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ORIGINAL ARTICLE

Lacticaseibacillus casei and *Lacticaseibacillus rhamnosus* cell-free supernatants induce apoptosis-related responses in PANC-1 human pancreatic cancer cells

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ABSTRACT

Background and Objectives: This study aimed to evaluate the anticancer potential of *Lacticaseibacillus casei* and *Lacticaseibacillus* rhamnosus cell-free supernatants (CFSs) against the PANC-1 human pancreatic cancer cell line, focusing on apoptosis, cell cycle modulation, and the expression of *BAX* and *BCL-XL* genes.

Materials and Methods: PANC-1 cancer cells and adult human dermal fibroblast (HDFa) cells were treated with various concentrations of individual or combined CFSs. Cell viability was assessed using the MTT assay. Apoptosis was evaluated through Hoechst/PI staining and flow cytometry, while cell cycle distribution was analyzed via flow cytometry. Gene expression of *BAX* and *BCL-XL* was measured by quantitative real-time PCR.

Results: At 20% (v/v), all CFS treatments significantly reduced PANC-1 cell viability while showing minimal effects on HDFa cells. Flow cytometry confirmed apoptotic rates of 39.33%, 42%, and 40.33% for *L. casei*, *L. rhamnosus*, and their combination, respectively, alongside a notable increase in S-phase cell population. Gene expression analysis showed a pro-apoptotic shift, characterized by *BAX* upregulation and *BCL-XL* downregulation.

Conclusion: CFSs from *L. casei* and *L. rhamnosus* showed anticancer effects on PANC-1 cells, inducing apoptosis, S-phase arrest, and a favorable shift in apoptosis-related gene expression. These findings highlight their potential as promising adjuvant candidates for pancreatic cancer therapy.

Keywords: *Lacticaseibacillus casei*; *Lacticaseibacillus rhamnosus*; Pancreatic cancer; *BAX*; *BCL-XL*

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INTRODUCTION

Cancer is the second leading cause of death worldwide, and there are many new ways to treat it to improve survival (1). Pancreatic cancer is considered the most aggressive cancer, with a 5-year survival rate of less than 10% (2). Pancreatic ductal adenocarcinoma (PDAC) has no distinct early symptoms or screening modalities and is usually not diagnosed until more advanced stages are reached (3). Mutations in *P53*, a pro-apoptotic gene in most cancer cells, and an imbalance between the pro- and anti-apoptotic genes; *BAX* and *BCL-XL* can lead to PANC-1. Owing to the important role of the pancreas in regulating metabolism and the difficulty of diagnosing early-stage pancreatic cancer, the search for new therapeutics is highly important (4).

Over the past few years, many studies have provided compelling evidence suggesting a potential association between pancreatic cancer and the oral and gut microbiota (3). Consequently, it is imperative to develop novel strategies for manipulating the gut microbiome with the primary objective of enhancing therapeutic outcomes for patients while minimizing any associated risks (5).

The human microbiota consists of 10–100 trillion commensal microbial cells hosted by each human, most of which are bacteria in the gut. However, the human microbiome is composed of the genes harbored by those cells (6). Research has shown that the ratio of microorganisms to human cells in the body is almost 1:1. The majority of these microorganisms reside in the colon, making the gut the body's largest repository of microorganisms (7). The gut microbiota plays a crucial role in generating bioactive peptides, such as neurotransmitters, converting secondary bile acids, producing short-chain fatty acids (SCFAs), metabolizing branched-chain amino acids, and synthesizing intestinal hormones (8). Probiotics have shown effectiveness in the treatment of various conditions, such as dermatitis, inflammation, halitosis, diarrhea, irritable bowel syndrome, hypercholesterolemia, obesity, urogenital infections, and certain types of cancers. In particular, probiotics have gained attention for their potential to regulate cancer signaling.

The anticancer effects of probiotics can be attributed to several mechanisms. First, they can induce apoptosis, a form of programmed cell death, in cancer cells. For example, probiotic-derived proteins have been shown to induce apoptosis in colorectal cancer cells by upregulating specific genes (9). Second, they inhibit mutagenic activity, reducing the risk of genetic mutations that can lead to cancer development (10). Third, probiotics can reduce the expression of oncogenes, which are genes associated with promoting cancer growth (11). Additionally, probiotics have been found to increase autophagy, a process that helps remove damaged cellular components and suppresses cancer formation (12). They can also inhibit kinases, enzymes that play a role in cancer cell growth and division (13). Moreover, probiotics have the potential to reactivate tumor suppressor genes, which are responsible for suppressing tumor growth (14). Furthermore, probiotics have been shown to play a role in preventing metastasis and the spread of cancer cells to other parts of the body (15). The anticancer effects of probiotics are attributed mainly to their metabiotic activities, including the production of bioactive compounds and the modulation of the gut microbiota. Metabiotics refer to complex mixtures of metabolic products derived from probiotic bacteria, including enzymes, secreted proteins, short-chain fatty acids, vitamins, amino acids, peptides, and organic acids. These bioactive molecules, either alone or in combination, exert functional effects on host physiology, including anticancer properties (16). Importantly, while probiotics have demonstrated promising results in preclinical and clinical studies, further research is needed to fully understand their mechanisms of action and optimize their therapeutic applications in the field of cancer treatment (8). The Cell-Free Supernatant (CFS) of lactic acid bacteria (LAB) contains both low- and high-molecular-weight compounds, collectively referred to as metabiotics. Low-molecular-weight compounds, including diacetylene, hydrogen peroxide, reuterin, organic acids, and carbon dioxide, and high-molecular-weight compounds, consisting of bacteriocins and bacteriocin-like substances, play a significant role in the biological activities and potential health-promoting properties. Understanding the composition and function of these metabiotics can provide valuable insights into their therapeutic applications and contribute to the development of novel strategies for promoting human health (17). *Lacticaseibacillus rhamnosus* GG (LGG) is a bacterium that has been extensively studied in the field of oncology. One notable mechanism involves the use of LGG as a "time-release capsule" to deliver radioprotective

lipoteichoic acid (LTA) specifically to intestinal crypts. This targeted delivery allows LGG to selectively protect normal colon cells while minimizing its impact on tumor cells during radiotherapy. This phenomenon has been observed in laboratory studies conducted in vitro and animal studies conducted in vivo. In another study, *ferrichrome*, a *siderophore* produced by *Lacticaseibacillus casei*, was identified as a tumor-suppressive molecule. It exhibited significant tumor-suppressive effects on colon cancer cells, which were comparable to or even stronger than those of currently available anticancer medications (18). Multiple studies support the use of multistrain probiotics for the prevention and treatment of various conditions, ranging from noninfectious to infectious diseases (19). Previous studies have demonstrated the anti-cancer properties of both *Lactobacillus casei* and *Lactobacillus rhamnosus* in various cancer types. For example, *L. casei* KK378 has shown significant tumor growth inhibition in mouse models of head and neck squamous cell carcinoma by activating host immune responses and increasing cytokine production such as TNF-α and IFN-γ (20). Additionally, *L. casei* and *L. rhamnosus* have been reported to induce apoptosis and reduce viability in colon cancer cell lines through mechanisms including downregulation of cyclin D1 and BCL-2, as well as cell cycle arrest at the G0/G1 phase (21, 22). These findings highlight the broad anti-cancer potential of these probiotic strains and underscore the novelty of investigating their effects specifically on pancreatic cancer cell lines such as PANC1. The current study aimed to examine the effects of *L. casei* and *L. rhamnosus* CFSs, as well as their combined effects, on the PANC-1 cell line. This study assessed various factors, including cell viability, apoptosis, the cell cycle, and *BAX* and *BCL-XL* gene expression, in vitro.

MATERIALS AND METHODS

Cell culture. The cell lines used in this study, including the human pancreas cancer cell line PANC-1 (IBRC C10156) and the normal human dermal fibroblast line HDFa (IBRC C10506), were purchased from the National Center for Genetic and Biological Resources of Iran. PANC-1 and HDFa cells were cultured in 25-cm^2 flasks in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine. The cultures were maintained in a humidified atmosphere with 5% CO_2.

***Lactobacillus* culture.** *L. rhamnosus* (IBRC-M 10711) and *L. casei* (IBRC-M 11410) as probiotics were purchased from the Iranian Biological Resource Center (IBRC). These *lactobacillus* strains were cultured in Man-Rogosa-Sharpe (MRS) broth (Difco Laboratories, Detroit, MN) at 37°C for 24 hours until reaching an optical density (OD) of 0.7-0.8 at 600 nm (OD600), corresponding to ~10^9 CFU/mL. Subsequently, the bacteria were transferred to serum-free DMEM for an additional 24-hour culture.

CFS extraction. Cell-free supernatant (CFS) was prepared following Bayoumi and Griffiths (2012) (23). Briefly, bacterial cultures were centrifuged at 6000 × g for 10 minutes at 4°C. The supernatant was filtered through a 0.22 μm sterile membrane, and the pH was adjusted to 7.2 post-filtration using 1 M NaOH. The CFS was used immediately for further experiments.

Treatment group preparation. For each test, the bacterial cell-free supernatant (CFS) was freshly prepared. A mixture of 50% L. casei and 50% *L. rhamnosus* (*L. cas /rha*) CFSs was prepared. Serial dilutions of *L. casei*, *L. rhamnosus*, and *L. cas/rha* CFSs were prepared in serum-free DMEM at concentrations of 5%, 10%, 20%, and 40% (v/v). These dilutions were freshly prepared and used for the experiments.

MTT assay. PANC-1 and HDFa cells were seeded in 96-well plates at densities of 4×10^4 and 2×10^4 cells/well, respectively. After 24 hours of pre-incubation, cells were treated with CFS dilutions for 24 hours (timeframe optimized for measurable cellular responses). The serum and cell-free DMEM served as control. Post-treatment, 20 μL MTT solution (5 mg/mL) was added, and absorbance was measured at 490 nm using an ELISA reader (Awareness PN142610) (22). The IC_{50} value was defined as the concentration of the tested treatment that reduced cell viability to approximately 50% relative to the untreated control, as determined from the MTT assay.

Differential nuclear staining (DNS) assay. Hoechst 33342 (Invitrogen, USA) and propidium iodide (MP Biomedicals, Solon, OH) were mixed in the cell culture media at final concentrations of 10 ng/ml for Hoechst and 1 μg/ml for PI and added to the wells.

Apoptosis assay. A total of 5×10^5 cells from each cell line were seeded in a 60 mm dish 24 h before treatment. Then, the cells were incubated with the IC_{50} concentration of CFS for 24 h. After incubation, the cells were stained with an Annexin-V MAB Tag kit, and apoptosis was detected by fluorescence-activated cell sorting (FACS) Caliber (Becton Dickinson, San Jose, CA). The cells population were recorded in the forward light scatter/side light scatter (FSC/SSC) dot plot, then a gate was used to select single cells form aggregates and debris. The data were analyzed by Cell Quest Pro software.

Cell cycle analysis. After each cell line in the groups was treated with the IC_{50} for 24 hours, the cells were trypsinized and resuspended in ice-cold 75% ethanol for 1 hour. The fixed cells were subsequently resuspended in a staining solution containing propidium iodide (50 µL), RNase A (100 µg/ml), and Triton X-100 (0.5%) and incubated for 20 minutes in the dark at room temperature. To remove aggregated cells, the cell suspension was filtered through 40 µm nylon mesh. A total of 5×10^5 stained cells were subsequently collected by fluorescence-activated cell sorting (FACS) (Becton Dickinson, San Jose, CA), and the data were analyzed by Modfit software.

Gene expression analysis. Total RNA was extracted from PANC-1 and HDFa cells using TRIzol reagent following the manufacturer's instructions. For cDNA synthesis, 1 µg of total RNA was used with random hexamer primers and the RevertAid™ H First Strand cDNA Synthesis Kit (Takara Bio Inc., Kusatsu, Shiga, Japan). Real-time PCR was performed using SYBR Green PCR Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan) on a Thermal Cycler Abi (Corbett, Mortlake, Australia) according to the manufacturer's protocol. The gene expression of *BAX* and *BCL-XL* was normalized to the expression level of the *B-actin* gene. All measurements were performed in triplicate using three separate samples, and data analysis was conducted using the 2^{-ddCt} method. The primer sequences used are listed in Table 1.

Statistical analysis. The effects of different treatments on the cell cycle of PANC-1 and HDFa cells were analyzed using one-way ANOVA with SPSS (Version 26.0; IBM Corp., Armonk, NY) statistical software, incorporating three independent replicates for each sample. Differences in means were assessed using the Tukey HSD test, and statistical significance was considered at the 5% level, with a p-value of less than 0.05 indicating significant differences.

RESULTS

Cell viability. The MTT assay was employed to assess the effects of cell-free supernatants (CFSs) from *L. casei*, *L. rhamnosus*, and their combination (*L. cas/rha*) on the viability of PANC-1 and HDFa cells after 24 hours of treatment. The results demonstrated a significant reduction in cell viability with an increase in the concentration of the CFSs, particularly at 10%, 20%, and 40% (v/v). Notably, this decrease in cell viability was more pronounced in the cancerous PANC-1 cells compared to the HDFa cells treated with the same concentrations of CFS and control group. The control group, which included both PANC-1 and HDFa cells, was not treated and served as a baseline for comparison. The IC_{50} values for *L. casei*, *L. rhamnosus*, and the *L. cas/rha* CFSs against PANC-1 cells were determined to be 20% (v/v), corresponding to cell viabilities of 52%, 47.33%, and 54.66%, respectively ($p \leq 0.05$) (Fig. 1).

Hoechst/Pi nuclear staining. In the present study, the inhibitory effects of *L. casei* and *L. rhamnosus* CFSs on the viability of PANC-1 cells and HDFa were investigated. The Hoechst/PI nuclear staining images provided visual evidence supporting these findings. Compared with those in the control group, the viability of PANC-1 cells (indicated by blue dots) decreased, and the number of dead cells (indicated by red dots) in the treatment groups increased (Fig. 2). On the other hand, the cell viability of the HDFAs treated with CFS

Table 1. Primers used for real-time PCR.

Gene	Primer F (5′-3′)	Primer R (5′-3′)	AT	Product size	Accession number
B-Actin	AGATGCGTTG TTACAGGAAG	TGTGTGGAC TTGGGAGAG	60	92	NM_001101.3
BAX	CGCCCTTTT CTACTTTGC	CGGAGGAAG TCCAATGTC	57	103	GENE ID:581
BCL-XL	CCCTTTCCTT CCATCCCTAC	TAGCCAGTCC AGAGGTGAG	60	120	GENE ID:5071

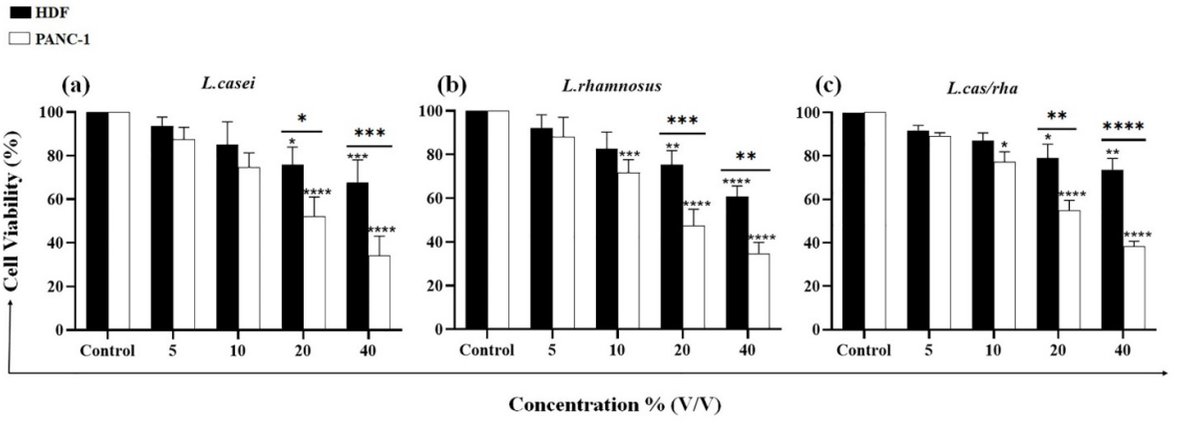

Fig. 1. Cytotoxic effects of *L. casei* and *L. rhamnosus* CFSs on PANC-1 and HDFa normal cells after 24 h. Untreated PANC-1 and HDFa normal cells were considered a control group ($p < 0.05$).

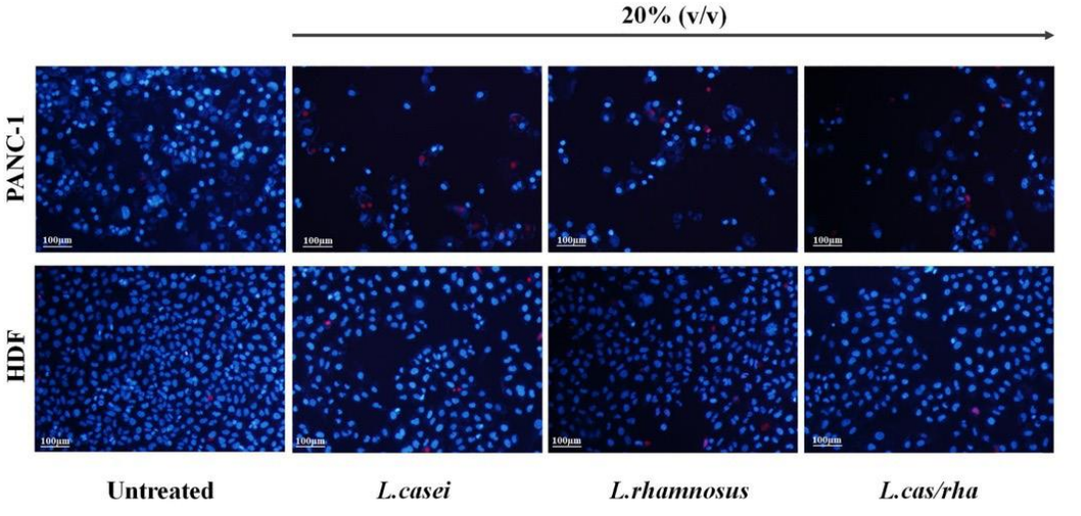

Fig. 2. Double staining of PANC-1 and HDFa cells with Hoechst and PI After 24 h control, cells treated with *L. casei*, *L. rhamnus* and *L.cas/rha* CFSs. Nuclei were stained with Hoechst 33342 (blue). Dead cells were stained using PI and shown in red (Scale bar: 100 µm).

was not significantly different from that of the control group (Fig. 2). These results further demonstrate the selective inhibitory effects of the CFS on PANC-1 cells while sparing HDFa cells.

Apoptosis induction. Apoptosis analysis after 24 hours of treatment revealed significant induction of apoptosis in PANC-1 cells treated with *L. casei*, *L. rhamnosus*, and *L. cas/rha* CFSs (39.33%, 42%, and 40.33%, respectively), compared with untreated cells with 10% apoptosis ($p \leq 0.05$, Fig. 3). However, there were no significant effects of CFSs on HDFa cells ($p \geq 0.05$, Fig. 3). The data showed that the effects of the CFSs were comparable when applied individually or in combination (Fig. 3).

Cell cycle assay. Cell cycle analysis was performed after 24 hours to evaluate the effect of CFS on the cell cycle of PANC-1 and HDFa cells (Fig. 4). The results revealed that treatment with *L. casei* and *L. rhamnosus* individually increased the proportion of PANC-1 cells in the S phase to approximately 35% (Figs. 4a and b), compared to 18% in control cells, indicating a significant accumulation of treated cells in this phase. However, the combined treatment group showed a lower S-phase percentage of 24.6% (Fig. 4c). This suggests a possible slowdown in DNA synthesis ($p \leq 0.05$, Figs. 4a and b). In contrast, the cell cycle analysis of HDFa cells treated with CFS revealed no significant increase in the proportion of cells in the S phase ($p \geq 0.05$, Figs. 4a-c).

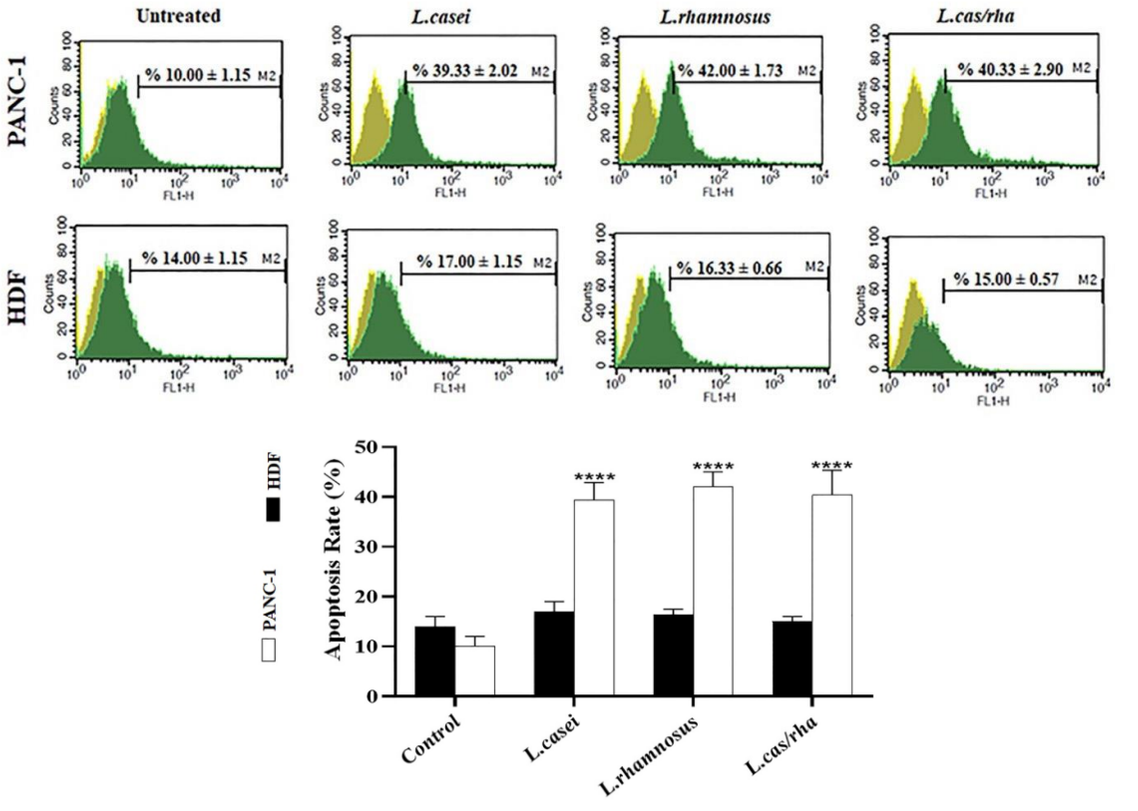

Fig. 3. Flow cytometry analysis of PANC-1 and HDFa cells following treatment with the IC_{50} concentration of *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus* CFSs for 24 h. The plot illustrates the percentage of apoptotic cells, highlighting significant differences in apoptosis induction between treated and control groups. Statistically significant differences ($p < 0.05$) are indicated by asterisks (*).

Gene expression. The expression levels of the apoptosis-related genes *BAX* and *BCL-XL* were assessed using real-time PCR. The results demonstrated that in PANC-1 cells treated with CFS from *L. casei* or *L. rhamnosus*, there was significant upregulation of the *BAX* gene and downregulation of the *BCL-XL* gene ($p \leq 0.05$, Fig. 5). However, no significant changes in the expression levels of the *BAX* and *BCL-XL* genes were detected in HDFa cells upon treatment with the CFSs ($p \geq 0.05$, Fig. 5).

DISCUSSION

Previously, several studies have shown that *L. casei*, and *L. rhamnosus* CFSs have inhibitory effects on cancer cells, but to the best of our knowledge, no study has investigated the simultaneous effects of *L. casei* and *L. rhamnosus* CFS to determine the inhibitory or combined effects of CFS on each other. This study evaluated the apoptotic effects of *L. casei* and *L. rhamnosus* CFSs. We performed an MTT assay to assess the inhibitory effects of CFS on PANC-1 cells. The data revealed that CFS had greater inhibitory effects on cancer cells than on normal cells. These findings were consistent with the results obtained from Hoechst/PI staining. Our findings demonstrated that treating PANC-1 cells with 20% (v/v) CFS for 24 hours significantly reduced cancer cell viability, while no significant effects were observed on HDFa cells. These results are consistent with a previous study in which cell extracts of *L. rhamnosus* exhibited inhibitory effects on DLD-1 and HGC-27 cell lines, reducing cell viability by 65% and 55%, respectively (24). Another study demonstrated that heat-killed *Lactobacillus* can significantly reduce the viability of HT-29 and Caco-2 cells by 62% and 73%, respectively (25). Research on multiple cancer cell lines has shown that heat-killed *L. rhamnosus* reduces cancer cell viability (26). An in vitro study confirmed that *L. casei* can decrease HT-29 cell viability to 78% within 24 hours (21). While numerous studies

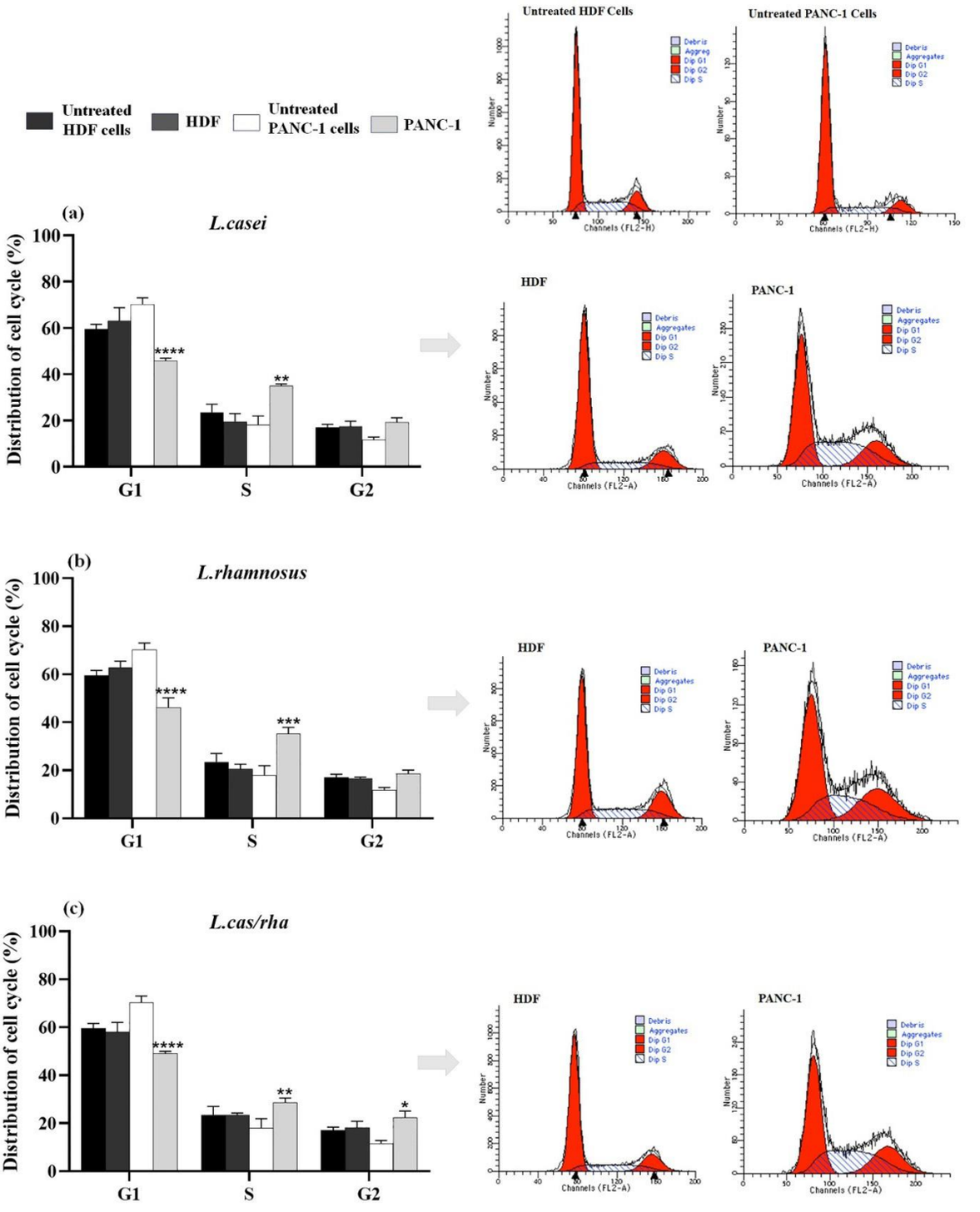

Fig. 4. Cell cycle analysis of PANC-1 and HDFa cells treated with or without the IC_{50} concentration of CFSs. The distribution of cell cycle phases in PANC-1 and HDFa cells following treatment with *Lacticaseibacillus casei* CFS (a), *Lacticaseibacillus rhamnosus* CFS (b), and a combination of both (c) for 24 h. Statistically significant differences ($p < 0.05$) are indicated by asterisks (*).

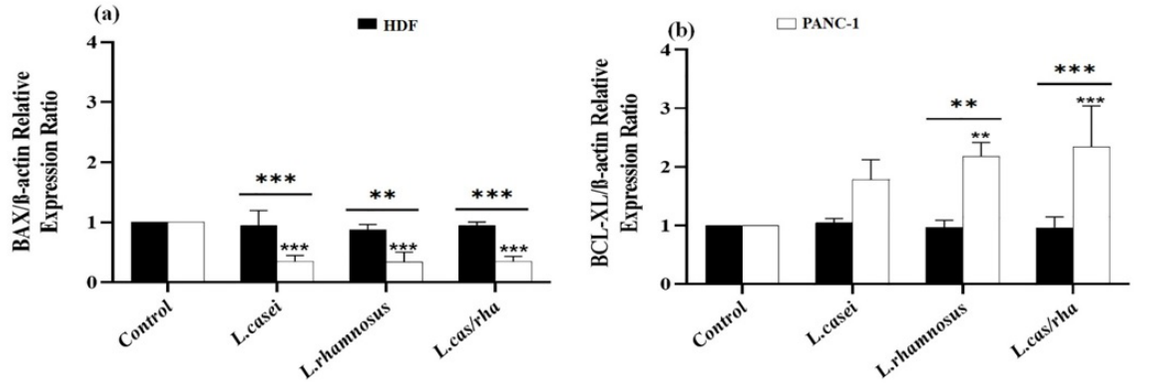

Fig. 5. Relative expression ratio of *BAX* and *BCL-XL* with different concentrations of *L. casei*, *L. rhamnosus*, and of *L. cas/rha* CFSs on PANC-1 and HDFa cells after 24 h ($p \leq 0.05$).

have highlighted the inhibitory effects of probiotics on cancer cells, there is still a lack of research on their effects on normal cells. However, studies have demonstrated that the IC_{50} concentration of *Lactobacillus coagulans* does not significantly affect normal cells compared with the MCF7 cell line (22). Nuclear staining, which has been previously utilized to assess the viability of adherent nerve cells and count live and dead cells, was employed in this study (27). The staining results of PANC-1 cells and fibroblast cells validated the ability of CFSs to reduce the survival of cancer cells but had no significant effect on fibroblast cells. These findings were consistent with the results obtained from the MTT assay. For the assessment of CFS-induced apoptosis, DMEM was utilized for culturing bacteria and purifying the CFS. The investigation of the in vitro effect of CFS on PANC-1 cancer cell apoptosis yielded results that aligned with our expectations, demonstrating a greater level of apoptotic cells than untreated cells. This observation can be attributed to the presence of externally secreted compounds in *L. casei* and *L. rhamnosus* CFS, such as glycoproteins and organic acids (acetic, butyric, lactic, and pyruvic acids), which possess anticancer properties (28). Our findings indicated that treatment of PANC-1 cells with 20% (v/v) CFS for 24 hours induced apoptosis in cancer cells, while no significant effects on HDFa cell apoptosis were observed. Previous studies have also demonstrated the apoptotic and inhibitory effects of probiotics on cancer cells (29). In line with these findings, a study demonstrated that the CFS of *Lactobacillus acidophilus* can induce apoptosis in HeLa and HT-29 cancer cells but does not affect normal cells (30). Another study revealed that the CFS of *Lactobacillus brevis* inhibits breast cancer cell proliferation and induces apoptosis (31). Similarly, *L. casei* SR1, SR2, and *Lactobacillus paracasei* SR4 were found to inhibit cervical cancer cells by inducing apoptosis through the intrinsic mitochondrial pathway (32).

In our study, we performed cell cycle analysis to confirm the ability of CFS to induce apoptosis. Consistent with previous research, our data demonstrated that CFS can arrest the cell cycle of PANC-1 cells in the S phase but does not affect HDFa cells. Previous studies have reported similar findings. Ferrichrome has been reported to exert inhibitory effects on pancreatic cancer cells in vivo and in vitro by arresting the cell cycle in the S phase (33). Treatment of HT-29 and Caco-2 cells with *Lactobacillus pentosus* and *Lactobacillus plantarum* CFS led to cell cycle arrest in the G1 phase (34). Additionally, studies have shown that the cytoplasm of *Lactococcus lactis* can halt the cell cycle of SNUC2A cells in the S phase (35). *Bifidobacterium* can inhibit the growth of colorectal cancer (CRC) cells by arresting them in the G0/G1 phase and increasing the activity of alkaline phosphatase (36). In our study, we observed that treatment with CFS increased the expression of the pro-apoptotic gene *BAX* and decreased the expression of the antiapoptotic gene *BCL-XL* in PANC-1 cells but not in HDFa cells.

The role of short-chain fatty acids (SCFAs) secreted by probiotics in inhibiting cancer cells through epigenetic regulation of tumor-inhibitory gene expression and oncogenes has been demonstrated (37, 38). Bacteriocins, which are antimicrobial peptides produced by probiotics, have also been implicated in cellular responses, and studies have shown their selective binding to negatively charged cancer cells while

sparing normal cells, which are either charge-neutral or slightly positive (39). Investigating the effect of *Lactobacillus fermentum* CFS on HCT-116 and HT-29 cells revealed a decrease in the expression of the antiapoptotic gene *BCL-2* and an increase in the expression of the pro-apoptotic gene *BAX* (40). *L. rhamnosus* isolated from human milk has been shown to induce apoptosis in HeLa cells through the positive regulation of *BAX*, *BAD*, and *Caspase 3/8/9* expression and the negative regulation of *BCL-2* expression (41). *L. coagulans* CFS can increase the expression of the *BAX* and Caspase 3/9 genes while decreasing the expression of the *BCL-2* gene (22). Previously, the antitumor effect of ferrichrome obtained from *L. casei* CFS on colorectal and gastric cancers was reported to be more potent than that of drugs such as 5-fluorouracil (5-FU) and cisplatin (33). In our study, the combined treatment with CFS did not result in a statistically significant enhancement or reduction of the observed effects compared with individual treatments in PANC-1 and HDFa cells. Therefore, no conclusions regarding synergistic or inhibitory interactions can be drawn from the current data, and the results are reported solely as observed combined effects without formal synergy analysis. It is possible that overlapping metabolic pathways or competition for cellular targets limit the combined efficacy. Such strain-specific interactions highlight the importance of understanding individual probiotic actions before designing combination therapies (21, 42). Compared with monocultures, *Lactobacillus sanfranciscensis DPPMA174* and *Pediococcus pentosaceus 2XA3* inhibited the growth of *L. plantarum DC400*, resulting in an increased number of dead/damaged cells. This effect was attributed to the biosynthesis of the pheromone PlnA by *L. plantarum DC400*, both in monoculture and co-culture conditions (43, 44). Despite the promising anticancer effects of *Lactobacillus casei* and *L. rhamnosus* CFSs, identification and characterization of specific bioactive compounds responsible for these effects are essential for future therapeutic applications. Potential candidates include short-chain fatty acids (SCFAs) and bacteriocins, which have demonstrated anticancer properties through various mechanisms such as epigenetic regulation and selective cytotoxicity towards cancer cells (45). Further studies focusing on isolating and elucidating these molecules will enhance our understanding and facilitate the development of targeted probiotic-based therapies. Future research should also address the mechanistic pathways and in vivo efficacy of these compounds to fully harness their therapeutic potential.

One limitation of this study is the lack of detailed characterization of the cell-free supernatant (CFS). Parameters such as pH, lactic acid concentration, and the proteinaceous nature of the bioactive compounds were not assessed. Another constraint is that only the S phase of the cell cycle was examined. Although this phase is directly relevant to apoptosis assessment, a comprehensive cell cycle analysis including the G0/G1 and G2/M phases would provide a more complete understanding of the treatment effects. Future investigations should therefore include full cell cycle profiling.

CONCLUSION

In summary, our study examined the effects of *L. casei* and *L. rhamnosus* CFS on PANC-1 cancer cells in vitro. The results showed that these CFSs effectively induced apoptosis in PANC-1 cancer cells compared with untreated cells while having minimal effects on normal cells. These findings suggest that probiotic CFS has the potential to be used as a promising approach for cancer inhibition by upregulating the expression of proapoptotic genes and downregulating the expression of antiapoptotic genes. The use of these CFSs may offer a promising avenue for the treatment of PANC-1 cancer.

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