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Ochratoxin A biosorption onto genetically improved of *Lactobacillus delbrueckii* mutants

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Abstract

Eight lactic acid bacterial strains were evaluated for Ochratoxin A (OTA) biosorption. Results showed that *Lactobacillus delbrueckii* NRRL B-1024 is the most effective strain for OTA biosorption of 50 ng/ml within one hour. It was selected and mutagenized using ethyl methanesulfonate (EMS) to induce genetic variations in relation to improve OTA biosorption. Seven of 19 mutants exhibited OTA biosorption higher than the wild-type strain. The maximum capacity was achieved using the mutant EMS-9, which recorded 82.62 ng/ml of OTA uptake within one hour. The mutant EMS-9 appears to be the most superior strain for OTA biosorption since 88% of the initial OTA concentration was removed using 40% (v/v) inoculum concentration within one hour after studying the effect of inoculum concentration on OTA uptake. Finally, the application of the Random Amplified Polymorphic DNA (RAPD) technique by polymerase chain reaction (PCR) on some selected mutants lead to correlate the genetic characteristics of the mutants with the results obtained from the biosorption process in comparison with the wild type strain. Differences in RAPD patterns confirmed the evidence of genetic variability induced in *Lb. delbrueckii* NRRL B-1024 genome after EMS-treatment.

Introduction

The contamination of food and feed with different types of mycotoxins (e.g., aflatoxins, fumonisins, deoxynivalenol, zearalenone….etc.) has been a world environmental concern, particularly in developing countries. Ochratoxin A (OTA) naturally occurs in a large variety of foodstuff since it has been detected in cereals, beans, spices, dried fruits, grapes, coffee, fermented beverages and medicinal plants (Yang *et al*., 2010; Janati *et al*., 2012). There are eighteen isomers of ochratoxin including OTA, which is considered the most toxic ochratoxin among them. It is produced by fungal species such as *Aspergillus* and *Penicillium* (Ahn *et al*., 2016). Based on the classification of the International Agency for Research on Cancer (IARC), OTA belongs to group 2B (IARC, 1993). It is well known that OTA causes many toxic effects in humans and in animals and mainly affects the kidney (Gayathri *et al*., 2015). OTA is considered a source of some types of cancers (Kumar *et al*., 2012) and induced embryotoxicity, teratogenicity, carcinogenicity, nephrotoxicity, immunotoxicity, and hepatotoxicity (Chen and Chan, 2009; Zhang *et al*., 2009; Chopra *et al*., 2010; Islam *et al*., 2012; Anninou *et al*., 2014; Jo *et al*., 2016). Ensuring safe and healthy food is urgently needed to avoid the health hazards associated with the presence of mycotoxins in foodstuffs such as cereals, which are considered as popular foods in Egypt. As the contamination of foodstuffs with different mycotoxins has harmful effects on human and animal health, several approaches including physical, chemical and microbiological methods have been suggested and approved for removal or reduction of mycotoxins (Huwig *et al*., 2001; Amézqueta *et al*., 2009; Quintela *et al*., 2013). Many previous studies used adsorbent agents to remove or to reduce the toxic effects of different mycotoxins. Activated carbons derived from dates' stones as well as Egyptian montmorillonite clay were used as protective agents against deoxynivalenol toxicity in vivo study (Abdel-Wahhab *et al*., 2015). The adsorption efficiency of sorbent agents varies according to the types of mycotoxins. For example, phyllosilicate clay Hydrated Sodium Calcium Aluminosilicate (HSCAS) was found to be very strong adsorbent agent against aflatoxin B_1. However, it had limited adsorption capacity with other mycotoxins such as zearalenone and OTA and has no effect with T-2 toxin and deoxynivalenol (Huwig *et al*., 2001).

OTA is partially degraded during the technological processing, which reflects that the manufactured commodities may still contain a significant amount

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of OTA. Initial studies for microbiological treatments demonstrated that although aflatoxin biosynthesis can be inhibited using different strains of Lactic Acid Bacteria (LAB), the removal efficiency of aflatoxin from media is not appreciable enough (Coallier-Ascah and Idziak 1985; Thyagaraja and Hosono 1994). Twenty-nine strains of *Lactobacillus* and *Lactococcus* genera were tested against the toxicity of OTA and their ability to reduce the concentration levels of this toxin by Piotrowska and Zakowska (2005). About 50% reductions of the initial concentration of OCA were obtained with *Lb. acidophilus* CH-5, *Lb. rhamnosus* GG, *Lb. plantarum* BS, *Lb. brevis* and *Lb. sanfranciscensis* (Piotrowska and Zakowska, 2005). The same trends were observed by Del Prete *et al.* (2007) since removal of OTA from wine using LAB was very limited (8- 28%).

Development of biosorbent through isolating organisms with high capacity and specificity or by using genetically modified organisms via mutagenesis could be achieved as stated by Pazirandeh *et al.* (1998); Bae *et al*. (2002); Huang *et al*. (2003); Yan *et al*. (2007). The current work deals with screening *in vitro* adsorption of OTA onto different strains of lactic acid bacteria and generation of induced mutants by EMS mutagenesis from the wild type *Lb. delbrueckii* NRRL B-1024 and evaluation of its OTA biosorption. Application of the random amplified polymorphic DNA (RAPD) technique by Polymerase Chain Reaction (PCR) on the selected mutants in comparison with the wild-type strain was also done.

Materials and Methods

Chemical and reagents

Ochratoxin A (OTA) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA) as a crystalline powder. All solvents and reagents were HPLC grade. A stock solution was prepared by dissolving 1 mg OTA in 1 ml of toluene: acetic acid mixture (99:1, v/v) and kept at -20ºC in the dark for further usage for preparation of working solutions. For preparation of phosphate buffer saline, 0.2 g KCL, 0.2 g KH_2PO_4, 1.16 g Na_2PO_4 anhydrous and 8 g NaCl were dissolved in 900 ml distilled water.

Lactic acid bacterial strains

Lactic acid bacterial strains (*Lactobacillus delbrueckii* B-1024, *Lactobacillus casei* B-441, *Lactobacillus rhamnosus* B-445 and *Lactobacillus reuteri* B-14141) were obtained from the culture collection of Northern Regional Research Laboratory (NRRL), 1815 North University St., Peoria, USA. The other strains were obtained as follows:. Lactobacillus plantarum-77 from Food Technology and Dairy Products department, National Research Centre (NRC), Egypt; Enterococcus faecium-EF11, *Leuconostoc lactis*-LL12 and P*ediococcus pentosaceus*- PP11 from Applied Microbial Genetics Lab., Genetics and Cytology Dept., NRC, Egypt. These strains were maintained and cultivated on De Man, Rogosa and Sharpe (MRS) medium (De Man *et al*. 1960) and freshly sub-cultured before usage in the biosorption experiments. The strains were transferred into a fresh medium every month.

Ethyl methanesulfonate (EMS) mutagenesis

Three ml of overnight *Lb. delbrueckii* NRRL B-1024 cells were centrifuged at 8000 rpm for 5 min and resuspended in a phosphate buffer 0.1 M at pH 7. The suspended bacterial cells were treated with EMS mutagen for 60 min and then the suspension was diluted and spread to the surface of MRS medium contained OTA (200 ng/ml) for selection of highly OTA resistant colonies. Plates were then incubated for 2 days at 37ºC. The growing colonies with high growth rate were transferred on slants for further studies. Mutants with high growth rate were then recultured on the same medium plates for 2 days at 37ºC.

Biosorption processes of OTA

After the wild strain and mutants were grown on MRS medium for 48 h at 30ºC and shaken at 120 rpm. The flasks were incubated with OTA (100 ng/ml) for 1, 3 and 5 hrs under the same conditions. OTA uptake was determined for each mutant as well as the wild strain. To study the effect of inoculum concentration (v/v) on OTA uptake, 100 ng/ml OTA was inoculated with 10, 20 and 40% (v/v) of bacterial suspension for 1hr.

Determination of OTA using high performance liquid chromatography (HPLC)

The determination of OTA was carried out according to the condition mentioned by Hussein *et al.* (2012). Briefly, a symmetry C_{18} (5 µm particle size, 150 mm X 4.6 mm i.d) from Waters corporation (USA) equipped with fluorescence detector ($\lambda_{excitation}$ = 330 nm, $\lambda_{emission}$ = 470 nm). The separation was performed at ambient temperature at a flow rate of 1 ml/min using a mobile phase of Acetonitrile: Water: glacial acetic acid with ration of 55:43:2, respectively. The calibration curve was done with 5 different concentration levels of OTA (0.05, 0.10, 0.25, 0.50 and 1.0 ng/ml). For quality assurance, triplicate injections were carried out for all samples; matrix spiked was running with recovery ranged

from 78 to 93%.

Isolation of total DNA from mutant strains

Total DNA was isolated according to i-genomic BYF DNA extraction Mini Kit, iNtRON Biotechnology Inc., South Korea. The quantity and purity of the obtained DNA were determined based on UV-absorbance at 260 and 280 nm using spectrophotometer (Shimadzu UV-VIS model UV-240) (Sambrook *et al.*, 1989).

Molecular analysis of superior mutants by PCR

For PCR analysis, PCR-GOLD Master-Mix Beads (BIORON, Germany, Cat. No. 10020-96) were used. Each bead contains all of the necessary reagents, except primer and DNA template, for performing 25 µl PCR amplification reactions. Three different primers were used in the present study. The first primer sequence was 5′-GGG GTT TGC CAC TGG-3'. The second primer sequence was 5′-GTG TTG TGG TCC ACT-3'. The third primer sequence was 5′-TGA GTG GTC TAC GTG-3'. All primers were supplied by Operon Technologies Company, Netherlands. To each PCR bead, 12 ng of the used random primer and 40 ng of the purified DNA sample were added. The total volume of the amplification reaction was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as follows: Denaturation at 95ºC for five min. Thirty-five cycles each consists of the following segments: Denaturation at 95ºC for one min; primer annealing for two min. according to GC ratio of each primer and incubation at 72ºC for two min. for DNA polymerization. Then, 72ºC for 7 min., at the end, hold the PCR at 4ºC till analysis. The amplified DNA products from RAPD analysis were electrophorated on 1.5% agarose gel and 1 X TBE buffer at consistent 100 volt for about 2 hrs. The different band sizes were determined against 100 bp ladder (Pharmacia Biotech.) and the separated bands were stained with 0.5 µg/ml ethidium bromide and photographed using Gel Documentation System with UV-Transeliminator.

Analysis of RAPD-data

Amplification profiles for the five mutants as a result of RAPD application were compared to each other as well as to compare them with the wild type of *Lb. delbrueckii.* DNA fragments were scored as a binary data, where (1) means presence and (0) means absence of band. The distance coefficients were calculated based on the following statistical equation: F= 2Nxy/ (Nx + Ny). Where, F is the distance coefficient; Nx and Ny are the numbers of fragments in genotypes x and y, respectively, and Nxy is the number of fragments differed by the two genotypes (Lynch, 1990). The electrophoretic patterns of the reproducible banding patterns of each primer produced by RAPD were chosen for analysis. Pair wise comparisons between mutants were made to calculate the Jukes-Cantor coefficient using PAST program (PAleontological Statistics Version 1.94b) adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a denderogram using unweighted pair-group method with arithmetical average (UPGMA).

Results and Discussion

Evaluation of the collected strains and OTA biosorption

The present study was designed to obtain new Lactic Acid Bacteria (LAB) with high OTA biosorption through chemical mutagenesis treatment with ethyl methansulfonate (EMS). All the collected strains combined with three incubation periods were evaluated to examine and determine the efficiency and the optimum incubation period for sequestration of OTA. *Lb. delbrueckii* NRRL B-1024 was found to be the most effective strain for OTA biosorption (uptake of 50 ng/ml of OTA within 1 hr). So, it was selected and mutagenized using ethyl methanesulfonate (EMS) as a chemical mutagen to induce genetic variations in relation to improve OTA biosorption. Other *Lactobacilli strains* (*L. casei* B-441, *L. rhamnosus* B-445, *L. reuteri* B-14141 and *L. plantarum*-77) were able to absorb 43.7, 31, 44.7 and 40.2 ng/ml of OTA within 1 hr, respectively. In case of *E. faecium*-EF11, 39.8 ng/ml of OTA biosorption was achieved within 1 hr. However, 31.7 ng/ml of OTA biosorption was observed for *L. lactis*-LL12 strain. No OTA biosorption is observed by *P. pentosaceus*- PP11 strain after 1 hr (Table 1).

In the same respect, four strains of LAB and probiotic were tested for their ability to reduce the concentrations of aflatoxins during the baking process of bread by Elsanhoty *et al.* (2013). The results demonstrated that *Lactobacillus rhamnosus* TISTR541 was the most effective strain for sequestration of total aflatoxins (B_1, B_2, G_1 and G_2). Heating process had a synergistic effect in the ability of LAB to reduce aflatoxins (Elsanhoty *et al*., 2013).

EMS- mutagenesis and OTA biosorption

The production of mutations generally is done by using agents that interact with nucleic acids. So, the change in DNA nucleotide sequence is basically produced by mutation. The induced mutation

Table 1. Effect of different incubation times on OTA uptake of the original bacteria cultured in submerged fermentation inoculated with 10% (v/v).

Strain Code No.	OTA uptake (ng/ml)/ initial concentration of 100 ng/ml		
	Incubation period (hrs)		
	1hr	3 hrs.	5 hrs.
Lactobacillus delbrueckii B-1024	49.9 ± 1.4	44.0 ± 2.0	49.7± 2.1
Lactobacillus casei B-441	43.7 ± 0.6	38.7 ± 1.5	34.3 ± 1.5
Lactobacillus rhamnosus B-445	31.0 ± 1.0	36.6 ± 0.6	37.3 ± 1.2
Lactobacillus reuteri B-14141	44.7 ± 1.5	41.0 ± 1.0	42.3 ± 1.5
Lactobacillus plantarum-77	40.2 ± 1.3	32.7 ± 1.2	32.3 ± 1.5
Enterococcus faecium-EF11	39.8 ± 1.6	32.5 ± 1.4	39.3 ± 1.6
Leuconostoc lactis-LL12	31.7 ± 1.5	33.6 ± 1.3	32.5 ± 1.5
Pediococcus pentosaceus- PP11	0.0	33.6 ± 0.7	38.1± 0.5

Table 2. Ochratoxin A uptake of selected ochratoxin A resistant mutants of *Lb. delbrueckii* after EMS mutagenesis under initial OTA concentration of 100 ng/mL with 10% (v/v) inoculum concentration.

Mutant No.	OTA uptake (ng/mL)	OTA uptake % to W.T	Mutant No.	OTA uptake (ng/mL)	OTA uptake % to W.T
W.T	49.8 ± 0.5	100.00	EMS-10	72.3 ± 1.9	144.72
EMS-1	30.7 ± 2.0	61.42	EMS-11	64.9 ± 1.3	129.80
EMS-2	21.4 ± 1.8	42.80	EMS-12	39.4 ± 2.1	78.60
EMS-3	0	0.00	EMS-13	28.6 ± 1.9	57.20
EMS-4	0	0.00	EMS-14	0	0.0
EMS-5	28.6 ± 1.5	57.20	EMS-15	66.2 ± 2.3	132.28
EMS-6	0	0.00	EMS-16	14.3 ± 1.7	28.60
EMS-7	63.4 ± 1.7	126.92	EMS-17	14.3 ± 0.8	28.60
EMS-8	54.5 ± 1.2	108.92	EMS-18	35.7 ± 2.2	71.40
EMS-9	82.6 ± 1.9	165.24	EMS-19	67.9 ± 2.6	135.80

depends on the type of change in DNA sequence (base pair substitution, insertion, deletion etc.). Mutations are induced randomly in a microbial DNA by the application of chemical and physical mutagenic agents. The most popular mutagen used to improve bacterial strain is EMS, which typically produces a variety of point mutations in the bacterial DNA. Through these point mutations, the different mutants are produced at high levels in comparison with the natural mutations. Mutagenesis of industrial microbial strains is widely used to enhance the biosorption and biodegradation (Chang *et al*., 1995; Ramsay and Gadd, 1997; Gharieb and Gadd, 1998; Hua *et al*., 2008; Khattab *et al*., 2012).

Sixty minutes treatment with EMS resulted in 19 mutants, which are resistant to the high concentration of OTA (200 ng/ml), but 7 mutants exhibited OTA biosorption higher than the wild strain (Table 2). Therefore, mutants were only isolated of highly OTA resistant colonies (200 ng/ml). The highest uptake of OTA (82.6 ng/ml) was achieved with mutant EMS-9 with 165.2% improvement of OTA biosorption, followed by mutant EMS-10, which had an uptake 72.3 ng/ml with 144.7% improvement of OTA biosorption. However, 8 mutants proved to be lower OTA biosorption than their original strain. Four mutants lost their ability of OTA biosorption compared with their parental strain.

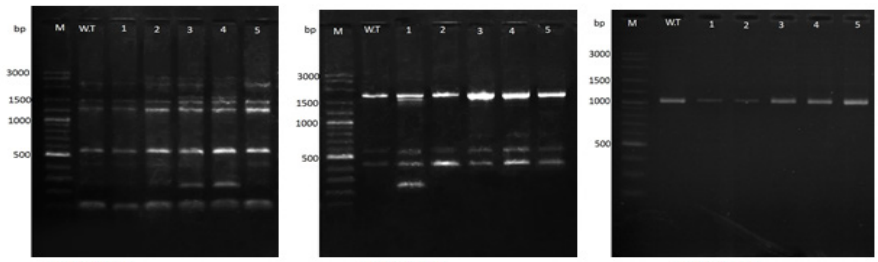
To study the effect of inoculum concentration (v/v) on OTA biosorption, three superior strains EMS-9, EMS-10 and EMS-19 were selected compared with W.T strain. OTA biosorption for all tested strains increased by increasing the inoculum concentration from 10% to 40% (v/v) except of EMS-10 and EMS-19 strains, which had decreased in OTA biosorption at 40% (v/v) of inoculum concentration by 3.6 % for each (Table 3). The rate of the change in OTA biosorption of every single tried strain when the inoculum concentration expanded from 10% to 20% (v/v) was high in examination of the rate of the change in OTA biosorption when the inoculum concentration was expanded from 20% to 40% (v/v).

Molecular analysis of some mutants in relation to OTA biosorption

To detect and examine the genetic impact of EMS-mutagenesis on the DNA nucleotide sequence of the obtained mutants in correlation with the wild type strain, three random primers got from Operon Technologies were utilized to recognize the genetic variability among the five remarkable mutants. All primers were effectively amplified particular fragments of the genomic DNA. The RAPD technique was used to test if genetic markers could be associated with the OTA biosorption. Two used primers were effectively generated reproducible polymorphic and

Table 3. Effect of inoculum concentration (v/v) on ochratoxin A uptake of three superior OTA uptake mutants in comparison with W.T strain in the presence of OTA (100 ng/mL).

Strain code	OTA uptake (ng/mL) Inoculum concentration (v/v)		
	10%	20%	40%
W.T	49.9 ± 0.53	76.3 ± 1.5	78.7 ± 2.1
EMS-9	82.5 ± 2.3	86.5 ± 2.0	88.1 ± 3.6
EMS-10	72.3 ± 1.9	81.2 ± 2.1	78.3 ± 2.6
EMS-19	67.9 ± 2.4	79.03 ± 2.8	76.2 ± 1.6



(RAP1) (RAP3) (RAP5)

Figure 1. Photographs of DNA amplified banding patterns based an RAPD for five mutant strains in comparison of W.T strain and VC100 bp Plus DNA ladder, Vivantis NL 1407(Lane M) using primers (RAP1), (RAP3) and (RAP5). Mutant strains sequence as follows: 1 = EMS-9; 2= EMS-10; 3= EMS-19; 4= EMS-2 and 5= EMS-16.

scorable bands. A photograph of the polymorphic amplified DNA bands based on RAPD analysis for the original strain (Lane W.T) and five tested mutants (lanes 1-5) using 15-mer primers RAP1 is exhibited in Figure 1. The bands sizes were detected against 100 base pair ladder marker Lane (M).

Results showed clear differences at bands number and size between the original strain and its derivatives using primer RAP1, it was clearly noticed that seven amplified bands were occurred when the original strain DNA was utilized as a template. The bands sizes were 2200, 1500, 1300, 550, 450, 250 and 100 bp as appeared in Lane W.T. All the five tested mutants contained the above seven amplified bands were occurred when the original strain DNA was used as a template but the mutant strain lane 2 contained also 2500 bp new band. On the other hand, two mutant's strains (lanes 3 and 4) exhibited new bands (2500 bp) and the band with size 250 bp converted from faint in the W.T into very distinct band.

The random amplified banding patterns of the original strain and the tested five mutants with primer RAP3 is proved in Figure 1. The obtained results showed that five mutants, produced the same amplified bands as their original strain. Three bands were detected for all of these six purified DNA with sizes of 1750, 600 and 250 bp. Moreover, additional bands with size of 1600 and 250 bp were occurred for the mutant (lane 1). Furthermore, the three mutants (lanes 3, 4 and 5) shared with the 1500 and 700 bp very faint bands which did not detected with the original strain.

The application of primer RAP5 (Figure 1) against strain utilized within RAPD investigation promoted only one monomorphic amplified band with size of 1050bp. It was interested to notice that mutants lane 1 and lane 2 which have about 55% OTA uptake more than the original strain, exhibited faint band. The other mutants and original strain exhibited high intensity band.

The obtained results showed excellent harmony with those reported by Thompson *et al*. (1997). They 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.

The obtained results are in good harmony with those reported by Schlick *et al.* (1994) who illustrated that some differences and similarities in RAPD profile between mutants in *T. harzianum* gotten following gamma radiation. Moreover, Barcelos *et al.* (2011) reported that, RAPD markers were useful for identifying genetic variability among isolates of *C. lindemuthianum.* In the same regards, Khattab and Abd El-Salam (2012) observed some differences

Table 4. Distance matrix among the five different mutants and the wild type strain of *Lb. delbrueckii* NRRL B-1024 based on RAPD analysis.

WT	EMS-9	EMS-10	EMS-19	EMS-2	0.00
0.90	0.91	0.82	0.69	0.75	EMS-16
0.82	0.69	0.75	0.92	1.00	EMS-2
0.75	0.64	0.83	1.00		EMS-19
0.90	0.75	1.00			EMS-10
0.82	1.00				EMS-9

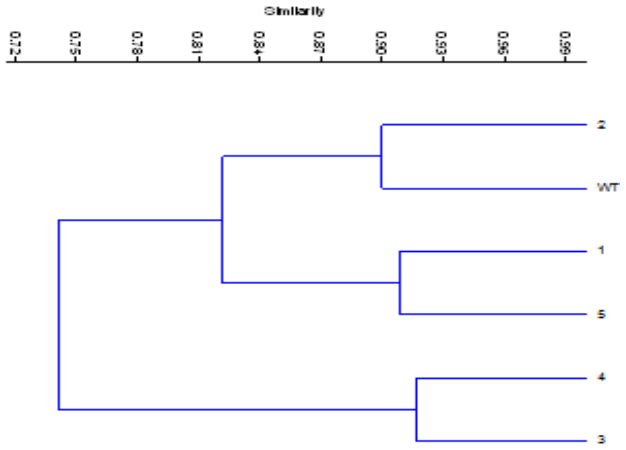


Figure 2. RAPD based dendogram of the five different mutants and the wild type strain of *Lb. delbrueckii* NRRL B-1024 constructed using unweighted pair–group arithmetic average (UPGMA) and similarity matrices.

in mutant strains in comparison with the original strain. These differences in RAPD profiles confirmed the evidence of genetic variations of mutants and parent genome after Ultra Violet mutagenesis. On the other hand, Khattab *et al.* (2012) exhibited that the application of RAPD mechanism by PCR on some selected excellent mutants lead to correlate the genetic features of the superior mutants with the results obtained from the biosorption experiments in correlation with the wild type. Differences in RAPD patterns confirmed the evidence of genetic variability induced in *R. toruloides* genome after UV-treatments. Therefore, cluster analysis was used to divide the studied genotypes into groups which could be reflecting the genetic diversity of the phenol biosorption mutants.

In the current study, the distance matrices resulting from the RAPD products data were performed to generate correct relationships based on RAPD analysis. The highest percentage of genetic distance was detected between EMS-19 and EMS-2 (92%) followed by genetic distances between EMS-9 and EMS-16 (91%) and then between EMS-10 and W.T (90%). The Lowest genetic distance (64%) was between EMS-9 and EMS-19 (Table 4).

The dendrogram based on RAPD distance indices separated the five different mutants and the wild type strain of *Lb. delbrueckii* NRRL B-1024 into two main clusters as shown in Figure 2. The first cluster was divided into two groups; the first group contained the mutants EMS-9 and EMS-19, while the second group contained W.T and EMS-10. Whereas, the second cluster contained EMS-19 and EMS-2 which they were highly diverged than other mutants and the wild type strain.

Conclusion

The current study was performed to examine the biosorption capacity of OTA using eight different strains of LAB at initial concentration level of 100 ng/ml. The investigated strains showed that the biosorption capabilities within 1 hr ranged from 31% for *Lb. rhamnosus* B-445 to 50% for *Lb. delbrueckii* B-1024, which are considered the most promising strain for OTA sequestration. However, *P. pentosaceus*- PP11 strain had no effect within 1 hr for OTA biosorption. *Lb. delbrueckii* B-1024 was selected to promote genetic variation in relation to improve OTA biosorption. Among 19 mutants that were found to be resistant to OTA, three superior strains, which recorded 82.5, 72.3 and 67.9 ng/ml OTA uptake were selected for further investigation. By increasing the inoculum concentration from 10% to 40%, OTA uptake was increased from 82.5 to 88.1 ng/ml and from 72.3 to 78.3 ng/ml for EMS-9 and EMS-10, respectively. However, the OTA uptake for EMS-19 was increased from 67.9% to 79.2% by increasing the inoculum concentration from 10% to 20%. The application of the RAPD technique by PCR on the selected mutants lead to correlate the OTA uptake of the mutants with the results obtained from the biosorption process in comparison with the wild-type strain. The results indicated that differences in RAPD profiles confirmed the changes of DNA sequence in the genome of *Lactobacillus delbrueckii* NRRL B-1024 after EMS-treatment are induced.

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