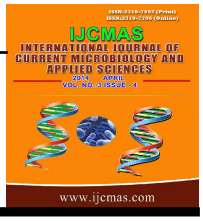
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 4 (2014) pp. 269-282**
http://www.ijcmas.com



Original Research Article

Application of Plackett-Burman Design for improved cold temperature production of lipase by psychrotolerant *Pseudomonas* sp. AKM-L5

Abhas Kumar Maharana* and Pratima Ray

Department of Microbiology, C.B.S.H., Orissa university of agriculture and technology, unit 7, surya nagar, Bhubaneshwar, Odisha-751003, India.

*****Corresponding author*

Keywords

Cold active lipase; Psychro-tolerant; *Pseudomonas* sp. AKM-L5; Plackett-Burman; experimental design.

A B S T R A C T

In this study, Plackett-Burman experimental design was used to develop a useful medium for the cold temperature production of lipase by a psychrotolerant *Pseudomonas* sp. AKM-L5 isolated from soil of Jammu city. The maximum lipase activity was found on 72 h of incubation (7.46 U/mL) at 15°C by taking tributyrin oil as substrate and at pH 8.0. Parameters like nitrogen sources, carbon sources, mineral chlorides and solid substrates were investigated by this experimental design. Fit of the model was evaluated by determination of R^2 and regression equation and main effect of each variable on lipase production were analyzed. Parameters like calcium nitrate, lactose, glucose, almost all mineral chlorides except sodium chloride and potassium chloride, and oil cakes influenced the lipase production significantly with very high confidence levels. Optimized medium formulated by Plackett-Burman experimental design enhances the lipase production by bacteria 3.45 -fold higher over that of un-optimized medium at cold temperature by using oil cakes. The findings of this study indicate the possibility that the isolated strain produce novel lipolytic cold active enzymes that are able to degrade lipid substrates at low temperature.

Introduction

Lipases (EC 3.1.1.3) are the hydrolytic enzymes those act on carboxylic ester bonds of triglycerides and give diglycerides, monoglycerides, fatty acids and glycerol as end product. Lipases occur widely in nature, but only microbial lipases are commercially significant as they are more stable than their corresponding plant and animal enzymes (Wiseman, 1995).

Lipases can be used in brewing and wine making, dairy processing, fruit, meat, and vegetable processing, starch modifications, leather processing, pulp and paper manufacture, detergents and cleaning agents, synthesis of amino acids and bulk chemicals, and also in wastewater treatment (Sharma et al., 2001).

Among the bacterial isolates more work is focused on lipases from *Pseudomonas* because of their versatility, stability and reactivity (Gao et al., 2000). Many researches were done on *Pseudomonas* lipase (Andersson, 1980; Aoyama et al., 1988; Tan et al., 1996; Choo et al., 1998; Dieckelmann et al., 1998; Rashid et al., 2001; Alquati et al., 2002) by taking refrigerated food(Andersson, 1980), milk samples (Dieckelmann et al., 1998), Alaskan soil (Choo et al., 1998) and subterranean environment (Rashid et al., 2001) as sources but there was a little work where the source was soil samples from sub-tropical Jammu region with the aim of production of cold active lipase.

Several of factors are regulating the enzyme activity in a production medium. Traditionally workers were acquainted with 'one factor at a time' method of optimization process. But this method has its own limitations like more consumption of time, labor intensive and maximum errors (Tari et al., 2006). To overcome these problems, there are many statistical techniques which can be used for effective and commercially important results. Plackett-Burman experimental design (PBD) is a tool to improve quality control processes and product by minimizing the wastage of time and money and limited number of experiments with efficient estimation. Analysis of PBD enlightens the most important factors by eliminating the inhibitors from production medium, which ultimately enhance the product production and formulation of new media. The former method was unable to distinguish the effects of all factors which can be done by PBD even if there was a little effect. The present study used PBD for production optimization of different carbon and nitrogen sources, mineral chlorides and solid substrates for cold active lipase production by psychrotolent *Psedomonas* sp. AKM-L5.

Materials and Methods

Lipase producing isolate

The studied cold active lipase producing bacterium *Psedomonas* sp. AKM-L5 (GenBank Accession Number KF649130) was isolated from soil of Jammu city (32° 432' 483" N latitude and 74° 522' 123" E). Screening for lipase production was done by taking tributyrin agar medium incubated at 15°C for 72-96 h (Maharana and Ray, 2013). Twenty four hour old culture in nutrient broth medium, incubated at 15°C was taken as inoculum for the production of lipase.

Lipase production by submerged fermentation

Lipase production was done by using 250mL Erlenmeyer flasks. Production medium used was the slight modification of mineral salt medium consisting (gL^{-1}) of yeast extract (1.0), NaCl (2.0), $MgSO_4$ (0.4), $(NH_4)_2SO_4$ (0.5), K_2HPO_4 (0.3), KH_2PO_4 (0.3), $CaCl_2$. $2H_2O$ (0.1), gum arabic (2.0), $NaNO_3$ (0.5) and 0.5% (v/v) tributyrin oil with pH 8.0 (Lee et al., 1999). Production was done at cold temperature i.e. 15°C for 72 h and lipase activity was determined from supernatant collected by samples in the interval of 24 h after centrifugation at 12,000 rpm for 20 min at 4°C.

Lipase production by solid state fermentation

Solid substrates were collected and sliced. Then it was spread on trays and oven dried at 70±2°C for 24 h. The dried slices were grounded and sieved through standard

mesh sieves to obtain particles ranging in size from 200-2400 µm, and stored in polyethylene bags at room temperature (30±2°C) until use (Singh et al., 2012). For production of lipase solid substrates with the above mentioned mineral salt medium was used having moisture content 75% and 10% inoculum (1.4×10^7 CFU/mL).

Lipase Assay

Lipase activity was measured spectro-photometrically using p-nitrophenyl palmitate (Sigma–Aldrich Co.) as substrate and formation of p-nitrophenol was measured at 400 nm (Gupta et al., 2002). Slight modification was done by incubating the reaction mixture at 15°C for 30 min. One unit of lipase activity is defined as the amount of enzyme releasing one micromole of p-nitrophenol per minute per milliliter under assay condition.

Fractional factorial design

To optimize lipase production by Pseudomonas sp. AKM-L5 Plackett-Burman statistical experimental Design (PBD) was used (Plackett and Burman, 1946). The main effect of each variable on lipase activity was calculated by using the following:

$$\text{Main effect} = [\Sigma Y_{(+)}/ n_{(+)}] - [\Sigma Y_{(-)}/ n_{(-)}] \quad (1)$$

The positive value of effect of variable indicates there will be a significant influence at the high concentration of the component and negative value shows the impact will be more at low concentration of the medium component. Parameters like carbon sources, nitrogen sources, mineral chlorides and various solid substrates were investigated by using the above design. The lower (-1) and higher (+1) level of each variable was given in respective tables for each parameters. Plackett-Burman experimental design is based on the first order polynomial model:

$$Y = \beta_0 + \Sigma \beta_i X_i \quad (2)$$

Where, Y is the response (lipase activity), β_0 is the model intercept, β_i is the linear coefficient, and X_i is the level of the independent variable. All experiments were conducted in triplicates and averages of the results were taken as the response.

Statistical analysis

All the above experiments were statistically analyzed by regression tool present in Microsoft Office Excel 2007 for coefficient determination, t-value, R^2 and adjusted R^2 and p-value. Factors having over 95% confidence level were considered to be highly significant effect on lipase production where as factors having 70-95% confidence level were consider to be effective otherwise factors were insignificant.

Results and Discussion

Evaluation of optimum incubation period for lipase production

The psychrotolerant *Pseudomonas* sp. AKM-L5 was investigated for the better production of lipase in varied incubation period from 12-96 h which revealed that maximum activity was shown on 72 h of incubation (7.46 U/mL) at 15°C by using tributyrin oil as substrate. After that there was a slight fall of lipase activity which may be due to lack of nutrients and changes in medium pH (Fig. 1). The inoculum added for production was 5% (1.1×10^7 CFU/mL). There was a positive

and significant correlation among the incubation period and lipase activity (r =0.96) and they were significantly differ at 0.05 probability level. For all the other experiments 72 h was taken as optimum incubation period for the given bacteria to produce cold active lipase. Another work was done on *Pseudomonas* sp. for lipase production at 30°C where maximum activity was shown on 72 h of incubation period (Tembhurkar et al., 2012). Besides many workers reported varied optimum incubation period for lipase production where various medium composition and isolates were concerned.

Influence of different nitrogen sources

In this experiment, a total of 12 experiments (in triplicates) were conducted by taking six variables having two levels each i.e., '+1' denotes 5.0 g/L and '-1' denotes 1.0 g/L of the variables in production medium. The experimental matrix with their observed and predicted responses was given in Table 1. The variation in lipase activity was varied from 4.65 to 9.38 U/mL showing the significant influences in each runs. The Pareto graph (Fig. 2) showed the main effect of all nitrogen sources on lipase activity calculated from the Equation-1. The maximum effect was given by calcium nitrate among all the others. Positive value shows the significant effect was due to high level of the variables and vice versa. Statistical analysis revealed that only calcium nitrate has highly significant effect having 99.99% confidence level (Table 2). Fit of the model was evaluated by determination of R^2 and regression equation. This model gave a high R^2 value of 98.85% and adjusted R^2 value of 97.48% for lipase activity. The high value of determination of coefficient (0.9885) indicates that only about 1.15% of the total variations were not satisfactorily explained by this model. The regression equation was formulated by the significant variables coefficients into Equation-2.

$$Y= 3.5151 + 0.0628 (X3) + 0.0741 (X4) + 0.95X6) \quad (3)$$

Where, Y is lipase activity in U/mL, X3 peptone, X4 is $NaNO_3$ and X6 is Ca $(NO_3)_2.4H_2O$ with their coefficients.

Here inorganic nitrogen sources are better stimulants for lipase production rather than organic nitrogen sources, which were also in accordance with the results of Tembhurkar et al (2012) while working on *Pseudomonas* sp. The variables having insignificant effect (confidence level < 70%) were omitted from the regression equation. Another work reported about a fungal lipase by solid state fermentation of palm Kernal cake where organic nitrogen sources were best over inorganic nitrogen sources (Imandi et al., 2010) and the same was reported by Rajendran et al (2007) where *Pseudomonas fluorescens* produce lipase at 30°C by using olive oil as substrates.

Influence of different carbon sources

A total of 12 experiments (in triplicates) were conducted by taking six carbon sources having two levels each i.e., '-1' denotes 2.0 g/L and '+1' denotes 10.0 g/L of the variables in production medium. The experimental matrix with their observed and predicted responses was given in Table 3. The variation in lipase activity was varied from 6.62 to 11.2U/mL showing the significant influences in each runs. The Pareto graph (Fig. 3) showed the main effect of all carbon sources on lipase activity. The maximum effect was given by lactose among all the others. Statistical

analysis revealed that only lactose has highly significant effect having 99.99% confidence level (Table 4). Fit of the model was evaluated by determination of R^2 and regression equation. This model gave a high R^2 value of 97.61% and adjusted R^2 value of 94.73% for lipase activity. The high value of determination of coefficient (0.9761) indicates that only about 2.39% of the total variations were not satisfactorily explained by this model. The regression equation was formulated by the significant variables coefficients into Equation-2.

$$Y = 6.4562 + 0.0875\,(X1) - 0.0686\,(X2) - 0.0484\,(X4) - 0.0371\,(X5) + 0.4651\,(X6) \quad (4)$$

Where, Y is lipase activity in U/mL, X1, X2, X4, X5 and X6 are glucose, xylose, sucrose, maltose and lactose respectively with their coefficients. Equation revealed that there is an increase of lipase activity with increase in lactose and glucose concentration in production medium. Imandi and coworkers reported that glucose affects a lot in lipase production in spite of lactose which showed negative response for fungal lipase production (Imandi et al., 2010). The present study was not in accordance with the mentioned work.

Influence of different mineral chlorides

A total of 12 experiments (in triplicates) were conducted by taking six variables having two levels each i.e., '-1' denotes 1.0 g/L and '+1' denotes 5.0 g/L of the variables in production medium. The experimental matrix with their observed and predicted responses was given in Table 5. The variation in lipase activity was varied from 4.49 to 10.25 U/mL showing the significant influences in each runs. The Pareto graph (Fig. 4) showed the main effect of all mineral chlorides on lipase activity. The maximum effect was given by $MnCl_2.4H_2O$ among other variables. Positive value shows the significant effect was due to high level and vice versa. Statistical analysis revealed that only $MnCl_2.4H_2O$ has highly significant effect having 99.73% confidence level (Table 6). Fit of the model was evaluated by determination of R^2 and regression equation. This model gave R^2 value of 95.98% and adjusted R^2 value of 91.16% for lipase activity. The value of determination of coefficient (0.9598) indicates that 4.02% of the total variations were not satisfactorily explained by this model. The regression equation was formulated by the significant variables coefficients into Equation-2.

$$Y = 4.8452 + 0.2049(X3) + 0.6295(X4) + 0.2263(X5) - 0.3578(X6 \quad (5)$$

Where, Y is lipase activity in U/mL, X3, X4, X5 and X6 are $CaCl_2$, $MnCl_2.4H_2O$, $MgCl_2$, and $BaCl_2.2H_2O$ respectively with their coefficients.

Minerals were essential for bacterial growth and lipase production. Here results showed except KCl and NaCl all other variables were affecting lipase production by *Pseudomonas* sp. AKM-L5. The present study is not in accordance with the study of Rajendran et al (2007) where both $CaCl_2$ and $MnCl_2.4H_2O$ drastically affect lipase production by *Pseudomonas fluorescens.*

Influence of different solid substrates

A total of 12 runs (in triplicates) were conducted by taking six variables having two levels each i.e., '-1' denotes 0.5 and '+1' denotes 1.0 g/experiment of the

variables in production medium. The experimental matrix with their observed and predicted responses was given in Table 7. The variation in lipase activity was varied from 7.9 to 23.11 U/mL showing the significant influences in each runs. The Pareto graph (Fig. 5) showed the main effect of all substrates on lipase activity. The maximum effect was given by ground nut oil cake among all. Positive value shows the significant effect was due to high level and vice versa. Statistical analyses revealed that coconut oil cake, ground nut oil cake, Teal oil cake have highly significant effect having 99.74%, 99.96%, 95.09% confidence levels respectively (Table 8). Fit of the model was evaluated by determination of R^2 and regression equation. This model gave R^2 value of 95.75% and adjusted R^2 value of 90.64% for lipase activity. This value of determination of coefficient (0.9575) indicates that about 4.25 % of the total variations were not satisfactorily explained by this model. The regression equation was formulated by the significant variables coefficients into Equation-2.

$$Y = -7.2106 + 8.4384 (X1) + 12.835 (X2) + 3.927 (X3) + 2.601 (X5 \quad (6)$$

Where, Y = lipase activity (U/mL), X1= coconut oil cake, X2= ground nut oil cake, and X3= teal oil cake and X5= wood chips. It can be concluded from equation - 6 that high level of all above factors will increase the responses (lipase activity) significantly.

Production of lipase by optimized conditions

Production of lipase was determined by taking all significantly affecting parameters ($p< 0.05$) with mineral salt medium as described earlier. There was significant fold increase with the various sources used where high fold 3.45 was observed in case of solid substrates (Table 9). Results revealed that additional sources to the production medium influences the lipase activity significantly.

It can be conclude from the above study that Plackett-Burman experimental design was found appropriate tool to enhance production of lipase by *Pseudomonas* sp. AKM-L5. The variables having much impact on lipase production were Ca $(NO_3)_2.4H_2O$, lactose, $MnCl_2.4H_2O$ and ground nut oil cake. Besides there was a negative impact of $BaCl_2.2H_2O$ in lipase production which reduces the lipase activity in medium therefore, it is better to be eliminated. Among all the sources, solid substrates were regarded as better when fold increase is concerned. Regression equations were made by taking all the results having higher confidence level (above 70%) but it can be modified if $p< 0.05$ is considered by eliminating the factors having below 95%.

The uniqueness of the present study was the production temperature. Cold active lipase was the important tool in various fields like pharmaceutical preparations, cosmetics, food production, waste management and also detergent making for cold washing of delicate clothes. These experimental outcomes can be used for high production of cold active lipase applied in varied industries and biotechnological purposes and it will give a new idea to the experimenters for better use.

Figure.1 Effect of incubation period on lipase activity

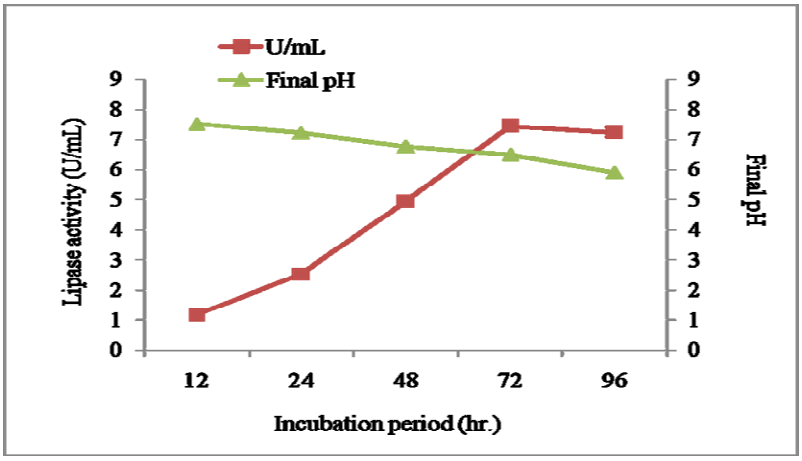


Figure.2 Pareto graph showing effect of various nitrogen sources on lipase production based on the observation of PBD

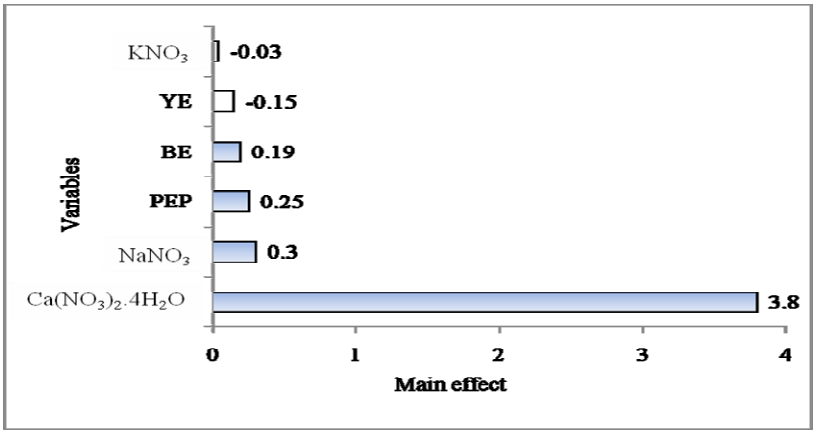


Figure.3 Pareto graph showing effect of various carbon sources on lipase production based on the observation of PBD

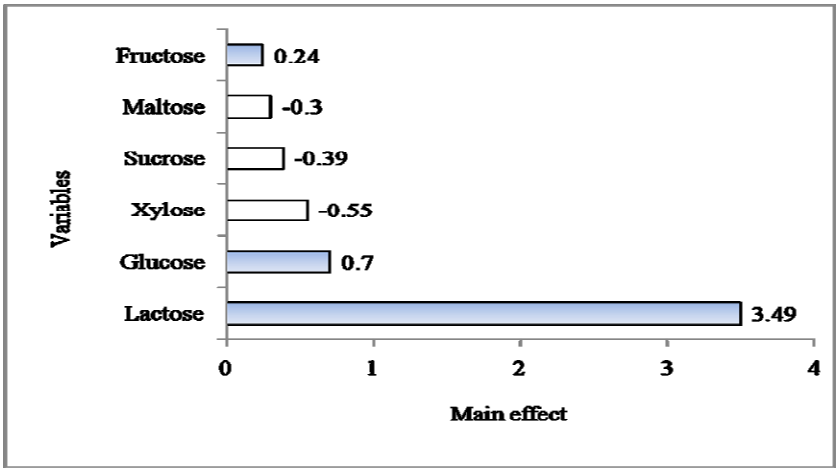


Figure.4 Pareto graph showing effect of various mineral chlorides on lipase production based on the observation of PBD

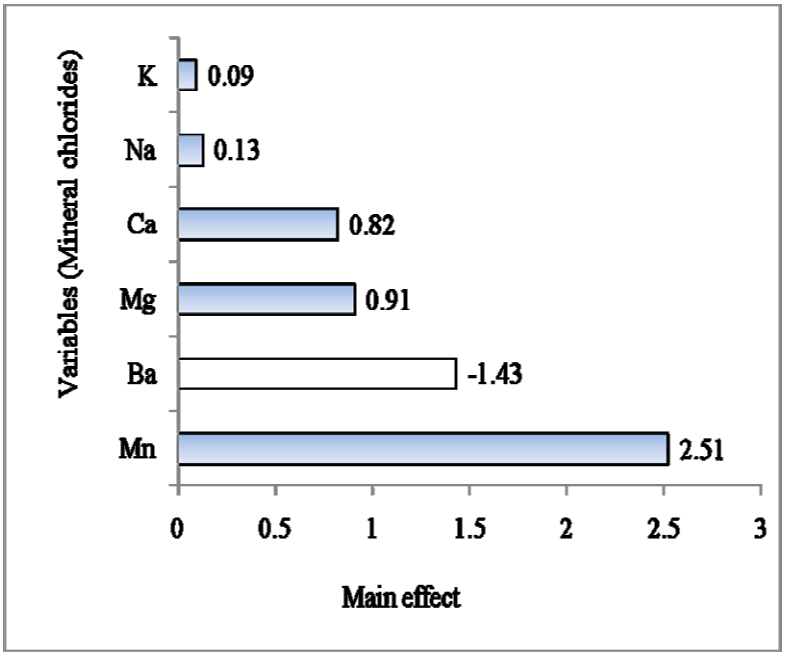


Figure.5 Pareto graph showing effect of various solid substrates on lipase production based on the observation of PBD

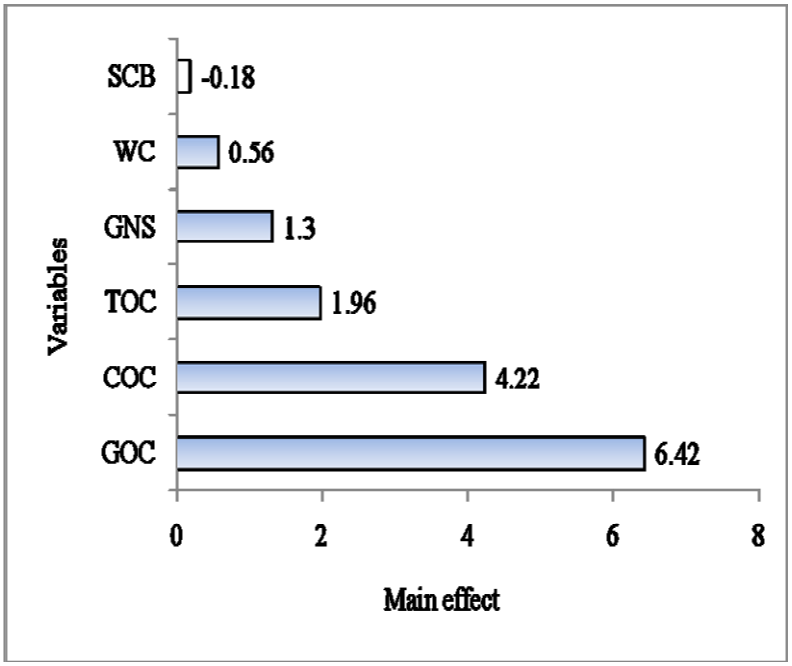


Table.1 PBD matrix for screening of various nitrogen sources for lipase production

Run order	Variables (g/L)						Lipase activity (U/mL)[b]	
	X1 YE*	X2 BE*	X3 PEP*	X4 $NaNO_3$	X5 KNO_3	X6 $Ca(NO_3)_2.4H_2O$	Observed[a]	Predicted
1	5	1	5	1	1	1	4.99	4.71
2	5	5	1	5	1	1	4.96	4.94
3	1	5	5	1	5	1	4.9	5.01
4	5	1	5	5	1	5	8.47	8.8
5	5	5	1	5	5	1	4.89	4.91
6	5	5	5	1	5	5	8.79	8.66
7	1	5	5	5	1	5	9.38	9.14
8	1	1	5	5	5	1	4.89	5.12
9	1	1	1	5	5	5	8.98	8.66
10	5	1	1	1	5	5	8.13	8.22
11	1	5	1	1	1	5	8.32	8.59
12	1	1	1	1	1	1	4.65	4.60

[a]The observed values were mean of triplicates.
*YE, Yeast extract, BE, Beef extract, PEP, Peptone
[b] r= 0.99 ($p< 0.05$)

Table.2 Statistical analysis of PBD (Nitrogen sources)

Variables	Lipase activity (U/mL)			
	Coefficient	t-stat	p-value	Confidence level (%)
Intercept	3.5151	10.027	0.0001	99.98
YE	-0.0366	-0.795	0.4624	53.76
BE	0.0471	1.023	0.3529	64.71
PEP	0.0628	1.363	0.2308	76.92
$NaNO_3$	0.0741	1.608	0.1685	83.14
KNO_3	-0.0084	-0.181	0.8627	13.72
$Ca(NO_3)_2.4H_2O$	0.95	20.627	0.0000	99.99

R^2= 98.85%, adjusted R^2= 97.48%

Table.3 PBD matrix for screening of various carbon sources for lipase production

Run order	Variables (g/L)						Lipase activity (U/mL)[b]	
	X1 Gluc	X2 Xylos	X3 Fructos	X4 Sucros	X5 Maltos	X6 Lactos	Observed[a]	Predicted
1	10	2	10	2	2	2	8.72	8.2
2	10	10	2	10	2	2	6.98	7.02
3	2	10	10	2	10	2	6.62	6.66
4	10	2	10	10	2	10	11.07	11.31
5	10	10	2	10	10	2	6.77	6.72
6	10	10	10	2	10	10	10.53	10.85
7	2	10	10	10	2	10	10.03	10.06
8	2	2	10	10	10	2	6.92	6.817
9	2	2	2	10	10	10	10.23	10.07
10	10	2	2	2	10	10	11.2	11.16
11	2	10	2	2	2	10	10.59	10.2
12	2	2	2	2	2	2	6.66	7.26

[a]The observed values were mean of triplicates.
[b] r= 0.987 ($p< 0.05$)

Table.4 Statistical analysis of PBD (Carbon sources)

Variables	Lipase activity (U/mL)			
	Coefficient	t-stat	p-value	Confidence level (%)
Intercept	6.4562	13.275	0.0000	99.99
Glucose	0.0875	2.739	0.0408	95.92
Xylose	-0.0686	-2.149	0.0843	91.56
Fructose	0.0304	0.952	0.3847	61.52
Sucrose	-0.0484	-1.516	0.1899	81.01
Maltose	-0.0371	-1.161	0.2978	70.21
Lactose	0.4368	13.681	0.0000	99.99

R^2= 97.61%, adjusted R^2= 94.73%

Table.5 PBD matrix for screening of various mineral chlorides for lipase production

Run order	Variables (g/L)						Lipase activity (U/mL) [b]	
	X1 KCl	X2 NaCl	X3 $CaCl_2$	X4 $MnCl_2.4H_2O$	X5 $MgCl_2$	X6 $BaCl_2.2H_2O$	Observed[a]	Predicted
1	5	1	5	1	1	1	6.29	6.51
2	5	5	1	5	1	1	8.15	8.34
3	1	5	5	1	5	1	6.9	7.45
4	5	1	5	5	1	5	7.61	7.6
5	5	5	1	5	5	1	9.43	9.24
6	5	5	5	1	5	5	6.65	6.11
7	1	5	5	5	1	5	7.46	7.64
8	1	1	5	5	5	1	10.25	9.85
9	1	1	1	5	5	5	7.35	7.59
10	5	1	1	1	5	5	4.84	5.17
11	1	5	1	1	1	5	4.49	4.3
12	1	1	1	1	1	1	5.98	5.60

[a]The observed values were mean of triplicates.
[b] r= 0.979 ($p< 0.05$)

Table.6 Statistical analysis of PBD (Mineral chlorides)

Variables	Lipase activity (U/mL)			
	Coefficient	t-stat	p-value	Confidence level (%)
Intercept	4.8452	8.839	0.0003	99.96921
KCl	0.0228	0.317	0.7642	23.57804
NaCl	0.0317	0.44	0.6783	32.1662
$CaCl_2$	0.2049	2.847	0.0359	96.40494
$MnCl_2.4H_2O$	0.6295	8.746	0.0003	99.96763
$MgCl_2$	0.2263	3.144	0.0255	97.44547
$BaCl_2.2H_2O$	-0.3578	-4.97	0.0042	99.579

R^2= 95.98%, adjusted R^2= 91.16%

Table.7 PBD matrix for screening of various solid substrates for lipase production

Run order	Variables* (gram/experiment)						Lipase activity (U/mL)[b]	
	X1	X2	X3	X4	X5	X6	Observed[a]	Predicted
	COC	GOC	TOC	SCB	WC	GNS		
1	1	0.5	1	0.5	0.5	0.5	13.73	13.25
2	1	1	0.5	1	0.5	0.5	17.27	17.52
3	0.5	1	1	0.5	1	0.5	15.73	16.75
4	1	0.5	1	1	0.5	1	13.13	13.63
5	1	1	0.5	1	1	0.5	19.08	18.83
6	1	1	1	0.5	1	1	23.11	21.53
7	0.5	1	1	1	0.5	1	15.58	15.83
8	0.5	0.5	1	1	1	0.5	9.85	10.15
9	0.5	0.5	0.5	1	1	1	9.79	8.75
10	1	0.5	0.5	0.5	1	1	11.58	13.15
11	0.5	1	0.5	0.5	0.5	1	13.73	14.04
12	0.5	0.5	0.5	0.5	0.5	0.5	7.9	7.07

[a]The observed values were mean of triplicates.

[b] $r = 0.978$ ($p < 0.05$)

*COC, coconut oil cake; GOC, ground nut oil cake; TOC, Teal oil cake; SCB, sugarcane bagasse; WC, wood chips; GNS, ground nut shell

Table.8 Statistical analysis of PBD (solid substrates)

Variables	Lipase activity (U/mL)			
	Coefficient	t-stat	p-value	Confidence level (%)
Intercept	-7.2106	-2.561	0.051	94.94
COC*	8.4384	5.557	0.003	99.74
GOC*	12.835	8.453	0.0003	99.96
TOC*	3.927	2.586	0.049	95.09
SCB*	-0.3603	-0.237	0.823	17.81
WC*	2.601	1.713	0.147	85.26
GNS*	1.1169	0.736	0.495	50.49

$R^2 = 95.75\%$, adjusted $R^2 = 90.64\%$

*COC, coconut oil cake; GOC, ground nut oil cake; TOC, Teal oil cake; SCB, sugarcane bagasse; WC, wood chips; GNS, ground nut shell

Table.9 Fold increase in lipase activity (U/mL) after PBD experiment

Parameters	Variables			
	Nitrogen sources	Carbon sources	Mineral chlorides	Solid substrates
Un-optimized[a]	7.07	7.69	6.76	7.46
Optimized[b]	11.98	13.19	14.37	25.71
Fold increase	1.69	1.72	2.13	3.45

[a]Mineral salt medium only

[b]Mineral salt medium with respective significantly activating sources with eliminating inhibitors if any (determined from production experiments)

Acknowledgment

The authors would like to thank Prof. (Dr.) Bibhuti Bhusan Mishra, Head, Department of Microbiology, C.B.S.H., Orissa University of Agriculture and Technology, Bhubaneswar, for the laboratory support.

References

Alquati, C., L. De-Gioia, G. Santarossa, L. Alberghina, P. Fantucci and Lotti M. 2002. The cold-active lipase of *Pseudomonas fragi* : Heterologous expression, biochemical characterization and molecular modeling. Eur. J. Biochem. 269, 3321-3328.

Andersson, R. E., 1980. Microbial lipolysis at low temperatures. Appl Environ Microbiol. 39, 36-40.

Aoyama, S., N. Yoshida and Inouye, S. 1988. Cloning, sequencing and expression of the lipase gene from *Pseudomonas fragi* IFO-12049 in *E. coli*. FEBS Lett. 242, 36-40.

Choo, D.W., T. Kurihara, T. Suzuki, K. Soda and Esaki N. 1998. A cold-adapted lipase on an Alaskan psychrotroph, *Pseudomonas* sp. strain B11-1: Gene Cloning and Enzyme Purification and Characterization. Appl Environ Microbiol. 64, 486-491.

Dieckelmann, M., L. A. Johnson and Beacham, I.R. 1998. The diversity of lipases from psychrotrophic strains of *Pseudomonas*: A Novel Lipase from a highly lipolytic strain of *Pseudomonas fluorescens.* J Appl Microbiol. 85, 527-536.

Gao, X-G., S-G. Cao and Zhang, K-C. 2000. Production, properties and application to non aqueous enzymatic catalysis of lipase from a newly isolated *Pseudomonas* strain. Enzyme Microb Tech. 27, 74-82.

Gupta, N., P. Rathi and Gupta, R. 2002. Simplifiedpara-nitrophenyl palmitate assay for lipases and esterases. Anal Biochem. 311, 98-99.

Imandi, S.B., S.K. Karanam and Garapati, H.R. 2010. Optimization of media constituents for the production of lipase in solid state fermentation by *Yarrowia lipolytica* from palm Kernal cake (*Elaeis guineensis*). Adv Biosci Biotech. 1, 115-121.

Lee, D.W., Y.S. Koh, K.J. Kim, B.C. Kim, H.J. Choi, D.S. Kim, M.T. Suhastono and Ryu, Y. R. 1999. Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. FEMS Microbiol Lett. 179, 393-400.

Maharana, A. K., and Ray, P. 2013.

Isolation and screening of cold active extracellular enzymes producing psychrotrophic bacteria from soil of Jammu City. Biosci Biotech Res Asia. 10(1): 267-273.

Plackett, R.L., and Burman, J.P. 1946. The design of optimum multi factorial experiments. Biometrika. 33(4): 305-325.

Rajendran, A., M. Thirugnanam and Thangavelu, V. 2007. Statistical evaluation of medium components by Plackett-Burman experimental design and kinetic modeling of lipase production by *Pseudomonas fluorescens.* Indian J Biotech. 6, 469-478.

Rashid, N., S. Yuji, E. Satoshi, A. Haruyuki and Tadayuki, I. 2001. Low-temperature lipase from psychrotrophic *Pseudomonas* sp. Strain KB700A. Appl Environ Microbiol. 67, 4064-4069.

Sharma, R., Y. Chisti and Banerjee, U.C. 2001. Production, purification, characterization, and applications of lipases. Biotech Adv. 19, 627-662.

Singh, A.K., A.K. Maharana, H. Masih, Y. Kumar and Mishra, S.K. 2012. Production, optimization and purification of bacterial cellulase by solid state bio-processing of agro biomass. Res J Pharm Bio Chem Sci. 3(2): 977-989.

Tan, S., A.R.K. Owusu and Knapp, J. 1996. Low temperature organic phase biocatalysis using cold-adapted lipase from psychrotrophic *Pseudomonas* P38. Food Chem. 57, 415-418.

Tari, C., H. Genckal and Tokatli, F. 2006. Optimization of a growth medium using a statistical approach for the production of an alkaline protease from a newly isolated *Bacillus* sp. L21. Process Biochem. 41, 659-665.

Tembhurkar, V.R., M.B. Kulkarni and Peshwe, S.A. 2012. Optimization of lipase production by *Pseudomonas* spp. in submerged batch process in shake flask culture. Sci Res Rep. 2(1): 46-50.

Wiseman, A. 1995. Introduction to principles. In: A Wiseman Handbook of enzyme biotechnology, 3rd edn, edited by Padstow & Cornwall, (UK: Ellis Horwood Ltd. T.J. Press Ltd.), pp. 3-8.