Biotechnology and Bioprocess Engineering **2004**, 9: 405-413

Physicochemical and Rheological Properties of a Novel Emulsifier, EPS-R, Produced by the Marine Bacterium *Hahella chejuensis*

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Abstract The rheological properties of an exopolysaccharide, EPS-R, produced by the marine bacterium *Hahella chejuensis* strain 96CJ10356 were investigated. The E_{24} of 0.5% EPS-R was 89.2%, which was higher than that observed in commercial polysaccharides such as xanthan gum (67.8%), gellan gum (2.01%) or sodium alginate (1.02%). Glucose and galactose are the main sugars in EPS-R, with a molar ratio of ~1:6.8, xylose and ribose are minor sugar components. The average molecular mass, as determined by gel filtration chromatography, was 2.2×10^3 KDa. The intrinsic viscosities of EPS-R were calculated to be 16.5 and 15.9 dL/g using the Huggins and Kraemer equations, respectively, with a 2.3 dL/g overlap. In terms of rigidity, the conformation of EPS-R was similar to that of caboxymethyl cellulose (5.0×10^{-2}). The rheological behavior of EPS-R dispersion indicated that the formation of a structure intermediate between that of a random-coil polysaccharide and a weak gel. The aqueous dispersion of EPS-R at concentrations ranging from 0.25 to 1.0% (w/w) showed a marked shear-thinning property in accordance with Power-law behavior. In aqueous dispersions of 1.0% EPS-R, the consistency index (K) and flow behavior index (n) were 1,410 and 0.73, respectively. EPS-R was stable to pH and salts.

Keywords: exopolysaccharide, *Hahella chejuensis*, marine bacterium, rheological properties

INTRODUCTION

Microbial extracellular polysaccharides (EPSs) have been used in a wide variety of industrial applications such as emulsification, gel formation, absorption, film formation and anticancer treatment [1-3]. Some microorganisms are able to produce bio-surfactants, which may enhance hydrophobic substrate utilization and/or detoxification processes, as they are excellent emulsifying, dispersing and solubilizing agents [4]. EPSs have significant commercial value, particularly in gel production and for the modification of the rheological properties of aqueous solutions. There is potential for microbial EPSs to replace the plant and macroalgal exopolysaccharides traditionally used in the food, pharmaceutical, textile and oil industries [5-6]. EPSs from marine microorganisms, including *Zoogloea* sp., *Pseudomonas* sp., *Vibrio fischeri*, *Cyanothece* sp. and *Alteromonas maleolii*, have been reported to be very promising as new biomaterials [7-11]. The transport properties and rheological behaviors of complex materials such as polysaccharide systems can be significantly affected by several factors, mainly related to their molecular and super-molecular features. These special characteristics of polysaccharides result in specific behaviors on both molecular and super-molecular scales, and different classes of materials can be identified on the basis of these behaviors [12]. The rheological behavior of exopolysaccharide results from its ordered conformation and intermolecular interactions usually observed in aqueous solutions. Rheological properties are informative with respect to the relationship between microstructure and physical properties (useful in optimizing formulations) and when analyzing the texture of commercial products [13-15]. Biosurfactants from microorganisms have a number of advantages over their chemical counterparts; namely, they are biodegradable, can be synthesized under user-friendly conditions (*e.g.*, low temperatures and pressures) and are effective over a wide range of temperature, pH and salinity conditions. Owing to their diverse biosynthetic capabilities, microorganisms are also likely to produce emulsifiers [16].

An EPS-producing marine bacterium, strain 96CJ10356, was isolated from the coastal region of Cheju Island. It was a gram negative, rod-forming bacterium that required 1~5% salt in the medium for growth, which was identified as *Hahella chejuensis* [17]. The EPS produced by this microorganism was designated EPS-R [18]. Herein some of the biochemical and rheological characteristics of the purified EPS-R produced by this strain are reported.

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MATERIALS AND METHODS

Microorganism and Culture Conditions

The marine bacterium strain 96CJ10356 was used and deposited as *Hahella chejuensis* (KCTC 2396^T = IMSUN 1157^T). A culture was grown in STN medium containing 20 g sucrose, 10 g tryptone, 10 g NaCl, 5 g $MgSO_4$, 1 g $CaCl_2$, 83 mg KH_2PO_4, 67 mg K_2HPO_4, 5 mg $FeCl_2$, 1 mg $MnCl_2$, 1 mg $ZnCl_2$ and 1 mg $NaMoO_4$ per liter at pH 7.0. The cultivation was carried out in 3 L volumes at 25°C in a 5 liter jar fermentor (Ko-Biotech Co. Ltd., Korea) with an aeration rate of 0.17 vvm [18,19].

Purification of EPS-R

Cell suspensions were harvested at the late stationary phase of growth (120 h) and heated to 60°C in the presence of 0.1 M NaOH and 0.03 M Na_2-EDTA (pH 10). The culture broth was diluted five times with deionized water, and the cells removed by centrifugation at 10,000 × *g* at 4°C for 45 min. The pigment produced by the strain was removed from the culture broth with methanol and chloroform and the precipitate obtained by centrifugation at 10,000 × *g* at 4°C for 45 min, which was then diluted with deionized water. The crude EPS-R solution was dialyzed against five volumes of deionized water, using Viva-Flow (Sartorius, Germany), and was finally lyophilized. The crude EPS-R was dissolved in deionized water and reprecipitated by the addition a 3% solution of cetyltrimethylammonium bromide (Cetavolon, Merk, Germany). The precipitated complex was collected by centrifugation (10,000 × *g*, 20 min, 4°C), redissolved in a 10% NaCl solution and recovered by the addition of three volumes of ethanol. The extracted EPS-R was dissolved in deionized water and dialyzed against deionized water for two days. Further purification was achieved by gel chromatography on a Sepharose 4B column (Sigma, USA), followed by elution with 0.4 M NaCl. The carbohydrate-containing fractions were collected, dialyzed using Viva-Flow and lyophilized.

Assay of Emulsification Activity

The emulsification activity was evaluated according to the method described by Cameron *et al.* [20]. A 4 mL of aqueous 0.5% (w/v) EPS-R solution was mixed with 6 mL of kerosene in a test tube and then vortexed for 2 min. After 24 h, the proportion of kerosene emulsified was compared with the total volume of kerosene added. Parameter (A) was an estimate of the percentage of the emulsified kerosene phase. The E_{24} was calculated as the height of the emulsion layer divided by the total height × 100. The kerosene content of the emulsion (B) was calculated by dividing the volume of kerosene in the emulsion phase by the total emulsion volume. No emulsion was generated when STN medium was mixed with kerosene.

Analytical Procedures

The sugar composition of EPS-R was identified by thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC). EPS-R was hydrolyzed with 2 M sulfuric acid at 100°C for 5 h. Then, the solution was neutralized with saturated $Ba(OH)_2$. TLC was performed on cellulose plates F_{254S} (Merk, Germany) using two solvent systems, *n*-butanol-water-acetic acid (60:20:20) and ethylacetate-pyridine-water-acetic acid (100:35:25:5). Sugar spots were visualized by spraying the plates with a solution of aniline-phthalic acid in water-saturated butanol, followed by incubation at 100°C for 5 min. HPLC analysis was performed on a YMC-Pack NH_2 column (4.6 × 250 mm YMC Co., Japan). Elusion was performed with water-acetonitrile (15:85) at a flow rate of 1.5 mL/min, using a refractive index detector (HP 1047A, Hewlett Packard, Germany) at 35°C.

The average molecular weight of EPS-R was determined by gel permeation chromatography. A 0.5 mL sample containing 5 mg of EPS-R was layered onto a glass column (Bio-Red, 2 × 87 cm) packed with Sephadex G-200 (Pharmacia, Sweden) and eluted with 0.4 M NaCl solution at 12 mL/h. The collected fractions were analyzed for total carbohydrate content using dextran standards (71, 580, and 2,000 KDa). The shown data are the averages of two independent determinations, with a standard deviation of < 10%. Infrared (IR) spectra were analyzed as KBr discs with an FT-IR spectrophotometer (Nicolet Magma 550).

A differential scanning calorimeter (model DSC 910, Dupont, Wilmington, DE, USA) equipped with a cooling system was used to record thermograms of single and mixed gels during heating and cooling over the temperature range 5~300°C at a scan rate of 2.5°C/min. An empty, sealed pan was used as the thermal reference. The thermal transition temperature and enthalpy were determined using an internal curve integration program. Two replicate measurements were made for each experiment.

Rheological Characterization of EPS-R

To measure the intrinsic viscosity [η] of each EPS-R solution in deionized water, Huggins and Kraemer plots of the relative viscosity and specific viscosity against concentrations were used in the range 0.01-1.0 g/dL, with measurements made on an Ubbelohde capillary viscometer (536 13/Ic, SCHOTT-GERÄTE, Hofheim, Germany) at 25°C. After measuring the flow time of EPS-R against deionized water, the relative viscosity (η_r), specific viscosity (η_{sp}), reduced viscosity (η_{red}), and inherent viscosity (η_{Inh}) were calculated [21].

The overlap concentration of EPS-R was measured by the following equation [12]:

$$\log \eta_{sp} = \log a + b \log C[\eta]$$

The concentration of a dilute solution was less than

Table 1. Emulsification activity of EPS-R relative to commercial polysaccharides after 24 h

Polysaccharide (0.5%, w/v)	E_{24}[a] (%)	A[b] (%)	B[c] (%)
EPS-R	89.22 ± 2.31[d]	96.17 ± 3.15	64.17 ± 2.23
Xanthan gum	67.80 ± 3.44	49.67 ± 2.96	43.95 ± 3.84
Gellan gum	2.01 ± 0.11	1.67 ± 0.32	49.34 ± 0.12
Sodium alginate	1.02 ± 0.01	0.12 ± 0.03	0.13 ± 0.01

a. emulsification index after 24 h, b. amount of emulsified kerosene phase, c. amount of kerosene phase in emulsion, d. Means ± standard deviations of three replicate experiments

that of the overlap solution: $C < C^*$ and $1.1 < b < 1.6$, where C^* is the overlap concentration, which can be assumed as a boundary concentration between dilute and concentrated solutions. The concentration of a concentrated solution was greater than that of the overlap solution: $C > C^*$ and $1.9 < b < 5.6$.

The chain stiffness of EPS-R was calculated using the Smidsrφd and Hang equation [22]:

$$[\eta] = A + B\,[\eta]_{0.1}^{1.3}\,Cs^{-1/2}$$

where, A is a constant, B is the chain stiffness, $[\eta]_{0.1}$ is the intrinsic viscosity at 0.1 M NaCl, and Cs is the salt concentration.

To investigate the steady shear flow properties, the lyophilized crude EPS-R was solubilized in distilled water. Agitation was maintained overnight at 4°C. The concentration of the EPS-R stock solution was determined by the phenol-sulfuric acid method [23]. This stock solution was diluted to the desired concentration (ranging from 10 to 0.01 g/L) and homogenized. All polysaccharide solutions were centrifuged at 10,000 × g for 10 min to remove air bubbles prior to measurements.

Apparent viscosity measurements of the solutions were achieved using a rotational spindle viscometer LTV fitted with a small sample adaptor (DV-III, Brookfield, USA). The measurements of the apparent viscosity were performed at different shear rates for each concentration (0.01, 0.02, 0.1, 0.2, and 0.5%, w/v) of EPS-R and xanthan gum fluids. Spindle SC4-32 measured from 0.28 to 56.0 sec^{-1} and SC4-18 from 1.32 to 132 sec^{-1}.

The pH of the two polysaccharide solutions was controlled with 1 M HCl and 1 M NaOH, and the effect of temperature and heat treatment investigated over the ranges 20-90°C and 121-25°C. The effects of the addition of different salts (NaCl and $CaCl_2$) over a range of concentrations (0.25-5% w/v in a constant volume of 1% EPS-R) on the rheological properties of EPS-R were also investigated.

RESULTS AND DISCUSSION

Emulsification Activity of EPS-R

The purified emulsifier EPS-R was tested for the production of stabilizing emulsions and compared with xanthan gum, gellan gum and sodium alginate as a commercial emulsifying agent (Table 1). An E_{24} of 0.5% EPS-R was able to form an emulsion with an emulsified kerosene content of 89.2%. This kerosene content was higher than those emulsified by the commercial emulsifying agents, xanthan gum (67.8%), gellan gum (2.01%) and sodium alginate (1.02%). The emulsification stability (Fig. 1) was compared with that of xanthan gum using a different EPS-R concentrations (0.1 and 0.5%) and time periods (1 h, 24 h, and 72 h). The EPS-R emulsions were stable relative to those of xanthan gum, although slight decreases in emulsion stability (< 10%) were observed between 24 and 72 h. EPS-R 0.5% (w/v) emulsified a variety of tested oil and organic solvents (Table 2), but maximal emulsifying activity was observed with kerosene (89.2%) and n-hexane (83.5%). Limited emulsifying activity was observed with cyclohexane (15.1%) and methylene chloride (13.2%), but no emulsifying activity was observed with n-butanol. EPS-R seems to be an effective emulsifier, as complete emulsification of kerosene was obtained at 0.5% (w/v). In most cases, the emulsification stability of a polysaccharide is considered to be a non-adsorbing 'depletion stabilization'. Polysaccharides are known to have significantly less surface activity compared to proteins [24]. Chemically modified polysaccharide derivatives such as highly-substituted methyl cellulose have similar levels of surface activity at the oil-water interface as those of other food emulsifiers [25]. Substan-

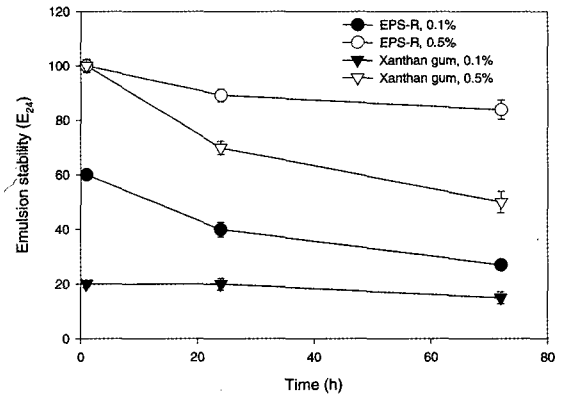

Fig. 1. Stability of EPS-R and xanthan gum emulsions over time (a. E_{24} was calculated as the height of the emulsion layer divided by the total height × 100).

Table 2. Emulsification of organic solvents by EPS-R 0.5% (w/v) after 24 h

Non-aqueous phase	E_{24}[a] (%)	A[b] (%)	B[c] (%)
Kerosene	89.22 ± 2.31[d]	96.17 ± 3.15	64.17 ± 2.23
n-hexane	83.52 ± 3.45	96.43 ± 1.34	69.88 ± 2.82
Benzene	30.21 ± 2.25	33.23 ± 1.33	66.67 ± 1.22
Ethyl acetate	10.27 ± 2.13	8.30 ± 0.92	50.11 ± 3.24
Cyclohexane	15.12 ± 2.03	8.30 ± 1.43	33.33 ± 3.74
Methyl chloride	13.22 ± 2.34	11.36 ± 1.12	53.85 ± 1.72
Chloroform	15.10 ± 1.01	13.30 ± 2.14	53.33 ± 2.73
Butanol	1.12 ± 0.02	0.17 ± 0.01	10.02 ± 0.17

a. emulsification index after 24 h, b. amount of emulsified non-aqueous phase in emulsion, c. amount of non-aqueous phase in emulsion, d. Means ± standard deviations of three replicate experiments

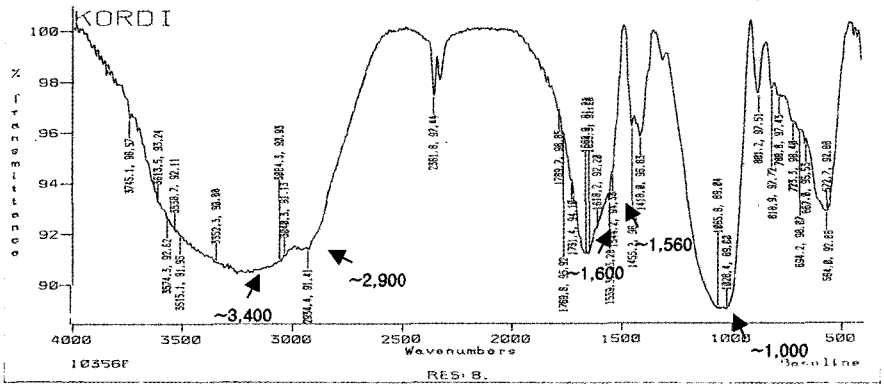

Fig. 2. Infra-red absorption spectrum of EPS-R produced by *Hahella chejuensis* strain 96CJ10356 (1. Infrared (IR) spectra were analyzed as KBr disics with an FT-IR spectrophotometer (Nicolet Magma 550)).

tial surface activity at the air-water interface has been reported with 1.0% (w/v) solutions of bacterial xanthan gum [26].

Chemical Characteristics of EPS-R

The compositional analysis of EPS-R hydrolysates revealed a heteropolysaccharide consisting of glucose and galactose in the molar ratio 1:6.8, with xylose and ribose as minor sugars. The molecular weight of EPS-R as determined by gel permeation chromatography was approximately 2.2×10^5 KDa.

The analysis of the polysaccharide chemical group characteristics from the infrared spectrum of EPS-R showed -OH stretching at ~3,400 cm^{-1}, C-H stretching at ~2,900 cm^{-1}, stretching vibrations of the carboxylate group at ~1,600 cm^{-1} and the amine group ($-NH_3$) at 1560 cm^{-1}, C-O-C antisymmetrical stretching or a polysaccharide sugar ring at ~1,000 cm^{-1}, and CO_2 in air at 2,400 cm^{-1}, which was similar to typical polysaccharide IR spectra (Fig. 2).

DSC analysis enabled the pyrolytic characterization of EPS-R (Fig. 3). The pyrolysis of EPS-R was investigated

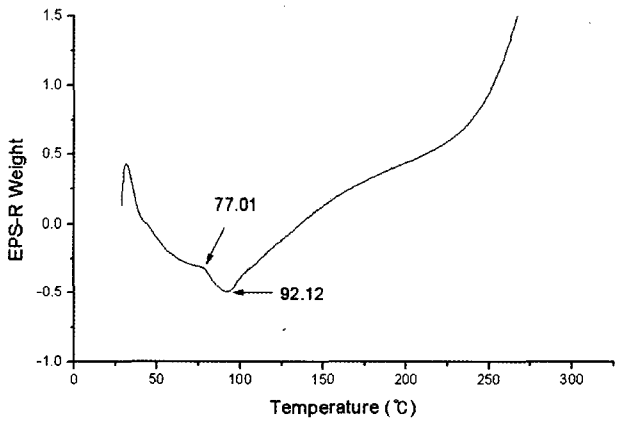

Fig. 3. Differential scanning calorimeter (DSC) thermogram of EPS-R produced by *Hahella chejuensis* strain 96CJ10356 (1, Differential scanning calorimeter (model DSC 910, Dupont, Wilmington, DE, USA) equipped with a cooling system was used; 2, Thermogram was recorded during heating and cooling over a temperature range of 5~300°C; 3, Scan rate was 2.5°C/min).

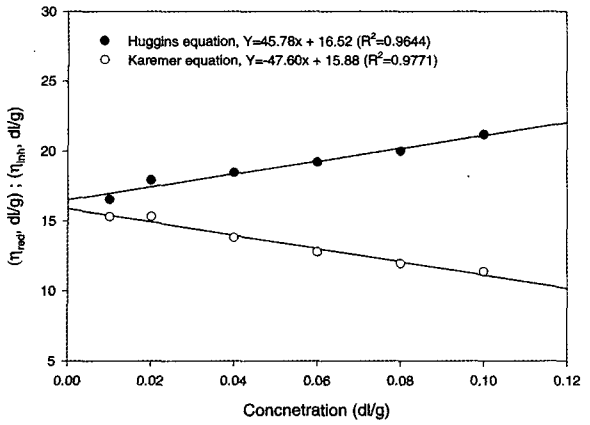

Fig. 4. Intrinsic viscosity of EPS-R fluid calculated by the reduced viscosity and inherent viscosity equations (1. Intrinsic viscosity [η] was measured by Huggins and Kraemer plots of relative viscosity; 2. Specific viscosity against concentrations in the range of 0.01-1.0 dL/g; 3, Ubbelohde capillary viscometer (536 13/Ic, SCHOTT-GERÄTE, Hofheim, Germany) at 25°C.).

from 77.0 to 92.1°C. The first transition was associated with the dissociation of aggregates, and the second transition represented melting. Further studies of the structure of EPS-R are in progress.

Intrinsic Viscosity of EPS-R

The intrinsic viscosity was determined from the capillary viscometer of EPS-R at concentrations ranging from 0.01 to 0.1% (w/v). Here, the viscosity relative to that of water (the solvent) was within the range of $1.2 < \eta_r < 2.0$. The results of the experiments carried out at high concentrations and with different shear rates suggest that, under these conditions, the solution viscosity is essentially Newtonian [27]. The results for the reduced and inherent viscosity are shown in Fig. 4. The intrinsic viscosities of EPS-R were 16.5 and 15.9 g/dL, as calculated using the Huggins equation and the Kraemer equation, respectively. The intrinsic viscosity of EPS-R was η (*i.e.*, $\eta=16.5$ g/dL), which implies the solutions of EPS-R have viscous effects at low concentrations.

The pH and Salt Dependency of EPS-R

The pH dependency of the reduced viscosity (η_{sp}/C) was highest at pH 9 with EPS-R 0.1% (w/v), but there was little variation. The salt dependency (η_{sp}/C) in EPS-R 0.1% (w/v) suddenly decreased as the concentration approached 0.01 M NaCl and remained constant at higher NaCl concentrations (Fig. 5).

Overlap Concentration of EPS-R

The overlap concentration was measured by log plots of the intersection of two linear branches (Fig. 6). The logarithmic representation of η_{sp0} as a function of the EPS-R concentration exhibited behavior typical of polysaccharide solutions. The C* of EPS-R was 0.15 g/dL. At $C_c < C^*$ (diluted solution), the b value (slope) was 1.3 ($1.1 < b < 1.6$), and the degree of coil overlap of EPS-R fluid was low. The EPS-R coil did not interact with

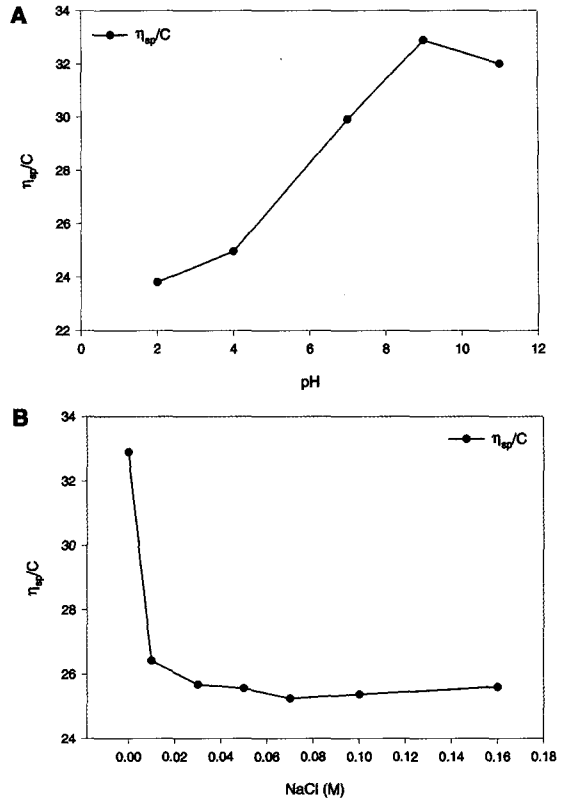

Fig. 5. The pH and salt dependency of the intrinsic viscosity of EPS-R produced by Hahella chejuensis 96Cj10356 (A, pH salt dependency of the intrinsic viscosity; B, salt (NaCl) dependency of the intrinsic viscosity).

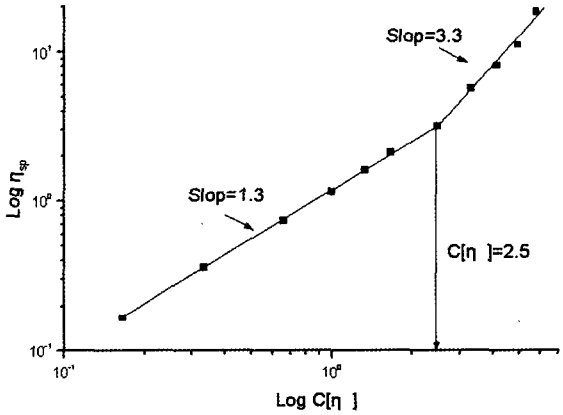

Fig. 6. Double logarithmic plots of concentrated-dependence of 'zero-shear' viscosity of EPS-R fluid (1. The overlap concentration was measured by plotting logarithm into the intersection of two linear branches. 2. Variation of specific viscosity (η_{sp}) with space occupancy c[η] for EPS-R fluid).

Table 3. Overlap concentrations and chain stiffness of EPS-R and other polysaccharides

Polysaccharide	b(dilution)[a]	b(concentration)[a]	C[η][b]	B[c]
EPS-R	1.3	3.3	2.5	4.8×10^{-2}
Xanthan gum	1.1	4.0	3.2	5.0×10^{-3}
Alginate	1.4	3.3	4.0	4.0×10^{-2}
Carboxymethyl amylose	1.4	3.3	4.0	2.0×10^{-1}
Carboxymethyl cellulose	1.3	3.2	3.2	5.0×10^{-2}

a, exponent (b) in diluted and concentrated fluid; b, degree of overlap; c, chain stiffness parameter (B).

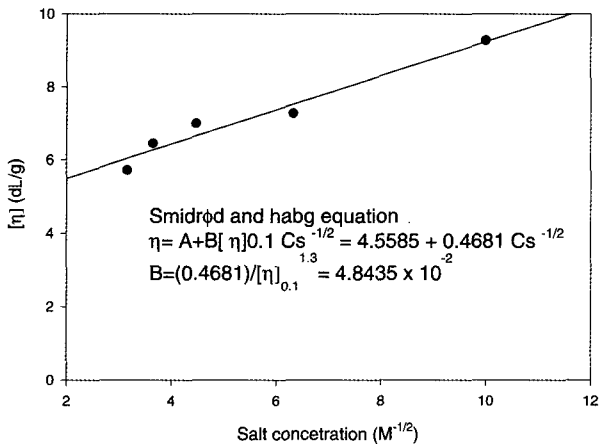

Fig. 7. Dependency of intrinsic viscosity on salt concentration for EPS-R (1. The chain stiffness of EPS-R was calculated using the Smidsrϕd and Hang equation; A is a constant, B is the chain stiffness, $[\eta]_{0.1}$ is the intrinsic viscosity at 0.1 M NaCl, and Cs is the salt concentration).

neighboring coils, but had hydrodynamic interactions with polymeric coils. At $C_c > C^{**}$ (concentrated solution), the b value was 3.3 ($1.9 < b < 5.6$). At these concentrations the EPS-R molecule overlapped and the entanglement increased. Comparisons of the EPS-R' exponent (b) in the diluted and concentrated fluids and C[η] with the values for reported polysaccharides are shown in Table 3. The degree of overlap between polymeric coils is termed C[η], and its value for EPS-R was 2.5.

Chain Stiffness of EPS-R

The chain stiffness of EPS-R could be estimated by the Smidsrϕd and Hang equation, using the calculated intrinsic viscosity. The chain stiffness parameter (B) of EPS-R was calculated as 4.8×10^{-2} (Fig. 7) using the Huggins equation. The B values of polyelectrolytic polysaccharides generally range from 0.05 to 0.24 (Table 3). Low B values for polysaccharides indicate a rigid rod form, while high B values indicate a flexible conformation [28]. In polysaccharides, the chain stiffness depends on the side chain units owing to the interactions between the side chains and molecular backbone. In terms of rigidity, the EPS-R conformation appeared to be similar to that of caboxymethylcellulose (5.0×10^{-2}).

Steady Shear Flow Property of EPS-R

Most non-Newtonian fluids without residual tension are represented by the Power-law equation, in which the apparent viscosity is,

$$\eta_a = kr^{n-1}$$

where, k is the consistency index of the fluid and n is the flow behavior index.

In this model the parameter η constitutes a physical property that characterizes the fluid's degree of non-Newtonian behavior: when $n < 1$, the fluid is pseudoplastic. The parameter k indicates the relative viscosity or thickness of a fluid: the higher the value of k, the thicker or more viscous the fluid. EPS-R solutions showed characteristic non-Newtonian behaviors in terms of fluid properties (Fig. 8). In aqueous dispersions of 1% EPS-R, the consistency index (K) and flow behavior index (n) were 1,410 and 0.73, respectively. According to the Power-law model, EPS-R solutions were pseudoplastic fluids. The rheological properties of EPS-R were influ enced by the salt concentration, pH, temperature and the presence of ionic compounds.

The shear stress of EPS-R decreased rapidly with increasing shear rate at all concentrations (Fig. 9A). The pH dependency of EPS-R is shown in Fig. 9B. The shear stress was measured at 25°C and that of EPS-R was especially similar in both acidic and alkaline ranges (pH 2-12), showing stability across a wide pH range. Dilute solutions of macromolecules carrying dissolvable groups (COOH, SO_3H, NH^{3+}, *etc.*) behave differently in media with very different dielectric constants. In media with low dielectric constants, the ionization-dissolvable group is suppressed, and the macromolecules assume random-coil configurations typical of non-ionic polymers. In media with both high and low dielectric constants, the dissolvable groups are ionized and macromolecules assume extended configurations between charged groups. Electrostatic interactions appear to be important or dominant factors for determining behavior, along with polymer-solvent interactions and static and van der Waals interactions between chain segments. Therefore, it could be anticipated that EPS-R contains an ionic group. The effects of different salts on the rheological properties of EPS-R are shown in Figs. 9C and 9D. NaCl at concentrations ranging from 0.25 to 5.0% (w/v) and $CaCl_2$ at concentrations ranging from 0.25 to 2.0% (w/v) were tested in a

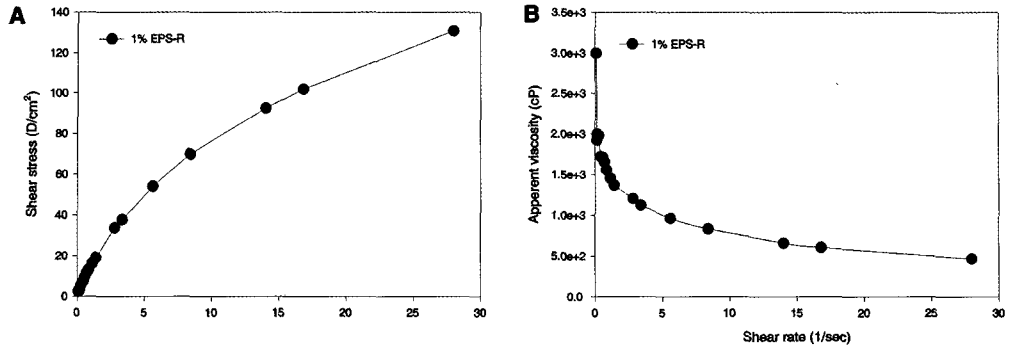

Fig. 8. Variation in shear stress and apparent viscosity of EPS-R 1.0% (w/v) fluid (A, Relationship between shear stress and shear rate of EPS-R 1.0% solution; B, Relationship between apparent viscosity and shear rate of EPS-R 1.0% solution).

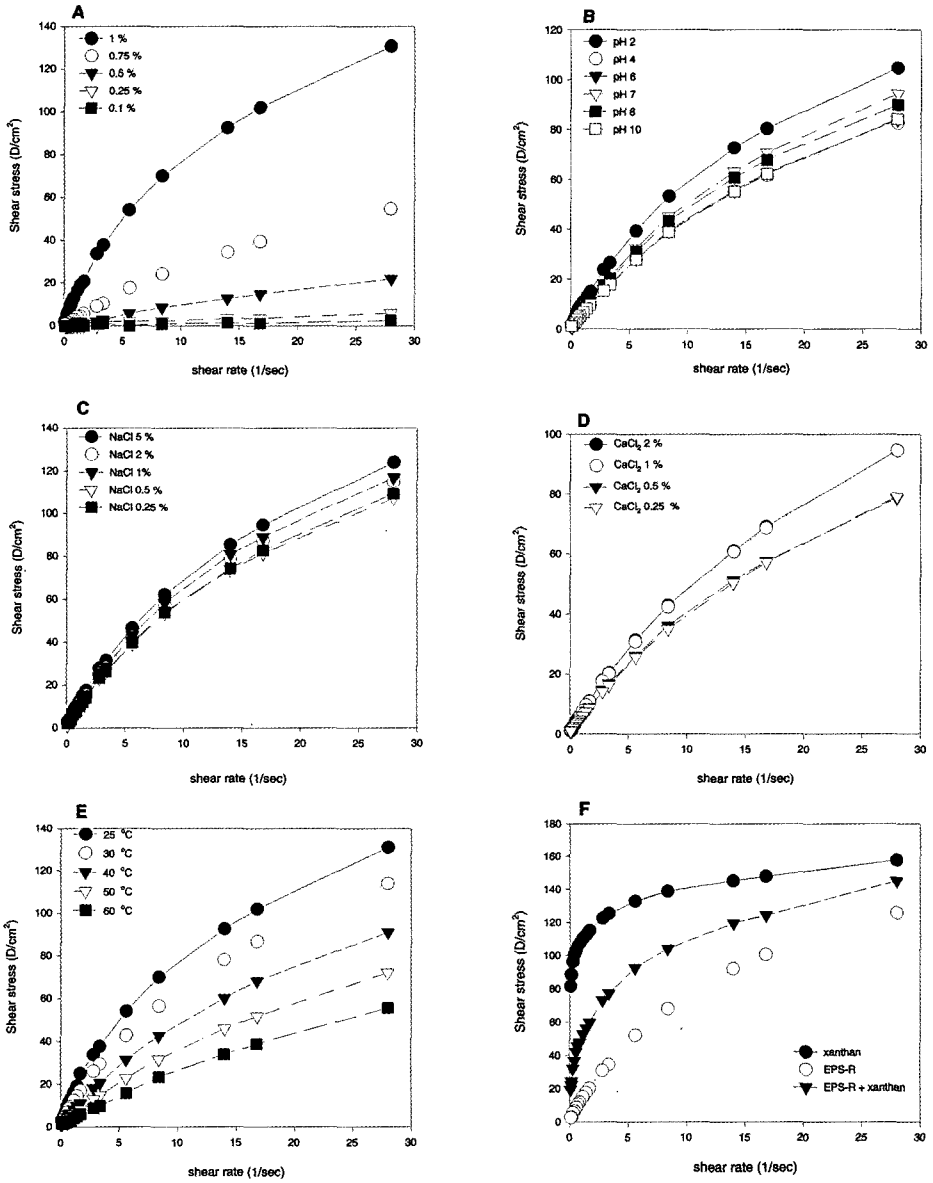

Fig. 9. Comparison of shear stresses and shear rates of EPS-R fluids (A, EPS-R concentration; B, pH; C, NaCl; D, $CaCl_2$; E, temperature; F, mixed with xanthan gum).

constant volume of 1% EPS-R fluid. The shear stress of EPS-R was similar following the additions of NaCl and $CaCl_2$, and its molecules appeared to be stable toward salts. Interactions between the counter ions (Na^+ and Ca^{2+}) within charged sites on the macromolecular backbone are important in determining the properties of a polyelectrolytic solution [29]. The observed changes in the rheological properties of EPS-R with changing temperature are described in Fig. 9E. The shear stress of EPS-R decreased with increasing temperature from 25 to 60°C, and increased with the addition of xanthan gum (Fig. 9F). Therefore, EPS-R was investigated to be stable in the variation of pH and salt concentration.

In conclusion, EPS-R is a novel emulsifier produced by the marine bacterium *Hahella chejuensis* strain 96CJ10356. Studies on the yield optimization will be requiring before estimations of its commercial potential can be made.

Acknowledgement This study was supported by a grant to HKL from the Development of Novel Marine Compounds Program of the Korean Ministry of Maritime Affairs and Fisheries.

Abbreviations *k*, consistency index; n, flow behavior index; E_{24}, emulsification index (24 h); A, percentage of kerosene; B, kerosene content of emulsion; η_{app}, apparent viscosity; η_r, relative viscosity; η_{sp}, specific viscosity; η_{red}, reduced viscosity; η_{Inh}, inherent viscosity; C[η], degree of overlap; Cetavolon, cetyltrimethylammonium bromide.

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[Received July 28, 2004; accepted September 8, 2004]