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Cytotoxic effects of enterococci isolated from traditional Iranian dairy products on breast cancer cell line SK-BR-3

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ABSTRACT

Breast cancer is a prevalent and heterogeneous disease affecting women worldwide. With its complex nature, effective treatment options remain challenging to develop. Probiotics, known as functional foods, have emerged as potential candidates in the fight against breast cancer, both in vivo and in vitro. Lactic Acid Bacteria (LAB), a group of probiotics, have been shown to have beneficial effects on host health and possess the potential to serve as adjuvant therapy for breast cancer prevention and treatment. This can be achieved through modulating immune responses and influencing the breast microbial community. This study aimed to screen and characterize enterococci from Iranian traditional cheese and detect its cytotoxic effects on SK-BR-3 breast cancer cell line. The Lactic Acid Bacteria were isolated from two Iranian cheese samples after enrichment culture in Lactobacillus de Man, Rogosa and Sharpe (MRS) broth and then streak plate method on MRS agar. The LAB isolates were identified using macroscopic, microscopic, and molecular analyses. The cytotoxicity of LAB on SK-BR3 human breast cancer cell line was assessed using the MTT assay with bacterial concentrations of 100, 250, 500, and 1000 µg/ml. Molecular analysis revealed that the two isolates obtained in this study were closely related to *Enterococcus* species. They were designated as *Enterococcus faecalis* HBM-IAUF-3 and *Enterococcus hirae* HBM-IAUF-5, and their 16s-rDNA genome sequences were deposited in GenBank, NCBI under the accession numbers MG757697 and MG757702, respectively. Both *Enterococcus faecalis* HBM-IAUF-3 and *Enterococcus hirae* HBM-IAUF-5 exhibited the highest cytotoxicity against SK-BR3 cancer cells at a concentration of 1000 µl/ml after 72 hours.This study reports the identification of *Enterococcus* strains from traditional Iranian dairy products that demonstrated cytotoxic effects on breast cancer SK-BR-3 cells. Encouraging the consumption of traditional organic dairy products and locally fermented foods may significantly reduce the risk of cancer. Moreover, the increased application of probiotics in the food industry holds promise for promoting health and combating cancer development. Probiotics can also be explored as potential beneficial agents in food and medical biotechnology.

1. INTRODUCTION

Breast cancer continues to be a significant cause of illness and death globally (1). Numerous factors, such as obesity, a diet high in sugary foods, alcohol intake, family history of breast cancer, age at menopause, and the use of hormonal therapy during menopause, have been linked to an increased risk of developing breast cancer (2-5). Despite advancements in treatment options like chemotherapy and radiation therapy, breast cancer rates and mortality rates remain high (6). Breast cancer patients often experience immune dysfunction, characterized by weakened immune responses, including delayed sensitivity, decreased ability to destroy harmful cells, reduced immune cell production, and reduced production of immune system signaling molecules (7). Therefore, the consumption of substances that boost the immune system is crucial for controlling breast cancer. Probiotics, live microorganisms that provide health benefits to the body when taken in sufficient quantities, have gained popularity for their potential to promote health and prevent and treat early-stage cancers. Long-term probiotic consumption has been associated with a significant reduction in breast cancer formation and proliferation (6-7). Laboratory-based studies using animal models and human breast cancer cells have demonstrated the anti-tumor effects of probiotics. Probiotics can enhance cellular, nonspecific immune responses by activating macrophages, natural killer cells, and antigen-specific cytotoxic T-lymphocytes, as well as releasing various cytokines in a dosage- and strain-dependent manner (8). Probiotics exert immunomodulatory functions through the regulation of gene expression and signaling pathways in host cells, as well as the activation of toll-like receptors (9). The development of chemotherapeutic and synthetic drug-resistant tumors, along with their life-threatening side effects and the adverse effects of surgery and hormone therapies, has led to increased research on the anticancer potentials of probiotics (10). Several probiotic strains, such as *Bifidobacterium animalis* Bb-12, *Lactobacillus casei Shirota, Bifidobacterium lactis* DR10, *Lactobacillus johnsonii* La1, and *Lactobacillus rhamnosus* GG, are known for their immunomodulatory characteristics (8). Probiotic strains, including *Bifidobacterium animalis*, *Lactobacillus acidophilus, Bifidobacterium infantis, Lactobacillus paracasei*, and *Bifidobacterium bifidum*, have been found to reduce cancer cell growth in MCF7 cells (10). Administration of fermented milk with *Lactobacillus helveticus* R389 has demonstrated an immunoregulatory response in mice with breast cancer, suggesting its potential use as an immune adjuvant therapy for malignancies (11). The administration of *Lactobacillus Plantarum* enriched with selenium nanoparticles not only increased levels of interferon (IFN)-γ and IL-2, but also improved natural killer cell activity in mice (12). Various studies have shown the anti-carcinogenic effects of LAB against different cancer cell lines. For example, *Lactobacillus bulgaricus* and *Streptococcus thermophiles* have been effective against gastrointestinal cancers, while *Lactobacillus acidophilus* and *Lactobacillus casei* have shown positive results against colorectal tumor LS315 cell line. Similarly, *Lactobacillus casei* and *Lactobacillus paracasei* have exhibited anti-cancer effects against bone marrow cancer K562 cell line, and *Enterococcus lactis* IW5 and *Enterococcus faecalis* are effective against breast cancer cells (10, 13-17). Numerous health benefits have been associated with the consumption of probiotics, as proven by researchers (18-19). Several reports indicate that enterococci have antiproliferative effects on cancer cell lines. The anti-cancer effects of *Enterococcus faecium* on ovarian cancer cells as well as different human cancer cell lines have been reported (20-21). Recently the probiotic properties as well as antibacterial effects of tree strains of *Enterococcus faecium* isolated from dairy products against pathogenic bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* have been indicated (22). The goal of this study was to isolate and identify probiotic Enterococci strains from

cheese products and assess their cytotoxic effects on the SK-BR-3 breast cancer cell line.

2. MATERIALS AND METHODS

Collection and Isolation of LAB

Samples of traditional cheese were collected from Hafshejan City, Chaharmahlo Bakhtiyari province, and Yahyaabad village, Isfahan province, in Iran. Sterile tubes were used for the collection process. The collected samples were then taken to the microbiology laboratory. To create bacterial suspensions from each cheese sample, 10 grams of the cheese were combined with 100 ml of sterile distilled water and shaken for 30 minutes. After that, 10 ml of the bacterial suspension from each sample was transferred to 200 ml of lactobacillus de Man, Rogosa and Sharpe (MRS) broth and incubated anaerobically at 37°C for 24 hours. Dilutions were then made from each of the samples, and 10 µl from each dilution was streaked on MRS medium. The streaked plates were incubated at 37°C for 72 hours. For colony purification, individual colonies were isolated using a sterile loop and streaked on MRS agar. These plates were then incubated at 37°C for 24 hours (13). Identification of the Enterococcus spp. was done through macroscopic, microscopic, and biochemical examinations. Only the colonies that were Gram-positive and catalase-negative cocci were selected. These selected colonies were preserved at -80°C in MRS broth containing 25% (v/v) glycerol (13, 15).

DNA Extraction and Amplification of LAB 16S-rDNA Gene Sequences

The colonies of pure LAB were transferred from the culture medium to 10 ml of sterile distilled water (15). Then, this suspension was transferred to a sterile 15 ml Falcon tube and centrifuged at 5000 g for 15 minutes. After removing the supernatant, the pellet was transferred to another sterile tube. DNA extraction was carried out using a DNA extraction kit (Bioneer, South Korea) from 1 mg of bacterial mass. PCR amplification was performed using LAB-specific universal primers that have been described previously (22, 23), (Table 1) in a thermo cycler system (Bio Rad). Each PCR reaction included the following components: 1 µl of each primer (200 pM), 0.75 µl of $MgCl_2$ (1.5 mM), 0.5 µl of dNTP mix (200 mM), 0.2 µl of Taq polymerase (1 U), 2.5 µl of PCR Buffer with $MgCl_2$, 2 µl of DNA template, and 19.05 µl of distilled water. The PCR program consisted of an initial denaturation at 94°C for 5 minutes, followed by 32 cycles of amplification. Each cycle involved denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension step was done at 72°C for 5 minutes. The PCR products were separated by electrophoresis in a 1% (w/v) agarose gel and visualized by staining with ethidium bromide. Subsequently, the PCR products were purified and sent for sequencing to Taligene Pars Co., located in Isfahan Science and Technology Town, Isfahan, Iran. The similarity of the sequences was determined using GenBank BLASTN analysis (23).

The MTT Assessment of LAB Cytotoxic Effects on SK-BR-3 Cell Line

The SK-BR-3 breast cancer cell line was acquired from the Pasteur Institute of Iran. To maximize bacterial quantity, isolated bacteria were initially cultured in MRS broth for 24 hours (cfu/ml). The microculture MTT assay was used to evaluate the cytotoxic effect of the metabolites secreted by the isolates on the SK-BR-3 cell line. In each well of a microplate, 180 µl of RPMI growth medium containing 5×10^3 cells in suspension was added. The cells were treated with three replications, receiving 20 µl of different bacterial concentrations (100, 250, 500, and 1000 µl/ml). As a negative control, the first well in each row was treated with 20 µl of RPMI 1640 medium, while the second well in each row received 20 µl of Doxorubicin (200 µg/ml) as a positive control (23). The remaining wells were treated with varying concentrations of bacterial suspensions, excluding the negative and positive controls. After incubating all plates at 37°C for 24, 48, and 72 hours, 20 µl of MTT solution was added to each plate and incubated at 37°C for 3 hours. The supernatant was then gently removed, and 150 µl of DMSO was added to dissolve the formazan crystals in the wells. The optical

density (OD) of each well was measured at 540 nm using a microplate ELISA reader (24).

Preparation of Different Concentrations of Bacterial Suspension

To prepare different concentrations of bacterial dilutions (100, 250, 500, and 1000 µl/ml), the purified bacteria were cultivated in MRS broth for 24 hours for optimal growth. RPMI 1640 medium was used to make the desired concentrations. Each bacterial concentration was added to microplate wells with three replications. The preparation of different concentrations was fulfilled for turbidometry analysis as described previously (10-12).

Statistical Experiments

The statistical analysis was done using SPSS 19.0 software. Differences among multiple groups were determined using one-way Anova, Two-way Anova, Friedman test, and Kruskal Wallis test. Multiple mean comparisons were carried out using Mann-Whitney U test and Wilcoxon signed ranks test. The data were presented as mean ± standard deviation, with a significance level of $P \leq 0.05$ (25).

3. RESULTS

LAB Macroscopic and Microscopic Traits

The examination of the isolated LAB strains from the cheese samples showed coccobacilli characteristics. The colonies appeared round, mucoid, and milky in MRS agar. Gram staining confirmed that the strains were Gram-positive and spore-free.

Molecular Characterization of Isolated LAB Using 16S-rDNA Sequencing

The strain isolated from the Hafshejan cheese sample was identified as *Enterococcus faecalis*. The sequence analysis showed a 353 bp nucleotide sequence, with a 99% similarity to the *Enterococcus faecalis* gene in NCBI GenBank. This isolate was named *Enterococcus faecalis* HBM-IAUF-3, and its partial sequence was deposited in GenBank under accession No. MG757697. The phylogenetic tree of *Enterococcus faecalis* HBM-IAUF-3 is shown in Figure 1.

The strain isolated from the Yahyaabad cheese sample was identified as *Enterococcus hirae* based on 16S-rDNA sequencing. The sequence analysis revealed a 703 bp nucleotide sequence, with a 99% similarity to the *Enterococcus hirae* gene in NCBI GenBank. This isolate was named *Enterococcus hirae* HBM-IAUF-5 (accession No: MG757702). The phylogenetic analysis supported the identification of *Enterococcus hirae* HBM-IAUF-5, as shown in Figure 2.

MTT Evaluation of LAB Cytotoxic Effects on SK-BR-3 Cell Line

The MTT Assay is a method commonly employed to assess cell cytotoxicity, proliferation, and viability in a well plate format. This study investigated the cytotoxic effects, exposure duration, and concentrations of lactobacilli sourced from cheese samples of Hafshejan and Yahyaabad over different durations: 24, 48, and 72 hours. Notably, the findings revealed significant differences ($P<0.05$) in mean values between two concentrations, 500 and 1000 µl/ml, of *Enterococcus faecalis* HBM-IAUF-3 compared to the control after a 24-hour duration. Moreover, the results indicated that the highest cell survival percentage of SK-BR-3 cell lines after 24 hours of treatment with varying concentrations of *Enterococcus faecalis* HBM-IAUF-3 suspensions was observed at 100 µl/ml (226.33 ± 20.54), while the lowest cell survival rate was recorded at 1000 µl/ml (109.13± 84.99). After 48 hours, the results indicated a noteworthy contrast between mean values at concentrations of 500 and 1000 µl/ml of *Enterococcus faecalis* HBM-IAUF-3 compared to the control, signifying significant differences. Within this timeframe, the lowest recorded cell survival percentage among SK-BR-3 lines subjected to various concentrations of *Enterococcus faecalis* HBM-IAUF-3 suspensions was at 1000 µl/ml (42.9 ± 34.48), while the highest survival rate was observed at 500 µl/ml (129.50 ± 31.68). The calculated IC50 for this isolate after 48 hours was 573.60 ± 0.37 µl/ml. Moving to the 72-hour mark, across all concentrations, no notable distinction was found compared to the control treatment.

During this duration, the least cell survival percentage among SK-BR-3 cell lines exposed to different concentrations of *Enterococcus faecalis* HBM-IAUF-3 suspensions was at 1000 µl/ml (25.30 ± 51.39), whereas the highest survival rate was noted at 500 µl/ml (57.80 ± 04.17). The measured IC50 for *Enterococcus faecalis* HBM-IAUF-3 was determined as 559.30 ± 0.38 µl/ml (Figure 3). Across all concentrations and time points (24, 48, and 72 hours), there were significant differences in the mean values of SK-BR-3 cell survival percentages. The lowest survival percentage for SK-BR-3 cell lines after 72 hours of exposure to various concentrations of *Enterococcus faecalis* HBM-IAUF-3 suspension was recorded at 1000 µl/ml (25.51 ± 3.39), while the highest survival rate was observed at 1000 µl/ml after 24 hours of treatment (226.20 ± 33.54). The measured IC50 for this strain at different time points remained consistent at 559.30 ± 0.38 µl/ml (Figure 4). As for *Enterococcus hirae* HBM-IAUF-5, the assessment after 24 hours indicated significantly higher SK-BR-3 cell survival percentages at concentrations of 100, 250, and 500 µl/ml compared to the control treatment. The lowest cell survival percentage after 24 hours of treatment with various concentrations of *Enterococcus hirae* HBM-IAUF-5 suspension was noted at 1000 µl/ml (98.19 ± 66.80), while the highest survival rate was observed at 500 µl/ml (190.13 ± 90.51). The IC50 measured for this isolate after 24 hours was 801.70± 0.42 µl/ml. The findings for *Enterococcus hirae* HBM-IAUF-5 at 48 hours revealed significantly different mean values in all concentrations except for 1000 µl/ml when compared to the control treatment. Among various concentrations of *Enterococcus hirae* HBM-IAUF-5 suspensions, the lowest cell survival percentage for SK-BR-3 cell lines after 48 hours was observed at 1000 µl/ml (119.35 ± 11.46), whereas the highest survival rate was noticed at 500 µl/ml (251.40 ± 17.65). The calculated IC50 for this isolate at 48 hours was 528.9± 0.44 µl/ml. Moving on to the 72-hour evaluation, the SK-BR-3 cell survival percentage at 1000 µl/ml was significantly lower than the control treatment. Specifically, the lowest survival percentage for SK-BR-3 cell lines after 72 hours of exposure to various concentrations of *Enterococcus hirae* HBM-IAUF-5 suspension was observed at 1000 µl/ml (49.30 ± 60.97), while the highest survival rate was noted at 100 µl/ml (95.40 ± 60.61). The IC50 for this isolate at 72 hours was calculated as 774.5± 0.39 µl/ml (Figure 5). Across all concentrations and time points (24, 48, and 72 hours), there were significant differences in the mean values of SK-BR-3 cell survival percentages. Specifically, the lowest cell survival percentage for SK-BR-3 cell lines after 72 hours of exposure to various concentrations of *Enterococcus hirae* HBM-IAUF-5 suspension was observed at 100 µl/ml (49.60 ± 3.97), while the highest survival after 24 hours of treatment was seen at 500 µl/ml (190.90 ± 13.51) (Figure 6).

4. DISCUSSION

The multifaceted mechanisms behind the anticancer effects of probiotics encompass a broad spectrum, including the suppression of microbiota growth linked to mutagen and carcinogen production, modulation of carcinogen metabolism, safeguarding DNA from oxidative damage, generation of anti-tumorigenic compounds, reduction of carcinogenic impact by binding or limiting mutagen absorption in the intestines, and the augmentation and regulation of the immune system (26, 27). In assessing the efficacy of anticancer drugs, their capability to detect and selectively induce apoptosis in cancer cells is pivotal. Identifying compounds that trigger apoptosis is crucial in screening for beneficial agents in cancer treatment (28). Research by Malik et al. (2018) revealed that *Enterococcus lactis* IW5 produces metabolites with cytotoxic potential against diverse cancer cells. These metabolites, following a 24-hour incubation, notably suppressed breast cancer cell growth. The cytotoxic impact of these metabolites demonstrated a positive disparity from the untreated control group, indicating an antiproliferative effect distinct from the *E. lactis* IW5 treated group (29). Moreover,

studies involving milks fermented by various LAB and bifidobacteria strains (such as *B. infantis, B. bifidum, B. animalis, L. acidophilus,* and *L. paracasei*) showcased in vitro inhibition of breast cancer cell line growth (8). Nami et al. assessed the anticancer activity of *Lactobacillus acidophilus* 36YL across breast, stomach, cervical, and colorectal cancer cell lines. Their findings highlighted that metabolite secreted by this strain exhibited notably potent cytotoxic effects, particularly against human colorectal cancer cells (HT-29) and Human Cervical Cancer Cells (HeLa) (19). These studies collectively underscore the potential of probiotics and their metabolites in combatting cancer through targeted inhibition of cancer cell growth and induction of cytotoxic effects. The research by Liu and Pan shed light on the impact of local lactobacilli strains, specifically *L. paracasei ssp. paracasei* NTU101 and *L. plantarum* NTU102, on human breast adenocarcinoma cells (MDA-MB-231). Their findings revealed that these strains induced a substantial arrest of cells at the G0/G1 phase in the cell cycle. Notably, they observed varied inhibitory effects on cancer cells among the different LAB strains, highlighting the robust anticancer activities exhibited by their Thailand local lactobacilli strains (30). De Moreno de LeBlanc et al. conducted research utilizing an in vivo breast cancer model in BALB/c mice, where the mice received subcutaneous injections of 4T1 mouse mammary adenocarcinoma cells. Their study demonstrated that feeding the mice fermented milk containing *Lactobacillus helveticus* R389 resulted in reduced tumor growth. Furthermore, they observed an increase in cytokines such as interleukin (IL)-10 and IL-4, indicating potential immune modulation associated with the reduction in tumor growth (31). Additionally, the suspensions of *Enterococcus faecium* CH3 exhibited robust inhibition of cellular proliferation in myeloma cells, as documented by a study conducted by researchers (32). These studies collectively highlight the potential of specific lactobacilli strains and *Enterococcus faecium* in exerting substantial anticancer effects, whether through cell cycle modulation, immune response modulation, or direct inhibition of cellular proliferation in cancer cells. Hassan et al. (2015) explored the potential of live, heat-killed cells (HKC) containing antioxidant extracts, and cytoplasmic fractions (CF) derived from *Enterococcus faecalis* and *Staphylococcus hominis* as agents against breast cancer. They treated the MCF-7 cell line with different concentrations (25, 50, 100, and 200 µg/mL) of live, HKC, and CF of these bacteria and assessed cytotoxicity over 24, 48, and 72 hours using the MTT assay. Their results showcased a significant reduction in MCF-7 cell proliferation by all three forms of bacteria, up to 33.29%, in a concentration- and time-dependent manner. They noted a robust anti-proliferative effect with substantial sub-G1 accumulation (up to 83.17%) in treated MCF-7 cells, along with a reduced number of cells in the G0/G1 phase (by 74.39%) (10). Sharma et al. (2018) reported that metabolites secreted by *Enterococcus hirae* 20c, *Enterococcus faecium* 12a, and *Enterococcus faecium* L12b selectively inhibited the in vitro proliferation of various human cancer cell lines such as HeLa, HCT-15, and A549 as well as normal human cells (PBMC) in a dose-dependent manner. Notably, these metabolites exhibited no activity against normal human peripheral blood monocytes (33). In a study novel lactococci as well as lactobacilli were isolated and identified from traditional Iranian dairy products such as yogurt and cheese, demonstrating anti-carcinogenic effects on the SK-BR-3 breast cancer cell line. It has been reported that *Lactobacillus plantarum* exhibited the highest cytotoxicity against SK-BR3 cancer cells after 72 hours at a concentration of 1000 µl/ml, while *Lactococcus lactis* showed the highest cytotoxicity after 72 hours at a concentration of 500 µl/ml. The authors encouraged the consumption of traditional organic dairy products and suggested the use of probiotics in the food industry could potentially contribute to cancer prevention and serve as beneficial components in food and medical biotechnology. These findings suggest a potential for specific bacterial components or metabolites in hindering cancer cell proliferation selectively, raising prospects for

targeted cancer therapies (34). So far there is no report of anti-carcinogenic effects of *Enterococcus faecalis* and *Enterococcus hirae* strains against breast cancer cell lines, so this study seems to be breaking new ground by exploring the anti-carcinogenic effects of *Enterococcus faecalis* HBM-IAUF-3 *and Enterococcus hirae* HBM-IAUF-5 strains against breast cancer cell lines. Identifying these strains from local dairy products and assessing their cytotoxic effects on SK-BR-3 cells provides valuable insights. The observed cytotoxicity of the secretion metabolites on the SK-BR-3 cells, as seen through microscopic analysis, highlights the potential of these strains to affect cancer cell viability. Using the MTT method to assess the cytotoxic effects further validated these strains' potential, revealing significant anti-cancer effects on SK-BR-3 cells. The concentration of *Enterococcus faecalis* HBM-IAUF-3 appeared to influence the cytotoxicity in a dose-dependent manner, while *Enterococcus hirae* HBM-IAUF-5 exhibited time-dependent effects. This difference might be attributed to varying metabolite secretion or the cellular response of cancer cells to these metabolites over time. The disparity in cytotoxic effects despite the low genetic distance between the two isolates raises interesting questions about their genetic divergence and potential variations in their mechanisms of action against cancer cells. This study opens avenues for further investigation into the molecular and cellular mechanisms underlying the anti-cancer effects of these *Enterococcus* strains, potentially offering novel insights into targeted cancer therapies.

5. CONCLUSION

This research represents a milestone in discovering and characterizing novel Enterococci strains from traditional Iranian cheese, *Enterococcus faecalis* HBM-IAUF-3 and *Enterococcus hirae* HBM-IAUF-5, which display significant anti-carcinogenic effects on the SK-BR-3 breast cancer cell line. The 16s-rDNA partial sequences of these isolates have been deposited in GenBank, NCBI, with accession numbers MG757697 and MG757702 for *Enterococcus faecalis* HBM-IAUF-3 and *Enterococcus hirae* HBM-IAUF-5, respectively. The highest observed cytotoxicity against SK-BR3 cancer cells was achieved after 72 hours at a concentration of 1000 µl/ml for both *Enterococcus faecalis* HBM-IAUF-3 and *Enterococcus hirae* HBM-IAUF-5. This groundbreaking discovery highlights the potential health benefits of incorporating traditional organic dairy products and locally fermented foods into diets as a means to significantly reduce cancer risk. Furthermore, the study emphasizes the potential of probiotics in the food industry, not only for their potential to mitigate cancer development but also for their promising role in medical and food biotechnology. This research opens avenues for future studies, promising new strategies for cancer prevention and potential applications in medical and food-related fields.

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TABLES

Table1. The sequences and the names of universal primers used in PCR (10-12).

Primer name	Primer Sequences
BU1(Forward)	**5'-**AACTGGAGGAAGGTGGGGAT**-3'**
BU1(Reverse)	**5'-**AGGAGGTGATCCAACCGCA**-3'**

FIGUREURE LEGENDS

Fig. 1. The distance tree of the 16s-rDNA gene of *Enterococcus faecalis* HBM-IAUF-3 generated by Mega X software using the neighbor-joining tree method.

Fig. 2. The distance tree of the 16s-rDNA gene of *Enterococcus hirae* HBM-IAUF-5 generated by Mega X software using the neighbor-joining tree method.

Fig. 3. The impact of *Enterococcus faecalis* HBM-IAUF-3 supernatant on the viability of SK-BR-3 cells at different concentrations and incubation times after 24, 48, and 72 hours of treatment. The mean viability ratio ± SD is reported.

Fig. 4. The effects of various concentrations of *Enterococcus hirae* HBM-IAUF-5 supernatant on the viability of SK-BR-3 cells compared to the exposure time. The mean viability ratio ± SD is presented.

Fig. 5. The effects of *Enterococcus faecalis* HBM-IAUF-3 supernatant on the viability of SK-BR-3 cells at different concentrations after 24, 48, and 72 hours of treatment. The mean viability ratio ± SD is reported. The lowest survival percentage for SK-BR-3 cell lines after 72 hours of exposure to various concentrations of *Enterococcus hirae* HBM-IAUF-5 suspension was observed at 1000 µl/ml (49.30 ± 60.97), while the highest survival rate was noted at 100 µl/ml (95.40 ± 60.61). The IC50 for this isolate at 72 hours was calculated as 774.5± 0.39 µl/ml

Fig. 6. The effects of various concentrations of *Enterococcus hirae* HBM-IAUF-5 supernatant on the viability of SK-BR-3 cells concerning the exposure time. The mean viability ratio ± SD is presented.

FIGURES

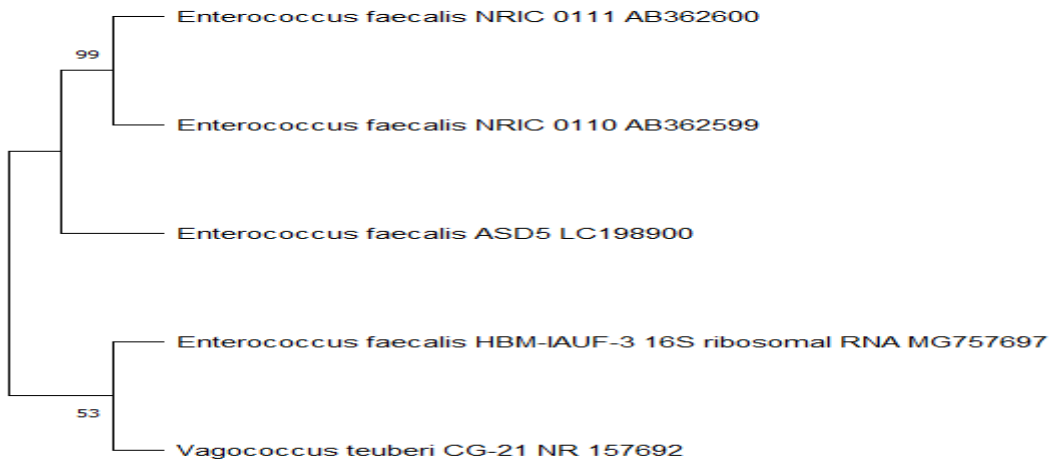


Figure 1.

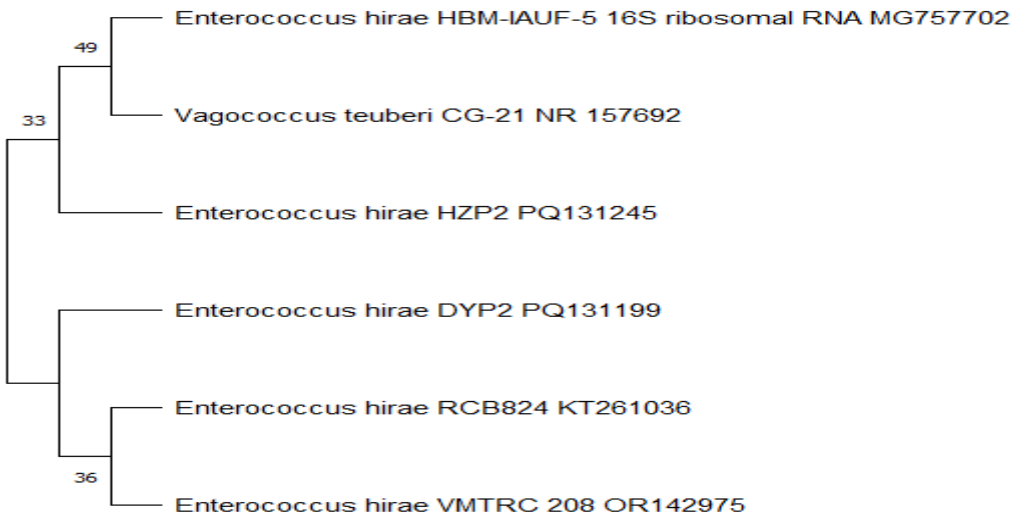


Figure 2.

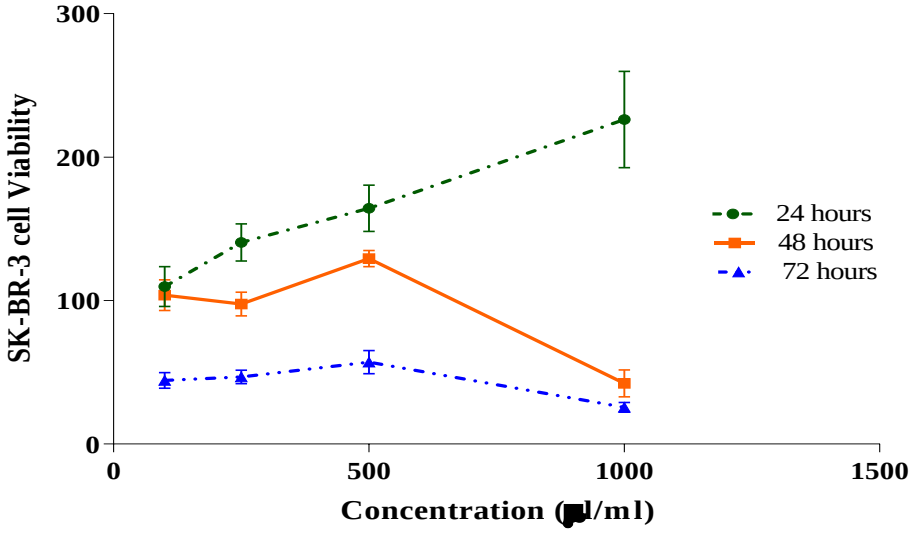


Figure 3.

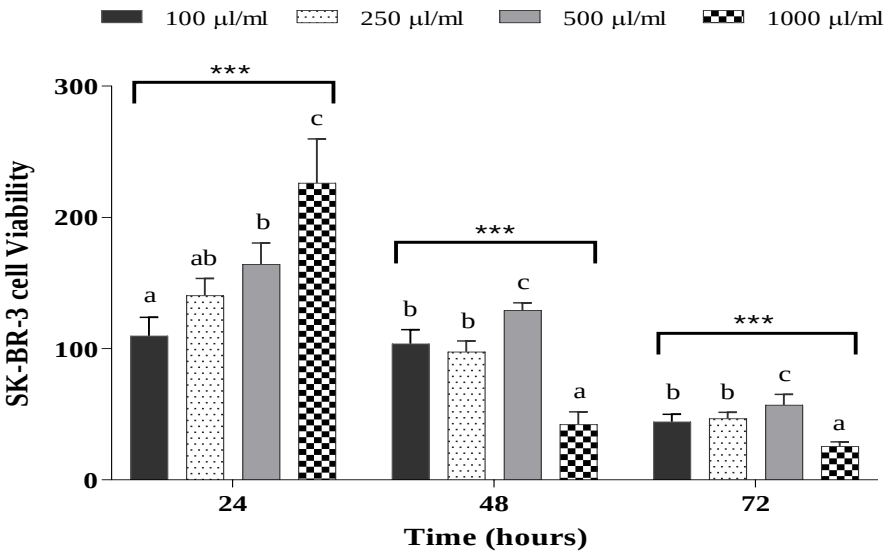
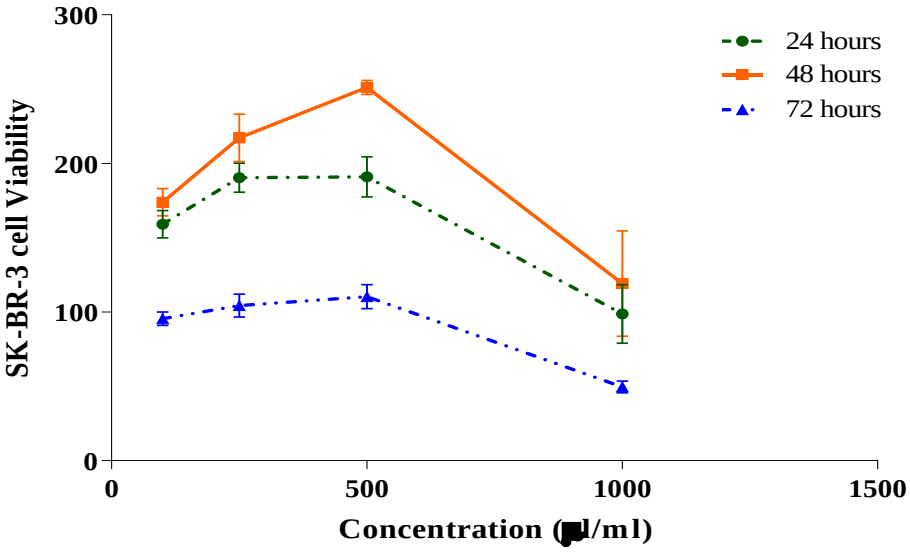


Figure 4.



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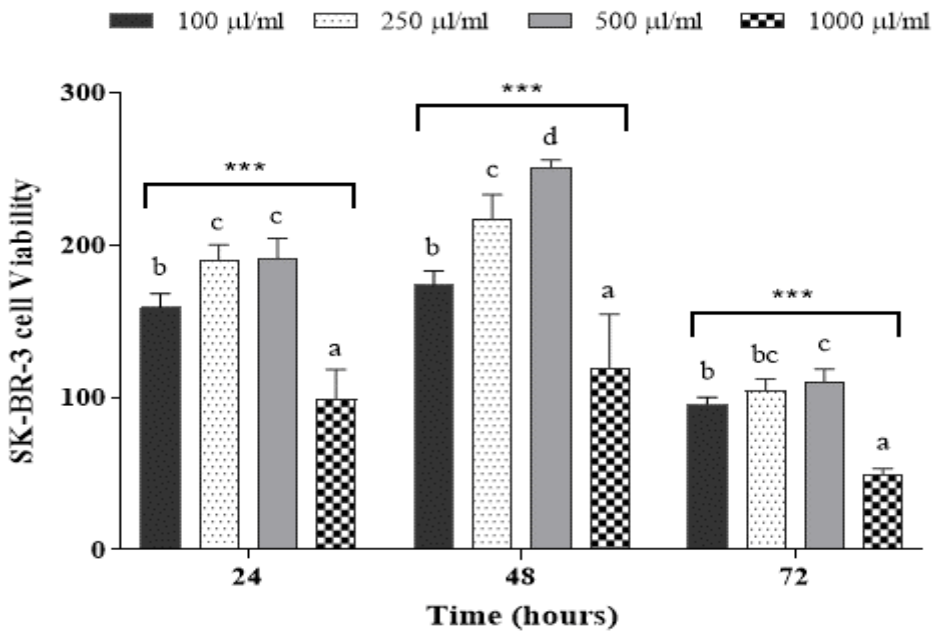
100 µl/ml
250 µl/ml
500 µl/ml
1000 µl/ml
SK-BR-3 cell Viability
300
200
100
0
***
***
***
b
c
c
a
b
c
d
a
b
bc
c
a
24
48
72
Time (hours)

Figure 6.

مجله علوم و صنایع غذایی ایران

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مقاله علمی-پژوهشی

اثرات سایتوتوکسیک انتروکوک های جدا شده از لبنیات سنتی ایران بر رده سلولی سرطان سینه SK-BR-3

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چکیده

سرطان سینه یک بیماری پراکنده و ناهمگن است که زنان در سراسر جهان را تحت تأثیر قرار می‌دهد و با طبیعت پیچیده‌ای که دارد، گزینه‌های درمانی موثر برای آن همچنان چالش‌برانگیز هستند. پروبیوتیک‌ها که به عنوان غذاهای کارآمد شناخته می‌شوند، به عنوان گزینه بالقوه در مبارزه با سرطان سینه، هم در محیط زنده و هم در محیط آزمایشگاهی، معرفی شده‌اند. باکتری‌های اسید لاکتیک (LAB)، یک گروه از پروبیوتیک‌ها، اثرات مفیدی بر روی سلامت میزبان دارند و توانایی پیشگیری و درمان سرطان سینه را دارند. این امر از طریق تنظیم پاسخ‌های ایمنی و تأثیر بر جامعه میکروبی قابل دستیابی است. هدف از این مطالعه، غربالگری و شناسایی پروبیوتیک‌های انتروکوک از پنیر سنتی ایرانی و تشخیص اثر سیتوتوکسیک آن ها بر روی سلول‌های سرطانی پستان SK-BR-3 بود. باکتری‌های لاکتیک از دو نمونه پنیر ایرانی پس از غنی سازی در محیط کشت MRS و سپس روش کشت خطی روی محیط کشت MRS آگار جداسازی شدند. جدایه‌های باکتری لاکتیک با استفاده از روش های میکروسکوپی و مولکولی شناسایی شدند. سایتوتوکسیسیته جدایه های لاکتیک اسید با استفاده از آزمون MTT با غلظت‌های ۱۰۰، ۲۵۰، ۵۰۰ و ۱۰۰۰ میکروگرم بر میلی‌لیتر بر روی سلول‌های سرطانی SK-BR3 بررسی شد. آزمون های مولکولی نشان داد که دو جدایه دریافت شده در این مطالعه به صورت نزدیکی مرتبط با جنس *انتروکوکوس* هستند. آن ها به ترتیب به عنوان آنتروکوکوس فکالیس HBM-IAUF-3 و آنتروکوکوس هیرائه HBM-IAUF-5 نامگذاری شدند و توالی ژنوم 16s-rDNA آن ها در بانک جهانی ژن در پایگاه NCBI تحت شماره‌های دسترسی MG757697 و MG757702 ثبت شد. هر دو سویه *آنتروکوکوس فکالیس* HBM-IAUF-3 و *آنتروکوکوس هیرائه* HBM-IAUF-5 بیشترین سمیت سلولی را در برابر سلول های سرطانی SK-BR3 در غلظت ۱۰۰۰ میکرولیتر بر میلی لیتر پس از ۷۲ ساعت نشان دادند. این مطالعه شناسایی سویه‌های جدید انتروکوکوس از لبنیات سنتی ایران را گزارش می‌کند که اثرات سیتوتوکسیک بر روی رده سلولی SK-BR-3 سرطان پستان را نشان دادند. لذا تشویق به مصرف لبنیات ارگانیک سنتی و غذاهای تخمیر شده محلی به طور قابل توجهی خطر ابتلا به سرطان را کاهش می دهد. علاوه بر این، افزایش کاربرد پروبیوتیک ها در صنایع غذایی نویدبخش ارتقای سلامت و مبارزه با توسعه سرطان است. پروبیوتیک ها همچنین می توانند به عنوان عوامل مفید بالقوه در مواد غذایی و بیوتکنولوژی پزشکی مورد بررسی قرار گیرند.