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Postbiotic Compounds Produced by Lactobacillus fermentum as Effective Antiproliferative Agents against HeLa Cells with Favorable Biocompatibility

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Abstract

Conventional chemotherapeutic regimens used in cervical cancer management are frequently associated with considerable adverse effects on the human body. Consequently, increasing attention has been directed toward microbial-derived bioactive compounds, particularly postbiotics, as alternative therapeutic options for malignant disorders. The present study explored the underlying mechanisms associated with two promising postbiotic-producing Lactobacillus isolates, Lactobacillus fermentum CH and L. fermentum KH, obtained from traditional Iranian dairy products. Identification of the isolates was achieved using 16S rDNA sequencing, followed by morphological and biochemical characterization. The biological effects of the derived postbiotics on a cervical cancer model were evaluated using cytotoxicity assays and apoptosis analyses. Furthermore, anticancer activity was examined by quantitative real-time PCR (qPCR) and subsequently validated through flow cytometry. The findings demonstrated that these postbiotics exerted notable anticancer effects on HeLa cells by upregulating the expression of BAX, caspase8, and caspase9, while downregulating BCl-2, IKB (Inhibitor of nuclear factor kappa-B), and RelA. Collectively, these results indicate that postbiotic derivatives from Lactobacillus strains isolated from indigenous Iranian dairy products may represent a potential topical therapeutic approach with a favorable therapeutic index for the treatment of cervical malignancy.

Keywords: Apoptosis, Cervical cancer, Indigenous dairy products, Postbiotics

Introduction

Cervical cancer represents one of the most prevalent malignancies affecting women, ranking as the third most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality globally [1–3]. This disease originates in the cervical epithelium as a result of uncontrolled cellular proliferation with the capacity to invade surrounding tissues and metastasize to distant organs [4]. Early stages of cervical cancer are often asymptomatic; however, advanced disease may manifest with symptoms such as pelvic discomfort, dyspareunia, and abnormal vaginal bleeding [5].

The human microbiota comprises a vast array of bacterial species that play a crucial role in preserving host health [6, 7]. Approximately 9% of the total human microbiome is associated with the urogenital tract [6], highlighting the importance of a balanced interaction between the host immune system and the urogenital microbiome in maintaining physiological homeostasis. Disruption of this microbial equilibrium may predispose individuals to urogenital infections, which can ultimately contribute to cervical carcinogenesis [8, 9]. Currently available chemotherapeutic strategies for cervical cancer are largely cytotoxic and may cause substantial harm to normal tissues. Therefore, identifying alternative anticancer interventions with fewer adverse effects remains a critical objective [10, 11].

Species of Lactobacillus fermentum are prominent members of the gastrointestinal microbiota and play an

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essential role in promoting vaginal health by lowering susceptibility to infections [12, 13]. These bacteria are also widely recognized as probiotic organisms [14, 15]. Probiotics are defined as live microorganisms that confer health benefits to the host when administered in sufficient quantities [16, 17]. Their anticancer properties are attributed to multiple mechanisms, including inhibition of pro-carcinogen conversion, neutralization of mutagenic compounds, suppression of pro-carcinogenic microbial populations, reduction of mitogen absorption, and enhancement of immune-mediated antitumor responses [14, 18].

More recently, the concept of "postbiotics" has emerged as a complementary term to probiotics [19]. According to the International Scientific Association of Probiotics and Prebiotics (ISAPP), postbiotics are defined as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" [20]. This definition emphasizes that non-viable probiotic cells and their metabolites can still exert beneficial biological effects. Importantly, several systematic investigations have reported the anticancer potential of postbiotics [21–24]. A major advantage of postbiotics is that their activity does not depend on bacterial viability within the gastrointestinal tract; rather, they consist of preformed bioactive metabolites and cellular components capable of directly interacting with host tissues. This feature may render postbiotics more stable and reproducible in their biological effects compared with live probiotics. Nevertheless, the precise molecular mechanisms underlying postbiotic-mediated anticancer activity remain insufficiently understood.

Apoptosis, or programmed cell death, is a fundamental physiological process responsible for maintaining cellular homeostasis [25–27]. In cancer, dysregulation of apoptosis-related genes leads to uncontrolled cell proliferation, making apoptosis a primary target in non-invasive anticancer strategies [28–31]. Accordingly, the present study assessed anticancer efficacy by examining changes in the expression of key apoptosis-associated genes, including BAX/Bcl2, Casp8, Casp9, RelA, and IKB (Inhibitor of nuclear factor kappa-B).

Despite their benefits, the safety of administering live probiotic bacteria, including Lactobacillus species, has not been fully established, particularly in vulnerable populations. Caution is advised for individuals with compromised immune systems, elderly patients, those with cardiac valve disorders, and patients with short bowel syndrome [32]. Moreover, live probiotics must survive gastrointestinal conditions and be delivered in adequate concentrations to elicit biological effects. In contrast, postbiotics offer comparable anti-inflammatory benefits, extended shelf life, and improved stability, while significantly reducing the risk of infection and adverse effects in immunocompromised individuals [33–35].

As a result, increasing research efforts have focused on postbiotics as safer alternatives to live probiotics [36, 37]. Although probiotic use is limited in cancer patients with severe immunodeficiency, studies investigating the anticancer properties of postbiotics remain scarce. Therefore, this study aimed to evaluate the effects of postbiotics derived from two Lactobacillus strains isolated from traditional Iranian dairy products on cell proliferation and apoptosis in both normal (HUVEC; human umbilical vein endothelial cells) and cervical cancer (HeLa) cell lines.

Materials and Methods

Bacterial isolation and growth conditions

A total of 68 samples of traditional dairy products, including yogurt, were collected from ten locations in Gilan province, Iran, as illustrated in the flowchart **(Figure 1)**. One gram of each sample was homogenized in 9 mL of 2% (w/v) peptone water and vigorously vortexed for 30 seconds. Subsequently, 1 mL of the suspension was inoculated into 9 mL of MRS (Man, Rogosa, and Sharpe) broth (Merck, Germany) and subjected to serial dilution. Aliquots (0.01 mL) of the diluted samples were plated onto MRS agar (Merck, Germany) supplemented with 0.05% cysteine and incubated for 48 hours. Distinct colonies were selected and transferred into 15 mL of MRS broth, followed by incubation at 37 °C for 24 hours. Two isolates were randomly chosen for further analysis, including assessment of morphology, motility, oxidase activity, catalase activity, and Gram staining. Isolates exhibiting non-motility, negative catalase and oxidase reactions, Gram-positive staining, and bacillus morphology were identified as Lactobacillus species. These isolates were preserved at −70 °C in a cryoprotective medium containing 30% (w/v) glycerol and 10% (w/v) skim milk for subsequent experiments.

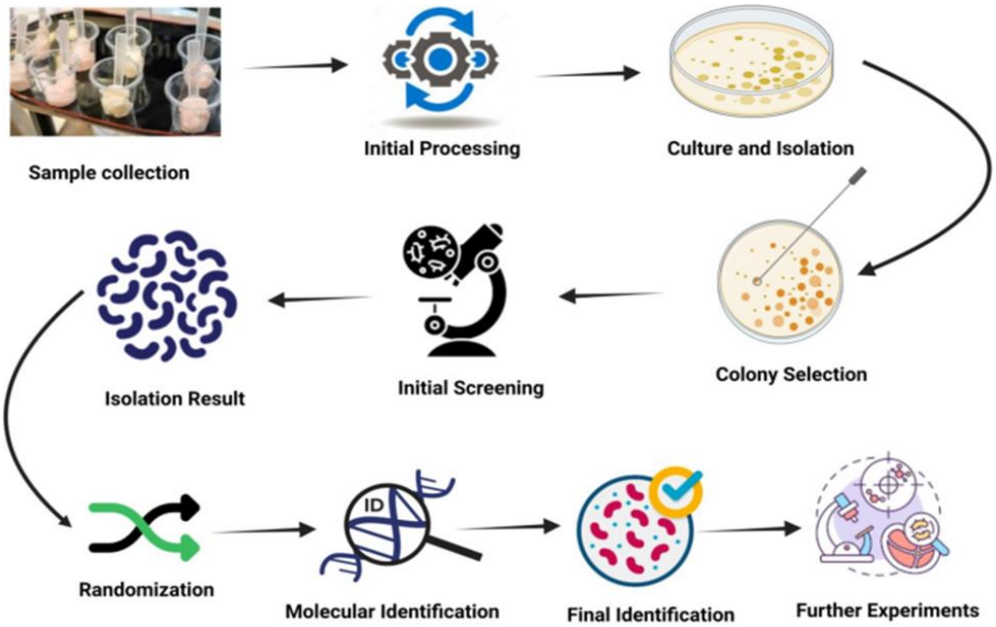


Figure 1. Methodological flowchart illustrating the isolation and analytical procedures for Lactobacillus fermentum.

Identification of lactobacillus isolates

Molecular identification of the selected isolates was performed using genomic techniques. Genomic DNA was extracted from the Lactobacillus isolates employing the boiling method as previously described [38]. Amplification of the 16S rDNA region was carried out using the primers RW01 (5′-AACTGGAGGAAGGTGGGGAT-3′) and DG74 (5′-AGGAGGTGATCCAACCGCA-3′) [39, 40]. PCR amplification was conducted in a Bio-Rad thermocycler (USA) under the following conditions: initial denaturation at 94 °C for 2 min; 30 cycles consisting of denaturation at 94 °C for 40 s, annealing at 50 °C for 30 s, and extension at 72 °C; followed by a final extension step at 72 °C for 10 min. The amplified products were electrophoresed on agarose gel and subsequently sequenced by Cinnagen Co. (Tehran, Iran). Sequence identity was confirmed using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI).

Preparation of cell-free postbiotic extract

Overnight cultures of Lactobacillus isolates (10%) were inoculated into fresh MRS broth and incubated for 48 h at 37 °C under microaerophilic conditions. Following incubation, cultures were centrifuged at 5000 rpm for 30 min, and the supernatants were discarded. The resulting bacterial pellets were washed with phosphate-buffered saline (PBS, pH 7.2). To obtain cell-free postbiotic extracts, the pellets were adjusted to various optical densities at 600 nm (OD600 = 0.5, 0.75, 1.0, 1.5, and 2.0) corresponding to different bacterial concentrations (cfu/mL). The suspensions were then subjected to sonication at 12 W for 30 s with 60-s intervals between cycles [41]. Sterilization of the supernatant was achieved through filtration, and sterility was verified by the absence of bacterial growth in MRS broth after 48 h of incubation at 37 °C. All prepared samples were stored at −80 °C until further use.

Cell culture handling

HeLa cells (Human Cervical Adenocarcinoma Cells, IBRC C11311) and Human Umbilical Vein Endothelial Cells (HUVEC, C554) were obtained from the Iranian Biological Resource Center (IBRC). Both cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cell morphology, viability, and density were routinely monitored using an inverted microscope. Upon reaching approximately 70% confluence, cells were detached using 0.05% trypsin and centrifuged at 1500 rpm for 5 min. Cell viability was assessed using Trypan blue staining and counting with a Neubauer hemocytometer. Only cell suspensions

exhibiting viability above 90% and free from contamination were used in subsequent experiments [42].

MTT assay

The cytotoxic effects of postbiotics derived from the two indigenous Lactobacillus isolates were evaluated on HeLa and HUVEC cell lines using the MTT assay. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well in 100 µL of culture medium and incubated for 24 h. Control wells received RPMI-1640 medium alone, identical to the solvent used for postbiotic preparation, without postbiotic supplementation. Based on previous reports, a range of postbiotic concentrations was selected to encompass sublethal to cytotoxic doses. Cells were treated with postbiotic concentrations corresponding to OD600 values of 1.2×10^8, 1.8×10^8, 2.4×10^8, 3.6×10^8, and 4.8×10^8 cfu/mL and incubated for an additional 24 h. Subsequently, 100 µL of MTT solution (0.5 mg/mL; Sigma, Germany) was added to each well, and plates were incubated for 4 h at 37 °C in 5% CO_2 [43]. Formazan crystals formed during the reaction were solubilized using dimethyl sulfoxide (DMSO), and absorbance was measured at 540 nm using an ELISA reader (BioTek, PowerWave, Winooski, VT, USA). Cell viability percentages and half-maximal inhibitory concentration (IC_{50}) values were calculated accordingly.

Assessment of apoptosis by flow cytometry

Apoptotic cell death was analyzed using an Annexin V-FITC apoptosis detection kit (Sigma-Aldrich, UK) in accordance with the manufacturer's instructions. HeLa and HUVEC cells were treated with IC_{50} concentrations of postbiotics from both isolates for 24 h. Apoptosis was quantified using a BD FACSCalibur flow cytometer (Erembodegem, Belgium).

Gene expression analysis by real-time PCR

Total RNA was isolated from HeLa and HUVEC cells treated with postbiotics for 24 h using a commercial RNA extraction kit (Pars Tous, Mashhad, Iran), following the manufacturer's guidelines. Complementary DNA (cDNA) synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Norgen Biotek, Canada). The expression levels of apoptosis-related genes (RelA, Casp9, Casp8, BAX/Bcl2, and IKB) were quantified using SYBR Green-based semi-quantitative real-time PCR on a Bio-Rad system (USA). Primer sequences used in this analysis are provided in **Table 1**.

Table 1. primer sequences employed for qPCR analysis

Gene	Forward (5′ → 3′)	Reverse (5′ → 3′)
BAX	ATCCAGGATCG AGCAGGGCG	GGTTCTGATCAG TTCCGGCA
Bcl-2	GTTCCCTTTCCT TTCCATCC	TAGGCCAGTCC AGAGGTGAAG
Caspase 8	CTGGGAAGGAT CGACGAATTTA	CATGTCCTGCAT TTTGATGGG
Caspase 9	AGCCAGATGCT GTCCCATAC	CAGGAGACAAA ACCTGGGAAA
RelA	CTGTGCGTGTC TCCCATGCA	TCGTCTGTATCT GGCAGGTA
IKB	GCTGAAGAAGG AGGCGGCTAC	TCGTACTCCTCG TCTTTTCAT
β-actin	ATGATGATATC GCCGGCCGCTC	CCCACCATCACG CCCTGG

The PCR conditions were as follows: 94 °C for 2 min, followed by 35 cycles at 94 °C for 15 s, 55 °C for 30 s, annealing at 68 °C for 60 s, and a final extension at 72 °C for 5 min. The analysis of outcomes was performed using the delta–delta Cq approach. A melting curve was applied to analyze reaction specificity. In the end, the relative gene expression of the RelA, Casp9, Casp8, BAX/Bcl2, and IKB were calculated with the $\Delta\Delta C_t$ formol. So all the data were analyzed using the $2^{-\Delta\Delta Ct}$ formol, wherever $\Delta\Delta C_t = (C_{t,\ Target} \_ C_{t,\ Control})_{Time\ x} \_ (C_{t,\ Target} \_ C_{t,\ Control})_{Time\ 0}$ is each sample's treatment time and Time 0 represents the non-treatment control sample. The fold change in the target gene (RelA, Casp9, Casp8, BAX/Bcl2, and IKB), regulated to the reference gene (B-actin) and also relative to the expression in the non-treatment samples, was calculated for each sample using the $2^{-\Delta\Delta Ct}$ formol.

Statistical analysis

All experimental procedures were independently repeated three times, and results are reported as mean ± standard deviation. Statistical differences among experimental groups were evaluated using one-way analysis of variance (ANOVA). Data processing and statistical evaluation were performed using SPSS software (version 22, NY, USA), with statistical significance defined as $P < 0.05$.

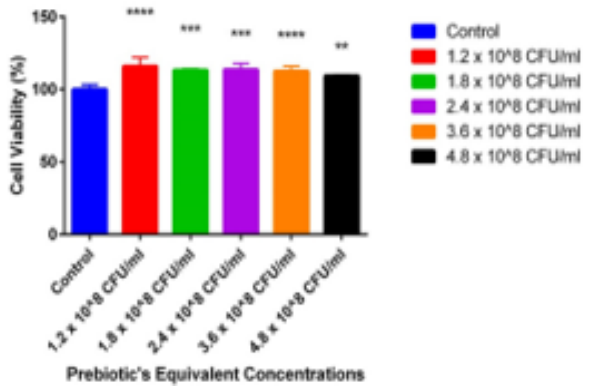
Results and Discussion

Isolation and molecular identification of bacterial strains

From the collected traditional dairy samples, a total of 25 bacterial isolates were initially recovered. Based on colony morphology consistent with Lactobacillus species on MRS agar, two isolates were randomly selected for detailed analysis. Microscopic and biochemical characterization revealed that both isolates were Gram-positive, rod-shaped bacteria forming short chains, lacking motility, and exhibiting negative catalase and oxidase activities. Subsequent molecular identification using 16S rRNA gene sequencing demonstrated that the selected strains, designated CH and KH, were classified as Lactobacillus fermentum, sharing approximately 99% sequence similarity with reference strains. The corresponding nucleotide sequences were submitted to the NCBI database under accession numbers OP168796 and OP164565.

Evaluation of cell proliferation

The impact of postbiotic preparations derived from L. fermentum CH and L. fermentum KH on cellular viability was assessed in both malignant and non-malignant cell models using the MTT assay. Exposure of HUVEC cells to increasing concentrations of postbiotics from either isolate resulted in cell viability values exceeding baseline levels, indicating an absence of cytotoxicity toward normal endothelial cells. Notably, escalating postbiotic doses did not negatively influence HUVEC survival. In contrast, treatment of HeLa cells with elevated postbiotic concentrations (3.6×10^8 and 4.8×10^8 cfu/mL equivalents) produced a marked reduction in cell viability, demonstrating a clear dose-dependent antiproliferative effect **(Figure 2)**. The half-maximal inhibitory concentration (IC_{50}) for both postbiotic preparations against HeLa cells following 24 h of exposure was determined to be 10 µg/mL.



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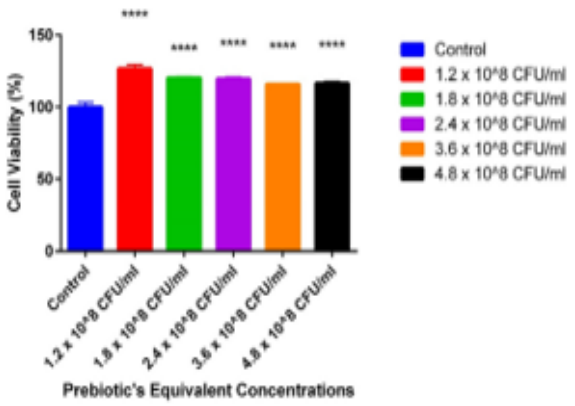

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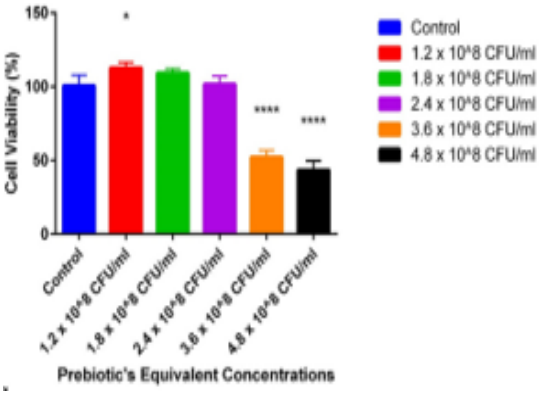

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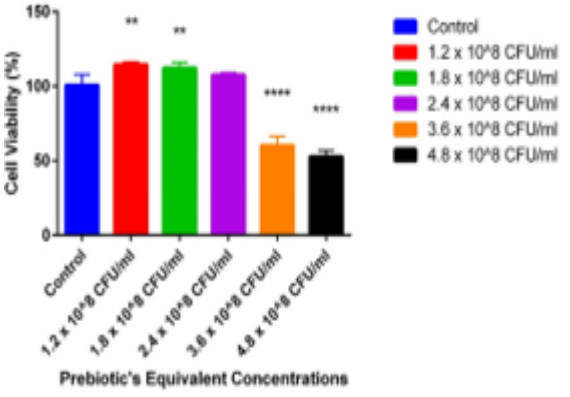

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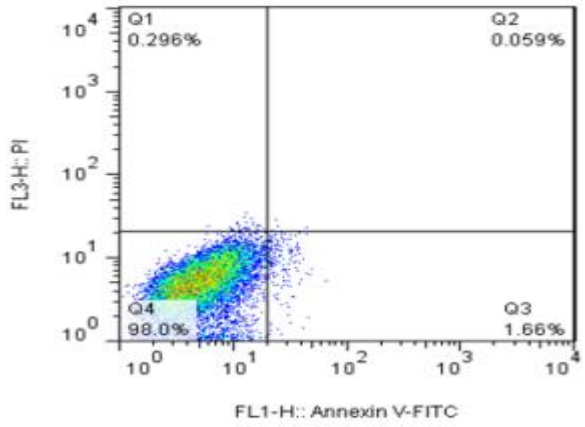
Figure 2. Cell viability following postbiotic exposure in the evaluated cell lines. (a) HUVEC cells treated with postbiotic derivatives from Lactobacillus fermentum CH; (b) HUVEC cells treated with postbiotic derivatives from L. fermentum KH; (c) HeLa cells exposed to postbiotics derived from L. fermentum CH; and (d) HeLa cells treated with postbiotics obtained from L. fermentum KH. Results are expressed as percentages of cell survival relative to untreated control groups analyzed in triplicate (n =

5). Statistical significance is indicated as ****P < 0.0001, ***P < 0.001, **P < 0.01, and *P < 0.05. Data are presented as mean ± SD.

Assessment of apoptosis by flow cytometry

The apoptotic response of HeLa and HUVEC cell lines following exposure to postbiotics at IC_{50} concentrations is summarized in **Figure 3**. Quantitative apoptosis analysis was performed using flow cytometry after 24 h of treatment. A marked induction of apoptosis was observed in HeLa cells treated with postbiotics derived from L. fermentum CH and L. fermentum KH, with total apoptotic cell populations reaching 18.95% and 14.15%, respectively. In contrast, exposure of HUVEC cells to the same IC_{50} concentrations resulted in minimal cell death, accounting for only 2% and 3.3% of apoptotic cells for L. fermentum CH and KH postbiotics, respectively. Furthermore, necrotic cell populations in HeLa cells treated with CH- and KH-derived postbiotics were limited to 1.06% and 1.44%, respectively, compared with the corresponding control values of 0.29% and 1.18%.



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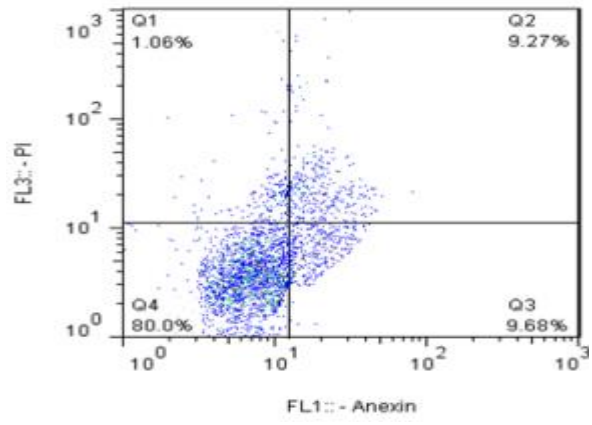

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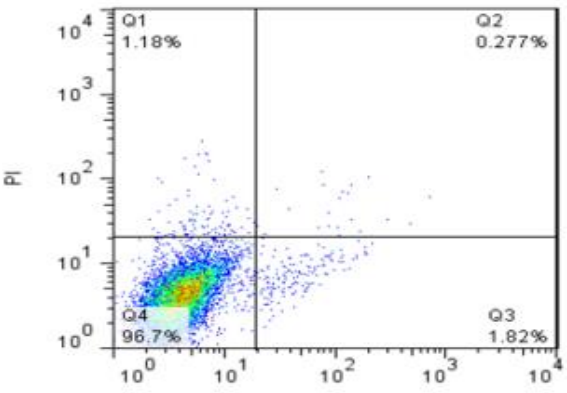

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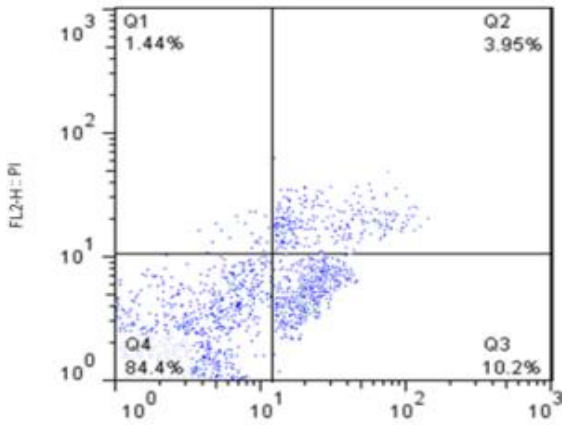

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Figure 3. Representative flow cytometry dot plots illustrating apoptosis induced by postbiotic preparations derived from Lactobacillus species following 24 h of exposure. Panels show: (a) untreated control cells, (b) cells treated with the IC_{50} concentration of postbiotics obtained from L. fermentum CH, (c) untreated control cells corresponding to the KH isolate, and (d) cells exposed to the IC_{50} concentration of postbiotics derived from L. fermentum KH. Apoptotic cell populations were identified using dual staining with Annexin V and propidium iodide (PI). Early apoptotic cells are represented in quadrant Q3, while late apoptotic cells are indicated in quadrant Q2.

Gene expression analysis by real-time PCR

To elucidate the molecular pathways underlying postbiotic-induced apoptosis in the cervical cancer model, transcriptional changes in key apoptosis- and survival-related genes (RelA, Casp9, Casp8, BAX/Bcl2, and IKB) were quantified in HeLa cells using real-time PCR. The housekeeping gene β-actin, selected for its stable expression across all experimental conditions,

served as the internal reference. Exposure of HeLa cells to IC_{50} concentrations of postbiotic extracts derived from both CH and KH isolates resulted in marked alterations in gene expression profiles relative to untreated control cells. Notably, transcript levels of Caspase 9, Caspase 8, and BAX were significantly elevated following 24 h of treatment ($P < 0.001$), indicating activation of apoptotic signaling cascades. Conversely, a pronounced suppression of anti-apoptotic and pro-survival genes (Bcl2, RelA, and IKB) was observed in treated cells compared with controls ($P < 0.001$), as illustrated in **Figure 4**. Importantly, evaluation of the BAX/Bcl2 expression ratio—a key indicator of apoptotic commitment—revealed a statistically significant increase after 24 h of postbiotic exposure ($P < 0.05$), further confirming the pro-apoptotic effect of the tested postbiotic preparations.

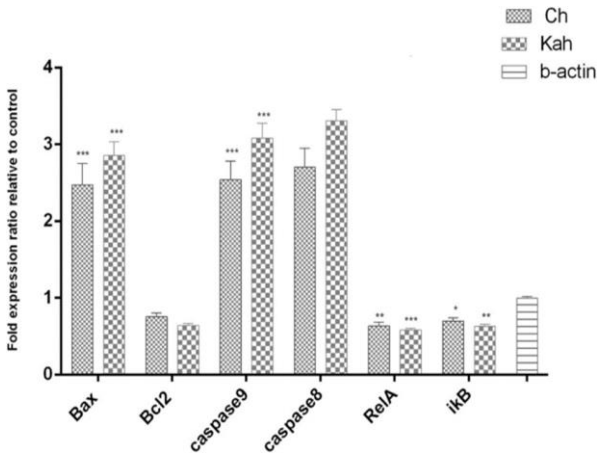

Figure 4. Relative mRNA expression levels of apoptosis-related genes in HeLa cells following 24 h of treatment with IC_{50} concentrations of postbiotic extracts from Lactobacillus fermentum CH and KH. Data represent the mean ± SD of three independent experiments (n = 3). Statistical significance is indicated as ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.

Cervical cancer ranks as the third most prevalent malignancy and the fourth leading cause of cancer-related mortality among women worldwide [44]. A hallmark of cancer cells is their evasion of apoptosis coupled with uncontrolled proliferation, making the induction of programmed cell death a key strategy in anticancer therapy.

While probiotics are widely recognized for their health-promoting effects through microbiome modulation, their practical application in pharmaceutical contexts is limited by factors such as reduced stability and short shelf life [21, 45]. Consequently, attention is shifting toward non-viable postbiotic products, which offer similar health benefits without the constraints associated with live bacteria. Postbiotics comprise a complex mixture of microbial metabolites, including secreted enzymes, proteins, vitamins, short-chain fatty acids, peptides, amino acids, organic acids, and biosurfactants, present either in the culture supernatant or cell extracts [22]. Although probiotics are generally safe in healthy individuals, adverse effects have been reported in immunocompromised or otherwise vulnerable patients, underscoring the need for careful evaluation of alternatives such as postbiotics [46]. Accordingly, this study investigated the effect of postbiotics on cervical cancer in vitro.

Previous research has demonstrated the anticancer and protective properties of postbiotics across in vitro, in vivo, and epidemiological studies [21, 33, 35, 41]; however, their effects on cervical cancer remain unexplored. In this study, two L. fermentum strains (CH and KH), isolated from indigenous Iranian dairy products, were assessed for their anticancer potential. Dose-response experiments revealed that higher postbiotic concentrations (3.6×10^8 and 4.8×10^8 cfu/mL) were required to suppress HeLa cell proliferation. In contrast, the same concentrations had no inhibitory effects on HUVEC cells, indicating selective activity against cancer cells. Postbiotics from the CH isolate showed slightly stronger inhibition than those from KH, though the difference was not statistically significant. Overall, both postbiotic preparations exhibited dose- and strain-dependent antiproliferative effects on HeLa cells.

Apoptosis, or programmed cell death, can be initiated via intrinsic or extrinsic pathways, which converge on cysteine proteases and caspases to eliminate damaged cells. The intrinsic pathway is activated by internal stressors, such as DNA damage or oncogene activation, and is tightly regulated by the Bcl-2 protein family [47, 48]. Pro-apoptotic members like Bax and Bak promote mitochondrial outer membrane permeabilization, triggering cytochrome c release. Cytochrome c then binds to apoptotic protease activating factor-1 (APAF1), facilitating caspase-9 activation, which subsequently activates downstream caspases including caspase-8 and caspase-3, culminating in apoptosis [11, 49, 50].

In the present study, postbiotic treatment of HeLa cells induced apoptosis rather than necrosis. As shown in

Figure 4, pro-apoptotic genes (Bax, Caspase-8, and Caspase-9) were upregulated, while the anti-apoptotic gene Bcl-2 was downregulated following exposure to CH and KH postbiotics. The elevated Bax/Bcl-2 ratio further confirms the activation of intrinsic apoptosis, highlighting the susceptibility of cervical cancer cells to postbiotic-induced programmed cell death as a primary anticancer mechanism.

In addition, the expression levels of IKB and RelA were evaluated by qPCR, revealing a 0.5-fold decrease relative to untreated controls **(Figure 3)**. RelA, also known as p65, is a key subunit of the NF-κB complex, which is normally retained in the cytoplasm in an inactive form bound to inhibitory IκB proteins [51, 52]. Under physiological conditions, IκBα sequesters NF-κB in the cytoplasm. Bacterial components, such as lipopolysaccharides (LPS), can trigger signaling that induces IκBα ubiquitination, allowing NF-κB translocation into the nucleus to promote transcription of proliferation and pro-inflammatory genes. Supporting this, previous work by Sambrani *et al*. demonstrated downregulation of Bcl-2 and RelA following probiotic treatment [53]. Notably, IκBα has also been reported at the outer mitochondrial membrane (OMM), where it inhibits apoptosis by stabilizing the hexokinase II (HKII)–VDAC1 complex, preventing Bax recruitment and cytochrome c release [54]. Consistent with these findings, our results suggest that the reduction of IκBα by the postbiotics contributes to apoptosis induction in HeLa cells.

Flow cytometric analysis using Annexin V/PI staining further confirmed apoptosis as the primary mode of cell death. The results revealed a significant increase in the population of early apoptotic cells in postbiotic-treated HeLa cells compared with controls, indicating that the antiproliferative effect of the postbiotics is predominantly mediated through apoptosis rather than necrosis **(Figure 2)**.

While the current study highlights the anticancer potential of postbiotics derived from Lactobacillus fermentum isolates, several limitations must be acknowledged. First, the scope of the study is limited to two specific isolates from Iranian dairy products, which may constrain generalizability. Broader evaluations involving multiple Lactobacillus strains and other probiotic species are warranted to fully understand postbiotic effects on cervical cancer. Second, the in vitro experimental model does not fully replicate the complexity of in vivo tumor biology; thus, animal studies and clinical trials are necessary to validate these findings. Moreover, although several apoptosis-related genes were investigated, more comprehensive molecular analyses could provide deeper insights into the mechanisms of postbiotic action. Evaluating long-term exposure and potential development of resistance in cancer cells is also essential, as the present study focused on short-term effects. Expanding testing to additional normal and cancerous cell lines would further clarify specificity and safety profiles. Future research should also consider in vivo studies to determine optimal delivery methods and bioavailability of postbiotics.

Taken together, these findings suggest several promising research directions. Expanding investigations to additional Lactobacillus strains and other probiotic species, combined with in vivo validation, could strengthen the translational potential of postbiotics. Comprehensive analyses of molecular mechanisms, including proteomics and metabolomics, may identify novel therapeutic targets. Long-term studies assessing sustained exposure and resistance development, along with evaluation across multiple cell lines, will be crucial for defining the clinical utility of postbiotics. In particular, postbiotics may offer advantages over live probiotics in immunocompromised patients or those with gastrointestinal vulnerabilities, where live bacteria may be contraindicated.

Conclusion

In conclusion, this study demonstrates that postbiotics derived from Iranian indigenous L. fermentum isolates induce apoptosis in HeLa cells through downregulation of IKB, Bcl-2, and RelA, alongside upregulation of Bax, Caspase-8, and Caspase-9. This regulation promotes cytochrome c release via the mitochondrial pathway, elevates the Bax/Bcl-2 ratio, and effectively triggers apoptotic cell death. Importantly, cell viability assays showed minimal toxicity in normal HUVEC cells, indicating selective anticancer activity. These findings suggest that indigenous postbiotics represent promising candidates for cervical cancer therapy due to their potent apoptotic effects and favorable safety profile.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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