

Full Length Research Paper

Enhanced lipase production by mutation induced *Aspergillus japonicus*

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Accepted 9 June, 2006

The purpose of the present investigation is to enhance production of the biomedically important enzyme, lipase, by subjecting the indigenous lipase producing fungal strain *Aspergillus japonicus* MTCC 1975 to strain improvement and random mutagenesis (UV irradiation, HNO₂ and N-methyl-N'-nitro-N-nitroso guanidine). The isolation of mutants and the lipolytic activity of selected mutants were described. The best UV selectant (AUV₃) showed 127% higher lipase activity than the parent strain. The lipase yield of the best HNO₂ mutant (AHN₃) was 139% higher than UV mutant (AUV₃) and 177% higher than the parent strain. Also, the lipase yield of the best NTG mutant (ANT₄) was 156% higher lipase activity than the HNO₂ mutant (AHN₃) and 217% higher than the UV mutant (AUV₃) and 276% higher lipase activity than the parent strain. The results indicated that UV, HNO₂ and NTG treatment were effective physical and chemical mutagenic agents for strain improvement of *Aspergillus japonicus* for enhanced lipase productivity.

Key words: Lipase, *Aspergillus japonicus*, UV Irradiation, HNO₂ and N-methyl-N'-nitro-N-nitroso guanidine.

INTRODUCTION

Lipases (triacyl glycerol acyl hydrolases, (E.C. 3.1.1.3) are versatile catalysts, which are used for diverse purposes by (Kajandjian et al., 1986). Fungal lipases have received attention because of their potential use in food processing, pharmaceuticals, cosmetics, detergents and leather industry was reported (Sugiara, 1984). Rao et al. (1993) showed new applications, such as the resolution of racemic mixtures to produce optically active compounds, which arise from the stereo specific activity of lipase.

The exponential increase in the application of lipases in various fields in the last few decades demands extension in both qualitative improvement and quantitative enhancement. Quantitative enhancement requires strain improvement and medium optimization for the overproduction of the enzyme, as the quantities produced by culture strains are low. Strain improvement is an essential part of process development for fermentation products. Develop-

ed strains can reduce the costs with increased productivity and can possess some specialized desirable characteristics. Such improved strains can be obtained by mutation and selection depends on the alternate processes of diversification, selection and rediversification, so that better strains are successfully picked out and further improved (Rowlands, 1984). The method used for diversification is mutation. This process involved changes in the nucleus of the organism, which leads to increased productivity. A mutant microbial strain is a strain derived from multiplication of a single haploid cell containing a mutant gene (Bapi Raju et al., 2004).

The aim of the present investigation is to enhance lipase productivity of the fungal strain *Aspergillus japonicus* by subjecting it to improvement by random mutagenesis (UV Irradiation, HNO₂ and N-methyl-N'-nitro-N-nitroso guanidine, NTG).

MATERIALS AND METHODS

Microorganism

Aspergillus japonicus MTCC 1975 was purchased from IMTECH, Institute of Microbial Cultures, Chandigarh, India. This fungal strain

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shows good lipolytic activity. The strain was grown on malt-agar media (malt extract, 2 g; agar 2 g; Triton X-100, 0.1 ml; distilled water, 100 ml) slants at 28°C for 5 days and stored in the refrigerator at 4°C until further use.

UV Irradiation

A completely sporulated slant of *A. japonicus* (5 days old slant) was taken. The spores were scrapped off in to 5 ml of sterile water. The spore suspension was serially diluted up to 10^{-5} dilution. A 0.1 ml quantity of spore suspension was poured aseptically on the medium contained in petriplates. The suspension was uniformly distributed using a sterile spreader.

The spore suspension was exposed to UV light, was carried out in a UV Illuminator fitted with TUP 40w Germicidal lamp which has about 90% of its radiation at 2540-2550 Å°. The exposure was carried out at a distance of 16 cm away from the center of the Germicidal lamp (UV light source) with occasional shaking. The exposure times were 60, 120, 180, 240 and 300 s. each UV exposed spore suspension was stored overnight to avoid photo reactivation. The plates were incubated for 5 days at 28°C and the numbers of colonies in each plate were counted. A total of 24 colonies were obtained and 6 isolates were selected from the colonies. The 6 isolates were streaked on Malt-Agar Slants and incubated for 5 days at 28°C. Among the selectants, the best UV mutant strain was used for further studies.

HNO₂ treatment

To 9 ml of 10^{-6} dilution of the best UV irradiated *A. japonicus* lipase producing spore suspension, 1 ml of sterile stock solution of 0.01 M sodium nitrate was added. One ml aliquots of samples were withdrawn at intervals of 10 min up to 60 min. 0.5 ml of phosphate buffer was added to each sample and was neutralized with 0.5 ml of 0.1 M NaOH. 0.1 ml of this exposed suspension was plated on malt-agar medium and the suspension was uniformly distributed using a sterile spreader. Each HNO₂ exposed spore suspension was stored overnight to avoid photo reactivation. The plates were incubated for 5 days at 28°C. After 5 days, 17 colonies were obtained and among them, 6 isolates were selected from the plates on the basis of their morphology, size and shape. These isolates were streaked on malt-agar slants and incubated for 5 days at 28°C.

N-methyl-N'-nitro-N-nitroso guanidine treatment

The best HNO₂ mutant (AHN₃) was used for N-methyl-N'-nitro-N-nitroso guanidine (NTG) treatment. The spore suspension was prepared in the same manner as described earlier. To a 9 ml of spore suspension, 1 ml of NTG (3 mg ml⁻¹ in phosphate buffer) was added. The reaction was allowed to proceed. Samples were withdrawn from the reaction mixture at intervals of 30, 60, 90, 120, 150, 180 min and immediately centrifuged for 10 min at 5000 rpm and the supernatant solution was decanted. Cells were washed three times with sterile water and resuspended in 10 ml of sterile phosphate buffer. The samples were serially diluted in the same buffer and plated over malt-agar as mentioned earlier. A total of 15 colonies, 5 isolates were selected from the plates showing less than 1% survival rate (120 and 150 min NTG treated spore suspension) and tested for lipase production.

Growth and lipase production

The fungi cultivated on malt-agar slants was scrapped with 5 ml of sterile distilled water and transferred into 250 ml Erlenmeyer flasks

containing 50 ml of production medium (malt extract 20 g, peptone 5 g, yeast extract 3 g and sodium chloride 5 g per liter of distilled water). The flasks were incubated 28°C for 5 days on a rotary shaker (120 rpm).

On the fifth day of fermentation, the enzyme was extracted from broth. The broth was filtered through cotton gauze for removing the cell mass. The filtrate was saturated with ammonium sulphate (40%) and then centrifuged at 10,000 rpm for 20 min at 5°C. The lipase enzyme precipitated was dissolved in phosphate buffer (pH 7).

Lipase assay

The activity of lipase was determined as described in the literature (Winkler and Stuckman, 1979) with some modifications. 1 ml of isopropanol containing 3 mg of *p*-nitrophenyl palmitate (pNPP) was mixed with 9 ml of 0.05 M Tris-HCl buffer (pH 8.0), 40 mg of Triton X-100 and 10 mg of gum Arabic. Liberation of *p*-nitrophenol at 28°C was detected in UV Spectrophotometer at 410 nm. One enzyme unit was defined as 1 μmol of *p*-nitrophenol enzymatically released from the substrate per minute (Raman et al., 1998). All the fermentations and activity calculations were carried out in duplicate and the mean value was presented.

RESULTS AND DISCUSSION

Hopwood et al. (1985) suggested that 99.9% kill is best suited for strain improvement as the fewer survivors in the treated sample will have undergone repeated or multiple mutations which may lead to the enhancement in the productivity of the culture. The plates having less than 1% survival rates (120 and 180 s) were used to select for mutants. A total of 6 mutants (AUV₁–AUV₆) were selected and tested for the lipase production and the results were represented in Figure 1. AUV₃ showed maximum lipase activity than parent strain (7.44 U/ml). Ellaiah et al. (2002) reported 156% fold increase in lipase yield of *A. niger* by UV mutagenic treatment whereas in the present investigation, the best UV mutant (AUV₃) showed 118% higher lipase activity than the parent strain.

The UV mutant (AUV₃) was selected and was subjected to further strain improvement by HNO₂ treatment. HNO₂ is considered to be very effective chemical mutagen. The selected HNO₂ treated isolates, AHN₁ to AHN₆, were obtained from plates having less than 1% survival rates (30 and 45 s). AHN₃ shows maximum lipase activity (9.47 U/ml) than parent strain as depicted in Figure 2. The lipase yield of the best HNO₂ mutant (AHN₃) was 139% higher than UV strain (AUV₃) and 177% higher than the parent strain.

The HNO₂ mutant (AHN₃) was selected and was subjected to further strain improvement by NTG treatment, a well known mutagenic technique (Cerado-Olmedo and Hanawalt, 1968; Adelberg et al., 1965). Plates having less than 1% survival rates (120 and 150 min) were selected for the isolation of mutants and the lipolytic activity. ANT₄ showed maximum lipase activity (13.2 U/ml) than parent strain was represented in Figure 3. ANT₄ has 156% higher lipase activity than the HNO₂

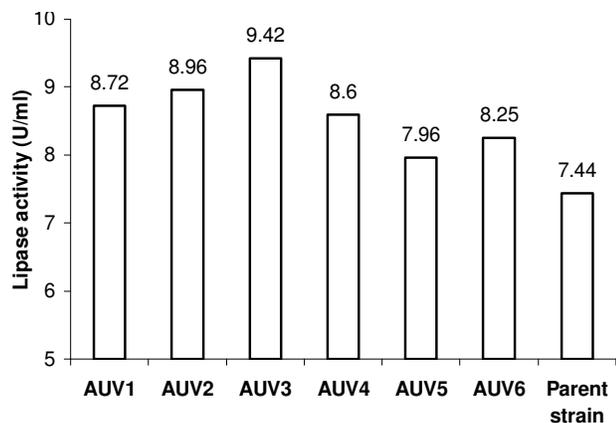


Figure 1. Effect of UV irradiation on lipase activity produced by *Aspergillus japonicus* MTCC 1975. AUV1 to AUV6 are selected irradiated isolates.

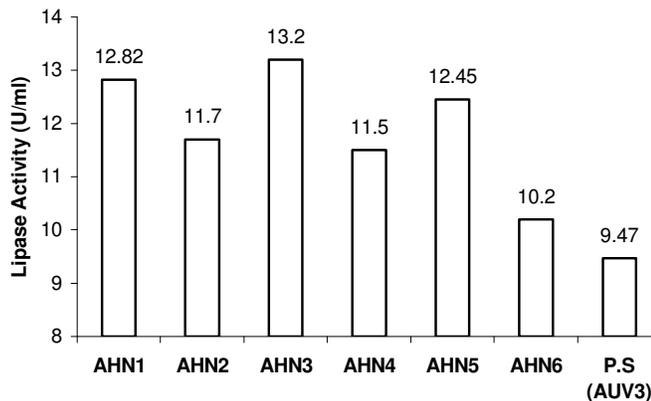


Figure 2. Effect of HNO_2 treatment on lipase activity produced by *Aspergillus japonicus* MTCC 1975. AHN1 to AHN6 are selected irradiated isolates.

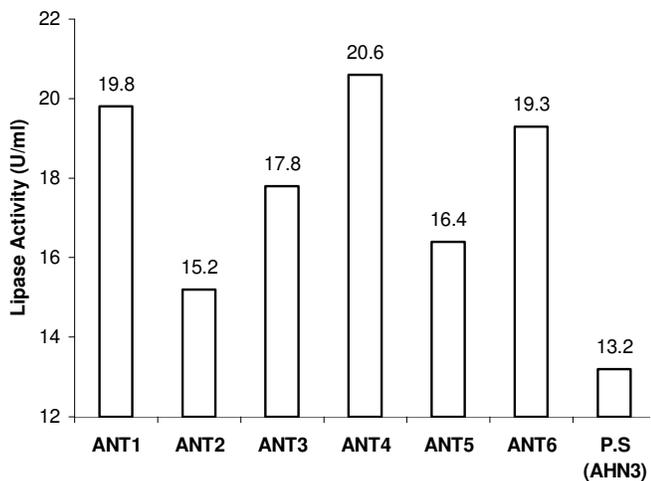


Figure 3. Effect of N-methyl-N'-nitro-N-nitroso guanidine (NTG) treatment on lipase activity produced by *Aspergillus japonicus* MTCC 1975. ANT1 to ANT6 are selected irradiated isolates.

mutant (AHN₃), 217% higher than the UV strain (AUV₃) and 276% higher lipase activity than the parent strain.

Caob and Zhanga (2000) reported an increase in lipase production of 3.25-fold by using a *Pseudomonas* mutant generated by UV, HNO_2 and NTG. Also, a 200% increase in lipase yield by *Aspergillus niger* mutant from UV and NTG treatments was reported by (Elliah et al., 2002). In the present investigation, a 276% increase in lipase production was achieved by strain improvement of indigenous isolate *A. japonicus* by induced mutations employing UV, HNO_2 and NTG.

Improvement of microbial strains for the overproduction of industrial products has been the hallmark of all commercial fermentation processes. Such improved strains can reduce the cost of the processes with increased productivity and may also possess some specialized desirable characteristics. Effectiveness of UV Irradiation (physical mutagen) and HNO_2 , NTG treatments (Chemical mutagen) in strain improvement for enhanced lipase productivity was demonstrated in the present investigation. It is hoped that the high yielding fungal mutant strain of the isolate *A. japonicus* (ANT₄) can be exploited commercially for large-scale industrial production of lipase.

ACKNOWLEDGEMENT

We are very thankful to the Head of the Center for Biotechnology and Department of Chemical Engineering, Andhra University, Visakhapatnam, Andhra Pradesh for providing us laboratory facilities.

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