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Original Article

Optimization of exopolysaccharides production by *Weissella confusa* NH 02 isolated from Thai fermented sausages

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Abstract

Exoploysaccharides (EPSs) producing lactic acid bacteria (LAB) were isolated from Thai traditional fermented foods. Colonies exhibiting a clear zone and ropiness on modified MRS agar containing 2% (w/v) sucrose and 0.02% (w/v) bromocresol purple were selected for Gram staining and catalase test. From 140 isolates, 120 isolates were identified as LAB. However, only 14 strains were able to produce a large amount of EPSs (>5.9 g/l) when cultivated in modified MRS broth containing 2% (w/v) sucrose at 37ºC for 24 hrs. The highest viscosity and yield of EPSs were obtained from isolate NH 02. Based on 16S rDNA analysis, strain NH 02 was identified as *Weissella confusa*. Sucrose (40 g/l), an initial pH of modified MRS at 7.0, and a cultivation temperature of 37ºC were the optimum conditions, in which EPSs were produced at levels up to 18.08 g/l within 12 hrs. EPSs produced by *W. confusa* NH 02 contained only glucose and had a molecular mass of 1.13×10^6 Da.

Key words: Lactic acid bacteria, Exopolysaccharides, Thai fermented food, *Weissella confusa*

1. Introduction

Exopolysaccharides (EPSs) are long-chain polysaccharides that are secreted mainly by bacteria and microalgae into the environment during growth and that are not permanently attached to the surface of the microbial cells. EPSs are responsible for the slime-forming or mucoid trait of many microorganisms. A second group of polysaccharides that are structurally similar but that are permanently attached to the cell surface are classified as capsular polysaccharides (Agira *et al.*, 1992; De vuyst and Deggest, 1999). Apart from natural polysaccharides plants, they can be produced by bacteria with novel characteristics. Among commercial EPSs, xanthan, gellan, and dextran are common. Xanthan and gellan are produced from *Xanthomonas campestris* and *Pseudomonas elodea*, respectively. However, products containing those polysaccharides must be labeled since these bacteria are plant pathogens (Ludbrook *et al.*, 1997).

Lactic acid bacteria (LAB) are found in a range of fermented foods and can produce natural products including EPSs. LAB producing EPSs have gained considerable attention in the fermented dairy industry because of their potential application as viscosifiers, texturizers and emulsifying agents (Grobben *et al.*, 1996). In addition, EPSs such as β-D-glucan have been found to possess antithrombotic, antitumoral, or immnunomodulatory activity (Sutherland, 1998). Consequently, the influence of β-D-glucan producing strains of *Lactobacillus brevis*, *L. delbrueckii* ssp. *bulgaricus* and *Pediococcus parvulus* 2.6 has been investigated on elaboration and intake of functional oat-based products (Martensson *et al*., 2002). EPSs produced by those food-grade microorganisms with GRAS (generally recognized as safe) status offer an alternative source of microbial polysaccharides for wider use in food formulations. Piermaria *et al*. (2009) demonstrated the ability to form a film of an EPS isolated from LAB. However, the production of EPSs by LAB

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is too low to make the production process economically feasible. In general, the relative concentration of carbon and nitrogen, and the temperature have been found to be the important parameters for EPSs production (Cerning *et al.*, 1992; Kimmel *et al.*, 1998; Velasco *et al.*, 2006; Lin *et al.*, 2007).

The objectives of this study were to isolate and screen EPSs producing LAB from various Thai traditional fermented foods and to examine the influence of environmental factors on growth and EPSs production of selected strain.

2. Materials and Methods

2.1 Isolation and screening for EPSs producing LAB

Various Thai traditional fermented foods including *Nham* and *Sai-Krok-Isan* (fermented pork sausage); *Som-Fag* (fermented fish sausage); *Nhang* (fermented meat); *Kapi* (fermented shrimp paste); *Hoy-Dong* (fermented clam); *Kung-Som* (fermented shrimp); *Pla-Ra*, *Pla-Som*, *Budu*, and *Pla-Pang-Dang* (fermented fish), and *Tai-Pla* (fermented fish viscera) were used as microbial sources. Twenty-five grams of each sample were homogenized with 225 ml of 1.0% (w/v) sterile physiological saline in a Waring blender (Connecticut, USA.) for 1 min and serially diluted using the same diluents. One hundred microliters of these dilutions were spread-plated on a modified MRS agar containing 2% sucrose (Ajax Finechem, New South Wales, Australia) instead of glucose and 0.02% bromocresol purple (Fisher Scientific, Leicester-shire, UK) following the recipes of Merck (Smitinont *et al.*, 1999). Plates were then incubated at 37ºC for 1-2 days. Slimy colonies exhibiting clear zones were selected and re-streaked on modified MRS agar 2-3 times for purification check.

The pure cultures were characterized using Gram stain, cell morphology, and catalase reaction tests. Gram-positive and catalase-negative isolates were stored at -20ºC in MRS broth supplemented with 25% (v/v) glycerol (Ajax Finechem, New South Wales, Australia). For routine analysis, strains were subcultured twice in modified MRS broth for 24 h at 37ºC. The selected isolate was further identified by 16S rDNA sequence.

2.2 Conditions of cultivation

Growth studies and EPSs production were done in 250 ml flasks containing 100 ml MRS broth at 37ºC for 48 hrs. The effects of carbon sources (glucose, fructose (Asia Pacific Specialty Chemicals Limited, New South Wales, Australia), sucrose and lactose (Ajax Finechem, New South Wales, Australia)), and concentrations (0, 10, 20, 30, 40, 50, and 60 g/l), initial pHs (5.0, 6.0, 6.5, 7.0, and 7.5), temperatures (15, 20, 25, 30, and 37ºC), and agitation rates (0, 50, 100, and 150 rpm) on growth and EPSs production were studied. Experiments were done in triplicate.

Cell growth was monitored by measuring the optical density of the culture fluid at 660 nm (Genesys^{TM} 10-S, Thermo Electron Corporation, Madison, USA) and the total cell protein by using the Lowry method (Lowry *et al.*, 1951). Prior to the analysis, cells were collected by centrifugation (5415 R, Eppendorf, Hamburg, Germany) at 11,000 x g for 15 min. Then, wet cells were re-suspended in 0.1N NaOH (Lan Scan, Bangkok, Thailand) and boiled at 100ºC for 20 min (Shabtai, 1990).

2.3 Isolation and analysis of EPSs

The EPSs-producing strain (*W. confusa* NH 02) with high yield was grown in modified MRS media containing a suitable carbon source at optimal cultivation conditions. Removal of cells and EPSs from the culture broth was performed according to a modified method of Smitinont *et al.* (1999). The supernatant was mixed with an equal volume of chilled ethanol and stored at 4ºC for 24 hrs. EPSs were collected by centrifugation (9,000 ×g for 15 min at 4ºC) (SCR20B, Hitachi, Tokyo, Japan). The precipitated EPSs were re-dissolved in distilled water and dialyzed (molecular weight cut-off 8,000) (Viskase, Illinois, USA) against 4 l of distilled water at 4ºC. Dialysate containing EPSs were then recovered by freeze-drying (Fts System Inc., New York, USA) and stored at room temperature.

The total sugar content was determined by the method of Dubois *et al.* (1956) using glucose as standard. Protein content was determined according to the method of Bradford (1976) using bovine serum albumin (Sigma Company, St. Louis, USA) as standard. For the sugar analysis, 25 mg of dried EPSs were dissolved in 2 ml of 3 M trifluoro acetic acid (Fluka, Steinheim, Germany) in a tightly sealed test tube. The solution was incubated at 100ºC in a water bath (W350, Memmert, Schwabach, Germany) for 8 hrs. After being cooled and neutralized, the clear solution was collected and kept at -20ºC until analysis. The determination of the monosaccharides was performed using a HPLC (2000ES, Alltech, Illinois, USA) with Lichrocart-NH_2 (250×4 mm) column (Merck, Darmstadt, Germany) and a refractive index detector using 90% aqueous acetonitrile (Lab Scan, Bangkok, Thailand) as mobile phase.

The molecular weight of EPSs was estimated on the basis of the calibration curve obtained by the HPLC (Waters, Madison, USA) with Ultrahydrogel linear 1 Column (Waters, Madison, USA) using a 0.05 M sodium carbonate buffer (pH 11) (Lab Scan, Bangkok, Thailand) as the mobile phase (column temperature 30ºC; flow rate 0.6 ml/min). The column was standardized with pullulans (Polymer Laboratories, Shropshire, UK) of diverse molecular mass. A RI detector was used (Waters 410, Madison,USA).

2.4 Viscosity measurements

An EPSs solution (5%, w/v) was prepared and the apparent viscosity of the solution was measured at 25ºC using a Brookfield viscometer equipped with spindle No.18 (Brookfield, Middleboro, USA).

2.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA). A comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). All statistical analysis was performed using the Statistical Package for Social Science (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL).

3. Results

3.1 Isolation and screening for EPSs producing LAB

One hundred and twenty ropy colonies of LAB were isolated from various Thai traditional fermented foods. Only 14 isolates were able to produce EPSs in modified MRS broth at levels more than 5 g/l (data not shown). Among the EPSs solutions (5% w/v) from the 14 isolates, four had viscosity values higher than 400 cP (Table 1). The highest viscosity with the highest EPSs yield was obtained from isolate NH 02, which was isolated from *Nham*, fermented minced pork sausage. From 16S rDNA analysis, the strain NH 02 was identified as *Weissella confusa*. The 16S rDNA sequence (approx. 1.5 kb) was deposited in DDBJ/EMBL/GenBank with accession number AB425970.

3.2 Growth and EPSs production

The maximum production of total cell protein was obtained when a medium containing glucose was used as a carbon source ($p<0.05$) (Table 2). However, the EPSs production showed the highest values when sucrose was used ($p<0.05$). EPSs with an apparent viscosity of 177 cP were obtained at a level of 2.75 g/l in MRS containing sucrose as the carbon source. EPSs were produced with a lower extent when glucose, fructose, and lactose were used. As a consequence, the viscosity of those EPSs was non-detectable with the equipment used. The maximum sucrose concentration for both cell growth and EPSs production was achieved at 40 g/l ($p<0.05$) (Table 2). Results revealed that sucrose was the preferable carbon source, compared to other sugars for EPSs production.

As shown in Table 2, the initial pH of medium had an effect on cell growth and EPSs production. At pH of 7.0 and 7.5, the highest EPSs production (about 17.67 g/l) was obtained ($p<0.05$).

From the temperature study, it was found that *W. confusa* NH 02 could grow under a temperature range from 15ºC to 45ºC. The incubation temperature ranging from 15-42ºC had no affect on the growth of *W. confusa* NH 02 ($p>0.05$). However, the incubation temperature affected the EPSs production of this strain (Table 2). *W. confusa* NH 02 cultivated at 37ºC produced EPSs with a maximum yield (about 20.25 g/l) ($p<0.05$).

The effect of agitation rate on growth and EPSs production of *W. confusa* NH 02 is illustrated in Table 2. Growth and EPSs production of *W. confusa* NH was governed by the agitation rate. The highest EPSs production was achieved at 150 rpm agitation ($p<0.05$), however the viscosity of the resulting EPSs solution had the lowest value (Table 2). High agitation might result in chain breaking, yielding the short chain EPSs. Therefore cultivation without agitation was selected to culture *W. confusa* NH 02 for EPSs production.

Time course studies were conducted on growth and EPSs production by *W. confusa* NH 02 in modified MRS medium (pH 7.0) using 4% sucrose as the carbon source for 60 hrs at 37ºC (Figure 1). The bacterium grew rapidly within the first 12 hrs, which was correlated with the rapid decline in pH. Sugar was suggested to be metabolized by cells and acidic metabolites and EPSs were subsequently produced. The highest EPSs yield (18.08 g/l) was obtained at 12 hrs of cultivation, which was in the early stationary phase. The optimal period for the production of cell biomass and EPSs from *W. confusa* NH 02 appeared to be dependent on its growth.

The molecular weight of EPSs from *W. confusa* NH 02 was determined to be 1.13×10^6 Da (data not shown). Glucose was the major sugar in EPSs, accounting for 96.8% of the total sugar (Figure 2). No protein was detected in the EPSs from *W. confusa* NH 02.

Table 1. Exopolysaccharides, viscosity, and yield of exopolysaccharides obtained from lactic acid bacteria isolated from fermented foods. Lactic acid bacteria were cultivated in modified MRS broth (4 ml) containing 2% sucrose and incubated 24 hrs at 37ºC.

Fermented food	Isolates	Exopolysaccharides (g/l)*	Viscosity (cP)*	Total cell protein (g/l)*	Yield (g/g total cell protein)
Som-Fag	SF 10	5.833±0.629^{b}**	420±20.380^{c}**	0.361±0.017^{a}**	16.19^{b}**
Sai-Krok-Isan	SE 16	6.500±0.250^{a}	808±45.846^{b}	0.387±0.017^{a}	16.78^{ab}
Nham	NH 02	6.667±0.289^{a}	2,341±65.238^{a}	0.367±0.008^{a}	18.18^{a}
Nham	NH 08	6.583±0.144^{a}	434±54.736^{c}	0.415±0.006^{a}	15.88^{b}

*Values are given as mean±SD from triplicate determinations.
**Different letters in the same column indicate significant differences ($p<0.05$).

Table 2. Effects of selected factors on growth, exopolysaccharides, and viscosity of exopolysaccharides produced from *W. confusa* NH 02, which was cultivated in modified MRS broth (100 ml) and incubated 24 hrs.

Factors	Growth (Total cell protein, g/l) *	EPSs (g/l) *	Viscosity (cP)
Carbon sources (2%)			
Glucose	0.31±0.014^{a}**	0.58±0.144^{c}**	ND
Fructose	0.22±0.017^{c}	0.75±0.250^{c}	ND
Sucrose	0.26±0.023^{b}	2.75±0.250^{a}	177±68.158
Lactose	0.10±0.020^{d}	1.17±0.144^{b}	ND
Sucrose concentrations (g/l)			
0	0.04±0.003^{c}	0.42±0.144^{d}	ND
10	0.29±0.018^{ab}	2.92±0.144^{c}	197±48.537^{c}**
20	0.31±0.032^{ab}	3.83±0.382^{ab}	224±58.423^{b}
30	0.30±0.016^{ab}	4.08±0.878^{ab}	279±29.861^{b}
40	0.32±0.016^{a}	4.67±0.629^{a}	437±76.668^{a}
50	0.32±0.005^{ab}	3.75±0.25^{b}	466±87.462^{a}
60	0.28±0.013^{b}	3.83±0.013^{b}	90±27.189^{d}
Initial pH			
5.0	0.26±0.016^{c}	8.08±0.946^{c}	820±37.569^{b}
5.7	0.36±0.030^{b}	11.50±1.090^{bc}	823±59.348^{b}
6.0	0.38±0.028^{b}	12.08±1.809^{bc}	920±49.376^{a}
6.5	0.44±0.011^{a}	13.75±4.562^{ab}	978±34.586^{a}
7.0	0.45±0.014^{a}	17.67±1.665^{a}	959±42.853^{a}
7.5	0.47±0.063^{a}	17.67±2.184^{a}	956±61.285^{a}
Incubation temperature (^{o}C)			
15	0.53±0.125^{a}	12.92±3.608^{b}	894±43.579^{b}
20	0.50±0.037^{a}	16.92±1.809^{ab}	854±38.497^{b}
25	0.48±0.087^{a}	14.67±3.166^{ab}	850±29.834^{b}
30	0.48±0.009^{a}	13.08±4.481^{b}	934±41.264^{a}
37	0.47±0.038^{a}	20.25±1.146^{a}	958±35.761^{a}
42	0.41±0.038^{a}	3.39±0.19^{c}	ND
45	0.29±0.013^{b}	0.33±0.12^{d}	ND
Agitation rate (rpm)			
0	0.47±0.005^{a}	18.92±0.144^{b}	923±64.972^{a}
50	0.47±0.022^{a}	18.00±0.433^{c}	524±48.924^{b}
100	0.43±0.010^{b}	17.92±0.520^{c}	513±38.469^{b}
150	0.45±0.025^{ab}	21.50±0.250^{a}	180±27.953^{c}

*Values are given as mean±SD from triplicate determinations.
**Different letters in the same column within the same parameter studied indicate significant differences ($p<0.05$).
ND: Non-detectable.

4. Discussion

Several LAB strains contribute to the texture development and viscosity of fermented products via the synthesis of EPSs. LAB, like many other bacteria, are able to produce several types of polysaccharides that are classified according to their location relative to the cell. Those excreted outside the cell wall are called exocellular polysaccharides or EPSs. These formed as an adherent cohesive layer are referred to capsular polysaccharides. EPSs also can either be loosely attached or be completely excreted into the environment as slime (Madigan *et al.*, 1997). In this study, *W. confusa* NH 02 produced EPSs and excreted them into the culture broth. The bacterial EPSs are not used as energy sources by the producer microorganism. They probably have a protective function in the natural environment, e.g. against desiccation, phagocytosis and predation by protozoa, phage attack, antibiotics or toxic compounds and osmotic stress (Ruas-Madiedo and de los Reyes-Gavilan, 2005).

*Weissella s*trains have been isolated from a variety of

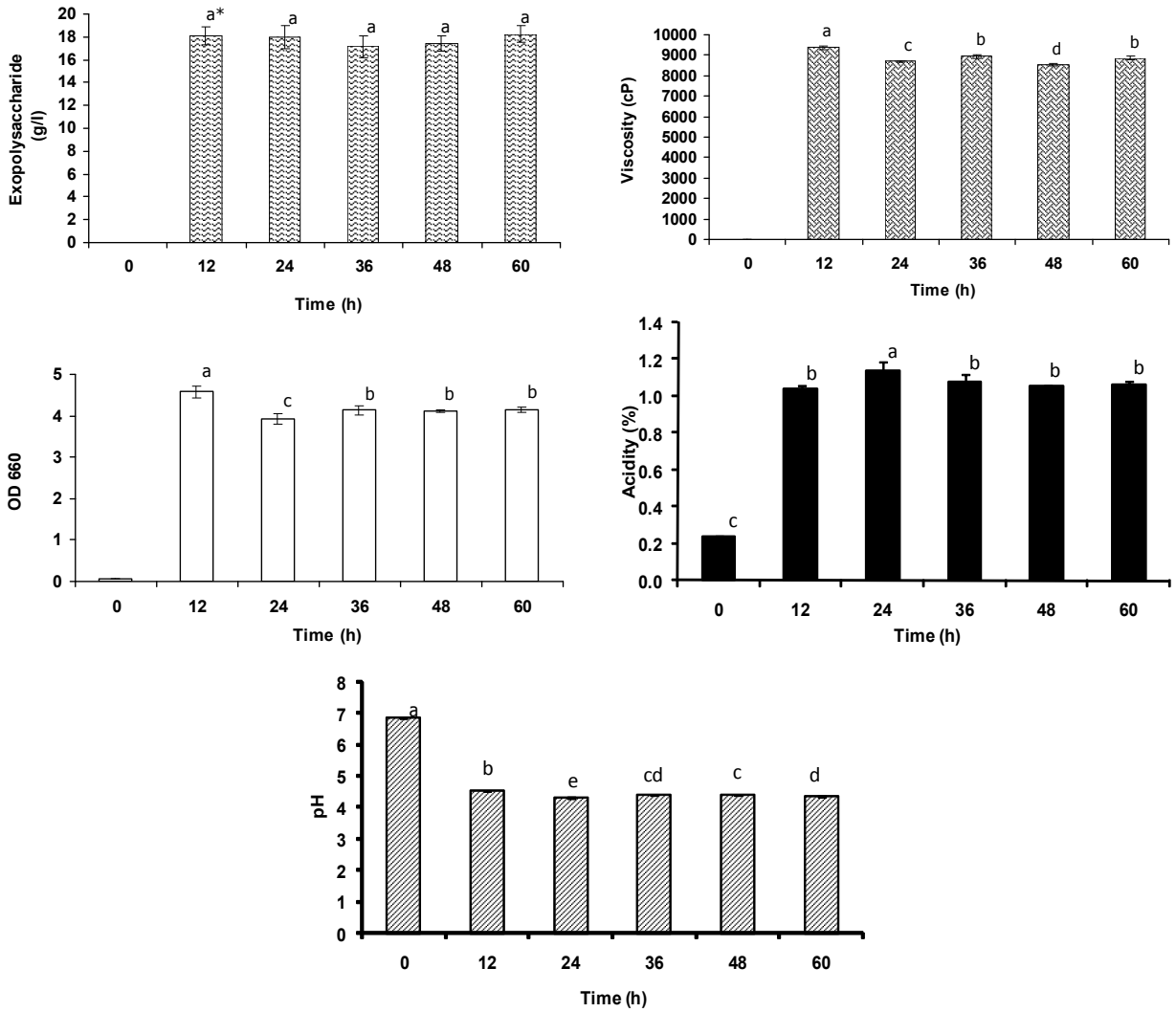

Figure 1. Time course of cell growth, exopolysaccharides production and viscosity of exopolysaccharides (5%, w/v) obtained from *W. confusa* NH 02 at various periods. *W. confusa* NH02 was cultivated in MRS broth containing 4% sucrose and incubated at 37°C. * Different letters in the same parameter studied indicate significant difference ($p<0.05$).

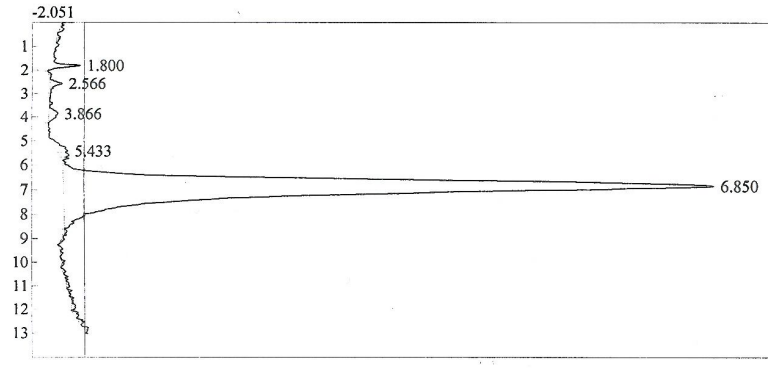

Figure 2. HPLC chromatogram of EPSs produced by *W. confusa* NH 02.

sources. *Weissella confusa* have been isolated from fish, vegetable products and fermented beverages (Corsetti *et al.*, 2001; Booysen *et al.*, 2002; Mugula *et al.*, 2003; Muyanja *et al.*, 2003; Lee *et al.*, 2005; Santos *et al.*, 2005; Thapa *et al.*, 2006). *Weissella paramesenteroides* is one of the predominant species found in fresh vegetables and it plays an

important role in the first stage of silage fermentation (Dellaglio *et al.*, 1984; Dellaglio and Torriani, 1986). *Weissella thailandensis* was isolated from fermented fish products in Thailand (Tanasupawat *et al.*, 2000). Most LAB producing EPSs have been isolated in primary stages of fermentation (van den Berg *et al.*, 1993). In contrast, the bacteria producing EPSs in this study were isolated from the product within the final stage of fermentation.

Fermentation conditions had a great impact on the EPSs production. In this study, it was found that *W. confusa* NH 02 metabolized all tested sugars, but only sucrose gave a remarkable EPSs production yield. The results were in accordance with a previous study that *Lactobacillus reuteri* ATCC 55730 had the ability to metabolize sucrose, glucose, and fructose; however the EPSs production was observed only when sucrose was used (Årsköld *et al.*, 2007). The best carbon source for the EPSs production varied with the species of LAB. Glucose was the most efficient carbon source for EPSs production by *Lactobacillus casei* CG11, whereas lactose was not an efficient carbon source (Cerning *et al.*, 1994). No difference in the quantity of EPSs produced was reported when glucose and lactose were used as carbon source by *Lactobacillus rhamnosus* 9595M and *Lactobacillus paracasei* Type V, except *Lactobacillus rhamnosus* R (Dupont *et al.*, 2000). The sugar concentration had a marked effect on the EPSs production yield. With increasing sucrose concentration, an increased EPSs production by *W. confusa* NH 02 was obtained. The maximum EPSs production occurred with 40 g of sucrose per liter in the medium. Since 20 g of sucrose per liter was an excessive carbohydrate source for normal bacterial growth, sucrose most likely stimulated the EPSs production. It has been reported previously that EPSs are produced even when little or no growth occurs (Bryan *et al.*, 1986; Cerning *et al.*, 1986). Generally, there appeared to be some relationship between the amount of EPS and the viscosity. However, it does not seem to be a direct proportional relationship sine differences in viscosity were much greater than differences in the amounts of EPS. In addition, due to the low sensitivity of the viscometer the degree of error of these results is probably higher (Ludbrook *et al.*, 1997).

Optimum temperatures for EPSs synthesis have been reported for a number of LAB. For *W. confusa* NH 02, EPSs production was highest at 37ºC, while the maximum growth was observed at 15ºC. However, temperature had no affect on growth of *W. confusa* NH 02 until the temperature rose up to 45ºC. van den Berg *et al.* (1995) reported that when *L. sake* 0-1 was cultivated at lower temperatures, the EPS production per gram of biomass increased from 600 mg at 20ºC to 700 mg at 10ºC. Cerning *et al.* (1992) have shown that the optimum temperature for EPSs synthesis was 25ºC and less than 37ºC for *L. lactis* and *S. thermophilus*, respectively. *L. rhamnosus* produced a high content of EPSs at 20-25ºC, whereas the optimal temperature for its growth was in the range of 30-37ºC (Gamar *et al.*, 1997). The incubation temperature affected the polysaccharide production (Table 2). Other studies have shown that incubating LAB below the optimum growth temperature resulted in a greater production of EPSs (Cerning *et al.*, 1992; Gancel and Novel, 1994). Schellhaass and Morris (1985) indicated that lower incubation temperatures resulted in slower growth rates, which promoted longer logarithmic and stationary phases. The association of low cell mass with greater polysaccharide production has been explained by Sutherland (1972). When cells were grown slowly, the synthesis of the cell wall polymer was also slower, making more isoprenoid phosphate available for the exopolymer synthesis. Generally, at suboptimal growth temperatures, more undecaprenol (C_{55}) lipid carrier is available for the synthesis of the repeating unit of the EPSs. Undecaprenol plays a key role in the biosynthesis of EPS but also in that of peptidoglycan, lipopolysaccharide, and teichoic acid. It may therefore be expected that at higher growth rates, more undecaprenol will be used for the production of cell wall material than for the biosynthesis of EPS, resulting in decreased slime production. On the other hand, it is also possible that at optimal growth temperatures, the organisms imply uses excess sugar in the cell wall biosynthesis.

A previous work (van den Berg *et al.*, 1995) has shown that pH is a critical parameter influencing the bacterial growth as well as the polysaccharide synthesis. However, no general conclusion can be drawn as the LAB respond to environmental pH in different ways (Nikkila *et al.*, 1996). Optimal initial pH of 7.0-7.5 was retained to improve both growth and EPSs production of *W. confusa* NH 02. Although unfavorable culturing conditions may trigger slime formation by the cells as a form of self-protection, it greatly depends on the parameter tested.

Agitation rate had influence on the growth of *W. confusa* NH 02 as well as on the EPSs production. An agitation rate at 150 rpm gave the highest yield but the resulting EPSs showed the lowest viscosity. Previous studies using LAB have shown that maximal EPSs production occurred under aeration (Cerning, 1990). Agitation and dissolved oxygen (DO) control are important in aerobic fermentation processes, particularly in polysaccharide production, since the broth becomes highly viscous and limits mass and oxygen transfer, which then influences the cellular activities and the secondary metabolite production (Banik and Santhiagu, 2006). Generally, agitation neither influences growth nor heteropolysaccharide production distinctly. However, it affects the viscosity, probably by breaking some molecular associations among the heteropolysaccharide, certain medium components, and the bacterial surface (Torino *et al.*, 2000).

The maximum EPSs production by *W. confusa* NH 02 was in the early stationary stage of growth which confirmed previous findings (Velasco *et al.*, 2006). The production of EPSs appears to occur during the logarithmic phase, and for some LAB, continues into the stationary phase (Cerning *et al.*, 1994; van Geel-Schutten *et al.*, 1998). Fermentation time is one of the critical environmental parameters affecting content, molecular mass, and sugar composition of EPS. Pham *et al.* (2000) reported that the content of EPS produced

by *L. rhamnosus* declined with prolonged fermentation time, due to the presence of various glycohydrolases. Shu and Lung (2004) observed that the proportion of high molecular weight EPSs produced by *Antrodia camphorate* declined with fermentation time, possibly due to the presence of an endoamylase capable of partially hydrolyzing EPSs (Catley, 1980). This was consistent with results obtained by Cerning *et al.* (1988) who observed that high molecular mass EPSs produced by *Strep. thermophilus* decreased after 48 hrs, probably due to the presence of either lactate and/ or hydrolytic enzymes . While Bouzar *et al.* (1996) reported that the sugar composition of EPSs from *L. delbrueckii* subsp. *bulgaricus* CNRZ 1187 changed during the fermentation cycle, the sugar composition of *Strep. thermophilus* LY03 remained constant during the whole batch fermentation process (De Vuyst *et al.*, 1998). Under optimal culture conditions, maximum EPSs production by *W. confusa* NH 02 was 18.08 g/l. Generally, a higher yield of EPSs production was obtained from LAB isolated from dairy products such as *Leuconostoc citreum* 4.11 (17.2 g/l, raw milk) (Van der Meulan *et al.*, 2007). An EPSs production of 6.0 g/l was achieved by *Pediococcus pentosaceus* AP-1 isolated from meat products (Smitinont *et al.*, 1999).

EPSs produced by LAB can be divided into two categories, homopolysaccharides, containing only one type of monosaccharide, and heteropolysaccharides, composing of repeating units, varying in size from di-to heptasaccharides, and non-sugar molecules. The homopolysaccharides can be clustered into four groups, α-D-glucans, β-D-glucans, fructans, and others, like polygalactan (Ruas-Madiedo *et al.*, 2002). The molecular weight of EPSs from *W. confusa* NH 02 was 1.13×10^6 Da. This was in agreement with earlier studies of Cerning (1990) who reported that homopolysaccharides have a higher molecular weight. These biopolymers are produced in large quantities. For example, a strain of *Lb. reuteri* LB 121 is able to produce nearly 10 g/l of two types of homopolysaccharides mainly comprising D-glucose or D-fructose (van Geel-Schutten *et al.*, 1999). When comparing the previous reports, *W. confusa* NH 02 produced the highest EPS yield (18.08 g/l). However, the type and structure of homopolysaccharide should be elucidated. In addition, further work in determining the mechanisms responsible for the control and regulation of EPSs biosynthesis, both at the level of genes and of proteins, is required.

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