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STRAIN IMPROVEMENT THROUGH MUTAGENESIS AND OPTIMIZATION OF PROTEASE PRODUCTION BY *ASPERGILLUS TERREUS* CJS-127 USING *JATROPHA* SEED CAKE AS SUBSTRATE

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ABSTRACT

Jatropha curcas seedcake (JSC), a byproduct of the biodiesel industry was utilized for the production of protease. To enhance the protease production, the parent strain of *Aspergillus terreus* CJS-127 subjected to UV (15, 30, 60, 90, 120 and 150 min) and chemical mutagenesis by using ethyl methanesulphonate (EMS-125, 250 and 500 mg). Two mutant strains designated as UV-6 and EMS-5 selected for optimization of protease production using JSC medium by submerged fermentation. Under the optimized conditions of pH 7.0, temperature 30°C, incubation period 72 h, inoculum 10^5/ml spores, 150 rpm agitation, JSC at 2% level, the maximum protease production was 172.32 and 163.57 U/ml for EMS-5 and UV-6 mutant strains respectively. The addition of maltose (2%) was found to enhance the protease production in the mutant strains. The protease activity increased by 2.30 and 2.41 folds for UV 6 and EMS 5 mutant strains after optimization of cultural conditions.

Keywords: Strain improvement, *Jatropha* seedcake, Protease, *Aspergillus terreus* CJS-127, mutagenesis

INTRODUCTION

Microorganisms play a vital role in technology for the production of intracellular and extracellular enzymes on an industrial scale **(Gupta *et al.*, 2002)**. Proteases constitute one of the most important groups of enzymes, and their annual sales account for 60% of the total world enzyme market **(Turk, 2006)**. In recent years, there is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic process but also have gained considerable attention in their commercial application. Proteases are industrially important due to their wide applications in leather processing, detergent industry, food industries, pharmaceutical, textile industry, etc. **(Jellouli *et al.*, 2009; Deng *et al.*, 2010).**
Jatropha curcas is a plant of economic importance owing to its nonedible oil being used as biofuel. *Jatropha* seed cake (JSC) is a waste by-product generated during biodiesel production. The JSC is very rich in protein; however, the presence of antinutrients and toxins limits its application in animal feeds. Hence, there is a need to utilize this seed cake, as a large quantity of it is generated while processing for biodiesel **(Sharath *et al.*, 2014)**. Conversion of wastes into useful biomass by microorganisms and their enzymes is a new trend, and new protease-producing microorganisms and suitable fermentation technology are needed to meet the ever-growing demand for this enzyme **(Rathakrishnan *et al.*, 2012).**
Aspergillus terreus is a saprotrophic fungus prevalent in tropical and subtropical regions. The fungus has dominated the biological production of the "blockbuster" drugs known as statins. The statins are a class of drugs that inhibit HMG-CoA reductase and lead to lower cholesterol production. **(Subhan *et al.*, 2016)**. The statins are the largest selling class of drugs throughout the world. Sales for statins in 2005 were $25 billion **(Endo, 2010)**. *A. terreus* found as the potential strain for production of tannery protease in submerged fermentation **(Chellapandi, 2010)**. In this study, *A.terreus* CJS-127 a locally isolated strain has been selected to generate mutants for enhanced production of protease. This particular fungus was selected as it was able to grow on JSC and produce protease. Strain improvement is an essential part of process development for fermentation products. Improved strains can be achieved by inducing genetic variation in the natural strain and subsequent screening. The mutagenesis and selection after random screening are still cost effective procedure, and reliable short-term strain development is frequently the method of choice **(Rowlands, 2002; Iftikar *et al.*, 2010)**. The enzyme production could be further enhanced by optimization of physiological and biological conditions.
The aim of this study was to isolate protease overproducing strains of *A. terreus* CJS-127 by mutagenesis using physical mutagens like UV radiation and a chemical mutagen -EMS (Ethyl methanesulphonate). The present study was also focused on the optimization of cultural conditions for higher yields of extracellular protease using JSC as substrate by submerged fermentation.

MATERIALS AND METHODS

Culture and growth condition

The microorganism used in the present study was *A. terreus* CJS-127, and the culture was isolated at CFTRI, Mysore and the culture was deposited at MTCC, India. The culture was grown on Czapek Dox agar (CDA) medium for 7 days at 25°C (Himedia, India). The subculture of the organism was done once in 15 days.

Mutagenesis

UV mutagenesis

For UV radiation mutagenesis, spores (10^5spores/ml) of *A. terreus* CJS-127 parent strain were prepared using saline solution. 4 ml of this suspension was taken in a sterile 9 cm Petri plate and kept in a sterile UV chamber with lids open and exposed to 254 nm UV radiations. The exposure was carried out at a distance of 20 cm. Petri plates were exposed to UV light for 15, 30, 60, 90, 120 and 150 min. After taking out from UV chamber, Petri plate lids were closed; wrapped with black paper and kept in dark for 12 h. Next day, the treated spore suspension was serially diluted, 100µl of each dilution was spread on CDA plates and incubated for 7 days. The surviving colonies were counted. After 7 days of incubation, six colonies were picked up based on colony morphology variation and designated as UV 1, UV 2, UV 3, UV 4, UV 5 and UV 6 and subcultured. These morphological variants were used for screening protease production.

Chemical mutagenesis using Ethyl methanesulphonate (EMS)

One ml of 10^5 spores/ml of spore suspension was treated with different concentration (125, 250 and 500 mg) of chemical mutagen EMS (SRL, India) for a period of 30 and 60 min respectively. The treated spore suspension was kept on a shaker incubator at 150 rpm. In another set of experiment 1 ml spore, suspension with a constant concentration of EMS (125 mg) was incubated for time periods of 15, 45 and 90 min. After treatment, the spore suspension was washed thrice with sterile distilled water, resuspended in saline and stored in dark for 12 h. Next day, the mutation induced spore suspension was serially diluted

and plated on CDA plates. After EMS treatment, the plates were incubated at 30°C for 5 or 7 days until the colonies appeared. The surviving colonies were counted. Then any colonies showing morphology different from that of the parent strain were picked out, followed by inoculation and cultivation as a single colony on a CDA plate. The resulting strains were subjected to protease assay. Untreated spores were also plated as the control. The per cent survival rate of colonies was calculated as follows:
Survival rate = (Number of test colonies/ No. of control colonies) X 100.

Submerged Fermentation

The *Jatropha curcas* seeds were procured from local market, and the *Jatropha* seed cake was prepared after the extraction of oil. The dried and ground JSC powder was taken at 2% level in a150 ml Erlenmeyer flask with 25 ml of distilled water and sterilized for 30 min at 121°C. Spores suspension was prepared using 0.85% saline with 0.1% of Tween 20 and JSC medium was inoculated with 1 ml of the spore suspension of 10^5/ml. The flasks were kept on rotary shaker at 25°C at 150 rpm. The time course of protease production was checked by growing the culture for a period of 7 days.
The selection criteria for mutants were based on morphological deviation of the colony structure from that of the wild type culture. A small portion of the colony variant was taken out in a loop and subcultured on to the slants. This subcultured mutant slants were used for checking the protease production.

Enzyme Assay

Protease enzyme was assayed in the reaction mixture containing 3.0 ml of 0.6% freshly prepared Casein Hammarsten (SRL chemicals, India) in 0.05 M sodium phosphate buffer pH 7.50. The reaction mixture was incubated for 20 min at 37°C. The reaction was terminated by adding 3 ml of 110 mM trichloroacetic acid. The precipitate was filtered using Whatman No.1 filter paper and 2 ml of filtrate was collected in fresh tubes. 5 ml of 500 mM sodium carbonate and 1 ml of Folin-Ciocalteau reagent (1:1 diluted) were added and incubated at room temperature for 30 min. The filtrate was collected using Whatman no.1 paper, and tyrosine liberated during casein hydrolysis was estimated by using **Lowry's method (1951)**. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μmol tyrosine/ml per min.

Optimization studies for protease production

Effect of inoculum concentration

The cultures used for optimization studies were UV 6 and EMS 5 mutant strains. To optimize the inoculum level, spore suspension containing 10^4, 10^5, 10^6, 10^7 and 10^8/ml were prepared in sterile saline individually and inoculated into conical flasks having 25 ml JSC (2%) medium.

Effect of incubation temperature

Flasks with protease production medium (2% JSC) were inoculated with 10^5 spores/ml and incubated at various temperatures such as 25°C, 30°C and 35°C up to 144 h and the enzyme activity was determined.

Effect of incubation period

The effect of incubation period was carried out by incubating the culture for different periods of time in JSC medium (2%). The culture media were kept at 30°C for 24, 48, 72, 96, 120 and 144 h at 150 rpm. The protease activity was checked at the above period.

Effect of pH

150 ml conical flasks with 25 ml of 2% JSC was adjusted to different pH using sodium citrate buffer for pH 5.0 and sodium phosphate buffer for pH 6.0, 7.0 and 8.0. The enzyme assay was carried out.

Effect of *Jatropha* seed cake concentration

The effect of JSC concentration was carried out using 1%, 2%, 2.5%, 3%, 3.5% and 4% in the medium and the protease was checked after 72 h.

Effect of agitation

Effect of agitation on protease production was carried at 100, 150 and 200 rpm on a rotary shaker and the protease was estimated at 72 h.

Effect of different carbon and nitrogen sources on protease production

The influence of other carbon sources in JSC medium was investigated using glucose, maltose and soluble starch (2%). Similarly, the effect of nitrogen sources on protease production was studied using peptone, sodium nitrate and ammonium sulphate at 2% level along with JSC medium.

RESULTS AND DISCUSSION

Isolation of UV mutants for enhanced protease production

The parent strain of *A. terreus* CJS-127 was subjected to UV irradiation for different periods of time. UV treatment affected the morphology of the mutant colonies (Figure 1). The wild type strain *A. terreus* is brownish and gets darker as it ages on culture media. On Czapekdox agar media wild type colonies grow rapidly and have smooth, rigid margin colonies. The UV-6 mutant colonies were tiny minute colonies and cream colored colonies when compared to the wild type strains. The EMS-5 mutant colonies were more yellowish compared to wild type culture on Czapekdox media. The EMS-5 mutant colonies were floccose with hair like soft tufts. The plates having less than 1.00 % of survival over the control (without UV treatment) showing colony morphological variation were selected for the screening of hyper-protease production. The per cent survival of UV treated cultures was in the range of 0.1-6%. The data on survival of culture on UV treatment in response to exposure time is presented in Table 1. The results obtained in the present study indicated the highest spore-killing rate of 0.005% when the spores were exposed to UV for 90 min. The minimum killing rate of 5.8% was observed when exposed for 15 min, and with an increase in the exposure time, the killing rate increased. There was no growth of colonies, when the spores were treated with UV for 120 and 150 min. There was a reduction in survival per cent of colonies with increased exposure to UV. Similar trend has been reported by other investigators **(Leng & Yan 2011; Yadav *et al.,* 2011).** The Six putative UV mutants were selected based on the morphological variation of colonies. The morphological variations observed were wrinkled, striated, filamentous, irregular, rhizoid, undulate, filiform, lobate and curled. The six UV mutants were grown on JSC medium, and they were screened for protease production.
The protease production by parent strain of *A.terreus* CJS-127 was maximum on the 6th day, i.e., 71.33±0.72U/ml. The protease production by different mutant strains and parent strain is given in Figure 2. The protease production was maximum in UV 6 strain on 5th day, i.e., 92.64±0.72

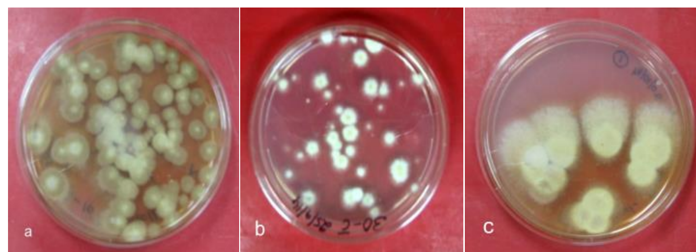

Figure 1 Morphology of a). *Aspergillus terreus* CJS-127 (wild type), b). UV-6 mutant colonies, c). EMS-5 mutant colonies

Table 1 Per cent survival of *A. terreus* after UV induced mutagenesis

Mutant strains	Treatments	No. of colonies	Survival (%)
CJS-127	Control	$1.7x10^4$	100
UV 1	UV-15 min	$1x10^3$	5.80
UV 2, UV 3	UV-30 min	46	0.27
UV 4, UV 5	UV-60 min	24	0.24
UV 6	UV-90 min	1	0.005
UV 7	UV-120 min	0	0
UV 8	UV-150 min	0	0

U/ml when the culture was grown at 25°C. The protease production was 29.8% higher than the wild strain which produced maximum protease on the 6th day under the same conditions (Figure 2). The least protease activity was observed in UV 5 with 26.4 ± 0.43 U/ml. A mutant strain of *A. niger* with lipase productivity of 2-fold higher was obtained after UV mutation **(Ellaiah *et al.,* 2002a)**. Earlier reports have shown that 2 fold increase in acid protease production by UV mutant strain of *A. niger* in solid state fermentation **(Radha *et al.,* 2012).**

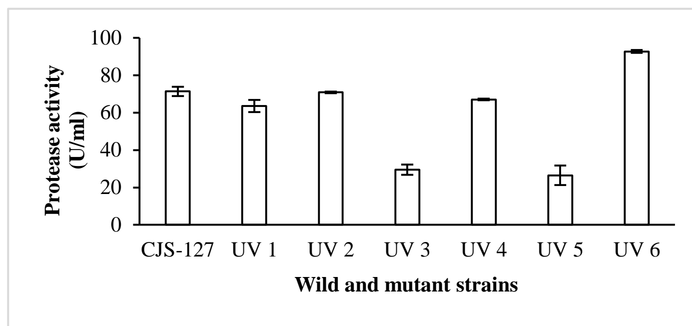

Figure 2 Protease production by UV mutant strains.

Isolation of EMS mutants for enhanced protease production

The strain improvement was carried out using different concentrations of chemical mutagen EMS. Morphological changes of colonies were observed with spore suspension exposed to EMS (Figure 1). The plates having less than 1.00 % of survival rate over the control (without chemical treatment) were selected for the screening of hyper-protease producing mutants. The per cent survival of the culture is given in Table 2. The results showed a highest spore killing rate of 99% when 500 mg of EMS was used and the lowest killing rate of 12% when treated with 125 mg of EMS. The killing percentage increased with the increase in the concentration of EMS and exposure time of spores (Table 2). Thus, from the results, it was observed that the killing percentage of the spores was proportional to the concentration of both EMS and exposure period. The per cent survival of EMS treated cultures was in the range of 0.5-18%. The survival of colonies was least in the case of spores treated with 500 mg of EMS for 60 min.

Table 2 Per cent survival of *A. terreus* after chemical mutagenesis (EMS)

Mutant No.	Treatments	No. of colonies	Survival (%)
CJS-127	----	$1.7x10^4$	100
EMS 7	100 µl-15 min	$3x10^3$	17.64
EMS 1, EMS 8	100 µl-30 min	$1.9x10^3$	11.17
EMS 9	100 µl-45 min	$1.2x10^3$	7.05
EMS 2, EMS 10	100 µl-60 min	$1.09x10^3$	6.41
EMS 11	100 µl-90 min	$7.6x10^2$	4.47
EMS 3	200 µl-30 min	$1x10^3$	5.80
EMS 4	200 µl-60 min	$7.4x10^2$	4.35
EMS 5	400 µl-30 min	$3x10^2$	1.76
EMS 6	400 µl-60 min	$1x10^2$	0.58

A total of eleven morphological variants were selected and screened for protease production using JSC in submerged fermentation. The colony morphological variations observed were circular, wrinkled, striated, filamentous, irregular, undulate, filiform, lobate, curled and floccose with hair like soft tufts. The colonies were distinctly different from that of the parent culture, and the colonies were streaked on to the slants for making spore suspension and then used for checking protease production. Initially, the mutant strains were screened for maximum protease production and the strain which produced maximum protease was selected for further optimization for enhanced protease production.
The protease production by the EMS mutant strains is given in Figure 3. Among several strains screened for protease, EMS 5 produced maximum protease on the 5th day with 86.77±3.64 U/ml. Figure 1C represents the mutant strain of EMS-5. EMS 7 strain produced the least protease with 52.3 ±0.72 U/ml. The EMS-5 mutant strain produced protease 21.6% higher than the wild strain. But the activity was less when compared to the mutant strain obtained after UV mutagenesis. **Radha *et al.*, (2012)** have reported similar results after mutagenesis. Mutation frequency was mentioned to be high when the survival rates were between 10 and 0.1% **(Hopwood *et al.*, 1985)**.

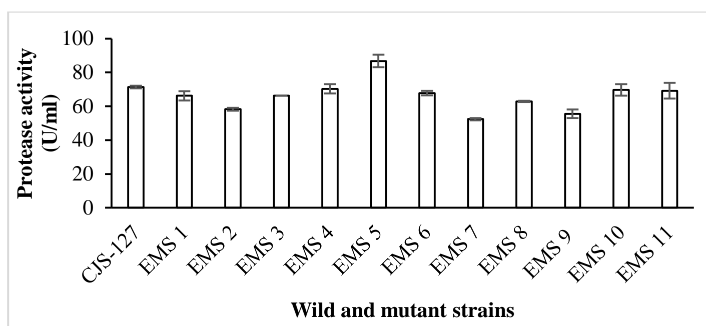

Figure 3 Protease production by EMS mutant strains

Optimization Studies

The yield of protease production was influenced by various physical and chemical parameters. To enhance the production of protease, mutant strains UV 6 and EMS 5 were selected for optimizing the cultural conditions.

Effect of temperature

The incubation temperature has a significant influence on the production of the enzyme in any microorganism. The submerged fermentation was carried out at 25, 30, and 35°C. The optimum temperature for protease enzyme production was found to be 30°C and the enzyme activity for UV 6 and EMS 5 was 97.89±3.34 U/ml and 95.42±1.31 U/ml respectively. When the cultures were incubated at 25°C the maximum protease activity of both UV 6 and EMS 5 was 92.64 ± 0.72 U/ml and 86.77±3.64 U/ml respectively. At 35°C, there was a decrease in protease production among the mutant cultures were observed (Figure 4). As the temperature increased beyond 30°C, the enzyme production was decreased. Higher temperature may adversely affect the metabolic activity and enzyme production. The enzyme gets denatured at high temperature and loses its catalytic properties due to stretching and breaking of weak hydrogen bonds within enzyme structure **(Conn *et al.*, 1987)**. **Raj *et al.*, (2012)** have found that 37°C was the optimum temperature for the production of protease by *Pseudomonas aeruginosa* by submerged fermentation. The incubation at temperatures other than 37°C was found to decrease the production of protease. Most of the fungi showed an optimum temperature for protease production between 28°C and 30°C **(Chutmanop *et al.*, 2008)**.

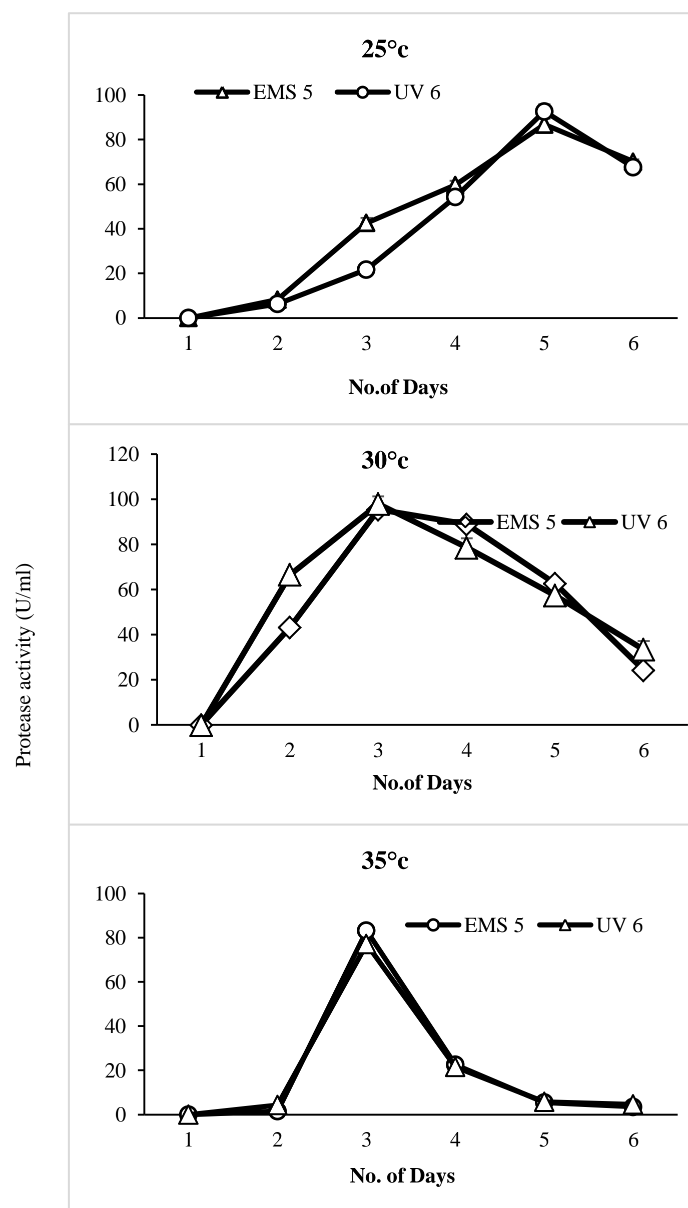

Figure 4 Effect of temperature on protease production

Effect of incubation period

The enzyme production varies with the incubation time, as it is dependent on the growth of the organism. The mutant cultures were incubated for 24-120 h at 30°C and screened for protease production. Figure 5 illustrates the effect of incubation period on protease production. The maximum enzyme production was observed at 72 h for UV 6 and EMS 5 strains. The maximum protease activity for UV 6 and EMS 5 was 97.89±3.34 and 95.42±1.31 U/ml respectively. The maximum amount of enzyme production was reported to be at 48 h for *P. aeruginosa* (**Raj *et al.,* 2012**). The maximum protease of 95 U/ml was reported by **Kranthi *et al.,* (2012)** using ground nut oil seed cake at 72 h by *Rhizopus stolonifer* under solid state fermentation. It is essential to detect the optimum incubation time at which an organism exhibits highest enzyme activity since organisms show considerable variation at different incubation periods **(Kumar *et al.,* 2012).**

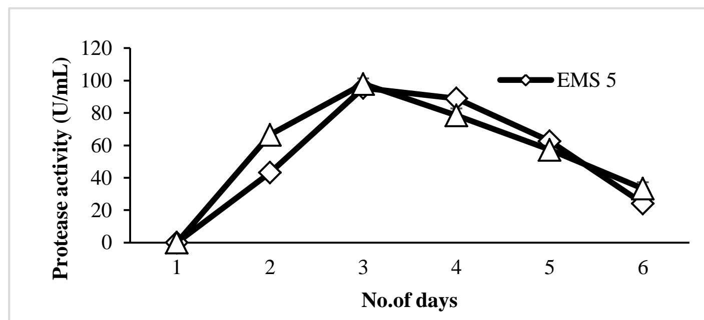

Figure 5 Effect of incubation period on protease production by mutant strains

Effect of pH

The effect of pH on protease production using JSC as a substrate is given in Figure 6. The metabolic activities of microorganisms are sensitive to pH changes, and the pH of culture media has marked effect on the type and amount of enzyme produced. Protease production by microbial strains depends on the pH because culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes, which in turn support the cell growth and product production **(Ellaiah *et al.,* 2002b)**. The optimum pH for protease production was recorded at 7.0 with an activity of 98.51±2.4 for UV 6 and 99.44±2.9 U/ml for EMS 5 respectively (Figure 6). A notable decline in protease productivity was observed at both higher and lower pH. The optimum pH for the production of the protease was recorded at 7.0 when solid state fermentation of rice was carried out using *A. niger* **(Paranthaman *et al.,* 2009)**. Optimum medium pH of 7–8 has been reported for maximum protease production by various species of *Bacillus* **(Wang *et al.,* 2008; Mahajan *et al.,* 2012; Mahmoud *et al.,* 2011)**. **Radha *et al.,* (2012)** studied the production and optimization of acid protease by *Aspergillus* sp. from the soil. They reported a gradual increase in protease at pH from 3.0 to 5.0, whereas, it declined at neutral and alkaline pH.

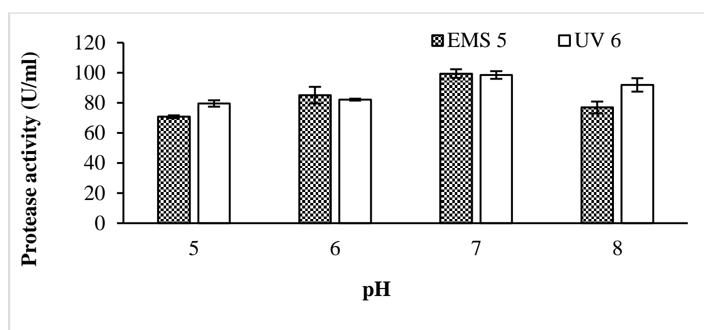

Figure 6 Effect of pH on protease production

Effect of inoculum level

The effect of the size of spore inoculum on the production of protease by mutant strains is illustrated in Figure 7. The size of inoculum ranged from 10^4 to 10^8. The result showed that maximum protease (95.42±1.31 and 84.61±0.58 U/ml) was produced by EMS 5 and UV 6 respectively when 10^5 spores/ml was added to the JSC medium. An appropriate inoculum concentration is important for optimum growth and enzyme production by the microorganism. When the inoculum size was increased, it rapidly consumed the available substrate for growth and hence enzyme synthesis was decreased **(Carlile *et al.,* 2001)**. The optimum level of inoculum of 10% was found to be favoring the production of alkaline protease from a mutant *A.flavus AS2* **(Rojarani *et al.,* 2012)**.

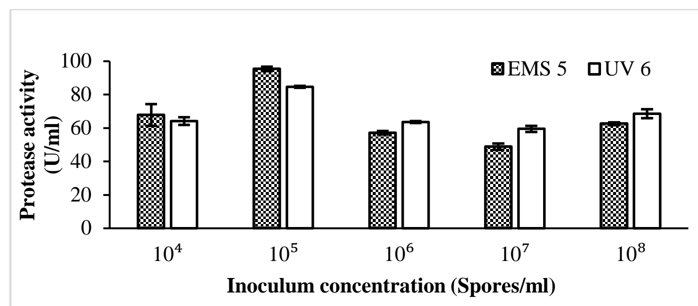

Figure 7 Effect of inoculum concentration on protease production by EMS 5 and UV 6 strains

Effect of substrate concentration

The *Jatropha* seed cake used in the medium for the production of protease had the proximate composition of protein- 21, lipid- 13, crude fibre- 8, ash- 4.5 and carbohydrate 53%. Optimization of JSC level was carried out by varying the amount of substrate (1-4%) in the fermentation process. The results showed that 2% of substrate yielded maximum protease production of 99.44±2.91 and 98.5±2.47 U/ml by EMS 5 and UV 6 strains respectively (Figure 8). It indicated that by increasing the concentration of substrate there was a decrease in protease production. Protease from a bacterial isolate *B. cereus* NS-2 was optimized by employing agricultural residues like wheat bran and cotton cake at 1%, w/v each in the medium. Wheat bran supported fibrinolytic protease production (148U/ml) and cotton cake enhanced fibrinolytic protease production to 315U/ml. **(Bajaj *et al.,* 2013)**.

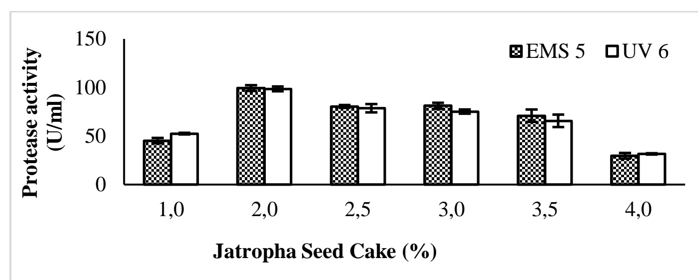

Figure 8 Effect of substrate percentage on protease production

Effect of agitation

The growth and production of the enzyme are dependent on the aeration/agitation given to the microorganism. The effect of various agitation rates of culture on protease production is shown in Figure 9. The results revealed that maximum protease was produced by EMS 5 (99.44± 2.91 U/ml) and UV 6 (98.51±2.47 U/ml) at 150 rpm. Further increase in agitation rate decreased the protease production. **Panta *et al.,* (2015)** have reported that 180 rpm was optimal for higher protease activity by *B. subtilis.* The similar observation has been made for the production of alkaline protease from mutant *A. flavus* **(Roja rani *et al.,* 2012)**. They have reported that 110 rpm as the optimum agitation rate for the production of alkaline protease by *A. flavus.*

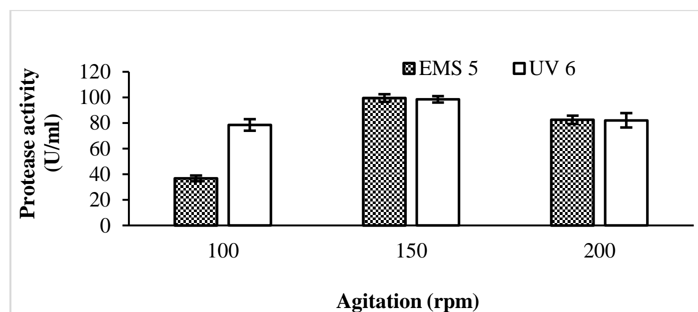

Figure 9 Effect of agitation on protease production

Effect of carbon sources

The raw material for the production of any enzyme is a significant cost determining factor. Hence, agricultural wastes are favored over synthetic media components which add to the cost of production. Production of protease is dependent on the carbon and nitrogen source available in the medium. A few carbon sources were added separately to the JSC medium to study their effect on

enzyme production. The additional carbon sources such as glucose, maltose and soluble starch with 2% concentration were studied for the production of protease by *A.terreus* mutant strains EMS 5 and UV 6. The addition of maltose to JSC medium enhanced protease production, i.e., 172.32 ±0.29 U/m and 163.57±0.72 U/ml by EMS 5 and UV 6 strains respectively (Figure 10). Fast metabolizable carbon sources lead to reduced enzyme production due to catabolite repression. The crude substrates are conditioned carbon sources and realize slow release of carbon and obviate catabolite repression **(Bajaj & Singh, 2010)**. In contrast, however, maltose enhanced protease production, in EMS-5 and UV-6 mutant strains. **Raj *et al.,*** (2012) have reported that maltose was the best carbon source among the other sugars tested with protease activity of 293 U/ml. Similar results were reported where maltose enhanced protease production by three-fold from *B. subtilis* ICTF-1 **(Mahajan *et al.,* 2012).** The shrimp shell powder was found to be an excellent substrate for protease production by *B. cereus* TKU006 **(Wang *et al.,* 2009)**.

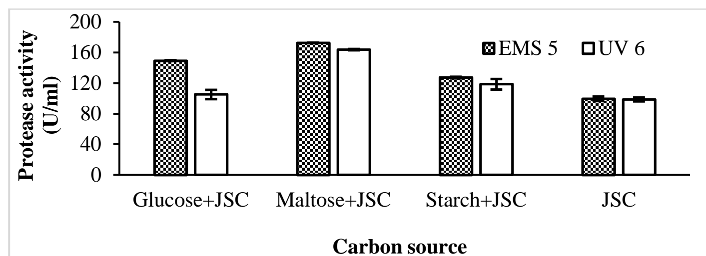

Figure 10 Effect of carbon sources on protease production (JSC=*Jatropha* seed cake)

Effect of nitrogen source

JSC is an excellent source of protein (21%) and supports the protease production by the mutant strains. To study the effect of other nitrogen sources like peptone, sodium nitrate and ammonium sulphate were added to the JSC medium and the impact on the production of protease by the mutants was observed. The maximum protease production by EMS 5 and UV 6 was 99.44± 2.91 U/ml and 98.51±2.47 U/ml when JSC was used in the medium. The additional nitrogen sources like peptone, sodium nitrate and ammonium sulphate did not enhance the protease production when compared to the JSC medium (without any additional source of nitrogen). Some reports indicate cotton seed cake served as a reasonably good nitrogen source for protease production from various microorganisms **(Kranthi *et al.,* 2013).** Yeast extract and soy/casein peptone enhanced the fibrinolytic protease production from *B. subtilis* ICTF-1 **(Mahajan et al., 2012). Raj *et al.,* (2012)** have tested the effect of various nitrogen sources on protease production, and it was found that yeast extract gave the highest enzyme activity of 317 U/ml.

CONCLUSION

Most of the reports are on the production of protease by solid state fermentation or with soluble medium **(Chutmanop *et.al.,* 2008; Kranti *et al.,* 2012; Ellaiah *et al.,* 2002b)**. In this study, an attempt has been made to use JSC in submerged fermentation for the production of protease. The advantages of submerged fermentation over solid state fermentation are it is easy to scale-up the process parameters. The process parameters like pH, temperature, agitation can be controlled in submerged fermentation. The recovery and purification of the product are better with submerged fermentation. Mutagenesis followed by the subsequent selection of a strain producing superior yields is an important technique in increasing the fermentative yield of enzymes. The EMS and UV irradiation are known to affect DNA leading to thymine dimers that cause mispairing during DNA replication. This would lead to permanent mutation in the organism if there is no DNA repair. The mutation is a frequently employed technique for improving the industrial microorganisms to increase the yield of various products. There is a vast quantity of JSC generation after biodiesel production in the future and disposal of JSC is a major environmental problem. Nonedible oil cakes such as *Jatropha* are potentially very useful for production of industrially important enzymes. The mutant strains EMS-5 and UV-6 were found to produce higher protease enzyme when compared to the parent strain *A. terreus* CJS-127. However, it was observed that protease production was slightly higher with EMS-5 compared to the UV-6 strain. The repeated subculturing did not affect the yield by EMS-5 and UV-6 strains and these strains were found to be genetically stable. The fermentation period was also reduced to 3 days in case of mutant strains instead of 6 days after optimization of growth conditions. The result of the present study elucidated that JSC can be an economical source for the production of protease by submerged fermentation. Because enzyme production by microorganisms is under the influence of various growth conditions, the present study determined the optimum physical and chemical parameters for maximum production of protease from the mutant strains. The use of cheap agricultural and food-processing by-products such as oil cakes, as feedstock, is highly favored to improve the commercial feasibility of bioprocess technology. The promising strains in this study could be used in various economic, industrial applications.

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