DETECTION OF GAMMA RADIATION EFFECT INDUCED BY COBELT-60 ON *ESCHERICHIA COLI* CELLS

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Abstract

Effect of ionizing radiation induced by coblet-60 (Co-60) on *Escherichia coli* cells was investigated. A cellular survival curve and mutant frequencies versus absorbed doses were determined. The results showed that the level of bacteria in survival curve decrease to (2) Log with gamma ray doses increase and D-value was equals (0.079) Gy. After irradiation the mutant frequencies increased with doses increase, the greatest mutant frequency was 3.4×10^{-12} (1 mutation for 0.29×10^{12} CFU for 150 μg of rifampicin) induced by(1) Gy, while the smaller was 0.39×10^{-12} (1 mutation for 3.41×10^{12} CFU for 150 μg of rifampicin) induced by(0.1) Gy.

Keywords: *Escherichia coli*, coblet-60, survival curve, mutant frequencies.

Introductions

The prokaryotes are interesting group of microorganisms. They possess intrinsic properties, such as reduced generation time and low cost of culture and maintenance so can be used as a tool for the scientific investigations to obtain important parameters for metabolic and genetic characterization of cells of more complex organisms (1), for example, using of bacterial cells as biosensor to sense the effect of ionizing radiation biologically, such as *Escherichia coli* (a common bacterium of the intestinal tract of human and animals) (2).

Microorganism can be inactivated directly or indirectly by radiation due to impairment of important molecules or organelles, such as DNA and cytoplasmic membrane (3).

Ionizing radiation induce mutagenesis by generated reactive oxygen species (ROS) that react with DNA, RNA and their precursors lead to damage nucleic acids and nucleotides by detectable deletions, major rearrangements and into point mutation(4)(5).

Rifampicin a potent inhibitor antibiotic of prokaryotic transcription initiation has long been used to study transcription and mutant frequency in bacteria and also has been used as highly effective drug. Resistance to rifampcin can arise from mutation in the *rpoB* gene encoding the B subunit of RNA polymerase, *E. coli* chromosome *rpoB* gene region A contains the two clusters responsible for rifampcin resistant (4) (6).

In this study, we try to determine the effect of gamma rays doses that reduces the level of *E. coli* and mutant frequency of the bacteria.

Material and Methods

One colony of *E. coli* (from laboratory of Department of Biology, Collage of Science, University of Baghdad) was cultivated in nutrient broth and incubated at 37 °C for 24 hours (0.5) ml of this culture was taken and added to (50) ml nutrient broth then incubated at 37 °C until reach log phase (4). One ml of cell broth suspension was distributed in the test tubes (duplicated for each dose) then irradiated with different doses of gamma rays from Co-60 gamma ray source ranged from (0.1-1) Gy For (15) min.(7).

Following irradiation, both control and irradiated tubes were cultured by inoculating (0.1) ml of them in nutrient agar plate for surviving bacteria and (0.2) ml in nutrient agar plate supplemented with rifampicin (150μg/ ml) for mutant frequency. All plates incubated at 37 °C for 24 °C to obtain the result (2).

Result and Discussion

After an incubation period, the colony forming unit (CFU) of irradiation and non irradiated bacteria were counted in petridishes with and without rifampicin, and then the number of cell per milliliter was calculated.

Survival curves of *E. coli* irradiated with Co-60 gamma rays (Fig.(1)) showed a decrease in the level of bacteria to (2) Log and the inactivation appeared to be linear; D- value (radiation doses needed to decrease a microbial population by 90%) was determined by the reciprocal of the survivor curve slope. D-value was equal to (0.079)Gy.

Chung *et al* (8) reported decrease in level of *E. coli* below (3) Log and D-value was (0.42) KGy in Kimbab food after irradiation with Co-60 gamma ray, also Buchaneun *et al* (9) show decrease in bacteria to (5) Log and D-value was (0.21) KGy in juice after irradiation with Cs-137 gamma ray.

These differences could be attributed to the environmental factors that affect the survival of irradiated cells like temperature, phase of growth, the nature of gaseous environment, water activity, small PH and chemical composition of the medium (10).

The results showed decrease in number of bacterial cell with gamma dose increase giving negative direct correlation (r = –0.946), also showed (0.1) Gy gamma ray dose caused reduction in *E. coli* level about(0.02×10^2) times, while the (1) Gy gamma ray dose reduced *E. coli* level about (2.1×10^2) times in contrast to the control (Fig.(2)).

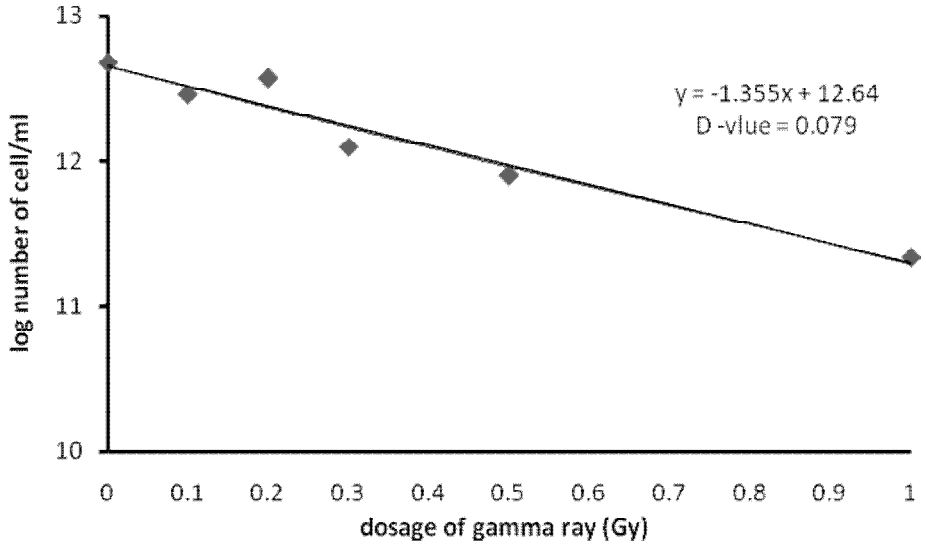

Fig.(1): Survival curves of E. coli irradiated with Co-60 gamma rays.

In a study on spinach leaves, it was found that (2) KGy gamma ray caused reduction 500 times in *E. coli* level while (0.5 KGy) cause 5 times reduction (11). The reduction of bacterial level by ionizing radiation due to damage biological molecules such as DNA, cytoplasmic membrane and proteins including enzymes by thermal denaturation caused by radiation energy (2).

The chromosomal *rpoB* gene was chosen as a mutagenesis target because most mutations detected by phenotypes selection (rifampicin resistance) have been found to occur in limited regions sequencing (12).

The mutant frequency was calculated by multiplying the mean number of colonies which resistant to (150) µg of Refampicin by (5) to obtain the number of mutant colonies in (1) ml of overnight culture then calculate the number of mutation and mutant frequency for each dose (13). Fig.(3) shows mutant frequencies of *E. coli* cells irradiated by various dosages of Co-60 gamma rays.

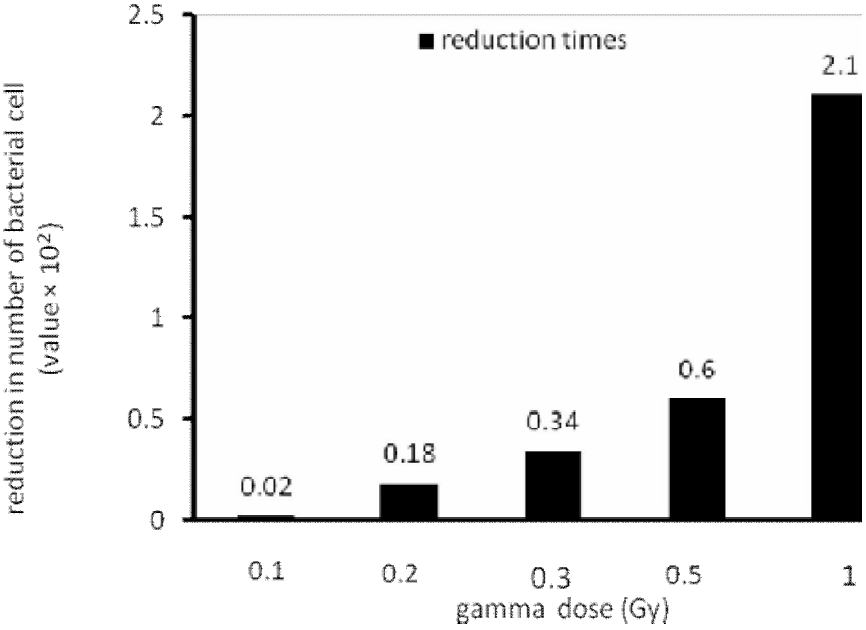

Fig.(2): Reduction times in E.coli level after irradiated by various dosages of Co-60 gamma rays.

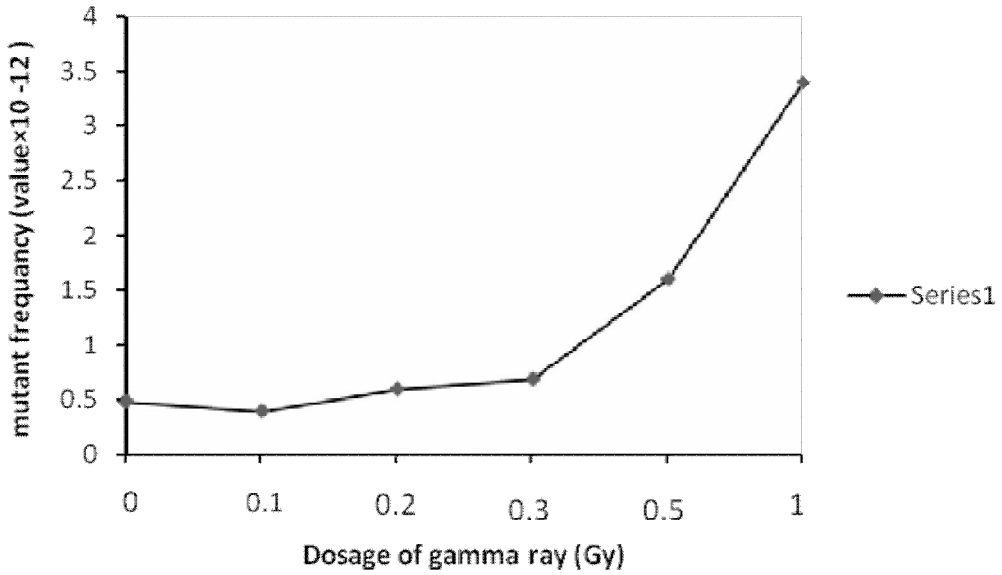

Fig.(3): Mutant frequencies of E. coli cells irradiated by various dosages of Co-60 gamma rays.

Generally *E. coli* strains have an average spontaneous mutation rate to Rifampicin resistance of about 0.48×10^{-12} (1 mutation for 2.04×10^{12} CFU). Spontaneous frequency for *E. coli* in other studies were about 1×10^{-8} (4) and 2×10^{-9} (14). The high frequency of mutation in our study compared with other studies may be referring to our protocols and also depend on the strain of *E. coil* used in experiment.

The mutant frequencies induced by Co-60 gamma ray doses increased with dose increased (r = 0.836). It showed that greatest mutant frequency was 3.4×10^{-12} (1 mutation for 0.29×10^{12} CFU for 150 µg of rifampicin) induced by(1) Gy, while the smaller was

0.39×10^{-12} (1 mutation for 3.41×10^{12} CFU for 150 μg of rifampicin) induced by(0.1) Gy.

Hori *etal* found that treatment of *E. coli* with 2-0H-d ATP (oxidized and damaged deoxy ribouncleotides) induce mutant frequency about 12.1×10^{-7} (12), thus the using of gamma ray clearly increased the frequency of mutation in the *rpoB* gene. The gamma radiation induced mutation by direct and/or indirect DNA damaged by formation a reactive species such as OH and there is an evidence indicating that ROS can react with DNA and cause mutation, also effecting of cytoplasm by radiation might play important role in the induction of mutation that studied in mammalian system (15)(16).

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الخلاصة

درست تاثير اشعة كاما الناتجة من العنصر المشع كوبلت –60 في خلايا بكتريا القولون *Escherichia coli*، وتم تعيين منحنى البقاء للبكتريا والتردد الطفوري للخلايا بعد التعرض الى جرعات الاشعة المؤينة. اظهرت النتائج ان مستوى بكتريا القولون في منحنى البقاء قد انخفض بمقدار دورتين لوغارتميتين وانخفاضه بازدياد كمية الجرعة المعرضة لها البكتريا وقيمة D-value كانت مساوية لـ Gy (0.079) .

كذلك سجلت النتائج زيادة في التردد الطفوري مع ازدياد الجرعة حيث ظهر اعلى تردد طفوري عند الجرعة Gy (1) وكانت 3.4×10^{-12} (1 طفرة لكل 0.29×10^{-12} وحدة مكونة للمستعمرة لـ 150µg من المضاد الحيوي الرفامبسين) بينما اقل تردد طفوري كانت عند الجرعة Gy (0.1) وكانت 0.39×10^{-12} (1 طفرة لكل 3.4×10^{-12} وحدة مكونة للمستعمرة لـ 150µg من المضاد الحيوي الرفامبسين).