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

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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.

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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; di-ammonium 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, KH_2PO_4 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}), KH_2PO_4 (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 Y_1 and Y_2 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; β_0, linear coefficients:β_1, β_2, β_3, β_4, squared coefficients: β_{11}, β_{22}, β_{23}, β_{33}, β_{44}, interaction coefficient: β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{33}. 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.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{11} AA + \beta_{22} BB + \beta_{33} CC + \beta_{44} DD + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC + \beta_{14} AD + \beta_{24} BD + \beta_{33} CD \quad \text{Eq. 1}$$

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_2HPO_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 ×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 ×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-Benhnken 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≤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 ($Y_{cell\ mass}$)	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				

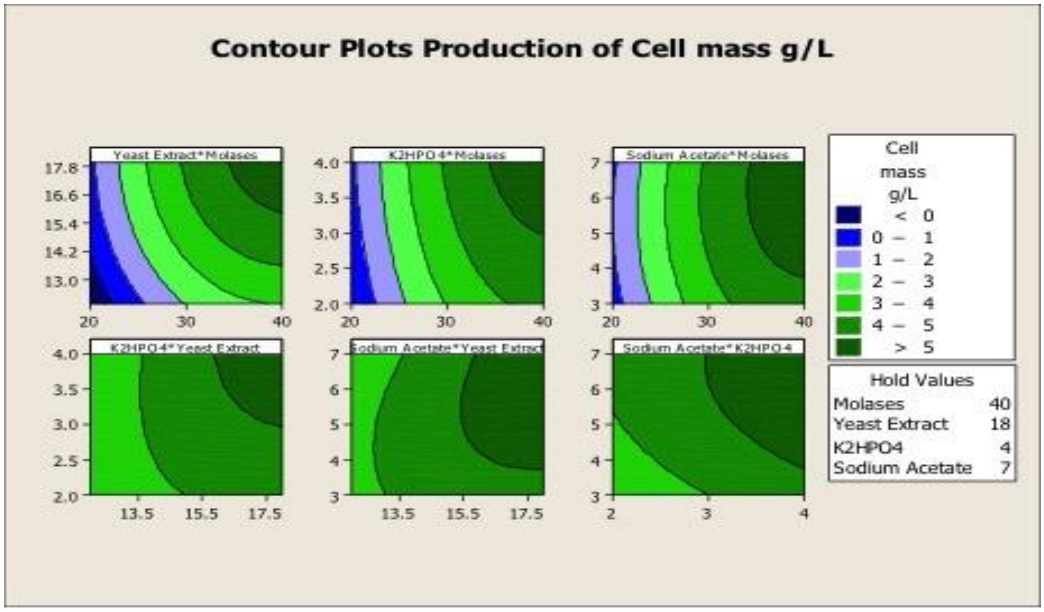

Figure 1. Contour plot interaction of all variables when the response is cell mass by *L. plantarum* ATCC 8014 after 72 h cultivation.

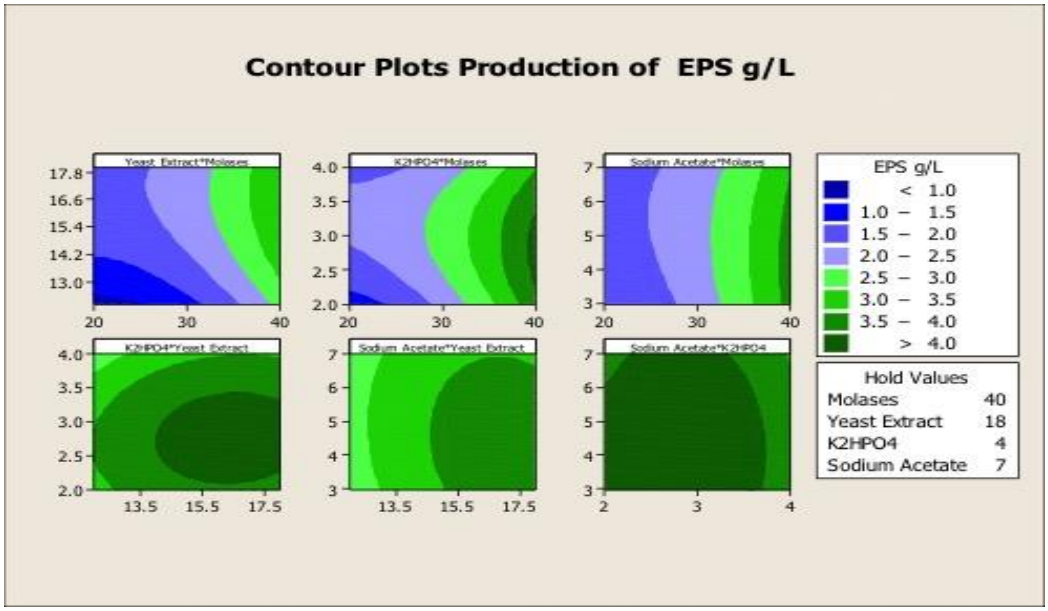

Figure 2. Contour plot interaction of all variables when the response is EPS production by *L. plantarum* ATCC 8014 after 72 h cultivation.

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

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

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 Camelini 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). Camelini 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 g l^{-1} 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_2PO_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.

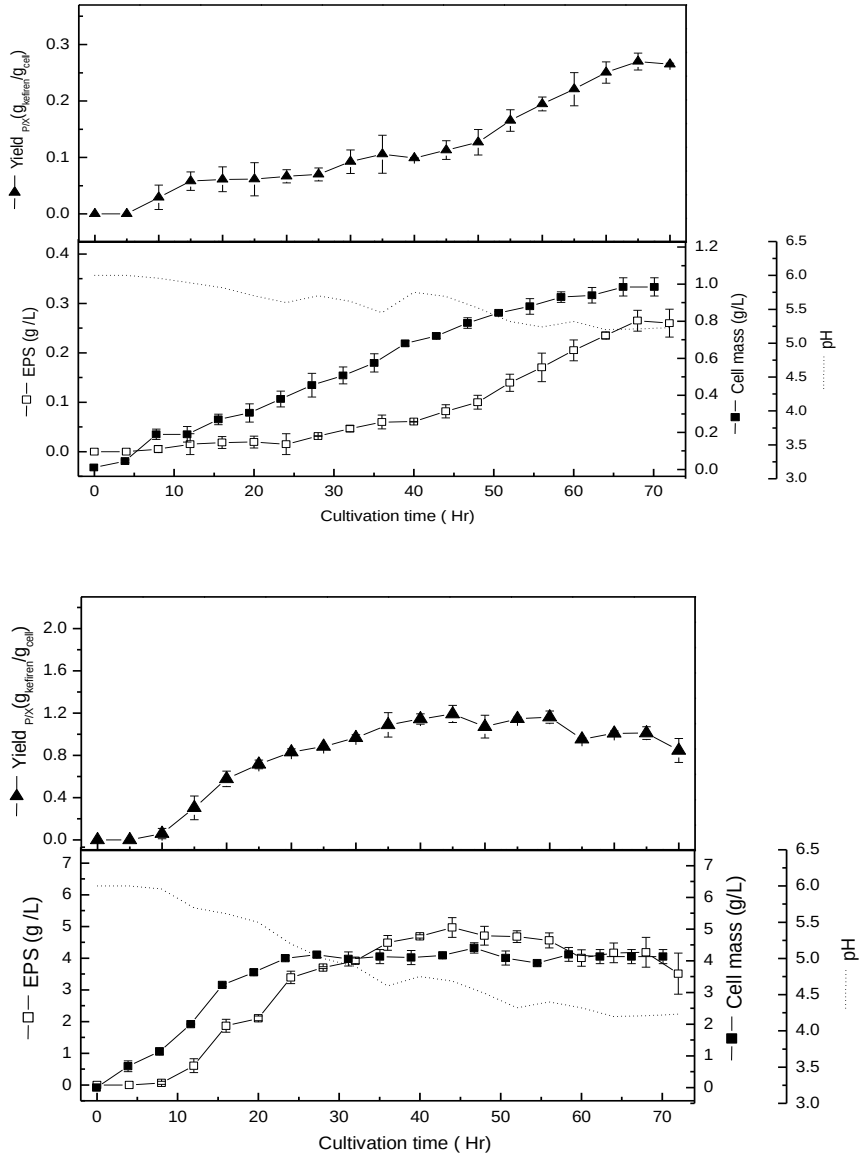

Figure 3. Kinetics of cell growth and EPS production by *L. plantarum* ATCC 8014 in shake flask cultivations using un-optimized (left) and optimized (right) medium.

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.

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6. Conflict of Interest

The authors declare no conflict of interest.

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

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چکیده

سابقه و هدف: *لاکتوباسیلوس پلانتاروم* ATCC 8014 به‌عنوان یک تولید کننده خوب پلی‌ساکارید خارج سلولی قابل حل در آب شناخته می‌شود. بنابراین، هدف از این مطالعه بهینه‌کردن ترکیبات محیط کشت، برای تولید همزمان توده سلولی متراکم و پلی‌ساکارید خارج سلولی توسط *لاکتوباسیلوس پلانتاروم* 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 پلی‌ساکارید 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 پلی‌ساکارید خارج سلولی متشکل از ($g\ L^{-1}$) ملاس ۴۰؛ عصاره مخمر ۱۶/۸؛ فسفات ۲/۷۲؛ سدیم استات ۳/۹۸ بود. معنی‌دار 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 پلی‌ساکارید خارج سلولی در محیط کشت جدید به‌ترتیب به میزان $4/40\ g\ L^{-1}$ و $4/37\ g\ L^{-1}$، پس از ۴۴ ساعت رشد بهینه شد. در نتیجه، تولید توده سلولی و پلی‌ساکارید 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 پلانتاروم* ATCC 8014 برای تولید توده سلولی متراکم و پلی‌ساکارید خارج سلولی می‌تواند باشد.

تعارض منافع: نویسندگان اعلام می‌کنندکه هیچ تعارض منافعی وجود ندارد.

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- توده سلولی
- پلی‌ساکارید خارج سلولی
- بهینه‌سازی محیط کشت
- زیست‌یار

***نویسنده مسئول**

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