J. Microbiol. Biotechnol. (2004), **14**(6), 1134–1141

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Isolation and Characterization of Marine Bacterium Producing Arylsulfatase

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Received: July 31, 2003
Accepted: June 11, 2004

Abstract A bacterial strain capable of hydrolyzing sulfate ester bonds in *p*-nitrophenyl sulfate and agar was isolated from the Southeast coast of Korea. The isolated strain (AS6330) is aerobic, Gram-negative, rod-shaped, and motile. Octadecanoic acid was the major cellular fatty acid in the isolate. An almost complete 16S rDNA sequence of the isolate was determined and the sequence similarity of the 16S rDNA with those of known *Sphingomonas* spp. was found to be at most 96.4%, implying that the isolate was a new *Sphingomonas* species. The organism was grown optimally at NaCl concentration of 1.5–3.5%. Optimum culture conditions were determined to be 30°C and pH 7.0 for 48 h fermentation using a laboratory fermentor under constant culture conditions. Partially purified arylsulfatase through Q-Sepharose and phenyl-Sepharose chromatographies catalyzed hydrolysis of sulfate ester bonds in agar, and 97% of sulfates in agar were removed after 4 h reaction at 45°C and pH 7.0. The arylsulfatase from the isolated bacterium might be useful for the removal of sulfate groups in agar.

Key words: Agar, arylsulfatase, *Sphingomonas*, sulfate

Agar is a structural polysaccharide found in cell walls of red algae such as *Gelidium* and *Gracilaria* and is generally considered to be a mixture of about 70% agarose and 30% agaropectin [33]. Agarose consists of a repeating unit of alternating 1,4-linked 3,6-anhydro-α-L-galactosyl residues and 1,3-linked α-D-galactosyl residues, which can be named as agarobiose [25]. The structure of agaropectin is like agarose but additionally contains sulfate groups in the C6 position of galactosyl residues and forms L-galactose-6-sulfate. Incorporation of sulfate groups in agarose residues causes weakened gel strength due to avoidance of double helix structure during gelation.

Most studies on the microorganisms producing sulfatase have been limited to microorganisms isolated from the terrestrial environment, and arylsulfatases play an important role in sulfur assimilation and dissimilation in filamentous yeast [18]. There have been several studies reporting that various sulfatases have been isolated from such bacteria as *Klebsiella pneumoniae* [19], *Salmonella typhimurium* [21], and *Pseudomonas aeruginosa* [2]. Several marine animals that feed on algae are known to secrete carbohydrate sulfatases as digestive enzymes, and cleave the sulfate ester bonds in dietary polysaccharides to improve digestion and absorption of marine polysaccharides [11]. The digestive glands of various mollusks were found to be a rich source of carbohydrate sulfatase [20].

Marine microorganisms that hydrolyze marine polysaccharides have been of interest for their potential in the utilization of polysaccharides in the food, cosmetic, or pharmaceutical industry. Screening of new marine microorganisms producing novel enzymes that change the structure of the polysaccharide have been mainly focused on the lyases of agar [29], alginate [12], and chitin [17]. Previously, we screened for an arylsulfatase-producing marine bacterium, which was confirmed by the hydrolysis of NPS and sulfate ester bonds in agar [26]. The enzyme showed high specificity against agar rather than other sulfated marine polysaccharides such as carrageenan, fucoidan, and porphyran. Arylsulfatase hydrolyzed sulfate ester bonds in agar without any glycosidase activities, which is demonstrated by increasing of gelling strength of enzyme-treated agar [26]. The enzyme can enhance gelling strength of agar by removing sulfate groups in agar, and further reaction would be exerted on the conversion of agar to agarose. This may replace the solvent fractionation step using solubility differentiation between agarose and agaropectin

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in agarose processing. The enzyme process would alleviate the use of organic solvent and lessen process expenditure. Also, the process would be an environmentally favorable one due to reduced solid waste and water discharge.

We isolated three kinds of arylsulfatase-producing bacteria from seaweeds. One of the bacteria was identified as *Sphingomonas* sp. AS6330 as determined by 16S rDNA sequence analysis. This paper reports the identification of the strain and establishment of culture conditions of the isolate for the growth and production of arylsulfatase.

MATERIALS AND METHODS

Chemicals

Q-Sepharose and phenyl-Sepharose were purchased from Pharmacia Biotech (Uppsala, Sweden). κ-Carrageenan, *p*-nitrophenyl sulfate (NPS), *p*-nitrophenyl α-D-galactopyranoside, and *p*-nitrophenyl β-D-galactopyranoside were obtained from Sigma (St. Louis, MO, U.S.A.). Tryptone, peptone, and yeast extract were purchased from Difco Laboratories (Detroit, U.S.A.). Porphyran and fucoidan were generously provided by Dr. S. B. Kim (Pukyong National University, Busan, Korea). Agar used for the enzymatic reaction was purchased from Myoung-Sin Agar Co. (Kyoungnam, Korea).

Screening and Isolation of Bacteria

Seaweed, *Gelidium* and *Gracilaria*, having pathogenic damage were collected at the Southeastern coast of Korea and blended with 5 volumes of autoclaved seawater. The homogenate was diluted 100, 1,000, and 10,000 times with sterilized seawater and plated onto ZoBell 2216E agar plate (5 g bacteriological peptone, 2 g yeast extract, 10 mg $FePO_4$, 15 g agar, 250 ml distilled water, and 750 ml seawater) containing 5 mM NPS. Bacterial colonies showing a yellow circle were isolated after 4 days incubation at 30°C. Isolated strains were stored as a glycerol stock at 80°C for further experiments.

Phenotypic Tests

Cell morphology was observed by optical microscopy of Gram-stained preparation. Motility of the AS6330 was examined using wet mounts. Oxidase activity was determined by the method of Kovacs [15]. Peroxidase test was performed by using 0.5% hydrogen peroxide with a colony taken from ZoBell 2216E agar plate. Assimilation of carbon sources was determined using Biolog GN microtiter plates (Biolog Inc., Hayward, CA, U.S.A.) with an inoculum grown on TSA plates. Oxidation of carbon sources was detected indirectly by observing reduction of tetrazolium dye with Biolog computer software after 24 h incubation at 25°C.

Cellular Fatty Acid Analysis

Cells were cultivated 24 h in marine broth at 30°C with gentle shaking. Harvested cells were saponified in 1.0 ml of saponification reagent (15 g of NaOH, 50 ml of methanol, 50 ml of water) at 100°C for 30 min, followed by methylation with 2 ml of methylation reagent (325 ml of 6 N HCl and 275 ml of methyl alcohol) at 80°C for 10 min. After cooling, an equal volume of water was added and the fatty acid methyl esters were extracted twice with n-hexane. The extracted fatty acid methyl esters were analyzed by gas chromatography.

DNA Amplification and Sequencing

DNA was prepared by the method of Sambrook *et al.* [24]. Seven DNA primers were used in the amplification and sequencing reactions. These primers corresponded to the following position in the *Escherichia coli* sequence: primer 8F (positions 827), primer 330F (330348), primer 515R (515-535), primer 803R (803-785), primer 927R (927-942), primer 1107F (1107-1088), and primer 1492R (1492-1510). rDNA gene was amplified using forward primer 8F and reverse primer 1492R [5]. The amplified 16S rDNA was purified using a Wizard PCR prep (Promega, Madison, U.S.A.). The DNA sequence was determined using BigDye terminator cycle sequencing kit (PE Applied Biosystems, Foster City, U.S.A.) according to the manufacturer's instruction. The data collection was performed on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, U.S.A.).

Phylogenetic Analysis

The sequences of each PCR product were manually aligned with sequences of genus *Sphingomonas* from the GenBank databases. Phylogenetic trees were inferred by using the Fitch-Margoliash [8] and neighbor-joining [23] methods. Evolutionary distance matrices for the neighbor-joining and Fitch-Margoliash methods were generated according to the model of Jukes and Cantor [13]. The trees were rooted using *Erythrobacter longus* as an out-group. The PHYLIP package [7] was used for all analyses. The resultant neighbor-joining tree topology was evaluated by bootstrap analyses [6].

Determination of Enzyme Activity

Arylsulfatase activity was determined by measuring the amount of *p*-nitrophenol released from NPS by cell homogenate. The assay mixture containing 1 ml of enzyme solution and 250 μl of 25 mM NPS (pH 7.0) was incubated at 45°C for 60 min. The reaction was stopped by adding 1 ml of 0.5 M NaOH solution, and *p*-nitrophenol was quantified spectrophotometrically at 410 nm. Sulfatase activity (unit) was defined as the absorbance equivalent of 1 mol *p*-nitrophenol produced per min per ml of enzyme solution at 45°C.

Sulfatase activity using agar, porphyran, fucoidan, or carrageenan was determined by the method of Dodgson and Price [4]. The assay mixture that contained 1 ml of

enzyme solution and 9 ml of 0.5% agar suspended in Tris-HCl buffer (pH 7.0) was incubated at 45°C for 60 min and centrifuged at 3,000 ×*g* for 5 min. Nine-hundred microliter of supernatant was mixed with 0.2 ml of concentrated HCl and 0.6 ml of precipitating solution containing 13.3% $BaCl_2 \cdot 2H_2O$ and 2.67% Tween-80. After the mixture had been left at room temperature for 30 min, the absorbance was measured at 420 nm. The amount of sulfate was calculated using a standard curve established in the range of 2-20 μg/ml of H_2SO_4.

Glycosidase activity was determined with the method described for arylsulfatase activity using *p*-nitrophenyl α-D-galactopyranoside and *p*-nitrophenyl β-D-galactopyranoside as substrates.

Determination of Culture Conditions

Optimum incubation conditions for the growth and sulfatase activity were determined with basal medium (10 g tryptone, 20 g sucrose, 4 g $MgSO_4$, 7 g $CaCl_2$, 0.07 g KH_2PO_4, 0.08 g K_2HPO_4, 5 mg $FeCl_2$, 1 mg $MnCl_2$, 1 mg Na_2MoO_4, 1 mg $ZnCl_2$, 25 g NaCl in 1 l distilled water). Erlenmeyer flasks (500 ml) containing 100 ml of the medium adjusted within the pH interval between 4.0 and 9.5 were sterilized at 121°C for 15 min. The influence of initial medium pH on the growth and activity was determined at incubation for 3 days after inoculation of 1 ml of glycerol stock. The effect of incubation temperature on the growth and activity was performed at 20, 25, 30, 35, and 40°C incubation of basal medium adjusted to pH 7.0 for 3 days after inoculation of 1 ml of glycerol stock. The effect of salt concentration on the growth and activity was determined by incubation at 30°C for 3 days with basal medium (pH 7.0) containing different concentration of NaCl. In order to investigate the effect of incubation time on the growth and activity, 8 l of basal media adjusted to pH 7.0 was sterilized and incubated at 30°C for 4 days with 1.0 vvm after inoculation of 50 ml of overnight culture. After or during incubation, enzyme activity was determined with NPS as previously described and cell growth was determined by measuring absorbance at 600 nm. At least three tests were carried out in parallel for each experiment.

Hydrolysis of Sulfate Ester Bonds in Agar

Cell pellet of an 8-l culture from the fermentor was harvested by centrifugation at 8,000 ×*g* for 20 min. The cell pellet was suspended in 20 mM Tris-HCl buffer (pH 7.0) containing 1 mM PMSF and 0.1% Triton X-100 and sonicated for 20 min in an ice water bath. The sonicated cell homogenate was centrifuged at 10,000 ×*g* for 20 min and 30 ml of streptomycin (0.5 mg/1 g cell) in 20 mM Tris-HCl was added to the supernatant. After removal of DNA by centrifugation at 12,000 ×*g* for 20 min, the supernatant was loaded onto a Q-Sepharose column (2.5×12 cm) equilibrated with 20 mM Tris-HCl buffer (pH 7.0). The enzyme was eluted with a 400 ml linear gradient ranging from 0.1 to 0.5 M NaCl in the equilibration buffer. The sulfatase fractions were pooled and mixed with the same volume of 2.0 M ammonium sulfate (AS) in the equilibration buffer to make a final 1.0 M concentration of AS. The solution was applied to a phenyl-Sepharose column (1.5×12 cm) that had been previously equilibrated with 1.0 M AS in 20 mM Tris-HCl buffer (pH 7.0). The enzyme was eluted with 300 ml of linear gradient solution from 1.0 to 0.0 M AS in 20 mM Tris-HCl buffer (pH 7.0). The sulfatase fractions were pooled and dialyzed overnight against 20 mM Tris-HCl (pH 7.0) and stored at -20°C.

One-hundred grams of agar were suspended in 500 ml of 20 mM Tris-HCl buffer (pH 7.0) and vigorously stirred (350 rpm) at 45°C for 30 min. Fifty units of dialyzed enzyme were added to the agar mixture and incubated for 12 h. During incubation, 20 ml aliquots of samples were taken at 0, 1, 2, 4, 8, and 12 h incubation times and centrifuged at 3,000 ×*g* for 5 min to separate supernatant and precipitate for assay of sulfate and sulfur, respectively. The amount of sulfate in the supernatant was determined by the method of Dodgson and Price [4]. The amount of sulfur in the agar was measured using a CHNS analyzer (EA 1110, Italy). The agar precipitate after centrifugation was washed with 50% ethanol and dehydrated with acetone. Two milligrams of dried agar were analyzed for sulfur content at the incineration temperature of 1,000°C for 10 min. The amount of sulfur was determined by comparison to the standard curve, which was obtained through CHNS analysis standard kit.

RESULTS

Isolation of Sulfatase-Producing Bacterium

Three Gram-negative bacteria capable of degrading NPS on the ZoBell 2216E agar plate were isolated from seaweed samples, and tentatively designated AS6330, AS6331, and AS6332. The AS6330 strain showed the largest yellow circle around the colonies on the agar plate produced by the release of *p*-nitrophenol, which points toward it having the highest sulfatase activity. The activity from isolate AS6330 was further confirmed by hydrolysis of NPS and sulfate ester bonds in agar with cell homogenate obtained from liquid culture.

Phenotypic and Biochemical Properties

The isolated strain was found to be Gram-negative and rod-shaped. The strain was motile and oxidase and catalase-positive (Table 1). The strain formed creamy yellow colonies on ZoBell 2216E agar and tryptic soy agar plates after 3 days incubation at 30°C. The color of colonies changed from yellow to deep yellow after 4 days. Physiological characteristics of the isolated AS6330 are summarized in

Table 1. Phenotypic properties of isolated AS6330.

Characteristic		AS6330
Oxidation of	Dextrin	w
	Tween-40	+
	Tween-80	+
	L-Arabinose	+
	Cellobiose	+
	D-Galactose	+
	Gentiobiose	+
	α-D-Glucose	+
	D-Maltose	w
	L-Rhamnose	+
	Turannose	w
	Methylpyruvate	+
	α-Hydroxybutyric acid	+
	α-Keto butyric acid	+
	α-Keto valeric acid	w
	D,L-Lactic acid	w
	Quinic acid	w
	Succinic acid	w
	Alaninamide	+
	L-Alaninyl-glycine	+
	L-Alanine	+
	L-Aspargine	w
	L-Aspartic acid	+
	L-Glutamic acid	+
	Glycyl-L-glutamic acid	+
	L-Leucine	+
	L-Proline	+
	L-Threonine	w
Morphology		Rods
Motility		+
Oxidase		+
Catalase		+
Color of colony		Deep yellow

+, Positive; w, weakly positive.

Table 1. Strain AS6330 oxidized 19 of the 95 different carbon sources tested with the Biolog identification system (Table 1). The substrates oxidized by strain AS6330 in the Biolog system were Tween-40, Tween-80, L-arabinose, cellobiose, D-galactose, gentiobiose, α-D-glucose, L-rhamnose, methylpyruvate, α-hydroxybutyric acid, α-keto butyric acid, alaninamide, L-alaninyl-glycine, L-alanine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, and L-proline.

Cellular Fatty Acid Composition

Fifteen fatty acids with 14-18 carbon atoms were detected (Table 2). Among those fatty acids analyzed, octadecenoic acid (C18:1) was the major fatty acid in AS6330 and 2-hydroxymyristic acid (C14:0 2OH) was present as a dominant hydroxylated fatty acid.

Phylogenetic Analysis

The sequence of 16S rDNA from strain AS6330 was similar to the sequences from *Sphingomonas* species.

Table 2. Cellular fatty acid composition of isolated AS6330.

Fatty acid	Composition (%)
C14:0	1.29
C15:0	0.26
C14:0 2OH	8.50
C16:1w5c	2.23
C16:1w7c/C15:0 iso 2OH	7.27
C16:0	7.74
C15:0 2OH	0.26
C17:1w6c	3.00
C16:1 iso 3OH	0.51
C16:0 2OH	0.42
C18:1w7c	62.50
C18:1w5c	2.74
C18:0	0.30
11 Methyl 18:1w7c	2.68
C18:1 2OH	0.32

Phylogenetic analysis was done using the sequence data of 24 type strains of *Sphingomonas* species and *Erythrobacter longus* strain as an outgroup microorganism. The results of the phylogenetic analysis show that strain AS6330 belongs to the genus *Sphingomonas* (Fig. 1). The similarity with *Sphingomonas xenophaga* strain was 96.4%, which suggested that strain AS6330 was the closest to *S. xenophaga*. The sequence of 16S rDNA from AS6330 was registered in GenBank (accession No. AY206698) as *Sphingomonas* sp. AS6330 and the isolated strain was deposited in the Korean Collection for Type Cultures (KCTC 2817).

Optimum Culture Conditions

Basal media adjusted to pH 4.0-9.0 were inoculated with glycerol stock of AS6330 and incubated at 30°C for 72 h with 150 rpm in rotary shaker. Cell growth was higher in the neutral pH range and sulfatase activity was highest at pH 7.0. Sulfatase activity increased with the increase of pH until 7.0 and decreased in the alkaline pH range (Fig. 2). Cell growth was not affected by alkaline pH medium; however, enzyme activity decreased compared to cell growth in the alkaline pH range.

The isolated strain was inoculated in basal medium (pH 7.0) and incubated at 20, 25, 30, 35, and 40°C for 72 h with the same incubation conditions used for pH optima determination. Cell growth and enzyme activity increased until incubation at 30°C, which showed the highest cell growth and enzyme activity (Fig. 3). Incubation above 35°C retarded cell growth and eliminated enzyme activity.

Several α-subclasses of *Proteobacteria* isolated in coastal areas required NaCl for their growth. The isolate had an absolute requirement of NaCl for growth and failed to grow when NaCl was lower than 0.5%. The isolated AS6330 had optimum salt concentration for growth and enzyme activity in the range of 1.5-3.5% NaCl (Fig. 4).

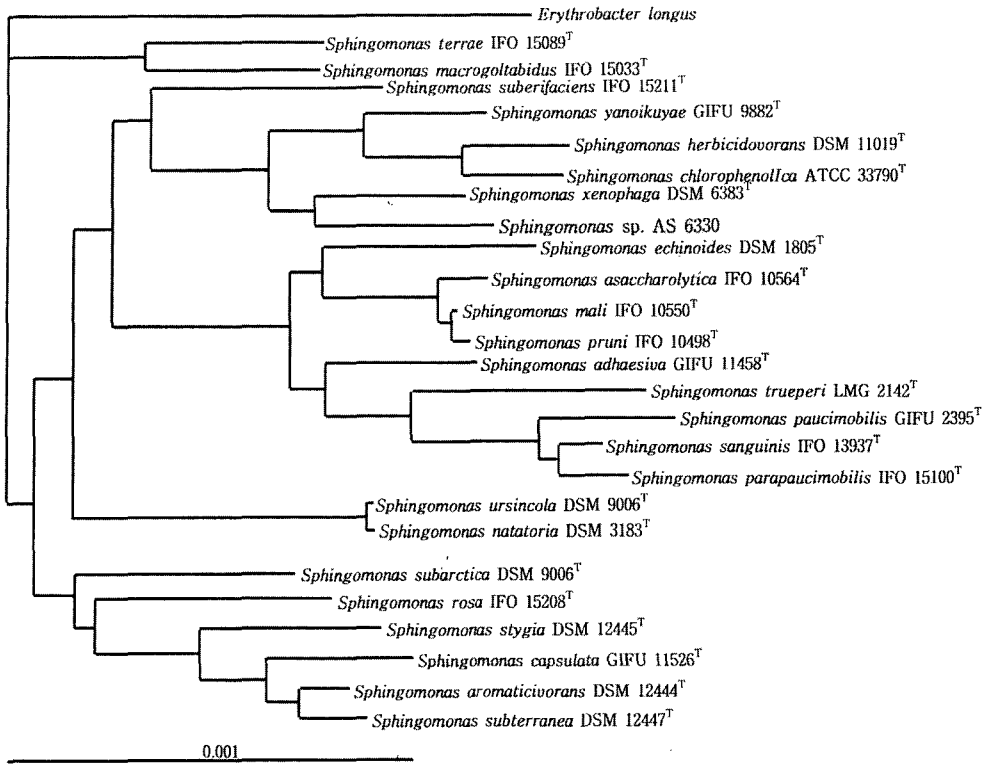

Fig. 1. Phylogeny of strain AS6330 based on 16S rDNA. Representatives of the most closely related groups of the *Sphingomonas* are included for comparison. The dendrogram was constructed by analyzing 1,429 bp of the sequence with the neighbor-joining method contained in the PHYLIP package.
Bar=Jukes-Cantor distance of 0.01. The type strain of *Erythrobacter longus* was used as an outgroup organism.

Enzyme activity was higher in the presence of 2.0-3.5% NaCl in the culture medium.

Overnight culture of glycerol stock (50 ml) was inoculated in a 10 l fermentation vessel with basal medium (pH 7.0). Fermentation time was prolonged until 4 days with 1.0 vvm at 30°C and cell growth and enzyme activity were determined every 24 h. As shown in Fig. 5, cell growth and enzyme activity were maximum after 48 h fermentation time and

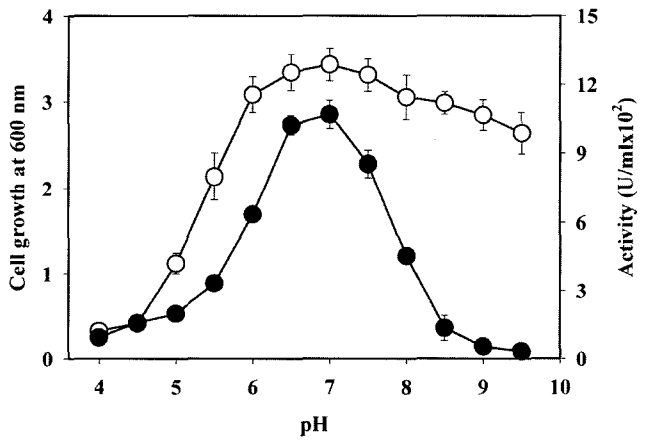

Fig. 2. Effect of initial medium pH on the growth of AS6330 and sulfatase activity.
Basal media adjusted to 4.0-9.5 were sterilized and incubated at 30°C with 150 rpm shaking after inoculation of glycerol stock. Cell growth and enzyme activity were determined after incubation for 3 days. Symbols: (○), cell growth; (●), arylsulfatase activity.

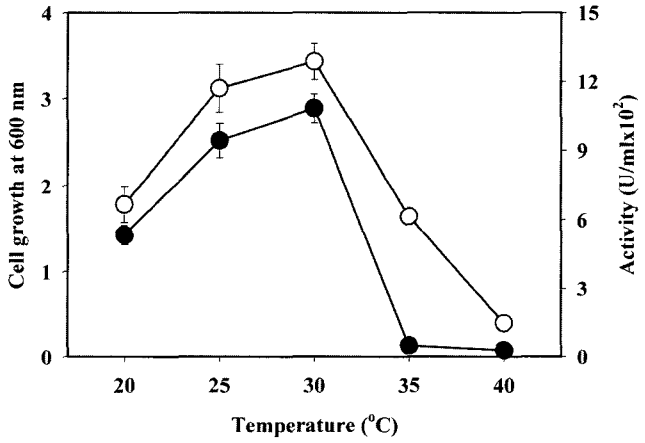

Fig. 3. Effect of incubation temperature on the growth of AS6330 and sulfatase activity.
Basal media adjusted to pH 7.0 were sterilized and incubated at the indicated temperatures with 150 rpm shaking after inoculation of glycerol stock. Cell growth and enzyme activity were determined after incubation for 3 days. Symbols: (○), cell growth; (●), arylsulfatase activity.

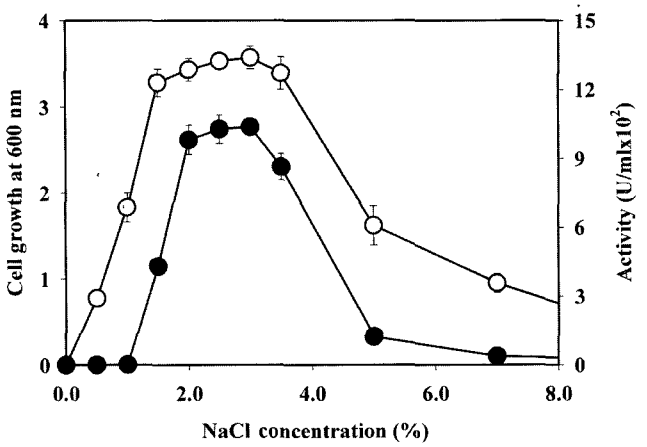

Fig. 4. Effect of NaCl concentration on the growth of AS6330 and sulfatase activity.
Basal media (pH 7.0) containing different concentrations of NaCl were sterilized and incubated at 30°C with 150 rpm shaking after inoculation of glycerol stock. Cell growth and enzyme activity were determined after incubation for 3 days. Symbols: (○), cell growth; (●), arylsulfatase activity.

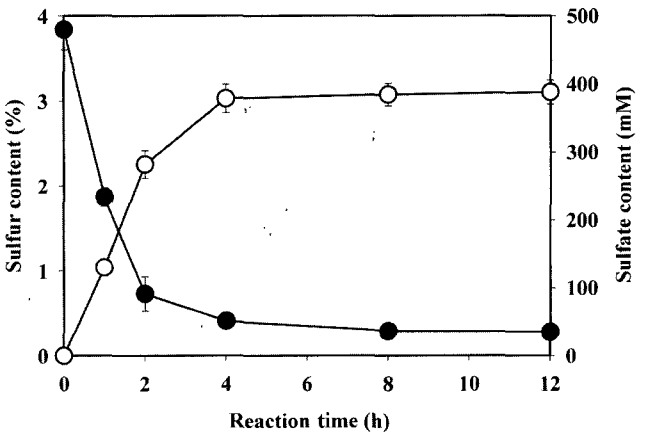

Fig. 6. Changes in sulfate in reaction solution and sulfur content in agar during the enzyme reaction.
Fifty unit of the arylsulfatase was used for 100 g of agar. The reaction samples were taken from the mixture according to the time course and centrifuged. Sulfate content in the reaction supernatant was measured through the barium precipitation method and sulfur content in the agar was analyzed using a CHNS analyzer. Symbols: (○), sulfate content; (●), sulfur content.

enzyme activity was not changed with prolonged fermentation until 4 days.

Enzyme Reaction with Agar

Figure 6 shows the change of sulfur content in agar and sulfate content in the reaction solution released from agar during enzymatic reaction. In the progress of reaction, hydrolysis of sulfate ester bonds in agar was increased by enzymatic catalysis. Sulfate ester bonds in the agar rapidly hydrolyzed and were released to reaction solution at the initial reaction time up to 4 h. After 4 h reaction, the sulfur content in agar did not decrease and sulfate content in the reaction solution did not increase until 12 h reaction. With 4 h reaction with the enzyme, 97% of sulfate in the agar was hydrolyzed.

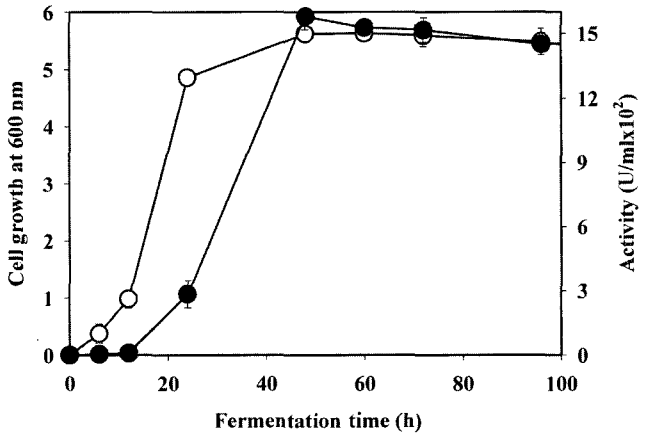

Fig. 5. Effect of fermentation time on the growth of AS6330 and sulfatase activity.
Basal medium (pH 7.0) containing 2.5% NaCl was sterilized and inoculated with overnight culture of AS6330. Fermentation was performed at 30°C with 250 rpm and 1.0 vvm aeration. Cell growth and enzyme activity were determined at indicated times. Symbols: (○), cell growth; (●), arylsulfatase activity.

Substrate Specificity

Sulfated marine polysaccharides were used as substrates to determine substrate specificity of the enzyme. As shown in Table 3, the enzyme showed highest activity against hydrolysis of sulfate ester bonds in agar; activity against fucoidan and carrageenan was less than 5% of that against agar, while that against porphyran was 24% of that against agar.

Synthetic substrates, *p*-nitrophenyl α-D-galactopyranoside and *p*-nitrophenyl β-D-galactopyranoside, were used for the determination of glycosidase activity of the enzyme. As shown in Table 3, the enzyme did not show glycosidase activity, which suggested the enzyme did not hydrolyze agar.

Table 3. Substrate specificity of arylsulfatase towards marine sulfated polysaccharides and synthetic substrates. Activities were determined using 0.5% different substrates at 45°C and pH 7.0.

Substrate	Relative activity (%)
Agar	100 (±7.2)
Porphyran	28.2 (±3.0)
Fucoidan	3.71 (±0.27)
κ-Carrageenan	4.92 (±0.38)
p-Nitrophenyl α-D-galactopyranoside	0
p-Nitrophenyl β-D-galactopyranoside	0

DISCUSSION

A number of marine bacteria that belong to the α-subclass of the *Proteobacteria* have been reported and their abundance were reduced with decreasing salinity and were not detected in low-salinity or freshwater samples [9]. Isolates of α-subclass of the *Proteobacteria* from marine environment have been found to have a salt requirement for the growth [9, 10]. The strain AS6330 was a Gram-negative, aerobic bacterium and the strain had an absolute requirement for NaCl and failed to grow when NaCl was lower than 0.5%. Furthermore, the 16S rDNA sequence analysis suggested that the isolated strain belongs to the α-subclass of the *Proteobacteria*.

The information obtained from the 16S rDNA sequence provides powerful evidence to classify bacterial species as well as genus [31]. Microorganisms with less than 97% similarity in the 16S rDNA gene sequence do not yield DNA reassociation values of more than 60% [28] and belong to different species [30]. The fatty acid compositions of AS6330 were most similar to *Sphingomonas paucimobilis* [32]. However, the fatty acid profiles of AS6330 did not closely match with the profiles of fatty acids in the microorganism identification system (Microbial ID Inc., Newark, DE, U.S.A.). To identify the isolated AS6330, the 16S rDNA sequence was analyzed and the resulting sequence was the closest to *S. xenophaga* with a similarity between strains of 96%. Comparison of the 16S rDNA sequence of AS6330 with 24 different *Sphingomonas* species showed less than 97% of 16S rDNA gene nucleotide sequence similarity, thus, the isolated strain in this study represents a new species of *Sphingomonas*.

The marine α-subclass of *Proteobacteria* previously isolated from marine environments have diverse metabolic capabilities such as lignin degradation [10], aerobic sulfite oxidation [27], organic sulfur compound degradation [16], and polyaromatic hydrocarbon degradation [3]. A group of marine bacteria that may be of particular interest for studies of organic sulfur cycling in the ocean is the α-subclass of *Proteobacteria* in coastal seawater [9]. The isolate in this study is also involved in the degradation of organosulfur compounds, especially agar, in marine environment.

Degradation of sulfonated or sulfated compounds by bacterial enzyme has been reviewed in detail [14]. Sulfated polysaccharides were degraded to sulfated disaccharides by lyase, followed by desulfatation of the resultant disaccharides and hydrolysis to the monosaccharide level by a glucosidase. Another family of bacterial carbohydrate sulfatases has been identified, which cleave the sulfate ester bonds from mucin [22]. The carbohydrate sulfatase of the marine Gram-negative aerobe *Alteromonas carrageenovora* has been proposed to play a role in degradation of sulfated polysaccharides from brown algae [1]. Most bacterial carbohydrate sulfatases are involved in pathways related to the utilization of polysaccharides as carbon sources. The isolate AS6330 in this study showed activity in the hydrolysis of sulfate ester bonds in agar without hydrolysis of glycoside bonds as determined by synthetic substrates (Table 3).

Conventional methods for commercial agarose production are limited in the separation of agarose and agaropectin using their solubilities in organic solvents. Furthermore, separation of agarose from agar with organic solvents requires time and is a labor-consuming process that results in a low yield. Enzymatic hydrolysis of sulfate groups in agar simplifies the process of agarose preparation. The arylsulfatase in this study could be used for agarose production with a high yield by converting agar to agarose through the removal of the sulfate group in agaropectin.

Acknowledgment

This work was supported by Korea Research Foundation Grant (KRF-2001-015-HP0001).

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