academicJournals

Vol. 7(27), pp. 3494-3500, 5 July, 2013
DOI: 10.5897/AJMR12.1911
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Optimization of medium composition for α-galactosidase production by Antarctic bacterial isolate, *Bacillus* sp. LX-1 using response surface methodology

Jaekoo Lee[1#], Inkyung Park[1#], Sangbuem Cho[2#], Sooki Kim[1], Yunjaie Choi[3], Jaiesoon Cho[1]*

[1]Department of Animal Science and Technology, College of Animal Bioscience and Technology, Konkuk University, South Korea.
[2]Department of Animal Science, Chonbuk National University, Jeonju, 664-14, South Korea.
[3]Department of Agricultural Biotechnology, Seoul National University, South Korea.

Accepted 26 April, 2013

The optimization of medium composition for α-galactosidase production by an Antarctic bacterial isolate, *Bacillus* sp. LX-1 was investigated in flask fermentation. The Central composite design (CCD) with 3 variables (galactose, peptone and $MnSO_4$) was employed to derive statistical model (Response surface methodology: RSM) for optimizing the medium composition. The results show the optimum medium composition of 16.5 g/L galactose, 6.2 g/L peptone and 0.0018 g/L $MnSO_4$ with predicted α-galactosidase activity, 1.17 U/mL and observed activity from the fermentation with optimized medium was 1.06 U/mL. The optimized value was 6.3-fold higher than that (0.168 U/mL) previously obtained under basal conditions. Thus, medium optimization using RSM is suggested to play a significant role in enhancing the production of α-galactosidase by *Bacillus* sp. LX-1.

Key words: α-Galactosidase, *Bacillus* sp. LX-1, medium optimization, central composite design (CCD), response surface methodology (RSM).

INTRODUCTION

α-Galactosidase (EC 3.2.1.22) is an exoglycosidase that initiates the hydrolysis of terminal α-1,6-linked D-galactose moieties from α-galactooligosaccharides such as mellibiose, raffinose and stachyose which primarily exist in soybeans and other legumes as well as the polysaccharides of galactomannans and galactoglucomannans (Gote et al., 2004; Naumoff, 2004; Patil et al., 2010). α-Galactosidase is of particular interest in view of many potentials of their biotechnological and medicinal applications. For examples, the removal of raffinose by the enzyme helps to increase the yield of sucrose in beet sugar industry (Shibuya et al., 1995) and to upgrade the nutritional value of legume-based product in food and animal feed processing (Guimaraes et al., 2001). Furthermore, in medicinal field, it is also involved in blood-type conversion (Goldstein et al., 1982), the treatment of Fabray's disease (Utsumia et al, 2005), and xenotransplantation (Fuller et al., 2004; Olsson et al., 2004).

Until now, a number of α-galactosidases have been derivedfromvariousorganismssuchasanimals(Gitzelmann and Auricchio, 1965), plants (Kang and Lee, 2001), and microorganisms (Jin et al., 2001) and characterized (Cao et al., 2010, Lee et al., 2012, Park et al., 2012). α-Galactosidases produced by a few *Bacillus* strains were reported to have pH (6~7.5) and temperature (30~45°C) optimum in the neutral range (Lee et al., 2012, Park et al., 2012), but little information about the optimization of this

*Corresponding author. E-mail: chojs70@konkuk.ac.kr. Tel:+82-2-450-3375. Fax:+82-2-455-1044.

#These authors contributed equally to this work.

enzyme production by submerged fermentation, using *Bacillus* species, is available in the scientific literature (Shivam and Mishra, 2010; Anisha et al. 2008a). The convenience of *Bacillus* strains is due to the fact that they secrete large amounts of enzyme in very short period of time into the fermentation broth (Asif et al., 2012).

The production and characterization of an enzyme are necessary for its industrial application. The first step in achieving this goal is the establishment of a suitable enzyme production technology (Anisha et al., 2008c). The conventional method for optimization of enzyme production involves varying one factor at a time and keeping the others constant (Adinarayana and Ellaiah, 2002). This laborious and time-consuming method often does not guarantee determination of optimal conditions, and designing experiments with every possible factorial combination of the test variables is impractical because of the large number of experiments required (Sen, 1997). Thus, response surface methodology (RSM) can investigate experimental variables without having to increase the number of experiments to the extreme (Montgomery, 1997).

RSM is a collection of statistical techniques for designning experiments, building models, evaluating the effects of variables and searching for the optimum conditions (Shivam and Mishra, 2010). At present, RSM is widely used in bioprocess technology for optimization of different types of fermentation media (Desai et al., 2008, Sunitha et al., 1999).

In our previous study, galactose, peptone and $MnSO_4$ were found to be crucial factor in enhancing α-galactosidase formation in submerged fermentation conditions by *Bacillus* sp. LX-1 (Lee et al. 2012), but it did not deal with statistical optimization of the process. Thus, the objective of this work is to develop an optimal culture medium using RSM for the production of α-galactosidase in submerged fermentation conditions by *Bacillus* sp. LX-1.

MATERIALS AND METHODS

Reagents

Unless otherwise stated, the chemicals and medium ingredient used in this study were purchased from Sigma Chemical Co. (St. Louis, Mo, USA) and BD Bioscience (Le Pont de Claix, France). All other chemicals were also of analytical grade.

Organism

An Antarctic bacterial isolate, *Bacillus* sp. LX-1 previously described (Lee et al., 2012) was used as an α-galactosidase producer for the present study. The strain LX-1 was streaked onto tryptic soy plate added to Bacto agar (1.5% w/v) to obtain single colonies and the plates were maintained at 28°C.

Inoculum preparation and culture condition

The isolated single colony on tryptic soy plate was inoculated directly into a glass test tube with screw cap (Pyrex brand Corning 9825, 16 X 150 mm) containing 5 mL of tryptic soy broth (BD, Le Pont de Claix, France), then incubated at 28°C for 24 h on the rotary shaker (220 rpm). In order to extend inoculum capacity, 50 mL of tryptic soy broth in 250 mL Erlenmeyer flask was aseptically inoculated with 1% of the preculture and aerobically incubated with vigorous shaking (220 rpm) at 28°C for 24 h. The cultures were used as inoculum for this RSM study, and inoculum size was 1.0% (v/v, based on the experimental medium). After inoculation, the experimental medium was incubated at 28°C for 24 h on the rotary shaker (220 rpm).

Experimental medium for RSM study

In order to produce α-galactosidase in experimental medium, 5.0 g/L yeast extract and 10.0 g/L tryptone were fundamentally fixed in all medium composition. On basis of the medium composition, experimental medium was compounded with adding various concentrations of galactose, peptone and $MnSO_4$.

Enzyme extraction

The culture medium containing secreted α-galactosidase was centrifuged at 5,000 X g at 4°C for 20 min. The resultant supernatant was used as the α-galactosidase source throughout this work.

Enzyme assay

α-Galactosidase assay was carried out by the modified method of Dey and Pridham (1969) and Anisha and Prema (2007). Unless otherwise stated, α-galactosidase activity was measured at 40°C for 15 min by assaying the release of *p*-nitrophenol from *p*-nitrophenyl-α-D-galactopyranoside with final concentration of 1 mM in 1 mL of 50 mM sodium phosphate (pH 7.0). The reactions were then stopped by adding 1 mL of 1 M Na_2CO_3, and color development was measured at a wavelength of 405 nm. One unit (U) of enzyme activity was defined as the amount of enzyme required to produce 1 µmol of *p*-nitrophenol per minute under the given assay conditions.

Optimization of enzyme production

Characterization of the different factors for α-galactosidase production was optimized by applying RSM. Then statistical model was obtained using Central composite design (CCD) with three independent variables [galactose concentration (X_1), peptone concentration (X_2) and $MnSO_4$ concentration (X_3)]. CCD maximizes the amount of information that can be obtained while limiting the number of individual experiments (Kunamneni and Singh, 2005). Each factor in the design was studied at five different levels (Table 1). A set of 20 experiments were performed. All variables taken at a central coded values was listed in Table 2. Upon completion of experiments, the average of α-galactosidase production was taken as the dependent variable or response.

Statistical analysis and modeling

The results obtained from RSM on α-galactosidase production were

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1\beta_1 X_1^2 + \beta_2\beta_2 X_2^2 + \beta_3\beta_3 X_3^2 + \beta_1\beta_2 X_1 X_2 + \beta_1\beta_3 X_1 X_3 + \beta_2\beta_3 X_2 X_3 \quad (1)$$

Table 1. Experimental range and levels of each variable studied using CCD in terms of actual factors for the production of α-galactosidase by *Bacillus* sp. LX-1

Independent variable	Coded level				
	-1.633	-1	0	+1	+1.633
Galactose (g/L, X_1)	3.670000	10.000	20.00	30.000	36.330000
Peptone (g/L, X_2)	1.835000	5.000	10.00	15.000	18.165000
$MnSO_4$ (g/L, X_3)	0.001835	0.005	0.01	0.015	0.018165

Table 2. Experimental design and results of the CCD.

Run number	Coded level			α-galactosidase (U/mL)	
	X_1	X_2	X_3	Observed	Predicted
1	-1	-1	-1	0.7874	0.7528
2	1	-1	-1	0.5805	0.5438
3	-1	1	-1	0.7622	0.7025
4	1	1	-1	0.4687	0.4244
5	-1	-1	1	0.3914	0.4300
6	1	-1	1	0.1164	0.1704
7	-1	1	1	0.6301	0.6611
8	1	1	1	0.3034	0.3323
9	0	0	0	0.6639	0.6068
10	0	0	0	0.6416	0.6068
11	0	0	0	0.5215	0.6068
12	0	0	0	0.5770	0.6068
13	-1.633	0	0	0.5407	0.5532
14	1.633	0	0	0.1179	0.1141
15	0	-1.633	0	0.5632	0.5475
16	0	1.633	0	0.6143	0.6386
17	0	0	-1.633	1.0755	1.1803
18	0	0	1.633	0.9377	0.8416
19	0	0	0	0.7132	0.7389
20	0	0	0	0.7906	0.7389

X_1, X_2 and X_3 mean galactose, peptone and $MnSO_4$, respectively.

used to fit a second-order polynomial equation (Equation 1) as it represents the behavior of such a system more appropriately: where Y is the dependent or response variable, β_0 is intercept, β_1, β_2, and β_3 are linear coefficients, $\beta_1\beta_1$, $\beta_2\beta_2$, and $\beta_3\beta_3$ are squared coefficients, $\beta_1\beta_2$, $\beta_1\beta_3$, and $\beta_2\beta_3$, are interaction coefficients and X_1, X_2, X_3, X_1^2, X_2^2, X_3^2, X_1X_2, X_1X_3, and X_2X_3 are levels of independent variables. Statistical significance of the model equation was determined by P-value. A regression model was made with analysis regres-sion coefficients, a variance analysis table (ANOVA), P-values and F-values. A quality assessment of the polynomial model was expressed by coefficient of determination R^2. The Minitab v.14 software (State College, PA, USA) was used for statistical design, data analysis, and 3D plots.

Validation of the model

To verify the predicted results, validation experiment was performed in triplicate tests under the optimized condition.

RESULTS AND DISCUSSION

CCD and analysis

A CCD with 6-star points and 6 replicates at center point was used for optimizing α-galactosidase production for galactose (X_1), peptone (X_2), and $MnSO_4$ (X_3) factors. CCD combines a two-level full or fractional factorial design with additional points (star points) and at least one point at the center of the experimental region, selected to obtain properties such as rotarability or orthogonality, in order to fit quadratic polynomials (Ferreira et al., 2004). For fitting quadratic response models, CCD is a better alternative to full factorial three-level design because its performance is comparable at a lower cost (Ferreira et al., 2007). Therefore, it has been the most accepted experi-

Table 3. Results of regression analysis of the CCD.

Term	Coefficient	*T*-value	*P*-value
Intercept	0.84	3.526	0.006
X_1	0.05	4.663	0.001
X_1X_1	-0.00152	-7.301	0.000
X_2	0.03	1.229	0.250
X_2X_2	-0.0022	-2.627	0.027
X_3	-125.44	-5.484	0.000
X_3X_3	4081.02	4.902	0.001
X_1X_2	-0.0003	-0.647	0.534
X_1X_3	-0.25	-0.473	0.647
X_2X_3	2.81	2.631	0.027

X_1, X_2 and X_3 mean galactose, peptone and $MnSO_4$, respectively and each coefficient was calculated with uncoded of variables.

Table 4. Analysis of variance (ANOVA) for the model regression representing α-galactosidase production

Source	DF	SS	MS	*F*	*P*
Model	9	0.94724	0.105249	18.40	<0.0001
Linear	3	0.39471	0.136697	23.90	<0.0001
Quadratic	3	0.50927	0.169756	29.67	<0.0001
Interaction	3	0.04326	0.014419	2.52	0.124
Residual Error	9	0.05149	0.005721		
Lack-of-Fit	5	0.03599	0.007197	1.86	0.284
Pure Error	4	0.01550	0.003875		
Total	19	1.08250			

R^2 = 95.2%; DF, degrees of freedom; SS, sum of squares; MS, mean square.

mental design for optimization of biological process (Anisha et al., 2008b).

The coded values of factors in CCD were shown in Table 2. Table 2 showed the design of this experiment and experimental results. Regression analysis was conducted to fit the response function with the experimental data.

The regression coefficients and their significance for α-galactosidase production were shown in Table 3. The data in Table 3 and Equation 2 showed positive effects of X_1, X_2, X_3^2, and X_2X_3, and negative effects of X_3, X_1^2, X_2^2, X_1X_2, and X_1X_3. *P* values less than 0.05 indicate that the model terms are significant, while values greater than 0.1000 indicate that the model terms are not significant (Li et al., 2008). In this study, X_1, X_3^2, and X_2X_3 were very significant with a probability of over 97%. By applying multiple regression analysis on the experimental data, the following 2nd-order polynomial equation (Equation 2) was found to explain the α-galactosidase production:

The statistical significance of the 2nd –order model

$$Y = 0.84 + 0.05X_1 + 0.03X_2 - 125.44X_3 - 0.00152X_1^2 - 0.0022X_2^2 + 408.02X_3^2 - 0.0003X_1X_2 - 0.25X_1X_3 + 2.81X_2X_3 \quad (2)$$

equation was checked by an *F*-test (ANOVA) and the data were shown in Table 4. Smaller *P* value and larger magnitude of *F*-value show high significance of the corresponding variables (Karthikeyan et al., 1996). ANOVA of the model showed high *F*-value (18.40) and *P*-value (<0.0001), which explained that the model itself is significant (Tanyildizi et al., 2006). Joglekar and May (1987) and Manikandan et al., (2009) have suggested for good fit of a model, R^2 should be at least 80%. The determination coefficient (R^2) implied that the sample variation of 95.2% for α-galactosidase production was attributed to the independent variables, and only about 4.8% of the total variation wasn't able to be explained by the model. The ANOVA result showed that this model was

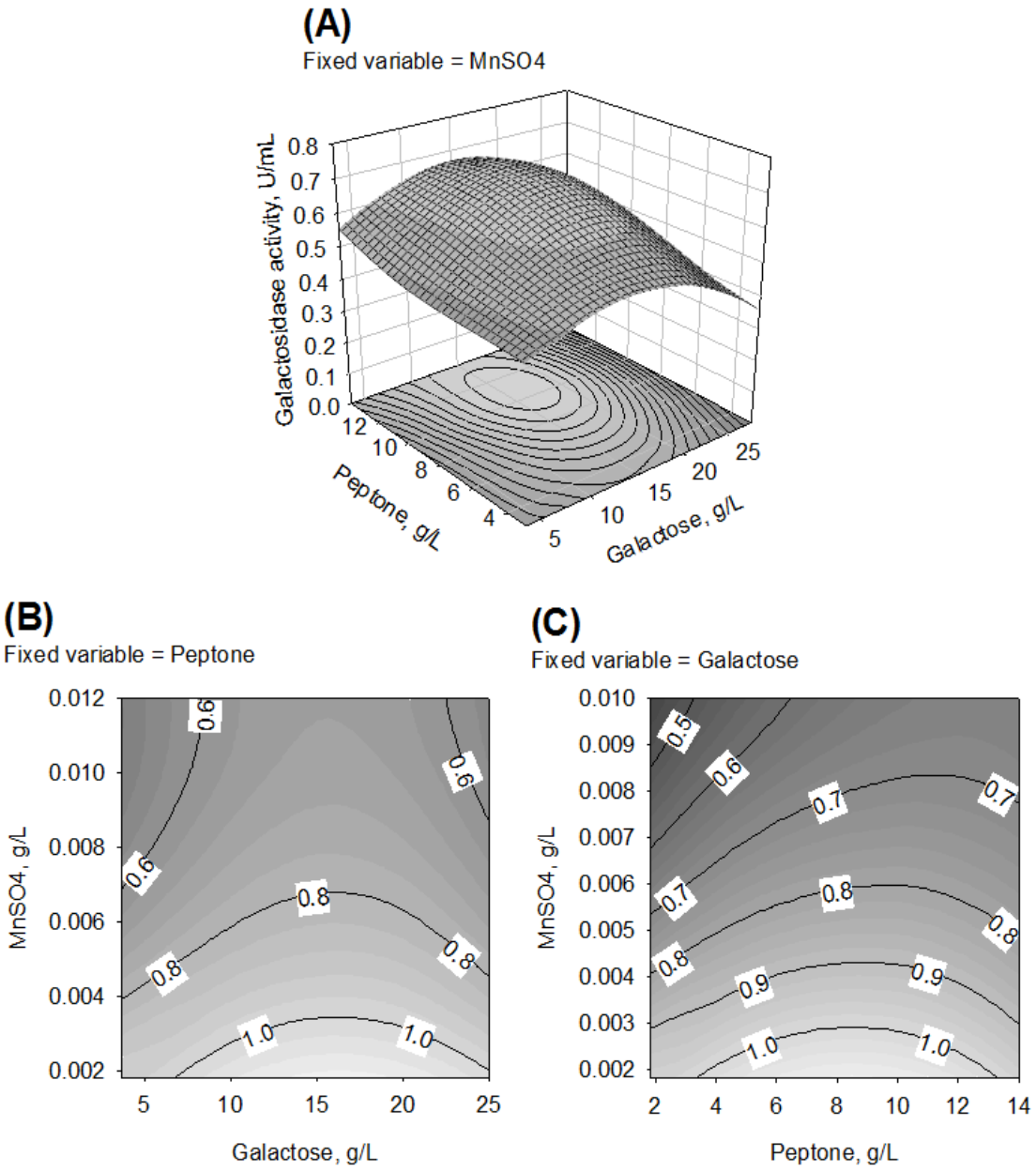

Figure 1. Interactions among variables. (A) is 3-dimensional plot for the interaction between galactose and peptone on α-galactosidase activity with fixed level of $MnSO_4$ at center point (0.01 g/L); (B) and (C) are contour plots for the interaction between galactose and $MnSO_4$ and between peptone and $MnSO_4$, respectively, with fixed levels of remaining variables (peptone = 10 g/L, galactose = 20 g/L).

appropriate, because the model showed statistically insignificant lack of fit (*P*=0.284). A non-significant value of lack of fit indicates that model is significant (Box et al., 1978; Li et al., 2007). It was also suggested that the quadratic and linear terms of galactose, peptone, and $MnSO_4$ of the model primarily determined α-galactosidase production by *Bacillus* sp. LX-1.

Three-dimensional response surface and contour plots of galactose, peptone, and $MnSO_4$ against α-galactosidase can further explain the results of the statistical and mathematical analysis, and were shown in Figure 1. Response surface was generated by plotting the response (α-galactosidase production) on the z-axis against any two independent variables while keeping the other independent variable at zero level. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Muralidhar et al., 2001). Therefore, three response surfaces were obtained by considering all the

Table 5. Experimental verification of combined effect of optimized medium on the response of α-galactosidase production.

Substrate	Concentration of optimization (g/L)	Predicted response (U/mL)	Observed response (U/mL)
Galactose (X_1)	16.5		
Peptone (X_2)	6.2	1.17	1.06
$MnSO_4$ (X_3)	0.0018		

possible combinations. Figure 1A represents a 3-D diagram and a contour plot of calculated response surface from the interaction between the concentration of galactose and peptone, while keeping the other variable (concentration of $MnSO_4$) at '0' level. A linear increase in the enzyme production was observed when galactose concentration was increased up to 15 g/L and thereafter it declined. When the level of pepone concentration was increased up to 11 g/L, a linear increase in α-galactosidase production was recorded. At the '0' level of peptone concentration (Figure 1B), the response between galactose concentration and $MnSO_4$ concentration indicated that galactose concentration of 15 g/L was maximum for α-galactosidase production, and $MnSO_4$ concentration was less than 0.002 g/L. The interaction between the remaining two parameters (concentration of peptone and concentration of $MnSO_4$) was shown in Figure 1C. Maximum concentration of peptone for α-galactosidase production was from 8 g/L to 10 g/L, and $MnSO_4$ was similar to Figure 1B.

Validation of the optimized condition

On the basis of medium optimization, the quadratic model predicted that the maximum production of α-galactosidase by *Bacillus* sp. LX-1 was 1.17 U/mL, when the concentration of galactose, peptone, and $MnSO_4$ were 16.5 g/L, 6.2 g/L, and 0.0018 g/L, respectively (Table 5). To verify the predicted results, validation experiment was performed in triplicate tests. Under the optimized condition, the observed experimental activity of average α-galactosidase was 1.06±0.009 U/mL, suggesting that experimental and predicted values of α-galactosidase production were in good agreement. As the result of the optimization of the culture conditions, a 6.3-fold increase in α-galactosidase activity was achieved compared to that value (0.168 U/mL) previously obtained under basal conditions (Lee et al., 2012). This result therefore corroborated the predicted values and the effectiveness of the model, indicating that the optimized medium favors the α-galactosidase production by *Bacillus* sp. LX-1.

CONCLUSION

In order to optimize culture media for maximal α-galactosidase production by *Bacillus* sp. LX-1 in this study, a CCD approach was used. The factorial design approach to medium development relies on 2 stages of experimentation: optimization and verification. Optimization experiments are designed to provide in-depth infor-mation about a few variables (galactose, peptone and $MnSO_4$) identified as having the greatest impact on α-galactosidase production. As verification experiments are used to validate the predicted results under experimental conditions for maximum enzyme production, RSM has been suggested to play a significant role in enhancing the production of α-galactosidase by *Bacillus* sp. LX-1.

ACKNOWLEDGMENT

This research was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Project No. 610005031SB130).

REFERENCES

Adinarayana K, Ellaiah P (2002). Response surface optimization of the critical medium components for the production of alkaline protease from a newly isolated *Bacillus subtilis* PE-11. J. Pharm. Pharma. Sci. 5:281-287.

Anisha GS, Prema P (2007). Production of α-galactosidase by a novel actinomyces *Streptomyces griseoloalbus* and its application in soymilk hydrolysis. World J. Microbiol. Biotechnol. 23:859-864.

Anisha GS, Rojan PJ, Nicemol J, Niladevi KN, Prema P (2008a). Production and characterization of partially purified thermostable α-galactosidase from *Streptomyces griseoloalbus* for food industrial applications. Food Chem. 111:631-635.

Anisha GS, Sukumaran RK, Prema P (2008b). Evaluation of α-galactosidase biosynthesis by *Streptomyces griseoloalbus* in solid-state fermentation using response surface methodology. Lett. Appl. Microbiol. 46:338-343.

Anisha GS, Sukumaran RK, Prema P (2008c). Statistical optimization of α-galactosidase production in submerged fermentation by *Streptomyces griseoloalbus* using response surface methodology. Food Technol. Biotechnol. 46:171-177.

Asif M, Hussain A, Ali MA, Rasool M (2012). Scale up studies for the production of protease enzyme using *Bacillus subtilis* adopting response surface methodology. Afr. J. Microbiol. Res. 6:2120-2128.

Box GEP, Hunter WG, Hunter JS (1978). Statistics for experimenters: an introduction to design. Data Analysis and Model Building, John Wiley and Sons, New York, pp. 291-334.

Cao Y, Yuan T, Shi P, Luo H, Li N, Meng K, Bai Y, Yang P, Zhou Z, Zhang Z, Yao B (2010). Properties of a novel α-galactosidase from *Streptomyces* sp. S27 and its potential for soybean processing. Enzyme Microb. Tech. 47:305-312.

Desai KM, Survase SA, Saudager PS, Lele SS, Singhal RS (2008). Comparison of artificial neural network (ANN) and response surface methodology (RSM) in fermentation media optimization: case study of fermentative production of scleroglucan. Biochem. Eng. J. 41:266-273.

Dey PM, Pridham JB (1969). Purification and properties of α-galactosidases from *Vicia faba* seeds. Biochem. J. 113:49-55.

Ferreira SLC, dos Santos WNL, Quintella CM, Neto BB, Bosque-Sendra JM (2004). Doehlert matrix: a chemometric tool for analytical chemistry–review. Talanta 63:1061-1067.

Ferreira SLC, Korn MDAG, Ferreira HS, da Silva EGP, Araujo RGO, Souza AS, Macedo SM, Lima DDC, de Jesus RM, Amorim FAC, Sendra JMB (2007). Application of multivariate techniques in optimization of spectroanalytical methods. Appl. Spectrosc. Rev. 42:475-491.

Fuller M, Lovejoy M, Brooks DA, Harkin ML, Hopwood JJ, Mickle PJ (2004). Immunoquantification of α-galactosidase evaluation for the diagnosis of Fabray's disease. Clin. Chem. 50:1979-1985.

Gitzelmann R, Auricchio S (1965). The handling of soy α-galactosidase by a normal and galactosemic child. Pediatrics 36:231-235.

Goldstein J, Siviglia G, Hurst R (1982). Group B erythrocytes enzymatically converted to group O survive normally in A, B, and O individuals. Science 215:168-170.

Gote M, Umalkar H, Khan I, Khire J (2004). Thermostable α-galactosidase from *Bacillus stearothermophilus* (NCIM 5146) and its application in the removal of flatulence causing factors from soymilk. Process Biochem. 39:1723-1729.

Guimaraes VM, Rezende ST, Moreira MA, Barros EB, Felix CR (2001). Characterization of α-galactosidase from germinating soybean seed and their use for hydrolysis of oligosaccharides. Phytochemistry 58:67-73.

Jin FX, Li Y, Zhang CZ, Yu HS (2001). Thermostable α-amylase and α-galactosidase production from the thermophilic and aerobic *Bacillus* sp. JF strain. Process Biochem. 36:559-564.

Joglekar AM, May AT (1987). Product excellence through design of experiments. Cereal Foods World 32:857-868.

Kang HC, Lee SH (2001). Characteristics of an α-galactosidase associated with grape flesh. Phytochemistry 58:213-219.

Karthikeyan RS, Rakshit SK, Varadarajan A (1996). Optimization of batch fermentation conditions for dextran production. Bioprocess Eng. 15:247-251.

Kunamneni A, Singh S (2005). Response surface optimization of enzymatic hydrolysis of maize starch for higher glucose production. Biochem. Eng. J. 27:179-190.

Lee J, Park I, Cho J (2012). Production and partial characterization of α-galactosidase activity from an Antarctic bacterial isolate, *Bacillus* sp. LX-1. Afr. J. Biotchnol. 11:12396-12405.

Li XY, Liu ZQ, Chi ZM (2008). Production of phytase by a marine yeast *Kodamaea ohmeri* BG3 in an oats medium: optimization by response surface methodology. Bioresour. Technol. 99: 6386-6390.

Li Y, Liu Z, Zhao H, Xu Y, Cui F (2007). Statistical optimization of xylanase production from new isolated *Penicillium oxalicum* ZH-30 in submerged fermentation. Biochem. Eng. J. 34:82-86.

Manikandan M, Pasic L, Kannan V (2009). Optimization of growth media for obtaining high-cell density cultures of *halophilic archaea* (family *Halobacteriaceae*) by response surface methodology. Bioresour. Technol. 100:3107-3112.

Montgomery DC (1997). Response surface methods and other approaches to process optimization. In Montgomery DC (eds) Design and Analysis of Experiments, John wiley and Sons, New York, pp. 427-510

Muralidhar RV, Chirumamila RR, Marchant R, Nigam P (2001). A response surface approarch for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. Biochem. Eng. J. 9:17-23.

Naumoff DG (2004). Phylogenetic analysis of α-galactosidases of the GH27 family. Mol. Biol. 38:388-399.

Nikerel IE, Ates Ö, Öner ET (2008). Effect of bioprocess conditions on growth and alkaline protease production by Halotoleratn *Bacillus lichenifromis* BA17. Appl. Biochem. Microbiol. 44:487-492.

Olsson ML, Hill CA, Dela Vega H, Liu QP, Stroud MR, Valdinocei J (2004). Universal red blood cells-enzymatic conversion of blood group A and B antigens. Transfus. Clin. Biol. 11:33-39.

Patil AG, Praveen Kumar SK, Mulimani VH, Veeranagouda Y, Lee K (2010). α-Galactosidase from *Bacillus megaterium* VHM1 and its application in removal of flatulence-causing factors from soymilk. J. Microbiol. Biotechnol. 20:1546-1554.

Park I, Lee J, Cho J (2012). Partial characterization of α-galactosidic activity from the Antarctic bacterial isolate, *Paenibacillus* sp. LX-20 as a potential feed enzyme source. Asian-Aust. J. Anim. Sci. 25:852-860.

Sen R (1997). Response surface optimization of the critical media components for the production of surfactin. J. Chem. Technol. Biotechnol. 68:263-270.

Shibuya H, Koboyashi H, Park GG, Komatsu Y, Sato T, Kaneko R, Nagasaki H, Yoshida S, Kasamo K, Kusakabe I (1995). Purification and some properties of α-galactosidase from *Penicillium purpurogenum*. Biosci. Biotech. Biochem. 59:2333-2335.

Shivam K, Mishra K (2010). Response surface optimization of the critical medium components for the production of α-galactosidase from *Aspergillus parasiticus* MTCC-2796. Folia Biologica 56:78-82.

Sunitha K, Lee JK, Oh TK (1999). Optimization of medium components for phytase production by *E. coli* using response surface methodology. Bioproc. Eng. 21:477-481.

Tanyildizi MS, Elibol M, Ozer D (2006). Optimization of growth medium for production of α-amylase from *Bacillus amyloliquefaciens* using response surface methodology. J. Chem. Technol. Biotechnol. 81:618-622.

Utsumia K, Mitsuhashib F, asahic K (2005). Enzyme replacement therapy for Fabry disease: morphologic and histochemical changes in the urinary sediments. Clin. Chim. Acta. 360:103-107.