

## Research Article

# Rapid Mass Spectrometric Analysis of a Novel Fucoidan, Extracted from the Brown Alga *Coccophora langsdorfii*

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The novel highly sulfated (35%) fucoidan fraction **Cf2**, which contained, along with fucose, galactose and traces of xylose and uronic acids was purified from the brown alga *Coccophora langsdorfii*. Its structural features were predominantly determined (in comparison with fragments of known structure) by a rapid mass spectrometric investigation of the low-molecular-weight fragments, obtained by “mild” (5 mg/mL) and “exhaustive” (maximal concentration) autohydrolysis. Tandem matrix-assisted laser desorption/ionization mass spectra (MALDI-TOF/TOFMS) of fucooligosaccharides with even degree of polymerization (DP), obtained by “mild” autohydrolysis, were the same as that observed for fucoidan from *Fucus evanescens*, which have a backbone of alternating (1 → 3)- and (1 → 4) linked sulfated at C-2 and sometimes at C-4 of 3-linked  $\alpha$ -L-Fucp residues. Fragmentation patterns of oligosaccharides with odd DP indicated sulfation at C-2 and at C-4 of (1 → 3) linked  $\alpha$ -L-Fucp residues on the reducing terminus. Minor sulfation at C-3 was also suggested. The “exhaustive” autohydrolysis allowed us to observe the “mixed” oligosaccharides, built up of fucose/xylose and fucose/galactose. Xylose residues were found to occupy both the reducing and nonreducing termini of FucXyl disaccharides. Nonreducing galactose residues as part of GalFuc disaccharides were found to be linked, possibly, by 2-type of linkage to fucose residues and were found to be sulfated, most likely, at position C-2.

## 1. Introduction

The quantity of scientific papers, concerning various biological activities [1–4] of fucoidans, sulfated polysaccharides from brown algae, is constantly growing. However, one cannot notice the same about the works regarding the structural features of these unique carbohydrate polymers. Fucoidans are known [5] to be mainly built up of sulfated  $\alpha$ -L-fucopyranose ( $\alpha$ -L-Fucp) residues. Other monosaccharides, like D-galactose, can occupy significant percentage in the monosaccharide composition in galactofucans or reside in minor ones, like xylose [6–11]. In order to unambiguously establish the structure of such complex polysaccharides by powerful spectroscopic techniques, like  $^{13}\text{C}$  NMR, time-consuming multiple-step chemical modification procedures, coupled with purification steps, are required. These methods are offering low yields and thus they are requiring a large number of samples. Recently [12] it was found that modern methods

of mass spectrometry, offering high sensitivity, accuracy, and speed, enabled the analysis of low-molecular-weight (LMW) oligosaccharides, left by solvolytic desulfation reaction as a side product. It was found that minor constituents of fucan-sulfate (Xyl, Gal, and GlcA residues) from the brown alga *Fucus evanescens* are incorporated into the backbone, probably, as branching points [12].  $^{13}\text{C}$  NMR technique that was employed for the analysis of the same fucoidan [13] was unable to observe these fragments, just because they were rejected at purification step or the method was not sensitive enough. Hence, using mass spectrometry as a complementary tool to the spectroscopic techniques and methods of carbohydrate chemistry is essential for the elucidation of the exact structure of fucoidans. However, in some cases using known mechanisms of mass spectrometric fragmentation, it is possible to elucidate some structural features of fucoidans by mass spectrometry only. In this way glycosaminoglycans and carrageenans were successfully investigated by the analysis of

their fragments, obtained either by enzymes or by acid hydrolysis [14–17]. Unfortunately, fucoidanases are still to come, they are not widely available [18]. Despite of that, a polymer chain must be “softly” depolymerized to save “intact” positions of labile sulfate groups and other substitutes. An autohydrolysis (“autohydrolysis” is used here to denote acidic polysaccharide hydrolysis under very mild conditions using the  $-SO_3H$  groups of the compound as the source of acid) procedure was developed as an alternative to the mild acid hydrolysis decomposition technique. It was found that it is a reproducible method for the preparation of multisulfated oligosaccharides, well reflecting the structure of the source polysaccharide. In this way, fragments of fucoidans from brown algae *Saccharina cichoriooides* [19], *F. evanescens* [1], and *Costaria costata* [20] were successfully analyzed by tandem ESI- and MALDI-TOFMS. Structural features of oligosaccharides matched with that observed previously for corresponding fucoidans using independent methods [13, 21].

Herein we report mass spectrometric elucidation of the structural properties of oligosaccharides, obtained by “soft” and “exhaustive” autohydrolysis from a novel fucoidan, purified from the brown seaweed *Coccophora langsdorfii*.

## 2. Experimental

**2.1. Materials.** Brown alga *Coccophora langsdorfii* was collected from the Troitsa Bay (Sea of Japan) at the Sea Experimental Station of Pacific Institute of Bioorganic Chemistry (PIBOC) (<http://goo.gl/maps/UMRce>), Far-East Branch, Russian Academy of Sciences (Primorsky region of Russia) in July of 2008. The algae species were identified by Dr. A. Skriptsova (A. V. Zhirmunsky Institute of Marine Biology FEB RAS, Russia).

A MALDI-TOFMS matrix, 2,5-dihydroxybenzoic acid (DHB), was purchased from Sigma (USA). All experiments were performed using ultrapure water, produced with Direct-Q 3 equipment (Millipore, USA).

**2.2. Instruments.** MALDI-TOFMS spectra were recorded with an Ultra Flex III MALDI-TOF/TOF mass spectrometer (Bruker, Germany) with a nitrogen laser (SmartBeam, 355 nm), reflector, and potential LIFT tandem modes of operation.

Infrared spectra were recorded with a Vektor-22 (Bruker, Germany) FT-IR spectrometer.

Monosaccharide composition was determined on a Biotronik IC-5000 carbohydrate analyzer (Wissenschaftliche Geräte, Germany) using a Shim-pack ISA-07/S2504 ( $0.4 \times 25$  cm) column eluted with a potassium borate buffer at an elution rate of 0.6 mL/min. Detection was carried out by bicinchoninate method and integration on a Shimadzu C-R2 AX system. Monosaccharides (rhamnose, ribose, mannose, fucose, galactose, xylose, and glucose) were used as standards for HPLC.

**2.3. General Methods.** The content of carbohydrates was determined using the phenol-sulfuric method [22]. The content of sulfates in the polysaccharides was determined by a turbidimetric method [23].

**2.3.1. Extraction of Polysaccharides.** The fresh samples of *C. langsdorfii* have been washed with deionized water in order to remove salt, epiphytes, and sand attached to the surfaces of the samples and dried between sheets of paper. Then, the algal biomass (1065 g) was treated at  $40^\circ C$  with ethanol (96% (v/v)) for 24 h to remove colored matter, washed with acetone, filtered, and vacuum dried to yield 230 g of defatted biomass. The milled defatted alga biomass (60 g) was extracted at  $20-22^\circ C$  with 0.1 M HCl (ratio 1:20) for 3 h. The extracts were combined, neutralized, dialyzed, and lyophilized to give crude polysaccharide fraction **Cf0** with yield 1.17 g (2% of dry defatted biomass). The monosaccharide composition of **Cf0** (mol%) is Fuc, 45.0, Xyl, 6.0, Man, 16.2%, Gal 18.0, Glc, 8.8, Uronic acids, and 6.0. The sulfate content of **Cf0** is 18.2% from the fraction weight.

**2.3.2. Anion Exchange Chromatography.** A solution of polysaccharides **Cf0** in 0.04 N HCl (1.0 g in 20 mL) was applied onto a DEAE-cellulose column (Cl<sup>-</sup> form,  $3.0 \times 14$  cm) equilibrated with 0.04 N HCl. Sulfated polysaccharides were successively eluted using NaCl-water solution liner gradient of  $0 \rightarrow 2$  M. The fractions were dialyzed by ultrafiltration and lyophilized to get the polysaccharide preparations **Cf1** (43.5 mg) and **Cf2** (650 mg). The monosaccharide composition of the **Cf1** fraction (mol%) is Fuc, 45.8, Xyl, 8.0, Man, 16.2, Glc, 7.0, and Gal 23.0. The sulfate content of the **Cf1** fraction was not determined due to the low yield. The monosaccharide composition of the **Cf2** fraction (mol%) is Fuc, 86.0, Xyl, 3.0, Gal 6.9, and uronic acids 4.1. The sulfate content of the **Cf2** fraction was 35% from the fraction weight.

**2.3.3. Depolymerization of Fucoidan by Autohydrolysis.** Autohydrolysis procedure [19] was used to obtain oligosaccharides suitable for MS analyses: an aliquot of 10 mL of fucoidan **Cf2** (5 mg/mL in water) was changed to the H<sup>+</sup> form using a minicolumn of cation exchange (Timberlite CG-120, 200–400 mesh, Serva, Germany) and left for 72 h at  $37^\circ C$ . The mixture was then neutralized with 5% NH<sub>4</sub>OH solution in water and lyophilized to obtain **Cf2-AH** preparation. An exhaustive autohydrolysis was performed to obtain partially desulfated oligosaccharides: an aliquot of 10 mL of fucoidan **Cf2** (10 mg/mL in water) was changed to the H<sup>+</sup> form as described above and lyophilized. Then, the sample was left at room temperature for 2 weeks (humidity ~80%) to obtain **Cf2-exAH** preparation.

**2.3.4. Derivatization of Oligosaccharides.** NaBD<sub>4</sub> reagent (Sigma, USA) of 50  $\mu$ L, 0.05 M NaBD<sub>4</sub> in 0.01 M NaOH, was added to the lyophilized oligosaccharides (~50  $\mu$ g), and the reduction was carried out at  $4^\circ C$  overnight as described [24]. The solution was then neutralized with 0.1 mL AcOH and evaporated. Boric acid was removed by repeated coevaporation with MeOH.

**2.3.5. Mass Spectrometric Analysis.** Instrument settings are as follows: accelerating voltage, 25 kV; laser power, 30  $\mu$ J; pulsewidth, 6 ns; number of shots, 100; laser shot rate, 66 Hz. Sample preparation is as follows: a mixture containing 1  $\mu$ L of

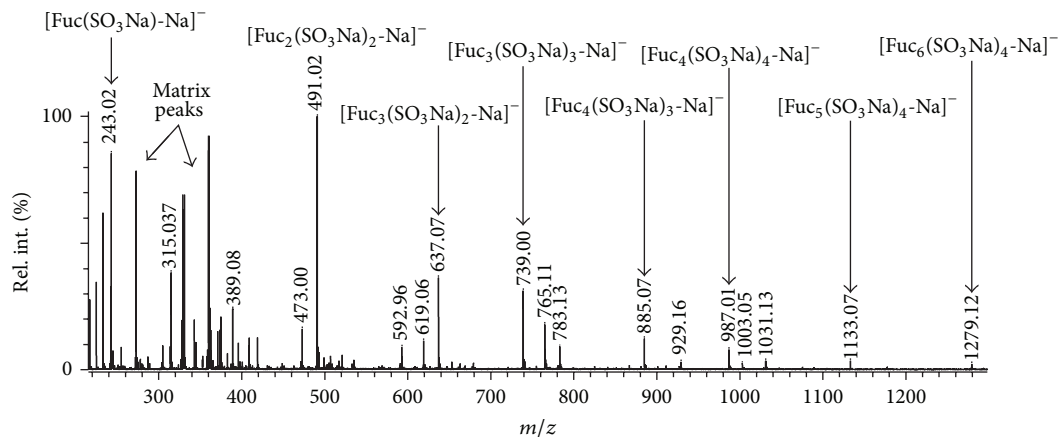


FIGURE 1: Negative-ion MALDI-TOFMS of Cf2-AH sample: product of “soft” autohydrolysis of Cf2 fucoidan fraction from the brown alga *C. langsdorfii*.

sample (0.1 mg/mL) and 1  $\mu$ L of 0.5 M DHB matrix solution in MeOH was introduced onto the sample plate and air dried.

### 3. Results and Discussion

The present work is devoted to the preliminary characterization of a fucoidan from *C. langsdorfii* by the means of mass spectrometry. The brown alga *C. langsdorfii* (Order Fucales, Family Sargassaceae) is a perennial plant. It grows most actively in spring. Its biomass reaches maximum in June and January [25]. This alga is distributed in the Sea of Japan and in northern part of the East China Sea and can be found along the coasts of the Russian Far East [26, 27], Japan [25], and Korea [28]. The chemical composition of this alga is poorly studied. The polysaccharide composition of the *C. langsdorfii* is also studied at preliminary level [29].

**3.1. Purification of Water-Soluble Polysaccharide Fractions and Preliminary Analysis.** The crude polysaccharide preparation Cf0, extracted by cold extraction from *C. langsdorfii*, has heterogeneous monosaccharide composition (Section 2.3.1). The glucose content is low, thus; no laminaran was extracted, as it was reported previously [29]. The fraction Cf0 was purified using anion-exchange chromatography with NaCl gradient (Section 2.3.2). Cf1 (1 M NaCl) and Cf2 (2 M NaCl) fractions were obtained. Fucoidan fraction Cf1 had low yield and heterogeneous monosaccharide composition and thus it was put aside. The purified highly sulfated (35%) fucoidan fraction Cf2 contained 14% of other monosaccharides: galactose (7.9), xylose (3.0), and uronic acids (4.1). It must be noted that high sulfate content also had a galactofucan from *Sargassum mclurei* that was recently examined by mass spectrometry [7]. The IR spectrum (not shown) of Cf2 contained an intense absorption band at 1254  $\text{cm}^{-1}$  (S = O) common to all the sulfate esters. An additional sulfate absorption band at 826  $\text{cm}^{-1}$  (C–O–S, secondary equatorial sulfate) and a shoulder at 846  $\text{cm}^{-1}$  (C–O–S, secondary axial sulfate) indicated that sulfate groups occupied positions 2 and/or 3 and position 4 of fucopyranose residues.

**3.2. Mass Spectrometric Investigation of Fucoidan Fraction Cf2.** Fucoidan fraction Cf2 with simple monosaccharide composition and high sulfate content was subjected to autohydrolysis of two different conditions to obtain LMW fragments, suitable for mass spectrometric investigation (Section 2.3.3).

The elucidation of the structural features of sulfated oligosaccharides by a negative-ion tandem mass spectrometry was carried out using the following observations, estimated in the former studies of glycosaminoglycan- [30], sulfated galactan- [16, 31, 32], and sulfated fucan-derived fragments [33, 34]. It was shown that the product ion spectra of  $[M-\text{Na}]^-$  (where M represents the sodium salt of oligosaccharides) featured an extensive series of B- and C-type (according to nomenclature, suggested by Domon and Costello [35]) glycosidic cleavages, whereas the Y-type cleavage occurred mainly at the sulfated residues [16, 30]. Fragment ions from crossing cleavage of  $^{0,2}\text{X}/^{0,2}\text{A}$ -type were observed only for mono/oligosaccharides that were not 3 linked [12, 16, 30, 32, 34, 36]. As recently shown by ESIMS/MS and computational chemistry methods on C-2 and C-4-sulfated fucose residues [34], the mechanism of formation of the  $^{0,2}\text{X}/^{0,2}\text{A}$  fragment ions required unsubstituted proton on the C-3 hydroxyl group. Hence, in case of 3-linked sulfated fucans/galactofucans from Laminariales [19, 20, 37] and enzymatically obtained oligosaccharides from carrageenan [14], when all protons of the C-3 hydroxyl groups are substituted, no  $^{0,2}\text{X}/^{0,2}\text{A}$ -type ions could be produced during CID MS/MS.

**3.2.1. Mass Spectrometry of Cf2-AH Sample from “Soft” Autohydrolysis.** The obtained preparation Cf2-AH, which was made in “soft” conditions (to avoid unwanted sulfate rearrangement effects [38]), due to negative-ion MALDI-TOFMS, contained a set of multiply sulfated (up to 4) oligosaccharides (DP 2–6), including monosulfated fucose residue (Figure 1). The main component of the mixture was doubly sulfated fucobiose, similarly as it was observed for the fucooligosaccharides that were derived from fucansulfate of the brown alga *F. evanescens*. As the structure of the backbone of fucansulfate from *F. evanescens* was shown [13] to have a linear core of

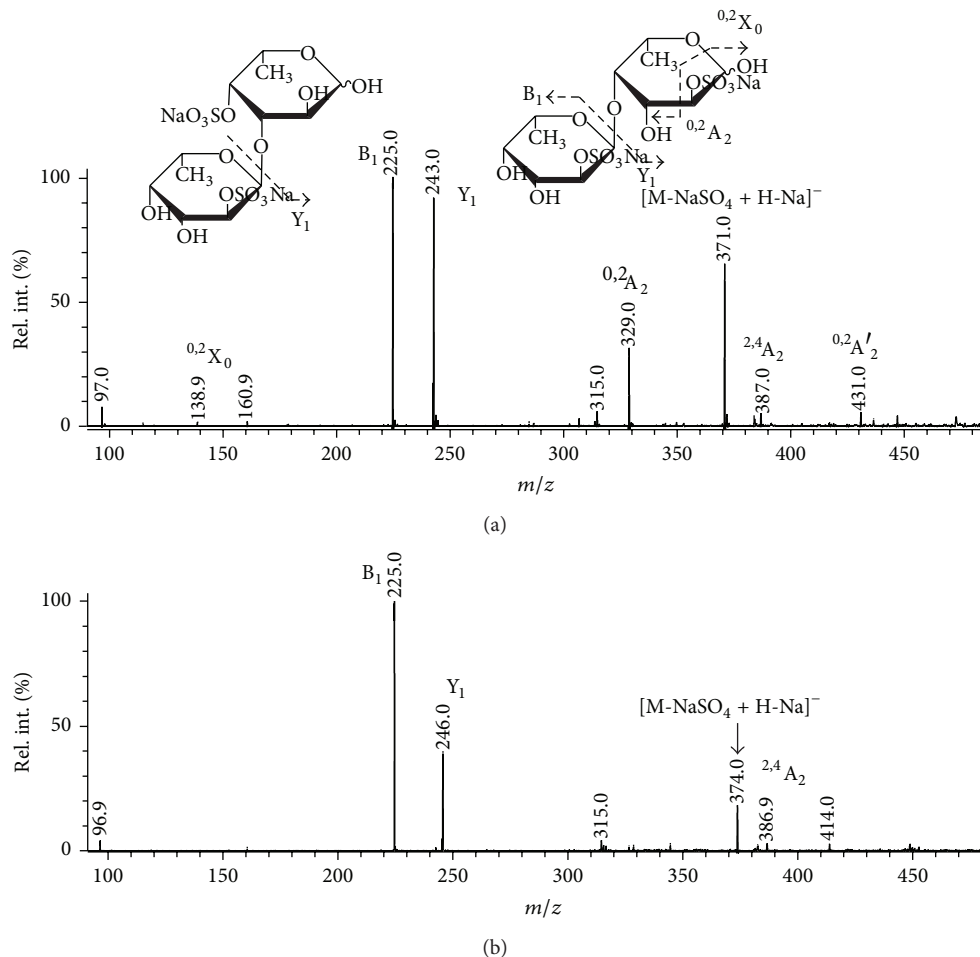


FIGURE 2: Negative-ion tandem MALDI-TOFMS ion at  $m/z$  491.02. M represents sodium salt of oligosaccharide.

repeating  $\rightarrow 4$ - $\alpha$ -L-Fucp-(1  $\rightarrow$  3)- $\alpha$ -L-Fucp-(1  $\rightarrow$  block, it was found that during autohydrolysis 3-linked  $\alpha$ -L-Fucp residues hydrolyzed faster and thus oligosaccharide mixture contained monosaccharide and fragments with even DP only [1]. No sulfation at C-3 was observed for *F. evanescens* samples [1, 12, 13]. In our case, the origin of fragments with odd DP that were detected at  $m/z$  637.01, 739.00, 929.16, 1031.13, and 1133.07 (Figure 1), as it was observed in autohydrolysis mixture of fucoidan from *Silvetia babingtonii* (Fucales) is unclear. The fucooligosaccharides from *S. babingtonii*, possibly, contained  $\alpha$ -L-Fucp residues, sulfated at position C-3 and/or insertions of 3-linked chains with DP = 2 [39], and were not yet examined by other method is but mass spectrometry. It is worthy to mention that **Cf2-AH** sample did not contain any signals corresponding to “mixed” oligosaccharides, built up of fucose and other sugars that were detected in monosaccharide composition, as it was observed in the samples from Fucales *F. evanescens* and *S. babingtonii* [1, 39], as well as *Sargassum mclcrei* [7], and Laminariales *C. costata* and *S. gurjanovae* [6, 20]. The selected conditions were assumed to be too soft for the cleavage of “mixed” oligosaccharides.

However the “softness” of the selected conditions is not soft enough to keep all the sulfates. The fact of the excess

cleavage of sulfates from highly sulfated (>30%) fucans/galactofucans during autohydrolysis (and/or in the ion source of the instrument) which have a backbone with 3 type of linkage ( $\alpha$ -L-Fucp chain) is known [7, 19, 20].

A tandem MALDI-TOFMS of the ion at  $m/z$  243.02 (not shown) did not exhibit any substantial difference of that observed for *F. evanescens* sample, except that  $^{0,3}X$ -signal at  $m/z$  168.9 was detected [34], like in the sample from *S. babingtonii* [39]. Minor sulfation at C-3 was also observed for fucoidans of the order Fucales: *Fucus serratus* [40] and *Fucus distichus* [41]. Furthermore, a signal that indicated sulfation at C-4 at  $m/z$  182.9 ( $^{0,2}A$ ) had higher intensity than the signal, corresponding to sulfation at C-2 at 138.9 ( $^{0,2}X$ ). The observation of the prevalence of sulfation at C-4 was observed recently for the galactofucan from brown alga *Sargassum polycystum* [42].

A tandem MALDI-TOFMS of the ion at  $m/z$  491.02 (Figure 2(a)) and its reduced variant (reduction with NaBD<sub>4</sub>, Section 2.3.4, Figure 2(b)) had differences in comparison to that observed for *F. evanescens* sample. Fragment ions from cross-ring cleavages  $^{0,2}A_2$  at  $m/z$  329 and  $^{0,2}A'_2$  at  $m/z$  431, suggesting structural variants  $\alpha$ -L-Fucp-(2 or 4-OSO<sub>3</sub><sup>-</sup>)-(1  $\rightarrow$  4)- $\alpha$ -L-Fucp-2-OSO<sub>3</sub><sup>-</sup> and  $\alpha$ -L-Fucp-(1  $\rightarrow$  4)- $\alpha$ -L-Fucp-2,4-di-OSO<sub>3</sub><sup>-</sup>, had the lowest intensities along the samples from





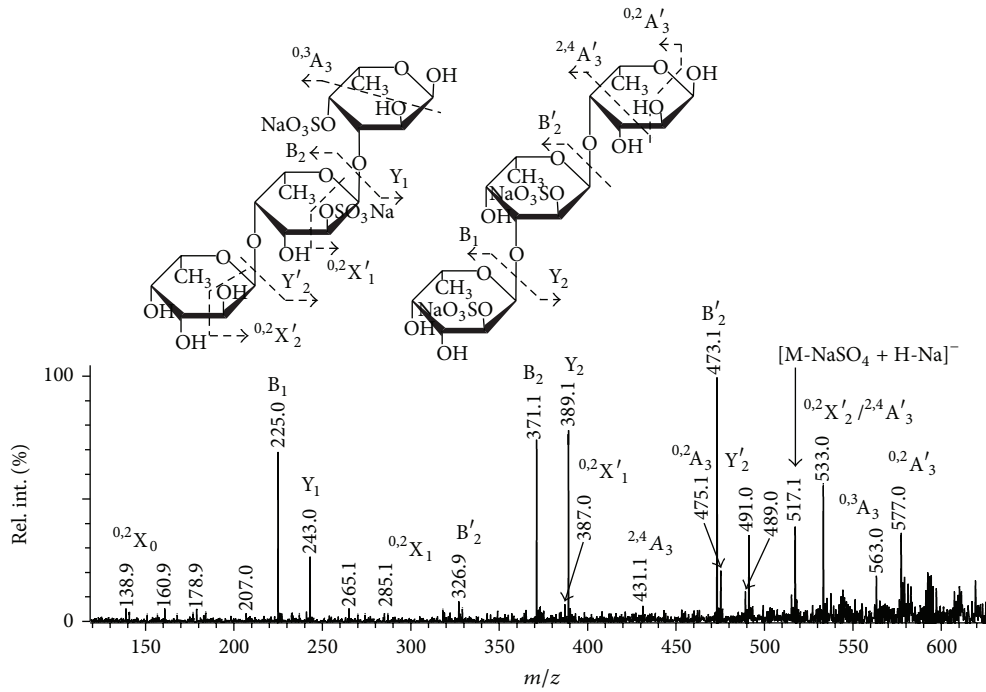


FIGURE 3: Negative-ion tandem MALDI-TOFMS ion at  $m/z$  637.07. M represents sodium salt of oligosaccharide.

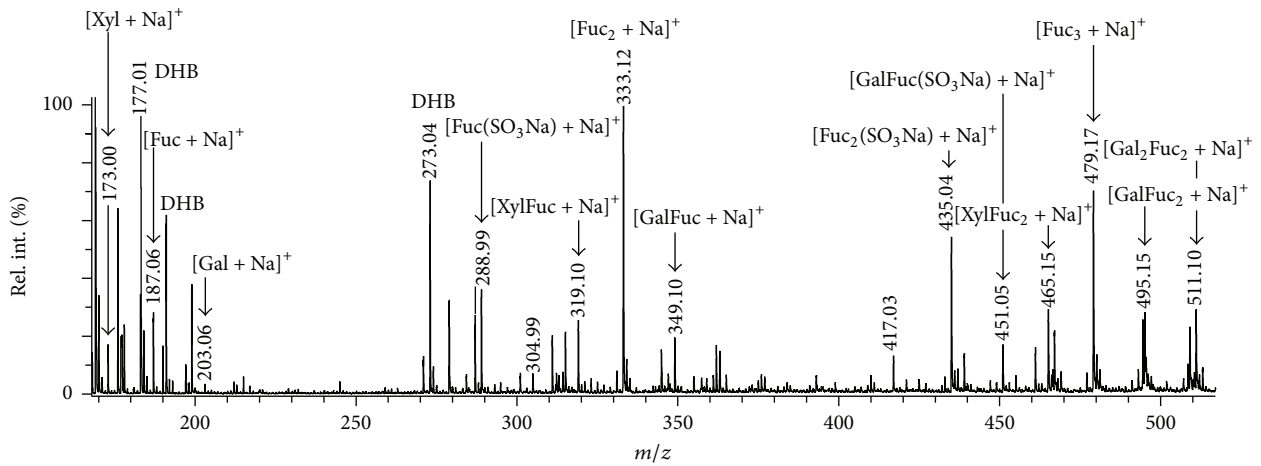


FIGURE 4: Positive-ion MALDI-TOFMS of **Cf2-exAH** sample: product of “exhaustive” autohydrolysis of **Cf2** fucoidan from the brown alga *C. langsdorfii*.

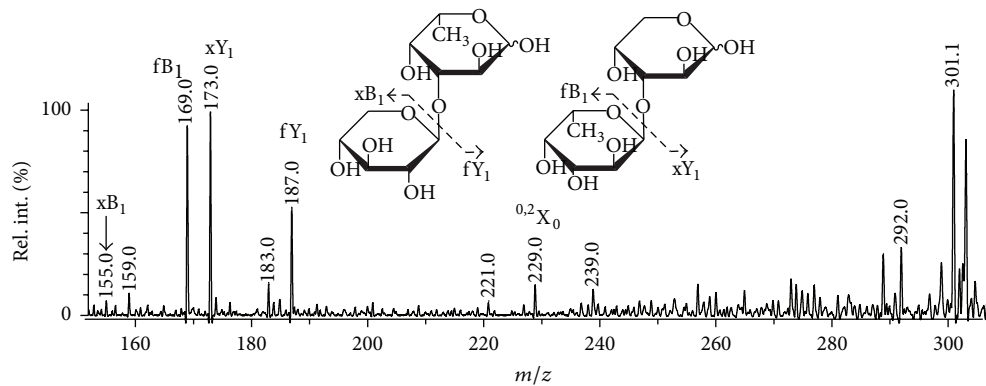
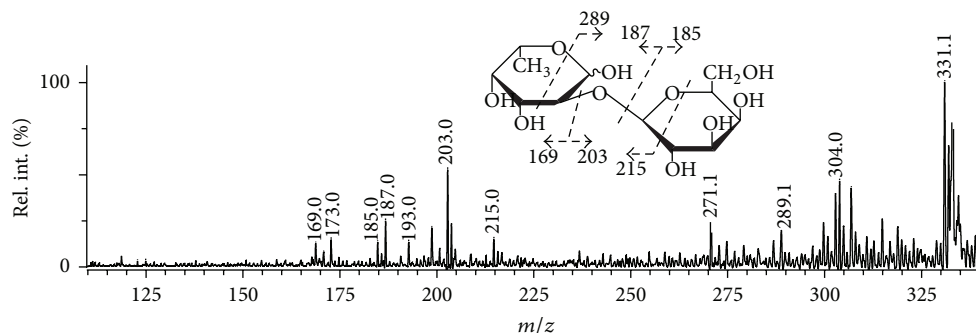
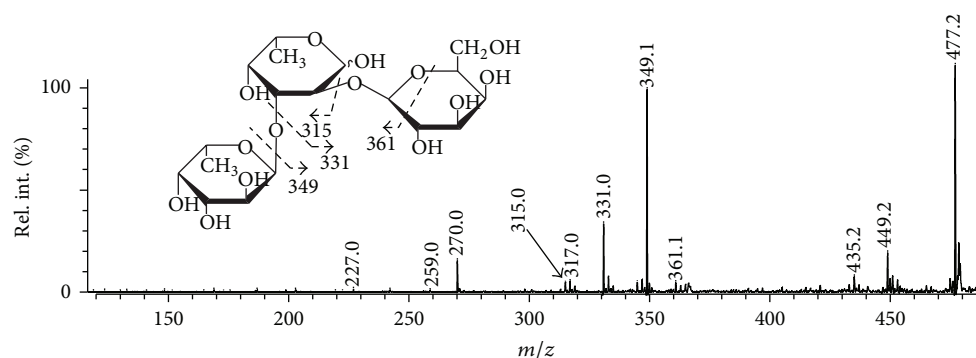


FIGURE 5: Positive-ion tandem MALDI-TOFMS ion at  $m/z$  319.10.

FIGURE 6: Positive-ion tandem MALDI-TOFMS ion at  $m/z$  349.10.FIGURE 7: Positive-ion tandem MALDI-TOFMS ion at  $m/z$  495.15.

and “exhaustive” autohydrolysis. Tandem MALDI-TOFMS of fucoligosaccharides with even DP (degree of polymerization) were exactly the same as that observed for fucoidan from *F. evanescens*, which have a backbone of alternating sulfated (1 → 3)- and (1 → 4) linked sulfated  $\alpha$ -L-Fucp residues [13]. Fragmentation patterns of oligosaccharides with odd DP indicated sulfation at C-2 of 4-linked  $\alpha$ -L-Fucp residues and at C-4 of (1 → 3) linked  $\alpha$ -L-Fucp residues on the reducing terminus. Minor sulfation at C-3 was detected by investigation of monosulfated fucose, and also a prevalence of the sulfation at C-4 over sulfation at C-2 was observed. The “exhaustive” autohydrolysis (maximal concentration in minimal volume) allowed us to obtain a mixture, enriched with minor constituents of the Cf2 fucoidan—xylose and galactose. It was found that unsulfated xylose residues could be, probably, 3- or 4-linked to fucose residues and could reside both on the reducing and nonreducing termini. Non-reducing galactose residues were found to be linked (in GalFuc disaccharides), possibly, by 2-type of linkages to fucose residues that were sulfated, most likely, at position C-2. Structural features of trisaccharides Gal<sub>2</sub>Fuc were not established due to strong overlapping of peaks. Fragments, including uronic acids, were not detected, being probably, minor impurities.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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### References

- [1] S. D. Anastyuk, N. M. Shevchenko, S. P. Ermakova et al., “Anticancer activity in vitro of a fucoidan from the brown alga *Fucus evanescens* and its low-molecular fragments, structurally characterized by tandem mass-spectrometry,” *Carbohydrate Polymers*, vol. 87, no. 1, pp. 186–194, 2012.
- [2] T. V. Alekseyenko, S. Y. Zhanayeva, A. A. Venediktova et al., “Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea *Fucus evanescens* brown alga,” *Bulletin of Experimental Biology and Medicine*, vol. 143, no. 6, pp. 730–732, 2007.
- [3] S. Ermakova, R. Sokolova, S.-M. Kim, B.-H. Um, V. Isakov, and T. Zvyagintseva, “Fucoidans from brown seaweeds *Sargassum horneryi*, *Cladonia cava*, *Costaria costata*: structural characteristics and anticancer activity,” *Applied Biochemistry and Biotechnology*, vol. 164, no. 6, pp. 841–850, 2011.
- [4] S. R. Khilchenko, T. S. Zaporozhets, N. M. Shevchenko et al., “Immunostimulatory activity of fucoidan from the brown alga *Fucus evanescens*: role of sulfates and acetates,” *Journal of Carbohydrate Chemistry*, vol. 30, no. 4–6, pp. 291–305, 2011.

- [5] J. Conchie and E. G. V. Percival, "Fucoidin. Part II. The hydrolysis of a methylated fucoidin prepared from *Fucus vesiculosus*," *Journal of the Chemical Society*, pp. 827–832, 1950.
- [6] N. M. Shevchenko, S. D. Anastuyuk, N. I. Gerasimenko, P. S. Dmitrenok, V. V. Isakov, and T. N. Zvyagintseva, "Polysaccharide and lipid composition of the brown seaweed *Laminaria gurjanovae*," *Russian Journal of Bioorganic Chemistry*, vol. 33, no. 1, pp. 88–98, 2007.
- [7] P. D. Thinh, R. V. Menshova, S. P. Ermakova, S. D. Anastuyuk, B. M. Ly, and T. N. Zvyagintseva, "Structural characteristics and anticancer activity of fucoidan from the brown alga *Sargassum mcclurei*," *Marine Drugs*, vol. 11, pp. 1456–1476, 2013.
- [8] M. I. Bilan, A. A. Grachev, A. S. Shashkov et al., "Further studies on the composition and structure of a fucoidan preparation from the brown alga *Saccharina latissima*," *Carbohydrate Research*, vol. 345, no. 14, pp. 2038–2047, 2010.
- [9] B. Li, X.-J. Wei, J.-L. Sun, and S.-Y. Xu, "Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed, *Hizikia fusiforme*," *Carbohydrate Research*, vol. 341, no. 9, pp. 1135–1146, 2006.
- [10] A. V. Skriptsova, N. M. Shevchenko, T. N. Zvyagintseva, and T. I. Imbs, "Monthly changes in the content and monosaccharide composition of fucoidan from *Undaria pinnatifida* (Laminariales, Phaeophyta)," *Journal of Applied Phycology*, vol. 22, no. 1, pp. 79–86, 2010.
- [11] J. Wang, Q. Zhang, Z. Zhang, H. Zhang, and X. Niu, "Structural studies on a novel fucogalactan sulfate extracted from the brown seaweed *Laminaria japonica*," *International Journal of Biological Macromolecules*, vol. 47, no. 2, pp. 126–131, 2010.
- [12] S. D. Anastuyuk, N. M. Shevchenko, E. L. Nazarenko, P. S. Dmitrenok, and T. N. Zvyagintseva, "Structural analysis of a fucoidan from the brown alga *Fucus evanescens* by MALDI-TOF and tandem ESI mass spectrometry," *Carbohydrate Research*, vol. 344, no. 6, pp. 779–787, 2009.
- [13] M. I. Bilan, A. A. Grachev, N. E. Ustuzhanina, A. S. Shashkov, N. E. Nifantiev, and A. I. Usov, "Structure of a fucoidan from the brown seaweed *Fucus evanescens* C.Ag," *Carbohydrate Research*, vol. 337, no. 8, pp. 719–730, 2002.
- [14] S. D. Anastuyuk, A. O. Barabanova, G. Correc et al., "Analysis of structural heterogeneity of  $\kappa/\beta$ -carrageenan oligosaccharides from *Tichocarpus crinitus* by negative-ion ESI and tandem MALDI mass spectrometry," *Carbohydrate Polymers*, vol. 86, no. 2, pp. 546–554, 2011.
- [15] A. Antonopoulos, J. Hardouin, P. Favetta, W. Helbert, A. F. Delmas, and M. Lafosse, "Matrix-assisted laser desorption/ionization mass spectrometry for the direct analysis of enzymatically digested kappa- iota- and hybrid iota/nu-carrageenans," *Rapid Communications in Mass Spectrometry*, vol. 19, no. 16, pp. 2217–2226, 2005.
- [16] G. Yu, X. Zhao, B. Yang et al., "Sequence determination of sulfated carrageenan-derived oligosaccharides by high-sensitivity negative-ion electrospray tandem mass spectrometry," *Analytical Chemistry*, vol. 78, no. 24, pp. 8499–8505, 2006.
- [17] J. Zaia and C. E. Costello, "Tandem mass spectrometry of sulfated heparin-like glycosaminoglycan oligosaccharides," *Analytical Chemistry*, vol. 75, no. 10, pp. 2445–2455, 2003.
- [18] M. I. Kusaykin, Y. V. Burtseva, T. G. Svetasheva, V. V. Sova, and T. N. Zvyagintseva, "Distribution of O-glycosylhydrolases in marine invertebrates. Enzymes of the marine mollusk *Littorina kurila* that catalyze fucoidan transformation," *Biochemistry*, vol. 68, no. 3, pp. 317–324, 2003.
- [19] S. D. Anastuyuk, N. M. Shevchenko, E. L. Nazarenko et al., "Structural analysis of a highly sulfated fucan from the brown alga *Laminaria cichorioides* by tandem MALDI and ESI mass spectrometry," *Carbohydrate Research*, vol. 345, no. 15, pp. 2206–2212, 2010.
- [20] S. D. Anastuyuk, T. I. Imbs, N. M. Shevchenko, P. S. Dmitrenok, and T. N. Zvyagintseva, "ESIMS analysis of fucoidan preparations from *Costaria costata*, extracted from alga at different life-stages," *Carbohydrate Polymers*, vol. 90, pp. 993–1002, 2012.
- [21] T. N. Zvyagintseva, N. M. Shevchenko, A. O. Chizhov, T. N. Krupnova, E. V. Sundukova, and V. V. Isakov, "Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the developmental conditions," *Journal of Experimental Marine Biology and Ecology*, vol. 294, no. 1, pp. 1–13, 2003.
- [22] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350–356, 1956.
- [23] K. S. Dodgson, "Determination of inorganic sulphate in studies on the enzymic and non-enzymic hydrolysis of carbohydrate and other sulphate esters," *The Biochemical Journal*, vol. 78, pp. 312–319, 1961.
- [24] H. Kogelberg, V. E. Piskarev, Y. Zhang, A. M. Lawson, and W. Chai, "Determination by electrospray mass spectrometry and 1H-NMR spectroscopy of primary structures of variously fucosylated neutral oligosaccharides based on the iso-lacto-N-octose core," *European Journal of Biochemistry*, vol. 271, no. 6, pp. 1172–1186, 2004.
- [25] N. Nakabayashi and K. Taniguchi, "Seasonal changes and productivity of the *Cocophora langsdorffii* population off the coast of Oga Peninsula in the Japan Sea," *Nippon Suisan Gakkaishi*, vol. 68, no. 5, pp. 659–665, 2002.
- [26] L. P. Perestenko, *The Seaweeds of Peter the Great Bay*, Akademia NAUK SSSR, Leningrad, Russia, 1980.
- [27] E. A. Titlyanov and T. V. Titlyanova, *Marine Plants of the Asian Pacific Region Countries, Their Use and Cultivation*, Dalnauka & A.V. Zhirmunsky Institute of Marine Biology Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia, 2012.
- [28] S. M. Cho, S. M. Lee, Y. D. Ko, L. Mattio, and S. M. Boo, "Molecular systematic reassessment of *Sargassum* (Fucales, Phaeophyceae) in Korea using four gene regions," *Botanica Marina*, vol. 55, pp. 473–484, 2012.
- [29] A. I. Usov, G. P. Smirnova, and N. G. Klochkova, "Polysaccharides of algae. 55. Polysaccharide composition of several brown algae from Kamchatka," *Bioorganicheskaya Khimiya*, vol. 27, no. 6, pp. 395–399, 2001.
- [30] O. M. Saad and J. A. Leary, "Delineating mechanisms of dissociation for isomeric heparin disaccharides using isotope labeling and ion trap tandem mass spectrometry," *Journal of the American Society for Mass Spectrometry*, vol. 15, no. 9, pp. 1274–1286, 2004.
- [31] A. G. Gonçalves, D. R. B. Ducatti, T. B. Grindley, M. E. R. Duarte, and M. D. Nosedá, "ESI-MS differential fragmentation of positional isomers of sulfated oligosaccharides derived from carrageenans and agarans," *Journal of the American Society for Mass Spectrometry*, vol. 21, no. 8, pp. 1404–1416, 2010.
- [32] T. Minamisawa and J. Hirabayashi, "Fragmentations of isomeric sulfated monosaccharides using electrospray ion trap mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 19, no. 13, pp. 1788–1796, 2005.



- [33] R. Daniel, L. Chevolut, M. Carrascal, B. Tissot, P. A. S. Mourão, and J. Abian, "Electrospray ionization mass spectrometry of oligosaccharides derived from fucoidan of *Ascophyllum nodosum*," *Carbohydrate Research*, vol. 342, no. 6, pp. 826–834, 2007.
- [34] B. Tissot, J.-Y. Salpin, M. Martinez, M.-P. Gageot, and R. Daniel, "Differentiation of the fucoidan sulfated L-fucose isomers constituents by CE-ESIMS and molecular modeling," *Carbohydrate Research*, vol. 341, no. 5, pp. 598–609, 2006.
- [35] B. Domon and C. E. Costello, "A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates," *Glycoconjugate Journal*, vol. 5, no. 4, pp. 397–409, 1988.
- [36] J. M. Daniel, V. V. Laiko, V. M. Doroshenko, and R. Zenobi, "Interfacing liquid chromatography with atmospheric pressure MALDI-MS," *Analytical and Bioanalytical Chemistry*, vol. 383, no. 6, pp. 895–902, 2005.
- [37] W. H. Jin, J. Wang, S. M. Ren, N. Song, and Q. B. Zhang, "Structural analysis of a heteropolysaccharide from *saccharina japonica* by electrospray mass spectrometry in tandem with collision-induced dissociation tandem mass spectrometry (ESI-CID-MS/MS)," *Marine Drugs*, vol. 10, pp. 2138–2152, 2012.
- [38] S. D. Anastyuk, N. M. Shevchenko, P. S. Dmitrenok, and T. N. Zvyagintseva, "Investigation of a sulfate transfer during autohydrolysis of a fucoidan from the brown alga *Fucus evanescens* by tandem ESIMS," *Carbohydrate Research*, vol. 346, no. 18, pp. 2975–2977, 2011.
- [39] S. D. Anastyuk, N. M. Shevchenko, P. S. Dmitrenok, and T. N. Zvyagintseva, "Structural similarities of fucoidans from brown algae *Silvetia babingtonii* and *Fucus evanescens*, determined by tandem MALDI-TOF mass spectrometry," *Carbohydrate Research*, vol. 358, pp. 78–81, 2012.
- [40] M. I. Bilan, A. A. Grachev, A. S. Shashkov, N. E. Nifantiev, and A. I. Usov, "Structure of a fucoidan from the brown seaweed *Fucus serratus* L.," *Carbohydrate Research*, vol. 341, no. 2, pp. 238–245, 2006.
- [41] M. I. Bilan, A. A. Grachev, N. E. Ustuzhanina, A. S. Shashkov, N. E. Nifantiev, and A. I. Usov, "A highly regular fraction of a fucoidan from the brown seaweed *Fucus distichus* L.," *Carbohydrate Research*, vol. 339, no. 3, pp. 511–517, 2004.
- [42] M. I. Bilan, A. A. Grachev, A. S. Shashkov et al., "Preliminary investigation of a highly sulfated galactofucan fraction isolated from the brown alga *Sargassum polycystum*," *Carbohydrate Research*, vol. 377, pp. 48–57, 2013.