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Physicochemical Properties of the Exopolysaccharides Produced by Marine Bacterium *Zoogloea* sp. KCCM10036

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Abstract The physicochemical properties of the exopolysaccharide (EPS) produced by marine bacterium *Zoogloea* sp. KCCM10036 were investigated. Two types of isolated EPSs were shown to have average relative molecular masses (M_r) of 4.07×10^6 of CBP (cell-bound polysaccharide) and 3.43×10^6 of WSP (water-soluble polysaccharide), respectively. When the CBP was utilized as an emulsifier, it stabilized the emulsion for up to 148 h. Compared with other commercially available hydrocolloids such as xanthan gum, the Tween series, and Triton, the CBP showed much better emulsifying capability on a water-in-oil system. Phase separation occurred in the Tween series after 24 h, whereas the emulsion was better stabilized by the CBP. The CBP thus has potential as an emulsifying agent in commercial emulsions. The flocculating activity was also greatest at 0.01% (w/v) and decreased at higher concentrations than the optimized concentration of the WSP and CBP. The results also showed that both types of exopolysaccharides from *Zoogloea* sp. had excellent flocculating activity.

Keywords: Physicochemical properties, exopolysaccharide, *Zoogloea* sp. KCCM10036, marine bacterium

Microbial exopolysaccharides (EPS) are finding an increasing number of applications in a considerable diversity of industrial roles. Until recently, the number of industrially useful polysaccharides has been restricted, and the most widely available natural polymers are from the bacterial sources, such as xanthan gum and dextran, and the fungal polysaccharide like scleroglucan [3, 7, 8, 18, 20, 21, 25, 26]. Several new polysaccharides with interesting physicochemical and rheological properties have been reported, including an attractive gel-forming biopolymer that can be used as an alternative to agar [19]. Microbial EPSs have been utilized as new biomaterials in a wide range of industrial applications such as in textiles, detergents, adhesives, microbial enhanced oil recovery (MEOR), wastewater treatment, dredging, brewing, downstream processing, cosmetology, and pharmacology, and as food additives [1, 12, 24]. Furthermore, polysaccharides are natural polymers from renewable sources; therefore, peculiar features like biocompatibility, biodegradability, bioadhesivity, and nontoxicity, coupled with wide availability and usually low costs, account for their steadily increasing exploitation in the formulation of products for food, biomedical, and cosmetic applications [2].

Recently, an exopolysaccharide produced by *Zoogloea* sp. has been investigated in details. In the previous reports [5, 16], a new bacterial strain producing exopolysaccharides was isolated from the marine environment and identified as *Zoogloea* sp. KCCM10036. This strain produced two types of physically separable exopolysaccharides: water-soluble polysaccharide (WSP) and cell-bound polysaccharide (CBP) [4, 15]. Both exopolysaccharides contained glucose, galactose, and mannose as sugar components in common, but their molar ratios were different (2:2:3 for WSP and 1:2:2 for CBP) and half of the sugar components existed as uronic acid form [16]. These exopolysaccharides showed antitumor activity [4], and useful characteristics as an effective adsorbent of heavy-metal ions and as a new matrix for enzyme immobilization [15]. Both WSP and CBP had non-Newtonian, pseudoplastic, fluid behavior, and the solutions had low activation energy [10]. However, the physicochemical properties of the exopolysaccharides from this strain have not been studied yet. Therefore, in this study, we thoroughly investigated the physicochemical properties and potential applicability of the exopolysaccharides isolated from *Zoogloea* sp. KCCM10036 culture.

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MATERIALS AND METHODS

Bacterial Strain and Culture Conditions

The bacterial strain used in our studies, a marine bacterium *Zoogloea* sp. KCCM10036, was isolated from a surface layer of the seaweed, *Undaria* sp. collected from the south sea of Korea [16]. To prepare the inoculum, a single colony on an agar plate was transferred to 50 ml of a modified marine medium in a 250-ml baffled flask, and cultivated in a rotary shaking incubator at 30°C for 12 h. Then, 2% (v/v) culture broth was transferred to the fresh growth medium and cultivated for another 12 h under the same conditions. This preculture was used as an inoculum to cultivate the bacterial cells in a batch-type 5-l fermentor (KF-500, Korea Fermentor Co., Korea). The fermentation conditions were as follows: working volume, 3 l; stirring speed, 400 rpm; air flow rate, 2 ml/min; temperature, 30°C; pH, uncontrolled [9].

Isolation and Preparation of Exopolysaccharide Solutions

The crude WSP and CBP were isolated from the culture broth of *Zoogloea* sp. (KCCM10036) using the previously reported procedure (Fig. 1) [2, 5]. Briefly, the exopolysaccharide separation process was based on centrifugation, and acetone and cetylpyridinium chloride (CPC) precipitation. The WSP was isolated from the supernatant and the CBP was obtained from the precipitates. The exopolysaccharide solutions were prepared by complete dissolution of the freeze-dried WSP and CBP in distilled water, using a magnetic stirrer at a dissolution temperature of 30°C, and the dispersed air bubbles in the solutions were removed. Each solution was filtered through a 0.2 mm Whatman membrane filter before further analysis.

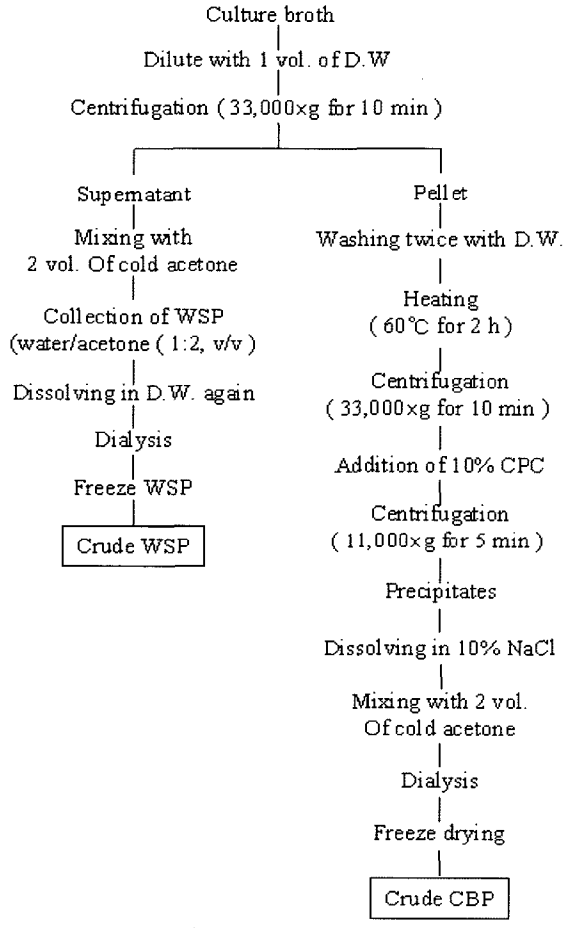


Fig. 1. Preparation procedure for crude exopolysaccharides.

Molecular Mass Determination High-Performance Size-Exclusion Chromatography (HPSEC)

The relative molecular mass (M_r) of the EPSs was measured by high-performance size-exclusion chromatography (HPSEC) using Zorbax PSM 60, 300, and 3,000 columns on an HP 1100 series HPLC system equipped with an RI-4 refractive index detector (Agilent, U.S.A.). Deioinized water (18 MΩ) was used as an eluent at the flow rate of 1 ml/min. Dextran standards (Sigma-Aldrich Chem Co., MO, U.S.A.) were used to construct a calibration curve from which M_rs of EPSs were calculated with no further correction.

Analysis of Thermal Properties

The thermal properties of EPS were analyzed by using a differential scanning calorimeter (DSC Pyris 1, Perkin-Elmer Co., U.S.A.). After placing 4.2 mg of Dried EPS sample in an aluminum pan, it was sealed and analyzed, using empty pan as a reference, for determining the melting point and enthalpy change. The heating rate was 10°C per min from 20 to 300°C.

Water-Holding Capacity

Water-holding capacity (WHC) of the EPS was determined following the method of Chen *et al.* [6]. Briefly, the dispersion of 5–25 g/l ESP (dry basis) in distilled water was agitated for 1 h. Then, it was transferred to a 250-ml centrifuge tube and spun down at 19,000 ×*g* at 25°C for 15 min. The wet exopolysaccharides were drained for 15 min to remove the free water and then weighed both before and after drying in an oven at 80°C for 24 h. The WHC was calculated based on the following equation: WHC (%)=[(the amount of wet EPS–the amount of dried EPS)/the amount of dried EPS]×100.

Emulsion Stability

The emulsion stability of the isolated EPS was measured and compared with other microbial gums (xanthan gum and gellan from Sigma-Aldrich Chem Co., U.S.A.) and chemical emulsifiers by adopting the following method [22, 24]. Four ml of olive oil was mixed with 6 ml of the EPS dispersion (1.0%, w/v) in a test tube and the mixture

was homogenized at 18,000 rpm for 10 min. The homogenized mixture was left to stand at room temperature up to 10 days, and the emulsion stability was measured at 24-h intervals.

Flocculating Activity

The flocculating activity was measured by following the method of Nakamura *et al.* [23] with slight modification. Charcoal-activated carbon that was used as a testing material was suspended in deionized water at a concentration of 5 g/l. In a test tube, 10 ml of a charcoal-activated carbon suspension was added and mixed with 0.1 ml of $CaCl_2$ solution (6.8 mM). To this mixture, various amounts of EPS were added and vortexed for 30 sec and allowed to stand for 10 min at room temperature. The turbidities of the upper 1 ml phase were measured at 550 nm. A control experiment without the EPS was also pursued in the same manner. The flocculating activity (%) was defined and calculated according to the following equation:

Flocculating activity=[(B-A)/B]×100×dilution rate

A: Turbidity of EPS-containing suspension
B: Turbidity of control suspension

RESULTS AND DISSCUSSION

Measurement of Molecular Mass

When the EPS produced by *Zoogloea* sp. was subjected to high-performance size-exclusion chromatography (HPSEC), it eluted from a series of size exclusion columns as a single peak. The relative molecular masses (M_rs) of WSP and CBP at peak were determined to be 4.07×10^6 and 3.43×10^6, respectively (Fig. 2). These values were very close to what have been reported for most microbial exopolysaccharides studied [13].

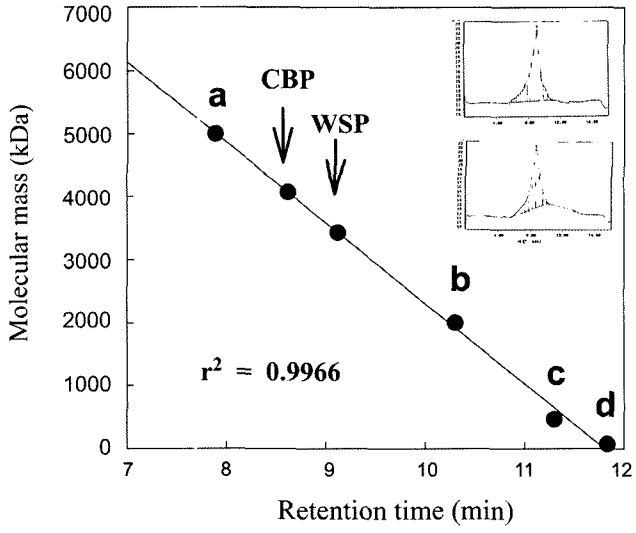


Fig. 2. Estimation of molecular mass of WSP and CBP by high-performance size-exclusion chromatography.
Standards a: Dextran (M_r 5×10^6); b: Dextran (M_r 2×10^6); c: Dextran (M_r 4.64×10^5); d: Dextran (M_r 7.14×10^4).

Table 1. Thermal properties of WSP and CBP by differential scanning calorimetry (DSC).

	Peak temperature	Enthalpy (cal/g)
WSP	162.82	453.49
CBP	166.05	529.78

Analysis of Thermal Properties

As for the thermal characteristics of exopolysaccharides, heat absorption and emission are accompanied with the physical change by deformation of polymer structure or melting of crystalline polysaccharides. The melting of WSP and CBP started at about 155.90°C and 159.34°C, respectively, and the endothermic enthalpy change (ΔH) required to melt 1 g of WSP and CBP were 453.49 and 529.78 cal, respectively (Table 1). Thus, the two two exopolysaccharides produced by *Zoogloea* sp. displayed different thermal characteristics from each other. As for the exopolysaccharides obtained from a mutant of *Bacillus polymyxa*, the melting point was 183.25°C, and enthalpy was 100.3 cal/g, and 177.87°C, 113.5 cal/g for xanthan gum, respectively [17].

In an earlier report, the measurement of the thermal characteristics of levan synthesized with levansucrase showed the highest melting point to be 178.4°C with an enthalpy of 1.66 cal/g, similar to the thermal characteristics of the exopolysaccharides derived from legacy microorganisms [11].

Water-Holding Capacity

The characteristic of water-holding capacity (WHC) comprises penetration and absorption of moisture, which comes from the material's physical structural factors such as swelling and surface porosity. In food processing, the water sorption and adsorption phenomena are a critical operating factor that requires engineering solution. Specifically, in the course of refrigeration after freezing, usually there is 20–25% moisture loss, which may be accompanied with the loss of flavor components and soluble proteins, and cause quality degradation when thawed. Thus, xanthan gum is the most common material in the food industry to control the WHC of processed products. The WHC measurements were carried out over an exopolysaccharide concentration range of 0.5–2.5% (w/v). The results showed that the WHC decreased with an increase in exopolysaccharide concentration (Fig. 3). It was speculated that the water content per unit mass of high molecular substances decreased owing to the physical transformation of the high molecules' chain units when added in high

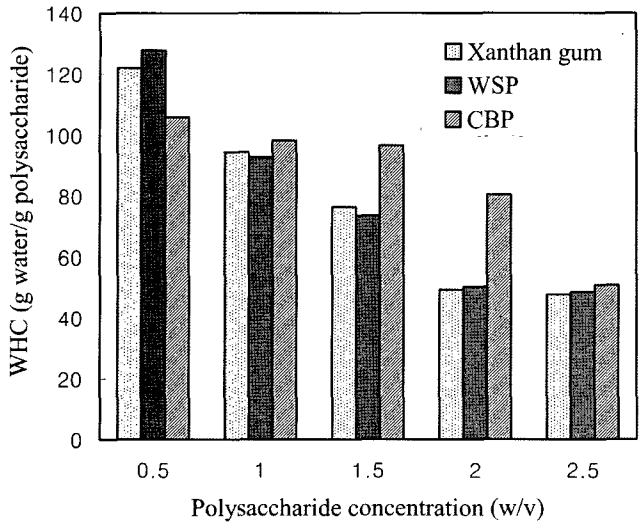


Fig. 3. Effects of WSP, CBP, and xanthan gum concentrations on water-holding capacity (WHC).

concentrations. The CBP showed substantially better stability of WHC than that of the xanthan gum up to 2.0% (w/v), and the WSP at 0.5% (w/v) showed the greatest WHC of 127 g water/g exopolysaccharide.

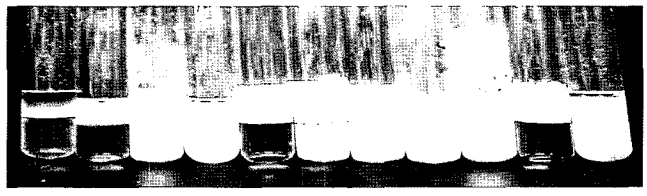
Emulsion Stability

The emulsion stabilities of WSP and CBP were compared with various commercial polysaccharides and synthetic emulsifiers (Table 2). The isolated EPSs in this study formed an emulsion with olive oil more efficiently than did other commercial polysaccharides and emulsifiers. In the case of alginate, water and oil began to separate after 30 min from the setting point, with complete separation by 24 h. Gellan and carrageenan did not show any significant emulsifying capacity due to gelling. The tween series and Triton X-100 showed full emulsifying capability in the early stage (0–1 h) of measurement, and a slow decrease in their emulsion stability could be visually observable thereafter; the stability began to rapidly decrease after 24 h. Xanthan gum and CBP showed excellent emulsifying stability in the early stage, which also remained up to 144 h, whereas the stability of the WSP sample decreased after 48 h. It is noteworthy that the CBP sample showed superior emulsifying stability even up to 144 h, which means that the exopolysaccharides derived from *Zoogloea* sp. have a potential in the emulsifier industry. Various types of the oils and fats should be extensively investigated to develop new useful emulsifiers out of the EPS derived from *Zoogloea* sp.

Flocculating Capability

Flocculating capability test was performed at EPS concentrations ranging from 0.1 to 2.0 mg/l in 5.0 g/l dispersion of charcoal-activated carbon containing 6.8 mM $CaCl_2 \cdot 2H_2O$ (Fig. 4). The flocculating capability of the isolated EPS was compared with that of xanthan gum. This capability was greatest at the EPS concentration range of 0.1 to 0.3 mg/l and decreased as the EPS (flocculant) concentration increased thereafter. The optimal flocculant concentration in the test solution was determined to be

Table 2. Emulsion stability test of the exopolysaccharides and other commercial polymers.



	Emulsifier	Emulsion stability (h)										
		0.1	0.5	1	3	5	12	24	48	72	122	144
A	Water (standard)	-	-	-	-	-		-	-	-	-	-
B	Na-alginate	+++	++	+	+	+	+	-	-	-	-	-
C	Xanthan gum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
D	Gellan gum	++	+	+	-	-	-	-	-	-	-	-
E	Tween 20	+++	+++	+++	++	++	++	+	+	+	+	+
F	Tween 40	+++	+++	+++	++	++	++	+	+	+	+	+
G	Tween 80	+++	+++	+++	+++	+++	++	+	+	+	+	+
H	WSP	+++	+++	+++	+++	+++	+++	++	+	+	+	-
I	CBP	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
J	Triton X-100	+++	+++	++	++	++	++	++	+	-	-	-
K	Carragenan	+++	+++	+	-	-	-	-	-	-	-	-

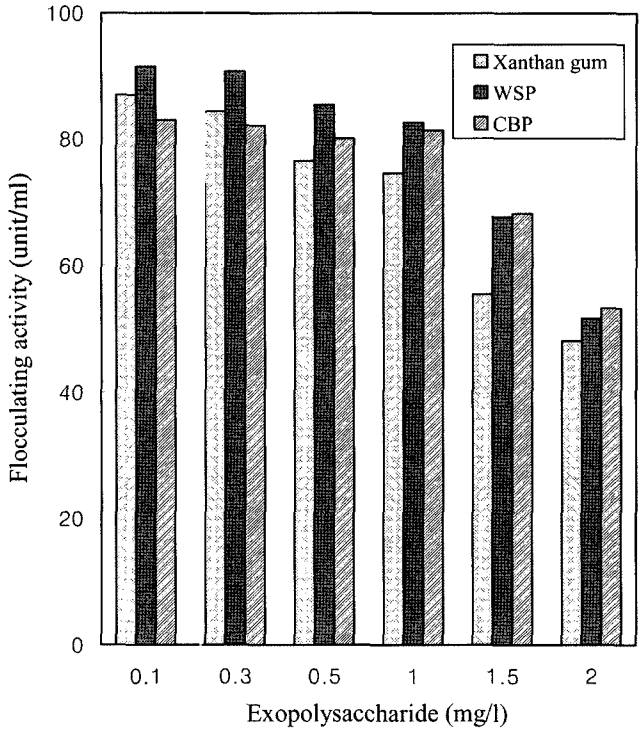


Fig. 4. Flocculating capacity of the WSP, CBP, and xanthan gum.

0.1 mg/l. The flocculating capability of xanthan gum was greatest between 0.1 and 0.3 mg/l as well. As shown in Fig. 4, the flocculating capability initially maintained at the relatively low concentration range, but then decreased as the adsorption of excess flocculants destabilized the particles. Because of incomplete dispersion of excess flocculants, only particles around flocculants participated in the flocculating reaction at that moment.

A large-molecular-weight flocculant is usually long enough and has a sufficient number of free functional groups that can act as bridges to bring many suspended particles together, and hence causes a larger floc size in the flocculation reaction. The flocculating activities of both WSP and CBP were shown to be higher than that of xanthan gum. These EPSs are expected to be useful flocculating agents in the areas of wastewater treatment, drinking water processing, and downstream processing in the food industry because of their biodegradability and harmlessness toward humans and the environment.

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