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# Isolation and Characterization of Marine Bacterial Strain Degrading Fucoidan from Korean *Undaria pinnatifida* Sporophylls

**Kim, Woo-Jung[1], Sung-Min Kim[1], Yoon-Hee Lee[1], Hyun Guell Kim[1], Hyung-Kwon Kim[1], Seong Hoon Moon[2], Hyun-Hyo Suh[3], Ki-Hyo Jang[4], and Yong-Il Park[1]***

*[1]Department of Biotechnology and Biomaterial Engineering Research Center, The Catholic University of Korea, Bucheon 420-743, Korea*
*[2]Biotechnology Policy Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-333, Korea*
*[3]Department of Environmental Engineering, Jinju National University, Jinju 660-758, Korea*
*[4]Department of Food and Nutrition, Kangwon National University, Samcheok 245-711, Korea*

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**In spite of an increasing interest in fucoidans as biologically active compounds, no convenient commercial sources with fucoidanase activity are yet available. A marine bacterial strain that showed confluent growth on a minimal medium containing fucoidan, prepared from Korean *Undaria pinnatifida* sporophylls, as the sole carbon source was isolated and identified based on a 16S rDNA sequence analysis as a strain of *Sphingomonas paucimobilis*, and named *Sphingomonas paucimobilis* PF-1. The strain depolymerized fucoidan into more than 7 distinct low-molecular-mass fucose-containing oligosaccharides, ranging from 305 to 3,749 Da. The enzyme activity was shown to be associated with the whole cell, suggesting the possibility of a surface display of the enzyme. However, a whole-cell enzyme preparation neither released the monomer L-fucose from the fucoidan nor hydrolyzed the chromogenic substrate *p*-nitrophenyl-α-L-fucoside, indicating that the enzyme may be an endo-acting fucoidanase rather than an α-L-fucosidase. Therefore, this would appear to be the first report on fucoidanolytic activity by a *Sphingomonas* species and also the first report on the enzymatic degradation of the Korean *Undaria pinnatifida* sporophyll fucoidan. Moreover, this enzyme activity may be very useful for structural analyses of fucose-containing polysaccharides and the production of bioactive fucooligosaccharides.**

**Keywords:** Fucoidan, fucoidanase, marine bacterium, fucooligosaccharides

Fucoidans are a group of marine sulfated polysaccharides in the cell-wall matrix of brown algae that contain large proportions of L-fucose and sulfate, along with minor amounts of other sugars, like xylose, galactose, glucose, mannose, uronic acids, and rhamnose [6, 19]. The main skeleton of fucoidans is α1,3-linked-L-fucose-4-sulfate [26], but a repeating structure of alternating α(1→3) and α(1→4) glycosidic bonds is also frequently observed depending on the algal species [9, 11]. Owing to the lack of any standard method for the analysis of polysaccharides, details on the chemical structure of fucoidans have not yet been elucidated, with only reports on some average or partial structures [6]. Nonetheless, these acidic polysaccharides are known to exhibit a wide range of physiological and biological activities, including medically useful activities, such as anti-inflammatory [24], antiviral [5, 16, 20], anticoagulant [22, 23], antitumor [27, 42], and antiangiogenesis [15] behavior. Fucoidans also prevent *Helicobacter pylori* infection and reduce the risk of associated gastric cancer [32]. Thus, the production and application of fucoidans as therapeutic agents have become increasingly important topics of intensive research [6], although their high molecular mass and viscous nature continue to hamper their application, especially as a therapeutic agent. Therefore, a reliable fucoidan-degrading enzyme preparation for producing fucooligosaccharides would certainly help to overcome these problems. Meanwhile, the investigation of fucoidanases and enzymatic decomposition of fucoidan would assist in elucidating the structure and mechanisms of the biological activity of fucoidans. Despite an increasing number of reports on the fucoidanolytic activities of marine mollusca and microorganisms towards various fucoidans [2, 6, 11, 12, 29, 38, 40], available data on purified fucoidanases remain scarce and no convenient commercial sources with fucoidanolytic activity are yet available for the degradation of these polysaccharides [6, 14, 40].

Accordingly, in an attempt to search for such enzymes, this study reports on the isolation, identification, and culture conditions for a marine bacterial strain capable of

*Corresponding author
Phone: 82-2-2164-4512; Fax: 82-2-2164-4846;
E-mail: yongil382@catholic.ac.kr

degrading fucoidan purified from the sporophylls of Korean *Undaria pinnatifida*. As such, this would appear to be the first report on fucoidanolytic activity by a *Sphingomonas* species, and the first report on the enzymatic degradation of Korean *Undaria pinnatifida* sporophyll fucoidan.

## MATERIALS AND METHODS

### Preparation of Fucoidan

The *Undaria pinnatifida* sporophylls used as the source of the algal fucoidan in this study were collected from the southern coastal area of Wando, Korea. Following the method of Tako *et al.* [36], fucoidan was extracted from the dried sporophylls mainly by 0.1 N HCl extraction and ethanol precipitation. The crude fucoidan obtained was further purified through DEAE-cellulose column chromatography (since data on the extraction, purification, and structure of fucoidans is not the scope of this paper, details will be published elsewhere). The lyophilized purified fucoidan was then used as a substrate to screen for fucoidanolytic activity.

### General Methods

The total carbohydrate in the purified fucoidan was determined using the phenol-sulfuric acid method [13], with L-fucose as the reference, and the amount of sulfate residues was determined using the $BaCl_2$-gelatin method [34], with $Na_2SO_4$ as the standard. The uronic acid content was determined using a carbazole reaction [7], with D-glucuronic acid as the standard, and uronic acid was identified using the method of Sakai *et al.* [29]. The neutral sugar composition was determined using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD), where a quantity of fucoidan dissolved in $dH_2O$ was acid hydrolyzed with an equal volume of 4 M trifluoroacetic acid (TFA) at 100°C for 2 h. After removing the TFA by repeated evaporation using a Speed-Vac (Biotron, Korea), the dried material was dissolved in $dH_2O$ and analyzed on a CarboPac PA-1 column (Dionex, USA). The protein was quantified using the Bradford method [8]. A commercially available fucoidan (Sigma) was also analyzed, as described above, and its composition compared with that of the newly isolated fucoidan. The average relative molecular mass of the fucoidan was estimated using HPLC (Dionex, U.S.A.) on a size-exclusion column (Shodex OHpak SB-806HQ, 8.0×300 mm; Showa Denko Co., Japan) with dextrans as the relative molecular mass markers: 464, 188, 162, 143, 71, 43 kDa (Sigma).

### Isolation of Fucoidan-Degrading Bacterium

Samples of seawater, mud, and seaweed (floating and embedded in mud) were collected at several coastal sites on Jeju Island and used as sources of fucoidan-utilizing microorganisms. For pre-incubation of the microorganisms at the beginning of the isolation process, coastal seawater (NSW, natural seawater) collected separately from similar areas was used as the growth medium after removing all insoluble materials by filtration (0.2-μm Cellulose filter; Advantec MFS Inc., Japan). The seawater samples were inoculated (10%, v/v) into 50 ml of an isolation medium (medium A) containing 0.2% (w/v) fucoidan, 10 g of Bacto peptone, 1 g of yeast extract, 1 mM ferrous solution ($FeHNO_3$), 1.6 mg of ammonium nitrate, and 8 mg of disodium phosphate in 1 l of NSW (pH 7.8). The mud and other solid samples were suspended in sterilized seawater (1:1, w/v) and after precipitation had occurred, a portion of each supernatant was inoculated as above (10%, v/v). The cells were pre-incubated at 25°C for 5 days on a shaking incubator (180 rpm; JEIO TECH Co., Korea), and then 200 μl of each culture was spread on agar plates (medium B) containing 10 g of Bacto peptone, 5 mg of ammonium nitrate, a 1 mM ferrous solution, 10 g of fucoidan as the sole carbon source, and 15 g of agar per l and incubated at 30°C for another 4 days. The colonies developed were then transferred to agar slants of medium B, cultivated for 3 days, and stored at 4°C until further testing. To examine the fucoidan-degrading ability of these isolates, the cells were aerobically incubated at 30°C for 3 days in 10 ml of medium B, boiled for 10 min to stop any growth, and centrifuged in a benchtop microfuge for a few seconds, and each supernatant was examined for reducing sugars using the Somogyi-Nelson method [35].

Among all the bacterial isolates, several strains with increased reducing sugars in the culture medium were selected and examined further for fucoidan-degrading activity. One strain, tentatively named as PF-1, was then selected that exhibited the highest level of reducing sugar generation and grew well in just laboratory-distilled water ($dH_2O$) supplemented with only fucoidan (pH adjusted to 7.8 with 0.5 N NaOH). Thus, unless stated otherwise, subsequent experiments with this strain were performed in a minimal medium consisting of 0.2% fucoidan in $dH_2O$ (pH 7.8) (medium C) or 0.2% fucoidan with 2% Bacto peptone in $dH_2O$ (pH 7.8) (medium D).

### Fucoidanolytic Activity

The fucoidanolytic activity of the isolated strain was confirmed by measuring the reducing sugars released after incubation with an enzyme preparation, any reduction in the fucoidan viscosity, and a monosaccharide composition analysis of the degradation products after Bio-Gel P-4 size-exclusion chromatography. The PF-1 isolate was pre-incubated in 100 ml of medium C at 30°C for 3 days, and then 20 ml of this seed culture was inoculated into 1 l of medium D and cultivated for 4 days. After centrifugation at 6,000 ×*g* at 4°C for 30 min, the supernatant was concentrated to 50 ml using a UF system (MWCO 10,000; Amicon, U.S.A.) on ice, while the cells were washed twice and finally suspended in a 50 mM sodium acetate buffer (pH 5.6). To examine for the presence of fucoidan-degrading activity, 50 ml of the concentrated supernatant or cell suspension was mixed with equal volumes of 0.2% fucoidan (as the substrate) containing 0.02% sodium azide, and then incubated at 30°C for 120 h in a shaking water bath (120 rpm; Dong-A Scientific Co., Korea). Sodium azide was added to prevent any growth of PF-1 or any contaminating microorganism during the incubation with fucoidan. Aliquots of each reaction mixture were taken at several time intervals and checked for reducing sugars at 510 nm.

The fucoidanase activity of the PF-1 isolate was also confirmed by measuring any reduction in the fucoidan viscosity. The reaction mixture consisted of 2 ml of a 0.5% fucoidan solution, 2.95 ml of a 50 mM acetate buffer (pH 5.5), and 0.05 ml of the cell suspension ($10^6$ cells/ml) as the enzyme solution prepared in the same buffer. After incubation at 30°C in an Ostwald-type viscometer, the flow time was measured following the method of Kitamura *et al.* [17].

### Identification of Fucooligosaccharides

For final and clear evidence of fucoidanolytic activity by strain PF-1, a reaction mixture of fucoidan and an enzyme preparation (cell suspension) was fractionated using a Bio-Gel P-4 column (1.5×

95 cm), and each oligomer eluted from the column was analyzed for its monosaccharide composition and molecular mass. A reaction mixture of 40 ml of a 50 mM sodium acetate buffer (pH 5.6) containing 1% fucoidan and 40 ml of a crude enzyme preparation (cell suspension with 0.01% sodium azide) was incubated for 7 days at 30°C in a shaking water bath (120 rpm). After incubation, the cells were removed by centrifugation and the supernatant concentrated by freeze drying. The dried material was then dissolved in 5 ml of 50 mM sodium nitrate and 1 ml applied to a Bio-Gel P-4 column and eluted at 0.3 ml/min using the same solvent. The carbohydrate-containing fractions were pooled, freeze-dried, and subjected to a monosaccharide composition analysis using HPAEC-PAD after 2 M TFA hydrolysis, as described above. The carbohydrates in the acid hydrolysates were eluted through a CarboPac PA-1 column with 18 mM NaOH at 0.8 ml/min in an isocratic mode and identified by comparison with authentic monosaccharides [25].

### α-L-Fucosidase Assay

To determine whether the fucoidan hydrolytic activity of the PF-1 isolate was related to α-L-fucosidase activity, *p*-nitrophenyl-α-L-fucoside (Sigma) was tested as a substrate. The reaction mixture contained 0.25 ml of the 2 mM substrate in a 50 mM sodium acetate buffer (pH 5.6) and 0.05 ml of an enzyme solution (cell suspension, $5\times10^3$ cells). After incubation at 30°C in a shaking water bath, 2.2 ml of 0.55 M sodium carbonate was added to stop the reaction. The *p*-nitrophenol released was measured spectrophotometrically at 400 nm. Commercial α-L-fucosidase (Sigma) was used as the control.

### Biochemical and Growth Characteristics of Strain PF-1

The cellular morphological characteristics were investigated using general light microscopy (1,000×, Olympus BH-2) and electron microscopy. For the electron microscopy, the cells were grown for 2 days at 30°C in the minimal medium containing 0.2% fucoidan as the sole carbon source, and then negatively stained with 2% phosphotungstic acid and photographed using a transmission electron microscope (LIBRA 120; Carl Zeiss, Germany) operated at 120 kV. The Gram staining was performed during exponential cell growth on the LB plates. The oxidase activity was assayed with disks impregnated with dimethylparaphenylene diamine oxalate (Diagnostic Pasteur), whereas the catalase activity was determined by applying a few drops of 10% $H_2O_2$ onto the colonies. The other phenotyping tests were all performed using API 20 NE strips (API System, bioMerieux) and Biolog GN microplates (Micromer, France) [4]. To examine the growth behavior, the cells were grown under various culture conditions, such as differing pH and NaCl concentrations, in an LB medium.

### Identification of Strain PF-1

To identify strain PF-1, its DNA was extracted using the guanidine thiocyanate/phenol/chloroform method [10] and the 16S rDNA sequence examined. The 16S rDNA was amplified by a PCR using bacterial universal 16S rDNA primers: 27F (5'-AGAGTTTGA-TCCTGGCTCAG-3') and 1492R (5'-GGCTACCTTGTTACGACTT-3') [18]. After the PCR amplification, a total of 124 16S rDNA clones were sequenced by the chain termination method on an ABI Prism 370 automatic sequencer (Applied Biosystems, Foster City, CA, U.S.A.) using a T7 primer. All the sequences with approximately 700 bases were compared with sequences available in the EMBL/GenBank database using a BLAST search [1]. The sequences were aligned using the MegAlign Program (DNAstar Inc., U.S.A.) and phylogenetic trees constructed using the neighbor-joining method [27, 33].

## Results and Discussion

### Preparation of *Undaria* Sporophyll Fucoidan

The fucoidan used in this study was extracted and purified using dilute acid extraction, ethanol precipitation, $CaCl_2$ precipitation, and then anion-exchange chromatography (detailed experimental methods and data will be published elsewhere). The average molecular mass of the fucoidan was estimated to be about $2.1\times10^3$ kDa by size-fractionation HPLC and it consisted of neutral sugar (52.34% in mass), uronic acid (26.2%), and sulfate (7.4%). The HPAEC-PAD analysis of the neutral sugar composition of the fucoidan showed that fucose was its major neutral sugar (~70% molar ratio), whereas xylose (1.3%), galactose (14.8%), and mannose (10.9%) were present as minor components (data not shown), thereby suggesting that the fucoidan extracted from the Korean *Undaria pinnatifida* sporophylls was a sulfated, U-type fucoidan. This fucoidan was then used as the enzyme substrate to screen for fucoidan-degrading microorganisms.

### Isolation and Cultivation of Fucoidan-degrading Bacterial Strain

One hundred and eighty-five strains of microorganisms were isolated from the seawater, mud, and seaweed samples collected from several coastal sites on Jeju Island,

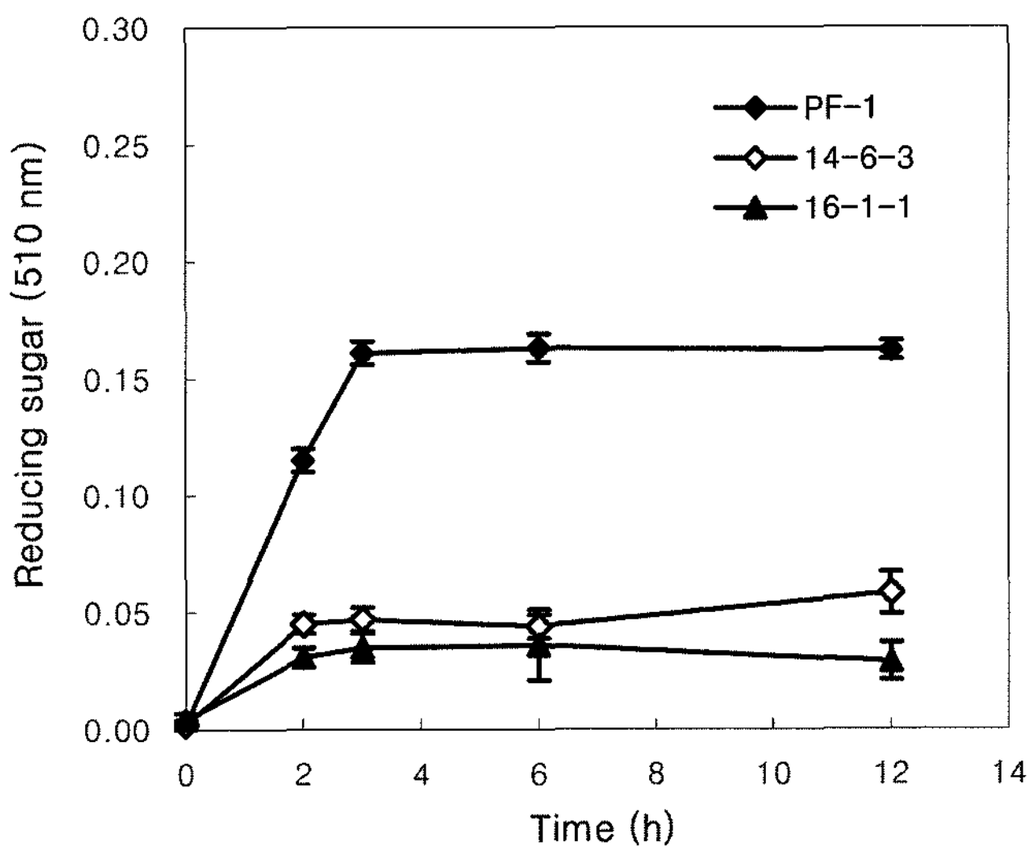


**Fig. 1.** Generation of reducing sugars by growth of bacterial isolates in culture medium containing fucoidan as the sole carbon source.

The cells were aerobically grown for 3 days at 30°C in an NSW medium (pH 7.8). The reducing sugars in the culture supernatant were quantified using the Somogyi-Nelson method [35]. PF-1, 14-6-3, and 16-1-1 were given as tentative names to the representative isolates during the initial isolation process.

Korea. Each isolate was cultivated in a medium containing fucoidan as the sole carbon source, and the strain that increased the reducing sugars in the medium to the highest level was selected as a bacterial source of fucoidan-digesting enzymes (Fig. 1). This strain, tentatively named PF-1, was isolated from a seawater sample taken at Sewha Beach, Jeju Island, Korea. The fucoidan-degrading ability of this strain was further examined in a minimal medium (medium D) containing only fucoidan (with no nitrogen sources added) in laboratory distilled water (pH 7.0 adjusted with 0.5 N NaOH). Whereas the growth of an enterobacterial strain of *E. coli* was negligible under these nutrient-deficient conditions, the PF-1 isolate showed slow, yet significant growth (data not shown). It was interesting that strain PF-1 was able to grow, although slowly, in just laboratory distilled water ($dH_2O$) containing only fucoidan as the carbon source and without any additional nitrogen sources. However, the growth of strain PF-1 was substantially enhanced when supplementing the minimal medium with 2% peptone as a nitrogen source (data not shown). Thus, unless indicated otherwise, all the subsequent cultivations were performed in medium D containing 2% peptone.

**Localization of Enzyme Activity**

A cell-free culture supernatant and intact cells of strain PF-1 were examined for the presence of fucoidanolytic activity. The increase in reducing sugars when adding the culture supernatant was negligible, suggesting that the culture supernatant did not contain any fucoidan-degrading activities (Fig. 2). In contrast, incubating the reaction mixture with the cell suspension showed a significant time-dependent increase in reducing sugars, clearly demonstrating that the fucoidanolytic enzyme activities of strain PF-1 were associated with its intact cells, most probably displayed on the cell surface. Therefore, the subsequent fucoidan degradation reactions were all performed with intact cells as the crude enzyme preparation.

**Fucoidanolytic Activity of PF-1 Strain**

The fucoidanase activity was determined by measuring the reducing sugars, and confirmed by measuring the reduction in the fucoidan viscosity and performing an HPLC analysis of the degradation products. As a result, from the time-course increase in the reducing sugars after incubating a 0.2% fucoidan solution with a suspension of intact cells as the crude enzyme preparation (Fig. 2), along with the ability to grow on media containing only fucoidan as the sole carbon source, it was evident that stain PF-1 was capable of degrading the fucoidan tested. This was also confirmed by the significant reduction in the fucoidan viscosity on an Ostwald viscometer (data not shown). The fucoidan incubated with the intact cells as the enzyme preparation showed a relatively rapid reduction in its viscosity by 27.4%, indicating that the enzyme obviously

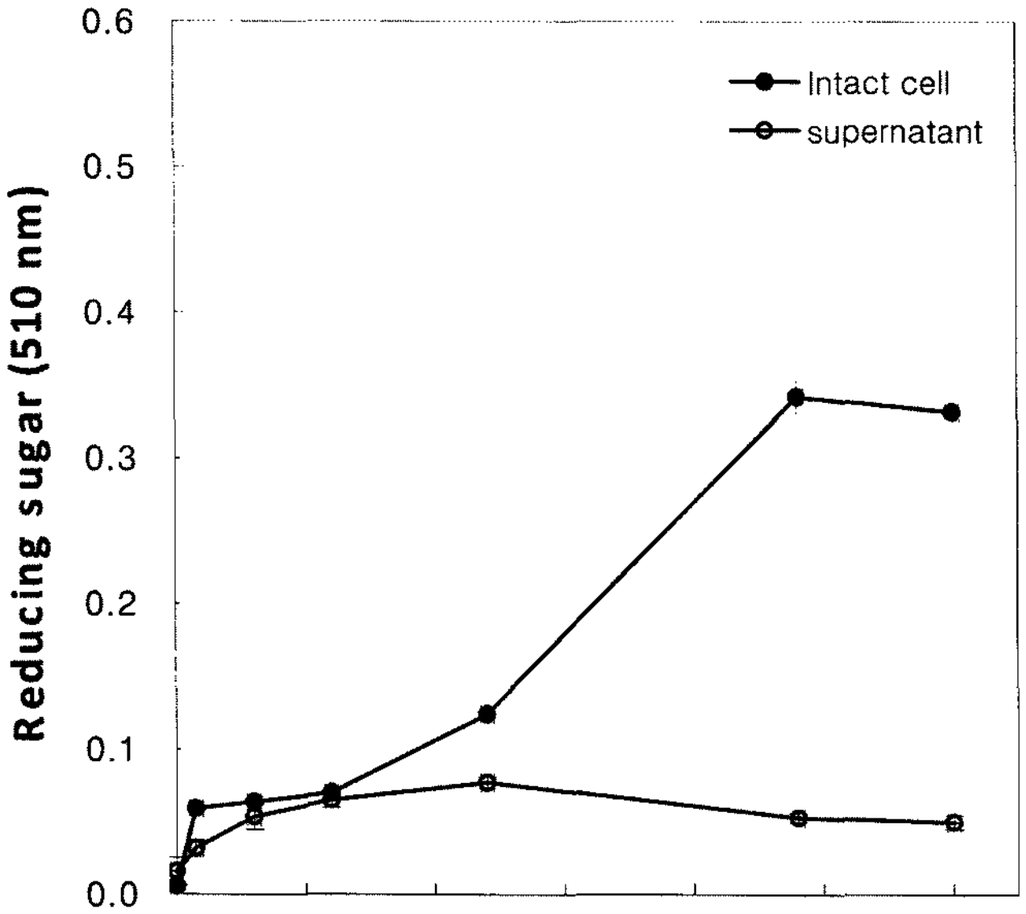


**Fig. 2.** Localization of fucoidan-degrading enzyme activities.
The PF-1 isolate was grown in a medium containing 0.2% fucoidan and 2% peptone (pH 7.0). The culture supernatant and cells were separately examined for fucoidan-degrading enzyme activities with 0.2% fucoidan as the enzyme substrate. The reducing sugars were measured as described in Fig. 1.

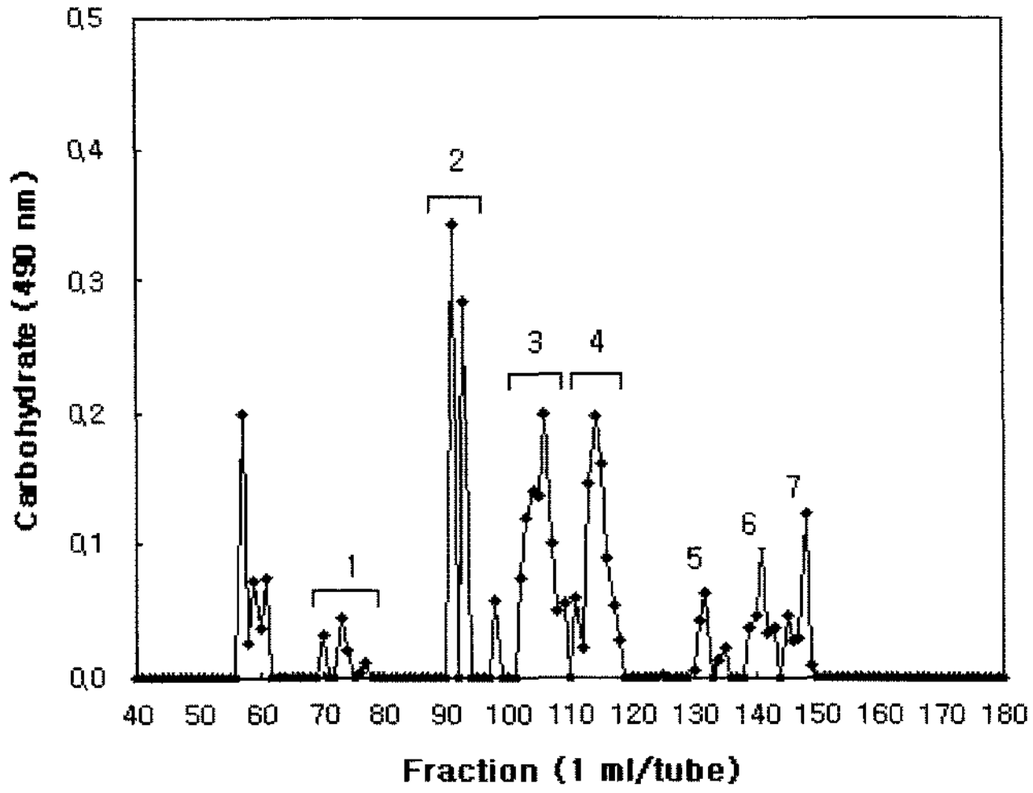


**Fig. 3.** Bio-Gel P-4 column chromatography of fragmented fucoidan after digestion by strain PF-1.
Intact cells of strain PF-1 were incubated with 1% fucoidan for 7 days at 30°C in a 50 mM sodium acetate buffer (pH 5.6) containing 0.01% sodium azide. The reaction products were eluted with 50 mM sodium nitrate at a flow rate of 0.3 ml/min. Each fraction was monitored for carbohydrates using the phenol-$H_2SO_4$ method at 490 nm with L-fucose as the reference. Arrows indicate the void volume of unhydrolyzed fucoidan and hydrolyzed products with a larger molecular mass over approximately 5,000 Da, which was the upper exclusion limit of the gels. Only major peaks (1 to 7) were collected and examined for their monosaccharide composition using an HPAEC-PAD analysis [25].

**Table 1.** Monosaccharide composition and relative molecular mass of fucooligosaccharides produced from fucoidan by strain PF-1.

| Peak No.[a] | $M_r$[b] (Da) | Monosaccharide composition[c] | Relative amount (%)[d] |
|---|---|---|---|
| 1 | 3,749–2,569 | Fucose, Galactose, Mannose (mole ratio, 8:7:4) | 4.5 |
| 2 | 2,122–2,059 | Fucose, Galactose, Mannose (mole ratio, 3:5:5) | 53 |
| 3 | 1,644 | Fucose, Galactose, Mannose (mole ratio, 3:5:2) | 15.3 |
| 4 | 1,389 | Fucose, Galactose, Mannose (mole ratio, 3:3:1) | 15.8 |
| 5 | 815 | Fucose, Galactose, Mannose (mole ratio, 2:1:1) | 4.8 |
| 6 | 528 | Fucose, Mannose (mole ratio, 2:1) | 4.3 |
| 7 | 305 | Fucose (2 moles) | 2.3 |

[a]Oligosaccharides were fractionated using a Bio-Gel P-4 column after digestion of fucoidan by isolated strain PF-1.
[b]Relative molecular mass of each oligosaccharide was estimated using maltooligosaccharides (1–7 glucose units; Sigma) as standard molecular markers.
[c]Monosaccharide composition was determined by HPAEC-PAD analysis [25].
[d]Carbohydrate amount of each peak was total neutral sugars determined by the phenol-sulfuric acid method, and relative amounts of each oligosaccharide were estimated by setting the sum of major oligosaccharides (peaks 1–7) as 100%.

hydrolyzed the fucoidan, although not completely. This incomplete digestion could be attributed to the intricate structure of the fucoidan used.

Finally, the fucoidan-degrading activity was also clearly confirmed by the fractionation of the degradation products using Bio-Gel P-4 size-exclusion chromatography and a monosaccharide composition analysis of the resulting oligosaccharides (Fig. 3). The oligosaccharides were resolved into 7 distinct low molecular mass fractions, peaks 1 to 7. From a comparison with the mass of standard maltooligosaccharides, the relative molecular masses of the fucoidan oligosaccharide fractions were determined to be 3,749–2,569 Da (peak 1), 2,122–2,059 (peak 2), 1,644 (peak 3), 1,389 (peak 4), 815 (peak 5), 528 (peak 6), and 305 Da (peak 7) (Table 1). On the basis of their levels within the total sugars, the 3 major fractions (peaks 2, 3, and 4) represented approximately 84% of the initial oligosaccharide mixture. From an HPAEC-PAD analysis after TFA hydrolysis, the constituent monosaccharides of the 7 major oligosaccharides were also determined: peak 1, fucose, galactose, and mannose, 8:7:4 (as the mole ratios); peak 2, 3:5:5; peak 3, 3:5:2; peak 4, 3:3:1; peak 5, 2:1:1; peak 6, fucose, mannose, 2:1; peak 7, fucose only (dimer) (Table 1). The uronic acid and sulfate contents of these fractions are not reported here (data with the complete structures of these oligosaccharides will be published elsewhere). The monosaccharide composition analysis of the fractions, especially the presence of fucose, clearly showed that the oligosaccharides were the degradation products of fucoidan and that the PF-1 isolate carried enzymes that could produce low-molecular-weight fucooligosaccharides from the Korean *Undaria pinnatifida* sporophyll fucoidan. Interestingly, the monomer L-fucose was not released from the fucoidan (Fig. 3), which was also confirmed by a TLC analysis of the enzyme reaction mixture (data not shown). In addition, whereas the commercial α-L-fucosidase released *p*-nitrophenol from the artificial substrate *p*-nitrophenyl-α-L-fucoside, the PF-1 cell suspension used as the enzyme preparation did not, demonstrating that the fucoidan-degrading activity of the PF-1 isolate was not related to α-L-fucosidase activity (data not shown). Thus, when taken collectively, the results strongly suggest that the enzyme is not α-L-fucosidase, but rather an endo-acting fucoidanase that randomly attacks the fucoidan chains [6, 17].

## Identification and Characterization of PF-1 Strain

The PF-1 strain was found to be a Gram-negative, short rod with four polar flagella (Fig. 4), developing bright brown colonies on the agar plates (Table 2). It was able to grow on a freshwater (distilled water) LB medium containing up to 3% NaCl, as well as on a seawater medium. It was also surprising that the strain could grow, although not vigorously, on a minimal medium containing only fucoidan dissolved in distilled water (data not shown).

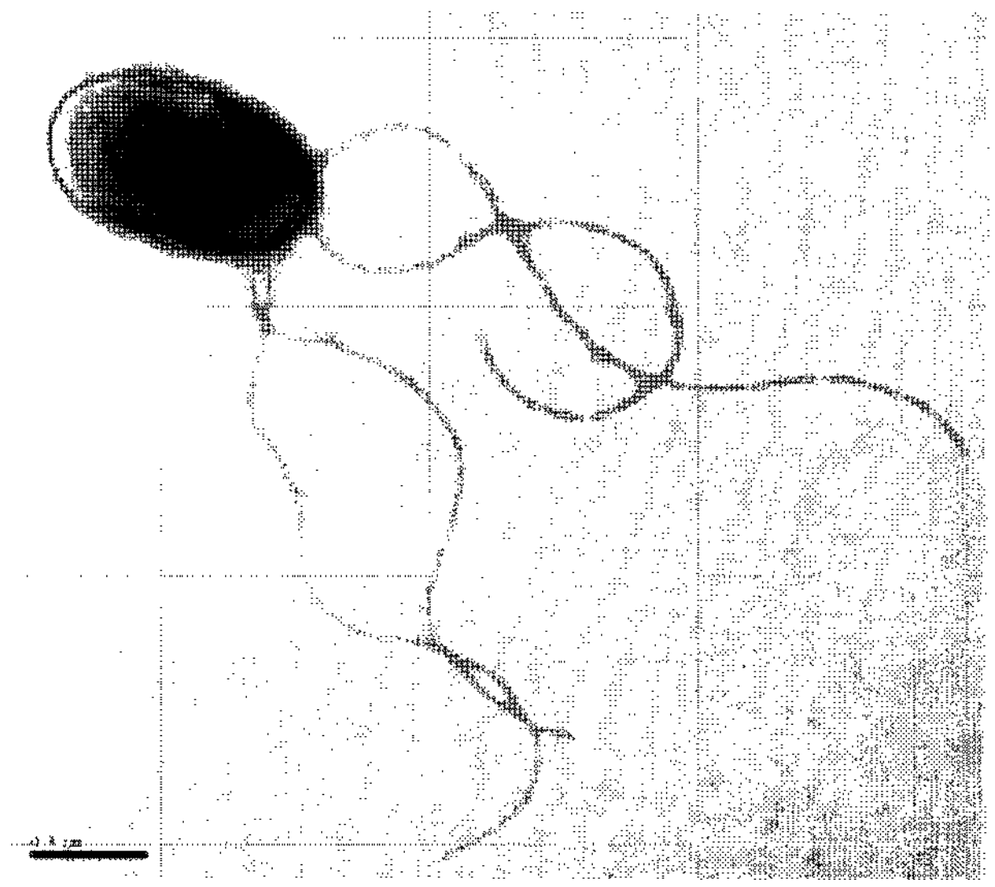

**Fig. 4.** Transmission electron micrograph of strain PF-1.
The cells were grown for 2 days at 30°C in the minimal medium containing 0.2% fucoidan as the sole carbon source, and then negatively stained and photographed at 120 kV. Bar=0.5 μm.

**Table 2.** Morphological and growth characteristics of fucoidan-degrading bacterial strain PF-1.

| Test | Result | Test | Result |
|---|---|---|---|
| Cell shape | Rods | 1% NaCl | +++ |
| Gram staining | – | 2% NaCl | +++ |
| Color of colony | Bright brown | 3% NaCl | ++ |
| Oxidase | – | 4% NaCl | – |
| Catalase | (+) | pH 3 | + |
| Growth at/in | | pH 4–10 | +++ |
| Fresh water | +++ | NSW broth | +++ |
| 0.5% NaCl | +++ | Motility (Flagellar) | (+) 4 |

Symbols: (+), positive; –, negative; +++, confluent growth; ++, moderate growth; +, poor growth; NSW, natural sea water.

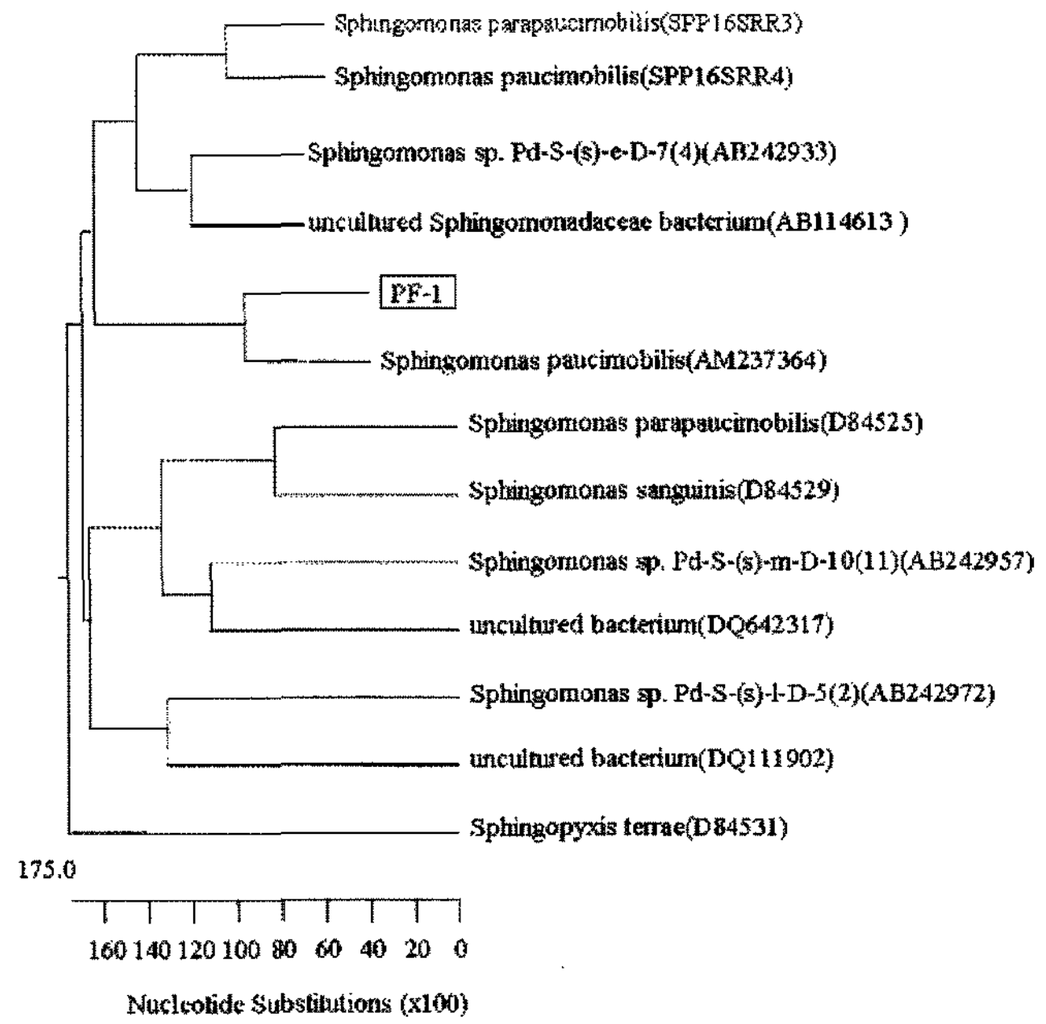


**Fig. 5.** Phylogenetic position of strain PF-1 among neighboring species of genus *Sphingomonas*.
The phylogenetic tree was constructed based on pairwise comparisons of the 16S rDNA sequences using the neighbor-joining method [28].

However, PF-1 was not able to grow on plates containing over 4% NaCl, suggesting it was a halotolerant, rather than halophilic, marine bacterium. The strain was also shown to be an aerobic, chemoorganotrophic, and heterotrophic organism, with an aerobic metabolism that used nitrate, rather than oxygen, as the electron acceptor (Table 3). The bacterium synthesized β-glucosidase and β-galactosidase, yet did not synthesize cytochrome oxidase, plus it was unable to degrade agarose. The pH for optimal growth on an LB medium ranged from 6.0 to 9.0 (data not shown).

The 16S rDNA sequence (309 bp) of strain PF-1 was found to be closely related, with sequence similarities of 99.9%, to strain *Sphingomonas paucimobilis* (AM237364) that belongs to the *Sphingomonads* family (Fig. 5). Furthermore, the physiological and biochemical characteristics, as well as a phylogenetic analysis of the 16S rDNA gene, suggested that strain PF-1 is a member of the *Sphingomonas* species. However, when compared with known strains of *Sphingomonas paucimobilis* [3], there were some different characteristics, such as the presence of four polar flagella and the ability to grow in a medium without NaCl and up to 3% NaCl. Thus, since it is generally accepted that nearly all known *Sphingomonas* species to date have no or only one flagellum [3], this suggests that strain PF-1 may be a new member of *Sphingomonas paucimobilis*. Consequently, strain PF-1 was named *Sphingomonas paucimobilis* PF-1 and deposited in the Korean Type Culture Collection under the accession number KCTC 11130BP.

Although this report did not present any indepth data about the physicochemical nature of the fucoidan-degrading enzymes of strain PF-1, it was clearly demonstrated that the strain produced fucoidan-degrading enzymes. Therefore, this would appear to be the first report on fucoidanolytic activity by a *Sphingomonas* species [2, 40]. The Korean *Undaria pinnatifida* sporophyll fucoidan was first cleaved into a number of low-molecular-weight fucose-containing

**Table 3.** Assimilation and biochemical characteristics of strain PF-1.

| Test | Reactions/Enzymes | Result | Test | Reactions/Enzymes | Result |
|---|---|---|---|---|---|
| $NO_3$ | Reduction of nitrates to nitrites | + | MNE | Assimilation of mannose | + |
| | Reduction of nitrates to nitrogen | – | MAN | Assimilation of mannitol | – |
| TRP | Indole production | – | NAG | Assimilation of *N*-acetyl-glucosamine | + |
| GLU | Acidification | – | MAL | Assimilation of maltose | + |
| ADH | Arginine dihydrolase | – | GNT | Assimilation of gluconate | – |
| URE | Urease | – | CAP | Assimilation of caprate | – |
| ESC | Hydrolysis (β-glucosidase) | + | ADI | Assimilation of adipate | – |
| GEL | Hydrolysis (protease) | + | MLT | Assimilation of malate | + |
| PNG | β-Galactosidase | + | CIT | Assimilation of citrate | + |
| GLU | Assimilation of glucose | + | PAC | Assimilation of phenyl-acetate | – |
| RAR | Assimilation of arabinose | – | OX | Cytochrome oxidase | – |

Symbols: +, positive; –, negative.

oligosaccharides (Fig. 3, Table 1), without any detectable level of the monomer L-fucose. In addition, the fucoidan-degrading activity of strain PF-1 was not related to α-L-fucosidase activity (data not shown). Thus, although still preliminary, the present results suggest that the enzyme is an endo-type fucoidanase that randomly attacks the fucoidan chains. Despite an increasing number of reports on fucoidan-degrading enzyme activities from various sources, including many herbivorous marine mollusca [6, 11, 17, 37] and marine microorganisms [2, 12, 14, 39, 40], most of these enzyme activities have been found to be exo-acting fucohydrolases and include the release of the monomer L-fucose. For example, Sakai *et al.* [29] recently reported on the isolation of a halophilic *Fucobacter marina* (*Flavobacteriacea*) that cleaved various fucoidans from Laminariales, yet not Fucales. The enzyme from this strain was further characterized as a sulfated fucoglucuronomannan lyase, rather than a hydrolase [30]. Unlike exo-type fucoidanases, reports on endo-acting fucohydrolases are rare [2, 12, 31]. Nonetheless, Descamps *et al.* [12] recently isolated a marine bacterium belonging to the family *Flavobacteriacea*, which degraded the fucoidan from the brown alga *Pelvetia canaliculata* in an endo-acting manner, producing tetrasaccharide and hexasaccharide oligomers. However, no convenient commercial sources with fucoidan-endohydrolase activity are available yet for the degradation of fucoidans [40]. Furthermore, no reports have yet been published on enzymes with fucoidanolytic activities towards the fucoidans from Korean alga, which may have different structures and therefore different bioactivities to the fucoidans from brown alga harvested in other countries. Thus, since the enzyme activity of strain PF-1 (*Sphingomonas* sp.) was found to digest the Korean *Undaria pinnatifida* sporophyll fucoidan into fucose-containing oligosaccharides in an endo-acting manner, it may be a more efficient tool, over exo-type hydrolases, for the elucidation of fucoidan structures and to manufacture fucooligosaccharides from fucoidans [6, 12, 19, 29]. The fucooligosaccharides produced by the strain PF-1 enzyme could also be investigated for their therapeutic potential with more feasibility, especially owing to their low viscosity and low molecular weights (smaller sizes), for development as potent drugs, such as anticoagulants, when compared with unhydrolyzed fucoidans with larger molecular masses [21, 41]. Further studies on the purification and chemistry of the strain PF-1 enzyme for fucoidan digestion are currently being conducted. Whether the enzyme can also catalyze the degradation of other fucoidans from other seaweeds remains to be elucidated.

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