Influence of UV Mutagenesis on β-Mannanase Production Potential of *Aspergillus glaucus* and *Rhizopus japonicus*

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**Author's contribution**

This work was carried out in collaboration between authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ABJ and DJA managed the analyses of the study and the literature searches. The authors read and approved the first manuscript.

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**ABSTRACT**

**Aim:** The present study was conducted to improve the β-mannanase production from *Aspergillus glaucus* and *Rhizopus japonicus* using UV mutation.

**Study Design:** The first experiment, spore suspensions of *A. glaucus* and *R. japonicus* was exposed to UV irradiation, while in the second experiment, wild types and mutant strains of *A. glaucus* and *R. japonicus* were screened for β-mannanase production in submerged fermentation.

**Place and Duration of Study:** Microbiology Research Laboratory Federal University of Technology, Akure, Ondo State, Nigeria between July and August, 2012.

**Methodology:** Mutants of *A. glaucus* and *R. japonicus* were generated by exposure of spores suspension to UV irradiation for a period of 110 minutes at a distance of 13 cm in dark from the centre of germicidal lamp (240 nm) at 10 min intervals, 1 ml spores suspension was withdrawn and plated on Malt Extract Agar (MEA). The developed mutants and wild parents were screened for β-mannanase production in submerged fermentation. Quantitatively, β-mannanase activity was determined using dinitrosalicylic acid method, while protein content was determined by Lowry method.
Results: Eleven UV mutant fungal strains were generated for each of the wild types (A. glaucaus and R. japonicus) within 110 min of spore exposure to UV irradiation. The amount of enzyme produced by the mutants varied with the time of exposure. Approximately 27% of the mutant of A. glaucaus (9A1UV30, 9A1UV50 and 9A1UV70) generated from 30, 50 and 70 min of exposure to UV irradiation showed higher increase in β-mannanase activities when compared with parent strain, while repression of enzyme biosynthesis was observed in other mutants. Of all the mutants generated, the 9A1UV30 mutant had the highest increase in mannanase activity with approximately 46% higher than the parent strain, while the mutant 9A1UV10 exhibited 0% enzyme activity. Beta-mannanase production potential was repressed in the mutants of R. japonicus except for mutant 9A2UV50 where unappreciable higher increase of enzyme activity of 100.90% was observed in comparison with the parent strain.

Conclusion: Enhanced β-mannanase production was obtained from mutant strains 9A1UV30, 9A1UV50 and 9A1UV70 of A. glaucaus and they could be exploited commercially for industrial production of β-mannanase to meet industrial demand. To the best of my knowledge, this is the first report on successful mannanase producer mutants of A. glaucaus and R. japonicus and it is suggested that molecular studies should be carried out on the improved mutants to reveal the mutation.

Keywords: Aspergillus glaucaus; β-Mannanase; UV mutagenesis; Rhizopus japonicus; strain improvement; submerged fermentation.

1. INTRODUCTION

Hemicelluloses are the second most abundant polysaccharide in nature after cellulose. The major constituents of hemicellulose are the hetero-1,4-β-D-xylans and hetero-1,4-β-D-mannans (galactoglucosamannan, galactomannan, and glucomannan). The heteroxylans are found mainly in grasses, cereals, and hardwoods (angiosperms). The mannans are more abundant in copra, palm, coffee, and locust bean endosperms [1,2,3].

Mannolytic microbes were found in soil, compost, and animal rumen [4]. Biodegradation of β-mannans is caused by β-mannanase (1,4-β-D mannan manohidrolase EC 3.2.1.78) produced by bacteria and fungi. The enzyme hydrolyses the β-(1,4)-linkages in backbone of β-mannan polymer producing short chain mannoligosaccharides. Then, these compounds can be further degraded by the action of β-mannanosidase (β-D-mannosidase EC 3.2.1.25) and a-α-galactosidase (EC 3.2.1.22) [5,6]. Mannan degradation from glucomannan and galactomannan produces mannooligosaccharide, mannobiase, and mannose. Mannan-degrading enzymes can be used for numerous applications in food, feed, pulp, and paper industries [7,8,9].

Today the major prospect of biotechnology is the production of enzymes by using various strains of bacteria and fungi in submerged and solid state fermentation. For commercial production of enzymes, filamentous fungi are mostly preferred because the enzymes produced by these fungi are more efficient as compared to those obtained from yeast and bacteria [10,11,12].

For efficient enzyme production, strains can be improved by mutagenesis which is a successful method. The process of mutagenesis involves laborious steps of procedures in performance [12,13]. Ultraviolet (UV) and gamma (γ) irradiations can also be used to obtain mutants yielding higher enzymes production from conidia of thermophilic fungi [14]. The improvement in few of the mutants for enzyme production could be attributed to ease of reconstitution of damaged genes by these strains with improved properties. For strain mutation UV rays are important inducers. Main effect of this light is to modify the structure of pyrimidine (cytosine and thymine) causing the formation of thymine dimmer which distort the structure of DNA helix and block the further replication process [15]. 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. [16] have reported lipase production 2.53 times more by UV mutants than the parental strain of Aspergillus niger [17] reported that Aspergillus strains can be treated with UV irradiations or chemicals such as N-methyl, N-nitro, N-nitrosoguanidine, dimethyl sulphate, EMS, ethidium bromide and nitrous acid to induce mutation for the improvement of amylglucosidase production. According to [18], UV radiation was a potent mutagen. UV irradiation was found to be best for the improvement of strains like Aspergillus niger for
maximum production of various enzymes [19]. UV mutagenic strains of Aspergillus niger have the ability to produce 156% more lipase production as compared to the wild strains. UV treated strains of Aspergillus niger UAM-GS1 increases the production of hemicellulolytic and cellulolytic activities [20]. [21] have reported that the strain improvement in Rhizopus oryzae by UV resulted in the production of more glucoamylase by a mutant than the parent strain. Some workers [22,23] obtained mutants, which produced cellulase enzymes with 5-fold increase in β-glucosidase and one fold increase in both CMCase and FPase, through chemical mutagens treatments.

The exponential increase in the application of β-mannanase 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 wild strains are usually too low. The spectacular success examples of strain improvement in industry are mostly attributed to the extensive application of mutation and selection. The aim of this work was to evaluate the effect of different exposure time of UV irradiation on Aspergillus glaucus and Rhizopus japonicus for β-mannanase production in submerged state fermentation.

2. MATERIALS

2.1 Raw Materials

The coconut residual cake was sourced from farm field in Akure, Ondo State, Nigeria and it was used as a carbon source for medium formulation. The residual were treated with petroleum ether and dried at 60°C for 2 h. After that, the residual were blended, milled and sieved to obtain uniform particle size of 0.5 mm. Locust Bean Gum (LBG) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2 Microorganisms

Two fungal strains Aspergillus glaucus and Rhizopus japonicus (from orange and pineapple peels respectively) previously confirmed to be positive for mannanase activity by plate assay were used in this study [24]. The fungal strains were identified by cultural characters and microscopic structure [24] in the Microbiology Research Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria. The fungal isolates were maintained on LBG agar plate and sub-cultured at regular intervals. They were incubated at 30±2°C until the entire plates were covered by active mycelium and stored at 4°C in refrigerator on agar slants until use.

2.3 UV mutagenesis

Ten ml spores suspension of A. glaucus and R. japonicus from 5 days old culture slant was exposed to UV irradiation for a period of 110 minutes at a distance of 13 cm in dark from the centre of germicidal lamp (240 nanometer), and at 10 min intervals, 1 ml spore suspension was withdrawn and plated on Malt Extract Agar (MEA) [13]. The developed mutants were maintained on MEA slant at 4°C in refrigerator until use.

2.4 Production and comparison of β-mannanase by wild types and mutant strains in submerged fermentation (SmF)

Beta-mannanase production was carried out for both wild types and mutant strains of A. glaucus and R. japonicus in SmF with Mandels and Weber’s medium modified by [24]. Enzyme production was performed in 250 ml Erlenmeyer flask containing 50 g of enzyme inducing medium (PM). This medium contained copra meal 10, NaNO₃ 2, KH₂PO₄ 1, MgSO₄·7H₂O 0.5, KCl 0.5, FeSO₄·7H₂O traces, pH 5.0. 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 types and mutant strains of A. glaucus and R. japonicus from MEA culture plates using cup borer. 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 activity was assayed in the reaction mixture composing of 0.5 ml of 50 mM potassium phosphate buffer pH 7.0 and LBG with 0.5 ml of supernatant at 45°C for 60 min (modified method of [24]). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) [25]. One unit of mannanase activity was defined as amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.
2.6 Determination of Fungal Biomass Yield

The cells were separated from the media by centrifugation at 6000 rpm, washed twice with distilled water and dried at 100°C to a constant weight.

2.7 Protein Determination

The amount of protein liberated in the fermentation media was evaluated according to the method designed by [26] using Bovine Serum Albumin (BSA) as the standard.

2.8 Statistical Analysis

Data presented on the average of three replicates (± SE) are obtained from their independent experiments. Experiment data was subjected to ANOVA of SPSS programming. Duncan’s multiple range tests was used to identify significant differences between means of treatments.

3. RESULTS

3.1 Screening of Wild Types and Mutant Strains of A. glaucaus and R. japonicus for β-mannanase Production

UV irradiation a conventional approach for mutant generation was adopted to develop mutant strains of A. glaucaus and R. japonicus for enhanced β-mannanase biosynthesis. The colonies of the mutants generated on MEA medium varied with the time of exposure, while the appearance remained intact when compared with wild strains (results not shown).

A total of eleven mutant strains for each of the wild types were developed within 110 min of exposure to UV irradiation. Beta-mannanase was synthesized by the mutants at varying quantities (Tables 1 and 2).

Beta-mannanase activity, protein content, biomass estimation, specific mannanase activity and percentage increase in mannanase activity is shown in Table 1. Approximately 27% of the mutant strains of A. glaucaus (9A1UV30, 9A1UV50 and 9A1UV70) generated from 30, 50 and 70 min of exposure to UV irradiation showed higher increase in β-mannanase activities when compared with parent strain, while repression of enzyme biosynthesis was observed in other mutants. Of all the mutants generated, mutant designated 9A1UV30 had the highest increase in mannanase activity with approximately 46% higher than the parent strain, while mutant coded 9A1UV10 exhibited 0% enzyme activity. In the fermentation broth, the mutants displayed protein content ranged from 0.01±0.09 mg/ml to 1.02±0.07 mg/ml with the highest protein content lied on mutant strain 9A1UV30. However, the protein content of mutant strains coded 9A1UV10, 9A1UV30, 9A1UV40, 9A1UV50, 9A1UV60, 9A1UV70, 9A1UV80, 9A1UV90 and 9A1UV110 was significantly (P<0.05) higher than the wild type. The biomass growth of the mutants varied with the time of exposure. The biomass growth of mutant designated 9A1UV100 (0.30±0.01 g/50 ml) was significantly (P<0.05) greater than the value obtained for parent strain (0.29±0.01 g/50 ml).

Table 2 shows β-mannanase activity, protein content, specific β-mannanase activity, biomass estimation and percentage increase in enzyme activity of mutants of R. japonicus. Potential production of β-mannanase was repressed in the mutants except for mutant 9A2UV50 where unappreciable higher increase of enzyme activity observed in comparison with the parent strain. The mutant (9A2UV70) generated from the dosage of UV for 70 min gave maximum protein content of 0.86±0.01 mg/ml, while other mutants exhibited appreciable protein content above 0.30 mg/ml. However, the protein content of parent strain was significantly higher than mutant designated 9A2UV60. In terms of biomass estimation, mutant 9A2UV30 generated from 30 min of exposure to UV irradiation gave the highest value of 0.27±0.01 g/50 ml, approximately 1.9 fold higher than what was obtained for parent strain. However, the biomass of parent strain was significantly higher than mutants 9A2UV20, 9A2UV50, 9A2UV60, 9A2UV80, 9A2UV90, 9A2UV100 and 9A2UV110.

4. DISCUSSION

Improvement of microbial strains for overproduction of industrial bio-products has been the hallmark of all commercial fermentation processes [12,13]. Strain improvement is an essential part of process development for fermentation products. Developed strains can reduce the costs with increased productivity and can possess some specialized desirable characteristics. Such improved strains can be achieved by inducing genetic variation in the natural strain and subsequent screening.
### Table 1. β-mannanase activity of wild type and UV mutant strains of *A. glaucus*

| Mutants         | Biomass (g/50 ml) | Protein content (mg/ml) | Mannanase activity (U/ml) | Specific activity (U/mg) | Increase in activity (%) |
|-----------------|-------------------|-------------------------|---------------------------|--------------------------|--------------------------|
| Wild Strains/Control | 0.29±0.01<sup>a</sup> | 0.05±0.01<sup>b</sup> | 61.08±2.51<sup>f</sup> | 1327.89±0.14<sup>j</sup> | 100                      |
| **Effect of UV radiation** |                   |                         |                           |                          |                          |
| 9A1UV10         | 0.23±0.00<sup>e</sup> | 0.07±0.01<sup>d</sup> | 0.00±0.00<sup>g</sup> | 0.00±0.00<sup>h</sup> | 0                        |
| 9A1UV20         | 0.27±0.00<sup>f</sup> | 0.01±0.00<sup>h</sup> | 39.94±0.92<sup>i</sup> | 7988.80±0.02<sup>j</sup> | 65.39                    |
| 9A1UV30         | 0.13±0.01<sup>a</sup> | 1.02±0.01<sup>j</sup> | 89.39±0.10<sup>a</sup> | 87.38±0.02<sup>k</sup> | 146.34                   |
| 9A1UV40         | 0.24±0.00<sup>b</sup> | 0.24±0.00<sup>e</sup> | 18.58±0.08<sup>a</sup> | 77.11±0.00<sup>b</sup> | 30.42                    |
| 9A1UV50         | 0.17±0.00<sup>b</sup> | 0.46±0.01<sup>h</sup> | 81.06±0.29<sup>i</sup> | 176.98±0.11<sup>j</sup> | 132.7                    |
| 9A1UV60         | 0.28±0.00<sup>b</sup> | 0.38±0.01<sup>j</sup> | 19.22±0.01<sup>b</sup> | 51.26±0.01<sup>b</sup> | 31.47                    |
| 9A1UV70         | 0.19±0.01<sup>c</sup> | 0.83±0.01<sup>c</sup> | 85.67±0.10<sup>j</sup> | 103.34±0.02<sup>j</sup> | 140.25                   |
| 9A1UV80         | 0.29±0.00<sup>b</sup> | 0.09±0.01<sup>d</sup> | 18.19±0.01<sup>b</sup> | 195.63±0.01<sup>d</sup> | 29.79                    |
| 9A1UV90         | 0.24±0.00<sup>b</sup> | 0.38±0.01<sup>d</sup> | 23.31±0.01<sup>c</sup> | 60.69±0.03<sup>d</sup> | 38.16                    |
| 9A1UV100        | 0.30±0.00<sup>c</sup> | 0.04±0.00<sup>d</sup> | 43.19±0.01<sup>a</sup> | 1028.43±0.00<sup>i</sup> | 70.71                    |
| 9A1UV110        | 0.24±0.00<sup>b</sup> | 0.22±0.01<sup>e</sup> | 18.53±0.09<sup>c</sup> | 84.99±0.07<sup>c</sup> | 30.33                    |

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

### Table 2. β-mannanase activity of wild type and UV mutant strains of *R. japonicus*

| Mutants         | Biomass (g/50ml) | Protein content (mg/ml) | Mannanase activity (U/ml) | Specific activity (U/mg) | Increase in activity (%) |
|-----------------|------------------|-------------------------|---------------------------|--------------------------|--------------------------|
| Wild Strains/Control | 0.14±0.01<sup>a</sup> | 0.37±0.02<sup>a</sup> | 95.45±0.02<sup>j</sup> | 260.85±0.02<sup>j</sup> | 100                      |
| **Effect of UV radiation** |                   |                         |                           |                          |                          |
| 9A2UV10         | 0.15±0.00<sup>ac</sup> | 0.36±0.00<sup>ac</sup> | 95.46±0.01<sup>i</sup> | 267.43±0.01<sup>i</sup> | 100                      |
| 9A2UV20         | 0.12±0.01<sup>bc</sup> | 0.39±0.02<sup>cd</sup> | 95.28±0.02<sup>bc</sup> | 241.89±0.02<sup>bc</sup> | 99.83                    |
| 9A2UV30         | 0.27±0.01<sup>bc</sup> | 0.41±0.01<sup>cd</sup> | 95.04±0.02<sup>bc</sup> | 235.89±0.02<sup>bc</sup> | 99.56                    |
| 9A2UV40         | 0.22±0.02<sup>cd</sup> | 0.40±0.01<sup>cd</sup> | 94.50±0.00<sup>bc</sup> | 234.49±0.02<sup>bc</sup> | 98.98                    |
| 9A2UV50         | 0.13±0.02<sup>cd</sup> | 0.36±0.01<sup>bc</sup> | 96.30±0.04<sup>bc</sup> | 266.86±0.01<sup>bc</sup> | 100.90                   |
| 9A2UV60         | 0.08±0.01<sup>a</sup> | 0.31±0.01<sup>a</sup> | 95.02±0.04<sup>bc</sup> | 293.21±0.06<sup>bc</sup> | 99.51                    |
| 9A2UV70         | 0.19±0.01f        | 0.86±0.01<sup>f</sup> | 30.15±0.14<sup>i</sup> | 35.17±0.01<sup>i</sup> | 31.71                    |
| 9A2UV80         | 0.11±0.02bc       | 0.52±0.01<sup>a</sup> | 89.67±0.12<sup>bc</sup> | 169.98±0.11<sup>bc</sup> | 94.01                    |
| 9A2UV90         | 0.13±0.00<sup>cd</sup> | 0.53±0.01<sup>bc</sup> | 87.33±0.01<sup>bc</sup> | 165.40±0.01<sup>bc</sup> | 91.48                    |
| 9A2UV100        | 0.12±0.01<sup>bc</sup> | 0.39±0.02<sup>bc</sup> | 93.17±0.01<sup>bc</sup> | 239.50±0.01<sup>bc</sup> | 97.59                    |
| 9A2UV110        | 0.11±0.00<sup>b</sup> | 0.36±0.01<sup>bc</sup> | 84.26±0.02<sup>b</sup> | 233.46±0.05<sup>d</sup> | 88.28                    |

Means with the same superscript letters down the column are not significantly different (P>0.05)
The use of mutation and selection to improve the productivity of cultures has been strongly established for over fifty years and is still recognized as a valuable tool for strain improvement of many enzyme producing organisms.

In recent years new procedures such as rational screening and genetic engineering have begun to make a significant contribution to this study but mutagenesis and selection—so-called random screening is still cost effective procedure, and reliable short term strain development is frequently the method of choice [12]. Mutation induction and/or selection techniques together with cloning and protein engineering strategies have been exploited to develop enzyme production [18].

The current practice of strain improvement by mutagenesis and selection is a highly developed technique drawing on the latest advances from a wide range of scientific and technical disciplines. Mutagenic procedures can be optimized in terms of type of mutagen and dose. Ultraviolet radiation is one of the well known and most commonly used mutagen. It is universally used to induce genetically improved strains.

There have been many efforts to screen large array microorganisms with ability to produce substantial volume of mannanase for industrial application [7,27]. Series of research had been conducted globally to improve microbial strains for enhanced enzyme production; however there is no information on improved microbial strains for enhanced mannanase production. Improved strains could be achieved via physical and chemical mutagenesis [22,28].

Mutant strains of A. glaucaus (9A1UV30, 9A1UV50 and 9A1UV70) generated from 30, 50 and 70 min of exposure to UV irradiation obtained in this study produced promising amounts of β-mannanase and can therefore be utilized at bioreactor level for commercial production. The variation in mannanase production between mutant strains might be due to some factors like damaged DNA and differences in their ability to repair damaged genes [28,29,30].

5. CONCLUSION

Depending upon these trials, it was suggested that mutants 9A1UV30, 9A1UV50 and 9A1UV70 of A. glaucaus could be exploited commercially for industrial production of β-mannanase to meet industrial demand. The mechanism behind this study is yet to be elucidated. To the best of my knowledge, this is the first report on successful mannanase producer mutants of A. glaucaus and R. japonicus and it is suggested that molecular studies should be carried out on the improved mutants to reveal the mutation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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