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Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7

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Abstract

A total of 188 carbohydrate polymer-producing bacterial strains were isolated from recycled sludge of five seafood processing plants. Among three selected isolates, identified as *Enterobacter cloacae* WD7, *Enterobacter agglomerans* WD50 and *Pseudomonas alcaligenes* WD22. *E. cloacae* WD7 generated a viscous culture broth exhibiting the highest flocculating activity and a crude polymer yield of 2.27 g/L after 3 days cultivation. Partial purification of this polymer was performed by precipitation with 95% ethanol, dialysis and freeze-drying. It was characterized as an acidic heteropolysaccharide, composed of neutral sugars (29.4%), uronic acids (14.2%) and amino sugars (0.93%). The functional group analysis by FT-IR spectroscopy showed the presence of hydroxyl, carboxyl, carbonyl and methoxyl groups. Thermal analysis by DSC showed the crystalline transition and the crystalline melting point (T_m) at 300 °C. This polysaccharide was soluble in water and insoluble in any organic solvents tested; gelation occurred under alkaline conditions in the presence of divalent cations in which copper as $CuSO_4$ gave the best result. Studies on the flocculation property revealed that this polysaccharide was stable at 4–60 °C and pH 5–7. The optimal concentrations for the flocculating activity were 2 mg/L polysaccharide and 40 mM $CaCl_2$ which played the synergistic effect on kaolin flocculation. Moreover, this polysaccharide could flocculate the kaolin suspension over a wide range of pH (pH 2–8) and temperature (4–50 °C) tested in the presence of $CaCl_2$.

Keywords: Screening; Characterization; Flocculating property; Polymer; *Enterobacter cloacae* WD7

1. Introduction

Environmental pollution has become one of the world problems. Water pollution caused by industrial pollutants has been recognized and both private and government sectors are now trying to mitigate this problem. One of the toxic pollutants is the polyacrylamide used together with aluminium sulfate to flocculate protein as well as oil and grease in the wastewater. Polyacrylamide is an organic synthetic high polymer containing acrylamide monomers which are both neurotoxic and strong human carcinogens (Yokoi, Natsuda, Hirose, Hayashi, & Takasaka, 1995). To substitute this synthetic flocculant, bioflocculants are required; polymers from microorganisms are especially suitable potential since they could be produced uniformly and reliably by fermentation processes.

With their different functional properties, microbial exopolymers especially polysaccharides are widely used as stabilizer, suspending agent, dispersant, thickener, film-forming agent, water retention agent, lubricant or friction reducer, etc. in many industries such as detergent and laundry products, textiles, adhesives, paper, paint, food, pharmaceutical, cosmetic and others (Sandford, 1979). Among these many functions, 'flocculation' is an interesting one and warrants development for wastewater treatment, water treatment, dredging, and downstream

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processes in fermentation industries (Kurane & Matsuyama, 1994) to replace the generally used synthetic polymers which are not friendly to the environments.

Many flocculants are used in food industries such as the removal of microbial cells from fermentation downstream stages. Microbial flocculants are mainly polysaccharide and produced mostly by bacteria such as *Alcaligenes cupidus* KT-201 (Toeda & Kurane, 1991), *A. latus* B-16 (Kurane & Nohata, 1991), and *Bacillus* sp. DP-152 (Suh et al., 1997).

This paper aims to search for bacterial isolate(s) producing carbohydrate polymers with high flocculating activities, and to characterize and study the flocculation properties of these polymers for further application.

2. Materials and methods

2.1. Isolation, selection and identification of polymer-producing bacteria

Polymer-producing bacteria were isolated from recycled activated sludge samples (pH 6.98–7.32) which were taken from five seafood processing plants in Songkhla region of Thailand. The procedure was the same as described previously (Dermlim, Prasertsan, & Doelle, 1999). Each isolated strain was cultivated in basal medium (50 ml) containing 1% w/v glucose (or sucrose), 0.05% $(NH_4)_2SO_4$, 0.5% K_2HPO_4, 0.2% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.01% NaCl on a rotary shaker (200 rpm) at 31 ± 2 °C for 3 days. At the end of cultivation, the viscous culture broth was measured for flocculating activity (Kurane, Takeda, & Suzuki, 1986; Suh et al., 1997). Strains with the viscous culture broth possessing the highest flocculating activity were chosen and identified according to Bergey's Manual of Determinative Bacteriology (Holt, Krieg, Sneath, Staley, & Williams, 1994), and biochemical tests (MacFaddin, 1980).

Time courses of the selected strains were carried out by cultivating in basal medium with the addition of 5% starter culture (12 h and viable cell count of 10^8 CFU/ml) on a rotary shaker at room temperature (30 °C) for 5 days. Samples were taken every 24 h to measure pH, dry cell weight, crude polymer yield and flocculating activity and flocculation rate as described previously (Dermlim et al., 1999). The strains which produced polymers with the highest flocculating activity were selected for further studies.

2.2. Partial purification of the biopolymer

The selected strain was cultivated for an optimal period (results from time course studies). The viscous culture broth (1.2 L) was diluted five times with distilled water, 1% formaldehyde was added then heated at 60 °C for 5 min and centrifuged at 12,846*g*. The supernatant (6 L) was concentrated (to 3 L) in a vacuum dryer at 50 °C for 7 h, then mixed with four volumes of cold 95% ethanol and left overnight. After centrifugation at 7600*g*, 5 °C for 15 min, the precipitated polymer was redissolved in 1 L distilled water and concentrated again. The concentrate (150 ml) was dialysed against distilled water at 4 °C for 24 h and the dialysate was freeze-dried to obtain the partially purified biopolymer (method modified from Smith & Pace, 1982; Yokoi et al., 1995).

2.3. Characterization of the partially purified biopolymer

Analysis of the partially purified biopolymer for its components using qualitative analysis (Plummer, 1978) and quantitative analysis (Chaplin & Kennedy, 1986), electric charge allocation (Scott, 1965), functional group analysis by Fourier-transform infrared (FT-IR) spectroscopy (Brauer & Kline, 1995) and thermal analysis by differential scanning calorimetry (DSC) (Hill, 1995) were the same procedures as described in previous paper (Dermlim et al., 1999). Additional analysis of the biopolymer included the solubility test in distilled water and several solvents such as acetone, carbon tetrachloride, ethanol, isopropanol, hexane, methanol and nitrobenzene (Collins, Bares, & Billmeyer, 1973), gelation propensity using metal salts (Shimada, Nakata, & Nakamura, 1997), using monovalent cations (NaCl and KCl) and divalent cations ($CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$). The procedure for gelation studies was that each cationic salt (2 mg) and 2 M NaOH (0.2 ml) were added into biopolymer solution (0.5% w/v, 1 ml). After mixing, the phase transition from solution to semi-solid phase of gel was visually in order to express it in terms of weak gelation and medium-strong gelation.

2.4. Flocculation property of the partially purified biopolymer

The effect of pH (2–12) and temperature (4, 20, 30, 40 and 50 °C) on the flocculating activity of the partially purified biopolymers was studied by measuring the flocculating activity of the reaction mixture containing the optimum concentration of biopolymer at the specified ranges of pH and incubated at different temperatures (Kwon et al., 1996; Lee, Lee, Jang, & Lee, 1995).

The pH stability of the biopolymers was determined (Lee et al., 1995) by measuring the residual activity after 24 h pre-incubation at various pH (pH 2–12) and compared with that of normal polymer solution at pH 6.3. Thermal stability of the biopolymer was determined (Kwon et al., 1996; Lee et al., 1995) by measuring the residual activity after 30-min incubation at various temperatures (4–120 °C under pressure) and compared with that of 30 °C. Furthermore, effects of polymer concentration (final concentration of 0–7 mg/L), as well as the cation types (NaCl, KCl, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$) and cation concentrations (0, 0.01, 0.1, 1, 10 and 100 mM) on the flocculating activity were also studied.

3. Results and discussions

3.1. Isolation, selection and identification of polymer-producing bacteria

A total of 188 slime-forming or mucoid colonies were isolated with 89 and 99 isolates were grown on the basal screening media (pH 7) with glucose or sucrose as carbon source. The screening medium contained high carbon to nitrogen ratio which is suitable for inducing growth and stimulate exopolymer formation (Sutherland, 1996). All of the isolates were found to be bacteria with convex, rounded, entire edge colonies with creamy or light brown colour. Some were irregular shapes or less convex as caused by the production of slime on the medium. Some slimes were highly ropy.

Cultivation in basal medium for 3 days revealed that most isolates grew well and only three isolates possessed a viscous culture broth which gave a low flocculating activity due to the fact that the high amount of polymers in the culture broth caused the kaolin to be suspended in the solution. So, the viscous culture broth had to be diluted. The optimum volume of each culture broth in the presence of 1% $CaCl_2$ giving the maximum flocculating activity or flocculation rate. The flocculating activity was calculated according to the equation:

$$(1/OD550)_s - (1/OD550)_c,$$

where $(OD550)_s$ was the absorbance of the sample and $(OD550)_c$ was the absorbance of the control, while the flocculation rate was calculated from the equation:

$$[(OD550)_c - (OD550)_s]/(OD550)_c.$$

The biopolymer sample was 50 μl for the isolate WD7 while they were 100 μl for the WD22 and WD50 strains. According to their morphological and biochemical characteristics (Table 1), the three selected isolates, WD7, WD22 and WD50, were Gram-negative bacteria, rod shaped with different size, and identified as *Enterobacter cloacae*, *Pseudomonas alcaligenes* and *Enterobacter agglomerans*, respectively.

The time courses on growth and polymer production of the three selected strains in basal medium using glucose (strain WD7 and WD22) or sucrose (strain WD50) as carbon source for 5 days are given in Fig. 1. All three strains exhibited a similar pattern of growth with fast growth within 1 day which correlated with a rapid decline in pH due to the metabolism of sugar and ammonium sulfate. The highest polymer yields of 2.27, 3.41 and 0.83 g/L, respectively, were obtained after 3 days cultivation. Nevertheless, the flocculating activity (Fig. 2) of *E. cloacae* WD7 and *P. alcaligenes* WD22 reached their maximum (10.28 and 1.43, respectively) after 3 days cultivation whereas it was 5 days (1.80) for *E. agglomerans* WD50. The rapid decline of the flocculating activity may indicate that both strains (WD7 and WD22) also possessed polymer-degrading enzymes (Kurane & Nohata, 1991). *E. cloacae* WD7 and *P. alcaligenes* WD22 possessed a flocculation rate of 91% and 55%, respectively, after 3 days cultivation and 60% after 5 days for *E. agglomerans* WD50. These results illustrated that *E. cloacae* WD7 was the most suitable strain for polymer production due to its high flocculating activity (10.28) and high flocculation rate (91%) although its polymer yield (2.27 g/L) was lower than that of *P. alcaligenes* WD22 (3.41 g/L).

3.2. Characterization of the partially purified biopolymer

Enterobacter cloacae WD7 was selected and cultivated in basal medium for 3 days, then the polymer was recovered, partially purified, and freeze-dried.

3.2.1. Components of the partially purified biopolymer

The partially purified biopolymer of *E. cloacae* WD7 was composed of neutral sugars (29.4%) and uronic acids (14.18%) as the major and minor components, respectively, with a little amount of amino sugar (0.93%) (Table 2). Neither alpha amino acids analysed by the ninhydrin reaction (L-leucine standard) nor aromatic amino acids analysed by the Xanthoproteic reaction (L-tryptophan standard) (Plummer, 1978) were detected indicating that it contained no amino acids or protein in its molecule. Therefore, the polymer produced by *E. cloacae* WD7 was classified as a polysaccharide. The uronic acid contained in its molecular structure might be glucuronic acid or galacturonic acid as is found generally in the acidic polysaccharides (Margaritis & Pace, 1985).

3.2.2. Electric charge property of the partially purified biopolymer

The precipitation which occurred after the addition of cetylpyridinium chloride (CPC) to the solution of partially purified biopolymer of *E. cloacae* WD7 indicates that it contained acidic groups in its structure due to the interaction with the quaternary ammonium cation (QN^+) of the CPC, resulting in the formation of a cetylpyridinium chloride polysaccharide complex (Scott, 1965). Therefore, this polymer was an acidic polysaccharide; its component acid can be one or more of the acidic groups of pyruvate, succinate, uronate, acetate or sulfate (Pace & Righelato, 1980; Sutherland, 1977). These acidic groups may play an important role causing the anionic (or acidic) charge of the polysaccharide (Margaritis & Pace, 1985).

3.2.3. Functional group analysis of the partially purified biopolymer

The infrared spectra of the biopolymer polysaccharide (Fig. 3) showed the presence of carbonyl (1716 cm^{-1}), hydroxyl (3455 cm^{-1}), carboxyl (1608 and 1400 cm^{-1}) and methoxyl (1136 and 1075 cm^{-1}) groups (Brauer & Kline, 1995; Sawyer, Heineman, & Beebe, 1984; Suh et al., 1997). This polysaccharide may be a partially methylated polysaccharide containing uronic acid due to the

Table 1
Taxonomical and biochemical characteristics of the three selected isolates

Test	WD7	WD22	WD50
Gram stain (24 h)	Negative	Negative	Negative
O_2 requirement	Facultative anaerobe	Strictly aerobe	Facultative anaerobe
Cell morphology	Short rod	Rod	Short rod
Motility	+	+	+
Oxidase	−	+	−
Catalase	+	+	+
Indole production	−	−	−
Methyl red	+	−	+
Voges–Proskauer	+	−	−
Citrate (Simmons)	+	+	+
TSI reaction	A/A,G	NC/NC	A/A,G
H_2S production (TSI)	−	−	−
Esculin hydrolysis	+	−	+
Urea hydrolysis	−	−	+
Gelatin hydrolysis	−	+	−
Starch hydrolysis	−	−	−
Lipid hydrolysis	−	+	−
Casein hydrolysis	−	−	−
Nitrate reduction	+	−	+
Lysine decarboxylase	−	NC	−
Arginine dihydrolase	+	NC	−
Ornithine decarboxylase	+	NC	−
KCN growth	+	−	+
Oxidation–fermentation	(O–F) F	Non-oxidizer	F
Litmus milk	Acid, clot	Peptonization	Acid, clot
Acid production from:			
Glucose	+	−	+
Galactose	+	−	+
Fructose	+	−	+
Ribose	+	−	+
Arabinose	+	−	+
Xylose	+	−	+
Rhamnose	+	−	+
Mannitol	+	−	+
Glycerol	−	−	+
Dulcitol	−	−	−
Sorbitol	+	−	+
Inositol	−	−	+
Maltose	+	−	+
Sucrose	+	−	+
Lactose	+	−	+
Cellobiose	+	−	+
Trehalose	+	−	+
Raffinose	+	−	+
Inulin	−	−	−
Identified as	*Enterobacter cloacae*	*Pseudomonas alcaligenes*	*Enterobacter agglomerans*

Note. +, positive result; −, negative result; A, acid; G, gas; NC, no change.

presence of O–H broad band at 3700–3000 cm^{-1} and the intensity of absorption due to O–CH_3 at 1150–1050 cm^{-1} as based on work by Churms (1995). Two absorption peaks (at 1608 cm^{-1} and near 1400 cm^{-1}) of carboxylate ions were the characteristic pattern for uronate of the polysaccharides produced by *Butyvibrio fibrisolvens* (Ha, Stack, Hespell, Gordon, & Bothast, 1991) and *Bacillus* sp. (Pfiffner, McInerney, Jenneman, & Knapp, 1986). The carboxylate functional groups are responsible for its physical and chemical properties, for example, carboxylate groups can serve as binding sites for divalent metal ions. In addition, the carboxylate groups of the polysaccharide might be used as functional groups to link the polysaccharide to starch or man-made polymers to form new polymers with unique properties (Ha et al., 1991).

3.2.4. Thermal analysis of the partially purified biopolymer

The thermal stability of this partially purified biopolymer polysaccharide determined by DSC is illustrated as a heat flow–temperature curve (Fig. 4). Initially, there was no exhibition of a glass transition in this polysaccharide since there was no indication of an

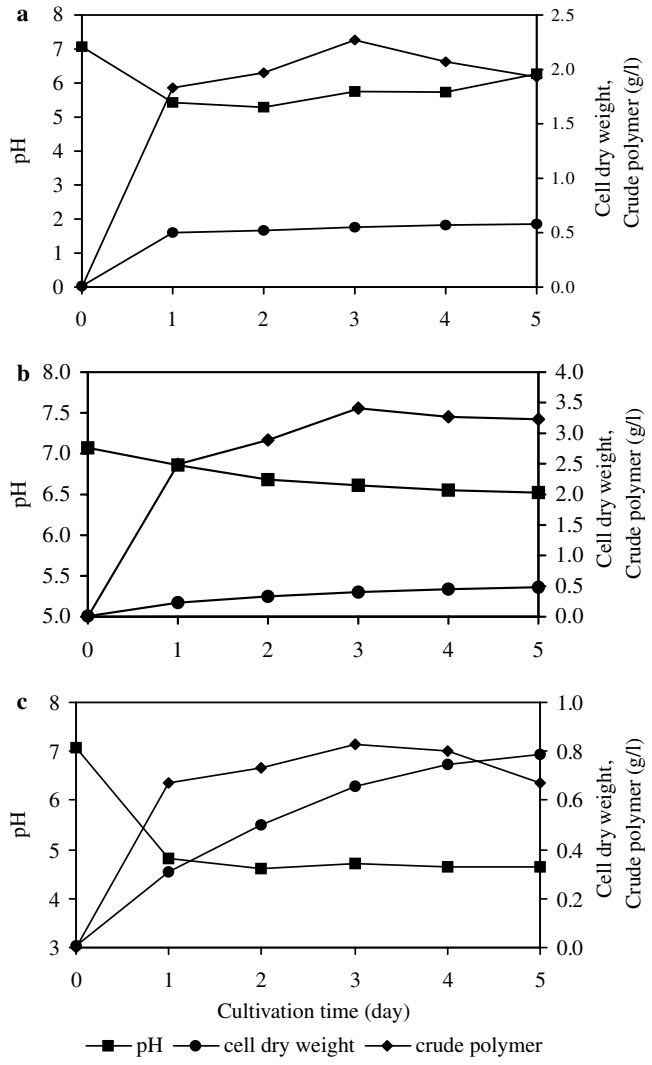

Fig. 1. Time course of polymer production by *E. cloacae* WD7 (a), *Pseudomonas alcaligene* WD22 (b) in basal medium (1% glucose as carbon source) and *E. agglomerans* WD50 (c) in basal medium (1% sucrose as carbon source).

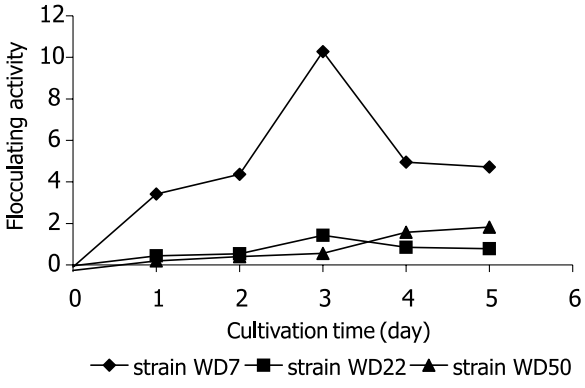

Fig. 2. Flocculating activity change during cultivation of three selected strains in basal medium (glucose basal medium for *E. cloacae* WD7 and *P. alcaligenes* WD22, sucrose basal medium for *E. agglomerans* WD50). *E. cloacae* WD7 (50 μl of culture broth), *Pseudomonas alcaligenes* WD22 (100 μl of culture broth) and *E. agglomerans* WD50 (100 μl of culture broth).

initial baseline shift (Brown, 1988; Hill, 1995). As the temperature slowly increased up to 272 °C, the solid polysaccharide may be recrystallizing resulting in the sharp drop in the specific heat curve (exotherm). At still higher temperatures, 282–390 °C, the crystalline polymer melted with a corresponding rise in the specific heat curve (endotherm) resulting in a peak at 314 °C, thus indicating the crystalline melting point (T_m) of this polysaccharide. According to the crystalline transition, the physical nature of this solid polysaccharide may be of a partially crystalline polymer consisting of crystalline and amorphous regions (Tadokoro, 1979). These crystalline and amorphous regions indicated a high, little or no degree of ordering of polymer chain interaction (Kroschwitz, 1990). Since crystalline polymers are strong, tough, stiff and generally more resistant to solvents and chemicals than their non-crystalline counterparts, it is possible to improve the desirable properties of this amorphous polysaccharide for material applications if it can be made to crystallize.

3.2.5. Solubility of the partially purified biopolymer

This polysaccharide was found to be soluble in water but insoluble in all tested organic solvents. Consequently, it can be recovered from the cell-free supernatant by precipitation with organic solvents such as ethanol. In an aqueous system, polysaccharide particles can take up water, swell and usually undergo partial or complete dissolution (BeMiller & Whistler, 1996). The abundance of hydroxyl groups builds up strong forces of attraction between polysaccharide molecules, and may result in relatively hard crystalline solids – where hydrogen bonding can occur. These forces are too great to be broken by organic solvents, so the polysaccharide was insoluble in organic solvents (James, 1986).

3.2.6. Gelation property of the partially purified biopolymer

This polysaccharide can form gels in the presence of divalent metal cations (available from their salts: $CaCl_2$, $MgSO_4$, and $CuSO_4$) at higher pH values (with the addition of NaOH). Only divalent metal salts, not monovalent, can form a gel. This may be due to the molecular chains of polysaccharide in alkaline solution spread owing to repulsion between dissociated acidic groups. In contrast, the reverse change occurs at high ionic strength or in the presence of salt (Shimada et al., 1997). The different types of divalent cations also gave the different appearances of gels with the same amount (2 mg of each divalent metal salts used with combination of 0.5% polysaccharide solution (1 ml) and NaOH (0.2 ml)). Thus, the ratio of polysaccharide to metal salt was 2.5:1 by weight. $CuSO_4$ (final concentration of 6.68 mM) gave a stronger gel than $CaCl_2$ and $MgSO_4$ (final concentration of 11.3 and 6.76 mM, respectively). Gelation of this polysaccharide with various metal cations was similar to that which occurred with an acidic polysaccharide from *Enterobacter* sp. (Shimada et al., 1997).

Table 2
Components of the partially purified biopolymer

Method	Analyzed item	% (w/w)
Qualitative test		
1. Ninhydrin reaction	α-Amino acids	Not detected
2. Xanthoproteic reaction	Aromatic amino acids	Not detected
Quantitative test		
1. Anthrone reaction	Neutral sugar	29.40
2. Phenol–sulfuric acid reaction	Total sugar	41.15
3. Carbazole–sulfuric acid reaction	Uronic acids	14.18
4. Elson–Morgan reaction	Amino sugars	0.93

Gelation is useful in various applications such as alginate, which undergoes gelation in the presence of Ca^{2+}, and therefore conversely can be used as an eliminator of Ca^{2+} from solution and as an immobilization agent.

The application of this biopolymer, therefore, for wastewater treatment would be effective due to the acidic groups in its molecule which can bind the metal ions and may be simultaneously enhance the formation of inter-particle bridging between biopolymer chains and particles in wastewater to larger agglomerate (floc) and effect settlement.

3.3. Flocculation property of the partially purified biopolymer

3.3.1. Effect of pH on the flocculating activity

The partially purified biopolymer had the optimum pH for flocculating activity at pH 6 and was not affected by the pH range of 2–8. At higher pH of 9–12, the

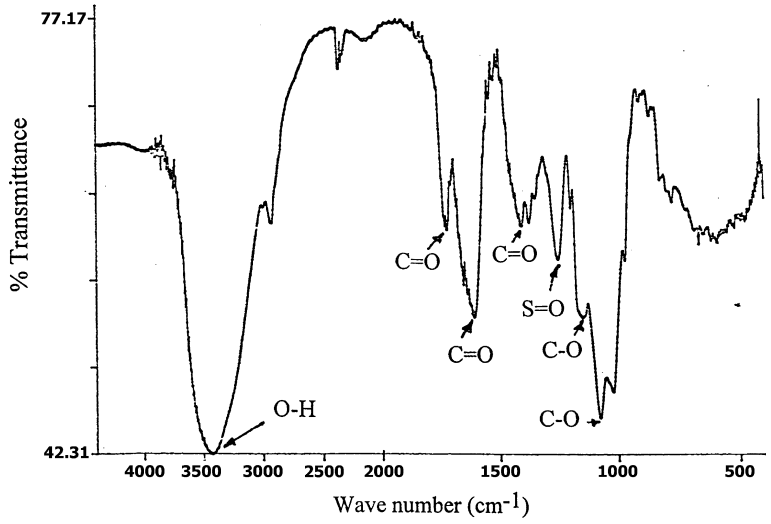

Fig. 3. FT-IR spectra of the partially purified biopolymer from *E. cloacae* WD7.

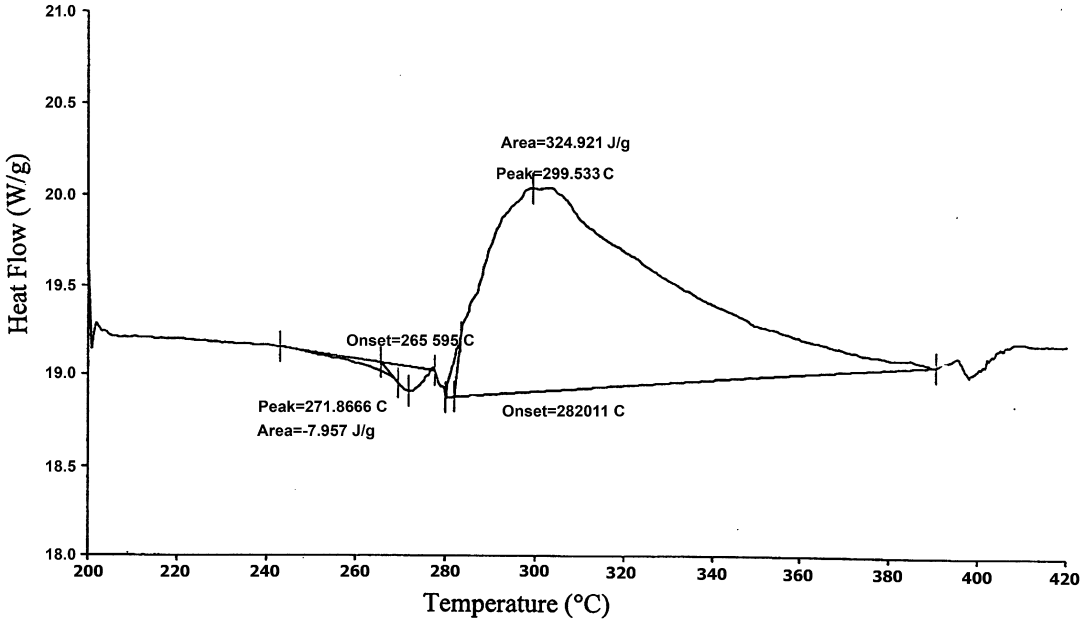

Fig. 4. Differential scanning calorimetry (DSC) curve of partially purified biopolymer from *E. cloacae* WD7.

flocculating activity decreased gradually. This suggested that the hydroxide ion (OH^-) may interfere with the complex formation of the polysaccharide and kaolin particles mediated by Ca^{2+}, consequently the kaolin particles were suspended in the mixture. The pH range for flocculation reaction of this polymer was wider than those of the polyglutamate from *Bacillus subtilis* PY-90 (pH range of 3–5) (Yokoi et al., 1995), and the cationic polysaccharide from *Paecilomyces* sp. I-1 (pH range of 4–8) (Takagi & Kadowaki, 1985).

3.3.2. Effect of temperature on the flocculating activity

The flocculating activity of the partially purified biopolymer increased with the increase of reaction temperatures in the range of 4–50 °C. The acceleration by high temperature (50 °C) of this polymer was better than the optimum flocculation reaction at 25 °C of the acidic polysaccharide produced by *Enterobacter* sp. BY-29 (Yokoi et al., 1997). However, this high temperature was lower than the flocculation temperature (100 °C) against cell suspension of *Escherichia coli* by cationic polysaccharide from *Paecilomyces* sp. I-1 (Takagi & Kadowaki, 1985) and the optimum temperature (approximately 70 °C) for flocculation of polyglutamate from *B. subtilis* IFO 3335.

3.3.3. pH stability

The pH stability tested within the pH range of 2–12 illustrated that the partially purified polysaccharide was most stable at pH 7.0 with an increase of 20% flocculating activity, compared to that of the control (polymer solution) at pH 6.3. More than 80% of its activity remained in the pH range of 5–7 with 65% residual activity at pH 4. However, in acidic solution (pH 2–3), the flocculating activity decreased to about 10% due to the glycosidic bonds in the polysaccharide chain being hydrolyzed. In basic solutions (pH 8–12), the flocculating activity decreased gradually (from 50% down to 28%) due to the alkaline degradation of polysaccharide causing several changes such as molecular rearrangement of its residue or fragmentation of the polysaccharide chain (Aspinall, 1982).

3.3.4. Thermal stability

Thermal stability studies in the range of 40–120 °C revealed that the partially purified biopolymer was stable up to 70 °C, compared to the control at 30 °C and the flocculating activity decreased thereafter with the total lost at 110 °C. The flocculating activity was highest at 60 °C due to the increase in entropy (Lee et al., 1995) or this temperature may be the optimum temperature for enhancing the polysaccharide chain to have highly effective flocculation. The lower flocculating activity of the polysaccharide at higher temperatures was due to the breaking down of the polysaccharide chain above 60 °C which led to the low potential to form bridges with the kaolin particles. The stability of this polymer up to 70 °C was the same as that of the acidic polysaccharide from *Pestalotiopsis* sp. (Kwon et al., 1996), but lower than that of the glycoprotein produced by *Arcuadendron* sp. which was stable up to 100 °C (Lee et al., 1995).

3.3.5. Effect of cation types and concentrations

Since the flocculating activity of the polysaccharide occurred in the presence of $CaCl_2$, the effect of cation alone and in combination with the polysaccharide was investigated. It was found that addition of a cation to the reaction mixture was necessary to induce the effective flocculation by forming complexes of the polysaccharide and kaolin clay mediated by a cation (Kurane & Matsuyama, 1994). $CaCl_2$ in the range of 10–100 and 1 mM $FeCl_3$ were more effective than other cations in kaolin flocculation. Although $CaCl_2$ at 10 mM possessed a slightly lower flocculating activity compared to $FeCl_3$, the Ca^{2+} enhanced the formation of the large and compact floc whereas Fe^{3+} formed the gelatinous precipitates which were more difficult to remove. $CaCl_2$, therefore, was chosen and its optimum concentration for flocculation of kaolin clay was found to be 40 mM.

The cation could stimulate the flocculation by neutralization and destabilization of residual negative charges of carboxyl groups of uronic acid in an acidic polysaccharide, forming bridges which bind kaolin particles to each other (Yokoi et al., 1997).

3.3.6. Effect of polysaccharide concentration on the flocculating activity

The polysaccharide dosage and the size of floc were related to each other at the increasing polysaccharide up to 2 mg/L which gave the highest flocculating activity, then decreased as the adsorption of excess polysaccharide restabilized the kaolin particles. The results could be explained in two ways: (1) the incomplete dispersion of excess polysaccharide, only the kaolin particles around the polysaccharides participated in the flocculation reaction, therefore, other kaolin particles did not participate in the reaction (Yokoi et al., 1997) and (2) the excess polysaccharide was oversaturated on many binding sites of the surface of kaolin particles, thus the attractive force of the other particles was reduced and the flocculating activity decreased (Kwon et al., 1996). Thus, either the deficiency

Table 3
Flocculation property of the partially purified polymer from *E. cloacae* WD7 polysaccharide

Parameter	Range tested	Optimum value	Accepted range
pH	2–12	6	2–8
Temperature (°C)	4–50	50	4–50
pH stability	2–12	7	5–7
Thermal stability (°C)	40–120	60	Up to 70 °C
$CaCl_2$ conc. (mM)	0–100	40	10–100
Biopolymer conc. (mg/L)	0–7	2	1–2.5

Table 4
Comparison on the flocculating activity at the optimum dosage of various flocculants

Microorganism	Polymer	Dosage (mg/L)	Flocculating activity	Reference
Bacillus sp.	Acidic PS[a]	1	43	Suh et al. (1997)
Bacillus subtilis	Polyglutamate	20	15	Yokoi et al. (1995)
B. subtilis	Polyglutamate	20	20	Yokoi et al. (1996)
Rhodococcus erythropolis	Protein	20	33	Takeda et al. (1991)
Enterobacter sp.	Acidic PS	20	125	Yokoi et al. (1997)
Arcuadendron sp.	Glycoprotein	2	17	Lee et al. (1995)
Pestalotiopsis sp.	Acidic PS	1	50	Kwon et al. (1996)
Zoogloea ramigera	Acidic PS	3	15	Suh et al. (1997)
Enterobacter cloacae	Acidic PS	2	105	This study

[a] Acidic polysaccharide.

or excess amount of polysaccharide and kaolin clay decreased or even prevented the flocculating activity (Lee et al., 1995).

Flocculation property of the partially purified polymer from *E. cloacae* WD7 polysaccharide was summarized in Table 3. This polysaccharide showed much higher flocculating activity than those of other flocculants previously reported (Table 4). Thus, it is possible that this polysaccharide could be substituted for a commercial polymer with respect to flocculation.

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