



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
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Research Article

Optimized Production of Bacteriocin from Cheaper Carbon and Nitrogen Sources Using Response Surface Methodology

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Abstract

Background and Objective: Bacteriocins are the protein molecules produced by bacteria that possess antimicrobial activities and play a major role as preservative in food industry. The widely used De-Man Rogosa and Sharpe (MRS) medium for the growth of lactic acid bacteria is quite expensive and is a barrier for the large scale economical production of bacteriocin. The present study aims at optimizing the medium composition of bacteriocin production using cheaper carbon and nitrogen sources. **Methodology:** *Lactobacillus plantarum* JX183220, isolated from goat milk was used for the production. Economical carbon and nitrogen sources such as molasses, red lentil were selected to replace the expensive dextrose and peptone respectively in the MRS Medium. The concentration of molasses as a carbon source, the concentration of red lentil as a nitrogen source and salt concentration were the three chosen process variables optimized by applying the box-behnken design of response surface methodology so as to obtain the maximum activity of bacteriocin. **Results:** The bacteriocin activity of 1839 AU was found at the optimum combination of process variables: Molasses concentration 37.7% (v/v), red lentil concentration 3.97% (w/v) and NaCl concentration 1.69 g L⁻¹. **Conclusion:** Molasses and red lentil are proved to be potential cheaper sources to replace the expensive carbon and nitrogen sources in the MRS medium for the production of bacteriocin from isolated *Lactobacillus plantarum* JX183220.

Key words: Bacteriocin, *Lactobacillus plantarum* JX183220, experimental optimization, box-behnken design, molasses, red lentil

Received: April 30, 2016

Accepted: August 22, 2016

Published: December 15, 2016

Citation: Sridevi Venigalla, Yasarapu N. Sindhuja, Kancharana Srujana, Silarapu Swathi, Yerri Naidu and Garapati H. Rao, 2017. Optimized production of bacteriocin from cheaper carbon and nitrogen sources using response surface methodology. Res. J. Microbiol., 12: 42-49.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacteriocins are the polypeptide molecules produced by many Gram positive and Gram negative bacteria during their growth. Bacteriocin, produced by Lactic Acid Bacteria (LAB), has broad spectrum of inhibition against pathogenic food spoilage microorganisms encountered in the manufacture of dairy, meat, vegetables and bakery products. One of the most important feature of these microbes is the extended shelf life of the product^{1,2}. Bacteriocins produced by lactic acid bacteria are considered as natural or bio preservatives^{1,3-5}. The cost of MRS medium used for the growth of lactic acid bacteria in the production of the bacteriocin is very high and is a barrier for its large scale economical production. Hence, studies are carried out not only on the production but also for the optimization of existing bacteriocins to address both biologic and economic concerns^{5,6}.

Bacteriocin production can be influenced by many factors like environmental factors^{7,8}, medium composition and other growth conditions^{6,9}. Very few studies have been reported on the production of bacteriocins using cheaper nitrogen and carbon sources^{10,11}. Optimization of many factors by conventional methods of changing one variable at a time is time consuming and often misses the interaction between the factors. Response Surface Methodology (RSM) is an efficient statistical design technique to optimize the conditions¹² synergistically.

Hence, the present study focuses on optimizing the medium composition of MRS medium using cheaper carbon and nitrogen sources by using box-behnken design of RSM for the cost effective production of bacteriocin using the new strain *Lactobacillus plantarum* JX183220 isolated from goat milk.

MATERIALS AND METHODS

Microorganism: *Lactobacillus plantarum* JX183220 isolated from goat milk in the Department of Biotechnology, ANITS, Visakhapatnam, India^{13,14} was used for the production of bacteriocin.

Culture maintenance: Stock cultures were maintained in frozen suspension in 20% glycerol. The culture was revived in MRS broth at 37°C for 24 h. A loop full of culture was streaked on MRS agar, grown for 24 h at 37°C and stored at 4°C for further use. Sub-culturing was performed once in every 15 days.

Inoculum preparation: The inoculum was prepared in 50 mL of MRS liquid medium (Dextrose 20 g L⁻¹, peptone 10 g L⁻¹, beef extract 10 g L⁻¹, yeast extract 5 g L⁻¹, tween-80 1 mL L⁻¹, sodium acetate 5 g L⁻¹, tri-ammonium citrate 2 g L⁻¹, magnesium sulphate 0.1 g L⁻¹, manganese sulphate 0.05 g L⁻¹ and di-potassium phosphate 2 g L⁻¹ pH -6.2) A loop full of *Lactobacillus plantarum* JX183220 was transferred to sterilized medium and incubated at 37°C and 100 rpm for 24 h in an orbital shaker.

Optimization of bacteriocin production: Cost effective production of bacteriocin in the present study focused on optimizing the MRS medium, replacing the carbon and nitrogen sources with cheaper sources and also the influence of sodium chloride. Molasses, baker's yeast and red lentil were tested to replace dextrose, yeast extract and peptone in the MRS medium. The parameters optimized in the preliminary studies were incubation time (1-3 days), pH (4-8), molasses concentration (25-50% v/v), baker's yeast concentration (0.3-6% w/v), red lentil concentration (0.8-1.0% and 2-6% w/v) and NaCl concentration (1-3% w/v). Molasses, red lentil and NaCl salt concentrations were chosen for further optimization by box behnken design of response surface methodology. About 2% inoculum of 24 h culture was inoculated into 100 mL of sterilized production medium and incubated at 37°C and 100 rpm for 48 h in an orbital shaker.

The samples were collected for every 24 h, centrifuged at 5000 rpm for 5 min and pH of cell free supernatant was adjusted to 5.0. The cell free supernatant was tested for bacteriocin activity by agar well diffusion assay.

Bacteriocin activity assay: Bacteriocin activity was tested by agar well diffusion assay. The indicator organism against which the activity to be tested was cultured in nutrient broth and pour plated. An aliquot of 100 µL cell free supernatant was added to the wells on the agar plate and left at room temperature for 1 h for the diffusion of bacteriocin. The plates were incubated at 37°C for 24 h and diameter of the inhibition zones were measured^{12,15,16}.

The activity of the bacteriocin was calculated by the equation (LZ -LS)/V and expressed in Activity Unit (AU).

LZ = Area of the inhibition zone = πR^2

R = Radius of the clear zone (mm)

LS = Area of the well = πr^2

r = Radius of the well (mm)

V = Volume of the sample loaded in the well (mL)

AU = Millimeter square per millimeter (mm² mL⁻¹)

Table 1: Inhibitory spectra of bacteriocin on different indicator organisms

Name of the organism	Source	Sensitivity for bacteriocin and zone of inhibition (mm)
<i>Escherichia coli</i>	King George hospital	+(12)
<i>Escherichia coli</i>	Indus hospital	-
<i>Bacillus cereus</i>	NCIM 2155	+(14)
<i>Bacillus subtilis</i>	NCIM 2010	+(12)
<i>Staphylococcus aureus</i>	NCIM 2079	-
<i>Staphylococcus aureus</i>	King George hospital	-
<i>Pseudomonas aeruginosa</i>	NCIM 2036	+(14)
<i>Pseudomonas aeruginosa</i>	King George hospital	+(12)
<i>Proteus vulgaris</i>	King George hospital	+(11)
<i>Klebsiella pneumonia</i>	Indus hospital	+(13)

The antimicrobial activity of bacteriocin produced by *Lactobacillus plantarum* JX183220 was tested using agar well diffusion assay against different indicator organisms to select indicator organism (Table 1).

RESULTS AND DISCUSSION

The present study aims at production of the bacteriocin with a low cost production medium using new strain *Lactobacillus plantarum* JX183220 isolated from the goat milk.

Selection of indicator organisms for the antimicrobial assay of bacteriocin:

Different micro organisms collected from NCIM and local hospital laboratories (King George Hospital (KGH), Visakhapatnam and Indus Hospital, Viskahapatnam) were screened to select an indicator organism for further studies. The results showed that organisms, *Bacillus cereus* (NCIM 2155), *Bacillus subtilis* (NCIM 2013), *Escherichia coli* (KGH), *Pseudomonas aeruginosa* (NCIM 2036), *Pseudomonas aeruginosa* (KGH) and *Klebsiella pneumonia* (Indus) are inhibited by the bacteriocin activity. While, *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (Indus) and *Staphylococcus aureus* (KGH) did not show inhibitory response for bacteriocin produced by *Lactobacillus plantarum* JX183220. The larger zones of inhibition by bacteriocin were observed against *Pseudomonas aeruginosa* (NCIM 2036), *Klebsiella pneumonia* (Indus) and *Bacillus cereus* (NCIM 2155). Among these, *Bacillus cereus* (NCIM 2155) which showed large inhibition zones was chosen as indicator organism for further studies (Table 1). Similar studies have been reported on the screening of the indicator organisms for the antimicrobial assay of bacteriocin^{9,17}.

Determination of optimum incubation period: *Lactobacillus plantarum* JX183220 showed an increase in bacteriocin activity from 24-48 h and a constant activity up to 72 h of

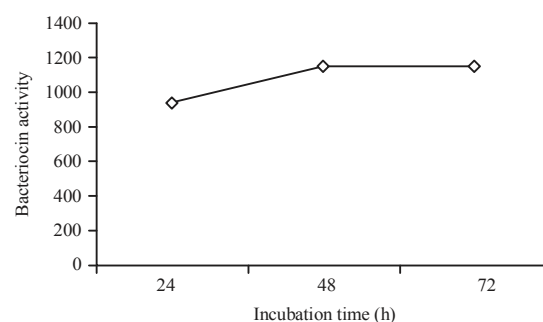


Fig. 1: Effect of incubation time on bacteriocin activity

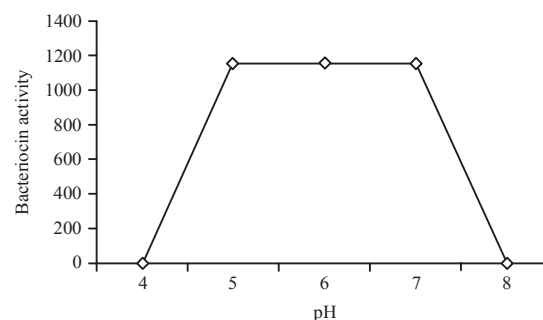


Fig. 2: Effect of pH on bacteriocin activity

incubation when tested against *Bacillus cereus*. Hence, incubation time of 48 h was maintained for bacteriocin production in the further studies (Fig. 1).

Determination of optimum pH: The activity of the bacteriocin also depends upon the pH of the production media. The results showed that a similar and maximum bacteriocin activity at the pH of 5.0, 6.0 and 7.0. Bacteriocin activity was not observed at pH of 4.0 and 8.0. The pH value of 6.0 was selected for the bacteriocin production by *Lactobacillus plantarum* JX183220 in further studies (Fig. 2).

Determination of range of NaCl concentration: Salt concentration helps in increase in biomass of microorganisms in the production media and also plays an important role in

the bacteriocin production¹⁰. In the present study, influence of NaCl concentrations of range 1.0-3.0 g L⁻¹ on bacteriocin activity was tested. The increase in concentration of NaCl showed an increase in the bacteriocin activity and highest activity was observed at 2.5 g L⁻¹ of NaCl. From the results it is evident that the NaCl concentration had influenced the bacteriocin activity which was considered to be optimized and the concentration range of 2.0-4.0 g L⁻¹ was selected for the box-behnken design (Table 2).

Determination of range of molasses concentration: Dextrose in the MRS medium was successfully replaced by molasses. The results showed that an increase in bacteriocin activity from 25-33.3% and a decrease from 41.6-50% of molasses concentration (v/v %). Highest activity was observed at

Table 2: Effect of salt (NaCl) concentration on bacteriocin activity

Medium constituents	Bacteriocin activity (AU)
Standard MRS without NaCl	1266.45
MRS+1.0 g L ⁻¹ NaCl	1266.45
MRS+1.5 g L ⁻¹ NaCl	1266.45
MRS+2.0 g L ⁻¹ NaCl	1382.34
MRS+2.5 g L ⁻¹ NaCl	1625.7
MRS+3.0 g L ⁻¹ NaCl	1625.7

Table 3: Effect of molasses on the bacteriocin activity

Medium constituents (v/v %)	Bacteriocin activity (AU)
Standard MRS with dextrose	1266.40
MRS without dextrose+25% molasses	1625.81
MRS without dextrose+33.33% molasses	1884.90
MRS without dextrose+41.66% molasses	1884.90
MRS without dextrose+50% molasses	1625.81

Table 4: Effect of baker's yeast concentration on bacteriocin activity

Medium constituents (%)	Bacteriocin activity (AU)
Standard MRS with yeast extract	1154.5
MRS (without yeast extract)+0.3% baker's yeast	-
MRS (without yeast extract)+0.5% baker's yeast	-
MRS (without yeast extract)+0.7% baker's yeast	746.12
MRS (without yeast extract)+2.0% baker's yeast	565.48
MRS (without yeast extract)+4.0% baker's yeast	942.47
MRS (without yeast extract)+6.0% baker's yeast	942.47

Table 5: Effect of red lentil concentration on bacteriocin activity

Medium constituents (%)	Bacteriocin activity (AU)
Standard MRS with peptone	1154.58
MRS (without peptone)+0.2% red lentil	1154.58
MRS (without peptone)+0.4% red lentil	1154.58
MRS (without peptone)+0.8% red lentil	1154.58
MRS (without peptone)+1.0% red lentil	1266.4
MRS (without peptone)+1.2% red lentil	1266.4

Table 6: Combination effect of red lentil with molasses and NaCl on bacteriocin activity

Medium composition	Bacteriocin activity (AU)
Standard MRS with peptone, dextrose and without NaCl	1154.58
MRS without peptone and dextrose+2 g L ⁻¹ NaCl+33.3 v/v % molasses+2% red lentil	1154.58
MRS without peptone and dextrose+2 g L ⁻¹ NaCl+33.33 v/v % molasses+4% red lentil	1266.45
MRS without peptone and dextrose+2 g L ⁻¹ NaCl+33.33 v/v % molasses+6% red lentil	1382.30

two different concentrations, 33.3 and 41.6%. So, the concentrations of 25, 41.6 and 58.33% were taken for the box-behnken design of response surface methodology (Table 3).

Determination of range of baker's yeast concentration:

Different concentrations of baker's yeast were tested replacing yeast extract in MRS medium. From results it is evident that the bacteriocin activity was found to be low in baker's yeast medium compared to the activity of standard MRS medium, though activity showed some increase with concentration. The studies revealed that baker's yeast was not effective in replacing the yeast extract in the MRS medium. So, yeast extract was retained in the MRS medium without replacing with baker's yeast in further studies (Table 4).

Determination of range of red lentil concentration:

Different concentrations of red lentil in place of peptone were used to test its effect on bacteriocin activity. Red lentil effectively replaced the peptone and showed an increase in its activity from 0.8-1.0%. So, red lentil was taken as a substitute of peptone and further studies were carried by increasing the concentration of red lentil (Table 5).

Determination of combination effect of red lentil with molasses and NaCl:

Combination effect of the three variables i.e., molasses, NaCl and red lentil was tested by varying the red lentil concentration. Bacteriocin showed an increase in activity with the increase in concentration of red lentil in combination with molasses and NaCl. The highest activity was observed at 6% red lentil with molasses and NaCl. So, the red lentil concentration range of 2-6% was used for box-behnken design (Table 6).

Application of box-behnken design: For optimizing a typical process with 3 variables at 3 levels box-behnken design of RSM is widely used. The three chosen variables are coded according to the relation:

$$x = (X - X_0) / \Delta X \quad (1)$$

where, x is coded variable, X is natural variable, X₀ is the middle point (zero level) and ΔX is the step change that represents the difference between the successive levels.

Table 7: Range of process variables for box-behnken design

Variable factors	Lower level (-1)	Middle level (0)	Upper level (+1)	Step change ΔX = Difference between levels
Molasses concentration (v/v %)	25	41.66	58.33	16.66
Red lentil concentration (%)	2	4	6	2
NaCl concentration (g L ⁻¹)	2	3	4	1

Table 8: Box-behnken design for maximizing bacteriocin activity

Run No.	Coded variables			Natural variables			Bacteriocin activity (Response)		
	x_1	x_2	x_3	X_1 (v/v)	X_2 (%)	X_3 (g L ⁻¹)	y_{exp} (AU)	$\log(y_{exp})$	$\log(y_{cal})$
1	-1	-1	0	25.00	2	3	942.47	6.48	6.87
2	-1	1	0	41.66	6	3	1046.50	6.953	6.932
3	1	-1	0	58.33	2	3	1502.07	7.315	7.336
4	1	1	0	58.33	6	3	842.33	6.736	6.797
5	-1	0	-1	25.00	4	2	1382.30	7.232	7.377
6	-1	0	1	25.00	4	4	842.33	6.736	6.673
7	1	0	-1	58.33	4	2	1382.30	7.232	7.294
8	1	0	1	58.33	4	3	1502.70	7.315	7.169
9	0	-1	-1	41.66	2	2	1625.80	7.394	7.31
10	0	-1	1	41.66	2	3	942.40	6.484	6.973
11	0	1	-1	41.66	6	2	1502.00	6.315	7.19
12	0	1	1	41.66	6	4	746.12	6.615	
13	0	0	0	41.66	4	3	1625.80	7.394	7.394
14	0	0	0	41.66	4	3	1625.80	7.394	7.394
15	0	0	0	41.66	4	3	1625.80	7.394	7.394

Table 9: Regression coefficients, their statistics and analysis of variance

Table 3: Regression coefficients, their statistics and analysis of variance					
Coefficient	Standard error	t-stat	p-value		
7.39370	0.083063	89.014	3.39E-09		
0.10344	0.050865	2.0336	0.09766		
-0.09829	0.050865	-1.9324	0.11115		
-0.20716	0.050865	-4.0727	0.00960		
-0.17081	0.071934	-2.3745	0.06359		
0.14461	0.071934	2.0103	0.10061		
-0.03859	0.071934	-0.5365	0.61460		
-0.17255	0.074872	-2.3046	0.06937		
-0.25807	0.074872	-3.4469	0.01829		
-0.09274	0.074872	-1.2387	0.27044		
Regression ANOVA source	df	SS	MS	F	p-value
Regr	9	1.0594	0.1177	5.687	0.035
Resid	5	0.1035	0.0207		
Total	14	1.1629			

Molasses concentration (X_1 , v/v %), red lentil concentration (X_2 , %) and NaCl (X_3 , g L⁻¹) were chosen as the three independent variables and the bacteriocin activity (y , g L⁻¹) was taken as the dependent variable and the range of the process variables is indicated in Table 7. Using the box-behnken method, 15 sets of experiments with appropriate combinations of molasses, red lentil and NaCl were conducted (Table 8).

The relation between the response (bacteriocin activity) with the three variables is represented by the following quadratic equation:

$$y \text{ (Bacteriocin activity)} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_1x_3 + b_6x_2x_3 + b_7x_1^2 + b_8x_2^2 + b_9x_3^2 \quad (2)$$

The experimental values of bacteriocin activity were listed in the 8th column of Table 8. Using MATLAB function 'Regstats', the following coefficients were estimated for Eq. 2:

$$y = 1625.8 + 127x_1 - 109.44x_2 - 232.37x_3 - 191x_1x_2 + 165.09x_1x_3 - 18.132x_2x_3 - 234.58x_1^2 - 307.89x_2^2 - 113.78x_3^2 \quad (3)$$

Optimization of the above equation resulted in the following values:

Coded scale	Natural scale
$x_{1 \text{ opt}} = -0.2385$	$X_{1 \text{ opt}} = 37.6842$ v/v % (molasses)
$x_{2 \text{ opt}} = -0.014193$	$X_{2 \text{ opt}} = 3.97161\%$ (Red lentil)
$x_{3 \text{ opt}} = -1.3007$	$X_{3 \text{ opt}} = 1.69931$ g L ⁻¹ (NaCl)
$y_{\text{opt}} = 1838.5195$ AU	

The estimated coefficients, their t-statistics and probability values along with analysis of variance (ANOVA) are summarized in Table 9. The model is highly significant as is evident from the model F-value and a very low probability value (P model: 0.05). The goodness of the model can be checked by the R² statistic, which is 0.911, suggesting that 91.1% total variation for bacteriocin activity is represented by the model.

The three dimensional response surface plots relating the bacteriocin activity with the three process variables were generated by MATLAB software using Eq. 3 and are presented in Fig. 3a-c. The shapes of the contours plot, circular or

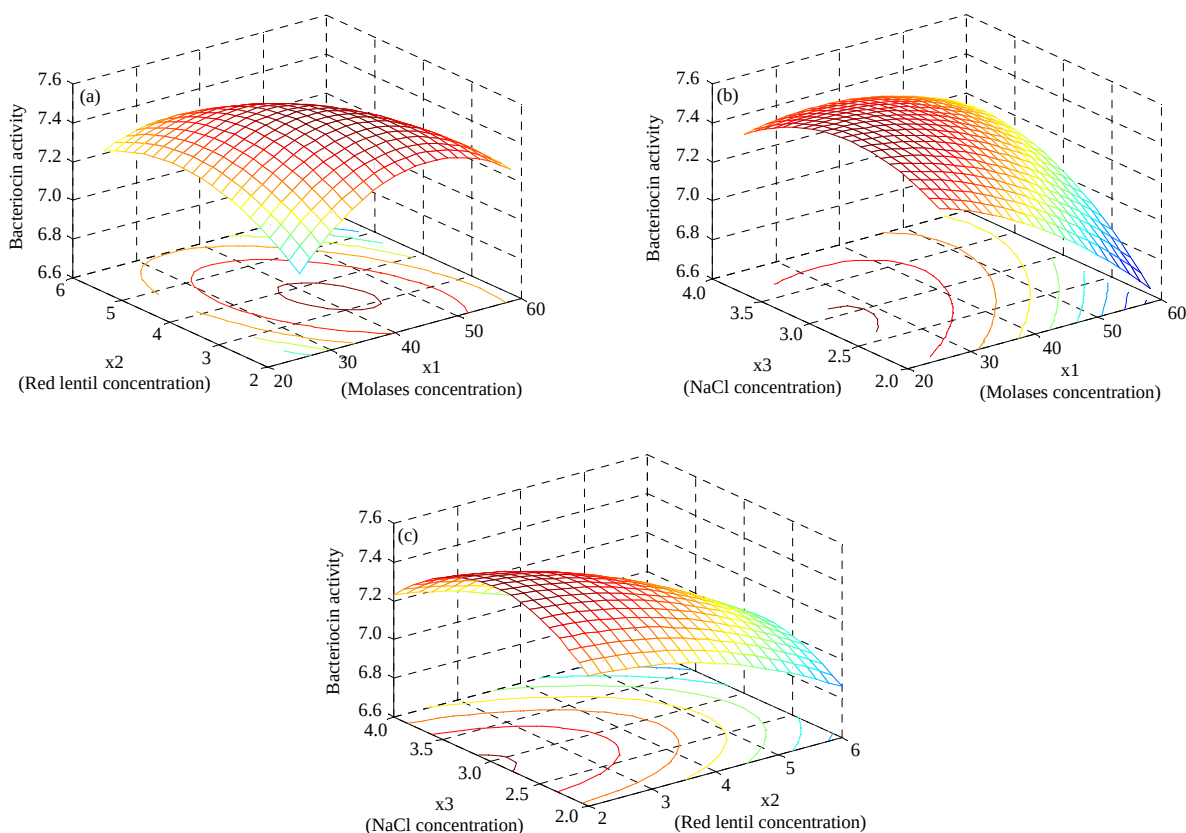


Fig. 3(a-c): 3-D surface plot of bacteriocin activity versus (a) Molasses and red lentil, (b) Molasses and NaCl concentrations and (c) Red lentil and NaCl concentrations

elliptical, indicate whether the mutual interactions between the variables are significant or not. A circular contour plot indicates that the negligible interactions between the variables. In contrast, elliptical plots are evidence of significant interactions¹⁸. The maximum response falls within the design boundary for all the figures. Figure 3a shows the response surface of bacteriocin activity when NaCl concentration is fixed at its optimum value and the other two variables (molasses and red lentil) vary in the experimental domain. As evident from the Fig. 3a, maximum bacteriocin activity was obtained from the center point of inner most contour (initial molasses concentration: 37.6842% and red lentil concentration: 3.97161%). At other concentrations of these two variables, bacteriocin activity decreased. Figure 3b shows the response surface plot of bacteriocin activity with respect to the combined effect of molasses concentration and NaCl concentration when red lentil concentration is fixed at its optimum value. From the Fig. 3b, maximum bacteriocin activity was obtained from the center point of inner most contour (initial molasses concentration v/v: 37.6842% and NaCl concentration: 1.69931 g L⁻¹). At other concentrations of these two variables, bacteriocin activity decreased.

Figure 3c shows the combined effect of red lentil concentration and NaCl concentration on bacteriocin activity when the third variable is kept constant. From the Fig. 3c, maximum bacteriocin activity was obtained from the center point of inner most contour (initial red lentil concentration: 3.97161% and NaCl concentration: 1.69931 g L⁻¹). At the other concentrations of these two variables, bacteriocin activity decreased. As evident from contour plots (the horizontal projections of Fig. 3a-c, the optimal values of the parameters conform to those values obtained from Eq. 3. The optimum values obtained from the box behnken design shows that the concentration of molasses and red lentil were nearer to the mid value of the fixed range, while the concentration of NaCl was found to be less compared to the range. The results infer that there might be an interaction effect when the different concentrations of molasses and red lentil were combined. Similar studies were carried out by Han *et al.*¹⁰ and Herranz *et al.*¹⁹. The present study showed that the molasses and red lentil efficiently replaced the dextrose and peptone, respectively, making the medium cheaper and cost effective for the production of bacteriocin.

The results obtained by the application of RSM were in good agreement with the previous results. Usmiati and Marwati¹² obtained a maximum bacteriocin activity of 638.803 mm² mL⁻¹ on *S. typhimurium*, 623.264 mm² mL⁻¹ on *E. coli* and 509.434 mm² mL⁻¹ on *L. monocytogenes*.

Validation of the model: To validate the maximum point, experiments using the best concentration of molasses, red lentil and NaCl were performed. The experimental values for bacteriocin activity were closer to the predicted values there by validating the model. So, the validity of the Eq. 3 and hence the application of RSM are justified.

CONCLUSION

A three-factor-three-level box-behnken design of RSM was employed in the present investigation to obtain the optimized production of bacteriocin by *L. plantarum* JX183220, isolated from goat milk. From a set of 15 experimental runs, the optimal molasses concentration, red lentil concentration and NaCl were found to be 37.7 v/v%, 3.97% and 1.69 g L⁻¹, respectively for the Bacteriocin activity of 1839 AU which is comparable with the literature values. The box-behnken design of RSM was found to be a useful tool for optimizing the biomass production with minimum resources and time. Molasses and red lentil are proved to be potential cheaper sources to replace the expensive carbon and nitrogen sources in the MRS medium for the production of bacteriocin from isolated *Lactobacillus plantarum* JX183220.

ACKNOWLEDGMENT

Authors are thankful to the management of ANITS, Visakhapatnam, India for providing infrastructural facilities to carry out this research study.

REFERENCES

- Chen, H. and D.G. Hoover, 2003. Bacteriocins and their food applications. *Comprehen. Rev. Food Sci. Food Saf.*, 2: 82-100.
- Joerger, R.D., D.G. Hoover, S.F. Barefoot, K.M. Harmon, D.A. Grinstead and C.G.N. Cutter, 2000. Bacteriocins. In: *Encyclopedia of Microbiology*, Volume 2, Lederberg, J. (Ed.). Academic Press, San Diego, USA., pp: 383-397.
- Appendini, P. and J.H. Hotchkiss, 2002. Review of antimicrobial food packaging. *Innov. Food Sci. Emerg. Technol.*, 3: 113-126.
- Jamuna, M. and K. Jeevaratnam, 2004. Isolation and characterization of lactobacilli from some traditional fermented foods and evaluation of the bacteriocins. *J. Gen. Applied Microbiol.*, 50: 79-90.
- De Vuyst, L. and F. Leroy, 2007. Bacteriocins from lactic acid bacteria: Production, purification and food applications. *J. Mol. Microbiol. Biotechnol.*, 13: 194-199.
- Von Mollendorff, J.W., S.D. Todorov and L.M.T. Dicks, 2009. Optimization of growth medium for production of bacteriocins produced by *Lactobacillus plantarum* JW3BZ and JW6BZ and *Lactobacillus fermentum* JW11BZ and JW15BZ isolated from Boza. *Trakia J. Sci.*, 7: 22-33.
- Todorov, S.D., 2008. Bacteriocin production by *Lactobacillus plantarum* AMA-k isolated from Amasi, a Zimbabwean fermented milk product and study of the adsorption of bacteriocin AMA-K to *Listeria* sp. *Braz. J. Microbiol.*, 39: 178-187.
- Mall, P., B.K. Mohanty, B.S. Tunga and R. Tunga 2011. Optimization of fermentation process parameter for Nisin production from *Lactococcus lactis* (MTCC-440) using a statistical experimental design technique. *Global J. Biotechnol. Biochem.*, 6: 13-24.
- Todorov, S.D. and L.M.T. Dicks, 2005. Effect of growth medium on bacteriocin production by *Lactobacillus plantarum* ST194BZ, a strain isolated from boza. *Food Technol. Biotechnol.*, 43: 165-173.
- Han, B., R. Zhang, Z. Yu, B. Liu and Q. Ma, 2011. Optimization of bacteriocin production by *Lactobacillus plantarum* YJG, isolated from the mucosa of the gut of healthy chickens. *Afr. J. Microbiol. Res.*, 5: 1147-1155.
- Verluyten, J., F. Leroy and L. de Vuyst, 2004. Influence of complex nutrient source on growth of and curvacin a production by sausage isolate *Lactobacillus curvatus* LTH 1174. *Applied Environ. Microbiol.*, 70: 5081-5088.
- Usmiati, S. and T. Marwati, 2009. Selection and optimization process of bacteriocin production from *Lactobacillus* sp. *Indonesian J. Agric.*, 2: 82-92.
- Sridevi, V., 2012. Isolation of Lactic acid bacteria with probiotic potential from goat milk and cow milk. Genbank Submission No. JX183220, *Lactobacillus plantarum*, Partial Sequence of 16s rRNA Gene. <http://www.ncbi.nlm.nih.gov/nuccore/Jx183220>
- Sridevi, V., R. Sirisha and S.N. Swapna, 2015. Screening of probiotic goat milk and cow milk isolates for acid resistance, antagonistic activity and tolerance to antimicrobial activity of spices: Molecular identification of potential probiotic goat milk isolate, G8. *Int. J. Curr. Microbiol. Applied Sci.*, 4: 406-421.
- Joshi, V.K., S. Sharma and N.S. Rana, 2006. Production, purification, stability and efficacy of bacteriocin from isolates of natural lactic acid fermentation of vegetables. *Food Technol. Biotechnol.*, 44: 435-439.

16. Rajaram, G., P. Manivasagan, B. Thilagavathi and A. Saravanakumar, 2010. Purification and characterization of a bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. *Adv. J. Food Sci. Technol.*, 2: 138-144.
17. Ogunbanwo, S.T., A.I. Sanni and A.A. Onilude, 2003. Influence of cultural conditions on the production of bacteriocin by *Lactobacillus brevis* OG1. *Afr. J. Biotechnol.*, 27: 179-184.
18. Montgomery, D., 2012. Design and Analysis of Experiments. 8th Edn., John Wiley and Sons, New York, USA.
19. Herranz, C., M.J. Martinez, J.M. Rodriguez, P. Hernandez and L. Cintas, 2001. Optimization of enterocin P production by batch fermentation of *Enterococcus faecium* P13 at constant pH. *Applied Microbiol. Biotechnol.*, 56: 378-383.