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Enhancing the Production of Therapeutic Enzyme Arginase from *Lactobacillus acidophilus* Using Response Surface Methodology

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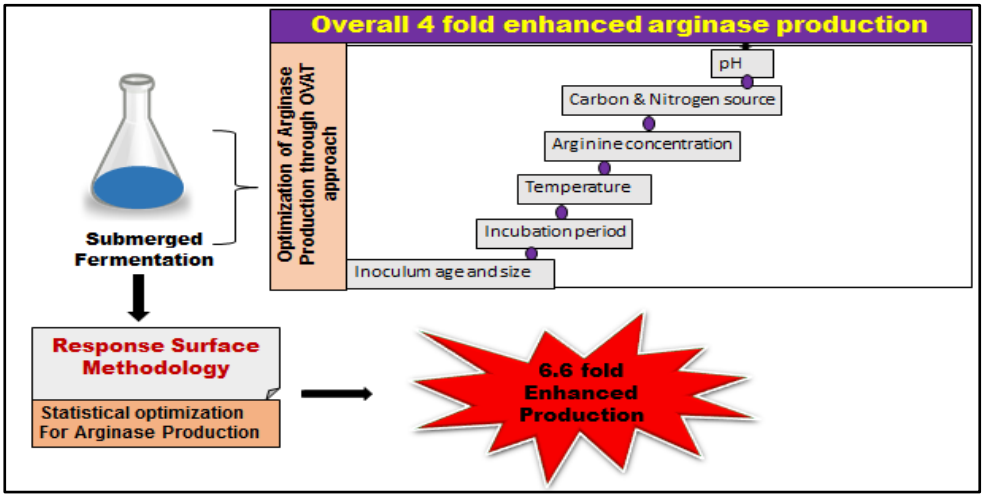
HIGHLIGHTS

- First report showing optimization study of arginase production by *Lactobacillus acidophilus.*
- Optimization by OVAT approach resulted in four-fold enhanced arginase production.
- Response surface methodology resulted in six-fold enhanced arginase production with 15 mM arginine, 1% Yeast extract and medium pH 5.5.

Abstract: Arginase plays an imperative role in the cell growth and proliferation of healthy cells under normal physiological conditions. Recently arginase has gained the sight of many researchers as it has emerged as a potential candidate for auxotrophic cancer treatment. Through one variable at a time approach (OVAT), 4-fold enhanced arginase production was observed compared to unoptimized cultural conditions. The highest arginase production (2 U/mL) was achieved when a 1% 10 h old inoculum was used to carry out submerged fermentation for 24 h. Further additional supplementation of media with arginine (15 mM), sucrose (1%), yeast extract (0.3%) and pH (6) also resulted in improved arginase production. Further optimization of cultural conditions by response surface methodology (3.3 U/mL) resulted in 6.6-fold improved arginase yield compared to unoptimized physiochemical cultural conditions. The present work is the first report regarding optimization of arginase production by *Lactobacillus acidophilus* by OVAT and the statistical approach using central composite design under submerged conditions. This study can further be extended to explore the anti-carcinoma properties of arginase produced by *Lactobacillus*, followed by its scaling up.

Keywords: *Lactobacillus acidophilus*; Submerged Fermentation; Arginase Production; Optimization; Response Surface Methodology.

GRAPHICAL ABSTRACT



INTRODUCTION

Arginase is a metalloenzyme, which requires manganese to catalyze the terminal step of the urea cycle by hydrolyzing L-arginine to non-amino acids L-ornithine and urea [1,2]. Arginase enzyme is widely present in lower organisms such as bacteria, fungi, and yeasts to higher complex organisms [3-5]. Among arginase-producing microbes, bacteria are the prominent ones, including bacilli like *Bacillus subtilis* [6], *Bacillus caldovelox* [7], *Rummeliibacillus pycnus* SK32.001 [8], *Mycobacteria* [9] and many others.

Various *in vitro* and in vivo studies have suggested that arginine depletion can be effective in treating tumors like hepatocellular carcinomas (HCCs) and melanomas [1,10,11]. The major mechanism behind the effectiveness of arginase therapy is the lack of expression of argininosuccinate synthetase-1 (ASS1) in arginine auxotrophic cancers due to which they need arginine from a nutritional pool of normal healthy cells. With the administration of arginase, these cancer cells will expose to deficient arginine concentration, which ultimately halts its growth and metastasis, while the normal cells remain unaffected [1,12-14]. Well-established previous studies have already shown the therapeutic effects of arginase in cancer therapy against hepatocellular carcinoma [15,16], leukemia [17], human prostate cancer cells [18], pancreatic cancer [19], and breast cancer [20]. Hence, its commercial production should be emphasized for its curative properties against cancers.

L-ornithine, a precursor for glutamate and several other polyamines required for cell growth, the product of arginase reaction, makes arginase commercialization more significant [7,21]. Previous studies were also evident that arginases can be provided extracellularly as neuroprotective [22]. It has also been identified as involved in cardiovascular and neural diseases [22,23]. In the present report, *Lactobacillus acidophilus* is being studied for its ability to produce arginase by optimizing the cultural conditions and media components. For the economic production of the therapeutic enzyme, media components should be optimized for higher production.

Optimization through one variable at a time (OVAT) approach provides a central idea about significant parameters of medium components. But this approach is laborious, time-consuming, and does not include the interactive studies between various parameters under study. Response surface methodology (RSM) is a popular mathematical and statistical model that helps identify a relationship between a response of interest and other control variables [24]. It is a method for designing a process model in the form of a non-linear regression equation by keeping in mind the effect of individual, square, and interactive terms of process variables on the output [25]. The present study utilizes RSM for modeling and optimization of arginase production by *Lactobacillus acidophilus* through submerged fermentation using arginine as an inducer.

MATERIAL AND METHODS

Chemicals and Microbial culture

The chemicals and reagents used in the present study were purchased from Himedia (Mumbai, India) and were of analytical grade. The culture of *Lactobacillus acidophilus* was procured from National Dairy

Research Institute, Karnal (Haryana). The culture was maintained on the MRS agar medium and stored at 4 °C for further application.

Production of the arginase enzyme

The culture of *Lactobacillus acidophilus* was revived in a sterile MRS broth. Minimal media composed of arginine (10 mM) along with 13.6 g/L Potassium dihydrogen phosphate (KH_2PO_4), 2 g/L Ammonium sulphate $(NH_4)_2SO_4$, 10 mg/L Calcium chloride ($CaCl_2.2H_20$), 0.5 mg/L Ferrous sulphate ($FeSO_4.7H_2O$) and 0.5 g/L Sucrose was used for the production of arginase enzyme. The (1%) pre-cultivated culture was seeded in minimal media and incubated for 24 h at 37 °C with continuous shaking. An aliquot of fermentation broth withdrawn from the flask was centrifuged at 4 °C at 6000 rpm for 15 min. The pellet after centrifugation was suspended in the lysis buffer containing lysozyme and additionally, the cells were sonically disrupted at 4 °C to extract the enzyme. The lysate after sonication was centrifuged again at 4ºC at 8000 rpm for 15 min to remove the cell debris and was then used for measuring arginase activity. Both pellet (after sonication) and the supernatant collected after centrifugation were further assayed for arginase activity.

Arginase enzyme assay

The crude arginase activity was estimated spectrophotometrically by measuring the amount of ornithine released at the end of the reaction. One unit of arginase activity was defined as the amount of enzyme that releases one micromole of ornithine per minute under standard reaction conditions [21,26].

Optimization of Arginase production using one variable at a time (OVAT) approach

Initially, various enzyme production parameters such as inoculum age (4-12 h with 1 h interval), inoculum size (0.5%-5%), incubation time (4 h-24 h), incubation temperature (20-40 °C), and arginine concentration (inducer 5-25 mM) were studied. Carbon source (glycerol, glucose, sucrose with one control having no carbon source), optimum sucrose concentration (0.5% to 3%), nitrogen sources (yeast extract, peptone and di-ammonium sulphate with one control), optimum yeast extract concentration (0.1% to 0.8%) and pH (4-7) was optimized in a stepwise manner to maximize the arginase production.

Modeling and optimization studies

The three variables and their values for the Central Composite Design of Response Surface Methodology were selected based on OVAT optimization data. The experiments for arginase production optimization were designed through the statistical software package 'Design-Expert version 10' Stat-Ease. In the present study, the response was measured in terms of arginase activity (U/mL), a dependent variable. Each independent variable in the design at three different levels, i.e., higher (+1), middle (0), and lower (-1), were used in this study as tabulated in Table 1.

Table 1. Experimental (low, mid, and high) range of variables for the central composite design in terms of actual and coded factors.

Variables	Symbol coded	Range of variables		
		Low (-)	Mid (0)	High (+)
Arginine (mM)	A	5	15	25
Yeast extract (%)	B	0.1	0.45	0.8
pH	C	4	5.5	7

Further, validation of the model was done by performing the sets of experiments under optimal conditions. 20 experimental sets based on the CCD (shown in Table 2) were implemented in triplicate runs and both experimental and predicted values were compared. The individual, square and interactive effects of sets of variables on arginase activity were studied through statistically significant P value and analysis of variance (ANOVA) tests. The acceptability of the developed model was further confirmed through R^2 and adjusted R^2 values. These statistical values showed the accuracy, aptness, and significance of our model [27].

RESULTS AND DISCUSSION

Production of the arginase enzyme

Arginase activity was observed in cell lysate (after sonication of cells; 0.5 U/mL), whereas no arginase activity was observed in the supernatant. This result demonstrates that arginase is intracellularly located. Prior studies also revealed that arginase is produced intracellularly by various organisms such as *B. subtilis*, *B. licheniformis* [28,29].

Optimization of production parameters

Effect of Inoculum Age and Inoculum Size

Inoculum age of 10 h (cells in log phase were used as inoculum) (0.79 U/mL) was optimized for arginase production from *L. acidophilus*. An increase in arginase production was observed with an increase in inoculum age and a decrease after 10 h old inoculum (Figure 1a), probably due to older inoculum being metabolically inactive. In a prior study,18 h old inoculum of *Bacillus* spp. was used to obtain a maximal intracellular arginase production [29].

Further, 1% inoculum concentration has shown the highest arginase activity (0.9 U/mL). Further increase in inoculum size results in a reduction in arginase activity (Figure 1b). This may be due to the addition of a higher initial concentration of cells leading to nutrient starvation in a shorter period. Even higher inoculum size, 10%, was reported in *Idiomarina sediminum* for arginase production [30]. Other studies over the *Streptomyces diastaticus* MAM5 observed higher extracellular and intracellular arginase productivity with 2.31% and 1.59% inoculum size [31].

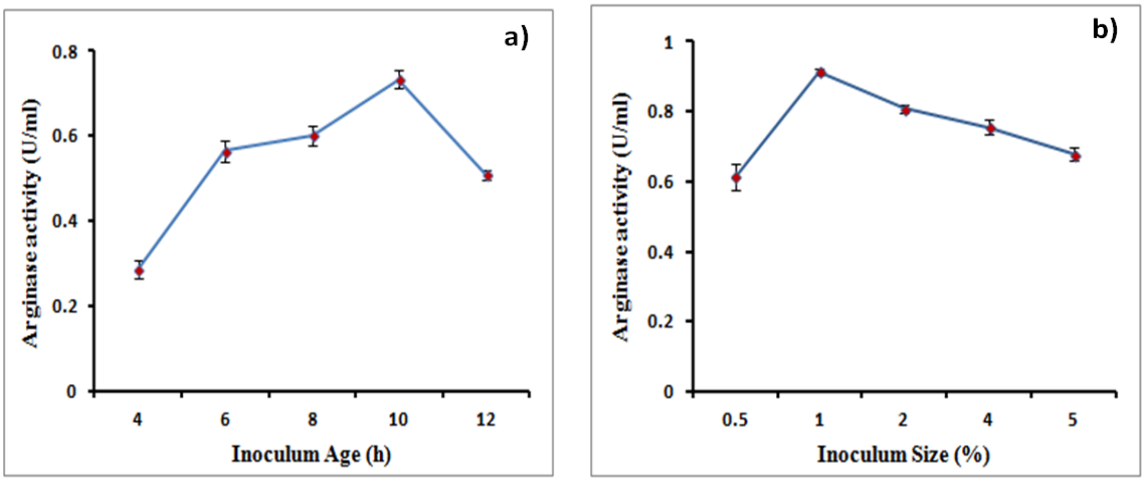


Figure 1. Effect of a) inoculum age and b) inoculum size on the arginase production

Effect of Incubation Time and Temperature

Enzyme activity was assayed at regular intervals for 30 h to check the optimum incubation period. Maximum arginase activity (0.92 U/mL) was observed at 24 h, and afterwards, a slight downfall in enzyme activity was observed (Figure 2a). The slight decline in arginase activity after 24 h may be due to the depletion of nutrients followed by ceased bacterial growth. Prior studies also observed the same pattern of 24 h incubation period for arginase production for *Bacillus thuringiensis* SK 20.001 [26] and *Pseudomonas sp.* strain PV1 [32]. Although a higher incubation period, i.e., 36 h, was also reported for arginase production in *Bacillus licheniformis* [29].

Incubation temperature is one of the most important parameter which affects enzyme production through modulating the exponential phase of bacterial growth. The maximum arginase activity was observed at 40 °C (1.24 U/mL) (Figure 2b). Similar to our finding, 37 °C temperature was used by *B. licheniformis* [29], *Idiomarina sediminium* [30], and *Pseudomonas* sp. strain PV1 [32] for arginase production. The temperature is an important consideration because it impacts metabolic activity during the development phase as well as contributes to the enzyme's stability. Enhancing the temperature beyond 40 °C resulted in a sharp dip in arginase production owing to denaturation of protein at higher temperatures [32,33].

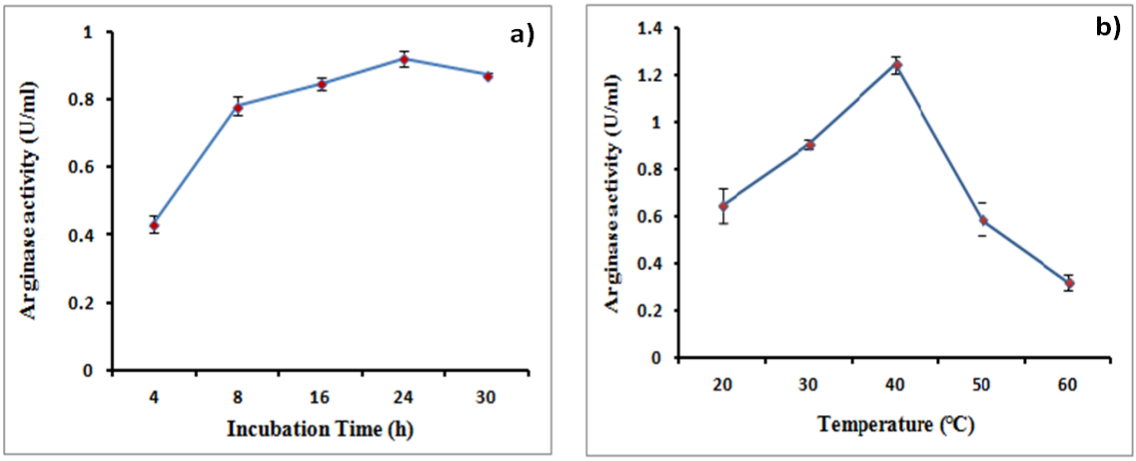


Figure 2. Effect of a) incubation time and b) incubation temperature on arginase production

Effect of Arginine concentration

Arginine concentration directly affects the biotransformation of arginine to ornithine. Arginine not only plays imperative role as the inducer but also acts as carbon and nitrogen source [7]. The effect of different arginine concentrations supplemented in production medium showed maximum amount of enzyme production in 15 mM arginine concentration (1.5 U/mL), whereas a further increase in concentration results in a sudden fall in arginase production (Figure 3). Arginine also supports the arginase production at a 2% concentration in *Idiomarina sediminium* [30]. In *Streptomyces diastaticus* MAM5, 0.32% arginine concentration maximizes the extracellular arginase productivity [31].

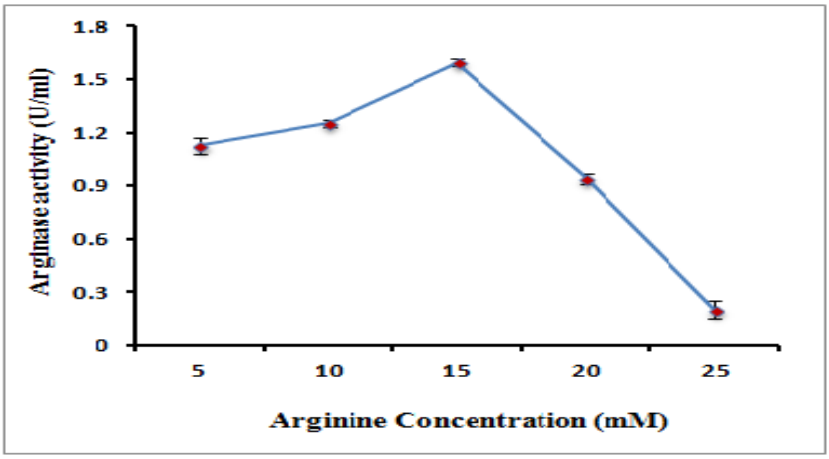


Figure 3. Effect of varied arginine concentration on arginase production

Selection of Carbon and Nitrogen source

Maximum arginase production was recorded when a medium was supplemented with sucrose (1.72 U/mL) whereas, supplementation of glycerol showed deleterious effects on arginase production. The previous report suggested that *Streptomyces diastaticus* MAM5 utilizes soluble starch as a carbon source [31]. Among different sucrose concentrations, it was observed that optimum arginase production from *L. acidophilus* is 1% (1.75 U/mL) sucrose. Further increase in sucrose concentration above 1% showed inhibitory effects on arginase production probably due to end product inhibition (Figure 4b). Earlier studies carried out also showed that *Pseudomonas* sp. strain PV1 utilized sugar alcohol i.e., mannitol for arginase production [32].

Among various nitrogen sources studied for optimum arginase production, medium supplemented with yeast extract (0.3%) resulted in higher arginase activity (1.80 U/mL) (Figure 4c). This enhanced production may be attributed to the complex and wide array of nutritional factors present in yeast extract. A similar finding showed yeast extract as an optimal nitrogen source in submerged fermentation was reported in *Pseudomonas* sp. strain PV1 [32]. Further, yeast extract concentration was varied from 0.1% to 0.8% to

obtain its optimal concentration. The maximum enzyme production was observed at 0.30% yeast extract (2 U/mL) (Figure 4d).

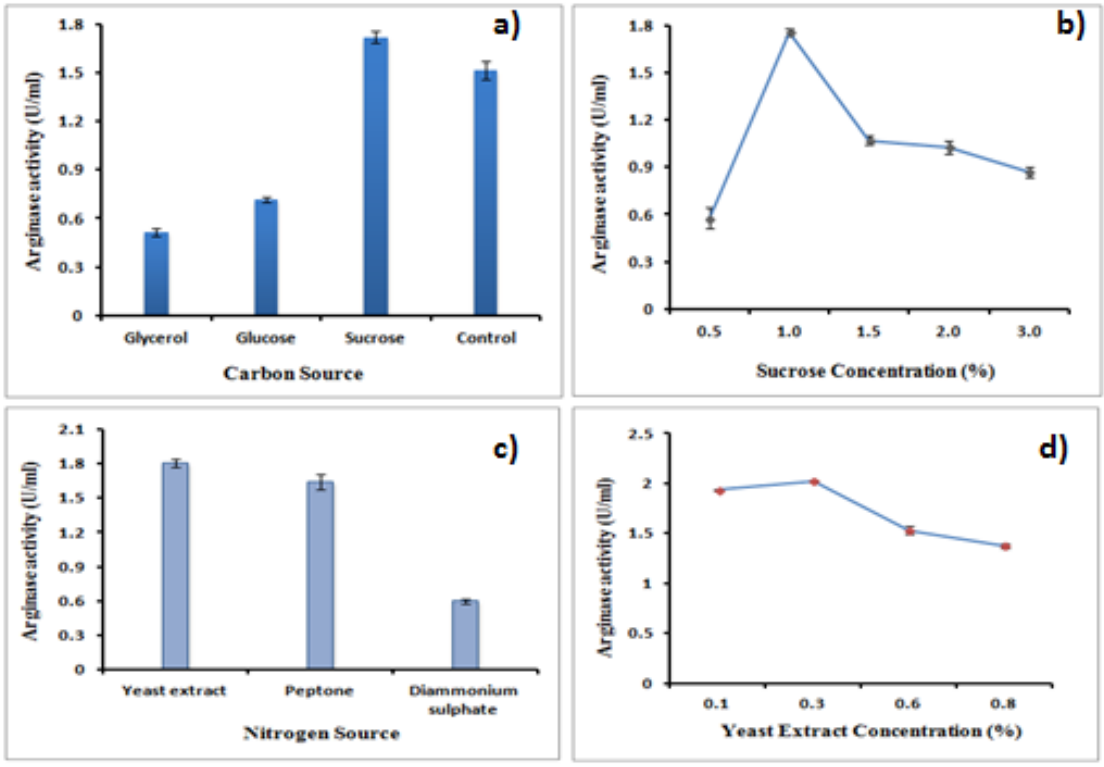


Figure 4. Effect of supplementation of a) different carbon sources b) sucrose concentration c) different nitrogen sources and d) yeast extract concentration on arginase production

Effect of pH

The pH regulates and changes numerous metabolic processes of the organism while also assisting in the stability of enzymes released in the production medium. Optimum pH for maximum arginase production was observed at pH 6 (2 U/mL) (Figure 5). A study over the *Streptomyces diastaticus* MAM5 observed higher extracellular and intracellular arginase productivity at pH 6.88 and 6.96, respectively [31], whereas the arginase from *B. thuringiensis* SK20.001 was procured at pH 7.0 [26].

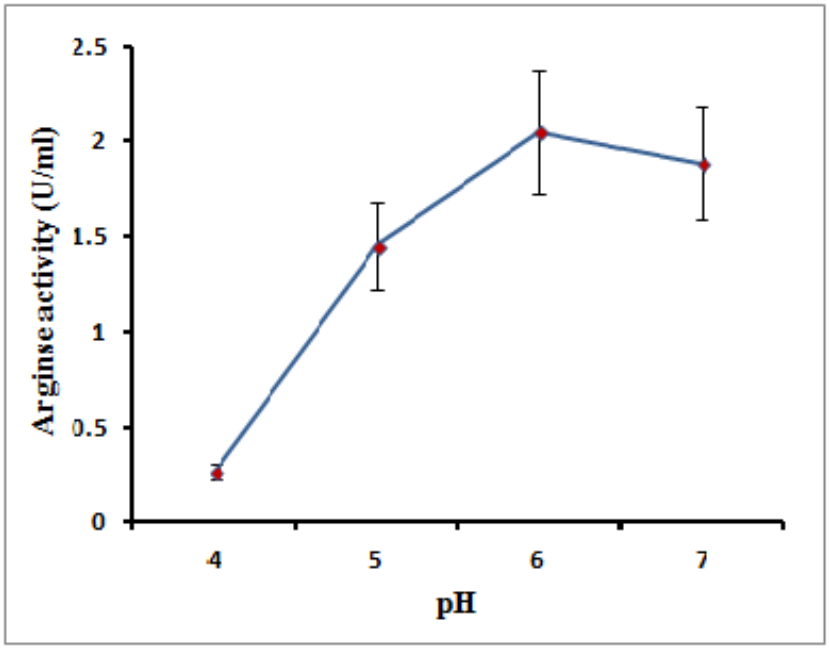


Figure 5. Effect of pH on arginase production

Enhancement of arginase production using Response Surface Methodology (RSM)

The experiment was designed and executed based on the Central Composite Design (CCD) of RSM. 20 experimental sets were tabulated in Table 2. The statistical model was validated by performing these experimental sets under predicated sets of conditions. The experimental output of arginase activity from the set of experiments and the predicted values are also tabulated in Table 2. The comparison between predicted and experimental values and their close values showed the accuracy of the RSM models [24,25]. Considering the individual, square, and interaction terms of SmF variables on output, the following non-linear regression equation (uncoded form) was developed for arginase activity.

Table 2. Composition of the various runs of the central composite design, actual and predicted values of the different parameters and their responses.

S. No.	Arginine concentration (mM)	Yeast extract concentration (%)	pH	Response (U/mL) (Exp.)	Response (U/mL) (Predict.)
1	15	0.45	5.5	2.4	2.41
2	15	1	5.5	3.3	2.42
3	15	0.45	3	0	-0.343
4	25	0.8	4	0.63	1.02
5	15	0.45	5.5	2.4	2.41
6	25	0.1	7	3.1	2.71
7	5	0.1	7	0.2	0.1
8	15	0.45	5.5	2.4	2.42
9	15	0.45	8	0.4	0.467
10	15	0	5.5	1.2	1.91
11	15	0.45	5.5	2.4	2.41
12	31	0.45	5.5	2.3	2.09
13	5	0.1	4	0	-0.14
14	15	0.45	5.5	2.4	2.41
15	5	0.8	7	0.3	0.43
16	0	0.45	5.5	0.52	0.71
17	15	0.45	5.5	2.4	2.41
18	25	0.1	4	0.32	0.48
19	5	0.8	4	1.00	1.68
20	25	0.8	7	0.56	0.43

Arginase activity (U/mL) =-13.06104+ 0.084920 * Arginine (mM) + 8.29276* yeast extract (%) + 4.41373 * pH – 0.11893 * Arginine (mM) * yeast extract (%) + 0.026750 * Arginine (mM)* pH – 0.89286 * yeast extract (%) * pH – 4.46114E-003 * Arginine^2 (mM) -1.21738* yeast extract^2 (mM) – 0.38841 * pH^2

This equation can be used to make predictions about the response for given levels of each factor.

Lack of fit test

The predictive ability of developed models and results of CCD were further confirmed through statistical significance tests and ANOVA [27,34,35]. Significance test results for arginase activity are tabulated in Table 3 and ANOVA test results are tabulated in Table 4.

Table 3. Results of significance test on the non-linear model –coefficients and standard error. Std. Dev. = 0.57, R-squared = 0.8673, Adj R-squared = 0.7478

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.41	1	0.23	1.90	2.93	
A-Arginine	0.45	1	0.15	0.10	0.79	1.00
B-Yeast extract	0.18	1	0.15	-0.17	0.52	1.00
C-pH	0.21	1	0.15	-0.13	0.55	1.00
AB	-0.42	1	0.20	-0.86	0.03	1.00
AC	0.40	1	0.20	-0.046	0.85	1.00
C	-0.47	1	0.20	-0.92	-0.02	1.00
A^2	-0.45	1	0.15	-0.78	-0.11	1.02
B^2	-0.15	1	0.15	-0.48	0.18	1.02
C^2	-0.87	1	0.15	-1.21	-0.54	1.02

Table 4. ANOVA for quadratic model for arginase (U/mL)

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.41	1	0.23	1.90	2.93	
A-Arginine	0.45	1	0.15	0.10	0.79	1.00
B-Yeast extract	0.18	1	0.15	-0.17	0.52	1.00
C-pH	0.21	1	0.15	-0.13	0.55	1.00
AB	-0.42	1	0.20	-0.86	0.03	1.00
AC	0.40	1	0.20	-0.046	0.85	1.00
BC	-0.47	1	0.20	-0.92	-0.02	1.00
A^2	-0.45	1	0.15	-0.78	-0.11	1.02
B^2	-0.15	1	0.15	-0.48	0.18	1.02
C^2	-0.87	1	0.15	-1.21	-0.54	1.02

The regression equation obtained after the analysis of variance (ANOVA) indicated the higher R^2 value of 0.867 (a value of R^2 0.75 indicates the aptness of the model), which ensured a satisfactory adjustment of the quadratic model to the experimental data and indicated that 86% of the variability in the response could be explained by the model [24,25,36]. An adequate precision of 7.782 indicates an adequate signal to measure the signal-to-noise ratio. This equation proved to be the best fit having a low standard deviation of 0.57 and was used for further analysis.

The model F-value of 7.26 implies that the model is significant [34,37]. In this case, the variables that seemed to have a significant effect ("Prob>F" is less than 0.0500) were an individual effect of arginine, interaction effect of yeast extract, and pH and squared effects of arginine and pH. Further, the interaction effects of variables selected on the production of arginase were studied by plotting three-dimensional surface curves to determine the optimum level of each variable for maximum enzyme activity [25,27]. These 3-D response surface plots describe the effect of individual variables and their combined effect upon response (Figure 6a-6c). It shows that increasing yeast extract concentration and decreasing arginine concentration resulted in a sharp decline in arginase activity (Figure 6a). The interaction between yeast extract and pH also showed a positive interactive effect, whereas both go down below optimum level; arginase activity gets declined (Figure 6b). The interaction between arginine concentration and pH showed a bell-shaped graph that depicts their positive correlation (Figure 6c).

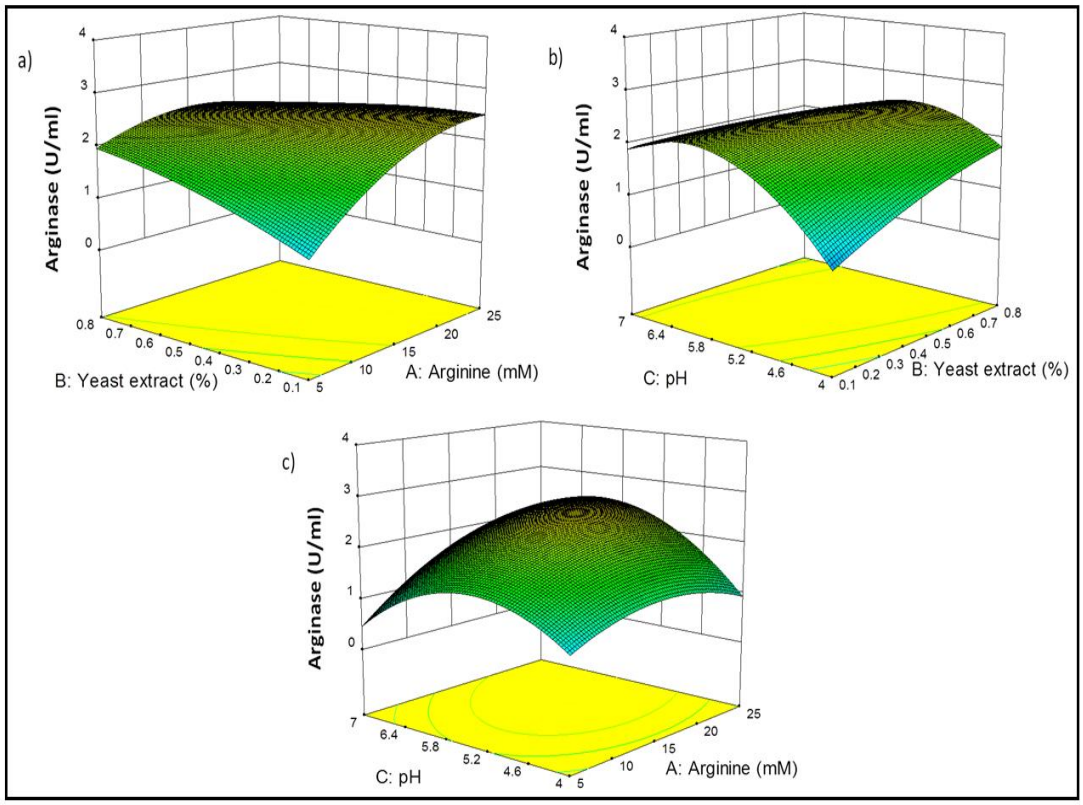


Figure 6. Effect of (a) yeast extract and arginine (b) pH and yeast extract (c) arginine and pH on arginase production

Validation of the model

The maximum activity was obtained by performing optimization of production parameters found to be 3.3 U/mL compared to the predicted value of 2.42 U/mL calculated by ANOVA analysis. The experiments were performed under optimum conditions at pH 5.5 with 1% yeast extract and 15 mM arginine in the media, whereas the optimization through the one variable at a time approach resulted in the maximum activity of 2 U/mL. Comparing data obtained from both approaches results in a 4-fold increase in enzymatic activity through the OVAT approach and a 6.6-fold increase in arginine activity through the RSM approach. Earlier studies have also shown that arginase extraction is enhanced by the RSM approach in *Bacillus licheniformis* [29]. Other studies revealed that 3.5 and 4.5-fold improved arginase production were observed for intra and extracellular arginase, respectively, in the *Streptomyces diastaticus* MAM5 using the RSM approach [31].

CONCLUSION

Despite studying arginase from many bacterial and fungal species in detail, there is no effort seen to obtain this enzyme from lactic culture. This is the first effort to obtain arginase from a lactic acid bacterium, *Lactobacillus acidophilus* and to enhance arginase productivity through OVAT and response surface methodology. The current study revealed that the concentration of arginine, yeast extract, and pH showed a major impact on arginine production. Maximum enzyme activity of 2.0 U/mL was obtained by OVAT with pH 6, yeast extract 0.3 %, and 15 mM arginine concentration. While with the RSM, a 6.6-fold increase in arginase production was observed. Further, these studies can be extended to its potential role as a therapeutic agent as well as for ornithine production.

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Conflicts of Interest: The authors state that there is no conflict of interest to disclose.

REFERENCES

1. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. Trends Pharmacol. Sci. 2015 Jun;36(6):395–405.
2. Munder M. Arginase: an emerging key player in the mammalian immune system. Br. J. Pharmacol. 2009 Oct;158(3):638–51.
3. Alabadi D, Aguero MS, Perez-Amador MA, Carbonell J. Arginase, Arginine Decarboxylase, Ornithine Decarboxylase, and Polyamines in Tomato Ovaries (Changes in Unpollinated Ovaries and Parthenocarpic Fruits Induced by Auxin or Gibberellin). Plant Physiol. 1996 Nov 1;112(3):1237–44.
4. Dzik JM. Evolutionary Roots of Arginase Expression and Regulation. Frontiers in Immunology. 2014 Nov 7;5.
5. Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. Comp. Biochem. Physiol. -B Biochem. Mol. Biol. 1996 May;114(1):107–32.
6. Nakamura N, Fujita M, Kimura K. Purification and Properties of L-Arginase from *Bacillus subtilis*. Agric. Biol. Chem. 1973 Dec 9;37(12):2827–33.
7. Song W, Niu P, Chen X, Liu L. Enzymatic production of l-ornithine from l-arginine with recombinant thermophilic arginase. J. Mol. Catal. B Enzym. 2014;110:1-7.
8. Huang K, Zhang T, Jiang B, Mu W, Miao M. Characterization of a thermostable arginase from *Rummeliibacillus pycnus* SK31.001. J. Mol. Catal. B Enzym. 2016 Nov;133:S68–75.
9. Zeller EA, van Orden LS, Kirchheimer WF. Enzymology of mycobacteria. VI. Enzymic degradation of guanidine derivatives. J. Bacteriol. 1954;67(2):153-58.
10. Yoon JK, Frankel AE, Feun LG, Ekmekcioglu S, Kim KB. Arginine deprivation therapy for malignant melanoma. Clin. Pharmacol. 2013, 5:11-19.
11. Changou CA, Chen YR, Xing L, Yen Y, Chuang FYS, Cheng RH, et al. Arginine starvation-associated atypical cellular death involves mitochondrial dysfunction, nuclear DNA leakage, and chromatin autophagy. Proc. Natl. Acad. Sci.. 2014 Sep 30;111(39):14147–52.
12. Phillips MM, Sheaff MT, Szlosarek PW. Targeting Arginine-Dependent Cancers with Arginine-Degrading Enzymes: Opportunities and Challenges. Cancer Res. Treat. 2013 Dec 31;45(4):251–62.
13. Patil MD, Bhaumik J, Babykutty S, Banerjee UC, Fukumura D. Arginine dependence of tumor cells: targeting a chink in cancer's armor. Oncogene. 2016 Sep 22;35(38):4957–72.
14. Kumari N, Bansal S. Arginine depriving enzymes: applications as emerging therapeutics in cancer treatment. Cancer Chemother. Pharmacol. 2021 Oct 26;88(4):565–94.
15. Cheng PNM, Lam TL, Lam WM, Tsui SM, Cheng AWM, Lo WH, et al. Pegylated Recombinant Human Arginase (rhArg-peg 5,000mw) Inhibits the In vitro and In vivo Proliferation of Human Hepatocellular Carcinoma through Arginine Depletion. Cancer Res. 2007 Jan 1;67(1):309–17.

16. Chrzanowska A, Krawczyk M, Barańczyk-Kuźma A. Changes in arginase isoenzymes pattern in human hepatocellular carcinoma. Biochem. Biophys. Res. Commun. 2008 Dec;377(2):337–40.
17. Hernandez CP, Morrow K, Lopez-Barcons LA, Zabaleta J, Sierra R, Velasco C, et al. Pegylated arginase I: a potential therapeutic approach in T-ALL. Blood. 2010 Jun 24;115(25):5214–21.
18. Hsueh EC, Knebel SM, Lo WH, Leung YC, Cheng PNM, Hsueh CT. Deprivation of arginine by recombinant human arginase in prostate cancer cells. J. Hematol. Oncol. 2012 Dec 30;5(1):17.
19. Glazer ES, Stone EM, Zhu C, Massey KL, Hamir AN, Curley SA. Bioengineered Human Arginase I with Enhanced Activity and Stability Controls Hepatocellular and Pancreatic Carcinoma Xenografts. Transl. Oncol. 2011 Jun;4(3):138–46.
20. Singh R, Pervin S, Karimi A, Cederbaum S, Chaudhuri G. Activity in human breast cancer cell lines: N(ω)-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. Cancer Res. 2000;60(12):3305-12.
21. Zhang X, Liu J, Yu X, Wang F, Yi L, Li Z, et al. High-level expression of human arginase I in *Pichia pastoris* and its immobilization on chitosan to produce L-ornithine. BMC Biotechnol. 2015;15(1).
22. Lange PS, Langley B, Lu P, Ratan RR. Novel Roles for Arginase in Cell Survival, Regeneration, and Translation in the Central Nervous System. J. Nutr. 2004 Oct 1;134(10):2812S-2817S.
23. Beleznai T, Feher A, Spielvogel D, Lansman SL, Bagi Z. Arginase 1 contributes to diminished coronary arteriolar dilation in patients with diabetes. Am. J. Physiol. -Heart Circ. Physiol. 2011 Mar;300(3):H777–83.
24. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley Interdiscip. Rev Comput Stat. 2010 Mar 19;2(2):128–49.
25. Myers RH, Montgomery DC, Anderson-Cook CM. Response Surface Methodology: Process and Product Optimization Using Designed Experiments [Internet]. Wiley; 2016. (Wiley Series in Probability and Statistics).
26. Zhang T, Guo Y, Zhang H, Mu W, Miao M, Jiang B. Arginase from *Bacillus thuringiensis* SK 20.001: Purification, characteristics, and implications for l-ornithine biosynthesis. Process Biochem. 2013;48(4):663-68.
27. Baş D, Boyacı İH. Modeling and optimization I: Usability of response surface methodology. J. Food Eng. 2007 Feb;78(3):836–45.
28. Yu JJ, Park KB, Kim SG, Oh SH. Expression, purification, and biochemical properties of arginase from *Bacillus subtilis* 168. J. Microbiol. 2013;51(2):222-28.
29. Momin B, Chakraborty S, Annapure U. Investigation of the cell disruption methods for maximizing the extraction of arginase from mutant *Bacillus licheniformis* (M09) using statistical approach. Korean J. Chem. Eng. 2018 Oct 20;35(10):2024–35.
30. Unissa R, Sudhakar M, Sunil Kumar Reddy A. Optimized production of L-arginase: A tumour inhibitor isolated from marine bacteria. Int. J. Pharma Bio Sci. 2015;6(3):506-17.
31. Abdelraof M, Abo Elsoud MM, Selim MH, Hassabo AA. L-arginine amidinohydrolase by a new *Streptomyces* isolate: Screening and statistical optimized production using response surface methodology. Biocatal. Agric. Biotechnol. 2020 Mar;24:101538.
32. Nadaf P, Vedamurthy AB. Optimization of l-arginase production by *Pseudomonas* sp. Strain PV1 under submerged fermentation. Int. J. Scient. Technol. Res. 2020;9(1):4390-94.
33. Kumari N, Bansal S. Production and characterization of a novel, thermotolerant fungal phytase from agro-industrial byproducts for cattle feed. Biotechnol. Lett. 2021 Apr 2;43(4):865–79.
34. Chauhan M, Garlapati VK. Modeling Embedded Optimization Strategy for the Formulation of Bacterial Lipase-Based Biodetergent. Ind. Eng. Chem. Res. 2014 Jan 15;53(2):514–20.
35. Kumari N, Bansal S. Statistical modeling and optimization of microbial phytase production towards utilization as a feed supplement. Biomass Convers. Biorefin. 2021 Jun 25.
36. Zainol N, Fakharudin AS, Zulaidi NIS. Model Optimization Using Artificial Intelligence Algorithms for Biological Food Waste Degradation. In: Adv. Waste Process. Technol. 2020.173-81.
37. Waheed A, Akram S, Ashraf R, Mushtaq M, Adnan A. Kinetic model and optimization for enzyme-assisted hydrodistillation of d-limonene-rich essential oil from orange peel. Flavour Fragr. J. 2020;35(5):561-69.