

ASSAY OF XYLANOLYTIC ACTIVITY OF MUTANTS OF *NEUROSPORA CRASSA* (EMA 5297) INDUCED WITH ULTRA-VIOLET RADIATION

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Abstract

Mutation was induced in *Neurospora crassa* (Ema 5297) with UV (having the wave length of 254 nm) irradiation. Three groups of biochemical mutants (*leucine*, *tryptophan* and *arginine*) were obtained. Ema and 7 *leucine* mutants were used to evaluate whether mutation has any considerable effect for the production of such enzyme. Enzymatic activity was expressed as International Unit (IU). It was found that *N. crassa* mycelia secrete xylanases to the culture supernatant. Mutant Y10 showed highest xylanase activity (1.663 IU) and wild *N. crassa* (Ema) showed lowest (0.416 IU) activity.

For commercial production of enzymes filamentous fungi are mostly preferred because the enzymes produced by these fungi are more efficient as compared to those obtained from yeast and bacteria (Bakri and Thonert 2003). *Neurospora crassa* (Ascomycetes), a non-pathogenic filamentous fungus, is of particular interest to biologists due to their ability to produce hydrolytic enzymes, such as cellulases, amylases and chitinases, which are of special interest due to their importance in biotechnological processes (Benoliel *et al.* 2005) and its use in the study of genetic and metabolic pathways. For efficient enzyme production strains can be improved by mutagenesis which is successful method. This process is mostly trial and error process involving laborious steps of procedures in performance (Baker 1991, Iftikhar *et al.* 2010). Ultraviolet light has been widely used in inducing mutations in the field of microbial breeding (Gadgil *et al.* 1995, Adsul *et al.* 2007). Kuek and Kidby (1984) improved the glucoamylase production over 140% after UV mutagenesis in *Aspergillus phoenesis*. Similar improvement related to cellulolytic enzymes was achieved by Szczodrak (1989) after mutagenesis of *Trichoderma reesii*. In this study, an attempt was taken to produce mutants of *N. crassa* for evaluation of xylonite activities compared to the wild type and also to evaluate whether mutation has any considerable effect for the production of such enzymes.

Wild type strains of *Neurospora crassa* Emerson a (5297) was received from Fungal Genetics Stock Centre, Kansas, USA. Five days old conidia of Ema (5297) were irradiated with UV light having the wave length of 254 nm for 120 and 180 seconds. Radiation was given at a distance of 15 cm. Vogel's minimal medium (Vogel 1956) supplemented with arginine, leucine, tryptophan and lysine were used for plating the irradiated conidia and isolating conidial cultures, respectively. The isolates were incubated at 25°C. After 5 days, the isolates were classified according to their nutritional requirements. For induction of mutation procedures of Catcheside (Catcheside 1954) were followed.

For the study of enzyme activities, wild and mutants of *N. crassa* were grown on VM liquid medium (supplemented for biochemical mutants) separately in which the glucose was replaced by xylan at 0.5% (w/v) concentration. The fungi were incubated at 30°C for 3 days in shake culture.

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The mycelia and other non-soluble material in the culture filtrates were separated by centrifugation at 15,000 rpm for 5 min. Then the culture supernatant was diluted three times before use. The clear supernatants were used for enzyme assays (0.2 ml supernatant with 1.8 ml substrate (1% xylan in citrate buffer, pH 6.5) in each case). For this purpose, standard curves of reducing sugar for xylanase assay were prepared. Reducing sugars were determined by measuring the absorbance at 540 nm. Hydrolytic activity was calculated by measuring the amount of reducing sugars released from xylan. The amount of reducing sugar produced was measured by the DNS method (Miller 1959) using xylose as standards for xylanase activities.

Mutation was induced with UV having the wave length of 254 nm. The treatment period of *Ema* conidia were 120 and 180 seconds. Three groups of biochemical mutants were obtained (Table 1). The highest frequency of the mutant was *leucine* (64.85%). The best treatment period was 120 seconds. Seven *leucine* bio-chemical mutants were selected for enzymatic studies. UV irradiated 120 seconds mutants were Y1, Y4, Y5, Y7, Y10 and 180 seconds were Y32 and Y41. These were used for extracellular xylanase production in submerged cultures. Enzyme activities were calculated by the amount of reducing sugar (Table 2) obtained from standard curve prepared for xylanase (Fig. 1).

Table 1. Classification of biochemical mutants on the basis of their nutritional requirement

Group	Name of the mutants	Time of treatment		Total number	Frequency (%)
		120"	180"		
A	<i>Leucine</i>	12	10	22	62.86
B	<i>Tryptophan</i>	09	03	12	34.29
C	<i>Arginine</i>	-	01	01	2.85

Table 2. Reducing sugar of xylanase obtained from standard curve.

Name and no. of mutants	Assay sample (OD)	Assay control (OD)	OD of xylanase	Reducing sugar of xylanase (mg/ml)
Ema	0.101	0.053	0.048	0.104
Y1	0.118	0.060	0.058	0.126
Y4	0.151	0.058	0.093	0.202
Y5	0.162	0.072	0.090	0.196
Y7	0.204	0.068	0.136	0.296
Y10	0.241	0.050	0.191	0.416
Y32	0.194	0.065	0.129	0.281
Y41	0.174	0.062	0.112	0.244

During the experiments it was found that *N. crassa* secretes xylanase to the culture supernatant, since activity was detected in mycelium cultures of the fungus. The cultures grown on xylan yielded detectable activities. But this xylanase activity of wild type was found to be lower than the UV ray induced mutated *N. crassa*. Wild (*Ema* 5297) showed lowest xylanase activity (0.416 IU), mutant-Y10 showed highest (1.663 IU) and mutant-Y7 showed second highest (1.184 IU) activity (Fig. 2). Although xylanase activity of wild type *N. crassa* (*Ema* 5297) found to be similar as reported earlier by Rahim (2001), Ray and Rahim (2012), the activity of xylanase enzyme of the UV ray induced morphological and biochemical mutants of *N. crassa* was found to be higher than the activity of wild type, is supported by some UV mutant studies (Sadana *et al.* 1980, Mohamed *et al.* 2011).

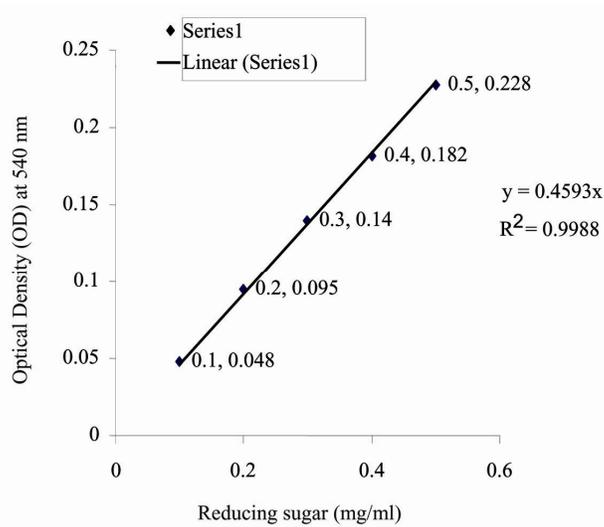
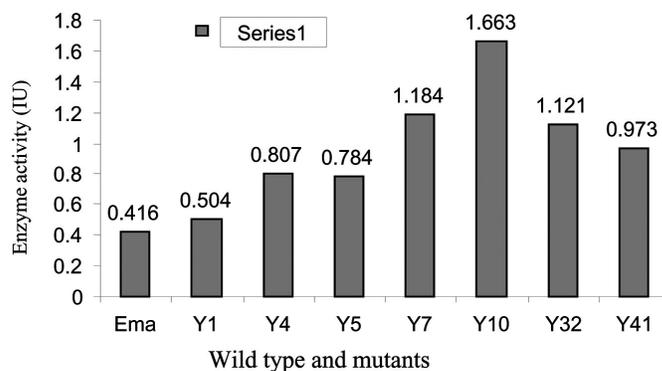


Fig 1. Standard curve of xylose.

Fig 2. Xylanase activity of wild type and *leucine* mutants of *N. crassa*.

The xylanase activity was found to be the most important hydrolytic activity, being related to the hydrolysis of hemicellulose. Xylan is the main component of hemicellulose (Thomson 1993). The enzymatic assays of this study indicate the presence of a hydrolytic system in *N. crassa*, which was able to grow in media containing xylan as the sole carbon source. Besides, these findings revealed that mutation is not deleterious in all cases but advantageous for pure research and applied field. UV mutation is a random mutation. So it may cause some unknown genetic change in the gene that is responsible for xylanase production. This change might be beneficial for xylanolytic activity of mutants of *N. crassa*.

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