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RESEARCH PAPER

Production and optimization of pterin deaminase from cyanide utilizing bacterium *Bacillus cereus* AM12

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Optimization

Abstract In a search of new-fangled antitumor chemotherapeutic agent, the studies are elucidating the enzymes, contributing to the metabolic reprogramming and their potential as therapeutic targets. This study was an endeavor to explore the potential of enzyme pterin deaminase from cyanide degraders. In preliminary and secondary screening, potent cyanide degraders were selected and estimated for pterin deaminase activity. The intracellular crude extract containing enzyme activity was found to be higher in potential strain *Bacillus cereus* AM12. The optimization conditions for the production of pterin deaminase from this selected strain was studied with the help of Box–Behnken design. The determination coefficient of the model (0.91) was highly significant and indicative of goodness of fit. This design found to be ideal for the selected parameters, such as temperature of 35 °C, pH 8, 0.005 M of pterin and 20% of glucose which yielded maximum production of pterin deaminase (9.99 U/ml). This study might make a major breakthrough in the field of medicine as well as in pharmaceutical compositions when the biological significance of this distinct enzyme is proven.

Introduction

Enzymes are invoked as chemotherapeutic drugs for disease conditions. It holds the peculiar affinity and binding properties toward precise targets with minimal toxicity. Pteridine has been recognized as imperative biomolecule for many biological processes, such as amino acid metabolism, nucleic acid synthesis, neurotransmitter synthesis, cancer, cardiovascular function, growth and development of all living organisms (Milstein, Kapatos, & Levine, 2002). It serves as essential cofactors in the process of cell metabolism and becomes a focal point of cancer screening research (Gamagedara, Gibbons, & Ma, 2011). In the metabolic pathway of pteridines, one of the most important unexplored enzyme is pterin deaminase. Pterin deaminase is an enzyme belonging to the family of hydrolases, which has been assigned the EC number of 3.5.4.11 and acts on carbon–nitrogen bonds other than peptide bonds of

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2-amino-4-hydroxypteridine. When it reacts with substrate in the presence of water, it produces 2,4-dihydroxypteridine with the liberation of ammonia. It has been highly stable against proteases, high temperature and alkaline pH (Bernstein & Van Driel, 1980). This enzyme controls the irreversible pterin catabolism by hydrolytic deamination of various pterin such as pterin, 6-carboxypterin, biopterin, 6-methylpterin, 7-methylpterin, xanthopterin, hydroxymethylpterin, sepiapterin, isosepiapterin, folic acid and 6,7-dimethylpterin to its corresponding lumazine (Curtius et al., 1983). This unique pterin deaminase was distributed as intracellular, membrane-bound or extracellular enzyme in a wide range of prokaryotic and eukaryotic sources (Ziegler, 2003). The first report on intracellular pterin deaminase was isolation of pterin deaminase from the bacterium *Alcaligenes metalcaligenes*. In bacteria, pterin deaminase might be produced for regulating the metabolism of pterins (Takikawa, Kitayamayokokawa, & Tsusue, 1979). In mammals, anabolic and catabolic steps of pterin metabolism might regulate as co-stimulators or inhibitors of lymphocyte activation and lymphoblast proliferation (Ziegler, Ulrike, & Berndt, 1983).

In 1976, Kusakabe and his colleagues discovered that pterin deaminase exhibits effective antitumor activity. Apart from drug resistance, there are limitations for therapeutics like insufficient drug concentrations, systemic toxicity and lack of selectivity among the normal and cancer cells. This has paved a way for novel therapy by targeting the folic acid directly, which is the precursor for DNA synthesis of cancerous cells. Chabner, Chello, and Bertino (1972) reported that carboxypeptidase produced pteroate by removing the terminal glutamate residue of the folate molecule and has been recognized as antitumor agent. However, pterin deaminase seems to be more effective than the carboxypeptidase because it completely inactivates folic acid by transforming pterin structure into biologically inactive lumazine structure and it does not catalyze any reaction related to the transformation of pterin moiety of folic acid (Kusakabe et al., 1976). Understanding the structure and function of the enzyme might provide solutions to several unsolved problems in the biological system. This is the first report on pterin deaminase production from cyanide utilizing bacterium *Bacillus cereus* AM12 which was supposed to produce pterin as a cofactor for cyanide oxidizing enzyme cyanide oxygenase (Cabuk et al., 2006). Cyanide oxygenase is an enzyme involved in the oxygenolytic conversion of cyanide to carbon dioxide and ammonia. It has been recently reported that cyanide oxygenase is a pterin-dependent hydroxylase.

Assuming that the cofactors production also increases with increased production of metabolic enzymes and cofactor regulating enzymes also would be produced, the first attempt has been made to isolate cyanide utilizing bacteria from the cyanide contaminated soil samples for pterin deaminase production which regulates the pterin concentration in the bacteria.

These enzymes are also the perpetrator in decreasing the BH_4 concentration leading to neuronal diseases such as Parkinson's disease, Alzheimer's disease and schizophrenia (Jayaraman et al., 2016). Inspite of the antitumor property and biological roles exhibited by pterin deaminase, lacunae in research with detailed biological and biochemical characterization is enigmatic. So understanding this unexplored enzyme might deal with several unsolved problems in the biological system. Thus, the present study was attempted to explore the production of pterin deaminase.

Materials and methods

Sample collection

Soil samples were aseptically collected in sterile polythene bags from three different places viz., sago factory (Attur), metal plating and solid waste disposal industry (SIDCO, Coimbatore). These samples were brought to laboratory within 24 h for the subsequent isolation of bacteria.

Isolation and identification of bacteria

Bacterial isolation from the soil samples were conducted by serial dilution plate method. Nutrient agar (Himedia Pvt. Ltd., Mumbai, India) was used for the isolation and enumeration of bacteria. Pure colonies were obtained by quadrant streaking method, sub-cultured and preserved for further studies.

Selection of cyanide degrading bacteria

Preliminary selection was carried out to exploit the ability of the bacteria to utilize cyanide as nitrogen source. Selection of cyanide utilizing bacteria was done in M9 minimal medium without ammonium and citrate (Luque-Almagro et al., 2005). The selection medium contains the following ingredients as per liter of distilled water: Na_2HPO_4 – 12.8 g/l, KH_2PO_4 – 3.0 g/l, NaCl – 0.5 g/l, $MgSO_4$ – 120 g/l, $CaCl_2$ – 110.98 g/l, sodium acetate – 4.10 g/l. 10 mL of 20% filter sterilized glucose was added. Various concentrations of membrane (0.2 μm) sterilized potassium cyanide solution (0.001–0.005 M) was added to the medium before plating and allowed to solidify (Maniatis, Fritsch, & Sambrook, 1982). Each bacterial strain were streaked onto potassium cyanide supplemented nutrient agar plates and incubated at 37 °C for 5 days. Bacterial strains capable of surviving in potassium cyanide plates were selected for further screening.

Screening of pterin deaminase producing cyanide utilizing bacteria

Cyanide utilizing bacteria capable of producing pterin deaminase were identified by adopting the method of Gulati, Saxena, and Gupta (1997) with slight modifications. The modified M9 medium supplemented with 0.1% bromothymol blue dye and 0.005 M pterin (sole source of carbon and nitrogen) were prepared aseptically and inoculated with selected bacterial strains. The ability of the bacteria to grow and change the media pH after 48 h of incubation, served as criteria for confirming pterin deaminase production. The bacterial strains which utilize pterin as a substrate were selected for pterin deaminase production.

Preparation of crude enzyme

After the growth for 3 days in modified M9 medium, the bacterial cells were harvested by centrifugation at 10,000 × *g* at 4 °C for 15 min, washed by suspending in a small volume of 0.05 M Tris Buffer, pH 8 and collected by centrifugation. The supernatant and the pellet were separated. The intracellular enzyme (pellet) was taken as a crude extract containing enzyme because of their higher enzyme activity. The dispersed pellet was sonicated using Ultra Sonicator (S-4000 MISONIX). After sonication the supernatant was collected from the sonicated sample and it was stored at −20 °C for at least 3 months without appreciable loss of enzyme activity (Levenberg & Hayaishi, 1959). Protein was assayed by the method of Lowry et al. (1951) using BSA as a standard.

Assay for pterin deaminase

The assay of pterin deaminase activity was adopted from the method described by Mashburn and Wriston (1964). The assay conditions used for the estimation are as follows; 340 μl of 0.005 M of pterin was incubated with 100 μl of crude extract containing enzyme at 30 °C in the presence of 40 μl of 0.05 M Tris buffer (pH 8). The reaction was terminated using 1.5 M tricholoroacetic acid. The resulting precipitate was centrifuged and estimated the ammonia suspended in supernatant using Nessler's reagent ($A_{480\,nm}$). Pterin deaminase activity was expressed as micromoles of ammonia released per minute per milliliter against pterin as a substrate. Corresponding heat inactivated crude extract for 10 min was used as blank for each assay.

Scanning electron microscopy

The potent bacterial culture morphology was studied using scanning electron microscopy. A drop of the granule suspension of bacterial culture was placed on air dried slide and fixed with 2% (v/v) glutaraldehyde for 2 h. The slide was washed with sodium carbonate bicarbonate buffer (0.05 M) and passed through a series of increasing acetone concentrations (50, 70, 90 and 100% (v/v)). The slide was dried using a critical point dryer (Gomes & Mergulhao, 2017). The sample was coated with a thin film of gold using a spimodule sputter coating device and observed under scanning electron microscope at 5000× magnification in high-vacuum mode at 10 kV (FEI Quanta 250).

16S rRNA gene sequence

The standardized method of Sambrook and Russell (2001) was used to extract DNA from bacterial culture. The genomic DNA of potent bacterial strain was taken through 16S rRNA gene amplification, sequencing, BLAST analysis and sequence comparison in Gen bank nucleotide databases. The 16S rRNA gene was amplified using the universal primers, forward (27F) 5′-AGAGTTTGATCMTGGCTCAG-3′ and reverse (1492R) 5′-ACGGYTACCTTGTTACGACTT-3′ (James, 2010). The gene sequence was analyzed for the similarity and homology with the existing sequences available in data bank of National Center for Biotechnology Information (NCBI) using BLAST search available at http://www.ncbi.nlm.nih.gov/. The sequences were aligned and incorporated into phylogenetic tree construct using maximum-likelihood method. The phylogenetic tree was revealed with the help of MEGA 5 and BioEdit Software.

Optimization for pterin deaminase activity

Response surface methodology (RSM) was a statistical model to analyze the interaction of factors in a set of experiments for determining the maximum influence on the chosen response by altering them concurrently. Box and Behnken (1960), a RSM design experiment, uses a group of measured responses and controlled experimental factor, based upon one or more selected criteria. The factors were selected from three different levels (−1, 0, +1) such as minimum, central and maximum values wherein six runs at the center of the design are devoted for appraising the pure error and sum of squares. In the present study, six independent variables including pH (A), temperature (B), inoculum size (C), incubation period (D), substrate–pterin (E) and carbon source-glucose (F) were selected for the optimum production of pterin deaminase from crude extract containing enzyme (chosen response).

Several experimental designs have been selected for the Box–Behnken design proposed by Box, Hunter, and Hunter (1978). The current experiments consisted of 6 factorial designs where 54 experiments were required in order to determine the most influencing parameters on the production of pterin deaminase (Kathiresan, Saravanakumar, Sahu, & Sivasankaran, 2014; Shanmugam, 2011). The chosen response (Y) on these selected variables could be expressed by quadratic polynomial equation as shown below:

$$\begin{aligned} Y = {} & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 \\ & + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} + \beta_{44} X_{42} + \beta_{55} X_{52} + \beta_{66} X_{62} \\ & + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 + \beta_{16} X_1 X_6 \\ & + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{26} X_2 X_6 + \beta_{34} X_3 X_4 \\ & + \beta_{35} X_3 X_5 + \beta_{36} X_3 X_6 + \beta_{45} X_4 X_5 + \beta_{46} X_4 X_6 + \beta_{56} X_5 X_6 \end{aligned}$$

where Y is the predicted response, β_0 is the constant, β_1, β_2, β_3 and β_4 are the linear co-efficient, β_{11}, β_{22}, β_{33} and β_{44} are the quadratic co-efficient and β_{12}, β_{13}, β_{23} and β_{24} and β_{34} are the cross-product coefficients.

Results

A total of forty seven bacterial isolates were obtained as pure cultures from soil samples and tested for the cyanide utilizing activity. The preliminary selection was made in M9 minimal medium with the addition of potassium cyanide solution (0.001–0.005 M). Among the forty seven strains, the eight strains were observed as cyanide degrading bacteria. These potent eight cyanide degraders were subjected to pterin deaminase production. Only three bacterial strains MP4, MP8 and AM12 were able to grow with pterin as sole substrate. Among the selected bacteria, the intracellular crude extract containing enzyme activity was found to be higher in strain AM12 (9.99 U/ml) followed by MP8 (2.45 U/ml) and MP4 (1.25 U/ml), respectively'. Thus, the

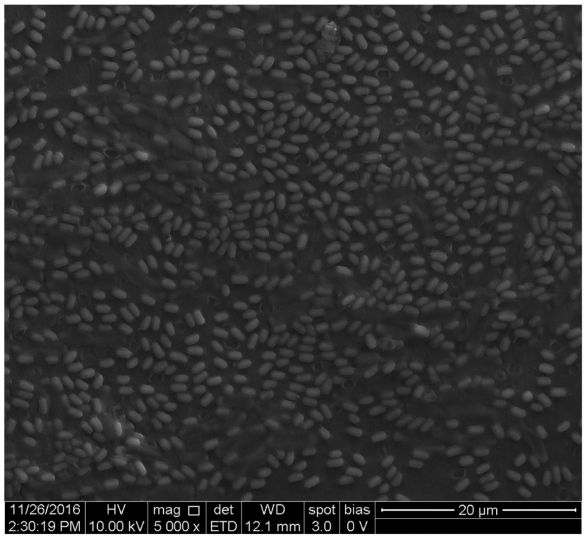

Figure 1 Scanning electron microscopyimage of *Bacillus cereus*.

efficient bacterial strain AM12 was used for further experiments.

The SEM image of AM12 exposed the structure of *Bacillus* species in Fig. 1. 16S rRNA sequencing revealed that the potential strain was *Bacillus cereus* AM12 and Genbank accession number as KX298861. The evolutionary relationship was deduced by using the maximum with outgroup (Fig. 2).

Determination of significant variables by Box–Behnken design

Table 1 represented ANOVA and multiple regression analysis of experimental results. The *F* value 9.73 of model exemplified significant nature of the model. The *F* value occurred due to noise was significantly nullified. Prediction of experimental results was found to be significant and estimated factor effects were real. This was supported from the high calculated F values than the tabulated value. High *F* value and a very low probability ($P > F$ less than 0.05) indicated that the present model was in a good prediction of experimental results and model terms were significant. The determination coefficient of the model (0.91) was highly significant and indicative of goodness of fit. The obtained results revealed that 6% cannot be explained by the model. The integrity of the model was expressed from the ''Pred R^2'' (0.81) which was in realistic agreement with the ''Adj R^2'' (0.62). The Lack of Fit *F* value (0.6) demonstrated that the model was significant and non-significant lack of fit was found as good in order to fit the model. Signal to noise ratio has been measured from ''Adeq Precision'' and the value of 10.9 indicated the desirability of the model. The response surface model provided clear outlook not only on optimum conditions for the production of pterin deaminase enzyme from crude intracellular extract but also helped to study the combination effects between selected variables on the chosen response. Using response surface model, the logarithmic values of enzyme yields and test variables in coded unit were expressed as follows:

$$\begin{aligned}\text{Enzymeactivity} = {} & +9.54 + 1.000\text{E}{-}002 * \text{A} - 0.023 * \text{B} \\ & + 0.023 * \text{C} + 0.11 * \text{D} + 0.39 * \text{E} + 0.42 * \text{F} \\ & - 0.027 * \text{AB} - 0.10 * \text{AC} + 0.034 * \text{AD} + 0.014 * \text{AE} \\ & - 0.061 * \text{AF} + 8.750\text{E}{-}003 * \text{BC} + 0.093 * \text{BD} \\ & + 0.21 * \text{BE} - 0.011 * \text{BF} + 0.048 * \text{CD} + 0.039 * \text{CE} \\ & - 0.042 - \text{CF} + 0.12\text{DE}{-}0.030 * \text{DF} + 0.29 * \text{EF} \\ & - 1.14 * \text{A}^2 - 0.53\text{B}^2 - 0.12 * \text{C}^2 + 0.34 * \text{D}^2 - 0.93 * \text{E}^2 \\ & - 0.68 * \text{F}^2\end{aligned}$$

The 3D contour plot was generated from plotting the response for optimum production of pterin deaminase. The response surface was selected based on the interaction between the two selected variables with another variable being at a fixed level. Fig. 3 represents the interaction effect between temperature and pH on the enzyme activity. It also

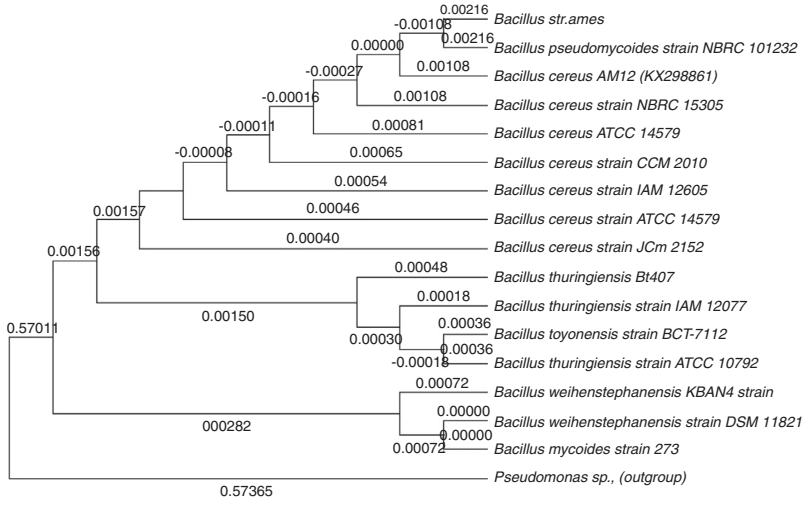

Figure 2 Phylogenetic analyzes of potent *Bacillus cereus* AM12.

Table 1 ANOVA for response surface quadratic model.

ANOVA for response surface quadratic model					
Source	Sum of squares	df	Mean square	*F* value	*p*-Value Prob > *F*
Model	36.80	27	1.36	9.73	<0.0001
A-pH	2.400E−003	1	2.400E−003	0.017	0.8969
B-Temperature	0.013	1	0.013	0.093	0.7625
C-Inoculum size	0.013	1	0.013	0.093	0.7625
D-Incubation period	0.30	1	0.30	2.15	0.1544
E-Pterin	3.67	1	3.67	26.23	<0.0001
F-Glucose	4.18	1	4.18	29.86	<0.0001
AB	6.050E−003	1	6.050E−003	0.043	0.8370
AC	0.086	1	0.086	0.61	0.4401
AD	0.019	1	0.019	0.13	0.7163
AE	0.16	1	0.16	1.14	0.2956
AF	0.030	1	0.030	0.21	0.6473
BC	6.125E−004	1	6.125E−004	4.372E−003	0.9478
BD	0.068	1	0.068	0.49	0.4907
BE	0.72	1	0.72	5.16	0.0317
BF	1.013E−003	1	1.013E−003	7.228E−003	0.9329
CD	0.018	1	0.018	0.13	0.7225
CE	0.012	1	0.012	0.086	0.7720
CF	0.028	1	0.028	0.20	0.6582
DE	0.11	1	0.11	0.81	0.3777
DF	7.200E−003	1	7.200E−003	0.051	0.8224
EF	0.69	1	0.69	4.93	0.0354
A^2	13.42	1	13.42	95.82	<0.0001
B^2	2.92	1	2.92	20.87	0.0001
C^2	0.14	1	0.14	0.98	0.3324
D^2	1.20	1	1.20	8.56	0.0071
E^2	8.84	1	8.84	63.09	<0.0001
F^2	4.73	1	4.73	33.73	<0.0001
Residual	3.64	26	0.14		
Lack of fit	2.60	21	0.12	0.60	0.8153
Pure error	1.04	5	0.21		
Core total	40.44	53			

illustrated that optimum interaction was observed for the selected variables for the production of pterin deaminase activity (response). Temperature also played a vital role in influencing the activity of pterin deaminase enzyme from crude intracellular extract (Fig. 3). Temperature was considered to exhibit the affirmative response along with the glucose concentration (Fig. 3). The pH also played optimum interaction with substrate concentration, pterin (Fig. 3) for the optimum production of pterin deaminase. Similarly, pH showed a positive influence with glucose concentration in the production of pterin deaminase activity (Fig. 3). The presence of a center point of the 3D plot strongly suggested the influence of the pterin and glucose concentration on the optimization of enzyme production (Fig. 3). Other chosen parameters were not found to show a positive influence on the optimization of production of pterin deaminase enzyme from crude intracellular extract.

The transformation of the response variable was an appropriate method for stabilizing the variance of the response. Generally, transformations are meant to stabilize the response, to make distribution of the response variable closer to the normal distribution and to improve the fit of the model to the data. The natural logarithm (ln) of the residual sum of square (SS) against λ declined suddenly with a minimum in the region with best optimum value of 0.9 (Fig. 3). The model exhibited the minimum and maximum confidence interval value of −0.34 to 3.94, respectively. The minimum value of the standard error existed in low and flat error around the centroid indicated the fitness of the design points and polynomial fitness (Fig. 4).

Comparison of observed and predicted response and validation of the model

A regression model could be used to compare and validate the observed and predicted model for the maximum activity of crude extract-containing the pterin deaminase enzyme. High resemblance was notified between the predicted data of the response (9.54 U/ml) from the empirical model and the experimental values (9.97 U/ml) which demonstrated the applicability of Box–Behnken of RSM to optimize the

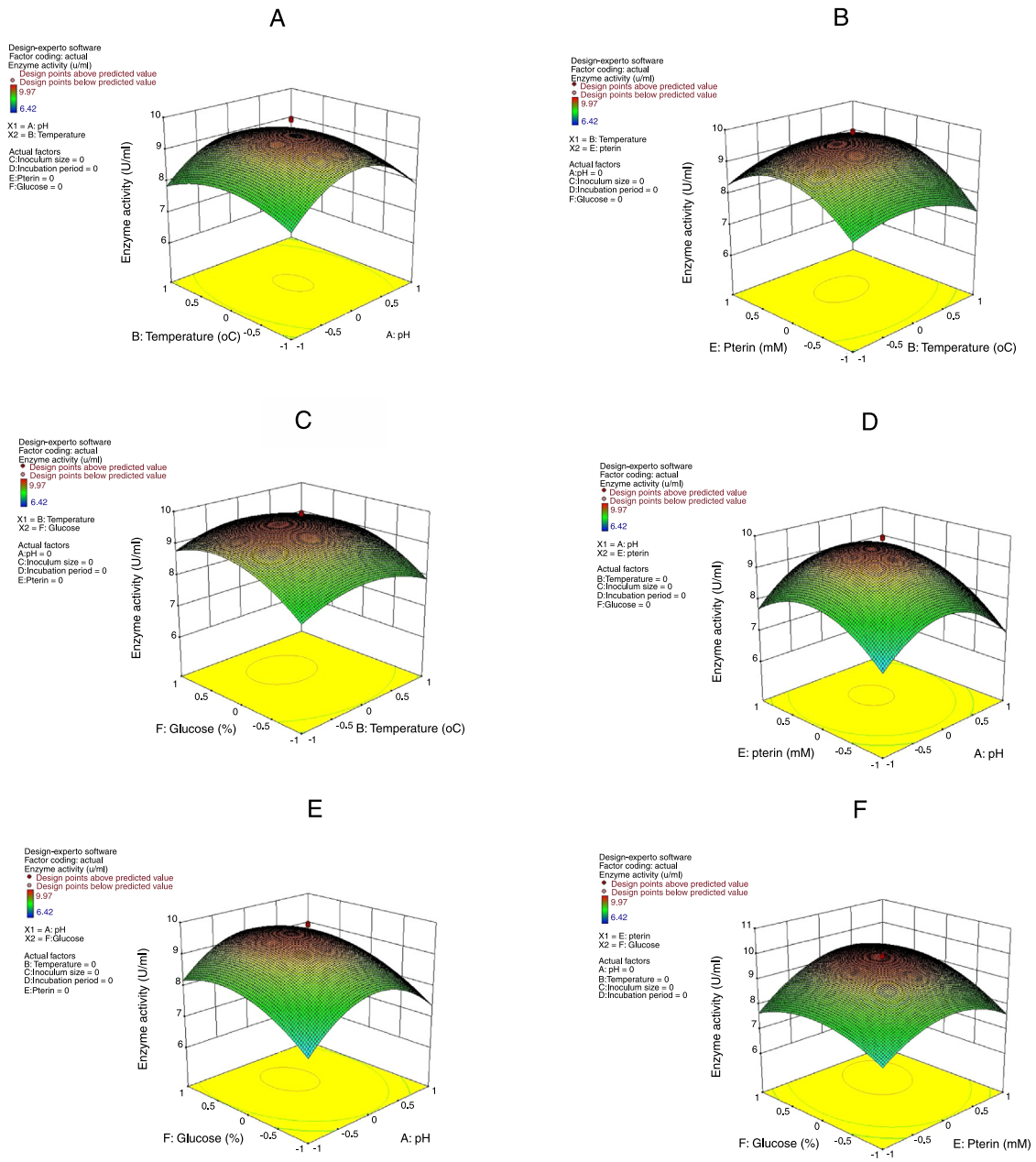

Figure 3 (A) The interaction effect between temperature and pH on the enzyme activity. (B) The interaction effect between temperature and pterin on the enzyme activity. (C) The interaction effect between temperature and glucose on the enzyme activity. (D) The interaction effect between pterin and pH on the enzyme activity. (E) The interaction effect between glucose and pH on the enzyme activity. (F) The interaction effect between glucose and pterin on the enzyme activity.

production of pterin deaminase enzyme. This design demonstrated the optimum conditions to be temperature of 35 °C, pH 8, 0.005 M of pterin and 20% of glucose which resulted in maximum production of pterin deaminase (9.97 U/ml).

Discussion

Literature review on pterin deaminase signifies that very few works has been attempted to characterize the enzyme and surprisingly in India, the study of pterin deaminase is nil. Furthermore, very few information on biological activity of pterin deaminase have been reported. Hence, this study was aimed to obtain pterin deaminase from bacterial source and scrutinize the production. Takikawa et al. (1979) reported that the pterins was regulated by the pterin deaminase enzyme which was produced in bacteria. These pterins were the cofactors for pterin-dependent hydroxylases, such as cyanide oxygenase (Kunz, Fernandez, & Parab, 2001) and monooxygenases viz. phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase (Fitzpatrick, 1999). So the oxygenolytic cleavage of cyanide was dependent on cyanide monooxygenase which obligatively required pterin cofactor for its activity (Durairaju et al., 2015). Based on

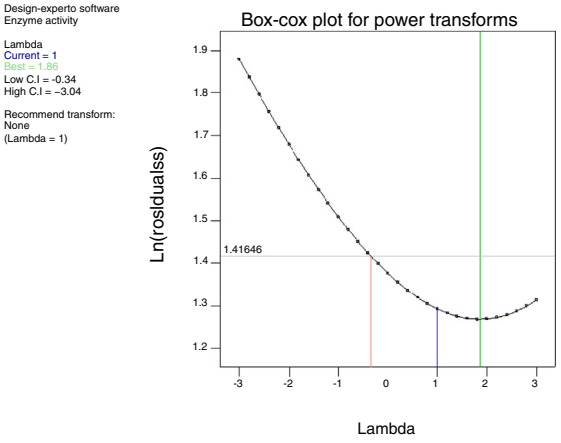

Figure 4 The fitness of the design points and polynomial fitness of enzyme activity.

this lane, a potent cyanide degrader was chosen for producing this unexplored enzyme pterin deaminase. Cyanide was used in gold mining, metal and jewelry industries, which generate waste with high cyanide concentrations (Igeno et al., 2007; Luque-Almagro et al., 2011; Murugesan et al., 2017). Also in cassava processing industries, large amounts of natural cyano glucosides released from the roots were hydrolyzed to cyanide (Balagopalan & Rajalekshmy, 1998). With this knowledge the present study focused on isolating cyanide assimilating microorganisms from cyanide contaminated places. .Among the forty seven bacterial isolates only eight strains were observed as potent cyanide utilizer. Similarly, Kumar and Bhalla (2015) have reported that the genera of cyanide degraders include *Bacillus*, *Pseudomonas*, *Fusarium*, *Trichoderma*, *Penicillium*, *Azotobacter* and *Serratia marcescens*. Park, Lee, Kim, and Park (2008) reported that the microbes destruct the cyanide into non-toxic products to create eco-friendly environment while others (Dubey & Holmes, 1995; Ebbs, 2004) reported that the microorganisms utilize cyanide as their nitrogen source. Consistent to the above reports, in the present investigation, the proficient strain AM12 which produced the highest amount of pterin deaminase was confirmed as *Bacillus cereus*. This study was correlated to the studies of Zhu, Han, Chen, and Han (2010) and Mekuto, Ntwampe, and Jackson (2015) where *Bacillus* sp. CN-22 was evaluated for biodegradation of cyanide and it tolerated the highest cyanide concentration of 700 mg l^{-1} under the optimal conditions. This study also endorsed the reports of Durairaju et al. (2015) in which, *Bacillus subtilis* (JN989651), a cyanide degrader had the ability of producing pterin derivatives.

Statistical tools play a vital role to select designs and parameters which exhibit great influence on the response in biotechnological researches. There are several reports for optimization of culture media, using statistical approaches (Faiza, Haq, & Saima, 2011; Kumari, Mahapatra, & Banerjee, 2009; Salihu, Alam, Abdul Karim, & Salleh, 2011). RSM was reported as remarkably powerful statistical tool used to optimize various parameters. RSM has been effectively applied in various optimization experiments including dye de-colorization, adsorption and production of various enzymes, medium compositions (Lee & Chen, 1997), conditions of enzymatic hydrolysis (Ma & Ooraikul, 1986) and fermentation processes (Sonia, Chadha, & Saini, 2005). The *F*-value of model (9.73) exemplified the significant nature of the model. The *F* value of model demonstrated significant factuality (Akhnazarova & Kafarov, 1982; Khuri & Cornell, 1987). Doddapaneni, Tatineni, Potumarthi, and Mangamoori (2007) suggested that value of determination coefficient closer to 1.0 indicated that the selected model was found to show strong ability to predict its competence in the responses. The determination coefficient of the model (0.91) was highly significant and indicative of goodness of fit. The obtained results indicated that 6% was not explained by the model. The adjusted coefficient, Adj. R^2 (81%) was also very high, which dictated a high applicability of the experiment (Akhnazarova & Kafarov, 1982; Box & Wilson, 1951; Cochran & Cox, 1957; Khuri & Cornell, 1987; Mak, Yap, & Teo, 1995; Yee & Blanch, 1993). Box et al. (1978) reported a higher value of the correlation coefficient ($R = 91\%$) signifies an excellent correlation between the independent variables (Box & Wilson, 1951; Cochran & Cox, 1957; Khuri & Cornell, 1987; Mak et al., 1995; Yee & Blanch, 1993)

The lack of fit *F*-value (0.6) demonstrated that the model was significant. On the basis of results obtained from ANOVA it can be concluded that the model was highly significant and sufficient to represent the actual relationship between the response and the significant variables and can be used successfully to navigate the design space. The yield of pterin deaminase was found to be improved after appropriate supplementation of culture medium suggested by Box–Behnken design. Hence, the optimized conditions were found to show three-fold increase in pterin deaminase production. The interactions of pH and temperature, temperature and concentration of pterin, temperature and concentration of glucose, pH and concentration of pterin, pH and concentration of glucose and concentration of pterin and concentration of glucose were found to be essential parameters on the response. The earlier report (Shanmugam, 2011) showed that, the optimum pH 8 and temperature 32 °C influenced the pterin deaminase production from *Bacillus cereus* AM12.

Overall result of this study has a major impact in large scale production of the enzyme. This study is all a matter of major breakthrough in the field of medicine as well as in pharmaceutical compositions when the therapeutic potential of enzyme is proved.

Conclusion

In conclusion, studies reported herein appear to throw light on the potential yet unexplored enzyme, pterin deaminase. Owing to the significance of the substrates (pteridines) of the enzyme in the biological system, the study on pterin deaminase would unveil numerous research avenues in medicine especially in neurology. Additional approach may be useful in the discovery of *in vitro* and*in vivo* functions of this unexplored enzyme.

Conflicts of interest

The authors declare no conflict of interest.

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