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GAMMA RAY INDUCED EFFECTS ON NEW SPECIES OF *STREPTOMYCES* (AEFO2) (HM775973.1GI:302495616) ISOLATED FROM EGYPTIAN SOIL

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Abstract: A new *Streptomyces* sp (AEFO2) isolated from Egyptian soil was exposed to increasing doses of gamma radiation up to 10 kG_y and this isolate showed high antimicrobial activities against bacteria, yeast, and fungi. The lethal dose of studied isolate was $5KG_y$. Dose level of 3.5 kG_y enhanced the antimicrobial activity, pigmentation, melanin production, utilization of carbon sources of this isolate and also increased their sodium chloride tolerance from 5 to 8% either at first or second generation after irradiation process. Dose level 3.5 kG_y showed decreasing of the amount of DNA in both first and second generations also.

Key Words: Gamma Radiation, *Streptomyces* sp (AEFO2), Antimicrobial activity.

INTRODUCTION

Actinobacteria are a group of Gram-positive bacteria. They were all believed to have high guanine and cytosine content in their DNA (Ventura et *al.,* 2007). The genus *Streptomyces* is the dominant among actinobacteria where, they are responsible for the production of about 50% of the discovered bioactive secondary metabolites (Berdy, 2005), antibiotics (Strohl, 2004), antitumor agents (Cragg *et al.,* 2005), immunosuppressive agents (Mann, 2001) and enzymes (Oldfield *et al.,* 1998).

Actinomycetes and their bioactive compounds show antibacterial and antimicrobial activity against various pathogens and multi drug resistant pathogens e.g. Vancomycin-Resistant Enterococci, Methicillin-Resistant *Staphylococcus aureus, Shigella dysenteriae, Klebsiella sp. and Pseudomonas aeruginosa* etc. (Servin *et al.,* 2008; Bhatnagar and Kim, 2011).

Streptomyces are obligate aerobes, chemoorganotrops that need only an organic carbon source (such as glucose, starch or glycerol), an inorganic nitrogen source, and a few mineral salts for growth. However, faster growth can be obtained in complex media containing, for instance, yeast extract, or other organic nitrogen sources. Trace elements contained in tap water are generally sufficient, but addition of iron manganese, zinc and ions can be beneficial (Gottlieb and Shirling, 1967).

Streptomyces generally required a good supply of free water for growth but unable to grow at high osmotic potentials. However, some soil isolates were able to grow in media at high osmotic potentials and a few strains can grow at 13% (w/v) NaCl salinity (Tresner *et al.,* 1968).

On solid medium a spore germinates, grows vegetatively as a substrate mycelium and then develops into an aerial mycelium, which segments into chains of spores (Ensign, 1978; Chater, 1984). The potential usefulness of scanning electron microscopy in studies of actinomycetes was demonstrated by Williams and Davies (1967).

Ionizing radiation was the first agent shown to be mutagenic (Muller, 1927). Also, this type of radiation has been studied as mutagenic agent by Wong and Smith (1978). Ultraviolet and gamma-irradiated actinomycete spores yield high proportions of mutant colonies, and that some of the changes occur as sectors (Newcombe, 1953).

Early experiments showed that the effect of ionizing radiation on microorganisms was by their direct and indirect effect, and the biological effect of radiation is amplified by the oxygen this is always present in the medium (Yarmonenko, 1988). Ionizing radiation induces mutagenesis by generated reactive oxygen species (ROS) that react with DNA, RNA and their precursors leading to damage of nucleic acids and nucleotides by detectable deletions, major rearrangements and into point mutation (Huang *et al.,* 2007; Seifried *et al.,* 2007).The gamma radiation induced mutation by direct and/or indirect DNA damaged by formation of a reactive species such as OH. There is evidence indicating that ROS can react with DNA and cause mutation (Hei *et al.,* 1997).

MATERIALS AND METHODS

A new *Streptomyces* sp (AEFO2) (HM775973.1GI:302495616), isolated from Egyptian soils and identified by El Shobaky *et al.,* (2010), (recorded at Genbank), exposed to increasing doses of gamma radiation up to 10 kG_y. The physiological

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properties and the amount of DNA of our isolate were studied at dose 3.5KG_y in both first and second generation.

Irradiation process: Co-60 gamma Indian chamber 400 irradiator source located at National Center for Radiation Research and Technology (NCRRT) Nasr City, Cairo-Egypt was used for irradiation of the spore suspension. The dose rate of this source at the time of experiment was 1.63 kG_y / hour (Moussa *et al.*, 2005).

Morphological properties

The morphology of spore chain of the studied isolate was examined using slide culture technique (Bergey's Manual of Determinative Bacteriology, 1989). After growth, the slide was taken, left in air to dry, and then examined under light microscope.

The spore surface ornamentation of the studied isolate was examined by the spore-print technique (Williams and Davies, 1967) applied on the prepared materials for scanning electron microscope (Jeol JSM LV 5500).

Antagonistic properties

The antimicrobial properties of the actinobacterial isolate were studied against a number of test organisms comprising dual culture method (Hamdali *et al.*, 2008).

a) Bacteria:

Gram-negative: *Escherichia coli, Klebsiella, Shigella, Erwinia carotovora, Proteus vulgaris and Pseudomonas aueroginosa*

Gram-positive: *Staphylococcus epidermidis, Streptococcus pyogenes* and *Staphylococcus aureus*

(Obtained from Botany department, Faculty of science and Plant Disease Department, Faculty of agriculture, Mansoura University.)

b) Yeast:

Candida boidinii NRRL 2332 (obtained from culture collection center, Ain Shams University)

c) Fungi:

Fusarium oxysporum, Fusarium solani, Aspergillus flavus, Aspergillus ochroceus, Trichothecium sp and *Penicillium purpurogenum*

(Obtained from Plant Disease Department, Faculty of agriculture, Mansoura University).

The actinomycete isolate was grown on starch-nitrate agar medium. After 6 days of incubation at 30°C, agar discs were cut off by a sterilized cork borer and transferred to the surface of agar plates freshly inoculated with the test organisms separately. The bacterial test-organisms were grown on nutrient agar whereas yeasts and fungi were grown on Sabaroud and Czapek's solution agar media respectively. All plates kept overnight at 4°C before incubation. The diameters of inhibition zones against the test organisms were measured after 24 hours for bacteria, and 72 hours for yeast and 6 days for fungi.

Physiological Properties of Streptomyces sp (HM775973.1GI:302495616)

A) Utilization of different carbon sources: The ability of isolates to utilize different carbon sources (Sucrose, D-Fructose, Raffinose, Maltose, L-lactose, Mannose, D+ xylose, L-Arabinose, Manitol, Sorbitol) was studied on Gauze No. 1 as a basal liquid medium (Devoid of starch). The tested carbon sources were sterilized through bacteriological filter (0.450 µm, not by heat), then added separately at a concentration of 2% (w/v). Test tubes containing 2 ml of these media were inoculated separately with spore suspension of the studied isolates and incubated at 28°C. The ability of certain strain to use a carbon source was determined by comparison of its growth intensity, recorded after 7 and 14 days of incubation, with that on the positive (glucose) and negative (no carbon source) controls (Shirling and Gottlieb, 1966).

B) Production of melanin pigments: The production of melanin (or melanoid) pigments was tested on peptone-yeast iron agar, tyrosine agar and tryptone-yeast broth. Each medium was inoculated with every isolate and incubated for 7 days at 28°C. The cultures were examined after 24-48 hours and the formation of diffusible brown to black pigments was considered as a positive reaction (Shirling and Gottlieb, 1966).

C) Hydrolysis of starch: Spore suspension of the isolate was streaked over the surface of starch-nitrate agar plates and incubated at 28°C. The amylolytic activity of isolates was tested after 3, 5, 7 and 14 days by pouring Gram's iodine on the surface of the culture. The presence of clear zones around the growth was considered to indicate positive activity (Shirling and Gottlieb, 1966).

D) NaCl tolerance: Sodium chloride tolerance determined by distributing starch-nitrate agar) containing different concentration of NaCl (1-12%) in sterile petri dishes. The selected organisms were streaked and incubated at 28°C for 7-14 days. After incubation period, the growth of tested organisms was examined (Kutzner, 1981).

E) Cellulose-decomposition: Test tubes containing strips (20 X 100mm) of Whatman filter paper No. 1 (cellulose source) and10 ml of Hutchinson liquid

medium were inoculated with the studied isolate. The inoculation was carried out by transferring a loop of spore suspension of the actinobacteria to the surface of the filter paper, as near as possible to the level of the liquid medium. After 15, 21, 30 and 45 days of incubation at 28°C, the filter paper strips were examined. The destruction or rupture of paper was considered to indicate positive ability of the organism to decompose cellulose (Shirling and Gottlieb, 1966).

F) Casein hydrolysis: Agar plates of starch casein agar were spot-inoculated with each isolate and incubated at 28°C. The appearances of clear zone around growth indicate positive casein hydrolysis (Mansour, 1972). After incubation (7-14days), zones of clearance were noted and confirmed by flooding the plates with acid mercuric chloride.

G) Coagulation and peptonization of milk: Test tubes containing sterile defatted milk broth were inoculated with the studied isolate and incubated at 28°C, the degree of either coagulation or liquefaction of milk, type of growth and pigmentation were recorded after 3, 7 and 14 days of incubation (Nonomura, 1974).`

H) Gelatin liquefaction: Test tubes containing 5 mL of gelatin-peptone broth were sterilized and inoculated by dipping a needle carrying the spores into the slants containing gelatin medium. The slants were incubated at 28°C for 3, 7, a 14 days. After incubation, the culture was put in a refrigerator for few hours and the results were recorded at room temperature. The degree of liquefaction of gelatin was determined by comparison with non-inoculated medium (Nonomura, 1974).

Extraction and quantification of DNA (using Nano drop instrument) from *Streptomyces sp* AEFO2 (HM775973.1GI:302495616). The standard phenol-chloroform DNA extraction procedure was performed as described in Massana *et al.*, (1997).

RESULTS AND DISCUSSION

Exposure of microbial cell to ionizing radiation set off a chain of reactions giving rise to physiological changes and antimicrobial activities. These changes depend on the absorbed does. So, radiation presents an additional stress to the cells, which tends to disturb their organization. As a consequence of this biochemical damage, a great variety of changes can be observed in irradiated cells, some are temporary and other is permanent. Difference in or modification to the probability that a cell will survive a particular dose of radiation is measured in terms of differences or changes in radiation sensitivity or radiation resistance (Tallentire, 1967). Early experiments showed that ionizing radiation kills microorganisms through their direct and indirect effect. Moreover, the biological effect of radiation is amplified by the oxygen always present in the medium (Yarmonenko, 1988). A variety of heritable changes affecting colony morphology and color are induced in a *Streptmnyces* sp. by exposure of the spores to ultraviolet and gamma rays according to Newcombe (1953). The effect of different increasing doses of gamma radiation on the studied isolate indicated that the lethal dose of this isolate was 5 $KG_{y.}$ So, the antimicrobial activity was studied at (0.5–4.5 kG_y), and the other properties (morphological and physiological) studied only at the dose of 3.5 KG_y the does that showing the highest antimicrobial activity.

Morphological properties

Morphology of spore chain: The spore chain morphology is recti flexibiles (RF.) when examined under light microscope, but there was no distinct difference between irradiated sample and non-irradiated one. That may mean there was no effect of gamma ray at the dose 3.5 KG_y on the spore chain morphology of *Streptomyces* sp (AEFO2).

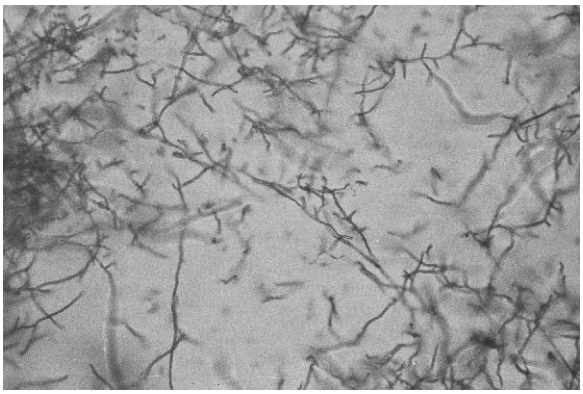

Figure 1: Morphology of spore chain of *Streptomyces* sp AEFO2 (X1200)

The spore surface ornamentation: The spore surface ornamentation appears smooth shape before and after irradiation but some deformations in the chain of conidia might happen for example; some spores appeared elongated and compressed in the middle to form bone shape and others enlarged in size (fig. 2).

On the other hand, increasing doses of gamma radiation increase the pigmentation of the organism either in the first or in the second generation. Fig. 3 showing pigmentation at the dose 3.5 KG_y for example. Accepted with this result physically induced mutations of *Aspergillus terrus* strain isolated and identified from Saudi Arabia were studied by exposing the conidia of this fungus to different doses of gamma rays resulted in the induction of autotrophic and color mutants of conidia of *A. terrus* (Mutwakil, 2011).

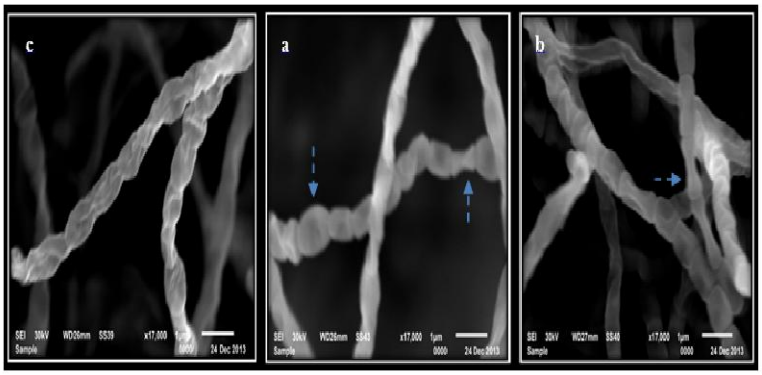

Figure 2: Scanning electron micrograph of spore surface of *Streptomyces* sp AEFO2. c: control, a: first generation after irradiation, b: second generation after irradiation.

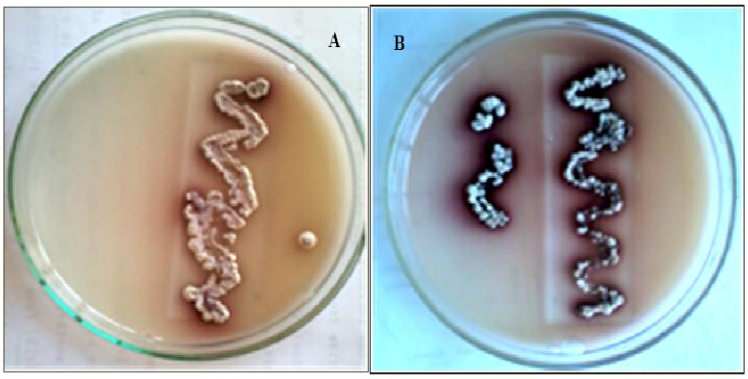

Figure 3: Effect of gamma radiation on the pigmentation of the *Streptomyces* sp (AEFO2) at the dose of 3.5 KG_y. A: control isolate, B: irradiated isolate.

Table 1: Effect of different doses of gamma radiation on the antimicrobial potency of *Streptomyces* sp. (AEFO2)

Dose Pathogen	Diameter of inhibition zone by mm, 1 is 1st and 2 is 2nd, dose of radiation by KG_y																			
	control		0.5		1		1.5		2		2.5		3		3.5		4		4.5	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Klebsiella	11.6	11.6	14	11	14	13	-	-	13	14	13	13	12	14	13	15	10	11	11	11
Pseudomonas aueroginosa	12	13	13	-	14	13	16	13	19	16	19	14	19	18	22	19	15	14	10	9
Staphylococcus aureus	12	13	15	-	14	14	14	-	-	13	14	14	-	15	16	16	-	17	-	15
Shigella spp	12.6	13	-	12	-	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	13	12	15	14	15	15	15	14	15	13	15	15	16	15	16	16	14	15	13	12
Streptococcus pyogenies	11	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erwinia carotovora	13	13	-	11	-	11	-	-	-	-	11	16	11	16	11	16	-	10	-	-
Staphylococcus epidermales	14	14	13	13	13	-	14	13	14	13	15	13	15	15	14	15	15	13	12	13
Proteus valgaries	16	16	12	18	-	18	15	15	13	16	-	16	14	14	15	15	14	13	11	10
Candida boidinii	13	13	-	-	11	-	11	-	-	-	-	-	-	15	-	14	-	-	-	-
Aspergillus flaviries	14	12	12	12	-	13	12	13	13	11	13	11	13	13	16	14	11	-	11	-
Aspergillus ochroceus	13	13	11	11	13	15	10	15	11	13	14	13	15	15	19	15	12	16	10	10
Trichothecium spp	13.6	13.6	21	-	21	-	15	-	15	-	25	-	25	-	25	-	13	-	-	-
Penicillium purpurgenium	-	-	-	-	8.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporium	-	-	11	-	-	-	11	-	-	-	-	-	12	-	13	-	-	-	-	-
Fusarium solani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Screening of Actinobacterial isolates for antibiotics producing potentiality

The antagonistic action of *Streptomyces* sp (AEFO2) against several pathogenic organisms based on degree of growth inhibition is summarized in the previous table. The antimicrobial activity of the studied isolate as shown in Table (1) increases with the increase of the radiation dose from 0.5KG_y to 3.5 kG_y .when compared with control, then begin to decrease after the dose of 3.5 kG_y which shows the highest antimicrobial activity with Gram+ve bacteria (*Staphylococcus aureus*), and Grame-ve bacteria (*Pseudomonas aueroginosa, Klebsiella, Escherichia coli* and *Erwinia carotovora*) and also with fungi (*Aspergillus flaviries* and *Aspergillus ochroceus*). *Streptomyces sp (AEFO2)* showed high antimicrobial activity with *Trichothecium spp* at different increasing doses of radiation which appeared only in the first generation but disappeared in the second generation. Previous studies on two thermophilic isolates identified as *Streptomyces albaduncus* and *S. erythogresius* showed the highest antimicrobial activities against bacteria, moulds and yeasts among them. Dose level 2 kG_y enhanced the antimicrobial activity of both isolates either at first or second generation. Relativity higher doses enhanced the utilization of carbon sources and increased their sodium chloride tolerance from 8 to 10% (Moussa *et al.*, 2005). Also the induced mutagenesis using gamma rays proved to be effective in enhancing antifungal metabolites production in three species of *Tichoderma* (Haggag and Mohamed, 2002).

Physiological Properties

A) Utilization of different carbon sources: Utilization of different carbon sources by the studied isolate at 3.5KGy had several changes, some was temporary and other was permanent. Maltose, mannose, sucrose, xylose and manitol represent the highest activity in both 1st & 2nd generations. Also presence of pigmented crystals observed mainly using glucose, maltose and sucrose as Carbone sources Table (2). Previous studies indicated the ability of

Streptomyces sp to utilize different carbon sources, and the enhancement of the biosynthesis of deoxyribonucleases of several actinomycetes (Wlachow, 1980). Also the gamma irradiation process enhanced the utilization of different carbon sources in two *Streptomyces* sp (Moussa *et al*., 2005).

B) *Production of melanin pigments:* The radiation process affects in the production of melanin pigment Table (3) at the radiation dose of 3.5KG_y mainly present in solid medium specially peptone yeast extract iron agar Fig.(4) in both first and second generation. Many studies have demonstrated that melanin pigment has been associated with microorganism radio resistance. For example; *Alternaria* spp was tolerant to doses of up to 4 kG_y because of its ability for melanin production (Mironenko *et al*., 2000). This agree with our results explained that production of melanin increases with the increase of radio resistance.

Table 2: Effect of dose (3.5KG_y) of gamma radiation on the utilization of different carbon sources

Sample	Control		1st generation		2nd generation	
Carbon sources	(1)	(2)	(1)	(2)	(1)	(2)
+ve control	+++	+++	++++	++++	++++	++++
-ve control	-	-	-	-	-	-
Sucrose	+++	+++	+++	+ ++	++ +	+ ++
D-Fructose	++	++	++	++	++	++
Raffinose	++	++	+	+	+	-
Maltose	++	++	+++	+++	+++	+++
L-lactose	++	++	++	++	+	+
L-Arabinose	+	+	++	++	++	++
Mannose	++	++	+++	+++	+++	+++
D+ xylose	++	++	+++	+++	++	++
D- manitole	+++	+++	+++	+++	+++	+++
Sorbitol	+	+	+	+	+	-
Starch	++	++	++	++	++	++

- no growth +: little growth, ++: moderate growth, +++: heavy growth, ++++very heavy growth, 1 and 2 refers to replica.

Table 3: Effect of dose (3.5KGy) of gamma radiation on the production of melanin pigment of studied isolate

Type of media	Control		1st generation		2nd generation	
	1	2	1	2	1	2
Glycerol tyrosine agar	+	+	++	++	++	+ +
Peptone yeast extract iron agar	+	+	+	++	++	++
Tryptone yeast extract broth	+	+	+	+	+	+

+ Less productivity of melanin, ++ high productivity of melanin, 1 and 2 refers to replica.

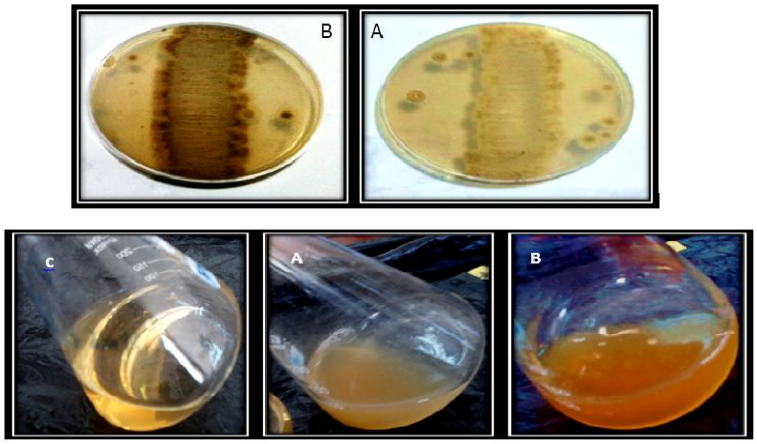

Figure 4: Effect of dose (3.5KG_y) of gamma radiation on the production of melanin pigment of *Streptomyces* sp (AEFO2) on peptone yeast extract iron agar and Tryptone yeast extract broth, A: control, B: irradiated and C: -ve control.

C) *Hydrolysis of starch:* The ability of the *Streptomyces* sp to utilize any substrate depends on its ability for produce enzymes for hydrolyzing this substrate. *Streptomyces* sp (AEFO2) has the ability to produce amylase enzymes which enhanced by gamma radiation at dose of 3.5KG_y Fig. 5. This result accepted with the results obtained by Moussa *et al*., (2005).

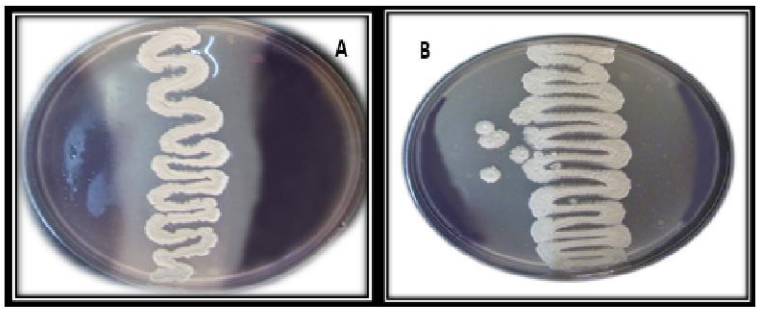

Figure 5: Starch hydrolysis activity of *Streptomyces* sp (AEFO2), A: control and B: irradiated

Table 4: Effect of dose (3.5 KG_y) on NaCl tolerance of *Streptomyces* sp (AEFO2)

Nacl (con.) / Sample	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
Control	+++	+++	++	++	+	-	-	-	-	-	-	-
1st generation	++++	+++	+++	+++	++	++	+	+	-	-	-	-
2nd generation	++++	+++	+++	++	++	+	+	+	-	-	-	-

- No growth, +: little growth, ++: moderate growth, +++: heavy growth, ++++very heavy growth.

D) *NaCl tolerance:* The increase in salinity tolerance due to radiation (3.5KG$_y$) represented in both generations from5% to 8% when compared with control Table (4). Moreover, dose level of 1-4 kG$_y$ increased the tolerance to NaCl. However, in case of 9-12% NaCl negative growth was occur in all treatments. The radiation induced effect on salt tolerance studied isolate is unclear. It is presumably that NaCl acted as salting-out agent that maintains the stability of different enzymes in both isolates (Shevchuk *et al.*, 1987). Moussa *et al.*, (2005) reported that the tolerance to NaCl of two *Streptomyces* sp was released to 8%.

E) *Cellulose-decomposition:* Data indicated that the *Streptomyces* sp (AEFO2) had the ability to cellulose hydrolysis due the production of cellulase enzymes (McCarthy, 1987), also irradiation process indicated that there is no enhancement of productivity of cellulase enzymes Table 5. Alike Moussa *et al.*, (2005), stated that no enhancement of productivity of cellulase enzymes was found on the *Streptomyces albaduncus*.

Table 5: Effect of dose (3.5KG$_y$) on Cellulose-decomposition of Streptomyces sp (AEFO2)

Sample	1	2
Control	+	+
1st generation	+	+
2nd generation	+	+

-, + indicate negative and positive results respectively, 1 and 2 refers to replica.

F) *Casein hydrolysis:* Previous studies indicate that actinomycetes are able to hydrolyze casein (Mohamedin, 1999), and that appeared previously with *Streptomyces* sp (AEFO2) which was able to hydrolyze casein by Caseinase enzyme increased by irradiation process. Does up to 3.5KG$_y$ enhanced the productivity of Caseinase enzyme of the *Streptomyces* sp (AEFO2). *Streptomyces albaduncus* and *S. erythogresius* showed similar result when irradiated by increasing doeses of gamma rays up to 5 KG$_y$ (Moussa et *al.*, 2005).

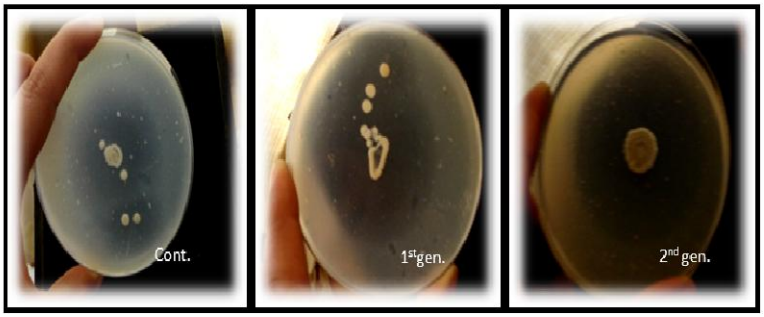

Figure 6: Increasing of casein hydrolysis activity in the first and second generations of *Streptomyces* sp (AEFO2)

G) *Coagulation and piptonozation of milk:* The effect of *Streptomyces malvinense* nov. on coagulation and peptonization of milk was demonstrated by Cercos (1977). The activity of production of proteolytic enzymes for *Streptomyces* sp (AEFO2) increased by the irradiation process in both 1st and 2nd generation (Table 6) alike that obtained Moussa *et al.*, (2005).

Table 6: Effect of dose (3.5KGy) on Coagulation and peptonization of milk (production of proteolytic enzymes).

Sample	1	2
Control	+	+
1st generation	++	++
2nd generation	++	++

+: moderate growth, ++: heavy growth, 1and 2 refers to replica

H) *Gelatin liquefaction*: Several actinomycetes had the ability for Gelatin hydrolysis and the others did not. *Streptomyces* sp (AEFO2) was able to hydrolyze Gelatin substrate and this ability increased by irradiation (Table7).

Table 7: Effect of dose (3.5KGy) on gelatin liquefaction

sample	1	2
Control	+	++
1st generation	+++	+++
2nd generation	+++	++

+ Little growth ++: moderate growth, +++: heavy growth, 1and 2 refers to replica

Extraction and quantification of DNA

The total amount of DNA for *Streptomyces* sp (AEFO2) decreased with the increasing doses of gamma radiation (Table 8). Similarly, Aly (1985) who reported that when *S. lipmani* cell was irradiated with gamma rays the amount of DNA was decreased. Also, all irradiation doses used (0.25-5.0 kGy) decreased the cell DNA content in both *S. albaduncus* and *S. erythogresius* with the exception of dose 2 kGy which increased the cell DNA content (Moussa *et al.*, 2005). That result indicated that DNA may increase or decrease depending on the dose or may also the type of radiation.

Table 8: Extraction and quantification of DNA from *Streptomyces* sp AEFO2 (HM775973.1G:302495616)

Sample	Amount of DNA (µg/gm fresh weight)
Control	16.18 %
First generation	5.05 %
Second generation	1.64 %

CONCLUSION

The ionizing radiation like gamma rays make a series of events on various physiological and morphological properties in addition to nucleic acid of the studied actinobacterium strain. So, gamma rays shows potent stress to the actinobacterial cells. AS a

result of this, great changes occur to the studied strain *Streptomyces* sp AEFO2 (HM775973.1G:302495616). The exposure to optimum gamma rays dose level of 3.5 KG_y stimulate antimicrobial activity, pigmentation, melanin production, carbon source utilization (specially maltose and sucrose), NaCl tolerance till 8% and causes decline in DNA amount using nano drop instrument analysis. All of previous results were performed in both first and second generations. So, finally we have a great variety of changes that can be observed in irradiated cells ether temporary or permanent.

REFERENCES

1. Aly MF. Studies on some actinomycetes isolates. Ph.D. Thesis, Microbiology, Faculty of Sci. Zagazig Univeristy, Zagazig, Egypt, (1985).

2. Berdy J. Bioactive microbial metabolites. J Antibiot, (2005). 58: 1–26.

3. Bergey's Manual of Determinative Bacteriology by William STR, Henslyl and Harriet Felscher, (1989). Volume 4. The Wilkins Col. Baltimore.

4. Bhatnagar I and Kim SK. Immense essence of excellence: marine microbial bioactive compounds. Marine drugs, (2011). 8: 2673-2701.

5. Cercos AP. Description of Streptomyces malvinense nov.sp. Isolated from soil of Islas Malvinas. Rev. Assoc. Argent Microbiol., (1977). 9: 17–21

6. Chater KF. Morphological and physiological differentiation in *Streptomyces*. In Microbial Development, Edited by R. Losick & L. Shapiro. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory, (1984). 89-115.

7. Cragg GM, Kingston DGI and Newman DJ. Anticancer Agents from Natural Products. Taylor & Francis, (2005). 23:676-686.

8. Ensign JC. Formation properties and germination of actinomycete spores. Annual Review of Microbiology, (1978). 32: 185-219.

9. Gottllieb D and Shirling EB. Cooperative description of type cultures of Streptomycetes. I. The International of Streptomyces Project. Int. Syst. Bacteriol., (1967). 17: 315–76.

10. Haggag WM and Mohamed HAA. Enhancement of antifungal metabolites production from Gamma-ray induced mutants of some Trichderma species for control onion white rote disease. Plant Pathol. Bull., (2002). 11:45-56.

11. Hamdali H, Hafidi M, Virolle MJ and Ouhdouch Y. Rock phosphate solubilizing Actinomycetes: screening for plant growth-promoting activities. World Microbiol. Biotechnol., (2008) 24: 2565-2575.

12. Hei TK, Wu LJ, Liu SX, Diane V, Waldren CA and Randers-Pehrson G. Mutagenic effects of a single and an exact number of alpha particles in mammalian cells. Proc Natl Acad Sci USA, (1997). 94:3765-3770.

13. Huang WY, Cai YZ, Xing J, Corke H and Sun M. Apotential antioxidant resource: endophytic fungi from medicinal plants. J. Econ. Bot. 61, 14–30. J. Microbial., (2007). 9: 30-36.

14. Kutzner HJ. The family Streptomycetaceae In: Starr MP, Stolp H Trüper, HG Balons and A Schlegel HG. (eds.): The Prokaryotes-A handbook on habitats, isolation and identification of bacteria Springer Verlag, Berlin. (1981). pp. 2028-2090.

15. Mann J. Natural products as immunosuppressive agents. Nat Prod Rep., (2001). 18: 417-430.

16. Mansour FA. Physiological and biochemical studies on some local *Streptomyces* with special reference to streptomycin production. M.Sc. Thesis, cairo university, cairo, Egypt,(1972).

17. Massana R, Murray AE, Preston CM and Delong EF. Vertical Distribution and Phylogenetic Characterization of Marine Planktonic Archaea in the Santa Barbara Channel. Applied and environmental microbiology, (1997). 63: 50–56.

18. McCarthy AJ. Lignocellulose–degrading actinomycetes. FEMS Microbio. Lett., (1987). 46: 136–45.

19. Mironenko NV, Alekhina IA, Zhdanova NN and Bulat SA. Intraspecific Variation in gamma-radiation resistance and Genomic Structure in the filamentous fungus *Alternaria alternata*: a Case study of strains inhabiting Chernobyl reactor no. 4.Ecotoxicology and Environmental Safety, (2000). 45: 177–187.

20. Mohamedin AH. Isolation, identification and some cultural conditions of a proteas–producing thermophilic Streptomyces strain grown on chicken feather as a substrate. Int. Biodegredation, (1999). 43: 13–21.

21. Moussa LAA, Mansour FA, Serag MS and Abou El–Nour, SAM. Effect of gamma irradiation on the

Physiological Properties and Genetic Materials of Streptomyces albaduncus and *S. erythogresius* Int. J. Agri. Biol., (2005). 7: 197–202.

22. Muller J. Artificial transmutation of the gene. Science, (1927). 66: 84-87.

23. Mutwakil MHZ. Mutation Induction in *Aspergillus terrus* Using N-Methyl-N'-Nitro-N-Nitrosoguanidine (NTG) and Gamma Rays. Australian Journal of Basic and Applied Sciences, 2011, 12: 496-500.

24. Newcomb HB. Radiation -induced Instabilities in Streptormyces. J. Microbial., (1953). 9: 30-36.

25. Nonomura H. Key for classification and identification of 458 species of the Streptomycetes included in ISP. J. Ferment. Technol., (1974). 52: 78 - 92.

26. Oldfield C, Wood NT, Gilbert SC, Murray FD and Faure, FR. Antonie Van Leeuwenhoek, (1998). 74:119-132.

27. Seifried HE, Anderson DE, Fisher EI and Milner JA, A review of the interaction among dietary antioxidants and reactive oxygen species. J. Nutr. Biochem., (2007). 18: 567–579.

28. Servin JA, Herbold CW, Skophammer RG and Lake JA. Evidence excluding the root of the tree of life from the actinobacteria. Mol. Biol. Evol., (2008). 25 (1): 1-4.

29. Shevchuk IA, Dubchenko YG and Grudneva GE. Solvent extraction isolation and separate determination of glyphosphate and dichlorvos in aqueous solution. J. Analyt. Chem., (USSR), (1987). 42: 267–70.

30. Shirling EB and Gottlieb D. The cultural, morphological and physiological Methods for characterization of streptomycetes characteristics of the experimental isolate appeared to be species. International J. Systematic Bacteriol, (1966). 16: 313-340.

31. Tallentire A. Radio sterilization of medical products, pharmaceuticals and bio products. Techl. Report Series No. 72, International Atomic Energy Agency, Vienna. (1967).

32. Tresner HD, Hyes JA and Backus EJ. Differential tolerance of streptomycetes to sodium chloride as a taxonomic aid. Appl. Microbiol., (1968). 16: 1134–6.

33. Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF and van Sinderen D. Genomics of Actinobacteria: Tracing the Evolutionary History of an Ancient Phylum. Microbiology and Molecular Biology Review. (2007). s71: 495–548.

34. Williams ST and Davies FL. Use of a Scanning Electron Microscope for the Examination of Actinomycetes. J. Microbiol., (1967). 48: 171-177.

35. Wlachow SS. Effect of carbon and nitrogen sources on the deoxyribonucle activity of actinomycetes. Zentralbl Bakteriol Naturwiss, (1980). 135: 70–67.

36. Wong TV and Smith KC. Enzymatic photoreactivation of *E. coli* after ionizing irradiation: Chemical evidence for the production of pyrimidine dimers. Rad. Res., (1978). 76: 540–8.

37. Yarmonenko SP. Radiobiology of Humans and Animals. Mir Publisher, Moscow, (1988).

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