

Statistical Optimization of Medium Compositions for High Cell Mass and Exopolysaccharide Production by *Lactobacillus plantarum* ATCC 8014

Othman, N.Z.^{1*}, Mohd Din, A.R.J.¹, Azam, Z.,¹ Rosli, M.A.¹, Sarmidi, M.R.^{1,2}

1- UTM Innovation Centre in Agritechology for Advanced Bioprocess, Universiti Teknologi Malaysia-Pagoh, 84600 Muar, Johor, Malaysia.

2- Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Skudai Johor, Malaysia.

Abstract

Background and Objective: *Lactobacillus plantarum* ATCC 8014 is known as a good producer of water soluble exopolysaccharide. Therefore, the aim of this study is to optimize the medium composition concurrently for high cell mass and exopolysaccharide production by *Lactobacillus plantarum* ATCC 8014. Since both are useful for food and pharmaceutical application and where most studies typically focus on one outcome only, the optimization process was carried out by using molasses as cheaper carbon source.

Material and Methods: The main medium component which is known significantly give high effect on the cell mass and EPS production was selected as variables and statistically optimized based on Box-Behnken design in shake flask levels. The optimal medium for cell mass and exopolysaccharide production was composed of (in g l⁻¹): molasses, 40; yeast extract, 16.8; phosphate, 2.72; sodium acetate, 3.98. The model was found to be significant and subsequently validated through the growth kinetics studies in un-optimized and optimized medium in the shake flask cultivation.

Results and Conclusion: The maximum cell mass and exopolysaccharide in the new optimized medium was 4.40 g l⁻¹ and 4.37 g l⁻¹ respectively after 44 h of the cultivation. As a result, cell mass and exopolysaccharide production increased up to 4.5 and 16.5 times respectively, and the maximal exopolysaccharide yield of 1.19 per gram of cells was obtained when molasses was used as the carbon source. In conclusion, molasses has the potential to be a cheap carbon source for the cultivation of *Lactobacillus plantarum* ATCC 8014 concurrently for high cell mass and exopolysaccharide production.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

Received 01 Dec 2017
Revised 07 Feb 2018
Accepted 10 Mar 2018

Keywords:

- Cell mass
- Exopolysaccharide
- Medium optimization
- Probiotic

*Corresponding author:

Nor Zalina Othman

UTM Innovation Centre in
Agritechology for
Advanced Bioprocess,
Universiti Teknologi
Malaysia-Pagoh, 84600
Muar, Johor, Malaysia.

Tel: +(6) 06-69742821
Fax: +(6) 06-9742996/98
Email: norzalina@utm.my

1. Introduction

The human intestinal tract constitutes a complex ecosystem which contains over 100 different species of bacteria, and their survival rate depends on the host's diet, the strength of the immune system, existing levels of bacteria, infections and the dosage of competing bacteria consumed each day [1]. The probiotic bacteria performs various types of metabolism in the intestine, thereby influencing the host's health by improving nutrition absorption, physiological function, immunological responses and resistance to infections and other stressors [2,3]. Each of the lactic acid bacteria possesses diverse enzymes, capable of converting substances into beneficial and detrimental compounds such as exopolysaccharide (EPS) [4,5]. Historically, *Lactobacillus* strain has been

stated as generally recognized as safe (GRAS) for human consumption [6,7,8] and different strains of LAB have been reported to be a good and safe producer of EPS. EPS is not only important as the bio thickeners in the food industries but it also has therapeutic effects on human health, such as antitumor, immuno-modulatory and antimicrobial activity [9,10]. For the industrial application, an alternative and cheaper carbon source is required since it is widely known that the carbon source is the main contributor for the synthesis of exopolysaccharides by *Lactobacillus* sp. [8] and its cost will greatly affect the main production cost. Therefore, alternative renewable carbon source such as molasses is used not only because it is a cheaper source but also due to the nutritious value

since it consists of a high concentration of organic and inorganic substances [11]. During the previous study, molasses was evaluated as a good substrate for the growth of *Lactobacillus* sp. for cell mass and lactic acid production with the aim of decreasing the cost of the process [12,13]. Nowadays, the live cells and lactic acid production are not the only useful compounds for us but the EPS in the fermentation broth is also a more economical alternative for the pharmaceutical and food industries. Therefore, the aim of this study is to optimize the main medium composition for high cell mass and exopolysaccharide production by *Lactobacillus* (*L.*) *plantarum* ATCC 8014. The experiment is designed to optimize medium components towards industrial application for both production of cell mass and EPS in submerged fermentation when molasses has been used as the carbon source. In this study, response surface methodology (RSM) is used as a collection of mathematical and statistical techniques useful for modeling and analyzing problems in which a response of interest is influenced by several variables to optimize the response.

2. Materials and Methods

2. Materials and methods

2.1 Microorganisms

L. plantarum ATCC 8014, was first propagated in Man Rogosa Sharpe's (MRS) broth (Merck, Darmstadt, Germany) consisting of (g l⁻¹): peptone from casein 10.0; meat extract 10.0; yeast extract 4.0; D(+)-glucose 20.0; dipotassium hydrogen phosphate 2.0; Tween 80 1.0; diammonium hydrogen citrate 2.0; sodium acetate 5.0; magnesium sulfate 0.2; manganese sulfate 0.04; agar-agar 14.0 and incubated at 30°C for 48 h. The arisen colonies were harvested by glycerol solution (50% w v⁻¹) and put in series of 2 ml cryogen vials. The vials were frozen at -20°C for 24 h followed by further storage in the working cell bank at -80°C for further use.

2.2 Optimization of production medium using RSM in the shake flask cultivation

A Box-Behnken of MINITAB 15 software was used as a statistical tool for the medium optimization to determine the optimum level of specific variable, with a total of 54 experiments used to optimize the cell mass and EPS production by *L. plantarum* ATCC 8014. Box Behnken experiment design was chosen because the advantage of this method is that the model takes into account the concentration of each compound within the boundaries and

eliminate the possibilities which are outside the boundaries called corner points. In this study, the optimization of the main medium components was molasses, yeast extract, KH₂PO₄ and sodium acetate monohydrate based on the medium modified from the studies of Dailin et al., [14]. However, lactose was replaced with molasses after screening different types of carbon source revealed that the highest cell mass and EPS production for *L. plantarum* ATCC 8014 was yielded when the carbon sources was molasses. The chemical composition of molasses used in this study contains total carbohydrate of 56 % with low nitrogen content only 5.8 % and the ash content was only 3.3 %. Each factor in the design was studied at three different levels; low, intermediate and high value as shown in Table 1; Molasses (A: 20-40 g l⁻¹), yeast extract (B: 12-18 g l⁻¹), KH₂PO₄ (C:2-4 g l⁻¹) and sodium acetate monohydrate (D:3-7 g l⁻¹). The responses were analyzed after 72 h of cultivation. The pH of the medium was adjusted to 6.0 before sterilization. The carbohydrate from the molasses was sterilized separately to avoid Millard reaction between reducing sugar (glucose) in the molasses with amino acid in nitrogen source. Then, mixed together in sterile condition before the inoculation of inoculum. The shake flasks cultivations were carried out in 250 ml Erlenmeyer flasks containing 50 ml broth and incubated in a rotary shaker (4230 Innova, New Brunswick, NJ, USA) at 150 rpm at 30°C with an inoculum size of 10 % (v v⁻¹).

The cell mass and EPS production by *L. plantarum* ATCC 8014 in the extracellular medium was taken as response or dependent variables of Y₁ and Y₂ respectively. The experimental value of the response was recorded in the experimental set up as presented in Table 1. A second order polynomial equation (Equation 1) was fitted and explained each of the response. Where Y, predicted response; intercept; β₀, linear coefficients: β₁, β₂, β₃, β₄, squared coefficients: β₁₁, β₂₂, β₃₃, β₄₄, interaction coefficient: β₁₂, β₁₃, β₁₄, β₂₃, β₂₄, β₃₃. From the Box-Behnken design, contour plots that delineate predicted responses over a certain range in the design surface can be plotted. The contour plots between the 4 factors were analyzed and the numerical optimization was chosen to generate optimal conditions by setting a goal as 'maximum' for both responses that were analyzed by the Box-Behnken design.

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_4D + \beta_{11}AA + \beta_{22}BB + \beta_{33}CC + \beta_{44}DD + \beta_{12}AB + \beta_{23}BC + \beta_{13}AC + \beta_{14}AD + \beta_{24}BD + \beta_{33}CD \quad \text{Eq. 1}$$

Table1. Actual levels for the experimental design with experimental values of the cell mass and exopolysaccharide production by *L. plantarum* ATCC 8014.

Expr. No	Variables: Actual concentration (g l ⁻¹)				Response (g l ⁻¹)	
	Molasses	Yeast Extract	K ₂ HPO ₄	Sodium acetate	Cell mass	EPS
1	20	15	4	5	0.75	1.53
2	30	15	4	7	3.23	2.2
3	30	15	4	3	3.70	2.09
4	40	12	3	5	3.80	3.97
5	30	12	3	7	2.15	1.6
6	30	12	2	5	2.90	1.32
7	30	12	3	3	2.93	1.579
8	30	18	2	5	2.30	2.5
9	30	18	3	3	2.70	2.6
10	20	15	3	7	0.65	1.9
11	20	12	3	5	0.15	1.23
12	30	18	2	5	2.50	2.53
13	40	15	3	7	4.35	4.65
14	30	12	3	3	2.93	1.6
15	30	18	4	5	3.50	2.9
16	40	15	3	3	4.20	4.71
17	30	18	3	7	3.60	2.39
18	40	15	2	5	4.16	3.8
19	20	15	2	5	0.80	1.6
20	30	15	3	5	3.63	3.17
21	30	15	3	5	3.62	3.15
22	30	15	3	5	3.52	3.15
23	20	18	3	5	0.80	1.77
24	30	12	3	7	2.63	1.56
25	40	18	3	5	5.42	4.23
26	40	15	3	7	4.02	4.6
27	30	15	2	7	3.10	2
28	30	15	3	5	3.20	1.33
29	20	15	3	3	1.10	1.43
30	30	18	3	7	3.93	2.37
31	30	15	2	7	3.65	2.02
32	20	15	3	7	0.56	1.93
33	20	12	3	5	0.02	1.2
34	20	18	3	5	0.90	1.75
35	30	15	2	3	3.60	2.09
36	40	15	2	5	3.94	3.88
37	30	15	4	3	3.55	2.09
38	20	15	3	3	1.24	1.32
39	30	15	4	7	3.46	2.3
40	20	15	4	5	0.60	1.47
41	30	12	4	5	3.28	1.43
42	40	15	3	3	3.94	4.59
43	30	15	3	5	2.37	2.2
44	30	18	3	3	2.93	2.66
45	30	12	2	5	3.26	1.29
46	30	15	2	3	3.31	2.07
47	40	12	3	5	3.90	4.02
48	30	12	4	5	3.46	1.49
49	40	18	3	5	5.53	4.335
50	20	15	2	5	0.75	1.63
51	30	18	4	5	4.83	2.93
52	30	15	3	5	3.22	3.11
53	40	15	4	5	4.56	3.83
54	40	15	4	5	4.61	1.63

2.5 Analysis

2.5.1 Biomass determination

The fermentation broth was centrifuged in 50 ml falcon tubes at $6339 \times g$ for 15 min to precipitate the cells. The supernatant was taken for EPS analysis. Cell pellets were then washed with sterile water and centrifuged again under the same conditions. After the second centrifugation cycle, the supernatant was discarded and the cells were dried at 65°C in an oven for 48 h.

2.5.2 Extraction of EPS

The cell-free clear supernatant was used for EPS determination. The crude EPS was then isolated by 95% ethanol precipitation at the ratio of 1:3. After centrifugation at $6339 \times g$ of 15 min, 4°C , the EPS pellet was dispersed in aqueous 95 % ethanol and centrifuged again. The final precipitate was dried to a constant weight at 55°C [15,16]. The EPS yield was measured.

2.5.3 Total carbohydrate analysis

Phenol-sulfate acid method was utilized to detect total sugar content using glucose as a standard [17].

3. Results and Discussion

3.1 Medium optimization using experimental design and statistical analysis (ANOVA) for high cell mass and EPS production by *L. plantarum* ATCC 8014

One of the strategies that can influence the production of cell mass and secretion of EPS into the extracellular medium was the modification of medium composition. By applying multiple regression analysis on the experimental data, the second-order polynomial equation explained the cell mass and EPS production as seen in Equation 2 and 3. The highest cell mass production ranging from 5.42 g l^{-1} to 5.53 g l^{-1} was seen in treatment runs 25 and 49 when the independent variables for molasses and yeast extract were at the high-level and independent variables for sodium acetate and KH_2PO_4 at the mid-level (Table 1). However, the highest EPS production were observed when the concentration of molasses and KH_2PO_4 were at high-level

and the concentration of yeast extract and sodium acetate were at mid-level with the EPS production ranging from 4.59 to 4.71 g l^{-1} (Table 1).

As shown in Table 2, ANOVA was used to determine the significance of the variables to the responses of the response surface methodology (RSM) by using Box-Behnken design. The calculated values of F were compared to the critical value of ($F_{(p-1, n-p, \alpha)}$) tabulated. The ANOVA showed that the calculated value of F for the cell mass and EPS production were 27.53 and 37.89, respectively. All of which were greater than the critical values for the F distribution table of $F_{3,49,0.05}=2.79$ (Table 2). If the calculated value of F exceeds the $F_{(3,49,0.05)}$, the null hypothesis was rejected at the level of significance, and it was inferred that the coefficient estimates are not all zero and the variation verified that the model was significantly greater than the unexplained variation [18]. Therefore, the p -value for all of the responses were below 0.05 which means that the 95 % confidence level of the models were significant. In this experiment, all responses gave r^2 above 90%, indicating that this study signifies a good correlation between the experimental data and the predicted values of which only 10% was not explained by the model. The acceptance of the model was supported by p -value of lack of fit which was insignificant ($p>0.05$) [10,16].

As shown in Table 3, the variables with the greatest impact ($F=27.39$) on the cell mass production was the square term of molasses (AA) followed by linear term of molasses (A). However, the greatest impact on the EPS production ($F=9.47, 7.0, 4.17$) by the square term of KH_2PO_4 (CC) followed by molasses (AA) and sodium acetate (BB) respectively. The regression coefficient with the values of probability less than 0.05 ($p \leq 0.05$) will significantly affect the cell mass and EPS production which indicated a high significance of the model [10]. The following equations were the second order polynomial equation (Equation 2 and 3) in term of coded variables and actual one. The acceptance of the model was supported by p -value of lack of fit which was insignificant ($p > 0.05$).

Table 2. Analysis of variance (ANOVA) of the response variables

Response	Source	DF ^{*1}	SS ^{*2}	MS ^{*3}	F value, F_{cal}	Prob(p)>F	r^2 (%)	F (0.05)
Cell mass ($Y_{\text{cell mass}}$)	Regression	4	40.867	10.217	27.53	0.000	91.12	2.79
	Residual error	49						
	Total	53						
	Lack of fit					0.341		
Exo- polysaccharide (Y_{EPS})	Regression	4	84.601	21.150	37.89	0.000	90.01	2.79
	Residual error	49						
	Total	53						
	Lack of fit					0.053		

*¹-Degree of Freedom, ²- Sum of Square, ³-Mean Square

As shown in Figure 1 and 2, a contour plot that explained the relationships of all variables illustrated by the slight curvilinear plot which means that molasses and yeast extract concentration have significant effects on the cell growth and EPS production. This correlated with the study by Manochai et al. [11] whereby they observed that the production of EPS improved by factor of two when sugarcane juice was used as the carbon sources when compared with growth in sucrose for *L. confusus*. As

studied by Imran et al, [10] and Mecado et al., [19], the organic nitrogen sources were inferred to yield a higher amount of cell mass and EPS. However, interaction of CB, DB and DC gave the elliptical nature of the contour plot indicating that the interaction of each independent variable to the response was significant at the mid-level for the maximum EPS production. The results demonstrate that the yeast has a strong influence on both cell growth and EPS production.

Table 3. Analysis of variance for the cell mass and exopolysaccharide production of *L. plantarum* ATCC 8014 using Box-Behnken design

Source	DF	Cell mass [g L ⁻¹]					EPS [g L ⁻¹]				
		Seq SS	Adj SS	Adj MS	F	P	Seq SS	Adj SS	Adj MS	F	P
Regression	14	97.925	97.925	6.995	19.440	0	49.069	49.069	3.505	13.69	0
Linear	4	84.601	4.961	1.240	3.450	0.017	40.867	2.002	0.501	1.95	0.121
A	1	81.058	3.750	3.750	10.420	0.003	35.954	0.003	0.003	0.01	0.921
B	1	2.361	0.125	0.125	0.350	0.559	4.749	1.141	1.141	4.46	0.041
C	1	1.154	0.143	0.143	0.400	0.532	0.029	1.262	1.262	4.93	0.032
D	1	0.027	0.079	0.079	0.220	0.641	0.135	0.219	0.219	0.86	0.361
Square	4	10.311	10.311	2.578	7.160	0	7.478	7.446	1.862	7.27	0
AA	1	8.558	9.853	9.852	27.390	0	4.678	1.791	1.791	7	0.012
BB	1	0.812	1.393	1.393	3.870	0.056	0.363	1.068	1.068	4.17	0.048
CC	1	0.028	0.226	0.226	0.630	0.433	2.267	2.424	2.424	9.47	0.004
DD	1	0.913	0.914	0.913	2.540	0.119	0.170	0.175	0.175	0.68	0.414
Interaction	6	3.014	3.014	0.502	1.400	0.241	0.724	0.724	0.121	0.47	0.826
AB	1	0.371	0.371	0.371	1.030	0.316	0.033	0.033	0.033	0.13	0.721
AC	1	0.201	0.201	0.201	0.560	0.460	0.495	0.495	0.495	1.93	0.172
AD	1	0.230	0.230	0.230	0.640	0.429	0.130	0.130	0.130	0.51	0.480
BC	1	1.088	1.088	1.088	3.020	0.090	0.030	0.030	0.030	0.12	0.734
BD	1	1.104	1.104	1.104	3.070	0.088	0.029	0.029	0.029	0.11	0.739
CD	1	0.020	0.020	0.020	0.060	0.813	0.007	0.007	0.007	0.03	0.874
Residual Error	39	14.031	14.031	0.360			9.986	9.986	0.256		
Lack-of-Fit	11	4.067	4.067	0.407	1.180	0.341	4.614	4.614	0.419	2.19	0.053
Pure Error	28	9.964	9.964	0.344			5.372	5.372	0.192		
Total	53	111.956					59.054				

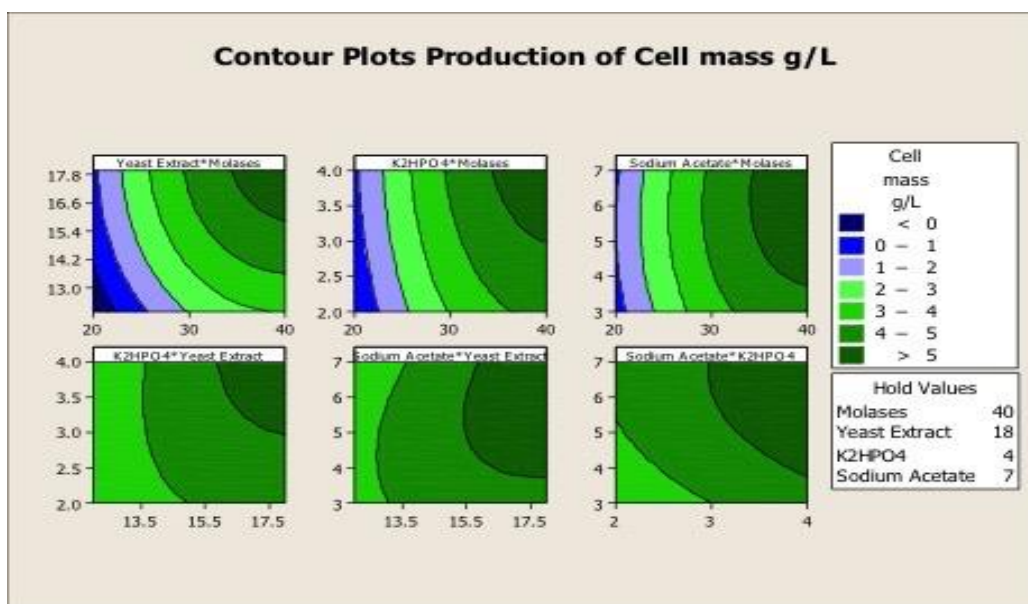


Figure 1. Contour plot interaction of all variables when the response is cell mass by *L. plantarum* ATCC 8014 after 72 h cultivation.

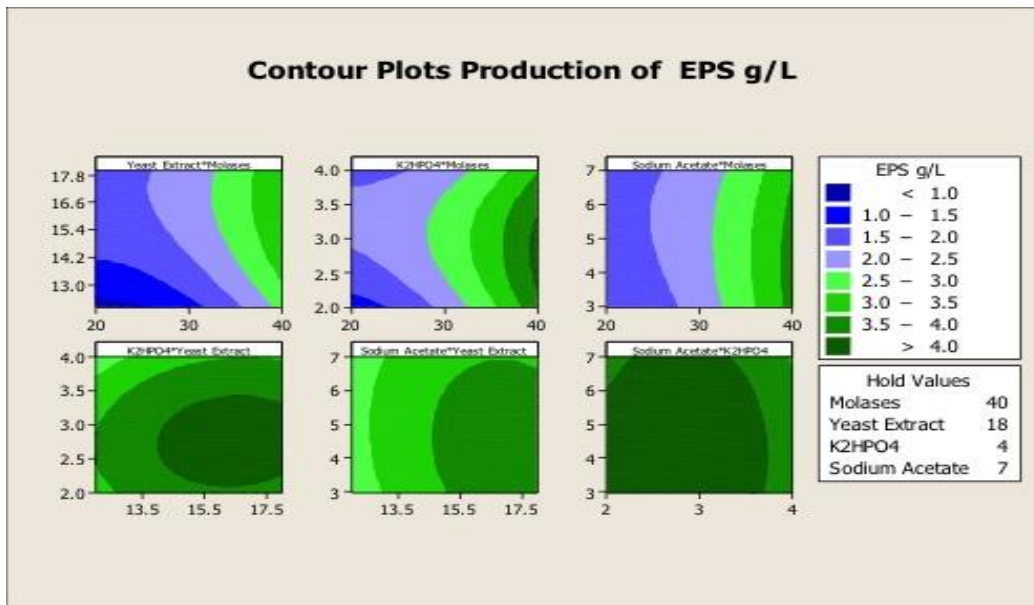


Figure 2. Contour plot interaction of all variables when the response is EPS production by *L. plantarum* ATCC 8014 after 72 h cultivation.

$$Y_{\text{cell mass}} = -8.9947 + 0.56282A \quad \text{Eq. 2}$$

$$Y_{\text{EPS}} = -16.3330 + 1.2582B + 3.2083C + 0.0041 A^2 - 0.0352B^2 - 0.4797C^2 \quad \text{Eq. 3}$$

3.2 Validation of optimization study and the growth kinetics studied between non-optimized and optimized medium composition

Based on the regression model, an optimization plot can be generated using the MINITAB 15 software to determine the optimum composition for the cell mass and EPS production by *L. plantarum* ATCC 8014. The optimum concentration of molasses, yeast extract, KH_2PO_4 , and sodium acetate obtained using statistical medium optimization were 40, 16.8, 2.72 and 3.98 (g l^{-1}), respectively (Table 4). As shown in Figure 3, cultivations in both un-optimized and statistical optimized medium showed significant differences in cell mass production with maximum cell mass production of 0.985 g l^{-1} and 4.40 g l^{-1} , respectively after 72 h and 48 h of cultivation. As mentioned by Camellini et al., [20], in the cultivation of *L. plantarum* ATCC 8014 as the cell mass increase so does EPS production even though under poor nutritional conditions. In this study, the specific growth rate (μ) of *L. plantarum* ATCC 8014 when cultivated in the un-optimized which is lactose as carbon source and in optimized medium with molasses as carbon source was 0.014 and 0.036 h^{-1} , respectively (Table 4). Camellini et al., [20] obtained biomass production of 2.7 g l^{-1} for *L. plantarum* ATCC 8014 cultivated in MRS medium when glucose as a carbon source with specific growth rate more longer (0.076 h^{-1}). Most of the studies showed that *Lactobacillus sp* were capable of growing and producing EPS despite the high growth-inhibiting heavy metal

content in the molasses [20]. From Figure 3, growth of *L. plantarum* ATCC 8014 in both medium showing the pH gradually decrease and mainly produced acid during the reproduction and growing phase [10,22] which was consistent with our result. As shown in Figure 3 for optimized medium, the pH of the broth changes from the initial value of 6.0 to about 4.3 after 72 h cultivation.

In this study, the maximum EPS production of 4.97 g l^{-1} at h 44 was achieved when the concentration of molasses was 40 g l^{-1} which fitted the predicted data by the models. The EPS production rate was 0.113 $\text{g l}^{-1} \text{h}^{-1}$ with the maximal EPS yield of 1.19 EPS g^{-1} cells was obtained when molasses was used as the carbon source when compared to the cultivation using lactose that only yielded 0.268 EPS g^{-1} cells (Table 4). Molasses is known as a good carbon source for EPS production as study by Abdul Razack et al. and Yilmaz et al. when change of carbon source to molasses during cultivation of *Bacillus sp* [23,24]. In addition, the concentration of yeast extract at 16.8 g l^{-1} was found to produce the maximum yield of 1.19 EPS g^{-1} which in agreement with study by Dailin et al, and Mecado et al. where using of yeast extract as efficient nitrogen source increased EPS production when compared with other types of organic nitrogen sources for *Lactobacillus sp*, [14,19]. cultivation. This new medium formula not only increased the cell mass and EPS volumetric production but also shorten the production time from 72 h to only 48 h.

Table 4. Kinetic parameters of cell growth and EPS production by *L. plantarum* ATCC 8014 in the un-optimized and optimized medium in the shake flask cultivation.

Parameters	Un-optimized	Validation of optimized medium
Carbon source (g l ⁻¹)	Lactose -50.0	Molasses- 40.0
Yeast extract (g l ⁻¹)	12.0	16.80
KH ₂ PO ₄ (g l ⁻¹)	0.25	2.72
Sodium acetate (g l ⁻¹)	5.0	3.98
Cell Growth parameters		
X _{max} [g l ⁻¹]	0.985	4.40
dx/dt[g l ⁻¹ h ⁻¹]	0.016	0.106
μ [h ⁻¹]	0.014	0.036
EPS Production parameters		
P _{max} [g l ⁻¹]	0.265	4.37
Q _{EPS} [g l ⁻¹ h ⁻¹]	0.004	0.113
Y _{p/x} [g g ⁻¹]	0.269	1.19

X_{max}: maximal cell dry weight; dx/dt: growth rate; μ: specific growth rate; P_{max}: maximal EPS production; Q_{EPS}: EPS production rate.

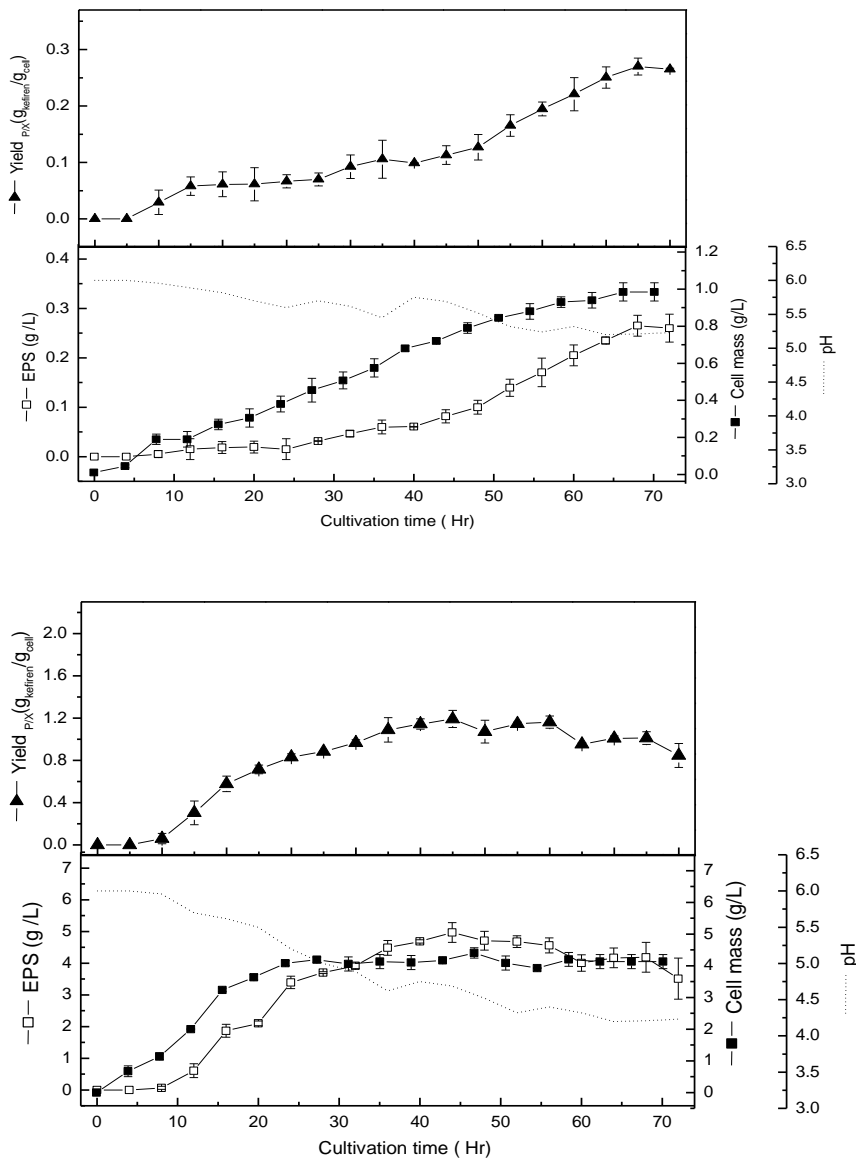


Figure 3. Kinetics of cell growth and EPS production by *L. plantarum* ATCC 8014 in shake flask cultivations using un-optimized (left) and optimized (right) medium.

4. Conclusion

In the present study, an attempt was made to optimize medium composition by statistical experimental design of Box-Behnken to improve concurrently high cell mass and EPS production from *L. plantarum* ATCC 8014. As a result, cell mass and EPS production increased up to 4.5 and 16.5 times, respectively. The data showed that molasses are good alternative for the major nutrient sources with addition of yeast extract for both cell mass and EPS production. The significant increase in both cell mass and EPS production using the new medium formulation make it attractive for further studies on production in industrial scale.

5. Acknowledgements

This work was funded by the HiCOE grant under Ministry of Higher Education, Malaysia. Project entitled: Development of high viability spray dried probiotics, Cost Center No: R.J130000.7809.4J270

6. Conflict of Interest

The authors declare no conflict of interest.

References

- Ozyurt VH and Ötles S. Properties of probiotics and encapsulated probiotics in food Acta Sci Pol Technol Aliment. 2014; 13(4):413-424. doi: 10.17306/J.AFS.2014.4.8.
- Panthavee W, Noda M, Danshiitsoodol N, Kumagai T, Sugiyama M. Characterization of exopolysaccharides produced by thermophilic lactic acid bacteria isolated from tropical fruits of thailand. Biol Pharm Bull. 2017; 40: 621-629. doi:10.1248/bpb.b16-00856
- Dilna SV, Surya H, Aswathy RG, Varsha KK, Sakthikumar DN, Pandey A, Nampoothiri KM. Characterization of an exopolysaccharide with potential health benefit properties from a probiotic *Lactobacillus Plantarum* RJF4. Food Sci Technol 2015; 64(2): 1179-1186. doi:10.1016/j.lwt.2015.07.040
- Zannini E, Waters DM, Coffey A, Arendt EK. Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl Microbiol Biotechnol. 2016; 100:1121-1135. doi: 10.1007/s00253-015-7172-2.
- Patel AK, Michaud P, Singhania RR, Soccol CR, Pandey A. Polysaccharides from probiotics: New developments as food additives. Food Technol Biotechnol. 2010; 48 (4): 451-463.
- Sasikumar K, Vaikkath D K, Devendra L, Nampoothiri KM. An exopolysaccharide (EPS) from a *Lactobacillus plantarum* br2 with potential benefits for making functional foods. Bioresour Technol. 2017; 241:1152-1156. doi: 10.1016/j.biortech.2017.05.075.
- Wang J, Zhao X, Yang Y, Zhao A, Yang Z. Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. Int J Biol Macromol. 2015; 74: 119-126. doi: 10.1016/j.ijbiomac.2014.12.006.
- Prathimal PC, Lulel VK, Tomar1 ST, Singh AK. Optimization of exopolysaccharide production by *Lactococcus lactis* NCDC 191 by response surface methodology. Int J Curr Microbiol App Sci. 2014; 3(5): 835-854.
- Wang X, Shao C, Liu L, Guo X, Xu Y, Lü X. Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus plantarum* KX041. Int J Biol Macromol. 2017; 103:1173-1184. doi: 10.1016/j.ijbiomac.2017.05.118.
- Imran MYM, Reehana N, Jayaraj KA, Ahamed AAP, Dhanasekaran D, Thajuddin N, Alharbi NS., Muralitharan G. Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20. Int J Biol Macromol. 2016; 93:731-745. doi: 10.1016/j.ijbiomac.2016.09.007.
- Manochai P, Phimolsiripol Y, Seesuriyachan P. Response surface optimization of exopolysaccharide production from sugarcane juice by *Lactobacillus confusus* TISTR 1498. Food Appl Bioscience 2014; 13(1): 425-438. doi: 10.12982/cmujns.2014.0046.
- Lee K., Kang SK, and Choi YJ. A Low-cost *Lactobacillus salivarius* L29 growth medium containing molasses and corn steep liquor allows the attainment of high levels of cell mass and lactic acid production. Afr J Biotechnol. 2013;12: 2013-2018. doi: 10.5897/AJB12.2597.
- Vidra A, Tóth AJ, Németh A. Lactic acid production from cane molasses. liquid waste recovery. 2017; 2: 13-16. doi: 10.1515/lwr-2017-0003.
- Dailin DJ, Elsaeyed EA, Othman NZ, Malek R, Phin HS, Aziz R, Wadaan M, El Enshasy HA. development of cultivation medium for high yield kefiran production by *Lactobacillus kefiranofaciens*. Int J Pharm Pharm Sci. 2015; 7: 159-163.
- Trabelsi I, Slima SB, Chaabane H, Riadh BS. Purification and characterization of a novel exopolysaccharides produced by *Lactobacillus* sp. Ca6. Int J Biol Macromol. 2015; 74:541-546. doi: 10.1016/j.ijbiomac.2014.12.045.
- Seesuriyachan P. Statistical modeling and optimization for exopolysaccharide production by *Lactobacillus confusus* in submerged fermentation under high salinity stress. Food Sci Biotechnol. 2012; 21(6): 1647-1654. doi: 10.1271/bbb.110905.
- Li W, Xiang X, Tang S, Hu B, Tian L, Sun Y. Effective Enzymatic Synthesis of Lactosucrose and its Analogues by *d*-galactosidase from *Bacillus circulans*. J Agric Food Chem. 2009; 57(9): 3927-3933. doi: 10.1021/jf9002494.
- Wonnacott T H, and Wonacott R J. Introductory Statistics. New York: John Willey and Sons.1990.
- Macedo MG, Lacroix C, Champagne CP. Combined effect of temperature and medium composition on exopolysaccharide production by *Lactobacillus rhamnosus* RW-9595M in a Whey Permeate Based Medium. Biotechnol Prog. 2002; 18:167-173. doi: 10.1021/bp0101637.
- Camelini CM, Rezzadori K, Benedetti S, Azambuja AA, Penha FM, Rossi MJ, Di Luccio M, Petrus JCC, Giachini AJ. Production of exopolysaccharides by *Lactobacillus plantarum* ATCC 8014 and Concentration by Nanofiltration. J Adv Biotechnol. 2016; 6 (2): 901-912
- Yenieli N, Beyatli Y, Aslim B, Nur Z. Designated production of exopolysaccharide (EPS) and lactic acid with sugarbeet

- molasses by some lactic acid bacteria. 2006. Sugar industry. 131(12):841-845.
22. Zhang Y, Hu P, Fan M, Liao Q. Study on effect elements of exopolysaccharide production of *Lactobacillus Kimchi* SR8 and DPPH radical scavenging activity. *J. Food Nutr Res.* 2017; 5(12): 928-934. doi: 10.12691/jfnr-5-12-8.
23. Abdul Razack S, Velayutham V, Thangavel V. Medium optimization for the production of exopolysaccharide by *Bacillus subtilis* using synthetic sources and agro wastes. *Turk J Biol.* 2013; 37: 280-288. doi:10.3906/biy-1206-50.
24. Yilmaz M, Celik GY, Aslim B, Onbasili D. Influence of carbon sources on the production and characterization of the exopolysaccharide (EPS) by *Bacillus sphaericus* 7055 strain. *J Polym Environ.* 2012; 20:152-156. doi:10.1007/s10924-011-0358-5.

بهینه‌سازی آماری ترکیبات محیط کشت برای توده سلولی متراکم و تولید خارج سلولی پلی ساکارید توسط لاکتوباسیلوس پلاننتاروم ATCC 8014

ن. ز. اوتمان^{۱*}، آ. ر. ج. محد دین^۱، ز. آزم^۱، م. آ. رسلی^۱، م. ر. سرمیدی^۲

۱- مرکز نوآوری در علوم کشاورزی UTM برای فرایند زیستی پیشرفته، دانشگاه صنعتی مالزی-پاگوه، ۸۴۶۰۰ ماآر، جوهور، مالزی.

۲- انستیتو توسعه فرآورده زیستی، دانشگاه صنعتی مالزی، ۸۱۳۱۰ اسکودای جوهور، مالزی.

تاریخچه مقاله

دریافت ۰۱ دسامبر ۲۰۱۷
داوری ۰۷ فوریه ۲۰۱۸
پذیرش ۱۰ مارچ ۲۰۱۸

واژگان کلیدی

- توده سلولی
- پلی ساکارید خارج سلولی
- بهینه‌سازی محیط کشت
- زیست‌یار

*نویسنده مسئول

نور زالینا اوتمن، مرکز نوآوری در علوم کشاورزی UTM برای فرایند زیستی پیشرفته، دانشگاه صنعتی مالزی-پاگوه، ۸۴۶۰۰ ماآر، جوهور، مالزی.

تلفن: +۶۰۶-۶۹۷۴۲۸۲۱

دورنگار: +۶۰۶-۹۷۴۲۹۹۶/۹۸

پست الکترونیک:

norzalina@utm.my

چکیده

سابقه و هدف: لاکتوباسیلوس پلاننتاروم ATCC 8014 به‌عنوان یک تولید کننده خوب پلی ساکارید خارج سلولی قابل حل در آب شناخته می‌شود. بنابراین، هدف از این مطالعه بهینه‌کردن ترکیبات محیط کشت، برای تولید همزمان توده سلولی متراکم و پلی ساکارید خارج سلولی توسط لاکتوباسیلوس پلاننتاروم ATCC 8014 می‌باشد. از آنجا که هر دو هدف کاربرد غذایی و دارویی مفیدی دارند و بیشتر مطالعات انجام شده فقط بر یک نتیجه یا هدف متمرکز بوده‌اند، فرایند بهینه‌سازی هر دو با استفاده از ملاس، به‌عنوان منبع ارزان کربن، انجام شد.

مواد و روش‌ها: ترکیب اصلی محیط کشت که تاثیر زیادی بر تولید توده سلولی و پلی ساکارید خارج سلولی دارد، به عنوان متغیر انتخاب و از نظر آماری با طراحی باکس بنکن در مقیاس ارلن لرزان بهینه شد. محیط کشت بهینه برای تولید توده سلولی و پلی ساکارید خارج سلولی متشکل از (g L⁻¹) ملاس ۴۰؛ عصاره مخمر ۱۶/۸؛ فسفات ۲/۷۲؛ سدیم استات ۳/۹۸ بود. معنی دار بودن مدل و سپس اعتبار سنجی آن با مطالعه سینتیک رشد در کشت ارلن لرزان حاوی محیط کشت بهینه و غیربهینه حاصل شد.

یافته‌ها و نتیجه‌گیری: بیشینه تولید توده سلولی و پلی ساکارید خارج سلولی در محیط کشت جدید به‌ترتیب به میزان ۴/۴۰ g L⁻¹ و ۴/۳۷ g L⁻¹ پس از ۴۴ ساعت رشد بهینه شد. در نتیجه، تولید توده سلولی و پلی ساکارید خارج سلولی به‌ترتیب تا ۴/۵ و ۱۶/۵ مرتبه افزایش یافت، بیشینه راندمان تولید پلی ساکارید خارج سلولی ۱/۱۹ به ازای یک گرم سلول به‌دست آمد که از ملاس به عنوان منبع کربن استفاده شد. در نتیجه، ملاس منبع بالقوه ارزان کربن برای کشت لاکتوباسیلوس پلاننتاروم ATCC 8014 برای تولید توده سلولی متراکم و پلی ساکارید خارج سلولی می‌تواند باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافی وجود ندارد.