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Full Length Research Paper

Growth and exopolysaccharide production by *Weissella* sp. from low-cost substitutes for sucrose

Chintana Tayuan[1]*, Gerald W. Tannock[2] and Sureelak Rodtong[1]

[1]School of Microbiology, Institute of Science, Suranaree University of Technology, Nakon Ratchasima, 30000, Thailand.
[2]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand.

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Homopolysaccharides produced from sucrose by lactic acid bacteria (LAB) are of interest as natural additives to improve textural properties of foods. The production of exopolysaccharide (EPS) by *Weissella* strains, which had been isolated from a traditional Thai food (plasom), was investigated using sugarcane molasses and white sugar from sugarcane as substrates in comparison to analytical grade sucrose. EPS production was evaluated at 30°C for 48 h. EPS production and bacterial growth were influenced by the type of carbon source used in culture medium. The highest EPS concentration was obtained in the medium supplemented with white sugar from sugarcane as a sole carbon source by *Weissella* sp. PSMS4-4. The optimal concentration and factors affecting the production of EPS, that is, cultivation temperature and initial pH of medium, were determined. The highest EPS production of 8.65 g/l was attained in culture with an initial pH of 7.0, temperature of 30°C, and white sugar concentration of 5%. This study shows the first report on the production of *Weissella* EPS using low-cost substitutes for sucrose and provides knowledge of factors influencing the production of the polymer.

Key words: Exopolysaccharide, sucrose, white sugar from sugarcane, lactic acid bacteria, *Weissella*.

INTRODUCTION

Microbial exopolysaccharides (EPS) are long-chain polysaccharides which are either adherent to the cell surface in the form of a capsule, or secreted into the extracellular environment as loose slime. The polymer may protect the cell against desiccation (Robertson and Firestone, 1992), metal ions, antibiotics, bacteriophages, and the cell wall-degrading enzymes (Costerton, 1999; Looijesteijn et al., 2001). Other possible functions of EPS include involvement in adhesion and biofilm formation (Roberts, 1996). Some generally recognized as safe (GRAS) bacteria, particularly lactic acid bacteria (LAB), propionibacteria and bifidobacteria, are known for their EPS production ability (Gorret et al., 2001; De Vuyst and Degeest, 1999; Andaloussi et al., 1995). *Weissella* sp. has also been reported to produce EPS. *Weissella* is Gram positive, obligate heterofermentative, and catalase-negative lactic acid bacteria which first described by Collins et al. (1993). EPS-forming strains of *Weissella* can be present in several habitats such as in (Kim et al., (2008), human saliva (Kang et al., 2009), soya (Malik et al., 2009) and sourdoughs (Di Cagno et al., 2006). Homopolysaccharides (that is, glucans and fructans made of glucose and fructose, respectively) are common exopolysaccharides synthesized by *Weissella* from sucrose (Di Cagno et al., 2006; Kang et al., 2006; Katina et al., 2009; Kim et al., 2008; Maina et al., 2008). Several studies reported that EPS-producing *Weissella* offer many promising applications in food industry. Kim et al. (2008) reported that the β-glucan produced by *Weissella* SKkimchi3 isolated from fermented food (kimchi) may be developed for a safe food additive. *Weissella confusa* has also been reported to be a promising strain for efficient *in situ* production of dextrans and isomaltooligosaccharides in sourdoughs (Katina et al., 2009). The *Weissella* EPS

*Corresponding author. E-mail: chobvijuk@hotmail.com. Tel: + 66 44 223 203. Fax: + 66 44 224 633.

Abbreviations: EPS, Exopolysaccharide; **LAB,** lactic acid bacteria; **GRAS,** generally recognized as safe; **HPLC,** high pressure liquid chromatography; **TCA,** trichloroacetic acid; **PCR,** polymerase chain reaction; **DNA,** deoxyribonucleic acid; **MRS,** De Man, Rogosa and Sharpe.

produced in sourdoughs improved the textural properties and quality of bread (Di Cagno et al., 2006). Galle et al. (2010) reported that *Weissella* strains are suitable starter cultures for wheat and sorghum sourdoughs and efficiently produce gluco-oligosaccharides and EPS.

In general, EPS production and concentrations can be influenced by the composition of the culture medium (carbon and nitrogen sources) (Tallon et al., 2003; De Vuyst et al., 1998; Gamar-Nourani et al., 1998). LAB strains can utilize carbohydrate sugars, such as glucose, lactose, sucrose, and mannose as the carbon source for EPS production (Aslim et al., 2005; Cerning et al., 1994; Gamar et al., 1997; Smitinont et al., 1999, Senini et al., 2004; Tallon et al., 2003; Bauer et al., 2009; Xu et al., 2010). It has previously been reported that EPS can also be produced from a wide range of carbon sources such as oat-based non-dairy medium (Martensson et al., 2000; Martensson et al., 2003), whey permeate medium (Macedo et al., 2002), sugarbeet molasses (Yeniel et al., 2006), and sago starch (Yeesang et al., 2008). In addition, the pH of the culture media and incubation temperature appear to be other important parameters in EPS production (Dueñas et al., 2003; Grobben et al, 1995; Kimmel et al., 1998; Tallon et al., 2003; Velasco et al., 2006; Årsköld et al., 2007).

Sucrose is a common carbon source for EPS production by *Weissella* sp. (Di Cagno et al., 2006; Kang et al., 2006; Katina et al., 2009; Kim et al., 2008; Maina et al., 2008). However, research on EPS produced by *Weissella* sp. using low-cost carbon sources has not previously been reported. Cheap and abundant carbon sources would be of interest for economical production of EPS, especially in developing countries.

In the present study, *Weissella* sp. were evaluated for EPS production using low-cost substitutes for sucrose under various growth conditions.

MATERIALS AND METHODS

Microorganism and culture conditions

Six strains of *Weissella* sp. were isolated from a traditional Thai fermented fish (plasom). Bacterial isolates were maintained in De Man, Rogosa and Sharpe (MRS) broth (De Man et al., 1960) and stored at -20°C with the addition of skim milk to 5% (v/v) final concentration. For cell propagation procedure, the stock cultures were taken from -20°C freezer, thawed at room temperature. Two hundred µl of each culture were used to inoculate 2 ml of MRS broth. After incubation for 18 h in an anaerobic chamber with a gas mixture of CO_2:H_2:N_2 (5:5:90%) (Shel LAB, Sheldon Manufacturing, Inc, U.S.A.), the culture was streaked onto MRS agar, and incubated under the same conditions for 48 h. Then, a single colony was subcultured for further study.

Influence of different carbon sources on exopolysaccharide (EPS) production

To obtain a suitable carbon source based on a cheap and abundant raw material, sugarcane molasses, white sugar from sugarcane purchased from a local supermarket (Mitr Phol Sugar Co., Ltd., Thailand), or analytical grade sucrose (Carlo Erba, Italy) were used to replace glucose in MRS medium (De Man et al., 1960) at a concentration of 20 g/l. The initial pH of the media was adjusted to pH 6.2. Ten ml of MRS medium were inoculated with 2% (v/v) of culture (approximately 10^6 CFU/ml) and incubated at 30°C under anaerobic condition for 48 h. Bacterial growth was measured by monitoring viable cell counts. Viable cell counts (CFU/ml) were estimated by plating serial dilutions using 0.1% peptone water of bacterial suspension on MRS agar. Plates were incubated anaerobically for 48 h in an anaerobic chamber. EPS concentration in the supernatant was measured after centrifugation at 10,000 rpm for 10 min at 4°C to deposit bacterial cells. Polysaccharides in the supernatant were isolated by precipitation with 3 volumes of chilled 80% (v/v) ethanol, and kept overnight at 4°C according to Dueñas et al. (2003). The precipitate was collected by centrifugation at 4,500 rpm for 20 min at 4°C. EPS quantity was determined by measuring total sugar content of the precipitates. The total sugar content was determined by the phenol-sulfuric acid method (DuBois et al., 1956), using glucose as a standard. The uninoculated MRS broth was employed as a control. The EPS concentration after subtracting the value for the control was expressed in g equivalent glucose/l. The specific EPS production, calculated by dividing the amount of EPS produced by the bacterial cell count, was expressed as pg EPS/cell. The isolate which produces the highest amount of EPS and the most promising carbon source were selected for further study.

Optimization of exopolysaccharide (EPS) production conditions

Optimum concentration was then determined for the carbon source selected in the previous stage. Carbon source concentrations of 20, 30, 35, 40, 45, 50, 55 and 60 g/l were tested to determine the optimal concentration for EPS production. The initial pH of the optimized medium for EPS production was then studied. The medium was adjusted to pH 4.5, 5.0, 6.0, 7.0, or 8.0 using 1 N HCl or 1 N NaOH, and then used for production of the polymer. The optimal initial pH of the medium was then used in further studies. Temperatures for EPS production were also investigated (30, 35, 37, 40, and 45°C).

To study the time course of growth, EPS production, change of pH, sugar consumption under optimal conditions, the fermentation was performed in 500 ml Duran bottles containing 350 ml of medium, for 48 h. Changes of pH, EPS concentration, and bacterial growth were measured after 0, 3, 6, 9, 12, 15, 18, 24, 30, 36 and 48 h of cultivation as described previously. Residual sugar concentration was measured at time intervals using high pressure liquid chromatography (HPLC) with a Refractive Index Detector (Waters, Waters Corporation, U.S.A). A 100 µl of the supernatant was injected into VertisepTM OA HPLC (Vertical chromatography, Thailand) (300×7.8 mm). A 0.005 M H_2SO_4 solution was used as the mobile phase at a constant flow rate of 0.4 ml/min. The EPS yield, calculated by dividing amount of EPS produced by the amount of sugar consumed, was expressed as %.

Purification and monosaccharide analysis

After EPS production under optimal conditions, the EPS was purified, hydrolyzed and analyzed by HPLC. The crude polysaccharide was purified by trichloroacetic acid (TCA) precipitation and protease digestion. Briefly, 20% TCA was added to the polysaccharide solution at a 1:1 volume ratio. Then, the precipitate was removed by centrifugation at 11,000 rpm for 10 min at 4°C. Supernatant containing EPS was dialyzed using

Snakeskin™ pleated dialysis tube of molecular weight cut-off 10,000 against sterile distilled water at 4°C for 3 days, with two daily changes of water, then freeze-dried. Pronase E (protease type XIV) from *Streptomyces griseus* and Proteinase K from *Tritirachium album* were then used for EPS purification according to Gancel and Novel (1994) and Zisu and Shah (2003), respectively. The residual proteins were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard. After purification, negligible amount of protein was detected.

Hydrolysis of the EPS was carried out by incubating the sample for 4 h in 2 M tri-fluoroacetic acid (Supelco, Sigma-Aldrich Chemical Company, U.S.A.) at a concentration of 1 mg/ml at 100°C. The sugar composition of the EPS was analyzed by HPLC with Refractive Index Detector (Waters, Waters Corporation, U.S.A) and Vertisep^{TM} OA HPLC (Vertical chromatography, Thailand) in which the flow rate was adjusted to 0.4 ml/min at 25°C. Deionized water was used as mobile phase. D-Glucose, D-galactose, D-mannose, N-acetylgalactosamine, N-acetylglucosamine and L-rhamnose were used as external standards (Merck KGaA, Germany).

Bacterial identification

Primary identification of EPS-producing isolates was including Gram-staining, cell morphology, cell arrangements, catalase activity, and production of CO_2 from D-glucose in MRS broth (Schillinger and Lücke, 1987). Carbohydrate fermentation profiles and biochemical tests were obtained using API 50-CHL system (Biome´rieux, RCS Lyon, France) according to the manufacturer's instructions. The results were recorded after 24 and 48 h of incubation at 30°C. Carbohydrate fermentation profiles and biochemical results were compared to those in the API50CH/CHL database (Biome´rieux, RCS Lyon, France). The selected isolate which produced the highest amount of EPS was confirmed by 16S ribosomal ribonucleic acid (rRNA)] gene sequence analysis according to Weisburg et al. (1991).

Briefly, genomic DNA was extracted from bacterial cells using a wizard genomic DNA purified kit (Promega). Polymerase chain reaction (PCR) was performed using a thermo electron corporation Px2 thermal cycler (Bioscience Technologies Division, U.S.A.) with a primary heating step for 2 min at 95°C, followed by 35 cycles of denaturation for 45 s at 95°C, annealing for 45 s at 55°C, and extension for 2 min at 72°C which was followed by a final extension at 72°C for 7 min. Each 25 µl of reaction mixture contained 2 µl of genomic DNA, 13.5 µl of MilliQ water, 2.5 µl of 10X PCR buffer (Invitrogen), 2.5 µl of 25 mM $MgCl_2$, 2.5 µl of 2 mM dNTPs mixture (Invitrogen), and 0.5 µl of Taq DNA polymerase (5U/µl) (Invitrogen). Amplification of 16S rRNA gene was performed with primers: fD1; 5'-AGAGTTTGATCCTGGCTCAG-3' and rP2; 5'-ACGGCTACCTTGTTACGACTT-3' (Weisburg et al., 1991). After agarose gel electrophoreses of the PCR amplicon, bands of approximately 1,500 bp were excised, purified with Witzard® SV Gel and PCR clean-up system (Promega) according the manufacturer's instructions.

DNA fragments were then cloned in *E. coli* pGEM-T easy vector (Promega) according the manufacturer's instructions. Sequencing of the 16S rRNA gene was performed using M13/pUC universal primers forward (5'-GTTTTCCCAGTCACGAC-3') and reverse (5'-CAGGAAACAGCTATGAC-3') (Messing, 1983) and also walking primers: f-LAB/seq (5'-TAACTACGTGCCAGCAGCC-3') and r-LAB/seq (5'-CGACAACCATGCACCACCTG-3') (Microbial Culture Collection and Application Laboratory, Suranaree University of Technology) with terminator ready reaction kit version 2.0 using ABI377 Automated DNA sequencer (Perkin Elmer, U.S.A.). A length of 1541 nucleotides was sequenced and compared to those available in GenBank databases using standard nucleotide-nucleotide BLAST program to identity the isolate.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). A comparison of means was carried out by Turkey's multiple-comparison test. Statistical analysis of the results was performed in GraPad Prism software package version 5.0.

RESULTS

Synthesis of exopolysaccharides (EPS) from different carbon sources

Low-cost sources of sucrose such as sugarcane molasses and refined but not laboratory grade white sugar from sugarcane were tested as a sole carbon source for the production of biopolymer. The initial experiment was carried out anaerobically in modified MRS medium with a carbon source concentration of 2% at 30°C for 48 h. All carbon sources supported bacterial growth and EPS production (Figure 1). White sugar from sugar cane gave higher amount of EPS ranging from 0.24±0.03 to 3.40±0.39 g/l than molasses (0.005±0.002 to 0.64±0.31 g/l) compared to analytical grade sucrose (1.88±0.37 to 3.78±1.06 g/l). *Weissella* sp. PSMS4-4 provided the highest specific EPS production of 75.91±3.87 pg/cell when cultured in medium containing white sugar. In addition, the EPS concentration of 3.40±0.39 g/l in this medium was not significantly different from 3.78±1.06 g/l in analytical grade sucrose. Result indicating that white sugar from sugar cane is a potential carbon source for EPS production by selected lactic acid bacterial isolates. The isolate PSMS4-4, produced high amounts of EPS in white sugar, was selected. From the economic point of view and EPS production potential, white sugar was the most suitable carbon source and was used in further experiments.

The effect of carbon source concentration

Effects of white sugar concentration on cell growth and EPS production were studied. Bacterial cell growth was not influenced by the sugar concentration (P>0.05) (Figure 2). For EPS production, total EPS was significantly increased by increasing the concentration of sugar from 20 to 35 g/l. No further increase was obtained by supplementing the medium with sugar concentrations of 35, 40 and 45 g/l. When sugar concentration increase to 50 g/l, a statistically significant increase in EPS production was observed and provided the highest EPS maximum concentration. However, increasing the sugar concentration from 55 to 60 g/l resulted in the significant reduction of EPS production by *Weissella* sp. PSMS4-4. The trend of the specific EPS production value, which was calculated by dividing the amount of EPS produced by the viable cell count, was similar to EPS production. Maximum specific EPS production of was also obtained at the 50 g of sugar per liter. Overall, a sugar

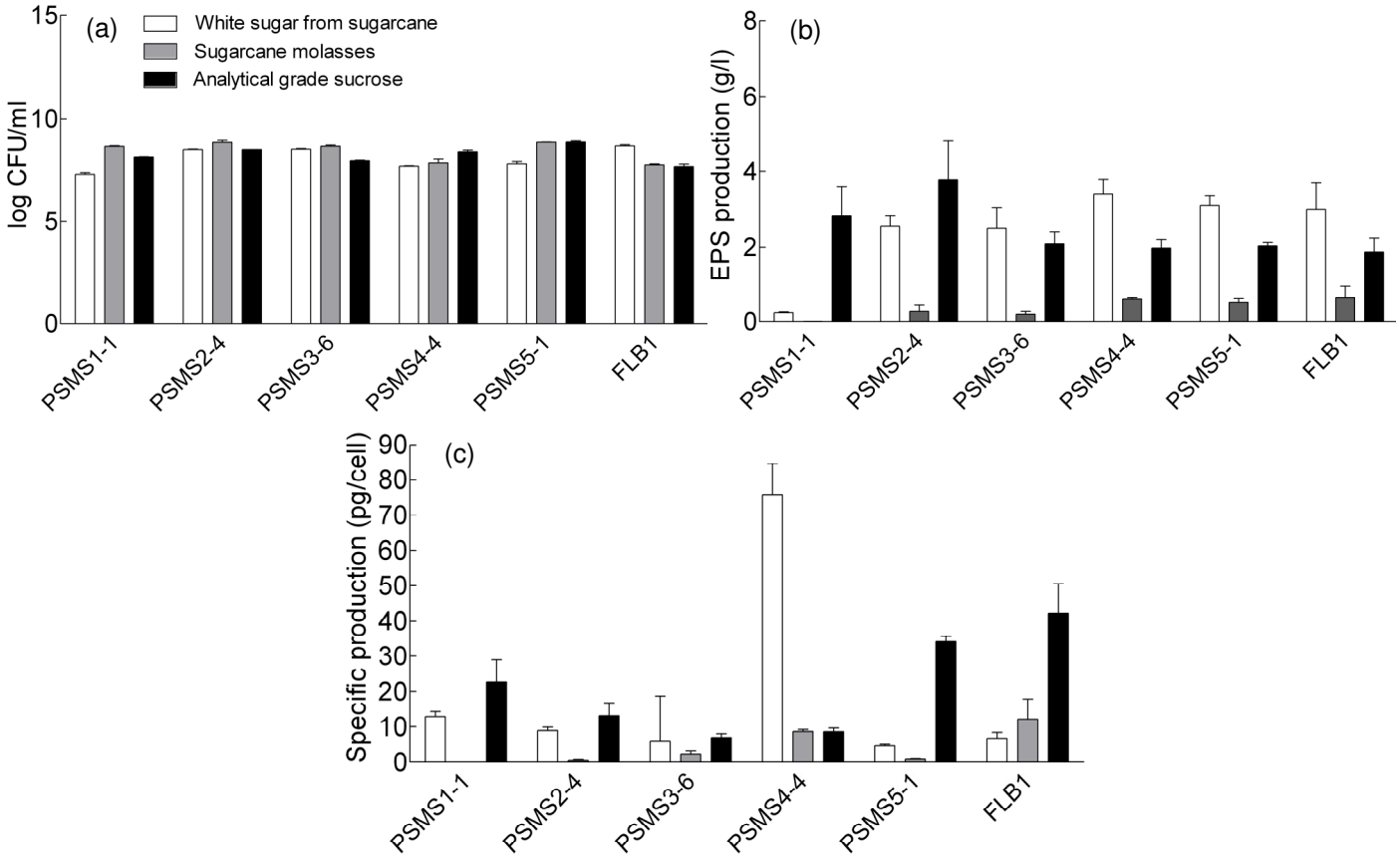

Figure 1. Effect of carbon sources (20 g/l): white sugar from sugarcane, sugarcane molasses, analytical grade sucrose on bacterial growth (a), EPS production (b), and specific EPS production (c) by selected isolates in modified MRS medium with an initial pH of 6.2 and incubated at 30°C for 48 h. Results shown as mean ± SD for duplicate samples.

concentration of 50 g/l was the most suitable for production of the biopolymer by *Weissella* sp. PSMS4-4.

The effect of initial pH of the culture medium

The influence of initial pH values of 4.5, 5.0, 6.0, 7.0, and 8.0 on EPS production by *Weissella* sp. PSMS4-4 was tested anaerobically in MRS broth containing 50 g/l of white sugar at 30°C for 48 h. As shown in Figure 3 the initial pH of medium had an effect on bacterial cell growth and EPS production. There was an increase in EPS production with increasing pH from 5.0 to 7.0. Maximum EPS synthesis was obtained when *Weissella* sp. PSMS4-4 was cultured at a pH of 7.0. While high specific EPS production was found at both pH of 5.0 and 7.0. Further increases in the medium pH did not affect EPS production and specific EPS production significantly. Bacterial growth was stimulated more at pH of 6.0. An increase in pH did not affect the growth of the bacteria (P>0.05). On the other hand, bacterial cell count decreased when the pH was reduced from 6.0 to 5.0 with no significant further decrease at pH 4.5. Hence, the pH of 7.0 was chosen for examining other variables such as temperature.

The effect of cultivation temperature

Five temperatures were tested: 30, 35, 37, 40, and 45°C. The influence of temperature on bacterial growth and EPS production is presented in Figure 4. There was an inverse relationship between both bacterial growth and EPS production, and incubation temperature. The maximum amount of EPS and specific EPS production were attained at 30°C. Increases in the cultivation temperature led to a reduction in growth and EPS production.

The time course of exopolysaccharide (EPS) production

Weissella sp. PSMS4-4 was cultured in MRS medium containing 5% white sugar as a sole carbon source at an initial pH of 7.0 for a period of 48 h at 30°C. The time course for the production of EPS, bacterial growth, sugar

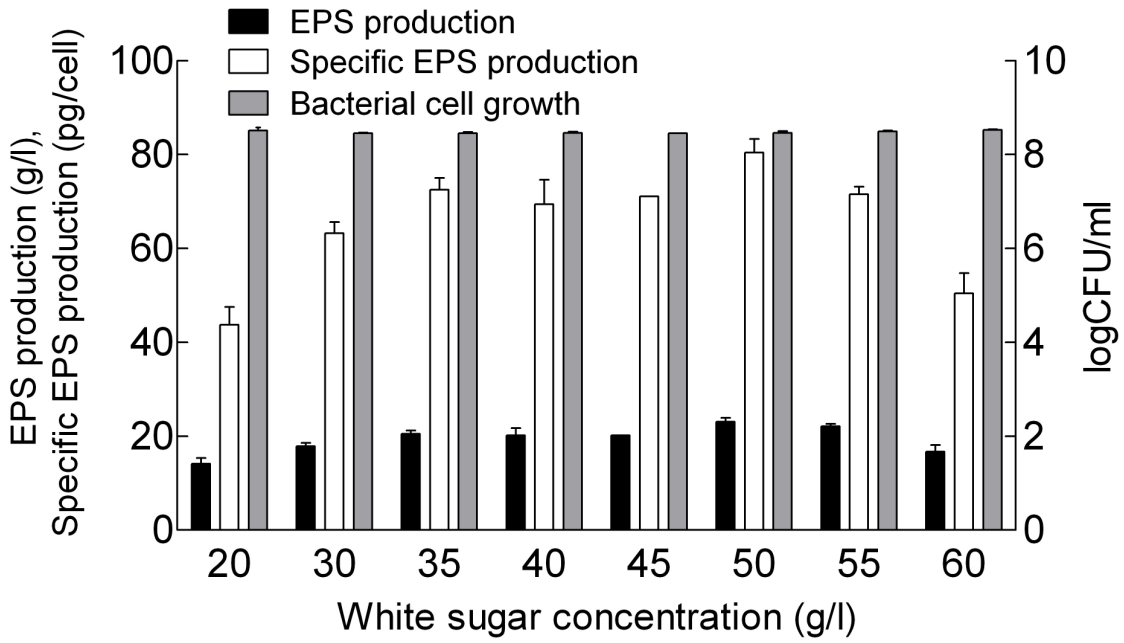

Figure 2. Effect of white sugar concentrations on bacterial growth and EPS production by *Weissella* sp. PSMS 4-4 in modified MRS medium at an initial pH of 6.2 and incubated at 30°C for 48 h. Results shown as mean ± SD for duplicate samples.

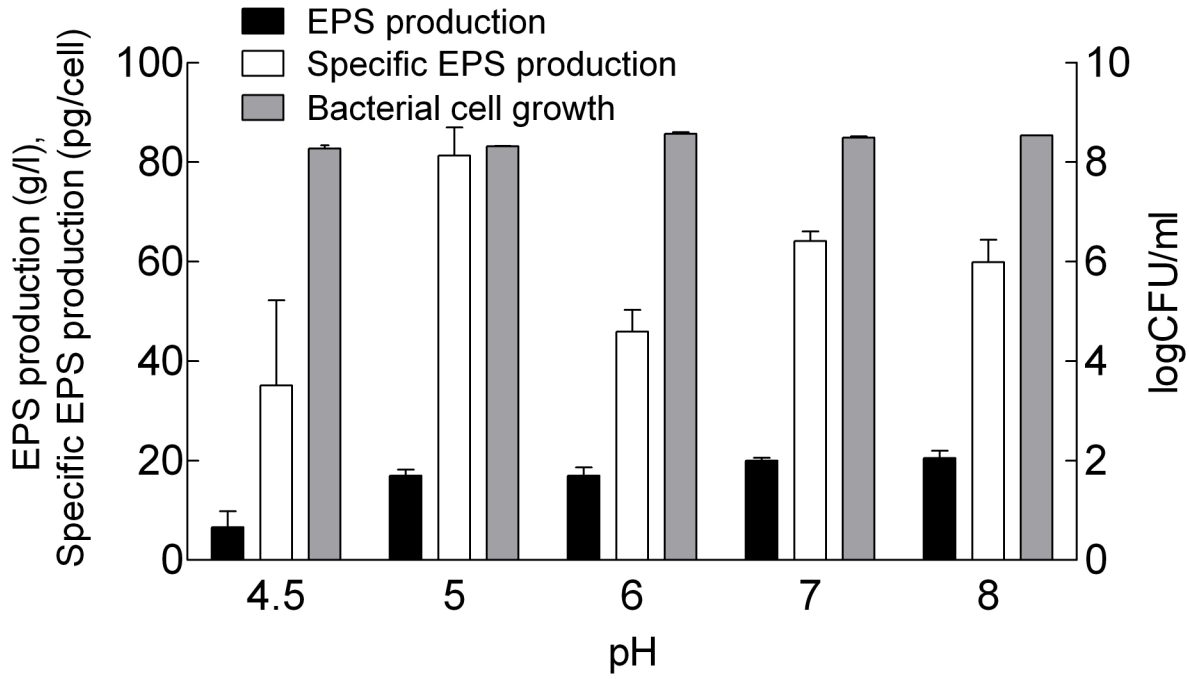

Figure 3. Effect of initial pH of medium on bacterial growth and EPS production by *Weissella* sp. PSMS 4-4 in modified MRS medium at 40°C for 48 h. Results shown as mean ± SD for duplicate samples.

consumption, and changes in pH are presented in Figure 5. Cell number increased exponentially until 6 h after incubation and then entered stationary phase. EPS production increased as the fermentation began and increased dramatically during the 18 h of incubation. Then, the EPS concentration continued to increase and

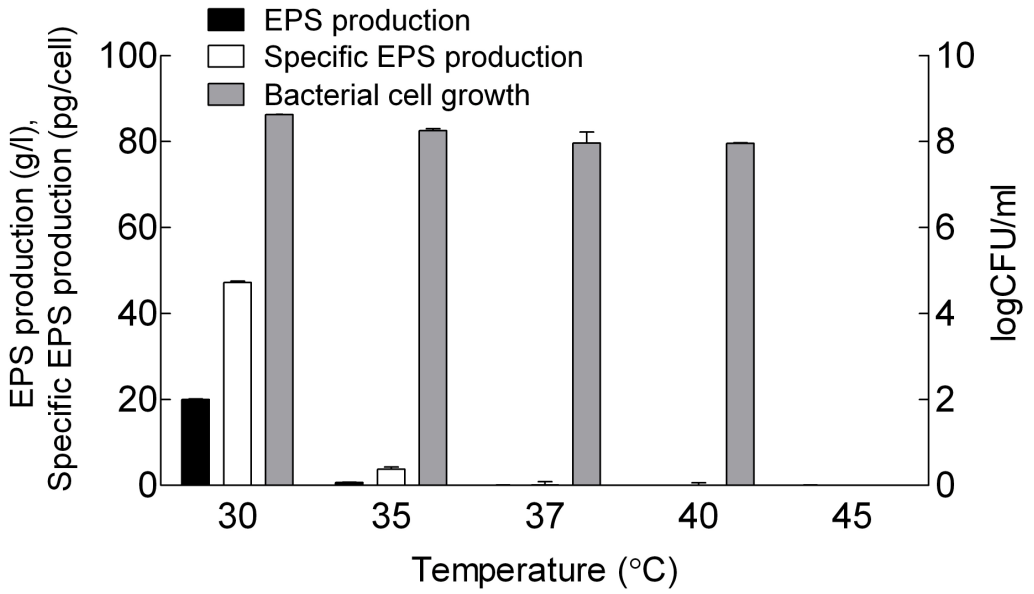

Figure 4. Effect of incubation temperature on bacterial growth and EPS production by *Weissella* sp. PSMS 4-4 in modified MRS medium at an initial pH of 7.0 for 48 h. Results shown as mean ± SD for duplicate samples.

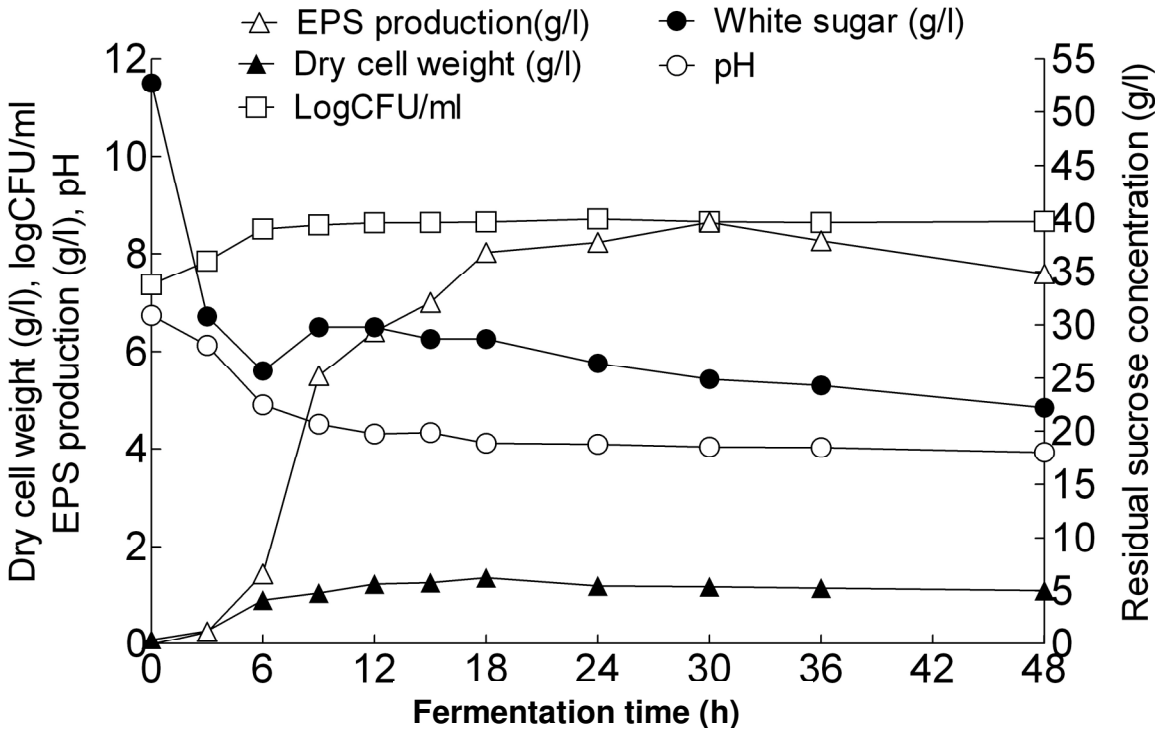

Figure 5. Time course of growth, EPS production, pH of culture medium, and sugar content in the culture of *Weissella* sp. PSMS 4-4 cultivated in modified MRS medium containing 50 g/l of white sugar from sugarcane, initial pH of 7.0, and incubation at 30°C for 48 h. Results represent means of duplicate samples.

reached a maximum level of 8.65 g/l after 30 h of fermentation. Acidification of the culture medium started almost simultaneously with the *Weissella* growth. The initial pH of the medium rapidly decreased from ca. 7.0 to

4.91 during the 6 h incubation and then slightly decreased to 3.93 at 48 h. Sugar consumption by *Weissella* sp. PSMS4-4 during the fermentation period was observed. A continuous reduction of sucrose concentration in the culture medium was accompanied by an increase in EPS concentration. There was a consumption of sucrose at ca. 30.55 g/l (57.95% sucrose consumed) at the end of 48 h of fermentation. The highest EPS yield of 31.09 was obtained after 30 h of fermentation.

Identification of isolate and characterization of exopolysaccharide (EPS)

All EPS-producing isolates were Gram-positive, short chain arrangements, non-spore forming, catalase activity negative, and heterofermentive rods (CO_2 production from glucose). Carbohydrate fermentation profiles and biochemical results were compared to those in the API50CH/CHL database (Biome´rieux, RCS Lyon, France). The isolates PSMS1-1, PSMS2-4, PSMS3-6, PSMS4-4, PSMS5-1 and FLB1 were identified as *W. confusa* with preciseness at 99.8, 99.9, 99.7, 99.2, 99.2 and 99.6% homology, respectively. PSMS4-4 was further identified using 16S ribosomal RNA gene sequencing technique (Weisburg et al., 1991). The gene was amplified from genomic DNA using fD1/rP2 primers. The size of amplified DNA fragments obtained was a 1,541 bp. The 16S rRNA gene sequence of PSMS4-4 was closest to those of *W. confusa* JCM 1093 and *Weissella cibaria* LMG 17699^T (99% similarity). The 16S rRNA gene sequence of *Weissella* sp. PSMS4-4 was deposited in GenBank under nucleotide accession number FJ611786. The EPS produced by *Weissella* sp. PSMS4-4 using 5% of white sugar from sugarcane under optimum conditions was a polymer of glucose.

DISCUSSIONS

Sucrose being one of the most suitable carbon sources for EPS production by LAB (Smitinont et al., 1999; Årsköld et al., 2007; Vijayendra and Babu, 2008; Zotta et al., 2008), including *Weissella* sp. (Di Cagno et al., 2006; Kang et al., 2009; Katina et al., 2009; Kim et al., 2008; Maina et al., 2008). However, cheaper substrates would reduce the cost of EPS production. The results obtained in this study indicate white sugar from sugarcane could be used as a low-cost substrate for EPS production by Weissella, particularly PSMS4-4. The sugar concentration in the medium has been reported to stimulate EPS production by LAB (Cerning et al., 1994). Increase in sugar concentration leads to more EPS production (Dueñas et al., 2003; Hassan et al., 2001; Kim et al., 2008). In this study, raising the white sugar concentration resulted in increased EPS production by *Weissella* PSMS4-4, the maximum EPS production occurred with 50 g of white sugar per liter in the medium. Similarly, EPS production by *Weissella. hellenica* SKkimchi3 was proportional to the amount of sucrose added to the culture (Kim et al., 2008). However, increasing the sugar concentration from 55 to 60 g/l resulted in the significant reduction of EPS production. Decreased EPS production was also found in other LAB, that is, *Lactobacillus salivarius* when cultivated in medium with a high concentration of lactose (Liu et al., 2009). In our study, bacterial growth was not influenced by the sugar concentration, in agreement with Kim et al. (2008).

Very few reports examined the effect of initial pH of medium and temperature on EPS production by *Weissella* sp. An initial pH of modified MRS at 7.0, and a cultivation temperature of 37°C were the optimum conditions for EPS production by *W. confusa* (Wongsuphachat et al., 2010). For *Weissella hellenica* SKkimch3, the maximal EPS was produced by grown on sucrose and pH 5 and at 20°C (Kim et al., 2008). However, the maximum EPS production by *Weissella* sp. PSMS4-4 occurred at an initial pH of the medium of 7.0 at 30°C. In addition, the pH and temperature found to be the important parameters for EPS production by *Weissella* sp. PSMS4-4. In this work, the optimal pH for bacterial growth was 6.0, whereas that for EPS was around 7.0. On the contrary, previous authors have shown that the optimal pH for EPS production by LAB strains was similar to those for optimal growth (Mozzi et al., 1996; De Vuyst et al., 1998).

As already shown by several lactic acid bacteria, temperature is known to have an influence on EPS production. Our result clearly showed that the optimal temperature for EPS production by the Weissella was similar to those for optimal growth. In contrast, Garcia-Garibay and Marshall (1991) found that specific polymer production by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in skim milk was greater at a temperature (48°C) than at the optimal temperatures for growth (37 to 42°C). Cerning et al. (1992) and Van den Berg et al. (1995) reported that the optimal EPS production takes place at temperatures below the optimal growth temperature. In *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains, the EPS production rate was lower at 30, 37 and 42°C than at 45°C (Aslim et al., 2005).

Weissella sp. PSMS4-4 synthesized EPS during growth and continued in stationary phase at 30°C in white sugar. EPS synthesis in LAB strains occurs at different growth phase. *Lactobacillus plantarum* EP56, *Lactobacillus salivarius* and *Bifidobacterium bifidum* were reported to continue producing EPS even after entering the stationary phase of growth (Tallon et al., 2003; Liu et al., 2009). Other researchers have found that EPS production increased during the exponential growth phase but no further production was observed in the stationary phase (De Vuyst et al., 1998; Aslim et al., 2005). Van den Berg et al. (1995) found that EPS

production by *Lactobacillus sake* 0-1 started in the early growth phase and stopped when the culture reached the stationary phase. In contrast, *Streptococcus salivarius* produced EPS only during stationary phase growth (Gancel and Novel, 1994).

Conclusion

The present work is the first study on the production of EPS by *Weissella* sp. PSMS4-4, most likely a strain of *W. confusa*, using low-cost white sugar from sugarcane as a carbon source. The carbon source concentration, initial pH of culture medium, and incubation temperature were the factors with a major impact on both bacterial growth and EPS production. The optimal conditions for production of the EPS by *Weissella* sp. PSMS4-4 were white sugar concentration of 5% with an initial medium pH of 7.0 and incubation temperature of 30°C. Under these conditions, the largest amount of EPS of 8.65 g/l (yield of EPS of 31.09%) was attained after 30 h of fermentation. The strain PSMS4-4 produced a homoplysaccharide composed of glucose. This information may provide alternative production conditions of the EPS produced by *Weissella* sp. for food industry applications.

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