

# Optimization of Culture Conditions for Enhanced Bacteriocin Production by *Lactococcus lactis* MT186647 Using Response Surface Methodology

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**Abstract** Bacteriocins produced by various lactic acid bacteria (LAB) have found enormous use in the food industries as biopreservatives. This study evaluated the effect of varied culture conditions (temperature, pH, and NaCl concentration) on bacteriocin production by *Lactococcus lactis* MT186647 isolated from fermenting African oil bean seeds (*Pentaclethra macrophylla* Benth) using response surface methodology. A three-factor central composite design (CCD) was adopted with the interest of estimating the optimal conditions for its production using the response surface regression model, which evaluated the linear, squared, and interactive relationship between the response variables. The analysis of variance (ANOVA) using Minitab statistical software version 14.13 showed an  $R^2 = 0.869$  variations in the response variable for culture conditions. It was accounted for by the predictors suggesting that the model was adequate. The optimal culture condition for bacteriocin production by *L. lactis* was estimated at 30.5°C, pH 5.9, 1.94% NaCl concentration at  $Y = 12.31$  mm where Y represents the response (zone of inhibition) against *Staphylococcus aureus* ATCC 19095 using the agar well diffusion assay method. Validation of optimal values according to the regression model, produced an inhibition zone at  $Y = 13.33 \pm 0.29$  mm. There was a 19.33% increase in bacteriocin activity compared to an OFAT optimized medium from a previous study.

**Keywords:** *Lactococcus lactis* MT186647, Bacteriocin, optimization, central composite design, response surface methodology

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## 1. Introduction

Bacteriocins are antimicrobial proteins that are ribosomally synthesized by bacteria and possess a wide range of antagonistic activity towards genetically related bacterial species [1,2]. However, there have been reports of bacteriocin activity against some Gram-negative bacteria, especially those from lactic acid bacteria [3,4]. Bacteriocins from lactic acid bacteria (LAB) have presented substantial potential applications in the prevention and treatment of many diseases of the gastrointestinal tract [5,6], as well as in food industries as bio-preservatives [7,8].

Bacteriocin production by LAB largely depends on bacterial growth during optimum metabolic activity associated with maximum cellular growth [9,10]. However, certain environmental factors, such as pH,

temperature, and media composition, can influence bacteriocin production [11,12,13,14]. Various components, such as salts, carbohydrates, surfactants included in the fermenting medium, can interfere with the bacteriocin production by LAB [15,16,17].

The successful application of surface response methodology (RSM) in many fields, especially in biotechnology, has facilitated the study of distinct factors that influence bacteriocin production [18,19,20]. Numerous studies have employed RSM in evaluating the effect of environmental conditions and medium components on the antibacterial activity of bacteriocins of *Pediococcus acidilactici* BA28 [21], *Lactobacillus acidophilus* [22], *Lactobacillus brevis* DF01 [23], and *Lactobacillus plantarum* [24].

This study aimed to optimize bacteriocin production by *Lactococcus lactis* MT186647 in MRS broth containing different supplements, pH levels, temperatures, and salt concentrations using RSM.

## 2. Materials and Methods

### 2.1. Bacterial Strains

*Lactococcus lactis* LB10 was isolated from fermenting African oil bean seeds (AOBS). The isolate was identified by sequencing the 16S rRNA gene followed by BLAST and nucleotide sequence deposited with the NCBI under the accession number MT186647.

### 2.2. Indicator Strain and Culture Media

The indicator strain *Staphylococcus aureus* ATCC 19095 obtained from the Federal Institute of Industrial Research, Oshodi (FIRO), Lagos, Nigeria, was cultured on Mannitol salt agar (HiMedia) at 37°C. The strain was then sub-cultured on Nutrient broth and stored at -20°C with 20% (v/v) glycerol.

### 2.3. Bacteriocin Activity Assay

*Lactococcus lactis* MT186647 was grown in MRS broth (HiMedia) at 35°C for 24 h. The culture broth was centrifuged at 10 000 rpm for 15 mins; at 4°C. The pH of the cell-free supernatant (CFS) was adjusted to 6.5 using 1 mol/l NaOH then partially purified according to [25]. Bacteriocin precipitation was achieved by adding ammonium sulfate and then centrifuged at 10 000 rpm for 20 mins. The residue was then re-dissolved in double-distilled water and filtered through a 0.22 µm syringe filter. Bacteriocin activity was determined using the agar well diffusion method, according to [26].

### 2.4. Experimental Design

The optimization of bacteriocin production by *L. lactis* MT186647 was done using RSM. Culture condition variables (temperature, pH, and sodium chloride concentration) were optimized for enhanced antibacterial activity using the central composite design (CCD). A 2-level, 3-factor ( $2^3$ ) response surface methodology was adopted to estimate the optimal culture conditions and supplementation on bacteriocin production by the isolate. Bacteriocin production was estimated using a response surface regression model to estimate the linear, squared, and interactive relationship between the response variable and the predictors. Optimization comprises of 20 experimental trials (8 factorial points with six axial and six central points). Fermentations were in random order involving three variables (pH =  $X_1$ , temperature =  $X_2$ , and NaCl concentration =  $X_3$ ) with five coded levels (-1.68, - $\alpha$ , 0,  $\alpha$  and 1.68) to determine the effect of independent variables on response variable (antibacterial activity) according to [27].

### 2.5. Statistical and Mathematical Analysis

In developing the regression equation to determine the relationship between the independent and dependent variables for the RSM, Minitab statistical software 14.13 was used to determine the effects of linear, squared, and interactive terms of the independent variables.

Given the values of the minimum and maximum level of temperature, pH, and Sodium Chloride concentration to be 28 and 32°C, 5.5 and 6.5, and 1.5 and 2.5%, respectively, we obtain the uncoded units for the design.

$$X = b(\text{coded unit}) + a$$

$$\text{where: } a = \frac{X_{\max} + X_{\min}}{2}; b = \frac{X_{\max} - X_{\min}}{2}$$

where X is the absolute value,  $X_{\min}$  = the minimum level value,  $X_{\max}$  is the maximum level value.

The response factor ( $Y_i$ ) was modeled into the regression analysis as a mathematical function of a few continuous factors. The response was then expressed using a second-order polynomial equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Where Y is the predicted response (bacteriocin activity in terms of zone of inhibition),  $\beta_0$  the intercept,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the linear coefficients,  $\beta_{11}$ ,  $\beta_{22}$ ,  $\beta_{33}$  are the squared coefficients,  $\beta_{12}$ ,  $\beta_{13}$ ,  $\beta_{23}$  are interaction coefficients,  $X_1$  the temperature,  $X_2$  the pH and  $X_3$  sodium chloride concentration.

A three-dimensional surface plot was drawn to show the interactive effect of the independent variables on bacteriocin production. The optimal values were obtained by solving the regression equation and analyzing the surface contour plots. The coefficient of determination ( $R^2$ ) was employed for the goodness of fit of the model equation [28]. All experiments were conducted in triplicates. Obtained values were analyzed using Fisher's test, and differences were considered statistically significant at  $p < 0.05$ .

### 2.5. Validation of the Experimental Model and Optimum Condition

To verify the optimization results and accuracy of the model, experiments were carried out in duplicates using the optimal culture conditions obtained from the RSM and compared to results obtained using one factor at a time OFAT, according to [14].

## 3. Results

The results of 20 runs from CCD experiments for studying the effect of culture conditions (pH, temperature, and NaCl concentration) on bacteriocin production by *Lactococcus lactis* MT186647 from fermenting African oil bean seeds (AOBS) is as shown in Table 1. The maximum experimental value for bacteriocin production was 12.87 mm based on RSM.

Equation 2 shows the regression analysis data obtained were fitted into a quadratic model to generate a second-order regression equation for the full actual model on bacteriocin production. Where Y is bacteriocin activity (zone of inhibition in mm), A is the temperature (°C), B is pH, and C is NaCl concentration (%).

An *F*-test checked the statistical significance of Eq. 2, and Table 2 shows the ANOVA table testing the model, linear, squared, and interaction effects of variables. The model produced an *F*-value (3.75), *P*-value (0.031), and an insignificant lack of fit ( $P_{\text{model}} > F$ ) at 0.102.

The regression equation generated after ANOVA indicated an  $R^2$  value of 0.7894. All linear and interactive effects were not significant at ( $P > 0.05$ ), while the squared effects of temperature (A) and NaCl concentration (C) were significant at ( $P < 0.05$ ).

**Table 1.** Coded experimental design and results for RSM of maximum bacteriocin production of *L. lactis* MT186647 as a function of temperature, pH, and NaCl concentration

Runs No.	Coded value order	A	B	C	Zone of Inhibition (mm)	
					Actual	Predicted
5	1	28	5.5	2.5	7.12	6.78
3	2	28	6.5	1.5	7.66	8.12
20	3	30	6.0	2.0	12.66	12.13
9	4	27	6.0	2.0	-	4.87
1	5	28	5.5	1.5	6.78	7.15
10	6	33	6.0	2.0	10.12	8.20
7	7	28	6.5	2.5	6.22	6.84
12	8	30	6.8	2.0	11.81	10.23
15	9	30	6.0	2.0	12.24	12.13
6	10	32	5.5	2.5	8.85	9.75
16	11	30	6.0	2.0	12.87	12.13
18	12	30	6.0	2.0	11.94	12.13
2	13	32	5.5	1.5	9.21	9.95
13	14	30	6.0	1.2	10.12	8.84
17	15	30	6.0	2.0	12.62	12.13
11	16	30	5.2	2.0	11.22	10.88
14	17	30	6.0	2.8	8.23	7.59
19	18	30	6.0	2.0	10.11	12.13
4	19	32	6.5	1.5	7.41	9.11
8	20	32	6.5	2.5	7.01	8.00

A is the temperature (°C), B is pH, and C is NaCl concentration (%)

$$\begin{aligned}
 Y = & -636 + 32.79A + 41.7B + 25.6C \\
 & -0.494A^2 - 2.22B^2 - 5.53C^2 \\
 & -0.452A^2B + 0.042A^2C - 0.91B^2C
 \end{aligned}
 \tag{2}$$

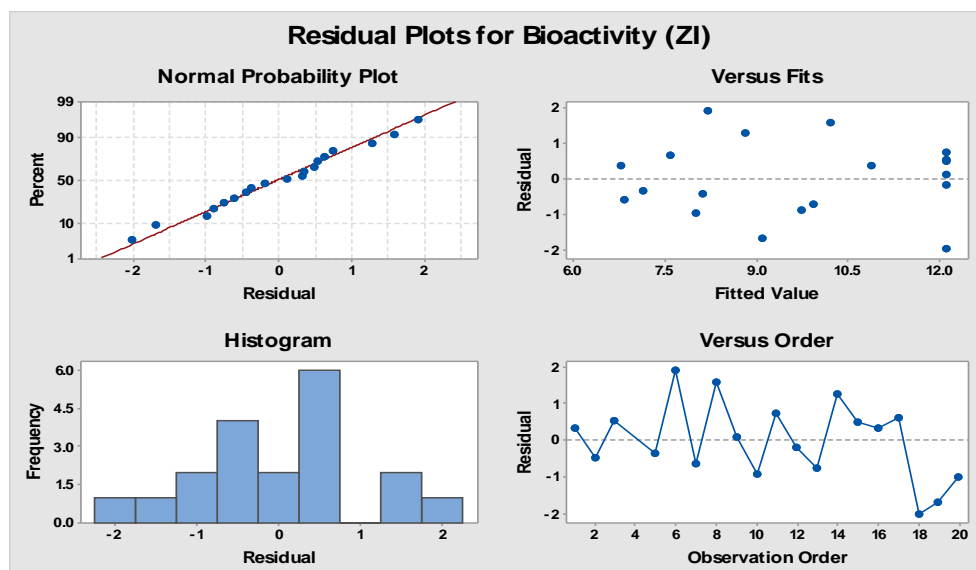
**Table 2.** Coded experimental design and results for RSM of maximum bacteriocin production of *L. lactis* MT186647 as a function of temperature, pH, and NaCl concentration

Source	DF	SS	MS	F-Value	P>F
Model	9	73.5346	8.1705	3.75	0.031*
Linear	3	11.1602	3.7201	1.71	0.235
A	1	8.7802	8.7802	4.03	0.076
B	1	0.5211	0.5211	0.24	0.637
C	1	1.8589	1.8589	0.85	0.380
Square	3	66.3421	22.1140	10.15	0.003*
A*A	1	32.8903	32.8903	15.09	0.004*
B*B	1	4.2395	4.2395	1.95	0.197
C*C	1	26.2394	26.2394	12.04	0.007*
2-way Interaction	3	2.0665	0.6888	0.32	0.814
A*B	1	1.6380	1.6380	0.75	0.408
A*C	1	0.0144	0.0144	0.01	0.937
B*C	1	0.4141	0.4141	0.19	0.673
Error	9	19.6129	2.1792		
Lack of fit	4	14.4350	3.6087	3.48	0.102
Pure error	5	5.1779	1.0356		
Total	18	93.1475			
Model summary					
S	R <sup>2</sup>	R <sup>2</sup> (adj.)	R <sup>2</sup> (pred.)		
1.47621	78.94%	57.89%	0.00%		

Figure 1 shows the validation (graphical) of the regression model using residual plots. The probability plot showed linearity, residual versus fitted were randomly scattered; whereas, the histogram of frequency versus residual had a dumbbell shape, and residual versus observation order had a symmetrical pattern.

The 2D contour and 3D response surface plots of antibacterial activity of bacteriocin from *Lactococcus lactis* MT186647 against *S. aureus* are shown in Figure 2 and Figures 3a, b, and c respectively.

Figure 4 displays the predicted optimal level based on the regression model. An optimal response variable of 12.31 mm was obtained when temperature, pH, and NaCl concentration were fixed at 30.5°C, 5.9, and 1.94%, respectively.



**Figure 1.** Residual plots for bacteriocin activity (mm) from *L. lactis* MT186647

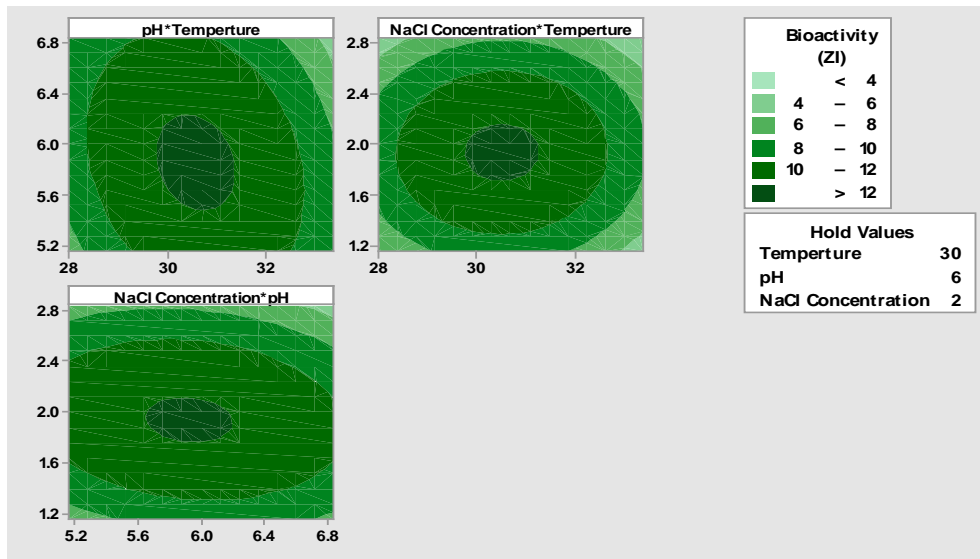


Figure 2. Response surface of bacteriocin production by *Lactococcus lactis* MT186647 estimated by a zone of inhibition as a function of pH\*Temperature, NaCl\*Temperature, and NaCl\*pH

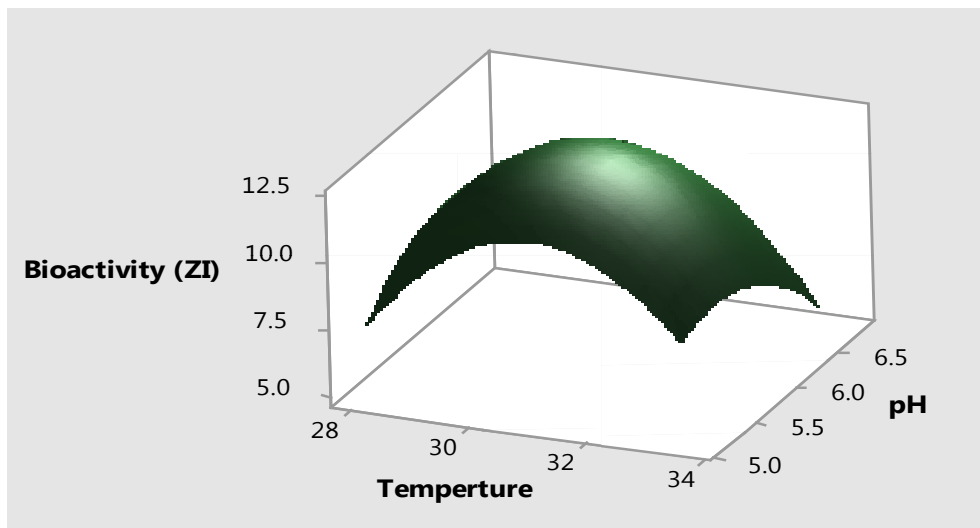


Figure 3a. Effect of pH versus Temperature (3-D Surface plot) on Bacteriocin Production by *L. lactis* MT186647

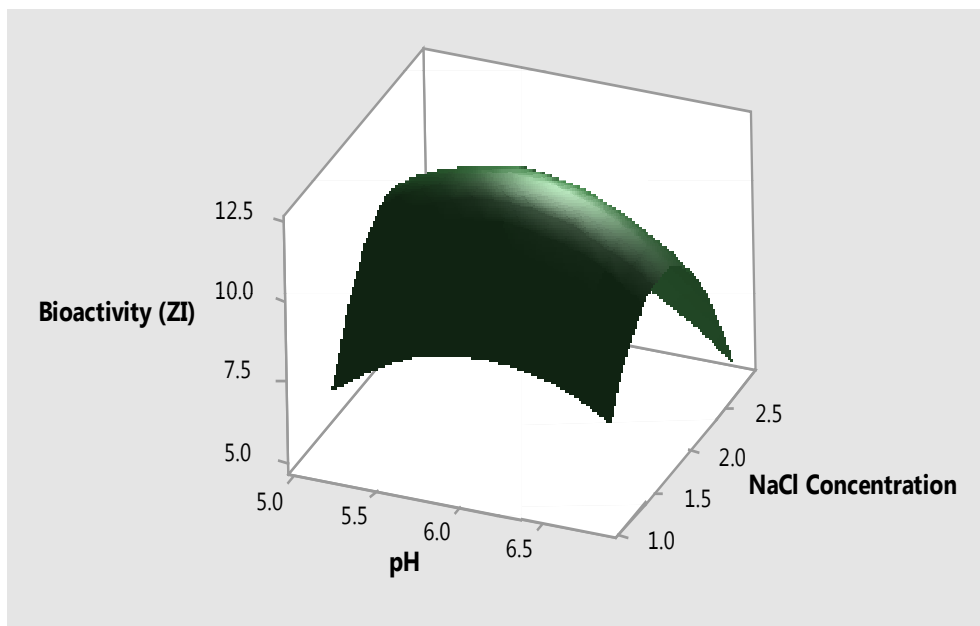


Figure 3b. Effect of pH versus NaCl concentration (3-D Surface plot) on Bacteriocin Production by *L. lactis* MT186647

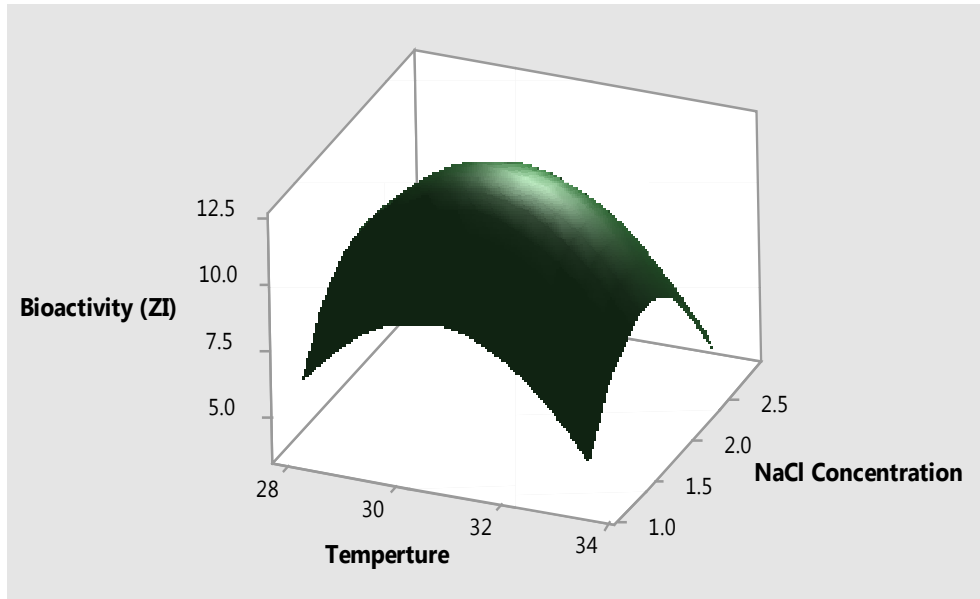


Figure 3c. Effect of Temperature versus NaCl concentration (3-D Surface plot) on Bacteriocin Production by *L. lactis* MT186647

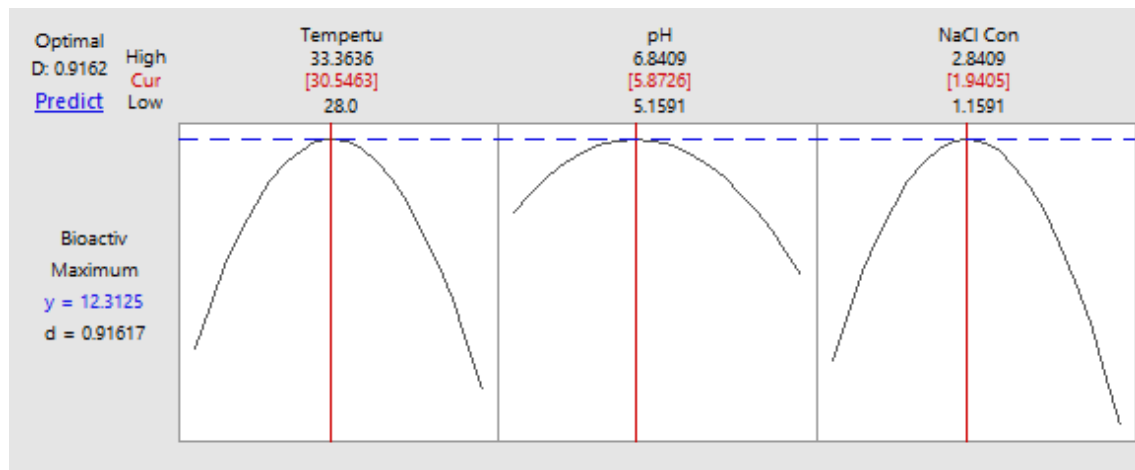


Figure 4. An optimal plot of bacteriocin production by *L. lactis* MT186647

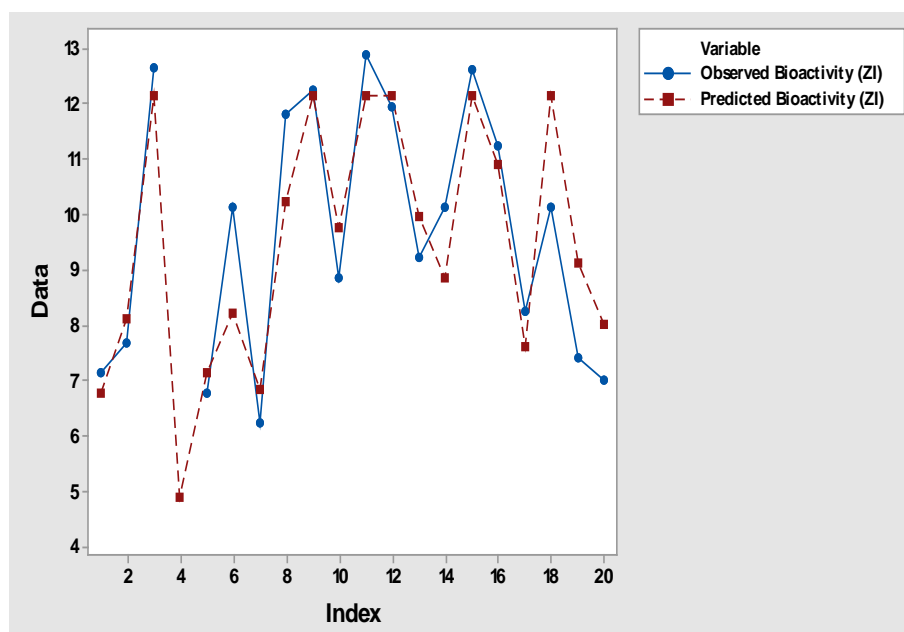


Figure 5. Time series (Parity) plot of observed versus predicted response variable of bacteriocin production by *L. lactis* MT186647

The correlation between actual and predicted bacteriocin activity is presented in the parity plot in Figure 5. Actual and predicted values are closely aligned.

## 4. Discussion

### 4.1. Response Surface Quadratic Model

The ANOVA result shows the statistical analysis for the level of significance of all variables. The model adequacy was analyzed using the  $R^2$  coefficient, correlation, and model significance ( $F$ -value). The  $P$ -value  $> F$  of less than 0.005 shows that the model is statistically significant and means that the model can predict experimental results [23,29,30]. All axial points with zero coded values were repeated six times to estimate the pure error for lack of fit as predictions can be desirably made with an insignificant lack of fit. The model produced a negligible lack of fit ( $P_{\text{model}} > F$ ) at 0.102. The regression equation generated after ANOVA indicated an  $R^2$  value of 0.7894 (a value of  $R^2 > 0.75$  indicates aptness of the model). These coefficient values ensured a fine adjustment of the quadratic model to experimental data and explained 78.94% of the variability in the response [29].

The linear coefficient values of all variables showed no impact on bacteriocin production; whereas, the quadratic effect of variables were all insignificant. The squared effect coefficient of temperature ( $p=0.004$ ) and NaCl concentration ( $p=0.007$ ) were significant, while others were not significant, as bacteriocin production decreased as values of variables increased. Alteration of temperature and NaCl concentration had a strong influence on bacteriocin production. Similar results were observed by [31] as the two factors significantly affected bacteriocin production by *Lactobacillus brevis* OG1.

### 4.2. Correlation of Actual and Predicted Values for Bacteriocin Production

A comparison of actual (experimental) and predicted values detailed a good correspondence as there was proximity indicating an adequate description of the relationship between variable and response owing to the empirical model derived from the RSM [29].

### 4.3. Residual Analysis for Regression Assumptions

The residual plot analyzes the error pattern in data gotten from experiments. Linearity in the error pattern of the probability plot indicates little or no issues in data and verifies the regression model. The representative graphical validation of the regression model with the help of residual plots showed that the residual is symmetry (normally distributed) and contains a constant variance. This continuous variation indicates the results from this model can be used for inferential purposes following the central limit theorem [27]. The standard probability plot was linear, residual versus fitted values scattered around zero (0) point. There was a dumbbell shape in the frequency versus residual plot, while there was a

symmetrical pattern observed in the residual versus order plot.

### 4.4. Prediction of Optimal Levels

The interaction of factors shown graphically with the 2-D and 3-D response surface plots showed that culture conditions should not exceed specified values. The Maximum bacteriocin production was achieved at 30.5°C, pH 5.9, and 1.94% NaCl concentration, producing a zone of inhibition of 12.31 mm against *Staphylococcus aureus* ATCC 19095, as shown in the optimal plot. The statistical optimization of culture conditions showed that temperature and NaCl concentration significantly affected bacteriocin production. Different temperatures affect the ability of LAB to produce bacteriocins [28,32]. One factor at a time optimization of temperature, pH, and NaCl concentration for bacteriocin production by *Lactobacillus* species showed peak values of  $11.17 \pm 0.33$  mm,  $10.50 \pm 0.29$  mm,  $10.70 \pm 0.17$  mm, respectively [14]. The increase in bacteriocin activity indicates the effect of optimized conditions for fermentation by LAB as a 10.20% increase in activity was observed when CCD was employed.

### 4.5. Validation of the Experiment

Validation of obtained results was carried out applying the optimal culture variable conditions as follows: temperature 31°C, pH 6.0, and 1.9% NaCl concentration in a shaker incubator. The maximum antibacterial activity obtained experimentally was  $13.33 \pm 0.29$  mm diameter of inhibition against 12.31 mm predicted from RSM and agreed with the model prediction. Therefore, the developed model is considered reliable for predicting enhanced bacteriocin production in *Lactococcus lactis* MT1186647. More so, comparing the OFAT optimized medium, according to [23], showed a 19.33% increase in bacteriocin activity, which means RSM is a useful tool for optimization.

## 5. Conclusion

The enhancement of bacteriocin production by *Lactococcus lactis* MT1186647, using CCD by optimizing some culture conditions (temperature, pH, and NaCl concentration), resulted in the antibacterial activity of  $13.33 \pm 0.29$  mm. The statistical optimization resulted in antifungal activity of  $13.33 \pm 0.29$  mm against *S. aureus*, which was 19.33% higher than that of the OFAT optimized medium ( $11.17 \pm 0.17$  mm). The experimental results were in correlation with predicted values, hence, validating the experiment and confirming the accuracy of the RSM model.

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## References

- [1] Arnison, P. G., Bibb, M. J., Bierbaum, G., Bowers, A. A., Bugni, T. S., Bulaj, G., Camarero, J. A., Campopiano, D. J., Challis, G. L., Clardy, J., Cotter, P. D., Craik, D. J., Dawson, M., Dittmann, E., Donadio, S., Dorrestein, P. C., Entian, K. D., Fischbach, M. A., Garavelli, J. S., ... Van Der Donk, W. A., Ribosomally synthesized and post-translationally modified peptide natural products: Overview and recommendations for a universal nomenclature. *Natural Product Reports*, 30(1). 108-160. 2013.
- [2] Papagianni, M., Ribosomally synthesized peptides with antimicrobial properties: Biosynthesis, structure, function, and applications. *Biotechnology Advances*, 21(6). 465-499. 2003.
- [3] Elayaraja, S., Annamalai, N., Mayavu, P., and Balasubramanian, T., Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. *Asian Pacific Journal of Tropical Biomedicine*, 4. 305-311. 2014.
- [4] Shelburne, C. E., An, F. Y., Dholpe, V., Ramamoorthy, A., Lopatin, D. E., and Lantz, M. S., The spectrum of antimicrobial activity of the bacteriocin subtilisin A. *Journal of Antimicrobial Chemotherapy*, 59(2). 297-300. 2007.
- [5] Egan, K., Ross, R. P., and Hill, C., Bacteriocins: antibiotics in the age of the microbiome. *Emerging Topics in Life Sciences*, 1(1). 55-63. 2017.
- [6] Nigam, A., Gupta, D., and Sharma, A., Treatment of infectious disease: Beyond antibiotics. *Microbiological Research*, 169 (9-10). 643-651. 2014.
- [7] García, P., Rodríguez, L., Rodríguez, A., and Martínez, B., Food biopreservation: Promising strategies using bacteriocins, bacteriophages and endolysins. *Trends in Food Science and Technology*, 21(8). 373-382. 2010.
- [8] Parada, J. L., Caron, C. R., Medeiros, A. B. P., and Soccol, C. R., Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. *Brazilian Archives of Biology and Technology*, 50(3). 521-542. 2007.
- [9] Chapot-Chartier, M. P., and Kulakauskas, S., Cell wall structure and function in lactic acid bacteria. *Microbial Cell Factories*, 13(11). 59-81. 2014.
- [10] König, H., and Fröhlich, J., *Lactic acid bacteria*. In: *Biology of Microorganisms on Grapes, in Must and Wine*. Springer International Publishing. 2017, 3-41.
- [11] Abbasiliasi, S., Tan, J. S., Tengku Ibrahim, T. A., Bashokouh, F., Ramakrishnan, N. R., Mustafa, S., and Ariff, A. B., Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. *RSC Advances*, 7(47). 29395-29420. 2017.
- [12] Bogovic Matijasic, B., Rogelj, I., Batič, M., and Raspor, P., Influence of pH on bacteriocin production by *Lactobacillus* K7 during batch fermentation. *Periodicum Biologorum*, 103(2). 163-167. 2001.
- [13] Himelbloom, B., Nilsson, L., and Gram, L., Factors affecting production of an antilisterial bacteriocin by *Carnobacterium piscicola* strain A9b in laboratory media and model fish systems. *Journal of Applied Microbiology*, 91(3). 506-513. 2001.
- [14] Onwuakor, C.E., Nwaugo, V.O., Nnadi, C.J., and Emetole, J.M., Effect of Varied Culture Conditions on Crude Supernatant (Bacteriocin) Production from Four *Lactobacillus* Species Isolated from Locally Fermented Maize (Ogi). *American Journal of Microbiological Research*, 2(5), 125-130. 2014.
- [15] Castro, M. P., Palavecino, N. Z., Herman, C., Garro, O. A., and Campos, C. A., Lactic acid bacteria isolated from artisanal dry sausages: Characterization of antibacterial compounds and study of the factors affecting bacteriocin production. *Meat Science*, 87(4). 321-329. 2011.
- [16] Todorov, S. D., and Dicks, L. M. T., Effect of medium components on bacteriocin production by *Lactobacillus pentosus* ST151BR, a strain isolated from beer produced by the fermentation of maize, barley and soy flour. *World Journal of Microbiology and Biotechnology*, 20(6). 643-650. 2004.
- [17] Vázquez, J. A., Cabo, M. L., González, M. P., and Murado, M. A., The role of amino acids in nisin and pediocin production by two lactic acid bacteria: A factorial study. *Enzyme and Microbial Technology*, 34(3-4). 319-325. 2004.
- [18] Kumar, M., Jain, A. K., Ghosh, M., and Ganguli, A., Statistical optimization of physical parameters for enhanced bacteriocin production by *L. casei*. *Biotechnology and Bioprocess Engineering*, 17(3). 606-616. 2012.
- [19] Radha, K. R., and Padmavathi, T., Statistical optimization of bacteriocin produced from *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from yoghurt. *International Food Research Journal*, 24(2). 803-809. 2007.
- [20] Yolmeh, M., and Jafari, S. M., Applications of Response Surface Methodology in the Food Industry Processes. *Food and Bioprocess Technology*, 10(3). 413-433. 2017.
- [21] Kaur, B., Garg, N., and Sachdev, A., Optimization of bacteriocin production in *Pediococcus acidilactici* BA28 using response surface methodology. *Asian Journal of Pharmaceutical and Clinical Research*, 6(SUPPL.1), 192-195. 2013.
- [22] Nikbakht Kashkooli, T., Joyandeh, H., Tahmoozi Dide Ban, S., and Samavati, V., Optimizing of the production process of synbiotic dahi containing *Lactobacillus acidophilus*, tragacanth and inulin using Surface Response Methodology. *Food Science and Technology*, 14(62). 103-189. 2017.
- [23] Lee, Y. M., Kim, J. S., and Kim, W. J., Optimization for the maximum bacteriocin production of *Lactobacillus brevis* DF01 using response surface methodology. *Food Science and Biotechnology*, 21(3). 653-659. 2012.
- [24] Le, N. T. T., Bach, L. G., Nguyen, D. C., Le, T. H. X., Pham, K. H., Nguyen, D. H., and Thi, T. T. H., Evaluation of factors affecting antimicrobial activity of bacteriocin from *Lactobacillus plantarum* microencapsulated in alginate-gelatin capsules and its application on pork meat as a bio-preservative. *International Journal of Environmental Research and Public Health*, 16(6). 2019.
- [25] Tulini, F. L., Gomes, B. C., and Martinis, E. C. P. de., Partial purification and characterization of a bacteriocin produced by *Enterococcus faecium* 130 isolated from mozzarella cheese. *Ciência e Tecnologia de Alimentos*, 31(1). 155-159. 2011.
- [26] Delgado, A., Brito, D., Fevreiro, P., Tenreiro, R., and Peres, C., Bioactivity quantification of crude bacteriocin solutions. *Journal of Microbiological Methods*, 62(1). 121-124. 2005.
- [27] Suganthi, V., and Mohanasrinivasan, V., Optimization studies for enhanced bacteriocin production by *Pediococcus pentosaceus* KC629718 using response surface methodology. *Journal of Food Science and Technology*, 52(6). 3773-3783. 2015.
- [28] Cladera-Olivera, F., Caron, G. R., and Brandelli, A., Bacteriocin production by *Bacillus licheniformis* strain P40 in cheese whey using response surface methodology. *Biochemical Engineering Journal*, 21(1). 53-58. 2004.
- [29] Sarabia, L. A., and Ortiz, M. C., Response Surface Methodology. *Comprehensive Chemometrics*, 1. 345-390. 2009.
- [30] Wang, L., Zhang, M., Li, Y., Cui, Y., Zhang, Y., Wang, Z., Wang, M., and Huang, Y., Application of response surface methodology to optimize the production of antimicrobial metabolites by *Micromonospora* Y15. *Biotechnology and Biotechnological Equipment*, 31(5). 1016-1025. 2017.
- [31] Ogunbanwo, S. T., Sanni, A. I., and Onilude, A. A., Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology*, 2(8), 223-235. 2003.
- [32] Malheiros, P. S., Sant'Anna, V., Todorov, S. D., and Franco, B. D. G. M., Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. *Sakei* 2a. *Brazilian Journal of Microbiology*, 46(3). 825-834. 2015.
- [33] Leães, F. L., Vanin, N. G., Sant'Anna, V., and Brandelli, A., Use of Byproducts of Food Industry for Production of Antimicrobial Activity by *Bacillus* sp. P11. *Food and Bioprocess Technology*, 4(5). 822-828. 2011.
- [34] Monafathia, N.R., M., and Widanami., Optimization of bacteriocin production from *Lactobacillus plantarum* IN05 by using response surface methodology. *Pakistan Journal of Biotechnology*, 15(3). 785-791. 2018.

