Response Surface Optimization of Exopolysaccharide Production from Sugarcane Juice by *Lactobacillus confusus* TISTR 1498

Phayungsak Manochai[1], Yuthana Phimolsiripol[1*] and Phisit Seesuriyachan[2]

[1]Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand
[2]Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand

**Corresponding author. E-mail: yuthana.p@cmu.ac.th*

ABSTRACT

Lactobacillus confusus TISTR 1498, isolated from Thai traditional fermented pork (Nham), could secrete large amounts of exopolysaccharides (EPS). Response surface methodology was applied to optimize the culture conditions for EPS production using Central Composite Design (CCD). The effects of three variables including pH (4-7), temperature (20-40°C) and nitrogen source (0.25-1.75 PYB) on EPS yield and biomass were investigated. The 1PYB was the mixtures of 5 g/L peptone (P), 2.5 g/L yeast extract (Y) and 2.5 g/L beef extract (B). Response surface methodology showed that the data were adequately fitted to a second-order polynomial model via quadratic regression relationships. The optimal culture conditions for EPS production in sugarcane juice were pH of 5.55, 29.75°C and 1.22PYB, which was composed of 6.1 g/L peptone, 3.05 g/L yeast extract and 3.05 g/L beef extract. Under the optimum condition, the predicted maximum EPS production was 107.5 g/L and the predicted biomass was 1.92 g/L. In submerged fermentation, sugarcane juice enhanced EPS yield twice as well as the modified MRS sucrose medium. In addition, the cost of medium can be lowered to 0.53 THB/g EPS, which was lower than that of the medium from the mixtures of the PYB (0.78 THB/gEPS).

Keywords: *Lactobacillus confusus*, exopolysaccharides, optimization, response surface methodology, sugarcane juice

INTRODUCTION

Exopolysaccharides (EPS) produced by lactic acid bacteria have important applications (Welman and Maddox, 2003), including as thickeners, stabilizers, emulsifiers, bodying and gelling agents in food, pharmaceutical and chemical products (Prasertsan et al., 2008). Some generally recognized as safe (GRAS) bacteria, particularly lactic acid bacteria (lactic acid bacteria), propionibacteria and bifidobacteria, are known for their EPS production ability (Andaloussi et al., 1995; De Vuyst and Degeest, 1999; Gorret et al., 2001). EPS derived from

lactic acid bacteria plays an important role in improving the rheology, texture, and mouth feel of fermented food formulations (Monde et al., 2013) and in conferring beneficial physiological effects on human health, such as antitumor activity, immune-modulating bioactivity, anti-carcinogenicity (Doleyres et al., 2005) and slowing carbohydrate digestion and glucose absorption to attenuated postprandial hyperglycemia (Okuno et al., 2010).

Lactobacillus confusus, recently known as *Weissella confusa,* is one of the lactic acid bacteria that can produce EPS (Maina et al., 2008). Most lactic acid bacteria are fastidious microorganisms. They require several growth factors for bioactivity, such as yeast extract, beef extract and peptone, which are expensive nutrients. However, alternative cheaper substrates can be substituted to reduce the production cost (Seesuriyachan et al., 2010). Thus, an alternative renewable carbon source is required to sustain EPS production.

Sugarcane, cultivated in several parts of Thailand, is used primarily for sugar production. Sugarcane juice is an alternative material for EPS production, because it has a high concentration of sucrose, mineral salts and vitamins, which gives an exceptional nutritional value. Sugarcane juice is comprised of approximately 80% water and 20% total soluble solids. The primary sugars in sugarcane juice are 17% sucrose (17%), glucose (0.4%) and fructose (0.2%) along with organic non-sugars (mineral including potassium, calcium, magnesium, sulphate, chloride and phosphate). It also contains 0.44-1.06% protein (Martini et al., 2011). In general, high water content, pH range of 5.0-5.5, high concentration of organic and inorganic nutrients and maintenance at 25-30ºC are favorable conditions for a great and diverse microbial community (Martini et al., 2011). In a previous study, the *Lactobacillus confusus* TISTR 1498 only produced EPS in the presence of sucrose as a carbon source (Kuntiya et al., 2008). A high concentration of EPS was also found when sugarcane juice and coconut water were used (Seesuriyachan et al., 2010). However, the effect of temperature on EPS production by *Lactobacillus confusus* in liquid fermentation using sugarcane juice has not been reported.

Therefore, the objective of this research was to determine the optimal temperature, pH and nitrogen source (peptone, yeast extract and beef extract) for EPS production by *Lactobacillus confusus* TISTR 1498 using sugarcane juice, an alternative source of carbon, via an application of response surface methodology.

MATERIALS AND METHODS

Bacterial strain and culture condition

The freeze dried *Lactobacillus confusus* TISTR 1498 was purchased from the Thailand Institute of Scientific and Technological Research (TISTR) culture collection (accession number TISTR 1498). It was transferred into 10 mL of MRS broth and incubated at 37°C for 24 h. An optical density measured at 650 nm (OD_{650}) of the resulting suspension was adjusted to 0.8 before further use (Kuntiya et al., 2010).

Effect of initial sucrose concentration

EPS production was investigated using the modified MRS medium containing sucrose (mod-MRS medium) instead of glucose, which is usually present in the MRS medium. The mod-MRS medium consisted of (g/L): peptone - 10.0; beef extract (BE) - 5; yeast extract (YE) - 5; K_2HPO_4 - 2.0; di-ammonium hydrogen citrate - 2.0; $CH_3COONa.\ H_2O$ - 7.6; $MgSO_4$ - 0.1; and $MnSO_4$ - 0.4. Then, 1 mL/L of Tween 80 was added (Seesuriyachan et al., 2011). The mod-MRS-sucrose medium was prepared using different sucrose concentrations. The initial sucrose concentrations were 100, 200, 300 and 400 g/L. The pH was controlled at 5.5 with 1M HCl or 5M NaOH, as necessary. The constant agitation speed was kept at 50 rpm. All media were sterilized by autoclaving at 121°C for 15 min. Batch fermentations were carried out in a 4-L bioreactor (Braun Biostat, Biotech International, Germany) with 2 L working volume at 35°C. The bioreactor was inoculated with 10% (v/v) inoculums. Samples were taken after 24 h of cultivation and analyzed for EPS and biomass.

Culture medium

In the study on optimization of pH, temperature and nitrogen sources on EPS production, the 100% sugarcane juice with added crystallized sucrose up to desired concentration at 300 g/L and agitation speed at 50 rpm was used for EPS production. The media was sterilized by autoclaving at 121°C for 15 min. Batch fermentations were carried out in a 4-L bioreactor with 1 L working volume. The bioreactor was inoculated with 10% (v/v) inoculums. Samples were cultivated after 24 h before further analysis.

Optimization of EPS production from sugarcane juice

To find the optimal cultivation condition for EPS production from the sugarcane juice, central composite design (CCD) was used for locating the true optimum conditions of pH, temperature and nitrogen source (PYB). The pH, temperature and 1PYB (5.0 g/L peptone; 2.5 g/L yeast extract; 2.5 g/L beef extract) were varied from 4-7, 20-40°C and 0.25-1.75PYB, respectively. A full 23 factorial design with six axial points ($\alpha = 1.682$) and three replications of the center point were applied for a total of 17 test runs.

Biomass determination

The fermented broth was centrifuged at 10,000 x g for 10 min at 4°C to separate cell pellet and supernatant. Cell pellet was washed twice with distilled water and re-suspended before measuring optical density at 650 nm with reference to a standard curve. The absorbance of culture samples for biomass determination was quantitated at 650 nm by a spectrophotometer (T80 UV/VTS spectrometer, PG Instruments Ltd.), and the corresponding biomass concentration was calculated from a standard calibration curve.

Determination of EPS yield

The fermented broth was centrifuged at 10,000 x g for 10 min at 4°C and the cell-free clear supernatant was used for EPS determination. Before the EPS

was precipitated with ethanol, a supernatant was added with 30% (v/v) trichloroacetic acid and stored at 4°C for 30 min, to inactivate EPS degrading enzymes and to precipitate the proteins. The crude EPS was then isolated by 80% ethanol precipitation at the ratio of 1:3. After centrifugation (3,500 x g, 15 min, 4°C), the EPS pellet was dispersed in aqueous 80% ethanol and centrifuged again. The process was repeated three times. The final precipitate was dissolved in distilled water and the pellet was dried to a constant weight at 55°C (Duenas et al., 2003; Seesuriyachan et al., 2011). The EPS yield was measured.

Statistical analysis

Output variables including pH, temperature and medium source were used for optimization. The goal of this research was the highest content of biomass and EPS content with the lowest medium cost. The results of the CCD experiment were expressed as the following second-order polynomial (Equation 1) using a multiple regression technique:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (1)$$

where Y is the predicted response, β_0 the intercept term, β_i the linear coefficients, β_{ii} the quadratic coefficients, β_{ij} the interactive coefficients and x_i and x_j the coded independent variables.

Experimental designs and the polynomial coefficients were calculated and analyzed using Design-Expert software (version 6.0.10). Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The validation data of both biomass and EPS yield were separately analyzed using the SPSS software (version 16).

RESULTS

Effect of initial sucrose concentration

Initial sucrose concentration was varied at 100, 200, 300 and 400 g/L by adjusting with crystalline sucrose. Fermentation was performed at 35°C, pH 5.5 and an agitation speed of 50 rpm. EPS yield significantly increased with increasing initial sucrose concentration from 100-300 g/L, as shown in Figure 1A. The EPS yields at 300 and 400 g/L of sucrose concentration were not significantly different ($p>0.05$), ranging from 55.63-59.60 g/L. The cell growth significantly decreased with increasing sucrose concentration (Fig. 1B). Our results showed that the EPS yield obtained from the treatment using 300 g/L of sucrose concentration had the highest productivity. Therefore, this sucrose concentration was chosen for further testing of culture optimization.

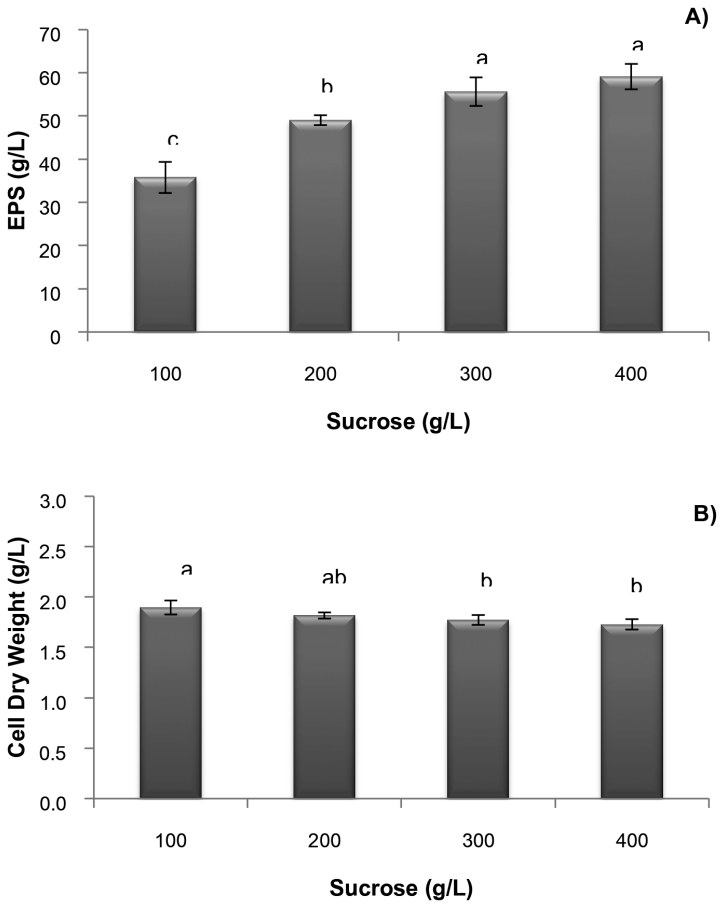

Figure 1. Effect of sucrose concentration on (A) EPS yield and (B) cell growth by *L. confusus* TISTR 1498 in mod-MRS-sucrose medium under *static condition at* 35°C, pH 5.5 and 50 rpm.

Optimal condition of EPS production from sugarcane juice

The optimal level of the key variables (pH, temperature and PYB) and the effect of their interactions on biomass and EPS yield were further explored using response surface methodology. The design matrix and the corresponding experimental data to determine the effects of three independent variables are shown in Table 1. At the central point of the full factorial design with pH 5.5, 30°C and 1PYB (peptone 5.0 g/L; yeast extract 2.5 g/L; beef extract 2.5 g/L), the highest EPS yield was obtained, with a range of 99.50-110.07 g/L, whereas biomass production was 1.79-1.93 g/L.

Low-pH culture conditions yielded small amounts of EPS and biomass.

Overall high temperatures yielded low EPS and biomass. For example, in sample 11 (pH 5.5, 13.2oC and 1PYB), EPS and biomass production were 16.51 and 1.27 g/L, respectively, whereas, in sample 12 (pH 5.5, 46.8°C and 1PYB), lower yields of EPS and biomass were found at 5.54 and 1.25 g/L, respectively. At 30°C, the EPS yield seemed to be higher than other temperatures.

Table 1. EPS yield (g/L) and biomass of different fermentation conditions.

Sample	pH	Temperature (°C)	PYB	Biomass (g/L)	EPS (g/L)	Cost (Baht/gEPS)
1	4.0	20.0	0.25	1.50	1.59	29.83
2	4.0	20.0	1.75	1.56	1.83	54.78
3	4.0	40.0	0.25	1.28	1.54	30.79
4	4.0	40.0	1.75	1.43	1.63	61.51
5	7.0	20.0	0.25	1.70	10.66	4.45
6	7.0	20.0	1.75	1.84	12.07	8.31
7	7.0	40.0	0.25	1.61	6.54	7.25
8	7.0	40.0	1.75	1.79	7.79	12.87
9	3.0	30.0	1.00	0.92	1.18	62.69
10	8.0	30.0	1.00	1.28	4.60	16.08
11	5.5	13.2	1.00	1.27	16.51	4.48
12	5.5	46.8	1.00	1.25	5.54	13.35
13	5.5	30.0	0.00	1.71	67.91	0.57
14	5.5	30.0	2.26	2.78	114.72	1.03
15	5.5	30.0	1.00	1.85	105.30	0.70
16	5.5	30.0	1.00	1.93	99.50	0.74
17	5.5	30.0	1.00	1.79	110.07	0.67

Note: 1PYB = peptone 5.0 g/L; yeast extract 2.5 g/L; beef extract 2.5 g/L.

Low content of PYB yielded small amounts of EPS and biomass, except the zero PYB (sample 13) with conditions of pH 5.5, 30°C and 0PYB, which provided medium yield of EPS at 67.91 g/L.

The multiple regression analysis of the experimental data is shown in Table 2. The fit of the model was checked by the adjusted coefficient of determination (Adj. R^2), which was 0.782 for biomass and 0.806 for EPS production, indicating 78.2% and 80.6% of the variability in the response. This result indicated that the models' equations were adequate for predicting the biomass and EPS yield under any combination of values of the variables. All these results showed a good agreement between the experimental and predicted values and implied that the mathematical models were suitable for the simulation of biomass and EPS yield in the present study.

Table 2. Regression coefficient estimates for EPS yield and biomass of strain TISTR 1498.

Properties	Equation	Adj. R^2	*p*-value
Biomass	$-3.173 + 1.250X_1 + 0.101X_2 - 0.451X_3 - 0.112\ X_1^2 - 0.002X_2^2 + 0.273\ X_3^2 + 0.002\ X_1X_2 + 0.012\ X_1X_3 + 0.002\ X_2X_3$	*0.782*	*0.0076*
EPS	$-844.202 + 207.515\ X_1 + 23.487\ X_2 + 43.893\ X_3 - 18.543\ X_1^2 - 0.388\ X_2^2 - 18.592\ X_3^2 - 0.068\ X_1X_2 + 0.259\ X_1X_3 - 0.005\ X_2X_3$	*0.806*	*0.0052*

Note: X_1 is pH, X_2 is temperature (°C) and X_3 is PYB.

The contour plots and three-dimensional response surfaces were generated by Design Expert program to study the interactions among the three factors tested and to visualize the combined effects of factors on the biomass and EPS yield (Fig. 2). The response surface plots showed biomass and EPS yield by *Lactobacillus confusus* TISTR 1498 as a function of two factors, with the other variables set at their zero levels, i.e., 1PYB, 30°C and pH 5.5. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. In this case, the mutual interactions between every two of the three variables were significant. The optimal values for maximum EPS yield of three variables were 5.55 for pH, 29.75°C for incubation temperature and

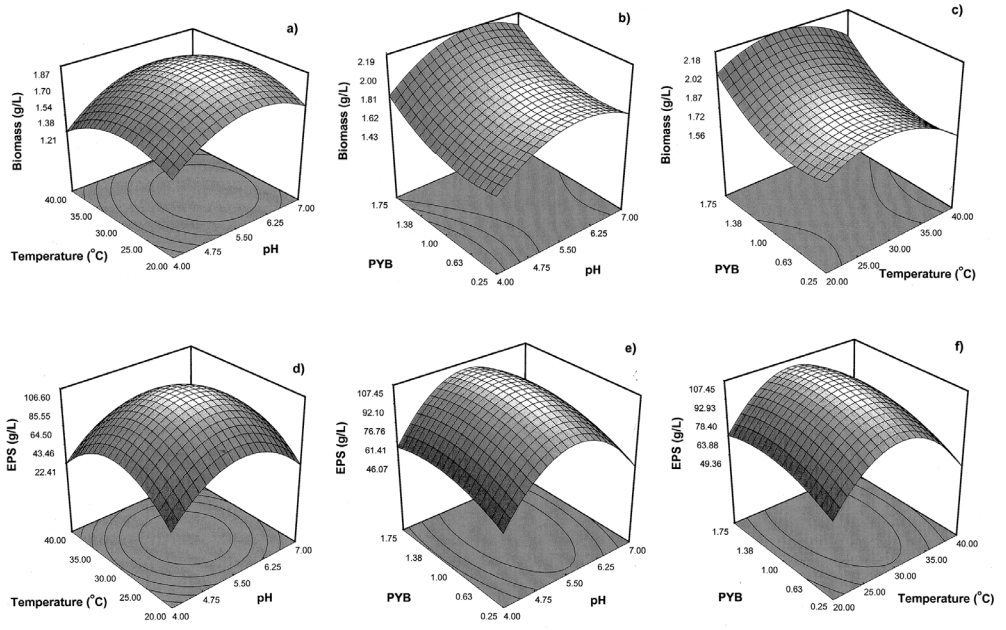

Figure 2. Contour plots and corresponding three-dimensional plots of the effect of three variables on biomass (a, b and c) and EPS yield (d, e and f). When the effect of two variables was plotted, the other variable was set at central levels. a and d indicate pH and temperature, b and e indicate pH and PYB, c and f indicate temperature and PYB.

1.22 of PYB. Under the optimum condition, the predicted maximum EPS yield was 107.52 g/L and the predicted biomass was 1.92 g/L, higher than the maximum yield produced with the modified MRS medium containing only 300 g/L of sucrose (55.6 g/L of EPS and 1.8 g/L of biomass).

Validation of the models

In order to validate the adequacy of the model equations, five verification experiments were carried out under various conditions (within the experimental range). Table 3 presents the design matrix of the independent variables in natural units along with the experimental results and predicted values of EPS yield and biomass from the model (Table 2). The percentage error between the experimental and predicted values for EPS yield was measured in a range of 1.95-6.76% and 2.9-12.5% for biomass. This indicated that the EPS yield of experimental values was in good agreement with the predicted ones, suggesting a satisfactory and accurate model. The optimal condition of 5.5 pH, 29.75°C and 1.22PYB yields EPS, biomass and Yp/x of 105.42 g/L, 1.68 g/L and 62.75, respectively. The mathematical models and response surface methodology via a central composite design revealed that high sucrose concentrations could enhance EPS production in the sugarcane juice media.

Table 3. Model validation experiments.

No	X_1 (pH)	X_2 (temperature)	X_3 (PYB)	*Biomass (g/L)*			*EPS (g/L)*		
				Pred.	*Exp.*	*Error (%)*	*Pred.*	*Exp.*	*Error (%)*
1	5.55	29.75	1.22	1.92	1.68	12.5	107.52	105.42	1.95
2	5.00	35	0	1.74	1.79	2.9	64.13	67.45	5.18
3	5.16	30	0	1.85	1.77	4.1	77.39	73.21	5.40
4	6.00	30	1.59	2.10	1.88	10.7	101.09	96.88	4.16
5	4.93	25.72	0	1.81	1.59	12.2	66.74	62.23	6.76

Note: Pred. are predicted values from the optimal model. Exp. are the experimental values.

DISCUSSION

Sucrose is one of the most suitable carbon sources for EPS production by lactic acid bacteria (Årsköld et al., 2007) including *Lactobacillus confusus* (Kuntiya et al., 2010). The sugar concentration is another essential factor that influences EPS production (Tayuan et al., 2011). Cheaper substrates would reduce the cost of EPS production. Our results indicated that sugarcane juice could be used as a low-cost substrate for EPS production by *Lactobacillus confusus* TISTR 1498. The sugar concentration in the medium has been reported to simulate the EPS production by lactic acid bacteria (Cerning et al., 1994). The high content of sugar concentration increases the EPS yield (Dueñas et al., 2003). Our study also confirmed that increase in sucrose concentration resulted in increased EPS yield. The maximum EPS yield was found using 400 g/L of sucrose in the medium. However, the EPS yield did not vary significantly with sucrose concentrations

of 300 and 400 g/L. The EPS production from lactic acid bacteria had a positive effect when operated under high sucrose concentrations due to reduction in the growth of *Lactobacillus confusus* TISTR 1498 and metabolism changes. Nevertheless, EPS production in mod-MRS-sucrose media can be fermented with high EPS yield (above 50 g/L). This is consistent with the report of Cerning et al. (1994), who found an optimal sugar concentration between 20-100 g/L for several lactic acid bacteria. High sugar content in the modified-MRS medium led to a decrease in osmotic stress, which affected the yield of EPS and biomass production (Prasertsan et al., 2008). This pattern was also found in the previous report of Xu et al. (2010) that indicated the reduction of cell growth due to the high osmotic stress from high sugar concentration. EPS are biopolymers with a protective property for living cells under stress conditions (Cerning, 1990). High sucrose concentration also shows the inhibitory influence on the yield of dextran produced by *Leuconostoc* sp. due to limitation of mass transfer between nutrients and microbial cells (Shaileshkumar and Lele, 2010). It was clearly observed in this study that EPS yield was independent of the carbon source or the carbon and nitrogen (C/N) ratio under conditions with a high concentration of NaCl, although the influence of the C/N ratio on yield has generally been reported (Prasertsan et al., 2008; Kuntiya et al., 2010). Dols et al. (1997) also found high levels of sucrose induced dextran production during the cultivation of *Leuconostoc mesenteroides* NRRL B-1299. Moreover, the high C/N ratio had a positive effect on the enhancement in EPS production by *Lactobacillus casei* (Cerning et al., 1994) and *Lactobacillus* LB 121 (van Geel-Schutten et al., 1998; De Vuyst and Degeest, 1999).

The sugarcane juice can be partially replaced as a carbon source (sucrose) in the modified MRS-sucrose medium (initial sucrose concentration at 300 g/L) for EPS production by *Lactobacillus confusus* TISTR 1498. The optimal culture conditions for EPS production were: pH 5.55, temperature 29.75°C and 1.22PYB (composed of 6.1 g/L peptone, 3.05 g/L yeast extract and 3.05 g/L beef extract), providing the highest EPS yield (107.52 g/L) and highest biomass (1.92 g/L). This is consistent with the previous results of Seesuriyachan et al. (2010) who found that the EPS yield by *Lactobacillus confusus* in solid state and liquid fermentation was carried out using sugarcane juice and coconut water as renewable wastes. High yields of EPS of 62 g/L sugarcane juice and 18 g/L of coconut water were produced in solid-state fermentation when nitrogen sources (0.20PYB) were reduced by about five fold from the original medium (1PYB).

A circular contour plot of response surfaces indicates that the interaction between the corresponding variables can be ignored, while an elliptical or saddle nature of the contour plot suggests that the interaction between the corresponding variables is significant (Muralidhar et al., 2001; Xu et al., 2008). Our study showed that higher EPS yields were observed when operation temperature ranged from 25-35°C. Previous reports from Shu and Yang (1990) and Shu and Yang (1991) revealed higher values of EPS at 30-33°C. Similarly, Esgalhado et al. (1995) referred to an optimum temperature from 25-30°C, with the highest yield of EPS (39.26 mg/L) at 27°C. At the general growth temperature of 37°C, EPS biosynthesis from *Lactobacillus paracasei* HCT was slightly lower (Xu et al., 2010). An increase of EPS yield at low temperature seems to be a common feature for meso-

philic EPS-producing lactic acid bacteria, in which suboptimal growth conditions resulted in improvement of EPS production (Degeest et al., 2001).

In general, the optimal medium pH for cell growth is around 2.0 to 4.0, but the optimal medium pH for exopolysaccharide formation is around 5.0 to 7.0 (Shu and Lung, 2004). In some lactic acid bacteria, the optimal initial pH for *Streptococcus thermophilus* S22 was 7.0 for lactose and 5.5 for sucrose as the carbon source (Gancel and Novel, 1994). The effect of initial pH on growth and EPS formation from *Lactobacillus paracasei* HCT was examined at 37°C for 60 h. The highest EPS yield (35.24 mg/L) was found at pH 5.90, while the cell growth (log CFU/mL = 8.06) was the highest at pH 6.70 (Xu et al., 2010). Wongsuphachat et al. (2010) reported that optimal initial pH of 7.0-7.5 was retained to improve both growth and EPS yield of *Weissella 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. Moreover, Gamar-Nourani et al. (1998) also found that the kinetics of EPS production by *Lactobacillus rhamnosus* C83 were not different at pH 5.5, 6.2 or 7.2 and initial pH 7.2. EPS yield was linked to growth, reached a maximum (100-150 mg/L) after 40 h of fermentation and subsequently decreased. For *Weissella* sp. PSMS4-4, the highest EPS yield of 8.65 g/L was attained in culture with an initial pH of 7.0, temperature of 30°C, and sucrose concentration of 5% (Tayuan et al., 2011). However, higher EPS yield are obtained when fermentations are carried out under controlled pH conditions. A maximum EPS yield was obtained by *Lactobacillus casei* CRL 87 when the pH was controlled at 6.0 (Mozzi et al., 1994). Kuntiya et al. (2010) found that the favorable pH level for EPS yield from *Lactobacillus confusus* TISTR 1498 was 5.5, the EPS production being at a maximum of 12.95 g/L after a 24 h incubation period in coconut water and in modified MRS-sucrose medium.

The present work describes the over-biosynthesis of EPS by *Lactobacillus confusus* TISTR 1498 under high sucrose stress in sugarcane juice fermentation. Results indicated that sugarcane juice could enhance EPS production in submerged fermentation by about two fold when compared to the modified MRS sucrose medium experiments. Additionally, it could reduce production cost to 0.53 THB/g EPS, which was lower than the application of peptone yeast extract and beef extract (0.78 THB/g EPS). Biosynthesis of EPS by *Lactobacillus confusus* TISTR 1498 was independent of biomass production (Seesuriyachan, 2012). The C/N ratio appears to be important for EPS production. It has been suggested that high-energy generation with the high sugar concentration and the relatively low and moderate amount of nitrogen (low C/N ratio) of sugarcane juice resulted in high EPS yield and relatively high cell-mass production (Seesuriyachan et al., 2010). This study confirmed that the production of *Lactobacillus confusus* EPS using sugarcane juice could be used as a low-cost substitute for sucrose.

CONCLUSION

Sugarcane juice, a naturally renewable resource, can be partially replaced as a carbon source (sucrose) in the modified MRS-sucrose medium (initial sucrose concentration at 300 g/L) for EPS production by *Lactobacillus confusus*

TISTR 1498. The optimal culture conditions for EPS production were: pH 5.55, temperature 29.75°C and 1.22PYB (composed of 6.1 g/L peptone, 3.05 g/L yeast extract and 3.05 g/L beef extract). These conditions provided the highest EPS yield (107.52 g/L) and highest biomass (1.92 g/L). This was higher than the maximum yield produced with the modified MRS medium containing only 300 g/L of sucrose (55.6 g/L of EPS and 1.8 g/L of biomass). The sugarcane juice improved the EPS yield by a factor of two when compared to the modified MRS sucrose medium. In addition, the cost of medium is lower (0.53 THB/g EPS) than a medium from the mixtures of peptone, yeast extract and beef extract (0.78 THB/g EPS). Future research is required to investigate the mechanisms of EPS production from sugarcane juice and to understand the functional properties of EPS produced by *Lactobacillus confusus* in some foods, such as starchy food.

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