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Production and Characterization of Extracellular Polysaccharide Produced by *Pseudomonas* sp. GP32

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A strain GP32 which produces a highly viscous extracellular polysaccharide was conducted with soil samples and identified as *Pseudomonas* species. The culture flask conditions for the production of extracellular polysaccharide by *Pseudomonas* sp. GP32 were investigated. The most suitable carbon and nitrogen source for extracellular polysaccharide production were galactose and $(NH_4)_2SO_4$. The optimum carbon/nitrogen ratio for the production of extracellular polysaccharide was around 50. The optimum pH and temperature for extracellular polysaccharide production was 7.5 and 32℃, respectively. In batch fermentation using a jar fermentor, the highest extracellular polysaccharide content (15.7 g/l) was obtained after 70 hr of cultivation. The extracellular polysaccharide produced by *Pseudomonas* sp. GP32 (designated Biopol32) was purified by ethanol precipitation, cetylpyridinium chloride (CPC) precipitation, and gel permeation chromatography. Biopol32, which has an estimated molecular weight of over 3×10^7 datons, is a novel polysaccharide derived from sugar components consisting of galactose, glucose, gulcouronic acid and galactouronic acid in an approximate molar ratio of 1.85 : 3.24 : 1.00 : 1.42. The solution of Biopol32 showed non-Newtonian characteristics. The viscosity of Biopol32 exhibited appeared to be higher at all concentration compared to that of zooglan from *Zoogloea ramigera*. An analysis of the flocculating efficiency of Biopol32 in industry wastewater (food, textile, and paper wastewater) revealed chemical oxygen demand (COD) reduction rates 58.4-67.3% and suspended solid (SS) removal rates 82.6-91.3%. Based on these results, Biopol32 is a possible candidate for industrial applications such as wastewater treatment.

Key words : Apparent viscosity, bacterial polysaccharide, flocculating efficiency, polysaccharide production

Introduction

Microbial extracellular polysaccharides are produced by various genera of microorganisms. Many microorganisms (bacteria, yeast and fungi) have an ability to synthesize extracellular polysaccharides and excrete them out of the cell either as soluble or insoluble polymers [7]. Because of their wide diversities in structure and physical properties of microbial extracellular polysaccharides have found a wide range of applications in the food, pharmaceutical and other industries [7, 11]. These polysaccharides produced by bacteria have characteristic rheological and physiological properties which are different from those of natural gums and synthetic polymers. They are biodegradable and generally not harmful to the environment. Some of these applications include their use as emulsifiers, stabilize, binders, gelling agents, flocculants, lubricants, and film former, thickening and suspending agents [3, 19]. These biopolymers are rapidly emerging as a new and industrially important source of polymeric materials which are gradually becoming economically competitive with traditional natural gums produced for marine algae and other plants [13]. Owing to their diversity in structure, physico-chemical properties and rheological properties, microbial extracellular polysaccharides have found a wide range of application. Especially, xanthan gum produced by *Xanthomonas compestris* is in commercial production and have been in uses for oil drilling and recovery, paint, detergent, adhesive, rheology control, flocculant, plasma expander [1, 3, 21, 22]. Therefore, there have been lots of efforts to search new and novel polysaccharides of microbial origin, which are able to replace the hydrophilic gums of plants and algae.

Organic synthetic high-poymer floccuants are frequently used because they are inexpensive and highly effective [8]. Some of them are not easily degraded in nature and some

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of the monomers derived from synthetic polymers are harmful to the human body [8, 9]. In recent years, to solve these environmental problems, utilization of flocculants produced by microorganisms has been anticipated due to their biodegradability and the harmlessness of their degradation intermediates to the environment [9]. Naturally occurring, generally safe and biodegradable flocculants are used for improvement of food process, but have only weak flocculating activities. Therefore, developments of biodegradable and safe flocculants that do not cause problems in environmental pollution and toxicity and also have strong flocculating activities are required. Microbial flocculants have shown to be very efficient in coagulation of kaolin and in removal of microorganisms in various fermentation industries. Kurane *et al*. [9, 10] reported that *Rhodococcus erythropolis* produced a flocculant which was effective for various colloidal suspensions and pigments. A polysaccharide that was obtained from *Alcaligenes latus* B-16 showed flocculating and water-absorbing activities [17].

In this paper, the screening and characteristics of extracellular polysaccharide from bacteria are reported. This extracellular polysaccharide was purified, characterized, and examined for its flocculating activity for postulating its possible industrial applications.

Materials and Methods

Screening of strain producing extracellular polysaccharide

Extracellular polysaccharide-producing bacteria were isolated from soil samples. Soil samples were collected from many places in Korea. After serial dilution of collected samples with 0.85% physiological saline solution, aliquot of each preparation was inoculated in screening medium (glucose 30 g/l, NH_4NO_3 1.0 g/l, K_2HPO_4 0.5 g/l, KH_2PO_4 0.5 g/l, $MgSO_4 \cdot 7H_2O$ 0.1 g/l, Agar 15 g/l). The culture temperature and initial pH were 30℃ and 7.0, respectively. After incubating at 30℃ for 3 days, mucoid colonies were selected. One loop of mucoid colonies was transferred to 250 ml Erlenmeyer flask containing 50 ml of screening medium and cultivated at 30℃ for 3 days, at 150 rpm in rotary shaking incubator. Finally, strain producing high viscous extracellular biopolymer was selected.

Identification of isolated strain

Identification of isolated strain was based on morphological and physiological characteristics of strain, according to Bergey's Manual of Systematic Bacteriology [18]. The chemotaxonomical characteristics of the strain were examined by the procedures of MacFaddin [12], and Tamaoka and Komagata [24].

Measurement of cell growth

The optical cell density was measured at a wavelength of 660 nm using a spectrophotometer (UV-160A, Shimadzu, Japan) for estimation of the cell growth. Dry cell weight was measured as follows ; 10 ml of culture broth was centrifuged at 9,000×*g* for 10 min, and packed cells were washed with saline solution followed by distilled water. The washed cells were dried by desiccation at 105℃ to a constant weight prior to measuring the dry weight.

Determination of extracellular polysaccharide amount

The total accumulated carbohydrate in the culture broth was measured by the phenol-sulfuric acid method [4] after cells were removed by centrifugation. The amount of extracellular polysaccharide was estimated using an equivalent amount of glucose as the standard.

Isolation and purification of extracelluar polysaccharide

In order to remove the bacterial cells, the culture broth was diluted to with ten volume of distilled water. The most of the bacterial cells were removed by centrifugation at 9,000×*g* for 30 min. The cell free culture broth was precipitated by addition of two volume of ethanol. The precipitated crude extracellular polysaccharide was dried with vacuum evaporator, and redissolved in distilled water. Then, 10% cetylpyridimium chloride (CPC) solution was added until no more precipitate was formed. The insoluble extracellular polysaccharide-CPC complex was collected by centrifugation, and redissolved in a 10% sodium chloride solution. The extracellular polysaccharide was dialyzed against distilled water for desalting. Purified extracellular polysaccharide was obtained by freeze-drying and used as a sample for further analyses.

Measurement of apparent viscosity and molecular weight

The apparent viscosity of cell-free solution was measured by Brookfield Digital Rheometer (model DVⅢ, USA) equipped with a much-sample adapter (spindle SC4-34) and small-sample adapter (spindle SC4-18). Unless otherwise

stated, measurements were carried out at 25℃ with the sample volume of 8 ml. A high performance liquid chromatography (HPLC) (LC10, Shimadzu, Japan) equipped with PL-GFC 1000 Åcolumn (8 μ, 10×325 mm) (Polymer Laboratory, U.S.A.) and refractive index detector (RID-6A, Shimadzu, Japan), was employed for investigating the molecular weight (MW) distribution of Biopol32. Ten microliters of the sample solution were injected and eluted with HPLC grade water at a flow rate of 1 ml/min at room temperature. The molecular weight markers used were dextrans (Sigma Chem. Co., U.S.A.) of 2,000, 580, 143, 73, and 15 kDa. The molecular weight of Biopol 32 was determined by comparing its retention time with those of the standards.

Preparation of Biopol32 hydrolysate

Purified Biopol32 was dissolved in 20 ml of distilled, deionized water (ddH_2O, 0.2%, w/v) and then hydrolyzed at 121℃ for 1 hr after adding an equal volume of 4.0 M trifluoroacetic acid (TFA). Insoluble materials were removed by centrifugation at 35,000× *g* for 30 min and supernatant was filtered through a 0.2-μm pore-size filter. The filtrate was dried on Speed Vac and redissolved in ddH_2O.). The completely remove residual TFA, this procedure was repeated 4 times. For thin-layer chromatographic (TLC) analysis of the TFA-hydrolysate, the TLC plate used was plate coated with silica gel as the solid support and detection reagent used was the mixture contained diphenylamine, aniline and phosphoric acid. The loading volume of standard sugars was conducted by 20 μl of standard sugar solution (1%, w/v) and separating solvent system was acetonilrile (HPLC grade) : water (HPLC grade), 85 : 15 (v/v). The component test of TFA-hydrolysate was performed by HPLC (LC10, Shimadzu, Japan) fitted with refractive index detector. The sugar components of the hydrolysate was analyzed byμ-spherogel carbohydrate column 6.5 mm×300 mm (Beckman Co. U.S.A.). The solvent was HPLC grade water and injection volume was 20 μl. The flow rate of solvent was 0.5 ml/min and the operating temperature was 80℃.

Measurement of flocculating activity

To measurement of flocculating activity was based on the flocculation process of wastewater treatment, and adjusted to laboratory the procedure, as depicted by Fig. 1. Standard substance used was kaolin clay (Junsei chemical Co.) and flocculating activity was investigated by flocculation of kaolin clay suspended solution by multi-point stirrer (PMC industries Inc.). Flocculating activity was measured as optical density of supernatant at 550 nm by spectrophotometer (UV-160A, Shimadzu, Japan) and the calculation of flocculating activity was based on the following equation. The control was treated with distilled water and the sample was treated with the supernatant of culture broth.

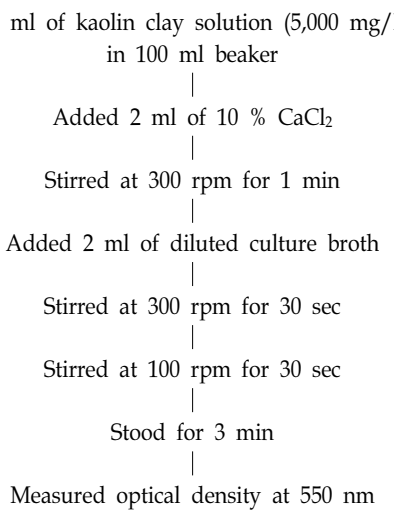

Fig. 1. Procedure of flocculating activity measurement.

Flocculating activity = 1/A − 1/B

Where A is the optical density of sample and B is that of a control.

Flocculation test for industrial wastewater

For the evaluation of industrial applicability of Biopol32, the flocculating efficiency was examined using industrial wastewater such as food wastewater, textile wastewater and paper waste water. Food wastewater, textile wastewater and paper waste water were analyzed before use for flocculating test. The chemical oxygen demand (COD) and suspended solid (SS) of food wastewater were 5,480 mg/l and 1,028 mg/l, respectively. The COD and SS of textile waster were 289 mg/l and 153 mg/l. The those of paper wastewater were 254 mg/l and 89 mg/l, respectively. And the flocculation conditions of Biopol32 was optimized for each wastewater.

Results and Discussion

Screening of the extracellular polysaccharide-producing bacteria

For the screening of extracellular polysaccharide-producing bacteria, more than 82 bacterial strains which excreted mucous material on the agar plate of the screening medium

were isolated from soil samples. A mucoid colony on the agar plate presumed extracellular polysaccharide producer was cultured in 50 ml of the liquid culture. For selection, materials produced from each isolate were tested for viscosity and extracellular polysaccharide productivity. Among the materials tested, mucoid from the strain GP32 showed the highest viscosity (4,170 cp) and extracellular polysaccharide production (5.61 g/l-broth). From these results, strain GP32 was selected to be the most suitable candidate as a practical biopolymer producer. The morphological and physiological characteristics of strain GP32 were investigated (Table 1). Colony types on common solid medium were large, convex and yellow tinge. Strain GP32 was rod shaped (0.6~0.8×1.5~3.0 μm) and Gram-negative. Growth occurred at 4℃, 25℃, and 40℃ but not at 50℃. The isolated strain was able to liquefy gelatin and to form nitrate. The strain showed a negative reaction in Voges-Proskauer, starch hydrolysis and indol reduction, and produced acid from glucose, fructose, sucrose, xylose and inositol. The mole% G plus C of the DNA of strain GP32 was found to be 62% ; the mol% G plus C of the DNA of *Pseudomonas* sp. ranges from 59 to 68 mole% [18]. When the keys to the genera listed in Bergey's Manual [18] were traced on the basis of the these results, strain GP32 was identified as *Pseudomonas* species, and designated as *Pseudomonas* sp. GP32.

Table 1. Taxonomic properties of strain GP32

Characteristics	Strains GP32
Morphological	
Gram staining	−
Shape	rod
Size (cell diameter, μm)	0.6~0.8
(cell length, μm)	1.5~3.0
Flagella number	1
Spore	−
Motility	+
Acid fast	−
Cultural	
Colony color	yellow-tinge
Growth in air	+
Growth anaerobically	−
Growth at 4℃	+
Growth at 25℃	+
Growth at 40℃	+
Growth at 50℃	−
Growth at 7% NaCl	−
Physiological	
Catalase	+
Oxidase	+
O/F (Oxidation/Fermentation)	O
Carbohydrates, acid form	
glucose	+
fructose	+
lactose	−
sucrose	+
xylose	+
inositol	+
Gelatin liquefaction	+
Starch hydrolysis	−
Voges-Proskuer reaction	−
Nitrate reduction	+
Indole reduction	−
Urase	+
Chemical	
Mol% G+C of DNA	62

Effect of carbon and nitrogen sources on the extracellular polysaccharide production

Carbon and nitrogen sources is the important factor in production of extracellular polysaccharide by microorganisms [14, 28]. The assimilation of various carbon and nitrogen sources by as *Pseudomonas* sp. GP32 was examined in the flask culture. To find a suitable carbon source for the extracellular polysaccharide production, *Pseudomonas* sp. GP32 was cultivated in the medium containing various carbon sources such as glucose, fructose, sucrose, galactose, lactose, mannose, rhamnose and soluble starch. Each carbon source was added to the medium at a concentration 20 g/l. When the cells were grown in the galactose containing medium, the extracellular polysaccharide production (7.8 g/l) was the highest among those tested (Fig. 2). On the other

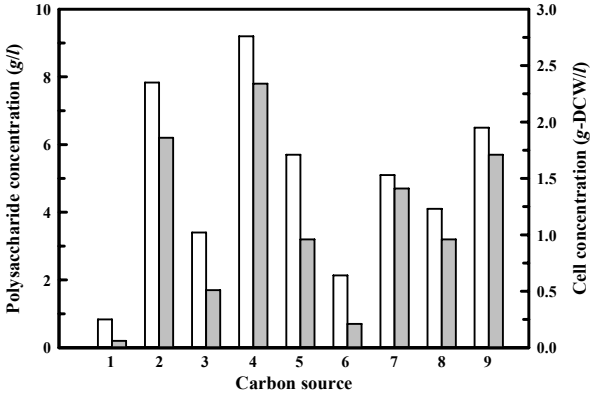

Fig. 2. Effect of carbon sources on the extracellular polysaccharide. Carbon sources were added to the basal medium at the concentration of 20 g/l. Initial pH and temperature were at 7.5 and 32℃, respectively. The numbers : 1(None), 2(glucose), 3(fructose), 4(galactose), 5(mannose), 6(Rhammnose), 7(Lactose), 8(sucrose), 9(soluble starch). The symbols : (□) cell concentration, (■) : polysaccharide concentration.

hand, fructose, mannose, sucrose and rhamnose we not much favorable for either extracellular polysaccharide production or cell growth. Therefore, galactose was used as the carbon source for extracellular polysaccharide production. Glucose and galactose have been reported to be good substrates for extracellular polysaccharide production *Alcaligenes cupidus* KT201 [26]. We further examined the effect of carbon concentration on the extracellular polysaccharide production since there have been reported broad ranges of optimal concentration depending on the microorganisms [8]. The effective concentration of galactose on the extracellular polysaccharide production was investigated with culture medium containing 10, 20, 30, 40, 50, and 60 g/l. The concentration of nitrogen source (NH_4NO_3) was fixed to 1.0 g/l. The highest extracellular polysaccharide production (10.9 g/l) was reached at 30 g/l galactose and cell concentration was 2.8 g/l. The addition of more than 40 g/l galactose depressed the production of extracellular polysaccharide. As nitrogen sources were the important factor in extracellular polysaccharide biosynthesis and cell growth [28], the effect of nitrogen sources on extracellular polysaccharide production and cell growth were investigated. Extracellular polysaccharide production and cell growth was maximum with $(NH_4)_2SO_4$, and nitrogen source was determined as $(NH_4)_2SO_4$, The nitrogen sources for *Pseudomonas* sp. GP32 differed from the nitrogen source (NH_4NO_3) for *Paecilomyces* sp. [25] and *Zoogloea* MP6 [27].

In general, extracellular polysaccharide production is favoured by a high ratio of carbon to nitrogen in many bacterial species and also in some fungi [27]. Production of extracellular polysaccharide was greatly influenced and stimulate by the limitation of nitrogen sources [1]. In order to determine the optimum carbon to nitrogen ratio for the production of extracellular polysaccharide by *Pseudomonas* sp. GP32, several media containing different C/N ratio with $(NH_4)_2SO_4$ as a nitrogen source were investigated. Initial C/N ratios were adjusted to 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100. The concentration of galactose was fixed to 30 g/l. The effects of C/N ratio on cell growth and extracellular polysaccharide production of *Pseudomonas* sp. GP32 are summarized in Fig. 3. The optimum C/N ratio for extracellular polysaccharide was obtained at around 50. Production of extracellular polysaccharide was greatly influenced and stimulated by the limitation of nitrogen sources. Consequently, it appears that the extracellular polysaccharide may have been produced under the condition of

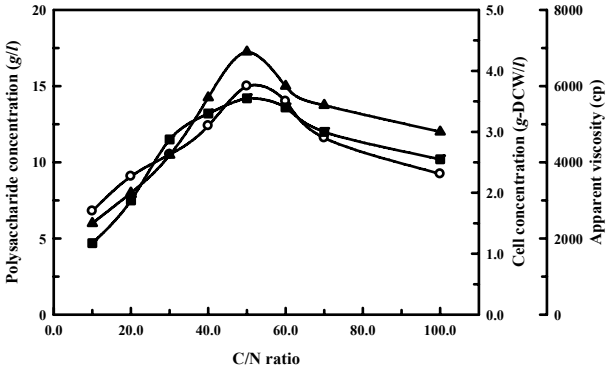

Fig. 3. Effect of C/N ratio on the extracellular polysaccharide production. Cells were incubated with shaking at 32℃ for 3 d in the culture medium containing $(NH_4)_2SO_4$ of various concentration. Symbols : (○) cell concentration, (■) polysaccharide concentration, (▲) apparent viscosity.

nitrogen limitation. This result suggests that the ratios of extracellular polysaccharide to cell is maintained high under the condition of relatively low nitrogen. Similar patterns have been also observed with other extracellular polysaccharides [15, 21].

Batch Fermentation for extracellular polysaccharide production

While the growth conditions of *Pseudomonas* sp. GP32 was similar to that of general bacteria as slightly acidic and neutral ranges of pH and mesophiles, the growth ranges of *Pseudomonas* sp. GP32. was as pH 5~9 and 25~35℃. The optimum medium at the flask level for the production of extracellular polysaccharide was as follows : 30 g/l galactose, 0.6 g/l $(NH_4)_2SO_4$, 1.5 g/l K_2HPO_4, 0.08 g/l KH_2PO_4, 0.15 g/l $MgSO_4 \cdot 7H_2O$, 0.1 g/l $MnSO_4 \cdot 4{\sim}5H_2O$, 0.05 g/l NaCl. The initial pH was 7.5 and the culture temperature was 32℃. By using this medium, the extracellular polysaccharide was produced in a yield 2.8 fold higher than that with the basal medium. To examine the pattern of the extracellular polysaccharide production in detail, batch fermentations were carried out in a jar fermentor (Fig. 4). In the jar-fermentor culture, the culture broth became progressively viscous with the production of extracellular polysaccharide. The cell concentration increased gradually with the extension of cultivation time and reached the maximum value of 4.25 g/l after 80 hr of cultivation. The concentration of extracellular polysaccharide increased in proportion to the increasing cell concentration and reached the maximum level of 15.7 g/l after 70 hr of cultivation. It was apparent that extracellular polysaccharide by *Pseudomonas* sp. GP32 was definitely

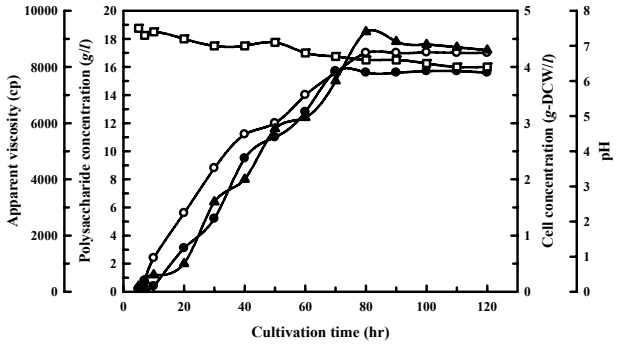

Fig. 4. Change of extracellular polysaccharide and apparent viscosity according to the culture time in a jar fermentor. Cells were incubated at 32℃ for 120 hr in the optimized medium. An optimized medium contained 30 g/l galactose, 0.6 g/l $(NH_4)_2SO_4$, 1.5 g/l K_2HPO_4, 0.08 g/l KH_2PO_4, 0.15 g/l $MgSO_4 \cdot 7H_2O$, 0.1 g/l $MnSO_4 \cdot 4{\sim}5H_2O$, 0.05 g/l NaCl. The initial pH was maintained at 7.5. Symbols : (○) cell concentration, (●) polysaccharide concentration, (▲) apparent viscosity, (□) pH.

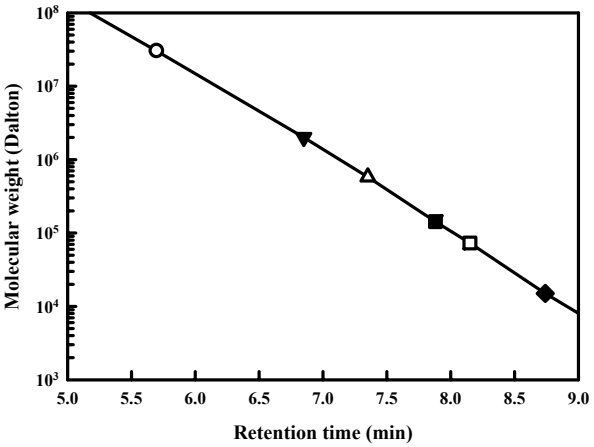

Fig. 5. Estimation of molecular weight of Biopol32 by gel-permeation HPLC with PL-GFC column. Symbols : (○) Biopol32 (▼) 2,000 kDa, (△) 580 kDa, (■) 143 kDa, (□) 73 kDa, (◆) 15 kDa.

growth-dependent. This result indicates that the concentration of extracellular polysaccharide from *Pseudomonas* sp. GP32 is much higher than that of other polysaccharide producing microorganisms [2, 23]. The viscosity of the culture broth at that time was 92,600 centipoise. The production of extracellular polysaccharide by *Pseudomonas* sp. GP32 was parallel to cell concentration, a large amount of extracellular polysaccharide was released at the exponential phase. In the case of *Rhodococcus erythropolis*, an extracellular polysaccharide appears to be released into the culture broth in proportion to cell growth in the stationary phase [10]. These results suggested that the production mechanism of extracellular polysaccharide of those microorganisms might be different.

Molecular weight and sugar component of Biopol32

The extracellular polysaccharide produced by *Pseudomonas* sp. GP32 was purified by ethanol precipitation and cetylpyridinium chloride (CPC) precipitation and gel permeation chromatography. The purified extracellular polysaccharide were combined and lyophilized (the final preparation was designated Biopol32). The molecular weight of Biopol32 was estimated by high performance gel permeation chromatography with dextrans as standards. Biopol32 is extremely high molecular weight carbohydrate polymer and was measured about $3{\times}10^7$ Da by comparison with standard dextrans (Fig. 5). Generally, the molecular weight of a polysaccharide is related to the chain length of the polymer, which is an important factor in the industrial applications. The molecular weight of Biopol32 in present study is similar to that of extracellular polysaccharide from *Penibacillus* sp. [20].

For component sugars analysis of Biopol32, chromatographic analysis was conducted after complete hydrolysis and the result was compared with authentic sugar standards. The acid hydrolysate was subjected to the thin layer chromatography (TLC) analysis. The loading volume of standard sugars was conducted by 20 μl of standard sugar solution (1%, w/v) and the separating solvent system was acetonitrile : water (85 : 15, v/v). The constituent sugars by TLC were determined as galactose, glucose, galactounonic acid and glucouronic acid. High performance liquid chromatography (HPLC) of Biopol32 was conducted after complete hydrolysis. As shown in Fig. 6, Biopol 32 was found to be heteropolysaccharide composed of galactose, glucose, glucouronic acid and galactouronic acid. The molar ratios of galactose, glucose, glucouronic acid and galactouronic acid were approximately 1.85 : 3.24 : 1.00 : 1.42.

Apparent viscosity of the extracellular polysaccharide

Apparent viscosity of polysaccharides is an important functional property in fluid. The study of apparent viscosity has practical significance in relation to process engineering, new product development, and quality control. Changes in apparent viscosity can be used to monitor the changes in physicochemical interactions between polysaccharide or conformational changes of component in solution of dispersion [6]. Among the rheological properties, apparent vis-

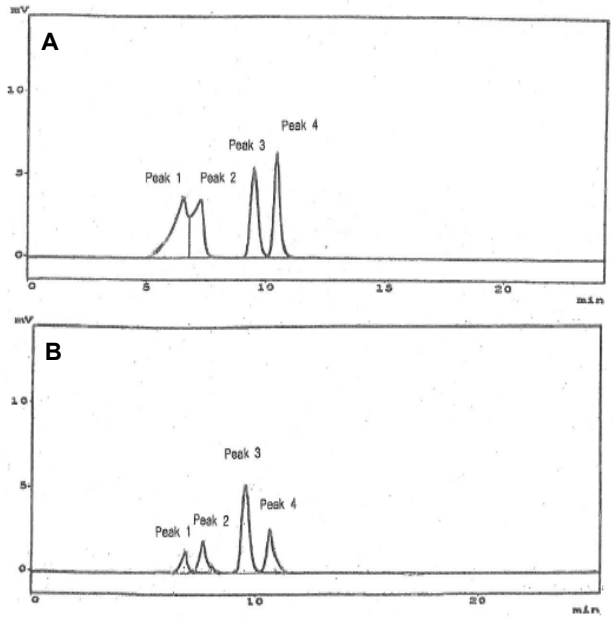

Fig. 6. Sugar component analysis of Biopol32 hydrolysate. A : HPLC chromatogram of standard carbohydrate, B : HPLC chromatogram of TFA-hydrlyzed purified Biopol 32. (Peak 1) glucouronic acid, (Peak 2) galactouronic acid, (Peak 3) glucose, (Peak 4) galactose.

cosity is an important factor which can measure rheological characteristics of polymer solutions. The characteristic flow behavior of the Biopol32 solution has been studied in comparison with the zooglan solution form *Zoogleoa ramigera*. The Biopol32 solution and zooglan solution were pseudoplastic non-Newtonian fluids at concentrations above 0.05 g/l, respectively. Fig. 7 shows a plot of an apparent viscosity

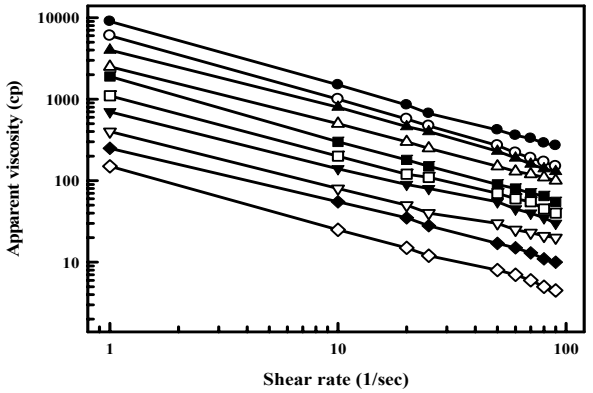

Fig. 7. Relationship between shear rate and apparent viscosity at the different concentration of Biopol32 from *Pseudomonas* sp. GP32 and zooglan from *Zoogloea ramigera*. Symbols : (◆) 0.05 % Biopol32, (◇) 0.05% zooglan, (▼) 0.1% Biopol32, (▽) 0.1% zooglan, (■) 0.2% Biopol32, (□) 0.2% zooglan, (▲) 0.3% Biopol32, (△) 0.3% zooglan, (●) 0.5% Biopol32, (○) 0.5% zooglan.

against the shear rate with varying concentrations of Biopol32. At each shear rate, the apparent viscosity of the extracellular polysaccharide solution was found to be about 3 times higher than the zooglan solution at each concentration. The values of apparent viscosity of different types of polysaccharides are remarkably different at the same concentration. It was proposed to be determined according to structural differences such as molecular weight, distribution of side chains, charge density, and shape of the linkage [16]. Apparent viscosity of Biopol32 solution decreased rapidly with increasing shear rate at all concentrations tested. A decrease in apparent viscosity with an increase in shear rate may indicate the degree of orientation of the molecule, the change in the shape of flexible molecules, and the effect of flow on intermolecular interaction. The thixotropic effect is more dominant at higher concentrations of extracellular polysaccharide. These phenomena indicated that Biopol32 and zooglan had the highest degree of pseudoplasticity, identified as shear thining, among the characters of non-Newtonian fluids. The effect of the shear rate on apparent viscosity is greater than on that of zooglan, indicating that the Biopol32 molecule is highly charged and asymmetric. From these rheological properties, Biopol32 can be considered to be a new polysaccharide which shows a high apparent viscosity. It has several important properties such as a high viscosity at low concentrations, low viscosity at high shear rates, and high viscosity at low shear rates.

Flocculating activity of the extracellular polysaccharide

Many different flocculants have been used in wastewater treatment and down stream processing techniques. The culture broth of *Pseudomonas* sp. GP32 was tested for its ability to flocculate kaolin clay. *Pseudomonas* sp. GP32 was showed high flocculating activity (flocculating activity (1/O.D) ; 6.23) on the test material. To elevate the industrial applicability of Biopol32, its flocculating efficiency was examined using wastewaters from the food, textile, and paper industries. In a jar test, coagulation and flocculation were accomplished at room temperature. The reagent dosing, mixing (destabilization), and the subsequent sedimentation (phase separation) took place in the same reaction chamber. The efficiency of the flocculation process in the jar test was evaluated on the basis of the chemical oxygen demand (COD) and suspended solids (SS) before and after treatment. The optimized condition and treatment efficiency on the food, textile, and paper wastewater were shown Table 2, re-

Table 2. Flocculation effect on the treatment of industrial wastewater of extracellular polysaccharide from *Pseudomonas* sp. GP32

Wastewater	Food wastewater	Textile wastewater	Paper wastewater
Optimal Treatment condition			
Coflocculant $CaCl_2$ concentration	15.0 mM	17.0 mM	12.0 mM
Flocculant Concentration	15.5 mg/l	21.3 mg/l	2.0 mg/l
Flocculation efficiency			
COD reduction rate	58.4%	47.7%	67.3%
SS removal rate	87.2%	82.6%	91.3%

spectively. The sorts and concentration of Biopol32 and coflocculant depend on the sorts of wastewater and at the optimal treatment condition, the concentration of each Biopol32 was 15.5 mg/l (food wastewater), 21.3 mg/l (textile wastewater), and 2.0 mg/l (paper wastewater) and the concentration of each coflocculants was 15.0 mM $CaCl_2 \cdot 2H_2O$ (food wastewater), 17.0 mM $CaCl_2 \cdot 2H_2O$ (textile wastewater), and 12.0 mM $CaCl_2 \cdot 2H_2O$ (paper wastewater). As shown in Table 2, the flocculating efficiency of Biopol32 in food wastewater was showed COD reduction rate (58.4%) and SS removal rate (87.2%). In case of textile wastewater, the COD reduction rate and SS removal rate of Biopol32 was 47.7 % and 82.6%, respectively. In case of paper wastewater, COD reduction rate was 67.3% and SS removal rate was 91.3%. Based on these results, extracellular polysaccharide (Biopol32) from *Pseudomonas* sp. GP32 was applicable considered a possible candidate for industrial application.

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초록 : *Pseudomonas* sp. GP32에 의해 생산된 세포 외 다당류의 생산 및 특성

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미생물유래 다당류 생산균주를 분리하기 위해 전국 각지의 토양시료로부터 가장 높은 점성과 다당류 생산성을 나타내는 균주 GP32를 분리하였으며, 분리균주 GP32의 동정을 분리균주의 형태학적, 생리학적 특성을 조사한 결과, *Pseudomonas* 속 세균으로 확인되었으며 최종적으로 *Pseudomonas* sp. GP32로 명명하였다. 플라스크 수준에서 *Pseudomonas* sp. GP32의 다당류 생산을 위한 가장 적합한 탄소원과 질소원은galactose와 $(NH_4)_2SO_4$를 이용하였을 때 가장 많은 다당류를 생산하는 것으로 확인되었으며, 다당류 생산을 위한 최적의 C/N ratio는 50이었다. 다당류 생산을 위한 최적 pH와 온도는 각각 7.5와 32℃였다. 최적화된 배지를 이용한 fermentor 배양에서 다당류 생산은 배양 70시간에 최고치를 나타내었으며, 이때 다당류 생산량은 15.7 g/l이었다. *Pseudomonas* sp. GP32로부터 생산된 다당류는 ethanol 침전, cetylpyridimium 침전과 gel permeation chromatography를 통하여 정제하였으며, 정제된 다당류는 Biopol32로 명명하였다. Biopol 32의 분자량은 3×10^7 datons이었으며, Biopol32가 함유하고 있는 구성당은 galactose : glucose : gulcouronic acid : galactouronic acid 등이 1.85 : 3.24 : 1.00 : 1.42의 몰비로 함유되어있다. Biopol32 용액은 의가소성 성질을 갖는 고분자 화합물로서 *Zoogloea ramigera*가 생산하는 생물고분자인 zooglan보다 모든 농도에서 높은 점성을 나타내었다. Biopol32의 실제 폐수처리현장에서 응집제로의 사용 가능성을 검토하기 위하여 식품폐수, 섬유폐수와 제지폐수를 대상으로 응집효율을 조사한 결과, 높은 COD 감소율(58.4~67.3%)과 SS제거율(82.6~91.3%)를 나타내어 실제 산업폐수에서 뛰어난 응집효율을 나타내었다.