International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Effect of Ultraviolet Radiation on Bacteria

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ABSTRACT

Ultraviolet (UV) radiation is known to inhibit cell growth and induce gene damage (1). For these reasons, UV radiation is used as a method to sterilize surgical instruments because it kills the bacteria present and disrupts bacterial reproduction (2). Infections acquired from hospitals, particularly post-surgical infections, have become increasingly common, and require the use of UV disinfection systems. In this study, we investigated the effect of UV light on Escherichia coli (E. coli). Specifically, this study explored the effects of the small UV lights currently used in school laboratories, in an attempt to extend UV radiation methods to common households. We used the number of colony-forming units (CFUs) to determine whether or not the UV light increases or decreases cell growth. E. coli were exposed to UV light with a wavelength of 254 nm. The number of CFUs under control and UV-exposed conditions were measured after 24 and 48 hours. We observed that UV light exposure at 254 nm from a small school laboratory light inhibits bacterial growth.

Keywords: Ultraviolet,Colony Forming Unit, Escherichia Coli, bacteria.

INTRODUCTION

Microbiology defined as the study of organisms too small to be seen with the naked eye. These organisms include viruses, bacteria, algae, fungi, and protozoa. Microbiologists are concerned with characteristics and functions such as morphology, cytology, physiology, ecology, taxonomy, genetic and molecular biology.

HISTORY OF MICROBIOLOGY :-

Ultraviolet (UV) light is the portion of the electromagnetic spectrum between visible light and x-rays, with a wavelength of 100 to 400 nm. Sources of UV radiation include the sun, lasers, tanning beds, and numerous medical instruments such as dental polymerizing equipment. In medical operating rooms, UV radiation is a method of infection control. The UV light is used to sterilize both operating rooms and surgical instruments, which reduces the risk of surgical wound contamination and postsurgical infections. According to a study conducted by Duke University Medical Center and the University of North Carolina Health Care, in which 229 environmental surfaces were sampled from the rooms of 39 patients over a 15-month period, infection rates in medical environments were reduced from 10% to 0.24% using a UV-C lamp . In comparison to the lamp used in the experiment, the wavelength of the UV-C lamp implemented by Duke University is capable of stronger penetration of bacteria. Therefore, the effect of UV-C radiation would be greater than that of the UV.lamp used in this experiment. UV-C radiation is emitted by the sun and is typically blocked by the atmosphere, particularly the ozone.

Fig 1 :E.Coli

MATERIALS AND METHOD

Pure culture procedure for preparation-

Pure culture consist of a population of any one species of microorganisms.The isolation of kid of microorganisms from mixture of many different kinds is called pure culture techniques.The method use for isolation of microorganisms are as follows:-

1) Streak Plate method

2)pour plate method

- Dilutiontechnique
- Serial dilution technique

3)Spread plate method

4)Micromanipulator method

5) Roll tube method

1) Streak Plate Method:-

Streak plate method is most widely used method for isolation of culture .Streak plate are prepared by streaking a small amount of mixed culture over the surface of the solid medium in Petri plate with a platinum wire loop. The sample is streaked in such a way as to provide successive dilution.

Fig 2:- Streak plate method

2) Pour plate method:-

In the pour plate method, a fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of a sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

1)The microorganism are trapped beneath and surface of the medium when solidifies.

2) Hence surface as well as substance colonies are develop & difficult to isolate and count the sub surface colonies.

3) This method is tedious time consuming and require skill.

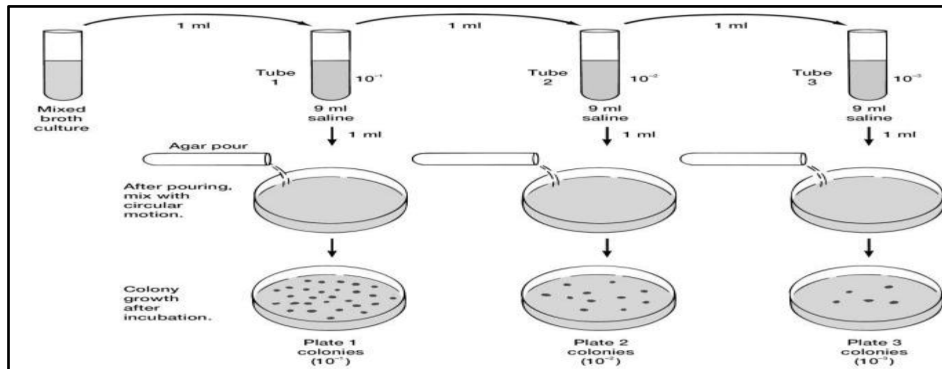


Fig 3:- Pour Plate Method

3) Spread Plate Method-

In this method the mixed culture is not diluted in culture medium.It is diluted in series of tubes containing sterile water or saline solution .A sample is remove from each dilution tube (0.1 ml) and place on to a surface of agar plate.The plate are incubate and isolated colonies are observed at 24 hours and counted.

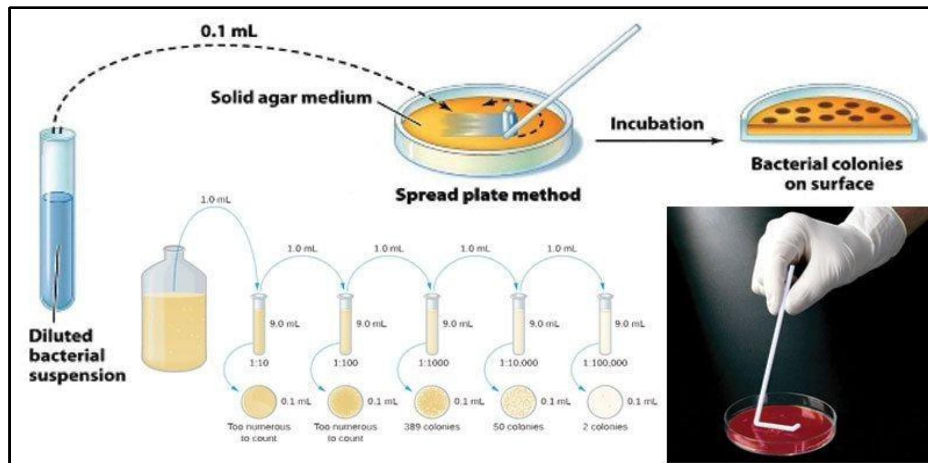


Fig 4:- Spread Plate Method

Advantages:-

1) It is simple Method & only surface colonies are formed

2) This method are also use for counting the microorganism present in the inoculum.

4) Micromanipulator Method :-

It is device that can pickup single microbial cell from a colony and mixed culture. It used in conjugation with microscope to pickup single bacterial cell from hanging drop preparation.

The single microbial cell is generally sucked into the micropipette and trasfered on to a large drop of sterile medium and another covership micromanipulation method makersone reasonably sure of pure culture coming from single cell.

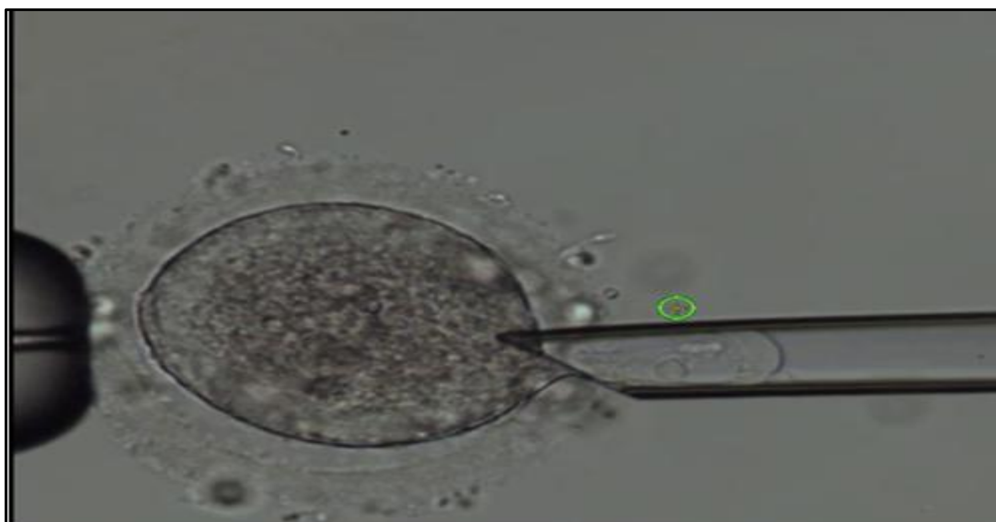


Fig 5:- Micromanipulator Method

5) Roll Tube Method:-

It is used for isolation of streingentanaerobes.This anaerobes culture tube is used for isolation which has coated with preeduced agar medium containing oxygen for nitroggen when stoppered is remove tube kept anaerobic by continously flushing with oxygen from carbon dioxide from gas.Inoculation done with transfer loop held against agar surface as tube being rotated by motar.Inoculation start from bottom and down loop gradually upword. After inoculation tubes restoppered and incubated anaerobically to get well isolated colonies.

Fig 6:- Roll Tube Method

Procedure for pure culture preparation :-

1) Label the name of organisim on bottom of the petriplate.
2) Sterlized the inoculating loop by holding it with right hand in flame till it red . 3) Hold the culture tube in left hand and remove the cotton plug with the help of little finger of right hand.

4) Sterilize the mouth of culture tube immediately in flame and taken culture with the sterilized inoculating loop. Replace the cotton plug and keep the tube in test tube rack.

5) Transfer the culture to label petriplate by streaking for the same. Hold the plate in left hand at angle 60c and open petriplate cover with the help of thumb.

6)Place the culture at one end of the plate and spread it slightly in rounded manner it is the primary inoculum.

7)Flame the loop and cool it,then streak the inoculum at angle to primary inoculum by making 5-6 parallel lines 1-2 lines should pass throgh primary inoculum.

8) The flaming of the loop result in disired dilution and fewer organism streaked in each sucessive area leading to final seperation of microbial cell.

9) Reflame and cool the loop.Turn the plate at a right angle.

10)Drag inoculum in several parallel lines across the agar surface by touching the inoculum in area 2 only once or twice.

11)Again rotate petridish at 90 c angle loop, touch the inoculum in area 3 and streak the inoculum several times in parallel line across the agar. Look at margine.

12) Without reflamimg the loop again turn the petri plate at angle and drag culture from previous streak series across area in a similar manner by making parallel lines.

13) Replace the lid on the petriplate and incubate the culture for 24 hours at 37c.

14) Observe plate for isolated colonies.

15) Pick and restreak the isolated colonies and another nutrient agar plate in similar manner to get the pure culture.

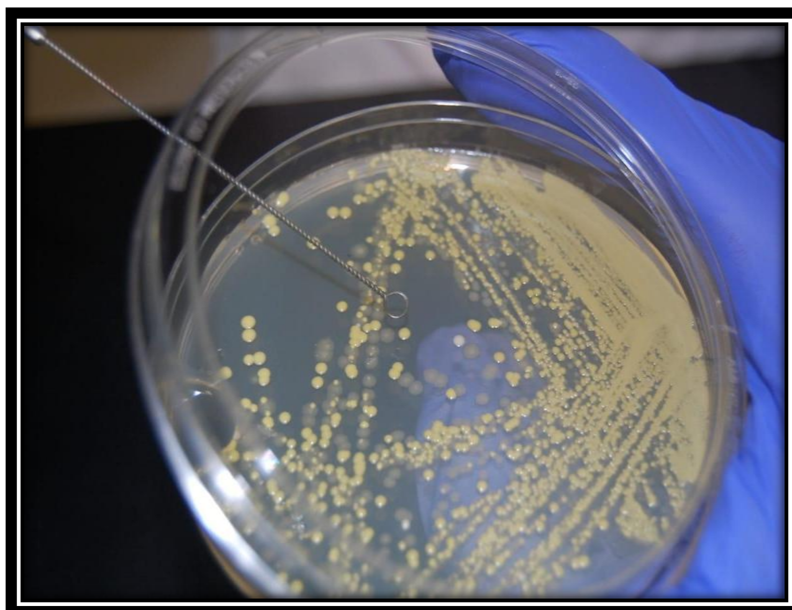


Fig 8 :- Pure Culture Media Effect Of UV Light On E-Coli:-

Effect Of UV Radiation On Bacteria Staphylococcus aureus & Klebsiella pneumoniae:-

Strains of bacteria Staphylococcus aureus and Klebsiella pneumoniae were obtained from microbiology laboratory of College of Applied Education and Health Sciences, Meerut. They had been isolated through basic microbiological techniques and confirmed through biochemical techniques. Biochemical test were done to confirm that the culture medium was free from contamination.Two sets were made namely Test and Standard. A total of eight plates of each bacteria were prepared from each of the isolates, In Control bacteria were not exposed to Ultra violet radiation, while in test,the bacteria were exposed to Ultraviolet radiation for 5 hours with the lid on dish and both Control and Test bacteria were incubated aerobically at 370C for 24 hours.

The same experiment was repeated in triplicates.

Determination of Bacterial Survival time :-

The bacteria survival rate was monitored by colony count of both the controlsand the experiments. The number of colonies on the control plates was used to compare those of the experimental treatment.

Antibiotic Sensitivity Test:-

Isolates that survived the exposures to Ultra Violet radiations were subjected to antibiogram test by planting them on nutrient agar and exposing them to the antibiotics of which they had been previously tested and showed resistance. Antimicrobial susceptibility testing was performed using the disk diffusion method. Antibiotics were selected to represent some major classes of antibiotics .Antibiotics used in study include Cefixime(200mg)Oflaxacin(200mg),Ornidazole(200mg),Amoxicillin(200mg),Moxifloxacin(200mg),Ciprofloxacin (200mg).

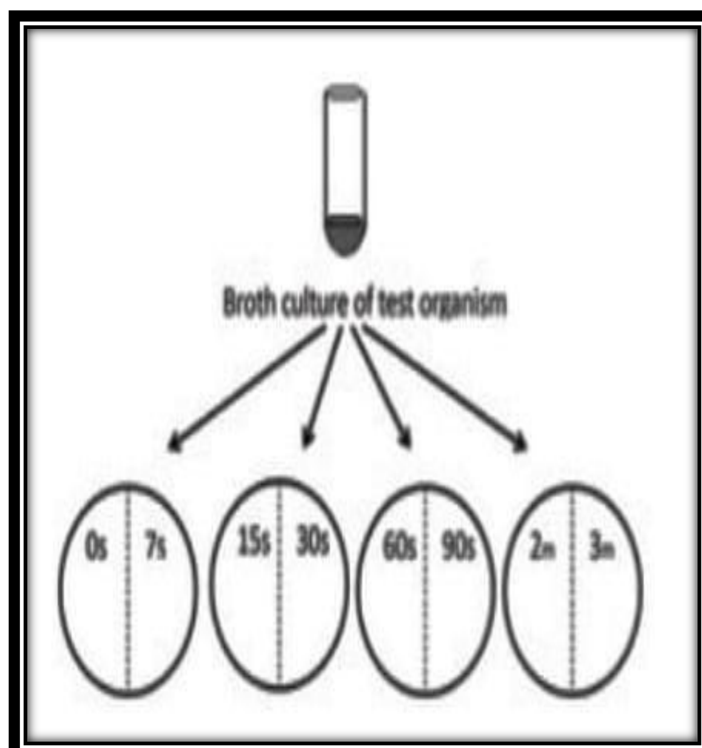


Fig 9:- Broth culture

Lactic Acid Bacteria Induced By UV Radiation:-

Microorganisms **:-**

The lactic acid bacteria Lactobacillus acidophilus GH 201, Lactobacillus delbrueckii MH10 and Lactococcus lactis GH 204 and Escherichia coli K-12 obtained from the Microbial Depository Center of NAS RA.

Media:-

LAPTg and Nutrient broth (Sigma and agar media were used). Phosphate buffer solution (PBS) (0.1 M), pH 6.8 was used in irradiation experiments.

Ultraviolet Sensitivity Assay :-

For ultraviolet sensitivity assay, LAB were grown at 37˚C in 20 ml of LAPTg and E. coli in NB up to OD 0.4 (600 nm). The cells were harvested by centrifugation at 5.000 g for 15 min and washed twice in 20 ml cold PBS. Aliquots of cell Suspensions (2 ml) was transferred to sterile Petri dishes (D=5 cm) and exposed to UV-light (254nm) for 5, 10, 20 and 40 seconds. After serial dilution, 0.1 ml cell suspensions from each treatment were spread on LAPTg agar and after growth enumerated cells amount. Cultures in flasks wrapped with aluminum foil and plates were kept in complete darkness to avoid photo-reactivation.

Mutagenesis and Selection of Antibiotic Resistant Mutants :-

LABs were grown at 37°C in LAPTg to optical density up to OD 0.6, cells were harvested by centrifugation at 5,000 x g for 15 min and resuspended in PBS.Cell suspensions 2 ml aliquots were poured in sterile petri dishes and irradiated with UV – light (254 nm) for 5, 10, 20 and 40 sec. Treated cells were diluted tenfold into fresh LAPTg broth and grown at 37°C for 4 h to permit 3 - 4 division cycles and spreated on LAPTg agar containing 100 µg/ml of appropriate antibiotic and incubated at 37°C till colonies appearance.

Statistical Analysis:-

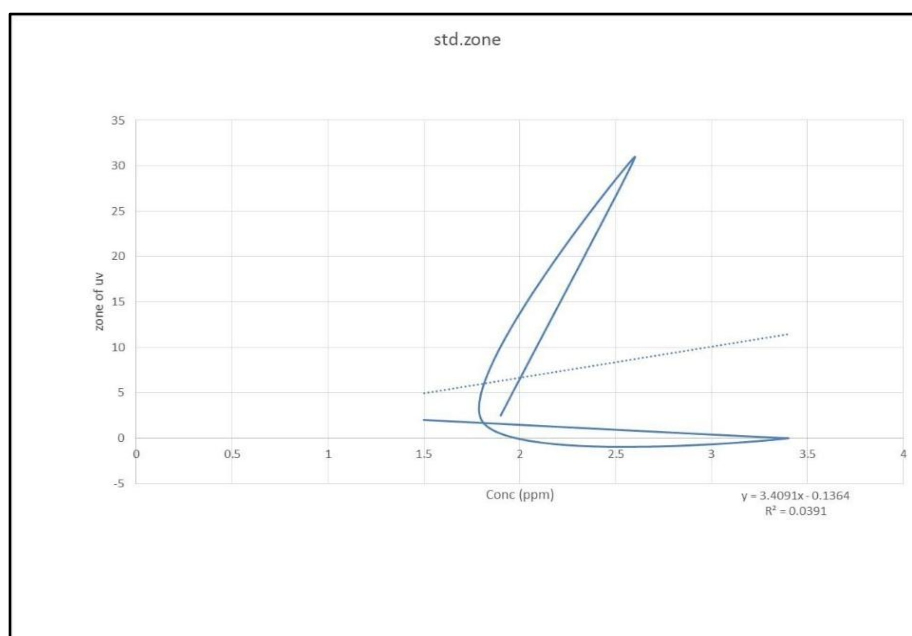
Statistical analysis was performed with Student's t-test and $P < 0.05$ was considered significant to indicate difference

RESULT

Table 1-The result of Ultra violet exposed bacteria effect against different antibiotics

BACTERIA (*Staphylococcus aureus)*

NAME OF ANTIBIOTICS	ZONE OF INHIBITION (TEST) UV EXPOSED	ZONE OF INHIBITION	EFFECT
Cefixime	1.9mm	2.5 mm	Decrease
Amoxicillin	2.6 mm	3.1 mm	Decrease
Oflaxacin	1.8 mm	2.0 mm	Decrease
Moxifloxacin	3.4 mm	0.0 mm	Increase
Ornidazole	1.5 mm	2.0 mm	Decrease
Ciprofloxacin	1.9 mm	0.1 mm	Increase

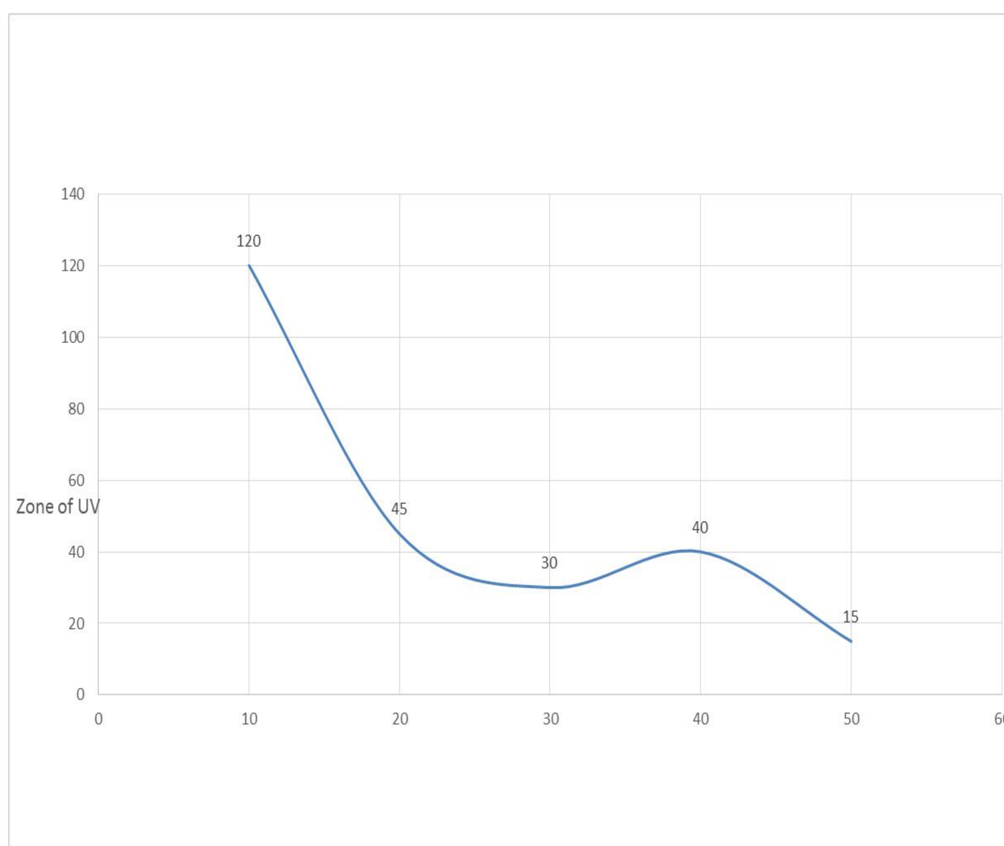


Graph1. Showing effect of Ultra Violet radiation on bacteria (Staphylococcus aureus).

Table 2.The result of Ultra violet exposed bacteria effect on against different antibiotics.

BACTERIA *(Klebsiella Pneumoniae)*

NAME OF ANTIBIOTICS	ZONE OF INHIBITION (TEST) UV EXPOSED	ZONE OF INHIBITION (STANDARD)	EFFECT
Cefixime	3.9mm	2.5mm	Increase
Amoxicillin	3.2mm	1.8 mm	Increase
Oflaxacin	3.1 mm	2.0 mm	Increase
Moxifloxacin	3.1 mm	2.6mm	Increase
Ornidazole	3.7 mm	2.1 mm	Increase
Ciprofloxacin	0.0 mm	1.1 mm	Decrease



Graph 2.Showing effect of Ultra violet radiation on bacteria(Klebsiella pneumoniae)

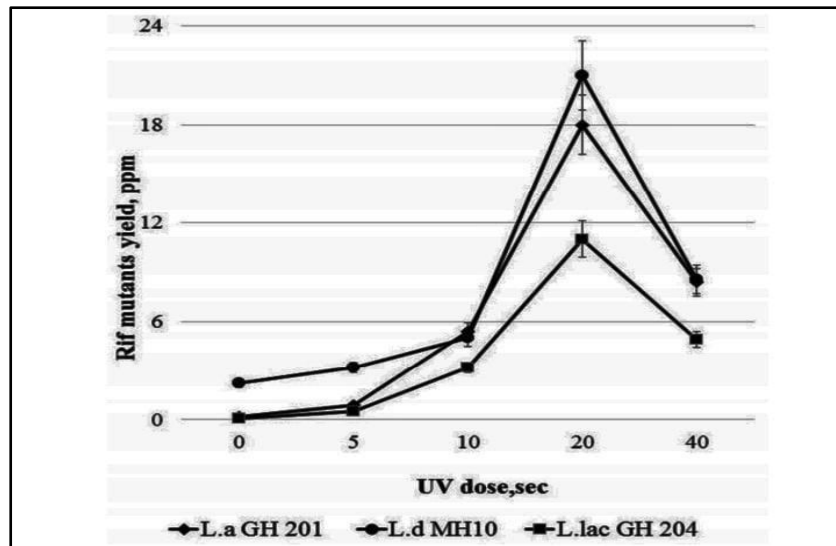
The result of observation for both the standard (bacteria with no UV exposure) and test (bacteria with exposure of ultra violet radiation) has been tabulated in terms of zone of inhibitions (mm) observed against both the strains.

Table 1: shows the result of UV exposed bacteria have an effect on its efficacy against different antibiotics. Recombinant bacteria Staphylococcus aureus show decrease in zone of inhibition against diffferent antibiotics used but Moxifloxacina (200mg) appear to showed increase zone of inhibition against recombinant Staphylococcus aureus.

Table 2. shows the result of UV exposed bacteria have an effect on its efficacy against different antibiotics. Recombinant bacteria Klebsiella pneumoniae show increase in zone of inhibition against diffferent antibiotics used but Ciprofloxacin (200mg) appear to showed decrease zone of

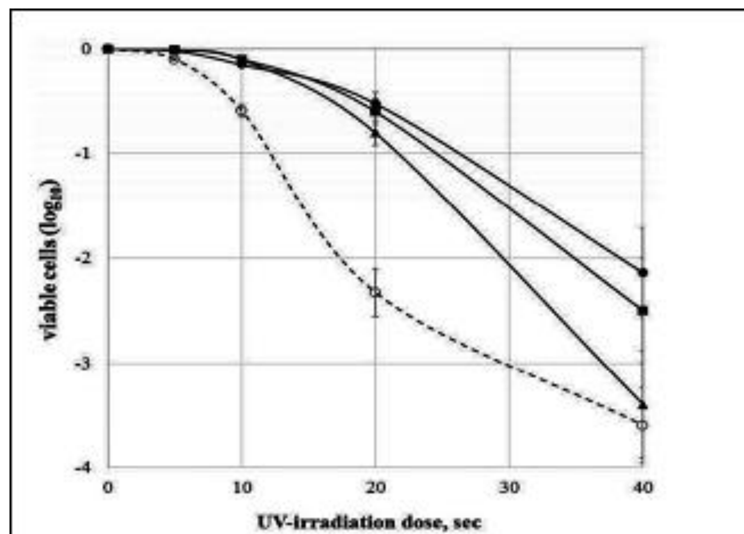
inhibition against recombinant Staphylococcus aureus.

The LAB species L. acidophilus, L. delbrueckii, L. lactisand E. coli K-12 exposed to UV light (5 sec, 10 sec, 20 sec and 40 sec) and survival curves were generated to estimate their relative sensitivity.

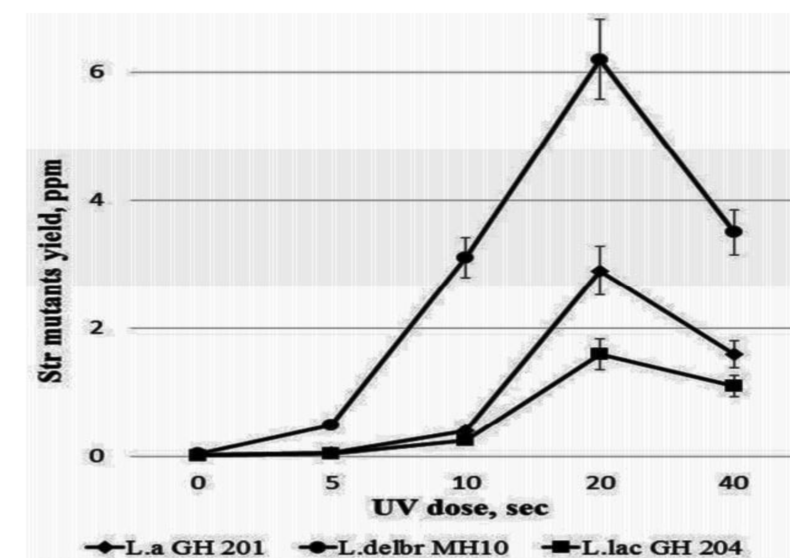


Graph1.1:- Survival rate of lactic acid bacteria and E.coli irradiated by UV light.

Graph1 shows the relative rate of survival of the lactic acid bacteria exposed to UV compared to those of E. coli K-12. The survival curves show that there was steady decrease in survival with increasing exposure to UV. The characters of survival curves of LAB strains as well as E. coli were sigmoidal. The lactic acid bacteria were1 – 2 log more resistant to UV than E. coli, but at dose 40 sec the difference in survival between L. lactis and E. coli was not so significant. In order to study UV mutagenicity, rifampicin and streptomycin were used as selective agents for these experiments because of relatively low frequency of their spontaneous resistance mutations . The mutation rates were derived after plating UV irradiated cultures LAPTg medium with antibiotic. The yield of Rif and Str mutants of LABs after various UV exposures was studied (Graph 2-3).



Graph 1.2:-The yield of Rif mutant of L.acidophilus By UV Radiation.



Graph 1.3:- The yield of Str mutants of L.acidophillus by UV Radiation

As the LABs survival rate reduced the mutation rategradually increased, reaches to maximum at dose of 20 sec (~0.1% of survivors). The UV induced yield of Rif & Str mutants were 1-2 log higher than of spontaneous yield. The yield of mutants in the lactobacilli cultures were 1.5 time superior the yield of mutants in L.lactis GH 204. In turn the yield of Rif mutants more than 5 time greater, than Str mutants. All obtained by UV mutagenesis Rif and Str mutants were table during serial cultivation.

DISCUSSION

It was revealed that LABs are 1-2 log more resistant to UV irradiation in comparison with E. coli. UV light causing damages in DNA by formation of covalent bonds (dimer) between neighbors pyrimidines, mainly between thymine.Beside of resistance to UV some of lactobacilli show enhanced stress resistance caused by absence of genes for sporulation, catalase, and other key enzymes of oxidative stress response. Also LAB high resistance was demonstrated by theincreased recovery of live lactobacilli from irradiated food by comparisonto staphylococcal and Salmonella species.

CONCLUSION

The results revealed that the UV exposure did decrease the number of bacterial colonies formed. In fact, there were fewer CFUs in the petri dishes exposed to UV for 48 hours than in those exposed for 24 hours. The longer the duration of exposure resulted in a lower amount of CFU's.

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