Molecular genetic study of *Pseudomonas aeruginosa* DNA repair system

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Abstract: Bacteria *Pseudomonas aeruginosa*, *E coli* and *Staphylococcus aureus* were exposure to different doses of ultraviolet radiation and survival curves drawn for each type, the results show that the bacteria *Pseudomonas aeruginosa* more resistant by UV radiation than *Ecoli* and *Staphylococcus 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 appear that it was to the antibiotics Chloramphenicol, Carbenicillin, Trimethoprim, Rifampicin The diameters of inhibition were (16,20,17,18) mm respectively and resistant to the antibiotics Amonoxicilin, Ampilicillin, 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.

Keywords: Molecular genetic , *Pseudomonas aeruginosa*, DNA 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 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.\(^\text{(7)}\)

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.

Materials and methods

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\(^\text{(10)}\). Presently, it is known that RecA protein also participates in genetic DNA exchange, in recF, recO, recR, recN and ruvABC-dependent recombinational DNA repair\(^\text{[11]}\), 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\(^\text{[12-14]}\).

b- **Detection of Photoreactivation 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 flouence. 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 37°C for 24-48 h. .01ml sample was taken from the second treatment, diluted and spread on nutrient agar (in the light). Plates were incubated at 37°C for 24-48 h. (20)

c- Survival of *Pseudomonas aeruginosa* different concentration of caffeine and acriflavin:
0.1ml sample of *Pseudomonas aeruginosa* at mid exponential phase, was diluted properly and spread on nutrient agar containing either caffeine at concentration (0.2,3,4.5,6 mg/ml) or acriflavin at concentration (0.2,0.4,0.6,0.8 µg/ml). All plates were incubate at 37°C for 24-48 h to determine the total viable count. (21)

d- Detection of excision and recombinant 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 flouence. 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 acriflavin at concentration (0,02,0,4,0.6,0.8 µg/ml). All plates were incubated at 37°C for 24-48 h . (22)

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. (23)

e - Test the resistant antibiotics to *Pseudomonas aeruginosa* .
Tested the sensitivity of the bacteria *Pseudomonas aeruginosa* to many antibiotics that inoculating one colony of bacteria in 5 ml of nutrient broth Incubated at 37°C for 24 hours and take 0.1 ml of bacteria and culture on a plate containing Nutrient agar and then placed disks of antibiotics and then incubated at 37°C 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.*
Bacteria *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* who 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) (23), on the other hand the inactivation of *Ecoli* and *Staphylococcus 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 *Staphylococcus 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 repair 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 apphotoreactivation 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 break 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 streptomycyes 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 a very 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 concentrations of caffeine and acriflavine. *Pseudomonas aeruginosa* was found to be resistant to the caffeine concentration used 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, 25mg/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 two 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 after exposure to the different doses of U.V. irradiation are show in (Fig3, Fig4). Survival of *Pseudomonas aeruginosa* on media containing 10, 15mg/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 (concentration 15 mg/ml from caffeine) the percentage of survival was reduced to about 2%. These results indicated that the presence of caffeine increases the killing effect of U.V. light very significantly.

The strategies used by bacteria to resist the antibiotics include:

- 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.

Reference
1- Zhiwei Fang,b Jiao Zhao,b Yuanqiang Zou,b Tianzhi Li,a Junfeng Wang,a Yinhua Guo,a De Chang,a Longxiang Su,a Peixiang Ni,b and Changting Liu,a (2012). Draft Genome Sequence of Pseudomonas aeruginosa Strain ATCC 27853. American Society for Microbiology Volume 194 Number 14 Journal of Bacteriology p. 3755
19- Piyush Tripathi, Gopa Banerjee, Shivani Saxena, Mahendra Kumar Gupta, and P. W. Ramteke (2011). Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection,


Fig1: show resistant bacteria against UV in different time

Fig2: show DNA repair system in *Pseudomonas aeruginosa*
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.

**Pseudomonas aeruginosa**

دراسة وراثية جزئية لنظام اصلاح DNA في بكتيريا الزواحف الزنجارية

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الخلاصة

عرضت بكتيريا Pseudomonas aeruginosa, E coli and Staphylococcus aureus إلى جرعات مختلفة من الأشعة فوق البنفسجية ورمز منحنى البقاء لكل منها عند مقارنة النتائج المتصلة. ومن خلال منحنى البقاء لوحظ أن البكتيريا في الدراسة كانت أكثر مقاومة للأشعة فوق U.V Light بtraîع البكتيريا إلى Pseudomonas aeruginosa, وتم دراسة نظام الاصلاح الضوئي للبكتيريا بنفس الدراسة لتحديد تأثير بعض الأشعة فوق البنفسجية وترويج مادة caffeine. وتحدد وجد ظل البكتيريا مقاومة إلى الدراسات من الجرعة الأولى ومعظمه البكتيريا تمت تلميح ذلك بتلك الدراسة، وتم دراسة حالة لتحديد وrack defective repair system

**Excision repair system**

استخدمت مادة Acvrivalvine لتحديد وrack defective repair system

**Recombination repair system**

استخدمت مادة Caffeine لتحديد وrack defective repair system

**Chloramphenical ,Rifampcin**

ما يدل على امتلاك البكتيريا لنظام الاصلاح (SOS)