*AsPac J. Mol. Biol. Biotechnol. 2024*
*Vol. 32 (4) : 63-73*

# Random amplified polymorphic DNA-based polymerase chain reaction is an effective tool to examine the genotoxic effects of some food colors

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*Received 9th July 2024 / Accepted 27th October 2024 / Published 30th November 2024*

**Abstract.** A large number of natural or synthetic dyes have been removed from both national and international lists of permitted food colors because of their mutagenic or carcinogenic activity. Therefore, this study aimed to use the Random Amplified Polymorphic DNA-Based Polymerase Chain Reaction (RAPD-PCR) assay as a feasible method to evaluate the ability of some food colors as genotoxin-induced DNA damage and mutations. *Lactiplantibacillus plantarum* was used as a bioindicator to determine the genotoxic effects by RAPD-PCR using M13 primer after treatment with some synthetic dyes currently used as food color additives, including Sunset Yellow, Carmoisine, and Tartrazine. Besides qualitative analysis, the bioinformatic GelJ software was used for cluster analysis to compare DNA fingerprints before and after treatment. The bacteria treated with the food colors showed the presence of polymorphism represented by DNA changes in the RAPD patterns, including variation in bands intensity, disappearance of normal bands, and appearance of new bands compared with the non-treated control. The GelJ program confirmed the presence of genetic variations between the bacteria treated with different concentrations of the food dyes and the bacteria without treatment. The RAPD approach can be applied for the detection of DNA damage and mutations induced by genotoxic compounds. Furthermore, *L. plantarum* and M13 are suitable as in vitro screening tools for detecting of potential genotoxicity of numerous compounds.

**Keywords:** carmoisine, food coloring agents, *Lactiplantibacillus plantarum,* RAPD-PCR, sunset yellow, tartrazine

## INTRODUCTION

Carcinogens are categorized into two classes, genotoxic and non-genotoxic. Genotoxic carcinogens are compounds that cause cancer by inducing mutations (Nohmi, 2018). Owing to their DNA interaction properties, there is thought to be no safe exposure threshold or dose (Nohmi, 2018). The International Agency for Research on Cancer (IARC) used a more complex classification scheme to classify a substance as a human carcinogen or reasonably anticipated to be a human carcinogen. Among these classes, the fourth category of concern is anthropogenic chemicals intentionally added to foods, such as food coloring, but they are not addressed as contaminants because they are added intentionally (Abnet, 2007). Color additives are defined as dyes, pigments or substances that can impart color when added or applied to food, drugs, or cosmetics and to certain medical devices, such as contact lenses (Macioszek & Kononowicz, 2004). Food color additives are used for a variety of purposes, such as restoring natural color variations in food and counteracting color loss during food processing or storage, and finally to improve a product's appearance in order to satisfy customer expectations and desires (Macioszek & Kononowicz, 2004).

Among the most common azo food dyes used in food manufacturing are Sunset Yellow (E110), Carmoisine (E122), and Tartrazine (E102) (Kaya *et al.,* 2021). The capital E in front of the number means that the additive has been passed for food

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use throughout the European Community (Abdelmigid, 2009). Over the years, improvements have been made to increase the efficiency and ensure the safety of all additives (Abdelmigid, 2009). Nevertheless, food additives have been reported to be genotoxic and carcinogenic among numerous compounds (Chakravarthy *et al.,* 2014). Several natural and synthetic dyes' mutagenic or carcinogenic properties have led to their removal from lists of approved food colors on both national and international levels (Abdelmigid, 2009). There is a great concern about the effect of genotoxic compounds on the structure and function of DNA, including adduct formation, DNA breakage and mutations (Lanzone *et al.,* 2016).

The establishment of simple, rapid, cost effective, and non-labor-intensive high-throughput screening techniques to assess the genotoxicity of various molecules ranks high on the priority list (Ranganatha *et al.,* 2016). Detection of genotoxic effects using *in vitro* systems can be extremely useful in risk assessment procedures. In addition to the avoidance of using animals, the advantages of using *in vitro* assays are related mainly to cost, versatility, volume of waste, and laboratory facilities required (Rocco *et al.,* 2014). Several genotoxicity tests, like the chromosome aberration assay and the micronucleus assay, produce positive results even in the absence of DNA damage, making it difficult to distinguish between them in practice (Armstrong *et al.,* 2000; Ramirez *et al.,* 2007). Random amplified polymorphic deoxyribonucleic acid analysis by polymerase chain reaction (RAPD-PCR) is a feasible method to evaluate genotoxin-induced DNA damage and mutations (Lanzone *et al.,* 2016). This technique has been used for the analysis of molecular characterization of several model organisms and as a tool to detect DNA alterations in environmental genotoxic studies (Rocco *et al.,* 2014). RAPD-PCR technology is reproducible and sensitive, and it can be used for the examination and estimation of genomic variation in genotoxic studies (Rocco *et al.,* 2014). The principal benefits of the RAPD method are its speed, adaptability to any organism (because it does not require knowledge of the nucleotide sequence, cell cycle, or complement of chromosomes), and sensitivity in identifying a broad spectrum of DNA damage and mutations (Lee *et al.,* 2007; Liu *et al.,* 2008; Noel & Rath, 2006). In addition, it is characterized by excellent reliability and is inexpensive because it does not require specialist and expensive equipment, and it also eliminates the use of radioisotopes (Atienzar & Jha, 2006). The RAPD-PCR assay involves the amplification of random segments of genomic DNA, using short arbitrary primers (Welsh & McClelland 1990; Williams *et al.,* 1990). It has been used in species and strain identification and genetic diversity analysis (Hasan & Lafta, 2021; Hasan *et al.,* 2023; Mraidi & Lafta, 2021), genetic marker-assisted breeding (Liu *et al.,* 1999), and genotoxicity evaluation of environmental pollutants (Rocco *et al.,* 2011, 2012). More extensive assessment of food dyes is warranted to study the DNA damaging potential of them on living cells and consequently on human health (Abdelmigid, 2009). *Lactiplantibacillus plantarum* is the most diverse lactic acid bacteria (LAB), belonging to the heterofermentative group and producing both L- and D-lactic acid (Saito & Sato 2021). This bacterium is a highly versatile species found in several ecological niches (Siezen *et al.,* 2010). Thus, this study aimed to use RAPD-PCR assay as a feasible method to detect the effects of the food colors as genotoxins-induced DNA damage and mutations in *L. plantartum*.

## MATERIALS AND METHODS

### *Lactiplantibacillus plantarum*

An isolate of *L. plantartum,* isolated from locally made dairy products and identified in the study of Qasim *et al.,* (2023) was used in this research. To perpare the bacterial inoculum, at least 1 to 2 well isolated colonies of an overnight culture of *L. plantarum* were transferred by a sterilized loop to a tube containing 4 to 5 ml of Mueller-Hinton broth (MHB, Himedia, India). A suspension equivalent to 0.5 McFarland Standard tube ($1.5\times10^8$ CFU/ml) was made from the bacterial growth.

### *Making different concentrations of Mitomycin C and food color additives*

A stock solution of the three food color additives, including Carmoisine, Sunset yellow, and Tartrazine (obtained from a local market in Baghdad) and the Mitomycin C (Zydus, india) was

made. Mitomycin C was used as a positive control to determine the sensitivity of *L. plantarum* to the genotoxic agents. A stock solution of Mitomycin C was prepared by dissolving 2 µg of its powder into 10 ml of MHB. Then, the solution was sterilized by Millipore filter (0.22 µm). Serial concentrations of 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml 12.5 ng/ml and 6.25 ng/ml were then made. Concerning the food color additives Carmoisine, Sunset yellow, and Tartrazine, different concentrations of 25,600 µg/ml, 12,800 µg/ml, 6,400 µg/ml, 3,200 µg/ml, 1,600 µg/ml, 800 µg/ml, and 400 µg/ml were prepared, and then sterilized by Millipore filter (0.22 µm).

***Bacterial exposure and growth conditions***

100 µl of the standardized bacterial inoculum was added to each stain concentration and the different concentrations of Mitomycin C made above and incubated overnight at 37°C for 24-36 h. In addition, a bacterial culture without treatment served as the negative control.

***RAPD-PCR***

Random Amplified Polymorphic DNA-based polymerase chain reaction (RAPD-PCR) involved the use of the lyophilized primer M13 (5'-GAGGGTGGCGGTTCT-'3) (Tofalo & Corsetti, 2017), which was manufactured by Macrogen (Korea). It was dissolved in nuclease-free dd$H_2O$ to give a final concentration of 100 pmol/µl as a stock solution, which was kept at -20°C. The bacterial genomic DNA was extracted from *L. plantarum* culture treated with Mitomycin C, Carmoisine, Tartrazine, Sunset yellow, or left without treatment as a negative control, according to the protocol of FavorPrep Total DNA Mini Kit (Favorgen, Korea). After that, the RAPD-PCR reaction components were prepared at a total volume of 25 µl, which was composed of 12.5 µl of 2x Master mix (Promega /USA), 1 µl (20 pmol/ml) of the M13 primer, 5 µl (50 ng/µl) of DNA template, as well as 6.5 µl of nuclease-free water.

Pertaining to the RAPD-PCR cycling conditions, they consisted of 1 cycle of initial denaturation at 94°C for 4 min, followed by 35 cycles of: 94°C for 30 sec (denaturation), 45°C for 20 sec (annealing), and 72°C for 2 min (extension). Finally, one cycle for 7 min at 72°C for extension was done. The agarose gel electrophoresis was performed to confirm the occurrence of DNA amplification and DNA fingerprints by the RAPD-PCR assay. The PCR amplicons (10 µl) were loaded into the solidified 1.5% agarose gel dissolving into the TAE buffer. Along with the PCR products, 100 bp (Intron, Korea) or 1 kb DNA ladder (CSL, UK) was loaded into the first lane of the gel. After covering the DNA samples at a certain level with 1×TAE buffer, which filled the electrophoresis tank as well, the electrophoresis was run firstly at 80 V for 30 min, and then at 100 V for 2 h. Finally, the power supply was switched off, and the gel was transferred to the ethidium bromide staining solution (0.5µg/ml). The gel was placed under Gel Imaging System (Major Science, Taiwan) supplied with UV transilluminator and photographs were captured.

***In silico analysis***

In order to perform a compiled comparison between the RAPD-PCR bands of the bacterial gDNA treated or not with various concentrations of the food dyes mentioned above, an *In Silico* analysis was done using GelJ version 2.0 software. This analysis was developed and updated later at the Department of Mathematics and Computer Science of the University of La Rioja, Spain, by Heras *et al.* (2015). The program was designed for analyzing DNA fingerprint images. Some of the outstanding features of GelJ were functionality for accurate lane- and band-detection, several methods for computing migration models and generating dendrograms, comparison of banding patterns from different experiments, and database support.

## RESULTS

***DNA Extraction***

The agarose gel showed the successful extraction of gDNA from *L. plantarum* incubated with different concentrations of Mitomycin C as well as various concentrations of the food colors (Carmoisine, Tartrazine, and Sunset yellow dyes), along with one negative control (bacteria without treatment) (Figure 1).

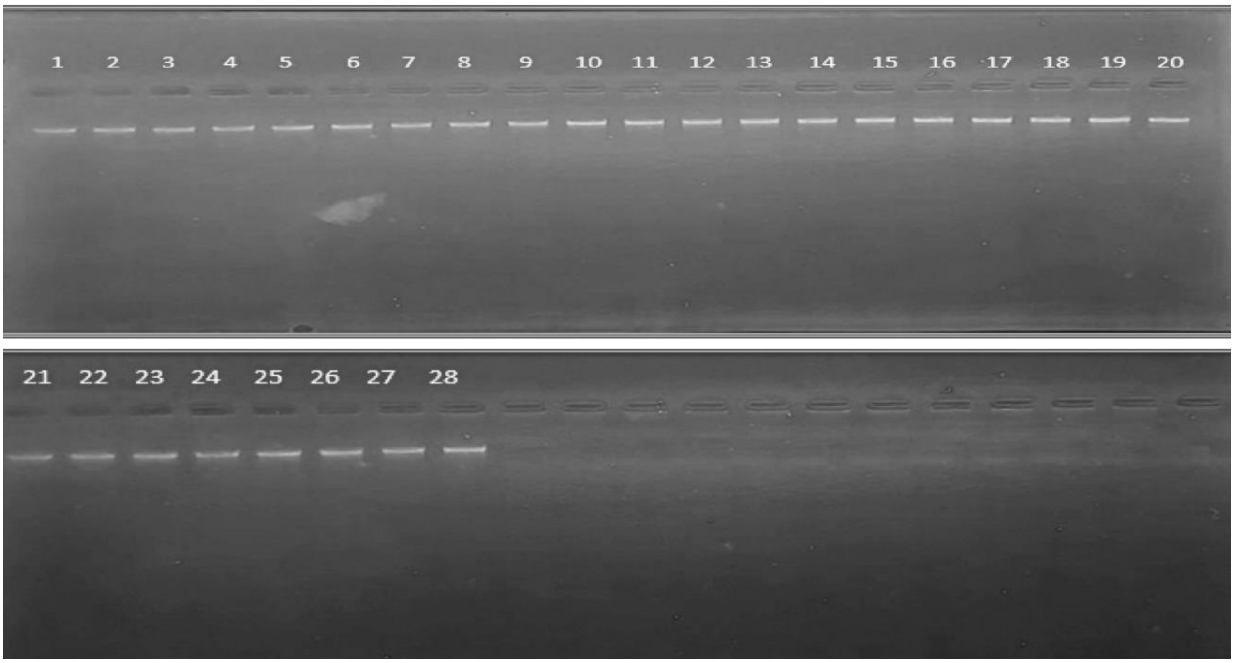


**Figure 1.** Agarose gel shows successful extraction of the genomic DNA from *L. plantarum* after incubation with different food coloring agents, Mitomycin, or negative control (bacteria without treatment). Lanes 1-6: Mitomycin C; Lanes 7-13: Carmoisine; Lanes 14-20: Tartrazine; Lanes 21-27: Sunset Yellow; Lane 28: bacterial gDNA without treatment (negative control). Electrophoresis on 1% agarose gel at 80 V for 1 h.

### *RAPD analysis of Mitomycin C*

*Lactiplantibacillus plantarum* treated with Mitomycin C showed complete disappearance of its gDNA at the high concentrations of that antibiotic, in particular 200 μg/ml and 50 μg/ml. In comparison with the bacterial cells without treatment with the antibiotic, a common band of ~1000 bp was seen in the no-treatment control as well as lanes 6, 5, 4, and 2 where the gDNA extracted from the bacterial cells and treated with the Mitomycin C at concentrations of 6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, and 100 μg/ml, respectively, were loaded. Moreover, another common faint band of ~2,000 bp appeared in the non-treated cells and lanes 6 (6.25 μg/ml), 5 (12.5 μg/ml), and 2 (100 μg/ml). However, the strong bands sized ~3,000 bp and ~1500 bp that appeared in the gDNA of the control cells were not present in the lanes of Mitomycin treatment (Figure 2). When examining the correlation between the drug concentration and the effect on the bacterial DNA, no relationship existed and all the concentrations even the least one (6.25 μg/ml) affected negatively and caused DNA damage represented by the lack of the sharp bands.

### *RAPD analysis of the food color agents Carmoisine*

In comparison with the no-treatment control, different DNA bands were observed when treating the bacterial cells with various concentrations of the food coloring agent Carmoisine. The intensity of some bands differed among the different concentrations of Carmoisine, and bands of different sizes either emerged or disappeared relative to the control cells (Figure 3). For example, the strong band of ~3,000 bp seen in the no-treatment control was slightly fluctuated at different Carmoisine concentrations. In Figure 3, it can be seen that the lanes of 12,800 μg/ml and 6,400 μg/ml were apparently similar, and the same was true for the concentrations 1,600 μg/ml and 800 μg/ml. Even the bacterial cells treated with the lowest concentration (400 μg/ml) showed different band patterns relative to the control cells. Overall, all concentrations of Carmoisine caused changes in the gDNA regardless of whether the concentration was high or low.

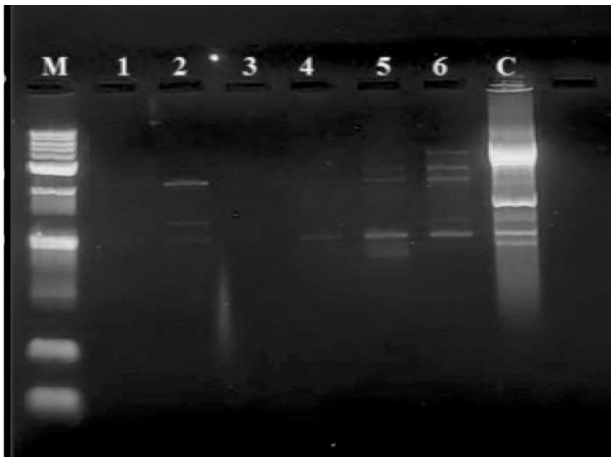


**Figure 2.** 1.5% Agarose gel shows bands of the gDNA of *L. plantarum* treated with Mitomycin C and amplified by RAPD-PCR. The bands were separated by electrophoresis at 80 volts for 1 h. Lane M: 1 KB DNA size marker. Lane 1: 200 μg/ml, Lane 2: 100 μg/ml, Lane 3: 50 μg/ml, Lane 4: 25 μg/ml, Lane 5: 12.5 μg/ml, Lane 6: 6.25 μg/ml and, Lane C: control (bacteria without Mitomycin C).

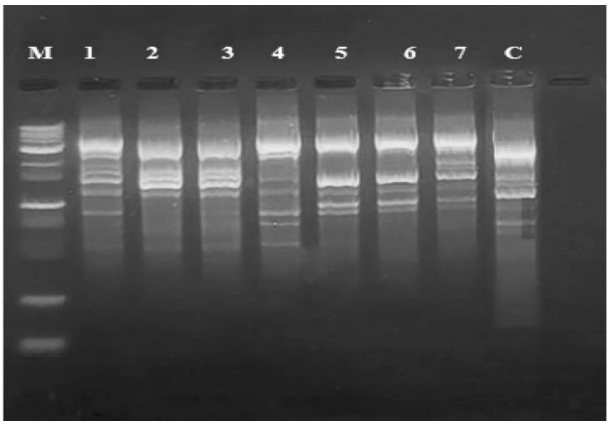


**Figure 3.** 1.5% Agarose gel shows bands of the gDNA of *L. plantarum* treated with Carmoisine and amplified by RAPD-PCR. The bands were separated by electrophoresis at 80 volts for 1 h. Lane M: 1 KB DNA size marker. Lane 1: 25,600 μg/ml, Lane 2: 12,800 μg/ml, Lane 3: 6,400 μg/ml, Lane 4: 3,200 μg/ml, Lane 5: 1,600 μg/ml, Lane 6: 800 μg/ml, Lane 7: 400 μg/ml, and Lane C: control (bacteria without treatment).

***Tartrazine***

The visual examination of the gel in Figure 4 reveals appearance of a common strong band of ~3000 bp in all of the lanes of treated and non-treated bacteria. Concerning the other popular band of ~1,500 bp, it disappeared only at the concentrations 3,200 μg/ml and 1,600 μg/ml, but new bands of ~2,800 bp occurred at the same concentrations (in lanes 4 and 5). A faint band of ~1,000 bp was present in the control as well as at the low concentrations of Tartrazine (800 μg/ml and 400 μg/ml), but it disappeared at the higher concentrations in lanes 1 to 5 (i.e. 25,600 μg/ml to 1600 μg/ml). Clearer two bands sized ~750 bp and ~500 bp occurred upon treatment with 3,200 μg/ml and 1,600 μg/ml of Tartrazine (Figure 4). No correlation was present between the dye concentration and the DNA alterations.

***Sunset Yellow***

No relationship existed between the different concentrations of the food color, Sunset Yellow, and the degree of the bacterial DNA damage. The bacterial cells treated with various concentrations of Sunset Yellow showed existence of common prominent bands of ~3,000 bp at all concentrations as well as in non-treated cells. Concerning the other prominent band of ~1,500 bp, it disappeared only in three lanes where the bacterial cells were treated with 3200 μg/ml, 1600 μg/ml, and 800 μg/ml compared to the other lanes (Figure 5). Furthermore, other bands that existed in the lane of the control were either absent or present at different intensities when various Sunset yellow concentrations were used, as shown in Figure 5.

***Compiled analysis for the food colors***

Importantly, Figure 6 confirms the consistency of the previous findings where the bacterial DNA was subjected to different dye concentrations or the drug along with the negative control and loaded separately on a gel. It further assured that no correlation occurred between the concentration and the impact on the DNA. Figure 6 reveals presence of big differences in the banding patterns between the bacterial gDNA without treatment (control) and that treated with various concentrations of the food color additives. Compared to the negative control

sample, a popular extra band more than 1500 bp in size was noticed in all the bacterial gDNAs treated with numerous concentrations of food coloring agents (Figure 6). Pertaining to the prominent 1500 bp band of the control, bands of the same size but less intensity occurred upon treatment with different concentrations of the three food color additives. Additionally, further bands were also noticed that sized approximately 1200 bp to 800 bp, especially upon treatment with certain concentrations of Carmoisine and Tartrazine (Figure 6).

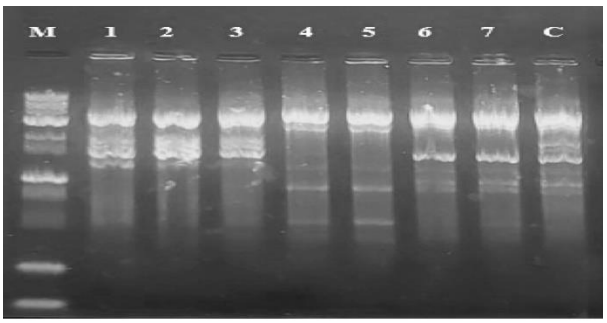


**Figure 4.** 1.5% Agarose gel shows bands of the gDNA of *L. plantarum* treated with Tartrazine and amplified by RAPD-PCR. The bands were separated by electrophoresis at 80 volts for 1 h. Lane M: 1 KB DNA size marker. Lane 1: 25600 µg/ml, Lane 2: 12800 µg/ml, Lane 3: 6400 µg/ml, Lane 4: 3200 µg/ml, Lane 5: 1600 µg/ml, Lane 6: 800 µg/ml, Lane 7: 400 µg/ml, and Lane C: Control (bacteria without treatment).

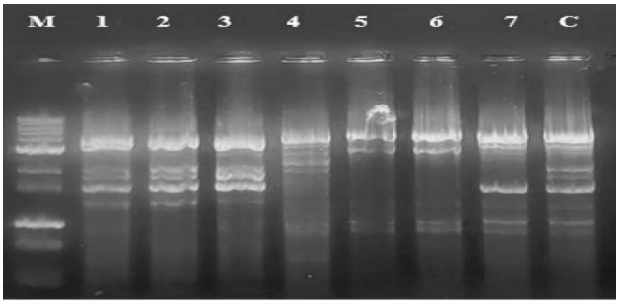


**Figure 5.** 1.5% Agarose gel shows bands of the gDNA of *L. plantarum* treated with Sunset Yellow and amplified by RAPD-PCR. The bands were separated by electrophoresis at 80 volts for 1 h. Lane M: 1 KB DNA size marker. Lane 1: 25600 µg/ml, Lane 2: 12800 µg/ml, Lane 3: 6400 µg/ml, Lane 4: 3200 µg/ml, Lane 5: 1600 µg/ml, Lane 6: 800 µg/ml, Lane 7: 400 µg/ml, and Lane C: Control (bacteria without treatment).

### *In silico* analysis of the RAPD results

*In silico* analysis was successful in showing similarities and variations among the DNA samples either treated or not with the food colors. It is clear in Figure 7 that the highest concentrations of the dyes, particularly Carmoisine 25,600 µg/ml and 12,800 µg/ml and Sunset Yellow (12,800 µg/ml) are the nearest to the bacterial DNA due to the occurrence of some common bands. Despite that, the similarity between the highest concentration of Carmoisine and the non-treated control was very low, approximately 65%. Interestingly, the lowest dyes concentrations were far from the control indicating the presence of fundamental differences in the DNA banding patterns (Figure 7).

## DISCUSSION

In this study, the gDNA of *L. plantarum* was successfully extracted without degradation, and the electrophoresis indicated that the size of the DNA obtained from the treated and control bacteria was the same. The purity and integrity of the DNA template are crucial for good RAPD analysis (Sharma *et al.,* 2010). The selection of appropriate primers represents a fundamental step in the development of RAPD-PCR methodology (Lanzone *et al.,* 2016). The results of the present study showed that the M13 primer used had a high efficiency in amplifying gDNA of lactobacilli under the PCR conditions applied here. This primer showed good discriminatory power, and the DNA profiles revealed differences between the control cells and those treated with various food colors, with visible changes in the number and size of amplified DNA fragments, and both increase and decrease of DNA band intensities. Previous studies reported that RAPD analysis was more sensitive than classic tests such as the comet and micronucleus assay, since RAPD analysis is capable of detecting temporary DNA changes at lower concentrations that may not finally manifest themselves as mutations (Atienzar & Jhr, 2006; Liu *et al.,* 2005).

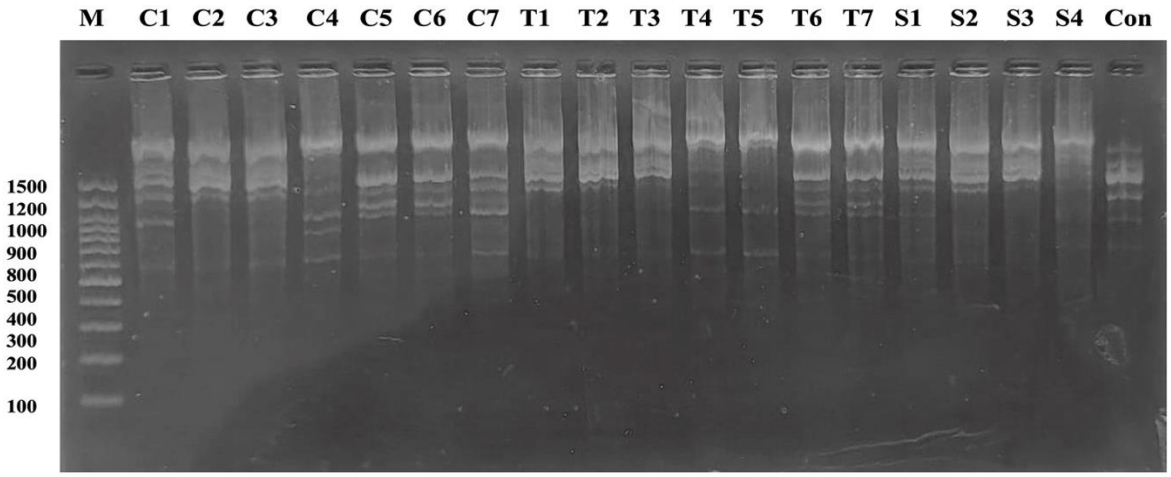


**Figure 6.** 1.5% Agarose gel shows bands of the gDNA of *L. plantarum* treated with numerous concentrations of the food dyes (C: Carmoisine, T: Tartrazine, S: Sunset yellow) and amplified by RAPD-PCR. The bands were separated by electrophoresis at 80 volts for 1 h. Lane M: 100 bp DNA size marker. Lane 1: 25,600 μg/ml, Lane 2: 12,800 μg/ml, Lane 3: 6,400 μg/ml, Lane 4: 3,200 μg/ml, Lane 5: 1,600 μg/ml, Lane 6: 800 μg/ml, Lane 7: 400 μg/ml, and Lane Con: Control (bacteria without treatment).

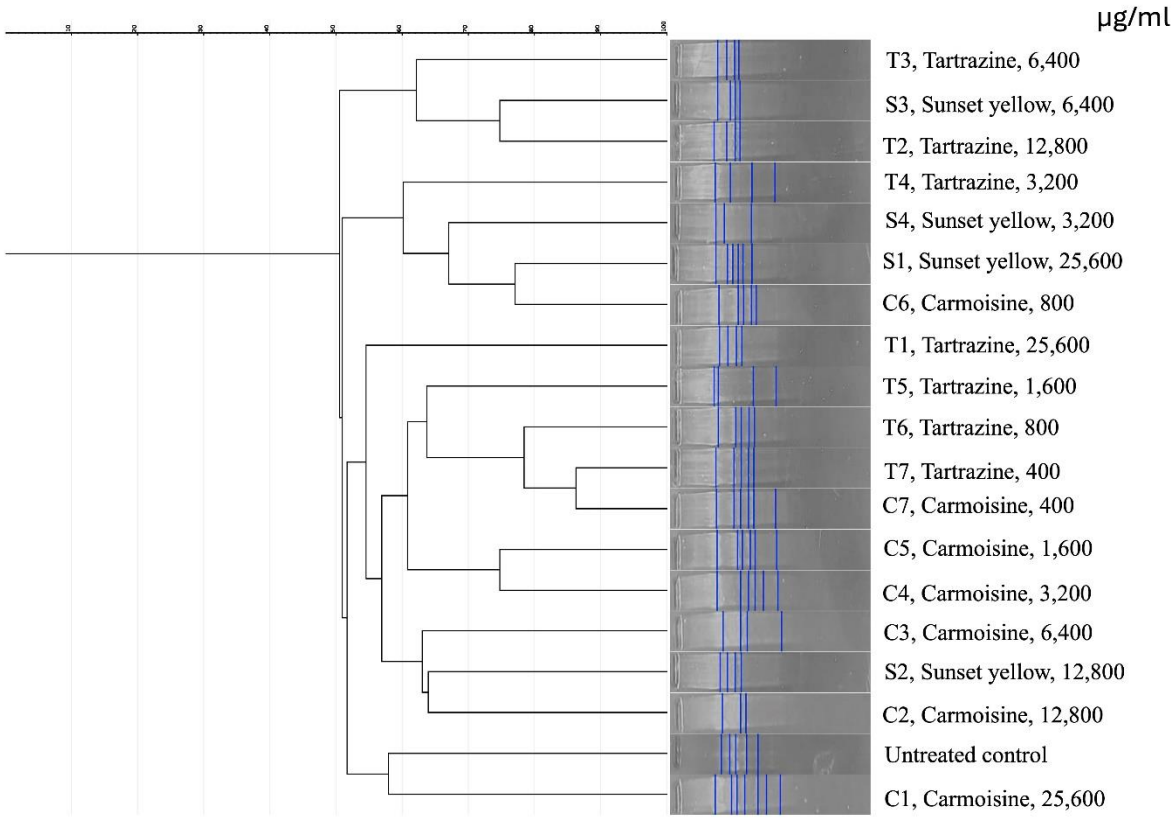


**Figure 7.** A dendrogram shows analysis of the RAPD-PCR products and electrophoresed gDNA of *L. plantarum* treated with various concentrations of Carmoisine (C), Sunset Yellow (S), and Tartrazine (T) at different concentrations: 25,600 μg/ml, 12,800 μg/ml, 6,400 μg/ml, 3,200 μg/ml, 1,600 μg/ml, 800 μg/ml, and 400 μg/ml, compared to the control (bacteria without treatment).

Generally, model microorganisms used for the detection of DNA damage and mutations are *Saccharomyces cerevisiae* (Frassinetti *et al.,* 2011) and *Escherichia coli* (Atienzar *et al.,* 2002). Here, *L. plantarum* was screened for DNA genetic alterations by DNA fingerprinting after treatment with food color additives. The use of Mitomycin C as a positive control in the current study supports the use of *L. plantarum* for the detection of DNA mutations by RAPD-PCR. The different concentrations of Mitomycin C used in this study caused changes in the genetic material represented mainly by the disappearance of high molecular weight bands and the appearance of new small molecular weight bands. The RAPD technique was successfully used to detect DNA alterations induced by Mitomycin C among other chemical agents and radiation (Abdelmigid, 2009). Mitomycin C is an extremely effective antibiotic that kills bacteria by selectively crosslinking complementary DNA strands (Tomasz, 1995). Interestingly, the antibiotic and anticancer mechanisms of Mitomycin C are quite similar (Giurini *et al.,* 2024). Mitomycin C undergoes a reductive activating reaction resulting in two alkylating reactions that occur in the cytosol of the cell. The first activating reaction covalently links the C1 of Mitomycin C to DNA, and the second links DNA to Mitomycin C at C10, culminating in the crosslinking of DNA (Giurini *et al.,* 2024). The interaction between DNA and Mitomycin C prevents access to DNA synthesis machinery, inducing cell cycle arrest and cell death (Tomasz, 1995). In the current investigation, the bacterial cells treated with the highest concentrations of Mitomycin C, in particular 200 μg/ml and 50 μg/ml, did not show any DNA band on the agarose gel. This result might be interpreted by the death of almost all of the bacterial cells and the inhibition of PCR amplification for the DNA from dead cells (Nocker *et al.,* 2006). However, the findings of this study showed that no correlation existed between the concentration of this drug and the degree of the DNA damage because even the low drug concentration was also effective and caused DNA changes represented by occurrence and lack of bands compared to the no-treated bacterial cells.

In the present study, different concentrations of the food dyes were examined extensively using the molecular technique, RAPD. In the current study no correlation was observed between the dye concentration and the occurrence of the DNA damage. Nevertheless, since genomic template instability may be related to different kinds of DNA damage, such as DNA adducts, mutations, rearrangements, and so on, it would be difficult to anticipate a dose-response relationship (Rocco *et al.,* 2014). Considerable inconsistency was found to exist between the control and color-treated bacterial cells in this study. In the case of Carmoisine, polymorphism was observed at all concentrations of the dye relative to the control. The intensity of some bands differed among the different concentrations of Carmoisine, and bands of different sizes either emerged or disappeared relative to the control cells. Even the bacterial cells treated with the lowest concentration (400 μg/ml) showed different band patterns compared to the control cells' DNA. Similarly, Carmoisine was found in animal studies to have either mutagenic or carcinogenic action (Tsuda *et al.,* 2001). Treatment of lactobacilli used in this investigation with different concentrations of Tartrazine caused various genomic alterations represented by a lack of normal bands or occurrence of new bands of high or low molecular weights. However, in the study of Das & Mukherjee (2004), Tartrazine was shown to be neither carcinogenic nor genotoxic in most short-term tests, including the Ames assay. Pertaining to the bacterial cells treated with various concentrations of Sunset Yellow, their DNA showed various alterations. These changes were either the presence of new bands of different molecular weights or the absence of normal bands already existing in the non-treated bacterial cells. Additionally, variations in the intensities of some bands relative to the negative control were also seen.

Many researchers have demonstrated that the differences in RAPD patterns occur due to many reasons, including band intensity, loss of normal bands, and appearance of new bands as compared with the control (Abdelmigid, 2009; Rocco *et al.,* 2014). The change in the number of bands and the variation in their intensity, in the RAPD-PCR profile, are associated with alterations of the genetic material (Rocco *et al.,* 2014). Such alterations *in vivo* are considered mutations that are produced by changes to, deletions of, or insertions of base pairs (Le Blanc & Bain, 1997).

These different types of DNA damage must be detected by changes in RAPD profiles (Abdelmigid, 2009). A reduction in the signal intensity of some bands in treated samples has been suggested to be attributed to a change in the number of copies of the sequence (Lanzone *et al.,* 2016). Band loss may not only be related to different types of DNA damage (e.g. single- and double-strand breaks, modified and oxidized bases, bulky adducts, DNA–protein cross links), and point mutations but also complex chromosomal rearrangements induced by genotoxins (Atienzar *et al.,* 2002). A loss of PCR amplicon can only be the outcome if the same structural changes occur in 75-90% of the cells or if the same mutations arise in the same percentage of cells (Atienzar, 2000). However, it is unlikely that mutations occur in a large portion of cells, because most of the DNA damage, which can lead to mutations during DNA replication, will be efficiently repaired (Abdelmigid, 2009). It is anticipated that bulky adducts and other DNA lesions may negatively impact RAPD profiles (Abdelmigid, 2009). Not only can they induce structural changes, but they can also reduce the polymerization of the DNA and/or block the *Taq* DNA polymerase (Nelson *et al.,* 1996). This will lead to a reduction in band intensity, or in the event of significant DNA damage, the absence of amplified products (Atienzar, 2000). In the current study, many new bands were observed in the DNA of the treated bacteria. This leads one to think that these bands are the result of a food-color-directed action on specific points of the genomic DNA. This is in accordance with the study of Liu *et al.,* (2008) and Selvi *et al.,* (2007). Extra bands are produced by significant deletions that bring two pre-existing annealing sites closer together, homologous recombination that juxtaposes two sequences that match the primer sequence, and mutations that may result in novel annealing events (Atienzar *et al.,* 2000). The appearance of new bands can also be explained as the result of DNA structural changes (breaks, transpositions, deletions, and so on) (Atienzar *et al.,* 2001). Furthermore, a gain of new bands may be the result of genomic template instability related to the level of DNA damage, and the efficiency of DNA repair and replication (Atienzar *et al.,* 1999).

The dendrogram constructed by the GelJ bioinformatic software used in the present study was successful in showing variations among the DNA samples either treated or not with various concentrations of the food colors. This program does not consider just the presence or absence of the bands, but it also based on the variance between two fluorescence density values. Cluster analysis of *L. plantarum* revealed that the DNA exposed to numerous concentrations of food colors formed four distinct clusters using a similarity level of 85%. Considering what was mentioned above, all the food colors investigated in the current study are considered mutagenic. This is partially consistent with other investigators, e.g., Das & Mukherjee (2004) and Tsuda *et al.,* (2001) who reported that the food colors are well-known mutagenic and clastogenic agents. Finally, the data provided in this study proved the RAPD technique as a promising tool for the *in vitro* detection of DNA alterations produced by genotoxic agents, enabling a closer look at the agent's mechanisms of action.

## CONCLUSION

In conclusion, the levels of DNA damage can be measured in *L. plantarum* gDNA by using RAPD-PCR technique, which is rapidly becoming one of the most used methods in genetic toxicology. Differences in RAPD-PCR patterns were seen in the DNA treated with different concentrations of the food color additives compared to the control. These alterations represented by changes in band intensity, disappearance of bands, and appearance of new bands of amplified DNA. Although no correlation existed between the dye concentration and the influences on the bacterial DNA. Finally, the lactic acid bacteria exemplified by *L. plantarum*, represent a good experimental model to assess the genotoxic effects of chemical components, including food dyes, by means of the RAPD-PCR assay. Therefore, we recommend its use as a tool for the detection of genotoxic compounds because it is the most diverse lactic acid bacteria, a highly versatile species present in different environments, and has been proved to be effective in the detection of DNA damage and mutations induced by some genotoxic chemicals.

The assessment of parameters at the molecular level is valuable for detecting the specific influences of agents interacting with the DNA. Thus, certain rules or laws must be enforced to prevent the effects of the food colors.

## ACKNOWLEDGEMENTS

Many thanks should go to Prof. Dr. Falah M.K. Al-Rekabi for his suggestions and scientific collaboration concerning toxicology.

## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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