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Isolation of Exopolysaccharide-Producing Lactic Acid Bacteria from Pa-Kimchi and Characterization of Exopolysaccharides

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Three lactic acid bacteria (LAB) producing exopolysaccharides (EPSs) were isolated from Pa (green onion)-kimchi, and identified as *Weissella confusa* (SKP 173), *Weissella cibaria* (SKP 182), and *Leuconostoc citreum* (SKP 281), respectively by 16S rRNA gene sequencing. The yields of EPS were 21.27, 18.53, and 15.4 g/l for EPS from SKP 173, 182, and 281, respectively when grown in MRS broth containing sucrose (5%, w/v). Total sugar contents were 64.39, 62.84, and 65.16% (w/w) for EPS from SKP 173, 182, and 281, respectively while the protein contents were 0.33, 0.31, and 0.25% (w/w), respectively. EPSs from *W. confusa* SKP 173 and *W. cibaria* SKP 182 contained glucose only but EPS from *L. citreum* SKP 281 contained glucose and glucitol. Viscosities of the 2% (w/w) freeze-dried EPS solution were 9.60, 8.00, and 8.20 centipoise (cP) for EPS from SKP 173, 182, and 281, respectively. Viscosities of culture grown in MRS broth with 5% sucrose (no glucose) were 92.98, 57.19, and 18.8 cP, respectively. The average molecular weights of EPSs were larger than 2×10^7 Da. Fourier transform infrared spectroscopy (FT-IR) analyses of EPSs showed typical carbohydrate peaks, suggesting that the EPSs consisted of pyranose saccharides with α-(1,6) and α-(1,3) glycosidic linkages. *L. citreim* SKP 281 was used as the starter for yogurt fermentation, and EPS production was confirmed.

Keywords: **Exopolysaccharides, Lactic acid bacteria, *Weissella, Leuconostoc,* Yogurt**

Introduction

Lactic acid bacteria (LAB) produce several important metabolites during growth on foods, which confer health-promoting effects for human beings [1, 2]. The metabolites include bioactive-peptides, vitamins, exopolysaccharides (EPSs), mannitol, γ-aminobutyric acid (GABA), bacteriocins, and et cetera [3]. Many LAB species (spp.) have been utilized in food and related industries as starters for various fermented foods, probiotics, and hosts for production of special compounds due to their GRAS (generally regarded as safe) status and health beneficial effects [4]. LAB have been used for a long time to improve the preservation, organoleptic properties, rheological properties, and nutritional values of foods, such as milk, vegetable, and meat [5–7].

Some LAB strains secrete EPSs, long-chain polysaccharides consisting of repeating units of sugars or sugar derivatives [8]. Dextran is the most well-known EPS produced by LAB (LAB EPS), consisting of D-glucose units connected via α 1→6 glycosidic bond mostly [3]. LAB EPSs have some known health-beneficial properties, such as immune stimulation, anti-cancer, and anti-viral activities [9–11]. To improve the sensorial properties of fermented foods, such as yogurt, the contents of fat, sugars, or proteins are increased or stabilizers are included [8]. Pectin, starch, alginate, and gelatin are commonly used stabilizers. LAB EPSs have a potential as natural

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stabilizers replacing currently used stabilizers, and can improve the rheology, texture, and mouthfeel of fermented products [12]. Therefore LAB spp. producing EPSs could be used as an alternative to chemical stabilizers when incorporated into foods as starters.

In this study, 3 LAB strains producing EPSs were isolated from pa (green onion)-kimchi, and the characteristics of EPSs were studied. Yogurt was prepared by using one isolate and the properties of yogurt were examined.

Materials and Methods

Isolation of EPS-producing LAB from Pa-kimchi

Pa-kimchi was purchased at a local market in Sacheon, Gyeongnam, republic of Korea in July, 2020, and homogenized by using a stomacher®80 (Seward, UK). Diluted homogenates were spreaded onto de Man, Rogosa, and Sharpe (MRS, Becton Dickinson Co., USA) agar plates containing 1% $CaCO_3$ and 0.006% bromocresol purple. Colonies with yellow color and surrounding clear zones were selected as putative LAB after 48 h incubation at 30℃, and tested for EPS production. Colonies were spotted onto MRS agar plates (5% sucrose and no glucose), and mucoid colonies were selected as EPS producers after 48 h incubation at 30℃ [13].

16S rRNA gene sequences of EPS producers were determined and analyzed by basic local alignment search tool (BLAST) at national center for biotechnology information (NCBI, USA) as described previously [14].

Extraction of EPSs

MRS broth (200 ml with 5% sucrose and no glucose) was 1% inoculated (v/v) with each isolate and cultivated for 48 h at 30℃. Trichloroacetic acid (Sigma, USA) was added to a final concentration of 4% (w/v). After 2 h at 4℃, culture was centrifuged (9,950 ×*g*, 25 min) to remove cells and proteins. Two volumes of 95% cold ethanol was added to the supernatant, and the mixture was stood for 15 h at 4℃. EPS was recovered by centrifugation at 9,950 ×*g* for 25 min, and the remaining ethanol was removed by evaporation. EPS was dissolved in distilled water, dialyzed using a membrane (MW cut-off 12,000–14,000) for 24 h at 4℃, and freeze-dried [15].

Growth properties of EPS-producing strains

Each strain previously grown in MRS broth for 24 h was used to inoculate fresh MRS broth (1%, v/v). Inoculated culture was cultivated for 72 h under different conditions: incubation temperature (4–45℃). initial pH (pH 4–8) of MRS broth, and NaCl content (0–7%) of MRS broth. Growth of each culture was monitored by measuring the absorbance at 600 nm (UV-1601, Shimadzu, Japan).

Resistance of EPS-producing isolates against acidic pH and bile salts

Resistance of each isolate against low pH and bile salts was examined as described previously [16]. Each strain was cultivated in MRS broth until the OD_{600} reached 1.5, and then 1 ml of culture was centrifuged at 12,000 ×*g* for 5 min at 4℃. The cell pellet was resuspended in MRS broth where the pH was previously adjusted to 2, 3, 4 or 6.5 (control), respectively. Viable cells were counted by standard plate method after 2 h at 30℃. Cell pellet obtained as described above was washed and resuspended with 1 ml of MRS broth supplemented with 0.3% bile salts (Fluka, USA). Viable cells were counted after 2 h at 30℃. All measurements were done in triplicate.

Total sugar and protein contents and monosaccharide compositions of EPSs

Sugar contents of EPSs were measured by phenol-sulfuric acid method using glucose (Duchefa Biochemie, Netherlands) as a standard [17]. One mg of lyophilized crude EPS was dissolved in 1 ml of distilled water. One ml of 50% phenol (Daejung Chemicals & Metals, Korea) was added, and 5 ml of concentrated sulfuric acid (Junsei, Japan) was added rapidly. The mixture was stood for 10 min, shaken, and placed in a water bath at 25℃ for 20 min. Absorbance of the characteristic yellow, orange color was measured at 490 nm. Protein contents of EPSs were measured by Bradford method using BSA (bovine serum albumin, BioRad, USA) as a standard [18]. Lyophilized crude EPS (0.4 mg) was dissolved in 800 µl of distilled water. Bradford assay reagent (200 µl, BioRad) was added and the mixture was stood on ice for 5 min. The protein content was calculated by measuring the absorbance at 595 nm.

Monosaccharide compositions of EPSs were analyzed by gas chromatography (GC-2010 Plus, GCMS-TQ 8030, Shimazu) employing a DB-5 MS column (30 m × 0.25 mm

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id, 0.25 um film thickness, J & W Scientific, USA) after acid hydrolysis. Acid hydrolysis was done by adding 1 ml of 2 N sulfuric acid to 20 mg freeze- dried EPS and standing for 5 h at 100℃ in a heating block (VWR Co., USA). The hydrolyzate was neutralized to pH 7 with 1 N NaOH, filtered through a 0.45 μm filter (Advantec, Japan), and used as the sample. GC analysis was done as described previously [19].

Viscosity of EPS containing solution

Viscosities of EPS-containing samples were measured. Samples were fermentation broth, crude EPS solution, and crude EPS solution with 2% (w/v) concentration. Fermentation broth was a culture grown on MRS broth with 5% sucrose (no glucose) for 48 h at 30℃. Crude EPS solution was prepared by dissolving freeze-dried EPS in distilled water. The amount of each freeze-dried EPS was the same with the EPS yield of each strain, which was observed when the strain was cultivated in MRS broth with 5% sucrose (no glucose) for 48 h at 30℃. Crude EPS solution with 2% concentration was prepared by dissolving 1 g of each freeze-dried EPS in 50 ml distilled water. A viscometer (Brookfield, USA, spindle LV2) was used [20].

Determination of average molecular weight and structural characterization of EPSs

The average molecular weight of EPSs were determined by MALS (multi-angle light scattering) (Dawn Heleos II, Wyatt Technol., USA), and HPLC (Shimadzu) with PL aquagel-OH MIXED-H column. NaCl solution (150 mM) was used as the buffer. Buffer and the same concentration of sample were filtered through 0.22 um syringe filter before injected. Injection volume was 100 μl when the flow rate was 0.5 ml/min. Molecular weight measurements were performed using the LS-RI method and ASTRA 6 software.

Freeze-dried EPSs were analyzed by a FT-IR spectrometer (Thermo Fisher, USA). For FT-IR measurements, EPSs were dried on an ATR crystal (built-in Diamond) and the absorption spectra between 1,000 and 7,800 cm^{-1} were measured by co-adding 65 scan/sec [21].

Yogurt fermentation

Reconstituted milk was prepared by dissolving whole milk powder (Seoul milk Co., Korea) with distilled water (11.5%, w/v) at 40℃. Reconstituted milk was homogenized for 10 min, fortified with sucrose (5%, w/v) and heat treated for 10 min at 90℃. Two commercial yogurt starters, *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*L. bulgaricus*), were obtained from National Institute of Agricultural Sciences (Wanju, Jeonbuk, Republic of Korea) and used as a control. Total 6 yogurt samples were prepared: yogurt fermented with *L. citreum* SKP 281 with 5% sucrose (sample 1) or 0% sucrose (sample 2), yogurt fermented with *S. thermophilus* and *L. bulgaricus* (control) with 5% sucrose (sample 3) or 0% sucrose (sample 4), yogurt fermented with 2 commercial starters plus *L. citreum* SKP 281 with 5% sucrose (sample 5) or 0% sucrose (sample 6). All starters were inoculated at 5×10^6 CFU/ml. Fermentation was carried out for 24 h at 37℃. After fermentation, yogurts were stabilized for 24 h at 10℃, and then stored for additional 24 h at 4℃.

Aliquots of samples were collected at 0, 6, 12, 24 (during fermentation), 48 (after stabilization), and 72 h (after storage). pH, TA, viable cells, EPS contents, viscosity and syneresis were measured. EPS content, viscosity, and syneresis were measured for 72 h samples. EPS content was determined as follows: yogurt (10 g) was centrifuged (2,650 ×*g*, 10 min, 4℃) to recover EPS. EPS content (%) was expressed as weight of EPS (weight of freeze-dried yogurt with 5% sucrose - weight of freeze-dried yogurt with 0% sucrose) per weight of yogurt (10 g) × 100. Viscosity was measured as described above. Syneresis was determined as follows: 10 g of each sample was spreaded across a Whatman no. 1 filter paper as a thin layer to cover the surface, and stood for 10 min. The amount of liquid that passed through the filter paper was measured. Syneresis (%) was expressed as weight of drained whey per weight of yogurt (10 g) × 100 [21].

Statistical analysis

All data were expressed as the mean ± standard deviation of triplicate experiments. All data obtained from measurements were evaluated by ANOVA in SAS, ver. 3.8 (SAS Institute Inc., USA). Significance of a difference between the means of measured values was analyzed by Duncan's multiple range test ($p < 0.05$).

Results and Discussion

Isolation of EPS-producing LAB from Pa-kimchi

A total of 500 tentative LAB isolates were obtained from fermented foods including pa-kimchi. Three isolates, SKP 173, SKP 182, and SKP 281, from pa-kimchi produced EPS profusely on MRS agar plates with 5% sucrose (no glucose). They were identified as *Weissella confusa* (SKP 173), *Weissella cibaria* (SKP 182), and *Leuconostoc citreum* (SKP 281) by 16S rRNA gene sequencing. Genbank accession numbers are ON 651652, ON 651654, and ON 651653 for the 16S rRNA gene from SKP 173, 182, and 281, respectively. The yields of freeze-dried EPS were 21.27, 18.53, and 15.4 g/l for *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281, respectively. *W. confusa* SKP 173 produced more EPS than other isolates.

Growth properties of EPS-producing strains

Two *Weissella* strains grew rapidly at 25–45℃, slowly at 15℃, and did not grow at 4℃ (Fig. 1). *L. citreum* SKP 281 grew quicker at 4℃ and 15℃ and slower at 37℃ and 45℃ than *Weissella* strains. The optimum growth temperature for *L. citreum* SKP 281 was 25-30°C, and the highest cell numbers (OD_{600} = 1.5–1.6) reached in 12 h. All strains grew well at the initial pH of 6.0–7.0, and grew slowly at lower pH. All strains grew well at 3% and lower NaCl concentration.

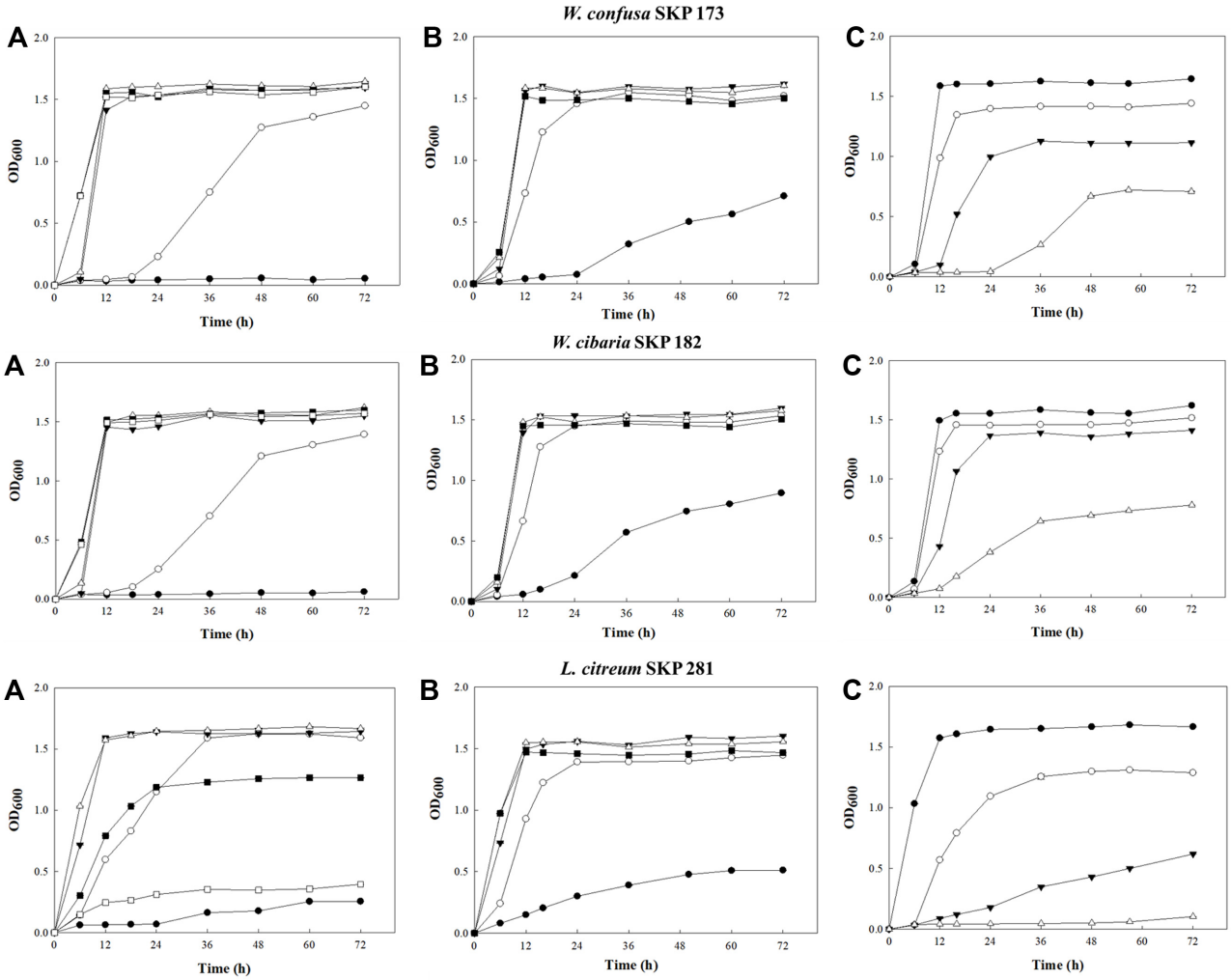

Fig. 1. Growth of EPS-producing LAB on MRS broth under different conditions. (A) temperature: ●, 4℃; ○, 15℃; ▼, 25℃; △, 30℃; ■, 37℃; □, 45℃. (B) initial pH: ●, pH 4; ○, pH 5; ▼, pH 6; △, pH 7; ■, pH 8. (C) NaCl content: ●, 0%; ○, 3%; ▼, 5%; △, 7%.

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Table 1. Acid tolerance of EPS-producing three isolates.

Strains	control[a] (CFU/ml)	pH 4.0 (CFU/ml)	SR[b] (%)	pH 3.0 (CFU/ml)	SR[b] (%)	pH 2.0 (CFU/ml)	SR[b] (%)
W. confusa SKP 173	2.7×10^9	2.37×10^9	87.78	2.05×10^9	75.93	5.33×10^5	0.02
W. cibaria SKP 182	1.8×10^9	1.17×10^9	65	1.07×10^9	59.44	4.23×10^3	0.00
L. citreum SKP 281	1.37×10^9	1.04×10^9	75.91	6.6×10^8	48.18	1.27×10^5	0.01

[a] control, cells in pH 6.5.
[b] SR(survival ratio, %), viable cells exposed to acidic pH in MRS/viable cells in pH 6.5 x 100

Table 2. Bile acid tolerance of EPS-producing three isolates.

Strains	control[a] (CFU/ml)	0.3% bile salts (CFU/ml)	SR[b] (%)	pH 3.0 + 0.3% bile salts[c] (CFU/ml)	SR[b] (%)
W. confusa SKP 173	2.7×10^9	1.81×10^9	67.04	2.05×10^9	66.67
W. cibaria SKP 182	1.8×10^9	1×10^9	55.56	1.07×10^9	48.17
L. citreum SKP 281	1.37×10^9	5.63×10^8	41.09	6.6×10^8	36.72

[a] control, cells in pH 6.5.
[b] SR(survival ratio, %), viable cells exposed to 0.3% bile salts or pH 3.0 + 0.3% bile salts/viable cells in pH 6.5 × 100
[c] cells exposed to pH 3 first and then exposed to 0.3% bile salts.

Resistance of EPS-producing isolates against acidic pH and bile salts

The ability of an organism to survive under low pH environments is an important prerequisite for a probiotic. *Weissella* strains showed higher than 50% viabilities after 2 h exposure at pH 3.0 and pH 4.0 (Table 1). *L. citreum* SKP 281 showed higher than 70% viability after 2 h exposure at pH 4.0 and lower than 50% viability at pH 3.0. All strains showed near 0% viabilities after 2 h exposure at pH 2.0. *Weissella* strains survived better than *L. citrum* SKP 281. When exposed to 0.3% bile salt for 2 h at 30℃, survival ratios were 67.04, 55.56, and 41.09% for *W. confusa* SKP 173, *W. confusa* SKP 182, and *L. citreum* SKP 281, respectively (Table 2). The ratios decreased further to 66.67, 48.17, and 36.72% for *W. confusa* SKP 173, *W. confusa* SKP 182, and *L. citreum* SKP 281, respectively, when first exposed at pH 3 for 2 h followed by 2nd exposure to 0.3% bile salt for 2 h. Considering the results, 3 strains possessed reasonable degree of resistance against low pH and bile salts, and could be used as probiotics.

Total sugar and protein contents and monosaccharide composition of EPSs

Total sugar contents were 64.39, 62.84, and 65.16% (w/w) for EPS from *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281, respectively. Total protein contents were 0.33, 0.31, and 0.25% (w/w) for EPS from *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281, respectively. The results showed that 3 EPSs had similar sugar contents and very small amounts of proteins.

When acid hydrolyzates were analyzed by GC, a single peak corresponding to glucose was observed from EPSs of *W. confusa* SKP 173 and *W. cibaria* SKP 182 (Fig. 2). An additional peak corresponding to glucitol (sorbitol) was observed from *L. citreum* SKP 281 hydrolyzate. The results indicated that the EPSs from *Weissella* strains were homopolymers, consisting of glucose, and most likely dextran. The EPS from *L. citreum* SKP 281 was a heteropolymer, consisting of glucose and glucitol (sorbitol).

Viscosities of EPS containing solution

The viscosities of EPS containing solution were measured (Table 3). The viscosities of the 48 h culture of *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281 were 92.98, 57.19, and 18.8 cP, respectively. The viscosities of the crude EPS solution of *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281 were 10.80, 6.60, and 6.40 cP, respectively. The viscosities of the crude EPS solution (2% concentration, w/v) of *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum*

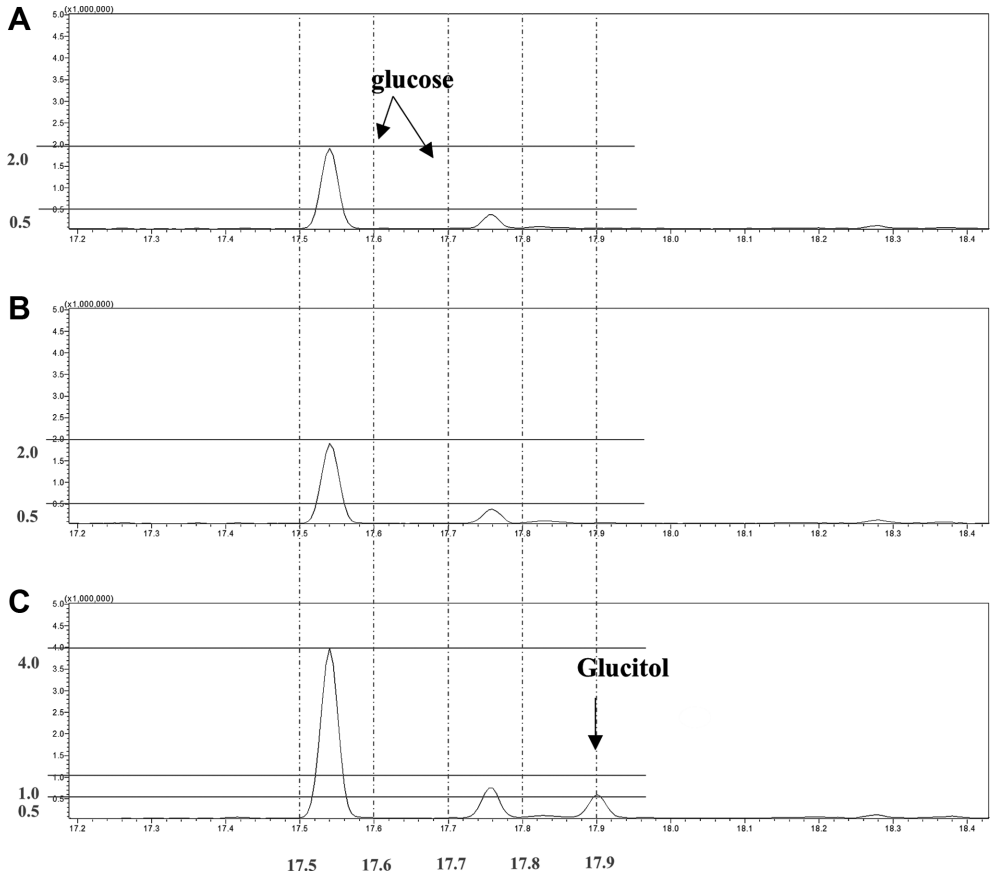

Fig. 2. GC analysis of EPSs after acid hydrolysis. (A) 8-fold diluted acid hydrolysis product of EPS from *W. confusa* SKP 173 (B) 8-fold diluted acid hydrolysis product of EPS from *W. cibaria* SKP 182 (C) 4-fold diluted acid hydrolysis product of EPS from *L. citreum* SKP 281.

Table 3. Viscosity of the fermentation medium and EPS solution.

Strains	Fermentation medium (cP)	Crude EPS (cP)	2% Crude EPS (cP)
W. confusa SKP 173	92.98 (±25.60)	10.80 (±1.04)	9.60 (±0)
W. cibaria SKP 182	57.19 (±0.92)	6.60 (±0.6)	8.00 (±0.35)
L. citreum SKP 281	18.8 (±0.69)	6.40 (±0.69)	8.20 (±0.35)

* cP: centipoise

SKP 281 were 9.60, 8.00, and 8.20 cP, respectively, The results showed that the EPS from *W. confusa* SKP 173 showed the highest viscosity and the EPS from *L. citreum* the lowest. Crude EPS solution from *W. confusa* SKP 173 showed higher viscosity than 2 other samples even at the same 2% concentration.

Determination of average molecular weight and structural characterization of EPSs

The average molecular weight of EPSs were found to be 3.8×10^7, 4.7×10^7, 2.8×10^7 Da for EPS from *W. confusa* SKP 173, *W. cibaria* SKP 182, and *L. citreum* SKP 281, respectively. Bounaix *et al.* (2009) reported production of glucan greater than 10^6 Da in size from *W. cibaria* and *W. confusa* [22]. *Leuconostoc citreum* N21 from dried milk cake was reported to produce EPS with molecular mass of 6.07×10^6 [23]. Gu *et al.* (2015) reported that a *Leuconostoc* spp. isolated from kimchi produced dextrans with the molecular size of 5×10^5 – 2×10^6 Da [24]. Compared with these reports, our iso-

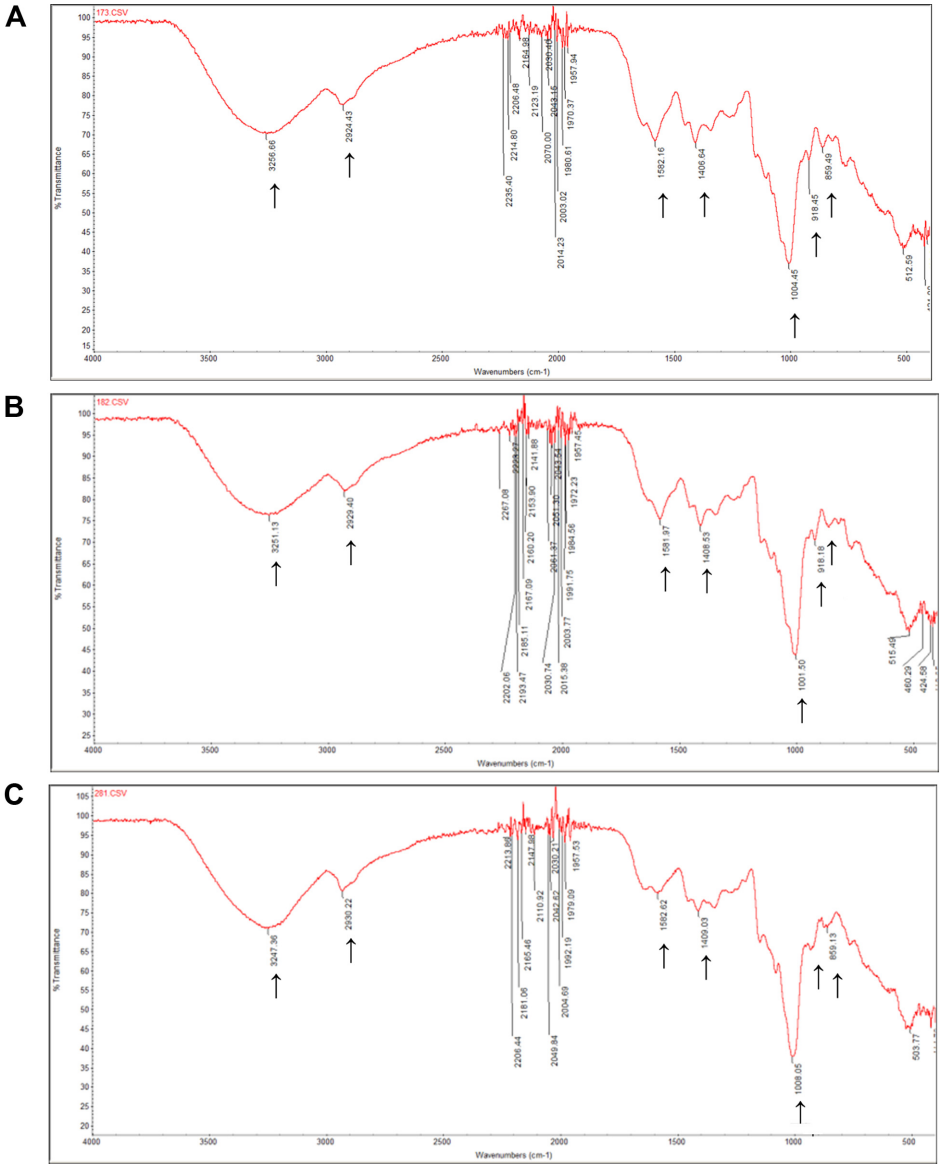

Fig. 3. FT-IR spectra of 3 EPSs. (A) *W. confusa* SKP 173, (B) *W. cibaria* SKP 182, (C) *L. citreum* SKP 281.

lates produce EPSs with higher molecular weights. Dextran with higher molecular weight and few branch linkages is considered a good quality dextran [25].

All EPSs showed typical carbohydrate peaks by FT-IR spectroscopy (Fig. 3). FT-IR spectra showed O-H stretching at ~3200 cm^{-1}, C-H stretching at ~2900 cm^{-1}, 1400 cm^{-1} [26], and the signal at 1580 cm^{-1} might be associated with water bending vibration [15]. The area between 800 and 1200 cm^{-1} was termed as the fingerprint area for carbohydrates and provided a good indication of the structural differences. The absorption peak at 918 cm^{-1} indicated the α-pyranose form of the glucose residue. The peak at 1000 cm^{-1} indicated the presence of the α-(1,6) glycosidic linkages and the peak at 859 cm^{-1} was characteristic of the α-(1,3)-D-glucan [27, 28]. Therefore the FT-IR spectra suggested that EPSs consisted of pyranose saccharide in α-configuration connected via α-(1,6) and α-(1,3) glycosidic linkages. These results also indicated that EPSs from *Weissella* strains are most likely dextran. Dextran consists of α-D-glucose units connected via α-1→6 glycosidic bonds with a few branches connected via α-1→3 bonds, and occasionally α-1→2 or α-

1→4 linkages [3]. More studies are necessary for the accurate determination of structures of 3 EPSs.

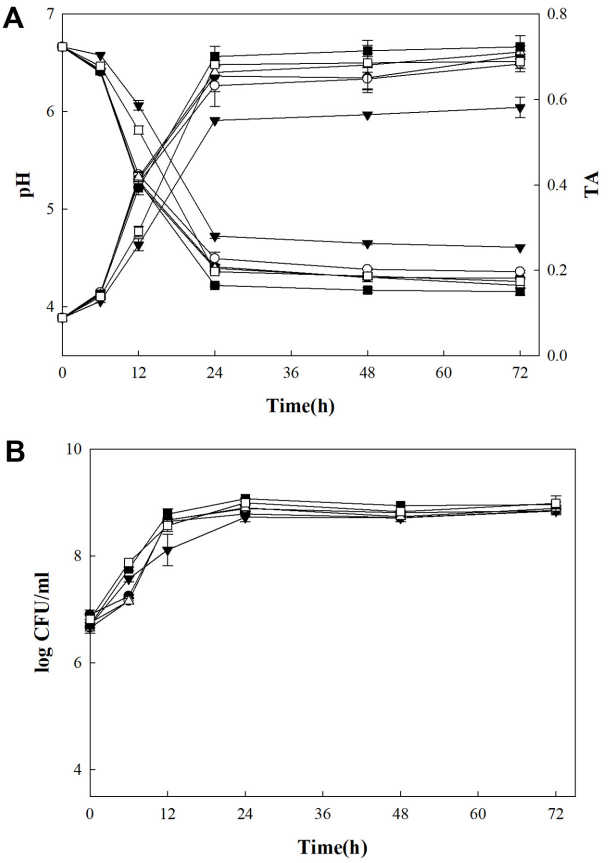

Fig. 4. pH, TA, and LAB counts of yogurt samples during fermentation. (A) pH and TA, (B) LAB counts. ●, yogurt 1 (*L. citreum* SKP 281 with 5% sucrose); ○, yogurt 2 (*L. citreum* SKP 281 with 0% sucrose); ▼, yogurt 3 (control, *S. thermophilus* and *L. bulgaricus* with 5% sucrose); △, yogurt 4 (*S. thermophilus* and *L. bulgaricus* with 0% sucrose); ■, yogurt 5 (control plus *L. citreum* SKP 281 with 5% sucrose); □, yogurt 6 (control plus *L. citreum* SKP 281 with 0% sucrose).

Yogurt fermentation

L. citreum SKP 281 was chosen as the starter for yogurt fermentation because this strain caused less phase separation during yogurt fermentation although the strain produced less EPS than *Weissella* strains. pH of all 6 yogurt samples decreased during fermentation (Fig. 4A). The pH of yogurt 1 (*L. citreum* SKP 281 and 5% sucrose) was 4.36 ± 0.01 at 24 h. The pH of yogurt 2 (*L. citreum* SKP 281 and no sucrose) was 4.72 ± 0.02 at 24 h. The pH of yogurt 3 (2 commercial starters and 5% sucrose) was 4.40 ± 0.04 and that of yogurt 4 (2 commercial starters and no sucrose) was 4.41 ± 0.03 at the same time. The pH of yogurt 5 (2 commercial starters plus *L. citreum* SKP 281 and 5% sucrose) was 4.22 ± 0.01 and that of yogurt 6 (2 commercial starters and no sucrose) was 4.50 ± 0.06. Two commercial starters seemed to produce acids from lactose rather than sucrose whereas *L. citreum* SKP281 produced acids from sucrose. During the storage period, pH of all samples remained constant. TA increased with fermentation time, and a significant increase occurred during the first 24 h, similar to the pH changes but in a reverse direction (Fig. 4A). Yogurt 5 showed the highest TA value of 0.72 ± 0.03 at 72 h, whereas yogurt 2 showed the lowest TA of 0.58 ± 0.02.

The initial LAB count was 5×10^6 CFU/ml, and the number increased during 24 h of fermentation at 37℃ (Fig. 4B). Yogurt 5 showed the highest LAB count of 1.18×10^9 CFU/ml at 24 h, whereas yogurt 2 showed the lowest count of 5.4×10^8 CFU/ml. Following fermentation, LAB counts of all samples remained nearly constant.

After 1 day storage at 4℃ (72 h), EPS content of yogurt 1 was 24 ± 1.41 g/l and that of yogurt 5 was 21 ± 0.00 g/l (Table 4). EPS was not detected from other samples. The results indicated that *L. citreum* SKP 281 produced EPS

Table 4. Properties of yogurt samples after 24 h of storage.

	Yogurt (no sucrose)			Yogurt (5% sucrose)		
	control[1]	control + 281[2]	281[3]	control	control + 281	281
EPS contents (g/l)	ND[4]	*ND	*ND	*ND	21 (±0.00)	24 (±1.41)
Viscosity (cP)	102.84 (±2.58)	39.85 (±3.03)	39.42 (±3.64)	93.12 (±12.16)	137.68 (±2.76)	139.11 (±4.40)
Syneresis (%)	39.95 (±0.21)	41.25 (±0.49)	46.1 (±0.14)	37.5 (±4.38)	36.7 (±0.71)	31.9 (±4.24)

[1] control, 2 commercial yogurt starters were used.
[2] control + 281, 2 commercial yogurt starters plus *L. citreum* SKP 281 were used.
[3] 281, *L. citreum* SKP 281 was used as a single starter.
[4] ND, not detected.

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from sucrose, and two commercial starters did not produce EPS. Viscosity of yogurt 4 (102.84 ± 2.58 cP) was higher than those of yogurt 2 (39.42 ± 3.64 cP) and 6 (39.85 ± 3.03 cP). Viscosity of yogurt 1 (139.11 ± 4.40 cP) and 5 (137.68 ± 2.76 cP) were higher than that of yogurt 3 (93.12 ± 12.16 cP). Yogurt 2 was less viscous than yogurt 4. But *L. citreum* SKP 281 produced more viscous yogurt in the presence of sucrose. Zhao *at al.* (2022) reported that coinoculation of a EPS producing *Lactobacillus plantarum* MC5 together with *S. thermophilus* and *L. bulgaricus* for yogurt fermentation improved the viscosity, consistency, and cohesiveness of yogurt [29], which agreed with our observations. Syneresis of yogurt 4 (39.95 ± 0.21%) was lower than those of yogurt 2 (46.1 ± 0.14%) and 6 (41.25 ± 0.49%). Syneresis of yogurt 1 (31.9 ± 4.24%) was also lower than those of yogurt 3 (37.5 ± 4.38%) and 5 (36.7 ± 0.71%). Further studies are necessary to understand the effects of EPS on the texture of yogurt fermented with *L. citreum* SKP 281.

The optimum conditions for EPS production by 3 isolates should be studied in the future. The topics will include the optimum sucrose content, the ratio between sucrose and glucose, optimum growth time, and other compounds encouraging the growth of hosts and EPS production. Kareem *et al.* reported that the best conditions for dextran production by lactobacilli isolated from human were 24 h incubation at 30℃ with 15% sucrose and 4% inoculum size [30]. Another topic should be the improvement of functionalities of yogurt with EPS, such as antioxidant activity and antibacterial activity compared to regular yogurt [31].

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Conflict of Interest

The authors have no financial conflicits of interests to declare.

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