# MUTANT OF ALOCAL ISOLATE OF LACTOBACILLUS PLANTARUMBY UV LIGHTIN COMPARISION WITH ASTANDARD STRAIN THE EXTENT OF ITSABILITY TO LOWER CHOLESTEROL LEVELS[1]

Sinan J. Abdul-Abbas*; Aswan H. Aboud Al-bayyar**; Adnan E. Al-Badran***

* Department of Food Science ,College of Agriculture,University of ,Basrah,Iraq

**Department of Food Science ,College of Agriculture,University of Baghdad, Baghdad ,Iraq

Department of Biology ,College of Science,university of Basrah,Basrah,Iraq

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## ABSTRACT

Local strain bacteria of *Lactobacillus plantarum* IRQ12 isolated from cucumber sativuspickle registered in (GenBank LN871447) and another standard strain, IRQ12 strain was mutated by UV light (254 nm) for (50 cm) distance for different periods 20, 25, 30, 35 and 60 second, RAPD – PCR test was done to investigate the effect of UV light on*L.plantarum* genes and determine the genetic distant. The ability ofstandard and local mutated bacteria to decrease the amount of cholesterol was determined in MRS broth–EY mediawhich was (4.6 mg/ml), the cholesterol amount was decreased at 25s which was0.35 mg/ml in a decreased percentage 92.3%, followed by 60s and 35s which were 0.43and 0.45mg/ml in a decreased percentage 90.6% and 90.2%, respectively.

## INTRODUCTION

In biology, a mutation is a change occurs in the genetic information - bio genetic information encoded in DNA sequences and chromosomes that contain DNA or RNA sequences, as in some viruses, mutation makes changes in the sequences of nucleotide bases in different ways, it may change the arrangement sequences of nucleotides, or it may change its number by deletion or insertion or mutations caused by viruses(2, 6,9). Mutationcan occur as a result of exposure of mutagenic chemicals and radiation and errors that occur during DNA doubled,or spontaneously, can be caused by organismitself by cellular processes such as hyper mutation (26).The mutation is one of the methods

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used widely to improve the bacterial strains which are used in industrial microbiology(3). Ultra-violet (UV) light has strong effects on DNA genes, occurrence of mutations,in the worst case it may cause the growth of cancer; sun is the main source of UV (31, 8). Random mutations by ultraviolet rays used to improve certain properties of the strain, as the efficiency of the formationmutations depends on the type of damage (replace the pair of base, deletion and insertion..Etc.)Which causes a certain change in the DNA and the mechanism to repair this damage, mutation resulted from the use of UV light is more stable in the long time of generation (11, 25, 36). (15, 10) studied the effect of random mutations of three genes of*L.plantarum*bacteria which encode proteins related to stress, studies were indicated that these mutations can cause the distinctive changes could be observed on the cell surface when grow and thus affect their ability to live symbiotically (16, 7, 10). (27)Was indicatedthatthe range of UV impact in the DNA depends on the dose ofradiation as its intensity,time exposure and the distance between the sample and the source.The cholesterol is the main cause of heart disease and hardening of thearteries,(37)illustrated that there is a relationship between cholesterol and heart disease and blood vessels.There are two types of cholesterol, one of them is because the food contain of a high rate of cholesterol like meat, milk and eggs, and this is called the outer cholesterol (Exogenous cholesterol) The second type is the internal cholesterol (Endogenous cholesterol) which is synthesis within the body and is made in the liver (24).Many studies have indicated that Lactobacillus bacteria have the ability to lower cholesterol through many mechanisms like deconjugation (22).This is done through the ability of therapeutic bacteria to absorb the cholesterol and decreased its level in addition to its ability to produce ferulic acid(38, 39). (37) found that *L.plantarum* bacteria have the ability to metabolic the cholesterol in a rate 28.3% in amediainclude the cholesterol in a rate 100 Mμ gm/ml when treated for 24 hours, whilein MRS media which include the intestinal fluid 100 Mμ gm/ml of cholesterol metabolism is 20.5%.

The aim of this studyis to investigatethe ability of the *Lactobacillus plantarum*bacteria to decrease the cholesterol levels and using the mutation of bacteria by UV light to increase its ability to decrease the cholesterol levels.

## MATERIALS AND METHODS

The local strain bacteria *Lactobacillus plantarum* IRQ12 isolated from Cucumbersativus pickle which registered in (GenBank LN871447) was used in this study, while the standard strain thatused as starter was suppliedbyChr-Hansen's Denmark / UK. The mutation process for these bacteriadone by using UV frequency (254nm) for distance 50 cm with various periods (20, 25, 30, 35 and 60) seconds according to the method of (19, 20)which wasmodified by (34) usingTryptone soy broth (TSB) media.

**Detection of the identical genes**

DNA was extractedusing Automated Nucleic Acid Extraction system following the manufacturer instructions. Theelectrophoresis gel wasprepared for the detection of DNA molecules(1).

**Random Amplified Polymorphic DNA (RAPD) byPCR**

RAPD-PCR was performed as previously described (33).

**Table (1) Identification RAPD – PCR primers**

| Primers | Sequence | Length | Optimizing TA |
|---|---|---|---|
| 1283 | 5'-GCGATCCCCA-3' | 10 | 36˚C |

**Table (2) Materials and quantities used for the amplification of RAPD (20µl) in PCR**

| Reagent | Volume |
|---|---|
| primer | 1.5 (30 pmol) |
| Go Taq Green Master Mix. 2x | 5µl |
| DNA template | 2.5µl |
| Free nuclease water | 11 µl |
| Total volume | 20µl |

**Table (3) the conditions for PCR cycles for RAPD reaction**

| Steps | Temperature | Time | Number of cycles |
|---|---|---|---|
| Initial denaturation | 95$^{o}$ C | 3 min. | 1 |
| Denaturation | 94$^{o}$C | 1 min. | 45 |
| Annealing | 36$^{o}$C | 1 min. | |
| Extension | 72$^{o}$C | 2 min. | |
| Final extension | 72$^{o}$C | 5 min. | 1 |

**Data Analysis**

- The RAPD bands of each individual strain were calculated for theirdistances based on the ladder's bands.
- The data of the RAPD patterns of all strains according to (29) were transformed to the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm program creating and modifying by (18).
- RAPD patterns of individual strains were compared based on the index of similarity between samples (12), providing a mathematical model by calculating a similarity matrix and transforms similarity coefficients into a distance matrix (Distance Matrix value "0.000" indicating identical strains) and makes a clustering to construct a dendrogram from a set of variables, to study genetic variation especiallywith difficult or closelyrelated RAPD patterns.

**Cholesterol determination**

The mediaMRS-Egg yolk (MRS-EY) was preparedas previously described(4).

**Determination of cholesterol rate**

The rate of cholesterolof all media used for testing the ability of *Lactobacillus* to decrease the cholesterol was determinedas previously described (17).

## RESULTS AND DISCUSSION

**Study the rate of geneticvariation for *L.plantarum* IRQ12isolate before and after induction of mutation**

Figure (1) showed theresults of using RAPD – PCR between the non-mutated strain in line 2 and the mutated strain with the exposure periods 20, 25, 30, 35 and 60s in the lines 3, 4, 5, 6 and 7 respectively, which are distinctlydifferent and there is a mismatch between bands. The genetictree (Dendogram) (Figure.2) illustrate that the periodof radiation exposure led tomakevarietymutations in all periods, and this indicate that the irradiation process led to make a genetic mutation.It was also absolved the lack of any

genetic match between the allmutation periods for IRQ12 strain, as each time showed individual mutation genetic.

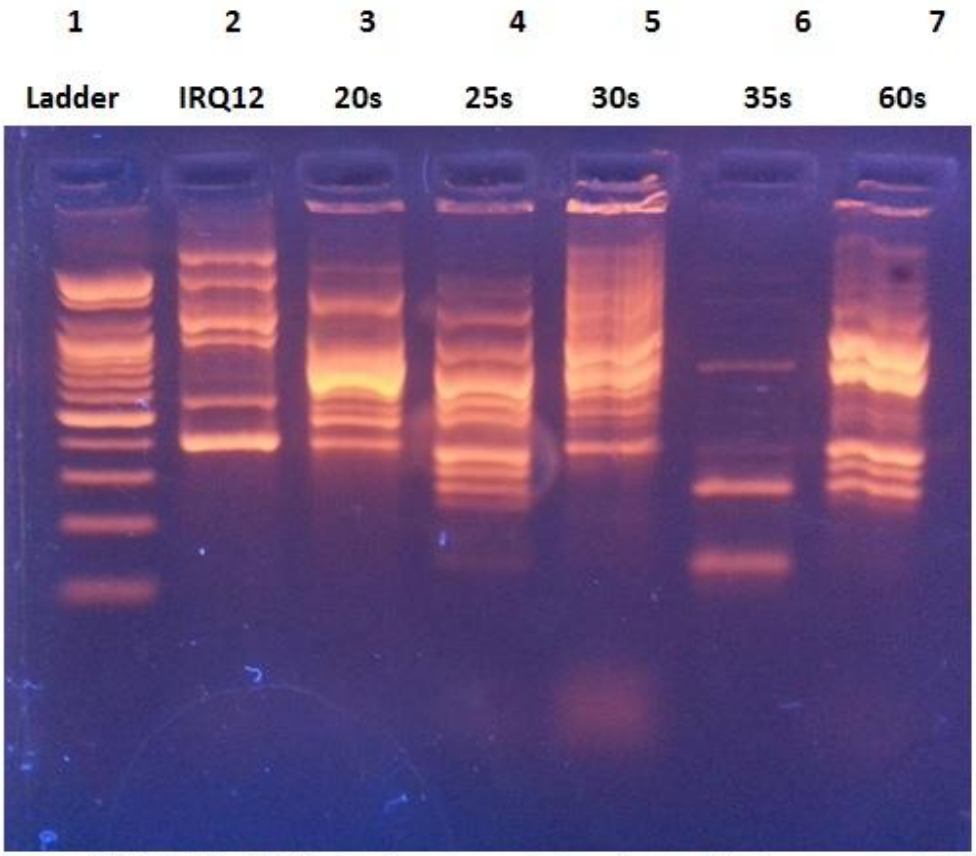


**Figure (1) RAPD – PCR electrophoresis bands on 2% Agarose gel, 70 volts for *L. plantarum* exposed to UV light for different periods**

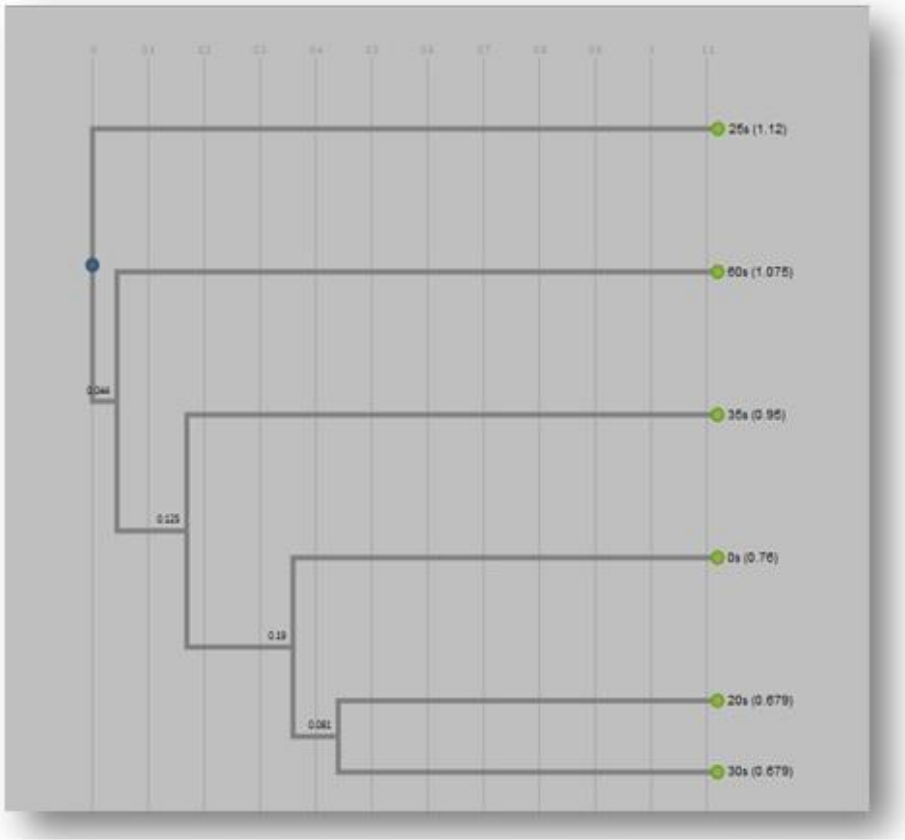


**Figure (2) the genetic tree (Dendogram) and genetic distant for different periods of *L. plantarum* strain exposure to UV light**

**Table (4) Effect of different exposure periods of *L. plantarum* IRQ12 strain for UV light**

| Strain | 0s | 20s | 25s | 30s | 35s | 60s |
|---|---|---|---|---|---|---|
| 0s | 0 | 1.509 | 2.526 | 1.532 | 1.986 | 2.186 |
| 20s | | 0 | 2.423 | 1.359 | 1.886 | 1.930 |
| 25s | | | 0 | 2.384 | 1.933 | 2.280 |
| 30s | | | | 0 | 1.742 | 2.049 |
| 35s | | | | | 0 | 2.213 |
| 60s | | | | | | 0 |

Table (4) showed that *L.plantarum* IRQ12 strain exposed to irradiation for 25s was moreaffected on bacteria DNA and this is because of ahigh mutation rate for strain which was 2.52, while it was less affectedfor irradiation exposure (less genetic distance),at 30s and 20s as it was 1.359.

Figure (3) indicate the local and standard strains ability to decrease the cholesterol rate of (4.6 mg/ml) after growth on MRS broth – EY media at 37°C for 24 hours. The cholesterol rate in broth media for IRQ12 and the standard strains before induction of mutation was 0.52, 0.733 mg/ml.It was absolved that IRQ12 have less rate of cholesterol (Figure4) in the media with decreasing the percentage 88.69%, while the decrease percentage of thestandard strain was 84.06%, the results of mutation for *L.plantarum* IRQ12 bacteria after grow on MRS broth – EY media at 37°C for 24 hours indicate that the cholesterol rate in varying depending on the radiation dose which was used, the less amount of cholesterol in the media for mutation isolate for 25s which was 0.35 mg/ml with a high decreasing percentage 92.3%, Followed by the strain treated for 60s with amount 0.43 mg/ml and with decreasing percentage 90.6%, then the mutation strain for 35s with amount 0.45 mg/ml and with decreasing percentage 90.2%, while the mutation strains treated for 30s and 20s the cholesterol amount in the media were approached IRQ12 strain before mutation which were 0.541 and 0.537 mg/ml with the decreasing percentage 88.23% and 88.3%, respectively. It was noted by the results mentioned above, find that *L.plantarum* bacteria has the ability to decrease cholesterol levels were high when it compared with other results from previous studies.

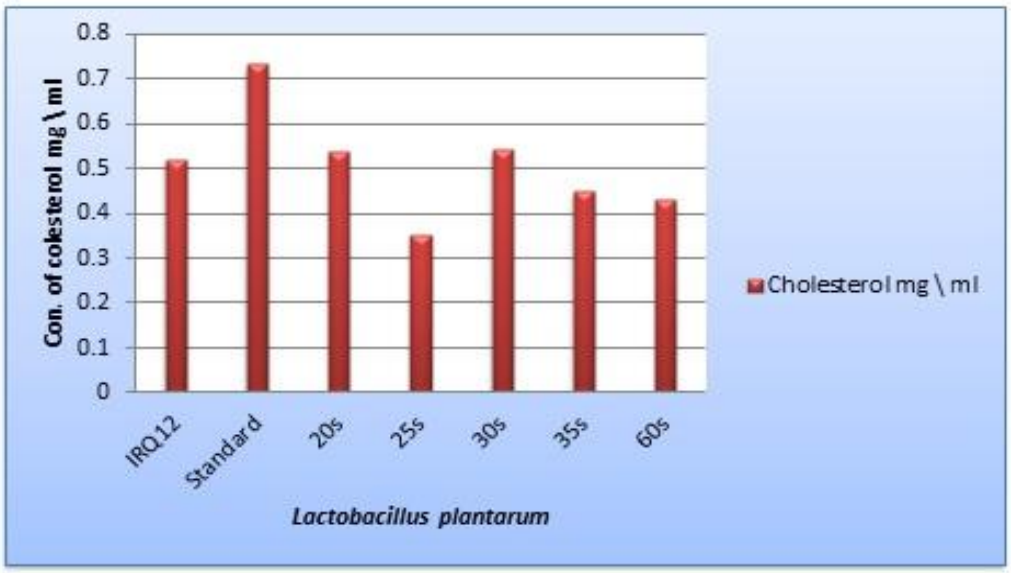


**Figure (3) the ability of local and standard *L. plantarum* bacteria to decrease cholesterol amount mg/ml in MRS broth – EY media**

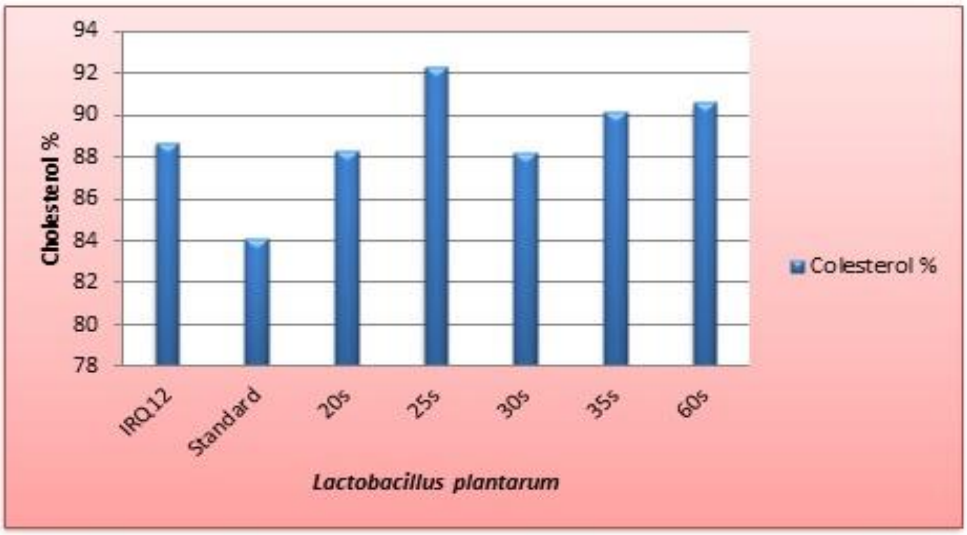


**Figure (4) Decreasing percentage of cholesterol for local and standard *L. plantarum* in MRS broth – EY media**

The current results isagreement with (32) in a study included 15 isolated bacteria *L.plantarum* from different sources which observed bacteria ability to absorb and decrease cholesterol between 56.52 - 95.65%.It is also agree with (30)when *L.plantarum* strain capable to decrease cholesterol levels between 26.74 - 85.41% in growth media

after 20 hours in incubator.While as for the isolation of mutation isolate IRQ12 which showed a significant increase in the percentage of decrease cholesterol at 25s,35s and 60s and this result are approach with (5), whichwas investigated the *L.caesi*ability to decreasethe cholesterol when grown in MRS broth - EY media, the percentage decrease was 92.5%.The increasingof the cholesterol decreasing percentage in mutationisolates maybe due tothe increasingability for the cell membrane of bacteria to absorb more than the amount of cholesterol in the media(23). It must be pointed out that the bacteria's ability to absorb cholesterol affected according to their growth rate in the media (14). (28)Investigatedthat proposed mechanism for reducing cholesterol in the lactic acid bacteria involves removing some of cholesterol through the cell membrane during growth, so the useful of a high amount of live bacteria to increase ability to absorb more amount of cholesterol,thereby reducing the rate of absorption of cholesterol in the intestine and transfer to the blood(21). The absorb cholesterol through the cell membrane estimated 20% (13).Whilethecholesterol as a whole did not absorbed through the cell membrane, assome researchers believe that free cholesterol maybe compoundwith cells so maybe disposal out throw thedead cells, this characteristic may increase due to the occurrence of some of the changes that may lead to increase the tensile strength of the external membrane of the bacteria, which increase the deconjugation(21).Also, the efficiency of the binding process between the cholesterol molecules and the outside member of bacteria are strongwhich cannot separate, even when washing several times(35).

# تطفير العزلة المحلية لبكتريا *Lactobacillus plantarum* بوساطة الأشعة فوق البنفسجية ومقارنتها مع سلالة قياسية لتقدير مدى قابليتها على خفض الكولسترول[2]

سنان جودة عبد العباس* ،أسوان حمد الله عبود** ،عدنان عيسى البدران***
*قسم علوم الأغذية ، كلية الزراعة ، جامعة البصرة ،البصره ،العراق.

** قسم علوم الأغذية ، كلية الزراعة ، جامعة بغداد ،بغداد ،العراق.

*** قسم علوم الحياة ، كلية العلوم ، جامعة البصرة ،البصره ،العراق.

## الخلاصة

استخدمت سلالة محلية من بكتريا *Lactobacillus plantarum* IRQ12 معزولة من مخلل خيار القثاء ومسجلة في بنك الجينات (GenBank LN871447) وسلالة أخرى قياسية، تم تطفير السلالة IRQ12 بوساطة الأشعة فوق البنفسجية بطول موجي (254 nm) ولمسافة 50 cm عن النماذج وعلى مدد تطفير مختلفة 20 و 25 و 30 و 35 و 60 ثانية، وجرى فحص التضخيم العشوائي متعدد الأشكال – RAPD PCR لبيان مدى تأثير الأشعة الآيونية على جينات بكتريا *L.plantarum* وتقدير البعد الوراثي بين الجينات. جرى تقدير قابلية البكتريا القياسية والسلالة المحلية المطفرة على خفض مستوى الكولسترول (4.6 ملغم كولسترول / مل) في وسط مرق MRS – EYإذ أظهرت النتائج انخفاض مستوى الكولسترول للبكتريا المطفرة بمدة التطفير 25 ثانية وبلغ 0.35 ملغم كولسترول / مل وبنسبة مئوية للانخفاض بلغت 92.3 تلتها مدة التطفير 60 و35 ثانية بمقدار 0.43 و 0.45 ملغم كولسترول / ملوبنسبة مئوية للانخفاض بلغت 90.6 و 90.2 على التوالي.

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