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Apoptotic Effects on HepG2 Cells and Antifungal Activity Against *Candida* spp. of *Lactobacillus paracasei* Postbiotics

Lactobacillus paracasei Postbiyotiklerinin HepG2 Hücrelerinde Apoptotik Etkisi ve *Candida* spp.'ye Karşı Antifungal Aktivitesi

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ABSTRACT

Aim: This study aimed to evaluate the antiproliferative effects of postbiotics derived from *Lactobacillus paracasei subsp. paracasei* (*L. paracasei*) on the HepG2 cell line and their antifungal activity against *Candida* species.

Materials and Methods: Cell-free supernatants (CFS) were obtained from *L. paracasei* isolates, and their cytotoxic effects on HepG2 cell lines were assessed using the MTT assay. Fluorescent staining and cell cycle analyses were performed on the cells at the determined inhibitory concentration 50 (IC_{50}). The antifungal activities of the postbiotics were evaluated by determining the minimum IC_{50} and minimum fungicidal concentration values on *Candida albicans* (*C. albicans*) ATCC 90028 and *Candida glabrata* (*C. glabrata*) ATCC 2950 strains.

Results: Live and heat-inactivated *L. paracasei* CFS were found to exhibit significant cytotoxic effects on HepG2 cells. Inactivated CFS produced stronger antiproliferative effect with lower IC_{50} value (live: 20.13%; inactive: 10.81% ± standard deviation). Acridine orange/propidium iodide staining revealed an increase in apoptotic cells, while cell cycle analysis revealed a significant increase in the Sub-G phase (control 0.67%; live CFS 6.47%; inactivated CFS 12.67%). In antifungal tests, both CFS strains were found to be effective against *C. albicans and C. glabrata*. While inactivated CFS (6.25%) was more potent in *C. albicans*, live CFS (12.5%) was found to be more effective in *C. glabrata*.

Conclusion: This study demonstrates that *L. paracasei* CFSs possess anticancer and antifungal effects. The fact that these effects vary depending on the CFS form and target yeast species suggests that the postbiotic response is biologically driven and specific. Elucidating the active components of CFS will significantly contribute to the development of new postbiotic-based treatment strategies.

Keywords: *L. paracasei*, HepG2, *Candida*, anticancer, antifungal

ÖZ

Amaç: Bu çalışma, *Lactobacillus paracasei subsp. paracasei* (*L. paracasei*) elde edilen postbiyotiklerin HepG2 hücre hattı üzerindeki antiproliferatif etkilerini ve *Candida* türlerine karşı antifungal aktivitelerini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: *L. paracasei* izolatlarından hücresiz süpernatantlar (CFS) elde edilmiş ve HepG2 hücre hatları üzerindeki sitotoksik etkileri MTT testi kullanılarak değerlendirilmiştir. Belirlenen inhibitör konsantrasyon 50 (IC_{50}) hücreler üzerinde floresan boyama ve hücre döngüsü analizleri gerçekleştirilmiştir. Postbiyotiklerin antifungal aktiviteleri, *Candida albicans* (*C. albicans*) ATCC 90028 ve *Candida glabrata* (*C. glabrata*) ATCC 2950 suşları üzerinde minimum IC_{50} ve minimum fungisidal konsantrasyon değerleri belirlenerek değerlendirilmiştir.

Bulgular: Canlı ve ısıyla inaktif edilmiş *L. paracasei* CFS'lerinin HepG2 hücreleri üzerinde anlamlı sitotoksik etkiler gösterdiği bulunmuştur. İnaktif edilmiş CFS, daha düşük IC_{50} değeri ile daha güçlü antiproliferatif etki oluşturmuştur (canlı: %20,13; inaktif: %10,81 standart sapma). Akridin oranj/propidyum iyodür boyaması apoptotik hücrelerde artış olduğunu gösterirken, hücre döngüsü analizi Sub-G fazında anlamlı bir artış olduğunu

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ortaya koymuştur (kontrol %0,67; canlı CFS %6,47; inaktif CFS %12,67). Antifungal testlerde her iki CFS'nin *de C. albicans ve C. glabrata'*ya karşı etkili olduğu belirlenmiştir. İnaktif CFS (%6,25) *C. albicans* üzerinde daha güçlü bulunurken, canlı CFS'nin (%12,5) *C. glabrata* üzerinde daha etkili olduğu saptanmıştır.

Sonuç: Bu çalışma, *L. paracasei* CFS'lerinin antikanser ve antifungal etkilere sahip olduğunu göstermektedir. Bu etkilerin CFS formuna ve hedef maya türüne bağlı olarak değişmesi, postbiyotik yanıtın biyolojik olarak yönlendirildiğini ve özgül olduğunu düşündürmektedir. CFS'nin aktif bileşenlerinin aydınlatılması, yeni postbiyotik temelli tedavi stratejilerinin geliştirilmesine önemli katkılar sağlayacaktır.

Anahtar Kelimeler: *L. paracasei,* HepG2, *Candida*, antikanser, antifungal

INTRODUCTION

Cancer is a serious disease that develops when cells multiply uncontrollably and spread to different parts of the body, causing high mortality rates worldwide[1]. Cancer, which has many different subtypes such as breast, liver, skin, and prostate cancer, continues to be a significant public health problem as it causes one in every six deaths today[2]. Although treatment approaches such as radiotherapy, chemotherapy, and immunotherapy are widely used among treatment options, the wide side effect profile of chemotherapeutic agents, the development of drug resistance, and the risk of relapse constitute important limitations in treatment[3]. Liver cancer is among the most common malignancies, with hundreds of thousands of new cases worldwide each year. Hepatocellular carcinoma (HCC), which constitutes the majority of primary liver cancers, has a high mortality rate due to its aggressive course and lack of early symptoms[4]. Challenges in the diagnosis and treatment of HCC include the lack of specific biomarkers and the limited efficacy of current treatment options. HepG2 cell line is one of the widely used models in liver cancer research due to its high proliferation capacity and ease of culturing[5,6].

Probiotics are defined as live microorganisms that provide benefits to the host health when consumed in adequate amounts. *Lactobacillus* and other lactic acid bacteria species are one of the most commonly used probiotic groups due to their microbiota-supporting properties[7,8]. Postbiotics are metabolites or cell-free supernatants (CFS) produced by probiotics that do not contain living cells and are considered a safer alternative in immunosuppressed individuals[9,10]. Although the exact mechanisms of these components are not yet clear, they are thought to support host health. Studies have indicated that some *Lactobacillus* strains suppress tumor development through antiproliferative effects, apoptosis induction, and anti-inflammatory mechanisms[11,12]. Apoptosis is a programmed cell death mechanism involved in maintaining tissue homeostasis. Cancer cells generally suppress apoptosis and continue uncontrolled proliferation[13]. Therefore, reactivation of apoptosis is considered an important target in cancer treatment. Postbiotic components are reported to promote cell death by triggering apoptosis in cancer cells through their cytotoxic properties[14-16]. In addition to this therapeutic potential, postbiotics are also considered important biological agents in combating the increasing number of fungal infections in recent years. Opportunistic fungal pathogens, especially *Candida albicans* (*C. albicans*) and *Aspergillus fumigatus*, cause life-threatening infections in conditions where the immune system is weakened, such as AIDS or immunosuppression after organ transplantation, and the incidence of these infections is increasing. Although the innate immune response plays a critical role in the early stages of fungal infections, the emergence of resistant strains limits the effectiveness of current antifungal agents[17]. In recent years, the antifungal activities of *Lactobacillus paracasei* (*L. paracasei*) strains have attracted attention. Probiotic metabolites and postbiotic compounds from *L. paracasei* have been shown to inhibit biofilm formation, especially against *Candida* species, suppress hyphal growth, and disrupt fungal cell wall integrity by lowering the pH of the medium[18-20]. It has also been reported that postbiotics obtained from *L. paracasei* have an antifungal effect by causing structural damage to the *C. albicans* cell wall[21,22]. In this study, it was aimed to investigate the antiproliferative effects as well as antifungal activities of CFSs from *L. paracasei subsp. paracasei* (*L. paracasei*) on HepG2 cell line.

MATERIALS AND METHODS

This study was reviewed and approved by the Tekirdağ Namık Kemal University, University Non-Interventional Clinical Research Ethics Committee (approval number: 2025.58.03.16, date: 25.03.2025).

Bacterial Culture and Inactivation

The *L. paracasei* isolate was incubated in de Man-Rogosa-Sharpe (MRS Broth, Biolife, Milan, Italy) broth medium at 37 °C in an anaerobic environment for 24-48 hours. Then, single colony culture was performed on MRS (Agar, Biolife, Milano, Italy) solid medium under the same conditions for 24 hours. A single colony obtained from the purified *L. paracasei* isolate was seeded in MRS broth and incubated. After growth was observed, the culture was inactivated in a water bath at 100 °C for 30 minutes. After the incubation period was completed, the cultures were centrifuged at 4000 rpm for 15 minutes to obtain the supernatant[12]. This liquid phase was sterilized by passing through a 0.22 µm membrane filter (Isolab, Eschau, Germany) and stored at -80 °C.

Cell Culture

HepG2 cells used in this study were cultured in Dulbecco's Modified Eagle Medium (DMEM, Euroclone, Pero (MI), Italy] containing 10% fetal bovine serum (FBS) (FBS, Capricorn Scientific, South America) and 1% penicillin-streptomycin at 37 °C, 5% CO_2, and under humidified conditions.

MTT Viability Test

Passaging was performed when the cell density covered approximately 80-90% of the T75 (Tissue Culture Flask 75, TPP, Switzerland) culture flask surface and cell viability was assessed with trypan-blue (Gibco, New York, USA). Then, cells were added to 96-well flat-bottom cell culture plates (Tissue Culture Test Plate 96F, TPP, Switzerland) containing 1×10^4 cells per well[23,24]. Plates were incubated for 24 hours at 37 °C, 5% CO_2 and 95% relative humidity. Cell adherence to the surface was observed using an inverted microscope. Afterwards, serial dilutions of live and inactivated CFSs (0.39-100%) were applied to the cells and incubated for 24 hours. For the MTT assay, 10 µL of a 5 mg/mL solution of reagent (Merck, Darmstadt, Germany) was prepared and added to each well, and the plates were incubated at 37 °C for 4 hours. The medium formed after incubation was removed and 100 µl DMSO (Dimethyl Sulfoxide, Biofroxx, Germany) was added to each well and gently shaken for 5 minutes. Each test was performed in triplicate, and absorbance values were measured at 570 nm wavelength using a microplate reader (BioTek-800-TS absorbance reader, Agilent, Santa Clara, United States). Cell viability rates were calculated using the obtained data. Inhibitory concentration 50 (IC_{50}) values were determined using GraphPad Prism 8.0 software[25].

Cell Cycle Analysis

To investigate the cell cycle, 6-well cell plates were seeded with 5×10^5 cells per well. Cells were treated with CFSs at the determined IC_{50} concentrations for 24 hour. At the end of the incubation period, cells were lifted with 0.25% trypsin (ThermoFisher, Paisley, United Kingdom) and centrifuged at 1200 rpm for 3 minutes. After washing, the supernatant was removed and the pellet was resuspended in 1 mL Dulbecco's Phosphate-Buffered Saline (D-PBS) [Euroclone, Pero (MI), Italy]. Then, 8 mL of 70% cold ethanol was added dropwise on the vortex. Following vortexing, the cells were incubated overnight at +4 °C for fixation. After incubation, the fixed cells were centrifuged and transferred to tubes suitable for flow cytometry analysis. 500 µL propidium iodide (PI) (PI, Invitrogen, United States of America) and staining buffer (1 mL D-PBS, 5 µL of 20% Triton X, 6.6 µL RNase A and 20 µL PI) were added to the cells. This mixture was incubated for 30 minutes in dark conditions[12]. In the final step, cells were analyzed by flow cytometry using a FACSCalibur (BD Biosciences) device.

(AO/PI) Fluorescent Staining

For the evaluation of live and dead cells, a staining solution was prepared by carefully mixing 10 g sodium-ethylenediaminetetraacetic acid , 4 mg PI, 50 mL FBS and 4 mg acridine orange (AO) (dissolved in 2 mL 99% ethanol). A homogeneous mixture was achieved by adding sterile distilled water to a final volume of 200 mL. Cells were seeded in 96-well plates with 2×10^4 cells per well in triplicate. Plates were incubated for 24 hours at 37 °C in an environment containing 5% CO_2 to allow cells to adhere to the surface. Cells were treated with CFSs at the determined IC_{50} concentrations, and after the incubation period was completed, 10 µL of AO/PI staining mixture was added to each well and left for 5 minutes[26]. Then, apoptotic cells were examined morphologically under a fluorescence microscope (version 7.5; Genetix; Leica Microsystems).

Assessment of Antifungal Activity

Antifungal susceptibilities of yeast strains were examined using the broth microdilution method according to CLSI guidelines. *C. albicans* ATCC 90028 and *Candida glabrata* (*C. glabrata)* ATCC 2950 were used as reference strains. Serial dilutions of live and heat-inactivated *L. paracasei* CFSs were prepared and the final concentration range was adjusted to 0.39-100%. Each yeast strain was standardized to 1.5×10^3 colony forming unit/mL and 10 µL of the suspension was added to microplate wells containing different concentrations of CFS. Microbial growth was visually assessed after incubating the microplates at 35 °C for 24 hours. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined for each strain. For MFC determination, samples at the concentration were plated on sabouraud dextrose agar. The concentration at which no growth was observed was designated as MFC[27]. The antifungal effects of CFSs were compared with the reference antifungal agent Micafungin (8 µg/mL). All experiments were repeated in triplicate.

Statistical Analysis

All experiments were conducted with three independent replicates, and results are presented as the mean ± standard deviation Graphs were created using Microsoft Office 365 Excel and GraphPad Prism 8. Statistical analysis of the data was performed using ANOVA, and post-hoc Tukey test was applied for multiple comparisons. Statistical significance was determined using p-values; results with $p\leq0.05$ were considered significant and are indicated with asterisks in the graphs.

RESULTS

Comparative Cytotoxicity of Live and Heat-inactivated *L. paracasei* CFSs Against HepG2 Cells

To evaluate the cytotoxic potential of *L. paracasei* isolate on HepG2 human hepatocarcinoma cells, both live and heat-inactivated CFSs were applied in the concentration range between 0.39% and 100%. Cell viability was determined by MTT assay after 24 hour incubation period. The findings showed that both forms of CFS decreased cell viability in HepG2 cells in a dose-dependent manner. In particular, inactivated CFS was found to have a significantly stronger cytotoxic effect compared to the live form (****$p<0.0001$). IC_{50} values were calculated as 20.13±0.22% for live CFS and 10.81±0.13% for inactivated CFS (Figure 1).

Apoptosis Detection by AO/PI Dual Staining

Morphological changes in HepG2 cells treated with IC_{50} concentrations of live and heat-inactivated *L. paracasei* CFS for 24 hours were examined under a fluorescence microscope. AO/PI dual staining method was used to distinguish viable and apoptotic cells. Green fluorescent cells represent AO-stained live cells (white arrows), while orange/red fluorescent cells represent PI-stained apoptotic cells (yellow arrows). A higher number of apoptotic cells were observed in HepG2 cells treated with both live and inactivated CFS of *L. paracasei* compared to the control group (Figure 2). Furthermore, inactivated CFS was found to have a relatively greater apoptotic effect compared to live CFS.

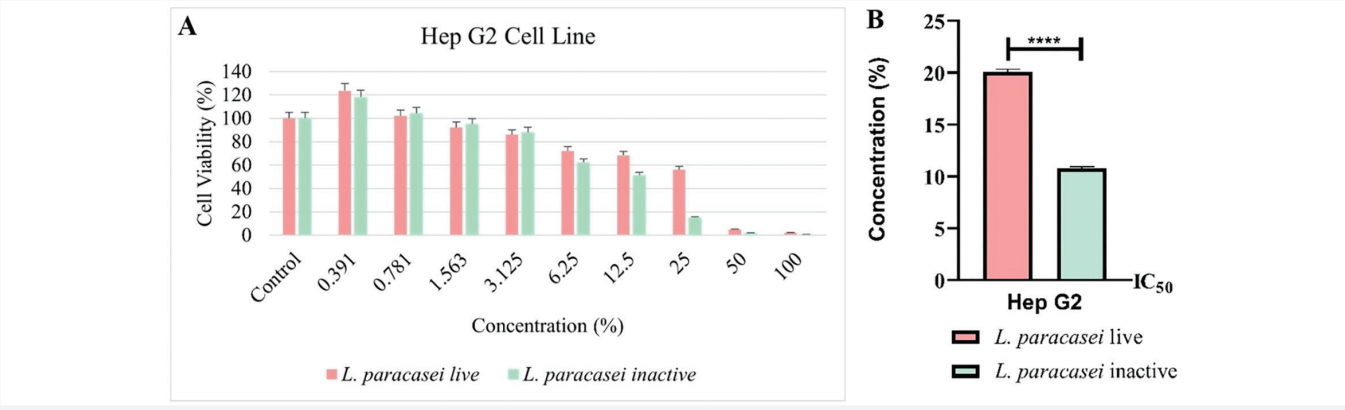


Figure 1. Percentages (A) and IC_{50} values (B) of live and inactivated *L. paracasei* CFS in HepG2 cell after 24 hours of treatment

IC_{50}: Inhibitory concentration 50, CFS: Cell-free supernatants, L. paracasei: Lactobacillus paracasei

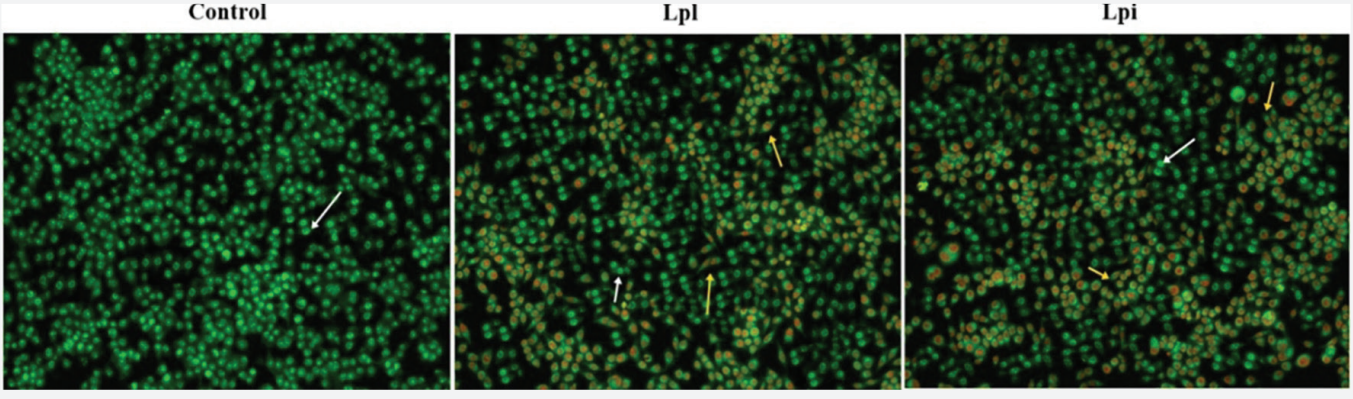


Figure 2. Fluorescence microscope images (20x) of HepG2 cells treated with live and heat-inactivated *L. paracasei* CFSs at IC_{50} concentrations for 24 hours. White arrow shows AO-stained live cells (green), while the yellow arrow indicates PI-stained apoptotic cells (orange/red)

CFSs: Cell-free supernatants, IC50: Inhibitory concentration 50, AO: Acridine orange, L. paracasei: Lactobacillus paracasei, LPL: L. paracasei live, LPI: L. paracasei inactive

Cycle Analysis in HepG2 Cells

Cell cycle analyses were performed by flow cytometry and cell distributions in Sub G, G0-G1, S and G2-M phases were evaluated (Figure 3). According to the findings, while the proportion of cells in Sub G phase was 0.67% in the control group, this proportion increased to 6.47% in cells treated with live *L. paracasei* CFS and to 12.67% in cells treated with inactive CFS. This increase indicates that both treatments caused a significant accumulation of cells in the Sub G phase. While the proportion of cells in the G0-G1 phase was 69.41% in the control group, it slightly increased to 71.49% in the live CFS treatment and decreased to 65.27% in the inactive CFS group. In contrast, the cell population in the S phase was 13.51% in the control group, but decreased to 8.68% and 8.24% after live and inactive CFS applications, respectively. Similarly, the G2-M phase decreased from 15.22% in the control group to 11.27% in the live CFS group and 9.29% in the inactivated CFS group. These findings indicate that *L. paracasei* CFS suppresses cell cycle progression and arrests a significant portion of cells in the sub G phase, inducing apoptosis. The significant increase in the sub G phase and the decrease in the G2-M phase observed with inactivated CFS suggest that this form triggers cell death mechanisms more potently than live CFS.

Antifungal Effects of Live and Inactive *L. paracasei* CFSs

In this study, the antifungal activities of live and inactivated CFS obtained from *L. paracasei* were evaluated against the clinically important strains *C. albicans* 90028 and *C. glabrata* 2950 (Table 1; Figure 4). Antifungal activity was measured by MIC and MFC values, and the results revealed that both forms of CFS exhibited significant antifungal activity on these pathogens. Against *C. albicans*, the MIC and MFC values of inactivated CFS were 6.25% and 12.5% for the live form, respectively. For *C. glabrata*, the MIC and MFC values of inactivated CFS were 25% and 12.5%

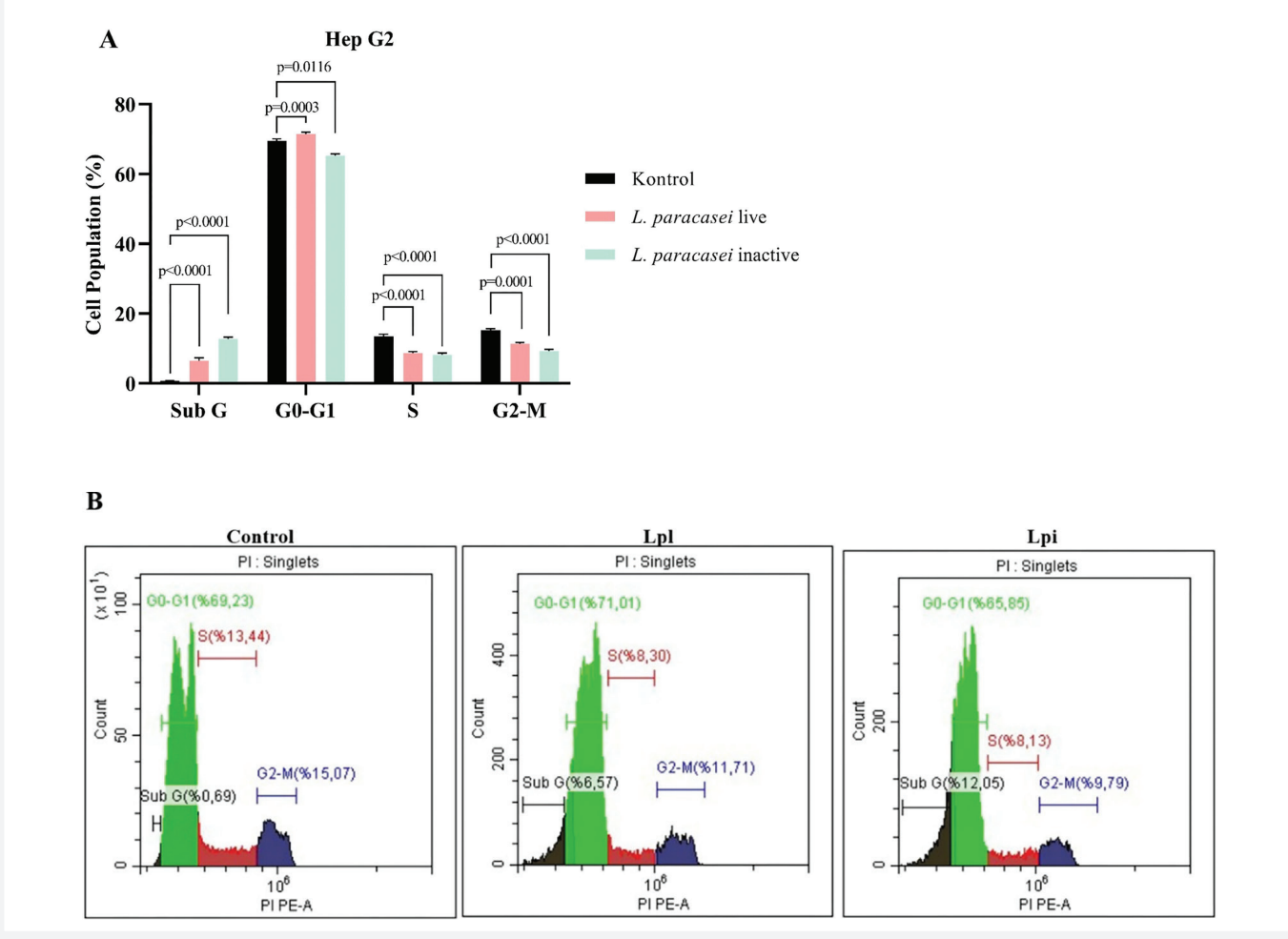


Figure 3. Cell cycle distribution of HepG2 cells treated with IC_{50} concentrations of live and inactive supernatants from *L. paracasei*, showing changes (A) Sub G, G0-G1, S and G2-M phases; (B) Flow cytometry image

IC_{50}: Inhibitory concentration 50, L. paracasei: Lactobacillus paracasei, LPL: L. paracasei live LPI: L. paracasei inactive

Table 1. MIC and MFC values (%) of *L. paracasei* live and inactivated CFSs against *Candida strains*

Fungi strains	Live		Inactive		MF	
	(%)		(%)		(µg/mL)	
	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans ATCC 90028	12.5	12.5	6.25	6.25	0.015	0.015
Candida glabrata ATCC 2950	12.5	12.5	25	25	0.015	0.03

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration, MF: Micafungin, *L. paracasei*: *Lactobacillus paracasei*

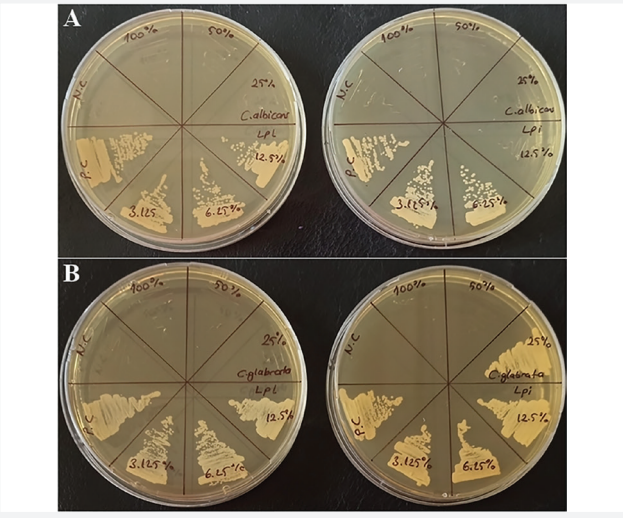


Figure 4. MFC petri dish images of live and inactive *L. paracasei* CFSs. (A) *Candida albicans* 90028. (B) *Candida glabrata* 2950

MFC: Minimum fungicidal concentration, CFSs: Cell-free supernatants, L. paracasei: Lactobacillus paracasei, NC: Negative control; PC: Positive control

for the live form. These results indicate that both forms have antifungal potential, but the level of efficacy varies depending on the *Candida* species.

DISCUSSION

Cancer continues to be one of the diseases with the highest mortality rate worldwide, and current treatment options often cause serious toxic effects on healthy tissues. Although conventional chemotherapeutic agents target tumor cells, their low selectivity leads to increased side effects during treatment. This situation increases the need for safer and more effective alternative therapeutic strategies[1,28,29]. In recent years, intensive research has been carried out on the anticancer potential of probiotic microorganisms and their CFSs. Studies have shown that CFSs derived from probiotic bacteria, such as *Lactobacillus* spp., can suppress tumor cell proliferation, induce apoptosis, and regulate the cell cycle, leading to anticancer effects[29-33]. These findings suggest that *Lactobacillus*-derived CFS may be considered as a potential anticancer agent not only in colon cancer but also in HCC, breast cancer and other types of cancer. HCC is the most common primary liver malignancy worldwide and represents a significant health problem in terms of cancer-related mortality[34]. The fact that HCC mostly develops on the background of chronic liver diseases such as cirrhosis, viral hepatitis, alcohol consumption and metabolic steatohepatitis and that early diagnosis is rare limits the treatment options in the advanced stages. However, the frequent occurrence of chemotherapy resistance in HCC makes treatment success difficult. Different studies demonstrate the anticancer potential of probiotics and postbiotics on HepG2 cells. Mubeen et al.[35] revealed that sea buckthorn and monk fruit beverage fermented with lactic acid bacteria exhibited high antioxidant capacity and significant cytotoxic activity in HepG2 cells. Similarly, it has been reported that cranberry proanthocyanidins increased mitochondrial pathway-dependent cytotoxicity and inhibited HepG2 cell proliferation in a dose- and time-dependent manner as a result of biotransformation by *Lactobacillus rhamnosus (L. rhamnosus)*[36].

Additionally, *L. rhamnosus* GG-derived extracellular vesicles have been shown to induce apoptosis and create cytotoxicity by increasing the Bax/Bcl-2 ratio[37]. Similarly, *Lactobacillus fermentum* BGHV110 postbiotics were reported to reduce acetaminophen-induced hepatotoxicity and provide cytoprotective effects by activating PINK1-dependent autophagy[38]. In addition, it has been reported that exopolysaccharides from lactic acid bacteria and Bifidobacterium have a cytotoxic effect on HepG2 cells and increase the expression of Bax, Caspase-3/8 and p53[39].

In vivo studies have also demonstrated the beneficial effects of probiotics on hepatocytes. For example, *L. paracasei* HY7207 was shown to suppress genes associated with lipogenesis and apoptosis in palmitic acid (PA)-treated HepG2 cells and to reduce inflammation, fibrosis, and hepatic steatosis in non-alcoholic fatty liver disease (NAFLD) mouse models[40]. Similarly, *Lactobacillus plantarum* (*L. plantarum*) MG4296 and *L. paracasei* MG5012 were reported to alleviate insulin resistance in PA-induced HepG2 cells and improve metabolic parameters in mice on a high-fat diet[41]. Additionally, *L. plantarum* LP158, *Lactobacillus helveticus* HY7804 and *L. paracasei* LPC226 strains were shown to suppress lipogenesis genes in PA-treated HepG2 cells, increase β-oxidation and reduce fatty liver and inflammation in NAFLD mouse models[42].

In this study, the cytotoxic effects of live and inactive CFS obtained from *L. paracasei* isolates on HepG2 cells were evaluated by MTT assay and it was determined that both CFS forms caused significant cytotoxicity in cancer cells. Analyses

using AO/PI fluorescent staining methods revealed that CFS applications significantly increased the number of cells undergoing apoptosis in HepG2 cells.

Cell cycle analyses are reported in a limited number of studies in the literature[12,43-47]. Dehghani et al.[44] showed that *L. rhamnosus* CFSs decreased IC_{50} values in human colon cancer (HT-29) cells in a dose- and time-dependent manner and arrested the cells in the G0-G1 phases of the cell cycle[44]. Similarly, Erfanian et al.[45] reported that *Lactobacillus acidophilus* (*L. acidophilus*) CFSs produced antiproliferative and anti-migration effects in HT-29 cells by arresting the cell cycle in the G1 phase and leading to a reduction in the S and G2-M phases. In another study, *L. plantarum* UL4 strain was reported to cause cell accumulation in the G0-G1 phase in breast cancer cells[43].

In two different studies, Liu et al.[46] revealed that fermented grape skin extracts stopped the cell cycle in HepG2 cells and inhibited proliferation by inducing apoptosis[47]. Erdal et al.[12] reported that *L. paracasei* live and inactive CFSs stopped apoptosis and cell cycle in the Sub G phase in glioma cells (U-87); also indicated that inactivated CFSs exhibited a more selective anticancer effect against normal human embryonic kidney (HEK293T) cells.

In this study, live and inactivated *L. paracasei* CFSs applied to HepG2 cells significantly affected cell cycle progression and induced apoptosis. Both CFS forms accumulated in the Sub G phase, while mild changes were observed in the G0-G1 phase, and a decrease in the S and G2-M phases indicated suppression of DNA synthesis and mitotic division. In particular, inactivated CFS induced apoptotic cell death more strongly than live CFS. These findings indicate that probiotic-derived CFSs have the capacity to arrest the cell cycle and induce apoptotic processes in HepG2 cells, and are consistent with the results reported in the literature. Studies in the literature demonstrate that probiotic-derived CFSs are not limited to anticancer effects but also possess potent antifungal properties. For example, one study showed that *L. acidophilus* and *L. plantarum* CFSs suppressed the growth of oral *Candida* species isolated from HIV/AIDS patients, and provided particularly significant inhibition on *Candida* krusei[48]. Similarly, it has been reported that *L. plantarum* and *Lactobacillus* coryniformis metabolites isolated from rice washing water exhibited strong antifungal activity against Aspergillus species and this activity was mainly due to organic acids and fatty acids[49].

Dube et al.[50] showed that *L. rhamnosus* cell-free extract suppressed hyphae formation, protease/phospholipase production, and drug efflux pumps *in C. albicans*, thus reducing both virulence and antifungal drug resistance. Rossoni et al.[19] reported that clinical *Lactobacillus* isolates strongly inhibited *C. albicans* biofilms and this activity was associated with the downregulation of biofilm-related genes such as ALS3, HWP1, EFG1 and CPH1. Additionally, Coman et al.[22] demonstrated that the combination of *L. rhamnosus* IMC 501, *L. paracasei* IMC 502 and SYNBIO® provided broad-spectrum inhibition against both bacterial and fungal pathogens. In another study by García-Gamboa et al.[21], it was determined that *L. paracasei* and *L. plantarum* CFSs combined with inulin-type fructans significantly reduced both growth and biofilm formation in *C. albicans*. In a study conducted on multidrug-resistant *Candida auris*, it was reported that postbiotic fractions derived from *L. paracasei* 28.4 strongly inhibited all planktonic, biofilm and persister cells and exhibited therapeutic potential by enhancing the host immune response in in vivo models[51]. Additionally, Spaggiari et al.[20] showed that CFSs from different *Lactobacillus species* significantly reduced the capacity of *Candida parapsilosis* (*C. parapsilosis*) to adhere to epithelial cells and establish infection in both monolayer and transwell models.

Erdal et al.[12] evaluated the antifungal effects of live and inactivated CFSs from *L. paracasei* on *C. albicans* 10231 and *C. parapsilosis* ATCC 22019 and reported that inactivated CFSs, in particular, showed significant fungistatic and fungicidal activity at lower concentrations. In this study, the antifungal activities of live and inactivated CFSs obtained from *L. paracasei* isolates were investigated on *C. albicans* 90028 and *C. glabrata* 2950 strains, and both forms were determined to exhibit significant activity. Activity levels were observed to vary among species, with the inactivated form exhibiting stronger antifungal activity on *C. albicans*, while the live form exhibited stronger antifungal activity on *C. glabrata*.

Study Limitations

This study has several limitations. The data obtained were evaluated only in the HepG2 cell line, which limits its generalizability to different tumor models. Furthermore, the specific bioactive compounds responsible for the observed antitumor and antifungal activity were not isolated, and mechanisms could not be analyzed at the proteomic or metabolomic level.

CONCLUSION

In this study, the bioactive effects of live and inactivated CFSs obtained from *L. paracasei* isolates on both the HepG2 liver cancer cell line and *Candida species* were comprehensively evaluated. The findings reveal that CFSs carry dual therapeutic potential in terms of both cancer biology and microbial pathogen control. The cytotoxicity, increased apoptosis, and cell cycle arrest observed in HepG2 cells suggest that inactivated CFSs are more effective than the live form. Similarly, the antifungal effects observed on *C. albicans* and *C. glabrata* indicate that live and inactivated CFSs exhibit varying levels of activity depending on the species. These findings suggest that the stable and safe structures of CFSs may offer innovative

and applicable biological strategies in both anticancer and antifungal therapies.

Ethics

Ethics Committee Approval: This study was reviewed and approved by the Tekirdağ Namık Kemal University, University Non-Interventional Clinical Research Ethics Committee (approval number: 2025.58.03.16, date: 25.03.2025).

Informed Consent: No human participants were involved; therefore, informed consent was not required.

Footnotes

Authorship Contributions

Concept: B.Y., B.E., Design: B.Y., B.E., Data Collection or Processing: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Analysis or Interpretation: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Literature Search: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Writing: B.Y., B.E.

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