Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (11): 542-550, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.542.550
© 2015 Academic Journals Inc.

UV Mutagenesis of *Aspergillus flavus* for Enhanced Mannanase Synthesis and Catabolite Activation Studies

[1]Oladipo Oladiti Olaniyi, [2]Ibitoye Oluyemisi Folasade, [1]Taye Victor Familoni and [3]Emmanuel Olufemi Bankefa

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria
[2]Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria
[3]Department of Microbiology, Federal University, Oye Ekiti, Ekiti State, Nigeria

Corresponding Author: Oladipo Oladiti Olaniyi, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria

ABSTRACT

The present investigation was conducted to generate catabolite activation mutants of *Aspergillus flavus* through UV mutagenesis. Mutants of *A. flavus* were generated by exposure of spores suspension to UV irradiation at a distance of 13 cm in dark from the centre of germicidal lamp (240 nm). Quantitatively, mannanase activity was determined using dinitrosalicylic acid method, while protein content was determined by Lowry method. Mannanase production by the mutants varied with time of exposure to UV irradiation. All the mutants except for mutant designated AFUV90 showed higher specific mannanase activity in comparison with the parent strain. The isolated mutants were screened for catabolite activation studies in the presence of different mannose and glycerol concentrations (1 and 1% w/v) as carbon sources. The supplementation of 0.1 and 1% (w/v) mannose in the fermentation media caused activation of mannanase biosynthesis in 100 and approximately 91% of the mutants, respectively. The inclusion of 0.1 and 1% (w/v) glycerol induced an improvement in approximately 82 and 55% of the mutants, respectively in terms of mannanase biosynthesis. The generation of catabolite activation mutants through UV mutagenesis might be considered as a break through in the industrial production of mannanase.

Key words: UV irradiation, mutagenesis, catabolite activation, mannanase, *Aspergillus flavus*

INTRODUCTION

Enzymes are known to be nature's catalysts. Enzymes sourced from microorganisms are often more useful than enzymes derived from plants or animals because of the great variety of catalytic efficiencies, high production rate, stability at extreme conditions, ease of genetic manipulation of producing strains, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms or inexpensive media (Iftikhar *et al*., 2007, 2008; Treichel *et al*., 2010).

The exponential increase in the application of mannanase in various industrial processes has necessitated expansion both in qualitative improvement and quantitative enhancement. Quantitative expansion requires strain improvement and optimization of process parameters for the overproduction of the enzyme as the quantities produced by wild strains are usually too low (Haq *et al*., 2010). The spectacular success examples of strain improvement in industry are mostly attributed to the extensive application of mutation and selection of microorganisms (Bapiraju *et al*., 2004).

For efficient enzyme production, strains' improvement can be achieved by mutagenesis which is a successful method. The process of mutagenesis involves series of laborious steps of procedures in performance (Bapiraju *et al*., 2004; Iftikhar *et al*., 2010). Mutants with higher yielding enzyme capacity can be obtained through Ultraviolet (UV) and gamma (γ) irradiations from conidia of thermophilic fungi (Irfan *et al*., 2011). The improvement in few of the mutants for enzyme production could be attributed to ease of re-constitution of damaged genes by these strains with improved properties (Olaniyi *et al*., 2014). Ultraviolet rays are important mutagenic agent of mutation in potential microbial strains and the main effect of this light is to modify the structure of pyrimidine (cytosine and thyamine) causing the formation of thyamine dimmer which distort the structure of DNA helix and block the further replication process (Sambrook and Russell, 2001). In most cases, UV mutation are very harmful but at sometimes it may lead to better adaptation of an organism to its environment with improved biocatalytic performance. An improvement in terms of enzyme production has been reported for large array of microorganisms. Mala *et al*. (2001) reported higher lipase production by UV mutant of *Aspergillus niger* with 2.53 times more than the parental strain. According to Agrawal *et al*. (1999), UV radiation was a potent mutagen. The UV irradiation was found to be best for the improvement of fungal strains for maximum production of relevant industrial enzymes (Olaniyi *et al*., 2014).

The biosynthesis of enzymes is regulated by induction and repression genes within the genome of the enzyme-producing strains. The inducer is usually the substrate for or some structurally related compound (Prescott *et al*., 1999). In most organisms, enzyme production is repressed in the presence of high concentration of readily metabolized carbon source. The rate of enzyme-mediated hydrolysis of the cellulose has been reported to be inhibited by products of hydrolysis and fermentation products, particularly when hydrolysis and fermentation are carried out at the same time (Lynd *et al*., 2002; Olaniyi *et al.,* 2014). As a result of presence of repressing substance and end product associated with the fermentation of wastes cellulose, this had led to an increase in the production cost of microbial enzymes. Research articles had been published on catabolite repression of cellulase biosynthesis however, there is scanty research article on catabolite repression or activation of mannanase biosynthesis in mannanase producing microorganisms.

The challenge of catabolite repression associated with mannanase production in mannanase producing microorganisms justified this study and series of attempt have been made to generate mutant strains with high catabolite activation potential. It is believed that the use of catabolite activation mutants might avert the repression of mannanase synthesis and allow production of high yields of mannanase in direct microbial fermentation. The aims of the present study were to generate UV mutants from *Aspergillus flavus* for improved mannanase production and screen them for catabolite activation potential.

MATERIALS AND METHODS

Fungal strain: *Aspergillus flavus* strain was provided by the culture collection of Research Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria. The strain whose origin was cotton seeds previously confirmed to be positive for mannanase activity by plate assay technique was used in this study (Arotupin and Olaniyi, 2013). This strain was selected based on its performance in terms of quantitative mannanase production. The identity of this strain was ascertained to ensure its purity and viability through cultural characters and microscopic structure. The culture was maintained on Locust Bean Gum (LBG) containing agar slant at 4°C throughout the study.

Inoculum preparation: The inoculum of *A. flavus* grown for 96 h at 30°C on LBG agar medium slants was prepared by adding 10 mL of sterile distilled water which contained 0.1% (v/v) Tween 80 to the agar slant and shook vigorously. The spore suspension was adjusted to the spore concentration of 10^3 spores mL^{-1} (as the initial inoculum size) (Ibrahim *et al*., 2012).

Mutant generation by ultraviolet irradiation: Ten milliliter L spores suspension of *A. flavus* from prepared inoculum (10^3 spores mL^{-1}) was transferred in a sterilized Petri plate. The Petri plate was exposed to UV irradiation for a period of 110 min at a distance of 13 cm in dark from the centre of germicidal lamp (240 nm) and at 10 min intervals, 1 mL spore suspension was withdrawn and plated on Malt Extract Agar (MEA) (Bapiraju *et al*., 2004). The developed mutants were maintained on MEA slant at 4°C in refrigerator until use. The developed mutants and wild parents were screened for mannanase production in submerged fermentation using mannanase production medium (Olaniyi *et al*., 2014).

Media preparation and comparison of mannanase production by wild type and mutant strains: The mutants and the wild type of *A. flavus* were screened for quantitative mannanase production under submerged state fermentation. Enzyme production was performed in 250 mL Erlenmeyer flask containing 50 mL of enzyme Production Medium (PM). This medium contained (g L^{-1}) LBG 10, $NaNO_3$ 2, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, $FeSO_4 \cdot 7H_2O$ traces, pH 6.8. The medium was sterilized at 121°C for 15 min. After sterilization, each flask was inoculated with 2 discs of 8 mm diameter of both wild type and mutant strains of *A. flavus* (Olaniyi *et al*., 2014). The cultures were harvested after 5 days of incubation by centrifugation at 6000 rpm for 15 min at 4°C using refrigerated centrifuge. The supernatants were used as the crude extracellular enzyme source. Each treatment was carried out in triplicates and the results obtained throughout the work were the arithmetic mean of at least 3 experiments.

Mannanase assay: Mannanase activity was assayed in the reaction mixture composing of 0.5 mL of 1% LBG prepared in 50 mM potassium phosphate buffer pH 6.8 and 0.5 mL of supernatant at 45°C for 60 min modified method of Youssef *et al.* (2006). The control tube contained the same amount of substrate and 0.5 mL of the enzyme solution heated at 100°C for 15 min. Both the experimental and control tubes were incubated at 45°C for 60 min. At the end of the incubation period, tubes were removed from the water bath (Lamfield Medical England Model DK-600) and the reaction was terminated by addition of 2 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube. The tubes were incubated for 5 min in a boiling water bath for colour development and were cooled rapidly. The activity of reaction mixture was measured against a reagent blank at 540 nm. Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) (Miller, 1959). One unit of mannanase activity was defined as amount of enzyme producing 1 μmole of mannose per minute under standard assay conditions.

Screening of mutants for catabolite activation: The mutants and wild type of *A. flavus* were subjected to catabolite activation studies in basal medium containing varying concentrations of mannose and glycerol as energy sources (0.1 and 1% w/v). The cultures were incubated for at 30±2°C in static condition for 5 days (has described above under enzyme production). Mannanase synthesis by each mutant was induced by using 1% (w/v) LBG. Mutants which exhibited appreciable enzyme activity at screening stage were referred to as catabolite activation mutant (repression resistant mutants) (Olaniyi *et al*., 2014).

Statistical analyses: The statistical analysis was performed using the general linear model function of Statistical Package for Social Science (SPSS), version 16.0. All data generated were subjected to one-way ANOVA while statistical differences of treatment were determined using Duncan's Multiple Range.

RESULTS AND DISCUSSION

Screening of wild type and UV mutant strains of *A. flavus* for mannanase production: A total of eleven mutant strains were developed from *A. flavus* (wild type) within 110 min of exposure to UV irradiation. Mannanase activity, protein content, biomass estimation and specific mannanase activity are shown in Table 1. Mannanase was synthesized by the mutants at varying quantities. Mutant with a code AFUV80 generated from 80 min of exposure to UV irradiation showed highest specific mannanase activity when compared with other mutants. All the mutants except for mutant designated AFUV90 showed higher specific mannanase activity in comparison with the parent strain. Strain improvement is an essential part of process development for bio-products formation. Developed strains can reduce the costs with increased productivity and can possess some improved and desirable properties. Such improved strains can be achieved by mutagenesis in the natural strain and subsequent screening. Thus a major effort of industrial research in producing enzymes is directed towards the screening programs. Mutation is the primary source of all genetic variation and has been used extensively in industrial improvement of enzyme production (Ghisalba *et al*., 1984). The increased yield in mannanase production in the mutants is a proof of true strain improvement. This was in agreement with works of Kang *et al*. (1999) and Adegoke *et al*. (2012), who reported an improvement in enzyme production in *A. niger* and *Pluerotus pulmonarius*-LAU through UV light mutation. Similarly, an improvement in cellulase production was reported by Shahbazi *et al*. (2014) for UV mutants of *Trichoderma reesei*. Strains' improvement had been reported (Adsul *et al*., 2007; Ramzan and Mehmood, 2002; Rasul *et al*., 2011) for many relevant extracellular enzymes production through mutagenic processes, however, scanty or no information is available for mannanase. The Ultra Violet (UV) irradiation is the most convenient of all mutagens to use and it is also very easy to take effective safety precautions against it. The UV light is the best studied mutagenic agent in prokaryotic organisms. It gives a high proposition of pyrimidine dimers and includes all types of base pair substitutions (Meenu *et al*., 2000). The biomass growth of the mutants varied with the time of exposure. Of all the mutants generated, mutant AFUV80 had the highest biomass weight of 1.12±0.00 g/50 mL which is approximately 509% higher than the value obtained for the wild type.

Table 1: Mannanase activity of wild type and UV mutant strains of *Aspergillus flavus*

Mutants	Biomass (g/50 mL)	Protein content (mg mL^{-1})	Mannanase activity (U mL^{-1})	Specific activity (U mg^{-1})
Wild strains/control	0.22 ± 0.36^{a}	0.55 ± 0.02^{f}	42.31 ± 0.02^{a}	76.07 ± 0.06^{b}
Effect of UV radiation				
AFUV10	0.23 ± 0.15^{b}	0.36 ± 0.01^{d}	57.74 ± 0.05^{b}	157.77 ± 0.02^{g}
AFUV20	0.57 ± 0.01^{ef}	0.23 ± 0.02^{b}	65.25 ± 0.07^{e}	276.36 ± 0.01^{i}
AFUV30	0.26 ± 0.01^{c}	0.18 ± 0.11^{a}	63.92 ± 0.02^{e}	382.75 ± 0.03^{j}
AFUV40	0.63 ± 0.04^{g}	0.38 ± 0.15^{d}	69.43 ± 0.21^{i}	182.66 ± 0.13^{h}
AFUV50	0.74 ± 0.25^{i}	0.56 ± 1.5^{f}	68.24 ± 0.01^{h}	121.66 ± 0.65^{d}
AFUV60	1.07 ± 0.00^{j}	0.47 ± 0.08^{e}	65.35 ± 0.02^{f}	137.02 ± 0.01^{e}
AFUV70	0.58 ± 0.01^{f}	0.33 ± 0.15^{c}	65.36 ± 0.00^{f}	137.02 ± 0.01^{e}
AFUV80	1.12 ± 0.00^{k}	0.17 ± 0.10^{a}	66.04 ± 0.17^{g}	395.37 ± 0.01^{k}
AFUV90	0.54 ± 0.03^{d}	0.91 ± 0.02^{h}	64.22 ± 0.02^{d}	70.82 ± 1.25^{a}
AFUV100	0.57 ± 0.01^{e}	0.47 ± 0.06^{e}	70.42 ± 0.00^{k}	149.20 ± 0.01^{f}
AFUV110	0.69 ± 0.45^{h}	0.72 ± 0.02^{g}	70.12 ± 0.01^{j}	97.12 ± 0.01^{c}

Means with the same superscript letters down the column are not significantly different ($p>0.05$)

There were an appreciable improvement in mycelia cell and sporophore of *Rhizopus oryzea*, *P. florida* and *P. sajor-caju* when subjected to varied dosage of UV (Soden *et al*., 2002).

There was an appreciable improvement in terms of enzyme biosynthesis in all the mutants in the media supplemented with 0.1% mannose (Table 2). The specific enzyme activity ranged from 208.24±0.01-836.03±0.01 U mg^{-1} with the highest specific mannanase activity lied on mutant AFUV80. The lowest specific enzyme activity achieved for mutant AFUV40 was approximately 31% higher than the value obtained for the parent type. Seven mutants out the eleven mutants generated (AFUV20, AFUV30, AFUV50, AFUV60, AFUV70, AFUV90 and AFUV100) had higher biomass weight than the wild type, while the remaining mutants had 0.42±0.00 g/50 mL and below.

There was an appreciable improvement in specific mannanase activity in approximately 91% of the mutants as the mannose concentration increased to 1% (Table 3). The mutant AFUV90 generated from 90 min of exposure to UV yielded highest specific mannanase activity of 326.14±0.03 U mg^{-1}, while lowest of 20.89±0.00 U mg^{-1} was obtained from mutant AFUV10. Specific mannanase activity of mutant AFUV90 was 16.3 fold higher when compared with the wild type. The mutant AFUV50 generated from 50 min of exposure to UV yielded maximum protein content of 3.14±0.03 mg mL^{-1}, while other mutants exhibited protein content above 1.2 mg mL^{-1}. Approximately 82% of the mutants (AFUV20, AFUV30, AFUV40, AFUV50, AFUV60, AFUV80, AFUV90, AFUV100 and AFUV110) generated had higher biomass yield than the parent strain.

The supplementation of 0.1% glycerol in enzyme production media caused repression of specific mannanase activity in approximately 82% of the mutants (AFUV10, AFUV20, AFUV30, AFUV40,

Table 2: Supplementation of 0.1% (w/v) mannose on mannanase production by wild type and UV mutants of *Aspergillus flavus*

Mutants	Biomass (g/50 mL)	Protein content (mg mL^{-1})	Mannanase activity (U mL^{-1})	Specific activity (U mg^{-1})
Wild strains/control	0.42±0.00^{c}	1.13±0.00^{j}	180.33±0.01^{d}	159.57±0.01^{a}
Effect of UV radiation				
AFUV10	0.01±0.00^{a}	0.63±0.01^{e}	181.52±0.02^{e}	286.29±0.02^{d}
AFUV20	0.53±0.02^{e}	0.58±0.01^{d}	279.16±0.01^{i}	482.13±0.02^{k}
AFUV30	0.51±0.02^{d}	0.92±0.01^{h}	336.52±0.02^{l}	371.02±0.02^{g}
AFUV40	0.31±0.01^{b}	0.96±0.00^{i}	199.51±0.02^{f}	208.24±0.01^{b}
AFUV50	0.59±0.01^{f}	0.82±0.02^{g}	314.00±0.00^{k}	372.50±0.02^{h}
AFUV60	0.71±0.02^{gh}	0.82±0.01^{g}	303.65±0.02^{j}	368.52±0.01^{f}
AFUV70	0.71±0.01^{g}	0.63±0.01^{e}	238.66±0.01^{g}	376.44±0.01^{i}
AFUV80	0.42±0.01^{c}	0.18±0.00^{a}	154.67±0.01^{b}	836.03±0.01^{l}
AFUV90	0.73±0.00^{i}	0.54±0.01^{b}	152.51±0.02^{a}	286.64±0.02^{e}
AFUV100	0.70±0.01^{g}	0.56±0.01^{c}	241.51±0.02^{h}	424.42±0.02^{j}
AFUV110	0.01±0.00^{a}	0.71±0.02^{f}	158.81±0.02^{c}	230.21±0.01^{c}

Means with the same superscript letters down the column are not significantly different (p>0.05)

Table 3: Supplementation of 1% (w/v) mannose on mannanase production by wild type and UV mutants of *Aspergillus flavus*

Mutants	Biomass (g/50 mL)	Protein content (mg mL^{-1})	Mannanase activity (U mL^{-1})	Specific activity (U mg^{-1})
Wild strains/control	0.63±0.00^{c}	2.03±0.00^{b}	76.34±0.01^{b}	37.63±0.01^{b}
Effect of UV radiation				
AFUV10	0.60±0.01^{a}	2.15±0.01^{d}	45.16±0.01^{a}	20.89±0.00^{a}
AFUV20	0.70±0.01^{e}	2.30±0.01^{e}	108.51±0.01^{d}	47.44±0.01^{d}
AFUV30	0.74±0.01^{f}	2.65±0.01^{h}	157.65±0.03^{e}	59.61±0.02^{g}
AFUV40	1.08±0.00^{k}	2.13±0.01^{c}	191.52±0.03^{i}	90.52±0.02^{j}
AFUV50	0.67±0.01^{d}	3.14±0.03^{k}	183.35±0.02^{g}	58.92±0.02^{f}
AFUV60	0.96±0.00^{j}	3.08±0.02^{j}	227.15±0.02^{j}	73.91±0.01^{h}
AFUV70	0.61±0.01^{b}	2.31±0.01^{e}	189.16±0.01^{h}	81.72±0.01^{i}
AFUV80	0.82±0.00^{h}	3.02±0.00^{i}	170.16±0.01^{f}	56.38±0.01^{e}
AFUV90	0.86±0.00^{i}	1.24±0.01^{a}	409.33±0.01^{l}	326.14±0.03^{l}
AFUV100	1.18±0.00^{l}	2.38±0.01^{f}	340.65±0.02^{k}	142.91±0.02^{k}
AFUV110	0.77±0.01^{g}	2.43±0.01^{g}	93.81±0.02^{c}	38.46±0.00^{c}

Means with the same superscript letters down the column are not significantly different (p>0.05)

Table 4: Supplementation of 0.1% (w/v) glycerol on mannanase production by wild type and UV mutants of *Aspergillus flavus*

Mutants	Biomass (g/50 mL)	Protein content (mg mL^{-1})	Mannanase activity (U mL^{-1})	Specific activity (U mg^{-1})
Wild strains/control	2.12±0.01^{d}	0.11±0.01^{c}	72.22±0.01^{i}	744.56±0.00^{j}
Effect of UV radiation				
AFUV10	2.12±0.00^{d}	0.15±0.01^{d}	0.00±0.00^{a}	0.00±0.00^{a}
AFUV20	1.43±0.01^{b}	0.30±0.01^{f}	61.24±0.01^{h}	209.76±0.01^{f}
AFUV30	2.51±0.01^{f}	0.32±0.01^{f}	139.71±0.01^{l}	431.23±0.01^{i}
AFUV40	3.21±0.01^{i}	0.62±0.01^{k}	13.62±0.01^{b}	22.28±0.00^{b}
AFUV50	1.83±0.01^{c}	0.34±0.01^{g}	123.74±0.01^{k}	356.62±0.01^{h}
AFUV60	2.61±0.02^{g}	0.22±0.01^{e}	54.44±0.00^{f}	255.61±0.00^{g}
AFUV70	2.43±0.01^{e}	0.61±0.01^{j}	32.77±0.00^{d}	54.90±0.00^{c}
AFUV80	2.74±0.01^{h}	0.52±0.01^{i}	41.40±0.01^{e}	81.31±0.01^{d}
AFUV90	2.12±0.01^{d}	0.45±0.01^{h}	54.58±0.01^{g}	119.18±0.01^{e}
AFUV100	1.82±0.01^{c}	0.01±0.01^{a}	111.53±0.01^{j}	12392.00±0.00l
AFUV110	1.12±0.00^{a}	0.03±0.01^{b}	31.94±0.01^{c}	760.57±0.01^{k}

Means with the same superscript letters down the column are not significantly different (p>0.05)

Table 5: Supplementation of 1% (w/v) glycerol on mannanase production by wild type and UV mutants of *Aspergillus flavus*

Mutants	Biomass (g/50 mL)	Protein content (mg mL^{-1})	Mannanase activity (U mL^{-1})	Specific activity (U mg^{-1})
Wild strains/control	0.83±0.01^{g}	2.46±0.01^{d}	61.33±0.00^{e}	24.86±0.01^{f}
Effect of UV radiation				
AFUV10	0.31±0.02^{a}	1.53±0.00^{b}	119.16±0.01^{h}	78.00±0.01^{j}
AFUV20	0.61±0.01^{b}	0.65±0.00^{a}	201.51±0.01^{k}	308.54±0.03^{l}
AFUV30	1.12±0.01^{j}	2.33±0.02^{c}	46.49±0.01^{c}	20.12±0.01^{c}
AFUV40	1.03±0.01^{i}	3.52±0.02^{j}	84.33±0.01^{f}	23.86±0.01^{e}
AFUV50	0.70±0.01^{e}	3.13±0.02^{g}	92.33±0.01^{g}	29.45±0.01^{g}
AFUV60	0.67±0.00^{d}	3.20±0.02^{i}	284.83±0.01^{l}	89.30±0.01^{k}
AFUV70	0.63±0.01^{c}	3.16±0.00^{h}	41.33±0.01^{b}	13.12±0.01^{b}
AFUV80	0.85±0.01^{h}	2.48±0.01^{d}	132.52±0.02^{i}	53.31±0.01^{h}
AFUV90	0.75±0.00^{f}	3.55±0.01^{k}	2.00±0.00^{a}	0.55±0.01^{a}
AFUV100	1.21±0.00^{k}	2.87±0.00^{f}	180.82±0.02^{j}	63.11±0.01^{i}
AFUV110	0.84±0.01g^{h}	2.79±0.01^{e}	57.02±0.02^{d}	20.42±0.00^{d}

Means with the same superscript letters down the column are not significantly different (p>0.05)

AFUV50, AFUV60, AFUV70, AFUV80 and AFUV90) with lowest repression occurred in mutant AFUV10 (Table 4). All the mutants generated had higher protein content than the wild type except for mutants AFUV100 and AFUV110 where lower values were obtained. Of all the mutants evaluated, mutant AFUV40 had the highest biomass growth of 3.21±0.01 g/50 mL while the lowest value of 1.12±0.00 g/50 mL was achieved with AFUV110.

The supplementation of 1% glycerol in enzyme production media led to higher specific mannanase activity in six of the mutants (AFUV10, AFUV20, AFUV50, AFUV60, AFUV80 and AFUV100), while the remaining mutants (AFUV30, AFUV40, AFUV70, AFUV90 and AFUV110) exhibited varied degrees of repression when compared with the wild type (Table 5). Of all the improved mutants, mutant designated AFUV20 had the highest specific mannanase activity with 42.14% higher than the parent strain. The highest protein content of 3.55±0.01 mg mL^{-1} was achieved with mutant AFUV90, while the lowest value of 0.65±0.00 mg mL^{-1} was obtained for AFUV20. Mutants AFUV10, AFUV20 and AFUV30 had lower protein content than the parent strain. The supplementation of 1% glycerol into basal media caused higher biomass yield in approximately 46% of the mutants (AFUV30, AFUV40, AFUV80, AFUV100 and AFUV110) when compared with parent strain. The mutants with higher mannanase production potential in different concentrations of carbon sources might be regarded as catabolite resistant mutants. Low enzyme production is expected because the wild type and mutants already have carbon source-rich substrate in enzyme production media and hence do not need to secrete the hydrolytic enzymes (proteins) in which mannanase is one. Generally, enzyme biosynthesis in microorganisms and

its catalytic ability are under the Control of Catabolite Repression (CCR) mechanisms (Vinuselvi *et al*., 2012). The basic principle underlying CCR is universal in all microbes, that is, the most energy efficient cognate substrate is the most preferred carbon source. This is usually achieved through the inhibition of expression of genes encoding for enzymes involved in the catabolism of carbon sources other than the preferred ones (Portnoy *et al*., 2011). The development of catabolite resistant mutants might be connected with the alteration of catabolite gene activation protein (CAP) or cyclic AMP receptor protein (CRP). The cyclic AMP-CRP complex facilitates binding of RNA polymerase to the promoter site of catabolite sensitive operon which alleviates the repression state. The molecular machinery behind CCR varies widely across the species, with CCR being enforced and operable at different levels including transcriptional (Kimata *et al*., 1997), post-transcriptional (Gorke and Vogel, 2008), translational (Parker *et al*., 1997) and biochemical regulations (Kim *et al*., 2009) which has fascinated scientists for over half a century.

CONCLUSION

The development of UV mutants of *A. flavus* induced appreciable yield of mannanase production in producing strain and this could be employed to solve challenges facing industrial processes where mannanase is being utilized in large amount. CCR is important for the metabolic activity in microorganisms and thus CCR remains as a positive selection force in microorganisms in evolution. However, CCR would reduce their ability of an organism to perform as an efficient enzyme producer. Hence, it becomes necessary to develop strategies to overcome CCR without disturbing the evolutionary fitness of the microbes. The activation in UV mutants by supplementation of different concentrations of mannose and glycerol might curb CCR in industrial production of mannanase. The use of catabolite activation mutants might circumvent the repression of mannanase biosynthesis and allow production of high yields of mannanase in direct microbial fermentation. The molecular knowledge behind catabolite activation in these mutants is not yet elucidated. Therefore, further molecular studies on catabolite activation mutants will be necessary to reveal the alteration in their genetic makeup.

REFERENCES

Adegoke, A.E., O.J. Kola, A. Yadav and B.T. Chandral, 2012. Improvement of laccase production in *Pluerotus pulmonarius*-LAU 09 by mutation. J. Microbiol. Res., 2: 11-17.

Adsul, M.G., K.B. Bastawde, A.J. Varma and D.V. Gokhale, 2007. Strain improvement of *Penicillium janthinellum* NCIM 1171 for increased cellulase production. Bioresour. Technol., 98: 1467-1473.

Agrawal, R., N.U.A. Deepika and R. Joseph, 1999. Strain improvement of *Aspergillus* sp. and *Penicillium* sp. by induced mutation for biotransformation of α-pinene to verbenol. Biotech. Bioeng., 63: 249-252.

Arotupin, D.J. and O.O. Olaniyi, 2013. Screening and identification of mannanase-producing fungi isolated from selected agricultural wastes. Br. Microbiol. Res. J., 3: 635-644.

Bapiraju, K.V.V.S.N., P. Sujatha, P. Ellaiah and T. Ramana, 2004. Mutation induced enhanced biosynthesis of lipase. Afr. J. Biotechnol., 3: 618-621.

Ghisalba, O., J.A.L. Auden, T. Schupp and J. Nuesch, 1984. The Rifamycins: Properties, Biosynthesis and Fermentation. In: Biotechnology of Industrial Antibiotics, Vandamme, E. (Ed.). Maecel Decker Inc., New York, pp: 281-327.

Gorke, B. and J. Vogel, 2008. Noncoding RNA control of the making and breaking of sugars. Gene. Dev., 22: 2914-2925.

Haq, I., S. Ali, M.M. Javed, U. Hameed, A. Saleem, F. Adnan and M.A. Qadeer, 2010. Production of alpha amylase from a randomly induced mutant strain of *Bacillus amyloliquefaciens* and its application as a desizer in textile industry. Pak. J. Bot., 42: 473-484.

Ibrahim, D., H. Puspitaloka, R. Abdul Rahim and L.S. Hong, 2012. Characterization of solid state fermentation culture conditions for growth and mananase production by *Aspergillus niger* USM F4 on rice husk in tray system. Br. Biotechnol. J., 2: 133-145.

Iftikhar, T., M. Niaz, M. Afzal, Ikram ul-Haq and M.I. Rajoka, 2008. Maximization of intracellular lipase production in a lipase-overproducing mutant derivative of *Rhizopus oligosporus* DGM 31: A kinetic study. Food Technol. Biotechnol., 46: 402-412.

Iftikhar, T., M. Niaz, S.Q. Abbas, M.A. Zia, I. Ashraf, K.J. Lee and I.U. Haq, 2010. Mutation induced enhanced biosynthesis of lipases by *Rhizopus oligosporus* var. *Microsporus*. Pak. J. Bot., 42: 1235-1249.

Iftikhar, T.M., M.A. Niaz, M.A. Zia, S.O. Qadeer, M. Abbas, I. Rajoka and I.U. Haq, 2007. Screening of media and Kinetic studies for the production of lipase from a mutant strain of *Rhizopus oligosporus*. Proceedings of Biotech World 2007, November 24-25 , 2007, Oran Algerie,.

Irfan, M., J. Javed and Q. Syed, 2011. UV mutagenesis of *Aspergillus niger* for enzyme production in submerged fermentation. Pak. J. Biochem. Mol. Biol., 44: 137-140.

Kang, S.W., E.H. Ko, J.S. Lee and S.W. Kim, 1999. Over-production of β-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. Biotechnol. Lett., 21: 647-650.

Kim, J., J. Yeom, C.O. Jeon and W. Park, 2009. Intracellular 2-keto-3-deoxy-6-phosphogluconate is the signal for carbon catabolite repression of phenylacetic acid metabolism in *Pseudomonas putida* KT2440. Microbiology, 155: 2420-2428.

Kimata, K., H. Takahashi, T. Inada, P. Postma and H. Aiba, 1997. cAMP receptor protein-cAMP plays a crucial role in glucose-lactose diauxie by activating the major glucose transporter gene in *Escherichia coli*. Proc. Nat. Acad. Sci.U.S.A., 94: 12914-12919.

Lynd, L.R., P.J. Weimer, W.H. van Zyl and I.S. Pretorius, 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol. Mol. Biol. Rev., 66: 506-577.

Mala, J.G.S., N.R. Kamini and R. Puvanakrishnan, 2001. Strain improvement of *Aspergillus niger* for enhanced lipase production. J. Gen. Applied Microbiol., 47: 181-186.

Meenu, M., D. Santhosh, C. Kamia and S. Randhir, 2000. Production of alkaline protease by a UV-mutant of *Bacillus polymyxa*. Ind. J. Microbiol., 40: 25-32.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Olaniyi, O.O., B.J. Akinyele and O.F. Ibitoye, 2014. Mutagenesis of *Klebsiella edwardsii* for mannanase synthesis. Innov. Romanian Food Biotechnol., 15: 40-50.

Parker, C., N. Peekhaus, X. Zhang and T. Conway, 1997. Kinetics of sugar transport and phosphorylation influence glucose and fructose cometabolism by *Zymomonas mobilis*. Applied Environ. Microbiol., 63: 3519-3525.

Portnoy, T., A. Margeot, R. Linke, L. Atanasova and E. Fekete *et al*., 2011. The CRE1 carbon catabolite repressor of the fungus Trichoderma reesei: A master regulator of carbon assimilation. BMC Genomics, Vol.12. 10.1186/1471-2164-12-269

Prescott, L.M., J.P. Harley and D.A. Klein, 1999. Microbiology. 4th Edn., McGraw-Hill Companies, New Delhi, India, pp: 151-310.

Ramzan, M. and T. Mehmood, 2002. Enhanced production of glucose oxidase from UV mutant of *Aspergillus niger*. Afr. J. Biotechnol., 8: 288-290.

Rasul, S., M.A. Zia, M.A. Sheikh and T. Iftikhar, 2011. Enhanced production and characterization of a novel β-D-glucose:Oxygen-1-oxidoreductase by using *Aspergillus niger* UV-180-C mutant strain. Afr. J. Biotechnol., 10: 14522-14533.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., USA., ISBN-13: 9780879695774, Pages: 2344.

Shahbazi, S., K. Ispareh, M. Karimi, H. Askari and M.A. Ebrahim, 2014. Gamma and UV radiation induced mutagenesis in *Trichoderma reesei* to enhance cellulases enzyme activity. Int. J. Farm. Allied Sci., 3: 543-554.

Soden, D.M., J. O'Callaghan and A.D.W. Dobson, 2002. Molecular cloning of a laccase isozyme gene from *Pleurotus sajor*-caju and expression in the heterologous *Pichia pastoris* host. Microbiology, 148: 4003-4014.

Treichel, H., D. de Oliveira, M.A. Mazutti, M. di Luccio and J.V. Oliveira, 2010. A review on microbial lipases production. Food Bioprocess Technol., 3: 182-196.

Vinuselvi, P., M.K. Kim, S.K. Lee and C.M. Ghim, 2012. Rewiring carbon catabolite repression for microbial cell factory. BMB Reports, 45: 59-70.

Youssef, A.S., M.Y. El-Naggar, S.A. El-Assar and E.A. Beltagy, 2006. Optimization of cultural conditions for β-mannanase production by a local *Aspergillus niger* isolate. Int. J. Agric. Biol., 8: 539-545.