A THESIS FOR THE DEGREE OF
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Preparation of fucose-containing monosaccharides from *Undaria pinnatifida* sporophyll and synthesis of fucosylated oligossacharide using *Bifidobacterium longum* RD47

Undaria pinnatifida sporophyll으로부터 fucose 함유물의 추출과 *Bifidobacterium longum* RD47을 이용한 푸코실 올리고당의 합성

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Abstract

Preparation of fucose-containing monosaccharides from *Undaria pinnatifida* sporophyll and synthesis of fucosylated oligossacharide using *Bifidobacterium longum* RD47

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Human milk oligosaccharides (HMOs) are mostly fucosylated at their non-reducing termini. HMOs are known to function as a prebiotic for bifidobacteria. L-fucose is major component of HMOs but very costly. Fucoidan is a group of marine sulfated polysaccharide of the cell-wall matrix of brown algae, containing large proportions of L-fucose. In this research, L-fucose

containing monosaccharides (FCMs) were obtained from fucoidan. For the experiment, fucoidan was extracted from *U. pinnatifida* sporophyll using 0.03 M HCl. Crude fucoidan was purified by ion-exchange chromatography and hydrolyzed to obtain FCMs. The analysis of monosaccharides composition was performed by Bio-LC. FCMs were applied to gel permeation column and used as a substrate to synthesize FO using the crude enzyme extracts from *Bifidobacterium longum* RD47. The synthesized FO was detected by thin layer chromatography. In the application aspect, the FO may provide a new constituent for cost-effective prebiotic food ingredient.

Keywords: *Bifidobacterium*, oligosaccharide, sporophyll of *U. pinnatifida*, L-fucose

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B. longum RD47; *Bifidobacterium longum* supsp. *longum* RD47

U. pinnatifida; *Undaria pinnatifida*

FCM; Fucose-containing monosaccharide

TLC; Thin layer chromatography

1-D TLC; 1-dimentional thin layer chromatography

2-D TLC; 2-dimentional thin layer chromatography

FO; Fucosylated oligosaccharide

GO; Galactosyl oligosaccharide

1. Introduction

L-fucose is a hexose without hydroxyl group on the carbon at the 6-position [1, 2]. Fucose is abundant in brown algae such as *Fucus, laminaria, Sargassum* and *Undaria* as a constituent of fucoidan [2]. Fucoidan is a group of marine sulfated polysaccharides of the cell-wall matrix of brown algae, containing large proportions of L-fucose, sulfate-fucose and galactose together with minor sugars [3]. The structures of fucoidans from different brown algae vary from species to species [2]. Recent studies have shown the existence of three types of algal fucoidans. The first one consists of (1→3)-α-L-fucose residues [4, 5]. The second one contains alternating (1→3)- and (1→4)-α-L-fucose residues [6, 7] whereas the third type of fucoidans is built up of (1→3)-or (1→3, 1→4)-linked α-L-fucose and β-D-galactose residues [8]. *Undaria pinnatifida*, commonly called Miyeok in Korea, is a common edible brown seaweed plentiful on the shores of the Korean peninsula. In particular, the sporophyll of *Undaria pinnatifida* contains relatively higher amount of fucoidan [8].

Fucose exists as a component of human milk oligosaccharides

(HMOs) which promote the growth in the intestinal tracts of the infants and provide protection against pathogens [9]. HMO contains different oligosaccharide components and various structures. Approximately 200 molecular species have been identified in pooled human milk samples. These structures consist of neutral and acidic oligosaccharides containing a high degree of fucosylation. HMOs are terminated by fucose, and even in this aspect vary in the range of 50-70% fucosylated [10, 11, 12, 27]. Fucosylated oligosaccharide (FO), the major component of HMOs exerts probiotic effects. FO can promote the selective growth of *Bifidobacterium* which is a well-known beneficial bacterium in the large intestine [13, 14]. Also, HMO can inhibit the adhesion of pathogens on epithelial cell [13].

Previously, it was shown that the galactosidases have the enzymatic activity toward not only hydrolysis but also transglycosylation. Crude enzyme extracts from *B. longum* RD47 were found to have strong hydrolysis and transglycosylation activity to produce FO using L-fucose and lactose as substrate. In the process of hydrolysis and transglycosylation catalyzed by β-galactosidase in *B. longum* RD47 crude enzyme extract, L-fucose functions as acceptor in the formation of FO [15]. Therefore, L-

fucose could be used as a substrate for the enzymatic synthesis of FO that may provide new opportunities in the development of future prebiotics.

Despite of these possibilities, commercial L-fucose is very expensive so there are limitations in synthesizing FO. In this study, we extracted L-fucose from fucoidan of *U. pinnatifida* sporophyll and used this extract as the raw material for synthesis of FO using *B. longum* RD47.

Here, we present the preparation method of FCMs from *U. pinnatifida* sporophyll and synthesis of FO using *B. longum* RD47 crude enzyme extracts.

2. Materials and Methods

2.1. Materials

2.1.1. Source of extraction

U. pinnatifida sporophyll grown in Wando, Korea was purchased from Jook-do market (Pohang, Korea).

2.1.2. The microorganism strains and culture condition

B. longum RD47 were activated by two successive preculture in MRS medium (Difco, Detroit, MI, USA) with 0.05% (w/v) cysteine-HCl at 37℃ for 18 h. The activated *B. longum* RD47 was inoculated in 8 ml MRS containing 0.05% (w/v) cysteine-HCl and grown at 37℃ for 18 h under anaerobic condition.

2.1.3. Chemicals and reagents

All reagents were purchased from Sigma-Aldrich (Sigma, St, Louis, MO, USA) unless specifically described. All chemicals used in this study were of analytical grade.

2.2. Extraction and purification of fucoidan

The purification of fucoidan from *U. pinnatifida* sporophyll was performed as described by Kim et al. with several modifications. [16]

U. pinnatifida sporophyll was ground by food grinder and refluxed with a mixture of methanol/chloroform/water 4/2/1 (v/v/v) using rotary evaporator to remove colored matter and phenol compounds prior to extraction [17]. After pretreatment, the mixture was centrifuged at 10,000 x g for 20 min at 4℃, and the supernatant was discarded.

Then, 250 g of dried *U. pinnatifida* sporophyll was extracted in 4 L with 0.03 M HCl for 4 h at 90℃. The extract was filtered through a Whatman No.1 filter paper (Whatman Ltd., Maidstone, United Kingdom). The filtrate was neutralized with 1 N NaOH, and the solution was precipitated with 3 volumes of ethanol. After centrifugation at 6,000 x g for 30 min at 4℃, the precipitate was dissolved in distilled water. pH of the suspension was adjusted to 2.0 with 1 N HCl and $CaCl_2$ was added up to the final concentration of 2 M. After centrifugation at 6,000 x g for 30 min at 4℃, the precipitate was removed. The supernatant was treated with 3

volumes of ethanol and repeated three times. The final precipitate was dissolved in distilled water and dialyzed through MWCO 3500 membrane (Spectrum Laboratories Inc, LA, USA) at 4℃ in distilled water for 48 h and then freeze-dried. This product was designated as crude fucoidan. The yield of crude fucoidan was calculated on the basis of a following formula.

Yield (%) = [amount of crude fucoidan (g) / amount of *Undaria pinnatifida* sporophyll (g)] x 100

The extract was further purified by column chromatography. One gram of crude fucoidan was dissolved in 10 ml of distilled water applied to a DEAE-cellulose column (100 ml) pre-equilibrated with distilled water (pH 7.0 adjusted with 0.1 M NaOH) and eluted with the same buffer containing increasing concentrations of NaCl (0.1, 0.5, 1.0, 1.5, 2.0 M) until no more carbohydrate was detected. Each fraction was assayed for carbohydrates by thin layer chromatography (TLC). The carbohydrate-positive fractions were pooled together and dialyzed through MWCO 3500 membrane for 24 h in distilled water and then freeze-dried. This product was designated purified fucoidan.

2.3. Preparation of the purified fucoidan hydrolysate

To obtain fucose-containing monosaccharides (FCMs), 10 mg of fucoidan was dissolved in 1 ml of distilled water and equal volume of 0.2 N HCl was added and allowed to stand for 1 h at 120℃ in autoclave. After the reaction, the mixture was neutralized with 1 M NaOH and filtered through 0.45 μm syringe filter and vacuum dried using Speed-Vacuum.

Sulfate, phosphate, other ions and uronic acid present in hydrolysate were removed by ion exchange resin on IRA-400 (Chloride Form, Sigma, USA), DOWEX 50XW4 (Hydrogen form, Sigma, USA) open column. One gram of dried hydrolysate was dissolved in 50 ml of distilled water and loaded onto IRA-400 open column. The column was eluted by distilled water and fractions were collected and loaded onto a DOWEX 50XW4 open column. The column was eluted by distilled water and fractions were collected and concentrated by a speed vacuum concentrator (ScanSpeed 40, LaboGene, Denmark).

2.4. Preparation of FCMs

Impurities present in hydrolysate were removed by gel permeation chromatography (GPC) on PD Miditrap G-10 (5.3 ml, GE Healthcare, USA). One ml of hydrolysate dissolved in sterilized water was loaded onto a gravity column of PD Miditrap G-10. The column was eluted by 2 ml of sterilized water. Fractions containing monosaccharides were detected by TLC. Fucose-positive fractions were collected and concentrated by a speed vacuum concentrator.

2.5. Chemical Analysis

2.5.1. Analysis by TLC

The carbohydrates in the extract were determined by TLC. The extract was loaded on the silica gel plate 60 (Merck, Darmstadt, Germany). The mobile phase was composed of 1-propanol, distilled water, ethyl acetate (7/2/1, v/v/v). The sulfuric acid-ethanol (1/9, v/v) solution was sprayed and dried. Developing solvent of 2-D TLC was composed of ethyl-acetate, 1-propanol, DW, acetic acid (4/2/2/1, v/v/v/v). The silica gel plate was heated at 110℃ for 5 min for visualization.

2.5.2. Determination of monosaccharide composition by Bio-LC

The extract (dried) was dissolved to distilled water. The extracted solution was filtered through a 0.2-㎛ PVDF membrane filter and Bio-LC was performed to determine the composition of monosaccharides.

The Bio-LC analysis was carried out on a Dionex-2500 ion chromatography instrument equipped with a pulsed amprometry detector. All separation procedures were carried out on a

CARBOPAC_PA1 column (4 x 250 mm) from Dionex. The flow rate was kept at 1.0 ml/min. Mobile phase was 2 mM of potassium hydroxide and the solvent composition was performed as follows: 2 mM (1−35 min), 2−100 mM (35−36 min), 36−56 min (100 mM), 56−57 min (100−2 mM), 58−63 min (2 mM). Standard solutions were prepared in distilled water to calculate the concentration of monosaccharides in the samples. Assay conditions were shown in table. 1

Table 1. Operation conditions of Bio-LC for the analysis of hydrolyzed fucoidan from *U. pinnatifida* sporophyll

Parameters	Conditions
Mobile phase	Potassium Hydroxide
Solvent Composition	1-35 min 2 mM 35-36 min 2-100 mM 36-56 min 100 mM 56-57 min 100-2 mM 58-63 min 2 mM
Column	CARBOPAC_PA1 (4 x 250 mm, Dionex, USA)
Flow rate	1.0 ml/min
Inj. Volume	10 μl
Detector	Pulsed amprometry, ED40, Gold electrode
Instruments	Ion Chromatograph, Dionex-2500, USA

2.5.3. Determination of anion composition by anion chromatography

The anion chromatograph analysis was carried out to check the existing state of sulfate. The sample used for ion exchange chromatography was the same as that of previous Bio-LC. The analysis was carried out on a Dionex ICS3000 (Dionex, USA) instrument equipped with a suppressed conductivity detector ASRS URTRA Ⅱ (4 mm, recycle mode). All separations were carried out on a IONPAC AS20 column (4 x 250 mm, Dionex, USA) at 30℃. The flow rate was kept at 1.0 ml/min. Mobile phase was potassium hydroxide and the solvent composition was performed as follows: 12 mM (0-8 min), 20 mM (8-12 min), 12-17min (30 mM), 17-18 min (12 mM), 18-20 min (12 mM). Assay conditions were shown in table 2.

Table 2. Operation conditions for the anion analysis of hydrolyzed fucoidan from *U. pinnatifida* sporophyll

Parameters	Conditions
Mobile phase	Potassium Hydroxide
Solvent Composition	0−8 min 12 mM
	8−12 min 30 mM
	12−17 min 30 mM
	17−18 min 30 mM
	18−20 min 12 mM
Column	IONPAC AS20 (4 x 250, Dionex, USA)
Column temperature	30℃
Flow rate	1.0 ml/min
Inj. Volume	25 μl
Detector	Suppressed conductivity, ASRS URTRA Ⅱ (4 mm), Recycle mode
Instruments	IONPAC AS20 Anion−exchange column

2.6. Synthesis of fucosylated oligosaccharide using *B. longum* RD47

2.6.1 Preparation of crude enzyme extracts

B. longum RD47 grown in the MRS medium at 37℃ for 18 h were centrifuged at 16,000 x g for 5 min. Cells were harvested by centrifugation and washed twice in 50 mM sodium phosphate buffer (PB, pH 6.6) and the supernatant was discarded. For the preparation of crude enzyme extracts, washed cells were resuspended in 1 volume of PB (pH 6.6) and disrupted with a sonicator (Sonicator 500, Q-Sonica, USA) in 1.0 s on /1.0 s off intervals for 5 min. Supernatant was used after centrifugation at 16,000 x g for 12 min at 4℃.

2.6.2. Determination of β-galactosidase activity in crude cell extracts

Enzyme activity was measured by using the para-nitrophenol (*p*NP) D-β-galacto-pyranosides as substrate. Enzyme solution (80 μl, 5 μl of crude enzyme extracts in 75 μl of PB) was added to 20 μl of 5 mM *p*NP-D-galactoside in 50 mM PB (pH 6.6). The mixture was incubated at 37℃ for 10 min. The reaction was stopped by adding 100 μl of 1 M Na_2CO_3. Enzyme activity was

measured in microplates at 405 nm in a spectrophotometer. Specific activity (enzyme activity level relative to cell mass) was determined as units of β-galactosidase activity. One unit was determined as the amount of product converted by 1 ml of *B. longum* RD 47 crude enzyme over 1 min. One unit means the relative enzyme activity.

2.6.3. Synthesis of fucosylated oligosaccharide using FCMs

To synthesize fucosylated oligosaccharide, FCMs (1 g) and lactose (400 mg) were mixed and finally 1 ml solution in PB (pH 6.6) was prepared for reaction. The enzyme extract (40 μl) was added to sugar solution. The mixture was incubated at 37℃. After 24 h incubation, the reaction was terminated by boiling for 10 min.

The carbohydrates in the reaction product were determined by one dimentional or two dimentional TLC as previously described.

3. Result and Discussion

3.1. Yield of crude fucoidan from *U. pinnatifida* sporophyll

Crude fucoidans were isolated from *U. pinnatifida* sporophyll. From 100 g each of dried samples, 5.51±1.3 g of crude fucoidans were obtained with the yield of 5.5 % in dry mass. The yield of crude fucoidan from *U. pinnatifida* sporophyll in this work was somewhat lower compared to the 3.2%−16.0% yield that was previously reported [18, 19, 20]. Since fucoidan has seasonal variation in content from about 5−15%, the yield of the crude fucoidan in this work was shown adequate [21].

3.2. Bio-LC Analysis of the purified fucoidan hydrolysate

The analysis of monosaccharides composition was performed by Bio-LC analysis. Each monosaccharide was quantified by comparing the peak area of sample sugar to that of standard monosaccharide of known amount. (Fig. 1) The monosaccharide composition of the purified fucoidan was shown to be galactose, fucose, glucose, mannose, xylose, and arabinose (Table 4). The major compounds were galactose and fucose with a molar ratio of 1.2:1. The molar ratio of galactose and fucose from *U. pinnatifida* sporophyll in this work was somewhat different compared to the 1.1:1 that was previously reported. This might be because of the chemical composition of algae that varies depending on the harvest season. [21]

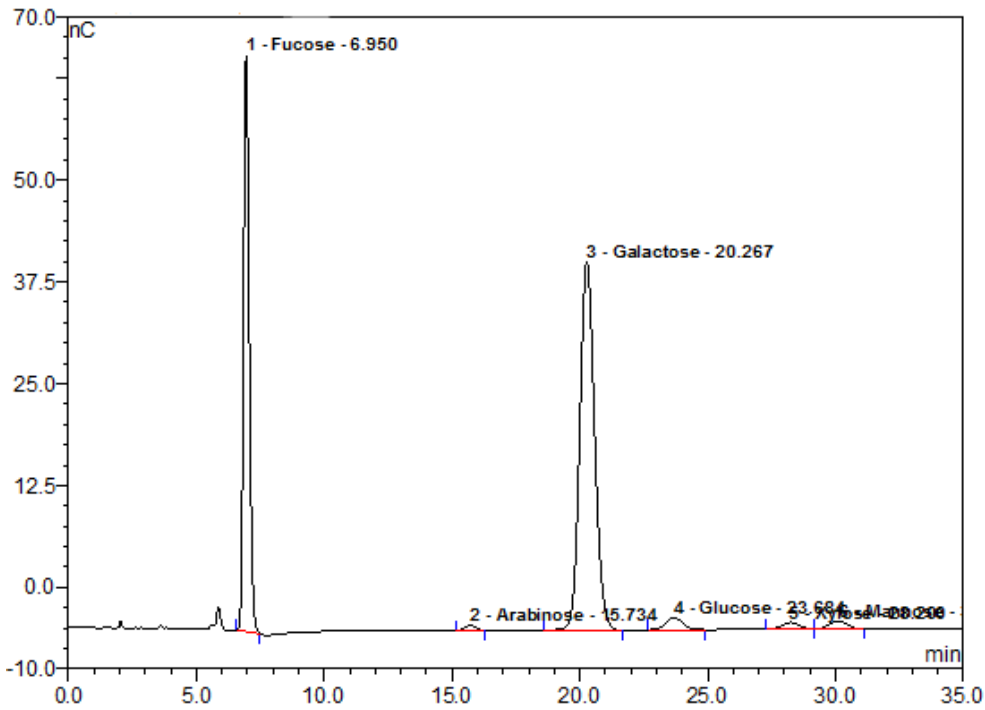

Fig 1. Bio-LC analysis for monosaccharide composition of the purified fucoidan hydrolysate. Chromatogram of acid hydrolysate of purified fucoidan. 1. Fucose; 2. Arabinose; 3. Galactose; 4. Glucose; 5. Xylose; 6. Mannose.

Table 3. Molar ratio of monosaccharides from the purified fucoidan hydrolysate

Molar ratio of monosaccharides					
Fucose	Galactose	Glucose	Mannose	Xylose	Arabinose
1	1.2	0.04	0.03	0.02	0.01

3.3. Anion analysis of the purified fucoidan hydrolysate

Fig. 2 shows the anion composition of hydrolyzed crude fucoidan by revealing the peaks of chloride, sulfate and phosphate. The result indicated that sulfate from the fucose was released by acid hydrolysis. The molar ratio of fucose and sulfate in this work was 1:0.8. That ratio between fucose and sulfate was more or less higher than those of other fucoidans [18, 19, 21].

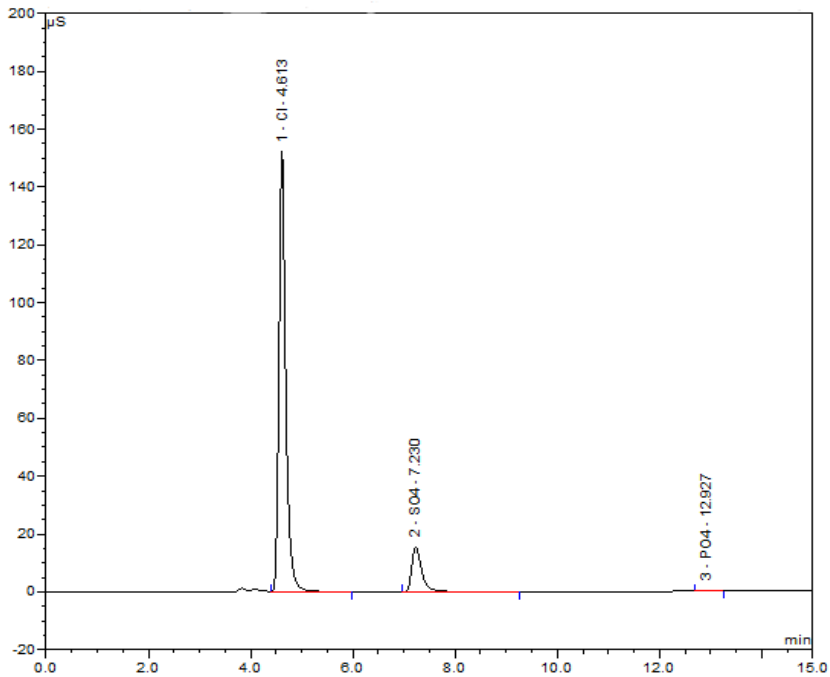

Fig. 2 Anion chromatography analysis for the anion of the purified fucoidan hydrolysate.

Chromatogram of acid hydrolysate of purified fucoidan. 1. Chloride; 2. Sulfate; 3. Phosphate.

Table 4. Anion proportion of the purified fucoidan hydrolysate and the molar ratio of fucose and sulfate

Proportion of anion (%)			Molar ratio	
Chloride	Sulfate	Phosphate	Fucose	Sulfate
82.4	16.8	0.7	1	0.83

3.4. Determination of FCMs

After hydrolysis, contaminants that might have been generated in the process of hydrolysis were detected by TLC. To prepare FCMs, gel permeation chromatography was performed following ion exchange chromatography. Gel permeation chromatography and ion-exchange chromatography do not influence the polysaccharide except the spatial pattern of the target compounds [18]. The result was shown in Fig. 3 and impurities were removed. Fucose and galactose were detected after 2.0 ml (Fig. 3 F2, F3, F4, F5) and these fractions were gathered to synthesize FOs.

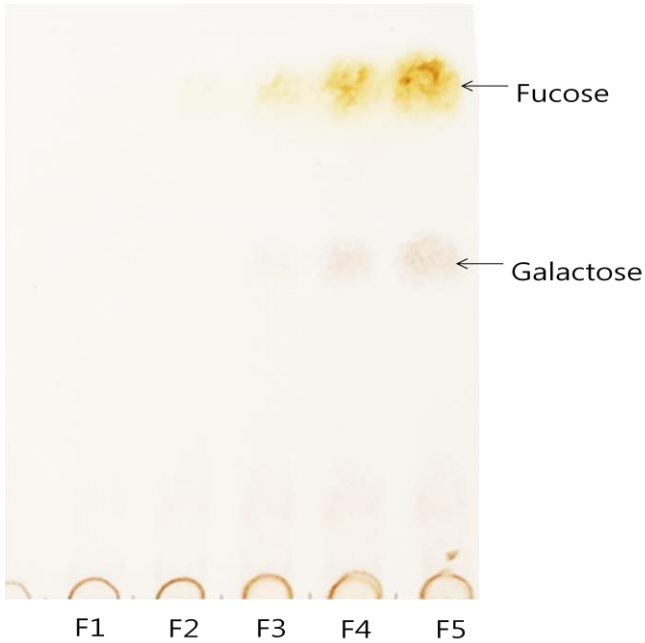

Fig. 3 Determination of FCMs by TLC following PD Miditrap-G10 column. Fractions were eluted from PD miditrap G-10. F; Fraction.

3.5. Determination of oligosaccharides synthesized by crude enzyme extracts from *B. longum* RD47

Crude enzyme extracts of *B. longum* RD47 hydrolyzed *p*NP-β-galactoside (Table 4).

Fig. 4 shows the reaction products using FCMs and lactose (Fig. 4A), and lactose only (Fig. 4B) as substrate. Lactose was hydrolyzed into galactose and glucose by β-galactosidase. Galactosyl oligosaccharides (GOs) with various degrees of polymerization were detected as shown Fig. 4A and 4B [15].

The Fig. 4A results are the same as these shown previously where commercially available L-fucose was used as a fucose substrate for the synthesis of FOs. Furthermore, the synthesized FOs was previously confirmed to be composed of fucose and galactose by MALDI-TOF and LC-ESI/MS. The analysis showed that the synthesized oligosaccharides had degree of polymerization from 2 to 7 [15]. Taken together, the fucose contained in FCMs which was obtained from *U. pinnatifida* sporophyll was successfully used for the synthesis of FOs.

On the TLC plate, the amount of FOs synthesized from FCMs and lactose at 48 h was lower than that of 24 h reaction suggesting that

reaction rate was retarded (Fig. 5). Previous report showed that the optimal condition for the transglycosylation reaction was similar to that of hydrolytic condition [22]. Since hydrolysis of synthesized oligosaccharides competes with transglycosylation, transglycosylation can be enhanced at high substrate concentration, controlled temperature, and lower water activity [23]. In addition, it has been indicated that transglycosylation of glycosidases is enhanced at a donor– acceptor ratio of 1:1 or excess of galactosyl–acceptor [24, 25]. This may be due to the fact that L–fucose has lower Michaeis constant, which is susceptible to the attack by the β–D–galactosidase [26]. For this reason, synthesized FOs were temporarily formed and the subsequently subjected to hydrolysis, which can be more accelerated toward the end of the reaction when the donor lactose was depleted. Therefore, it is crucial that reaction is terminated before the synthesized oligosaccharide is hydrolyzed again.

Table 5. Determination of β-galactosidase activity of crude enzyme extracts from *B. longum* RD47 by *p*NP analysis

Substrate	Total Activity (unit)	Specific Activity (/cell mass)
Cell extracts	5.31	1.275

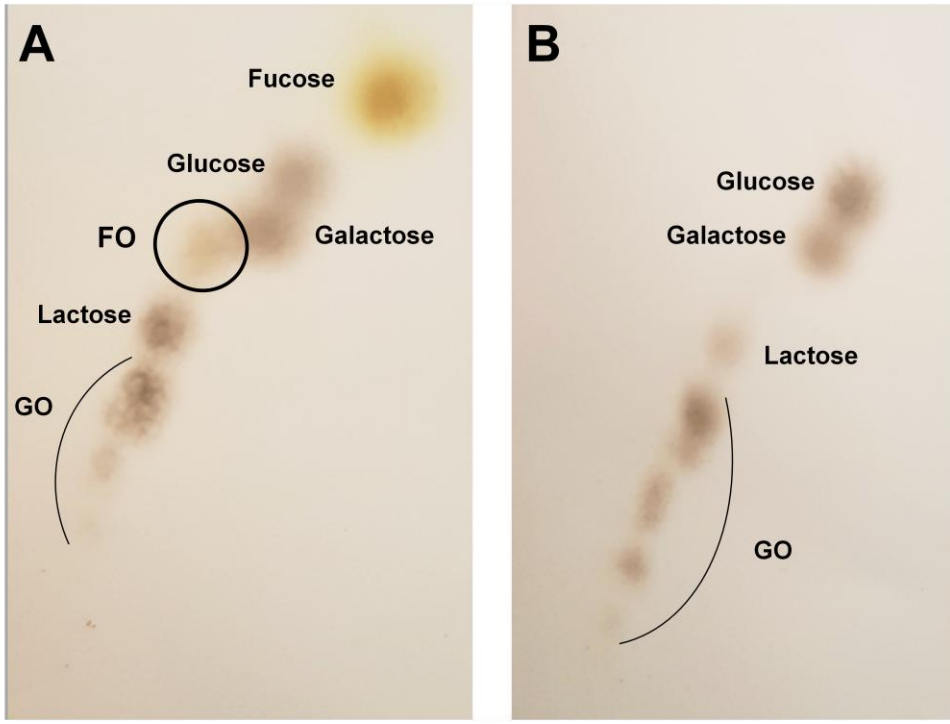

Fig 4. Determination of synthesized oligosaccharides by 2-D TLC using crude enzyme extracts from *B. longum* RD47 (24 h).

Both FCMs and lactose were added as substrates (A). Only lactose was added as a substrate (B). Crude enzyme extracts were mixed to produce transglycosylation products. Reactions were progressed over 24 h. Analysis was performed by TLC using a 1^{st}-dimentional solvent of 1-propanol:DW:ethyl-acetate (7:2:1) following using a 2^{nd}-dimentional solvent of 1-propanol:ethyl-acetate:DW:acetic acid (4:2:2:1).

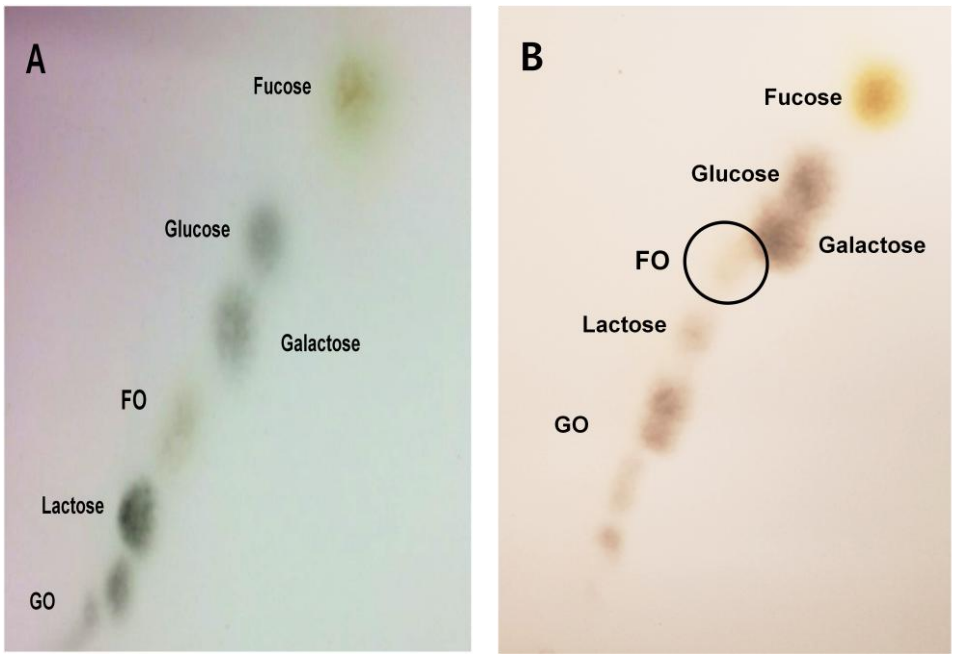

Fig 5. Determination of synthesized oligosaccharides by 2-D TLC using crude enzyme extracts from *B. longum* RD47 (12 h, 48 h).

FCMs, lactose and crude enzyme extract were mixed to produce transglycosylation products. Reactions were progressed over 12 h (A), 48 h (B). Analysis was performed by TLC using a 1st-dimentional solvent of 1-propanol:DW:ethyl-acetate (7:2:1) following using a 2nd-dimentional solvent of 1-propanol:ethyl-acetate:DW:acetic acid (4:2:2:1).

4. Conclusion

In this study, the fucose containing polysaccharide which is known as fucoidan was extracted from *U. pinnatifida* sporophyll. The purified fucoidan was hydrolyzed and used to synthesize FOs using crude enzyme extract from *B. longum* RD47. The synthesis of FO was assessed by 2-D TLC and this result indicated that fucose from *U. pinnatifida* sporophyll was used as a substitute for the commercial L-fucose. Commercial L-fucose is very expensive so there are limitations in synthesizing FO. From this study, L-fucose from fucoidan of *U. pinnatifida* sporophyll was identified as the raw material for synthesis of FO using *B. longum* RD47. This is the first report to produce FCMs and use of FCMs for the synthesis of FO which is relevant for prebiotics.

In the application aspect, the FO may provide a new constituent for cost-effective prebiotic food ingredient.

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국문초록

L-fucose는 6번 탄소에 hydroxyl group이 없는 hexose이다. Fucose는 다시마, 모자반, 미역 등의 갈조류에 fucoidan이라는 다당류를 이루며 존재한다. Fucoidan은 갈조류의 세포벽 성분이며 다시마와 미역의 경우 건조중량 대비 약 3~16%의 fucoidan을 함유하고 있다. Fucoidan은 L-fucose와 이에 황산기가 결합된 sulfated-fucose를 주요 구성성분으로 하여 galactose 및 미량의 기타 당류로 이루어진 다당류이다. L-fucose는 모유 올리고당의 구성성분이며 모유 올리고당은 *Bifidobcterium* spp. 과 같은 장내 유익균주를 선택적으로 증가시키는 프리바이오틱스로서의 기능이 알려져있다. 모유 올리고당의 구조 및 구성성분은 다양하며 그 중 fucose를 함유하는 퓨코실 올리고당이 약 75%를 차지한다.

이전 연구로부터 *B. longum* RD47의 β-galactosidase가 hydrolysis, transglycosylation 활성을 가지는 것이 밝혀졌다. *B. longum* RD47에서 추출한 조효소를 L-fucose와 D-lactose를 기질로 하여 반응하였을 때 퓨코실 올리고당이 합성됨이 확인되었다. 이는 조효소 중의 β-galactosidase에 의해서 lactose가 가수분해되면서 L-fucose가 galactosyl moiety의 acceptor가 되어 transglycosylation 된 결과이다. 그러나 기질로 이용되는 L-fucose의 비용으로 인해 퓨코실 올리고당을

합성하는데에 한계점이 있었다. 따라서 본 연구에서는 *B. longum* RD47의 조효소를 이용하여 갈조류 중 fucoidan의 함량이 타 갈조류에 비해 높은 것으로 알려진 미역포자엽(sporophyll of *Undaria pinnatifida*)으로부터 fucoidan을 추출 및 정제하였다. 이후 fucoidan을 가수분해하여 fucose를 함유한 추출물을 이용하여 이를 기질로하는 퓨코실 올리고당이 생성되는지를 알아보고자 하였다.

건조된 미역포자엽으로부터 5.51±1.3%의 crude fucoidan을 얻었으며 이를 가수분해 후 Bio-LC로 구성당의 성분을 확인한 결과 해당 fucoidan은 galactose와 fucose를 주요 구성당으로함을 확인했다. 가수분해물을 정제한 후, lactose와 함께 이를 transglycosylation의 기질로 하여 24시간 동안 반응을 진행했고 2-D TLC를 통해 transglycosylation product를 확인했다. 2-D TLC plate 상의 해당 밴드는 이전 연구에서 MALDI-TOF, LC-ESI/MS를 통해 퓨코실 올리고당으로 밝혀진 밴드와 일치했으며 따라서 미역포자엽 추출물로부터 퓨코실 올리고당이 합성됨을 확인하였다.

본 연구를 통해 합성된 올리고당은 고비용의 L-fucose를 대체할 수 있는 모유 올리고당 성분으로서 향후 경제성이 확보된 식품 소재의 프리바이오틱스로 활용될 수 있다.

주요어: 비피토박테리움, 올리고당, 미역포자엽, 푸코오스

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