JJBS

Jordan Journal of Biological Sciences

Volume 11, Number 5,December 2018
ISSN 1995-6673
Pages 557 - 562

Screening Wild and Mutant Strains of *Aspergillus flavus* and *Aspergillus niger* Isolated from Plantain Stalks for Amylase Production

Oluwatoyin F. Olukunle* and Oluwadamilola E. Ajayi

Department of Microbiology, Federal University of Technology, P.M. B. 704, Akure, Nigeria

Received April 9, 2018; Revised May 29, 2018; Accepted June 7, 2018

Abstract

This study is conducted to determine the amylase activity of wild and mutant strains of *Aspergillus flavus* and *Aspergillus niger* isolated from plantain stalks. The isolation of the fungal species was carried out using standard microbiological methods. Strain improvement of the fungal isolates was carried out by exposing the wild fungal species to ultraviolet (UV) radiation at 240nm for ten, twenty, and thirty minutes. The amylase production from the wild and mutant strains was examined quantitatively while the effects of pH and temperature on the amylase activities of the wild and mutant strains were determined. Three mutant strains were obtained from each of *Aspergillus flavus* and *Aspergillus niger*. The wild and mutant strains of the fungal isolates showed variations in the amylase production. The amount of amylase produced by the fungal strains ranged from 2.849 mg/mL/min to 3.263 mg/mL/min of which the mutant strains of *Aspergillus niger*, exposed for ten minutes showed the highest amylase production. Furthermore, the amylase activities of the wild and mutant strains of the fungal species were sensitive to changes in pH and temperature. Amylase was optimally produced from all the fungal strains at pH 6 and 25^{o}C. This study has revealed the amylase producing potential of wild and mutant (irradiated) strains of *Aspergillus flavus* and *Aspergillus niger*. Therefore, the mutated strains of *Aspergillus flavus* and *Aspergillus niger* could be employed in the commercial production of amylase. Findings from this study are promising; however, further intensive studies are still needed on the improved strains as well as the purification and characterization of the enzyme.

Keywords: Amylase, Wild strains, Mutant strains, Plantain stalk, *Aspergillus flavus*, *Aspergillus niger*, Enzyme activity.

1. Introduction

Enzymes are potential biocatalysts for a large number of reactions and they form parts of the most important products needed to meet human needs in the areas of industrial, environmental and food biotechnology through microbial sources (Chaudhri and Suneetha, 2012). Over five-hundred industrial products are being made using enzymes (Kumar and Sing, 2013). Industrial enzymes are highly demanded, and there is a need for a solution through which they can be produced on a large scale.

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Microbes are known to be one of the largest and useful sources of many enzymes (Demain and Adrio, 2008). Microbial enzymes are known to play a crucial role as metabolic catalysts, leading to their use in various industries and applications. The advantages of microbial enzymes over plant and animal enzymes are that they are more active and stable. In addition, microorganisms represent an alternative source of enzymes because they can be cultured in large quantities in a short time by fermentation owing to their biochemical diversity and susceptibility to gene manipulation. The main concern of industries is to get new microbial strains with the ability to produce different enzymes to fulfil the current enzyme requirements (Singh *et al.,* 2016).

The most widely used thermostable enzymes are the amylases in the starch industry (Emmanuel *et al*., 2000) and the enzymes obtained from fungal isolates are much more useful in industries for bakery, starch conversion and biofuel production. Developing countries depend on enzyme production from fungi because of the non-fastidious nutritional requirement and ubiquitous nature of the fungal enzymes (Gigras *et al*., 2002). A distinguishing characteristic of all the commercial fermentation processes is the improvement of microbial strains for a higher enzymatic yield. Such improved strains can reduce the cost of the process, and may also impact some specialized desirable characteristics of the products (Steensels *et al*., 2014; Rowland, 1984).

A variety of agricultural and food processing waste substrates can be employed in the production of industrially important enzymes. Fruits and vegetable wastes constitute agricultural wastes that are considered as a major source of environmental pollution (Garg and

* Corresponding author. e-mail: ofolukunle@gmail.com.

Ashfaque, 2010). Agricultural wastes are the source of starch containing substrates and necessary carbon and nitrogen sources for microbial metabolism (Siddiqui *et al*., 2014). Various agricultural wastes such as millet starch, potato and wheat bran are widely used for the production of enzymes (Sajjad and Choudary, 2012). In this study, Plantain (*Musa paradisiaca*) stalk wastes which are under-utilized in general, are exploited for the production of enzymes.

Microorganisms can be easily manipulated using genetic engineering or other means. They can be subjected to strain improvement, mutations, and other such changes by which the production of enzymes can be optimized (Ajita and Thirupathihalli, 2014). Ultraviolet (UV) light has been shown to be lethal and mutagenic in a variety of organisms, including fungi. Ultraviolet irradiation was found to be the best for the improvement of strains such as *Aspergillus niger* for a maximum production of various enzymes (Kang *et al*., 1999). In recent years, attempts have been made for the over production of microbial enzymes by induced mutagens. Novel enzymes were developed by the combined use of microbial screening and rational protein engineering (Sarikaya *et al*., 2000). Such improved strains can reduce the cost of the process and may also possess some specialized desirable characteristics.

Enzymes of fungal origin were found to be more stable than the bacterial enzymes on a commercial scale. Developing countries depend on enzyme production from fungi because of the non-fastidious nutritional requirements and ubiquitous nature of the fungal enzymes (Gigras *et al*., 2002). Various forms of amylases are used to convert starch into different sugars. A variety of industries employ microbial amylase, cellulase and lipase which are used in textile, paper and detergent industries (Das *et al*., 2011; Tsukagoshi *et al*., 2001). α- Amylases, β amylase and glucoamylase are commonly produced by various species of *Aspergillus* and are used as sources of industrial amylases. The most widely used thermostable enzymes are the amylases in the starch industry (Emmanuel *et al*., 2000). In fact, amylase has received a great deal of attention due to the technological and economic significance (Asrat and Girma, 2018). The enzymes obtained from fungal isolates are much more useful in starch conversion, bakery and fuel alcohol production.

The aim of this study is to subject the fungal strains isolated from plantain stalks to random mutagenesis by UV light for strain improvement and to compare the enzyme activities of the wild and the mutants after exposure to the UV light.

2. Materials and Methods

2.1. Study Area/Sample Collection

The Federal University of Technology, Akure, Nigeria lies between longitude 5.1^{o} East and latitude 7.2^{o} North. Its population is estimated at twenty two thousand. A lot of wastes have been generated, amongst which are the plantain stalk wastes. A plantain stalk waste sample was collected at a dump site at Obakekere at the Federal University of Technology, Akure, Nigeria and was placed in a clean sterile polythene bag and transported to the Departmental Laboratory for microbiological analyses.

2.2. Isolation and Identification of Fungal Isolates

One gram **(**1g) of the plantain stalk was cut using a sterile razor blade and was placed in 10mL of distilled water. This was shaken properly inside a test tube to obtain the stock culture. Serial dilutions, plating on Sabouraud dextrose agar (SDA) incorporated with chloramphenicol, incubation and subculturing were done using standard techniques. The colonies were counted as spore forming unit (SFU/g), while the identification was based on the microscopic and macroscopic features of the hyphal mass, nature of the fruiting bodies, and the morphology of cells and spores.

2.3. Inoculum Preparation

The inoculum of the fungal isolates grown for ninety-six hours at 30°C on SDA medium slants was prepared by adding 10 mL of sterile distilled water, containing 0.1% (v/v) Tween 80 to the agar slant and was shaken vigorously. The spore suspension was adjusted to the spore concentration of 10^3 SFU/mL as the initial inoculum size (Ibrahim *et al*., 2012).

2.4. Mutant Generation by Ultraviolet Radiation

The method described by Bapiraju *et al*., (2004) was modified and employed in preparing the mutant fungal strains. The Petri plates of the wild fungal isolates were exposed to UV irradiation for ten, twenty, and thirty minutes at a distance of 10 cm in the dark (to prevent photo reactivation) from the center of germicidal lamp (240 nm) with occasional shaking. Afterwards, 1mL spore suspension was withdrawn from each labelled plates and was plated on SDA medium. The developed mutants were maintained on SDA slants at 4°C in the refrigerator until use. The developed mutants and wild parents were quantified for amylase production.

2.5. Quantification for Amylase Production

Soluble starch (1 %) was prepared in 0.02M sodium phosphate of pH 6.9 containing 0.006M NaCl. Thereafter, 0.2mL of the enzyme solution (extract) was added to 0.2mL of substrate (starch) and incubated at 25^{o}C for three minutes. Afterwards, 1mL of 3-5 Dinitro salicylic Acid reagents (DNSA) was added. The mixture was then heated in a water bath (100^{o}C) for five minutes. After heating, the mixture was cooled and 10 mL of distilled water was added, and then read in a colorimeter at 540 nm against a blank containing buffer without enzyme with the aid of a spectrophotometer. A calibration curve was made with maltose to convert the colorimeter reading to the unit of activity (Sohail, 2005).

2.6. Effect of Physical Factors on Amylase Activity

The effect of temperature on the amylase activity was determined by the method described by Adekunle *et al*., (2014). This was performed by incubating an aliquot of the enzyme with the substrate at temperatures ranging from 25^{o}C to 60°C for twenty minutes. The residual amylase activity was plotted against the different temperatures.

The effect of pH on the amylase activity was investigated within the pH range of 3 - 9 at room temperature. For the measurement of pH stability, the enzyme was incubated at room temperature for one hour in

buffers at different pH values, and the residual activity was determined. (Adekunle *et al*., 2014).

2.7. Statistical Analysis

The experiments were carried out in replicates of three, and the results were expressed as mean ± standard error of three values. Data obtained were subjected to the one Way Analysis of Variance (ANOVA), and means were compared using New Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at $P<0.05$.

3. Results and Discussion

3.1. Enumeration and Identification of Fungal Isolates

The result of the total fungal load of the plantain stalk is presented in Table 1. The total fungal load was 10 SFU/g. The morphological and microscopic tests carried out on the fungal isolates from plantain stalk confirmed the identity of the isolates to be *Aspergillus niger* and *Aspergillus flavus* (Table 2).

Table 1. Enumeration of fungal isolates

Sample	Dilution factor	Total Count (SFU/g)	Species Description of fungal isolate
Plantain stalk	10^{-2}	10	Green and black colonies

Table 2. Cultural and morphological characteristics of the isolated fungal species.

Cultural characteristics	Microscopic characteristics	Organism
Brown mycelial growth	An upright conidiophore that terminates in a swelling, bearing phialides at the apex radiating from the entire surface: conidia are one celled. Spores are black.	*Aspergillus niger*
Greenish yellow mycelia growth and fully extended from the growth medium, conidiophores upright, simple terminating.	Branched hyphae and septate conidiophores, long upright aseptate and unbranched, no columella, terminate into globose vesicles surface contain many flask-shaped phialides with chains of conidia. Has yellowish green spores	*Aspergillus flavus*

3.2. Amylase Production by Wild and Mutant Fungal Isolates

Amylase production by the wild and mutant fungal isolates ranged between 2.849 and 3.263 mg/mL/min (Table 3).

When comparing the amylase activity of wild strains and the mutant strains exposed to the first dose of UV radiation, it was observed that the amylase production increased in the mutant strains. The amylase activity was found to be higher in mutants *Aspergillus flavus* exposed to UV for ten minutes (AFUV10) (3.243 mg/mL/min) and *Aspergillus niger* exposed to UV for ten minutes (ANUV10) (3.263 mg/mL/min). However, a decrease in amylase activity was observed in the mutant strains with prolonged exposure to UV radiation.

Aspergilus niger strains	Amylase quantity (mg/mL/min)	*Aspergillus flavus* strains	Amylase quantity (mg/mL/min)
ANW	3.162 ± 0.061^{c}	AFW	2.960 ± 0.030^{b}
ANUV10	3.263 ± 0.002^{d}	AFUV10	3.243 ± 0.004^{d}
ANUV20	3.246 ± 0.011^{d}	AFUV20	3.186 ± 0.002^{cd}
ANUV30	3.195 ± 0.003^{cd}	AFUV30	2.849 ± 0.003^{a}

Table 3. Amylase activity of wild type and UV mutant strains of *Aspergillus niger* and A*spergillus flavus.*

3.3. Effect of Temperature on Amylase Activity of Fungal Isolates

The effect of tempertaure on the wild and improved strains of *Aspergillus niger* and *Aspergillus flavus* is presented in Figure 1. The amylase activities of the fungal strains was found to be temperature-dependent. The fungal strains except for the improved strain of *A. niger* exposed to UV for ten minutes (ANUV10) produced amylase optimally (2.84 to 3.23 mg/mL/min). *A.niger* exposed to UV for thirty minutes (ANUV30) displayed the highest amylase activity (3.23mg/mL/min) while ANUV10 displayed the least amylase activity (2.84 mg/mL/min).

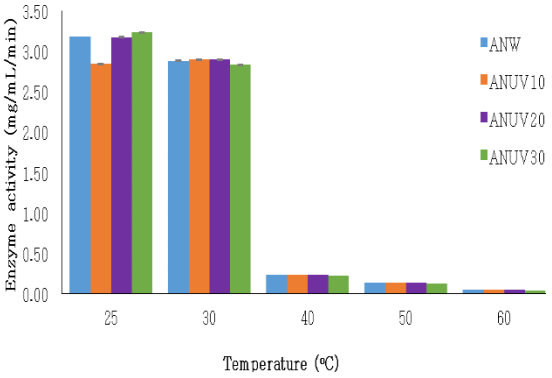

Figure 1. Effect of temperature on amylase activities of wild and mutant strains of *A. niger*. ANW: wide strain of *A. niger*. ANUV 10*: A. niger* exposed to ultraviolet light at 10 minutes ; ANUV20: *A. niger* exposed to ultraviolet light at 20 minutes; ANUV 30: *A. niger* exposed to ultraviolet light at 30 minutes.

Figure 2 also shows the effect of temperature on amylase activities of wild and mutant strains of *Aspergillus flavus*. The amylase activities of the fungal strains were found to decrease upon increasing temperature. The organisms produced varying amylase activities (0.04 to 3.24 mg/mL/min) within the temperature range of 25 ^{o}C to 60^{o}C. The optimal enzyme activity was at 25^{o}C with *A. flavus* exposed to 10 (AFUV10) and 20 (AFUV20) displaying the best activity.

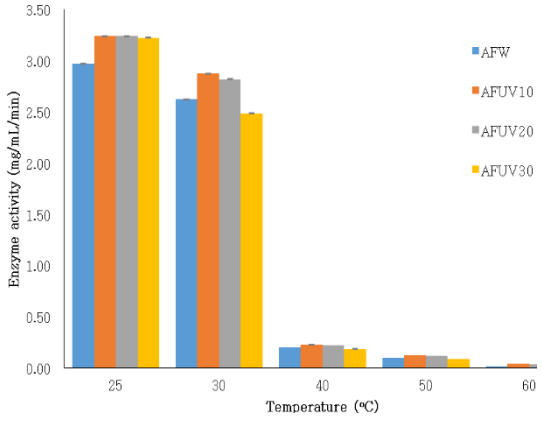

Figure 2. Effect of temperature on amylase activities of wild and mutant strains of *Aspergillus flavus*. AFW: wide strain of *A. flavus*. AFUV10*: A. flavus* exposed to ultraviolet light at 10 minutes. AFUV20: *A. flavus* exposed to ultraviolet light at 20 minutes. AFUV30: *A. flavus* exposed to ultraviolet light at 30 minutes.

3.4. Effect of pH on Amylase Activity of Fungal Isolates

The effect of pH on the amylase activity of the *A.niger* strains is presented in Figure 3 . The fungal strains were found to display pH-dependent enzyme activities. The enzyme activity of the fungal strains was found to be low (0.12 to 0.21 mg/mL/min) at pH 3 and pH 4. A sharp increase in the amylase activity was displayed by the strains of *A.niger* at pH 5 (2.23 to 2.30 mg/mL/min) and pH 6 (3.19 to 3.24 mg/mL/min). However, further increase in the pH values led to a sharp decline in the enzyme activity (0.32 - 0.14mg/mL/min**).** Similar observation was also made in the amylase activity of *A. flavus* strains when exposed to varying pH (Figure 4). Strains of *A. flavus* produced amylase optimally at pH 6 (2.84 to 3.23 mg/mL/min**).** The improved strain of *A. flavus*, AFUV10 displayed the highest enzyme activity (3.23 mg/mL/min) at pH 6.

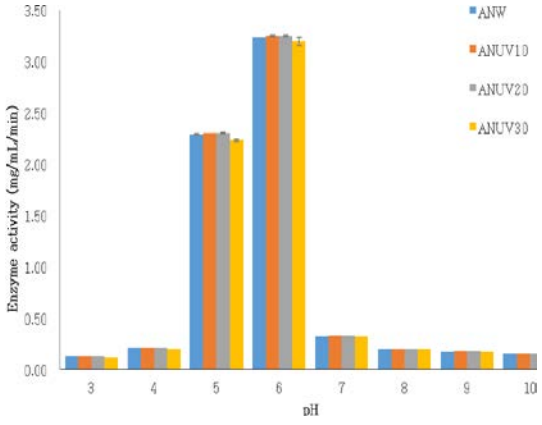

Figure 3. Effect of pH on amylase activities of wild and mutant strains of *A. niger*. ANW: wide strain of *A. niger*. ANUV10*: A. niger* exposed to ultraviolet light at 10 minutes. ANUV20: *Aspergilus niger* exposed to ultraviolet light at 20 minutes. ANUV30: *Aspergilus niger* exposed to ultraviolet light at 30 minutes.

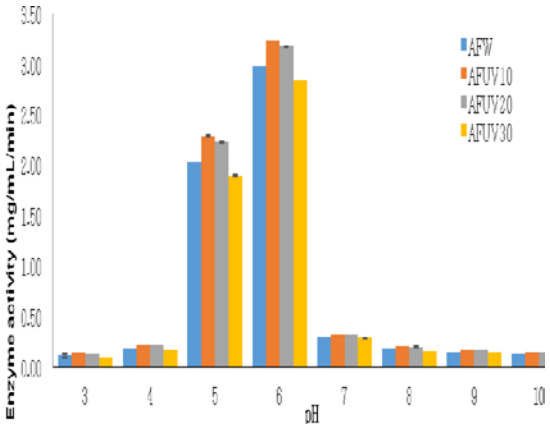

Figure 4. Effect of pH on amylase activities of wild and mutant strains of *A. flavus*. AFW: wide strain of *A. flavus*.. AFUV10*: A. flavus.* exposed to ultraviolet light at 10 minutes. AFUV20: *A. flavus.* exposed to ultraviolet light at 20 minutes. AFUV30: *A. flavus.* exposed to ultraviolet light at 30 minutes.

Results from the study showed two fungal species, *Aspergillus flavus* and *Aspergillus niger* isolated from the plantain stalks. These fungal species have been implicated in the biodegradation of agro-wastes and the production of industrially important enzymes (Thangaratham and Manimegalai, 2014). Hence, their presence on the plantain stalk indicates the biodegradation of the plantain stalk. The results of the current study also showed *Aspergillus flavus* and *Aspergillus niger* producing amylase at varying degrees. This observation is consistent with the findings of Oseni (2011) and El-Tablawy (2014). While studying the protease activity of some fungal isolates, Oseni (2011) suggested that protease production was directly linked to the organism involved, and the effectiveness and sustainability of the culture medium used. El-Tablawy, (2014) also observed different fungal species isolated from medicinal plants to produce extracellular enzymes at varying degrees.

Furthermore, *A. flavus* and *A. niger* mutant strains after exposure to 10 mins of ultraviolet radiation were found to produce higher amount of amylase in comparison with the wild strain. This suggests that strain improvement through irradiation by UV could lead to improved amylase production by the isolates. Thymine and cytosine are reportedly sensitive to modification when exposed to UV radiation as it can lead to the production of thymine dimers that can distort the DNA helix and block further replication (Sambrook and Russell, 2001). Several authors have reported enzyme production by microorganisms when exposed to UV radiation. For instance, a higher cellulase activity by UV mutant strains of *Trichoderma reesei* was reported by Shahbazi *et al*., (2014). However, longer exposure of the fungal strain led to a decline in the amylase production. This implies that longer exposure of the strains to UV radiation might be rather lethal than beneficial to the organisms in terms of amylase production.

The catalytic activities of enzymes are reportedly temperature and pH-sensitive (Pathak *et al.*, 2014). The use of optimal temperature for enzyme activity is an indispensable parameter especially in starch-processing industries (Sohail, 2005). The enzyme produced by the fungal strains used for this study displayed varying

thermostability, acted optimally at temperature of 25°C, and displayed the least thermostability at 60°C. This finding corroborates with the results of Alva *et al*., (2007) who reported similar thermostability range for amylase produced by *Aspergillus* species.

The findings of the current study showed that amylase produced by each fungal strain is pH- sensitive. For instance, amylase produced by each fungal strains was optimally active at acidic or near neutral conditions (pH 5 to 6). This indicates that the fungal strain prefers slightly acidic or neutral pH for its activity. This result concurs with the findings of Dutta *et al*. (2016). In their findings, they observed amylase produced by fresh water zooplankton, *Heliodiaptomus viduus*, had more than 50 % activity between pH 4.6 and 6.8. A similar observation was also made by Aydoğdu *et al*., (2012) who noted that the amylolytic activity of the fungal species used for the experiment falls between pH 4 and 6, while Asrat and Girma, (2018) observed a maximum activity at pH 6.0 in the production of the amylase enzyme by *Aspergillus niger* FAB- 211.

The ANOVA was used to compare the difference between the fungal isolates exposed to UV radiation for ten, twenty, and thirty minutes. Difference was considered significant at the 95 % confidence level ($p < 0.05$) (Table 3).

4. Conclusion

The current study has revealed the amylase producing potential of wild and irradiated strains of *Aspergillus flavus* and *Aspergillus niger*. The results from the study also showed improvement in amylase production after exposure to UV radiation after ten minutes. Furthermore, the amylase was seen to be optimally active at pH 6 and at 25°C. Therefore, these strains could be employed in the commercial production of amylase, and could help solve one of the challenges faced in the industrial setup where amylase utilization is high. The cost of disposing the plantain stalk will be reduced, and the environmental pollution arising from the decomposition of the wastes will also be reduced as well.

Findings of the current study are promising, however, there is more need for intensive further studies on the improved strains.

Acknowledgments

We are grateful to the Federal University of Technology, Akure, Nigeria for providing the laboratory space.

References

Adekunle TB, Isaac OA and Mufutau KB. 2014. Characterization of a novel α-amylase from *Bacillus macquariensis* isolated from Cassava Peels Dump site. *Am J Biochem.*, **4(4):** 84-92.

Alva S, Anupma J and Etha BS. 2007. Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp. JGI 12 in solid state fermentation. *Afr J Biotechnol.*, **6(5)**: 576-581.

Ajita S and Thirupathihalli PKM. 2014. α-Amylase Production and Applications: A Review. *J Appl Environ Microbiol.*,, **2 (4):** 166-175.

Aydoğdu H, Balkan B, Balkan S and Ertan F. 2012. Amylolytic activities of fungi species on the screening medium adjusted to different pH. *Fen Bilimleri Enstitüsü Dergisi Cilt-Sayı*, **5 (1)**: 1-13.

Asrat B and Girma A .2018. Isolation, production and characterization of amylase enzyme using the isolate *Aspergillus niger* FAB-211. *Inter J Biotechnol Mol Biol Res.*, **9 (2):** 7 -14.

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

Chaudhri A and Suneetha V. 2012. Microbially derived pectinases: A Review. *J Pharmacy and Biol Sci.*, **2 (2):** 1-5.

Das S, Singh S, Sharma V and Soni ML. 2011. Biotechnological applications of industrially important amylase enzymes. *Inter J Pharmaceut Biol sci.*, **1**: 486-496.

Demain AL and Adrio JL. 2008. Contributions of microorganisms to industrial biology. *Mol Biotechnol.*, **38**: 41–45.

Dutta TK., Jana M, Pahari P and Bhattacharya T. 2016. The effect of temperature, pH, and salt on amylase in *Heliodiaptomus viduus* (Gurney) (Crustacea: Copepoda: Calanoida). *Turk J Zool.*, **30**, 187-195.

El-Tablawy SY. 2014. Reduction of some enzymes produced by irradiated fungal strains isolated from certain medicinal plants. *J Natural Sci Res.*, **4(2):** 30-37.

Emmanuel L, Stephan J, Bernard H and Abdel, B., 2000. Thermophilic archeal amylolytic enzymes. *Enzyme Microbiol Technol.*, **26**: 3-14

Garg N and Ashfaque M. 2010. Mango peel as substrate for production of extracellular polygalacturonase from *Aspergillus fumigatus*. *Indian J Horticulture*, **67**: 140-143.

Gigras P, Sahai V and Gupta R. 2002. Statistical media optimization and production of ITS alpha amylase from *Aspergillus oryzae* in a bioreactor, *Current Microbiol.*, **45**: 203-208.

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

Kang SW, Ko EH, Lee JS and Kim SW. 1999. Over-production of β-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. *Biotechnol Lett.*, **21**: 647-650.

Kumar A and Singh S. 2013. Directed evolution: Tailoring biocatalysis for industrial application. *Crit Rev Biotechnol.*, **33**: 365–378.

Oseni OA. 2011. Production of Microbial Protease from Selected Soil Fungal Isolates. *Nig J Biotechnol.*, **23**:28 – 34.

Pathak SS, Kumar S, Rajak RC and Sandhu S 2014. Study of effect of temperature on amylase production by soil mycotic flora of Jabalpur region. *World J Pharmacy Pharmaceut Sci.*, **3(9):** 1448-1458.

Rowland RT. 1984. Industrial strain improvement: mutagenesis and random screening procedures. *Enzyme Microb.Technol.*, **6:** 224 – 230.

Sajjad M and Choudhry S. 2012. Effect of starch containing organic substrates on alpha amylase production in *Bacillus* strains. *Afr J Microbiol Res.*, **6**: 7285-7291.

Sarikiya E, Higassa T, Adachi M and Mikami B. 2000. Comparison of degredation abilities of α and β amylase on raw starch granules. *Proc Biochem.*, **35**, 711-715.

Sambrook J and Russel DW. 2001. **Molecular Cloning: A Laboratory Manual**. 3rd Ed., Cold Spring Harbor Laboratory Press, New York, USA. pp 2344.

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

Siddiqui A, Salahuddin T, Riaz A, Zohra RR and Naheed S. 2014. Production of amylase from *Bacillus* sp. AY3 using fruit peels as substrate. *Fuuast J Biol.*, **4(2):** 213-215.

Singh R, Kumar M, Mittal A and Mehta PK. 2016. Microbial enzymes: industrial progress in 21st century, *Biotech* **6(2):** 174.

Sohail M. 2005. Survey of amylolytic bacteria and fungi from native environment samples. *Pak J Bot.*, **37(I):** 155-161.

Steensels J, Snoek T, Meersman E, Nicolino MP, Voordeckers K and Verstrepen, K. 2014. Improving industrial yeast strains: exploiting natural and artificial diversity, *FEMS Microbiol Rev.*, **38 (5):** 947-995.

Thangaratham T and Manimegalai G. 2014. Optimization and production of pectinase using agro waste by solid state and submerged fermentation. *Inter J Current Microbiol Applied Sci.*, **3(9):** 357-365.

Tsukagoshi N, Kobayashi T and Kato M. 2001. Regulation of the amylotic and hemi-cellulolytic genes in *Aspergillus. J Gen Appl Microbiol.*, **47**: 1-19.