ORIGINAL ARTICLE

Physio-chemical and biological properties of partially purified exopolymers from newly isolated halophilic bacterial strain SM5

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Abstract

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An extremely halophilic bacterial strain SM5 producing exopolymers was isolated from Thai traditional fermented fish (Pla-ra). Morphological and biochemical properties and 16S rDNA sequencing revealed that the strain was *Halobacterium* sp. SM5. The exopolymers were identified as a water-soluble acidic polymer. They contained 70.40±0.70% (w/w) protein as a major component and 7.30±0.01% (w/w) sulfate as a minor component, as well as 5.02±0.30% (w/w) total sugar, 3.15±0.10% (w/w) neutral sugar and 2.05±0.10% (w/w) uronic acid. Fourier transform infrared (FT-IR) analysis showed that the exopolymers consisted of carboxyl, hydroxyl, amino and sugar derivative groups. Molecular weight of the exopolymers was $7.7x10^4$ and $2.5x10^5$ Da. Cytotoxicity effect of the exopolymers on the HT29 human cell lines revealed that the exopolymers concentration of 0.1 and 0.5 mg/ml gave the cell a viability of 93.48 and 98.46%, respectively. At the exopolymers concentration of 1.0, 1.5 and 3.0 mg/ml, the cell viability was 71.65, 74.28 and 57.77%, respectively. The exopolymers showed pseudoplastic property, resistance to shearing, and

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high viscosity (600 cP). Viscosity was increased with the exopolymers concentration (0.5-4.0% w/v). The exopolymers were stable over a wide pH range (6-8), and their viscosity decreased at temperatures above 60°C.

Key words : exopolymer, *Halobacterium* sp., halophilic bacteria, rheology

บทคัดย่อ

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สมบัติทางเคมี-กายภาพและชีววิทยาของพอลิเมอร์ภายนอกเซลล์ ที่ทำให้บริสุทธิ์บางส่วนจากแบคทีเรียชอบเกลือสายพันธุ์ SM5 ที่แยกได้ใหม่

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แยกแบคทีเรียชอบเกลือและผลิตพอลิเมอร์สายพันธุ์ SM5 จากอาหารหมักดั้งเดิม (ปลาร้า) จากลักษณะทางสัณฐานวิทยา สมบัติชีวเคมีและลำดับเบสของ 16 S rDNA ระบุว่าคือ *Halobacterium* sp. SM5 เมื่อนำพอลิเมอร์มาวิเคราะห์องค์ประกอบและศึกษาสมบัติต่าง ๆ พบว่า พอลิเมอร์ละลายน้ำมีประจุลบ ประกอบด้วยโปรตีน 70.40±0.70% โดยน้ำหนักเป็นองค์ประกอบหลัก และซัลเฟต 7.30±0.01% โดยน้ำหนักเป็นองค์ประกอบรอง รวมทั้งมีน้ำตาลทั้งหมด 5.02±0.30% โดยน้ำหนัก น้ำตาลนิวทรอล 3.15±0.10% โดยน้ำหนัก และกรดยูโรนิก 2.05±0.10% โดยน้ำหนัก ผลของ FT-IR แสดงว่าพอลิเมอร์ประกอบด้วยหมู่คาร์บอนิล ไฮดรอกซิล อะมิโน และอนุพันธ์ของน้ำตาล พอลิเมอร์มีน้ำหนักโมเลกุล 7.7x10^4 และ 2.5x10^5 ดาลตัน ผลการทดสอบความเป็นพิษของพอลิเมอร์ต่อเซลล์มะเร็ง (HT 29 human cell lines) พบว่าที่ความเข้มข้นของพอลิเมอร์ 0.1 และ 0.5 มก./มล. การรอดชีวิตของเซลล์มะเร็งเท่ากับ 93.48 และ 98.46% ตามลำดับ ส่วนที่ความเข้มข้น 1.0, 1.5 และ 3.0 มก./มล. การรอดชีวิตของเซลล์มะเร็งเท่ากับ 71.65, 74.28 และ 57.77% ตามลำดับ สารละลายพอลิเมอร์แสดงสมบัติเป็นซูโดพลาสติก ทนต่อแรงเฉือนและมีความหนืดสูง (600 cP) พบว่าความหนืดเพิ่มขึ้นตามความเข้มข้นของพอลิเมอร์ที่เพิ่มขึ้น (0.5-4.0% โดยน้ำหนัก/ปริมาตร) พอลิเมอร์มีความเสถียรที่พีเอชช่วงกว้าง (พีเอช 6-8) และเมื่อเพิ่มอุณหภูมิมากกว่า 60°C ความหนืดจะลดลง

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Exopolymers of microorganisms, either in capsule form or as slime layer, are mainly composed of sugar and protein. The exopolymer xanthan, which is widely used in industry, comes from *Xanthomonas campestris* (Crossman and Dow, 2004; Magnin *et al.*, 2004; Vendruscolo *et al.*, 2005). Others are gellan from *Sphingomonas panimobilis* (Lee *et al.*, 2004), curdlan from *Agrobacterium* (Saudager and Singhal, 2004), pullulan from *Aureobasidium pullulans* and *Kluyveromyces fragilis* (Seo *et al.*, 2004; Lppolito *et al.*, 2005) and levan from *Zymomonas mobilis* and *Erwinia herbicola* (Bekers *et al.*, 2005). Such microbial exopolymers can be used to modify the rheological property of aqueous systems and thus enhance the viscosity of the solution (Sutherland, 1986). Exopolymers are used to modify the flow characteristics of fluids, to stabilize suspensions, to flocculate particles, to encapsulate materials, to enhance oil recovery and as drag-reducing agents for ships.

Various types of exopolymers are in high demand for practical applications. However, only a small number of exopolymer-producing bacterial strains have been used for industrial applications to date. It is still necessary to isolate new pro-

ducers of exopolymers with useful chemical and physical properties. The potential use of extremely halophilic archaea to produce an exopolymer in biotechnological processes is mainly based on their extreme salt tolerance, which enables cultivation without sterile precautions. Moreover, extremely simple production systems can be developed, such as open pond, which obviously reduces production costs. (Anton *et al.*, 1988).

In previous paper, we used mixture design and response surface method to optimize exopolymer productivity from a halophilic bacterial strain SM5 (Lungmann *et al.*, 2007). In this paper the physical, chemical and biological properties of the partially purified exopolymers from the extremely halophilic bacterial strain SM5 are reported.

Materials and Methods

Strain identification

An extremely halophilic bacterial strain SM5 was isolated from Thai traditional fermented fish (Pla-ra) samples collected from the market in Songkhla, Thailand. The strain, SM5 was identified according to Bergey's Manual of Systematic Bacteriology (Grant *et al.*, 2001). Gram staining was performed by fixing the sample with 2% acetic acid (Dussault, 1955). Growth at different salt concentrations was determined by adding NaCl to the KS medium (Kamekura and Seno, 1990). Determination of catalase, oxidase, arginine dihydrolase, lysine decarboxylase, citrate and acetate utilization were performed according to the method described by MacFaddin (1980). Urease and gelatinase were tested using API 20E (BioMerieux, France). Carbohydrate utilization was tested in phenol red broth containing (per liter) 250g NaCl, 20g $MgSO_4 \cdot 7H_2O$ and 2g KCl. (Thongthai *et al.*, 1992). Temperature for growth of the bacterium was studied in the range of 20 to 50°C using the modified KS medium at pH 7.0. Growth at different pH (5 to10) was performed in the modified KS medium. The morphology of the cells was studied using scanning electron microscopy (SEM) as described by Denner *et al.* (1994). 16S rDNA was performed with extracted and purified DNA according to the method described by Sambrook *et al.* (1989). A primer pair was used to specifically amplify archaeal 16S rDNA fragments. Forward and reverse primers used for archaeal sequence were D30 (5'-ATTCCGGTTGATCCTGC-3') and D56 (5'-G(CT) TACCTTGTTACGACTT-3'), respectively. The amplified fragment was sequenced using an automated DNA sequencer (ABI 377). The sequence was compared with other sequences from the Gene Bank using the Blast (Basic Local Alignment Search Tool) search program of the National Center for Biotechnology Information. The phylogenetic tree was constructed by the neighbor-joining programs that form part of the PHYLIP package version 3.62.

Media and culture conditions

KS medium contained, per liter 250g NaCl, 10g yeast extract, 20g $MgSO_4 \cdot 7H_2O$, 7.5g vitamin casamino acid, 2.0g KCl and 0.05g $FeSO_4 \cdot 7H_2O$, pH of the medium was 7.0±0.2 (Kamekura and Seno, 1990). Modified KS medium contained, per liter, 250g NaCl, 7.425g glucose, 12.375g yeast extract, 17.325g $MgSO_4 \cdot 7H_2O$, 9.9g vitamin casamino acid, 2.475g KCl and 0.05g $FeSO_4 \cdot 7H_2O$ with a pH of 6.5±0.2 (Lungmann *et al.*, 2007).

The strain SM5 was transferred to 100 ml KS medium in a 250 ml Erlenmeyer flask and incubated on an orbital shaker (New Brunswick G25-KLG) at 200 rpm at 37°C until its growth reached early stationary phase. Adjustment was made to achieve an optical density (OD_{660}) of 0.5 with the KS medium, and 10 ml of cells suspension was then transferred into the modified KS medium. The culture was cultivated under the same conditions as mentioned above for 5 days. Growth was determined in terms of turbidity using a spectrophotometer (Hitachi U-2000) at a wavelength of 660 nm.

Harvesting and partial purification of the exopolymers

After removing the cell pellet by centrifugation, 5 ml of the supernatant was precipitated with

3 volumes of cold 95% ethanol and left standing overnight at -20°C. The precipitated exopolymers were re-dissolved in distilled water and transferred to a dialysis membrane with a molecular cut-off at 10,000 daltons. Dialysis of the sample was carried out against distilled water overnight at 4°C. Finally, the partially purified exopolymers were lyophilized.

Physical and chemical properties

The electric charge of the partially purified exopolymers was determined using a Zetasizer4 (Malvern Inst. Lim.), ZET 5104 angle detector (λ = 633 nm), and determined by precipitation with cetylpyridium chloride (CPC) (Scott, 1965). The infrared spectrum was measured using a Fourier transform infrared spectrophotometer (FT-IR Model Equirox-55, Bruker) and the partially purified exopolymers sample was prepared according to the potassium bromide (KBr) disk method. The average molecular weight of the partially purified exopolymers was determined using gel permeation chromatography. The analysis was carried out using a PL-GPC 110 system (Polymer Lab, UK) together with a refractive index (RI) detector and an ultrahydrogel linear column (300x7.8 mm ID, Water, USA). The universal calibration log (*Mp*) versus V_R where *Mp* is the peak molecular weight was obtained by using pullulan standards with molecular weight ranging from 5,900 to 788,000 Da. The partially purified exopolymers 1 mg was tested for its solubility in 1 ml of the following solvents: distilled water, acetone, ethanol, methanol, hexane, dichloromethane, isopropyl ether, 2-propanol and chloroform. Each exopolymers mixture was vigorously stirred and allowed to stand for 24 h before determining the solubility levels (Collins *et al.*, 1973).

Total sugar was determined by the phenol-sulfuric acid method (Dubois *et al.*, 1956) and expressed as a glucose equivalent. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin (Sigma) as a standard. Sugar derivatives were investigated by the carbazol-sulfate reaction for uronic acid, anthrone reaction for neutral sugar (Chaplin and Kennedy, 1986) and the turbidimetric method for sulfate (Dodgson and Price, 1962).

Biological property

Cytotoxicity tests of the partially purified exopolymers were carried out according to Carreno-Gomez and Duncan (1997). HT29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, BRL, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, BRL, USA) plus 100 µg/ml Antibiotic-Antimycotic (10,000 IU/ml, Gibco, BRL, USA). After the growth of HT29 cells reached a logarithmic phase, they were detached by the addition of 2 to 3 ml of a 0.05% trypsin (Gibco, 1: 250)-0.02% EDTA mixture and incubated for 2 to 5 min at 37°C. The 100 µl cell suspension (equal to 3,000 cells) was seeded into each well of microtitre-plates. Then 100 µl of the pasteurized partially purified exopolymers solution (60°C, 15 min) at a concentration in range of 0.1-3.0 mg/ml was added to the plates. The mixture was left for 4 days, then it was fixed and stained according to the method described by Skehan *et al.* (1990). Absorbance at a wavelength of 492 nm was read on a 96-well plate reader (Anthos-2001, Anthos labtech instruments, A-5022, Salzburg).

Rheological property

The partially purified exopolymers were dissolved in distilled water to give a 1 to 4% (w/v) solution. The viscosity of the solutions was measured with a Brookfield viscometer DVII with spindle SC4-18/13R. Effects of pH (in range of 5-12) and temperature (in range of 4-80°C) on the viscosity of the exopolymers solution were measured.

Results and Discussion

Strain identification

The extremely halophilic bacterial strain SM5 was isolated from fermented fish (Pla-ra), which is a proteinaceous salted product. The cells of strain SM5 were orange-red in color, Gram

Table 1. Main characteristics of strain SM5 members of *Halobacterium*

Characteristics	Strain SM5
Source of strain	Pla-ra
Colonies color	Orange- red
Cell shape	Oval
Gram-stain	-
Spore	-
Motile	+
Catalase	+
Oxidase	+
Growth condition	Facultative anaerobic
NaCl requirements (M)	
Range for growth	2.5 - 5.2
Optimal growth	3.5
pH	
Range for growth	6 - 8
Optimal growth	7
Temperature (°C)	
Range for growth	20- 50
Optimal growth	37
Enzyme activity	
Arginine dihydrolase	+
Lysine decarboxylase	+
Hydrolysis of :	
Gelatin*	+
Urea*	-
Acid production from	
Citrate	-
Acetate	-
Glucose	+
Xylose	+
Sucrose	-
Galactose	-
Fructose	-
Ribose	-
Mannitol	+
Rhamnose	+
Sorbitol	-
Arabinose	+

Remark : + = positive, - = negative
*** = biochemical tests were done using API 20E (bioMerieux)**

negative, catalase and oxidase positive, motile, ovoid-shaped and 0.1-1.0 μm in size (Figure 1 and Table 1). The strain SM5 required at least 2.5 M

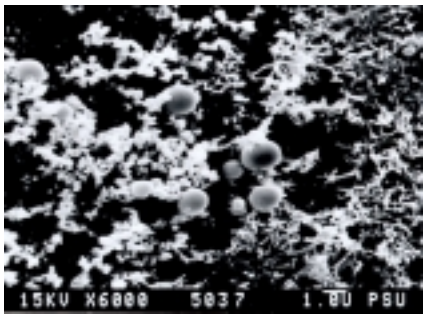

Figure 1. Scanning electron micrograph (X 6000) of *Halobacterium* sp. SM5; bar— 1.0 μm

NaCl for growth. The optimum and maximum NaCl required for growth were 3.5 and 5.2 M, respectively. In the modified KS medium, at a concentration of 3.5 M NaCl, the strain grew over the pH range of 6-8. The strain grew under both facultatively aerobic and anaerobic conditions. The optimum, maximum and minimum temperatures for growth were 37, 50 and 20°C, respectively. The strain could hydrolyze gelatin but not urea. The strain utilized glucose, xylose, mannitol, rhamnose and arabinose as carbon source. The main characteristics of strain SM5 are shown in Table 1. Therefore, the extremely halophilic bacterium strain SM5 resembles members of the genus *Halobacterium*.

Total DNA from the strain SM5 was subjected to PCR amplification with the forward and reverse primers to obtain a fragment from the 16S rDNA gene. The PCR product obtained was analyzed by using an automated DNA sequencer. Sequence-compose 1,346 bases and comparisons of 16S rDNA-encoding gene sequence had clearly indicated that it represented a distinct genus *Halobacterium*. A phylogenetic tree constructed by a neighbour-joining method is presented in Figure 2. Comparison of the *Halobacterium* sp. SM5 sequence with sequences for other halobacteria in the database indicated that it is most closely related to *Halobacterium salinarium* Cer6a, with 99% identity.

Physical and chemical properties

Figure 3 showed the zeta potential of the

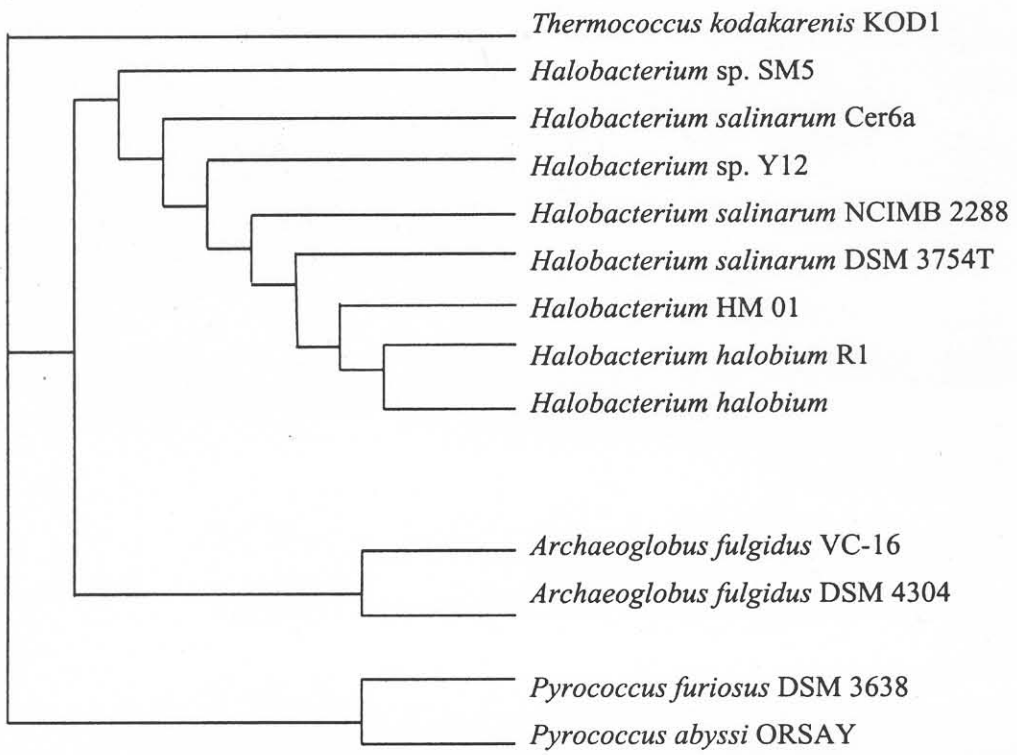

Figure 2. Neighbour-joining tree based on almost completed 16S rDNA sequenceing data. The result showed the relationships between *Halobacterium* sp. SM5 and member of the other *Halobacterium*

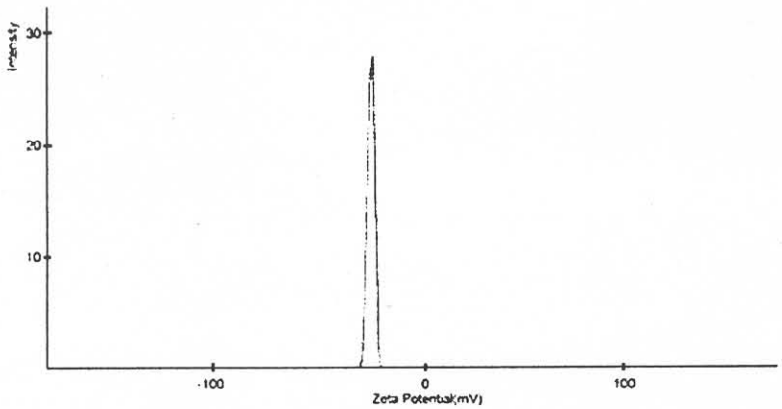

Figure 3. Response of negative charge of the partially purified exopolymers from *Halobacterium* sp. SM5

partially purified exopolymers dispersed in distilled water (pH 7.0) in which the viscosity of dispersing medium was 1.002 cP, at 25.0-25.3°C. The exopolymers are usually negatively charged at an average zeta potential of -25.16±0.44 mV.

Addition of CPC to the exopolymers solution caused precipitation. This suggested the presence of acid groups of pyruvate, succinate,

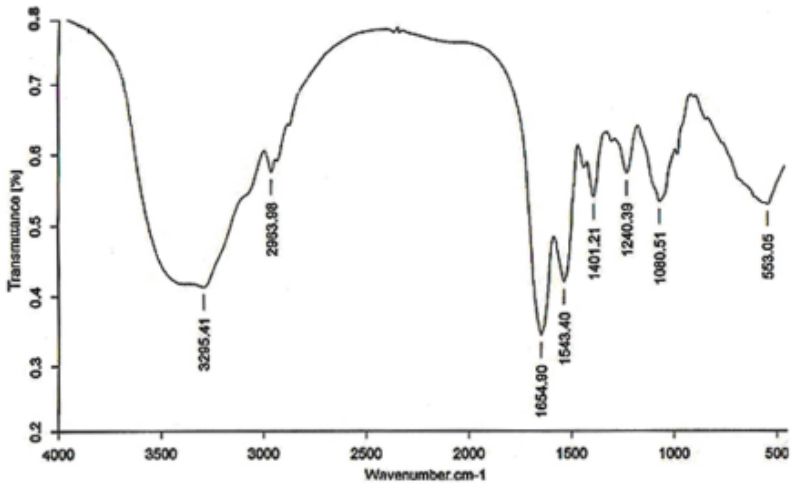

Figure 4. Infrared spectrum of the partially purified exopolymers from *Halobacterium* sp. SM5

uronic acid, acetate or sulfate and phosphate in the molecule (Sutherland, 1977; Sutherland, 2001). Scott (1965) reported that the interaction of acid groups with the quaternary ammonium ion (QN^+) of the CPC results in the formation of the exopolymer-CPC complex. The result also indicated that the exopolymers were an anionic polymer.

The infrared analysis of partially purified exopolymers (Figure 4) displayed a band at around 3295 cm^{-1} which was characteristic for hydroxyl and amine groups and a weak C-H stretching band at 2964 cm^{-1}. The absorption peak displayed in the range of 3000-3600 cm^{-1} indicated NH vibration (Li *et al.*, 2004). The absorption peak at 1543 cm^{-1} was O=C-N stretching indicated the presence of protein. The spectrum also displayed absorption bands of carboxylate groups at 1654 and 1401 cm^{-1}. The carboxylate groups can serve as binding sites for divalent metal ions. The absorption peak at 1250 cm^{-1} was S=O stretching indicated the presence of sulfate. Other bands observed in the range from 1000 to 1200 cm^{-1} are generally known to be typical characteristics of all sugar derivatives such as guluronic acid, manuronic acid and uronic acid (Suh *et al.*, 1997). The infrared spectrum of this partially purified exopolymers thus showed the presence of carboxyl, hydroxyl, amino and sugar derivative groups, which are the preferred groups for the exopolymers similar to that observed in an extracellular polymer from haloalkalophilic *Bacillus* sp. I-450 (Kumar *et al*., 2004).

Chemical analysis of the partially purified exopolymers revealed that the amount of the total protein and the total sugar of the exopolymers were found to be 70.40±0.70 and 5.02±0.03% (w/w), respectively. Hence, protein is the main component of these polymers. The sugar derivatives in the exopolymers are 3.15±0.10% (w/w) neutral sugar, 2.05±0.01% (w/w) uronic acid and 7.30±0.01% (w/w) sulfate. The high amount of protein in these polymers was similar to that of *Fusarium coccophilum* BCC2415 (about 69.22%) (Madla *et al.*, 2005).

Trace amounts of uronic acid, glucuronic acid or galacturonic acid were found in most acidic polymers (Kumar *et al.*, 2004). The uronic acids and hexosamines were also present in all the exopolysaccharides (EPS) from *Halomonas eurihalina* (Bejar *et al.*, 1998).

Data on the chemical composition of a polymer could indicate its applications. Among different types of polymer, there are very few reports on high protein content polymers. Glycoprotein polymers could be applied in the field of bio-and nanotechnology such as silicon wafers, lipid films and liposomes (Messner and Sleytr,

1992). Therefore, the protein polymer from the extremely halophilic bacterial strain SM5 could act as new biomaterials, and may be applied in many industrial sectors such as nanotechnology. In addition, sulfate polymers provide an interesting application for the pharmaceutical industry as antiviral agent (Okutani, 1992), antitumoral agent (Inoue *et al.*, 1988) and anticoagulant agent (Nishino *et al.*, 1989). The uronic acid in the EPS, from strain H96, was useful in biodetoxification and water treatment (Geddie and Sutherland, 1993).

The average molecular weight (*Mp*) of the partially purified exopolymers revealed two peaks which corresponded to exopolymers with apparent average molecular weight (*Mp*) of $7.7x10^4$ and $2.5x10^5$ Da, respectively. The molecular weight and the functional groups in the polymer molecule are important determinants for the flocculating activity (Kumar *et al.*, 2004). In case of protein bioflocculants, it is reported that the amino and carboxyl groups are responsible and effective flocculation groups (Kurane *et al.*, 1994). Singh *et al.* (2000) reported the flocculating efficiency of polymers increases with increasing molecular weight of polymer. Salehizadeh and Shojaosadati (2001) reported that the efficiency of the bridging mechanism in flocculation is related to the size of the biopolymer. Flocculation with high molecular weight biopolymer involves more adsorption points, stronger bridging, and higher flocculating activity than does flocculation with a low-molecular weight biopolymer.

From the solubility test, the exopolymers were only soluble in water but insoluble in all tested organic solvents. The solubility was based on the principle 'like dissolves like' (James, 1986). The exopolymers from *Halobacterium* sp. SM5 are composed of protein and sugar. The sugar component of glycoprotein can also have several other potential functions, and may help render the protein more soluble (Walsh, 2002). Therefore, the glycoprotein has a solubility in high-moisture or fluid-water systems, glycoprotein particles take up water, swell and may dissolve (BeMiller and Whistler,1997). The abundance of hydroxyl groups build up strong forces of attraction between exopolymer molecules, resulting in relatively hard crystalline solids. These forces are too great to be broken by organic solvents, so the exopolymer is insoluble in organic solvents (James, 1986).

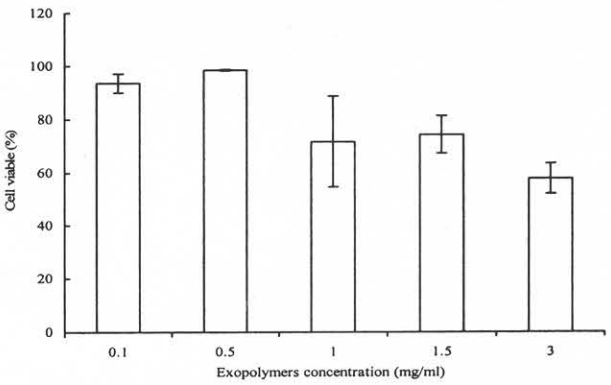

Figure 5. Effect of exopolymers at different concentrations on viability of HT 29 cell lines. All data represent the mean ± S.D; (n = 6)

Biological property

The biological property of the exopolymers solution was evaluated by cytotoxicity tests using the HT29 cell lines. The result of cell viability is shown in Figure 5. The effect of exopolymers solution to the HT29 cell lines was dependent on the concentration. After 4 days, exopolymers solution showed a cell viability of 93.48 and 98.46% at concentrations of 0.1 and 0.5 mg/ml, respectively. The cell viability dropped to 71.65, 74.28 and 57.77%, respectively when the concentration range was increased to 1.0, 1.5 and 3.0 mg/ml. The case of polymers affecting cells has been reported elsewhere (Fischer *et al.*, 2003; Kodaira *et al.*, 2004). In 1997 Carreno-Gomez and Duncan reported that the cytotoxicity of chitosan and its derivatives towards B16 F10 was concentration-dependent and varied according to the salt used and polymer molecule. From this data, it is clear that the exopolymers solutions showed some toxicity toward HT 29 cells at the concentration of 3 mg/ml after exposure to cells for 96 h.

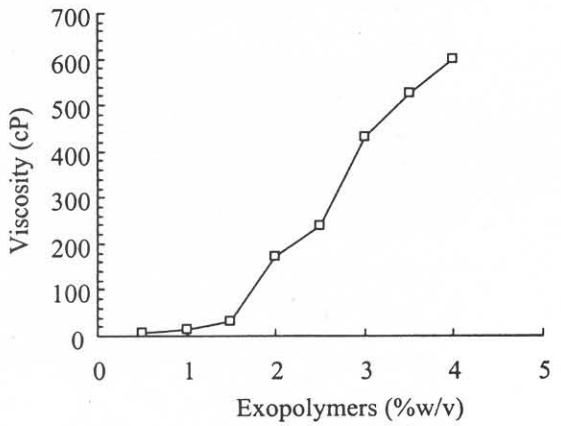

Figure 6. Viscosity of exopolymers at different concentrations. The samples were determined at 25°C at a shear rate of 1 rpm

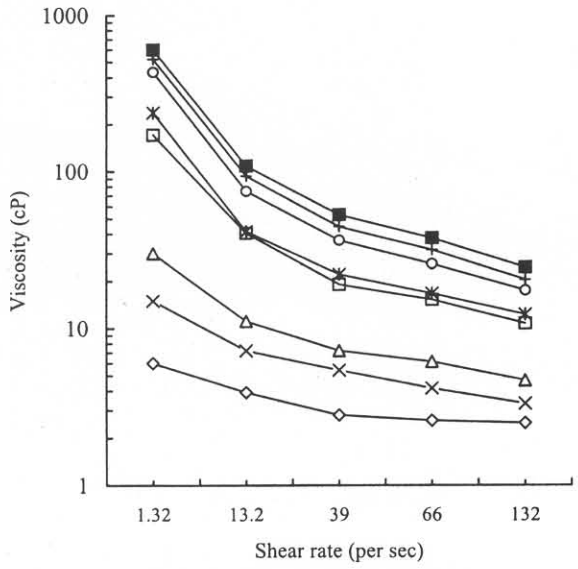

Figure 7. Effect of shear rate at various concentrations of exopolymers on the viscosity. The exopolymers were dissolved in distilled water (◇) 0.5%, (×) 1.0%, (Δ)1.5%, (□) 2.0%, (*) 2.5%, (○) 3%, (+) 3.5%, (■) 4.0%

Rheological property

The relationship at various concentrations of the exopolymers (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% w/v) on viscosity was investigated by using spindle No.18 at 25°C. The rheological properties indicated that viscosity increased markedly with the increase of polymers concentration (Figure 6). When increasing the shear rate from 1.32 to 132 sec^{-1} (1 to 100 rpm), the apparent viscosity decreased (Figure 7); therefore the exopolymers exhibited pseudoplastic behavior. The pseudoplasticity of this exopolymer is an advantageous flow property which may be useful, particularly when this exopolymer is mixed with other materials in commercial applications (Ha *et al.*, 1991). The rheological properties of the exopolymers were similar to those of the commercialized extracellular polymer from different strains such as *Bacillus* (Pfiffner *et al.*, 1986), *Haloferax mediterranei* (Anton *et al.*, 1988), and *Halomonas eurihalina* (Bejar *et al.*, 1998).

The relationship between temperature and viscosity of the exopolymers is shown in Figure 8. The exopolymers showed a gradual increase in viscosity when temperature decreased from 20°C to about 4°C followed by a plateau in viscosity from 20 to 60°C and a rapid decrease in viscosity from 60 to 80°C.

The apparent viscosity of the exopolymers solution was determined between pH 5 to 12 at 25°C, especially in the acidic to neutral range. Apparent viscosity of the exopolymers solution was high at pH 6 to 7. In alkaline range (pH 10 to 12), apparent viscosity of the exopolymers solution was lower and lowest at pH 12 (Figure 9).

Conclusion

The physico-chemical and biological properties of partially purified exopolymers produced by a newly isolated halophilic bacterium, classified as *Halobacterium* sp. SM5 were reported. The exopolymers were dissolved in water, containing protein as the major component and sulfate, total sugar, neutral sugar and uronic acid as minor composition. Infrared spectra revealed that the exopolymers consist of carboxyl, hydroxyl, amino and sugar derivative groups. Molecular weight of exopolymers was $7.7x10^4$ and $2.5x10^5$ Da. Effect of the exopolymers on HT29 human cell lines is

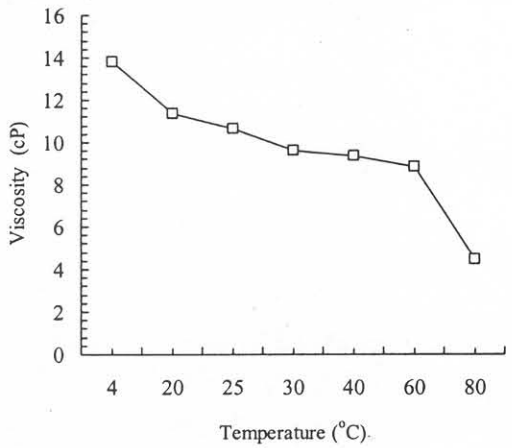

Figure 8. Viscosity of 2% exopolymers solution as a function of temperature. The solutions were measured at a shear rate of 100 rpm

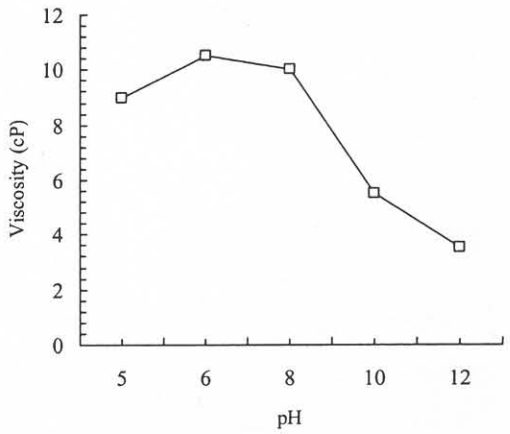

Figure 9. Viscosity of 2% exopolymers solution as a function of pH. The solutions were determined at 25°C at a shear rate of 100 rpm

concentration dependent. The rheological analysis of the exopolymers showed the pseudoplastic property, was resistant to shearing, and had a high viscosity. The viscosity increased markedly with the increase of exopolymers concentrations. The polymers were stable over a wide range of pH, and reversibly dropped on heating.

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