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Optimization of Media Compositions and Fermentation Conditions to Maximize Viable Cells and Biomass Production of *Lactiplantibacillus plantarum* DLBSK207 Using Response Surface Methodology

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Abstract

Background and Objective: This study aimed to increase the viable cells and biomass production of a potential probiotic strain of *Lactiplantibacillus plantarum* DLBSK207 by optimizing concentrations of key nutrients and fermentation conditions using response surface methodology with Box-Behnken design.

Material and Methods: Experiments investigated two key variables of media composition and fermentation conditions. Based on the one-factor-at-a time results, six factors were selected for the Plackett-Burman design to assess if the variables significantly affected the response. The media contained carbon (glucose) and nitrogen (yeast extract and peptone) sources, while the fermentation conditions included initial pH and temperature. The basal media, consisting of sodium acetate, $MgSO_4.7H_2O$, K_2HPO_4, $MnSO_4.H_2O$ and Tween 80, were set as constant. Using RSM, concentrations of glucose, yeast extract and peptone, as well as the initial pH and temperature, were optimized to maximize viable cell counts and biomass.

Results and Conclusion: The optimum media composition assessed by RSM included 33.76 g l^{-1} glucose, 32.59 g l^{-1} yeast extract and 28.38 g l^{-1} peptone at an initial pH of 6.0 and a temperature of 35 °C. Under these optimized conditions, this study achieved viable cell count of 9.30 log CFU ml^{-1} and dry cell weight of 4.32 g l^{-1}, which closely matched the predicted values of 9.30 log CFU ml^{-1} and 4.28 g l^{-1}, showing a 1.82-fold increase compared to standard MRS broth. A higher biomass production rate was achieved in scaling up using 10^{-1} bioreactor controlled at pH 6.0 that resulted in maximum viable cell count of 9.88 log CFU ml^{-1} and DCW of 5.82 g l^{-1} after 20 h of incubation.

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1. Introduction

Probiotic bacteria, such as lactobacilli, have been interested worldwide due to their numerous health benefits. These bacteria are critical in balancing gut microbiota, protecting against pathogens and enhancing immune functions [1]. Probiotics are live microorganisms that provide health benefits primarily by changing the microbiota community in the host's digestive system by increasing the number of good microbiotas; thereby, combating harmful microorganisms. The characteristics of probiotics depend on the strain of microorganisms. The most used probiotics in the industry are *Lactobacillus* and *Bifidobacterium* spp. [2], although other strains from the *Saccharomyces* and *Bacillus* genera have been used as probiotics [3]. In addition to antimicrobial activity against pathogenic bacteria, probiotics offer health beneficial-effects of probiotics that are attributed to cholesterol-lowering effect, antioxidant activity, blood sugar regulation effect and anticancer activity [4,5]. Probiotics produce bioactive compounds e.g. short-chain fatty acids, γ-amino-butyric acid, exopolysaccharides, acetylcholine, serotonin and vitamins that contribute to therapeutic effects [4].

Based on a report published by the Market Research Future, global sales of probiotic products have increased with the increase in demands for probiotic products. The compound annual growth rate for probiotics was 8.60%, 2020–2030, and is estimated to reach $73.90 billion, 2030 [6]. Research linked to the large-scale industrial production of probiotics is critical to meet the increasing demands. Therefore, optimizing the growth media by addressing growth parameters is vital. Designing novel media for enhanced biomass production can lead to further economical probiotic productions [7]. Lactic acid bacteria (LAB) are commonly detected in fermented foods and other food sources. Historically, *Lactobacillus* genus has been considered safe for human consumption [8]. The LAB are fastidious in their nutrient and environmental requirements. The most common medium for growing *Lactobacillus* is de Man Rogosa Sharpe (MRS). However, this medium does not provide the optimum conditions for maximizing growth of *Lactobacillus*, which includes complex and fastidious nutrient requirements, and the cost of MRS broth is relatively high [9]. Therefore, it is necessary to develop optimized media and fermentation conditions for specific strains of *Lactobacillus* to improve production of its viable cells and biomass. Increasing biomass production can help streamline recovery, decrease production costs and shorten fermentation time [4].

Several statistical methods such as factorial design, central composite design (CCD), Placket-Burman design (PBD), artificial neural networks, genetic algorithms, Taguchi method and Box-Behnken design (BBD) have been used for media optimization. In this study, BBD was selected for its efficiency in decreasing number of experimental runs while ensuring the high accuracy. The BBD is advantageous for investigating interactions between multiple variables used in the present study, which involves optimizing media compositions and fermentation conditions. Using BBD, the experimental trials are fewer than in factorial design because the BBD focuses on midpoints within the experimental design space to maximize the efficiency [10,11].

Lactiplantibacillus (*L.*) *plantarum* belongs to LAB commonly detected in fermented foods such as meats, vegetables, feeds and dairy products. The *L. plantarum* DLBSK207 isolated from goat colostrum has been detected to include anti-hypercholesterolemic effects by decreasing the uptake of cholesterol in intestinal cells and suppressing regulator genes, low-density lipoprotein receptor responsible for cholesterol uptake and 3-hydroxy-3-methylglutaryl-coA reductase responsible for cholesterol synthesis in hepatic cells, based on an *in vitro* assay [12]. While the functional characteristics of *L. plantarum* DLBSK207 demonstrated promising probiotic candidates, optimizing the growth media and fermentation conditions is vital to maximize viable count and biomass production. The efficacy of probiotics is derived from ingested viable cells; therefore, viable cells in the product are critical. The probiotic industry should provide sufficient quantities of viable cells in the products and produce probiotics cells sufficiently. This could be achieved using optimum production media. Therefore, optimizing the media for producing probiotics for large-scale industrial uses is critical for enhancing process efficiency and minimizing production costs while meeting the increasing demands for probiotic products in the market. Thus, this study aimed to optimize the media and fermentation conditions to increase the yield of viable cells and biomass production of *L. plantarum* DLBSK207 using BBD. Optimizing carbon and nitrogen sources, that are vital for the growth of the bacteria, as well as the initial pH and temperature of cultivation, provide an optimum way to produce viable cells of *L. plantarum* DLBSK207 as a novel probiotic candidate.

2. Materials and Methods

2.1 Bacterial culture preparation

The present study used *L. plantarum* DLBSK207 (GenBank accession no. OM004020.1), previously isolated from goat colostrum [13]. The colonies of 48 h cultivation on MRS agar were harvested, suspended in MRS broth containing 50% glycerol and distributed in-2 ml cryogen vials (CryoKING, China), followed by freezing at -20 °C for 24 h and storing at -80 °C (New Brunswick Scientific, USA). Prior to use, the culture was refreshed in MRS broth (HiMedia Laboratories, India) and incubated at 35 °C for 22 h.

2.2 Assessment of media and fermentation conditions using one-factor-at-a-time approach

One-factor-at-a-time (OFAT) approach was used to investigate various physicochemical parameters such as culture media, temperature of incubation, initial pH value and carbon and nitrogen sources. This method can estimate effects of each variable. Various carbon sources (glucose, galactose, lactose, maltose, sucrose and fructose) (Merck, Germany) were selected and six carbon sources were added individually to 100 ml of basal media containing 10 g l^{-1} yeast extract at a concentration of 20 g l^{-1}. Similarly, six nitrogen sources (yeast extract, peptone yeast, peptone, tryptone, skim milk and monosodium glutamate) (Kerry Ingredient and Flavors, UK) and peptone were added to 100 ml of basal media containing 20 g l^{-1} glucose at a concentration of 10 g l^{-1}. The effects of initial pH values (4.5, 5.5, 6.5, 7.5, 8.5 and 9.5) and temperature (20, 25, 30, 35, 40 and 45 °C) were assessed separately to produce *L. plantarum* DLBSK207. The inoculum for the fermentation included 1% (v v^{-1}) of a 24-h culture in MRS broth. Samples were collected for viable cell counts and dry cell weight (DCW) at 22 h of fermentation, where maximum response values were achieved. The best response provided viable cell counts and DCW served as the range point; at which, the RSM model was designed. The variables positively affecting biomass production at a confidence level higher than 95% were used for further experiments. All experiments were carried out in triplicate.

2.3 Plackett-Burman design

The experimental design for PBD was based on the 1st order model, which assumed no interactions between the fermentation media constituents (Eq. 1).

$$Y = \beta_0 + \sum \beta_{ixi} \qquad \text{Eq.1}$$

Where, Y was the estimated target function, xi was the parameter of study and βi was the regression coefficient. Based on the results of OFAT, three highly affecting variables were selected for each component. For this study, PBD was prepared using five selected variables (glucose, yeast extract, peptone, initial pH and temperature). The high and the low-level variables were coded as +1 and -1, respectively. The study was carried out in 12 runs and the results were transferred to Minitab 20 software (Minitab, USA) for statistical analysis. As PBD was only used as a screening tool, it was used as the only design tool to carry out the RSM optimization process, effectively. Hence, screened variables were further selected for the BBD study.

2.4 Box-Behnken design

Based on the results of PBD, the media composition and fermentation conditions were optimized using BBD. The BBD consisted of five variables (glucose, yeast extract, peptone, initial pH and temperature). The BBD was selected for the experiments based on its benefits in addressing the concentration of each compound within limits and eliminating possibilities that were included outside those limits (axial points). Each factor in the experiment was studied at three various levels of low, center and high with 10-U increases or decreases (15–35 U) for the media and 5.0-U increases or decreases in fermentation conditions pH (5.5–6.5) and temperature (30–40 °C).

2.5 Optimization of production medium and fermentation conditions

The industrial-grade materials included glucose (Merck, Germany), yeast extract and peptone (Kerry Ingredient and Flavors, UK). Basal media consisting of sodium acetate, $MgSO_4 \cdot 7H_2O$, K_2HPO_4, $MnSO_4 \cdot H_2O$, Tween 80 and glycerol (Merck, Germany) were used for all formulations. As much as 1% v v^{-1} (2.5 ml) of inoculum grown in similar media was added to each 250 ml media of 46 formula using Duran-Schott bottle and MRS broth was used as a control to reach 10^{-6} CFU ml^{-1}. Cultures were incubated at 35 °C for 24 h. Analyses were carried out for viable cell counts and DCW as cell biomass (Mettler-Toledo, China). The DCW was assessed according to Manzoor et al. [1] by collecting 100 ml of the culture broth and separating the cells by centrifugation at 8000× g for 10 min at 4 °C (Hermle, Germany), washing with distilled water, re-centrifuging and drying at 80 °C for 16–24 h to achieve a constant weight using drying oven (Memmert, Germany). Enumeration of viable cell counts were carried out on MRS agar plates, which were incubated at 37 °C (Percival Scientific, USA) for 48 h and calculated based on the bacteriological analytical manual (BAM) (2001) [14]. Each analysis were carried out in triplicates.

2.6 Assessment of optimum media composition and fermentation conditions

A three-level, three-factorial design was selected to assess the significant effects of glucose, yeast extract and peptone on L. plantarum DLBSK207. This statistical technique was used to assess the effects of individual and mutual interactions between various components in the media. A BBD was generated using Design Expert 13 to optimize the media and fermentation conditions. Furthermore, ANOVA was used to statistically analysis 46 experiments for the verification of validity. The BBD depended on the 2nd order polynomial of Eq. 2 as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j \qquad \text{Eq. 2}$$

Where, Y was the predicted response (g l^{-1}), x_i and x_j were the coded independent variables for factors, β_0 was the intercept term and β_i, β_{ii} and β_{ij} were the linear, quadratic and cross-interaction regression constant coefficients, respectively. The predicted responses achieved from the BBD were compared with the actual responses to estimate the model accuracy by independent testing. The optimum media were compared with MRS broth as well.

2.7 Scaling-up batch fermentation using 10-l bioreactor

Fermentation of *L. plantarum* DLBSK207 was carried out using optimum media and 10-l bioreactor (New Brunswick, Germany) with a working volume of 8.5 l. Media were inoculated with 1% (v v^{-1}) of 24-h culture in a similar media. Temperature was set at 35 °C and pH of the media was controlled during fermentation. The pH was set using sterile solution of NH_4OH at 25% (Merck, Germany). Agitation was set at 200 rpm and the mixture was incubated for 24 h. The MRS broth was used as a control under similar conditions. At the end of the fermentation, viable cell counts and DCW were carried out as described previously. The growth kinetics of *L. plantarum* DLBSK207 during fermentation in the 10-l bioreactor was predicted using ComBase, a systematically formatted database of quantified microbial responses to various environments (https://browser.combase.cc/DMFit.aspx). Information in ComBase were referred to as quantitative microbiological data, describing how levels of microorganisms changed over time. This type of information could be referred to as kinetic data.

2.8 Statistical analysis

Data were present as the mean ±SD (standard deviation). One-way analysis of variance and Duncan's multiple-range test were used to report significant differences. Values were reported significant when $P < 0.05$. All analyses were carried out using Minitab 20 software (Minitab, USA). Furthermore, BBD and RSM were carried out using Design Expert 13 software (Stat-Ease, USA).

3. Results and Discussion

3.1 Media and fermentation condition screening based on one-factor-at-a-time

The OFAT technique was used before using the RSM approach to identify critical factors that affected media and fermentation conditions. Effects of carbon source, nitrogen source, initial pH, temperature and other culture parameters were assessed (Table 1). When introduced to fermentation as a carbon source, glucose increased viable cell counts and DCW [15, 8]. Previous studies have shown that the addition of carbon and nitrogen sources includes a significant effect on increasing the quantity of biomass produced during fermentation. High quantities of nitrogenous sources such as yeast extract and peptone in the fermentation media have increased viable cell counts and biomass production. In the OFAT system, combined effects of factors on the response were not assessed [4]. Thus, RSM approach was used as an alternate statistical tool, which helped assess combined effects of all independent variables in a fermentation process, which might be resulted from their interaction. In the present study, six variables (glucose, maltose, yeast extract, peptone, initial pH and temperature) were addressed for the RSM approach based on results achieved in OFAT optimization. Table 1 shows the viable cell counts and biomass based on all components.

It is evident that various components in culture fermentation included various effects on response parameters. When monosodium glutamate was added to the media, *L. plantarum* DLBSK207 showed a greatly low viable cell count of 8.28 log CFU ml^{-1} with a biomass of 0.79 g l^{-1}. Yeast extract resulted in maximum response of 8.93 log CFU ml^{-1} with DCW of 1.23 g l^{-1} followed by peptone. According to Lee et al. [16], the highest cell density of *L. acidophilus* A12 was achieved in yeast extract media. Yeast extract and peptone are rich sources of nitrogenous, vitamins and growth factors and are effective in supporting LAB growth and biomass production. Thus, growth media of yeast extract could significantly increase viable cell counts and DCW.

Table 1. Effects of various component sources on culture production of *L. plantarum* DLBSK207

Component variables			Response parameter	
			Viable cell counts (CFU ml^{-1})	DCW (g l^{-1})
Carbon source	1	Sucrose	8.31 ± 0.02^{c}	1.05 ± 0.01^{c}
	2	Glucose	8.58 ± 0.02^{a}	1.07 ± 0.01^{a}
	3	Maltose	8.34 ± 0.01^{b}	1.07 ± 0.01^{ab}
	4	Fructose	8.28 ± 0.02^{dee}	1.01 ± 0.01^{d}
	5	Lactose	8.30 ± 0.02^{cd}	1.06 ± 0.01^{bc}
	6	Galactose	8.30 ± 0.01^{cd}	0.93 ± 0.01^{e}
Nitrogen source	1	Yeast extract	8.93 ± 0.03^{aa}	1.24 ± 0.01^{aa}
	2	Peptone	8.90 ± 0.02^{a}	1.08 ± 0.01^{b}
	3	Yeast peptone	8.84 ± 0.01^{b}	0.96 ± 0.02^{c}
	4	Tryptone	8.58 ± 0.02^{c}	0.83 ± 0.02^{d}
	5	Skim milk	8.56 ± 0.04^{c}	0.81 ± 0.01^{d}
	6	Monosodium glutamate	8.28 ± 0.01^{d}	0.79 ± 0.01^{e}
Initial pH	1	4.5	8.74 ± 0.02^{f}	0.41 ± 0.01^{f}
	2	5.5	9.28 ± 0.01^{b}	1.20 ± 0.01^{b}
	3	6.5	9.33 ± 0.01^{a}	1.29 ± 0.01^{a}
	4	7.5	9.24 ± 0.01^{c}	0.91 ± 0.02^{c}
	5	8.5	9.16 ± 0.01^{d}	0.86 ± 0.01^{d}
	6	9.5	9.02 ± 0.01^{e}	0.76 ± 0.01^{e}
Temperature (°C)	1	20°	8.33 ± 0.02^{f}	0.49 ± 0.01^{f}
	2	25°	8.75 ± 0.01^{e}	0.89 ± 0.01^{e}
	3	30°	9.06 ± 0.02^{c}	1.26 ± 0.02^{c}
	4	35°	9.33 ± 0.01^{a}	1.56 ± 0.01^{a}
	5	40°	9.27 ± 0.01^{b}	1.37 ± 0.02^{b}
	6	45°	8.86 ± 0.03^{d}	0.98 ± 0.01^{d}

*The result is expressed as mean ± SD of at least three independent experiments. Different letters (a–f) in one column represent significant differences ($p<0.05$) for comparison of all response parameters by one-way ANOVA.

Fermentation conditions such as initial pH and temperature affected the response. The highest response was achieved at initial pH 6.5 with 9.33 log CFU ml^{-1} viable cell counts and 1.29 g l^{-1} DCW. At 35 °C, this showed viable cell count of 9.33 log CFU ml^{-1} and DCW of 1.56 g l^{-1}, which decreased sequentially above and below this point. Low initial pH (< 4.4) and high initial pH (> 7.5) were detected to inhibit and slow down the growth rate of *L. plantarum* DLBSK207. Choi et al. [4] reported that higher pH decreases, defined as the difference between initial and final pH, enhanced biomass production until a final pH was inappropriate for LAB survivability and growth. Naturally, *Lactobacillus* spp. prefer a mildly acidic environment. If the pH is too low; the environment becomes stressful and adversely affects the bacterial growth. This inhibition occurs because an environment that is too acidic can disrupt cellular processes, damage cell membranes and disrupt enzyme activity necessary for cell metabolism and replication. Biomass production can be affected by low and high temperatures. Temperature below and above the optimum range can decrease growth rate and damage cellular structures such as membrane cells of LAB, leading to decreased viable cell counts. The maximum biomass (1.56 g l^{-1}) was achieved at 35 °C. *Lactobacillus* spp. generally grow at moderate temperatures of 30–40 °C, which are optimum for cell metabolic activity and growth [1].

3.2 Plackett-Burman design

Based upon the OFAT optimization, six independent variables (glucose, maltose, yeast extract, peptone, initial pH and temperature) were addressed in the viable cell counts and biomass production from *L. plantarum* DLBSK207. A 12-run model was created using PBD, which showed the response in ranges of 9.03–9.20 log CFU ml^{-1} for viable cell counts and 1.98–2.21 g l^{-1} for DCW in various runs (Table 2). The standardized effects of the variables represent as a single column on the Pareto chart (Figure 1).

The Pareto chart, described as a valuable tool for identifying the most important effects, was used to assess the significant factors. In this chart, length of each bar on a standardized Pareto chart was proportional to the absolute value of its associated regression coefficient or estimated effect. The vertical line through the column indicated if the variables were statistically significant. Where the column crossed over the lines and extended to the right showed that the variables greatly affected the response. The charts show that glucose concentration was the most effective factor, followed by other factors such as yeast extract and initial pH for viable cell counts and temperature and initial pH for DCW. Table 3 shows effects of the variables and analyses variance. The effects of glucose (X_1), maltose (X_2), yeast extract (X_3), peptone (X_4), initial pH (X_5) and temperature (X_6) included 0.063, -0.020, 0.040, 0.030, 0.040 and 0.030, respectively. Maltose included negative responses to viable cell counts and DCW within the six variables, suggesting that maltose was not a good carbon source for the bacteria. To assess authenticity of the model, ANOVA was used and P-value of the positive variables (glucose, yeast extract, peptone, initial pH and temperature) were selected as the most important factors for RSM. According to Elsayed et al. [15], the optimized major components for *L. delbrueckii* sp. *bulgaricus* WICC-B-02 was achieved when the media contained yeast extract (6.0 g l^{-1}) and peptone (6.0 g l^{-1}) at a similar ratio with initial pH 6.5 and temperature of 37 °C. The model from PBD was fit well because the assessment coefficient (R^2) was 0.9667 for viable cell counts and 0.9555 for DCW.

Table 2. Plackett-Burman experimental design and response values on *Lactiplantibacillus plantarum* DBSK207 production

Run Order	Variables^{a}						Response parameter	
	X_1 (g l^{-1})	X_2 (g l^{-1})	X_3 (g l^{-1})	X_4 (g l^{-1})	X_5	X_6 (°C)	Viable cell counts (log CFU ml^{-1})	DCW (g l^{-1})
1	35	35	15	35	6.5	35	9.15 ± 0.01^{d}	2.13 ± 0.01^{d}
2	35	35	35	15	6.5	40	9.18 ± 0.01^{b}	2.18 ± 0.01^{c}
3	35	15	15	15	6.5	40	9.17 ± 0.01^{c}	2.18 ± 0.01^{c}
4	15	15	35	35	6.5	35	9.15 ± 0.01^{d}	2.10 ± 0.01^{efg}
5	15	35	35	15	6.5	35	9.09 ± 0.01^{g}	2.08 ± 0.01^{h}
6	35	35	15	35	5.5	35	9.10 ± 0.01^{f}	2.09 ± 0.01^{gh}
7	35	15	35	35	5.5	40	9.20 ± 0.01^{a}	2.21 ± 0.02^{b}
8	35	15	35	15	5.5	35	9.13 ± 0.01^{e}	2.11 ± 0.01^{ef}
9	15	35	15	15	5.5	40	9.05 ± 0.01^{h}	2.01 ± 0.01^{a}
10	15	15	15	15	5.5	35	9.03 ± 0.01^{i}	1.98 ± 0.01^{i}
11	15	15	15	35	6.5	40	9.12 ± 0.02^{e}	2.11 ± 0.01^{de}
12	15	35	35	35	5.5	40	9.11 ± 0.01^{f}	2.09 ± 0.01^{fgh}

*Actual values are presented in parentheses. ($^{a}X_1$ = glucose; X_2 = maltose X_3 = yeast extract; X_4 = initial pH and X_5 = temperature). *The result is expressed as mean ± SD of at least three independent experiments. Different letters (a–h) in one column represent significant differences (p <0.05) for comparison of all response parameters by one-way ANOVA

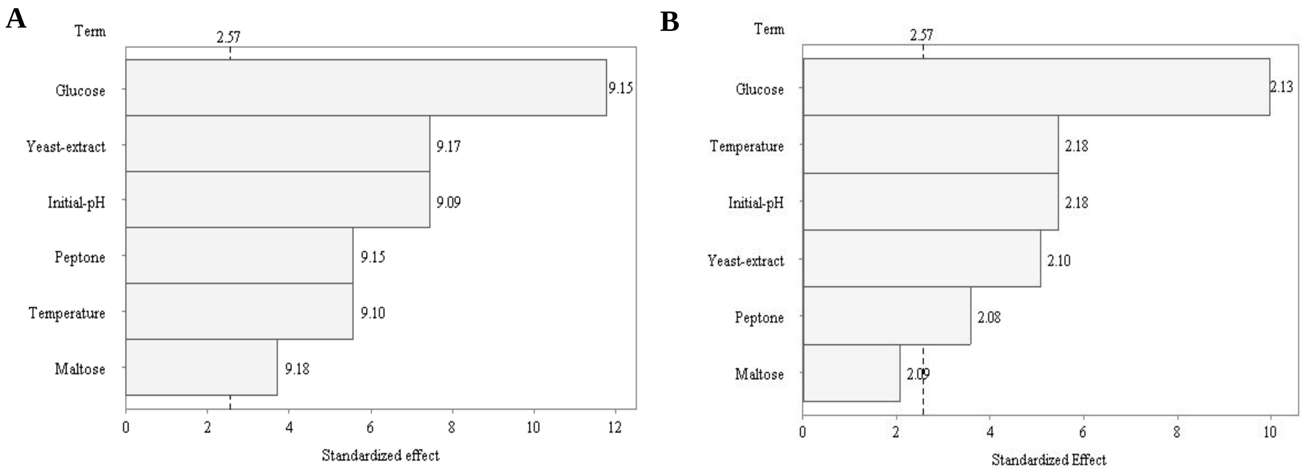

Figure 1. Pareto charts of the major effects of Plackett-Burman design on A, viable cell counts and B, dry cell weight (α = 0.05)

Table 3. Analysis of variables based on Plackett-Burman design

Variables	Viable cell counts response			
	Effect	Coefficient	*T*-value	*P*-value
Intercept		9.123	394.83	<0.001
Glucose (X_1)	0.063	0.031	11.78	<0.001
Maltose (X_2)	-0.020	-0.010	-3.72	0.014
Yeast extract (X_3)	0.040	0.020	7.44	0.001
Peptone (X_4)	0.030	0.015	5.58	0.003
Initial pH (X_5)	0.040	0.020	7.44	0.001
Temperature(X_6)	0.030	0.015	5.58	0.003
		DCW response		
Intercept		2.104	512.68	<0.001
Glucose (X_1)	0.088	0.044	10.78	<0.001
Maltose (X_2)	-0,018	-0.09	-2.21	0.078
Yeast extract (X_3)	0.045	0.002	5.58	0.003
Peptone (X_4)	0.032	0.016	3.96	0.011
Initial pH (X_5)	0.047	0.023	5.83	0.002
Temperature(X_6)	0.052	0.026	6.35	0.001

3.3 Optimization of composition media using Box-Behnken design

The number of viable cells and DCW produced in each formula of the production media varied (Table 4). The viable cell counts of LAB ranged 9.05–9.30 log CFU ml^{-1} and DCW ranged 2.47–4.27 g l^{-1}. The lowest viable cell counts were produced in formula 35 with a media composition of 25 g l^{-1} glucose, 15 g l^{-1} yeast extract and 25 g l^{-1} peptone. The lowest DCW was produced in formula 12 with a media composition of 25 g l^{-1} glucose, 25 g l^{-1} yeast extract and 25 g l^{-1} peptone. The highest viable cell counts and DCW were detected in formula 25 with a media composition of 35 g l^{-1} glucose, 35 g l^{-1} yeast extract and 25 g l^{-1} peptone.

Data in Table 4 show that the media with maximum glucose and yeast extract content produced high viable cell counts and DCW; however, peptone content in the media was needed up to a mid-range value of 25 g l^{-1}. Increasing concentrations of glucose and yeast extract to the highest concentrations increased viable cell counts and DCW during fermentation.

A 3D response surface plot was created to show how the two parameters (carbon and protein sources) interacted and assess the best concentration for maximum viable cell counts and biomass production (Figure 2). Glucose (X_1), yeast extract (X_2) and peptone (X_3) significantly affected the viable cell counts and DCW of *L. plantarum* DLBSK207 ($P < 0.05$). Glucose, yeast extract and peptone acted as barriers because the quadratic effect of these three variables was significant ($P < 0.05$), indicating that slight modification of their levels affected the DCW. The graph was created based on the model equation, showing infinite combinations of the two independent variables with the other variables at a constant level.

Naturally, LAB utilize glucose as a carbon source to produce energy by converting it into lactic acid [17] for synthesizing cell biomass [18]. In addition to glucose, yeast extract and peptone included a significant effect ($P < 0.001$) on the growth of *L. plantarum* DLBSK207. Organic nitrogen sources include proteins, free amino acids, peptides, fats and vitamins, which are important to support the growth of bacteria. Protein sources such as yeast extract contain B-complex vitamins. Yeast extract plays an important role in cell growth and production of secondary metabolites in cells owing to its specific growth factors. Addition of yeast extract to the media affects the production of biomass and secondary metabolites from *Lactobacillus* spp. [15, 8]. In addition, mineral salts such as manganese and magnesium are reported to increase production of DCW of *Lactobacillus* significantly but not viable cell counts [19]. The composition of 35 g l^{-1} yeast extract resulted in viable cell count of 9.30 log CFU ml^{-1} and a DCW of 4.27 g l^{-1} (Table 4).

Peptone is a good source of nitrogen and carbon. When combined with yeast extract, this helps excellent bacterial growth and optimizes lactic acid production by LAB [1]. In this study, addition of peptone and yeast extract increased viable cell counts and DCW. Formula 25 (35 g l^{-1} glucose, 35 g l^{-1} yeast extract and 25 g l^{-1} peptone) was the best medium to produce the highest rate of viable cells and DCW. The media formulation and optimum fermentation conditions suggested by Design Expert 13 software consisted of 33.76 g l^{-1} glucose, 32.59 g l^{-1} yeast extract and 28.38 g l^{-1} peptone with an initial pH of 6.0 and temperature of 35 °C. Choi et al. [4] achieved the optimum formula and conditions for *L. plantarum* 200655 biomass production using RSM with a composition of 31.29 g l^{-1} maltose, 30.27 g l^{-1} yeast extract and 39.43 g l^{-1} soytone with a pH of 6.5 and temperature of 30 °C.

Table 4. The actual levels for the experimental design with experiments of viable cell counts and dry cell weight

Run order	Nutrient variables and operating conditions levels					Response parameter	
	X$_1$(g l^{-1})	X$_2$ (g l^{-1})	X$_3$ (g l^{-1})	X$_4$	X$_5$ (°C)	Viable cell counts (log CFU ml^{-1})	DCW (g l^{-1})
1	-1 (15)	0 (25)	0 (25)	-1 (5.5)	1 (35)	9.19 ± 0.01^{jkl}	3.16 ± 0.01^{p}
2	0 (25)	0 (25)	0 (25)	1 (6.5)	1 (40)	9.15 ± 0.01^{pq}	2.55 ± 0.01^{aa}
3	0 (25)	0 (25)	0 (25)	0 (6.0)	1 (35)	9.29 ± 0.03^{ab}	3.80 ± 0.01^{f}
4	-1 (15)	0 (25)	1 (35)	0 (6.0)	1 (35)	9.25 ± 0.02^{d}	3.39 ± 0.01^{m}
5	0 (25)	1 (35)	0 (25)	-1 (5.5)	1 (35)	9.20 ± 0.02^{ij}	3.88 ± 0.02^{d}
6	-1 (15)	0 (25)	0 (25)	0 (6.0)	-1 (30)	9.06 ± 0.01^{uv}	2.76 ± 0.01^{x}
7	1 (35)	0 (25)	0 (25)	-1 (5.5)	1 (35)	9.18 ± 0.03^{klm}	3.56 ± 0.01^{j}
8	-1 (15)	0 (25)	0 (25)	1 (6.5)	1 (35)	9.13 ± 0.03^{qr}	2.82 ± 0.01^{v}
9	-1 (15)	0 (25)	0 (25)	0 (6.0)	1 (40)	9.20 ± 0.01^{ijk}	2.86 ± 0.01^{u}
10	1 (35)	0 (25)	0 (25)	0 (6.0)	-1 (30)	9.25 ± 0.01^{d}	3.21 ± 0.01^{o}
11	0 (25)	0 (25)	0 (25)	0 (6.0)	1 (35)	9.29 ± 0.01^{a}	3.80 ± 0.02^{f}
12	0 (25)	0 (25)	0 (25)	-1 (5.5)	-1 (30)	9.12 ± 0.01^{qr}	2.48 ± 0.02^{ac}
13	1 (35)	0 (25)	0 (25)	0 (6.0)	1 (40)	9.15 ± 0.02^{op}	3.45 ± 0.02^{l}
14	-1 (15)	-1 (15)	0 (25)	0 (6.0)	1 (35)	9.09 ± 0.03^{st}	2.99 ± 0.02^{s}
15	0 (25)	1 (35)	0 (25)	0 (6.0)	-1 (30)	9.16 ± 0.01^{mno}	3.16 ± 0.02^{p}
16	0 (25)	0 (25)	0 (25)	1 (6.5)	-1 (30)	9.12 ± 0.03^{r}	3.29 ± 0.01^{n}
17	0 (25)	1 (35)	-1 (15)	0 (6.0)	1 (35)	9.23 ± 0.02^{efg}	3.45 ± 0.01^{l}
18	0 (25)	0 (25)	0 (25)	1 (6.5)	-1 (30)	9.19 ± 0.01^{ijk}	3.72 ± 0.01^{g}
19	0 (25)	0 (25)	1 (35)	0 (6.0)	1 (40)	9.19 ± 0.01^{jk}	3.48 ± 0.01^{k}
20	-1 (15)	0 (25)	-1 (15)	0 (6.0)	1 (35)	9.15 ± 0.01^{nop}	2.72 ± 0.01^{y}
21	0 (25)	0 (25)	-1 (15)	0 (6.0)	-1 (30)	9.10 ± 0.03^{s}	2.60 ± 0.01^{z}
22	0 (25)	-1 (15)	0 (25)	1 (6.5)	1 (35)	9.10 ± 0.01^{s}	3.03 ± 0.01^{r}
23	-1 (15)	1 (35)	0 (25)	0 (6.0)	1 (35)	9.21 ± 0.02^{ghi}	3.22 ± 0.01^{o}
24	0 (25)	0 (25)	-1 (15)	-1 (5.5)	1 (35)	9.16 ± 0.02^{mno}	2.97 ± 0.01^{s}
25	1 (35)	1 (35)	0 (25)	0 (6.0)	1 (35)	9.30 ± 0.02^{abc}	4.27 ± 0.01^{a}
26	0 (25)	0 (25)	1 (35)	0 (6.0)	-1 (30)	9.20 ± 0.02^{hij}	3.44 ± 0.01^{l}
27	0 (25)	0 (25)	0 (25)	-1 (5.5)	1 (40)	9.12 ± 0.01^{r}	3.62 ± 0.01^{h}
28	0 (25)	1 (35)	1 (35)	0 (6.0)	1 (35)	9.26 ± 0.01^{cd}	4.01 ± 0.01^{c}
29	0 (25)	0 (25)	0 (25)	0 (6.0)	1 (35)	9.29 ± 0.01^{ab}	3.80 ± 0.01^{f}
30	0 (25)	0 (25)	1 (35)	0 (6.0)	1 (35)	9.21 ± 0.01^{ghi}	3.74 ± 0.01^{g}
31	1 (35)	0 (25)	1 (35)	0 (6.0)	1 (35)	9.27 ± 0.01^{bc}	4.12 ± 0.01^{b}
32	0 (25)	0 (25)	1 (35)	1 (6.5)	1 (35)	9.23 ± 0.01^{ef}	3.58 ± 0.01^{i}
33	0 (25)	0 (25)	0 (25)	0 (6.0)	-1 (30)	9.28 ± 0.01^{ab}	3.80 ± 0.01^{f}
34	1 (35)	-1 (15)	0 (25)	0 (6.0)	1 (35)	9.26 ± 0.01^{nop}	3.12 ± 0.02^{q}
35	0 (25)	-1 (15)	0 (25)	0 (6.0)	-1 (30)	9.05 ± 0.01^{v}	2.89 ± 0.01^{t}

Table 4. Cont.

Run order	Nutrient variables and operating conditions levels					Response parameter	
	X_1(g l^{-1})	X_2 (g l^{-1})	X_3 (g l^{-1})	X_4	X_5 (°C)	Viable cell counts (log CFU ml^{-1})	DCW (g l^{-1})
36	0 (25)	-1 (15)	0 (25)	0 (6.0)	1 (40)	9.07 ± 0.02^{tu}	2.59 ± 0.01^{z}
37	1 (35)	0 (25)	-1 (15)	0 (6.0)	1 (35)	9.25 ± 0.01^{de}	3.15 ± 0.02^{p}
38	0 (25)	0 (25)	-1 (15)	1 (6.5)	1 (35)	9.17 ± 0.02^{lmn}	2.82 ± 0.01^{v}
39	0 (25)	0 (25)	-1 (15)	0 (6.0)	1 (40)	9.16 ± 0.01^{nop}	2.80 ± 0.02^{w}
40	0 (25)	-1 (15)	1 (35)	0 (6.0)	1 (35)	9.16 ± 0.02^{nop}	3.54 ± 0.01^{j}
41	1 (35)	0 (25)	0 (25)	1 (6.5)	1 (35)	9.28 ± 0.01^{ab}	3.55 ± 0.01^{j}
42	0 (25)	0 (25)	0 (25)	0 (6.0)	1 (35)	9.28 ± 0.01^{ab}	3.80 ± 0.01^{f}
43	0 (25)	-1 (15)	0 (25)	-1 (5.5)	1 (35)	9.08 ± 0.01^{st}	2.97 ± 0.01^{s}
44	0 (25)	1 (35)	0 (25)	1 (6.5)	1 (35)	9.22 ± 0.01^{fgh}	3.48 ± 0.02^{k}
45	0 (25)	0 (25)	0 (25)	0 (6.0)	1 (35)	9.28 ± 0.01^{ab}	3.83 ± 0.01^{e}
46	0 (25)	-1 (15)	-1 (15)	0 (6.0)	1 (35)	9.08 ± 0.01^{tu}	2.53 ± 0.01^{ab}

[a] X_1, glucose; X_2, yeast extract; X_3, peptone; X_4, initial pH; X_5, temperature. Other composition included 5.0 g l^{-1} sodium acetate, 0.1 g l^{-1} $MgSO_4 \cdot 7H_2O$, 2 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MnSO_4 \cdot H_2O$, 1.0 g l^{-1} Tween 80. The result is expressed as mean ± SD of at least three independent experiments. Different letters (a–z) in one column represent significant differences ($p<0.05$) for comparison of all response parameters by one-way ANOVA.

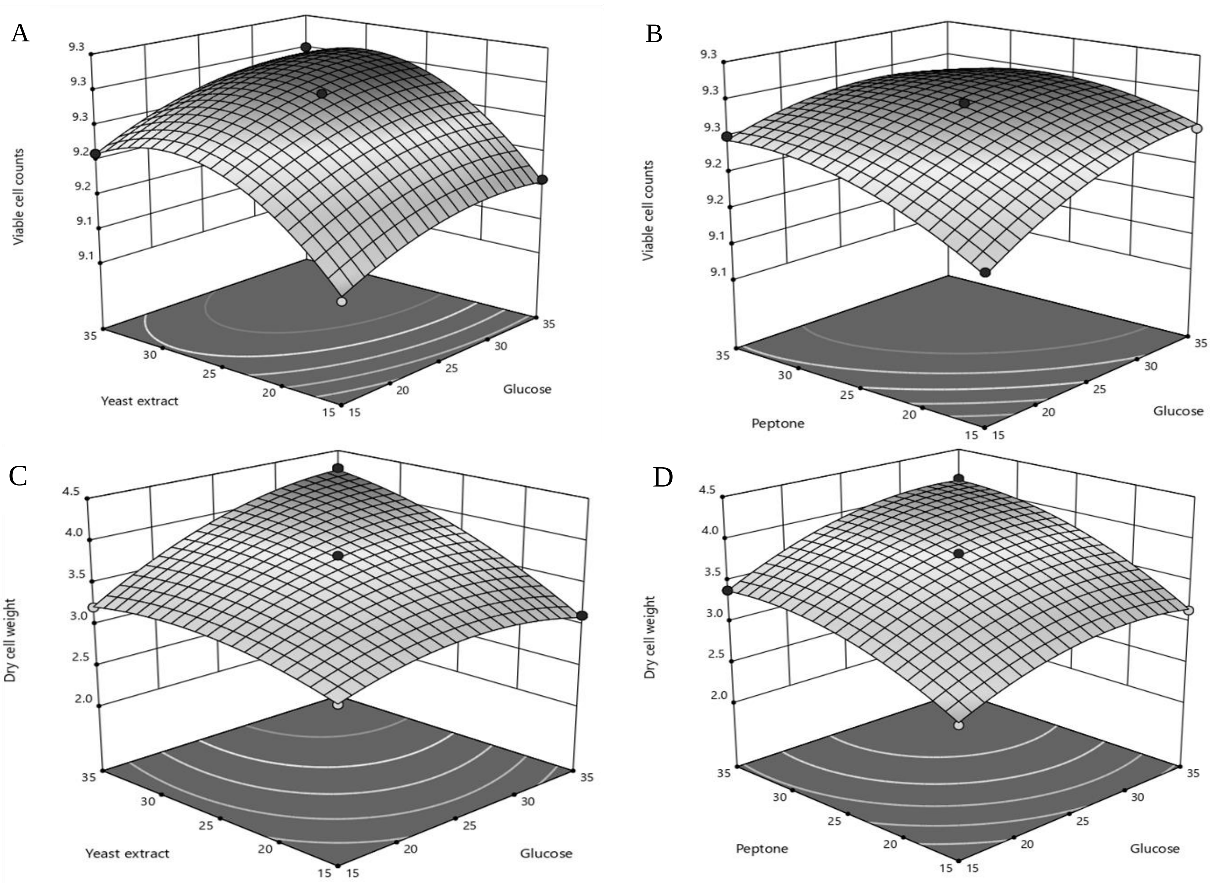

Figure 2. The 3D response surface plots of *Lactiplantibacillus plantarum* DLBSK207. A and C, Interaction between glucose (X_1) and yeast extract (X_2) and B and D, interaction between glucose (X_1) and peptone (X_3) at constant levels for viable cell counts (A, B) and dry cell weight (C, D)

3.4 Optimization of fermentation conditions using Box-Behnken design

Data from Table 4 shows that viable cell counts and the highest DCW (9.30 log CFU ml^{-1} and 4.27 g l^{-1}), respectively were achieved with an initial pH of 6.0 and a temperature of 35 °C. Physiochemistry such as pH and temperature with media formula included an important role in the growth of *L. plantarum* DLBSK207. The lower the initial pH of the media, the lesser the viable cell counts and DCW and the higher the fermentation temperature, resulting in lesser parameter values. Low pH (< 4.4) significantly inhibited the growth of *L. plantarum* DLBSK207. Naturally, the optimum pH for *Lactobacillus* spp. is in the range of 5.0–6.0. Low pH conditions can drastically slow the growth rate of LAB [19]. A buffer solution is added to the media to maintain an appropriate pH for the LAB growth. Sodium acetate, trisodium citrate and disodium glycerophosphate are common buffer substances in LAB growth media. Other compounds that act as buffers are disodium phosphate (Na_2HPO_4), ammonium citrate ($NH_4C_6H_5O_7$) and dipotassium phosphate (K_2HPO_4), which are widely used in MRS broth [19]. Sodium acetate and dipotassium phosphate were used in this study. Sodium acetate and ammonium citrate inhibit streptococci, molds and other microorganisms [19].

Minerals are vital for the growth of microorganisms, particularly LAB. These include essential roles in enzyme activity and are necessary in trace quantities for optimum cell growth and survival. Moreover, Mn^{2+} and Mg^{2+} are two essential elements for LAB growth and metabolic activities. The Mn^{2+} is involved in several key enzymes such as glutamine synthetase, RNA polymerase, lactate dehydrogenase and alkaline phosphatase. The Mg^{2+} promotes LAB growth and improves their survival. Therefore, addition of Mn^{2+} and Mg^{2+} to the minimal media of *L. plantarum* is critical to ensure its growth [19]. In the present study, the minerals were similar in all formula. A 3D response surface plot was created to show how pH and temperature interacted and to assess the best concentration for maximum viable cell counts and biomass production (Figure 3). The 3D model results in Figure 3 show that fermentation conditions significantly affected viable cell counts and DCW. Minor differences were seen in biomass production within a pH range of 5.5–6.5 and a temperature range of 30–40 °C. The optimum viable cell counts and DCW were observed at pH 6.0 and 35 °C that were the prime conditions for maximum biomass production by *L. plantarum* AS-14 according to Manzoor et al. [1]. Studies [1, 4, 8] have reported that the maximum biomass production of LAB is achieved at pH ranging 5.2–6.3 and incubation temperatures ranging 22–40 °C. These conditions are critical for the optimum growth of LAB [1].

A regression model estimated the maximum concentration of viable cell counts at 9.30 log CFU ml^{-1} and DCW concentration at 4.28 g l^{-1} with 95% confidence interval ranging 9.29–9.31 log CFU ml^{-1} and 4.24–4.32 g l^{-1}. Results showed a slight difference in biomass production in the pH range of 5.5–6.5 at 30–40°C. The optimum viable cell counts with the highest DCW were achieved when LAB were grown at a fermentation temperature of 35 °C and an initial pH of 6.0. Results were similar to those carried out by Manzoor et al. [1], where similar pH and temperature conditions produced the maximum biomass of *L. plantarum* AS-14. The ANOVA results for the BBD in Table 5 show no significant differences from the regression model lack-of-fit. However, Fisher *F*-test shows high significance levels ($P < 0.05$) for the regression. The model efficiency was assessed by investigating the viable cell counts, which included R^2 of 0.9970 and adjusted R^2 of 0.9947 and DCW, which included R^2 of 0.9983 and adjusted R^2 of 0.9969. Results showed that the model could explain the overall variation in viable cell counts by 99.70% and DCW by 99.83%. The model was reported as highly significant because of its good correlation between the data and the model, indicating good fits. A high R^2 value indicates that the test values approach the model predicted value, allowing the model to predict the responses better.

3.5 Response factors by Box-Behnken design

Results of BBD showed that the maximum viable cell count of 9.30 log CFU ml^{-1} and DCW response of 4.27 g l^{-1} were achieved in a run order of 25. Furthermore, multiple regression analysis of the results (Table 5) revealed that the investigated model was highly significant based on the P-values achieved for the assessed components. These results verified that the BBD was a powerful tool that could be used to generate accurate and reliable results for high-cell mass production.

Based on the experimental results fitted and explained by the second-order polynomial equation, the statistical significance of the model and the contribution of individual factors was derived using ANOVA (Eqs. 3 and 4):

$$\text{Viable cell counts} = 9.280 + 0.035X_1 + 0.061X_2 + 0.029X_3 + 0.008X_4 + 0.010X_5 + 0.005X_1X_2 - 0.020X_1X_3 + 0.040X_1X_4 - 0.060X_1X_5 - 0.012X_2X_3 + 0.001X_2X_4 + 0.002X_2X_5 + 0.002X_3X_4 - 0.017X_3X_5 + 0.007X_4X_5 - 0.0252X_1^2 - 0.077X_2^2 - 0.029X_3^2 - 0.063X_4^2 - 0.094X_5^2 \qquad \text{Eq. 3}$$

$$DCW = 3.802 + 0.280X_1 + 0.345X_2 + 0.391X_3 - 0.078X_4 + 0.0786X_5 + 0.231X_1X_2 + 0.074X_1X_3 + 0.081X_1X_4 + 0.033X_1X_5 - 0.113X_2X_3 - 0.112X_2X_4 + 0.215X_2X_5 - 0.002X_3X_4 - 0.040X_3X_5 - 0.471X_4X_5 - 0.227X_1^2 - 0.184X_2^2 - 0.225\ X_3^2 - 0{,}296X_4^2 - 0.513X_5^2 \qquad \text{Eq. 4}$$

Results of the ANOVA analysis of variance for BBD (Table 5) showed no significant differences in the lack-of-fit of the regression model. However, Fisher's F-test indicated a high level of significance ($P < 0.05$) for the regression. Efficiency of the model was assessed by investigating viable cell counts, which included $R^2 = 0.9970$ and adjusted $R^2 = 0.9947$ and DCW, which included $R^2 = 0.9983$ and adjusted $R^2 = 0.9969$. Results showed that the model could explain 99.70 and 99.83% variations in viable cell counts and DCW, respectively. The model was reported as highly significant since it included a good correlation between the data and the model, indicating good fits. The high R^2 value indicated that data were close to those predicted by the model and the model could better predict the responses.

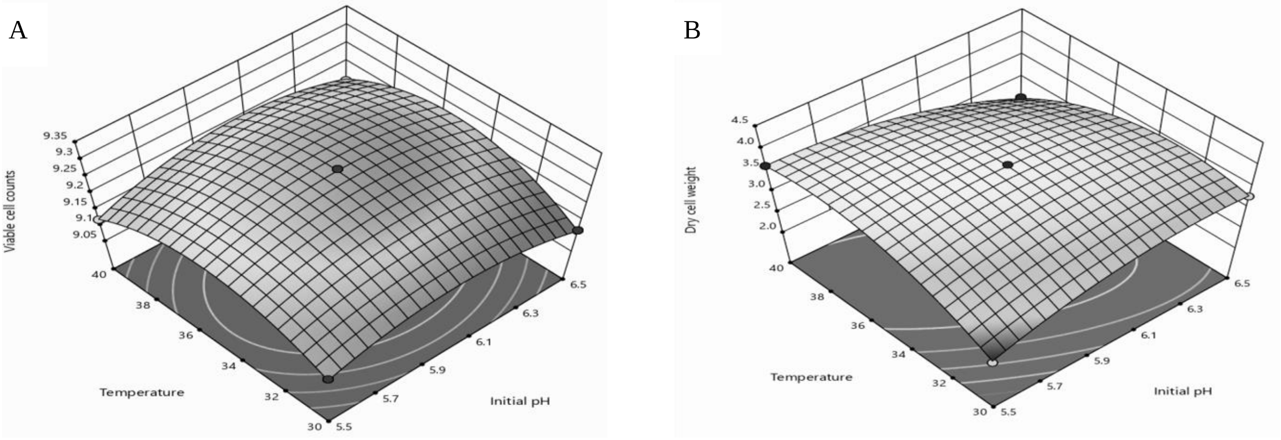

Figure 3. The 3D response surface plots of *Lactiplantibacillus plantarum* DLBSK207. A and B, Interaction between initial pH (X_4) and temperature (X_5) at constant level for viable cell counts (A) and dry cell weight (B)

Table 5. The ANOVA for the response surface of the full quadratic model of *Lactiplantibacillus plantarum* DLBSK207

Source	Viable cell counts					DCW			
	DF*	SS*	MS*	*F*-value	*P*-value	SS*	MS*	*F*-value	*P*-value
Model	20	0.2298	0.0115	420.39	<0.0001	9.85	0.4925	733.45	<0.0001
X_1	1	0.0196	0.0196	717.07	<0.0001	1.26	1.26	1878.86	<0.0001
X_2	1	0.0600	0.0600	2196.04	<0.0001	1.91	1.91	2850.36	<0.0001
X_3	1	0.0138	0.0138	505.11	<0.0001	2.45	2.45	3649.62	<0.0001
X_4	1	0.0012	0.0012	44.82	<0.0001	0.0980	0.0980	145.89	<0.0001
X_5	1	0.0018	0.0018	66.08	<0.0001	0.0991	0.0991	147.53	<0.0001
X_1X_2	1	0.0001	0.0001	3.66	0.0673	0.2139	0.2139	318.54	1.0000
X_1X_3	1	0.0016	0.0016	58.54	<0.0001	0.0221	0.0221	32.84	<0.0001
X_1X_4	1	0.0064	0.0064	234.15	<0.0001	0.0264	0.0264	39.32	<0.0001
X_1X_5	1	0.0144	0.0144	526.83	<0.0001	0.0045	0.0045	6.68	0.0159
X_2X_3	1	0.0006	0.0006	22.87	<0.0001	0.0518	0.0518	77.07	<0.0001
X_3X_5	1	0.0012	0.0012	44.82	<0.0001	0.0064	0.0064	9.53	0.0049
X_4X_5	1	0.0002	0.0002	8.23	0.0083	0.8883	0.8883	1322.83	<0.0001
X_1^2	1	0.0055	0.0055	202.90	<0.0001	0.4523	0.4523	673.50	<0.0001
X_2^2	1	0.0451	0.0451	1649.46	<0.0001	0.2962	0.2962	441.10	<0.0001
X_3^2	1	0.0075	00075	275.51	<0.0001	0.4434	0.4434	660.25	<0.0001
X_4^2	1	0.0352	0.0352	1289.15	<0.0001	0.7676	0.7676	1143.02	<0.0001
X_5^2	1	0.0777	0.0777	2843.81	<0.0001	2.30	2.30	3424.39	<0.0001
Residual	25	0.0007	0.0000		<0.0001	0.0168	0.0007		
Lack of fit	20	0.0005	0.0000	0.8889	<0.0001	0.0156	0.0008	3.22	0.0992
Pure error	5	0.0002	0.0000		0.6209	0.0012	0.0002		
Total	45	0.2305				9.78			

*DF : Degree of Freedom; SS: Sum of Square; MS: Mean Square. *The result were obtained for two response parameter $R^2 = 0.9970$; adjusted $R^2 = 0.9947$ – viable cell counts and $R^2 = 0.9983$; adjusted $R^2 = 0.9969$ – DCW

3.6 Verification of optimum media and comparison of biomass production

The optimum media and fermentation conditions (glucose, yeast extract and peptone at 33.76, 32.59 and 23.38 g l^{-1}, respectively, pH 6.0, 35 °C) were validated through five independent tests and then compared to MRS broth. Verification of these parameters resulted in maximum viable cell count of 9.30 log CFU ml^{-1} and DCW of 4.32 g l^{-1}, compared to the model predicted viable cell count of 9.30 log CFU ml^{-1} and DCW of 4.28 g l^{-1} (Figure. 4). The achieved model showed good agreements between the actual test results and the predicted values. The predicted values showed viable cell count of 9.30 log CFU ml^{-1} and DCW of 4.28 g l^{-1}, while MRS broth resulted in viable cell count of 8.97 log CFU ml^{-1} and DCW of 2.36 g l^{-1}. The optimum media resulted in 1.82-fold increases in DCW, compared to MRS broth (Figure 4).

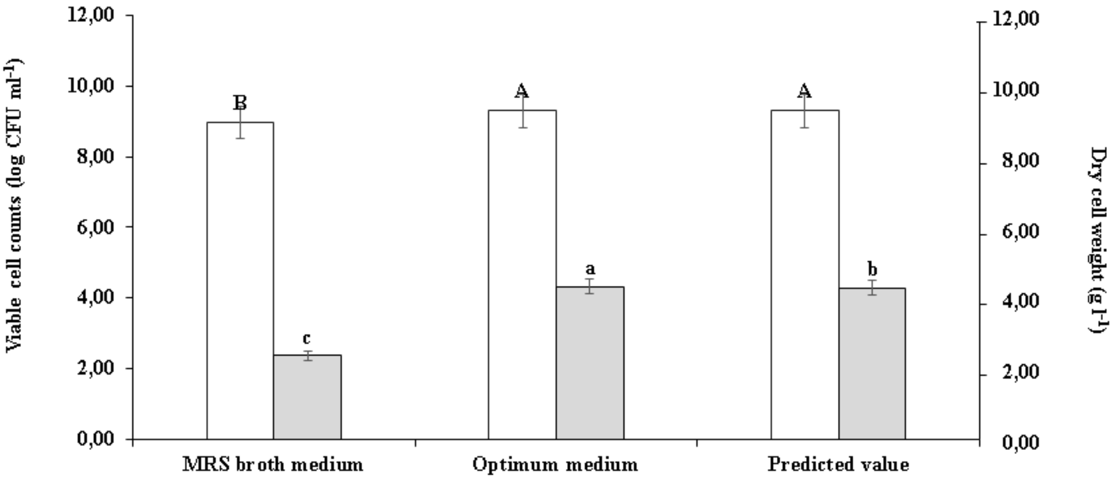

Figure 4. Comparison between the viable cell counts (□) and dry cell weight (▨) of *Lactiplantibacillus plantarum* DLBSK207 grown in MRS broth media, the optimum media and predicted value. Each value represents the mean ±SD (standard deviation) of experiments carried out in quintuplicate. Different letters on bars show significant differences ($P < 0.05$), compared to the MRS broth.

3.7 Large-scale fermentation with optimum media

Fermentation was carried out under optimum culture conditions with controlled pH and temperature. Figure 5 compares the viable cell counts and DCW response on a large scale (10 l) using the optimum media and MRS broth under optimum conditions (temperature of 35 °C, agitation of 200 rpm and controlled pH 6.0 for 24 h). Viable cell counts and DCW increased over time during incubation until the end of the logarithmic phase. The maximum viable cell counts and DCW were achieved from the two media after 20 h of incubation. The MRS broth produced viable cell count of 9.15 log CFU ml^{-1} and DCW of 2.50 g l^{-1}, while the optimum media produced viable cell count of 9.88 log CFU ml^{-1} and DCW of 5.82 g l^{-1}. The optimum media resulted in significant 2.32-fold increases in biomass. This suggests that the optimum media could be used on a larger scale for the production of viable cell of *L. plantarum* DLBSK207.

The cell growth under the controlled pH within 24 h is present in Figure 5. In the optimized media, cells immediately grew without adaptation with short logarithmic phase (0–6 h). A slight decrease was observed after 20 h of incubation. In contrast, cells in MRS broth adapted within the first 2 h and then significant growth occurred within 4–8 h.

The manually calculated specific growth rate (μ) and doubling time differed significantly from the predictions by ComBase (Table 6). In MRS broth, the observed growth rate was 1.33 h^{-1}, compared to the predicted 0.58 h^{-1} with a doubling time of 0.52 h, compared to the predicted 1.19 h. In the optimized media, the actual growth rate was 1.58 h^{-1}, whereas the predicted rate was 0.80 h^{-1} and the doubling time was 0.44 h, compared to the predicted 0.87 h. These discrepancies increased because ComBase incorporated data from the stationary phase, whereas manual calculations were based solely on logarithmic phase data. Regardless, growth in the optimized media was nearly twice that in MRS broth. Shorter doubling time in the optimized media suggested enhanced cell proliferation that were likely due to improved nutrient availability, stable pH and optimal environmental conditions. Results indicated that the optimized media significantly enhanced the cell growth, compared to that the MRS broth did. The optimized media created a further favorable environment for the cell proliferation, attributing to its further prosperous nutrient composition, greater energy source availability and controlled environmental factors such as pH and incubation temperature. The DCW production rates were significantly higher than those of MRS broth (Table 6). During fermentation, pH of the media was constant at 6.0 with the addition of 25% NH_4OH to prevent decreases in pH of the media; therefore, the exponential phase was longer. Controlling pH during fermentation not only affected the number of viable cells but also affected the biomass of *L. plantarum* DLBSK207. A significant difference in biomass production was observed with an increase of 1.34-fold, compared to uncontrolled pH. When comparing growth of the *Lactobacillus* strain in MRS broth and optimized media (Figure 5), it was evident that the logaritnic viable cell count and DCW in the optimized media were higher. The optimized media improved the microbial growth, significantly increased viable cell counts and DCW. The final viable cell count reached 7.57 × 10^9 CFU ml^{-1} and 2.32-fold increases of DCW were seen, compared to MRS broth. Although, pH was controlled.

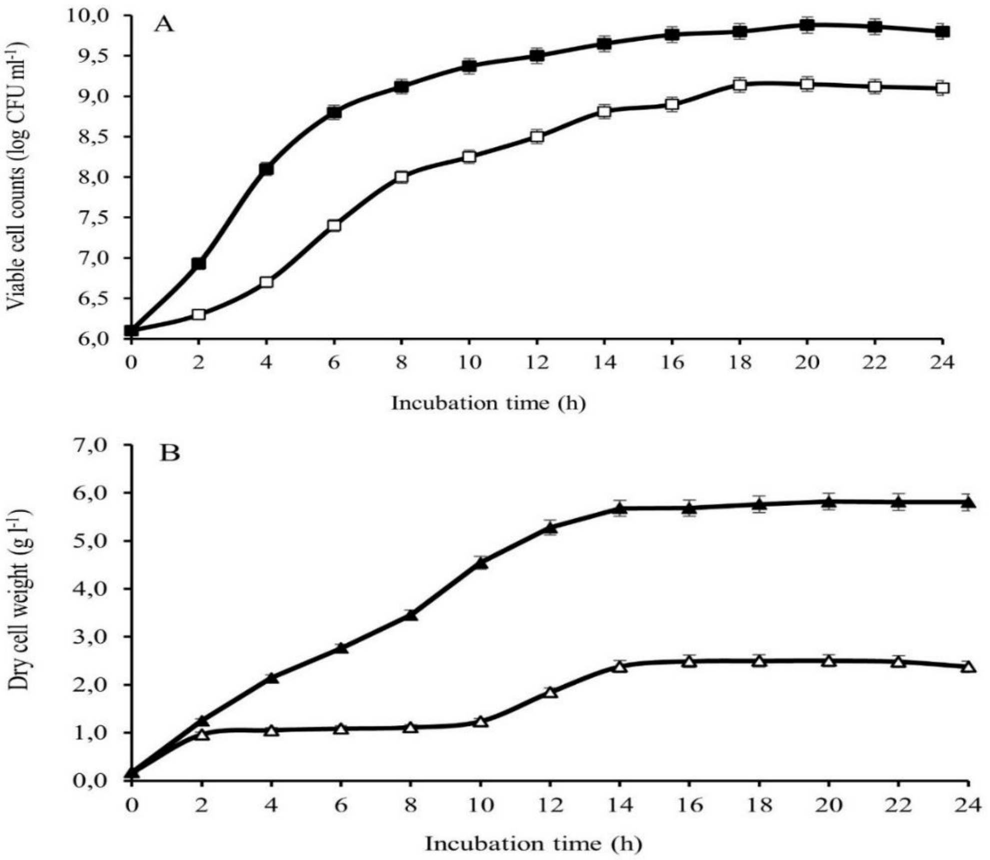

Figure 5. Viable cell count (A) and dry cell weight (B) of *Lactiplantibacillus plantarum* DLBSK207 using 10-l bioreactor. (A) Viable cell count (─□─ MRS broth, ─■─ optimum media) and (B) dry cell weight (─△─ MRS broth, ─▲─ optimum media)

Table 6. Assessment of growth parameters and kinetics of *Lactiplantibacillus plantarum* DLBSK207 using 10-l bioreactor

Parameters	MRS broth medium	Optimal medium
Glucose (g l^{-1})	20.00	33.76
Yeast extract (g l^{-1})	5.00	32.59
Peptone (g l^{-1})	20.00	28.38
Kinetic growth parameters of *L. plantarum* DLBSK207		
Actual values from experimental results		
Initial viable cell counts (log CFU ml^{-1})	6.10	6.10
Final viable cell counts (log CFU ml^{-1})	9.10	9.80
Initial DCW (g l^{-1})	0.72	0.72
Final DCW (g l^{-1})	2.38	5.80
Specific growth rate (μ) (h^{-1}) calculated during the logarithmic phase	1.33	1.58
Doubling time (0.693/μ) (h^{-1})	0.52	0.44
Predicted values based on ComBase		
Initial viable cell counts (log CFU ml^{-1})	5.99	6.18
Final viable cell counts (log CFU ml^{-1})	9.09	9.71
Initial DCW (g l^{-1})	0.56	0.54
Final DCW (g l^{-1})	2.52	5.82
Maximum rate (h^{-1}) calculated over 24 h of fermentation	0.58	0.80
Doubling time (0.693/μ) (h^{-1})	1.19	0.87
T$_{max}$ (h^{-1})	14.37	13.3
X$_{max}$ (g l^{-1})	2.50	5.82
Maximum rate (h^{-1})	0.31	0.43
Biomass production rate (g l^{-1} h^{-1})	0.07	0.21

*X$_{max}$: maximum viable cell counts or DCW; T$_{max}$: maximum time; μ: specific growth rate

Other researchers have optimized media and fermentation conditions to increase DCW. Selvamani et al. [20] achieved the maximum cell mass of *L. reuteri* DSM 20016^T after 48 h of fermentation in optimized media by RSM. The biomass of *L. reuteri* DSM 20016 increased by 3-fold (3.96 g l^{-1}) in optimized media, compared to unoptimized media (1.76 g l^{-1}). Fonteles et al. [21] reported that *L. casei* B-442 showed the highest number of viable cells (8.93 log CFU ml^{-1}) in fermented cantaloupe juice under optimized conditions (fermentation temperature at 31 °C and initial pH 6.1 for 24 h). The present study showed that biomass production by *L. plantarum* DLBSK207 could be optimized in media comprising of 33.76 g l^{-1} glucose, 32.59 g l^{-1} yeast extract and 28.38 g l^{-1} peptone with the addition of trace minerals, 5 g l^{-1} sodium acetate, 0.1 g l^{-1} $MgSO_4 \cdot 7H_2O$, 2 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MnSO_4 \cdot H_2O$ and 1 g l^{-1} Tween 80 under the culture conditions of 35 °C, pH 6.0 and agitation speed of 200 rpm. In conclusion, this study verified statistically optimized media composition and culture conditions that could be used to enhance biomass production of *L. plantarum* DLBSK207. In addition to the mineral mixture, composition of the optimized media was relatively simple, containing glucose, yeast extract and peptone. This composition can easily be used for commercial probiotic production under controlled pH and optimum temperature.

4. Conclusion

This study successfully achieved its objective of optimizing culture media and fermentation conditions for *L. plantarum* DLBSK207, resulting in significant improvements in viable cell counts and biomass production, compared to the standard media of MRS broth. Maintaining the fermentation conditions using 10-l bioreactor demonstrated greater yields. This indicated potential uses of the present finding for large-scale probiotic productions, particularly replying to the increasing demands of high-quality probiotics. Key challenges resolved in the present study included identifying the optimal nutrient composition and fermentation conditions that maximized biomass production while minimizing resources; thereby, enhancing efficiency and scalability of the production process. However, this study did not address specifically the challenge of cost-effectiveness, which is critical for the industrial use of the optimized media.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

7. Authors Contributions

BJS: Methodology designing, data curating, data analyzing and writing original draft of the manuscript; LNU: conceptualizing, methodology designing, supervising and reviewing and editing the manuscript; ABS: methodology designing, supervising and reviewing and editing the manuscript; RRT: supervising and reviewing the manuscript. All authors read and approved the final version of the manuscript

References

1. Manzoor A, Qazi JI, Haq IU, Mukhtar H, Rasool A. Significantly enhanced biomass production of a novel bio-therapeutic strain *Lactobacillus plantarum* (AS-14) by developing low-cost media cultivation strategy. J Biol Eng. 2017;11(1):1-10. https://doi.org/10.1186/s13036-017-0059-2
2. Hatti-Kaul R, Chen L, Dishisha T, Enshasy H El. Lactic acid bacteria: From starter cultures to producers of chemicals. FEMS Microbiol Lett. 2018;365(20):1-20. https://doi.org/10.1093/femsle/fny213
3. Li X, Wang Q, Hu X, Liu W. Current status of probiotics as supplements in the prevention and treatment of infectious diseases. Front Cell Infect Microbiol. 2022;12(3):1-12. doi:10.3389/fcimb.2022.789063.https://doi.org/10.3389/fcimb.2022.789063
4. Choi GH, Lee NK, Paik HD. Optimization of medium composition for biomass production of *Lactobacillus plantarum* 200655 using response surface methodology. J Microbiol Biotechnol. 2021;31(5):717-725. https://doi.org/10.4014/jmb.2103.03018
5. Nuraida L. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. Food Sci Hum Wellness.2015;4(2):47-55. https://doi.org/10.1016/j.fshw.2015.06.001
6. Bundele J, Deshmukh R. Probiotics market global opportunity analysis and industry forecast 2021-2030. Available from: https://www.alliedmarketresearch.com/press-release/probiotics-market.html [Accessed 10 July 2024].
7. Yu HS, Lee NK, Kim WJ, Lee DU, Kim JH, Paik HD. Optimization of an industrial medium and culture conditions for probiotic *Weissella cibaria* JW15 biomass using the Plackett-Burman Design and Response Surface Methodology. J Microbiol Biotechnol. 2022;32(5):630-637. https://doi.org/10.4014/jmb.2202.02020
8. Othman NZ, Mohd Din ARJ, Mohammad Z, Rosli MA, Sarmidi MR. Statistical optimization of medium compositions for high cell mass and exopolysaccharide production by *Lactobacillus plantarum* ATCC 8014. Appl Food Biotechnol. 2018;5(2):87-96.https://doi.org/10.22037/afb.v5i2.19299
9. Yeo S, Shin HS, Lee HW, Hong D, Park H, Holzapfel W, Kim EB, Huh CS. Determination of optimized growth medium and cryoprotective additives to enhance the growth and survival of *Lactobacillus salivarius*. J Microbiol Biotechnol. 2018;28(5):718-731.https://doi.org/10.4014/jmb.1801.01059

10. Ren H, Zentek J, Vahjen W. Optimization of production parameters for probiotic *Lactobacillus* strains as feed additive. Molecules. 2019; 24(18). https://doi.org/10.3390/molecules24183286

11. Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M, Tripathi CKM. Strategies for fermentation medium optimization: An in-depth review. Front Microbiol. 2017;7(1).https://doi.org/10.3389/fmicb.2016.02087

12. Tjandrawinata RR, Kartawijaya M, Hartanti AW. In vitro evaluation of the anti-hypercholesterolemic effect of *Lactobacillus* isolates from various sources. Front Microbiol. 2022;13(2).https://doi.org/10.3389/fmicb.2022.825251

13. Hwang CF, Chang JH, Houng JY, Tsai CC, Lin CK, Tsen HY. Optimization of medium composition for improving biomass production of *Lactobacillus plantarum* Pi06 using the Taguchi array design and the Box-Behnken method. Biotechnol Bioprocess Eng. 2012;17(4):827-834. https://doi.org/10.1007/s12257-012-0007-4

14. FDA. Bacteriological Analytical Manual. Chapter 3 aerobic plate count. *United States Food and Drug* Adm. 2001;(1):10.

15. Elsayed EA, Othman NZ, Malek R, Tang T, El Enshasy HA. Improvement of cell mass production of *Lactobacillus delbrueckii* sp. bulgaricus WICC-B-02: A newly isolated probiotic strain from mother's milk. J Appl Pharm Sci. 2014;4(11):8-14.https://doi.org/10.7324/JAPS.2014.4112

16. Lee NK, Park YL, Choe GJ, Chang HI, Paik HD. Medium optimization for the production of probiotic *Lactobacillus acidophilus* A12 using Response Surface Methodology. Korean J Food Sci Anim Resour. 2010;30(3):359-364. https://doi.org/10.5851/kosfa.2010.30.3.359

17. Heidarrezaei M, Dailin DJ, Malek RA, Hanapi SZ, El-Enshasy HA. Statistical medium optimization for enhancing high biomass production of *Lactobacillus reuteri* using Response Surface Methodology. Asia Pac J Sci Technol. 2022;27(6):10. https://doi.org/10.14456/apst.2022.94

18. Eyahmalay J, Elsayed EA, Dailin DJ, Ramli S, Sayyed RZ, El-Enshasy HA. Statistical optimization approaches for high cell biomass production of *Lactobacillus casei*. J Sci Ind Res. 2020;79(2-3):216-221. https://doi.org/10.56042/jsir.v79i3.68645

19. Hayek SA, Ibrahim SA. Current limitations and challenges with lactic acid bacteria: a review. Food Nutr Sci. 2013;(11):73-87. https://doi.org/10.4236/fns.2013.411A010

20. Selvamani S, Dailin DJ, Rostom M, Malek RA, Gupta VK, El-Enshasy HA. Optimizing medium components to enhance high cell mass production of biotherapeutic strain *Lactobacillus reuteri* DSM 20016^T by statistical method. J Sci Ind Res. 2020;79(9):798-803.https://doi.org/10.56042/jsir.v79i9.41715

21. Fonteles TV, Costa MGM, de Jesus ALT, Rodrigues S. Optimization of the fermentation of cantaloupe juice by *Lactobacillus casei* NRRL B-442. Food Bioprocess Tech. 2012;(5):2819-2826.https://doi.org/10.1007/s11947-011-0600-0

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بهینه‌سازی ترکیب محیط کشت و شرایط تخمیر برای به حداکثر رساندن سلول‌های زنده و تولید زی‌توده *لاکتوباسیلوس پلانتاروم DLBSK207* با روش سطح پاسخ

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چکیده

سابقه و هدف: این مطالعه با هدف افزایش سلول‌های زنده و تولید زی‌توده[۱] سویه بالقوه زیست‌یار[۲] *لاکتوباسیلوس پلانتاروم* DLBSK207 با بهینه‌سازی غلظت مواد مغذی اصلی و شرایط تخمیر با استفاده از روش سطح پاسخ با طرح باکس بانکن انجام شد.

مواد و روش ها: با آزمون‌ها دو متغیر اصلی ترکیب محیط کشت و شرایط تخمیر بررسی شد. بر اساس نتایج یک عامل در یک زمان، شش عامل برای طرح پلاکت برمن انتخاب شد تا ارزیابی شود که آیا متغیرها به‌طور قابل توجهی بر پاسخ تأثیر می‌گذارند یا خیر. محیط کشت حاوی کربن (گلوکز) و نیتروژن (عصاره مخمر و پپتون) بود، در حالی که شرایط تخمیر شامل pH و دمای اولیه بود. محیط کشت‌های پایه، متشکل از استات سدیم، $MgSO_4.7H_2O$، K_2HPO_4، $MnSO_4.H_2O$ و Tween 80، به میزان ثابت تنظیم شدند. با روش RSM، غلظت گلوکز، عصاره مخمر و پپتون، و همچنین pH و دمای اولیه، برای به‌حداکثر رساندن تعداد سلول‌های زنده و زی‌توده بهینه شدند.

یافته‌ها و نتیجه‌گیری: ترکیب بهینه محیط کشت توسط RSM شامل g l^{-1} ۳۳/۷۶ گلوکز، g l^{-1} ۳۲/۵۹ عصاره مخمر و g l^{-1} ۲۸/۳۸ پپتون در pH اولیه ۶/۰ و دمای ۳۵ درجه سلسیوس بود. تحت این شرایط بهینه، تعداد سلول‌های زنده log CFU ml^{-1} ۹/۳۰ و وزن سلول خشک g l^{-1} $^{۴/۳۲}$به‌دست آمد که با مقادیر پیش‌بینی‌شده log ۹/۳۰CFU ml^{-1} و g l^{-1} ۴/۲۸ مطابقت داشت که نشان‌دهنده افزایش۱/۸۲ برابری نتایج این محیط کشت در مقایسه با محیط کشت استاندارد MRS broth می‌باشد. میزان بیشتر تولید زی‌توده هنگام افزایش مقیاس با استفاده از یک بیوراکتور ۱۰ لیتری کنترل شده در pH ۶/۰، حداکثر تعداد سلول‌های زنده log CFU ml^{-1} ۹/۸۸ وg l^{-1} ۵/۸۲ DCW پس از ۲۰ ساعت گرمخانه‌گذاری بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- طرح باکس بنکن
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- طرح پلاکت برمن
- زیست¬یار
- افزایش مقیاس

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