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Enhancement of cellulase biosynthesis in *Aspergillus nomius* through application of mutagenesis

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Abstract

The fascinating structural alteration of fungi leads to its candidacy as major component for biosynthesis of cellulase enzyme that has major potency is included in several industries including Agriculture, bio-fuel, food, fodder and pharmaceutical sector. In view of its biological significance, newer approaches to maximize its production rate can be through the microbial strain improvement through application of mutagenesis (direct/random). Due to its wide availability, easy genetically alterability and higher ability to produce cellulase; The present study depicts an attempt for strain improvement in one of the fungal species *Aspergillus nomius* through applications of UV irradiation and induced mutation by chemical mutagen (ethyl methane sulfonate) application for higher yield of cellulase enzyme.From the result average evaluation index for UV treated mutant strains and EMS treated mutants clearly suggested superiority of SKUV7 and EMSSKU5 respectively and the mutant strains like EMSSKU5 and EMSSKU6 showed survival of 5% at 15 minutes of mutagen treatment. From the above study it could be opined that the physical mutagen (UV) treated mutant strain of *Aspergillus nomius*, SKUV7 and EMS treated mutant strain of *Aspergillus nomius*, EMSSKU5 are functionally more potent than the control strains of *Aspergillus nomius*in terms of production of cellulase enzyme.

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Introduction

Lignocellulose, structurally, is composed of cellulose, hemicellulose and lignin and is one of the prime components of plant cell wall (Hong Zhang, 2015). The basic structure of lignocellulose consists of intermediary hemicelluloses linkage to that of cellulose and lignin with higher polymerization degree and crystalline structure (Schirmaier et al., 2014). As major component of lignocellulose, cellulose structurally comprised of

linear homopolymericd-glucospyranose moieties connected by *β*-1, 4-glycosidic bonds with repeating cellobiose units (Cocinero et al., 2009). For delinking each of the cellulosic units the enzyme complex, cellulase is actively involved (Ghazanfar et al., 2019; Nazir et al., 2019).Most of the natural Cellulases are grouped by endoglucanases, β-glucosidases and cellbio hydrolases (Jayasekara and Ratnayake, 2019) and are widely produced and biosynthesized by several microorganisms like bacteria and fungi (Sharma et al., 2017; Singh et al., 2019). The wide applications of cellulytic enzymes in industrial sectors like food, beverages, textiles and pharmaceutics (Jerusik, 2010; Kuhadet al., 2011; Uzuner, 2019; Jayasekara and Ratnayake, 2019) is highly recommended. Basic industrial application of lignocellulose is the bioproduction of biofuel (Andlar et al., 2018; Li et al., 2020). Due to synergistic applications of cellulase and higher cost expenses during its synthetic productions, the biological synthesis mode is now-a-days gaining more priorities with a special attention towards microbial use like fungus for this. Due to the structural diversity, hyphal heterogeneity, habitat diversity and nutritional specificity of fungus group; these are widely recommended and involved in extracellular synthesis of cellulase enzyme.

In view of the biotechnological significance of cellulases, newer approaches to maximize its production rate can be through the microbial strain improvement through application of mutagenesis (direct/random) (Schulein, 2000; Wither, 2001 and Wilson, 2004). The present study depicts an attempt for strain improvement in one of the fungal species *Aspergillus nomius*through applications of UV irradiation and induced mutation by chemical mutagen (ethyl methanesulfonate) application for higher yield of cellulase enzyme. The microbes that are able to be genetically mutated can enhance the enzyme productivity and also elevates the activity of it and even stability also (Chen et al., 2018).

Materials and methods

Microorganism

In the above study *Aspergillus nomius*was selected for improvement of strain quality. Selection of species was done from successful screening of a total 120 no of fungal isolates in a chronological sequential manner through primary and secondary screening (Naik et al., 2023), with NCBI-Gene Bank accession no MW854331.1 and code SK1.

Growth of fungal strain

The selected fungal strain, *Aspergillus nomius*was grown on Mineral salts agar medium (MSM) for further culturing purpose (Nisar et al., 2020).

Substrate

Substrate optimization was done by taking saw dust, sugarcane bagasse, ground nut shells, corn cobs, wheat bran and rice bran as primary carbon sources. Amongst all wheat bran was optimized as carbon source for cellulase biosynthesis (Nisar et al., 2020). Dried wheat bran (sun drying followed by oven drying at 30^{o}C) was pulverized followed by vigorous washing with hot water for residual removal.

Random mutagenesis

The random mutation was achieved by two step method of application of Physical mutagenesis through UV irradiation followed by application of chemical mutagenesis through use of ethyl methane sulfonate.

Physical mutagenesis

To a 4 ml of *Aspergillus nomius*spore suspension, UV irradiation was done using Germicidal lamp with 90% radiation exposure at 2540-2550 A$^{\circ}$ for different exposure time intervals starting from 10 mins, 15 mins, 30 mins, 45 mins, 60 mins, 75 mins and 90 mins at a distance of 15 cm and 20 cm. All the UV irradiated spores were cultured on Mineral salts agar medium for 7days at 30°C after overnight incubation for delimitation of photo reactivation (Modified Pradeep et al., 2012). Out of all 14 colonies were tasted for study of cellulase production (Reddyet al., 2016; Nisar et al., 2020).

Chemical mutagenesis

The successful or identified fungal mutant from UV mutagenesis, SKUV7 (*Aspergillus nomius*) was used as basic fungal material for the mutation studies under chemical mutation. It was done through addition of 0.1% ethyl methane sulfonate to the isolate suspension in gradual time interval of 5-25 mins. After centrifugation, pellets were treated with 0.05M phosphate buffer at a pH of 7 (Burlacu et al., 2017; Nisar et al., 2020).

Submerged fermentation

To the fermentation medium in sterile condition 1ml of 2×10^8 conidia/ml inoculum was added and incubated at 30°C for 72 hrs. followed by centrifugation at 8000 rpm for 15min and used for cellulase production.

Enzyme assays

Carboxymethylcellulase (CMCase): Carboxymethyl cellulase activity was validated using 1% (w/v) CMC at 50 mM Na-acetate buffer with 5.3 pH incubated at 50 °C for 15 min (Casimir et al., 1996) followed by mixing 3, 5-dinitrosalicylic acid (Miller, 1959) and heating for 15 min followed by measuring the OD at 540 nm (Mandels et al., 1981).

β-glucosidase assay (BGL): β-glucosidase (BGL) enzymatic assay was done utilizing the substrate 0.1% *p*-nitrophenyl β-D glucopyranoside (Ghose and Bisaria, 1987) in 50 mM acetate buffer at pH 4.8 and incubated at 50 °C. The β-glucosidase activity was calculated at 410 nm.

Filter paper assay (FPase): The cellulase enzyme activity was validated through filter paper assay (IUPAC, Ghose, 1987). FPase activity was detected calculation of Glucose concentrated with cellulase using filter paper after 50 °C incubation through DNS method (Mandels et al., 1981).

Results and discussion

At present a variety of techniques (mutation, recombination and recombinant DNA technology) are available for improvement of strain with simultaneous decrease in the expenses of cellulase production. Strain improvement has become a main tool for enhancement of targeted item production through mutations and/or genetic engineering (Adrio and Demain, 2006). Researchers utilized the methodologies of mutation for strain improvement successfully to improve the production of cellulase (Wong and Maringer, 1999). However, Adebami and Adebayo-Tayo (2020) viewed that the methodology of mutation is deadening and seldom give equivocal results. Recently, biological engineering is suggested as easy method for strain improvement due to simple genetic makeup alteration of the targeted species (Toyosawa et al., 2017). However, the mutagenesis method has been opted due to higher efficacy and precision in the study. Mutation is definitive source of genetic variation.

The most popular mutagen agents utilised for fungal strain enhancement are the UV irradiation and the application of chemical mutagens. These two methods are ascribed as the most user safety (Nevalainen, 2001). In the present study, physical mutagen such as UV has been selected and the resultant mutant strain was further processed through a simple and effective chemical mutagen, the ethyl methanesulfonate (EMS) for examining further increase in cellulase enzyme production.

The prime mechanism of selection of individual fungal isolates that required two stage validations including primary selection through calculation of zone of clearance (Ribeiro et al., 2014) and secondary selection through biochemical evaluation of cellulolytic enzymes (carboxy methyl cellulase, filter paperase and β–glucosidase) and calculation of Evaluation Indexing (EI) (Mano et al., 1993). Through such verification processes *Aspergillus nomius*was selected for strain improvement steps (Naik et al., 2023).

For the development of successful procedure, to validate the biomass fermentation, identification of optimized fungal isolates was necessary (Naik et al., 2018). Amongst the studied 120 fungal strains through primary screening, considerable 14 fungal strains were identified. These were further screened for cellulase production. From the fungal isolates which are successful in secondary screening, 6 fungal isolates were subjected for optimum production of cellulase enzyme followed by strain improvement study for higher cellulase production.

Strain improvement through UV irradiation

The study validated the strain enhancement and efficacy increment of fungal isolate *Aspergillus nomius* carried out using random mutagenesis procedure for elevating the rate of cellulase production. *Aspergillus nomius* spores exposed to UV radiation for limited time duration with 5% survival rate were selected for further analysis. The hypothesis was that the survived spores at 5% survival, after exposure can give rise to colonies on MSM agar medium.

In general, the survival rate (%) was inversely related to the time of exposure to UV irradiation. However, three types of survival trends were observed in the

experimental spores. Some of the spores showed immediate mortality of 95% or above with UV exposure of mere 5 minutes (Fig. 1). The second group of spores showed a survival of 5% at 50 minutes UV exposure (Fig. 2). And the third group of spores was able to withstand up to an exposure of 75 minutes UV irradiation (Fig. 3) resulting in 5% survival. In all the three cases, the reaction of survival to duration of UV radiation was inversely related. However, the slop in first instance was sharp, in the second one it was moderate and in the third one it was slow due to fungal spore resistance to DNA repair (Cortesão et al., 2020).

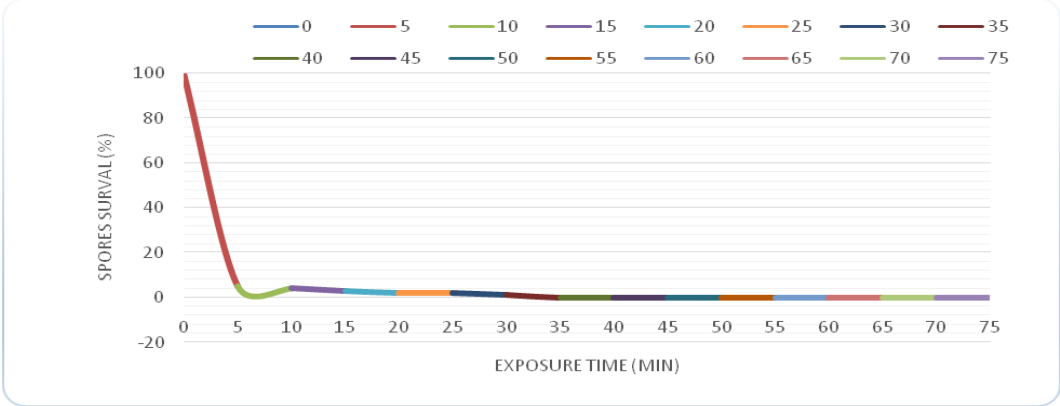

Fig. 1: Early attainment of low survival (%) of conidial spores of *Aspergillus nomius*, exposed to UV radiation.

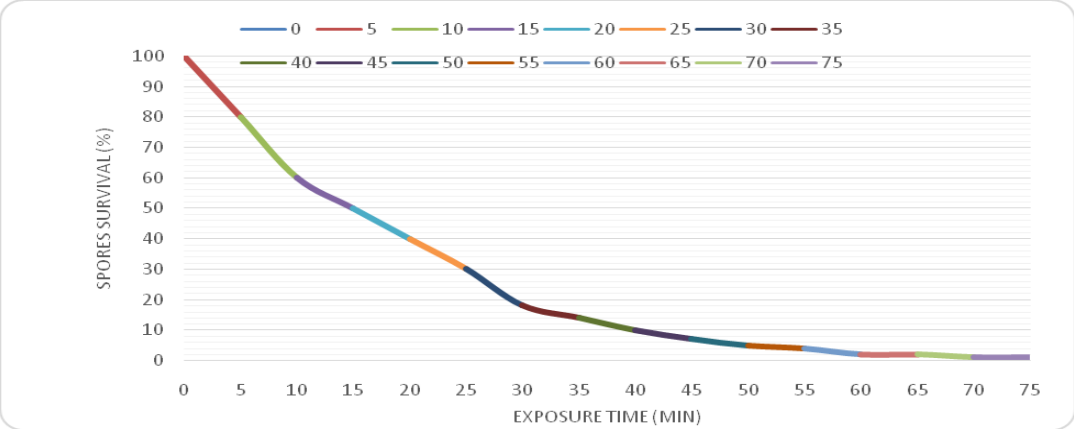

Fig. 2: Moderate attainment of low survival (%) of conidial spores of *Aspergillus nomius*, exposed to UV radiation.

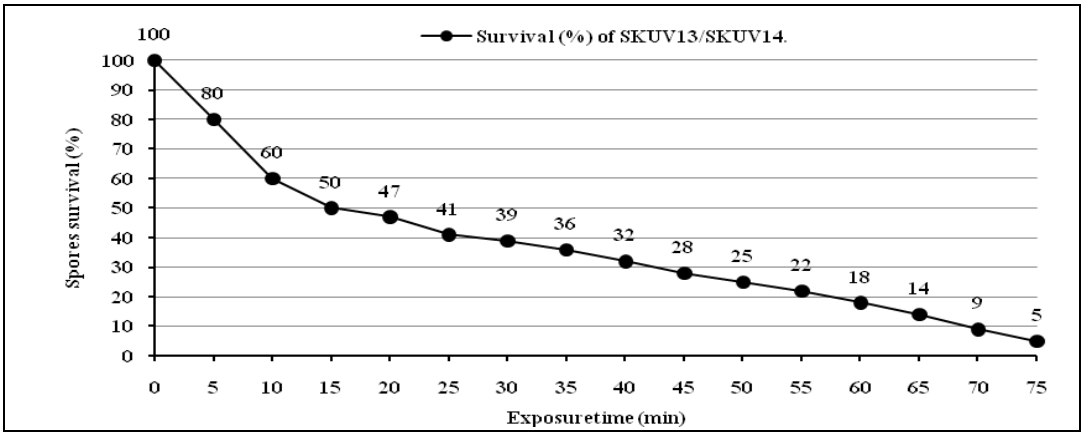

Fig. 3: Late attainment of low survival (%) of conidial spores of *Aspergillus nomius*, exposed to UV radiation.

Survival data for without UV exposure and UV-exposed spores were depicted in Figs. 1-3. Elevated UV exposure time leads to decrease in the spore survival rate. Almost 90% of fungal spores with 5 mins UV exposure had got only 5% survival rate. In this study, wild spores of *Aspergillus nomius* were exposed to UV irradiation and got 14 mutant strains, SKUV1 to SKUV14. Upon examination of survival percentage against the span of UV treatment, increase in the percentage killing of spores of *Penicillium chrysogenum* with increase in exposure time of UV radiation was reported (Veerapagu et al., 2008). Number of survivors of conidial spores of *Trichoderma viride* decreased with increase in exposure time of UV radiation (Shafique et al., 2011).

Zone of clearance in mutated strains of *Aspergillus nomius*

The strains resulted in UV radiation treatment were studied for zone of clearance. The results on mutant strains of *Aspergillus nomius* with which UV treatment exposure gave 5% survival (Table 1). Studies on zone of clearance in all 14 *Aspergillus nomius* mutant strains (SKUV1 to SKUV14) revealed high zone of clearance with SKUV7 and further confirmed by calculated EI values. In several studies, the clear zone of hydrolysis on Mandels medium with CMC was used for screening mutant strains for cellulase production (Shafique et al, 2011; Shahbazi et al., 2014, Elakkiya and Muralikrishnan 2014).

Table 1. Data on zone of clearance by mutant strains of *Aspergillus nomius* with which UV treatment exposure gave 5% survival.

Sl. No.	Name of *Aspergillus nomius* strain	Zone of clearance(mm)
1	Wild	0
2	$SKUV_1$	14
3	$SKUV_2$	28
4	$SKUV_3$	37
5	$SKUV_4$	32
6	$SKUV_5$	36
7	$SKUV_6$	43
8	$SKUV_7$	48
9	$SKUV_8$	45
10	$SKUV_9$	38
11	$SKUV_{10}$	27
12	$SKUV_{11}$	24
13	$SKUV_{12}$	12
14	$SKUV_{13}$	11
15	$SKUV_{14}$	05

From data, it was observed that thehighest evaluation index for strain SKUV7 with UV treated *Aspergillus nomius* mutant recorded more values in zone of clearance (48 mm).The data pertaining to activity of *Aspergillus nomius* mutation strains in terms of zone of clearance (mm) were statistically analyzed for evaluation index. Such evaluation index values are represented in Fig. 4 with the highest evaluation index of 69 mm for strain SKUV7. Of all mutants tested in this study, $EMSSKU_5$ displayed clear zone of hydrolysis with the largest diameter. Cellulase production of two isolates that gave highest zone of hydrolysis *P. chrysogenum* - zone 18mm and *T. reesei*– zone 12 mm (Kaur and Joshi, 2015). In our work EMSSKU4 and EMSSKU5 shows 43 mm and 56 mm clear zones,

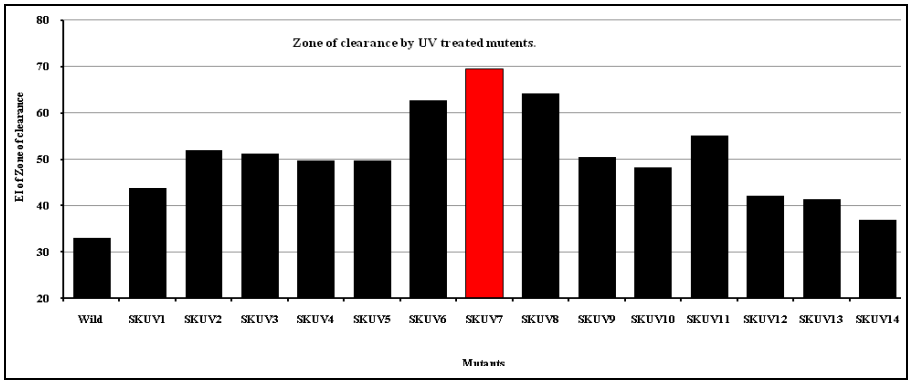

Fig. 4: Calculated Evaluation Index (EI) values for zone of clearance recorded for UV mutated strains of *Aspergillus nomius*.

Studies on production of cellulase enzyme in mutated strains of *Aspergillus nomius*

The mutated strains of *Aspergillus nomius* were studied for production of cellulase. All the three component enzymes of cellulase, carboxymethylcellulase, Filter paperase and β-Glucosidase were estimated and presented in Table 2.

Production data of component enzymes of cellulase, carboxymethylcellulase, Filter paper and β-glucosidase of mutant strains of *Aspergillus nomius* exposed to UV irradiation had depicted the high production of all the three component enzymes in mutant strain, SKUV7.

Table 2. Production of three component enzymes (CMCase, FPase and BGL) of cellulase by UV treated Isolates strains (*Aspergillus nomius*).

UV treated mutants	CMCase (IU/ml/min)	FPase (IU/ml/min)	BGL (IU/ml/min)
$SKUV_1$	7.908	2.161	1.921
$SKUV_2$	9.820	3.108	1.772
$SKUV_3$	11.244	3.353	1.315
$SKUV_4$	13.074	5.170	2.117
$SKUV_5$	13.567	6.732	2.121
$SKUV_6$	15.782	8.786	2.212
$SKUV_7$	16.923	9.982	2.256
$SKUV_8$	15.789	6.456	2.113
$SKUV_9$	11.890	3.782	1.670
$SKUV_{10}$	8.978	2.789	1.133
$SKUV_{11}$	6.909	4.245	1.345
$SKUV_{12}$	5.901	2.567	1.129
$SKUV_{13}$	4.567	3.678	0.956
$SKUV_{14}$	3.890	2.367	0.567

Data on the production of component enzymes of cellulase, CMCase, FPase and BGL of mutant strains of *Aspergillus nomius* exposed to UV irradiation were treated for evaluation index (EI) values. The evaluation index data for CMCase (Fig. 1), for FPase (Fig. 2) and for BGL (Fig. 3) were depicted individually with highest evaluation index for strain SKUV7. The data pertaining to average evaluation index are presented in Fig. 5 with the highest average evaluation index for strain SKUV7 in the case of average evaluation index values.

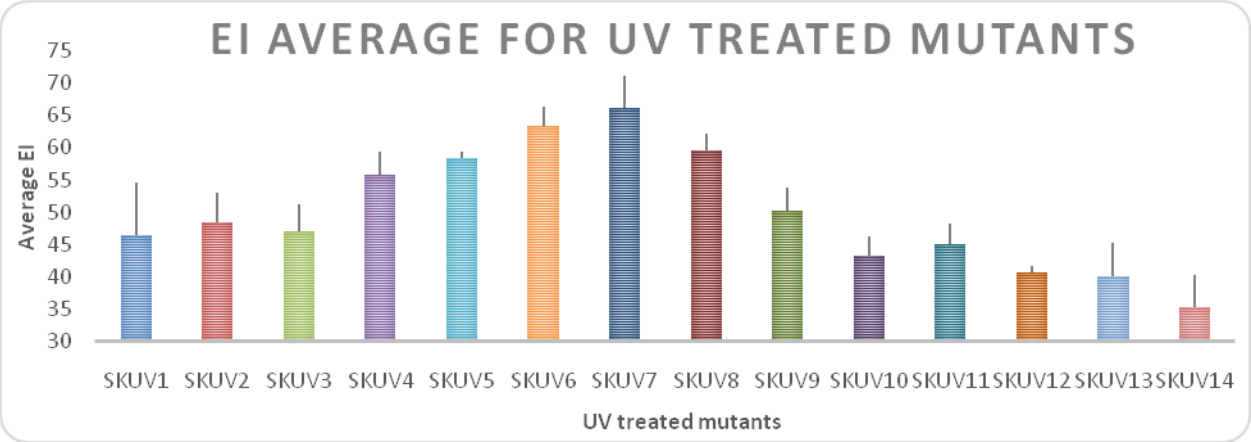

Fig. 5: Average Evaluation Index (AEI ± SD) values for three component enzymes (CMCase, FPase and BGL) recorded for mutated strains of *Aspergillus nomius.*

Based on the data recorded for zone of clearance, production of component enzymes of cellulase and further calculate evaluation index values (both individual and average), the mutant strain of *Aspergillus nomius* resulted from the UV treatment, SKUV7 has been selected for further studies, that is for improvement of strain through chemical mutagenesis. Experiments in the present study deal with inducing

mutation, improvement of dependent characters like production potentials of three different component enzymes of cellulase, CMCase, FPase and BGL in mutant strains of study, *Aspergillus nomius*. This situation warranted decisions making jointly on them. In the present, such method, called evaluation index as advocated by Mano et al. (1993) as described by Singh and Rao (1993) was inducted as the method was more appropriate.

Studies on UV mutant strain improvement through chemical mutagenesis

The main objective of such method was to eliminate the experimental errors. However, when the evaluation indexes of all the individual mutants are collated, the resultant average evaluation index for UV treated mutant strains and that for EMS treated mutants clearly demonstrated the perfect superiority of SKUV7 and EMSSKU5 respectively. The experimental error has been eliminated through all the statistical components like averages, standard deviation, standard value and value of preference cut-off. The calculation is the ration of difference between experimental value and average value over standard deviation, multiplied by a standard value and added by a preferred value of cut-off. Isolated mutated strain of *Aspergillus nomius*, SKUV7 from previous study (physical mutant with UV) was utilized for chemical mutagenesis studies also. Isolated colonies from plates in the chemical mutagenesis process studies were collected and were considered as mutants. Mutants derived after treatment with ethyl methane sulphonate (10 min duration) were labeled as $EMSSKU_1$-$EMSSKU_{10}$ so on All these mutants were screened on CMC plates for their cellulolytic activity based on formation of clear zones due to hydrolysis of CMC that was made visible with Congo red staining (Fig. 6).

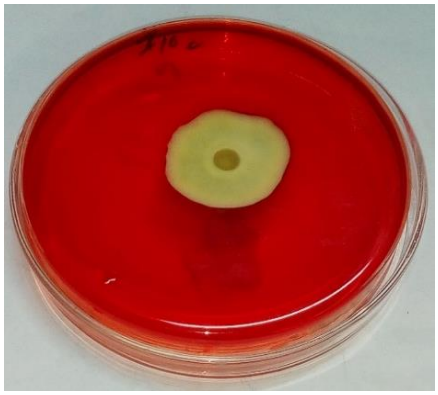

Fig. 6: *Aspergillus nomius* sp. showing clear yellow zone of clearance.

In the case of chemical mutagenesis treatment also, strains reacted differently in terms of survival and duration of chemical mutagen treatment, as observed for UV treatment. However, the treatment duration over 25 minutes resulted in 100% mortality. Therefore, survival studies were restricted to only 25 minutes with an interval of 5 minutes. With chemical mutagenesis also, 5% survival is considered as selection criteria. For survival percentage, strains interacted in three types. Mutant strains like EMSSKU1 and EMSSKU2 showed 5% survival at mere 5 minutes of treatment time. Further, mutant strains like EMSSKU5 and EMSSKU6 showed survival of 5% at 15 minutes of mutagen treatment. On the extreme side, mutant strains of EMSSKU9 and EMSSKU10 recorded survival of 5% with mutagen treatment duration of 25 minutes. In general, the interaction of survival in mutant strains was inversely related to time of chemical mutagen treatment, with three types of slopes in inversely curvilinear expression; the first type with high slope (EMSSKU1), the second with medium slope (EMSSKU5) and the third one with low slope (EMSSKU10), as observed in UV treatment (Fig. 7).

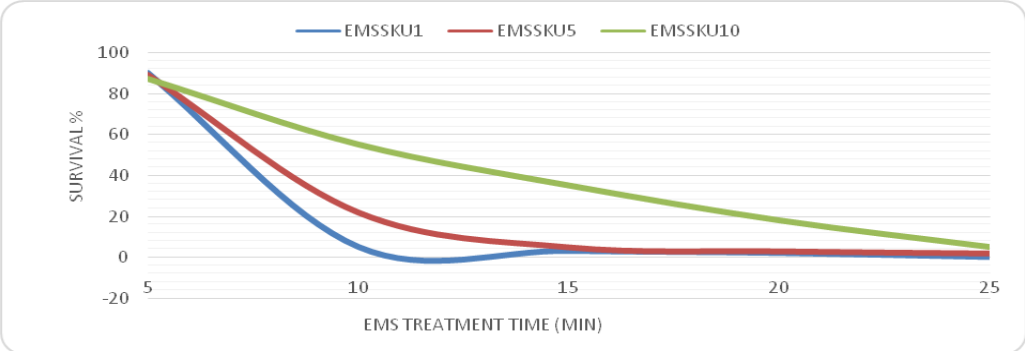

Fig. 7: Survival (%) of conidial spores of *Aspergillus nomius*, treated with EMS. Note inverse relation of survival % to time of EMS treatment and three types of slopes (high slope of EMSSKU1, medium slope of EMSSKU5 and low level of slope as EMSSKU10).

In the case of chemical mutagen treatment, data on duration of chemical mutagen treatment and zone of clearance is studied. The resulted mutants ($EMSSKU_1$ - $EMSSKU_{10}$) were tested for zone of clearance. Data on the zone of clearance by all 10 mutants against time span of treatment are presented in Table 1 that indicated that the *Aspergillus nomius*, treated with EMS and identified as EMSSKU5 has recorded maximum zone of clearance (mm). The data on zone of clearance was further treated for evaluation index. Such data on zone of clearance for all identified mutants (EMSSKU1 to EMSSKU10) are presented in Fig. 8. Killing of about 93% of spores of *Penicillium chrysogenum* at 1 mg/ml concentration of EMS was reported (Veerapogu et al., 2008). Number of surviving spores increased with decreasing concentration of EMS from 6 to 1 µg/ml (Shafique et al., 2011).

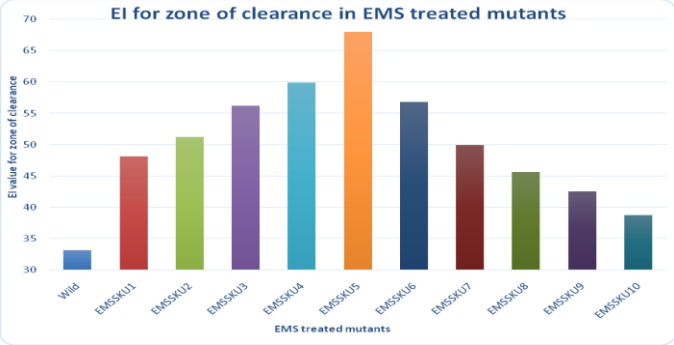

Fig. 8: Calculated Evaluation Index (EI) values for zone of clearance recorded for mutated strains of *Aspergillus nomius*, treated with EMS Note highest evaluation index for strain EMSSKU5.

In view of largest size of clear zone, subsequent studies were carried out with $EMSSKU_5$ mutant. The mutant, $EMSSKU_5$ was thus continued for production of cellulolytic enzymes on wheat bran in submerged fermentation. Data on the production of component enzymes of cellulase are presented in Table 3. Compared to other mutant strains of *Aspergillus nomius*, it is noted that the strain, EMSSKU5 produced maximum enzyme production of all three component enzymes of cellulase. Production of carboxy methylcellulase was highest with EMSSKU5 (26.58 IU/ml/min). Similarly, filter paperase was also highest in production by EMSSKU5 (15.73 IU/ml.min). The production of β-Glucosidase was also on higher side (4.0 IU/ml/min) compared to other mutants. The lowest production of component enzymes of cellulase (carboxymethylcellulase, filter paperase and β-glucosidase) was observed with mutant, EMSSKU10 (Table 2). High production of all the three component enzymes was found in mutant strain, EMSSKU5.

Table 3. CMCase, FPase and β-Glucosidase production of EMS treated muted strains of *Aspergillus nomius*

EMS treated mutants	CMCase (IU/ml/min)	FPase (IU/ml/min)	BGL (IU/ml/min)
$EMSSKU_1$	14.908	6.161	2.921
$EMSSKU_2$	16.820	8.108	2.772
$EMSSKU_3$	20.244	9.353	2.315
$EMSSKU_4$	22.074	11.170	3.187
$EMSSKU_5$	26.567	15.732	3.901
$EMSSKU_6$	14.782	12.786	2.589
$EMSSKU_7$	12.923	8.982	2.456
$EMSSKU_8$	9.789	7.456	1.657
$EMSSKU_9$	8.890	6.782	1.670
$EMSSKU_{10}$	6.978	3.789	1.903

Physical verification indicated that EMSSKU5 stood first in all the component enzymes of cellulase, other competing near mutants cannot be ignored. Therefore, data on individual component enzymes of cellulase were treated for evaluation index. Data on the EI for carboxymethylcellulase are presented in Fig. 4. In the case of filter paperase also, the EI calculated revealed and confirmed that the EMS treated mutant of *Aspergillus nomius* stood first in its secretion (Fig. 5). Similarly EMSSKU5 out yielded in stood first in β-glucosidase, a component enzymes cellulase, other mutants are also competing which cannot be ignored. Thus, data on BGL enzymes of cellulase were validated in terms of EI for β-glucosidase (Fig. 6).

In further supporting the hypothesis that the mutant strains of *Aspergillus nomius* treated with EMS, EMSSKU5 adjourned as the first in all the 10 mutants selected, the average index analysis (AEI) highly supported the assumption. Data on the average EI values for the mutant strains of EMS treated *Aspergillus nomiusi* are depicted in Fig. 7. Therefore, identification of SKUV7 and EMSSKU5 are justified as superior mutant strains of *Aspergillus nomius* treated with UV and EMS respectively. The production of component enzymes of cellulase for SKUV7 (UV treated mutant strain) and EMSSKU5 (EMS treated mutant strain) were compared. The data were further calculated for improvement in enzyme production of EMSSKU5 over SKUV7 (*Aspergillus nomius*). Such increase in enzyme production was reported by Hooi and Kuan (2015) and Nisar et al. (2020).

Comparison of cellulolytic enzymes production by mutant strains of *Aspergillus nomius*sp. SKUV7 and EMSKU$_5$

In the study of cellulolytic enzymes production by mutant strains of *Aspergillus nomius* sp. SKUV7 and EMSKU5 and control fungal strain, *Aspergillus nomius* (without any treatment); the results depicted in Table 4 suggested higher production rates of cellulase enzyme components (carboxymethylcellulase, filter paperase and β-Glucosidase) by a minimum of three-fold elevation. Both the mutant strains of *Aspergillus nomius*sp. SKUV7 and EMSKU5 showed better results than the control strain.

Table 4. Production of cellulase enzyme components by fungal mutant strain of *Aspergillus nomius* through UV radiation (SKUV7) and chemical mutagenesis (EMSSKU5) compared with wild (control) fungal isolate, *Aspergillus nomius.*

Strain	CMCase IU/ml/min.	FPaseIU/ml/ min.	BGL IU/ml/min.
SKUV7	16.923	9.982	2.256
EMSSKU5	26.567	15.732	3.901
Control	4.227	1.343	0.992
Increase over UV	300.3549	643.2614	127.4194
Increase over EMS	528.5072	1071.407	293.246

Data on production of cellulase enzyme components by the fungal mutant strain of *Aspergillus nomius* through UV radiation (SKUV7) and chemical mutagenesis (EMSSKU5) were compared. The data on component enzyme production of cellulase are statistically treated and the compared production is statistically highly significant ($p < 0.001$) with higher production of CMCase in EMSSKU5 compared to SKUV7. Derived data on percent increase in cellulase enzyme components (carboxymethylcellulase, filter paperase and β-glucosidase) by the fungal mutant strain of *Aspergillus nomius* of the EMS treated, EMSSKU5 over that treated with physical mutagen (UV) treated mutant strain, SKUV7 of fungal isolate, *Aspergillus nomius*with increased (%) of all the three component enzymes of cellulase are high in EMSSKU5 compared to SKUV7 fungal mutant strain.

From the above study it could be opined that the physical mutagen (UV) treated mutant strain of *Aspergillus nomius*, SKUV7 and EMS treated mutant strain of *Aspergillus nomius*, EMSSKU5 are functionally more potent than the control strains of *Aspergillus nomius* in terms of production of cellulase enzyme.

Improvement of strains was extended to few other cellulolytic organisms – *Cellulomonas biazotea* (Rajoka et al., 1998), *Bacillus pumilus* (Kotchoni et al., 2003), *Trichoderma aureoviridae* (Zaldivar et al., 2001), *Bacillus cereus* (Nanmori et al., 1983). Systematic improvement of production strains by mutagenesis and screening resulted in selection of mutant strains producing high levels of extracellular protein of over 40 g/liter with approximately half of the protein being the main cellulase, cellobiohydrolase I (Durani et al.,1988).

Conclusions

Cellulase is one of the enzymatic groups playing a vital role in the major hydrolysis cellulose by cleaving the β-1, 4-glycosidic linkage. The fascinating structural alteration of fungi leads to its candidacy as major component for biosynthesis of cellulase enzyme that has major potency as, solely or in combination with other enzymes. In view of its biological significance, newer approaches to maximize its production rate can be through the microbial strain improvement through application of mutagenesis (direct/random). The present study depicts an attempt for strain improvement in one of the fungal species *Aspergillus nomius* through applications of UV irradiation and induced mutation by chemical mutagen (ethyl methane sulfonate) application for higher yield of cellulase enzyme. From the result average evaluation index for UV treated mutant strains and EMS treated mutants clearly suggested superiority of SKUV7 and EMSSKU5 respectively and the mutant strains like EMSSKU5 and EMSSKU6 showed survival of 5% at 15 minutes of mutagen treatment.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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