Original Article | Iran J Med Microbiol. 2022; 16(5): 383-391

10.30699/ijmm.16.5.383

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Antiproliferative Potential of *Bacillus coagulans* Supernatant on SKBR3 Breast Cancer Cell Line

Masoumeh Dolati[1], Farzaneh Tafvizi[1*], Masoud Salehipour[1], Tahereh Komeili Movahed[2], Parvaneh Jafari [3]

1. *Department of Biology, Parand Branch, Islamic Azad University, Parand, Iran*
2. *Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran*
3. *Microbiology Department, Faculty of Science, Arak Branch, Islamic Azad University, Arak, Iran*

ABSTRACT

Background and Aim: Breast cancer is one of the most common types of cancer among Iranian women. To date, the usual cancer treatments have not been entirely effective. Therefore, creating anticancer products is of great importance. The aim of this study was to evaluate the cytotoxic, anticancer, and induction effects of *Bacillus coagulans* probiotic bacterial supernatant on SKBR3 cells.

Materials and Methods: The anticancer potential and cytotoxic effect of different concentrations of probiotic bacterial supernatants (1, 2, 3, 4, 5, 6 and 7 mg/mL) were evaluated on SKBR3 cells for 24, 48, and 72 h by MTT technique. QRT-RCR was used to assess the expression of *bax*, *bcl2, casp3,* and *casp9* genes, and flow cytometry was used to evaluate apoptosis in cancer cells.

Results: The inhibitory effect of dose- and time-dependent *B. coagulans* supernatant showed that the supernatant of this probiotic bacterium had a cytotoxic effect on SKBR3 cancer cells. On the other hand, analysis of flow cytometry results and increased expression of *bax*, *casp3,* and *casp9* pro-apoptotic genes and decreased *bcl2* expression in cancer cells showed induction of apoptosis.

Conclusion: The anticancer and cytotoxic effect of *B. coagulans* probiotic bacterial supernatant on SKBR3 cancer cells shows that with further research, this probiotic bacterium can be used as a new strategy for the possible treatment of breast cancer.

Keywords: Apoptosis, *Bacillus coagulation*, Breast cancer, Probiotic, SKBR3 cancer cells, Supernatant

Received: 2021/09/28; **Accepted**: 2022/06/10; **Published Online:** 2022/08/08

Corresponding Information: **Farzaneh Tafvizi**, *Department of Biology, Parand Branch, Islamic Azad University, Parand, Iran*
Email: tafvizi@piau.ac.ir; farzanehtafvizi54@gmail.com

Use your device to scan and read the article online

Dolati M, Tafvizi F, Salehipour M, Komeili Movahed T, Jafari P. Antiproliferative Potential of *Bacillus coagulans* Supernatant on SKBR3 Breast Cancer Cell Line. Iran J Med Microbiol. 2022; 16 (5) :383-91.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks
Send citation to: Mendeley Zotero RefWorks

1. Introduction

Probiotics are non-pathogenic living microorganisms considered essential in medical science due to their beneficial effects on the host body regarding the prevention and treatment of many chronic diseases, including cancer **(1, 2)**. The intestinal microbial flora plays a protective role against various diseases. An imbalance in the intestinal microbiome can lead to anti-inflammatory and immune reactions and the onset of pathogenic processes **(3)**. Research has shown that some types of probiotics can participate in the prevention and treatment of cancer, primarily by inducing apoptosis, modulating the intestinal microbiota, and creating an immune response **(4)**. The anticancer effects of probiotics are significant by preventing the conversion of procarcinogen to a carcinogen, binding and inactivating mitogen compounds, reducing the growth of carcinogenic bacteria, reducing mitogen uptake, inducing apoptosis, and enhancing immune function against cancer cells **(5)**. Induction of apoptosis is a critical way to reduce cell proliferation to prevent cancer cell development and increase cancer

treatments' effectiveness. Increasing the expression ratio of pro-apoptotic to anti-apoptotic genes (*bax*/*bcl2*) and activating the caspase cascade are essential in the onset and execution of apoptosis **(6)**.

Today, studied extensively in various fields, probiotic microorganisms belong mainly to bacterial genera such as *Lactobacillus* and *Bifidobacterium*, which produce lactic acid. However, other lactic acid-producing bacterial species have also been noticed by researchers **(7)**. Several Bacillus species have recently been reported as new probiotics **(8)**. *Bacillus coagulans* is a gram-positive, facultative anaerobic, non-pathogenic, catalase-positive, and negative oxidase bacterium and produces endospores. This bacterium can produce lactic acid, similar to *Lactobacillus* and *Bifidobacterium*, and since it produces spores, it is resistant to heat and pH. It can resist and survive while colliding with the stomach and bile acid. Madempudi *et al.* studied the effects of cytotoxicity, antiproliferative, and induction of apoptosis of this bacterium on human colon cancer cell line (COLO 205), cervical cancer (Hela), and chronic myeloid leukemia (CML) (K562) compared to the normal cell line of the human embryo (HEK 293) **(9, 10)**. Recent studies have shown that in addition to live probiotic bacteria, metabolic products of living probiotic bacteria, including bacterial cell-free supernatants **(11, 12)**, inactive bacteria (heat-killed bacteria) **(13)**, extracts of exo-polysaccharides **(14, 15)**, bacterial cell wall components **(16)**, cell extracts **(17)** and other cellular components have potential therapeutic, anti-tumor and anticancer activities and were also able to stimulate the immune system. According to previous studies, the benefits of metabolites released in the supernatant of probiotic bacteria can be anti-inflammatory, cytotoxic, antioxidant, and increasing the function of the immune system against cancer cells, as well as ease of use, stability, and longevity of these metabolic products compared to probiotic bacteria **(18, 19)**.

Cancer is the first or second leading cause of death in older adults in most countries worldwide **(20)**. Breast cancer is the first most common cancer (2.3 million people), the fifth leading cause of death (685,000) due to cancer worldwide, and the second most common cause of cancer death among women **(20, 21)**. To treat cancer, depending on the patient's age, genetics, and patient's physical condition, various treatment methods, including chemotherapy, radiation therapy, hormone therapy, and surgery, are performed **(22)**. However, besides killing cancerous tissue, these methods can cause damage to healthy tissue, tumor recurrence, and resistance of cancer cells to the drugs used during treatment. Therefore, studying the effect of probiotic bacteria or their metabolic products on inhibiting the growth of cancer cells without side effects can play an effective role in public health **(23, 24)**. To date, several studies have been performed on the effects of different strains of probiotics on the prevention and treatment of cancers, including breast cancer, by inhibiting proliferation and inducing apoptosis **(25)**. Evaluation of the efficacy of metabolites secreted by standard strains of *Lactobacillus* and *Bifidobacterium* on breast cancer cell lines (MCF-7) and gastric cancer (AGS) showed that metabolites could cause cancer cell death through the apoptosis pathway activation **(26)**.

Reviewing the literature, no report was found on the use of *B. coagulant* supernatant and cytotoxic effects on SKBR3 cancer. Therefore, this study was performed due to the therapeutic effect of probiotic bacteria on inhibiting the growth of cancer cells, with the aim of investigating the therapeutic effect of *B. coagulans* supernatant, including anticancer effects and induction of apoptosis, against breast cancer cells.

2. Materials and Methods

Cell Culture

Bacillus coagulans bacterial supernatant (GBI-30, 6068) was prepared according to the protocol reported in the previous study **(27)**. SKBR3 breast cancer cells (IBRC C10147) and HFF normal cells were purchased from Iran's National Center for Genetic and Biological Resources. Cells were cultures in DMEM High and DMEM (Gibco, UK) culture medium with 10% bovine fetal serum (FBS) (Gibco, USA) and 1% penicillin/streptomycin (Sigma, USA) at 37°C in a CO_2 incubator with 95% relative humidity (RH) and 5% CO_2 for 24 h. Then, by an inverted microscope, about 80% of the growth of cells inside the flasks and their health were examined and confirmed. Then they were washed using PBS. Trypsin-EDTA solution (0.25%) (Gibco, UK) was used for cell passage. Isolated cells were centrifuged, then the number and percentage of viable cells were determined by a slide neobar (hemocytometer) and Trypan Blue (Merck, Germany) **(28)**.

MTT Assay

In each 96-well plate, 200 µL containing 5000 cancer cells were cultured and incubated for 24 h in a CO_2 incubator with 5% CO_2 and 95% relative humidity (RH) at 37°C. Cells were treated with different dilutions of bacterial supernatants 1, 2, 3, 4, 5, 6, and 7 mg/mL. The plates were then incubated for 24, 48, and 72 h, similarly to the previous conditions. After removing the medium inside the wells, a new medium was added, and 20 µL of 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide at a concentration of 5 mg/mL (MTT) (Sigma Aldrich, Germany) was added in the dark and incubated for 4 h to form formazan

crystals. Then, the surface of the wells was washed, and 200 µL of DMSO (dimethyl sulfoxide) was added and placed on the shaker for 30 min in the dark and read by ELISA (Bio Tek, Synergy/HTX, Germany) at 570 nm, and the cell viability percentage was calculated by the following formula, and the graphs were drawn. Untreated SKBR3 cells with culture supernatant bacteria were used as a negative control.

Percentage of cell viability = (mean absorbance of treated cells/ mean absorbance of control cells) x 100

This formula calculated inhibitory concentration (IC_{50}) or the concentration of bacterial culture supernatant, which can inhibit the growth of 50% of cancer cells. All experiments were performed in three replications **(29)**.

Detection of Apoptosis Induction by Annexin V-FITC/PI

Flow cytometry was used to quantity primary, early and late apoptotic and necrotic cells. Apoptosis was assessed according to the protocol of the Annexin V-FITC/PI kit (MabTag GmbH, Germany). In summary, in each 6-well plate, 1×10^5 cells (SKBR3 and HFF) were cultured and, after 24 h, treated by IC_{50} of bacterial culture supernatant for 48 h, incubated at 37°C. Untreated cells were considered as negative controls. The cells were washed with PBS and centrifuged for 5 min. After adding 90 µL of binding buffer, 5 µL of Annexin-V solution was added and incubated in the dark for 10 min at room temperature. Then, 5 µL of PI was added. The resulting suspension was transferred to a flow cytometry tube and analyzed by flow cytometry (Thermo Fisher Scientific, USA) **(27, 30)**.

Real-Time PCR to Evaluate Gene Expression

Real-time PCR technique (Step One Plus, Applied Biosystems, USA) was used to evaluate the effect of *B. coagulans* supernatant on the expression of BCL2 associated X (*bax*), BCL2 apoptosis regulator (*bcl2*), caspase 3 (*casp3*), and caspase 9 (*casp9*) apoptotic genes on SKBR3 cancer cells. First, SKBR3 cells were prepared in a 6-well plate as in the previous step and treated with IC_{50} of bacterial culture supernatant for 48 h. Untreated cells were considered negative controls. RNA extraction, cDNA synthesis, and real-time PCR were performed according to the protocol reported in the previous study **(27)**.

Statistical Analysis

Statistical data analysis was performed by ANOVA (Analysis of Variance One-Way) and Paired-Samples T-test using GraphPad Prism 8.0.2 software. All experiments were performed in three times, and the results were analyzed in the form of Tables and bar graphs. In all analyses, the significant level of the tests was less than <0.05, and the data were presented as mean±standard deviation (Mean±SD).

3. Results

Bacillus coagulans was confirmed according to diagnostic tests of The Prokaryotes book and the Bergey's Manual. This bacterium was cultured in MRS agar; after gram staining, it was examined under a microscope, and Gram-positive bacteria were detected. The results of catalase and oxidase tests and sugar fermentation tests were reported in Table 1 **(31)**.

Table 1. Confirmation of *B. coagulans* probiotic bacterium by morphological and biochemical tests

Bacterium species	Spore	Gram staining	Sugar Fermentation	Catalase	Oxidase	Motility
B. coagulans			Glucose, Maltose, Mannitol, Trehalose, Sucrose			
	+	+	+	+	-	+

The Cytotoxic Effect of *B. coagulans* Supernatant on the Cell Viability of SKBR3 Cancer Cells

MTT assay showed that with increasing concentration of supernatant (1, 2, 3, 4, 5, 6, and 7 mg/mL) and treatment time (24, 48, and 72 hours), the cell viability of SKBR3 cancer cells decreased significantly (P<0.0001). The IC_{50} at 24, 48, and 72 h was 5.72, 4.98, and 3.49 mg/mL, respectively. Time has shown a significant effect on inhibiting the proliferation of SKBR3 cancer cells treated with supernatant.

Induction of Apoptosis in SKBR3 Cancer Cells

Analysis of the regions in Figure 2 showed that 36.02 ± 3.52% and 11.01 ± 1.78% of early and late apoptosis were observed in cancer cells, respectively. Of cancer cells, 0.1 ± 0.00% developed necrosis, which is negligible. The extent of induced apoptosis is summarized in Table 2.

Table 2. Results of induction of apoptosis in SKBR3 cells after treatment with *B. coagulans* for 48 h.

SKBR3	Control	IC_{50} (5 mg/mL)	P-value
early apoptosis	0.47 ± 0.10	36.02 ± 3.52	< 0.0001
late apoptosis	0.56 ± 0.32	11.01 ± 1.78	< 0.0001
necrosis	0.50 ± 0.61	0.1 ± 0.00	0.99

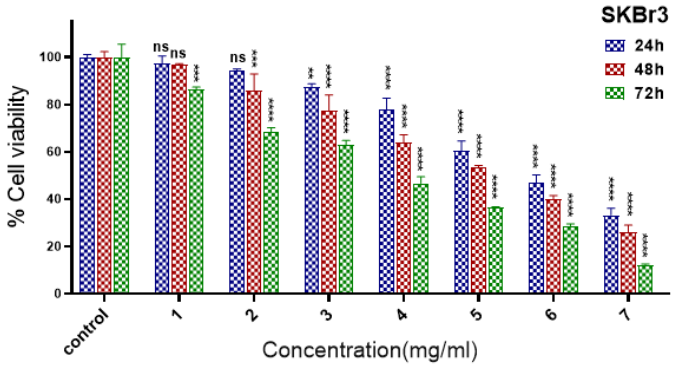

Figure 1. Survival percentage of SKBR3 cancer cells treated with different concentrations of *B. coagulans* culture supernatant at 24, 48, and 72 h compared to the control group (untreated SKBR3 cancer cells). Data are represented as mean ± SD of three replications.

(ns: non-significant, $P \leq 0.01$: **, $P \leq 0.001$: ***, $P \leq 0.0001$: ****)

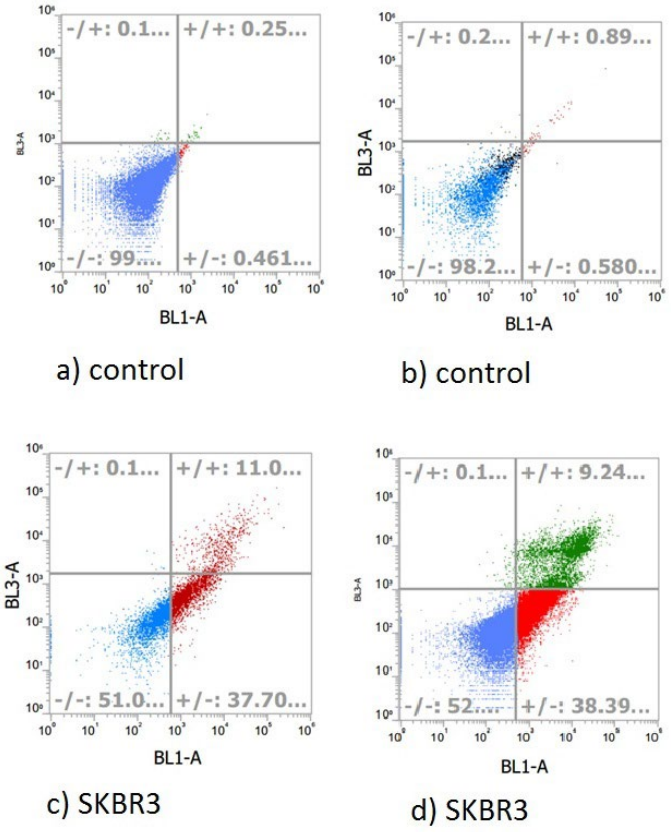

Figure 2. Flow cytometric diagrams of the effect of apoptotic supernatant of *B. coagulans* culture for 48 h on SKBR3 cancer cells. (a, b group of control cells (untreated SKBR3 cells), c, d) group of treated SKBR3 cancer cells. Lower left square: living cells (Annexin-$FITC^-/PI^-$), lower right square: early apoptotic cells (Annexin-$FITC^+/PI^-$), the upper right square of late apoptotic cells (Annexin-$FITC^+$ / PI^+), Upper left square of necrotic cells (Annexin-$FITC^-$/ PI^+)

Gene Expression Analysis

To evaluate the development of apoptosis in SKBR3 cancer cells, the expression levels of pro-apoptotic genes *bax*, *bcl2*, *caspase 3,* and *caspase 9* in cells treated by IC_{50} of *B. coagulans* culture supernatant for 48 h were evaluated by Real-Time PCR. After RNA extraction, its quantity was evaluated by nanodrop, and its quality was assessed by electrophoresis (Figure 3).

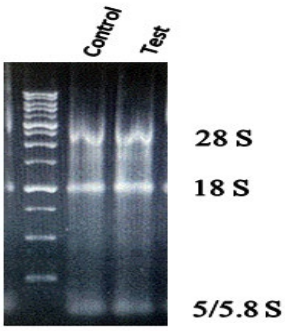

Figure 3. Quality of RNA extracted from SKBR3 cells on 2% agarose gel. The two 28S rRNA and 18S rRNA bands are observable with suitable quality due to staining by Safe Stain on 2% agarose gel.

Examining changes in the expression of different genes in SKBR3 cancer cells treated with IC_{50} concentration of *B. coagulans* culture supernatant confirmed the results of inducing apoptosis by flow cytometry. The *bax*, *casp3,* and *casp9* pro-apoptotic genes were up-regulated, and the anti-apoptotic *bcl2* gene was down-regulated after 48 h (Figure 4). The expression ratio of *bax* to *bcl2* genes increased over 48 h in treated SKBR3 cancer cells compared to control cells (Figure 4).

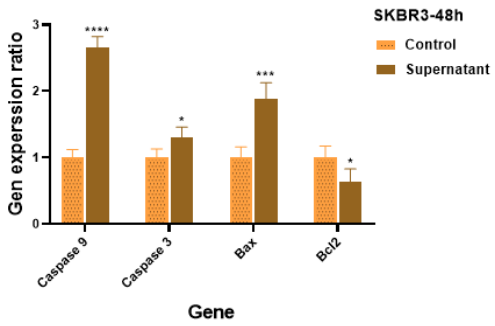

Figure 4. Expression of *bax*, *casp3, casp9*, and anti-apoptotic *bcl2* pro-apoptotic genes in SKBR3 cells treated with IC_{50} supernatant of *Bacillus coagulans* bacterial culture compared to the control group (untreated SKBR3 cancer cells), the data is presented as Mean ± SD of three dependent experiments is shown.

($P \leq 0.05$: *, $P \leq 0.001$: ***, $P \leq 0.0001$: ****)

4. Discussion

Today, probiotic supplements or compounds are increasingly being produced. These compounds are bacterial metabolites with beneficial properties related to community health, including anticancer properties **(32, 33)**. Studies have shown that probiotic bacteria have beneficial effects on inhibiting intestinal cancer by their location in the intestine. Also, they activate their protective properties against cancer by activating immune system cells and migrating them to more distant mucosa, including the mammary glands **(34)**. One of the most common cancers in the world is breast cancer, the second leading cause of death among women, with an increasing incidence and mortality rate each year **(20)**. Various methods are used to treat cancer, including chemotherapy, radiation therapy, hormone therapy and surgery. In all of these methods, in addition to killing cancer cells, some healthy cells are also destroyed, which is a side effect of chemotherapy **(22)**. Therefore, new strategies are needed to prevent and treat breast cancer. In various studies, the effects of live and heat-killed probiotic bacteria **(35)** and their supernatant **(11, 12)** through direct induction of apoptosis on various cancer cells have been investigated. Currently, the most commonly used lactic acid-producing bacteria are *Lactobacilli* and *Bifidobacteria*. Still, some yeasts and other bacilli have also been used **(36)**, including the bacterium *B. coagulans*, which produce not only lactic acid but also are resistant to temperature due to spore production's high pH. They can resist and grow against the pH of the stomach and intestines **(8, 9)**.

In the present study, the cytotoxic and anticancer effects and induction of apoptosis of *Bacillus coagulans* supernatant against SKBR3 breast cancer cells were evaluated due to the therapeutic effect of probiotic bacteria on inhibiting cancer cell growth. The results of this study showed that *B. coagulans* supernatant has a high potential to induce apoptosis and inhibit SKBR3 cell proliferation in a time and dose-dependent manner. The results of comparing the MTT test at 24, 48, and 72 h and in different concentrations (1, 2, 3, 4, 5, 6, and 7 mg/mL) on SKBR3 cancer cells showed that with increasing supernatant concentration and over time, the cell viability rate of SKBR3 cancer cells decreased significantly compared to the control group ($P<0.0001$) due to the increased cytotoxic concentration of supernatant on treated cancer cells.

On the other hand, the induction rate of apoptosis in treated cancer cells was estimated to be about 47%, indicating the strong apoptotic effect of bacterial culture supernatant on cancer cells. In addition, increased expression of *bax*, *casp3,* and *casp9* pro-apoptotic genes and decreased expression of *bcl2* anti-apoptotic gene in cancer cells treated for 48 h indicates strong apoptosis in cancer cells.

In the study by Madempudi *et al.,* the bacterial supernatant of *B. coagulans*, based on MTT assay, reduced cell proliferation in human colon (COLO 205), cervical (HeLa), and chronic myeloid leukemia (K562) cell lines, respectively, compared with normal human

embryonic kidney cells (HEK 293T). Similarly, in this study, a decrease in cell proliferation was observed in the SKBR3 cell line compared with normal HFF cells. Bacterial supernatant was also effective in inducing apoptosis by increasing BAX protein expression, decreasing BCL2 protein, decreasing mitochondrial membrane potential, increasing cytochrome c release, and increasing caspase 3 activity in cancer cell lines, especially in intestinal cancer cells **(10)**.

In this regard, Dehghani *et al.* showed that *Lactobacillus rhamnosus* supernatant, based on a time-dependent pattern (24, 48, and 72 h) and different concentrations of bacterial supernatant, has a high potential to inhibit HT-29 cancer cell proliferation and induce apoptosis. Thus, 99% inhibition of cancer cell proliferation was observed after 72 h at a 30 mg/mL concentration. According to this study, 7 mg/mL of *B. coagulans* bacterial supernatant in our study in 72 h inhibited the growth of about 90% of SKBR3 cells, which shows the strong effect of this supernatant compared to the study of Dehghani *et al.* Also, induction of apoptosis compared to the control group was investigated in 48 h, similar to this study. In both studies, strong apoptosis was induced after 48 h. *L. rhamnosus* supernatant and *Bacillus coagulans* supernatant (in this study) increased the expression of pro-apoptotic genes, including *bax*, *casp3,* and *casp9,* compared to the control group and decreased *bcl2* gene expression **(30)**.

The cytotoxic effect and induction of apoptosis of heat-killed bacterium Lactobacillus brevis (TD4) on HT-29 colon cancer cells and normal HEK-293 cells were investigated based on a time-dependent pattern. The results obtained by comparing the MTT test at 24, 48, and 72 h and different concentrations showed that heat-killed *L. brevis* had the highest cytotoxic effect of 1000 µg/mL dilution at 72 h compared with the control group. The control showed significant viability (P<0.001). The viability of HT-29 and HEK-293 cells was estimated to be 23% and 50%, respectively. The results of DNA extraction from the HT-29 cell line treated with different concentrations of TD4 bacteria also confirmed the induction of apoptosis in the HT-29 cell line **(37)**.

The cytotoxic effects of *Lactobacillus plantarum* HBM-IAUF-1 and *Lactococcus lactis* HBM-IAUF-8 supernatant on SKBR3 breast cancer cells have been reported in a time- and dose-dependent manner. *L. plantarum* HBM-IAUF-1 and *L. lactis* HBM-IAUF-8 had the highest cytotoxic effect on SKBR3 cancer cells compared with the control group at 1000 and 500 µg/mL, respectively, at 72 hours after treatment **(38)**.

The cytotoxic activity of different concentrations (5, 10, 15, 20 mg/mL) of supernatant of some gram-positive bacteria taken from the soil for different periods on PC-3 prostate cancer cells was examined. Among them, the *Bacillus licheniformis* strain, which had the best antimicrobial compounds, was selected for further investigation for survival and the effects of induction of apoptosis on PC-3 cell lines. MTS assay with an incubation time of 24, 48, and 72 hours of treated cells showed that survival was dose-dependent; increasing concentration could significantly reduce survival compared with the control group (P<0.05). The highest effect of cytotoxicity and apoptosis was observed on PC-3 cancer cells at a concentration of 20 mg/mL supernatant within 72 h after cell treatment **(39)**.

Elmanama *et al.* studied the anticancer effects of extracellular metabolites (supernatants) of eight bacterial species (*Escherichia coli*, *Staphylococcus aureus*, *Micrococcus*, *Pseudomonas aeruginosa*, lactic acid bacteria, *Klebsiella*, and *Proteus*). They dealt with the HeLa cell line. Their results showed a time- and concentration-dependent variation between the eight bacterial species in inhibiting proliferation against HeLa cells. *Pseudomonas* and *E. coli* with phage had significant anticancer activity compared to the others, with an inhibitory effect of 63% and 86% at a 1000 µg/mL concentration. The IC_{50} for these two bacteria was estimated to be 301 and 1395 micrograms per deciliter in 24 h, respectively. *Proteus* and *Micrococcus* showed less inhibitory effects, and *S. aureus* even increased HeLa cell proliferation at low concentrations **(40)**.

5. Conclusion

One of the critical features of anticancer drugs is their high effect on suppressing cancer cells with low side effects on normal cells. Studies to date have shown that metabolites produced by probiotic bacteria are effective in human health and could be used as a new treatment for cancer. The results obtained in this study, with the results obtained on other human cell lines, indicate that bacterial supernatants, which contain metabolites secreted by bacteria, can be a suitable model for anticancer tests. It seems that *B. coagulans* supernatant can induce apoptosis in cancer cells by increasing the *bax* pro-apoptotic gene expression and decreasing the anti-apoptotic *bcl2* gene expression. In the future, further studies on the cytotoxic and apoptotic effects of *B. coagulans* biomass, its various components, and heat-killed species on various cancer cells and laboratory animals may be proposed to determine the effective metabolite on cancer cells and take steps to create the proper medication that has less of an adverse effect on healthy cells.

Acknowledgment

The authors would like to acknowledge the Cellular and Molecular Research Center, Qom University of Medical Sciences, and the laboratory of Islamic Azad University.

Funding

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

Reference

1. Chondrou P, Karapetsas A, Kiousi DE, Vasileiadis S, Ypsilantis P, Botaitis S, et al. Assessment of the Immunomodulatory Properties of the Probiotic Strain Lactobacillus paracasei K5 in vitro and In Vivo. Microorganisms. 2020;8(5). [PMID] [PMCID] [DOI:10.3390/microorganisms8050709]
2. Śliżewska K, Markowiak-Kopeć P, Śliżewska W. The Role of Probiotics in Cancer Prevention. Cancers. 2020;13(1). [PMID] [PMCID] [DOI:10.3390/cancers13010020]
3. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell research. 2020;30(6):492-506. [PMCID] [DOI:10.1038/s41422-020-0332-7] [PMID]
4. Yavari M, Ahmadizadeh C. Effect of the Cellular Extract of Co-cultured Lactobacillus Casei on BAX and Human β-Defensin 2 Genes Expression in HT29 Cells. Intern Med Today. 2020;26(4):364-381. [DOI:10.32598/hms.26.4.3277.1]
5. Kahouli I, Tomaro-Duchesneau C, Prakash S. Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. J Med Microbiol. 2013;62(Pt 8):1107-23. [DOI:10.1099/jmm.0.048975-0] [PMID]
6. Ahagh MH, Dehghan G, Mehdipour M, Teimuri-Mofrad R, Payami E, Sheibani N, et al. Synthesis, characterization, antiproliferative properties and DNA binding of benzochromene derivatives: Increased Bax/Bcl-2 ratio and caspase-dependent apoptosis in colorectal cancer cell line. Bioorganic chem. 2019;93:103329. [DOI:10.1016/j.bioorg.2019.103329] [PMID]
7. Fathi-zavoshti H, Douroud N, Shahbazi N, Abbasi A. Evaluating the role of postbiotics as a new generation of probiotics in health and diseases. J Ardabil Univ of Med Sci. 2020;19(4):381-99. [DOI:10.29252/jarums.19.4.381]
8. Talebi S, Makhdoumi A, Bahreini M, Matin MM, Moradi HS. Three novel Bacillus strains from a traditional lacto-fermented pickle as potential probiotics. J Appl Microbiol. 2018;125(3):888-96. [DOI:10.1111/jam.13901] [PMID]
9. Adibpour N, Hosseininezhad M, Pahlevanlo A, Hussain MA. A review on Bacillus coagulans as a Spore-Forming Probiotic. Appl Food Biotechnol. 2019;6(2):91-100.
10. Madempudi RS, Kalle AM. Antiproliferative Effects of Bacillus coagulans Unique IS2 in Colon Cancer Cells. Nutr Cancer. 2017;69(7):1062-8. [DOI:10.1080/01635581.2017.1359317] [PMID]
11. Kalaycı Yüksek F, Gümüş D, Gündoğan G, Anğ Küçüker M. Cell-Free Lactobacillus sp Supernatants Modulate Staphylococcus aureus Growth, Adhesion and Invasion to Human Osteoblast (HOB) Cells. Curr Microbiol. 2021;78 (1):125-32. [DOI:10.1007/s00284-020-02247-1] [PMID]
12. De Marco S, Sichetti M, Muradyan D, Piccioni M, Traina G, Pagiotti R, et al. Probiotic Cell-Free Supernatants Exhibited Anti-Inflammatory and Antioxidant Activity on Human Gut Epithelial Cells and Macrophages Stimulated with LPS. J Evid Based Complementary Altern Med. 2018;2018:1756308. [DOI:10.1155/2018/1756308] [PMID] [PMCID]
13. Rasuli E, Tafvizi F, Tajabady Em. Antiproliferation Effect of Heat Killed Indigenous Probiotic Bacteria on AGS Cell Line. J Appl Microbiol Food Ind. 2017;3(1):42-52.
14. Vinothkanna A, Sathiyanarayanan G, Balaji P, Mathivanan K, Pugazhendhi A, Ma Y, et al. Structural characterization, functional and biological activities of an exopolysaccharide produced by probiotic Bacillus licheniformis AG-06 from Indian polyherbal fermented traditional medicine. Int J Biol Macromol. 2021;174:144-52. [DOI:10.1016/j.ijbiomac.2021.01.117] [PMID]
15. Mojibi P, Tafvizi F, Torbati MB. Cell-bound exopolysaccharide extract from indigenous probiotic bacteria induce apoptosis in HT-29 cell-line. Iran J Pathol. 2019;14(1):41. [DOI:10.30699/ijp.14.1.41] [PMID] [PMCID]
16. Fortin O, Aguilar-Uscanga B, Vu KD, Salmieri S, Lacroix M. Cancer Chemopreventive, Antiproliferative, and Superoxide Anion Scavenging Properties of Kluyveromyces marxianus and Saccharomyces cerevisiae var. boulardii Cell Wall Components. Nutr Cancer.

2018;70(1):83-96. [DOI:10.1080/01635581.2018.1380204] [PMID]

17. S AG, A HA. Potential anticancer activities of Rhus coriaria (sumac) extract against human cancer cell lines. Bioscie Rep. 2021;41(5): BSR20204384. [DOI:10.1042/BSR20204384] [PMID] [PMCID]
18. Adams CA. The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev. 2010;23(1):37-46. [DOI:10.1017/S0954422410000090] [PMID]
19. Orlando A, Refolo MG, Messa C, Amati L, Lavermicocca P, Guerra V, et al. Antiproliferative and pro-apoptotic effects of viable or heat-killed Lactobacillus paracasei IMPC2.1 and Lactobacillus rhamnosus GG in HGC-27 gastric and DLD-1 colon cell lines. Nutr Cancer. 2012;64(7):1103-11. [DOI:10.1080/01635581.2012.717676] [PMID]
20. Sung, H, Ferlay, J, Siegel, RL, Laversanne, M, Soerjomataram, I, Jemal, A, Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71(3):209-249. [DOI:10.3322/caac.21660] [PMID]
21. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. Int J cancer. 2021;149(4):778-89. [DOI:10.1002/ijc.33588] [PMID]
22. Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019;321(3):288-300. [DOI:10.1001/jama.2018.19323] [PMID]
23. Górska A, Przystupski D, Niemczura MJ, Kulbacka J. Probiotic bacteria: a promising tool in cancer prevention and therapy. Curr Microbiol. 2019;76(8):939-49. [DOI:10.1007/s00284-019-01679-8] [PMID] [PMCID]
24. Hassan Z. Anti-cancer and biotherapeutic potentials of probiotic bacteria. J Cancer Sci Ther. 2019;11:9-13. [DOI:10.4172/1948-5956.1000575]
25. Kamkar F, Haghighat S, Mahdavi M. Secreted Chemicals From Probiotic Bacteria Potentiate Th1 Pattern of Immune Cells and Apoptosis Induction in Breast Cancer and Gastric Adenocarcinoma Cell Lines. Immunoregulation. 2020;3(1):15-28. [DOI:10.32598/IMMUNOREGULATION.3.1.2]
26. Liu C-F, Pan T-M. In vitro effects of lactic acid bacteria on cancer cell viability and antioxidant activity. J Food Drug Anal. 2010;18(2). [DOI:10.38212/2224-6614.2287]
27. Dolati M, Tafvizi F, Salehipour M, Movahed TK, Jafari P. Inhibitory effects of probiotic Bacillus coagulans against MCF7 breast cancer cells. Iran J Microbiol 2021;13(6):839-47. [PMCID] [DOI:10.18502/ijm.v13i6.8089] [PMID]
28. Karimi Ardestani S, Tafvizi F, Tajabadi Ebrahimi M. Molecular detection of heat-killed probiotic bacteria and study of apoptosis induction on colon cancer HT-29 cell line. Iran J Med Microbiol. 2016;10(2):42-52.
29. Chuah L-O, Foo HL, Loh TC, Alitheen NBM, Yeap SK, Mutalib NEA, et al. Postbiotic metabolites produced by Lactobacillus plantarum strains exert selective cytotoxicity effects on cancer cells. BMC Complement Altern Med. 2019;19(1): 1-12. [PMID] [PMCID] [DOI:10.1186/s12906-019-2528-2]
30. Dehghani N, Tafvizi F, Jafari P. Cell cycle arrest and anticancer potential of probiotic Lactobacillus rhamnosus against HT-29 cancer cells. BIOIMPACTS. 2021;11(4):245-52. [DOI:10.34172/bi.2021.32] [PMID] [PMCID]
31. EKrami M, Rafiei Tabatabei R. Investigation about the Mutagenic and Carcinogenic Effects of Probiotice Bacteria with Using Salmonella typhimurium TA100 and Rat Liver Microsomes (S9). J Animals Physiol Dev. 2019;12(4):49-59.
32. Ranjbar S, Seyednejad SA, Azimi H, Rezaeizadeh H, Rahimi R. Emerging Roles of Probiotics in Prevention and Treatment of Breast Cancer: A Comprehensive Review of Their Therapeutic Potential. Nutr Cancer. 2019;71(1):1-12. [DOI:10.1080/01635581.2018.1557221] [PMID]
33. Lee JA, Ko JH, Jung BG, Kim TH, Hong JI, Park YS, et al. Fermented Prunus mume with probiotics inhibits 7,12-dimethylbenz[a]anthracene and 12-o-tetradecanoyl phorbol-13-acetate induced skin carcinogenesis through alleviation of oxidative stress. Asian Pac J Cancer Prev. 2013;14(5):2973-8. [DOI:10.7314/APJCP.2013.14.5.2973] [PMID]
34. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. J Pediatr. 2010;156(2 Suppl):S8-15. [DOI:10.1016/j.jpeds.2009.11.014] [PMID]
35. Hassan Z, Mustafa S, Rahim RA, Isa NM. Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of Enterococcus faecalis and Staphylococcus hominis isolated from human breast milk. In Vitro Cell Dev Biol Anim. 2016;52(3):337-48. [DOI:10.1007/s11626-015-9978-8] [PMID]

36. Er S, Koparal AT, Kivanc M. Cytotoxic effects of various lactic acid bacteria on Caco-2 cells. Turkish J Biol. 2015;39(1):23-30. [DOI:10.3906/biy-1402-62]

37. Karimi Ardestani S, Tafvizi F, Tajabadi Ebrahimi M. Heat-killed probiotic bacteria induce apoptosis of HT-29 human colon adenocarcinoma cell line via the regulation of Bax/Bcl2 and caspases pathway. Hum Exp Toxicol. 2019;38(9):1069-81. [DOI:10.1177/0960327119851255] [PMID]

38. Harandi, Z., Beheshti Maal, K., Monajemi, R. The High Anti-cytotoxic Effects of Novel Lactobacillus Plantarum HBM-IAUF-1 and Lactococcus Lactis HBM-IAUF-8 against SK-BR-3 Breast Cancer Cell Line. Biol J Microorganism. 2020; 9(36): 59-68.

39. Shahniani A, Bamzadeh Z, Mahmoudnia F, Rouhi L. In vitro Apoptosis Induction in Prostate Cancer Cells (PC-3) using Bacillus licheniformis Supernatant. Iran J Med Microbiol. 2020; 14(6): 618-42. [DOI:10.30699/ijmm.14.6.618]

40. Elmanama IA, Elmanama AA, Al Zaharna MM, Al-Reefi MR. In vitro Anticancer Activity Effect of Extracellular Metabolites of Some Bacterial Species on HeLa Cell Line. IUG J Nat Stud. 2020;28(2).