BIOCHEMICAL AND MOLECUIAR VARIABILITIES OF *LACTOBACILLUS CASEI* MUTANTS IN RELATION TO LACTIC ACID PRODUCTION

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ABSTRACT

High lactic acid producer strain ***Lactobacillus casei* subsp *rhamnosus* NRRL B-445** was subjected to induction of mutations using MNNG and UV-irradiation .Out of the obtained 366 isolates, about 106 isolates excelled the production of the original strain (as higher lactic acid producers). Six isolates yielded about 13-25% more lactic acid than the original strain .MNNG treatment induce higher numbers of auxotrophs than that obtained following UV-treatments but mutagenic treatments with UV-light proved to be more effective than MNNG for enhancement of lactic acid production .SDS-PAGE protein patterns showed that the high lactic acid producer isolates following both UV and MNNG treatments exhibited the highest number and intensity of bands in comparison with the other isolates and the original strain. Using RAPD–PCR analysis, many differences in RAPD banding patterns profile were detected as a result of mutagenic treatments.

INTRODUCTION

Microorganisms respond to sublethal doses of chemical and physical agents by synthesizing a variety of specific proteins and low molecular weight compounds. The newly synthesized cell components are thought to play a role as protectors or as signal compounds promoting the development of defensive reactions or tolerances **(Hartke *et al.* 1995)**.

Lactic acid producer bacteria (LAB) group contains extremely genetic divergent genera such as ***lactococcus, lactobacillus, Leuconostoc ,Streptococcus, Pediococcus*** and ***Enterococcus*** .All of these genra play an important role in human and animal food industry as well as human health benefits **[Pot *et al* .(1994) and Naidu *et al* .(1999)]**.

Differentiation of bacterial strains allows the diversity among the strains and within a species to be revealed. These strains can be

distinguished on the basis of biochemical and physiological properties, sensitivity to bacteriophages or antigenic properties. More recently, molecular techniques have been used to distinguish bacteria at the intrasubspecific level **(Quriberoni *et al.*, 1998)**. Among these techniques, Randomly Amplified Polymorphic DNA **(Welsh and McClelland, 1990; Williams *et al.*,1990)** has been found to be a rapid and sensitive method which can be applied to lactic acid bacteria for this purpose **(Schleifer *et al.*, 1995).**

The present investigation was directed to correlate between both biochemical and molecular analysis of both protein and DNA of some new constructed isolates from one side and their lactic acid productivity and genetic structure modifications from the other side.

MATERIALS AND METHODS

I- Micoorganism and Fermentation Medium

Out of ten lactic acid producer strains and four fermentation media, **Khattab (2002)** found that lactic acid bacterial strain *Lactobacillus casei* subsp. *rhamnosus* NRRL-445 with the fermentation medium of **Rincon *et al.*, (1993)** showed the highest combination in relation to lactic acid production. So, this strain used as the original wild type for lactic acid production on the previous fermentation meduim.

II. Growth Media

1. Man Rogosa Sharpe medium **(MRS)(De Man *et al* , 1960)** , it was used for routine culturing and preparing the seed inoculum for fermentation tests.
2. Basal medium **(BM)**: it was **Morishita *et al.*, (1981)** medium which used for mutant isolation.
3. Skim milk medium **(Lee–Wickner and Chassy, 1984)** was used for stock cultures maintenance of lactic acid bacterial strains.

III- Reagents and Buffers

a-Protoplast formation buffer (PB), **Lee–Wickner and Chassy (1984)**

b- Reagents , buffers and gels for total protein electrophoresis was the same as **Hames and Rickwood (1990)**

c- Buffers for isolation of total DNA from *Lactobacilli* was the same as **Anderson and Mckay (1983).**

d. Reagent and primers for molecular analysis;

1. Polymerase chain reaction (PCR) reagents For PCR technique, Ready-To-Go PCR Beads (Amersham, Pharmacia Biotech. No.

27-9555-01) were used. Each bead contains all of the necessary reagents, except primer and DNA template, for performing 25 µl PCR amplification reaction.

2. Primers: Table (1) presents six random 15-mer primers supplied by Gulf-Biotech. Laboratory.

Table (1): The nucleotide sequences of the applied primers.

Serial No.	Type of primer	Cat. No	Nucleotides sequence
1	Random	9050320	5'-GGG GTT TGC CAC TGG-3'
2	Random	9050321	5'-CAT ACC CCC GCC GTT-3'
3	Random	9050310	5'-GTG TTG TGG TCC ACT-3'
4	Random	9050322	5'-AAC CTC CCC CTG ACC-3'
5	Random	9050303	5'-TGA GTG GTC TAC GTG-3'
6	Random	9050314	5'-CCC CAA CGC CGA CTC-3'

Methods:

1. Fermentation and determination of lactic acid production was carried out according to the methods of **Barker and Summerson (1941)** and **Lunder (1972).**
2. Mutagenicity :UV-hight mutagenicity was carried out using a Phillips T- UV – 30 W Lamp No. 57413 P/40 at 20 cm distance for 0.5, 1.0 , 1.5 , 2.0 and 2.5 minutes. On the other hand , 100 µ g/ml of MNNG was used also to induce mutant for different exposure period , i.e. 10, 20, 30, 40, 50 , 60 minutes according to **Khattab (2002)** .
3. Total protein electrophoresis : The total protein was isolated from the protoplasts of some selected isolates according to **Khattab (2002).** Protein banding patterns were separated electrophoretically using sodium dedecyl sulfate discontinues polyacrylamide gel electrophoresis (SDS-Disc–PAGE) according to **Hames and Rickwood (1990)**.
4. Isolation of total DNA: according to **Anderson and Mckay (1983)** with some modifications as follows: the alkaline denaturation step was avoided and the samples were directly extracted with phenol saturated tris, followed by chloroform isoamylalcohol. After precipitation of DNA, it was resuspended in TE buffer (10/1) and purified from RNA and protein using RNase A and proteinase K.
5. Randome Amplified Polymorphic DNA (RAPD) analysis: To each ready To-Go- PCR bead, 12 ng of the used random primer and 40 ng of the purified DNA template were added. The total volume of the amplification reaction was completed to 25 µl

using sterile distilled water. The amplification protocol was carried out according to **Williams *et al.*, (1990)** with some modifications of cycling programme using Techne (Genius) Thermal Cycler. The cycling programme used was thirty–five cycles each consists of 94°C (1 min), 51-57°C (2 min) according to GC ratio of each primer **(Qiagen, 1997)** and72°C (3 min) for DNA polymerization . The RAPD – PCR products were analyzed on 1.5 % agarose gel and 1X TBE buffer against 100 bp ladder. The separated bands were stained with 0.5µg ml ethidium bromide and photographed using both Polaroid Instant Camera and UV transeliminator.

RESULTS AND DISCUSSION

A- Induction of Mutations and Lactic Acid Production

Data in Table (2) showed clearly that the highest percentage of the obtained auxotrophic mutant after UV- treatments was recorded following 2.0 min exposure time followed by those obtained from the bacterial suspensions exposed to 1.5 and 1.0 min. No auxotrophs were induced neither spontaneously nor following the lowest (0.5 min) and the highest (2.5 min) exposure periods. Three mutants proved to be single requiring auxotrophs, i.e., B_{26} and D_{32} required alanine and D_4 required methionine to grow on the enriched BM. One mutant (C_{20}) required two amino acids (lysine and alanine) to grow on enriched BM.

Table (2): Number of the counted colonies, survival percentages and induced auxotrophs (No. and %) following different exposure periods of *Lb. casei* subsp. *rhamnosus* No. 45 to UV-light.

Serial No.	Treatments in minutes.	No. of counted colonies	Survival %	No. and % of auxotrophs	
1	0.0	1808	100.00	0	0.00
2	0.5 (A)	961	53.15	0	0.00
3	1.0 (B)	461	25.50	1	0.22
4	1.5 (C)	135	7.47	1	0.74
5	2.0 (D)	62	3.43	2	3.23
6	2.5 (E)	28	1.55	0	0.00

Lactic acid productivity of randomly selected 147 isolates following exposure of the parental strain to different UV-exposure times indicated that the majority of the tested isolates proved to have a little bit more efficiency lactic acid productivity than the original strain. However, all of the auxotrophs obtained after different UV-treatments were the lowest lactic acid producers than

the original strain. The exposure to UV- light for 2 min proved to be the highest effective dose .While 26 out 34 isolates (76.5%) exhibited lactic acid productivity at least 10% higher than their untreated parental strain as shown in Table (3).

The obtained results were in agreement with **Wolfe and Mckay (1983)** they improved lactic acid production in *Streptococcus lactis* using UV-light and EMS mutagenesis. In addition, **Kristofikova *et al.* (1991)** and **Suntornsuk and Hang** 1994) used UV-lihgt to increase lactic acid productivity by *Rhizopus*. Morever, **Arihara and Itoh (2000)** treated *Lb. gasseri* JMC 1131 strain with UV- light and isolated mutant grown on meat contaning 3.3% sodium chloride and 200 ppm sodium nitrate medium.

On the other hand, data obtained following MNNG treatments (Table 4), showed that the numbers of the isolated auxotrophic mutants were 2,3,1 and 2 with percentages of 0.33, 1.03, 0.79 and 2.44 following 20,30,40 and 50 min MNNG treatments, respectively. The highest percentage of the auxotrophic mutants was induced after 50min followed by those obtained from the bacterial suspension treated for 30 min. Twenty minutes applied period induced the lowest percentage of the auxotrophic mutants. Meanwhile, no mutants could be isolated following 0, 10 and 6o min exposure time.

Auxanographic analysis of the eight mutants showed that , two mutants out of the eight auxotrophs lost the ability to grow on the enriched BM supplemented with one or more of the amino acids. Out of the rest three mutants required one amino acid only lysine; phenylalanine, alanine. On the other hand, three mutants required two amino acids to grow on the enriched BM (the first and the second ones need both methionine and alanine, while the third needs methionine and lysine)

Lactic acid productivity of randomly selected 194 isolates following MNNG treatments showed that out of the biochemical requiring mutants, no one produced lactic acid higher than the original strain. It appeared also that following some MNNG treatments i.e., 30, 40 and 50 min., one isolate in each lost completely its lactic acid production capacity. Table (5) presents the MNNG islates, which produced lactic acid at least 10%, more the original strain No. 45.

Table (3): Lactic acid productivity for the UV-isolates which proved to produce at least 10% more than the original *Lb . casei* subsp . *rhamnosus* No. 45.

Isolates	UV exposure time (mine)					Lactic acid productivity	
	0.5	1.0	1.5	2.0	2.5	g/L	% to W.T
W.T						87.5	100
B_{24}		√				97.0	110.9
C_{25}			√			99.3	113.5
C_{26}			√			102.9	117.6
C_{29}			√			97.0	110.9
C_{33}			√			101.3	115.8
C_{34}			√			99.8	114.1
C_{35}			√			96.7	110.5
C_{36}			√			97.8	111.8
D_1				√		109.8	125.5
D_2				√		107.2	122.5
D_3				√		106.6	121.8
D_5				√		100.9	115.3
D_6				√		104.1	118.9
D_7				√		104.8	119.8
D_8				√		98.5	112.6
D_{10}				√		97.8	111.8
D_{11}				√		104.1	118.9
D_{12}				√		105.0	120.0
D_{13}				√		97.8	111.8
D_{14}				√		104.8	119.8
D_{15}				√		106.6	121.8
D_{17}				√		101.4	115.9
D_{18}				√		99.6	113.7
D_{19}				√		98.3	112.3
D_{20}				√		108.2	123.6
D_{24}				√		103.2	117.9
D_{25}				√		103.9	118.7
D_{26}				√		109.3	124.9
D_{27}				√		102.9	117.6
D_{28}				√		100.5	114.9
D_{30}				√		102.4	117.0
D_{31}				√		102.5	117.1
D_{33}				√		110.0	125.7
D_{34}				√		109.6	125.3
Total	0	1	7	26	0		
No.of tested isolates	38	37	38	34	25		
% to tested isolates	.0	270	1842	765	.0		

Table (4): Number of the counted colonies, survival percentages and induced auxotrophic mutants (No.and%) after treatment of *Lb. casei* subsp. *rhamnosus* No. 45 with MNNG.

Serial No.	Exposure intervals (min)	No. of counted colonies	Survival %	No. and % of auxotrophs	
1	00	1514	100.00	0	0.00
2	10	1073	70.87	0	0.00
3	20	604	39.89	2	0.33
4	30	290	19.15	3	1.03
5	40	127	8.39	1	0.79
6	50	82	5.42	2	2.44
7	60	41	2.71	0	0.00

Table (5): Lactic acid productivity for the MNNG–isolates which produced at least 10% more than the original strain No.45.

Isolates	MNNG exposure time (min)						Lactic acid productivity	
	10	20	30	40	50	60	g/L	% to W.T
W.T.							87.5	100
20/9		√					96.5	110.29
20/11		√					99.4	113.60
20/21		√					98.2	112.23
20/25		√					100.5	114.86
30/2			√				98.5	112.2
40/15				√			98.1	112.11
40/20				√			99.6	113.83
40/26				√			96.5	110.29
Total	0	4	1	3	0	0		
No of tested isolates	34	30	37	29	31	33		
% to tested isolates	0	13.33	2.70	10.34	0	0		

The above results were in agreement with those obtained by **Honsa (1982); Kodota and Sakurai (1982) and Mayer *et al.*, (1995).** Furthermore, **Ibrahim and O'Sullivan (2000)** treated two *Bifidobacterium* species and one strain each of *Lb. delbrueckii* subsp. *bulgaricus* and *streptococcus thermophilus* with two chemical mutagens EMS and MNNG. They obtained seventy-five mutants, which showed enhancement in β-galactosidase activities from 70 to 222% when compared to the wild type strains. These results were in

a good harmony with those obtained in this study since the β-galactosidase enzyme cleaved lactose molecule into glucose and galactose during the first step of lactic acid biosynthesis from lactose.

In general, it could be concluded that the mutagenic treatments with UV-light was effective than MNNG for the enhancement of lactic acid production by *Lb.casei subsp . rhamnosus* NRRL B-445. **Kristofikova *et al.* (1991)** showed that using UV-light as a mutation inducer has been recommended as a method of choice for increasing lactic acid productivity.

B- Biochemical and Molecular Variation of Mutagenic Treated Isolates

I. Water Soluble Protein Analysis

An attempt was carried out in the present study to determine the biochemical variabilities between the original strain *Lb. casei* subsp. *rhamnosus* No. 45 and some of its derivatives treated with either UV or MNNG mutagens.

Figure (1) and Table (6) presented the photograph and banding patterns of the original strain No. 45 SDS-PAGE (Lane 4) and nine of its derivatives (lanes from 1 to 3 and lanes from 5 to 10) which were obtained following mutagenesis with UV-light.

Data in Table (6) and Figure (1) showed that no one of the tested UV-isolates exhibited the same number and intensity of the separated bands as its original strain. Although thirteen bands were detected for the original strain No. 45, however, eight different SDS-PAGE banding patterns were noticed for the different UV isolates. It was noticed that the high lactic acid producer isolates, i.e., Nos. D_{33} and D_{34} exhibited identical numbers and intensities of all bands. Moreover, both of the above isolates (lanes 5 and 6) as well as isolate No. D_1 (lane 9) showed an additional very faint band (band No. 9) which is not found neither in the original strain No. 45 nor the other isolates. In spite of isolate D_1 (Lane 9) classified as a higher lactic acid producer, however, it exhibited a little bit differences than D33 and D_{34} isolates (bands No. 3 and 8). Furthermore, the two isolates Nos. D_{20} (lane 8) and E_{20} (lane 10) exhibited an additional very faint band (band No. 6) which disappeared completely in the original strain and all other analysed UV-isolates. on the other hand, the low lactic acid producer isolates, i.e., D_{32} (lane 3) and D_4 (lane 7) exhibited low intensity for the majority of their separated bands and lost completely the bands Nos. 1 and 11 which appeared as a very faint bands in the original strain No. 45 and the high producer isolates.

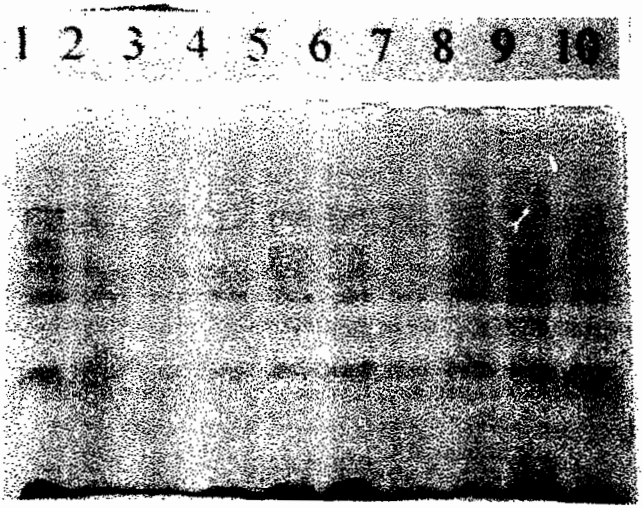

Fig. (1): Photograph of SDS-PAGE patterns of the original strain No. 45 and nine derivatives obtained after mutagenesis it with UV-light. Lanes; 1: B_{26}; 2:C_{26}; 3: D_{32}; 4: original strain No. 45; 5: D_{33}; 6: D_{34}; 7: D_4; 8: D_{20}; 9: D_1 and 10: E_{20}.

Table (6): SDS-PAGE banding patterns of the original strain No. 45 and nine of its derivatives obtained after mutagenesis of the original strain No. 45 with UV-rays.

Lane and isolate No. / Band No.	1 B_{26}	2 C_{26}	3 D_{32}	4 Original st. No. 45	5 D_{33}	6 D_{34}	7 D_4	8 D_{20}	9 D_1	10 E_{20}
1	a	a	-	a	a	a	-	-	a	-
2	a	a	a	b	a	a	a	a	a	a
3	a	a	a	b	a	a	a	a	b	a
4	c	a	a	b	c	c	a	a	c	a
5	a	a	a	b	c	c	a	a	c	a
6	-	-	-	-	-	-	-	a	-	a
7	c	a	a	b	c	c	a	a	c	a
8	a	a	a	b	c	c	a	a	b	a
9	-	-	-	-	a	a	-	-	a	-
10	c	c	a	c	c	c	a	b	c	b
11	-	-	-	a	a	a	-	-	a	-
12	a	-	-	a	a	a	a	a	a	a
13	c	c	c	c	d	d	b	c	d	c
14	a	a	a	a	a	a	-	-	a	-
15	a	-	a	a	a	a	a	a	a	a
Total bands No.	12	10	10	13	14	14	10	11	14	11

- = Not found a = Very faint b = Faint c = Dark d = Very dark

Figure (2) and Table (7) presented the banding pattern of the water soluble protein of the different *Lb. casei* subsp. *rhamnosus* No. 45 SDS-PAGE banding patterns (Lane 4) and nine of its MNNG treated isolates (lanes 1-3 and lanes 5-10). Results in Figure (2) and Table (7) showed that 13 bands were detected for the original strain No. 45. However, the nine different MNNG-treated isolates showed different SDS-PAGE banding patterns. The isolates No. 50/31 (lane 6) and No. 20/11 (lane 7) exhibited the highest banding number (15 bands). Like that which was reached concerning UV-isolate analysis, the high lactic acid producer isolates, i.e., 10/12, 20/25 and 20/11 (lanes 1, 5 and 7, respectively) exhibited the highest banding intensities in comparison with the other MNNG-isolates and the original strain No. 45. On the other hand, the low lactic acid producer isolates, i.e., Nos. 40/5, 40/6 and 60/22 (lanes 2, 3 and 10, respectively) and the non producer ones Nos. 30/32 and 40/4 (lanes 8 and 9) exhibited the lowest banding intensities in comparison with high lactic acid producer isolates.

The above biochemical variabilities between different treated isolates and their original strain was in parallel with **Grill *et al.* (2000)** since they reported that only one band, corresponding to the conjugated bile salt hydrolase enzyme, was observed in the mutant while four bands were detected in the wild type of *Lb. amylovorus* strain.

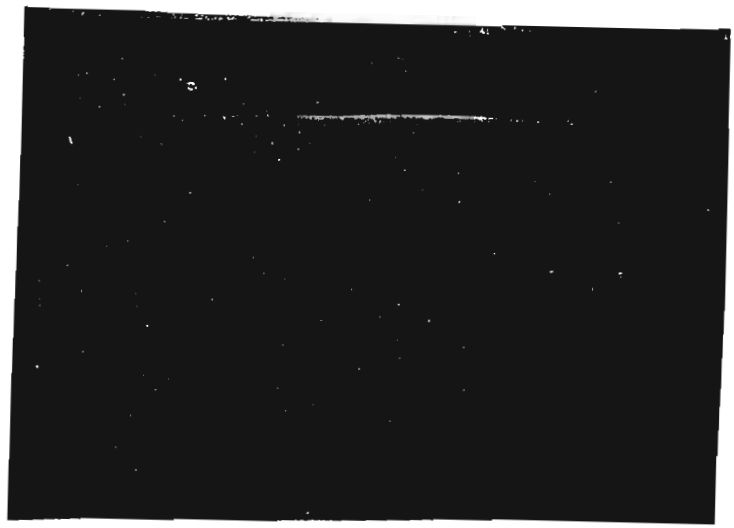

Fig. (2): Photograph of SDS-PAGE banding patterns of the original strain No. 45 and its nine derivatives obtained after treatment with MNNG mutagen. Lanes; 1: 10/12; 2: 40/5; 3: 40/6; 4: Original st. No. 45; 5: 20/25; 6: 50/31; 7: 20/11; 8: 30/32; 9: 40/4 and 10: 60/22.

Table (7): SDS-PAGE banding patterns of the original strain No. 45 and nine of its derivatives obtained after treatment it with MNNG mutagen.

Lane and isolate No. / Band No.	1 10/12	2 40/5	3 40/6	4 original st. No. 45	5 20/25	6 50/31	7 20/11	8 30/32	9 40/4	10 60/22
1	a	a	-	a	a	a	a	a	-	A
2	-	a	-	b	a	a	a	-	-	a
3	b	b	b	b	b	b	b	a	a	b
4	b	b	b	b	b	b	b	a	a	b
5	-	b	b	b	c	a	b	a	a	b
6	b	a	a	-	a	a	a	a	a	b
7	b	b	a	b	c	a	b	a	a	b
8	b	b	b	b	c	b	a	a	a	b
9	a	b	b	-	-	a	a	-	-	-
10	b	-	-	c	b	b	b	a	a	b
11	a	b	-	a	b	a	a	-	-	a
12	a	b	a	a	b	a	a	a	a	a
13	c	d	c	c	d	c	c	c	b	c
14	a	a	-	a	-	a	a	-	-	a
15	b	b	a	a	b	b	b	a	a	a
Total bands No.	13	14	10	13	13	15	15	11	10	14

- = Not found a = Very faint b = Faint c = Dark d = Very dark

II. Random Amplified Polymorphic DNA (RAPD) Analysis

An attempt was conducted to evaluate the genetic effects of mutagenic treatments on the DNA nucleotide sequence of the obtained isolates compared to the original strain *Lb. casei* subsp. *rhamnosus* No. 45. Six 15-mer random primers were applied using randomly amplified polymorphic DNA (RAPD) technique to detect the molecular variations between eleven mutagenic treated isolates compared to their original strain No. 45. The productivity of the lactic acid and pedigree of the selected isolates were presented in Table (8).

Table (8): Lactic acid productivity and pedigree of the selected isolates for molecular analysis.

Treated isolates					
UV			MNNG		
Isolate No.	Lactic acid productivity (g/L)	Lactic acid % to W.T.	Isolate No.	Lactic acid productivity (g/L)	Lactic acid % to W.T.
W.T.	87.6	100	W.T	87.5	100
D_1	109.8	125.34	20/11	99.4	113.60
D_{33}	110.0	125.57	20/25	100.5	114.86
D_4	0.4	00.46	40/5	25.0	28.57
D_{32}	48.7	55.59	40/6	27.2	31.09
			40/4	00.0	00
			50/6	00.0	00
			30/32	00.0	00

Figure (3) represents a photograph of the polymorphic amplified DNA bands based on RAPD analysis for the original strain (Lane 1) and eleven treated isolates (lanes 2-12) using 15-mer primers Nos. 1 (I) and 2 (II). Band sizes were detected against 100 base pair ladder marker Lane (M). Results in Figure (3) showed clear differences at bands number and size between the original strain No. 45 and its derivatives using both primers. Using primer No. 1, it was clearly noticed that five amplified bands were occurred when the original strain No. 45 DNA was used as a template. The bands sizes were 2200, 1900, 1500, 1300 and 600 bp as shown in Lane I-1. Out of the eleven tested isolates, four isolates (D_{33}, D_{32}, 40/5 and 50/6) did not contain the complementary sequences of the primer No. 1, since non of them showed any amplified bands as presented in lanes Nos. I-3, 7, 8 and 11, respectively. On the other hand, four isolates (20/11, 20/25, D_4 and 30/32) exhibited the same banding patterns of the amplified regions.

Four bands were detected with base pair sizes of 2300, 2000, 1700 and 1400 as shown in lanes Nos. I-4, 5, 6 and 12. Each of the rest three isolates, i.e., D_1, 40/6 and 40/4 presents specific banding pattern since 5, 6 and 8 bands were detected for each one respectively. Out of the above results non of the used 11 isolates resembled its original strain since no one exhibited the same random amplified banding pattern of it when the primer No. 1 was used. Moreover, there were no any correlation could be detected between the primer sequence and lactic acid productivity. The similar banding patterns obtained showed extreme variations of lactic acid production between the respective isolate.

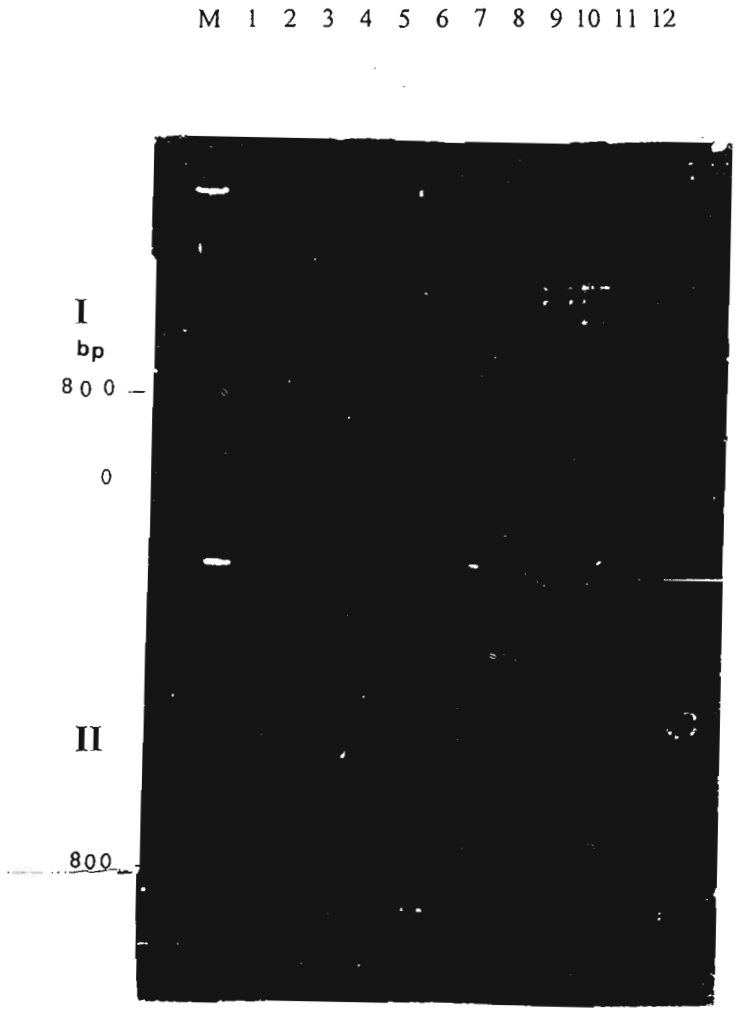

Fig. (3): Photographs of DNA amplified banding patterns based on RAPD for eleven isolates against original strain No. 45 (Lane 1) and 100-bp ladder marker (Lane M) using primer No. 1 (Upper) and No. 2 (Lower). Isolates sequence as follows: (Lanes 2 to 12), D_1, D_{33}, 20/11, 20/25, D_4, D_{32}, 40/5, 40/6, 40/4, 50/6 and 30/32(respectively).

Results in Figure (3-II) presented the random amplified banding patterns of the original strain No. 45 and the tested eleven isolates when primer No. 2 was used. The obtained results showed that four isolates, i.e., 20/11, D_4, 40/6 and 40/4 produced the same amplified bands as their original strain. Three bands were detected for all of these five purified DNA with sizes of more than 2600, 1300 and 700 bp. Moreover, an additional band with size of 500 bp was occurred for the last three isolates (D_4, 40/6 and 40/4). Regarding the lactic acid production of the above five isolates, it was 87.5 g/L for the original culture; 99.4 g/L for 20/11; 0.4 g/L for D_4; 27.2 g/L for 40/6 and zero for 40/4 isolate. The three lowest producers (D_4, 40/6 and 40/4) shared with the 500 bp band which did not detected with higher producers, i.e., the original culture and 20/11 isolate. On contrary, no amplified bands could be noticed as a result of using primer No. 2 with the purified DNA of the isolates D_{33} (the superior producers), D_{32}, 40/5, 40/4 (low producers) and 30/32 which were applied in the lanes Nos. II-3, 7,8 and 12, respectively. The isolates Nos. (higher producer) 20/25 and 50/6 (non producer) produced only one amplified band with size of more than 2600 bp (lanes Nos. 5 and 11) which appeared as a faint band when the DNA of the high producer isolate No. D_1 (lane 2) was used. In addition, two bands were also detected with 800 and 500 bp sizes for the same isolate, i.e., D_1.

Regarding the application of the primers Nos. 3 and 4 against the original strain and its derivatives of the mutagenic treated isolates, it was clearly noticed that all of the tested isolates proved to have special amplified banding patterns which differed than that of its original parents strain (Figure 4). With two exceptions of the low producer isolate No. 40/5 (lane I-8) which did not exhibit any amplified bands and isolates Nos. D_4, 40/6 and 40/4 (lanes I-6, 9 and 10) which showed identical banding patterns of six bands and proved to be low (40/5, D_4 and 40/6) or non (40/4) lactic acid producers, primer 3 proved to have variable repeated numbers of the complementary sequence of the rest tested isolates. Figure 5-II indicated that the original strain showed a major amplified band with size of 1100 bp and other two faint bands with sizes of 800 and 2000 bp as a result of using primer No. 4. The application of the same primer did not amplify any band when used against isolate 40/5 purified DNA. On the other hand, all of the rest isolates exhibited different numbers amplified bands ranged from one faint band with 1100 bp for the isolate D_{32} (lane II-7) to eight faint bands for the isolate 30/32 (lane II-12).

Figure (5-I and II) represents the random amplified polymorphic bands of the original strain and its eleven selected

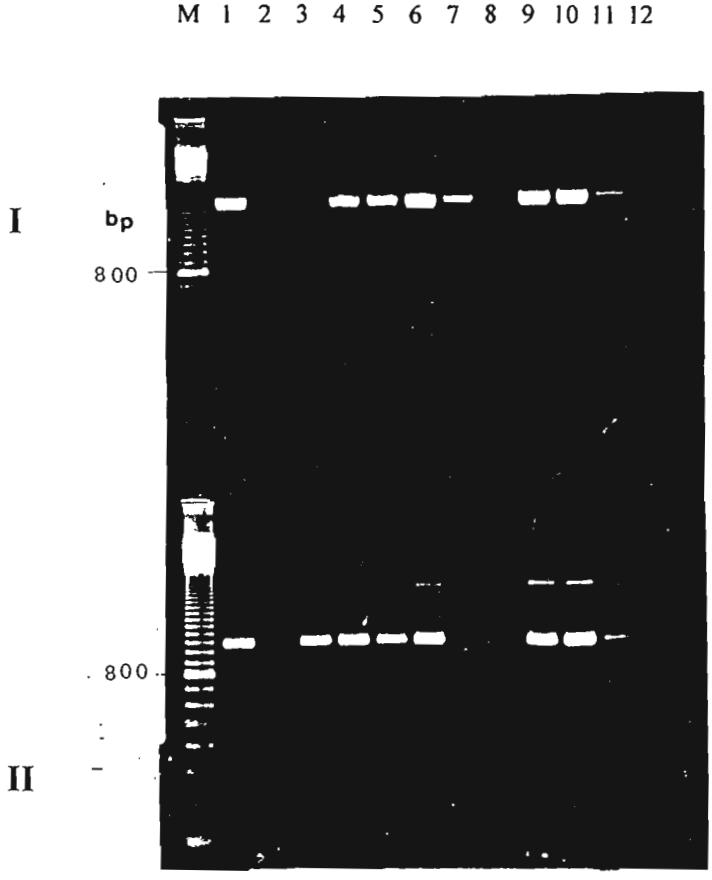

Fig. (4): Photographs of DNA amplified banding patterns based an RAPD for eleven isolates against original strain (Lane 2) and 100 bp ladder marker (Lane M) using primers No. 3 (upper) and No. 4 (lower). Isolates sequence (Lane 2 to 12), D_1, D_{33}, 20/11, 20/25, D_4, D_{32}, 40/5, 40/6, 40/4, 50/6 and 30/32.

derivatives DNA as a result of primers Nos. 5 and 6 application. The obtained banding patterns indicated that three bands were detected following the application of each primer against the original strain with sizes of 1400, 1200 and 1000 bp for primer No. 5 and 1300, 1100 and 500 bp for primer No. 6. No amplified bands could be obtained for the template DNA of the isolate D_{33}, 40/5 and 30/32 (lanes 6-I-3, 8 and 12) when primer No. 5 was applied. The same finding was detected when isolate Nos. D_{32} and 40/5 (lanes 7 and 8) was used against primer No. 6. The rest selected isolates exhibited different polymorphic banding patterns against the two adopted primers.

However, the above differences in RAPD profile confirmed the evidence of genetic variation in genomes after the mutagenic agents treatments. **Thompson *et al.* (1997)** studied the molecular analysis of antibiotic mutation by RAPD-PCR analysis in *Lactobacillus plantarum* and they found some differences and similarities in RAPD profile between streptomycin and other mutants. On the other hand, **Urbach *et al.* (1998)** suggested that the genetic variation among closely related strains could be detected by RAPD-PCR technique.

However, correlation between primers sequences repeated complementary sequences and the lactic acid productivities need extent studies in future.

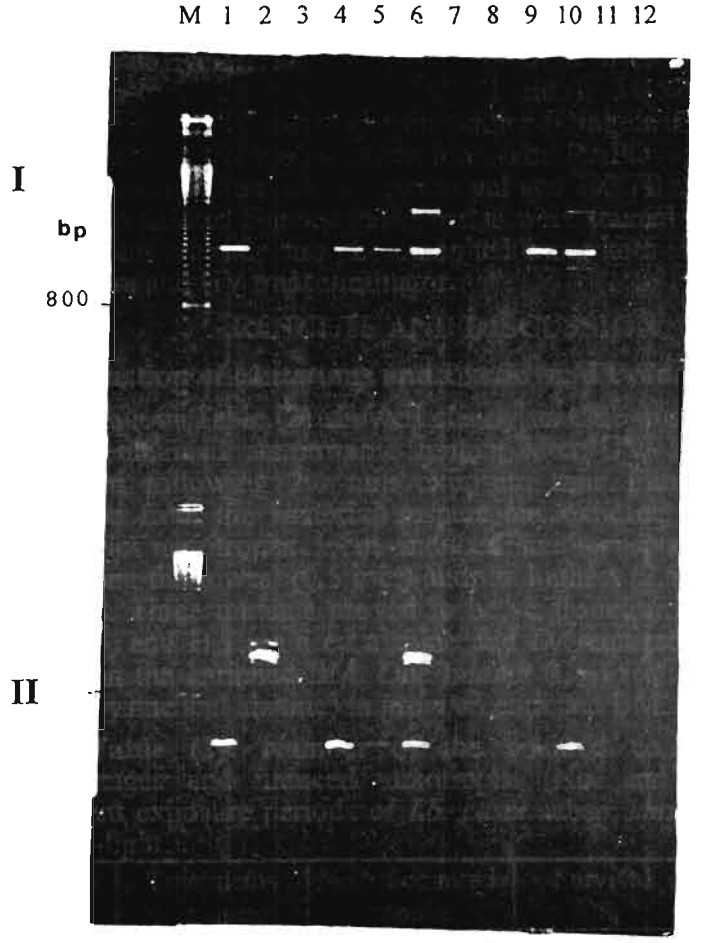

Fig. (5): Photographs of DNA amplified banding patterns based on RAPD for eleven isolates against original strain No. 45 (Lane 2) and 100 bp ladder marker (Lane M) using 15-mer random primers No. 5 (upper) and No. 6 (lower). Isolates sequence (Lane 2 to 12), D_1, D_{33}, 20/11, 20/25, D_4, D_{32}, 40/5, 40/6, 40/4, 50/6 and 30/32.

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علاقة التباينات البيوكيميائية والجزيئية لطافرات بكتريا اللاكتوباسيلس كيزى بإنتاج حمض اللاكتيك

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يعتبر حمض اللاكتيك من الأحماض العضوية ذات الأهمية الاقتصادية الكبيرة حيث يدخل فى العديد من الصناعات الغذائية والنسيجية والعلاجية وغيرها لذلك تم إختيار السلالة *Lb. casei* subsp. *rhamnosus* NRRL-B-445 لإجراء تجارب التحسين الوراثى لزيادة إنتاجية حمض اللاكتيك وتشير أهم النتائج المتحصل عليها إلى ما يلى :

١- أدى تعريض السلالة الأصلية لجرعات مختلفة من الأشعة فوق البنفسجية تراوحت بين صفر – ٢,٥ دقيقة (على مسافة ٢٠ سم) بالإضافة إلى استخدام المطفر الكيماوي النيتروزوجوانيدين بتركيز ١٠٠ ميكرو جرام / مل لفترات تعريض تراوحت بين ١٠-٦٠ دقيقة إلى عزل ٣٦٦ مستعمرة أظهرت ١٠ ٦ عزلة منها زيادة ملحوظة في إنتاجية حمض اللاكتيك عن السلالة الأصلية بينما تفوقت ست عزلات في معدل الإنتاج بنسب تراوحت بين ١٣- ٢٥%.

٢- تعتبر الأشعة فوق البنفسجية أكثر كفاءة فى تحسين إنتاجية حمض اللاكتيك بالمقارنة بالمطفر الكيماوي النيتروزوجوانيدين على الرغم من إستحداث الأخير لعدد أكبر من طفرات الإحتياج الغذائي .

٣- لتقييم العلاقة بين التغيرات الوراثية البيوكيماوية وإنتاج حمض اللاكتيك بإستخدام تحليل الفصل الكهربي للبروتين للسلالة الأصلية بالإضافة إلى ١٨ عزلة ناتجة عن المعاملة بكلا المطفرين أو ضحت النتائج أن العزلات عالية الإنتاجية أعطت عدد اكبر وكثافة شديدة للحزم بالمقارنة بالعزلات الاخرى والسلالة الأصلية.

٤- عند إجراء تفاعل البلمرة المتسلسل بإستخدام ستة بوادئ عشوائية لملاحظة التباينات الوراثية بين ١١ عزلة ناتجة عن المعاملة بالمطفرات أوضحت النتائج المتحصل عليها وجود العديد من الإختلافات في الحزم المتضاعفة ويمكن إستخدام بعض هذه الإختلافات كعلامات وراثية لصفة إنتاج حمض اللاكتيك .