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Mutagenic Effect of Gamma Irradiation on Phenotype and Enzyme Activities in *Aspergillus niger*

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THIS STUDY aimed at investigating the effect of gamma irradiation on the phenotypic characters of *Aspergillus niger* and its impact on the activities of some enzymes such as lipase, protease, cellulase, pectinase and amylase. Two phenotypically different mutants were obtained after gamma irradiation at doses of 1 and 3 kGy. SEM microscopy showed clear morphological changes in conidiophores, conidial heads and spores among all strains. The activities of lipase, protease and cellulase in both mutants become less than that of the parent strain, while, pectinase showed no significant difference among the tested strains. Amylase activity was enhanced by gamma-irradiation in the mutants. The obtained mutants were molecularly characterized using RAPD-PCR. The results demonstrated the occurrence of polymorphic pattern between parent and mutant strains due to change in the genetic makeup.

Keywords: Gamma Radiation; Mutagenesis; *Aspergillus niger*; Fungal Hydrolases.

Stored grains and seeds such as maize, rice, wheat and peanuts are regarded main targets for fungal contamination. The contaminating fungi can utilize carbohydrates, as energy sources; degrade lipids and proteins causing undesirable effects in these substrates. It was reported that fungal extracellular enzymes such as proteases, lipases and cellulases play an important role in the biodeterioration of the stored grains (Ribeiro *et al.,* 2011).

Aspergillus sp. mainly *A. niger* is considered one of the most important fungal species frequently isolated from these grains (Fraga *et al*., 2007 and Rosa *et al*., 2006). Using gamma radiation for decontamination of these substrates becomes an important issue in sterilization techniques. Gamma rays are the most energetic forms of ionizing radiation; they are characterized by their short wavelength so enabling them to penetrate deeply into the matter.

There are two different effects of gamma radiation, the direct and indirect effect. The direct effect results from the absorption of radiation energy by some molecules mainly DNA leading to their ionization, which may lead to mutations in some cases or killing of the cell. On the other hand, indirect effects caused by

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the mean changes occurred because of water radiolysis associated with formation of free radicals which cause single and double strand DNA breakages (Mcnamara *et al*., 2003).

Few studies reported the effect of gamma irradiation on survival of fungal pathogens (Aziz *et al.,* 1997 and Ferreira-Castro *et al*., 2007). Moreover, scare studies reported the influence of this radiation type in changing the morphological characteristics of fungi contaminating the grains. Therefore, this study aimed to investigate the effect of gamma radiation on the morphological characteristics of *Aspergillus niger*, furthermore, the activities of some enzymes such as lipase, protease, cellulase, pectinase and amylase were studied.

Experimental

Strain

Aspergillus niger was isolated from peanuts using the direct plate method (Food and Drug Administration, 2001).

Cultivation of *Aspergillus niger*

The purified and identified cultures of *A. niger* were maintained on potato dextrose agar (PDA) medium and stored at 4°C for further use.

Inoculum Preparation

Spore suspension was prepared by washing the spores from the surface of 7 day-old culture with 10 ml of spore buffer saline containing 0.9 g NaCl and 1 ml Tween-80 in 100 ml distilled water using sterile swaps. The final concentration of this suspension was adjusted by counting the spores using the haemocytometer to be ($5x10^6$) spore/ml.

Gamma Irradiation

Ten ml of fungal suspension of each fungal isolate was treated with gamma radiation. The suspension was transferred in clean sterile glass test tubes, sealed with cotton wool plug and wrapped with aluminum foil. These tubes were exposed to gamma irradiator. Different test doses of gamma radiation were selected 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, and 5 kGy. Irradiation process was carried out at Nuclear Research Center, Inshas, Egypt. The facility used was Co^{60} Gamma chamber (MC20, Russia). The average dose rate of this gamma radiation source was 1.1 kGy / h at the time of the experiment. Spores viability was determined following respective spore irradiation by plating of 0.1 ml of the irradiated suspension on PDA agar medium. The plates were incubated for 7 days at 28°C.

Survival rate of irradiated A. niger

After incubation, the survival rate was calculated by counting the colony forming units (CFU) of the treated and untreated fungal spores. Survival rate was calculated according to the following formula

Survival rate (%) = (A / T) x 100

Where A is the (CFU) of the survival colonies after irradiation by gamma rays and T is the (CFU) of the control (untreated) colonies.

Screening of morphologically different colonies of irradiated A. niger

The survivor colonies of the gamma irradiated spores were screened for changed colonial morphology, and then they transferred to fresh Czapek-Dox's agar plates, which composed of (g/ l): Sucrose: 30, NaNO3: 3.0, K2HPO4: 1.0, KCl: 0.5, MgSO4.7H2O: 0.5, FeSO4: 0.01, Agar: 20, pH was adjusted at 7. The plates were incubated at 28 ˚C. The growth and morphology were observed after 1-3 days incubation.

Phenotypic characters

Macroscopic assessment

Morphological characteristics of the selected colonies of *Aspergillus niger* treated by gamma irradiation were compared with the control. Color, texture, colony reverse and pigment production were observed macroscopically on CDA plates.

Microscopic assessment

Light microscopy

To compare the different morphological characteristics between the selected colonies with the control, slides were made from all colonies of untreated, and gamma treated *A. niger*. The slides were examined using light microscope with high magnification power (40 X).

Scanning electron microscopy (SEM)

All strains including the parent and the selected mutants were inoculated in Petri dishes containing Czapek-Dox's agar medium, then the plates were incubated at 28ºC for 48 hr. After incubation, pieces of approximately 1 cm^2 were cut and placed in 5ml vials containing 5% glutaraldehyde solution in 0.1 M phosphate buffer pH 7.3 and this for the primary fixation of the cells. The samples were then washed with buffer until complete removal of glutaraldehyde. After that, the samples were placed in 1% osmium tetraoxide in phosphate buffer 0.1 M, pH 7.3, for 2 hr. Osmium tetraoxide was then removed and the preparations were washed with phosphate buffer. Samples were dehydrated in several stages using alcohol with concentration gradients of (30, 40, 50, 70, 80, 90 and 100% v/v), and washed twice at 30 min. intervals. Samples were then air-dried and examined under (SEM, Qunta FEG 250, England). The electron photomicrographs were taken at the desired magnifications.

Enzymatic Analysis

Assay of lipase Assay

Lipase activity was studied by culturing the selected mutant strains and the parent one in medium contained (g/ l), KNO_3: 2.5, $KH2PO_4$: 1, $MgSO_4$: 0.5, olive oil: 10ml. pH adjusted to be 5.6. Culture filtrate was obtained by filtration

of the liquid medium through Whitman filter paper, then the filtrate undergo centrifugation at 10,000 rpm for 10 min. to remove any cell debris. This was done in triplicate and the culture filtrate was served as the enzyme solution. Lipase activity was assayed quantitatively by using *p*-nitrophenyl palmitate as the substrate (Winkler and Stuckmann, 1979; Gopinath *et al*., 2002, 2003b). The absorbance was measured at 410 nm. One enzyme unit was defined as 1µmol of *p*-nitrophenol enzymatically released from the substrate in milliliters per minute (ml/min).

Assay of protease activity

The basal medium used for protease production was the modified CZ-Dox medium containing (in g/l): K_2HPO_4:1.0, KCl: 0.5, Sucrose: 30.0, $MgSO_4$. $7H_2O$:0.5, $FeSO_4.7H_2O$:0.01, Casein: 1% as nitrogen source. The culture filtrate was centrifuged at 10,000 rpm for 10 min, then saved as an enzyme source. Protease activity in the culture filtrate was determined according to the method of Tsuchida *et al*. (1986) using casein as a substrate. The absorbance was measured at 660nm against a reagent blank using a tyrosine standard (Lowry *et al*., 1951). One unit of protease is defined as the amount of enzyme that releases 1µg of tyrosine per ml per minute under the standard conditions of supernatant solution.

Assay of cellulase activity

The tested fungal strains were cultured in 250 ml Erlenmeyer flasks, each containing 50 ml fermentation medium. The composition of the medium (g/l): $NaNO_3$: 5.0, Yeast extract: 2.0, $MgSO_4.7H_2O$: 0.05, $FeCl_3$: 0.001, KH_2PO_4: 1.0, Carboxy methyl cellulose (CMC): 1% concentration. The activity of CMC was determined in the centrifuged culture filtrate according to the method of Li *et al*. (2009) using carboxy methyl cellulose (CMC) as the substrate. The amount of released reducing sugars was determined according to Miller (1959) using 3, 5-dinitrosalicylic acid (DNS) reagent. The absorbance measured at 540 nm. Enzyme activity is expressed as µmol glucose released per ml/min of culture filtrate as enzyme solution.

Assay of pectinase activity: Media prepared for pectinase production contained (g /l): K_2HPO_4: 4, KH_2PO_4: 1.28, $(NH4)_2SO_4$: 2, Citrus pectin: 10, Yeast extract: 0.6, $MgSO_4.7H_2O$: 1.1; pH 5. Enzymatic activity was determined by measuring the release of reducing sugar, glucose from apple pectin using 3, 5-dinitrosalicylic acid (DNS) reagent assay according to Miller (1959), measure the absorbance at 550 nm.

Assay of amylase activity: Parent and mutants of *A. niger* were grown in 250 ml Erlenmeyer flasks, each containing 50 ml fermentation medium. The composition of the medium was (g/l): soluble starch: 5.0, Yeast extract: 2.0, $MgSO_4.7H_2O$: 0.5, KH_2PO4: 1.0. Centrifugation of the culture filtrate was carried out at 10,000 rpm for 10 min and the supernatant was used as a source of extracellular enzyme. The activity was assayed by the modified method of Ramakrishna *et al*. (1982) using soluble starch solution as a substrate. The

amount of reducing sugar was measured using (DNS) reagent according to Miller (1959). The absorbance measured at 540 nm .One unit of amylase activity was defined as the amount of enzyme resulting in release of 1 µmole of reducing sugars in one minute at 60 °C for 20 min.

Molecular study

Investigative studies were performed to elucidate the relationships or differences between mutant strains and wild type by RAPD-PCR.

DNA isolation procedure

The bulked DNA extraction was performed using DNeasy Fungi Mini Kit (QIAGEN).

Randomly Amplified Polymerase Chain Reaction (RAPD– PCR)

PCR was performed in 30-µl volume tubes according to Williams *et al*. (1990) containing 2 µl of genomic DNA template (25ng) , 2 µl of 10 pmol of random primer, 3 µl of 25 mM $MgCl_2$, 3 µl of 2.5 Mm dNTPs and 0.2 µl of Taq DNA polymerase. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 37º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min. The reactions were conducted using ten arbitrary 10 mer primers with 5´→ 3´ sequences shown in Table 1.

TABLE 1. Primers used in RAPD-PCR reaction

RAPD Primers	Sequence (5´-3`)
OP-A09	GGG TAA CGC C
OP-A12	GTG ATC GCA G
OP-A18	AGG TGA CCG T
OP-B04	GAT GAC CGC C
OP-B07	GAA AGG GGT G
OP-B11	GTA GAC CCG T
OP-C15	GAC GGA TCA G
OP-E15	ACG GCG TAT G
OP-Q18	GGG AGC GAG T
OP-Z07	CAC GAG TCT C

Data analysis

Each laboratory sample was divided into three analytical samples and then each sample was analyzed in triplicate and data are reported as mean of three replicates (±) standard deviation (SD).

Results and Discussion

Effect of gamma irradiation on survival rate

The effect of gamma rays on the survival rate of *A. niger* is illustrated in Fig 1. It has been found that gamma rays of doses between 0.1 and 0.5 kGy resulted in survival rate ranges between (98 to 78 %), while a sharp decrease was recorded at 3 and 5 kGy with survival rates of 0.8 and 0.1 %, respectively. The present results agreed with that of Shinohara *et al*. (2013) who studied the lethal action of gamma rays at doses of (0, 0.3, 0.1, 1 and 3 kGy) of fungus *Isaria fumosorosea*. They reported that the survival rate was slightly affected at doses below 0.3kGy (>78.1 %), but at 1 kGy the survival rate sharply decreased and reached (0.5 %). The complete killing effect was achieved at 3 kGy. Similarly, the dose response curve of *A. niger* AUMC 4301 which exposed to different gamma doses (0.25, 0.5, 0.75, 1, 1.25 and 1.5 kGy) showed a decrease in the fungal colonies in response to increased gamma doses (El-Fouly *et al*., 2012). Gamma radiation makes an additional stress to the cells resulting in disturbance to their organization. This disturbance is mainly dependent on the absorbed dose. The lethal action of gamma rays on microbial cells can be explained according to Tauxe (2001) who stated that the high energy rays of irradiation directly damage the DNA structure of living organisms inducing cross-linkages and other changes that make an organism unable to grow or reproduce. Moreover, when these rays of high energy content interact with water molecules, which represent 80 % of the vegetative cells composition, it resulted in radiolysis of water and generation of the active free radicals. These active radicals also caused an additional indirect damage to DNA.

Phenotypic characters of Aspergillus niger mutants

Mutants (M1 and M2) of irradiated *Aspergillus niger* were selected to be studied based on their different phenotypic pattern from the parent. These mutants appeared at doses of 1 and 3 kGy, respectively. They were cultivated on CDA plates to compare between them morphologically. It was found that they differ in some morphological characteristics such as spore color, texture and quantity, colony diameter, colony reverse and pigment formation. Fig. 2 demonstrated theses morphological differences among them. Colonies of *A. niger* parent strain appeared with compact yellow mycelia, covered with many spores which are carbon black or very dark brown spores.

The colony diameter was large and reached 8 cm; colony reverse was white associated with spread growth.

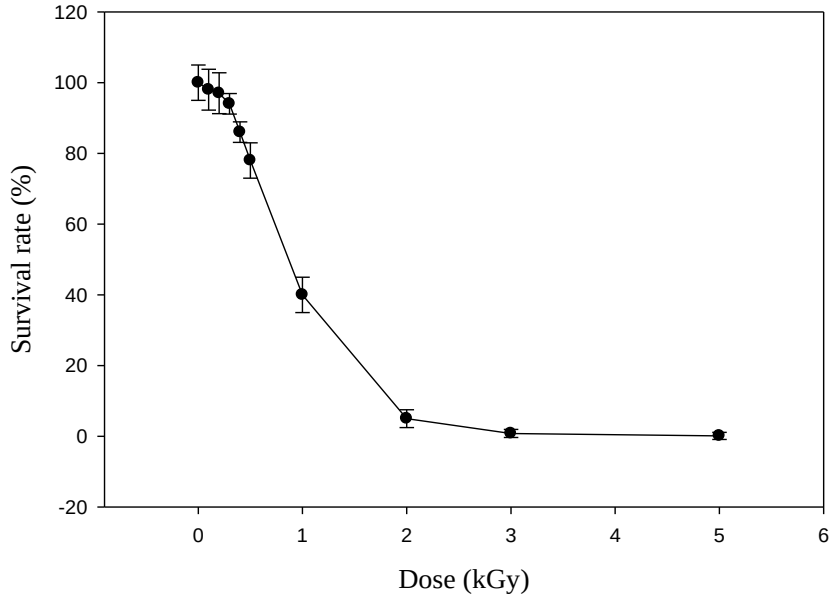

Fig. 1. Survival rate of *A. niger* spores in response to the increasing gamma doses.

The M1 strain showed a different morphological pattern from the parent, the colonies were pale yellow colored when they were young aged; then turned to dark brown after 7 days incubation. It showed less spore number and smaller colony of 5 cm diameter. For M2 strain, it had dark yellow colored colonies covered with spores. The colony diameter was 8 cm, with pale yellow colored reverse. The colony margins are yellow colored. It was observed that the growth of mutants M1 and M2 was delayed for 1 day. These changes were persistent for 5 generations indicating true mutations.

Our observations were found to be compatible with Otteheim *et al*. (2015); who obtained a mutant strain from *A. niger* (DSM 26641) using gamma irradiation. They found that the spore formation was delayed for 1 day associated with a yellow pigment formed in excess after 4 days. In addition, Borges *et al*. (2011) reported that the colonies of *A. ochraceus* exhibit fewer conidia, associated with slower growth rate with increasing gamma doses in the range of 2-4 kGy. Interestingly, Fang *et al*. (2012) explained the reason for the delaying sporulation in the generated mutants as the obstruction of the morphogenetic genes which involved in the conidiation process, so this physiological process was disrupted.

Figure 2 illustrated the light microscopy of all examined strains. *Aspergillus niger* parent strain showed a more widespread colony growth than the mutants. Large, globose, dark brown conidial heads, which become radiate, tend to split into several loose columns with age. Conidial heads are biseriate with the phialides

borne on brown, often septate metulae. Conidiophores are smooth-walled hyaline or turning dark towards the vesicle. Conidia are globose to subglobose, dark brown to black with rough-walled. Mutants M1 and M2 showed variations between them and with the parent one. M1 examination showed the conidial head which become deformed with reduced size and no discrimination between its parts; moreover the spores become reduced in its number and smaller than that of the parent strain. However, M2 was found to have the columnar conidial head resemble that of the parent, but it appeared also deformed. The spores are spherical and small sized compared to those of the parent.

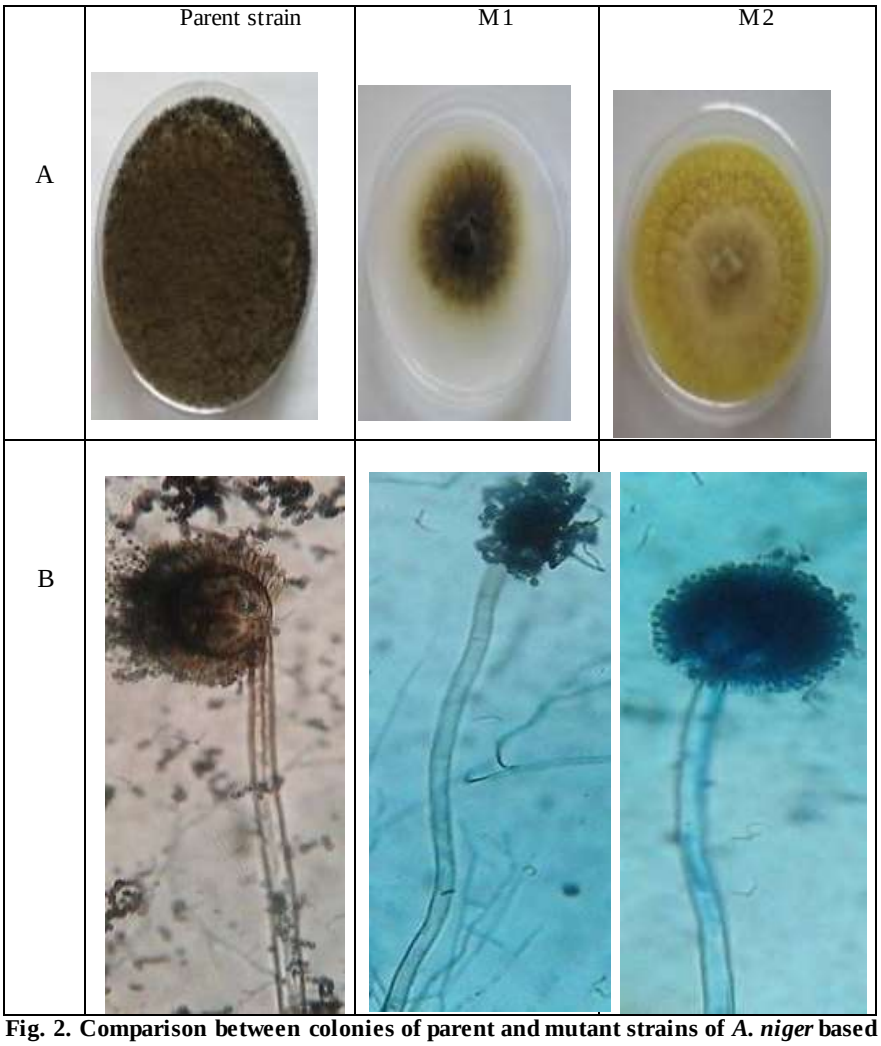

Fig. 2. Comparison between colonies of parent and mutant strains of *A. niger* based on phenotypic characters (A) and microscopic structure (B).

SEM analysis micrographs showed distinctive morphological variations appeared in the mutants compared to the parent strain (Fig. 3). It was observed that the mycelia of parent strain were thick, tube shaped, rigid and branched with rough surface. SEM analysis of M1 photomicrographs demonstrated rigid, tube-shaped mycelia with white spots giving the fish-scaled-appearance making its surface to be rough. While the photomicrographs of M2 showed a decrease in the thickness of the mycelia compared to the parent one. Also, appearance of white spots which are distributed on the mycelia and making its surface rough. Development of abnormal branching of the mycelia was observed. The SEM graphs of M1 sporangia showed the abnormal shape of the conidiophores, carrying disrupted conidial heads, which become smaller in size; the spores become smaller in size compared to the parent one. M2 micrographs illustrated the large sized spores compared with other strains, which carried on distorted conidiophore. The general observation of all examined mutants is the deformation of the conidial heads that became small in size as compared to the parent strain. Mutants M1 and M2 resulted from gamma irradiation showed a morphological variation from the parent one. Presence of white spots resemble the fish scales appeared on the mycelial surface, these may be resistant structures.

The advantages of SEM as compared with light microscopy are a better resolution, higher magnification, greater depth of field and greater versatility (Goodhew and Humpreys, 1998). It makes fungal structures and fungal growth more visible (Bacon *et al*., 1992). Our observations are supported by Braghini *et al*. (2009) who studied the effect of gamma irradiation on the fungal structures of *Alternaria alternata* using SEM. The radiation doses were 2, 5 and 10 kGy.

The SEM analysis demonstrated that the fungal structures were not changed after irradiation at 2 kGy, but the irradiation doses of 5 and 10 kGy make the mycelia twisted with marked alterations in the shape and surface of the hyphae associated with rupture of filaments.

According to Borges *et al*. (2011) these different appearance may be due to formation of resistant structures, which appeared on the irradiated mycelia of *A. ochraceus*. The appearance of such morphological changes in the produced mutants may be due to the mutagenic effect of the applied radiation, as it was stated that the radiation effect is random. Based on this, the radiation may mutate the genes responsible for the ordinary fungal phenotype and so become altered.

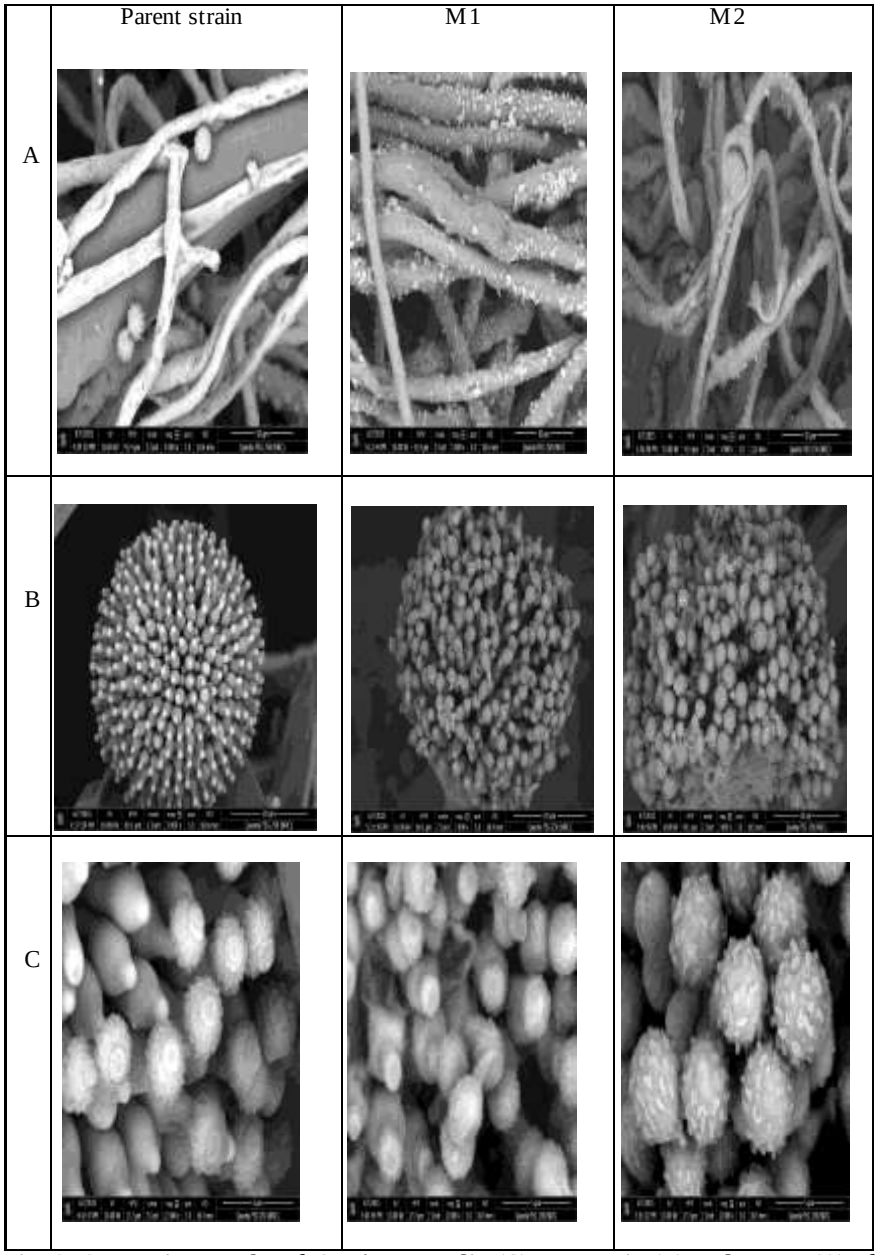

Fig. 3. SEM micrographs of *A. niger* mycelia (A), sporangia (B) and spores (C) of parent strain, M1 and M2.

Enzymatic study

In this work, the parent strain and the obtained mutants of *A. niger* were characterized biochemically through analysis of the activities of various important enzymes including lipase, protease, cellulase, pectinase and amylase after irradiation by gamma rays at 1 and 3 kGy.

Lipase activity

The lipolytic activity of mutants (M1 and M2) decreased compared with the parent one (Fig 4). The parent strain recorded the highest activity (86 U / ml), while the mutants activities were (83 and 80 U / ml) for M1 and M2 as a result of gamma irradiation at doses of 1 and 3 kGy, respectively. The statistical comparison among all strains displayed significant difference ($P < 0.05$). These observations extended allover 5 generations indicating the true alteration.

This was agreed with El-Tablawy and Araby (2014), who reported that the lipase activity decreased from 52 to 31 U / ml, in *Aspergillus fumigatus* at dose of 3 kGy, and from 68 to 36 U/ml in *Penicillium italicum* at dose of 5 kGy. In contrast, the enhancing effect of gamma radiation on lipolytic activity in *Aspergillus niger* was reported (Iftikhar *et al*., 2010).

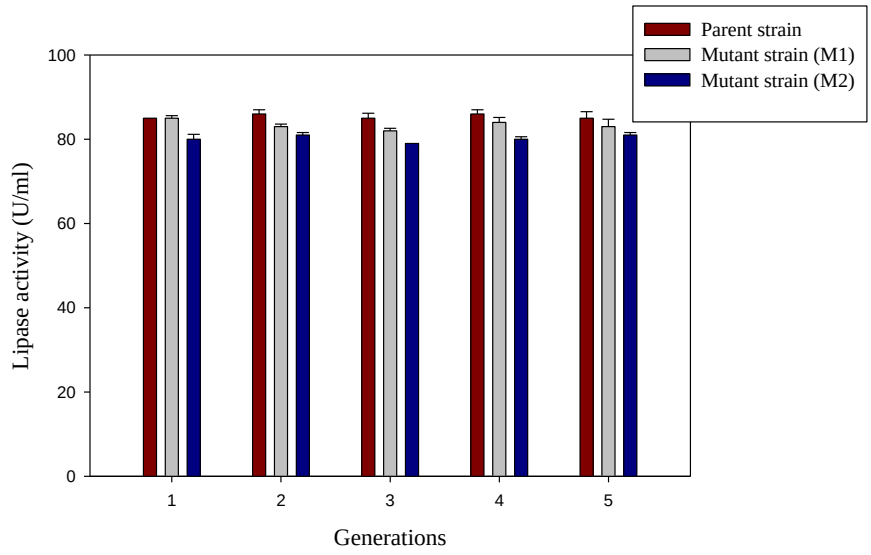

Fig. 4. Comparison between the lipase activity of parent and mutant strains (M1 and M2) irradiated at 1 and 3 kGy for five generations.

Protease activity

Figure 5 showed the protease activity of the parent and mutant strains (M1 and M2) for five generations. After exposure to 1 and 3 kGy, respectively it was observed that the activity decreased in both mutants in comparison with the parent strain. The activity of the mutant M2 (5.2 U/ml) was higher than that of

mutant M1 (2.5 U/ml), while the parent strain had the highest activity of (6.4 U/ml) which is significant difference. The enzymatic activity was stable till the fifth generation for the mutants revealing occurrence of true mutation. This data are in harmony with El-Tablawy and Araby (2014), who found that the protease activity of *A. niger* and *A. flavus* decreased after their irradiation by gamma rays at doses of 3 and 5 kGy, respectively. *A. niger* showed a decrease from 9.14 to 5.08 U/ ml at a dose of 3 kGy, while a decrease from 6.52 to 2.14 U/ml showed by *A. flavus* whent irradiated at dose of 5 kGy. This can be explained as the high doses of gamma rays were proved to be inhibitory to the enzymatic activities of microorganisms (El-Batal and Abdel-Karem, 2001; El-Batal and Khalaf, 2003).

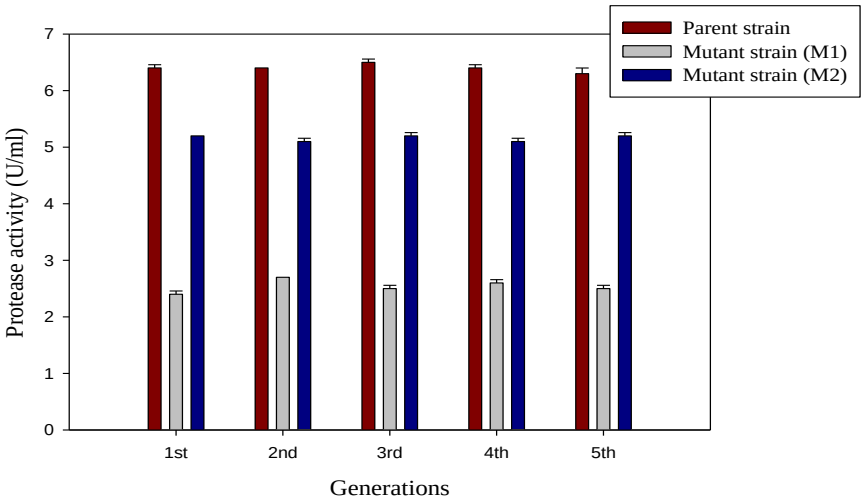

Fig. 5. Comparison between the protease activity of parent and mutant strains (M1 and M2) irradiated at 1 and 3 kGy for five generations.

Cellulase activity

Cellulase assays showed a decrease in the celluolytic activity for the two mutants irradiated by gamma rays at doses of 1 and 3 kGy with respect to parent one (Fig. 6). The stability of celluolytic activity was observed for mutant strains with the five generations. The average activities were (5.6, 14.4 and 15.3 U/ml) for M1, M2 and parent strains, respectively. In the activity of cellulase for mutants obtained from irradiation of *Pleurotus ostreatus* at 1 and 2 kGy was investigated. All obtained mutants (OP-5, OP-6, OP-14, OP-15 and OP-16) have higher cellulase activity compared to the parent. Only, mutant OP-6 recorded a decrease in the activity reaching 55 % in comparison with parent strain (Lee *et al*., 2000). Gherbawy (1998) reported the effect of low and high doses gamma radiation on the activity of cell wall degrading enzymes including polygalactronase, pectinmethylgalacturonase, cellulase and protease in *A. niger*. The results indicated that the low dose of radiation enhanced the production of all studied enzymes, while the higher dose reduced their production. On contrary, Mostafa (2012)

studied the effect of gamma radiation on the production of cellulase in *A. niger* isolated from wheat straw. The results demonstrated hyper-production of cellulase at dose of 2 kGy.

The inhibitory effect of gamma radiation on the enzymatic activities can be interpreted as the damage or deterioration occurs in the microbial vitality, as gamma rays causes rupturing in cell membrane. This major injury allows the extracellular fluids to enter into the cells, associated with leakage of the ions and nutrients. Membrane rupture results in decrease in the enzyme synthetic activity and death of the cell (El-Batal, 2012).

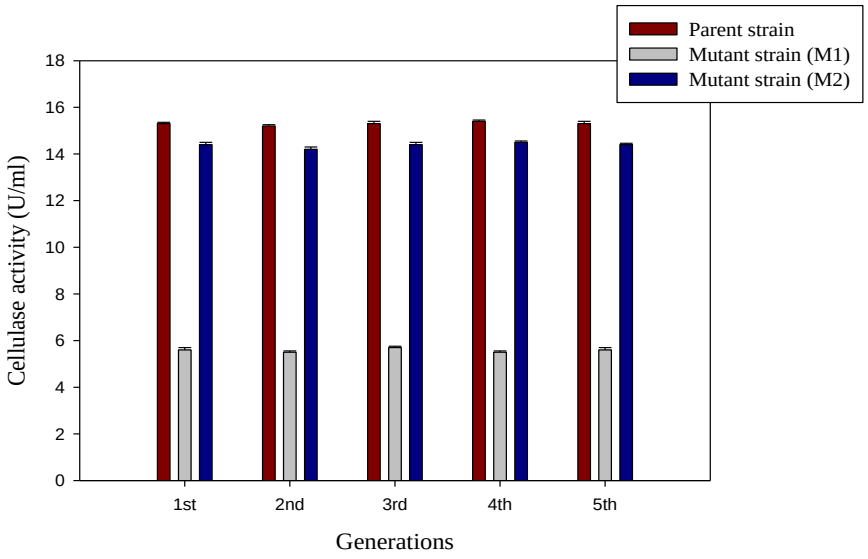

Fig. 6. Comparison between the cellulase activity of parent and mutant strains (M1 and M2) irradiated at 1 and 3 kGy for five generations.

Pectinase activity

The results of pectinase activity presented in Fig, 7 demonstrated no significant difference among all strains after gamma irradiation at doses of 1 and 3 kGy. Pectinase activity was observed to be stable until the fifth generation indicating true mutation. In contrast, the enhancing effect of gamma radiation on pectinase production by *Penicillium citrinum* was reported by El-Batal *et al.* (2013). They stated that the dose at which the activity increased was 0.7 kGy, and then a gradual decrease in the enzymatic activity was observed at doses of 1, 1.5 and 2 kGy.

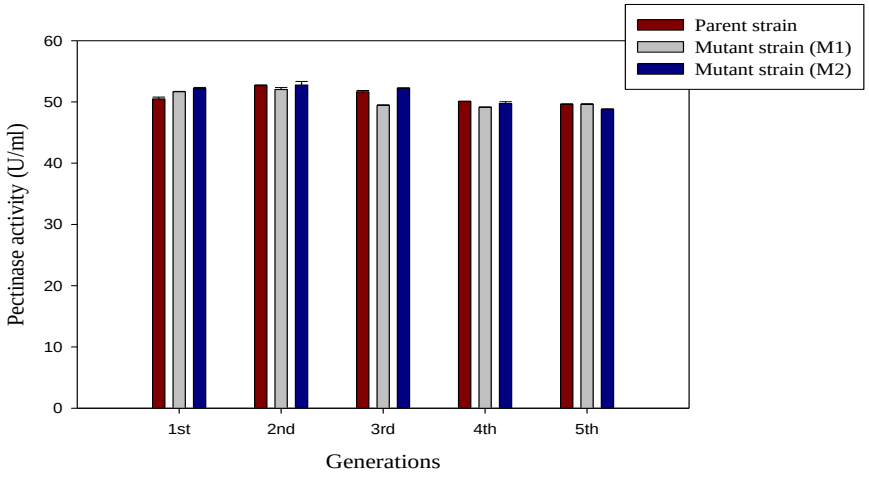

Fig. 7. Comparison between the pectinase activity of parent and mutant strains (M1 and M2) irradiated at 1 and 3 kGy for five generations.

Amylase activity

Amylase activity recorded a remarkable increase in mutant (M2) compared to the parent as a result of gamma irradiation at dose of 3 kGy (Fig. 8). The increase was 1.7 fold of the parent, while the mutant (M1) showed a decreased activity. Thus, the mutant M2 can be used for further studies to increase the production of amylase for industrial applications. Fixed changes allover 5 generations indicate true mutation. The enhancing effect of gamma rays on the production of α- amylase by *A. niger* was reported by Bedan *et al*. (2014), using different food wastes such as wheat bran, corn cob, banana peel and potato peel. They obtained a mutant with hyper production of amylase with increase percentage of 151 % of the control.

The inducing effect of gamma radiation on enzymatic production can be explained in correlation with the induction of genes responsible for amylase production an increase in gene copy number, or due to an improvement in the expression of genes or both in DNA (Gaedner *et al*., 1991; Rajoka *et al*., 1997 and Shaukat, 2004).

Genetic variability studies

Samples of genomic DNA of wild and mutant strains were analyzed by RAPD-PCR method to detect the genetic diversity of the selected mutants with the wild strain using 10 random primers. Each primer produced number of distinctive polymorphic bands in all strains tested, with different degree of polymorphism among all the genotypes (Fig. 9). Out of the 10 primers, four of them (OP.B04, OP.B07, OP-B11 and OP.A18) giving the highest degree of polymorphism between all strains (50, 75, 75 and 80%), respectively. A number of total bands were 52, of these 21 bands were polymorphic. The total degree of polymorphism was 40 % (Table 2).

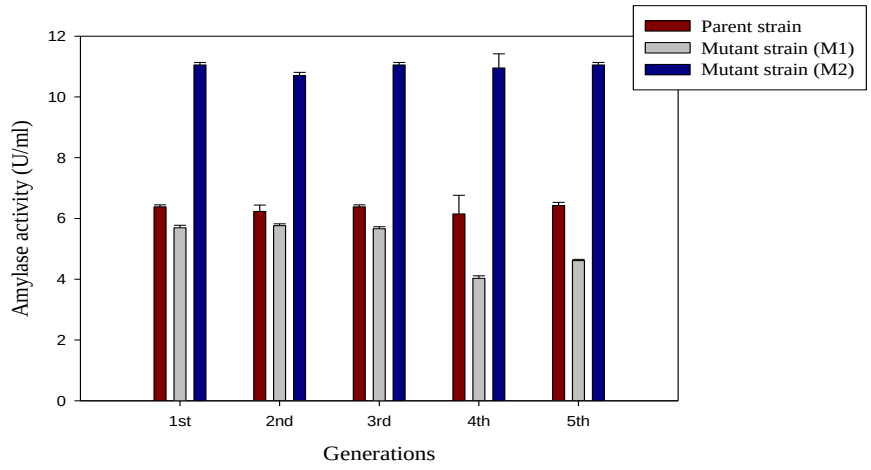

Fig. 8. Comparison between the amylase activity of parent and mutant strains (M1 and M2) irradiated at 1 and 3 kGy for five generations.

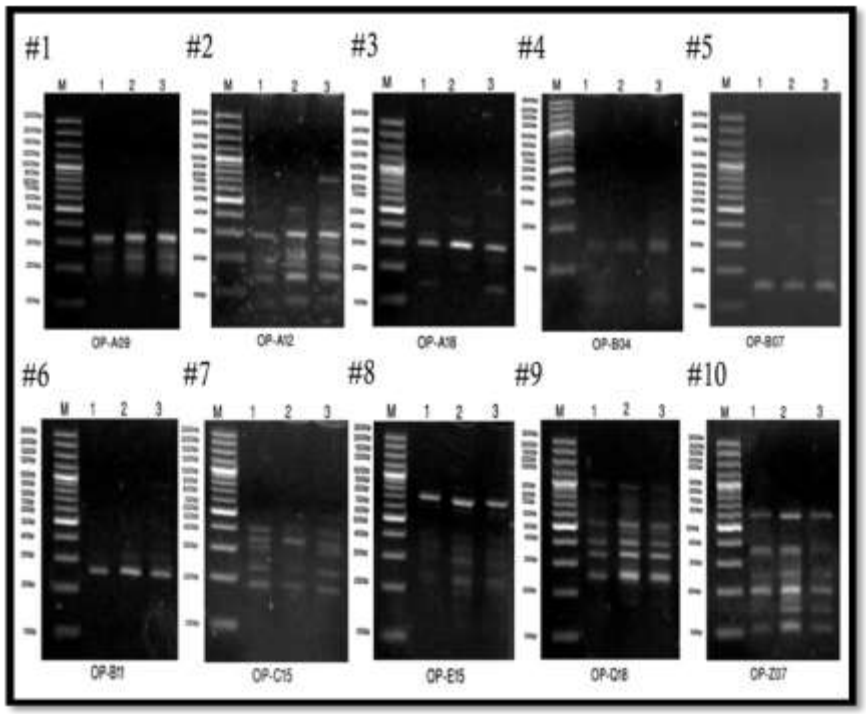

Fig. 9. RAPD fingerprints of the parent and mutant strains (M1 and M2) using ten different primers. Lane M indicates DNA marker, lane 1 indicates parent *Aspergillus niger*, lane 2 indicates mutant (M1) and lane 3 indicates mutant (M2).

TABLE 2. DNA polymorphism detected in mutant genotypes of *Aspergillus niger*

RAPD-primer	Total number of bands	Monomorphic bands	Polymorphic bands	Polymorphism percentage (%)
OP.A09	4	3	1	25
OP-A12	7	5	2	28
OP.B04	2	1	1	50
OP.B07	4	1	3	75
OP-B11	4	1	3	75
OP.C15	5	4	1	20
OP-Q18	7	5	2	29
OP-Z07	8	5	3	38
OP.A18	5	1	4	80
OP.E15	6	5	1	17

Zhang *et al*. (2009) reported that RAPD is a powerful tool to study the genetic diversity and relatedness between species. This technique was capable of differentiating between parent and mutant strains and producing pattern of the tested strains (Awan *et al*., 2011). In our study 40% the degree of polymorphism was calculated between the parental and mutant strains of *A. niger*.

Summary and conclusion

Gamma irradiation has a mutagenic effect on the morphological characteristics and enzymatic activities of lipase, protease, cellulase, pectinase and amylase in *A. niger*. The results can be summarized as follow:

- Gamma irradiation results in reducing the survival rate of *Aspergillus niger* spores which reached 0.3 % at a dose of 5 kGy.
- Two morphological mutants were obtained at dose of 1 and 3 kGy. These mutant strains were cultured on Czapek's-Dox (CDA) medium.
- Fixed changes in enzymatic activities allover 5 generations were detected indicating true mutation.
- The induction of some enzymes was different among them. The enzymes, lipase, protease and cellulase showed a decrease in their activity in comparison with the parent strain.

- Amylase activity increased in M2 recording an increase by 1.7 fold more than the parent strain. This mutant can be used in further studies in fermentation industries for production of amylase on large scale.
- The genetic diversity among all strains which characterized using RAPD-PCR demonstrated the occurrence of polymorphic pattern between wild and mutant strains due to change in the genetic makeup.

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التأثير الطفرى لأشعة جاما على النمط الشكلى و الانشطة الانزيمية فى أسبيرجيلس نيجر

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يهدف هذا العمل الى دراسة تأثير أشعة جاما على الصفات الشكلية لفطرالاسبيرجليس نيجر و نشاط بعض الانزيمات مثل الليبيز، البروتييز، السيليوليز ، البكتينيز و الاميليز. تم الحصول على متطفرين مختلفين شكليا عند جرعات اشعاعية واحد و ثلاثة كيلو جراى. أظهر الميكروسكوب الماسح الالكترونى تغيرات شكلية فى الحامل الكونيدى و الرؤوس الكونيدية و الجراثيم بين السلالات. كان هناك نقص فى أنشطة انزيمات الليبيز و البروتييز و السيليوليز فى كلا المتطفرين عنهم فى العزلة الام. فى حين لم يكن هناك فرقا معنويا فى نشاط البكتينيز بين السلالات المختبرة. ازداد نشاط الاميليز بزيادة اشعاع الجاما فى المتطفرات. تم توصيف المتطفرات باستخدامRAPD-PCR .أظهرت النتائج حدوث أشكال نمطية مختلفة بين السلالة الام و المتطفرة و هذا لحدوث تغير فى البناء الجينى.