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Improvement of Amylase Production by UV Mutagenesis of *Aspergillus flavus* FSS63 under Solid State Fermentation

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Abstract. Enhancement of the amylase productivity by *Aspergillus flavus* was investigated. Spores of strain were exposed to ultraviolet (UVC) radiation and 10 different mutants were selected and isolated from starch plate agar on the basis of the visible clearance zone around the colonies. The amylase production by selected mutants was evaluated under solid state fermentation. One mutant of *A. flavus* FSS63UV8 showed higher biosynthesis level of amylase (733 IU/g), which was 3.35 fold higher than that detected in the parental strain. Physical parameters optimisation revealed that the optimum pH and temperature for amylase production obtained by mutant are 7.0 and 35°C, respectively. Among several tested agricultural wastes , wheat bran was found to support the highest yield of amylase after 5 days of incubation**.** *A. flavus* FSS63UV8 strain proved to be a promising microorganism for a high amylase production in a simple medium.

Keywords: *Aspergillus flavus*, amylase, ultraviolet radiation

Introduction

Alpha amylase (EC3.2.1.1, 1,4-α-D-glucan-glucano-hydrolase) is an extracellular enzyme, which is involved in the starch processing industries where it breaks down starch into simple sugar constituents (Haq *et al.,* 2002; Akpan *et al.,* 1999). Amylase enzymes have also potential applications in a number of industries including brewing, baking, textile and detergent (Sundarram and Murthy, 2014; Gupta *et al.,* 2003). Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market (Das *et al.,* 2011). Today, new potential of using microorganisms as bio-technological source for production of industrially relevant enzymes has stimulated interest in exploring extra cellular enzymatic activity in several micro-organisms (Gupta *et al.,* 2003; Buzzini and Martini, 2002; Akpan *et al.,* 1999). Amylases are generally found in animals, plants, bacteria and fungi and their sources in yeast, bacteria and fungi and their properties have already been reported earlier (Bedan *et al.,* 2014; Singh *et al.,* 2013; Liu and Xu, 2008 Chi *et al.,* 2007). Among microorganisms, fungi have been recognized as a potential source of new enzymes with useful and/or novel characteristics (Singh *et al.,* 2009). Moreover, amylases from fungal origin were found to be more stable when compared with the bacterial enzymes (Abu *et al.,* 2005).

Several studies have been performed to investigate the use of different types of radiation on enzymatic activity of different microorganisms (Singh *et al.,* 2013; Vladimirov *et al.,* 2004). Improvement in enzymes production has been achieved through mutation, selection, or genetic recombination. However, in many cases, mutations are harmful, but occasionally may lead to a better adapted organism to its environment with improved bio-catalytic performance. The potential of a microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool. The real challenge is how to isolate those strains that are true mutants and thus carry the beneficial mutations (Parekh *et al.,* 2000). UV rays are important inducers of strain mutations. The pyrimidines (thymine and cytosine) are especially sensitive to modifications by UV rays absorption. This may result in the production of thymine dimers that distorts the DNA helix and block future replications (Sambrook *et al.,* 1989).

The objective of the present study was to improve the amylase production from *Aspergillus flavus* FSS63 through the use of ultraviolet radiation (UVC) as mutagenic agent and optimisation of cultural conditions.

Materials and Methods

Microorganism. *Aspergillus flavus* FSS63 utilized in this study was isolated from Syrian soil and identified in Center Wallon of Biology Industrial (CWBI), Belgium (Bakri *et al.,* 2009). The fungus was grown on potato-dextrose agar (PDA) plates at 30 °C.

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Ultra violet irradiation. The conidial suspension 10^8 spore/mL was prepared using sterile peptonic water, which consists of 5 g/L NaCl, 3 g/L peptone, 1 mL tween 80, and distilled water. Ten mL of this conidial suspension was irradiated by placing it in an opened sterilized plate which was on the inner base of ultraviolet radiation (UVC) apparatus (UVITEC) and 15 cm away from the UV lamps. The obtained dose was calculated based on this distance as 60 w/m^2 ±5. Serial dilutions were made up to 10^4, then 100 µL of the suspension was cultured on PDA plates. The same experiment was repeated changing the UVC exposure time to 10, 15, 20, 25 and 30 min. All plates were incubated at 30 °C for 24-48 h. The D^{10} was 20 min. Every grown spore was separately cultured in a new plate and made a conidial suspension as the parent strain. All new isolates and the parent were evaluated for amylase production (Table 1).

Amylase production medium. Amylase was produced by culturing 1-mL spore suspension (10^6 spores/mL) of each *A. flavus* isolates in Erlenmeyer flasks (100 mL) containing 5 g of wheat bran as carbon source and mineral salt medium (Na_2HPO_4 .$2H_2O$ (1% W/V), KCl (0.05% W/V), $MgSO_4.7H_2O$ (0.015 W/V) and (1% W/V) and (0.5% W/V) yeast extract as nitrogen source. The pH was adjusted to 6.5 before sterilization. After inoculation, flasks were incubated at 30 °C for 5 days.

Amylase assay. α-Amylase activity was determined as described by Okolo *et al.* (1995). Briefly the reaction mixture consisted of 1.25 mL of 1% soluble starch, 0.25 mL of 0.1M acetate buffer (pH 5.0), 0.25 mL of distilled water, and 0.25 mL of crude enzyme extract. After 10 min of incubation at 50 °C, the liberated reducing sugars (glucose equivalents) were estimated by the dinitrosalicylic acid (DNS) method of Miller (1959). The blank contained 0.5 mL of 0.1 M acetate buffer (pH 5.0), 1.25 mL of 1% starch solution and 0.25 mL of distilled water. One unit (IU) of α-amylase is defined as the amount of enzyme releasing 1 µmol glucose equivalent per minute under the assay conditions. All experiments were repeated twice in the same manner.

Results and Discussion

The effect of UVC exposure on *Aspergillus flavus* amylase production. The effect of UVC exposure of *A. flavus* FSS63 was tested at UV doses of 72, 90 and 108 J/cm^2 and a total of 10 colonies were selected on the basis of clear zones due to starch hydrolysis. At 90 J/cm^2 dose for 25 min, 1 mutant strain named AFFSS63UV4, was selected and showed an amylase production 626 IU/g which was 2.85 fold higher than the parental strain (Table 1). At 108 J/cm^2 dose for 30 min, 4 mutants were chosen to have an amylase production higher than their parents. One mutant strain AFFSS63UV8 produced 733 IU/g amylase which was around 3.35 fold higher than the parental strain.

Exposure of fungi spores to UV radiation produce mutant strains, this mutation depends on the type of damage in cell DNA, and on the mechanism of rebuilding this damage. Many researchers have used UV radiation to generate new mutant cells in order to increase enzymes production (Singh *et al.,* 2013; Adsul *et al.*, 2009; Bapiraju *et al.,* 2004; Haq *et al.,* 2002) and they succeeded in producing many mutant strains with higher enzyme production from many microorganisms such as *Penicillium, Aspergillus, Trichoderma, Bacillus*. Adsul *et al*. (2009) studied the effect of UV radiation on *Penicillium janthinellum* and isolated the mutant strains producing enhanced levels (3-5 folds) of FPA, CMCase, and xylanase in comparison to the parent strain. UV was also successfully used on *Fusarium oxysporum* and one mutant produced enzyme ability around 19.1 IU/mL/min compared with the parental strain ability 13.1 IU/mL/min (Singh *et al.,* 1995). Bapiraju *et al.* (2004) also reported an increase in lipase enzyme production reaching 180% more by the mutant BTUV3 than the original strain of *Rhizopus* sp.BTS-24.

The effect of pH on amylase production. Initial pH is one of the critical parameters, which correlates with the microbial growth directly. pH ranging from 3.0 to 8.0 was used to study its effect on amylase production by *A. flavus* FSS63UV8 was by varying the pH from 3.0 to 8.0. Results indicate that pH 7.0 was found to be

Table 1. The Effect of UVC on the mutant of *Aspergillus flavus* FSS63 and its amylase production

Dose J/cm2	Mutant strain	Amylase (IU/g)
0	Parental strain	219
72	AFFSS63UV1	187
90	AFFSS63UV2	209
90	AFFSS63UV3	196
90	AFFSS63UV4	626
90	AFFSS63UV5	186
108	AFFSS63UV6	622
108	AFFSS63UV7	162
108	AFFSS63UV8	733
108	AFFSS63UV9	512
108	AFFSS63UV10	612

the best for amylase activity (Fig. 1) and maximum amylase production was (780 IU/g). This might be due to an enhanced enzyme stability produced by the mutant strain. In contrast to these findings, Ivanova *et al.* (2001) achieved the optimal α-amylase production at a pH range of 8.0-9.5. Puri *et al.* (2013) found that the highest enzymes production by *A. oryzae* was obtained at pH of 5.0. Changing the pH from the optimum to extreme levels results in inactivation of the enzymes of the organisms which hinder saccharification of the substrate (Silva *et al.,* 2005).

The effect of incubation temperature on amylase production. Incubation temperature not only influences the growth of microorganisms but also their biological activities. Figure 2 reveals the effect of incubation temperature i.e. 25, 30, 35, 40 and 45 °C on amylase production by the mutant strain of *A. flavus* FSS63UV8 after 5 days of inoculation. It is clear from the results that a temperature of 35°C was found to be best suitable for amylase production. It might be due to the fact that 35°C is the optimal temperature for fungal growth and subsequently for enzyme production. In addition, high temperature might have reduced the moisture contents of the fermentation medium and organism growth resulting in a decreased enzyme production as pointed out by Markkanen and Suihko (1974).

The effect of carbon sources on amylase production. Different substrates like wheat bran, wheat straw, soybean cake, corn cobs hulls, potatoes peel, tomato pomace, beet pomace and cotton seed cake were used

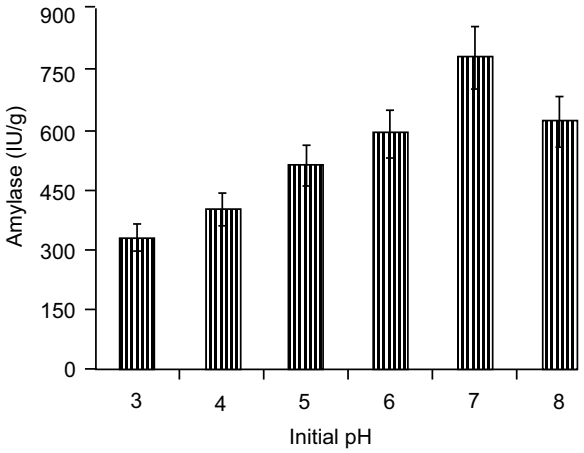

Fig. 1. Effect of pH on amylase production from *A. flavus* FSS6U8 mutant under solid state fermentation.

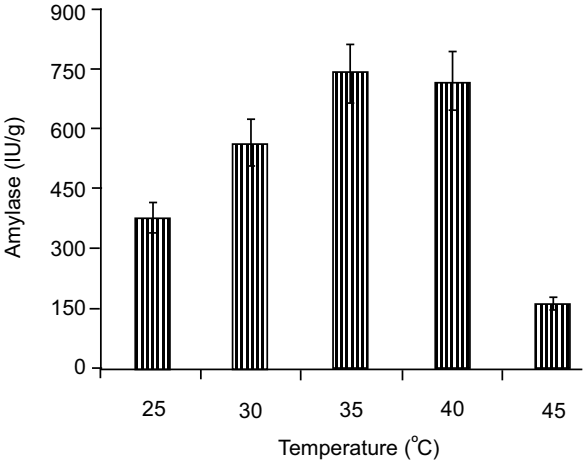

Fig. 2. Effect of incubation temperature on amylase prodution from *A. flavus* FSS63UV8 mutant under solid state fermentation

as carbon sources for amylase production by *A.flavus* FSS63UV8 under solid state fermentation. After inoculation and incubation for five days at 35 °C, the enzyme was extracted using phosphate buffer and was estimated for enzyme activity. Among the various substrates screened for amylase production, wheat bran gave the highest enzyme activity followed by potatoes peel (Fig. 3). This indicates that the nature of carbon source in culture media is important for production of extracellular amylase (Vijayaraghavan *et al*., 2011; Teodoro and Martins, 2000). Similarly, wheat bran was found to be the best substrate for α- amylase production

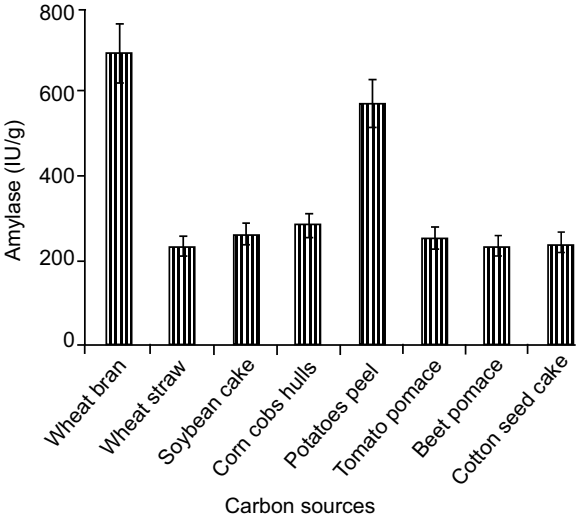

Fig. 3. Effect of carbon sources on amylase production from *A. flavus* FSS63UV8 mutant under solid state fermentation.

by a thermophilic fungus *Humicola lanuginose* and *A. niger* (Singh *et al.,* 2009). The highest activity in wheat bran may be due to its high carbohydrate contents and suitable texture.

Conclusion

In the present study, a mutant *A. flavus* FSS63UV8 was generated by UVC and proved to be an efficient producer of amylase under solid state fermentation. The optimal conditions for amylase production were pH 7 and temperature 35 ºC. Wheat bran was found to be less expensive substrate for an efficient amylase production (733 IU/g) after 5 days of incubation.

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