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# Molecular genetic study of *Pseudomonas aeruginosa* DNA repair system

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**ABSTRACT**

Bacteria *Pseudomonas aurginosa*, *E coli* and *Stapthylococcus aureus* were exposure to different doses of ultraviolet radiation and survival curves drawn for each type, the results show that the bacteria *Pseudomonas aurginosa* more resistant by UV radiation than *Ecoli* and *Stapthylococcus aureus* bacteria. The bacterium *Pseudomonas aeruginosa* was irradiated with different doses of U.V light via wave length( 254 nm ) for different periods ( 50 , 100 , 150 , and 200 sec ) .It appear that part of irradiated bacterial culture was exposed to sun light and the other part was kept in the dark . The survivors of the cells exposed to the sun light was more than the dark and this ensure possessing the bacterium photoreactivating repair system investigate the excision repair system, the minimal inhibitory concentration ( MIC) of caffeine against bacteria was studied by exposing the bacterium to different concentrations of caffeine (10 , 15 , 20 and 25 mg/ml ) and the MIC was 20 mg/ml , Furthermore the bacterium was exposed to different times of U.V. light in the presence of caffeine and the studying ensure that the survivors of the cells in the medium with caffeine was less than the medium with absence of caffeine and this leads to possess the bacterium excision repair system. To detect the recombination repair system , the bacterium was exposed to the concentrations( 0.1 ,0.2 , 0.3 , 0.4 µg / ml) of acrivlavine and the MIC was 0.3 µg / ml , then the bacterium was exposed to different times of U.V. light in the presence of acrivlavine . The survivors of the cells in the medium with acrivlavine was less compared with the absence of acrivlavine. It would seem that possessing bacterium recombination repair system . sensitivity test of the bacterium against antibiotics was established and the results appeare that it was to the antibiotics Chloramphenicol, Carpencillin, Trimethoprim, Rifampicin The diameters of inhibition were (16,20,17,18) mm respectively and resistant to the antibiotics Amoxicillin, Ampicillin, Clindomycin, Cloxacillin , Nalidixic acid , Cephaloxin , Tetracyclin and Tobromycin. To study SOS repair system the bacterium was mutated with direct mutagens represented with nitrous acid and indirect mutagens represented with U.V. light to isolate Rifampicin and Chloramphenicol mutants. It is quite likely that the sensitivity of bacterium for mutagenesis then possessing SOS repair system.

## Introduction

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals and humans. It is found in soil, water, skin flora, and most man-made environments throughout the world. It is an opportunistic pathogen for both humans and plants[(1)].

The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys [(2)].

DNA repair is an essential process in all living organisms, There are several genetic systems that avoid or repair the errors produced in DNA and so maintain the genome integrity. The genes involved in DNA repair are commonly known as mutator genes because their inactivation leads to increase in the mutation rate or

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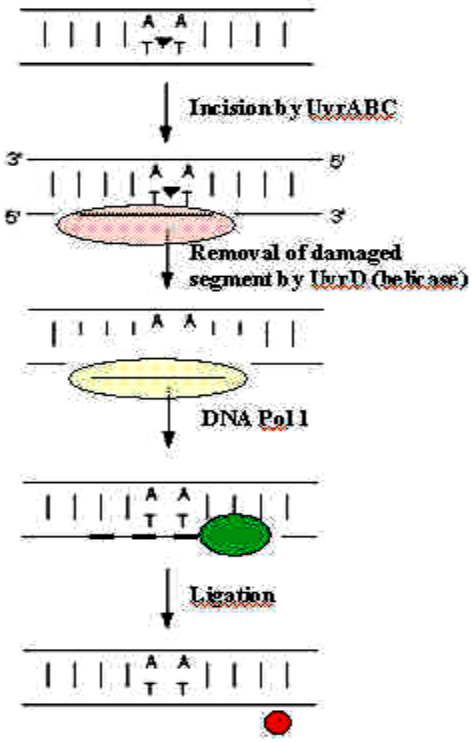
mutator phenotype[3,4]. DNA repair is an essential process in all living organisms, Mismatches, occurring during DNA replication or homologous recombination, are repaired by different mechanisms.[5] One of the most important DNA repair mechanisms in bacteria is that:

1-**photoreactivation is DNA repair** enzymes that repair damage caused by exposure to ultraviolet light. This enzyme mechanism[6] requires visible light, preferentially from the violet/blue end of the spectrum, and is known as Photolyases. Photolyases bind complementary DNA strands and break certain types of pyrimidine dimers that arise when a pair of thymine or cytosine bases on the same strand of DNA become covalently linked. These dimers result in a 'bulge' of the DNA structure, referred to as a lesion. The more common covalent linkage involves the formation of a cyclobutane bridge. Photolyases have a high affinity for these lesions and reversibly bind and convert them back to the original bases.(7)

2- **Excision Repair system:** There are three different types of repair mechanisms which use different enzymes but none-the-less follow the same basic principle as outlined in the figure below.




**Excision Repair**

3- The SOS response is a state of high-activity DNA repair, and is activated by bacteria that have been exposed to heavy doses of DNA-damaging agents. Their DNA is basically chopped to shreds, and the bacteria attempts to repair its genome at any cost (including inclusion of mutations due to error-prone nature of repair mechanisms). The SOS system is a regulon; that is, it controls expression of several genes distributed throughout the genome simultaneously.

The primary control for the SOS regular is the gene product of lexA, which serves as a repressor for recA, lexA(which means that it regulates its own expression), and about 16 other proteins that make up the SOS response. During a normal cell's life, the SOS system is turned off, because lexA represses expression of all the critical proteins. However, when DNA damage occurs, RecA binds to single-stranded DNA (single-stranded when a lesion creates a gap in daughter DNA). As DNA damage accumulates, more RecA will be bound to the DNA to repair the damage ,The recA and lexA genes were the first to be recognized as being involved in SOS induction. Mutations in these genes make cells highly sensitive to UV irradiation.The 27 kDa LexA and the 36 kDa RecA proteins were previously known as recombination proteins operating in the sexual life and genetic exchange of bacteria [10]. Presently, it is known that RecA protein also participates in genetic DNA exchange, in recF, recO, recR, recN and ruvABC-dependent recombinational DNA repair [11], and, together with LexA protein, plays a major role in the regulation of the SOS response. The down- and up-regulation of the SOS-induced genes is basically an interplay of two proteins, LexA repressor and RecA* where LexA is a transcriptional repressor protein, and RecA* is a coprotease aiding the autocatalytic selfcleavage of LexA [12-14]

## Materials and methods

**a- Culture, characterization and identification *of Pseudomonas* from burns samples.**

**Bacterial Strains:** A total of 30 specimens were isolated from Al-Ramadi Hospital from different clinical origins with positive culture results for *Pseudomonas aeruginosa* were included in the study. Cultures of the burn wounds were performed using swabs on the admission and all clinically indicated cultures such as

blood, tissue, and urine were evaluated as well. The samples were streaked on nutrient agar, MacConkey agar and blood agar. The plates were incubated at 37°C for 24 h as described by Cheesborough (2002). Isolates obtained after incubation were sub cultured using isolation media that *Pseudomonas* isolation agar.

The pure isolates of *Pseudomonas* were transferred to 1% nutrient agar slant and stored in the refrigerator at 4±1°C. Suspected *Pseudomonas* species were characterized and identified according to standard bacteriological methods, gram stains and biochemical tests such as oxidase, nitrate reduction, citrate utilization, oxidative fermentation, arginine, and growth at 42C°. [19]

**b- Detection of Photo reactivation systems**

Culture of *Pseudomonas aeruginosa* grown in nutrient broth until mid exponential phase, was pelleted from 20 ml by centrifugation at 4000 rpm for 10 min , U.V source was agermicidal lamp which emitted radiation primarily at 254 nm. The distance between the U.V lamp and irradiated suspension was adjusted to be 30 cm. irradiation was undertaken only when the lamp was emitting its maximum flounce. 10 ml sample in phosphate buffer were irradiation in sterilized petri dish with contain stirring for the following period (0,50,100,150,200,250 sec). 0.5 ml kept in sun light for 30 min , 0.1ml sample was taken for the first treatment, diluted in the dark and spread on nutrient agar . plates were warped in aluminum foil and incubated at $37C^0$ for 24-48 h .0.1ml sample was taken from the second treatment, diluted and spread on nutrient agar (in the light). Plates were incubated at $37C^0$ for 24-48 h . [20]

**c- Survival of *Pseudomonas aeruginosa* different concentration of caffeine and acriflavine:**

0.1ml sample of *Pseudomonas aeruginosa* at mid exponential phase, was diluted properly and spread on nutrient agar containing either caffeine at concentration (0,2.3.4.5.6mg/ml) or acriflavine at concentration (0,0.2,0.4,0.6,0.8 µg/ml). All plates were incubate at $37C^0$ for 24-48 h to determine the total viable count.[21]

**d- Detection of excision and recombine- tion repair systems**

Culture of *Pseudomonas aeruginosa* grown in nutrient broth until mid exponential phase, was pelleted from 20 ml by centrifugation at 4000 rpm for 10 min, U.V source was a germicidal lamp which emitted radiation primarily at 254 nm . The distance between the U.V lamp and irradiated suspension was adjusted to be 30 cm. irradiation was undertaken only when the lamp was emitting its maximum flounce. 10 ml sample in phosphate buffer were irradiation in sterilized petri dish with contain stirring for the following period (0,50, 100,150,200,250 sec). 0.1 ml sample was taken after each treatment, diluted in the dark and spread on nutrient agar containing either caffeine in the following concentration (0,2,3,4,mg/ml) or acriflavine at concentration (0,0.2, 0.4,0.6,0.8 µg/ml). All plates were incubated at 37 $C^0$ for 24-48h.[21]

**Detection of SOS repair systems**

In this method, used two mutant a direct nitrous acid and indirect (UV Light) to induce DNA damage cultured single colony in media contains 15 ml nutrient broth , and then identified the concentrations are sensitive to bacteria *Pseudomonas aeruginosa* through treatment with different concentrations of nitrous acid (HNO2) (0,20,40,60,80,100) µl , 0.001 N was added to growing cultures to nutrient broth then bacteria incubated at a temperature 37 ° C for a period of 100 min Samples were taken every 20 min.[22]

**e- Test the resistant antibiotics to *Pseudomonas aeruginosa* .**

Tested the sensitivity of the bacteria Pseudomonas aurginosa to many antibiotics that inoculating one colony of bacteria in 5 ml of nutrient broth Incubated at 37 C° for 24 hours and take was 0.1 ml of bacteria and culture on a plate containing Nutrient agar and then placed disks of antibiotics and then incubated at 37C° for 24 hours measured inhibition zones around discs with mm and diameters of inhibition compared with peers in the schedules of a private standard by which to set the sensitive bacteria and resistance to antibiotics.[23]

## Result and Discussion

**a- Culture, characterization and identification of *Pseudomonas from* burns samples.**

Bactera *Pseudomonas aeruginosa* was isolated from patients (burn swab) in AL-Ramadi hospital and the selected strain was identified by its physiological and biochemical characteristic (Table 1).

**b- Test the sensitivity of the bacteria *Pseudomonas aeruginosa* against UV**

The survival of *Pseudomonas aeruginosa* ,*Ecoli* and *Staphylococcus aures* whe exposure to different doses of U.V radiation is shown in Fig(1) . The survival curve of *Pseudomonas aeruginosa* has a shoulder , indicating that this bacterium is U.V resistance and can initially absorb radiation energy where it can accumulated sublethal damage for the forty three seconds for irradiation, followed by little loss of viability at the sixty six and 100 seconds. This result was similar to that obtained by Al-Dolaimi KJ(2012)**(17).** on the other hand the inactivation of *Ecoli* and *Stapthylococcus aures* by irradiation was exponential and were sensitive. It is obvious that *Pseudomonas aeruginosa* is more resistance to the U.V than of *Ecoli* and *Stapthylococcus aures* in this aspect *Pseudomonas aeruginosa* is relatively similar to the highly radiation resistance bacteria *Deinococcus radiodurans* (Duggan etal 1995; Tempest 1979) .

**c- Detection of Photo-reactivation repair systems**

survival of Pseudomonas aeruginosa after exposure to U.V irradiation for different intervals in light and darkness is show (Fig2) . The survival fraction of *Pseudomonas aeruginosa* irradiation in darkness for 100 seconds was about 60% of the survival fraction irradiation for the same periods in light. This result indicated that *Pseudomonas aeruginosa* possesses photoreactivation rpair system , because an increase in survival of *Pseudomonas aeruginosa* occurred following photoreactivation treatment in comparison to that in the darkness. this means that *Pseudomonas aeruginosa* contain aphotoreactivation enzyme similar to the photolyase (phr gene product of the E.coli) that can catalyze direct monomerisation of U.V induced pyrimidine dimers where is enzyme binds specifically to U.V irradiated DNA in darkness and in the presence of visible light breaks the covalent bond attaching two pyrimidines in a cyclo- butane ring ( saunder et al, 1987) . photoreactivation repair system ia an error- free system (set low, 1996) and found in several microorganisms like *streotomyces griseus* , *E.coli* , *penicillium notatum* . This system is absent in other microorganisms , for example the most U.V radiation resistant bacteria *Deinococcus radiodurans* lack this system but contain avery efficient excision repair system ( Moseley 1983) .

**d- Survival of *Pseudomonas aeruginosa* on different concentration of caffeine and acriflavine :**

survival of *Pseudomonas aeruginosa* after plating on media containing different concentration of caffeine and acriflavine . *Pseudomonas aeruginosa* was found to be resistant to the caffeine concentration use in this experiment, and the rate of loss of viability was remarkable slow. The survival of *Pseudomonas* aeruginosa on media containing 10mg/ml caffeine was similar to the control, while the survival fraction on the media containing 15,20, 25 mg/ml caffeine were about 98%, 95%, 87%respectively, in experiments where different concentration of acriflavine were used, the result showed that the rate of loss of viability of *Pseudomonas aeruginosa* was slow media contain 0.1,0.2,0.3,0.4 µg/ml acriflavine, where the survival fractions were about 100%, 93%, 84%, and 79% respectively .

The result obtained from these tow experiments employed to determine the appropriate concentrations of caffeine and acriflavine that are prerequisite for further investigation that involve U.V irradiation for the detection of excision and recombination repair system in the *Pseudomonas aeruginosa* .

**e- Detection of excision and recombination repair system in *Pseudomonas aeruginosa* .**

Survival *of Pseudomonas aeruginosa* on nutrient agar containing different concentration of caffeine and acriflavine aftr exposure to the different doses of U.V. irradiation are show in (Fif3,Fig4). survival of *Pseudomonas aeruginosa* on media contain 10, 15 mg/ml caffeine was decreased very sharply after 30 sec of irradiation where the survival fraction were about 20% and 13% respectively compared with about 100% ,98% survival on nutrient agar lacking caffeine and irradiation for the same period . after 60 sec of irradiation no survival were detected on nutrient agar containing 20 mg/ml caffeine ,while after 60 sec exposure to U.V irradiation (concentration15 mg/ml from caffeine) the percentage of survival was reduced to the about 2% . these results indicated that the presence of caffeine increases the killing effect of U.V. light very significantly. survival of *Pseudomonas aeruginosa* on media contain 0.1, 0.2µg/ml acriflavine was decreased very sharply after 30 sec of irradiation where the survival fraction were about 18% and 11% respectively compared with about 100% , 93% survival on nutrient agar lacking

acriflavine and irradiation for the same period . after 60 sec of irradiation no survival were detected on nutrient agar containing 0.3µg/ml acriflavine ,while after 60 sec exposure to U.V irradiation ( concentration 0.2µg/ml from acriflavine) the percentage of survival was reduced to the about 3%. these results indicated that the presence of acriflavine increases the killing effect of U.V. light very significantly.

Excision repair of pyrimidine dimers as described previously involves principally four enzymatic steps : (1) U.V specific endonuclease which include two enzymatic activity , the first recognize the damage by aglycoylase that clip damage bases out the DNA molecules by cutting the glycocylic bone between the pyrimidine and its sugar, the second enzyme activity include apurinic endonuclase that recognize the hole in the helix after the removal of dimer and make a cute across the phosphodiester bond to make anick.(2) DNA polymerase I 5-3 exonuclease degrades the damage portion.(3) polymerase I re-synthesizes the DNA through the opposite intact DNA template.(4) ligase seals off re-synthesized DNA (Frefelder, 1987; Fong and Bockrath, 1997) . It's know that the caffeine is selectively inhibit the excision repair process by binding tightly to the irradiation DNA , there by copeting with the dimer specific endonuclease( glycocylase) for the dimer and so inhibit the incision step leading to single strand DNA break formation (Braun and Grossman 1997, Fong 2000) . it's also known that there is no effect of the caffeine on the other types of DNA repair system like recombination repair while the percentage of survival after 60 sec of irradiation on media containing 0.2µg/ml acriflavine were about 3%. according this result obtained, it can be concluded that *Pseudomonas aeruginosa* possesses an excision and recombination repair system and these result indicated that the excision repair system of *Pseudomonas aeruginosa* is more efficient than recombination repair system .

**f- Test the resistant antibiotics to *Pseudomonas aeruginosa* .**

sensitivity test of the bacterium against antibiotics was established and the results appeared it was sensitive to the antibiotics Chloramphenicol ,Carpencillin, Trimethoprim ,Rifampicin The diameters of inhibition was (16,20,17,18) mm respectively and resistant to the antibitcs Amoxicillin, Ampicillin Clindomycin, Cloxacillin, Nalidixic acid, Cephaloxin, Tetracyclin and Tobromycin Bacteria can become resistant to one or even several classes of antibiotics and transfer their resistance to other bacteria and species via gene transfer. The strategies used by bacteria to resist the actions of antibiotics include[16]:

- Reduced membrane permeability to the antibiotic.
- increased efflux/decreased influx of antibiotic
- neutralization of the antibiotic by bacterial enzymes
- target modification by mutations and even
- target elimination

**G- SOS repair systems**

The minimum inhibitory concentration of nitrous acid and curved draw, as shown in Figure (5). nitrous acid is used frequently when using bacterial mutations Where this works mutagenic to remove amino group and bring oxygen replaced by three rules which guanine and adenine and cytosine It is well known that bacteria resistant to many antibiotics where some of which is carried on chromosome bacterial and less on plasmid were used as such to isolate mutants sensitive to antibiotics , although it before mutagenic were resistant to them , where he works nitrous acid Defect in the order of events gene which leads to damaging a gene that either be responsible for the production of enzymes for counter-analysis or through damaging the gene responsible for the composition of the recipients to embed a counter inside the bacterial cell The findings suggest that the antibiotic.

Amoxicillin using mutagenic more effective in the cells did not grow significantly indicating shift bacteria from resistance to any sensitive can cause high frequency of mutations in the gene responsible for antibiotic resistance Amoxicillin either frequency of mutations in the gene responsible for resistance to Ampicillin was in very small compared with the first We conclude that antibiotic resistance gene Amoxicillin is more sensitive than the rest of the other genes.

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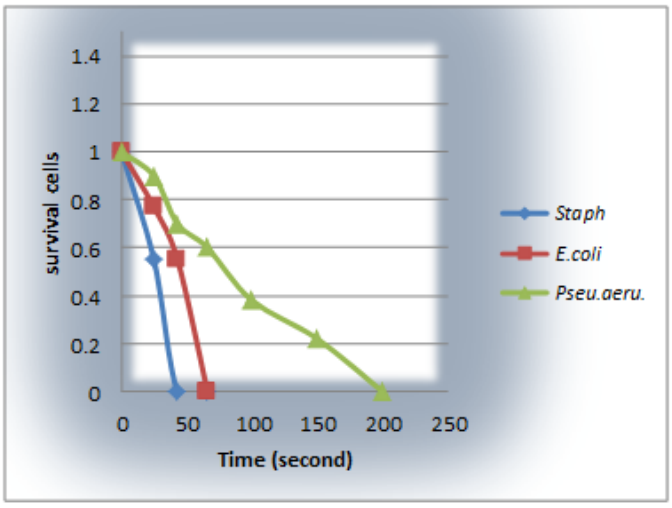


Fig1: show resistant bacteria against UV in different time

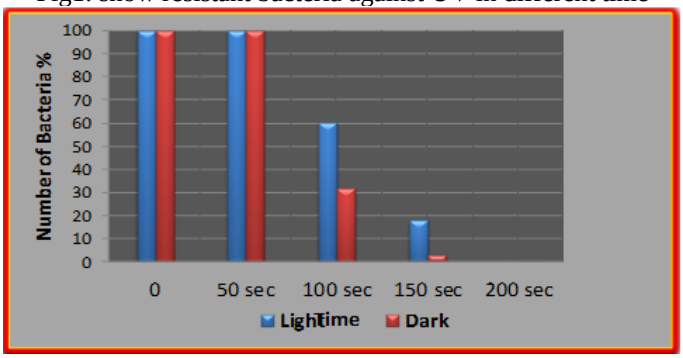


Fig2: show DNA repair system in Pseudomonas aeruginosa

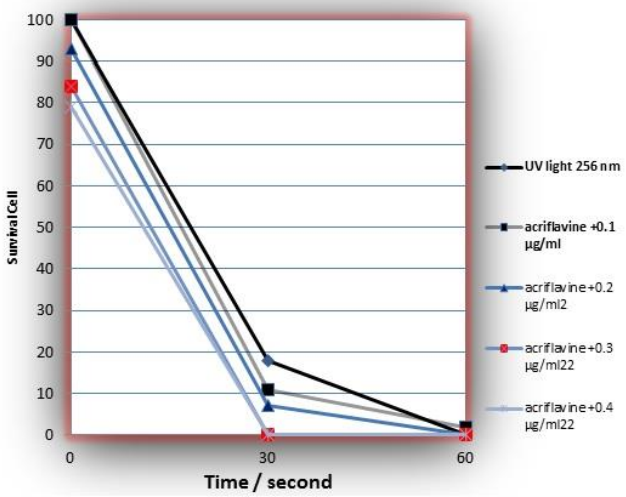


Fig (3): Survival curve to *Pseudomonas aeruginosa* in different concentration from acriflavine material after exposure to UV light

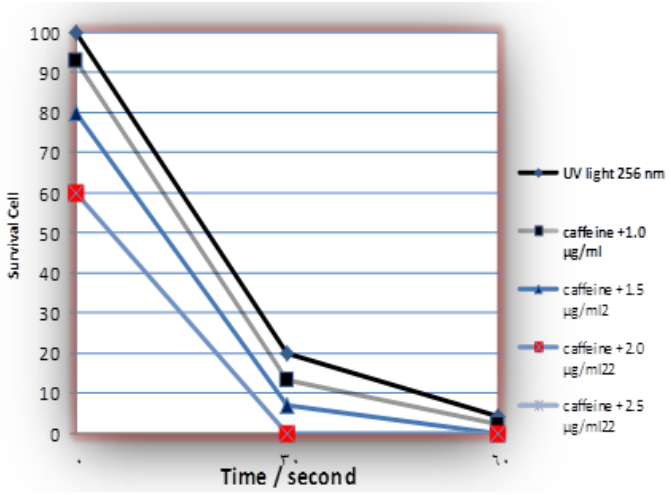


Fig (4): Survival curve to Pseudomonas aeruginosa in different concentration from caffeine material after exposure to UV light

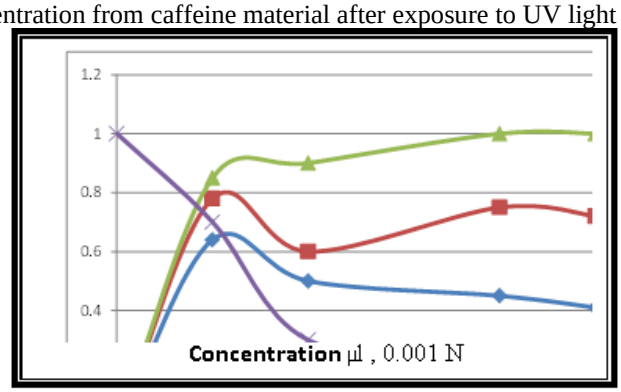


Fig (5): mutagenic effect to nitrous acid against Pseudomonas aeruginosa in different concentration from caffeine material after exposure to UV light

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# دراسة وراثية جزيئية لنظام اصلاح DNA في بكتيريا الزوائف الزنجاريةPseudomonas aeruginosa

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**الخلاصة**

عرضت بكتيريا Pseudomonas aurginosa, E coli and Stapthylococcus aureus الى جرعات مختلفة من الاشعة فوق البنفسجية ورسم منحني البقاء لكل منهما عند مقارنة النتائج المتحصلة ومن خلال منحني البقاء لوحظ ان البكتيريا قيد الدراسة كانت اكثر مقاومة للأشعة الفوق بنفسجية، وتمت دراسة نظام الاصلاح الضوئي للبكتيريا Pseudomonas aurginosa, بتعريض البكتيريا الى U.V Light بطول موجي 254 nm ولفترات مختلفة حفظ جزء من الخلايا المعرضة للإشعاع في الضوء اما القسم الاخر فحفظ في الظلام لوحظ ان نسبة بقاء الحلايا المعرضة لضوء الشمس اكثر من الخلايا التي حفظت في الظلام مما يدل على امتلاك البكتيريا نظام الاصلاح الضوئي. واستخدمت مادة caffeine لدراسة نظام excision حيث استخدمت تراكيز مختلفة لتحديد MIC حيث وجد ان التركيز المثبط الادنى كان 20mg/ml بعدها عرضت الخلايا البكتيرية الى الاشعة فوق البنفسجية بوجود وغياب مادة caffeine ولوحظ ان اعداد البكتيريا المعرضة للأشعة بوجود المادة اقل من الخلايا بغياب مادة caffeine وهذا يؤكد وجود نظامExcision repair system , ولتحديد وجود نظام Recombination repair system استخدمت مادة Acrivalvine وكان التركيز المثبط الادنى MIC هو 0.3 µg/ml واتبعت خطوات مادة Caffeine لتحديد وجود النظام وتم التصول الى وجود هذا النظام في البكتيريا ، واختبرت حساسية البكتيريا تجاه مجموعة من المضادات الحيوية ثم استخدمت ماة حامض النتروز ( مطفر مباشر) والأشعة فوق البنفسجية (مطفر غير مباشر) لإحداث طفرات تكون مقاومة للمضادات الحساسة لها وتم عزل خلايا بكتيرية كانت حساسة ثم تحولت بفعل المطفرات الى مقاومة والمضادات هي ( Chloramphenical ,Rifampcin) مما يدل على امتلاك البكتيريا لنظام الاصلاح SOS .