

THE USE OF RESPONSE SURFACE METHODOLOGY IN OPTIMIZATION PROCESS FOR BACTERIOICIN PRODUCTION

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ABSTRACT

The production of bacteriocin from MRS broth medium by *Lactobacillus* species LBC216 isolated in traditional dairy products was investigated. Response surface methodology was used to evaluate the effects of three main parameters (pH, Temperature, Incubation period) on the concentration of bacteriocin. Results of the statistical analysis showed that the fit of the mode was good in all cases. PH, temperature and Incubation period has a strong linear effect on bacteriocin production. The maximum bacteriocin titer of 11.200AUmL¹ was obtained at the optimization of process variables (pH 7.0, Temperature 37°C, Incubation period 24h). These values were obtained by fitting of the experimental data to the model equation. The response surface methodology was found to be useful in optimizing and determining the interaction among process variables in bacteriocin production using *Lactobacillus* spp LBC 216.

Keywords: *Lactobacillus* spp; MRS Medium; central composite design; response surface methodology

1. INTRODUCTION

Bacteriocins of lactic acid bacteria (LAB) are biologically active, ribosomally synthesized peptides that display an antagonistic activity against taxonomically related species and other bacteria of health or spoilage significance¹ and pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus*², these biological antimicrobial substances have attracted increasing research attention owing to their potential in food preservation as purified metabolites, through the use of their producer strains in starter culture or as adjunct starter culture³. Therefore there has been a continuous need to define the most appropriate conditions for bacteriocin production in fermentation media and food systems.

Optimization of bacteriocin production during fermentation has been the focus of intensive research work in the last decade. In addition to the control of the main

fermentation parameters (pH, Temperature and Incubation period). Nonetheless, control of this optimization of the fermentation medium remains the critical factors governing bacteriocin production and stability. The effect of medium composition on bacteriocin production has been extensively studied and there is general agreement the De man/Rogosa sharp (MRS) broth is one of the most suitable media for growth and bacteriocin production by LAB^{4,5}. It is usually produced in liquid substrates. The optimum conditions for bacteriocin production are affected by growth phase of the bacterium, pH of the medium, incubation temperature⁶. The production is increasing to optimum when pH of the substrate increases, and then decreasing. Mean while, the temperature increases bacteriocin production, but at the same time also kills the LAB⁷.

Response surface methodology (RSM) is an important statistical technique employed for multiple regression analysis by using quantitative experimental data obtained from properly designed experiments using central composite design (CCD)⁸. RSM can identify the various interactions among different parameters and it has been extensively applied for optimization of cultural medium conditions and other process parameters in bio processes⁹. The optimization of bacteriocin production from *Lactobacillus curvatus* CWBI-B28 using RSM has been described¹⁰. the production of bacteriocin from MRS medium by *Lactobacillus* species has been reported¹¹.

The present study examined bacteriocin production from MRS broth Medium by *Lactobacillus* species LBC 216. The effects of pH, Temperature and Incubation period were studied. RSM was used to optimize fermentation parameters to obtain maximum bacteriocin concentration. These parameters are among the most important factors for bacteriocin production. This study seems to work on both optimization and production of bacteriocin from de man rogosa sharpe (MRS) Medium using *Lactobacillus* species LBC 216.

2. MATERIALS AND METHODS

2.1. Microorganism:

Lactobacillus spp (LBC 216) used in this study was isolated from dairy products collected in the state of pondicherry. The strain was stored in de man rogosa and sharpe (MRS) broth with 20% (by volume) glycerol at -10°C.

2.2. Optimization of bacteriocin production process:

The process of bacteriocin production consisted of two steps, i.e. Preparing growth curve of the cells and optimization of bacteriocin production process. The first step was done to develop growth curves and to identify growth phases of the cells. The cells were first grown to MRS broth for 24hrs, and the bacterial growth were

observed every hour to measure optical densities of the culture using spectrometer with a 620nm wavelength. The changes in pH of the medium were measured¹². The bacteriocin production was done by growing the *Lactobacillus* on the MRS broth medium and adjust different pH (5.0 to 9.0), Temperature (20 to 45), incubation period (6 to 72) at a laboratory capacity using a 500ml Erlenmeyer flask with a 50 ml working volume. The bacteriocin harvest was done following the methods of^{13,14}.

2.3. Experimental design and statistical analysis:

The statistical analysis of the data was performed using Minitab Statistical Software (Release 15.00) details of RSM can be found elsewhere¹⁵. The levels of factors used in the experimental design are listed in Table 1. The data for the factors were chosen after a series of preliminary experiments. Twenty experiments were conducted using a face central composite statistical design for the study of three factors each at three levels (Tables 2). The levels were -1.682, -1, 0, 1, and 1.682, Where 0 corresponded to the central point. The response surface model was fitted to the response variable, namely bacteriocin production (AumL^{-1}).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + \dots + 1$$

Where X_1, X_2 and X_3 represent the level of the factors according to Table 1 and b_0, b_1, \dots, b_{23} represent Coefficient estimates with b_0 having the role of a scaling constant.

3. RESULT AND DISCUSSION:

The factors affecting the bacteriocin production from MRS broth Medium using *Lactobacillus casei* LBC216 isolated from Traditional milk products was studied using CCD experiments. The pH (X_1), The temperature (X_2) and the incubation period (X_3) were chosen as the independent variables, each at three levels and their

interactions on bacteriocin production were determined using a face centered design as shown in Table 1. bacteriocin production was chosen as the dependent output variable. twenty experiments based on the CCD were carried out with different combinations of variables and the results were presented in Table 2. It showed that the regression Coefficients of all the linear term and all Quadratic Coefficients of X_1 , X_2 and X_3 were significant at ($P > 0.001$). The individual effect of all the three parameters studied. Interaction effects between the temperature and pH were found to be significant from the response surface plots and contour shown in Figs 1, 2 and 3. P value of 0.001, hence an optimum combination of Incubation period and temperature is a must in order to get maximum production of medium to bacteriocin.

The ANOVA results of regression model for Y is described in Table 3. ANOVA of the regression model for Y demonstrated that the model was significant due to an F-value of 3.28 and a very probability value ($P < 0.001$). The P-values are used as a tool to check the significance of each of the Coefficients, which in turn indicate the pattern of the then it was more significant to the corresponding Coefficient. As shown in Table 4. R^2 is 0.899, which indicates that the model as fitted explained 90 % of the variability in bacteriocin production. These results show that the model chosen can satisfactorily explain the effects of optimization pH, Temperature and Incubation period on bacteriocin production by *Lactobacillus* spp., LBC216 using MRS broth Medium. The following model was fitted for bacteriocin production.

$$Y = 9888.9 + 616.2 X_1 - 1101.9 X_2 + 908.3 X_3 + 490.5 X_1^2 - 313.8 X_2^2 - 1672.9 X_2^3 + 1893.6 X_1 X_2 - 1029.6 X_1 X_3 + 1464.9 X_2 X_3 \dots \dots \dots (2)$$

Table 3 shows that pH, Temperature and Incubation period have a strong positive linear effect on the response, which is

bacteriocin concentration (p-value 0.001). There were also significant negative Quadratic effects of the above factors, indicating that bacteriocin production increases with increase in these parameters, but decreases as the above parameters are increased at optimum levels. Additionally, a significant negative interaction effect was observed between pH, Temperature, Incubation period. This indicated that bacteriocin production with the increase in these parameters.

Fig 1, 2, 3 and 4 shows surface plot and contour plot of bacteriocin production for each pair of factors whereas the third factor was kept constant at its middle level. The fitting of the experimental data to eqn (2) allowed to the determination of the concentration of pH (X_1 7.0), Temperature (X_2 37.0) and Incubation period (X_3 24hrs) giving a maximum bacteriocin production of $11.200 \text{ AU mL}^{-1}$ using RSM. The above data optimize production of bacteriocin from MRS broth Medium by *Lactobacillus* spp., (LBC) isolated from Dairy products.

CONCLUSION

In this study, bacteriocin was produced from *Lactobacillus* spp., LBC by using MRS broth Medium. RSM was used to determine the effects of three important factors (pH, Temperature and Incubation period) on bacteriocin production from broth. Linear, quadratic and interaction effects of these variables on bacteriocin production were determined. The model generated in this study by RSM satisfied all the necessary arguments for its use in optimization. By fitting the experimental data to a second-order polynomial equation, the optimum levels of the above-mentioned variables were determined. Using the optimum levels of fermentation parameters, a maximum bacteriocin production of $11.200 \text{ AU mL}^{-1}$ was obtained. This study indicates that the medium design using statistical technique such as RSM can be very useful in improving the production of bacteriocin by

Lactobacillus spp., LBC and in similar bioprocesses.

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Table 1=Codes and actual levels of the independent variables for design of experiment using CCD

Independent variables		-1.682 ^a	-1	0	1	1.682
pH	X ₁	5.0	6.0	7.0	8.0	9.0
Temperature/ ⁰ C	X ₂	20	30	37	40	45
Incubation period	X ₃	6	12	24	48	72

^a= coded levels

Table 2=Three level central composite design and the experimental responses of dependent variables bacteriocin production.

Runs order	pH	Temp (⁰ C)	Incubation period	(bacteriocin production AUmL ⁻¹)
1	1	-1	1	5250
2	0	1	0	11190
3	1	-1	-1	8500
4	0	0	-1.682	7800
5	1	1	1	5620
6	-1	1	1	3562
7	-1.682	0	0	6350
8	0	-1.682	0	5200
9	-1	-1	-1	6000
10	1.682	0	0	5420
11	1	1	1	10500
12	0	0	-1	11196
13	0	1	0	11102
14	0	0	0	11200
15	0	1.682	0	7450
16	0	-1.682	-1	8590
17	-1	-1	1	9820
18	0	0	-1.682	11000
19	1	0	0	11191
20	1	1	-1	9682

Table 3 = Analysis of Variance (ANOVA) for Quadratic Model

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-value	P-value
Regression	13460	9	14956	3.28	0.001
Linear	33032	3	11010	3.14	0.010
Square	47233	3	15744	2.90	0.042
Interaction	54334	3	18111	1.33	0.003
Residual Error	27137	10	27137	-	-
Lack of fit	10129	5	20258	0.12	0.438
Pure Error	17008	5	34016	-	-
Total	141738	19	-	-	-

$R^2 = 0.899$, $\text{adj } R^2 = 0.852$

Table 4: Estimated Regression Coefficients for bacteriocin production

Term	Coef	SE Coef	T	P
Constant	9888.9	671.9	12.520	0.001
(b ₁) P ^a	616.2	445.8	1.382	0.017
(b ₂) P ^b	-1101.9	445.8	1.382	0.014
(b ₃) P ^c	908.3	445.8	-2.472	0.069
(b ₁₁) P*P	490.5	433.9	1.130	0.019
(b ₂₂) T*T	-313.8	433.9	-0.723	0.013
(b ₃₃) I*I	-1672.9	433.9	-3.855	0.011
(b ₁₂) P*T	1893.6	582.4	3.251	0.003
(b ₁₃) P*I	-1029.6	582.4	-1.768	0.87
(b ₂₃) T*I	1464.9	582.4	2.515	0.013

Fig 1: surface plot of bacteriocin for temperature, pH

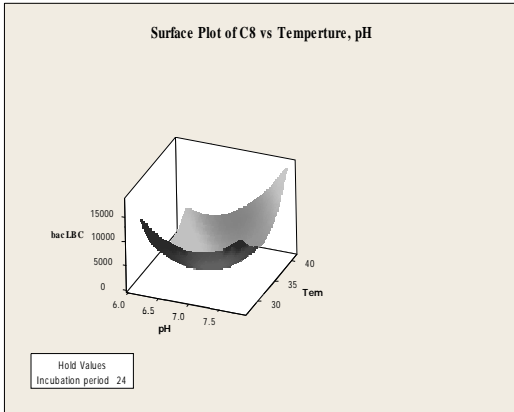


Fig 2: surface plot of bacteriocin for Incubation time, pH

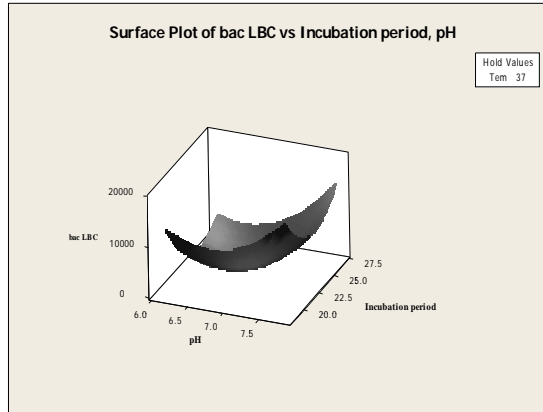


Fig 3 surface plot of bacteriocin for Incubation period, Temperature

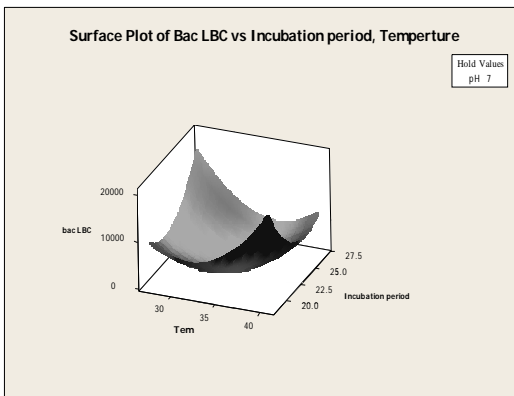


Fig 4: contour plot for Temperature, pH

