Production of Amylase by *Bacillus polymyxa* NCIM No. 2539 from Agro-industrial Wastes

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Abstract

Background: In the present study, *Bacillus polymyxa* NCIM No. 2539 was selected to utilize agro-industrial byproduct (orange peel) for amylase production under submerged fermentation conditions.

Material and Methods: Different agro-industrial byproducts like cane molasses, wheat bran, rice bran and orange peel were screened for maximum amylase production. Amylase activitiy of *Bacillus polymyxa* was studied using starch-agar plate method. MINITAB software Version 17 and central composite design were applied to evaluate effect of supplementation of substrate with different sulphur containing amino acids (cysteine, methionine and cystine) and vitamin thiamine on enzyme activity. Further optimization of the parameters viz. amount of substrate, concentration of amino acid and vitamin for maximum amylase production was studied by central composite rotatable design.

Results and Conclusion: Among 4 different agro-industrial substrates applied, orange peel showed maximum enzyme production (activity: 492.31 IU g^{-1} sample). Supplementation of the production media with cysteine showed maximum amylase production (515.38 IU g^{-1} sample) among all three amino acids and control. Supplementation with thiamine also showed more amylase production (469.23 IU g^{-1} sample) as compared to control (415.38 IU g^{-1}). Cysteine and thiamine proved to increase amylase production significantly. Maximum amylase production was obtained at 7.7 g orange peel, 37.29 mg cysteine and 34.23 mg per 10 ml thiamine.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

The starch degrading enzyme alpha amylase (α-1,4 glucan-glucanohydrolase (EC 3. 2.1. 1) is widely distributed in nature. This extracellular enzyme hydrolyses α-1,4 glucosidic linkages randomly throughout the starch mole-cule in endo-fashion producing oligosaccharides and mono-saccharides including maltose, glucose and alpha limit dextrin [1-3].

Alpha amylase can be derived from several sources such as plants, animals and microorganisms, but production from the first two groups is limited for several reasons [4]. The concentration of enzymes in the plant material is, generally, low so the processing of large amount of plant material is necessary; on the other hand, enzyme of animal origin is a by-product of meat industry. Microorganisms are chosen preferentially for amylase production due to the relative ease of handling, availability, favorable growth conditions, and cheap

nutrient requirement compared to other producers like plant and animal [5].

There are two types of fermentation methods used for microbial amylase production, i.e. submerged fermentation (SMF) and solid state fermentation (SSF). SSF had been proved to be a more cost effective method because of requirement of simpler equipment, higher volumetric productivity, higher concentration of products and lesser effluent generation [6].

With the advent of biotechnological innovations, especially in the area of fermentation technology and enzyme production, SSF with agro-industrial by-products such as wheat bran, spent brewing grain, maize bran, rice bran, rice husk, coconut oil cake, mustard oil cake, corn bran, citrus peel, etc., is, generally, used for amylase produc-tion [7-12]. The utilization of these agro-industrial wastes, on the one hand, provides alternative substrates

and, on the other, helps in solving pollution problems, which otherwise may cause their disposal [4]. Citrus peel is also an important agro-industrial by-product that can be a rich carbon source for microbial growth, and is used for simultaneous polygala-acturonase (PG) and xylanase produc-tion [13]. As citrus peel is rich in carbohydrates and minerals, it can be utilized as source for amylase production, and its solubility in water makes it suitable for submerged fermentation.

There are very few reports on amylase production by bacterial species under submerged fermentation process. Previously, α-amylase by *Bacillus* (*B.*) *megaterium* 16M [14], *B. licheniformis* M27 [14], *B. coagulans* [7], *B. subtilis* [3], *B. cereus* MTCC 1305 [15] and *B. amyloliquefaciens* [16] have been reported under SSF; however, the yield was found to be low and fermentation time was also extended.

Taking this into account what mentioned above, and since there are no previous reports available in this regard, in the present study, the influence of type of substrate, amino acid and vitamin, and the interactive effect of substrate level, amino acid and vitamin concentration on amylase production by *B. polymyxa* NCIM No. 2539 were studied.

2. Materials and Methods

2.1 Bacterial strain and growth media

B. polymyxa NCIM No. 2539 was procured from NCIM (National Collection of Industrial Microorganism), NCL, Pune, India, and was maintained on the nutrient agar media slants at $4°C$.

2.2 Determining the amylase activity of *B. polymyxa* NCIM No. 2539

Basal nutrient agar plates containing 0.3% w v^{-1} soluble starch were spot-inoculated with bacterial culture and incubated at $30°C$ for 48 h. The plates were flooded with Lugol's Iodine solution to detect the zones of clearance, which represents amylase activity. The area of the zone of clearance in the plates was measured to find out the extent of amylase production.

2.3 Inoculum preparation

Nutrient broth (containing (g l^{-1}) peptone: 10, beef extract: 10, and NaCl: 5, maintained at pH 7-7.5) was prepared. After autoclaving, the broth was inoculated by transferring 2-3 loops full of bacterial colonies from the slants to the broth. Then it was incubated at $30°C$ for 72 h and 150 rpm in incubator plus shaker, sub-cultured after every 15 days, and stored at $4°C$. Finally, this 72-hr old broth was used as inoculum.

2.4 Collection and treatment of agro-industrial waste samples

Agro-industrial waste products (i.e. wheat bran, rice bran and cane molasses) were collected locally from a farm in Varanasi, and orange peels were collected from juice shops and domestic sources. Orange peels were dried in tray driers at about $80°C$ for 3-4 days. Once completely dried and devoid of moisture, the orange peels were turned into powder form using a grinder, and further sieved to get finer powder. Wheat bran and rice bran were also grinded to a fine powder and sieved. The powder form of every sample was stored in air tight containers for further use. Cane molasses was kept in an air-tight colored bottle at $4°C$ and used as such without any pretreatment.

2.5 Production media composition

For submerged fermentation, the production media (100 ml) was prepared in 250 ml conical flask containing: starch or cane molasses: 10, and yeast extract: 5 in 100 ml mineral salt solution (g l^{-1}) (0.1 K_2HPO_4, 1.0; $(NH_4)H_2PO_4$, 0.5; $Mg-SO_4.7H_2O$, 0.1; $CaCl_2$, 0.1; $FeSO_4 .7H_2O$ and 0.1 $MnSO_4$) and pH 7.0 ± 0.2.

For SSF, the production media was prepared in 250 ml conical flask containing 5 g of solid agriculture waste utiliz-ed as substrate moistened with 12.5 ml of mineral salt solution (g l^{-1}) (0.1 K_2HPO_4, 1.0 $(NH_4)H_2PO_4$, 0.5 $MgSO_4 7H_2O$, 0.1 g l^{-1} $CaCl_2$, 0.1 g l^{-1} $FeSO_4 .7H_2O$ and 0.1 g l^{-1} $MnSO_4$) and pH 7.0 ± 0.2. The production media was autoclaved at $120°C$ and 15 psi for 15 min. 5 ml of a 72 h old inoculum broth was then added to each flask containing 100 ml of production media and incubated at 150 rpm, $30°C$ for 72 h. pH of the production media was kept at 7.0 ± 0.2.

2.6 Enzyme extraction

For submerged fermentation, after 72 h of fermentation, the fermented broth was filtered and then centrifuged at $12400 \times g$ at $4°C$ for 20 min. The supernatant was collected and used as crude enzyme extract for enzyme assay.

For SSF, after 72 h of fermentation, 50 ml of phosphate buffer was added to each flask; then the flasks were kept in a shaking incubator at $30°C$ for 1 h at 150 rpm for extraction of enzyme. This broth was then filtered and centrifuged at $12400 \times g$ at $4°C$ for 20 min. The supernatant was collected and used as crude enzyme extract for enzyme assay.

2.7 Enzyme assay

Alpha amylase activity of the extract was measured by DNS method [30]. In brief, the reaction mixture containing 0.5 ml of 1% soluble starch prepared in 50 mM phosphate buffer (pH 7.0) and 0.5 ml of supernatant was taken and incubated at $37°C$ for 20 min followed by the addition of 1 ml of 3, 5-dinitrosalicylic acid (DNS). The amount of the reducing sugar liberated during the assay was estimated by measuring color development at 540 nm by UV-VIS spectrophotometer. 1IU of amylase activity is defined as the amount of enzyme that liberates 1 µmol of glucose per minute per gram sample under standard assay conditions.

2.8 Effect of substrate type, amino acid and vitamin supplementation on amylase production

To ascertain the effect of culture conditions on amylase production, the present study was carried out on different substrates (agro-industrial by-products, including cane molasses, wheat bran, rice bran and orange peel powder). The solid substrate was supplemented with different amino acids (viz. cysteine, cysteine and methionine) and vitamin (thiamine) to study their effect on amylase production against the control (i.e. without any supplementation).

2.9 Optimization of parameters (amount of substrate, concentration of amino acid and concentration of vitamin)

The range of three selected factors (amount of orange peel, concentration of amino acid and concentration of vitamin) are shown in Table 1. These factors were then applied in MINITAB software (ver. 17) whereby full factorial Central Composite Design was employed to obtain the experimental runs as shown in Table 2. The data

obtained from the various experiments were recorded and subjected to statistical analysis as per method of "Analysis of Variance" by CCRD. The significance difference between the means was tested against the critical difference at 95% level of significance by using statistical tool RSM (MINI TAB 17) for data analysis. Statistical optimization of media was done by selecting three factors (amount of orange peel and concentration of amino acid and concentration of vitamin) and one response (enzyme activity). The experim-ental data obtained from the design were analysed by the response surface regression analysis using the following second order quadratic equation:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Where, Y_i is the predicted response, X_i and X_j are independent variables, β_0 is the offset term, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. The effect of individual variable was predicted based on the probability values. The study was carried out at 95% level of significance.

Table 1. Variables and their levels used for different parameters in RSM

Variables	Low level	High level
A(x^1) Orange peel powder(g)	5	10
B(x^2) Amino acid (Cysteine; mg/10ml)	10	50
C(x^3) Vitamin (thiamine; mg/10ml)	10	50

Table 2. CCRD design for optimization of three variables for production of amylase by *Bacillus polymyxa* NCIM No. 2539

Run Order	Orange peel powder (g)	Amino acid (mg/10ml)	Vitamin (mg/10ml)	Enzyme Activity(IU/g sample)
1	7.50	35.00	35.00	1133.33
2	7.50	35.00	35.00	1138.46
3	7.50	35.00	9.77	138.46
4	7.50	9.77	35.00	902.56
5	5.00	20.00	50.00	430.77
6	7.50	35.00	60.22	25.64
7	10.00	20.00	20.00	130.77
8	10.00	20.00	50.00	76.92
9	5.00	50.00	20.00	30.77
10	7.50	35.00	35.00	1128.21
11	10.00	50.00	20.00	596.15
12	7.50	35.00	35.00	1128.21
13	5.00	50.00	50.00	92.31
14	10.00	50.00	50.00	446.15
15	5.00	20.00	20.00	400.00
16	11.70	35.00	35.00	407.63
17	7.50	60.22	35.00	902.56
18	3.29	35.00	35.00	167.65
19	7.50	35.00	35.00	1123.08
20	7.50	35.00	35.00	1128.21

3. Results and Discussion

B. polymyxa NCIM No. 2539 procured from NCIM, Pune and was screened for the production of amylase by the starch hydrolysis test. Figure 1 shows the starch-agar plate containing the area of the zone of clearance of amylase activity. Previously, *B. polymyxa* was used for the produc-

tion of of β-amylase on Corn Steep-Starch-Salts Medium [19]. The effect of different carbon sources was studied on α-amylase production. Figure 2 shows enzyme activity of amylase for carbon sources. As shown in Figure 2, among all carbon sources used for study, starch showed maximum enzyme activity (492.31 IU g^{-1}).

Figure 1. Rapid screening of *Bacillus polymyxa* NCIM No. 2539 showing positive result

This was in correlation to previous report [20] where α-amylase was formed by *B. stearothermophilus* using starch as carbon source the total yield of α -amylase was (225 IU g^{-1}) which was less than obtained result in the present study [20]. But, among all agro-industrial waste sources, orange peel showed amylase production (423.08 IU g^{-1}) much more than the others, followed by cane molasses, wheat bran and then rice bran. Similarly, previously α-amylase on orange peel was produced and it showed the activity 53.72 U $ml^{-1}min^{-1}$ for unfermented substrate and was increased to 1128.2 U $ml^{-1} min^{-1}$ after 4 days of fermentation [21].
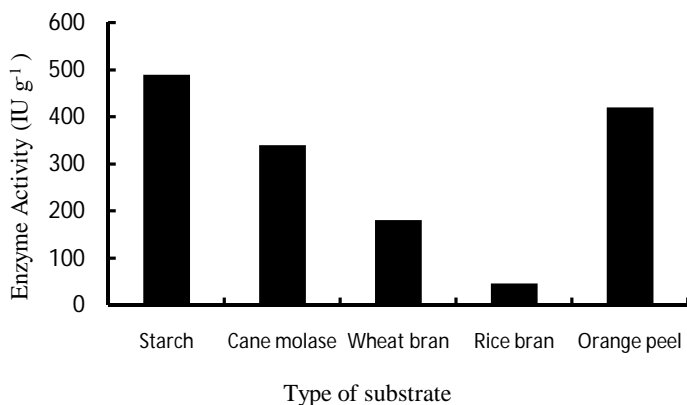

Figure.2. Effect of carbon source on amylase production

The effect of different sulphur containing amino acids was studied on α-amylase production. Figure 3 shows enzyme activity of amylase for amino acid supplementation. As shown in Figure 3, among all the three amino acids used, supplementation with cysteine showed enzyme activity (515.38 IU g^{-1}) much more than by the others. Similarly the supplementation of the L-cysteine in fermentation medium enhanced α-amylase production by 136% using *B. licheniformis* ATCC 12759 strain in submerged ferment-ation, followed by methionine and then cystine [22]. All the amino acid supplementation showed a positive effect on amylase production compared to the control.
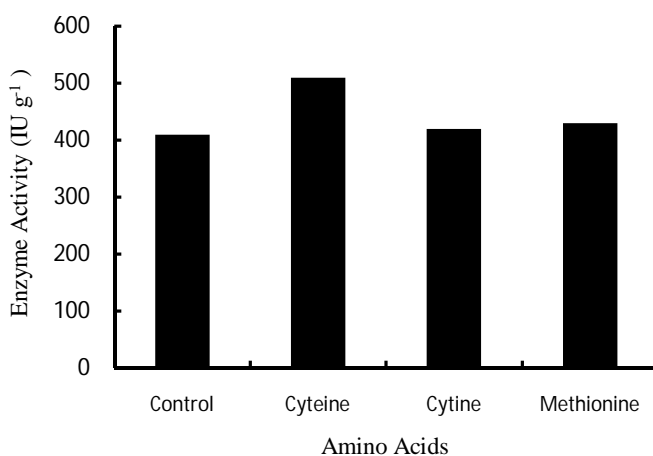

Figure 3. Effect of amino acids source on amylase production

The effect of vitamin thiamine was studied on α-amylase production. Figure 4 shows enzyme activity of amylase for vitamin supplementation. As shown in Figure 4, supplemen-tation with thiamine showed enzyme activity (469.23 IUg^{-1}) more than the control (415.38 IUg^{-1}) i.e. it showed a positive effect on amylase production. Contrary to current finding, media supplemented with nicotinic acid, ascorbic acid and thiamine, at all concentrations, decreased the enzyme prod-uction when compared to that of the control [23].
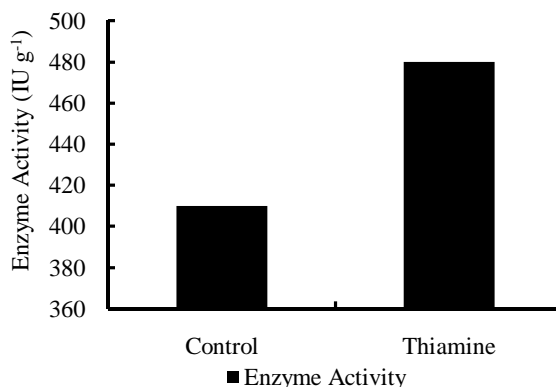

Figure 4. Effect of vitamin thiamine on amylase production

The experiments with different combination of amount of orange peel, amino acid and vitamin concentration were run and assayed for amylase activity. Optimization was done using RSM for three parameters i.e. amount of orange peel and concentration of amino acid and vitamin. A total of 20 experiments were run.

Fig.5A shows the interactive effect of amount of orange peel (g) and concentration of amino acid (mg per 10 ml) on amylase production by *B. polymyxa* NCIM No. 2539 (enzyme activity (IU g^{-1} sample)). It was observed from the graph that with the increase in amount of orange peel, the enzyme activity first increased exponentially and then beyond a certain level (7.5 g), the enzyme activity decreased with the further increase in amount of orange peel. Enzyme activity showed an increasing trend with increase in amino acid concentration till 60 mg per 10 ml (Fig 5 B). The dark-est green area indicates the contour where the enzyme activity was highest (>1000 IU). To maximize enzyme activity, amount of orange peel and concentration of amino acid should lie in the center of the plot i.e. 7.5 g orange peel and 35mg per 10 ml amino acid, taking vitamin concentra-tion constant at 35 mg per 10 ml.

Figure 5B shows the contour plot showing interactive effect of amount of orange peel (g) and concentration of vitamin (mg) on amylase production by *B. polymyxa* NCIM No. 2539 (enzyme activity (IU g^{-1} sample)).It was observed from the graph that with the increase in amount of orange peel, the enzyme activity first increased exponential-ly and the beyond a certain level (7.5 g), the enzyme activity again decreased with the further increase in amount of orange peel. Similarly, enzyme activity showed a increasing trend with increase in vitamin concentration till 40 mg per 10 ml and after that enzyme activity decreased. The darkest green area indicates the contour where the enzyme activity was highest (>1000 IU). To maximize enzyme activity, amount of orange peel and concentration of vitamin should lie in the center of the plot i.e 7.5 g orange peel and 35 mg per 10 ml of vitamin, taking amino acid concentration constant at 35 mg per 10

ml. Figure 5C shows the contour plot showing interactive effect of concentration of amino acid (mg per 10 ml) and vitamin (mg per 10 ml) on amylase production by *B. polymyxa* NCIM No. 2539 (enzyme activity IU g^{-1} sample). It was observed from the graph that with the increase in amino acid concentration, the enzyme activity first increas-ed exponentially and then beyond a certain level (40 mg per 10 ml), the enzyme activity decreased with the further increase in amino acid concentration. Similarly, enzyme activity showed a increasing trend with increase in vitamin concentration till 40 mg per10 ml and after that enzyme activity decreased. The darkest green area indicates the contour where the enzyme activity was highest (>1000). To maximize enzyme activity, concentration of amino acid and vitamin should lie in the center of the plot i.e. 35 mg per 10 ml of amino acid and 35 mg per 10 ml vitamin, taking amount of orange peel constant at 7.5 g.

The enzyme activity obtained corresponding to each run are shown in Table 2. It varies from 25.64 IU g^{-1} sample (lowest) to 1138.46 IU g^{-1} sample (highest). According to the results obtained, the maximum enzyme activity was obtained in Run 2 containing 7.5 g orange peel, 35 mg per 10 ml amino acid and vitamin (Table 2).

Response surface regression analysis was performed and results of estimated regression coefficients of second order polynomial model for optimization of amylase production by *B. polymyxa* using orange peel were shown in Table 3. The regression equation obtained after analysis gives the production of amylase. All the terms regardless of their significance were included in the second order polynomial equation:

$$Y= -3523 + 643.4*A - 4.71*B + 129.41*C - 51.22*A^2 - 0.4565B^2 - 1.7458C^2 + 5.141*AB - 0.987*AC - 0.036*BC$$

Where A= amount of orange peel (g); B= concentration of amino acid (mg per 10 ml); C= concentration of vitamin (mg per 10 ml).

Table 3. Coefficient of quadratic model of coded factors for enzyme activity

Term Constant	Effect	Coef	SE Coef	T-Value	P-Value
		1133.30	27.10	41.94	0.000*
A	172.30	86.20	30.20	2.86	0.017*
B	31.30	15.60	30.20	0.52	0.616
C	-74.20	-37.10	30.20	-1.23	0.247
A^2	-1810.80	-905.40	49.40	-18.34	0.000*
B^2	-581.00	-290.50	49.40	-5.88	0.000*
C^2	-2222.00	-1111.00	49.40	-22.50	0.000*
AB	1190.60	545.30	66.30	8.23	0.000*
AC	-209.40	-104.70	66.30	-1.58	0.145
BC	-46.20	-23.10	66.30	-0.35	0.734

*Significant terms in the model; Coef: coefficient, Model Summary: S: 66.26, R-sq: 98.84%, R-sq(adj): 97.80%
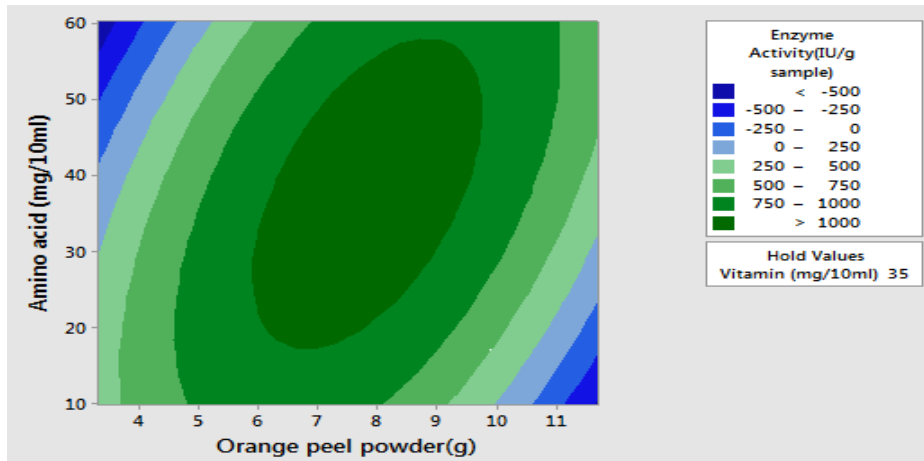R-sq(pred): 91.19%

Figure 5A. Contour plot showing interactive effect of amount of orange peel (g) and concentration of amino acid mg per 10 ml on amylase production by *Bacillus polymyxa* NCIM No. 2539
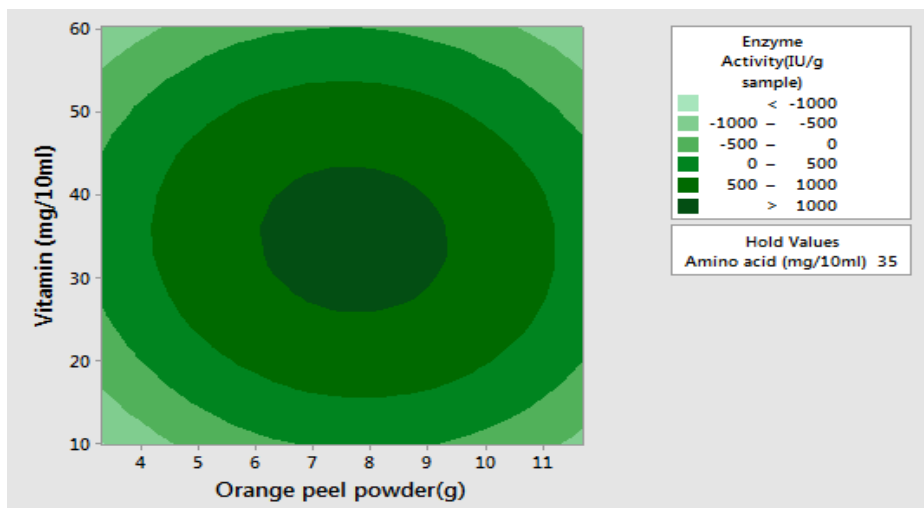

Figure 5B. Contour plot showing interactive effect of amount of orange peel (g) and concentration of vitamin mg per 10 ml on amylase production by *Bacillus polymyxa* NCIM No. 2539
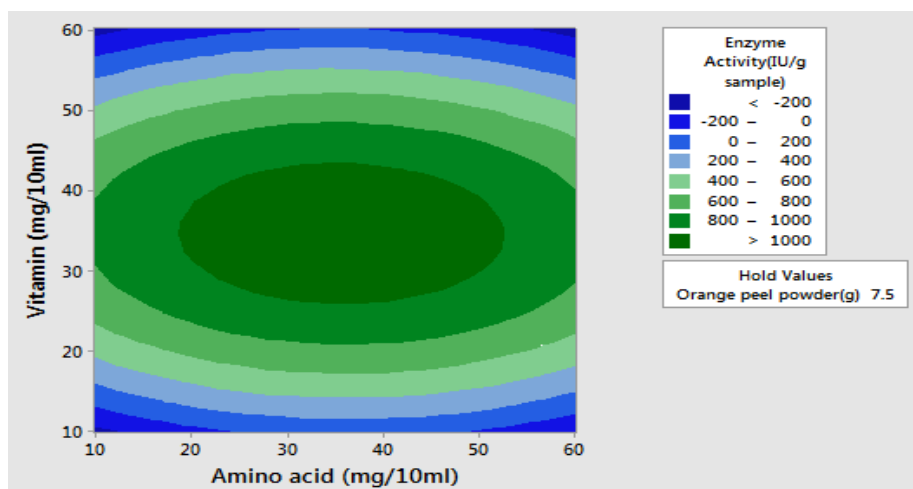

Figure 5C. Contour plot showing interactive effect of concentration of amino acid and vitamin mg per 10 ml on amylase production by *Bacillus polymyxa* NCIM No. 2539

By referring to Table 3, it was found that in linear effect factors, concentration of vitamin showed negative coeffi-cient, while amount of orange peel and concentration of amino acid showed positive coefficient. Square effect factors such as amount of orange peel (A^2); concentration of amino acid (B^2) and concentration of vitamin (C^2), all showed negative coefficients. Quadratic or Interactive effect factors such as amount of orange peel and concentration of vitamin (AC) and concentration of amino acid and concen-tration of vitamin (BC) showed negative coefficients, while amount of orange peel and concentration of amino acid (AB) showed positive coefficient

Student t-test and p-test were also performed and it was found that the linear effect factors A and B showed positive t-value, while C showed negative t-value. All Square effect factors i.e. A^2, B^2 and C^2 showed negative t-values. Quadr-atic or Interactive effect factors AB showed positive t-value, while AC and BC showed negative t-value. From Table 4, the square and 2-way interaction model was found to be significant (P<0.05). By referring to p-value (Table 4), it can be concluded that the amount of orange peel (A) had the most significant effect on the amylase production. For the square effect model, all the parameters found to have significant effect. For the two-way interaction model, interaction of amount of orange peel and concentration of amino acid (AB) was found to be significant (P=0.000).

The goodness of fit of the regression model was determined by the determination coefficient R^2 which provides a measure of how much variability in the observed values can be explained by the experimental factors and their interactions. The determination coefficient (R^2) of the model was calculated to be 0.9884 (a value of >0.75 indicates the fitness of the model). An R^2 value can be between 0 and 1, and the closer value is to 1, the better the model fits the experimental data. Thus the study indicates that 98.84% of the variation in the response (enzyme activity) was attributed to the independent variable, whereas 1.16% of the total variance could not be explained by the model. An adjusted R^2 was a corrected R^2 after the elimina-tion of unnecessary model terms. In this study, it was found that the adjusted R^2 was 0.978, which was high and very close to R^2. This high adjusted R^2 value was attributed to the absence of non-significant terms in the model and its close-ness to actual R^2 value showed good correlation between observed and predicted value responses. Both of the obtained values suggested that the model fits the data well.

ANOVA was also performed to test the significance and adequacy of the second order polynomial model. The results were summarized as in Table 4. The significance of the regression was evaluated by the Fischer F-value and P-value. The F-value predicts the quality of the entire model considering all design factors at a time. The P-value is the probability of the factors having very little or insignificant effect on the response. Larger F-values signifies better fit of the RSM model on the experimental data [17]. High F-value with a low P-value indicates high significance of the regression model [18]. However, the P-value should be lower than 0.05 for the model to be statistically significant. The square and interactive effect factors were found to be highly significant with high F-value and low P-value.

Table 4. Analysis of variance for RSM quadratic model for amylase production

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	9	3747755	416417	94.84	0.000*
Linear:	3	43679	14560	3.32	0.065
A	1	35853	35853	8.17	0.017*
B	1	1180	1180	0.27	0.616
C	1	6646	6646	1.51	0.247
Square:	3	3395240	1131747	257.76	0.000*
A^2	1	1476759	1476759	336.34	0.000*
B^2	1	152013	152013	34.62	0.000*
C^2	1	2223539	2223539	506.42	0.000*
2-way Interaction	3	308837	102946	23.45	0.000*
AB	1	297339	297339	67.72	0.000*
AC	1	10963	10963	2.50	0.145
BC	1	534	534	0.12	0.734
Error:	10	43907	4391	-	-
Lack of fit	5	43767	8753	312.04	0.000*
Pure error	5	140	28	-	-
Total	19	3791662	-	-	-

*Significant terms in the model; DF: degree of freedom; Adj SS: adjusted sum of squares; Adj MS: adjusted mean Square

Lack of fit test was also performed. An insignificant P-value (> 0.05) for lack of fit indicates high significance. In this study, P-value for lack of fit was found to be less than 0.05, which indicated that there might be some factors contributing in the regression-response relationship that are not accounted for in the model. Response optimizer was performed and the results for optimum condition for maximum amylase production were shown in Figure 6.

The amount of orange peel was varied from 3 g to 11.7 g; amino acid and vitamin was varied from 9.7 mg per10 ml to 60.2 mg per 10 ml in the experimental runs, but according to the response optimizer, the maximum amylase production (1137.81 IU g^{-1} sample) was obtained at orange peel amount of 7.79 g; amino acid concentration of 37.3 mg per 10 ml and vitamin concentration of 34.3 mg per 10 ml. At this level, desirability obtained was 0.999. According to this plot, it was clear that the enzyme production decreased at both very high and very low level of orange peel, amino acid and vitamin. In all the cases, the enzyme production and activity was first increased with the increase in amount or concentration of the parameters under study and after attaining a certain value, the enzyme activity decreased with the further increase in the concentration. The change in enzyme activity with change in amino acid concentration was less than that with orange peel and vitamin. It means that orange peel and vitamin had more pronounced effect on the enzyme production than amino acid. Further validation of optimized conditions by performing experiment at optimized conditions showed a clear increase in amylase production and activity from a predicted value of 1137.81 IU g^{-1} sample to an experimental value of 1310.66 IU g^{-1} sample.
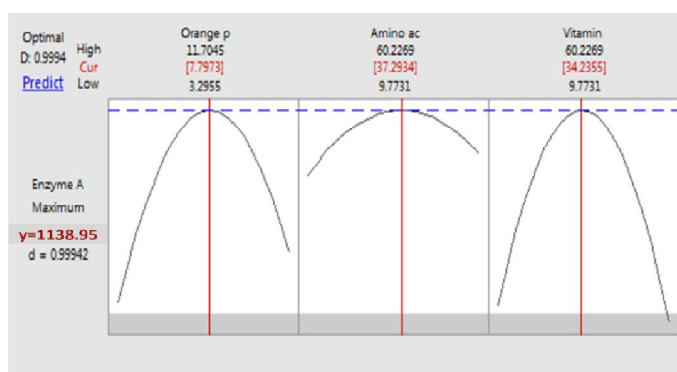

Figure 6. Optimization plot for production of amylase by *Bacillus polymyxa* NCIM No. 2539

4. Conclusion

From the present study, it was concluded that *B. polymyxa* NCIM No. 2539 can be used as a good source of amylase production. Also orange peel was proved to be a good substrate for amylase production. Cysteine and thiamine proved to increase amylase production significantly. The interactive effect of amount of orange peel and concentration of amino acid and concentration of vitamin was also found to be significant at some levels, and maximum amylase production was obtained at 7.7 gm orange peel, 37.29 mg per 10 ml concentration of cysteine and 34.23 mg per10 ml concentration of thiamine.

5. Acknowledgements

6. Conflict of Interest

The authors report no conflict of interest.

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تولید آمیلاز از *باسیلوس پلی میکسا* NCIM No. 2539 از پسماند کشت و صنعت پوست پرتقال

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چکیده

سابقه و هدف: در مطالعه حاضر *از باسیلوس پلی میکسا* NCIM No. 2539 به منظور استفاده از فراورده جانبی کشت و صنعت (پوست پرتقال) درتولید آمیلاز با تخمیر غوطهور استفاده شد.

مواد و روشها: فراوردههای جانبی گوناگون کشت و صنعت مانند ملاس نیشکر، سبوس گندم، سبوس برنج و پوست پرتقال به منظور تولید بیشینه آمیلاز غربالگری شدند. فعالیت آمیلاز *باسیلوس پلی میکسا* با استفاده از روش پلیت نشاسته- آگار مورد مطالعه قرار گرفت. برای ارزیابی اثر مکملافزایی سوبسترا با انواع آمینواسیدهای گوگرددار (سیستئین، متیونین و سیستین) و ویتامین تیامین بر فعالیت آنزیمی از نرم افزار مینی تب نسخه 17 و طرح مرکب مرکزی استفاده شد. سپس بهینهسازی پارامترها یعنی میزان سوبسترا، غلظت آمینواسید و ویتامین برای بیشینه تولید آمیلاز با طرح چرخشی مرکب مرکزی مورد مطالعه قرار گرفت.

یافتهها و نتیجهگیری: در میان 4 سوبسترای گوناگون کشت و صنعت مورد استفاده، پوست پرتقال بیشینه تولید آنزیم را از خود نشان داد (فعالیت: 492/31 واحد بین المللی به ازای گرم نمونه). مکمل سازی محیط کشت با سیستئین، بیشینه تولید آمیلاز را در میان تمام 3 آمینو اسید و شاهد به همراه داشت (515/38 واحد بین المللی به ازای گرم نمونه). مکمل سازی با تیامین تولید بیشتر آمیلاز (469/23 واحد بین المللی به ازای گرم نمونه) را در مقایسه با شاهد (415/38 واحد بین المللی به ازای گرم نمونه) نشان داد. سیستئین و تیامین افزایش قابل توجه تولید آمیلاز را اثبات کرد. تولید بیشینه آمیلاز با 7/7 گرم پوست پرتقال، 37/29 گرم سیستئین و 34/23 میلی گرم در 10 میلی لیتر تیامین به دست آمد.

تعارض منافع: نویسندگان اعلام میکنند که هیچ تعارض منافعی وجود ندارد.