ORIGINAL ARTICLE

Analyzing of colorectal cancerrelated genes and microRNAs expression profiles in response to probiotics *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* in colon cancer cell lines

Kosar Naderi Saffar[1] · Mohaddeseh Larypoor[1] · Maryam Bikhof Torbati[2]

Abstract

Background Colorectal cancer is the world's third most frequent cancer and the fourth cause of mortality. Probiotics play an important function in preventing metastasis as well as the growth and proliferation of malignant cancer cells.

Methods and results The study investigated the anticancer effect of *Lactobacillus acidophilus* supernatant and *Saccharomyces cerevisiae* yeast on colorectal cell lines, including HT29 and SW480 as a colorectal cancer model. The extract from the *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* standard probiotics were prepared, and probiotics characterization was confirmed by morphological and Biochemical tests. The viability of HT29 and SW480 colon cancer cell lines on effecting probiotic supernatant was evaluated by measuring the MTT colorimetric method. Comparison of the expression profile of several genes involved in apoptosis, cell cycle, and metastatic pathway in HT29 and SW480 cell lines with the treatment of probiotics extract showed an upregulation in the *BAX*, *CASP3*, and *CASP9* and down regulation *BCl-2*, *MMP2*, and *MMP9* genes. Also, a comparison of microRNA expression profiles indicated an increase of miR 34, 135, 25, 16, 195, 27, 98, let7 and a decrease of miR 9, 106b, 17, 21, 155, 221.

Conclusions and discussion The findings of this study indicate that probiotics can effectively suppress the proliferation of colorectal cancer cells and even reverse their development. Additionally, the study of cellular genes and miRNA profiles associated with colorectal cancer have demonstrated that our probiotics play a crucial role in CRC prevention by increasing the expression of tumor suppressor microRNAs and their target genes while decreasing oncogenes.

Keywords Colorectal cancer · Probiotics · miRNA · *Lactobacillus acidophilus* · *Saccharomyces cerevisiae*

Introduction

The effect of probiotic on digestive diseases such as irritable bowel syndrome, gastrointestinal disorders, prevention of Helicobacter infection, inflammatory bowel disease, diarrhea and allergic intolerance such as atopic dermatitis has been shown. The effect of probiotics on the treatment of obesity, insulin resistance syndrome, type 2 diabetes and non-alcoholic fatty liver disease has been proven. In addition, the positive effects of probiotics on human health have been shown by increasing and regulating the level of immunity of the body. The benefits of probiotics have been shown to prevent various types of cancer and cancer-related side effects [1].Currently, widespread research has been devoted to foods that contain probiotic strains. Probiotics are known as live microorganisms, and when consumed in appropriate amounts, have a beneficial effect on the health of the host. Although lactic acid bacteria and bifidobacteria are mainly probiotic microorganisms, several yeasts such as Saccharomyces and Kluyveromyces strains are also used for their properties as probiotics. Due to being resistant to antibiotics, yeasts also show the ability to exchange with probiotic bacteria [2]. Prebiotics are defined by Gibson and Roberfreud in 1995 as indigestible or poorly digestible compounds that grow against digestive enzymes in the human colon. According to this definition, a prebiotic has been selected as a fermented substance that has beneficial effects on the health

✉ Mohaddeseh Larypoor
  m.larypoor@iau-tnb.ac.ir

[1] Department of Biotechnology, Faculty of Biological Sciences, Tehran North Branch, Islamic Azad University, Tehran, Iran

[2] Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, YadegarEmam Khomeini Branch, Shahre-Rey, Iran

of the host by making specific changes to the composition or activity of the microbiota of the digestive system [3]. Intestinal flora probiotic bacteria not only improve digestion but also create chemicals and molecules like vitamins and antibiotics. The human intestine contains many types of beneficial and harmful bacteria that must be balanced in the human body; otherwise, in addition to disrupting the functions of the intestinal flora, it causes various clinical symptoms [4]. The composition of the intestinal flora of each person is specific and is also influenced by the environment [5]. About 400 to 800 different species of bacteria live in the intestines, and yeasts also make up a small part of this flora [6]. Most of the intestinal flora, for example, 35–50% of the colon (large intestine), consists of bacteria [4]. Bacteroides, Clostridium, Fusobacteria, Ruminococcus, Enterobacteriaceae, Lactobacillus, Escherichia coli, Bifidobacteria, Peptococcus are beneficial intestinal bacteria [7]. Cancer is known as one of the most important public health problems all over the world, which is estimated to reach an incidence rate of 22.2 million cases by 2030. Moreover, in 2020, 1,806,590 new cancer cases and 606,520 cancer deaths are approximately estimated to be recorded in the United States [8]. Cancer is regarded as the biggest cause of death worldwide, accounting for 13% of all mortality. Gastrointestinal cancers have the highest mortality rates among other kinds of cancer, such as colon, liver, and gastric cancers. Improving one's lifestyle (healthy diet, regular exercise, avoiding alcohol and tobacco use, etc.) or reducing exposure to chemicals and carcinogenic radiation can help prevent all types of cancer [9]. Colorectal cancer is the third most common cancer worldwide and the fourth leading cause of cancer-related deaths. It is a disease with multiple risk factors, including genetic, epigenetic, environmental, and lifestyle parameters [10].

It has been discovered that probiotics can inhibit cancer progression. Probiotics have been shown to have anticancer effects through a number of different mechanisms, including the facilitation of anticancer compounds, the enhancement of the immune system, the improvement of the intestinal barrier, the inhibition of cancer cell proliferation, and the induction of apoptosis in cancer cells [11].

Today, the anti-mutagenic and anti-carcinogenic effects of probiotics have been noticed. Experiments have shown that lactobacilli have anti-mutagenic action while expanding in specific culture mediums and can reduce the genotoxic effects of chemical substances [12]. Animal studies have demonstrated that specific strains of *Lactobacillus acidophilus* (*L. acidophilus*) inhibit the production of carcinogenic chemicals by decreasing the activity of enzymes such as beta-glucuronidase, azoreductase, and nitroreductase. In the meantime, Lactobacillus exerts resistance to colon cancer in several forms, including living and lethal strains, strain components, and metabolites of live strains.

Live lactic acid bacteria (LABs) activate cysteine aspartic protease, which causes colon cancer cells to undergo apoptosis. This, in turn, suppresses the proliferation of colon cancer cells such as Caco-2 [13]. However, the anti-mutagenic and anti-carcinogenic mechanisms of probiotic bacteria have not been clearly defined yet [14].

Saccharomyces cerevisiae is a unicellular yeast and one of the most valuable microorganisms in industry and genetic investigations [15]. Earlier research has demonstrated that representatives of the species Saccharomyces have probiotic and antibacterial characteristics [16]. This yeast has a round to spherical form with a thickness of 3 μm and a length of 2.5 to 10.5 μm that reproduces sexually and asexually [17]. Clinical study has indicated that *Saccharomyces cerevisiae* (*S. cerevisiae*) is regarded as a bio-therapeutic agent because of its antibacterial, antiviral, anti-carcinogenic, antioxidant, anti-inflammatory, and immune-modulating capabilities. Its oral or intramuscular injection can greatly improve the health of the host [18]. This strain is frequently utilized as a low-cost and efficient probiotic against gastrointestinal disorders such as inflammatory bowel disease and treating many forms of diarrhea [19].

The use of probiotics, particularly *Saccharomyces cerevisiae* var. *boulardii*, is the most frequent technique for the treatment of antibiotic-associated diarrhea (ADA) and *Clostridium difficile* infection (CDI), which a recently meta-analysis research has proven to be successful [20]. Crohn's disease (CD) is one of the inflammatory bowel disorders, and intake of this Saccharomyces can considerably lower the amount of CD, manage chronic inflammation and repair the intestinal epithelial tissue [21]. This probiotic yeast can decrease the tumorigenic effects of colorectal cells in humans and reduce the incidence of colorectal cancer by inducing the death of cancer cells. *Saccharomyces cerevisiae* var. *boulardii* dramatically lowers the expression of many tumor-inducing genes, including TNFα, interleukin-1β, and interleukin-17, and inhibits NF-κB and mTOR pathways [20].

Even though probiotics can modify the normal gut flora in the direction of balance or even preserve the proper balance of bacteria in the microbiome composition. They thus have a unique potential for both cancer prevention and cancer treatment. Micro RNAs, which are classified as small non-coding RNAs (ncRNAs) and have a length of about 22 nucleotides, regulate many cellular and developmental processes in biological systems by acting on epigenetic processes, chromatin structure, post-transcriptional gene silencing, or inhibition of translation. Alterations in the expression of micro RNAs in the cell are related to the occurrence of many diseases, including cancer.

Several studies have demonstrated that probiotics can inhibit the growth of tumor cells and metastasis through miRNAs, which is significant given the significant function

of miRNAs in modulating cellular activities. Yet, it is still unclear exactly how probiotics work on a biological level to treat cancers via miRNAs.

The anticancer effect of *L. acidophilus* and *S. cerevisiae* probiotics on two CRC cell lines is investigated in this study, as well as their effect on the expression of apoptosis genes, cell cycle, metastatic genes including BCL2 apoptosis regulator(*BCL2*), BCL2 associated X(*BAX*),caspase 3(*CASP3*),caspase 9(*CASP9*),matrix metallopeptidase 2(*MMP2*), matrix metallopeptidase9 (*MMP9*), and the expression profile of 14 related microRNA genes.

Materials and methods

L. acidophilus and *S. cerevisiae* culture

L. acidophilus(ATCC:4356) and *S. cerevisiae*(ATCC:9763) were purchased from Iranian Research Organization for Science and Technology (IROST).In order to prepare the supernatant, *Lactobacillus acidophilus* was cultured on autoclaved De Man–Rogosa–Sharpe (MRS) aerobically. *L. acidophilus* was inoculated into an MRS-broth culture medium (sigma;69966) and incubated at 37 °C for 4 h, and sub-cultured twice before the experiment.

Under these circumstances, the number of bacteria reached approximately 2.5×10^8 CFU. Following incubation, the strains were collected by centrifugation (6,000 g for 15 min at 4 °C). After that, they were washed three times in a buffer containing phosphate-buffered saline (PBS) with a pH of 7.4.

S. cerevisiae was incubated in PotatoDextroseBroth (PDB)culture medium at 30 °C for 24 h, and cell suspension at a concentration of 10^7 was obtained. Then obtained cells were centrifuged at 3000 g for 10 min. A 0.2 μm nylon filter (Milli-Q, Millipore, Germany) was utilized to filter the obtained supernatants. After then, obtained supernatants were kept at a temperature of − 20 °C until the conducting of the experiments.

Identification and antibiotic susceptibility of *L. acidophilus* and *S. cerevisiae*

L. acidophilus was identified by its Gram-positive, rod-shaped, catalase-negative and catalase-negative lactic acid bacteria [22]. Also *S. cerevisiae* is catalase and oxidase positive. Moreover, 18srRNA and 16srRNA primers [23] were used for molecular confirmation by Polymerase Chain Reaction (Table 1). Antibiotic sensitivity patterns of these isolates were determined by disk (Neo-Sensitabs, RoscoDiagnostica, Denmark) diffusion method using the CLSI 2014 guidelines [24].

Table 1 The sequence of 18srRNA and 16srRNA primers used in this study

		(bp)	Tm (°C)
18srRNA			
Forward	AGCTGGTTGATTCTGCCAG	19	53
Reverse	TGATCCTCCYGCAAGTTCAC	20	53
16srRNA			
Forward	AGAGGTTCCTGAGCTCAG	19	53
Reverse	ACAGCTTCCTTGTTACGATT	20	53

Cell culture

The HT-29 (Human Adenocarcinoma; Colorectal, C466) and SW480 (Human Adenocarcinoma; Colorectal, C506) Cell lines were obtained from the cell bank of the Iranian Pasteur institute (Tehran, Iran). Cells were grown in culture Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Fetal Bovine Serum, gibco, United Kingdom), 1% penicillin/streptomycin, and 1% Glutamax.

Co-culture of *L. acidophilus* and *S. cerevisiae* with HT-29 and SW480

Cells co-cultured with *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*were cultured in high-glucose DMEM cell culture media (without penicillin–streptomycin solution), supplemented with 10% FBS. For five minutes, cell suspensions were centrifuged at 1000 rpm. Cell culture incubated with 5 percent CO2 at 37 °C, the cell pellets were resuspended, plated into a 25-cm^2 culture flask, and allowed to develop. After 24 h, non-adherent cells were removed from the cell culture medium, which was then changed in accordance with the culture requirements. Cells were trypsinized with trypsin–EDTA after they had filled 80% of the culture flask in preparation for expansion and further tests.

MTT assay

The MTT assay is a colorimetric, cell-based assay used to measure cell viability and proliferation. It is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to a purple formazan product by mitochondrial dehydrogenases of metabolically active cells. The amount of formazan product generated is proportional to the number of viable cells in the sample.

Cells were seeded into a 96-well plate and allowed to adhere and grow overnight. After overnight incubation, the cells are treated with the *S. cerevisiae* and *L. acidophilus* supernatant (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/ml) and incubated for a 24, 48 and 72 h. After incubation,

MTT reagent is added to the wells and allowed to incubate for 4 h. The formazan product is then solubilized in a detergent solution and the absorbance is measured at 570 nm. The absorbance values are then compared to a control sample to determine the percentage of viable cells.

Analysis of *S. cerevisiae* and *L. acidophilus* genes expression on cell lines

Total RNA extraction

Total RNA extracted from the conditioned media-treated and untreated cell samples, it was performed using the Bon-yakhte reagent method suggested by the manufacturer (BON RNA Lysis buffer, BN-0011.33, Iran). Using Nano Drop (Nano Drop, Wilmington, USA) equipment, the RNA yield and purity were assessed.

cDNA synthesis

cDNA synthesizes to measure the expression of the selected miRNAs genes were performed by the BON-miR miRNA 1st-Strand cDNA Synthesis Kit (BN-0011.41) and to measure the expression of the mRNA genes used the cDNA synthesis Kit (BN-0011.37) according to the manufacturer's instructions by BON technology company, Iran.

Gene expression study

ABI-One step real-time PCR instrument was used to measure the expression level of miRNAs and mRNA genes. All primers were prepared by Bon Yakhte Technology Company, Iran with a concentration of 10 pmol (Table 2). Real-time PCR was performed to measure the expression levels of the target mRNAs using SYBR Master Mix reactions based on the use of SYBER green I dye were performed

Table 2 presents the primers used for real-time PCR

No.	Name		Seq	Size	Tm °C	Concentration
mRNA Genes						
1	GAPDH	F	CTTCCTTCCTGGGCAT	16	60	10 pmol
		R	GTCTTTGCGGATGTCCA	17		
2	BCL2	F	GATAACGGAGGCTGGGAT	18	60	10 pmol
		R	CAGGAGAAATCAAACAGAG	19		
3	BAX	F	CAAACTGGTGCTCAAG	16	60	10 pmol
		R	CACAAAGATGGTCACG	16		
4	CASP 9	F	GCCTCCACTTCCCCT	15	60	10 pmol
		R	GGTCCCTCCAGGAAAC	16		
5	CASP3	F	CACAGCACCTGGTTATTA	18	60	10 pmol
		R	TTGTCGGCATACTGTTTC	18		
6	H-MMP2	F	GCTCGTGCCTTCCAAGT	17	60	10 pmol
		R	AGTCCGTCCTTACCGTC	17		
7	H-MMP9	F	CGGACCAAGGATACAGTTT	19	60	10 pmol
		R	CTCAGTGAAGCGGTACATA	19		
miRNA Genes						
8	hsa-let-7-F		CGTGAGGTAGTAGGTTGTATA	21	60	10 pmol
9	hsa-miR-17-5p		CCAAAGGGCTTACAGT	16	60	10 pmol
10	hsa-miR-34a		ATGGTGGCAGTGTCTTA	17	60	10 pmol
11	hsa-mir-21-5p F		GGCTTGTCAGACTGATGT	18	60	10 pmol
12	H-miR-135-5P-F		TATGGCTTTTTATTCCTATG	20	60	10 pmol
13	hsa-miR-27a-3p		CTTCACAGTGGCTAAGTT	18	60	10 pmol
14	has-miR-98		GGTGAGGTAGTAAGTTGTATTG	22	60	10 pmol
15	Hsa-miR-25-3P		CATCGCACTTGTCTTGG	17	60	10 pmol
16	hsa-miR-106b-5p-F		AGTAAAGGGCTGACAGT	17	60	10 pmol
17	hsa-miR-93-3P-F		ATGACTACTGAGATAGCACT	20	60	10 pmol
18	hsa-miR-221-3p-F		ATTCAGGGCTACATTGTC	18	60	10 pmol
19	hsa-miR-195-5p-F		TTGGTAGCAGCACAGAAA	18	60	10 pmol
20	hsa-miR-155-5p		ACTTGGCTAATCGTGATAG	19	60	10 pmol
14	Forward 16–1		GGCATAGCAGCACGTAA	17	60	10 pmol
15	SNORD47 (F)		ATCACTGTAAAACCGTTC	18	60	10 pmol

using the kit of Bon Technology Company (Syber green Master Mix 2X, catalog number BN-0011.40) according to the manufacturer's instructions. Melt curves were obtained at 95 °C to confirm the amplification's specificity. To create the standard curves, qPCR amplification of cDNA was carried out using a serial dilution of the cDNA (10^{-1}–10^{-4}). To standardize the expression levels of the mRNAs/miRNA, GAPDH and SNORD47 were utilized as internal comparators in tandem with the control sample. The relative expression was calculated utilizing the difference in the CT values of the target RNAs after normalization to the RNA input level. The conventional $2^{-\Delta\Delta CT}$ computations were used to depict the relative quantification. All PCR reactions were carried out in triplicate.

Statistical analysis

The findings of investigations that were carried out in triplicate were given as the mean accompanied by the standard deviation. P value of less than 0.05 was established as the threshold for statistical significance. Using the Graph Pad Prism 8 program, nonlinear regression was performed to get the IC_{50} value, also known as the half-maximum inhibitory concentration (Graph Pad Software, Inc. La Jolla, CA, USA).

Results

Characterization of *S. cerevisiae* and *L. acidophilus*

Morphological characteristics

S. cerevisiae and *L. acidophilus* isolates grew on the medium after cultivation. To check the morphological characteristics, slides, and spreads were prepared from the single colonies grown on the culture medium, and after staining, they were observed with a light microscope, as shown in Fig. 1.

Biochemical characteristics

S. cerevisiae and *L. acidophilus* were confirmed according to standard Biochemical diagnostic tests (nitrogen absorption, oxidase, and catalase and antibiogram analysis). *S. cerevisiae* and *L. acidophilus* were cultured and examined with catalase, oxidase, nitrate and antibiogram tests. The results of catalase, oxidase, and nitrate tests and Antibiogram analysis are reported in Table 3.

Testing the influence of temperature, pH, and Bile salt on *S. cerevisiae* and *L. acidophilus* growth

All experiments were carried out in sterile tubes containing 10 mL of broth medium. After strains were cultured in broth

Fig. 1 Cell culture of **A** *S. cerevisiaes,* **B** *L. acidophilus,* andmorphological characteristics, of **C** *S. cerevisiae*), **D** *L. acidophilu* slides after staining, were observed with a light microscope
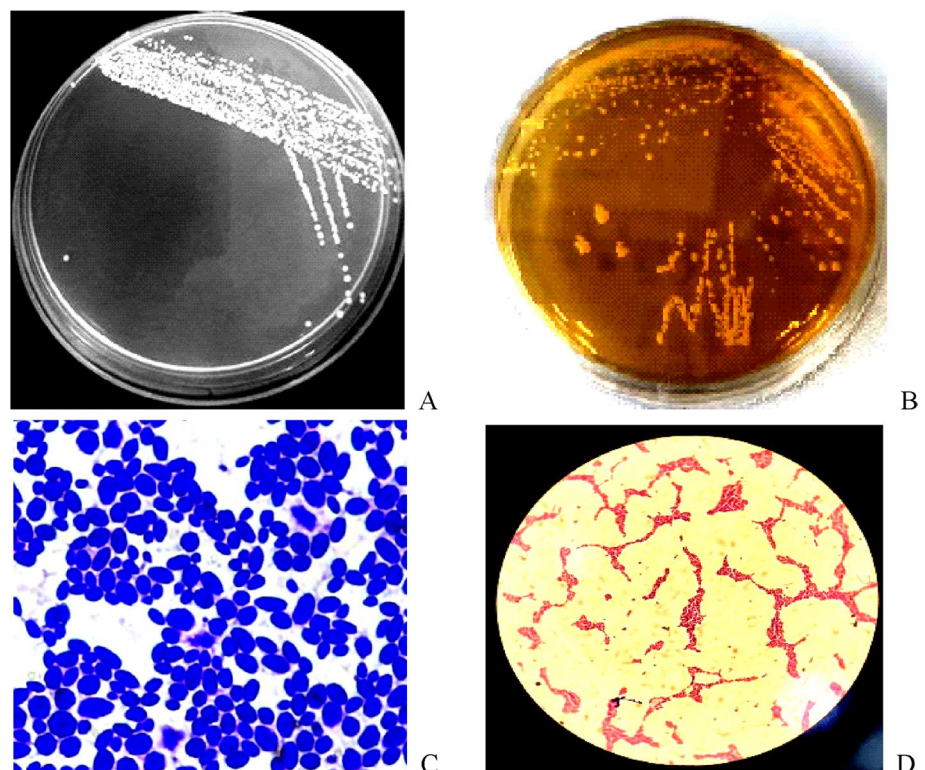
Table 3 Confirmation of *S. cerevisiae* and *L. acidophilus* probiotic organism by morphological and biochemical catalase, oxidase, nitrate tests, and antibiogram analysis

Experiment	Test	Species	
		L. acidophilus	*S. cerevisiae*
Absorption	Catalase	–	+
	Oxidase	–	+
	Nitrate	–	–
Antibiogram	Ketoconazole	–	Sensitive
	Amphotericin B	–	Semi sensitive
	Fluconazole	–	Resistant
	Tetracycline	Sensitive	Sensitive
	Clindamycin	Sensitive	–
	Chloramphenicol	Sensitive	–
	Gentamicin	Semi Sensitive	–
	Levofloxacin	Semi Sensitive	–
	ceftazidime	Resistant	–
	Streptomycin	Resistant	–
	Vancomycin	Resistant	–

medium, cells were extracted and resuspended in 0.9 percent sterile saline solution by centrifugation at 4000 g for 5 min at $4^{\circ C}$. The temperatures examined were 25 °C, 30 °C, 37 °C, and 42 °C. The pH was increased to the following values: 1.5, 2, 3, and 5. The bile salt test tubes were then incubated at 26 degrees Celsius for pH testing. CFU/mL was used to assess *S. cerevisiae* growth after 24 h of incubation. Growth Graph of heat resistance, pH, and bile salt on *S. cerevisiae* and *L. acidophilus* is displayed in Fig. 2.

S. cerevisiae and *L. acidophilus* molecular identification

Molecular identification of *S. cerevisiae and L. acidophilus* isolates was performed using PCR. *S. cerevisiae* strain was identified according to 18srRNA gene sequencing, and the *L. acidophilus* strain was identified according to 16srRNA gene sequencing. The gel electrophoresis images of both strains are shown in Fig. 3.

Fig. 2 Growth Graph of heat resistance, pH, and bile salt on *S. cerevisiae* and *L. acidophilus*. *S. cerevisiae* **A** heat resistance graph **B** bile salt **C** pH and *L. acidophilus* **D** heat resistance graph **E** bile salt **F** pH
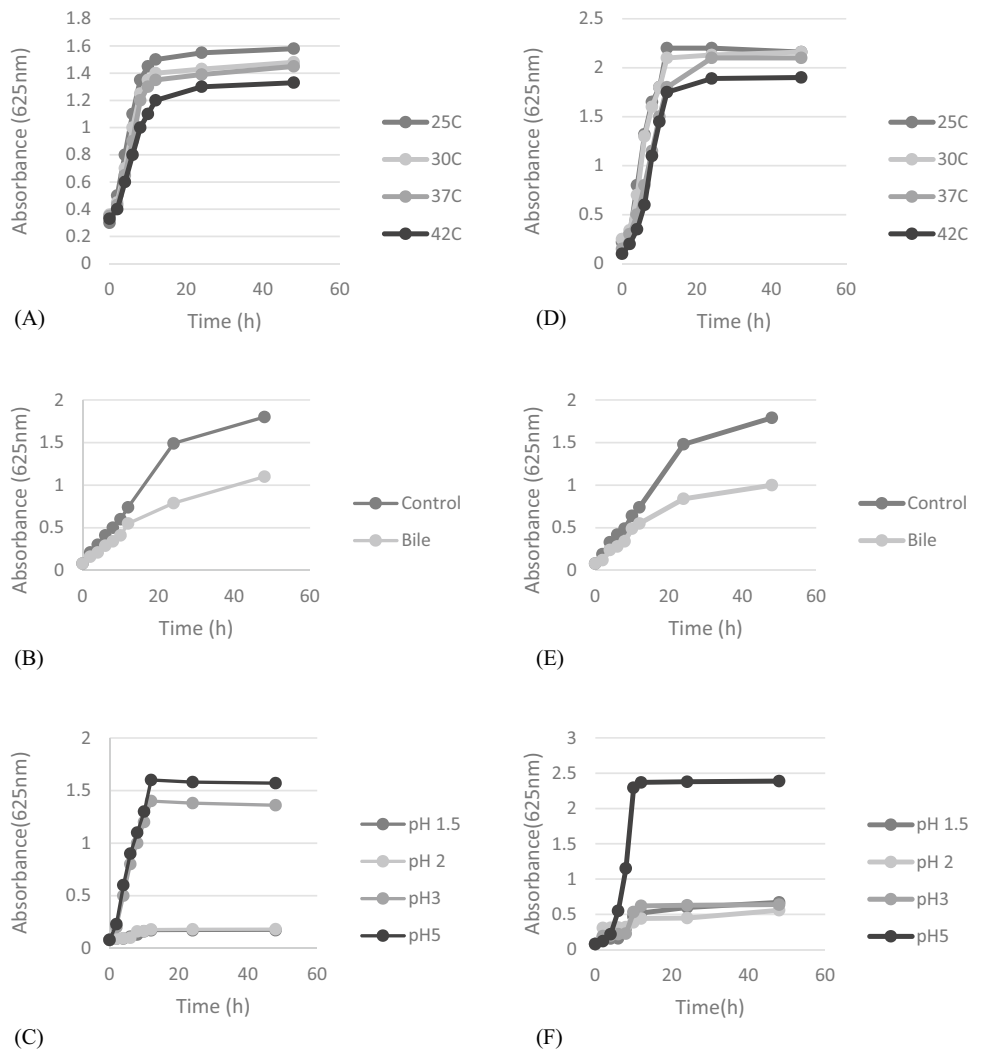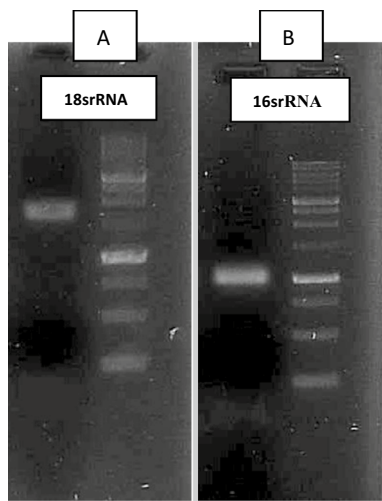
Fig. 3 Specific amplification band of **A** 18srRNA of *S. cerevisiae* and **B** 16srRNA of *L. acidophilus.* on 2% agarose gel. The two 28S rRNA and 18S rRNA bands are observable with suitable quality due to staining by Safe Stain on 2% agarose gel. *L; size marker, Sc: S. cerevisiae, La: L. acidophilus*

The cytotoxic effect of *S. cerevisiae* and *L. acidophilus* supernatant on the cell viability of HT29 cancer cells

After treating HT-29 cell line with *L. acidophilus* and *S. cerevisiae* probiotics, the viability of these cells was determined using the MTT colorimetric assay. The Cytotoxic Effect of *S. cerevisiae* demonstrated that as supernatant concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μg/ml) and treatment time (24, 48, and 72 h) increased, the cell viability of HT29 cancer cells decreased significantly ($P < 0.05$). At 24, 48, and 72 h, the half maximal inhibitory concentration (IC_{50}) was 49.23, 38.38, and 42.69 μg/ml, respectively. Time has been shown to have a significant effect on the proliferation of HT29 cancer cells treated with supernatant (Fig. 4A). Cell viability and proliferation rates were significantly reduced when *L. acidophilus* Supernatant concentrations were increased from 0 to 100 μg/ml after 24 h incubation ($p < 0.05$). Cell viability was significantly affected by treatment time when compared to the control group (0 mg/mL) (24, 48, and 72 h). However, the IC_{50} at 24, 48, and 72 h was 27.52, 16.66, and 20.04 μg/ml, respectively (Fig. 4B).
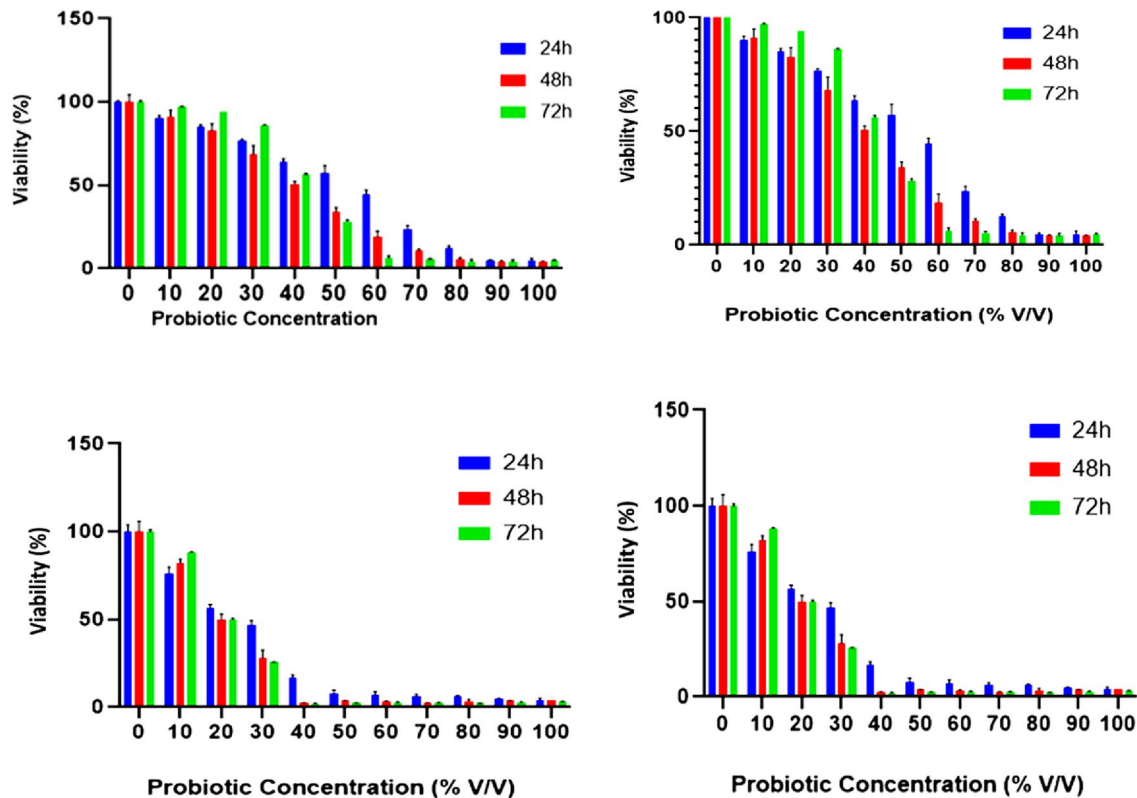

Fig. 4 Cell viability for treatment with different concentrations of *S. cerevisiae* and *L. acidophilus* supernatant for 24, 48, and 72 h on HT29 and SW480 cell lines. **A, B** Cell viability diagram after treatment with 0 μg/ml to 100 μg/ml of the *S. cerevisiae* and *L. acidophi-* *lus* supernatant on HT29 cell lines. **C, D** 0 μg/ml to 100 μg/ml of the *S. cerevisiae* and *L. acidophilus* supernatant on SW480. Values are reported as mean ± standard error (SE) of triplicate measurements

The cytotoxic effect of *S. cerevisiae* and *L. acidophilus* supernatant on the cell viability of SW480 cancer cells

S. cerevisiae and *L. acidophilus* Supernatant showed cytotoxicity with IC$_{50}$ values 19.26 μg/mL and 10.72 μg/mL on the SW480 cell line and could decrease the cell viability after 48 h. On the other hand, a little change had detected after 24 h and 72 h (Fig. 4C, D). At the highest dose (100 μg/mL), both *S. cerevisiae* and *L. acidophilus* supernatant inhibited SW480 cancer cell proliferation at a similar rate. The 100 μg/mL supernatants of *L. acidophilus* and *S. cerevisiae* demonstrated the cytotoxicity (%) toward SW480 cells. The results showed that the cytotoxicity of *S. cerevisiae* and *L. acidophilus* supernatant on SW480 cells after 48 h are dose-dependent, and a low concentration of *S. cerevisiae* and *L. acidophilus* supernatant could rapidly decrease SW480 cell viability.

qRT-PCR analysis of *S. cerevisiae and L. acidophilus* supernatant on HT29

To investigate the anti-cancer effect of *S. cerevisiae* and *L. acidophilus* supernatant on molecular leveland to realize the partial mechanism of cell death by apoptosis and the effect of their treatment on related microRNA dysregulation, we examined the expression of *BCL2, BAX, CASP3, CASP9, MMP2, MMP9,* and 14 related microRNA genes. The IC$_{50}$ dose of *S. cerevisiae* and *L. acidophilus* supernatant was used to assess the expression of *BAX, BCL2, CASP3, CASP9, MMP2,* and *MMP9* genes after 48 h and 72 h on HT29 cells; the data are shown in Fig. 5.

Evaluating the expression of apoptosis-related genes showed that *S. cerevisiae* and *L. acidophilus* supernatant treatment significantly increased mRNA expression of the *BAX, CASP3, and CASP9* genes in HT29 cancer cells,while in the same conditions, the expression of *BCl2, MMP2 and MMP9* genes decreases significantly (Fig. 5A).

Furthermore, the expression of fourteen microRNA genes in this study was evaluated, including 34, 135, 25, 16, 195, 27, 98, 106b, 17, 21, 155, 221, 93, and let7. The expression of eight selected microRNA genes (34, 135, 25, 16, 195, 27, 98, and let7) increased significantly within 48 h of exposing *S. cerevisiae* and *L. acidophilus* supernatant on HT-29 cells and continues for up to 72 h. Meanwhile, the expression of six selected microRNA genes (106b, 17, 21, 155, 221, and 93) decreased significantly under the same conditions (Fig. 5).

qRT-PCR analysis *S. cerevisiae and L. acidophilus* supernatant on SW480

The IC$_{50}$ dose of *S. cerevisiae* and *L. acidophilus* supernatant was used to assess the expression of *BAX, BCL2, CASP3,CASP9, MMP2,* and *MMP9* genes after 48 h and 72 h on SW480 cells; the data are shown in Fig. 5.Evaluating the expression of apoptosis-related genes showed that *S. cerevisiae* and *L. acidophilus* supernatant treatment significantly increased mRNA expression level of the *BAX, CASP3*, *and CASP9* genes in cancer cell lines,while in the same conditions, the expression of *BCl2, MMP2 and MMP9* genes decreases significantly (Fig. 5A).

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Discussion

Colorectal cancer (CRC) is the world's third most common type of cancer in men and the world's second most common type of cancer in women[25]. The 5-year relative survival rate for early-stage colon cancer (localized stage) is 90% [26]. The death rate from colon cancer has decreased over time as technology has advanced, so the survival rate in early-stage colon cancer is high. Finding ways to lessen the side effects of colon cancer therapies and increase the survival rates of patients is essential. There is a risk of severe complications and death from all types of cancer treatment, including surgery, chemotherapy, immunotherapy, and radiation therapy. In particular, Gastrointestinal-related adverse effects such as nausea, diarrhea, colitis, and gastrointestinal bleeding may negatively impact a patient's quality of life, prohibit them from continuing therapy, and in extreme cases, even be fatal [27]. The human microbiota is related to the 10 to 100 trillion symbiotic microbial cells that each individual nurtures, primarily in the gut, where they interact with the metabolism of food residues, intestinal secretions, and the gastrointestinal tract. The microbiota performs a variety of functions within the body that has both positive and negative effects on human health. A number of major research initiatives are currently investigating this complicated interaction. The heterogeneity of the human microbiome contributes to the difficulty of research studies [28].

Among the most common probiotic microbes, the Lactobacillus family is responsible for forming lactic acid, the primary metabolite of sugar metabolism. Recent research has demonstrated that lactobacilli are an effective treatment for diarrhea, food allergies, and inflammatory bowel disease (IBD). Lactobacilli's potential role and its beneficial effects
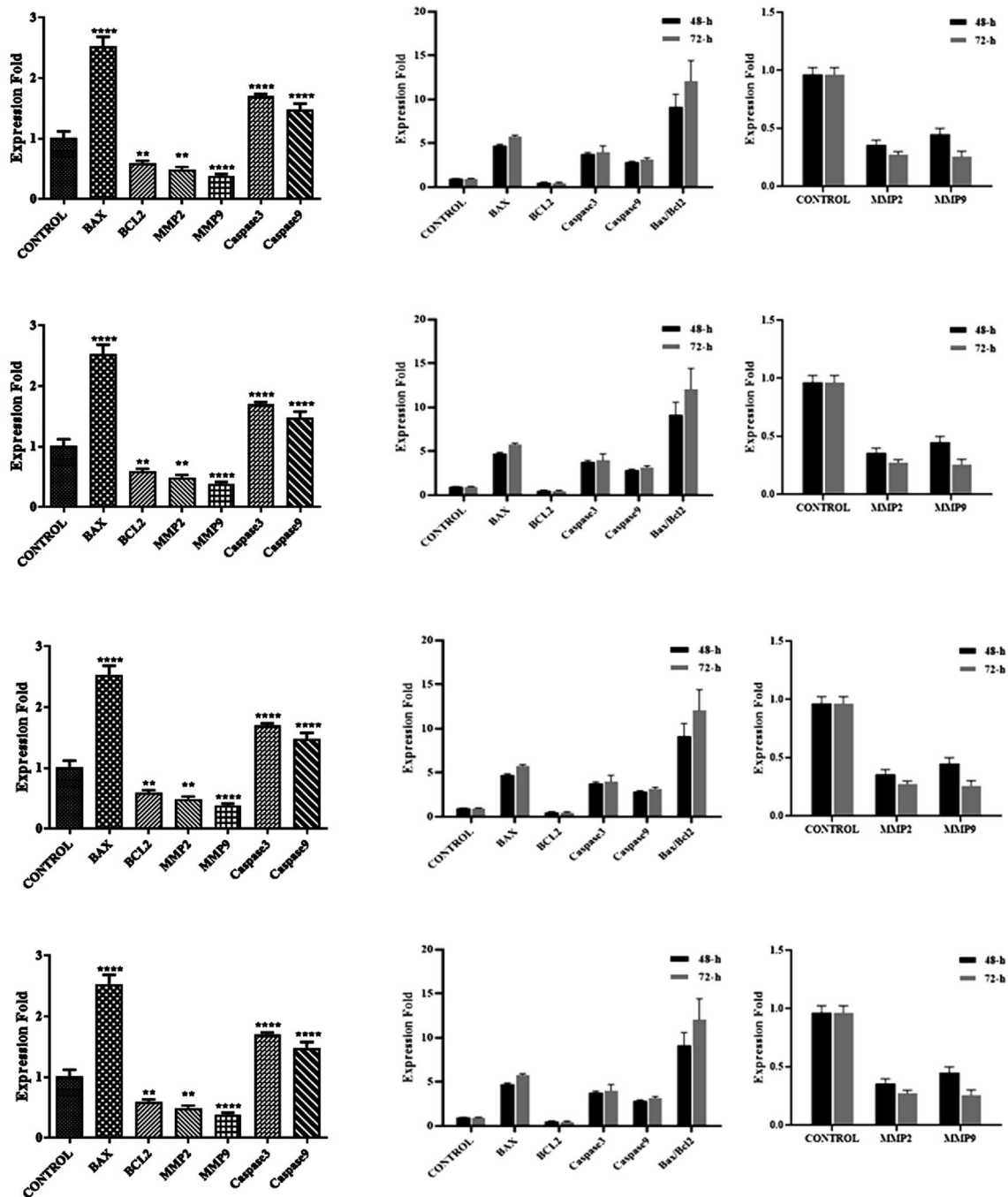
Fig. 5 qRT-PCR analysis for the gene expression levels of *BAX*, *BCL2, CASP3,CASP9, MMP2,* and *MMP9* genes; fourteen micro-RNA genes of 34, 135, 25, 16, 195, 27, 98, 106b, 17, 21, 155, 221, 93, and let7. in HT29 and SW480 cell lines. The data are expressed as the mean$\pm$SD, n$=$3 biologically independent measurements, *P$<$0.05, **P$<$0.01, ***P$<$0.001, *BCL2* BCL2 apoptosis regulator, *BAX*BCL2 associated X, *CASP3* caspase 3, *CASP9* caspase 9, *MMP2* matrix metallopeptidase 2, *MMP9* matrix metallopeptidase9

include enhancing the body's natural defense mechanisms and preventing gastrointestinal disorders has been extensively studied. Several studies have demonstrated that Lactobacilli play a crucial role in CRC prevention [28].

Although there is no general agreement on the role of Lactobacilli in treating CRC, it is generally accepted that certain Lactobacilli strains can activate anticancer mechanisms and regulate the host immune system. Although lactobacilli's role in treating colorectal cancer has been studied to some level, the mechanism of their effect, particularly via the miRNA network, has not been thoroughly studied [29]. Recent research has demonstrated that the pathogenic
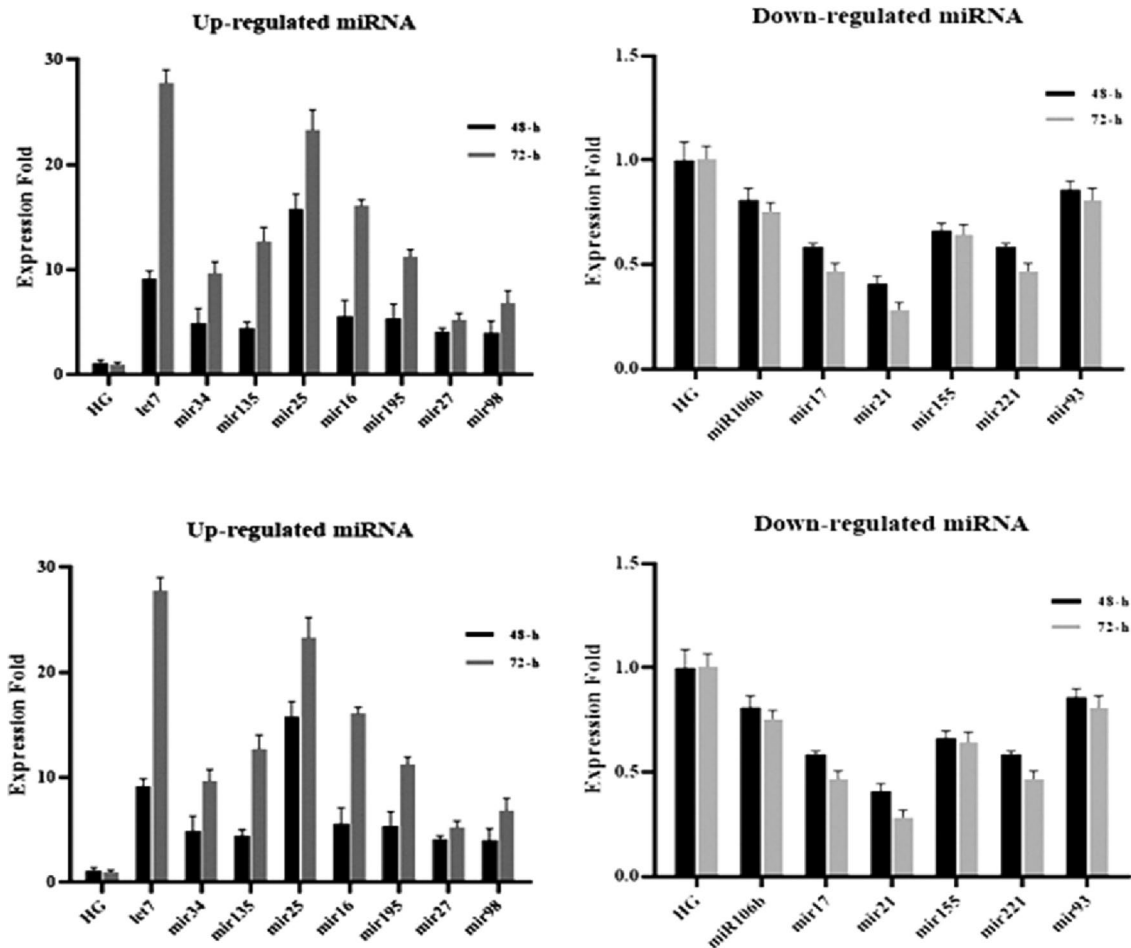
Fig. 5 (continued)

mechanisms of colorectal cancer are dependent on multiple signaling pathways, including p53, PI3K, RAS, MAPK, EMT transcription factors, and Wnt/-catenin. It has been discovered that miRNAs regulate the pathogenesis-related mechanisms in all of these pathways [30].

Alsomultiple studies have demonstrated the role of Saccharomyces in regulating and controlling the development and migration of cancer. *Saccharomyces cerevisiae*as a type of yeast,has been widely used in the production of alcoholic beverages, bread, and food industries. Some human cell signaling proteins, like cell cycle proteins and processing enzymes, are homologous to numerous yeast proteins [31].

HT-29 and SW480 are human colon adenocarcinoma cell lines that provide an ideal experimental system for studying the factors influencing the differentiation of epithelial cells. Under typical conditions, these cells form non-polar layers. These characteristics make them an ideal model for studying cell signaling pathways and therapeutic agents or approaches [30, 32]. Therefore, in the present study, the effect of *Lactobacillus acidophilus* and *Saccharomyces*

cerevisiae secretions (supernatant) on two colorectal cancer cell lines was investigated. The toxicity effect of the supernatant of these organisms was measured using the MTT assay, followed by the expression of 14 microRNAs and genes involved in apoptosis and metastasis; qRT-PCR was used to determine the mechanism by which these factors affect these cell lines.

MTT results from the present study demonstrated that exposure of SW480 and HT29 cell lines to *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* probiotics decreases cell survival and induces apoptosis. As an anti-apoptotic factor, the level of BCL2 gene expression decreases significantly under the influence of *Lactobacillus acidophilus* ($p < 0.05$) and Saccharomyces ($p < 0.05$) relative to the control sample. In the present study, it was shown that the expression level of BAX, CASP3, and CASP9 as apoptotic indicators changed significantly due to the treatment with both studied probiotics ($p < 0.05$).

Consistent with the findings of Li et al. in 2020, which demonstrated that *Saccharomyces cerevisiae* might play a

probiotic role in CRC by inducing cell apoptosis. Also, *Saccharomyces cerevisiae* has also been shown to induce apoptosis in other cancers, including breast and ovarian cancer [33–36].

Our results are consistent with those of Chen et al., who analyzed the effect of oral administration of *Lactobacillus acidophilus* (*L. acidophilus*) on colorectal cancer in mice; Their results showed that *L. acidophilus* reduced the severity of colorectal cancer and increased apoptosis in treated mice. In the next step, in order to investigate the effect of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* probiotics on the invasion and metastasis of SW480 and HT29 cell lines, *MMP2* and *MMP9* gene expression was investigated. After exposure to both probiotics, the expression level of matrix metalloproteinases(*MMP2* and *MMP9*) decreased significantly ($p < 0.05$), which can be an indicator of the effectiveness of probiotics in controlling colorectal cancer metastasis. Our results are in line with those of [37] FaizehMaqsood et al. [38], who found that *Lactobacillus acidophilus* significantly reduced MMP-9 gene expression and increased TIMP-1 expression in PMA-differentiated THP-1 cells ($P < 0.0001$) [38].

Furthermore, we focused on a panel of tumor suppressor miRNAs and key oncomiR associated with colorectal cancer to determine the molecular action mechanism of probiotics on miRNA expression regulation pathways. According to real time results, both *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* probiotics in SW480 and HT29 cell lines increase the expression of tumor suppressor miRNAs such as miR-135, miR-34, miR-25, miR-16, miR-195, miR-27, miR-98, LET-7 and on the other hand, it plays a significant role in reducing the expression of oncogenic miRNAs such as miR-17, miR-21, miR-155, miR-221, miR-93 and miR-106b. Researchers investigated how probiotics influence miRNA expression in another study. Consistent with our findings, a recent study found that adding *Lactobacillus acidophilus* and *Bifidobacteriumbifidum* to a colon cancer patient's diet increased the expression of tumor suppressor microRNAs and their target genes while decreasing oncogenes expression [37].

According to previous studies, MiR-34a inhibits colon cancer cell proliferation by increasing p53 and p21 levels, which play tumor suppressor proteins role; MiR-34a has been shown to suppress PAR2-induced cell growth, and its inhibition partially restores PAR2-induced cyclin D1 activation. PAR2 has been shown to increase cancer cell proliferation and induce the accumulation of Cyclin D1, a key player in tumorigenesis, by activating EGFR, MAPK, and other survival signals.

It has also been seen that MiR-34a has been shown to regulate apoptosis; the suppression of SIRT1 expression by MiR-34a results in increased levels of acetylated p53, which causes cell death. Furthermore, according to a functional study, after being transfected miR-34a into SW480 cells, miR-34a upregulated acetylated p53 and p21, resulting in a significant decrease in migration and invasion. Also shows that overexpression of miR-34a causes senescence-like phenotypes and cell growth arrest by upregulating the p53 pathway. MiR-34a has been shown to inhibit colorectal metastasis in a number of studies [35], and this suppression occurs via the EMT regulatory network of SNAIL/ZNF81 and IL6R/STAT3.

MiR-16 inhibits CRC cell growth and induces cell apoptosis by regulating the p53/survivin signaling pathway. Survivin is a direct target of miR-16 and plays an important role in colorectal cancer cell proliferation and survival. Survivin is expressed primarily during the G2/M phase of the cell cycle, so inhibiting Survivin expression can result in defective cytokinesis and cell cycle [39].

Anti-miR-135b (LNA) treatment was found to inhibit tumor progression in a mouse model and cause apoptosis in SW480 cells. The ability of HCT-116 cells to migrate and metastasize was found to be significantly diminished when miR-135b expression was elevated, as demonstrated in a study by Wu et al. Consequently, miR-135b may be a useful therapeutic target for colorectal cancer. The expression of miR-135b can be boosted by probiotics, which can assist in colorectal treatment [40].

Let-7 inhibits MAPK and PI3K/AKT signaling by inhibiting RAS. Numerous oncoproteins that are known to play an important role in CRC growth are directly repressed by Let-7. Intestinal epithelial cells and CRC cells are both encouraged to migrate and invade when the let-7 expression is reduced [41].

Let-7 inhibits cell growth by controlling Wnt signaling. Studies have shown that let-7 inhibits Wnt signaling in several cancers. Overexpression of LIN28 in the context of intestinal tumorigenesis has been shown to speed up the growth of intestinal tumors in ApcMin /+mice, and this tumor-promoting effect is dependent on let-7 [42].

The miR-17 levels in the plasma of patients with colorectal cancer are significantly higher than those in healthy people. The PTEN gene is a common target of miR-17, miR-21, and miR-92a in CRC. PTEN is a tumor suppressor gene that is frequently lost in human cancers.

High levels of miR-21 expression are observed in CRC, and aberrant miR-21 expression is associated to a poorer prognosis and a more rapid disease progression in CRC.

When miR-21 is overexpressed in HT-29 cells, the proportion of cells in G1/G0 decreases and the proportion in S phase rises. The opposite was seen when miR-21 expression was suppressed in HT-29 cells. These results demonstrate that miR-21 inhibits HT-29 cell proliferation and promotes apoptosis in CRC cells [43].

In addition to the previously observed aberrant expression of miR-155 in colorectal cancer, increased expression

levels of miR-155 have been detected in other human malignancies, such as lung cancer, cervical cancer, hematologic malignancies, and thyroid carcinoma. Once miR-155 is downregulated in HT-29 cells, proliferation, migration, and invasion are all significantly suppressed, while G0/G1 cell-cycle arrest and apoptosis are induced [44].

Multiple cancers, including ovarian, breast, lung, and thyroid, have been associated to the gene mir-221, which acts similarly to oncogenes in tumor initiation and progression. Overexpressed miR-221 can target tumor suppressor genes like P57, BIM, PUMA, TIMP3, and PTEN, which are highly expressed in tumor cells. Inhibiting these target genes activates AKT-induced bypass, initiates the cell cycle, suppresses ligands associated with apoptosis induction, and promotes tumor proliferation. Similar to how miR-211 can inhibit TIMP3 in colon cancer, it is overexpressed in this cancer. This suggests that miR-221 is critically important in colorectal cancer. Similarly, lowering miR-221 with probiotics can be helpful in treating colorectal cancer [45, 46].

In the functional study, miR-93 was found to inhibit both stem cell proliferation and colony formation in human colon cancer. Depending on the type of carcinoma, miR-93 can either act as an oncogene or a tumor suppressor [47]. On the other hand, the molecular mechanism behind this is not fully understood [48, 49].

This study's findings suggest that probiotics may regulate the expression of key miRNAs involved in colorectal cancer. This may explain their ability to decrease tumorigenesis, induce apoptosis, and prevent invasion and death. Probiotics have a significant role in limiting and controlling the growth and proliferation of colorectal cancer cells, as shown by an analysis of the expression level of related cell death and metastasis genes. Probiotics have been shown to have toxic properties against cancer cells in a number of previous studies; this study confirmed these findings and elucidated the molecular mechanism of action of Lactobacillus and Saccharomyces on the gene expression and miRNA panel of cells involved in colorectal cancer.

However, further study is required to reach clearer and more specific findings, such as assessing the mechanism of probiotic action using gene expression studies at both the transcript and protein levels identifying and extracting the beneficial *Lactobacillus acidophilus* and *saccharomyces cerevisiae* component, evaluating the effect of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* supernatant on in vivo and additional cell lines, the combination impact of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* supernatant on colorectal cancer is being studied the expression of additional genes associated with different cellular functions such as autophagy, necrosis, or inflammation.

Declarations

References

1. Larypoor M (2021) An overview of food synthetic dietary supplements. Food Hygiene 11(343):1–22
2. Abolghasemi H, Larypoor M, Hosseini F (2022) Probiotic effects of Metschnikowia isolated from dairy products aquatic environments. Int J Mol Clin Microbiol 12(2):1692–1703
3. Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P (2013) An overview of the last advances in probiotic and prebiotic field. LWT-Food Sci Technol 50(1):1–6
4. Torres-Maravilla E, Boucard AS, Mohseni AH, Taghinezhad SS, Cortes-Perez NG, Bermudez-Humaran LG (2021) Role of gut microbiota and probiotics in colorectal cancer: onset and progression. Microorganisms 9(5):1021
5. Dong Y, Xu M, Chen L, Bhochhibhoya A (2019) Probiotic foods and supplements interventions for metabolic syndromes: a systematic review and meta-analysis of recent clinical trials. Ann NutrMetab 74(3):224–241
6. Martín R, Miquel S, Ulmer J, Kechaou N, Langella P, Bermúdez-Humarán LG (2013) Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. Microb Cell Fact 12(1):1–11
7. Tannock G, Munro K, Harmsen H, Welling G, Smart J, Gopal PJA et al (2000) Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. Appl Environ Microbiol 66(6):2578–2588
8. Larypoor M, Bayat M, Zuhair MH, AkhavanSepahy A, Amanlou M (2013) Evaluation of the number of CD4^{+} CD25^{+} FoxP3^{+}treg cells in normal mice exposed to AFB1 and treated with aged garlic extract. Cell J 15(1):37–445
9. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA (2019) Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol 16(10):605–616
10. Arnold M, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA et al (2020) Global burden of 5 major types of gastrointestinal cancer. Gastroenterology. 159(1):335–349
11. Heydari Z, Rahaie M, Alizadeh AM (2019) Different anti-inflammatory effects of *Lactobacillus acidophilus* and

Bifidobactrumbifidioum in hepatocellular carcinoma cancer mouse through impact on microRNAs and their target genes. J Nutr Intermed Metab 16:100096

12. Gomes AM, Malcata FX (1999) *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci Technol 10(4–5):139–57

13. Thirabunyanon M, Boonprasom P, Niamsup P (2009) Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. Biotech Lett 31:571–576

14. Alander M, Satokari R, Korpela R, Saxelin M, Vilpponen-Salmela T, Mattila-Sandholm T, von Wright A (1999) Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. Appl Environ Microbiol 65(1):351–354

15. Branduardi P, Smeraldi C, Porro DJMP (2008) Metabolically engineered yeasts: 'potential' industrial applications. Mol Microbiol Biotechnol 15(1):31–40

16. Nayak SK (2011) Biology of eukaryotic probiotics Biology of Eukaryotic Probiotics. Springer, Berlin, pp 29–55

17. Ansari F, AlianSamakkhah S, Bahadori A, Jafari SM, Ziaee M, Khodayari MT et al (2023) Health-promoting properties of *Saccharomyces cerevisiae* var. boulardii as a probiotic; characteristics, isolation, and applications in dairy products. Crit Rev Food Sci Nutr 63(4):457–85

18. Abid R, Waseem H, Ali J, Ghazanfar S, Muhammad Ali G, Elasbali AM et al (2022) Probiotic yeast Saccharomyces: back to nature to improve human health. J Fungi 8(5):444

19. Palma ML, Zamith-Miranda D, Martins FS, Bozza FA, Nimrichter L, Montero-Lomeli M et al (2015) Probiotic *Saccharomyces cerevisiae* strains as biotherapeutic tools: is there room for improvement. Microbiol Biotechnol 99:6563–6570

20. Profir A-G, Buruiana C-T, Vizireanu C (2015) Effects of *S. cerevisiae* var. boulardii in gastrointestinal disorders. J Agroaliment Process Technol 21(2):148–55

21. Williams AJC (2008) Functional aspects of animal microRNAs. Cell Mol Life Sci 65:545–62

22. Falsen E, Pascual C, Sjödén B, Ohlén M, Collins D (1999) Microbiology E. Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: description of *Lactobacillus iners* sp. nov. J Syst Bacteriol. 49(1):217–21

23. Turenne CY, Sanche SE, Hoban DJ, Karlowsky JA, Kabani AM (1999) Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J Clin Microbiol 37(6):1846–51

24. Moore GW (2014) Recent guidelines and recommendations for laboratory detection of lupus anticoagulants. Semin Thromb Hemost 40:163

25. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin 71(3):209–249

26. Merrill RM, Hunter BD (2010) Conditional survival among cancer patients in the United States. Oncologist 15(8):873–82

27. Orlando A, Linsalata M, Russo F (2016) Antiproliferative effects on colon adenocarcinoma cells induced by co-administration of vitamin K1 and Lactobacillus rhamnosus GG. Int J Oncol 48(6):2629–38

28. Nozari S, Mohammadzadeh M, Faridvand Y, Tockmechi A, Movassaghpour A, Abdolalizadeh J (2016) The study of extracellular protein fractions of probiotic candidate bacteria on cancerous cell line. Arch Iran Med 19(11)

29. Chen Z-Y, Hsieh Y-M, Huang C-C, Tsai C-C (2017) Inhibitory effects of probiotic Lactobacillus on the growth of human colonic carcinoma cell line HT-29. Molecules 22(1):107

30. An J, Ha E-M (2016) Combination therapy of *Lactobacillus plantarum* supernatant and 5-fluouracil increases chemosensivity in colorectal cancer cells. Mol Cell Microbiol 26(8):1490–503

31. Nouri Z, Karami F, Neyazi N, Modarressi MH, Karimi R, Khorramizadeh MR et al (2016) Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. Cell J 18(2):127

32. Davoodvandi A, Marzban H, Goleij P, Sahebkar A, Morshedi K, Rezaei S et al (2021) Effects of therapeutic probiotics on modulation of microRNAs. Cell Commun Signal 19:1–22

33. Behroozi J, Shahbazi S, Bakhtiarizadeh MR, Mahmoodzadeh H (2020) Genome-wide characterization of RNA editing sites in primary gastric Adenocarcinoma through RNA-Seq data analysis. Int J Genom. https://doi.org/10.1155/2020/6493963

34. Pekarsky Y, Balatti V, Croce CM (2018) BCL2 and miR-15/16: from gene discovery to treatment. Cell Death Differ 25(1):21–6

35. Deng S, Calin GA, Croce CM, Coukos G, Zhang LJ (2008) Mechanisms of microRNA deregulation in human cancer. Cell Cycle 7(17):2643–6

36. Wei H, Cui R, Bahr J, Zanesi N, Luo Z, Meng W et al (2017) miR-130a deregulates PTEN and stimulates tumor growth. Mol Cancer 77(22):6168–6178

37. Song G-L, Xiao M, Wan X-Y, Deng J, Ling J-D, Tian Y-G et al (2021) MiR-130a-3p suppresses colorectal cancer growth by targeting Wnt family member 1 [WNT1]. Bioengineered 12(1):8407–18

38. Zhu Z, Huang J, Li X, Xing J, Chen Q, Liu R et al (2020) Gut microbiota regulate tumor metastasis via circRNA/miRNA networks. Med Oncol 12(1):1788891

39. Sambrani R, Abdolalizadeh J, Kohan L, Jafari B (2019) Saccharomyces cerevisiae inhibits growth and metastasis and stimulates apoptosis in HT-29 colorectal cancer cell line. Comp Clin Pathol 28(4):985–95

40. Saber A, Alipour B, Faghfoori Z, Khosroushahi AY (2017) Secretion metabolites of probiotic yeast, *Pichia kudriavzevii* AS-12, induces apoptosis pathways in human colorectal cancer cell lines. Nutr Adv Food Life Sci Res 41:36–46

41. Verma A, Shukla G (2014) Synbiotic (*Lactobacillus rhamnosus*+ *Lactobacillus acidophilus*+inulin) attenuates oxidative stress and colonic damage in 1, 2 dimethylhydrazinedihydrochloride-induced colon carcinogenesis in Sprague–Dawley rats. Eur Cancer Prev Org 23(6):550–9

42. Halper J, Leshin L, Lewis S, Li WI (2003) Wound healing and angiogenic properties of supernatants from Lactobacillus cultures. Exp Biol Med 228(11):1329–37

43. Pakbin B, PishkhanDibazar S, Allahyari S, Javadi M, Farasat A, Darzi SJFS et al (2021) Probiotic *Saccharomyces cerevisiae* var. boulardii supernatant inhibits survivin gene expression and induces apoptosis in human gastric cancer cells. Food Sci Nutr 9(2):692–700

44. Tang Q, Zou Z, Zou C, Zhang Q, Huang R, Guan X et al (2015) MicroRNA-93 suppresses colorectal cancer development via Wnt/β-catenin pathway downregulating. Mol Cancer 36:1701–1710

45. Chi Y, Zhou D (2016) MicroRNAs in colorectal carcinoma-from pathogenesis to therapy. J Exp Clin Cancer Res 35(1):1–11

46. Sivamaruthi BS, Kesika P, Chaiyasut C (2020) The role of probiotics in colorectal cancer management. Evid-Based Complement Altern Med. https://doi.org/10.1155/2020/3535982

47. Zhang N, Xianyu H, Yinan D, Juan D (2021) The role of miRNAs in colorectal cancer progression and chemoradiotherapy. Biomed Pharmacother 134:111099

48. Verma A, Shukla GJN (2013) Probiotics *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* suppresses DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. Nutr Cancer 65(1):84–91

49. Shehzeen N, Shaukat A, Shumaila R, Iqra S, Muhammad A, Ayesha S (2023) Chemopreventive role of probiotics against cancer: a comprehensive mechanistic review. Mol Biol Rep 50:799–814