International Journal of Research in Engineering, Science and Management
Volume-2, Issue-8, August-2019
www.ijresm.com | ISSN (Online): 2581-5792

Development of Mutant Fungal Strains of *Aspergillus flavus* for Enhanced Production of Keratinase Enzyme

R. V. Madhumitha Indu[1], P. Vanitha Pappa[2]
[1]*Research Scholar, Department of Zoology, Rani Anna Government College for Women, Tirunelveli, India*
[2]*Assistant Professor, Department of Zoology, Rani Anna Government College for Women, Tirunelveli, India*

***Abstract*: The keratinous wastes are increasingly accumulating in the environment mainly in the form of feathers, hair, horns, hooves and nails generated from various industries.**

Keratin is an insoluble macromolecule. Keratin comprises long polypeptide chains, which are resistant to the activity of non-substrate-specific proteases. The hydrolysis of keratin wastes by microorganisms is considered a biotechnological alternative for recycling the keratin. Keratinase is an extracellular proteolytic enzyme with the capability of degrading insoluble keratin substrates. Mutation is the ultimate source of genetic variation. The keratinase enzyme production was enhanced by altering the genetic nature of wild type fungal strain *Aspergillus flavus* by induced mutation. A physical method (UV irradiation) was employed to develop the mutant fungal stains. The activities of enzyme produced by different strains of fungi *Aspergillus flavus* were estimated using UV spectroscopic technique to identify the potential keratinase producing fungi. The study revealed that a strain of *Aspergillus flavus* which is irradiated in UV radiation for 20 mins and having dilution of 10-8 is producing the potential keratinase enzyme.

***Keywords*: *Aspergillus flavus*, Enhanced keratinolytic activity, Induced Mutation.**

1. Introduction

Keratin is highly rigid, strongly cross-linked structural polypeptide “keratin”. Keratin is recalcitrant to the commonly known proteolytic enzymes trypsin, pepsin and papain due to its cross linkage disulphide bonds, hydrophobic interactions and hydrogen bonding [1], [2]. Now a day a large amount of keratin by-products are wasted which is potential threat to the environment [3], [4].

Keratinous substrates are present in the environment in the form of hair, feather, wool, nail, horn etc. The continuous use of these sources for human needs and generation of loads of waste leads to the accumulation of waste in ecosystem. This is causing water and soil pollution. The traditional strategies used for the disposal of chicken feather are often burned in incineration, buried in landfills. However, these disposal methods are worse because it leads to emission of greenhouse gases which poses great danger to environment [5]. These methods of disposal may also direct to the environmental pollution and leading to the deterioration of a valuable resource [6]. Microbial keratinases have become biotechnologically important since they target the hydrolysis of this rigid keratin.

In the present study *Aspergillus flavus*, a potential producer of keratinolytic enzyme [7] is subjected to UV irradiation to produce mutant stains. In this study the keratinolytic activity of enzyme produced by the mutant stains were analysed to identify the potential enzyme producing strain for further study.

2. Materials and method

Soil samples were collected from feather dumping areas of Sankar Nagar in Tirunelveli district. The isolated fungi were identified as *Aspergillus flavus, Aspergillus niger and Fusarium* species by morphological, microscopic and sporological characteristics. Among the three species Aspergillus flavus is found to produces potential keratinase enzyme. This is confirmed by feather degradation experiment. Hence *Aspergillus flavus* is selected for this study. 7-day old slant is used for preparation of spore suspension. Fifteen samples of Aspergillus Flavus were subjected to exposure of UV light radiation with different exposure time.

A. Physical mutation by UV irradiation

Various serial dilutions of fungal suspension were prepared (10^{-1} to 10^{-10}). Dilution of 10^{-8} (S1, S4, S7, S10, S13), 10^{-9}(S2, S5, S8, S11, S14) and 10^{-10}(S3, S6, S9, S12, S15) were distributed into sterilized petriplates (2 ml in each plate). These were exposed to UV radiations for varying time periods ranging from 10 to 50 minutes in UV chamber keeping the distance of UV source at 15cm. After UV radiation they were kept in dark for stabilization of thymine-thymine (T-T) dimers. Parent type and UV treated fungal spore suspensions of 0.1 ml was inoculated into petriplate containing potato dextrose agar medium.

B. Determination of Keratinolytic Activity

1) Preparation of Chicken Feather Powder

Poultry feather was cut into small fragments washed extensively with water and dried in a hot air oven at 40°C for 72 hours. To prepare feather powder, the feathers were pulverized and passed through a small mesh grid to remove

coarse particles.

2) Inoculum Preparation

Spore suspension of the fungal isolates was prepared by adding 10 mL of sterilized water to 7 days old fungal isolates growing on plates of potato dextrose agar. The final concentration of the spore suspension was adjusted to about $2x10^6$ mL^{-1}.

3) Feather meal preparation

Feather meal medium contain (g L^{-1}); $MgSO_4.H_2O$; 0.5, KH_2PO_4; 0.1, $FeSO_4.7H_2O$; 0.01 and $ZnSO_4.7H_2O$; 0.005, the pH was adjusted to 7.5. The medium was supplemented with chicken feather powder as a sole source of carbon and nitrogen [8].

4) Degradation of feather by Isolated Fungi

In 100 mL conical flasks, 50 mL of the feather meal medium along with 1.5 g of poultry feather powder was added separately in each flask then autoclaved. After cooling, the flasks were inoculated by 5 mL of spore suspension and incubated under shaking (120 rpm) at 28°C for 7 days. At periodic intervals, final culture pH was determined and the cultures containing the hydrolysates were centrifuged (8000 rpm) and filtered through muslin cloth. The filtrate was recovered to determine the keratinase activity.

5) Keratinolytic Activity

Keratinase activity was assayed by using 20 mg of chicken feathers powder, suspended in 3.8 mL of 100 mM Tris-HCl buffer (pH 7.8) to which 300 µL of the culture filtrate (enzyme source) was added. The reaction mixture was incubated at 37°C for 1 h. After incubation, the assay mixture was dipped into ice-cold water for 10 min and the remaining feathers were filtered out. Then the absorbance of the clear mixture was measured at 280 nm -400nm using UV- spectrophotometer.

3. Result and discussion

A. Physical mutation by UV irradiation

Fig. 1. Colony morphology of different mutant strains of *Aspergillus flavus*

UV irradiated *A.flavus* strains were cultured on Potato Dextrose Agar with different dilutions and exposure time as shown in Figure 1. Figure 1 also indicated the morphology formed by 15 mutant strains of *A.flavus*. Growth rate was studied in each strain and it is observed that growth rate increases with the increase in exposure time till 30 minutes of exposure after which the growth rate decreases. Similar trend has been reported by some other investigators [9], [10].

B. Determination of Keratinolytic Activity

Figure 2 shows the graphical representation of absorbance of UV spectrum estimated between 200 to 400 nm by enzyme produced by different isolates. By analyzing the present study the enzyme produced by *Aspergillus flavus* mutant strain S4 with 10-8 dilution and exposure time of 20 minutes shows maximum keratinolytic activity.

Table 1 showed the absorbance of enzyme observed using UV spectrophotometer at 280nm. Figure 3 Graphical representation of Absorbance of keratinase enzyme produced by different mutant strains of *Aspergillus flavus* observed using UV spectrometer at 280nm. Enzyme activity in each strain was observed which increased with the increase of exposure time till 20 minutes of exposure after which the keratinase activity decreased. Dutta reported that protease produced by wild and UV-mutant strains were not the same, finally achieved 2.5 fold increased production with mutant Strain [11]. Similarly, in my study the maximum keratinase activity was observed in S4 mutant strain of *Aspergillus flavus* with absorbance of 2.2 units. So, this strain can be used for potential degradation of keratinouse substrates.

Table 1
Absorbance of keratinase enzyme produced by different mutant strains of *Aspergillus flavus* observed using UV spectrometer at 280nm

Spore concentration/ exposure time	10^{-8} dilution	10^{-9} dilution	10^{-10} dilution
10 minutes	2.1	1.9	1.2
20 minutes	2.2	1.4	1.2
30 minutes	2.0	1.8	1.4
40 minutes	1.8	1.3	1.2
50 minutes	1.2	1.0	0.9

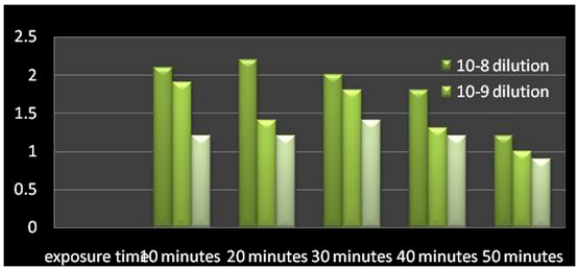

Fig. 3. Graphical representation of Absorbance of keratinase enzyme produced by different mutant strains of *Aspergillus flavus* observed using UV spectrometer at 280nm

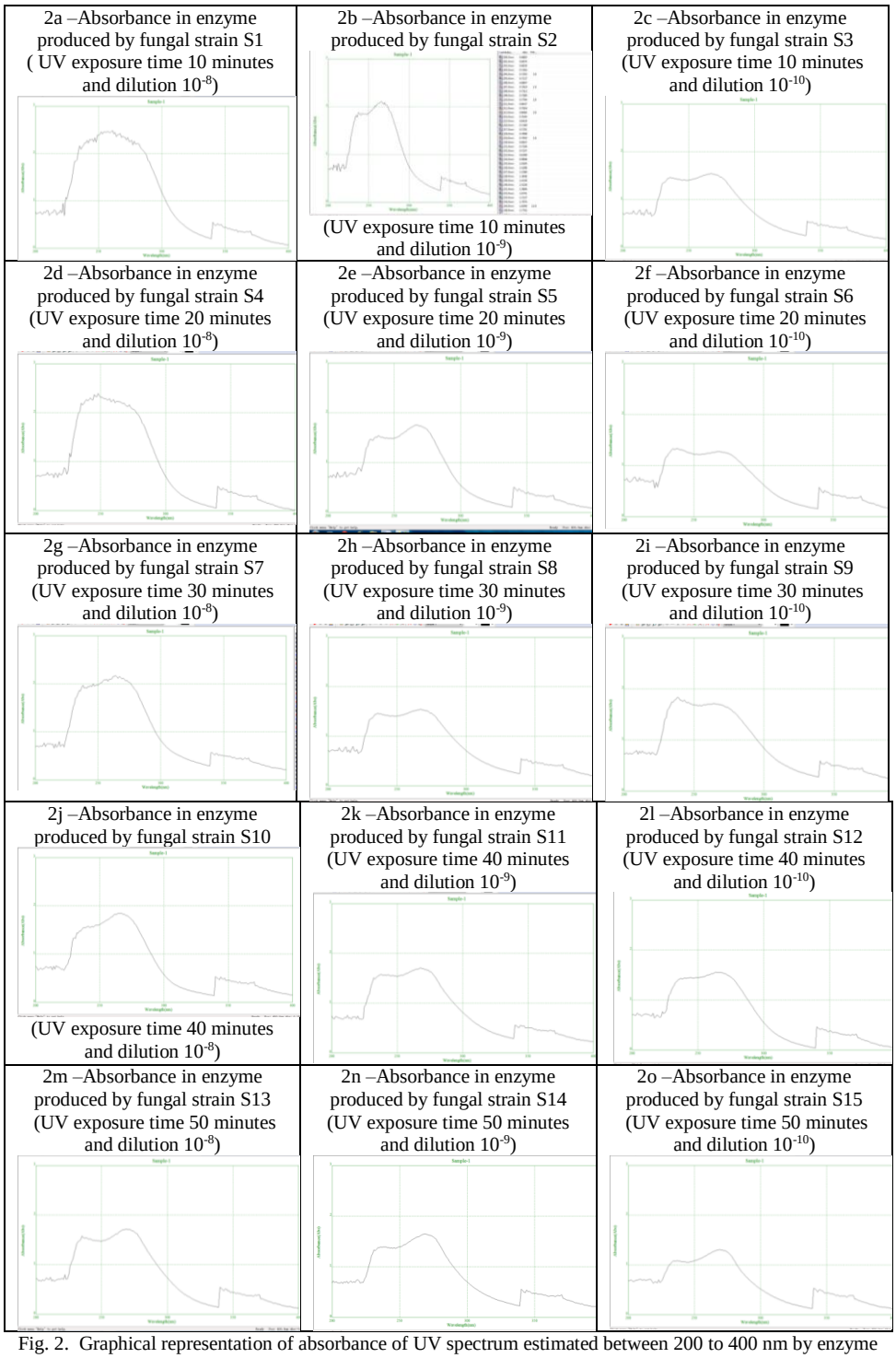

Fig. 2. Graphical representation of absorbance of UV spectrum estimated between 200 to 400 nm by enzyme produced by different mutant strains of *Aspergillus flavus*

4. Conclusion

Accumulation of keratinous substrates in the environment has now become a huge menace. Bioremediation is the only permanent solution for managing this issue. Moreover, keratin is a readily available as a source of amino acids like lysine, cystine, arginine and methionine. And it can also be effectively used in the production of bio fertilizers, bio feed and also used for the production of biogas. Hence, the enzyme keratinase not only leads a way to overcome environmental issue but also helps to use the kertin in beneficial way.

References

[1] K. K. Teresa, B. Justyna. "Biodegradation of keratin waste: Theory and practical aspects". Waste Manage., vol. 31, pp.1689-1701, 2011.

[2] S. Reichl, M. Borrelli, G. Geerling. “Keratin films for ocular surface reconstruction”. Biomaterials., vol. 32, pp. 3375-3386. 2011.
[3] I. A. Cavello, S. F. Cavalitto, R. A. Hours. “Biodegradation of a Keratin Waste and the Concomitant Production of Detergent Stable Serine Proteases from Paecilomyces lilacinus”. Appl. Biochem Biotech., vol. 167, pp. 945-958. 2012.
[4] M. Park, B. S. Kim, H. K. Shin, S. J. Park, H. Y. Kim, “Preparation and characterization of keratin-based biocomposite hydrogels prepared by electron beam irradiation”. Mat Sci. Eng. C-Mater., vol. 33, pp. 5051-5057, 2013.
[5] L. Verheyen, D. Wiersema, L. Hulshoff-Pol, P. Brandjes, P. Westra, J. Bos, J. Jansen, “Management of waste from animal product processing”. Livestock and Environment, finding a balance. Internal Agriculture centre, Wageningen, The Netherlands, 1996.
[6] S. Huda, Y. Q. Yang. “Composites from ground chicken quill and polypropylene”, Compos. Sci. Technol., vol. 68, pp. 790-798, 2008.
[7] J. D. Kim, “Keratinolytic activity of five Aspergillus species isolated from poultry farm soil in Korea”, Mycobiology, vol. 31, pp. 157-161, 2003.
[8] H. H. Elmayergi, R. E. Smith, “Influence of growth of streptomyces fradiae on pepsin-HCL digestibility and methionine content of feather meal”. Can J Microbial, vol. 17, no. 8, 1971.
[9] S. K. Yadav, B. Deepali, Shikha and S. D. Nandan. Afr. J. Biotechnol, vol. 10, pp. 8630-8640, 2011.
[10] X. W. Leng, and Yan Xu. Afr. J. Biotechnol, vol. 10, pp. 6824-6829, 2011.
[11] J. R. Dutta, and R. Banerjee, “Brazilian Archives of Biology and Technology, vol. 49, no.1, pp. 37-47, 2006.