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Effect of Gamma Radiation on the Physiological Properties and Genetic Materials of *Streptomyces albaduncus* and *S. erythogresius*

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ABSTRACT

Out of 14 isolates of actinomycetes, isolated from Egyptian soils, the two thermophilic isolates identified as *Streptomyces albaduncus* and *S. erythogresius* showed the highest antimicrobial activities against bacteria, moulds and yeasts among them. Both isolates were exposed to increasing doses of gamma radiation up to 5 kGy. All radiation doses used did not affect the physiological properties, but relativity higher doses enhanced the utilization of carbon sources and increased their sodium chloride tolerance from 8 to 10%. Dose level 2 kGy enhanced the antimicrobial activity of both isolates either at first or second generation. All radiation doses used increased the amount of RNA, while higher radiation dose levels (2-5 kGy) decreased the amount of DNA.

Key Words: Gamma Radiation; Actinomycetes; *Streptomyces albaduncus; S. erythogresius*

INTRODUCTION

Physiological criteria such as the ability to degrade different substrates, casine, and xanthine were used for genus determination. Streptomyces are obligate aerobes, chemoorganotrops that need only an organic carbon source (such as glucose, starch, and glycerol), an inorganic nitrogen source, and a few mineral salts for grow. 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 & Shirling, 1967).

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

The most interesting aspect of the antibiotic activity is the variety of their mechanisms of action. The mechanism of action of an antibiotic is the biochemical event by which the growth of sensitive microorganisms is inhibited. This is the result of the interference of the antibiotic with a molecule, called the target molecule, essential for the cell metabolism. Target molecules are normally macromolecules such as DNA, RNA, and enzymes, but are occasionally small metabolites, such as substrate of enzymatic reaction or membrane components. Strictly speaking, understanding the mechanism of action of an antibiotic implies the identification not only of the target molecules, but also of the site and of the type of interaction. This has been determined for a large number of antibiotics. However, it is easier to identify the metabolic pathway that is blocked than the specific molecule involved, and form the practical point of view, this is often sufficient. For this reason, one normally speaks of antibiotics that inhibit the synthesis, or cell membrane functions. The specificity of the mechanism of action is the main reason for the selectivity of action of the antibiotics. When, for instance the target molecule of the bacterial cell has no equivalent in mammalian cells, or the composition of its mammalian counterpart is substantially different, the antibiotic will in principle, be selectively active against bacteria and non toxic for higher organisms (Lechevalier *et al.*, 1988).

Streptomycetes, as other actinomycetes, are remarkable for a characteristic form of genetic instability. Many different traits, such as aerial mycelium and spore formation, antibiotic production and resistance, enzymes of arginine biosynthesis, are subjected to an irreversible loss with a frequency of 0.1-1% of the progeny of plated colonies. This loss of function is the result of the deletion of DNA segments, often associated with amplification of neighboring DNA segment. The mechanism underlying this and other forms of instability is at present being intensively studied (Giancarlo & Rolando, 1993). The Streptomyces genome (4-5 x $l0^9$ Dal-6000-7500 Kb) is about 1.5 times larger than that of the genome of *Escherichia coli* (4700 Kb), and it has a high GC content (71-76%) (Mohamedin, 1993). Early experiments showed that ionizing radiation kills microorganisms through their direct and indirect effect. The direct effects are mean changes such that appear as a result of the absorption of radiation energy by the molecules being studied "targets". But, the indirect effects are mean changes in the molecules in a solution caused by the free radicals and other reactive molecules which formed from radiation decomposition (radiolysis) of water or other solutes.

The free radicals and other end products formed upon

radiolysis of water, which in the bulk (up to 90%) of the mater in living cells, is the most important in the indirect effect of irradiation. Moreover, the biological effect of radiation is amplified by the oxygen always present in the medium (Yarmonenko, 1988). The death of microorganisms is a consequence of the direct ionizing action of high-energy radiation. Most studies indicate that the lethal damage of microbial DNA (resulting in loss of it's ability to reproduce) is a primary cause of lethality, beside the damage to other sensitive and critical molecules (e.g. in membranes). Indirect effect resulting from free radicals and other reactive molecules which formed from radiolysis of water in living cells or foods as well as direct effect of ionizing energy are responsible for the lethal damage to microorganisms. The destructive effects and mutations from radiation were originally thought to be due primarily to direct content of high-energy rays and particles with vital centers of microbial cells. Highly free radicals resulting from water hydrolysis are most important factors contributing to lethal and sublethal changes in microbial cells (Ingram & Roberts, 1980).

Ionizing radiation as a mutagenic agent has been studied (Wong & Smith, 1978). Morse and Carter (1949) found that in the irradiated cells of *E. coli*, RNA content per cell increased five to ten folds and DNA and nitrogen from two to three folds during the lag phase. Mutagenic effects are expected to increase as the irradiation dose increased; on the other hand, for such mutation to be expressed, the mutated microorganisms must survive and multiply. Low-dose irradiation of microorganisms may produce mutations which may conceivably be describe in producing products of ultimate importance such as antibiotic, organic acids, amino acids, vitamins, alcohol's etc. Casarett (1968) reported that, radiation doses required to produce measurable change in the common catabolic processes are higher than that necessary to decrease survival cells of microorganisms. Olive (1998) proved that, ionizing radiation produces many types of DNA lesions that have the potential of killing microbial cells.

MATERIALS AND METHODS

Microorganisms. Two thermophilic actinomycetes **(***Streptomyces albaduncus* and *S. erythogresius*) were isolated from egyptian soils and identified by Moussa *et al.* (2003)

Physiological Properties of Actinomycetes Isolates

Production of melanin pigments. The experimental isolates were cultivated on slants of glycerol- tyrosine agar, peptone-yeast extract-iron agar and tryptone-yeast extract broth. The colour of the substrate mycelium and media were recorded after 2, 3, 7, 14 and 21 days of incubation at 50°C. The production of brown or dark brown soluble pigments, at early stages of growth, indicated positive melanin production activity.

Utilization of different carbon sources. The ability of isolates to use different carbon sources and the effect of these carbon sources on the growth and pigmentation were studied on the basal mineral salts broth medium No. I (Shirling & Gottlieb, 1966). The carbon sources used are D-glucose (as positive control), L-arbinose, sucrose, D-xylose, I-inositol, D-fructose, L-rhamnose, raffinose, D-maonitol, melibiose, lactose, salicin and mannose. All of these carbon sources are chemically pure and free from contaminating materials, and separately sterilized without heat. An appropriate weight of the carbon source was spread as a shallow layer in a pasterilized Erlenmeyer flask with a loose cotton plug. Ethylether was added to cover the carbon source. The ether was allowed to evaporate at room temperature. After complete evaporation of the ether, sterile distilled water was added aseptically to make (10% w/v) solution of the carbon source. The sterilized carbon sources were added singly to the basal mineral salt broth medium to give a final concentration (1%). The pH of the medium was adjusted to 6.8-7.0 before autoclaving. After autoclaving of the basal medium it was, cooled to 60°C and then the sterile carbon sources were added aseptically to give a concentration of 1%, and then distributed into sterile test tubes. Test tubes containing the liquid medium were inoculated by the isolates under investigation and incubated at 50°C. After the incubation period (10-16 days), the growth was observed and compared with the positive control (basal medium with glucose) and the negative control (basal medium with no carbon source).

NaCI tolerance. Sodium chloride tolerance determined by distributing (gauze No. l agar medium) containing different concentration of NaCI (2-12%) in sterile petri dishes. The selected organisms were streaked and incubated at 50°C for 7 days. After incubation period, the growth of tested organisms was examined.

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 50°C for 7, 14, and 21 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.

Coagulation and peptonization of milk. Test tubes containing sterile defatted milk broth were inoculated with the studied isolates and incubated at 50°C, the degree of either coagulation or liquefaction of milk, type of growth and pigmentation were recorded after 3, 7,14, and 21 days of incubation.

Exoenzymes. The techniques employed for testing the activity of the exoenzymes are similar to those described by (Shirling & Gottlieb, 1966) and are summarized as follow:

Amylase activity. Starch-nitrate agar medium was distributed in sterile petri dishes and inoculated by streaking of the selected organisms and incubated at 50°C for 7, 14, and 21 days. After incubation, the plates were flooded with one half-strength Lugol's iodine solution, to determine the

amylase activity; the presence of clear zones around the growth indicated positive activity.

Caseinase activity. Caseinase activity was determined by distributing starch-casein agar medium in sterile petri dishes. The selected organisms were streaked and incubated at 50°C for 7, 14, and 21 days. After incubation, zones of clearance were noted and confirmed by flooding the plates with acid mercuric chloride.

Lipase activity. Starch-casein agar medium was distributed in conical flasks and autoclaved. The cotton seed oil, to which ether was added, heated on a water bath until the evaporation of ether and sterilization of oil. One-percent sterile cotton seed oil was introduced into each flask containing the sterile medium. The prepared medium was distributed into sterile petri dishes. The selected organisms were streaked and incubated at 50°C for 7, 14, and 21 days. Lipase activity was noted by a clearance of the fine oil droplets in the zone surrounding the growth of the organisms. The faint visible zone of lipase activity was accentuated by the addition of 20% copper sulphate solution to the plates.

Cellulase activity. Test tubes containing strips (l x 5 cm) of Whatman No. l filter paper and 10 mL of Hutchinson broth medium were inoculated with the studied isolates. The inoculation was carried out by transferring a loop of spore suspension to the surface of the filter paper, as near as possible to the surface of the filter paper, and as near as possible to the level of the liquid medium. After incubation at 50°C for 15, 21, 30, and 45 days, the filter paper strips were examined. Growth of the organism on the strips of the filter paper or appearing any destruction or rupture of paper was indicated positive ability of the organism to decompose cellulose. The type of growth and pigmentation of cultures were also recorded.

H_2S production. Peptone yeast extract-iron agar medium was distributed in sterile petri dishes and inoculated by streaking of the selected organisms and incubated at 50°C for 7, 14, and 21 days. The blackening of peptone yeast extract-iron agar indicates clearly the formation of H_2S by actinomycetes (Kuster & Williams, 1964).

Nitrate reduction. Nitrate reduction test was detected by using Griess-Ilosvay reagents I and II [sulphanilic acid (reagent I) & a-naphthylamine (reagent II)]. Test tubes containing 5 mL of Gilaty broth medium were inoculated with spore suspension of the studied isolates and incubated at 50°C for 7, 14, and 21 days. Three mL of each culture were transferred into sterile test tubes to which 1 mL of reagent I and 0.5 mL of reagent II were added. The appearance of a purple colour after 48 h incubation at 45C^o indicated the reduction of nitrate to nitrite. In the absence of a purple colour, zinc dust was added, the appearance of a purple colour indicated that no reduction of nitrate to nitrite happened; whereas, the absence of the colour indicated the reduction of nitrate to ammonia which was confirmed by the formation of brown precipitate after the addition of Nessler reagents (Allen, 1953).

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 kGy/hour.

Effect of gamma radiation on the antibiotic, biomass yield and the synthesis of nucleic acids. The irradiate actinomycetes spores which previously exposed to various irradiation doses were used in studying the effect of gamma radiation on the production of antibiotic-like substances and pigments as well as its effect on the biomass yield and the synthesis of nucleic acids of the investigated actinomycetes. One mL of irradiated actinomycetes spores (each dose) was inoculated in 250 mL conical flask contains 100 mL of starch-nitrate broth medium. The inoculated flasks were incubated at 50^{o}C for 15 days. For testing the antimicrobial activity resulting from the production of antibiotic-like substances and measuring biomass yield, the culture broth was filtered using preweight Whatman No. 1 filter paper. The biomass precipitated at Whatman No. 1 was determined by drying till constant weight, 0.2 mL of the filtrate was used for biological assay of antimicrobial agents as previously described**.**

Extraction of and quantification of DNA and RNA. The method reported by Renee and Joan (1993) and detailed by El-Fouly *et al.* (2002) was used.

RESULTS AND DISCUSSION

Exposure of microbial cell to ionizing radiation set off a chain of reactions giving rise to chemical and then to metabolic or physiological changes. So, radiation presents an additional stress to the cells, which tends to disturb their organization. The extent of this disturbance would depend partly on the amount of chemical changes, which in turn, will depend on the absorbed dose. 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 are measured in terms of differences or changes in radiation sensitivity or radiation resistance (Tallentire, 1967).

In the present work the effect of different increasing doses of gamma radiation (0.25–5.0 kGy) on the physiological, biochemical, nucleic acids (DNA and RNA) synthesis, antimicrobial activities and biomass yield of both isolates *S. albaduncus* and *S. erythogresius* were investigated at first and second generation.

All the irradiation doses used (0.25-5.0 kGy) showed no obvious effect on production of melanin pigment (Table I). For physiological properties tested, i.e, starch hydrolysis, casein hydrolysis, lipid hydrolysis. cellulose decomposition, gelatin liquefaction, coagulation and peptonization of milk, reduction of nitrate to nitrite and production of H_2S (Table II). The data show that the growth of both isolates *(S.*

albaduncus and S. ergthrogresius) gave positive results in case of starch hydrolysis, casein hydrolysis, lipid hydrolysis and coagulation and peptonization of milk indicating the ability of the isolates to hydrolysis them, i.e., having the ability to produce enzymes for hydrolyzing these substrate. Meanwhile, *S. albaduncus* failed to hydrolyse cellulose to reduce of nitrate to nitrite and produce H_2S. *S. ergthrogresius* gave negative results only in case of gelatin liquefaction. Other studies indicate that actinomycetes are able to hydrolyze cellulose (McCarthy, 1987), casein (Mohamedin, 1999), and lipid (Sharma *et al.*, 2001). The effect of *Streptomyces malvinense* nov. sp. on coagulation and peptonization of milk and uses different carbon and nitrogen sources was demonstrated by Cercos (1977). On the other hand, *S. albaduncus* may not contain the enzymes for cellulose hydrolysis and nitrate reduction, and similarly is *S. erythogresius* for gelatin liquefaction.

High irradiation doses relatively enhanced the utilization of the tested carbon sources (14 sources) especially in second generation (Table III). The data show

Table I. Effect of different doses of gamma radiation on the production of melanin pigment of *S. albaduncus* and *S. erythrogresius*

Type of media	Radiation doses (kGy)																																					
	S. a lbaduncus																				*S. erythrogresius*																	
	Control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0		control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Glycerol tyrosine agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peptone yeast extract iron agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryptone yeast extract broth	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Data of first 1 and second 2 generations-, + are negative and positive, respectively.

Table II. Effect of different doses of gamma radiation on the physiological properties of *S. albaduncus* and *S. erythrogresius*

Physiological tests	Radiation doses (kGy)																																					
	S. albaduncus																				*S. erythrogresius*																	
	control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0		control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipid hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellulose decomposition	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coagulation and peptonization of milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reduction of nitrate to nitrite	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Production of H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Data of first 1 and Second 2 generations -, + are negative and positive, respectively.

Table III. Effect of different doses of gamma radiation on the utilization of different carbon sources of *S. albaduncus* and *S. erythrogresius* which reflected on their growth

Carbon sources	Radiation doses (kGy)																																					
	S. albaduncus																				*S. erythrogresius*																	
	control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0		control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
D- glucose	b	b	b	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	b	a	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	a
D- fructose	b	b	b	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	c	a	c	a	c	a	c	a	c	a	a	a	a	a	a	a	a	a	a
D- melibiose	d	d	d	c	d	c	d	c	d	c	c	c	c	c	c	c	c	a	b	a	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	a	a
D- mannitol	b	d	b	d	a	d	a	c	a	c	a	b	a	b	a	b	a	b	c	d	c	d	c	d	c	d	c	d	c	c	c	c	c	c	c	c	c	c
D- xylose	b	c	b	c	a	c	a	c	a	b	a	a	a	a	a	a	a	a	c	e	c	e	c	e	c	e	c	e	c	c	c	c	c	c	c	c	c	c
L- rhamnose	b	b	b	b	a	b	a	a	a	a	a	a	a	a	a	a	a	a	d	e	d	e	d	e	d	e	d	e	d	d	d	d	d	d	d	d	d	d
L- arbinose	c	c	c	c	b	c	b	c	b	c	b	b	b	b	b	b	b	b	b	c	b	c	b	c	b	c	b	c	b	b	b	b	b	b	b	b	b	b
Lactose	b	b	b	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	b	a	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	a
I- inositol	d	b	b	b	b	b	b	b	b	b	b	a	b	a	b	a	b	a	d	e	d	e	d	e	d	e	d	e	d	d	d	d	d	d	d	d	d	d
Sucrose	d	d	d	d	c	d	c	d	c	d	c	c	c	c	c	c	c	c	b	e	b	e	b	e	b	e	b	e	b	c	b	c	b	c	b	c	b	c
Salicin	c	c	c	c	b	c	b	c	b	b	b	b	b	b	b	b	b	b	b	c	b	c	b	c	b	c	b	c	b	b	b	b	b	b	b	b	b	b
Galactose	b	c	b	c	b	c	b	c	b	c	b	b	b	b	b	b	b	b	c	d	c	d	c	d	c	d	c	d	c	c	c	c	c	c	c	c	c	c
Raffinose	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	a	b	a	b	a	b	a	b	a	b	a	a	a	a	a	a	a	a	a	a	a
Mannose	b	b	b	b	b	b	b	b	b	b	b	a	b	a	b	a	b	a	c	d	c	d	c	d	c	d	c	d	c	c	c	c	c	c	c	c	c	c

Data of first 1 and Second 2 generations. a= very high growth, b = high growth, c = moderate growth, d = week growth, e = very week growth, f = no growth.

that (2.0–5.0) kGy in first and second generations of *S. albaduncus* and *S. ergthrogresius* can utilize different carbon sources as general. It is also clear that both isolates can utilize D-glucose, D-fructose, D-melibiose, D-mannitol, lactose and mannose greater than utilization of L-inositol, sucrose and raffinose (Table III). Moderate utilization occurred in case of L-arbinose and salicin. In The second generation for *S. ergthrogresius* very low utilization of D-xylose, L-arbinose, 1-inositol and sucrose was observed. The biosynthesis of deoxyribonucleases of several actinomycetes was enhanced when different carbon sources were used (Wlachow, 1980), which is consistant with the utilization of *S. albaduncus* and *S. ergthrogresius* for the carbon sources used in this study.

The data of Table IV show that both isolates *S. albaduncus* and *S. ergthrogresius* can grow in medium containing NaCl up to 8%. High dose level (2-5) kGy increased the tolerance to NaCl for both isolates. However, in case of 11-12% NaCl negative growth was occure in all treatments. Using *Aspergillus candidus*, Shevchuk *et al.*

Table IV. Effect of different doses of gamma radiation on NaCl tolerance of *S. albaduncus* and *S. erythrogresius*

NaCl conc. (%)	Radiation doses (kGy)																															
	S. albaduncus																		*S. erythrogresius*													
	Control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0		control		0.25		0.5		1.0		1.5		2.0		3.0		4.0		5.0
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+
10	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Data of first 1 and second 2 generations. -, + are negative and positive, respectively.

Table V. Effect of different doses of gamma radiation on the antimicrobial activity and biomass yield of *S. albaduncus* and *S. erythrogresius.*

Radiation doses (kGy)	*S. albaduncus*				*S. erythrogresius*			
	Inhibition zone (mm of 0.2 mL broth)		Biomass yield (mg/100 mL)		Inhibition zone (mm of 0.2 mL broth)		Biomass yield (mg/100 mL)	
	1	2	1	2	1	2	1	2
0.0	35	35	375	375	33	33	385	385
0.25	30	35	363	374	33	33	380	384
0.5	28	34	342	374	34	33	380	385
1.0	32	34	335	375	35	33	377	385
1.5	37	33	325	374	36	33	365	384
2.0	40	39	319	399	38	37	355	355
3.0	35	35	270	370	32	30	345	350
4.0	22	20	292	290	28	28	320	317
5.0	18	18	210	209	22	20	278	275

Data of first 1 and second 2 generations.

Table VI. Effect of different doses of gamma radiation on the nucleic acids (DNA and RNA) of *S. albaduncus* and *S. erythrogresius* during different incubation periods

Radiation doses (kGy)	DNA and RNA (mg/gm fresh weight) & Incubation periods (day)															
	S. albaduncus								*S. erythrogresius*							
	DNA				RNA				DNA				RNA			
	4	6	8	10	4	6	8	10	4	6	8	10	4	6	8	10
0.0	2.9	3.2	5.7	4.7	4.4	8.6	13	13.5	2.8	3.2	4.5	5.9	5.2	5.4	6.9	7.3
0.25	2.8	3.2	5.7	3.6	6.2	22.0	23.0	23.3	2.7	3.0	3.9	4.73	5.9	7.2	21.7	29.2
0.5	2.8	3.0	5.3	3.2	8.8	22.0	24.0	23.0	2.4	2.9	3.5	2.7	7.6	7.9	23.8	29.8
1.0	2.6	2.7	5.1	2.7	14.4	22.5	24.0	22.0	2.1	2.6	3.1	1.9	14.8	18.4	25.7	32.3
1.5	2.2	2.4	5.1	2.3	22.0	26.0	27.0	25.1	1.9	2.3	2.9	1.6	19.6	19.6	29.2	36.7
2.0	3.0	3.8	3.8	4.6	32.0	38.0	30.0	26.2	3.3	4.9	5.2	5.7	30.1	30.3	35.4	39.7
3.0	2.5	2.8	3.5	4.7	36.2	38.0	37.0	37.0	2.19	2.5	3.6	3.8	34.3	35.2	38.6	42.3
4.0	1.2	1.5	4.2	1.4	39.0	42.0	43.0	43.0	1.1	1.3	1.3	1.4	36.0	36.2	40.2	43.4
5.0	0.9	1.3	2.4	1.3	39.0	54.0	47.0	48.1	0.8	1.0	0.95	1.2	38.1	39.0	41.2	46.2

(1987) reported that the fungal growth was positively correlated with salt concentration up to 8%. The radiation-induced effect on salt tolerance of both isolates 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).

Iradiation dose 2.0kGy enhanced the antimicrobial activity of the two isolates, while high dose level (4.0-5.0) highly decreased the antimicrobial activity (Table V). Meanwhile, increasing dose level from 0.25-5.0 kGy decreased that biomass yield in both isolates.

All irradiation doses used (0.25-5.0 kGy) increased the amount of cell RNA, and decreased the cell DNA in both *S. albaduncus* and *S. erythogresius.* (Table VI*).* For DNA content, 2 kGy was exception, i.e, It increased the cell DNA content. Our results are in agreement with those of Aly (1985) who reported that when *S. lipmani* cell was irradiated with gamma rays RNA content was increased while that of DNA was decreased.

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