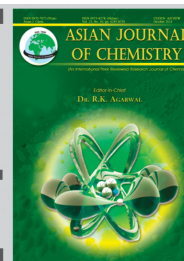
Asian Journal of Chemistry; *Vol. 24, No. 1 (2012), 451-454*

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Effects of Physical Mutagen on Catalase Production

MUHAMMAD SALMAN MUHSAN*, ANEELA AHMED and SHAZIA KHURSHID

Department of Chemistry, Government College University, Lahore, Pakistan

*Corresponding author: E-mail: salo_gcu@yahoo.com

(*Received*: 25 March 2011; *Accepted*: 27 September 2011) AJC-10457

The present study deals with the effects of physical mutagen on the production of catalse. The ultraviolet radiations were used as a physical mutagen. The effect of nitrous acid mutation on the catalase production was also observed. Some fermentation conditions such as fermentation period, concentration of substrate, pH, temperature, concentration of urea and KH_2PO_4 were optimized. It was resulted that activity of catalase from *Aspergillus niger* was maximum at 40 h of incubation, 8 % of glucose concentration (3.40 units/mg), at 30 ºC, at pH 7, at 0.2 % of urea concentration, at 0.4 % of KH_2PO_4. The mutation of nitrous acid decreased the catalase activity. Maximum activity of catalase after ultraviolet mutation, was found in mutant number 8 that was 5.983 unit/mg, which was exposed for 40 min under ultraviolet lamp.

Key Words: Catalase, *Aspergillus niger*, Mutation, Hydrogen peroxide.

INTRODUCTION

Catalase is a common enzyme which is found nearly in all living organisms which are exposed to oxygen, where the function of the catalase is to decompose the hydrogen peroxide into water and oxygen[1]. In food industry catalase is used to remove hydrogen peroxide from milk preceding to cheese manufacturing[2]. Catalase is also used in textile industry for the removal of the hydrogen peroxide to make sure that the fabric is peroxide-free[3]. Millions of molecules of hydrogen peroxide can be converted into water and oxygen by a single molecule of catalase[4]. A minute use is in the lenses is that, some cleaning products for lenses are having hydrogen peroxide and to use lens again a catalase containing solution is used for the decomposition of hydrogen peroxides[5]. Now-day's catalase is used in the aesthetics industry. Commercially catalase is produced from *Aspergillus niger* being a GRAS (generally regarded as safe) organism. *Aspergillus niger* is extensively used for the purpose of mutagenesis. Activity of catalase increases with the positive impact of mutation on active site of catalase. In the present study, isolated and pure cultures of *Aspergillus niger* were obtained and observed the activity of wild type of strain then caused the mutation in *Aspergillus niger* by ultraviolet (UV) and nitrous acid and then observed the effect of mutation on catalase production.

EXPERIMENTAL

Substrate: For the growth production of catalase the substrate used was glucose. Glucose serves as the carbon source for production of catalase.

Organism: The fungal species of *Aspergillus niger* were used for this kind of study and taken from the process development lab of Chemistry Department of G.C. University, Lahore. The fresh strains of *Aspergillus niger* were developed on potato dextrose agar (PDA) 30 ºC for 24 h in incubator and stored at 4 ºC in cold cabinet.

Inoculation: Inoculated the slants with the fresh strain of *Aspergillus niger* with help of inocculum needle. Then incubated the slants in the incubator at 37 ºC for 24 h. After every 2 weeks, propagation of strain on the fresh medium was continued. After incubation these pure and identified colonies of *Aspergillus niger* were stored in cold incubator/refrigerator at 4 ºC.

Submerged fermentation methodology: For the production of catalase from *Aspergillus niger*, the submerged fermentation was applied. The fermentation medium was used in 250 mL shake flasks. The composition of fermentation medium was as followed, 12.5 g of $NaNO_3$, 1.25 g of $MgSO_4 \cdot 7H_2O$, 0.025 g of $FeSO_4 \cdot 7H_2O$, 2.5 g of KH_2PO_4, 25 g of glucose, 1.75/25 mL H_2O of $CaCO_3$, 0.2 g of urea. The compound's composition used in flask was as follows, 1.75 g/25 mL of $CaCO_3$, 10 mL of glucose, 2 mL of urea, 2 mL of KH_2PO_4, 2 mL of $NaNO_3$, 2 mL of $MgSO_4 \cdot 7H_2O$, 2 mL of $FeSO_4 \cdot 7H_2O$, 5 mL of spore suspension. The suspension of spores was prepared by captivating the *Aspergillus niger* from slants and poured in distilled water. Laminar air flow cabinet was used to carry out this work. Six flasks of 250 mL each were taken and all the above contents were poured in these flasks. The spore suspension[6] (5 mL) was having about 107-108 spores/mL.

Assay method for activity of catalase: Essentially it was described thatin the disappearance of peroxide is followed spectrophotometrically at 240 nm. One unit decomposes one micromole of H_2O_2 per minute at 25 ºC and pH 7.0 under the specified conditions[7]. The formula to calculate the activity of catalase was,

$$\text{Units/mg} = \frac{\Delta A240/\text{min} \times 1000}{43.6 \times \text{mg enzyme/mL reaction mixture}}$$

whereas, mg enzyme/mL reaction mixture = 0.667. 0.05 M citrate phosphate buffer of pH 7. 0. 18 % hydrogen peroxide and 1M solution of glucose were used as reagents.

Optimization of fermentation conditions: Some fermentation conditions were optimized such as influence of period of fermentation, influence of glucose as a carbon source for the production of catalase, pH effects, influence of temperature, influence of urea, influence of KH_2PO_4.

Nitrous acid mutation: Mutation of nitrous acid was carried out by adding 0.1 mL of it sodium acetate buffer of 4.5 pH. Take out 0.5 mL of sample after every 5 min and for 24 h developed the colonies of *Aspergillus niger* on potato dextrose agar (PDA) plates at 30 ºC.

Mutation in *Aspergillus niger* strain by UV

(a) Twelve petri plates of *Aspergillus niger* on potato dextrose agar medium (PDA) were prepared, and subjected them under UV lamp (Desaga Sartedt-Gruppe Min-U-VIS) at 366 nm.

(b) First *Aspergillus niger* plate was taken out from UV lamp after 5 min of interval, 2nd after 10, 3rd after 15, 4th after 20, 5th after 25, 6th after 30, 7th after 35, 8th after 40, 9th after 45, 10th after 50, 11th after 55 and 12th plate after the interval of 60 min.

(c) Twenty four h of incubation was applied to all these mutated strains that were grown on freshly prepared PDA.

(d) Then subjected all the mutated strains to submerged fermentation by using the same procedure as that was for wild type of strain and incubated them for 40 h at 100 rpm at 30 ºC in shaking incubator.

(e) Then the absorbance at 240 nm in Cecil CE 7200 spectrophotometer was noted and activity of catalase was measured from mutated strains of *A. niger* by following the same method as for wild type *Aspergillus niger*.

RESULTS AND DISCUSSION

After 40 h of incubation at 30 ºC and 100 rpm partial enzyme was extracted by breaking down the mycelia. The activity of catalase was determined spectrophotometrically by examining the reduction of light absorption during decomposition of H_2O_2 by the enzyme at 240 nm.

Optimization factors: This study revealed that the catalase production from *Aspergillus niger* by using the optimization factors such as glucose concentration, fermentation period, pH, temperature, urea concentration, KH_2PO_4 concentration. By UV the mutation was caused in *Aspergillus niger* to check its influences on production of catalase. The obtained results of these influences are discussed below. After 48 h of incubation the partial enzyme was extracted by mycelia break down. That enzyme was intracellular which showed activity while filtrate of mycelia does not show any activity[8]. Activity of the enzyme was determined on the basis of reduction of hydrogen peroxide into water at 240 nm.

Influence of period of fermentation: This experiment was performed by optimizing the fermentation period. The media was taken in 2 flasks that were having 10 % glucose. After the incubation of 20, 40, 60 and 80 h the flasks were harvested. The activity of catalase was maximum after 40 h of fermentation (Table-1). The maximum activity of catalase reported[9] was after 48 h.

TABLE-1
INFLUENCE OF FERMENTATION PERIOD ON CATALASE PRODUCTION

Fermentation period (h)	Absorption	Enzyme activity (units/mg)
20	0.091	3.12
40	0.147	5.05
60	0.138	4.74
80	0.099	3.44
100	0.080	2.75

Influence of glucose concentration as a carbon source on catalase activity: As a carbon source, glucose is used for the production of catalase. There was used different concentrations glucose for fermentation media. To determine the effect of substrate on the activity of catalase, the used concentrations were 4 % up to 13 %. Maximum activity of catalase was shown at 8 %. It decreases on the further increase of concentration of glucose as shown in Fig. 1.

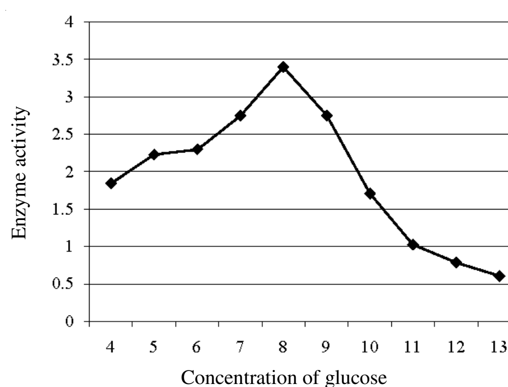


Fig. 1. Influence of glucose concentration as a carbon source on catalase activity

Influence of pH: It was noted that the activity of catalase is affected by pH and varies from 4-8. The catalase showed maximum activity at pH 7. There was very less activity of catalase during pH 7-8. Optimum pH 6 was for glucose oxidase and 5.7 was for catalase from *Aspergillus niger*[10]. It was reported that the mximum activity of catalase[11,12] was at pH 7. Present results are in accordance with the studies as shown in Fig. 2.

Influence of temperature: The temperature kept for the fermentation was optimized. And the temperature kept was 20, 30, 40, 50, 60 and 70 ºC. The Enzyme worked effectively from 20-40 ºC. At 30 ºC the maximum value of activity of catalase was observed. After 40 ºC its activity was decreased due to denaturation of enzyme (Table-2). The activity of catalase was maximum[13] at 24 ºC. Present results also suggest that

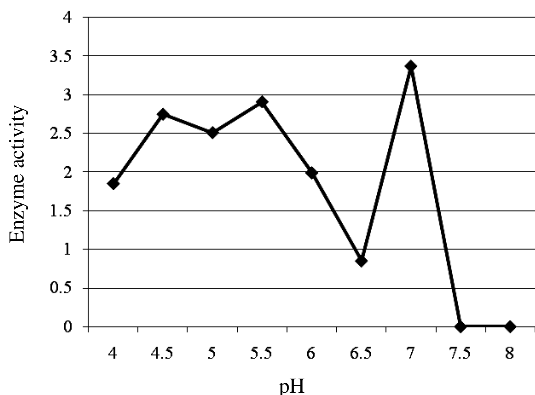


Fig. 2. Influence of pH

TABLE-2
INFLUENCE OF TEMPERATURE ON PRODUCTION OF CATALASE BY *A. niger*

Temperature (ºC)	Absorption	Enzyme activity (units/mg)
20	0.131	4.50
30	0.148	5.08
40	0.113	3.88
50	0.092	3.16
60	0.067	2.30
70	0.012	0.41

the activity was increased between 20-30 ºC. The maximum activity was recorded at 30 ºC .

Influence of urea: The nitrogen source concentration in medium has considerable effects on the enzyme activity. Activity of the catalase was observed maximum with 0.2 % urea (Fig. 3). Increasing the concentration of urea decreased further activity.

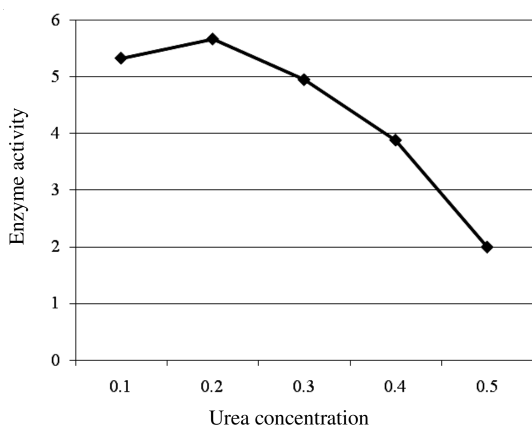


Fig. 3. Influence of urea concentration

Influence of KH_2PO_4: Different concentrations of KH_2PO_4 were observed which effect the production of enzyme. Maximum production was noted with 0.4 % KH_2PO_4. The concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 % were used as shown in Fig. 4.

Influence of nitrous acid mutation on activity of catalase: Activity of catalase was also observed by the mutation of nitrous acid. The activity was not noticeable. Maximum results by nitrous acid mutation was obtained by mutant No. 1 that was 2.09 units/mg (Fig. 5).

Enhancement of catalase production by UV mutation: The major source of the all genetic variation was mutagenesis.

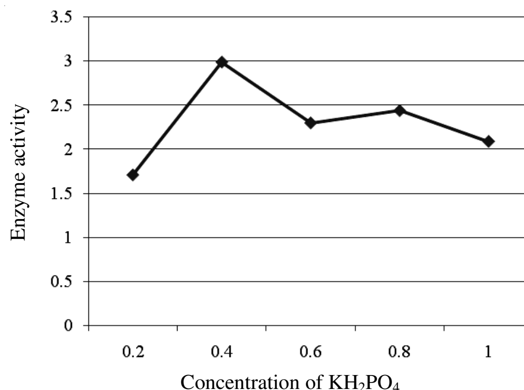


Fig. 4. Influence of KH_2PO_4

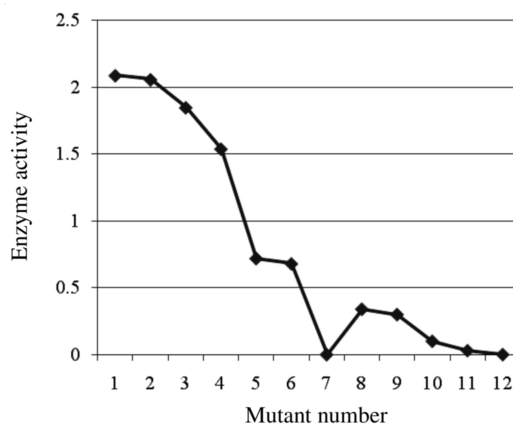


Fig. 5. Influence of nitrous acid mutation on catalase production

To produce the random mutation in genome of *Aspergillus niger*, UV radiations were used. The main purpose of the mutation was the selection of colonies of *Aspergillus niger* with the improved expression of catalase. The activity of catalase was 5.983 unit/mg with mutant No. 8 that was exposed for minutes 40 min to UV lamp as listed in Table-3 and shown in Fig. 6.

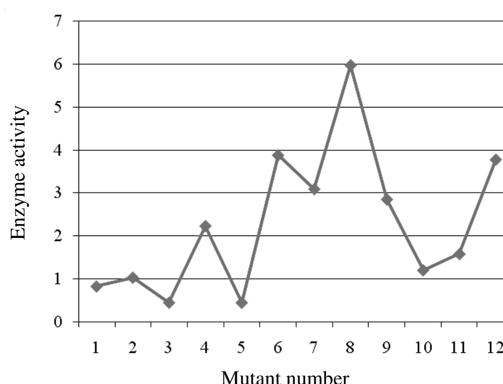


Fig. 6. Intracellular activity of catalase after UV mutation

Conclusion

Maximum activity of catalase was obtain when ultraviolet mutation was done. The maximum activity of catalase was shown by mutant number 8.0 which was exposed for 40 min under ultra violet lamp and the activity recorded was 5.983 unit/mg. Overall the minimum activity of catalse was obtain by mutation of nitrous acid that was 2.09 units/mg.

TABLE-3
INTRACELLULAR ACTIVITY OF CATALASE AFTER UV MUTATION

Mutant number	Time of UV exposure (min)	Absorbance	Enzyme activity (units/mg)
1	5	0.024	0.825
2	10	0.030	1.0316
3	15	0.013	0.447
4	20	0.065	2.235
5	25	0.013	0.447
6	30	0.113	3.885
7	35	0.090	3.094
8	40	0.174	5.983
9	45	0.083	2.854
10	50	0.035	1.203
11	55	0.046	1.581
12	60	0.110	3.782

REFERENCES

1. P. Chelikani, I. Fita and P.C. Loewen, *Cell. Mol. Life Sci*., **61**, 192 (2004).
2. C.C. Worthington, Enzyme Manual: Catalase. Worthington Biochemical Corporation (2009).
3. Case Study, Textile Industry, International Cleaner Production Information Clearinghouse, p. 228 (2009).
   T. Doppner and W. Hartmeirer, *J. Starch-Starke*, **36**, 283 (1984).
4. D.S. Goodsell, Catalase, Molecule of the Month, RCSB Protein Data Bank (2004).
5. J.N. Cook and J.L. Worsley, Compositions and Method for Destroying Hydrogen Peroxide on Contact Lens (1996).
6. J.A. Kolmer, E.H. Spaulding and H.W. Robinson, Approved Laboratory Techniques. Appelton, Inc. New York, edn. 5, pp. 55-60 (1959).
7. R. Beers and I. Sizer, *J. Biol. Chem.*, 195, 133 (1952).
8. T. Döppner and W. Hartmeier, *Starch*, **36**, 283 (1984).
9. A.W. Willis, Methods in Enzymology, Academic Press, New York and London, Vol. 9, pp. 83-84 (1966).
10. J.Z. Liu, H.Y. Yang, L.P. Weng and L.N. Ji, *Lett. Appl. Microbiol.*, **29**, 337 (1999).
11. A. Maehly and B. Chance, *Methods Biochem. Anal*., **1**, 357 (1954).
12. H.W. Zeng, Y.J. Cai, X.R. Liao, S.L. Qian, F. Zhang and D.B. Zhang, *Annals Microbiol*., **60**, 701 (2011).
13. S. Morgulis and M. Beber, *J. Biol. Chem*., **72**, 91 (1927).