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Influence of 805 nm Diode Laser on Plasmid Content of Some Locally Isolated *Escherichia coli* and *Proteus Mirabilis*

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Abstract: This work aimed to investigate the effect of Diode laser 805 nm on plasmid DNA and RNA contents of some Gram negative bacteria represented by *Escherichia coli* and *Proteus mirabilis* isolates .Plasmid extraction was done using two methods (Salting out and CTAB method).Different powers and pulse repetition rates for 805 nm Diode Laser were used to study this effect. Results revealed that the plasmid profile of the two species were highly affected using (2, 3) W at different frequencies including 5and 10 kHz as compared with 1 kHz while plasmids were gradually disappeared at 1W, 10 kHz. In the same time the shining of RNA was also decreased gradually then disappeared with increasing powers especially at 2W and 10 kHz causing disappearing of RNA while using 2 W (1, 5) kHz causes gradually disappearing. This may suggests using diode Laser as a successful method in plasmid curing.

Key words: Plasmid DNA, Gram negative bacteria, Diode Laser

Introduction

Escherichia coli, (family Enterobacterieace) is a major enteric pathogen particularly in developing countries. Their pathogenisity is due to their ability to produce many virulence factors including many enzymes and toxins which help bacteria to avoid immune response and resist antibiotics treatment (Keenth T., 2005)
,leading to different infections in many human body sites such as urinary tract infection, wound &burn infections and bacteremia if it reach the blood stream (Collee J.G. et al, 1991).
On the other hand, *Proteus mirabilis*,which belong to same family is an important causative agent of variety of opportunistic and nosocominal infections in humans. Virulence factors of *P.mirabilis* has been related to several potential factors including unease production ,active motility(swarming phenomena) mediated by flagella, beside wide spectrum resistant to antimicrobial agents mediated by different mechanisms (Krieg N., Holt J., 1984).

Susceptibility of bacteria to laser light is varied from one Genus to another ranging from highly effect on their metabolic mechanisms reaching killing by laser treatment (Gurzadgan G.G., et al 1981). used picoseconds Nd:YAG laser(266 nm) to study its effect on viruses and bacterial plasmids .They found that bacterial plasmids can be affected after using this wavelength of radiation .In the same year, (Steen S., Wolfgange A., and Helmut S., 1995). used 193 nm and 248 nm UV laser light to induce breakage in single strand DNA as well as double strands plasmid in *E.coli* . The frequency of strand breaks is increased at sites where at least two Guanines or, less frequently, a guanine and an adenine are adjacent to each other (AL-Dulaymi M.F., 2005), used He-Ne laser (632.8 nm) to cure DNA plasmid of *E.coli*, and it was done by this wavelength. Its is clear that most of the researches used He-Ne and UV laser

irradiation to study their effect on *E.coli* while Diode lasers can be used in wide range in medicine &biological researches &it may solves many problems in this field (Macrolf H.N., 1996). In the current study, chopped 805 nm Diode laser was used to investigate its effect on plasmid content &RNA in different frequencies on both *E.coli* and *P. mirabilis* isolates .

Materials and Methods

Bacterial isolates

Five isolates of *E.coli* and eight isolates of *P.mirabilis* were obtained from Al-Mustansiriyia university labs .They were recultured on MacConky and Blood agar. Different morphological and biochemical tests were used for identification and diagnosis bacterial isolate followed by using api 20E system.

Plasmid DNA extraction:

The extraction was done using CTAP-miniprep according to Sal *et al.* (Sal G.D., Manfioletti G., and Schneider G., 1989), and salting out method according to (Sambrook J. , Fritgah E., and Maniatis T. , 1989).

Agarose (8%) contains Ethidium bromide (final concentration 10mg/ml) were used for plasmid running, while UV source (Transillumenator) was used to verify plasmid& RNA content. Previously prepared DNA plasmid (10µL) was used with about (3-5) µL of the loading buffer and placed in the wells. The tray was put in a tank containing TBE buffer. A voltage of 70 v/cm² was allowed to pass through for 2 hours till the trapping dye nearly reached the edge of the gel, then examined under UV light source (336nm), and photographed by using camera with special filtration.

Samples Preparation for Irradiation

One isolate of each bacteria was chosen to study laser effect on plasmid profile &RNA contents. Single colony from each isolates (*E.coli* and *P.mirabilis*) was transferred from nutrient agar culture to brain heart infusion broth, incubated at 37˚C for 24 hours. Each culture broth was centrifuged at 6000 rpm for 10 minutes. Cell pellets were washed twice with physiological saline then mixed by vortex and re suspended in 5 ml of normal saline (AL-Dulaymi M.F., 2005).

Laser Device and Irradiation Setup

The 805nm chopped Diode (I.R) laser (Eltech S.R.Italy) was used in this study with aiming beam wavelength of 635-650 nm; the output power range from 1-4 W with spot size diameter of (6) mm for different frequencies 1 kHz, 5 kHz, 10 kHz. The exposure time was adjusted to 10 minutes. The set up of chopped diode laser is illustrated in Figure (1).

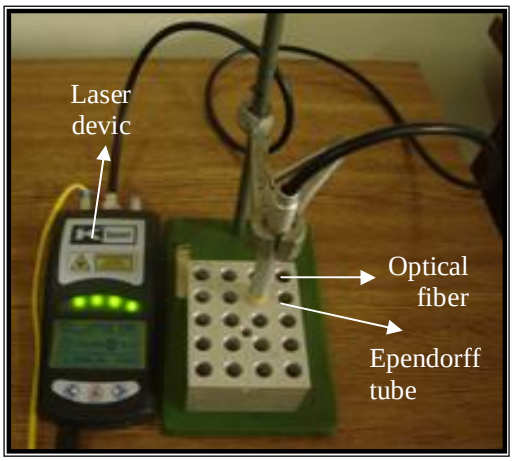

Fig. (1): The setup of irradiation method by Diode laser

Results and discussion

Tow isolates for(*E.coli* species) and one isolate for (*P.mirabilis*) were selected to detect the irradiation effect on plasmids content. Figure (2), shows the plasmid profile for *E.coli* isolate before and after laser irradiation using salting out method. Lane one represents the bacterial plasmid content before irradiation (control), lanes(2,3,4) show the effect of radiation using 1W and frequencies (1,5,10)kHz respectively, while lanes(5,6) represent the plasmid profile after irradiation with 2W and (1,5)kHz respectively .As it is clear in control(lane 1) the plasmid band was so shine and clear and somehow it remains as it is after treating the bacteria with 1W at 1kHz, 5kHz(lanes2,3), but it starts to be faint losing its brightness when the bacteria was irradiated with 1W ,10kHz (lane 4).

By using 2W (1kHz, 5kHz) the radiation causes complete loosing of plasmid band in this isolate as it is shown in lanes 5,6 respectively . The same result was observed with *P.mirabilis* isolate. Figure (3) representes the bacterial plasmid content for *P.mirabilis* isolate (also the extraction of plasmid DNA was done using salting out method).

Lane 1 represents the plasmid profile before irradiation (control), where the plasmid band was so bright, while lanes (2, 3, 4) showed the effect of irradiation using 1W for the three used frequencies (1, 5, 10) kHz. It is so clear that the plasmid start losing their brilliant appearance specially in lane 4, while irradiation with 2W (as represented in lanes 5,6,7 respectively) caused complete loosing of the plasmid bands and for sure the same result was obtained after irradiation with 3W and 1kHz as shown in lane(8). Generally form the previous two figures it will be clear that the laser irradiation had a major effect on plasmid bands specially after irradiating with powers (2, 3) W causes complete disappearing of the plasmid content after irradiation. According to Letokhov et *al.*(1991) said ,the idea of inducing DNA cleavage photo chemically has attracted attention, the strand breaks of plasmid DNA may be due to singlet oxygen which is generated through the irradiation process that causes plasmid to be destroyed due to the interaction with nitrogen base of DNA in particularly guanine bases, which may be act as sinks for electrons holes within DNA owing to their propensity to be oxidized. The electron holes induced by laser irradiation may be propagated via stacking interactions in the DNA helix until it encounters guanine cluster, where it may persist longer than at other sites (Letokhov V.S., et al., 1991 and Folwaczny M.J. 2000) (Al-khafaji A.S., 2002)

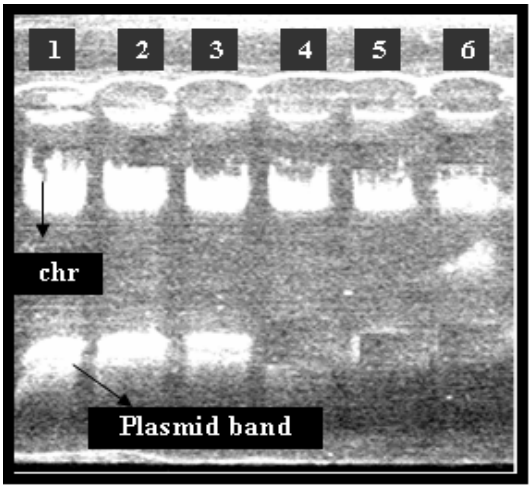

Fig. (2): Plasmid content for *E.coli* lane (1):plasmid contents without treatment(control) while lanes(2,3,4,5,6)represent plasmid content after irradiation with Diode laser[1W (1kHz,5kHz,10kHz) and 2W(1kHz,5kHz)]

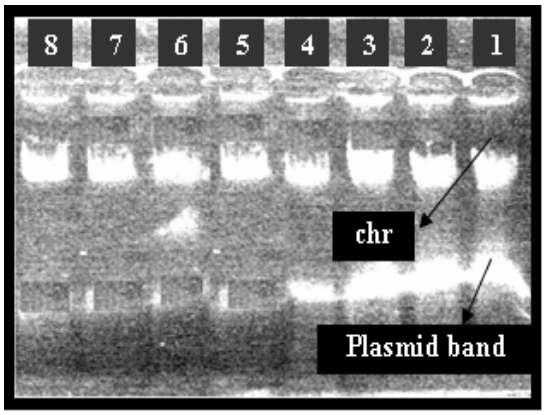

Fig. (3): Plasmid content for P.mirabilis isolate lane (1): plasmid content without treatment (control) while lanes (2, 3, 4, 5, 6, 7, 8): plasmid contents after irradiation with Diode laser 1W [1kHz, 5kHz, 10kHz], 2W [1kHz, 5kHz, 10kHz], 3W [1kHz]

Figure (4) represents the plasmid content for *P.mirabilis* isolate before and after irradiation with (1,2)W and (10)kHz. Lane (1) represents the bacterial plasmid& RNA content before irradiation(control) while lanes(2,3) represent laser effect after irradiation with (2,1)W respectively. As it is clear, the isolate starts losing its plasmid after using 1W but when 2W was used the isolate lost the RNA. In some cases there may be a lesion in DNA strand and the endonuclease. As *Cahaba*(2000) said the DNA makes Nicks on either side of the lesion which is then removed to leave a gap. This gap suppose to be filled with new nucleotides or nitrogen base by DNA polymerase and DNA ligase makes the final phosphodiester bond, the irradiation may affect these enzymes and make them loose their functions (Molna J.R, et al 2006, Cheba B.A. , 2000 and Alberto C., et al 1996).

Other fact which had been proved in this study was the effect of laser on RNA .It was clear that diode laser caused complete loosing of RNA band especially when 2W was used. These results were ensured using CTAB method for extraction .The effect on RNA can also be easily seen in Figure (5) which shows the complete disappearing of RNA after irradiation with (2W and 10 kHz). Lane (1) represent the plasmid profile before irradiation while lanes(2,3,4,5) represent the effect of laser on plasmid and RNA after irradiation with (1,2)W at frequencies(1,5,10)kHz respectively , the plasmids contents were affected using [1 W (5 kHz) and 2W(1, 5) kHz], beside the shining of RNA decreased gradually till it disappeared by

using 2W and (1,5)kHz. The effect of irradiation on large plasmid can be seen clearly in Figure (6), in this Figure another isolate of *E.coli* that contains large plasmids was taken to see the effect of irradiation. Lane (1) represents the plasmid profile before irradiation and lanes (2, 3, 4) represent the plasmid content after irradiation with [1 W (5, 10) kHz and 2W (10) kHz] respectively. The effect was obvious that by using 2 W (10) kHz the isolates lost their bacterial large plasmid content. The chemical composition of RNA differs from DNA that RNA is composed of one single strand as compared with DNA and the nitrogen base Thyaimin is replaced by Uracile in RNA (Candeias L.P., and Steenken S., 1992, White M.R.H., et al 2000, Harley P., Prescott M., and Klein A.D, 1999).

Disappearing of RNA strand may be due to the same reasons lead to disappearing DNA strands, beside RNA is composed of one strand which means it is more easily be broken by irradiation, and the free radicals including singlet oxygen which were formed during the process (Al-Jobori S.S. , 1997, Adenilson S., and Thiago O., 2010)

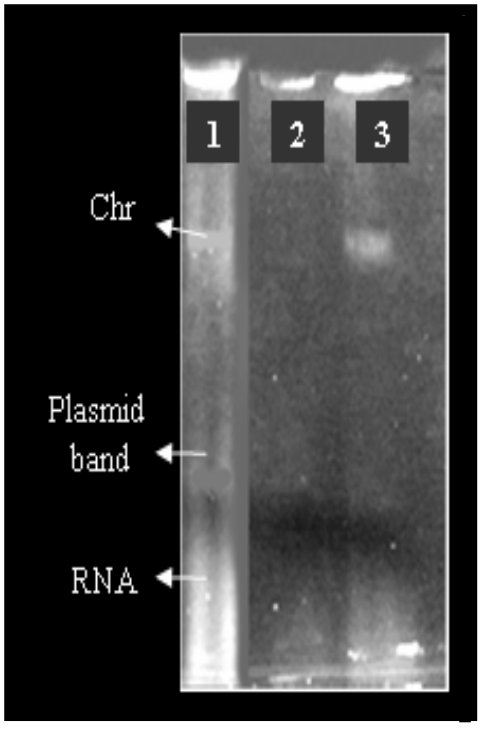

Fig. (4): Plasmid profile and RNA of *P.mirabilis* using CTAB method lane (1): plasmid and RNA contents without irradiation (control) while lanes (2, 3): plasmid and RNA contents after irradiation with Diode laser (2 Wand 1 W)

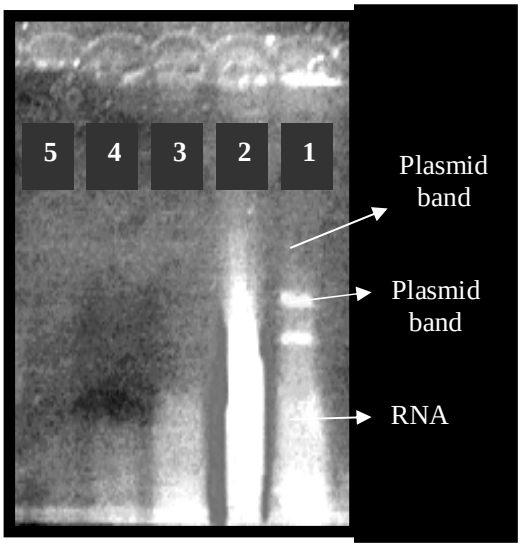

Fig. (5): Plasmid profile and RNA content for *E.coli* lane (1): plasmid and RNA contents of without treatment (control)while lanes (2,3,4,5): plasmid and RNA contents after irradiation with Diode laser (1,2)W at frequencies(1,5,10)kHz respectively .

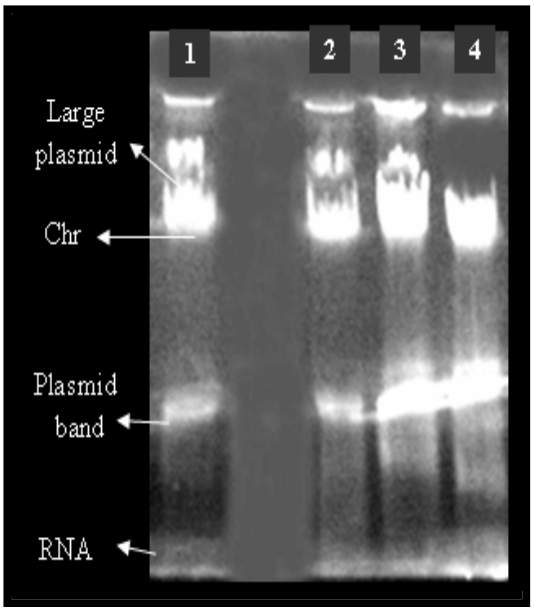

Fig. (6): Large plasmid profile of *E.coli* isolate lane (1): plasmid profile without treatment (control) while lanes (2, 3, 4) plasmid profile after irradiation with Diode laser[1 W (5, 10) kHz and 2W (10) kHz] respectively.

There is another reason that irradiation may be affected the DNA repair system. These results about disappearing of plasmid DNA and RNA of bacterial isolates have a good agreement with many researches which illustrated the similar effect of laser irradiation.

Conclusions

RNA and plasmids contents decrease gradually after laser irradiation this can be seen for small and large plasmid. This means diode laser could be used as a successful method in plasmid curing.

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تأثير ليزر دايود 805 نانومتر على محتوى البلازما لبعض القولونية المعزولة والمتقلبة

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الخلاصة يهدف هذا العمل الى دراسة تأثيرليزر الدايود 805 نانومتر على الحمض النووي الريبي البلازميد ومحتويات بعض الجراثيم السلبية التي يمثلها القولونية والمتقلبة يعزل البلازميد استخراج باستخدام طريقتين (تمليح بها وطريقة CTAB) مختلفة . واستخدمت معدلات تكرار النبضة 805 نانومتر لدراسة هذا التأثير. وكشفت النتائج التي تأثرت للغاية ملف بلازميد من هذين النوعين باستخدام (2 ، 3) واط على ترددات مختلفة بما في ذلك 10 كيلو هرتز ، مقارنة مع 1 كيلو هرتز بينما اختفت تدريجيا البلازميدات في W1 ، 10 كيلو هرتز. في الوقت نفسه مشرقة من الحمض النووي الريبي انخفض أيضا ثم اختفى تدريجيا مع زيادة خاصة في W2 والتسبب في اختفاء 10 كيلوهرتز من الحمض النووي الريبي أثناء استخدام 2 (1 ، 5) كيلو هرتز الأسباب تختفي تدريجيا. وهذا قد يوحي باستخدام الليزر الصمام الثنائي كوسيلة ناجحة في علاج البلازميد.