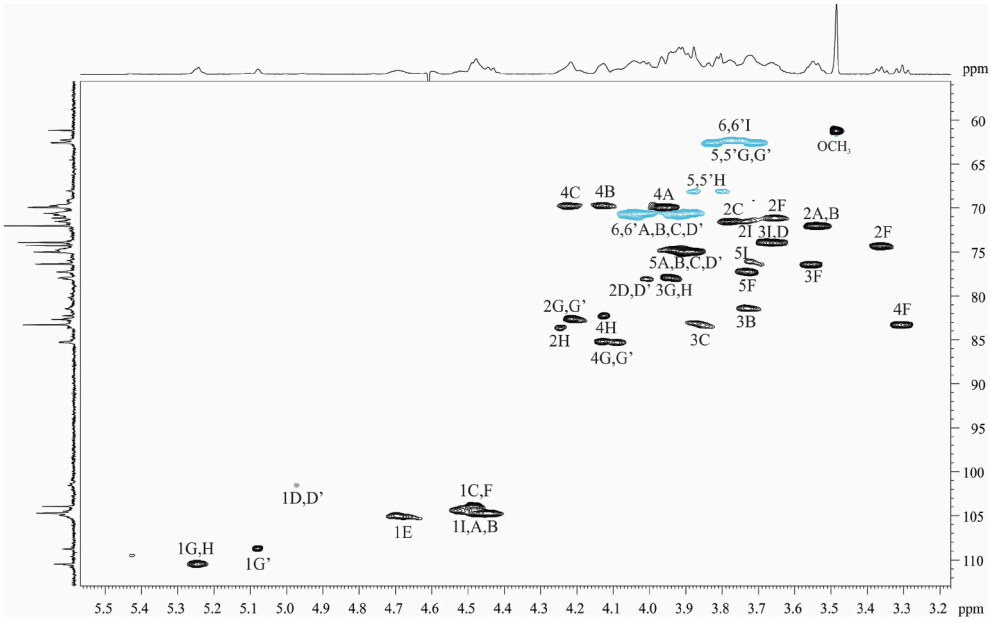

Fig. 4. HSQC spectrum of arabinogalactan AGF-1.

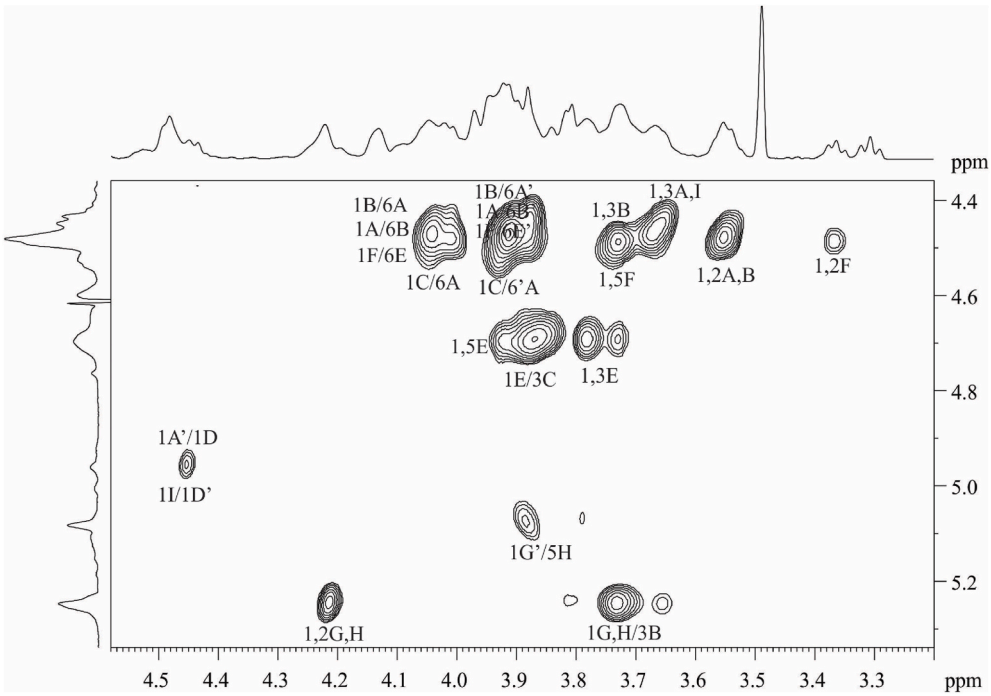

Fig. 5. Parts of the ROESY spectrum of arabinogalactan AGF-1.

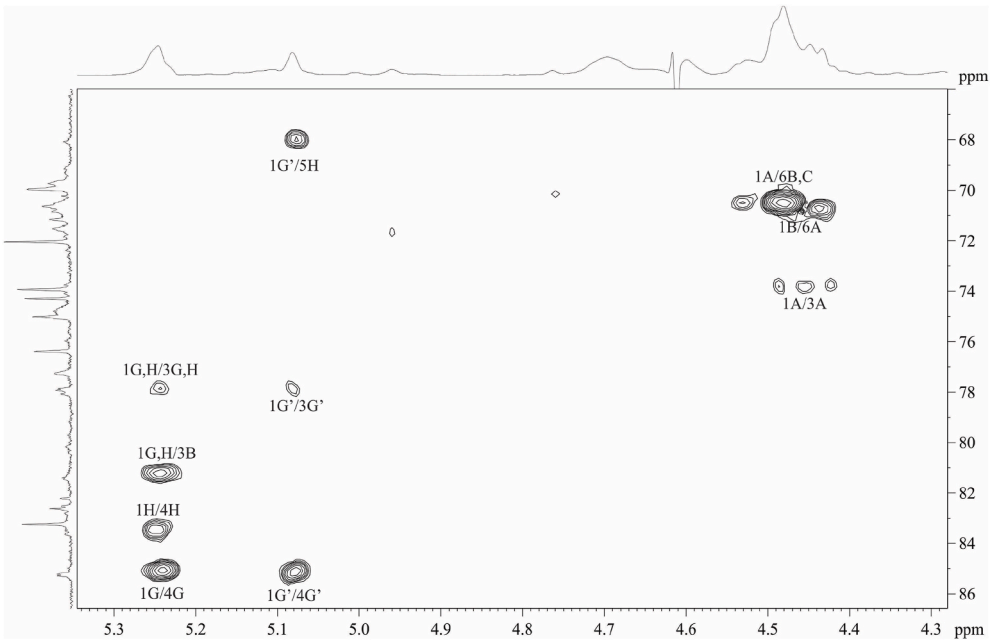

Fig. 6. Parts of the HMBC spectrum of arabinogalactan AGF-1. The Arabic numerals before the slash refer to protons, and after the slash, to the carbon atoms in the residues indicated by Latin letters in the formula in Table 2.

2.5. Prebiotic activity of arabinogalactan

We studied the effect of *F. kuhistanica* arabinogalactan AGF on the growth of an associative culture of bifidobacteria and some strains of lactobacilli. The data obtained showed that the introduction of arabinogalactan into the culture medium promotes their active use, thereby increasing the number of living cells. If we compare the growth of *Bifidobacterium longum* 17x + *Propionibacterium avidum* 1 on a balanced standard MRS medium and on media supplemented with a prebiotic, it can be seen that the latter provides a higher titer of living cells (Table 3). Table 3 shows that apple pectin at a concentration of 0.25% increases the growth of bifidobacteria and propionobacteria by 10.9%. The addition of AGF to the MRS broth was accompanied by more pronounced growth stimulation and amounted to 20.9%. This increase compared to apple pectin (PS-A) was statistically significant ($p<0.05\%$). An increase in the titer of living cells upon the addition of these prebiotics from 8.89 (control) to 9.86 and 10.75 (experiment) lg CFU/mL corresponded to an increase in acid production from 162 to 190° T.

The effect of AGF on the viability of *Lactobacillus del. subps bulgaricus* 906. As a result of this experiment, the viability of the monoculture of *Lactobacillus delbrueckii subps bulgaricus* 906 was assessed and the effect of AGF in comparison with apple pectin on the growth of lactobacilli was

Table 3
Influence of the studied prebiotics on the viability of *Bifidobacterium longum* 17x + *Propionibacterium avidum* 1.

Experimental conditions	lg CFU/mL	R	Effect, in %	Total titratable acidity, °T
MRS broth + glucose (control)	8.89 ± 0.23	–	100	150
MRS broth + PS-A	9.86 ± 0.28	<0.02	110.9	162
MRS broth+AGF	10.75 ± 0.27	<0.001	120.9	190

CFU-colony forming unit, R-reliability of research results.

Table 4
Influence of the investigated prebiotics on viability *Lactobacillus del. subps bulgaricus* 906.

N⁰	Experimental conditions	lg CFU/mL	R	Effect, in %	Total titratable acidity, °T
1.	MRS broth + glucose (control)	10.45 ± 0.48	–	100.0	160
2.	MRS broth + PS-A	12.03 ± 0.50	<0.05	115.2	165
3.	MRS broth+AGF-1	12.65 ± 0.35	<0.002	121.0	185

Table 5
Influence of the studied prebiotics on the viability of Lactobacillus rhamnosus 925 ak.

N⁰	Experimental conditions	lg CFU/mL	R	Effect, in %	Total titratable acidity, °T
1.	MRS broth + glucose (control)	10.33 ± 0.38	–	100.0	130
2.	MRS broth + PS-A	10.95 ± 0.42	>0.05	106.1	128
3.	MRS broth+AGF-1	12.4 ± 0.41	<0.002	120.0	165

determined (Table 4). For this purpose, the number of living cells in 1 ml of the cultured medium (lg CFU/mL) was counted. Stimulation of the growth of lactobacilli with the addition of: apple pectin was 15.2%, AGF – 21%. The titer of living cells also correlated with the total titratable acidity of the cultured medium (160–185 °T).

The increase in the number of living cells in the cultured medium with prebiotics in the *L. rhamnosus* 925ak culture was slightly lower (10.33–12.4 lg CFU/mL) than in the *L. delbruecrii* subsp *bulgaricus* 906 culture. In this case, when apple pectin was introduced, the effect reached 6.1%, and the effectiveness of AGF was 20% (the difference between these two groups was statistically significant, $p<0.05\%$). This culture is characterized by a slight acid formation, which increased with the addition of AGF to 165° T, which indicates the active utilization of the prebiotic by microorganisms (Table 5).

The results showed that the stimulating effect of arabinogalactan is superior to apple pectin on the viability of the association of cultures of bifidobacteria *Bifidobacterium longum* 17x and *Propionibacterium avidum* 1, as well as monocultures of *Lactobacillus rhamnosus 925ak* and is comparable to apple pectin. This indicates that the arabinogalactan of *F. kuhistanica*, in comparison with widely used apple pectin, exhibits more effective prebiotic activity [6,18].

3. Conclusion

It was established by chemical and spectral methods that the macromolecule of arabinogalactan isolated from *Ferula kuhistanica* consists of β-(1 → 6)-linked polygalactans. The side branches of arabinogalactan are represented by α-arabinofuranose and its 1,5-linked oligomers, as well as the disaccharide fragment β-Glc*p*A4-OMe-(1 → 6)-β-Gal*p*-(1 → . A small part of the 1,6-substituted residues of the main chain carries monosaccharide residues β-Gal*p*-(1→ in position C-2. Prebiotic activity of arabinogalactan on the growth of bifidobacilli and some lactobacilli monostrains was revealed.

4. Experimental

4.1. Plant material

The aerial part of *F. kuhistanica* was collected during the flowering period in the Samarkand region (Uzbekistan) in 2017.

4.2. Paper chromatography analysis

Paper chromatography (PC) was carried out on Filtrak-FN 13, 18 paper (Germany) in a solvent system *n*-butanol-pyridine-water (6:4:3), Compounds were detected by spraying the acidic aniline phthalate (for 5 min, at 100 °C) and bromophenol blue.

4.3. IR spectroscopy analysis

Polysaccharide sample was pressed in KBr tablets for infrared (IR) spectra analysis and IR spectrum was recorded in the wavelength range of 4000–400 cm^{-1} by a System 2000 IR Fourier transform spectrometer (PerkinElmer). Number of scans was 100.

4.4. NMR spectroscopy

NMR spectra were recorded on an Avance AV600 spectrometer (Bruker, Germany) with an operating frequency of 600 MHz for ^1H in D_2O, according to the company's standard methods. Chemical shifts were counted from TSP as an internal standard (δ_H 0.0, δ_C −1.6).

4.5. GC and GC-MS analyses

GC analysis of the samples was carried out on a Shimadzu GC-2010 chromatograph (Japan) with a flame ionization detector equipped with a Shimadzu Rxi-624Sil MS quartz capillary column (30 m × 0.25 mm × 1.40 μm). The mobile phase rate (N_2) was 1.5 mL/min, injector temperature was 260 °C, the detector temperature was 280 °C and column temperature was 230 °C. The samples were taken in the form of aldononitrile acetate.

GC-MS analysis of the obtained methyl products of arabinogalactan was carried out on an Agilent 5975C inert MSD/7890A GC gas chromatography-mass spectrometer. Separation of the mixture components was performed on an Agilent HP-INNOWax quartz capillary column (30 m × 250 μm × 0.25 μm) in a temperature mode: 100 °C (2 min) – 4 °C/min to 220 °C (5 min) – 15 °C/min to 250 °C (15 min). The injection volume of sample was 1.0 μL, the flow rate of the mobile phase (He) was 1.1 mL/min. The injector temperature was 240 °C. EI-MS spectra were obtained in the *m/z* range of 10–550 u. The components were identified by comparing the characteristics of the mass spectra with the data of electronic libraries (Wiley Registry of Mass Spectral Data-9th Ed., NIST Mass Spectral Library, 2011).

4.6. Isolation of water-soluble polysaccharides (WSPS)

100 g of raw materials were first degreased with a boiling mixture of chloroform-methanol (1:1) for 1 h (1:5), and then treated with 82% ethyl alcohol (1:3) under heating to remove low molecular weight compounds. WSPS was isolated by extraction with water at room temperature at hydromodule 1:10, 1:5 and 1:3. The aqueous extracts were separated by filtration, combined, and evaporated to half (900 mL) volume. To remove protein components, a mixture of 80 mL of chloroform and 16 mL of *n*-butanol was added to the extract, and the mixture

was stirred for 30 min. The reaction mixture was centrifuged and the protein components were removed. The centrifugate was evaporated to 300 mL and precipitated with alcohol 1:3. The precipitate that formed was separated, washed with alcohol, dehydrated with acetone, and dried in vacuum over P_2O_5. The WSPS yield was 6.8 g.

4.7. Fractionation of WSPS

3 g of WSPS was dissolved in 100 mL of water and 100 mL of alcohol was added dropwise with vigorous stirring. The precipitate that formed was separated by centrifugation, washed with alcohol, dehydrated with acetone, and dried in a vacuum over P_2O_5. The yield of fraction 1 was 0.83 g. Next, another 100 mL of alcohol was added to the supernatant solution, the precipitate formed was separated, treated in the same way, and the fraction 2 -arabinogalactan (AGF) was obtained with a yield of 1.54 g. Fraction 3 with a yield of 0.57 g was obtained by adding another 100 mL of alcohol to the supernatant solution, the precipitate was treated as described above.

4.8. Purification of the AGF

1.0 g of AGF was dissolved in 40 mL of water and passed through a DEAE cellulose (OH-form) column (42 × 3 cm). The column was eluted with 100 mL of water and the polysaccharide yield was monitored by the phenol-sulfuric acid reaction. Aqueous eluates were evaporated and added to a threefold volume of alcohol. The formed precipitate was separated, washed with alcohol, and dried. The yield of neutral polysaccharide (NPS) was 0.13 g. Then, the column was washed with 235 mL of 0.25 N NaOH, the alkaline eluate was dialyzed against distilled water, concentrated, and precipitated with alcohol. The precipitate was dissolved in distilled water and purified through columns with Sephadex G-50 (52 × 1.8 cm) to obtain AGF-1 with a yield of 0.825 g.

4.9. Determination of the molecular weight of arabinogalactan AGF-1

The molecular weight of polysaccharide was determined by high-performance exclusion chromatography on an Agilent 1260 Infinity liquid chromatograph using a PL Aquagel OH Mixed chromatographic column (USA) 300 mm long and 8 mm inner diameter. Concentrations of injected samples were 1–4 mg/mL, volume - 20 μL. Aqueous 0.1 N $NaNO_3$ solution was used as eluent. The volumetric flow rate of eluent was 0.8 mL/min. The temperature of the systems of columns and detectors was maintained at 25 °C. A differential refractometer was used as a detector. The chromatographic column was calibrated using linear, highly dispersed polymer standards of pullulan (Showa Denko, Japan).

4.10. Analysis of monosaccharide composition

The samples were hydrolyzed with 1 N H_2SO_4 at 100 °C for 8 h, the hydrolyzate was neutralized as described [11].

4.11. Smith degradation

0.0308 g of AGF-1 dried to constant weight was dissolved in 24.9 mL of water then was added 5.1 mL of 0.25 M $NaIO_4$ and kept at a temperature of +5 °C. Then the experiment was carried out as described [17]. In the final products of the Smith degradation by paper chromatography (solvent system: *n*-butanol-pyridine-water, 6:4:3) were found mainly glycerin.

4.12. Methylation of AGF-1

Arabinogalactan AGF-1 (0.1 g) was methylated according to the Hakomori method [14]. 0.5 mL of 0.5 N HCI in methanol was added to the permethylate and heated at 65 °C for 16 h. The obtained methyl glycoside was evaporated to dryness, and then the product was acetylated. To do this, 0.5 mL of absolute methanol, 10 μL of absolute pyridine, and 50 μL of acetic anhydride were added to the sample and the mixture was kept at room temperature for 15 min. The resulting acetyl derivative was dried in a desiccator over NaOH. Then, 50 μL of absolute pyridine, 10 μL of HMDS and 5 μL of TMSC were added to the sample and kept at room temperature for 30 min. The reaction mixture was centrifuged at 3000 rpm for 10 min, the precipitate was separated, the supernatant solution was dried, dissolved in hexane, and analyzed by GC-MS [15].

4.13. Determination of the prebiotic activity of arabinogalactan

To study the prebiotic activity of arabinogalactan, associations from local strains of bifidobacteria were used: *Bifidobacterium longum* 17x and *Propionibacterium avidum* 1, which form the basis of the preparation "Bifidumbacterin PL", as well as strains *Lactobacillus delbrueckii* subsp. *bulgaricus* 906, *Lactobacillus rhamnosus 925ak*.

The culture medium for bifidobacteria was based on standard Blaurock medium (Hi-Media, India), for lactobacilli - MRS broth (Hi-Media, India). The studied prebiotic was added to a sterile nutrient medium, and also apple pectin for comparison. The final concentration was 0.25%. The inoculum obtained by growing bifidobacteria and lactobacilli in MRS broth for 24 h at 38 °C was added in an amount of 1 mL per 9.0 mL of nutrient medium. The strains of *Bifidobacteria* and L. *rhamnosus* were grown at (38 ± 1)° C, *Lactobacillus delbrueckii* subsp. *bulgaricus* 906 strain at 43 °C in an anaerostat under nitrogen atmosphere for 24 h. This took into account the number of living cells in 1 mL of the cultured medium (lg CFU/mL). The experiments were carried out in five replicates. The results were processed statistically using the Student's *t*-test.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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