J Food Sci Technol (May–June 2010) 47(3):258–265
DOI: 10.1007/s13197-010-0040-2

ORIGINAL ARTICLE

# Simplification and optimization of deMan Rogosa Sharpe (MRS) medium for enhanced production of bacteriocin by *Weissella paramesenteroides* DFR-8

**Ajay Pal · Ramana K. V. · Bawa A. S.**

Revised: 21 September 2009 / Accepted: 28 September 2009


**Abstract** Complex growth medium such as deMan Rogosa Sharpe (MRS) medium, commonly used for cultivation of fastidious lactic acid bacteria (LAB) interfere in bacteriocin purification. Sometimes all the ingredients of a defined medium are not required by all LAB strains for bacteriocin production. In the present study, composition of the MRS medium for the production of bacteriocin by *Weissella paramesenteroides* DFR-8, an isolate from cucumber (*Cucumis sativus*), was simplified and optimized with a step-wise strategy. In the first step, production profile, effect of incubation temperature, various C and N sources were investigated. In the second step, central composite rotatable design was employed to decide the optimal concentration of 3 key components (glucose, tryptone and pH) and the experimental results were fitted with a second order polynomial regression equation. According to the set criteria, the predicted bacteriocin titer from a medium containing 7.99% glucose, 9% tryptone, pH 7.5 (91.9% desirability) was 540 AU/ml and the observed bacteriocin titer was 538 AU/ml that indicated the validity of the developed model. Using optimized medium, bacteriocin titer of 674.5 AU/ml could be achieved after 72 h of fermentation that is nearly 2.5 fold higher than that obtained from unmodified MRS medium.

**Keywords** Bacteriocin · Biopreservation · Optimization · *Weissella paramesenteroides*

Pal A. · Ramana K. V. · Bawa A. S.
Food Biotechnology Discipline,
Defence Food Research Laboratory,
Mysore - 570 011, India

Pal A. (✉)
E-mail: ajaydrdo@rediffmail.com

## Introduction

Lactic acid bacteria (LAB) produce a wide variety of antagonistic factors that include metabolic end-products such as lactic acid, antibiotic like substances and antimicrobial proteins or bacteriocins (Sudha et al. 2006, Delgado et al. 2007). In recent years, greater attention is being drawn towards application of bacteriocins as food biopreservative because they are innocuous due to their proteolytic degradation in gastrointestinal tract (Dominguez et al. 2007). Bacteriocins are small peptides with bactericidal or bacteriostatic activity against genetically closely related species. However, an exception to the rule i.e. activity against a range of Gram-negative bacteria has been reported (Jamuna and Jeevaratnam 2004).

The most important aspects in the bacteriocin study are production and purification. Most often, production of bacteriocin is very low and the complex growth media commonly used for bacteriocin production interfere in its purification (Mackay et al. 1997). Sometimes all the ingredients of a complex medium are not required for the production of bacteriocins. Therefore, optimization of growth conditions is very important for enhancing bacteriocin production.

Optimization of medium by the classical method involves changing one independent variable at a time while keeping others at a fixed level. This is extremely time consuming and expensive for a large number of variables (Adinarayana et al. 2003) and also may result in wrong conclusion (Oh et al. 1995). Response surface methodology (RSM) is the application of statistical techniques for designing experiments, building models, and evaluating the effects of factors for desirable response (Li et al. 2001, Puri et al. 2001). Hence, a combination of 'one-at-a-time' approach and RSM is well suited for the study of main and interaction effects of distinct factors in bacteriocin production.

*Weissella paramesenteroides* DFR-8 is a previously characterized bacterium isolated from cucumber (*Cucumis sativus*). As reported by us earlier, this strain produces

thermostable bacteriocin (Pal and Ramana 2009b) and non-bacteriocin antimicrobial compounds (Pal and Ramana 2009a) having broad spectrum of activity including against Gram-negative organisms which indicates their potential to be used as biopreservative in foods. The purpose of this study was to simplify the MRS medium, commonly used for the cultivation of LAB, for bacteriocin production from *W. paramesenteroides* DFR-8 using 'one-at-a-time' approach and then to optimize the key factors by using RSM so that it will be useful for further downstream processing of bacteriocin.

## Materials and methods

*Bacterial strains*: *Weissella paramesenteroides* DFR-8, the producer strain of bacteriocin was isolated from cucumber (*Cucumis sativus*). The strain was identified by sequencing the 708 bases of 16S rDNA gene in forward direction followed by BLAST homology search. The nucleotide sequences have been deposited with NCBI database under accession number FJ390112. The indicator strain used was *Staphylococcus aureus* MTCC 737.

*Effect of cultivation conditions on growth and optimization of bacteriocin production*: To study the optimum incubation time for the maximum production of bacteriocin, a series of 100 ml MRS media, pH 6.5 were inoculated with 1% v/v of young culture of strain under study and incubated statically at 35°C. At different incubation period, cultures were examined for viable LAB counts, pH and antibacterial activity. To study the effect of temperature on bacteriocin production, another set of flasks were incubated for 60 h at temperatures of 25–40°C and antibacterial activity was checked.

*Effect of C and N sources*: To test the influence of C sources, each C source (fructose, maltose, lactose, sorbitol, galactose, xylose and sucrose procured from Himedia, Mumbai) was added at 2% level to MRS medium, replacing 2% glucose. To study the effect of N sources, a modified MRS medium was used as a basal medium where yeast extract, beef extract, peptone and ammonium citrate were omitted. The basal medium was supplemented with each N source (peptone, casein peptone, $NH_4Cl$, soya peptone, yeast extract, tryptone, $(NH_4)_2SO_4$, beef extract, proteose peptone, urea and ammonium citrate procured from Himedia, Mumbai) at 2% level.

*Central composite rotatable design (CCRD)*: After selection of the growth substrate (C and N) by 'one-at-a-time' approach, the next step was to define the optimum conditions for bacteriocin production. Based on the result of Plackett-Burman Screening Design (data not shown), 3 variables chosen in this experiment were C source (glucose), N source (tryptone) and initial pH. Taking the above factors into consideration, CCRD based on 5 levels and 3 variables (glucose, tryptone and pH) was used to study their combined influence on bacteriocin production. The design consisted of 20 experiments with 8 factorial points, 6 axial points with α = 1 .682 and 6 center points for replication. In developing the regression equation, the test factors were coded according to the following equation:

$$x_i = X_i - X_0 / \delta X_i$$

where, $x_i$ is the dimensionless coded value of the *i*th independent variable; $X_i$ the natural value of the *i*th independent variable; $X_0$ the natural value of the *i*th independent variable at the center point and $\delta X_i$ the step-change value. The experimental results were fitted with a 2nd order polynomial function-

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

where, $Y$ is the predicted response, $b_0$ the intercept, $b_1, b_2, b_3$ the linear co-efficients, $b_{11}$, $b_{22}$, $b_{33}$ the squared co-efficients and $b_{12}, b_{13}, b_{23}$ the interaction co-efficients.

*Bacteriocin assay:* The antimicrobial activity of bacteriocin in culture medium was measured using the following procedure. The production medium (100 ml in 250 ml Erlenmeyer flask) was inoculated as described earlier and incubated for 60 h. After incubation, samples were heated at 70°C for 20 min to inactivate proteases and centrifuged. The cell free supernatant was treated with catalase (CAT) and lactate dehydrogenase (LDH) to remove $H_2O_2$ and lactic acid and concentrated to one-tenth of the original volume in a rotary vacuum evaporator. The pH of concentrate was adjusted to 6.5 and filter sterilized by passing through 0.22 μm pore size. The antibacterial activity was assayed according to agar well diffusion assay (Tagg and McGiven 1971) against *S. aureus*. Bacteriocin activity was expressed as AU/ml and defined as the reciprocal of the highest dilution showing a distinct zone of inhibition (Kim et al. 2000). The average bacteriocin activity of 3 independent experiments carried out in duplicates was taken as the dependent variable or response.

*Glucose estimation*: Residual glucose in modified medium was estimated using the glucose oxidase/peroxidase assay (Joshi et al. 1987).

*Data analysis:* Design expert 7.1.4 (Stat-Ease, Inc., Minneapolis, USA) was used for the regression analysis of the experimental data obtained. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, $R^2$, and its statistical significance was checked by Fisher's F-test. The significance level of each regression co-efficient was determined by Student's t-test. The level of significance was given as p-value.

## Results and discussion

*Effect of cultivation conditions on growth and optimization of bacteriocin production*: Growth and bacteriocin production time courses were determined in 250 ml flasks containing 100 ml media. The production of bacteriocin started at 12 h of incubation showing its primary metabolite kinetics. Fig. 1 shows that *W. paramesenteroides* DFR-8 grew and

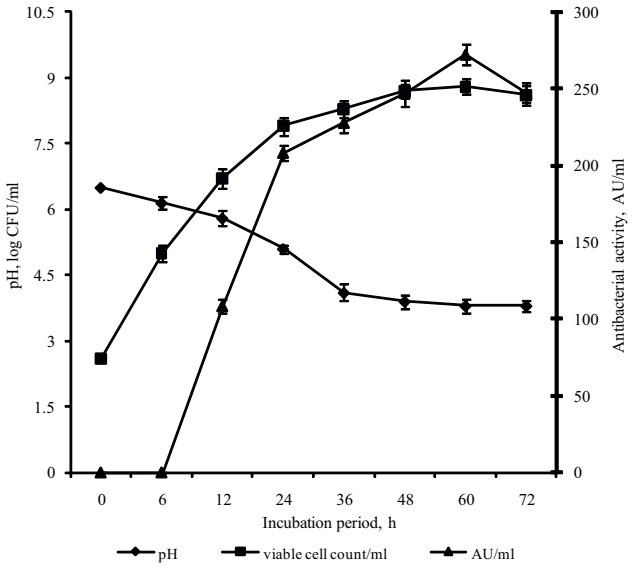


**Fig. 1** Effect of duration of incubation at 35$^0$C on the viable cell count, pH and antibacterial activity of *W. paramesenteroides* DFR-8 (n = 6)

produced acid at high rates between 12 and 36 h as indicated by increase in viable cell count and decrease in pH. Maximum yield of bacteriocin was during late logarithmic growth phase (at 60 h). These results suggest that a terminal pH < 5.5 along with a large cell density facilitates a high level of bacteriocin production. The data also implies that production of bacteriocin is associated with growth but is not apparently proportional to the growth rate or cell concentration. The prolonged incubation time (>60 h) perhaps led to digestion/degradation of bacteriocin by proteases co-secreted by producer organism in the medium thereby decreasing the bacteriocin titer. Most authors have noted that good cell growth frequently goes hand in hand with bacteriocin production (de Vuyst et al. 1996, Cabo et al. 2001). This is normally observed for bacteriocins of LAB. High correlations between biomass production and bacteriocin biosynthesis were reported for *Brevibacterium linens* (Motta and Brandelli 2003) and *Lactococcus lactis* (de Vuyst 1995). Nevertheless, optimal cell growth does not always result in high bacteriocin titer.

Fig. 2 shows that inhibitory activity was detected when the producer strain was incubated at 25, 30, 35 and 40°C, but maximum titer of inhibitory activity was obtained at 35°C. The optimal temperature for bacteriocin production was the same in the case of *Micrococcus* sp. GO5 (Kim et al. 2006), but differences in optimal temperatures were reported for organisms producing nisin and nisin-like bacteriocins (Matsusaki et al. 1996, Cheigh et al. 2002).

*Effect of C and N sources*: Glucose instigated highest bacteriocin production compared to other C sources (Fig. 3). Disaccharides, sugar alcohol (sorbitol) and pentose (xylose) were also utilized by bacteria but to a lesser extent thereby decreasing the bacteriocin production. The obser-

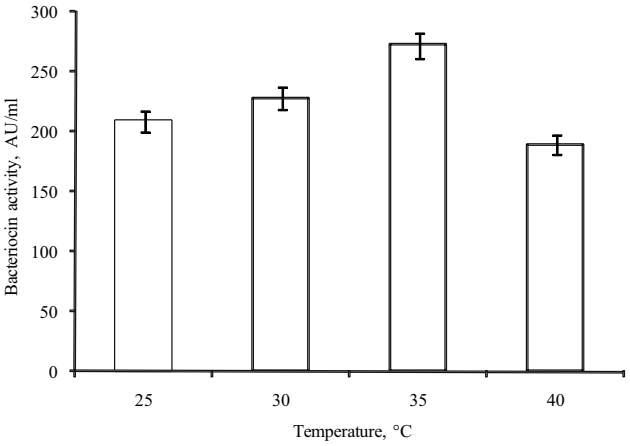


**Fig. 2** Effect of incubation temperature on bacteriocin production by *W. paramesenteroides* DFR-8 (n = 6)

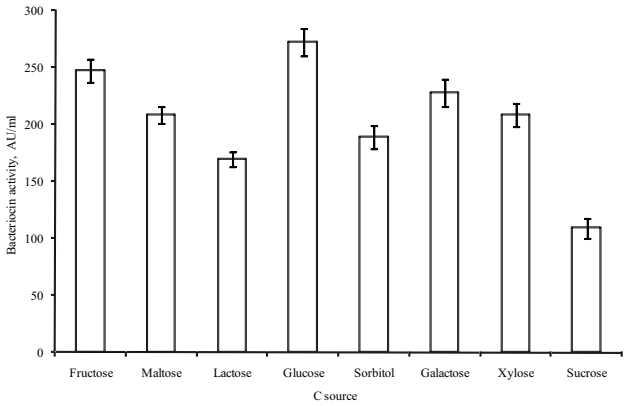


**Fig. 3** Effect of different C sources on bacteriocin production by *W. paramesenteroides* DFR-8 (n = 6)

vation agrees with earlier reports, which suggested that sources of C affected bacteriocin production (Matsusaki et al. 1996, Cheigh et al. 2002, Kim et al. 2006). Tryptone resulted in highest level of bacteriocin activity compared to other organic and inorganic N sources (Fig. 4). This result is in agreement with previous study (Kim et al. 2006) which reported an increased production of bacteriocin in the presence of tryptone. Additionally, effect of various combinations of different N sources were also tried for enhanced bacteriocin production (data not shown) but significant effect was not obtained indicating that multiple N sources were not required by the strain under study for enhanced bacteriocin titer.

*Central composite rotatable design (CCRD):* Based on 'one-at-a-time' approach and Plackett-Burman Screening Design (data not shown), glucose concentration, pH (pH is known to be important to cell growth as well as to bacteriocin production; aggregation, adsorption of bacteriocin to the cells and/or proteolytic degradation depend on pH and can affect the bacteriocin activity in culture supernatant) and tryptone concentrations were selected for further optimization. Preliminary experiments were carried out to

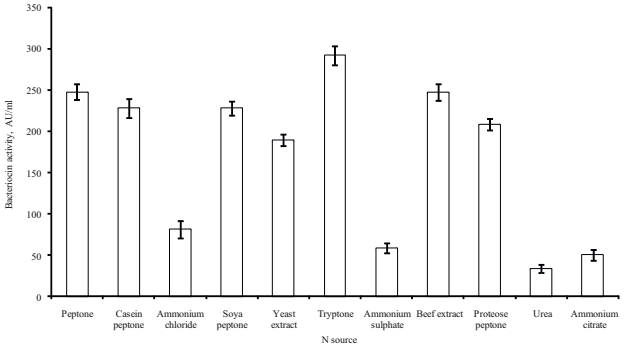


**Fig. 4** Effect of different N sources on bacteriocin production by *W. paramesenteroides* DFR-8 (n = 6)

**Table 1** Experimental domain

| Independent variables | Symbol | Range and level | | | | |
|---|---|---|---|---|---|---|
| | | –α | –1 | 0 | +1 | +α |
| Glucose, % | $x_1$ | 2.64 | 4 | 6 | 8 | 9.36 |
| pH | $x_2$ | 4.82 | 5.5 | 6.5 | 7.5 | 8.18 |
| Tryptone, % | $x_3$ | 3.64 | 5 | 7 | 9 | 10.36 |

determine the parameter range for 3 independent variables (Table 1). The results of CCRD experiments for studying the effects of 3 independent variables on bacteriocin production are represented in Table 2. Analysis of variance and Fischer's F-test showed that the value $F_{(9,10)} = 6.40$ which is greater than the tabulated value of 3.02 thereby demonstrating significance for the regression model (Table 3). The regression equation obtained indicated $R^2$ value of 0.85 (a value of $R^2$>0.75 indicates the aptness of the model). This value ensured a satisfactory adjustment of quadratic model to explain the experimental data and indicated that model could explain 85% of the variability in the response. The application of RSM yielded the following regression equation, which is an empirical relationship between bacteriocin activity and the test variables in actual units-

Bacteriocin activity (AU/ml) = 67.76 – 59.35 Glucose + 109.05 pH – 45.34 Tryptone + 3.56 Glucose$^2$ – 5.42 pH$^2$ + 2.20 Tryptone$^2$ + 1.73 Glucose X pH + 3.64 Glucose X Tryptone + 1.04 pH X Tryptone

The significance of each co-efficient was determined by Student's t-test (Table 4). The smaller the p-value and larger the t-value, the more significant is the corresponding co-efficient (Myers and Montogomery 2002). Student's t - test showed that all the linear co-efficients were significant ($p \leq 0.05$). These values suggest that these factors have a direct relationship on the production of bacteriocin. The interaction and quadratic terms were not statistically significant. The sign and magnitude of co-efficients indicate the effect of the variable on the response. Three-dimensional graphs were generated for the pair-wise combination of the three factors while keeping the other one at its centre levels

**Table 2** Experimental design and results

| Run number | Coded level | | | Response (AU/ml) | | Residual error |
|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | Observed | Predicted | |
| 1 | –1 | –1 | –1 | 291.67 | 292.00 | –0.33 |
| 2 | +1 | –1 | –1 | 341.67 | 335.24 | 6.43 |
| 3 | –1 | +1 | –1 | 447.22 | 392.32 | 54.90 |
| 4 | +1 | +1 | –1 | 477.78 | 450.50 | 27.28 |
| 5 | –1 | –1 | +1 | 316.67 | 313.86 | 2.81 |
| 6 | +1 | –1 | +1 | 391.67 | 334.54 | 57.13 |
| 7 | –1 | +1 | +1 | 447.22 | 423.60 | 23.62 |
| 8 | +1 | +1 | +1 | 569.40 | 540.02 | 29.38 |
| 9 | –α | 0 | 0 | 316.67 | 350.02 | –33.35 |
| 10 | +α | 0 | 0 | 477.78 | 485.44 | –7.66 |
| 11 | 0 | –α | 0 | 291.67 | 267.94 | 23.73 |
| 12 | 0 | +α | 0 | 391.67 | 456.94 | –65.27 |
| 13 | 0 | 0 | –α | 316.67 | 361.96 | –45.29 |
| 14 | 0 | 0 | +α | 447.22 | 449.82 | –2.60 |
| 15 | 0 | 0 | 0 | 391.67 | 377.74 | 13.93 |
| 16 | 0 | 0 | 0 | 366.67 | 377.74 | –11.07 |
| 17 | 0 | 0 | 0 | 391.67 | 377.74 | 13.93 |
| 18 | 0 | 0 | 0 | 366.67 | 377.74 | –11.07 |
| 19 | 0 | 0 | 0 | 391.67 | 377.74 | 13.93 |
| 20 | 0 | 0 | 0 | 366.67 | 377.74 | –11.07 |

(n = 6)

**Table 3** Regression analysis (ANOVA) for bacteriocin production

| Source | DF | SS | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 9 | 82536.39 | 9170.71 | 6.40 | 0.0038 |
| Residual | 10 | 14333.06 | 1433.31 | | |
| Total | 19 | 96869.45 | | | |

SS = Sum of square; DF = Degree of freedom; MS = Mean square

$R^2$ = 85.20%; Table $F_{9, 10 (1\%)} = 3.02$

**Table 4** Co-efficients of the regression equation

| Term | Co-efficient | t-value | p-value |
|---|---|---|---|
| Constant | 377.971 | 24.479 | - |
| Glucose | 40.177 | 3.922 | 0.003 |
| pH | 56.244 | 5.490 | 0.000 |
| Tryptone | 28.277 | 2.760 | 0.020 |
| Glucose$^2$ | 14.220 | 1.426 | 0.184 |
| pH$^2$ | –5.422 | –0.544 | 0.599 |
| Tryptone$^2$ | 8.818 | 0.884 | 0.397 |
| Glucose × pH | 3.467 | 0.259 | 0.801 |
| Glucose × Tryptone | 14.578 | 1.089 | 0.302 |
| pH × Tryptone | 2.078 | 0.155 | 0.880 |

for the bacteriocin production. An increase in bacteriocin yield with increase in glucose *versus* tryptone concentration (Fig. 5), pH *versus* glucose concentration (Fig. 6) and pH *versus* tryptone concentration (Fig. 7) was observed.

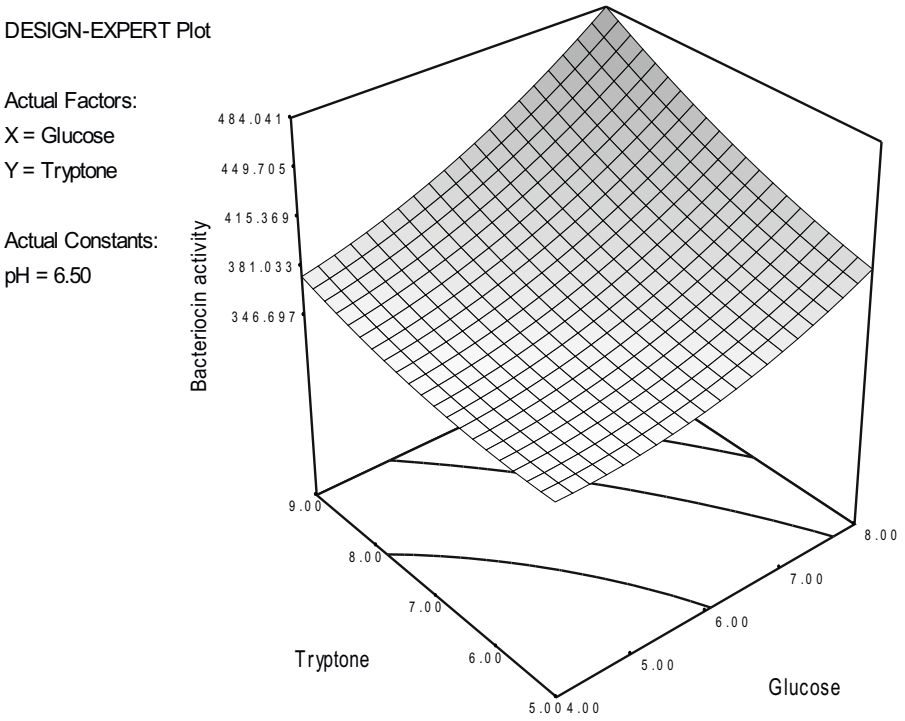


**Fig. 5** Response surface of bacteriocin production (AU/ml) by *W. paramesenteroides* DFR-8 as a function of tryptone (%) and glucose (%)

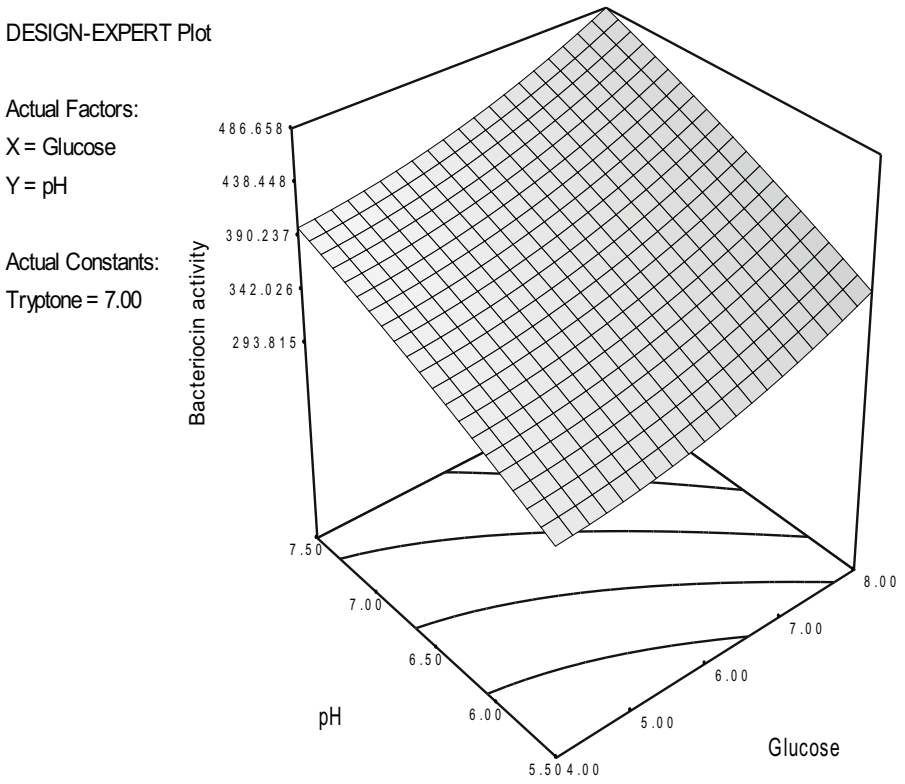


**Fig. 6** Response surface of bacteriocin production (AU/ml) by *W. paramesenteroides* DFR-8 as a function of pH and glucose (%)

The effect of variations in level of all 3 independent variables on bacteriocin production by the strain under study has been shown in the perturbation graph (Fig. 8). From the graph it can be concluded that at low pH, bacteriocin production drops down drastically. A plotting of the normal values *versus* residuals showed that data were very close to the straight line and situated at both sides of it indicating that model is fairly good (Fig. 9). Reports on the statistical optimization of bacteriocin from *W. paramesenteroides* are lacking to critically discuss our findings.

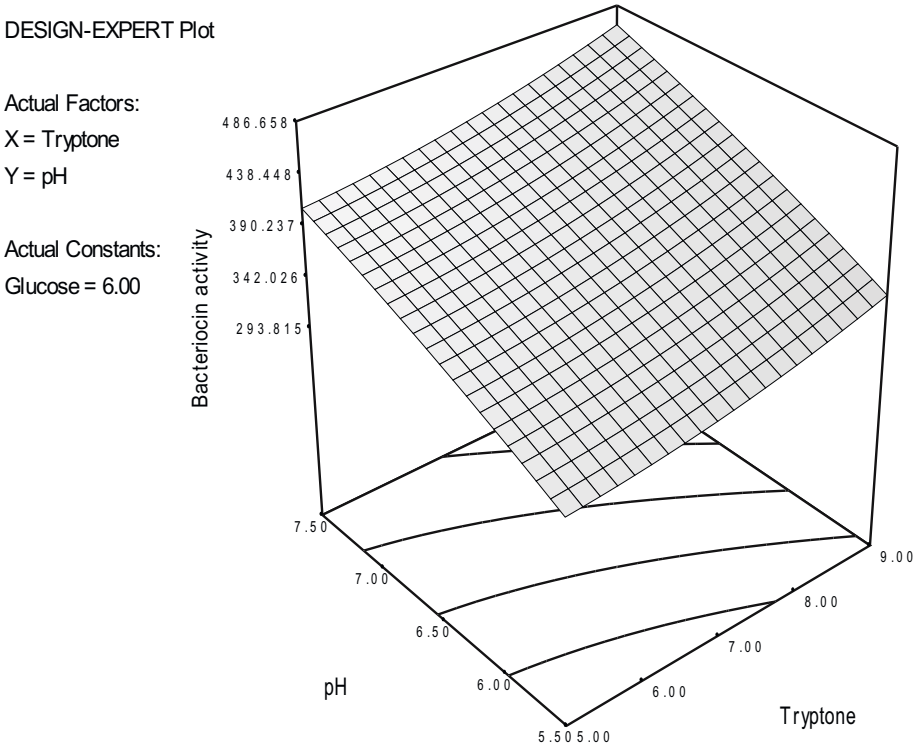


**Fig. 7** Response surface of bacteriocin production (AU/ml) by *W. paramesenteroides* DFR-8 as a function of pH and tryptone (%)

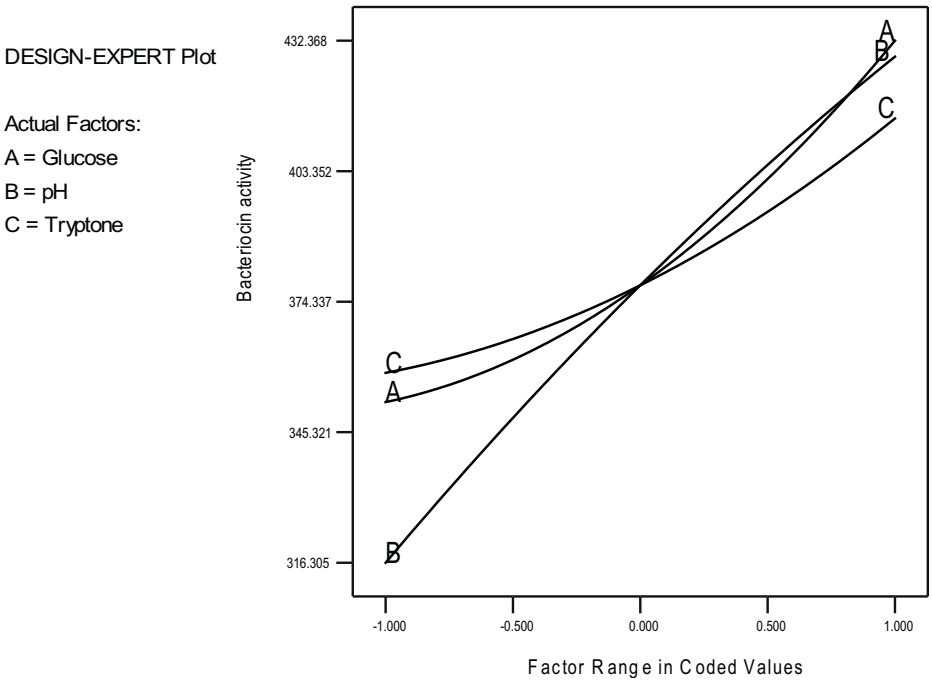


**Fig. 8** The perturbation graph showing the effect of all independent variables on bacteriocin production by *W. paramesenteroides* DFR-8

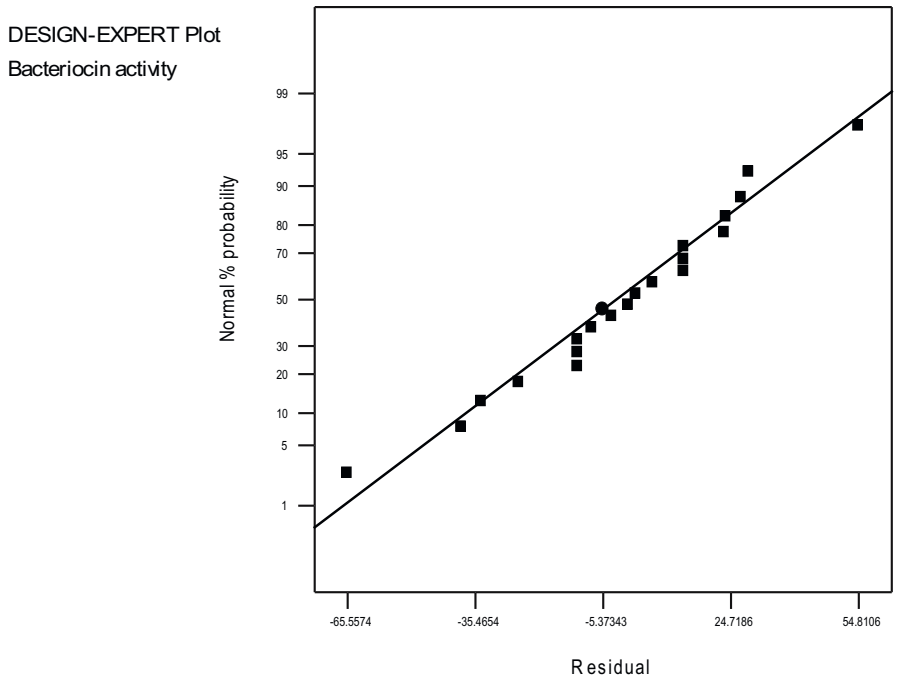


**Fig. 9** Plot between expected normal values *versus* residuals

**Table 5** Constraints, criteria for optimization, solution along with predicted and observed response value

| Constraints | Goal | Lower limit | Upper limit | Importance | Solution | Observed response* |
|---|---|---|---|---|---|---|
| Glucose, (%) | Maximize | 4 | 8 | 4 | 7.99 | - |
| pH | In range | 5.5 | 7.5 | 3 | 7.5 | - |
| Tryptone, (%) | Maximize | 5 | 9 | 4 | 9 | - |
| Activity, (AU/ml) | Target (550) | 291.67 | 569.4 | 5 | 540.19 | 538.89 ± 11.6 |

*(n=6)

*Validation of the model equation*: In order to determine the accuracy of the model, response was numerically optimized using Design-Expert software. The criteria used for optimization along with predicted and actual (observed) response value have been presented in Table 5. Our aim was to optimize the maximum level of glucose and tryptone concentration while keeping the pH value 'in range'. By using the given criteria, a solution having 91.9% desirability was selected and experiment was conducted. It was found that value of observed response (538 AU/ml) was almost equal to the predicted one (540 AU/ml) clearly proving the aptness of model.

*Bacteriocin production using modified MRS medium*: Bacteriocin production started after 12 h of fermentation in both the media and thereafter a progressive increase was recorded with incubation period (Fig. 10) with maximum of 674.5 AU/ml at 72 h in modified MRS medium. By this time, entire the glucose was utilized indicating that disposal of spent medium does not create environmental pollution. The decrease in bacteriocin titer afterward, observed to be due to its partial degradation by non-specific proteolytic enzymes. Using simplified and modified MRS medium, nearly 2.5 fold higher bacteriocin titer could be achieved. It is also important to mention that optimum incubation time

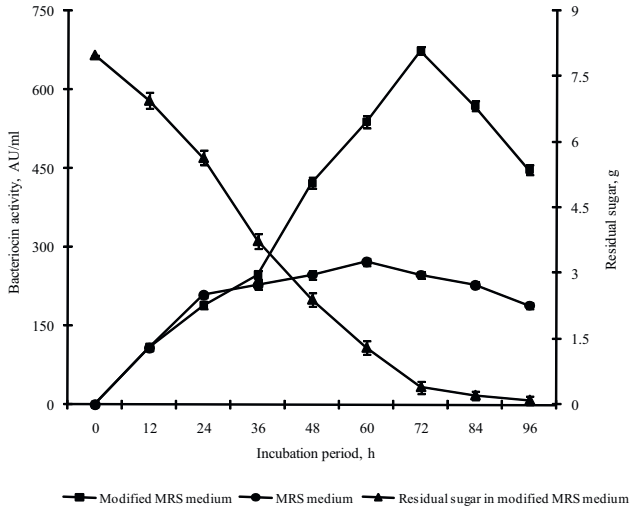


**Fig. 10** Time course behavior of bacteriocin production and glucose utilization by *W. paramesenteroides* DFR-8 grown on MRS and modified MRS medium (n = 6)

for bacteriocin production in modified medium has been shifted forward by 8 h as compared to its unmodified version. The results clearly indicate that fermentation should be stopped at 72 h for maximum recovery of bacteriocin.

## Conclusion

Conventional and statistical optimization methods were used efficiently and successfully to develop a regression equation for bacteriocin production by *W. paramesenteroides* DFR-8. The simplified and optimized MRS medium, obtained in two phases, showed nearly 2.5 fold higher bacteriocin yield and could be the starting point for scale up purposes and/or bacteriocin purification experiments.

## References


Adinarayana K, Ellaiah P, Srinivasulu B, Devi RB, Adinarayana G (2003) Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces marinensis* under solid-state fermentation. Process Biochem 38:1565–1572

Cabo ML, Murado MA, Gonzalez M, Pastoriza L (2001) Effects of aeration and pH gradient on nisin production, a mathematical model. Enz Microbial Technol 29:264–273

Cheigh CI, Choi HJ, Park H, Sim SB, Kook MC, Kim TS, Hwang JK, Pyun YR (2002) Influence of growth conditions on the production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from kimchi. J Biotechnol 95:225–235

Delgado A, Lopez FN, Brito D, Peres C, Fevereiro P, Fernandez AG (2007) Optimum bacteriocin production by *Lactobacillus plantarum* 17.2b requires absence of NaCl and apparently follows a mixed metabolite kinetics. J Biotechnol 130:193–201

de Vuyst L (1995) Nutritional factors affecting nisin production by *Lactococcus lactis* subsp. *lactis* NIZ022186 in a synthetic medium. J Appl Bacteriol 78:28–33

de Vuyst L, Callewaert R, Crabbe K (1996) Primary metabolite kinetics of bacteriocin biosynthesis by *Lactobacillus amylovorus* and evidence for stimulation of bacteriocin production under unfavorable growth condition. Microbiol 142:817–827

Dominguez APM, Bizani D, Cladera-Olivera F, Brandelli A (2007) Cerein 8A production in soybean protein using response surface methodology. Biochem Eng J 35:238–243

Jamuna M, Jeevaratnam K (2004) Isolation and characterization o lactobacilli from some traditional fermented foods and evaluation of their bacteriocins. J Gen Appl Microbiol 50: 79–90

Joshi MS, Gowda LR, Bhat SG (1987) Permeabilization of yeast cells (*Kluyveromyces fragilis*). Biotechnol Lett 9:549–554

Kim CH, Ji GE, Ahn C (2000) Purification and molecular characterization of a bacteriocin from *Pediococcus* sp. KCA 1303-10 isolated from fermented flat fish. Food Sci Biotechnol 19: 270–276

Kim MC, Kong YJ, Baek H, Hyun HH (2006) Optimization of culture conditions and medium composition for the production of micrococcin GO5 by *Micrococcus* sp. GO5. J Biotechnol 21:54–61

Li C, Bai J, Cai Z, Ouyang F (2001) Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. J Biotechnol 93:27–34

Mackay VC, Arendse G, Hastings JW (1997) Purification of bacteriocins of lactic acid bacteria: problems and pointers. Int J Food Microbiol 34:1–16

Matsusaki H, Endo N, Sonomoto K, Ishizaki A (1996) Lantibiotic nisin Z fermentative production by *Lactococcus lactis* 10-1: Relationship between production of the lantibiotic and lactate and cell growth. Appl Microbiol Biotechnol 45:36–40

Motta AS, Brandelli A (2003) Influence of growth conditions on bacteriocin production by *Brevibacterium linens*. Appl Microbiol Biotechnol 62:163–168

Myers RH, Montogomery RC (2002) Response surface methodology: process and product optimization using designed experiments. Wiley, New York

Oh S, Rheem S, Sim J, Kim S, Back Y (1995) Optimizing conditions for the growth of *Lactobacillus casei* YIT 9018 in tryptone-glucose medium by using response surface methodology. Appl Environ Microbiol 61:3809–3814

Pal A, Ramana KV (2009a) Isolation and preliminary characterization of a nonbacteriocin antimicrobial compound from *Weissella paramesenteroides* DFR-8 isolated from cucumber (*Cucumis sativus*). Process Biochem 44:499–503

Pal A, Ramana KV (2009b) Purification and characterization of bacteriocin from *Weissella paramesenteroides* DFR-8, an isolate from cucumber (*Cucumis sativus*). J Food Biochem (in press, jfbc-07-08-0183, Date of acceptance 2 Dec 2008)

Puri S, Beg QK, Gupta R (2001) Optimization of alkaline protease production from *Bacillus* sp. by response surface methodology. Current Microbiol 44:286–290

Sudha N, Rani SP, Agarwal R (2006) Studies on the stability and viability of a local probiotic isolate *Pediococcus pentosaceous* (MTCC 5151) under induced gastrointestinal tract conditions. J Food Sci Technol 43:677–678

Tagg JR, McGiven AR (1971) Assay system for bacteriocins. Appl Microbiol 21:125