Microbes and Infectious Diseases 2025; 6(3): 2081-2094

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

A preclinical evaluation of the response of repairing the DNA of MCF7 breast cancer cells after exposure to probiotic bacteria

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ARTICLE INFO

Article history:

Received 22 January 2025

Received in revised form 29 January 2025

Accepted 4 February 2025

Keywords:

Breast cancer

CAPS software

Comet assay

DNA damage

Probiotics bacteria

ABSTRACT

Background: Breast cancer is the most common cancer among women worldwide. The regulation of numerous cellular processes involved in DNA damage and repair have an impact on DNA damage response signaling. Probiotics may assist in repairing DNA damage by promoting tissue regeneration and activating DNA repair enzymes. **Methods**: The inhibition of MCF7 cells by *Lactobacillus planetarium* Treatment 1(T1) and a combination of 8 strains of probiotics Treatment 2(T2) was done. T1 and T2 were assessed by crystal violet assay, and genetic changes were conducted by comet assay. The repair of DNA damage was compared with control by using CASP software analysis image. **Results**: Both treatments, T1 and T2, showed no significant cytotoxic effect on breast cancer cell lines. The curve of images analysis demonstrated the potential role for T1 and T2 in breast cancer treatment, showing green small tail and red large head compared with control. MCF7 cells exposed to T1 for 48 h and 72 h showed a significant increase in L Tail ($P \leq 0.005$), Head DNA ($P \leq 0.001$), and Tail DNA ($P \leq 0.001$) compared with the control. In contrast to the control, MCF7 cells treated to T2 for 48 h and 72 h demonstrated a substantial increase in L Head ($P \leq 0.5$), L Tail ($P \leq 0.5$), L comet ($P \leq 0.5$), Head DNA ($P \leq 0.5$), and Tail DNA ($P \leq 0.5$). **Conclusion**, probiotics bacteria could support repairing DNA damage, making them a natural agent for the treatment of breast cancer. However, these results need more investigation to identify the signaling pathway that is used in the repair.

Introduction

Globally, breast cancer is the most prevalent malignancy cancer among women. Cancer has multiple etiologies. The possibility of survival can be increased with an early diagnosis and effective cancer treatment [1]. Epigenetic alterations affect transcription, DNA damage response signaling, and genomic stability—all of which are indicators of cancer—by controlling many cellular processes involved in DNA damage and repair [2]. DNA repair proteins and enzymes exist in S and G2 phases of cell cycle phases [3].

Since DNA is the fundamental genetic material, maintaining the integrity of DNA structure and function is essential to maintaining stable species traits and normal living activities [4,5].

Numerous correlations between gut microbiota and illness are connected to the specific types of microbes that contribute to the onset of disease in addition to the composition of the microbiota. It is unknown how gut microbiota and

DOI: 10.21608/MID.2025.354850.2476

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cancer are related [1]. Recent research has demonstrated that the microbiome affects breast cancer; the breast microbiota has been found to include a variety of microbial signatures, which exhibit varying patterns based on the stage and biological subgroups. The digestive tract of an adult human has about 100 trillion microorganisms. Research on the gut microbiota is a new area of study that has connections to several biological processes in a number of illnesses, such as cancer, rheumatoid arthritis, diabetes, obesity, and cardiovascular disease [1]. Studies using animal and cell models have shown that probiotics, as functional foods, may be able to prevent breast cancer; probiotics have the potential to prevent or treat breast cancer by modifying the immune system and gut microbiota. To validate the *in vitro* and *in vivo* outcomes, however, as well as to investigate the metabolic, immunological, and molecular pathways linked to probiotics in breast cancer, further clinical trials and research are required [6].

Probiotic organisms can be divided into two categories: fungal and bacterial strains that produce lactic acid or non-lactic acid. Common probiotics bacteria include *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *Enterococcus* [7]. Given the wide range of applications for lactic acid (LA), probiotic bacteria that produce LA are crucial. Because bacteria were assumed to have anticancer properties, *Lactococcus* and *Streptococcus* bacteria were more common in healthy breast tissue [8]. Certain strains of probiotics, including *Bifidobacterium animalis*, *L. acidophilus*, *B. infantis*, *L. paracasei*, and *B. bifidum*, have been demonstrated to prevent the proliferation of cancer cells in MCF7 cells, making them useful as natural agents for the treatment of cancer [9, 10]. The predominant bacteria in the breast microbiota of healthy people are *Lactobacillus* and *Streptococcus*, which regulate the growth of tumors via inducing natural killer (NK) cell activity. Furthermore, *Streptococcus thermophilus* generates antioxidants that by minimizing DNA damage and counteracting reactive oxygen species (ROS), have anticancer qualities [11,12]. Probiotics modify the immune system and cellular reactions by strengthening the barrier between cells and promoting the synthesis of anti-inflammatory, antioxidant, and anticarcinogenic substances, which lowers the incidence and spread of cancer [13].

It was discovered that there may be advantages in preventing obesity and dyslipidemia when *L.* with *B.* plus *S.* (ProLBS) with prebiotic fructooligosaccharides (FOS) is used by breast cancer patients, and ProLBS is used by breast cancer survivors. When combined, FOS with ProLBS reduces pro-inflammatory tumor necrosis factor (TNF-α) in breast cancer survivors and enhances the quality of life for people who have lymphedema related to breast cancer. A better strategy than probiotics alone could be to use prebiotics in addition to probiotics capsules (10^9 CFU) and to take them for ten weeks [14]. Probiotics may also directly counteract oxidative stress brought on by radiation exposure by acting as direct antioxidants [15]. Additionally, probiotics can support repair of DNA damage through the activation of DNA repair enzymes, stimulation of cell proliferation, and encouraging tissue regeneration and repair [16, 17]. The human body is impacted by ecology because it damages DNA, which leads to mutations and the development of cancerous cells. A quantitative technique for assessing DNA damage in eukaryotic cells is the comet assay. This approach is frequently utilized in fields including ecology, genotoxicology, and human biomonitoring. Another name for the comet assay is single-cell gel electrophoresis. Because of this, electrophoresis's results are examined under fluorescent light, making it comparable to comets. The intensity of the comet tail in relation to the head is compared in order to identify breaks in DNA strands [18]. Numerous programs are available for analysing comet images, and the results cover a wide range of parameters. The tail length (L Tail), tail moment (TM), and tail DNA percentage (Tail DNA) are the most often utilised parameters. Since L Tail tends to remain constant once formed, it can only be utilised at modest degrees of DNA damage. Then, as the damage is increased, the tail's intensity rises. Because it has a linear relationship with the breaking frequency, the percentage of DNA in the comet tail is another helpful metric. The most practical and widely used parameter is the TM, which combines the tail length and tail intensity into a single value. As it may detect differences in the distribution of DNA within the tail, the olive tail moment (OTM) has been shown to be very helpful in characterizing heterogeneity within a single cell population [19,20].

The current study aims to test the cytotoxic effects of *L. planetarium* and a combination of 8

strains of probiotics, including *L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *S. thermophilus*, *B. breve*, *B. longum*, and *B. bifidum* strains on MCF 7 breast cancer cells *in vitro* conditions. Also, changes in DNA of MCF7 cells after exposure to probiotic bacteria were studied, which could provide evidence for the possibility of using probiotic bacteria in the treatment of breast cancer. Cytotoxicity was performed by cell viability assay, and genetic changes were conducted by comet assay.

Materials and methods

Microorganisms

The probiotics bacteria supplements were chosen from the pharmacy randomly. One supplement contained one strain of bacteria, *L. planetarium* 299v (Probest company batch no. 22A535, 1×10^{10} CFU), and was used as treatment 1 (T1). The other supplement was a combination of 8 strains of probiotics bacteria, including *L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *S. thermophilus*, *B. breve*, *B. longum*, and *B. bifidum* (Probio 7 company, batch no. 06FP7uo6o519) and was used as treatment 2 (T2).

Preparation of probiotics bacteria

A total of 0.5 grams of powdered samples from each group, labeled T1 and T2, were carefully suspended in 10 milliliters of sterilized phosphate-buffered saline (PBS) to create a homogeneous mixture. This suspension was then transferred into De Man–Rogosa–Sharpe (MRS) broth (sourced from Oxoid Ltd., Basingstoke, Hampshire, UK) for microbial activation. The inoculated MRS broth was subsequently incubated at a controlled temperature of 37ºC within a CO2-enriched environment containing 5% carbon dioxide for 24 hours. To ensure the purity of the microorganisms, a purity check was performed by streaking a sample of the cultured broth onto MRS agar plates. Following this initial assessment, a single colony was selected from the agar plates and used to inoculate fresh broth media. This broth was incubated overnight under the same ideal conditions of 37 ºC and 5% CO2, allowing for further growth and development of the microorganism [7, 10].

The probiotics' biological density, measured in colony forming factors (CFU/mL)

Basically, the proportion of macrophages in tissue culture per milliliter determined the ratio of probiotic bacteria used in this investigation. Density, which was used 10:1 (probiotics/macrophages), according to [21-23].

Cell culture

The study utilized the human breast cancer cell line MCF 7. The Experimental Therapy Department of AL-Mustansiriyah University's Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR) in Baghdad kindly provided MCF 7 from May 2024 to July 2024. The MCF 7 cell line was cultivated in Roswell Park Memorial Institute (RPMI) 1640 (Gibco), which was supplemented with 10% FBS (fetal bovine serum) (Gibco) and incubated at 37 °C in a humidified environment with 5% CO_2. Two times a week, cells were passaged to ensure log phase. Within ten passages of cell recovery, experiments were carried out, and cells were seeded at a density of 2×10^5 cells per milliliter for the experimental protocols. The medium was removed once the cells had achieved 80–90% confluence. The cells were passed through the use of 1-3 mL of Trypsin-EDTA, and the cells were then incubated in the cell culture incubator for two to three minutes. To inhibit trypsin, media supplemented with 10% FBS was added, cells were centrifuged for five minutes at 1300 rpm, the supernatant was disposed of, and the pellet was resuspended in culture medium and aliquoted 1:4 in tissue culture flasks 75 cm^2. A hemocytometer was used to count the cells [21].

Viability assay and dose response

According to [24], to determine the cytotoxic effect, the crystal violate assay (CV) of cell viability was carried out on 96-well plates for 24 h, 48 h, and 72 h. MCF 7 cell lines were seeded at 1×10^4 cells/well. After 24 h, a confluent monolayer was performed, the concentrations of probiotics supplement T1 and T2 were (1×10^5 CFU/mL), the medium was disposed of after 24 hours, 48 hours, and 72 hours, 50 µL of CV dye solution with a concentration of 2 mg/mL was added to each well (Bio-World, USA). After 30 minutes of sitting, 50 µL of methanol had been added to eliminate the unused dye. Washed by tab water and left to dry overnight. Using a Fluorometer (BMG LABTECH, Germany), the optical density (OD) was measured at 492 nm [21].

Comet Assay

According to [25], modification, according to (ICCMGR) has been done [26]. Firstly, the suspension of cells in phosphate buffer saline (PBS) with 0.75% low melting point agarose

(USBiological, USA) was made. After being cast onto microscope slides, 0.5% normal melting agarose (USBiological, USA) was applied. After that, the cells were lysed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, and 10 mM Tris, pH 10) for one hour at 4°C. Following lysis, DNA was left to unwind in an electrophoretic solution (pH>13, 300 mM NaOH, 1 mM EDTA) for 40 minutes. 30 minutes of electrophoresis at 4°C and 0.73 V/cm (30mA) of electric field strength were performed. After neutralizing the slides with neutralization buffer (0.4 M Tris, pH 7.5), 100 µL of ethidium bromides (2 ug/mL) (Sigma Chemicals, USA) were added for staining, and coverslips were placed on top. A 200x magnification fluorescence microscope (Micros MCX 500, Austria) was used to examine the slides. It was attached to a CCD camera (Infinity Capturer, Micros, Austria) that was connected to a computer-based image processing system. Images were analyzed using CASP software (casp_1.2.3b1.exe). Each sample consisted of fifty randomly chosen photos, and the mean comet tail DNA of an MCF 7 cell was used to calculate endogenous DNA damage. The following measurements were made and noted. The product of the length of the tail and the percentage of total DNA in the tail is known as the olive tail moment. The comet tail length, which indicates the smallest observable size of moving DNA, and the intensity of DNA in the tail, which indicates the amount of relaxed or fragmented bits, are both included in the tail moment measurement. The tail length, which is used to calculate the amount of DNA damage, is defined as the distance that DNA migrates from the nuclear core center.

Statistics

Samples were run in triplicate for the cytotoxicity assay and comet test, and for publication, independent replicates were also run in triplicate. The significance between samples was determined using the f-test, where $p < 0.05$ was considered significant. The following measurements' values were shown as mean ±SD: head% DNA tail length, tail% DNA, tail moment, and olive tail moment.

Results

- **No cytotoxic effect of probiotic bacteria on MCF 7 cells**

Figures 1, 2, and **3** demonstrate the cytotoxicity of the probiotics bacteria on the breast cancer cell line according to the cell viability test. T1 and T2 treatments showed no significant cytotoxic effect on breast cancer cells. No identified probiotic treatment exposure time was found to be able to block at least 50% of the total number of cells.

- **Probiotics bacteria induced DNA repair of MCF 7 cells**

In the current study, the DNA changes that occurred in the MCF 7 cells following 48 h and 72 h exposure to 10^5 CFU/mL of live probiotics bacterial strains either *L. planetarium* 299v (T1) or mixture of strains including *L. casei, L. rhamnosus, L. acidophilus, L. plantarum, S. thermophilus, B. breve, B. longum & B. bifidum* (T2) were studied by comet assay. Images were analyzed using CASP software (casp_1.2.3b1.exe). By calculating the percentage of DNA contained in the tail in a comet experiment, the degree of damage can be quantified. As shown in **Table (1)**, there were no significant differences found in L head, L comet, and OTM among control, 48 h, and 72 h treated cells by T1. Meanwhile, MCF 7 cells exposed to T1 for 48 h and 72 h showed a significant increase in L Tail ($P \leq 0.005$), Head DNA ($P \leq 0.001$), and Tail DNA ($P \leq 0.001$) compared with the control. Furthermore, the Curve of images analysis demonstrated the potential role for T1 in breast cancer treatment as showing a green small tail and large red head compared with the control **(Table 1, Figure 4)**.

The results in **Table 2** showed that the TM and OTM did not significantly alter between control, 48 h, and 72 h treated cells by T2. In contrast to the control, MCF7 cells treated to T2 for 48 h and 72 h demonstrated a substantial increase in L Head ($P \leq 0.5$), L Tail ($P \leq 0.5$), L comet ($P \leq 0.5$), Head DNA ($P \leq 0.5$), and Tail DNA ($P \leq 0.5$) **(Table 2)**. Additionally, the examination of the curve of images showed that T2 may play a role in the treatment of breast cancer, as seen in **Figure 5**, green small tail and red large head compared with control which presented green tail equal to red head.

Table 1. Comparison of T1 effect on breast cancer cell line depending on exposure time. Parameters that are utilized to determine the comet assay are presented as the mean ±SE in pg/mL. The data presented is a representative experiment that compares the untreated (control) MCF 7 cell line to T1 48 h and T1 72 h. The experiment was done in triplicate n = 3. NS = Not significant, * = Significant and ** = Highly significant.

Groups **Parameters**	**Control**	**T1 48 h**	**T1 72 h**	**P value**
LHead Mean ±SD	67.7 ± 27.2	69 ± 76.3	186.3±85.4	NS
LTail Mean ±SD	70.3 ± 74.5	3.3 ± 0.6	20±24.2	P≤0.005*
L comet Mean ±SD	138 ± 5.7	72.3 ± 76.9	193±70.7	NS
Head DNA Mean ±SD	38.2 ± 5.7	99.9 ± 0.2	91.9±31.6	P≤0.001**
Tail DNA Mean ±SD	61.8 ± 76.3	0.08 ± 2.6	8.1±22.3	P≤0.001**
TM Mean ±SD	43.8 ± 96.2	1.5 ± 25.2	4.7±28.6	P≤0.5
OTM Mean ±SD	25 ± 14.1	6.6 ± 0.01	4.4±56.4	NS

Table 2. Comparison of T2 effect on breast cancer cell line depending on exposure time. Parameters that are utilized to determine the comet assay are presented as the mean ±SE in pg/mL. The data presented is a representative experiment that compares the untreated (control) MCF 7 cell line to T2 48 h and T2 72 h. The experiment was done in triplicate n = 3. NS = None significant

Groups **Parameters**	**Control**	**T2 48 h**	**T2 72 h**	**P value**
LHead Mean ±SD	67.5 ± 26.1	75 ± 43.2	195.3 ± 116	P≤0.5
LTail Mean ±SD	71.1 ± 73.4	18.5 ± 27.0	45.7 ± 44.2	P≤0.5
L comet Mean ±SD	137 ± 5.6	93.5 ± 33.6	236.5 ± 100.3	P≤0.5
Head DNA Mean ±SD	37.2 ± 5.8	78.4 ± 33.6	80.0 ± 34.5	P≤0.5
Tail DNA Mean ±SD	62.3 ± 75.3	21.5 ± 42.5	20 ± 30.7	P≤0.5
TM Mean ±SD	44.1 ± 95.1	12.6 ± 25.2	20.6 ± 28.6	NS
OTM Mean ±SD	24 ± 13.5	6.6 ± 13	11.3 ± 17.2	NS

Figure 1. Cytotoxic activity of T1 and T2 on MCF 7 cell line, which had incubated for 24 h and demonstrated no significant impact of exposure of MCF 7 cells into either T1 or T2 probiotics bacteria.

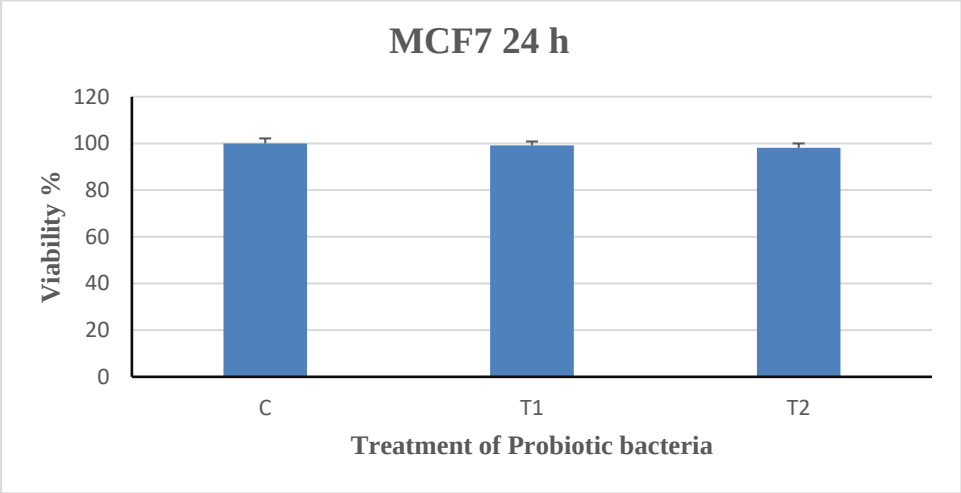


Figure 2. Cytotoxic activity of T1 and T2 on the MCF 7 cell line, which was incubated for 48 h, showed that exposure to either T1 or T2 probiotics bacteria had no important effect on MCF 7 cells.

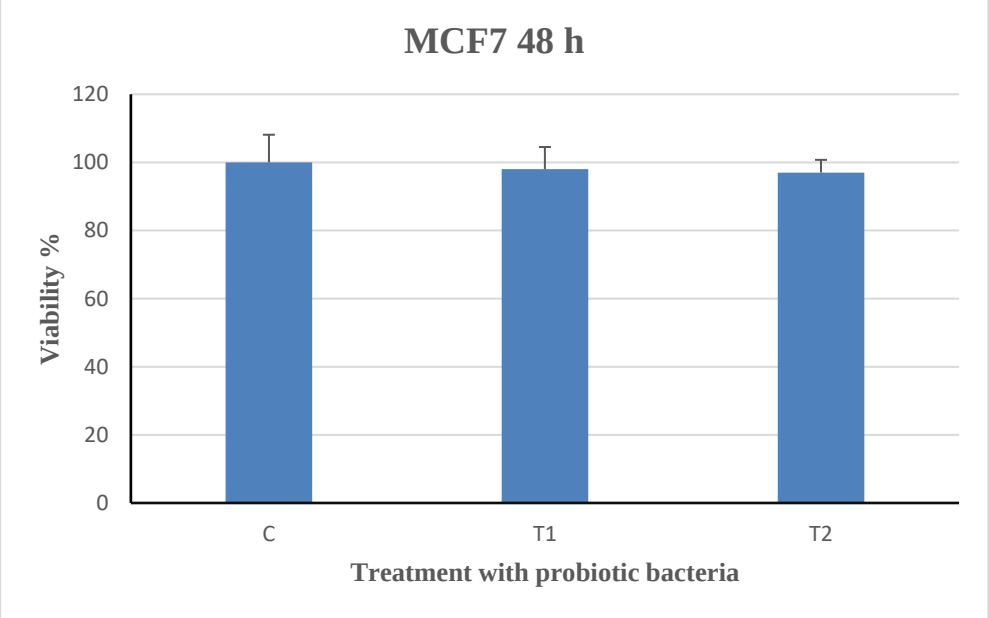


Figure 3. The MCF 7 cell line, which had been incubated for 72 h, showed no significant effects from exposure to either T1 or T2 probiotics bacteria; the cytotoxic activity of both T1 and T2 confirmed this.

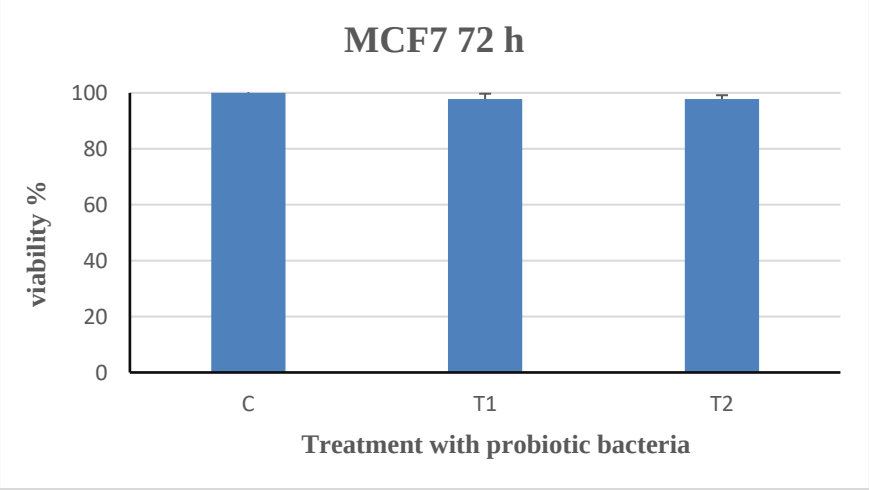


Figure 4. An illustration of the DNA distribution in the comet with a head-tail relationship is provided by the image analysis program of the T1. **A**, **C,** and **E** image analysis output of CASP program represented MCF 7 control, MCF 7 cells treated with T1 48 h exposure time and MCF 7 cells treated with T1 72 h exposure time, respectively. **B**, **D,** and **F** illustrate the curve of image analysis. The green small tail and red large head have seen in D and F compared to control B.

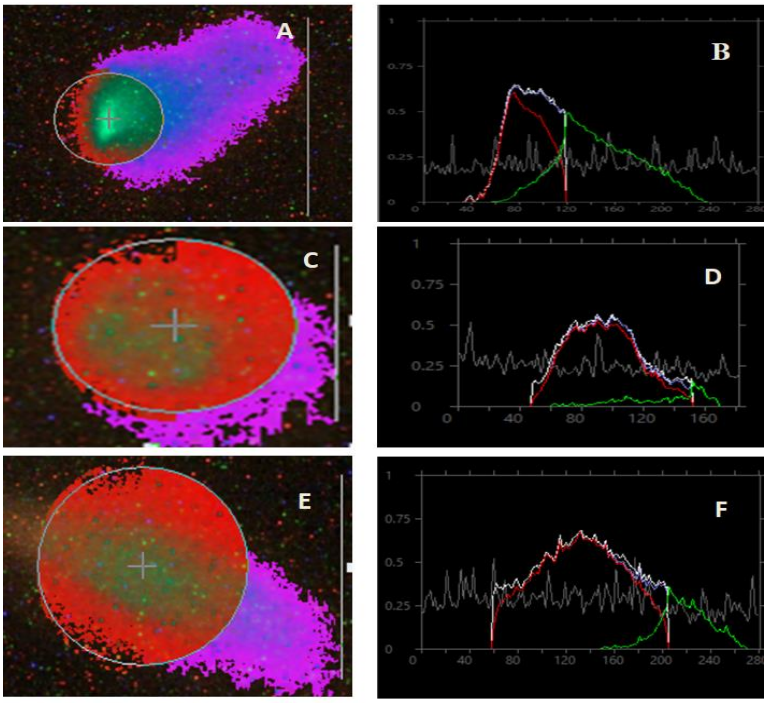
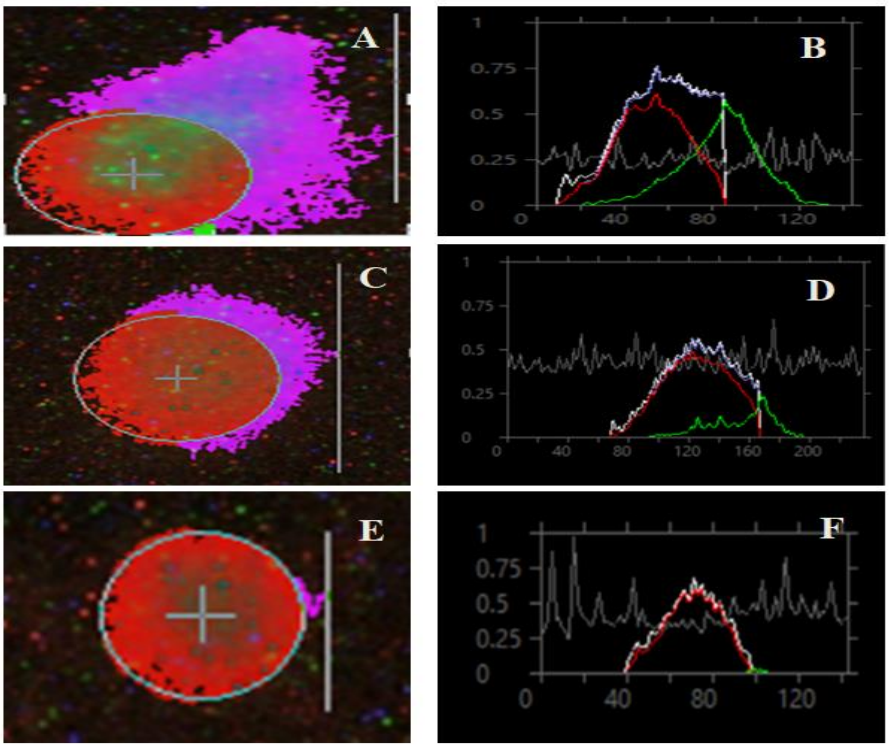


Figure 5. The image analysis software of the T2 provides an illustration of the head-tail connection of the DNA distribution in the comet. The CASP program's image analysis outputs **A**, **C**, and **E** showed MCF-7 cells control, MCF-7 cells exposed for 48 hours, and MCF-7 cells exposed for 72 hours, respectively. The analysis image curve is depicted in **B**, **D**, and **F**. When comparing D and F to control B, the green small tail and large red head are visible.



Discussion

Cancer can affect any organ in the body and is one of the leading causes of mortality globally. According to the World Health Organization (WHO), 2.26 million individuals with breast cancer were detected in 2020. The significant side effects of conventional treatments lower the quality of life or lead to drug resistance, which decreases patient survival. According to earlier findings, probiotic microorganisms may be a good therapeutic option [27].

In the current study, both treatments T1 and T2 demonstrate no significant cytotoxic effect on MCF 7 cells. In the exposure times of 24, 48, and 72 hours, there was no significant difference between the T1-treated cells and the untreated cell line, which was used as the control group. Also, the T2-treated cells and the untreated cell line utilized as the control group did not significantly vary during the exposure times of 24, 48, and 72 hours. In cancer, the cytotoxic effect depends on exposure time and dosage of probiotics bacteria. While *Bacillus coagulans* supernatants had fewer cytotoxic effects on normal HFF cells, they demonstrated concentration- and time-dependent inhibitory activity on MCF 7 cells. The stimulation of apoptosis in the cancer cells was supported by the flow cytometry data, as well as by the increases in the expression of the genes for *bax*, *caspase 3*, and *caspase 9*, as well as the decrease in the anti-apoptotic gene of *bcl2*. *Bacillus coagulans* supernatants demonstrated to possess a cytotoxic impact on breast cancer cells. This suggests that the bacteria may be a viable option for a novel therapeutic approach with fewer side effects, though more research is obviously needed [28]. Probiotics bacteria might be crucial in preventing DNA damage caused by oxidative stress, antioxidant enzymes that combat oxidative stress in lactic acid bacteria include glutathione S-transferase, glutathione reductase, glutathione peroxidase, feruloyl esterase, and catalase [29, 30]. According to reports, several *Lactobacilli* and *Bifidobacteria* in this situation have been shown to enhance the activity of antioxidant enzymes or modify signaling, protecting against oxidative stress [31].

A new preventive for preventing colorectal cancer in mice is *S. thermophilus*; at minimum, the secretion of b-galactosidase by *S. thermophilus* mediates the tumor-suppressive activity. Secreted by *S. thermophilus*, β-Galactosidase enhanced apoptosis, decreased colony formation, induced cell cycle arrest, and slowed the growth of CRC xenografts in cultured cells. The β-galactosidase-deficient mutant *S. thermophilus* failed to have tumor-suppressive properties. Through β-galactosidase, *S. thermophilus* also enhanced the abundance of recognized probiotics in the stomach, such as *Bifidobacterium* and *Lactobacillus*. The anticancer effects of *S. thermophilus* were predominantly mediated by β-galactosidase-dependent galactose synthesis, which disrupted energy balance to activate oxidative phosphorylation and downregulate the Hippo pathway kinases [32]. *Lactococcus* and *Streptococcus* are more common in healthy breast tissue because these bacteria were believed to have anticancer properties. To test the bacteria's capacity to cause DNA damage, human breast cancer cells were cultured with bacteria from malignant females; the growth of these malignant cells revealed a concerning circumstance of double-stranded DNA breakage brought on by three distinct bacterial strains: *Enterobacteriaceae*, *E. coli*, and *Staphylococcus* [33].

Probiotics bacteria have been shown in numerous *in vitro* and *in vivo* investigations to be useful in inhibiting the development of cancer cells [34,35]. With strains like *L. rhamnosus* demonstrated to inhibit proliferation and increase apoptosis in colon cancer cell lines, there is a good chance that these bacteria directly interact with the colonic epithelium, providing significant protective advantages against colon cancer [36,37]. The findings showed by Dehghani *et al*. explained that HT-29 cell proliferative activity was reduced, and cytotoxic effects were enhanced with increasing *L. rhamnosus* supernatant concentration and duration of treatment. Furthermore, at a dosage of 30 mg/mL, 99% inhibition was found in HT-29 cancer cells after 72 hours; after 24, 48, and 72 hours, the bacterial supernatant's IC50 values were 1.95, 0.25, and 0.053 mg/mL, respectively, the IC50 dropped with an increase in treatment duration [38].

As demonstrated in SKBR-3 cell lines, *Bifidobacterium* sp. also combats cancer indirectly by metabolizing lapachol and producing cytotoxic substances against BC. This bacterium functions effectively in combination with Bacteroides [39, 40]. The combination of the two microbiota has anti-breast cancer qualities since it inhibits BC cell angiogenesis, proliferation, and apoptosis [41]. These two bacteria together also enhance interferon

γ release, which causes tumor cells to lyse [37]. In the past few years, several publications have documented the application of commensal gut bacteria, such as *Bifidobacterium*, against sarcoma in *vivo* mouse xenograft models and tumors of the head, neck, and breast [42,43].

In the current study, the comet assay was utilized to evaluate genetic changes for probiotics bacteria on breast cancer cells. The results of the current comet study showed that in the control group, comets appeared with small heads and extremely big tails, signifying the big damaged fragments of DNA. The MCF 7 cells showed a substantial increase in L Tail (P≤0.005), Head DNA (P≤0.001), and Tail DNA (P≤0.001) when treated with *L. planetarium* 299v (T1) for 48 and 72 hours compared to the control. An increase in L Head (P≤0.5), L Tail (P≤0.5), L comet (P≤0.5), Head DNA (P≤0.5), and Tail DNA (P≤0.5) was also shown when the MCF 7 cells were treated with a mixture of strains, including *L. casei, L. rhamnosus, L. acidophilus, L. plantarum, S. thermophilus, B. breve, B. longum,* and *B. bifidum* (T2) for 48 and 72 hours. When *lactobacillus spp.* was added following bile damage, esophageal cells' NFκB-associated inflammation was decreased, and bile-induced DNA damage was repaired more quickly via recruiting pH2AX/RAD51. Lactobacilli have anti-genotoxic and anti-inflammatory properties, which makes them very interesting for preventing Barrett's esophagus and esophageal cancer in gastroesophageal reflux disease GERD patients [44]. DNA double strand breaks are repaired via homologous recombination (HR) or canonical non-homologous end joining [45,46]. While double strand break repair by canonical non-homologous end joining takes place throughout the cell cycle, but the HR pathway only functions during the S and G2 phases because it necessitates extensive DNA-end processing and a homologous DNA sequence from the sister chromatids to serve as a template for DNA-synthesis-dependent repair; as a result, it is regarded as being very accurate [47,48]. Both the preservation of genomic stability and the survival of cells depend on the RAD51 recombinase1. The primary function of RAD51 is to function as the key catalyst of homologous recombination (HR), which allows double-stranded DNA breaks (DSBs) to be repaired error-free between the S and G2 stages of the cell cycle [44,49]. Probiotics bacteria antigenotoxic activity has been extensively studied in relation to food and environmental genotoxic contaminants, including N-nitrosamines, aflatoxins (AFs), 2-nitrofluorene, 4-nitroquinoline 1-oxide (4-NQO), and polycyclic aromatic hydrocarbons (PAHs) [50-52]. Another study used food mutagen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), to induce DNA damage in mice; before food mutagen was administered, *L. rhamnosus* IMC501 suspensions have been administered orally to the mice for ten days. The comet assay, which was utilized to quantify the degree of DNA damage in colon and liver cells, explained the decrease in Tail Length and suggested that the *L. rhamnosus* IMC501 can work as an antimutagen food component when used as a dietary supplement [53]. Probiotics and gut microbiota will probably play a significant role in cancer prevention and treatment in the next years [54].

Conclusion

Probiotics bacteria showed efficacy in repairing DNA damage, which is evident from the small tail curve, while the head curve was large in the image analysis. Therefore, these results reflect the therapeutic potential of probiotics in treating breast cancer.

Recommendations

The results of the study gave insight in to the role of probiotic bacteria in the treatment of breast cancer. Further study on mice will support the results. Additionally, more investigations on DNA repair signaling pathways could provide a new tool to treat breast cancer.

Limitation

The current study was limited by the inability to utilize molecular techniques such as gene and protein expression due to resource constraints.

Abbreviations

T1	Treatment 1
T2	Treatment 2
LA	Lactic acid
NK	Natural killer
ROS	Reactive oxygen species
ProLBS	*Lactobacillus* with *Bifidobacterium* plus *Streptococcus*
FOS	fructooligosaccharides
MRS	De Man–Rogosa–Sharpe
ICCMGR	Iraqi Centre for Cancer and Medical Genetic Research
(RPMI) 1640	Roswell Park Memorial Institute 1640

OD	Optical density
WHO	World Health Organization
GERD	Gastroesophageal reflux disease
HR	Homologous recombination
AFs	Aflatoxins
4-NQO	4-nitroquinoline 1-oxide

Acknowledgments

The authors would like to thank the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR), AL-Mustansiriyah University (Baghdad, Iraq).

Author contribution:

Each author contributed significantly to the article's conceptualization and design, data collection, analysis, and interpretation; they also participated in its drafting or critical revision for key intellectual elements and agreed to submit it to the current journal.

Funding:

This research received no external funding.

Conflicts of Interest:

The authors declare no conflicts of interest.

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