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RESEARCH

Assessing the Potential Biological Activities of Postbiotics Derived from *Saccharomyces cerevisiae*: An In Vitro Study

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Abstract

A new biotherapeutic strategy involves the use of microbial bioactive substances (postbiotics) that exhibit optimum compatibility and intimate contact with the immune system of the host. This study was aimed at investigating the potential biological activities of postbiotics derived from *Saccharomyces cerevisiae* (PTCC 5269) (PSC) under in vitro circumstances. Based on the outcomes, the synthesized PSC possessing a high level of phenolic (102.46 ± 0.25 mg GAE/g) and flavonoid (19.87 ± 75.32 mg QE/g) content demonstrated significant radical scavenging activity (87.34 ± 0.56%); antibacterial action towards *Listeria monocytogenes*, *Streptococcus mutans*, *Salmonella typhi*, and *Escherichia coli* (in order of effectiveness) in both in vitro and food models (whole milk and ground meat); probiotics' growth-promoting activity in the fermentation medium; α-glucosidase enzyme-inhibiting and cholesterol-lowering properties in a concentration- and pH-dependent manner; reduction in the cell viability (with the significant IC_{50} values of 34.27 and 23.58 µg/mL after 24 and 48 h, respectively); suppressed the initial (G0/G1) phase of the cell's division; induced apoptosis; and increased the expression of *PTEN* gene, while the *IkB*, *RelA*, and *Bcl-XL* genes indicated diminished expression in treated SW480 cancer cells. These multiple health-promoting functions of PSC can be extended to medical, biomedical, and food scopes, as novel biotherapeutic approaches, in order to design efficient and optimized functional food formulations or/and supplementary medications to use as adjuvant agents for preventing or/and treating chronic/acute disorders.

Keywords Akt/NF-κB signaling pathway · Antimicrobial activity · Antioxidant activity · Hypocholesterolemic activity · Postbiotic · *Saccharomyces boulardii*

Abbreviations

PSC	Postbiotics derived from *Saccharomyces cerevisiae*
QS	Quorum sensing
YPD	Yeast peptone dextrose
YMB	Yeast malt broth
ABTS	2,2′-Azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
SDA	Sabouraud dextrose agar
MSA	Mannitol salt agar
EMB	Eosin methylene blue
PCA	Plate count agar
BHI	Brain heart infusion
LB	Luria Bertani
CT-SMAC	Cefixime tellurite sorbitol MacConkey
CFS	Cell-free supernatant
GAE	Gallic acid equivalent
QE	Quercetin equivalent

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DDA	Disc diffusion agar
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
WDA	Well diffusion agar
MEC	Minimal effective concentration
pNPG	4-Nitrophenly α-D-glucopyranoside
5-FU	5–Fluorouracil
PBS	Phosphate-buffered saline
PI	Propidium iodide
ANOVA	One-way analysis of variance
ROS	Reactive oxygen species
AFB_1	Aflatoxin B_1
CDC	Centers for Disease Control and Prevention

Introduction

The human digestive system is a complex microbial ecology comprised of trillions of fungi, archaea, bacteria, and viruses [1]. Starting at birth, a unique microbial population dominates one that plays a crucial function in host physiology throughout the lifespan. The host, the microbiota, the released metabolites, and the active metabolic pathways are all playing more important roles as progress has been achieved. The role of quorum sensing (QS), a sort of cell–cell interaction, in the gut microbiota and its influence on human metabolism and nutrition are still insufficiently understood [2]. Despite significant progress in the field of biology, more research has to be done to reveal the real role of bacteria in the gastrointestinal milieu, including their reaction to communicating with the host cells and creating a variety of bioactive substances. The optimal function of the intestinal microbiota is affected by a number of variables, including the host's microbiota, the host's food, and the host's health. Research also shows that dead or nonliving microbial cells, their biostructural components, and their metabolites may activate particular signaling pathways in host cells and perform certain biological or physiological actions [3]. Bioactive metabolites (postbiotics) made by intestinal microbiota are an intriguing strategy for offering therapeutic benefits that play an important role in creating eubiosis circumstances because they do things like prevent the growth of pathogens, keep the intestinal mucosa integrity and functioning properly, alter the composition of the intestinal microbiota, and moderate immune responses and certain key inflammatory pathways. Postbiotics, by virtue of their peculiar structure, interfere with the processes by which the immune system and the neurological system exert their controlling influence on host cells. Examples of this issue include improving the efficiency of the innate immune system, decreasing the inflammatory responses caused by the presence and activity of pathogenic germs with inflammation-inducing activity and carcinogenic agents (especially those derived from food processing), and bolstering the effectiveness of the intestinal barrier [4, 5].

The term "postbiotic" is used to describe the consumption of deactivated microbial cells, cellular bioactive elements/metabolites that are produced primarily through the fermentation process by potential probiotic microorganism cultures or in response to ecological context and, that when ingested in sufficient quantities, provide the host multiple biological health benefits [3, 6]. Furthermore, postbiotic compounds of specific chemical compositions may modify physiological mechanisms, regulatory pathways, and/or behavioral reactions associated with the host's commensal microbiota functioning. Exopolysaccharides, glycoproteins, peptides, proteins, peptidoglycans, linoleic acid, lactic acid, and short-chain fatty acids are only some of the many postbiotic metabolites that have been shown to have significant antioxidant, antiinflammation, and antibacterial effects [6–8].

Saccharomyces cerevisiae is a functional probiotic yeast that is often used as a fermenting dough starter and has been recognized for its probiotic and functional properties. Fungemia can occur in both humans and animals when exposed to certain strains of this yeast (under special conditions such as individuals with a suppressed immune system, dysbiosis status in the gut microbiome, etc.), which exhibited opportunistic pathogenic behavior. Several studies have lately suggested using the postbiotic form to take advantage of probiotics and health-promoting advantages in a safe and controllable manner [9].

Based on the aforementioned considerations, the present study characterized and investigated some of the main functional biologic dimensions of postbiotics derived from *Saccharomyces cerevisiae* (PSC) in generating significant antioxidant, antibacterial, α-glucosidase inhibition, cholesterol-lowering, cytotoxic, and apoptosis responses under in vitro circumstances.

Materials and Methods

Materials

Yeast peptone dextrose (YPD), yeast malt broth (YMB), agar–agar, absolute ethanol, and chloroform were purchased from Merck Millipore, (Darmstadt, Germany). ABTS (2,2′-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt), gallic acid, and quercetin were procured from Sigma-Aldrich Co. (St Louis, MO, USA). The culture media Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), Sabouraud dextrose agar (SDA), mannitol salt agar (MSA), eosin methylene blue (EMB), plate count agar (PCA), brain heart infusion (BHI), Luria Bertani (LB), and cefixime tellurite

sorbitol MacConkey (CT-SMAC) were purchased from Merck Co. (Darmstadt, Germany).

Preparation and Maintenance of Probiotic Yeast Strain

Iran's industrial center of bacteria and fungi collection (Iran's scientific, research, and industrial organization) supplied *Saccharomyces cerevisiae* (PTCC 5269) and was cultivated in YPD (20 g/L dextrose, 4 g/L yeast extract, 3 g/L bacterial peptone, 2 g/L triammonium citrate, 1 g/L polysorbate 80, 1 g/L KH_2PO_4, and 0.8 g/L $MgSO_4$) for 24–48 h at 30 °C while being stirred at 200 rpm. *Saccharomyces cerevisiae* suspensions were standardized using an ultraviolet–visible spectrophotometer (UV3600, Japan) at 600-nm wavelength region if needed [10].

Preparation of Yeast Cell-Free Supernatant Solution

The first stage in the generation of cell-free supernatant (CFS) postbiotics is the aerobic culture of *S. cerevisiae* in YMB for 48 h at a temperature of 37 ± 1 °C. This procedure was carried out in 2000-mL Erlenmeyer flasks in order to get sufficient and appropriate quantities of CFSs because different microbial strain growth circumstances and extraction techniques significantly affect the postbiotic efficiency. Briefly, CFS was extracted by 24–48 h of incubation at a temperature of 37 °C, followed by 10 min of centrifugation at 4 °C at 4500 rpm. The yeast medium supernatant was collected, the pH was modified to 7.2, and it was purified through a 0.22-µm Millipore filter (Sigma-Aldrich, MilliporeSigma Co., Germany) before being used to treat the samples [10]. The filtrate was collected for freeze-drying. After, the harvested CFS were frozen at − 80 °C for 24 h. The CFS was lyophilized (Lyophilization Systems, Inc, USA) from − 40 to − 30 °C, 0.2 mbar. The entire freeze-drying process was performed in 24 h, and the freeze-dried powders were stored at − 20 °C. They were then rehydrated with sterile deionized water prior to use.

Assessment of the Total Phenolic Content

An approach developed by Abeysekera and colleagues (2013) was used to evaluate the total phenolic content of the PSC using the Folin-Ciocalteu reagent [11]. Sodium carbonate solution (70 µL) and Folin–Ciocalteu reagent (110 µL) have been utilized to charge the PSC (20 µL). The mixture's absorbance was measured at 765 nm after being kept for 30 min at 25 °C. The standard curve was generated using gallic acid (0.06–1 mg/mL), and the total phenol content of PSC was calculated as mg gallic acid equivalent (GAE) per gram PSC.

Assessment of the Total Flavonoid Content

The aluminium chloride technique was used to determine PSC's total flavonoid content. After being incubated at 25 °C for 10 min, the combination of PSC (100 µL) and aluminium chloride mixture (100 µL; 2% w/v in methanol) was measured for absorbance at 367 nm. The total flavonoid concentration was reported as milligram quercetin equivalent (QE) per gram PSC, and quercetin (7.81–125 mg/mL) was used to generate the calibration curve [11].

Antioxidant Activity Assessment of the Prepared Postbiotics

The technique of Nantitanon and colleagues [12] was modified to test the antioxidant impact of PSC against ABTS free radicals. The 7 mM ABTS solution was oxidized with 2.45 mM $K_2S_2O_8$ to produce the ABTS radical monocation. After being kept at room temperature and kept in the dark for 12 h, the mixture was diluted with ethanol until it had an absorbance of 0.7 ± 0.2 at 750 nm. The ABTS solution (1.8 mL) was then mixed with the PSC and control (0.2 mL), and a spectrophotometer was utilized to determine the absorbance of the mixture at 750 nm. The following formula was used to determine PSC's antioxidant activity:

$$Antioxidant\ activity\ (\%) = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100 \quad (1)$$

Antibacterial Activity Assessment of the Prepared Postbiotics

Under In Vitro Condition

The disc diffusion agar (DDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and well diffusion agar (WDA) were employed to assess the postbiotics' antibacterial efficacy against *Salmonella typhi* ATCC 6539, *Escherichia coli* ATCC 25922, *Streptococcus mutans* ATCC 25175, and *Listeria monocytogenes* ATCC19112 using the techniques outlined by Sabahi and colleagues [13]. In the DDA test, the antibiotics tetracycline, gentamicin, and chloramphenicol were used to compare their antibacterial effects.

Under Food Models

The minimal effective concentration (MEC) of postbiotics was calculated using the same manner as previously stated by Hartmann and colleagues (2011) [14]. To get the final population to an adequate level (~ 3.2 and 4.1 (*E. coli*) and 3.5 and 4.6 (*L. monocytogenes*) $\log_{10}$ CFU/mL or gram of

milk and meat, respectively), *E. coli* ATCC 25922 and *L. monocytogenes* ATCC19112 were incorporated into 10 mL of pasteurized milk in a bottle and 100 g of ground meat in sterile wrappers, respectively. Both the milk and the meat samples, which had PSC added at doses ranging from 10 to 60 mg/mL, were fully homogenous. Six days were spent storing all samples at 4 °C. The examined pathogens were cultured and counted using cefixime tellurite sorbitol MacConkey agar and PALCAM *Listeria* selective agar. MEC was defined as the PSC concentration that decreases the initial microbial population to below the detection limit of 10 bacteria for milk and 100 bacteria for meat over the course of 3 days of preservation at 4 °C. Instead of a food matrix, comparable experiments were conducted using BHI broth and LB broth.

Determination of Growth-Promoting Properties of the Prepared Postbiotics

The effects of PSC on the proliferation of probiotic strains were studied to ascertain their growth-promoting qualities [15]. *Lactobacillus casei* L431, *Lactobacillus brevis* TD4, *Lactobacillus acidophilus* ATCC 4356, and *Bifidobacterium bifidum* ATCC 35914 were employed for this experiment. For 24 h, MRS broth medium was used to cultivate *Lactobacilli* and *Bifidobacteria* at 37 °C in anaerobic circumstances. We prepared sugar-free MRS (pH 5.7) with 0.5% PSC as the carbon source to determine whether it would stimulate growth. Positive controls consisted of an MRS medium comprising 0.5% glucose, whereas negative controls consisted of a sugar-free MRS medium. The cell density change was measured using a spectrophotometer (Cary-4000, Agilent, Technologies, USA) at 600 nm after 48 h of inoculation of probiotic strains (10^6 CFU/mL) from the previous culture into PSC-containing MRS media.

Determination of α-Glucosidase Inhibitor Activities of the Prepared Postbiotics

The activity of PSC to inhibit α-glucosidase was measured according to the protocol laid forth by Kazeem and colleagues [16]. This was accomplished by incubating 100 µL of α-glucosidase (1.0 U/mL, from *Saccharomyces cerevisiae*, Sigma, USA) with 50 µL of PSC (0.5, 1.0, and 2.0 mg/mL) at 37 °C for 10 min. The process was initiated by adding 50 µL of 3.0 mM 4-nitrophenly α-D-glucopyranoside (pNPG) diluted in 20 mM phosphate buffer, incubating for 20 min at 37 °C, and then adding 2 mL of 0.1 M Na_2CO_3. The wavelength of the yellow paranitrophenol emitted by pNPG was determined to be 405 nm (Multiskan Go, Thermo Fisher Scientific, Waltham, MA, USA). The percentage of α-glucosidase inhibition was determined using the following formula:

$$\alpha - glu\cos idase\ inhibitor\ activity\ (\%) = \left[\left(A_{Control} - A_{Sample}\right)/A_{Control}\right] \times 100 \quad (2)$$

where A_{Control} is the solution without the specimen and A_{Sample} is the solution with the various PSC concentration samples.

Determination of Cholesterol Removal Capabilities of the Prepared Postbiotics

The cholesterol-removing capacity of the synthesized PSC was evaluated according to the protocol laid forth by Soh and colleagues [17]. Different concentrations of 0.1% PSC (0.5, 1.0, and 2.0 mg/mL) and 30 µg cholesterol were combined in a 1-mL reaction mixture, which was then incubated at 25 °C for 20 min before 50 µL of hexadecyl trimethyl ammonium bromide was added. At 500 nm, the optical density of the supernatant was determined after centrifuging the mixture at 12,500 × g. The following equation was used to determine cholesterol-removing potential:

$$Cholesterol - removing\ capability\ (\%) = \left[\left(A_{Control} - A_{Sample}\right)/A_{Control}\right] \times 100 \quad (3)$$

where A_{Control} is the cholesterol solution without PSC and A_{Sample} is the cholesterol solution containing 0.1% PSC.

Determination of Anticancer Activity of the Prepared Postbiotics

Cell Culture

A 5% CO_2 incubator at 37 °C was used to cultivate SW480 colon cancer cell lines that had been obtained from the Pasteur Institute, National Cell Bank of Iran, Tehran, Iran. The medium included 10% (v/v) fetal bovine serum, 100 mg/mL of streptomycin, and 10 U/mL of penicillin. Cells were detached using trypsin at 80–90% confluency, and while seeding in the appropriate culture plates, the culture medium was changed at regular intervals over a period of 2–3 days. After 3–4 passages, we used the cells for experimental analyses [18].

Cytotoxicity Assay

To determine PSC's cytotoxic potential, our research used the cytotoxicity test described in [19, 20]. In brief, 96-well microtiter plates were seeded with the examined

cell lines (180 µL) at a density of 7×10^3 cells per milliliter, and the plates were incubated at 37 °C for 24 h. Then, 24-, 48-, and 72-h incubations at 37 °C were performed with PSC at doses of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL, along with a 7 µL/well addition of 5-Fluorouracil (5-FU) (50 mg/mL) as a positive control. Following the designated time in culture, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for another 4 h at the same temperature as the growth condition. After discarding the growth medium, we added 150 µL each of dimethyl sulfoxide (Sigma, St. Louis, MO, USA) and Sorenson buffer to the produced blue formazan crystals to dissolve them. The ELISA reader (ELx 800; Biotek, Winooski, VT, USA) was used to measure the absorbance at 570 nm. It is worth noting that every experiment was repeated three times.

Cell Cycle Analysis

Research into the anticancer properties of biomolecules produced from gut microbes sometimes includes cell cycle assay as a supplemental and confirmatory test. We used flow cytometry to examine the effects of 48 h of treatment with postbiotic metabolites on SW480 cells, looking for evidence of cell cycle arrest responses in addition to cytotoxicity [21]. It is important to note that the IC_{50} values obtained from the cytotoxicity test serve as the basis for selecting the time or/and concentration variables in this experiment. A six-well cell culture plate was seeded with 3×10^6 SW480 cells per mL, and the plate was placed in a 5% CO_2 incubator at 37 °C for 5 h. After 48 h in a growth medium, the cells were treated with 24 µL of postbiotic metabolites, harvested from the wells, centrifuged at 3000 rpm for 10 min, and given two washes. Following a final fixation step in cold ethanol (70%), the cells were incubated for 30 min at 37 °C in 1 mL of PI master mix solution comprising 950 µL of phosphate-buffered saline (PBS) (pH 7.4), 10 µL of RNase, and 40 µL of the PI solution. Following this, flow cytometry (Becton Dickinson, San Jose, CA) was used to analyze the cells' phases of division.

Annexin V/PI Binding Assay

Annexin V-FITC/propidium iodide (PI) cell apoptosis kit (eBioscience, BD Biosciences, San Diego, CA, USA) was used to quantify apoptosis in SW480 cell lines as per the manufacturer's instructions. In a nutshell, cells were seeded at a density of 0.5×10^6 cells per well on six-well culture plates and then treated to PSC (23.58 µg/mL) for 24 and 48 h. The cells were then removed, washed with PBS, incubated at room temperature in the dark for 30 min with annexin V-FITC binding solution, and then incubated for 5 min with PI [22]. FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) was used to measure annexin V and PI expression.

RNA Extraction and qRT-PCR

The SW480 cells were grown in RPMI medium with 10% fetal bovine serum. After adding the cell suspension (~2×10^5 SW480 cells) to the 6-well plates, we put them in an incubator at 37 °C, 5% CO_2, and humidified air for 48 h. PSC and 5-FU were used to treat the cells independently at the IC_{50} concentration. Total RNA was isolated from the cells using TRIzol Reagent (Invitrogen, USA) after 8, 24, and 48 h of incubation at 37 °C under the aforementioned conditions, and cDNA was generated as described below. After incubating at 65 °C for 5 min, 1 µg of mRNA, 1 µL of random hexamer primers, 1 µL of dNTP, and DEPC water up to 12.5 µL were combined. The reaction volume was adjusted to 20 µL with DEPC water before the addition of 1 µL of MMLV reverse transcriptase, 0.5 µL of RNase inhibitor, and 4 µL of reaction buffer. Both the 10-min and 60-min portions of the reverse transcriptions were performed at 25 °C. At 72 °C for 5 min; the process was stopped. Thermo Scientific was used for the procurement of all reverse transcription reagents.

SYBR Master Mix and a Bio-Rad IQ5 real-time PCR detection equipment (Bio-Rad, Hercules, CA, USA) were used to quantify the expression levels of the target mRNAs. First, the qRT-PCR was run at 94 °C for 10 min. Then, 45 cycles of annealing at various temperatures (Table 1) for 20 s and extension at 72 °C for 15 s were performed. Specificity was

Table 1 Sequence and annealing specifics of the primers utilized for qRT-PCR amplification

mRNA/miR	Sequence	Annealing (°C)
IκB	Forward: 5′-GCTGAAGAAGGAGCGGCTACT-3′	60
	Reverse: 5′-TCGTACTCCTCGTCTTTCATGGA-3′	
RelA	Forward: 5′-CTGTGCGTGTCTCCATGCA-3′	61
	Reverse: 5′-TCGTCTGTATCTGGCAGGTACTG-3′	
BAX	Forward: 5′-GATGCGTCCACCAAGAAG-3′	56
	Reverse: 5′-AGTTGAAGTTGCCGTCAG-3′	
BCL-XL	Forward: 5′-GTTCCCTTTCCTTCCATCC-3′	60
	Reverse: 5′-TAGCCAGTCCAGAGGTGAG-3′	
GAPDH	Forward: 5′-AAGCTCATTTCCTGGTATGACAACG-3′	63
	Reverse: 5′-TCTTCCTCTTGTGCTCTTGCTGG-3′	

Table 2 The mean inhibition zone diameter (mm) of postbiotics of *Saccharomyces cerevisiae* PTCC 5269 (PSC), on some pathogenic microorganisms by disc diffusion agar (DDA) method

	Antimicrobial substance				
Microorganism	PSC	In (Tet + PSC)	In (Chl + PSC)		In (Gen + PSC)
Salmonella typhi	12.25 ± 0.32c	20.34 ± 0.51c	23.21 ± 0.32b	26.29 ± 0.32a	
Escherichia coli	9.56 ± 0.45d	18.65 ± 0.35c	27.39 ± 0.15a	30.46 ± 0.34a	
Streptococcus mutans	16.81 ± 0.15b	24.75 ± 0.17b	14.42 ± 0.65d	25.36 ± 0.35a	
Listeria monocytogenes	22.41 ± 0.68a	34.32 ± 0.43a	21.26 ± 0.52c	23.34 ± 0.19b	

Means within the same row with different small letters differ significantly ($P < 0.05$)

determined once PCR was completed by analyzing melting curves. Amplification of cDNA at 10 different concentrations was performed using quantitative real-time PCR to create the standard curve. Using the PCR cycle number (C_T), we determined the expression level and then normalized the mRNA expression level using the GAPDH gene (as an endogenous control gene). Standard $2^{-\Delta\Delta CT}$ calculations demonstrated the ability to quantitatively express relationships. There were three separate runs of each response [23].

Statistical Analysis

All data were analyzed using GraphPad Prism (GraphPad Software; San Diego, California, USA). The Levene test was carried out to ensure the equality of variances. Distinctions between the groups were evaluated using unpaired, two-tailed *t*-tests or one-way analysis of variance (ANOVA) for normally distributed data. Results are shown as mean ± SD ($n = 3$). The threshold for significance was set at $P < 0.05$.

Results

Total Phenols and Flavonoids of the Prepared Postbiotics

The PSC has a high concentration of phenolic (102.46 ± 0.25 mg GAE/g) and flavonoid (19.87 ± 75.32 mg QE/g) components. Indeed, the content and quality of the final obtained PSC solution are affected by growth and extraction circumstances, along with primary stock preparation procedures. Taking into account all of the effective elements, the examined PSC included a large quantity of flavonoid and phenolic substances.

Antioxidant Activity of the Prepared Postbiotics

Since polyphenols contain natural redox capabilities that may neutralize free radicals, these bioactive substances have the ability to strengthen the immune system while also having preventative benefits on a number of degenerative illnesses, including diabetes and cardiovascular disorders [24]. The immediate oxidation of ABTS using $K_2S_2O_8$ to produce a stable form of $ABTS^{\bullet+}$ with blue color is the basis for the ABTS assessment. Next, at 750 nm, the radical solution's degree of decolorization (reduction) is assessed. The PSC had a remarkably strong antioxidant capacity (87.34 ± 0.56%).

Antibacterial Activity of the Prepared Postbiotics

Under In Vitro Condition

The antimicrobial property of the PSC was examined in this study using antimicrobial assays, such as DDA, WDA, MIC, and MBC, against some spoilage and pathogenic bacterial species. The type of yeast varieties affected the PSC's antibacterial activity, and the microbial byproducts frequently affected Gram-positive bacteria more than Gram-negative bacteria (Tables 2 and 3). For Gram-positive bacteria, the average inhibition zone was 19.61 mm and 23.24 mm in the DDA and WDA tests, respectively, whereas the mean inhibition zones for Gram-negative bacteria were 10.90 mm and 15.16 mm, in the DDA and WDA tests, respectively. Similar findings were obtained from the MIC and MBC assays, and less PSC was required to kill or limit the development of the Gram-positive bacteria than the Gram-negative bacteria (Table 3).

Under Food Models

The minimum effective concentration of an antimicrobial substance is the concentration required to prevent the development of a pathogen in a food product. Table 4 shows the estimated MECs of PSC against *L. monocytogenes* and *E. coli* in a variety of challenge matrices. It was found that the MECs

Table 3 The well diffusion agar (WDA), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the postbiotics of *Saccharomyces cerevisiae* PTCC 5269 (PSC) on some pathogenic microorganisms

Microorganism	WDA (mm)	MIC (mg/mL)	MBC (mg/mL)
Salmonella typhi	16.13 ± 0.31 c	9.51	36.52
Escherichia coli	14.20 ± 0.16 d	9.43	14.76
Streptococcus mutans	18.14 ± 0.15 b	2.52	4.62
Listeria monocytogenes	28.35 ± 0.48 a	2.44	4.85

Means within the same column with different small letters differ significantly ($P < 0.05$)

Table 4 Minimal effective concentration (MEC) (mg/mL) of the postbiotics of *Saccharomyces cerevisiae* PTCC 5269 (PSC) on *E. coli* and *L. monocytogenes* in whole milk, ground meat, and culture media

Inoculated bacteria	*E. coli*			*L. monocytogenes*		
	BHI broth	Whole milk	Ground meat	LB broth	Whole milk	Ground meat
PSC	30	50	50	20	30	40

of PSC were significantly higher in ground beef and milk in the *L. monocytogenes* inoculation model, respectively, but that the MECs varied widely among the various food models. Similar to in vitro conditions, PSC showed weak antibacterial activity (50 mg/mL in food models) when exposed to an *E. coli* inoculated model. This highlighted the significance of structural differences between Gram-positive and Gram-negative bacteria on the antimicrobial properties of PSC.

Growth-Promoting Properties of the Prepared Postbiotics

After 48 h of incubation, PSC was more effective than glucose at supporting *B. bifidum* ATCC 35914 growth. These findings suggested that PSC was used by *B. bifidum* ATCC 35914 for energy metabolism (Fig. 1). After 5 h, *L. casei* L431 began utilizing the synthesized PSC, and after 30 h, the cell density reached that of the positive control as well as the growth behavior of *L. casei* L431 to the presence of PSC in the media has been demonstrated in a diauxic growth curve form. However, cell density increased only slightly in the second logarithmic stage of the growth curve (Fig. 1). In regard to *L. brevis* TD4, it was detected that the PSC can be utilized as a carbon and energy source; however, its growth-promoting effect has significant difference ($P < 0.05$) from the glucose source in the positive control at the end of the incubation (Fig. 1). By the end of the incubation period, cell density had reached the same level as the positive control when PSC was added to the growth medium of *L. acidophilus* ATCC 4356. Therefore, it can be stated that in the case of *L. acidophilus* ATCC 4356, the growth and proliferation processes have demonstrated a strong positive response to the existence of PSC in the milieu, and in a way, it shows the importance of the capability of various strains to use PSC carbon sources, which can possess a significant impact like a glucose source (Fig. 1).

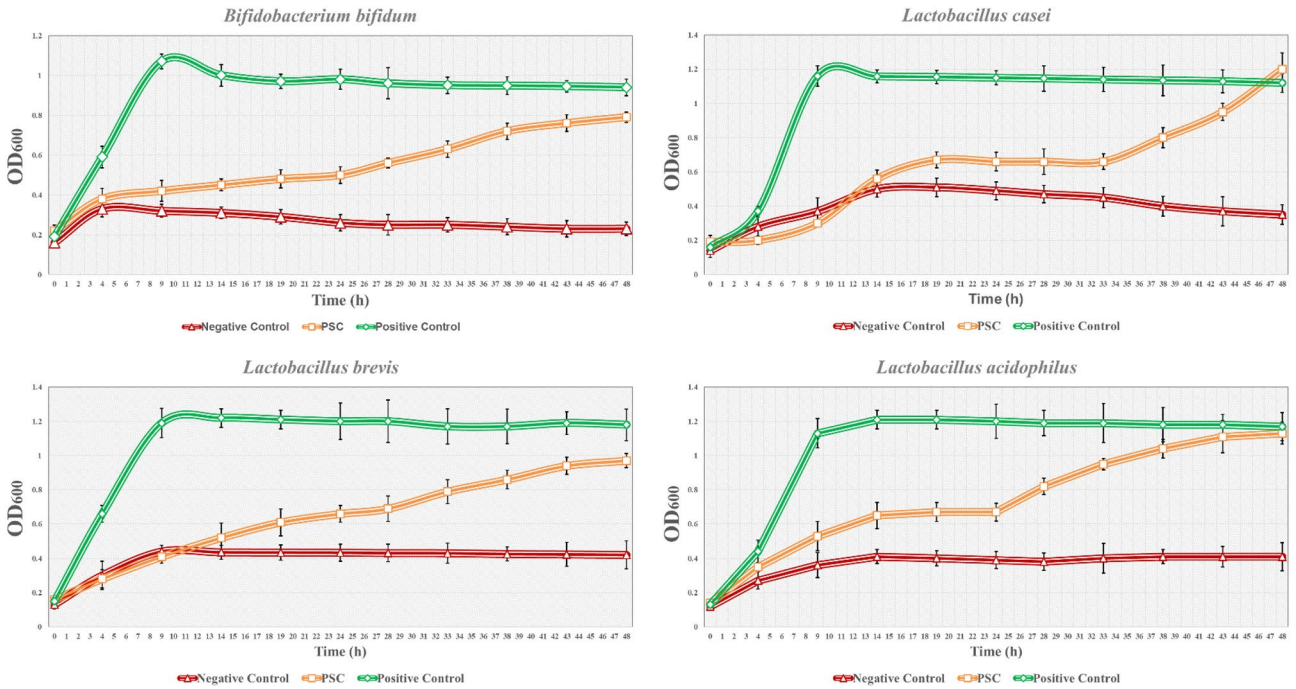

Fig. 1 The growth curve of treated probiotic strains with the MRS medium containing postbiotics derived from *Saccharomyces cerevisiae* (PSC), MRS medium containing 0.5% glucose (positive control), and sugar-free MRS medium (negative control). All the experiments were performed, at least, in triplicate. All the data are presented as mean ± SD ($n = 3$)

α-Glucosidase Inhibition Activities of the Prepared Postbiotics

In this research, PSC was synthesized and utilized to suppress α-glucosidase activity. There was a dose-dependent increase in enzyme inhibition in all replicates within the treatment group ($P < 0.05$) (Fig. 2A).

Cholesterol Removal Capabilities of the Prepared Postbiotics

Cholesterol was reduced by the tested PSC, although the degree to which it reduced cholesterol varied with PSC concentration and the milieu pH value (Fig. 2B). The maximum cholesterol-lowering activity (67.58%) was seen with 2 mg/mL PSC at pH 4.0, whereas the lowest activity (30.27%) was observed with 0.5 mg/mL PSC at pH 7.0 ($P < 0.05$).

Anticancer Activity of the Prepared Postbiotics

Cytotoxic Assay

The effect of different PSC doses on SW480 cell viability at 24 and 48 h is shown in Fig. 3. The IC_{50} of PSC was calculated to be 34.27 µg/mL after 24-h treatments and 23.58 µg/mL after 48-h treatments. There was a concentration-dependent, statistically significant ($P < 0.05$), and a significant decrease in colon cancer cell viability as a result of PSC's cytotoxic action. PSC significantly ($P < 0.05$) reduced cell viability of SW480 cells after 48 h compared

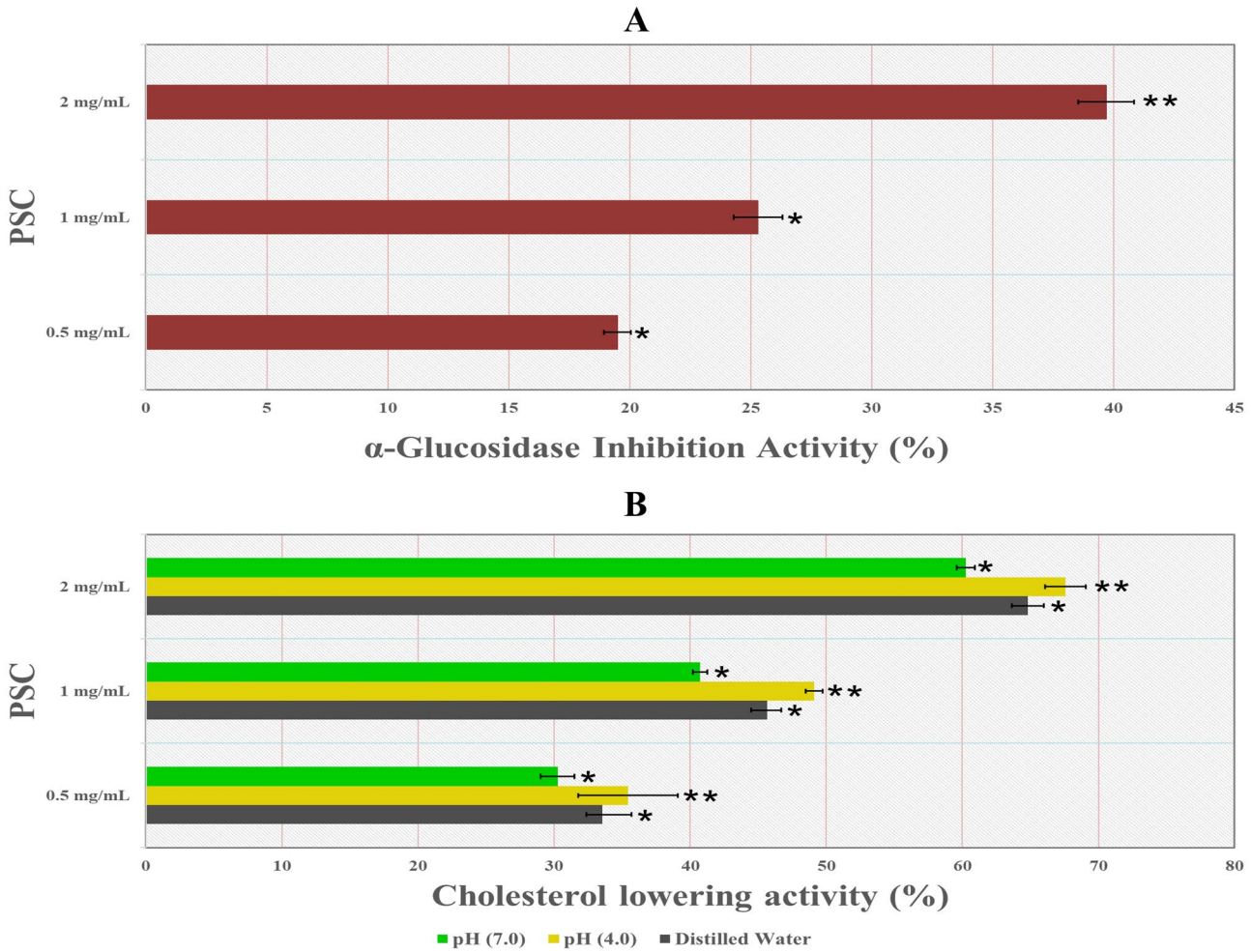

Fig. 2 The α-glucosidase inhibitory (**A**) and cholesterol-lowering (**B**) activities of postbiotics derived from *Saccharomyces cerevisiae* (PSC) at different concentrations (0.5, 1, and 2 mg/mL). All the data are presented as mean ± SD ($n = 3$). *$P \leq 0.05$ and **$P \leq 0.01$ indicate significant and highly significant differences between the concentrations at each PSC at **A** and PSC at **B**

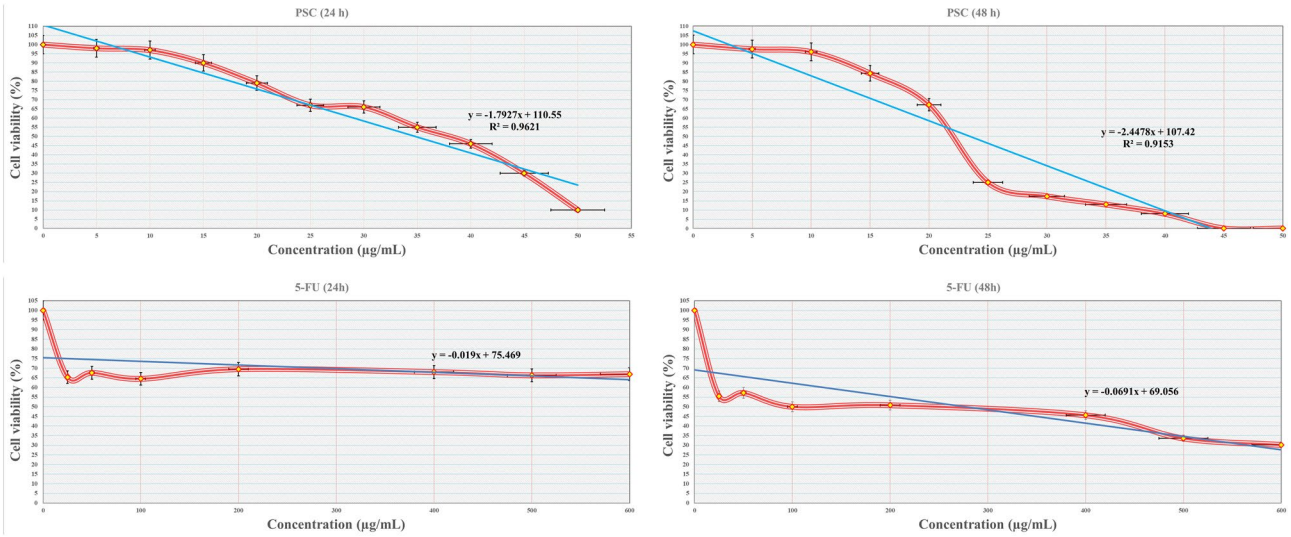

Fig. 3 MTT assay of different concentrations of postbiotics derived from *Saccharomyces cerevisiae* (PSC) and 5-FU (positive control) on SW480 cancer cell lines after 24- and 48-h treatment. All the experiments were performed, at least, in triplicate. All the data are presented as mean ± SD ($n = 3$)

to other circumstances (24-h treatment of PSC and 24–48-h exposure to 5-FU). This means that PSC is cytotoxic to human adenocarcinoma colon cells.

Cell Cycle Analysis

After 48 h of treatment with PSC, it was found that the majority of SW480 cells had entered the sub-G1 (G0/G1) (73.45%) and G2M (15.23%) phase of the cell cycle, indicating that exposure to postbiotic metabolites inhibited the division and growth of cancer cells in the early stages of their cell cycle (Fig. 4).

Annexin V/PI Binding Assay

The strongest barrier against the proliferation of cancer is apoptosis, the programmed death of cells that regulate homeostasis. The regulation of apoptosis, which might represent among the most significant techniques for avoiding colorectal cancer, may be triggered, according to various studies, by probiotic microorganisms and their generated biological metabolites. We labeled cells with annexin V-FITC/PI to show that PSC induced substantial apoptotic responses in SW480 cells (Fig. 5A). After being exposed to 23.58 µg/mL of PSC for 24 and 48 h, we found that the apoptosis rate was significantly greater in the treatment groups than in the control group ($P < 0.001$, Fig. 5B).

qRT-PCR Results

Cells were treated to an IC_{50} dosage of PSC and 5-FU for varying amounts of time before RNA was extracted, and qRT-PCR was done to determine the effects of the treatments on Akt/NF-κB-induced apoptosis. *IkB* mRNA expression peaked after 8 h and declined steadily during the next 48 h. At 48 h, there was a significant ($P < 0.01$) reduction in *IkB* mRNA expression in both treatment groups (Fig. 6). After 48 h of treatment, *PTEN* expression was observed to be statistically ($P < 0.01$) greater in the PSC- and 5-FU-treated groups compared to the untreated group. In addition, PSC was more effective than 5-FU at inducing *PTEN* mRNA expression (Fig. 6). At 8 h, the mRNA expressions of *RelA* were significantly ($P < 0.01$) reduced by both treatments, followed by a time-dependent upregulation (Fig. 6). The mRNA expression of *Bcl-XL* was higher in the PSC- and 5-FU treated group at 8 h compared to the untreated group due to cell resistance [27]. Both treatments resulted in a significant ($P < 0.01$) decrease of *Bcl-XL* at 48 h compared to 8 h, as was to be expected (Fig. 6).

Discussion

Reducing mortality from all causes is a top target for the Sustainable Development Goals in 2023. In this context, a unique therapeutic method is the use of microbial bioactive substances with features like high compatibility and

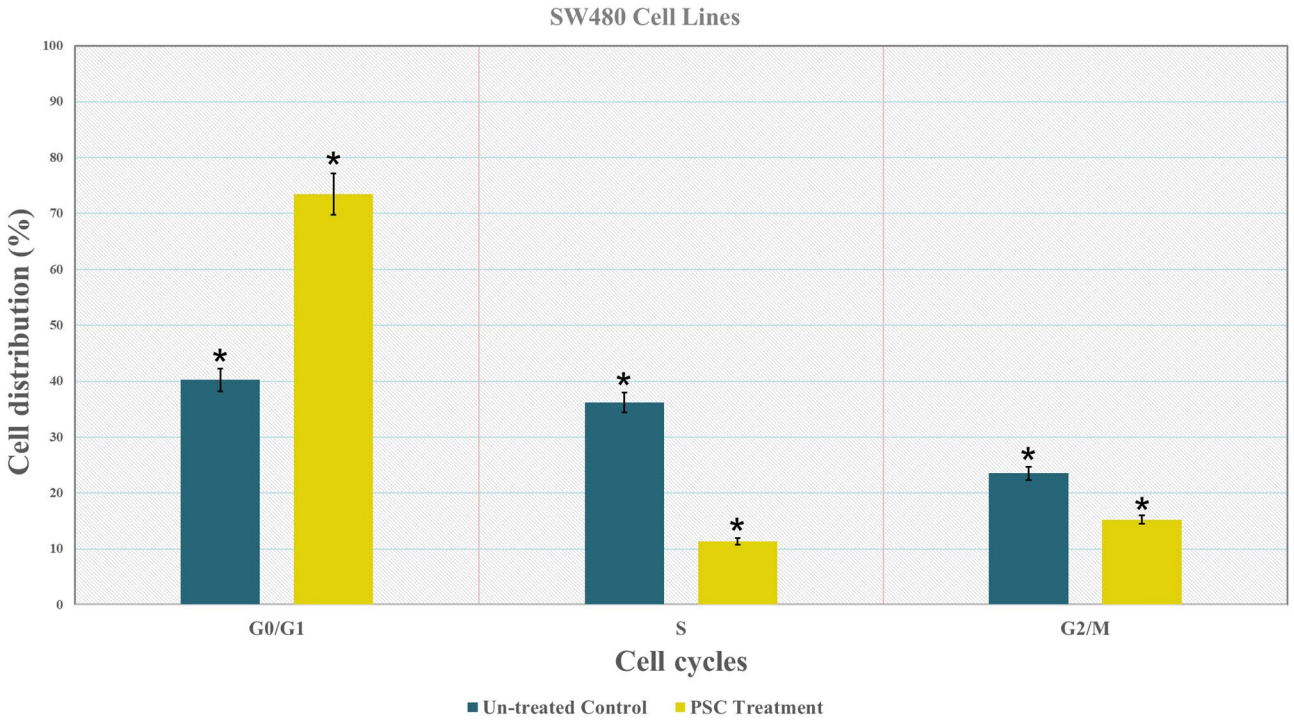

Fig. 4 Effect of postbiotics derived from *Saccharomyces cerevisiae* (PSC) and treatment on the cell cycle of SW480 cells by flow cytometry. Values are mean ± SD, *$P < 0.05$ v/s untreated cells

tight contact with the host immune system. Postbiotics with distinct structures and functions are critical mediators between the gut microbiota and human cellular processes/ metabolic pathways. Healthcare systems (especially in developing nations) will benefit greatly from a better understanding of the nature of parent microbial cells and

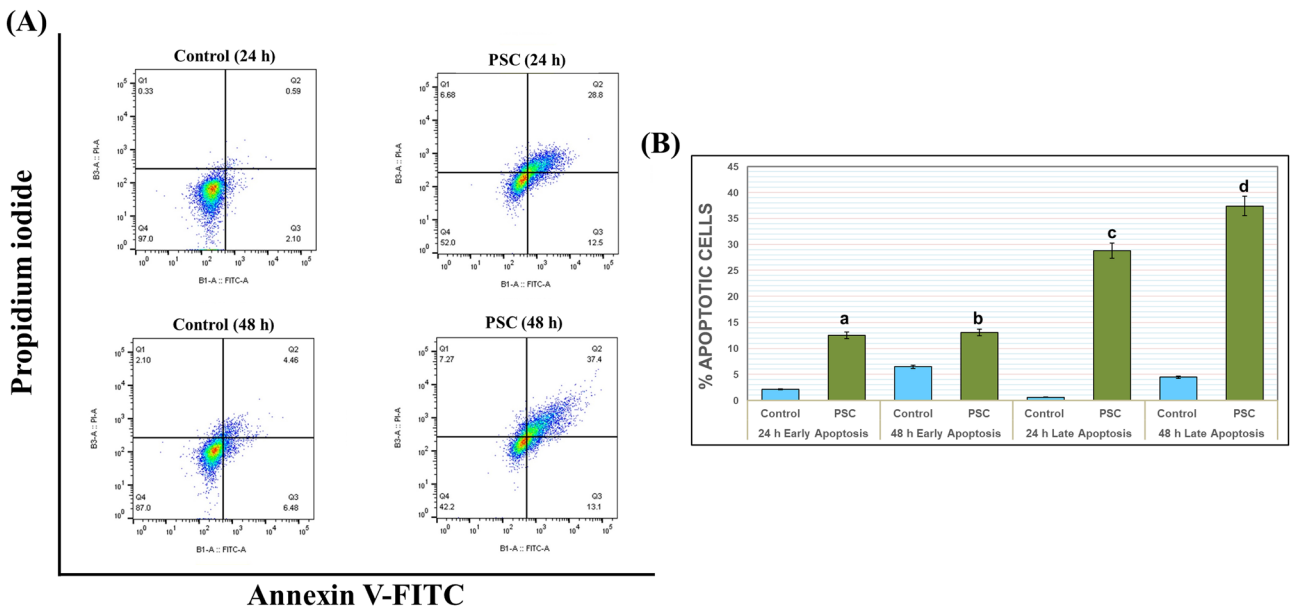

Fig. 5 Apoptotic effects of postbiotics derived from *Saccharomyces cerevisiae* (PSC), on SW480 cell line. SW480 cells were treated with the concentration of 23.58 µg/mL of PSC for 24 and 48 h. Apoptotic cells were stained with annexin V-FITC/propidium iodide (PI) for flow cytometry assay. **A** The raw flow cytometry figures. **B** The apoptosis rates. Data are expressed as mean ± SD ($n = 3$). [a,b]Means for each value without a common letter differ significantly ($P < 0.05$). [c,d]Means for each value without a common letter differ significantly ($P < 0.001$)

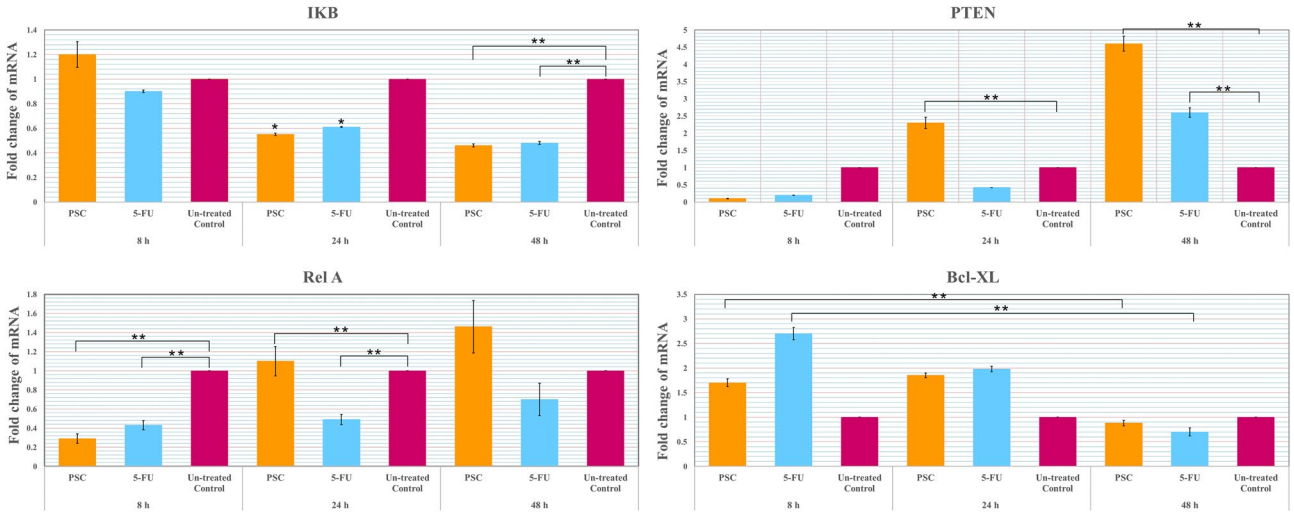

Fig. 6 The expression levels of *IkB*, *PTEN*, *RelA*, and *Bcl-XL* genes in the postbiotics derived from *Saccharomyces cerevisiae* (PSC)- and 5-FU-treated SW480 cells during 8, 24, and 48 h. The data are expressed as fold changes. Target genes were normalized to GAPDH as housekeeping control gene. All the experiments were performed, at least, in triplicate. All the data are presented as mean ± SD ($n = 3$). *$P \leq 0.05$ and **$P \leq 0.01$ indicate significant and highly significant versus the control group, respectively

elements affecting their metabolic pathways, as this will allow for the development of postbiotics with unique efficiencies [25]. In this context, the most usual yeast probiotic strain (*S. cerevisiae*) utilized in the fermentation process has been selected as parent microbial cells for generating postbiotic metabolites in this study, and in the following, their potential biological activities have been assessed under in vitro and food circumstances.

In regard to phenolic and flavonoid contents of the prepared postbiotics, it was demonstrated that the matrix of PSC possesses significant levels of these components. It is worth noting that phenolic and flavonoid components have a significant impact on PSC's biological activities [26]. Molska and colleagues (2022) investigated the concentration of phenolic elements and their impact on radical scavenging and anti-inflammatory activities, as well as dietary fiber, in transformed buckwheat sprouts. Buckwheat seeds were modified for this purpose by introducing *S. cerevisiae* var. *boulardii*. The altered buckwheat sprouts had a greater total phenol compound content (1526 µg/g d.w.) than the seed (672 µg/g d.w.) and control group (951 µg/g d.w.). The addition of probiotic yeast to the sprouts increased their nutritional content in addition to their anti-inflammatory and antioxidant activities [27]. In addition, Wang and colleagues (2022) used three commercial lactic acid bacteria (*L. plantarum* 90 (Lp90), *Lactobacillus helveticus* 76 (Lh76), and *L. acidophilus* 85 (La85)) to study the impacts on the phenolic compounds, antioxidant potentials, and flavor volatiles of kiwifruit juices made from two cultivars (*Actinidia chinensis* cv. Hongyang and *Actinidia deliciosa* cv. Xuxiang). The results indicated that *L. helveticus* 76 significantly boosted the total flavonoids and phenolics in Hongyang and Xuxiang juices. Moreover, protocatechuic acid and catechin concentrations (two newly generated phytochemicals in fermented kiwifruit juices) were meaningfully connected with antioxidant capabilities based on DPPH, ABTS, and FRAP techniques and considerably enhanced ($P < 0.05$) [28]. Hence, it can be stated that probiotic yeasts/bacteria and their postbiotic metabolites possess a significant level of phenolic and flavonoid compounds that directly influence their exhibited antioxidant activities.

Byproducts of cellular metabolism include reactive oxygen species (ROS), which may play a role in cell signaling as second messengers for a number of different biological processes. Cell and tissue damage results from an imbalance between ROS formation and protective antioxidants (oxidative stress), which may be exacerbated by environmental stressors including UV-B radiation and xenobiotic substances like mycotoxins. Multiple chronic diseases, such as cardiovascular disease and cancer, have been linked to this condition [29]. Various antioxidants from exogenous origins have been investigated for their protective effects toward oxidative stress due to the crucial role it plays in the development of such clinical conditions. Antioxidants from various sources have been explored [30], but the most essential ones come from vegetables and fruits and include vitamins (E, C), carotenoids, polyphenols (e.g., flavonoids), and minerals (e.g., selenium, zinc). Some probiotics have antioxidant activity, minimizing oxidative damage [31, 32]. Studies have demonstrated that probiotics exert this effect through antioxidant capacity, metal ion sequestration, microbiota modification, and/or modulating the expression of the

Nrf2 transcription factor via direct interaction with the host intestine epithelial cells; however, the exact mechanisms involved have not yet been established [33]. It is important to remember that these processes rely on microorganisms to function properly. In contrast, recent findings suggest that *Lacticaseibacillus casei* CRL 431's intracellular content may also mitigate the oxidative stress caused by aflatoxin B1 (AFB_1) in rats [34] and acrylamide in human erythrocytes [32]. In this regard, our results exhibited that the prepared postbiotics possess significant antioxidant activity under in vitro conditions, and flavonoids, phenols, and the primary chemical components of PSC all contribute to this remarkably strong antioxidant activity [48]. Several studies have reported the PSC's antioxidant properties [5, 35–37]. As a result, under in vitro or/and in vivo circumstances, the PSC with their safety profile might have been employed to postpone or block the free radical reactions and related oxidative impairments.

In regard to the antibacterial activity of the PSC, *L. monocytogenes* and *E. coli* earned the maximum and lowest inhibition zones, respectively, based on the results of the DDA and WDA tests. Moreover, the WDA method's inhibitory zone was much larger than the DDA assay's. This is largely because the former approach directly contacts the PSC with the bacterial species, while the latter method does not [38–40]. A synergistic impact between the PSC and antibiotics may also be worth mentioning; this combination had a typically stronger antimicrobial property than the PSC alone (Table 2). These outcomes have been attributed to variations in the bacterial cell wall structure based on the results of the MIC and MBC assessments. The mucopeptide layer is found to be thicker in the first group than in the second. Moreover, the primary components of the walls of Gram-negative bacterial species include lipoproteins and lipopolysaccharides, which contribute to their greater resistance to antimicrobial substances [41]. The PSC's capacity to prevent the production of vital enzymes produced by bacteria and/or harm the bacterium's cell wall is thought to be the reason for its antibacterial action [42, 43]. In this context, 42 yeast strains were recovered from *Bollo* batter by Pereira and colleagues (2021). Four yeast isolates (DABRP1, DABRP2, DABRP5, and DABRP12) were obtained in this study from the first screening of the isolates with probiotic capabilities and were identified as *S. cerevisiae* using D1D2-LSU-rDNA sequencing. The pathogenicity of the samples was assessed using the in vitro hemolysis assessment and measurement of gelatinase and DNase activities in order to assess the safety of the samples and their postbiotic metabolites. All of the isolates were regarded as possibly safe since none of them showed hemolysis or generated DNase or gelatinase. The strongest isolate of *S. cerevisiae*, DABRP5, demonstrated antimicrobial properties versus the investigated pathogens (*S. enterica* serovar *Typhi*, *P. aeruginosa*, and *E. coli*) [44].

The well-known foodborne pathogen *L. monocytogenes* can endure a wide variety of environmental circumstances, including temperature (1–45 °C), pH (4.3–9.8), and high salt levels [45]. One of the most serious foodborne diseases is listeriosis, which is induced by the bacterium *L. monocytogenes*. Pregnant women, newborns, and people with impaired immune systems are particularly vulnerable to listeriosis [46]. According to a report from the Centers for Disease Control and Prevention (CDC), listeriosis has one of the highest hospitalization rates of any disease. Each year, around 1600 individuals get listeriosis, and 260 people pass away from it [47]. According to our outcomes, postbiotics of *S. cerevisiae* possess a significant growth inhibitory effect on *L. monocytogenes* that in turn enable this probiotic yeast and their derived postbiotic metabolites to develop in food biotechnology practices, especially in meat and its microbial/plant analogues, to establish microbial safety. Li and colleagues (2021) found that marinating chicken breast strips in a marinade containing *S. boulardii*, a leucocin C bacteriocin producer, inhibited the proliferation of *L. monocytogenes* in raw chicken meat. The marinade preparation was proven to sustain its antibacterial effectiveness for 38 days in this experiment. After marinating chicken breast strips contaminated with *L. monocytogenes* in the antibacterial marinade preparation for an overnight period, the amount of *L. monocytogenes* that were eliminated was determined. Leucocin C marinating decreased the cell viability of *L. monocytogenes* by approximately 1.6 logs from $(2.2 \pm 0.6) \times 10^7$ CFU/g on day 24 and by 2.2 log from $(1.8 \pm 0.3) \times 10^5$ CFU/g on day 38 [48]. Thus, it can be concluded that marinade preparations containing *S. cerevisiae* and its effective postbiotic metabolites may be thought of as a promising tool for lowering the incidence of *Listeria* in chicken breast strips.

In regard to investigated food models, an isolate of *Leuconostoc* was shown to be effective against *E. coli* in ground beef in a case of CFS [49]. The organic acids produced by *Leuconostoc* were related by the authors to the bacterium's antibiotic properties. It has been found that a 1.0% concentration of CFSs from *L. acidophilus*, *B. bifidum*, and *L. plantarum* is antibacterial against *E. coli* [50]. They determined that the organic acids found in CFS may be responsible for this behavior. Organic acids and potentially other undiscovered bacteriocin-like components in *L. salivarius'*s CFS provide the necessary antibacterial activity against certain gram-positive bacteria (*L. monocytogenes*). For reasons including commodity complexity, solubility and adsorption of CFS to food matrix, and communications of CFS elements with food components [51], it is evident from this study that the effectiveness of CFS in culture media is significantly higher than in food matrices. Hartmann and colleagues (2011) studied the effects of CFS of several *Lactobacillus* spp. on the growth and survival of *L. monocytogenes* in different media and reported a greater MEC of

the CFS towards *L. monocytogenes* in ground beef and milk compared to culture broth [14].

Yeasts have long played an important role in the production of a variety of fermented foods, and due to their high potential in the synthesis of postbiotic compounds with a safe profile and impressive biological activities (e.g., antimicrobial activity), they may be useful in the development of functional, nonfermented foods as well.

In regard to the growth-promoting properties of PSC, all probiotic strains evaluated in this research showed increased growth, proving the study's hypothesis that PSC has a growth-promoting impact. The probiotic strains stimulated growth throughout the fermentation period of 48 h, but in the growth media, no fast growth was detected in the condition of the PSC compared to glucose. This was likely because the PSC's complex components took longer to hydrolyze and transfer during growth. Moreover, it is noteworthy that the growth-promoting effect of PSC is largely strain-dependent and according to this, various postbiotic preparations can be chiefly formulated for the target strain (particularly those utilized as starter culture in fermented food products).

In regard to the α-glucosidase inhibitory activity of PSC, the results demonstrated that this effect is largely concentration-dependent, and various investigated concentrations of PSC (0.5, 1.0, and 2.0 mg/mL) possess significant increments in the suppression of the enzymatic activity of α-glucosidase. Although the glucosidase inhibitory effect of *the L. plantarum* BR2 strain's high molecular weight postbiotics [52] was higher (67%) than our findings, the PSC was still significantly effective.

According to the results of the cholesterol removal capabilities of PSC, it can be stated that this effect is mainly concentration- and pH-dependent. It is noteworthy that the efficiency of PSC had established a direct and inverse relationship with the concentration and pH value, respectively. The mentioned consequences can be caused by the effect of different pH values on the charge density of polysaccharide compounds and the structural and functional changes of protein and peptide elements in PSC solution. Different postbiotics have been shown to have varying degrees of success in lowering cholesterol, from 31 to 48.81% [52, 53]. Our results showed that PSC's cholesterol-lowering activities were consistent with those found in the literature, whereas the 2-mg/mL concentration of PSC at pH 4.0 showed much higher levels of cholesterol-lowering capabilities than those found in the published literature. These results imply that the PSC's ability to remove cholesterol may vary with factors such as size, charge, structure, and the presence of functional groups under the investigated circumstances.

Colon cancer is the leading cause of cancer-related mortality globally and is one of the most frequent and aggressive malignancies of the digestive system. Due to its high incidence in the West, Iran ranks fourth for females and third for males. Scientific data reveal that *S. cerevisiae* and their biometabolites have significant growth and proliferation-inhibiting impact on the breast, bladder, stomach, and colon cancer cells [54]. *S. cerevisiae* has considerable antiproliferative and antioxidant capabilities, and it also modulates immune system responses and alters the composition of the gut microbiota. We showed that *S. cerevisiae* postbiotic metabolites exhibited a significant cytotoxic effect against cancer cells under study, further indicating the presence of biological molecules with antitumor function. These findings are consistent with those of previous studies showing that postbiotics of *B. adolescentis* SPM0212 inhibited the growth of HT-29, SW-480, and Caco-2 colon cancer cell lines to a greater extent than live/heat-inactivated. The findings demonstrated that the cytotoxic effects were not only time- and dose-dependent but also selective, with the strongest responses occurring after 48 h and at a concentration of 23.58 µg/mL.

Complex protein and carbohydrate molecules compose *S. cerevisiae*'s postbiotic metabolites. Cancer cell growth may be slowed or inhibited by taking yeast CFS, which contains beta-glucan and mannan [55]. Insoluble glucans and their derivatives are responsible for yeast cell wall extract's antiproliferative actions on cancer cells [56]. Postbiotics have been found to exhibit anti-inflammatory properties in a number of in vitro experiments employing THP-1 monocytes, Caco-2 cells, and HT-29 colonocytes in addition to their cancer-preventative effects [57]. The PSC was observed to decrease SW480 cell viability. We were prompted to finish our study by an interest in how PSC affects cell death and related gene expression in the context of these cancer cells. The strongest protection against cancer's spread is apoptosis, a programmed cell death process that maintains homeostasis. The present evidence suggests that yeast-based probiotics may be one of the most significant treatments for reducing colorectal cancer by stimulating the regulation of apoptosis. We used annexin V-FITC/PI staining to show that PSC induces apoptosis in SW480 cells (Fig. 5A). After 24 and 48 h of exposure to 23.58 µg/mL of PSC, the apoptosis rate in the treatment groups was significantly greater than in the control group ($P < 0.001$, Fig. 5B).

The Akt/NF-κB, MAPK (mitogen-activated protein kinase), pro-apoptotic, and antiapoptotic signaling pathways are often modified by probiotics. In this work, we looked at the signaling pathways involved in the antiproliferative and pro-apoptotic actions of PSC and 5-FU on a colorectal cancer cell line. Figure 7 shows that NF-κB and Akt are abnormally active in cancer cells [58]. Host cell surface receptors may be used to activate the positive effects of the probiotic bacteria or its postbiotics on host signaling pathways. Decreased levels of p-Akt induce apoptotic responses [59] when postbiotic metabolites or/and 5-FU are present. Caspase activation (caspase 3 and 9), the mitochondrial

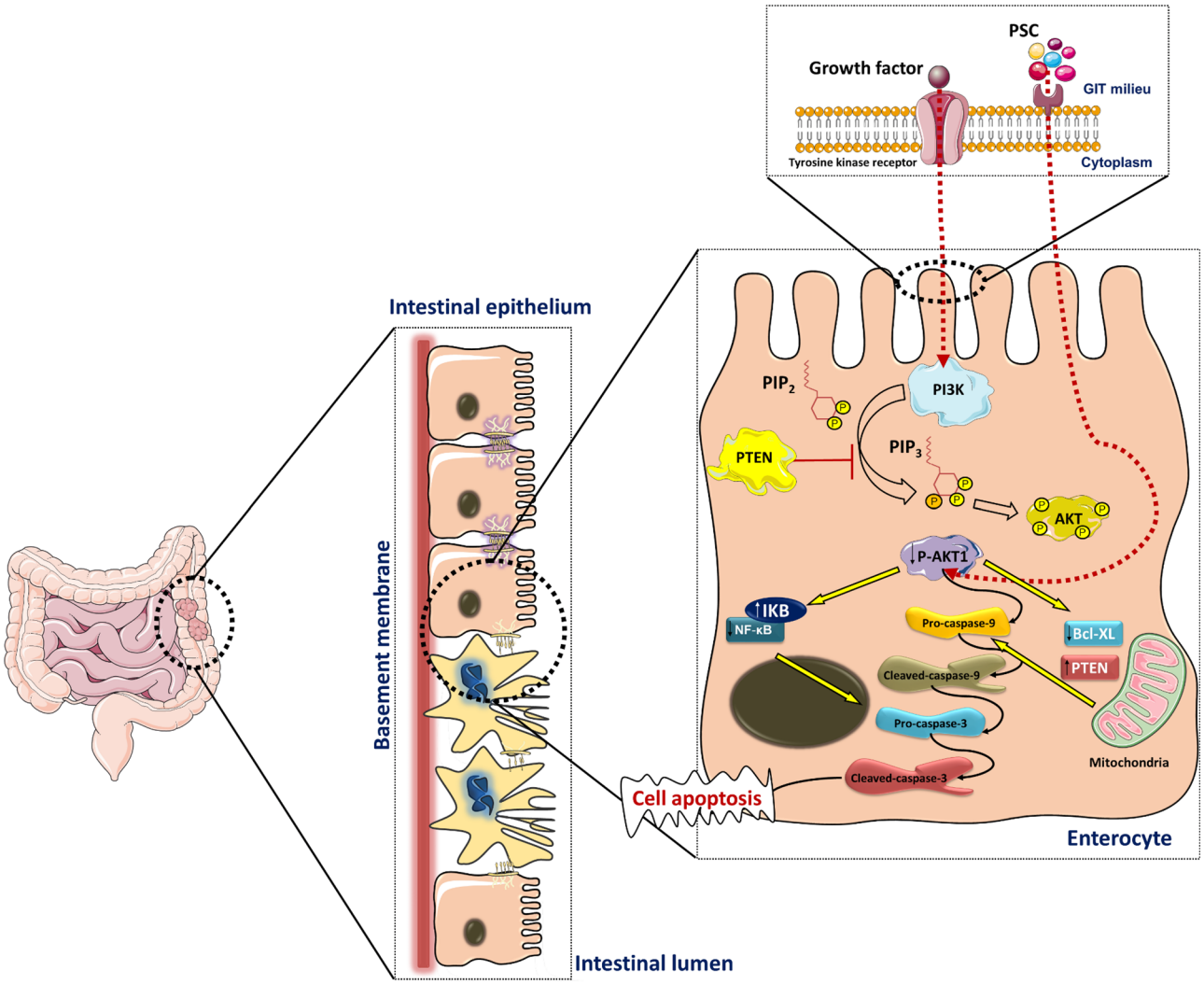

Fig. 7 Schematic illustration of the proposed signaling pathway involved in the anticancer activity of the postbiotics derived from *Saccharomyces cerevisiae* (PSC) on colon cancer cells. In cancer cells, PI3K-Akt and NF-κB-AKT pathways are overactivated. In response to extracellular stimuli (e.g., chemokines, growth factors, presence of insulin), PI3K is activated by tyrosine kinase receptors or G-protein-coupled receptors and it phosphorylates PIP2 to generate PIP3 which in turn phosphorylates and activates Akt. The main function of *PTEN* consists in the regulation of this pathway. *PTEN* is a lipid phosphatase that antagonizes the action of PI3K by dephosphorylating PIP3 to generate PIP2 (thus blocking the PI3K signaling cascade). On the other hand, protein-based postbiotic metabolites can be recognized by receptors on the host cell surface and triggers its beneficial effects on the host signaling pathways. The PSC induces apoptosis through the proposed Akt-1 modulating effect on the NF-κB signaling pathway. p-Akt1 reduction can downregulate *RelA* mRNA through an increased expression of *IkB*. Reduction of p-Akt-1 and NF-κB activates apoptosis cascade

apoptosis pathway (*Bcl-XL* and *PTEN*), and the nuclear factor kappa B (NF-κB) pathway (*RelA* and *IkB*) were all profoundly impacted by the lowering of p-Akt [60].

In addition, the mRNA expression level of *RelA* and its inhibitor gene (*IkB*) was evaluated by qRT-PCR to clarify the mechanism of NF-κB-induced apoptosis, and it was discovered that after 8 h of treatment with PSC and 5-FU, the *RelA* level was significantly reduced as compared with the untreated control cells, as a result of *lkB* upregulation. It has been shown that *IkB* mRNA has a very short half-life (~ 30 min) [61]. After peaking at 8 h, *IkB* levels in this research steadily declined over the subsequent 48 h. At 8 h, the *RelA* level (as a functioning subunit of NF-κB) was low, which is consistent with the beginning of the apoptosis cascade given the presence of *IkB* and its role as an inhibitor of *RelA*. The paradoxical impact of NF-κB on cancer cell proliferation may be shown by the elevated level of RelA 24 and 48 h after therapy. A thorough analysis of the paradoxical impact was published in 2002 by Karin and colleagues [62]. They spoke about how decreasing NF-κB activity initiates apoptosis and how increasing NF-κB activity might encourage the body's immune system to prevent cancer. Further research

is required to understand the complex role of NF-κB, which may be associated with cancer inhibition via stimulation of the immune system, as suggested by the elevated levels of NF-κB in our investigation throughout 24 and 48 h.

The expression levels of *PTEN* (a pro-apoptotic gene) and *Bcl-XL* (an antiapoptotic gene) were also measured to see whether PSC therapy may induce apoptosis through the intrinsic route (mitochondrial pathway). qRT-PCR results demonstrated an influence of PSC on the mitochondrial apoptotic pathway, with increased *PTEN* mRNA and decreased *Bcl-XL* mRNA [63]. Interestingly, the PSC-treated cells' increase in *PTEN* expression level was significantly greater than that seen in the 5-FU-treated cells. Previous research has documented an increased *Bcl-XL* mRNA level as cellular resistance to apoptosis [64]; however, this was shown to be incompatible with apoptosis in the first 8 h. In conclusion, our results suggest that postbiotic metabolites from *S. cerevisiae* PTCC 5269 may induce apoptosis in a colorectal cancer cell line through the Akt/NF-κB signaling pathway.

Conclusion

Biostrategies comprised of probiotics-derived bioactive substances have recently been recognized as a potential tool in preventing and complementary treatment of a broad variety of acute/chronic disorders, according to the existing data gained from preclinical and clinical investigations. Because they are derived from safe probiotics, postbiotics are structurally and functionally compatible with host cells and contribute to a number of cellular processes essential to restoring homeostasis. According to the results of this study, the postbiotic metabolites derived from *Saccharomyces cerevisiae* (PTCC 5269) possess multiple health-promoting effects due to their significant antioxidant capacity ($87.34 \pm 0.56\%$), antibacterial action toward both of the Gram-negative (*S. typhi*, *E. coli*) and Gram-positive (*L. monocytogenes*, *S. mutans*) bacteria under in vitro and food models (whole milk and ground meat), probiotics' growth-promoting activity, and cholesterol-lowering and α-glucosidase enzyme-inhibiting properties, as well as their capability to cause significant cytotoxicity effect (IC_{50} values of 23.58 µg/mL for 48 h), arrest the cell cycle in cancer cells (suppressed the initial G0/G1 phase), and induce apoptotic responses, which this effect mediated by molecular mechanisms with a positive influence on the expression levels of genes involved in the Akt/NF-κB signaling pathway–induced apoptosis process. Therefore, it can be accomplished to develop certain mixtures of postbiotics with significant efficiency by learning more about lactic acid bacteria, using developed and optimized techniques in extraction, verification, characterization, implementing metabolomic and proteomic studies. This will substantially enhance the efficacy of health systems toward a broad spectrum of acute/chronic diseases.

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Author Contribution All of the authors contributed to the conception and design of the study and revised the manuscript. AA and SA conducted all the experiments and statistical analysis. AA, SS, and AYA drafted the first version of the manuscript. HH and SS reviewed the draft manuscript, and all the authors revised the final version of the manuscript.

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Data Availability The data presented in this study are available on request from the corresponding author.

Declarations

Ethics Approval and Consent to Participate This study was approved by the ethics committee of the research and technology deputy of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1400.609). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Rajakovich LJ, Balskus EP (2019) Metabolic functions of the human gut microbiota: the role of metalloenzymes. Nat Prod Rep 36(4):593–625
2. Bhardwaj S et al (2021) Growing emergence of drug-resistant Pseudomonas aeruginosa and attenuation of its virulence using quorum sensing inhibitors: a critical review. Iran J Basic Med Sci 24(6):699
3. Sabahi S et al (2022) Postbiotics as the new frontier in food and pharmaceutical research. Crit Rev Food Sci Nut 1–28
4. Abbasi A et al (2023) A critical review on the gluten-induced enteropathy/celiac disease: gluten-targeted dietary and non-dietary therapeutic approaches. Food Rev Intern 1–41
5. Ozma MA, Abbasi A, Sabahi S (2022) Characterization of postbiotics derived from Lactobacillus paracasei ATCC 55544 and its application in Malva sylvestris seed mucilage edible coating to the improvement of the microbiological, and sensory properties of lamb meat during storage. Biointerface Res Appl Chem 13(3)
6. Abbasi A, Saadat TR, Saadat YR (2022) Microbial exopolysaccharides–β-glucans–as promising postbiotic candidates in vaccine adjuvants. Intern J Biol Macromole
7. Abbasi A, Sheykhsaran E, Kafil HS (2021) Postbiotics: science, technology and applications. Bentham Sci Pub
8. Moradi M et al (2020) Postbiotics produced by lactic acid bacteria: the next frontier in food safety. Compr Rev Food Sci Food Saf 19(6):3390–3415
9. Evers MS et al (2023) Thiamine and biotin: relevance in the production of volatile and non-volatile compounds during Saccharomyces cerevisiae alcoholic fermentation in synthetic grape must. Foods 12. https://doi.org/10.3390/foods12050972

10. Rahbar Saadat Y et al (2020) Modulatory role of exopolysaccharides of Kluyveromyces marxianus and Pichia kudriavzevii as probiotic yeasts from dairy products in human colon cancer cells. J Funct Foods 64:103675
11. Abeysekera W, Premakumara G, Ratnasooriya W (2013) In vitro antioxidant properties of leaf and bark extracts of ceylon cinnamon *(Cinnamomum zeylanicum Blume)*
12. Nantitanon W, Chowwanapoonpohn S, Okonogi S (2007) Antioxidant and antimicrobial activities of Hyptis suaveolens essential oil. Sci Pharm 75(1):35–54
13. Sabahi S, Abbasi A, Mortazavi SA (2022) Characterization of cinnamon essential oil and its application in Malva sylvestris seed mucilage edible coating to the enhancement of the microbiological, physicochemical and sensory properties of lamb meat during storage. J Appl Microbiol 133(2):488–502
14. Hartmann HA, Wilke T, Erdmann R (2011) Efficacy of bacteriocin-containing cell-free culture supernatants from lactic acid bacteria to control Listeria monocytogenes in food. Int J Food Microbiol 146(2):192–199
15. Wang K et al (2014) Characterization of a novel exopolysaccharide with antitumor activity from Lactobacillus plantarum 70810. Int J Biol Macromol 63:133–139
16. Kazeem M, Adamson J, Ogunwande I (2013) Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of Morinda lucida Benth leaf. BioMed Res Intern
17. Soh H-S, Kim C-S, Lee S-P (2003) A new in vitro assay of cholesterol adsorption by food and microbial polysaccharides. J Med Food 6(3):225–230
18. Faghfoori MH et al (2020) Anticancer effect of X-Ray triggered methotrexate conjugated albumin coated bismuth sulfide nanoparticles on SW480 colon cancer cell line. Int J Pharm 582:119320
19. Pang H et al (2021) Exosomes derived from colon cancer cells and plasma of colon cancer patients promote migration of SW480 cells through Akt/mTOR pathway. Pathol Res Prac 222
20. Abbasi A et al (2023) Antigenotoxicity and cytotoxic potentials of cell-free supernatants derived from Saccharomyces cerevisiae var. boulardii on HT-29 human colon cancer cell lines. Probiot Antimicro Prot 1–13
21. Vahed SZ et al (2017) Leuconostoc mesenteroides-derived anticancer pharmaceuticals hinder inflammation and cell survival in colon cancer cells by modulating NF-κB/AKT/PTEN/MAPK pathways. Biomed Pharmacother 94:1094–1100
22. Nozari S et al (2019) Potential anticancer effects of cell wall protein fractions from Lactobacillus paracasei on human intestinal Caco-2 cell line. Lett Appl Microbiol 69(3):148–154
23. Amirsaadat S et al (2017) Silibinin-loaded magnetic nanoparticles inhibit hTERT gene expression and proliferation of lung cancer cells. Artif Cells Nanomed Biotechnol 45(8):1649–1656
24. Sabahi S et al (2022) Production of functional ice cream fortified by immunoglobulin Y against Escherichia coli O157: H7 and Helicobacter pylori
25. Ozma MA et al (2022) Postbiotics as the key mediators of the gut microbiota-host interactions. Infez Med 30(2):180
26. Wang R et al (2022) Solvents effect on phenolics, iridoids, antioxidant activity, antibacterial activity, and pancreatic lipase inhibition activity of noni (*Morinda citrifolia L.*) fruit extract. Food Chem 377:131989
27. Molska M et al (2022) Analysis of phenolic compounds in buckwheat (Fagopyrum esculentum Moench) sprouts modified with probiotic yeast. Molecules 27(22):7773
28. Wang Z et al (2022) Fermentation of kiwifruit juice from two cultivars by probiotic bacteria: bioactive phenolics, antioxidant activities and flavor volatiles. Food Chem 373:131455
29. Doi K, Uetsuka K (2011) Mechanisms of mycotoxin-induced neurotoxicity through oxidative stress-associated pathways. Int J Mol Sci 12(8):5213–5237
30. Faustino M et al (2019) Agro-food byproducts as a new source of natural food additives. Molecules 24(6):1056
31. Amaretti A et al (2013) Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Appl Microbiol Biotechnol 97:809–817
32. Guerrero-Encinas I et al (2021) Protective effect of Lacticaseibacillus casei CRL 431 postbiotics on mitochondrial function and oxidative status in rats with aflatoxin B 1–induced oxidative stress. Probiot Antimicro Prot 1–11
33. Wang Y et al (2017) Antioxidant properties of probiotic bacteria. Nutrients 9(5):521
34. Aguilar-Toalá J et al (2019) Modulatory effect of the intracellular content of Lactobacillus casei CRL 431 against the aflatoxin B 1-induced oxidative stress in rats. Probiot Antimicro Prot 11:470–477
35. Li H et al (2021) Study on the nutritional characteristics and antioxidant activity of dealcoholized sequentially fermented apple juice with Saccharomyces cerevisiae and Lactobacillus plantarum fermentation. Food Chem 363:130351
36. Chen Q et al (2021) Characterization and antioxidant activity of wheat bran polysaccharides modified by Saccharomyces cerevisiae and Bacillus subtilis fermentation. J Cereal Sci 97:103157
37. Yu Y-H et al (2020) Successful biosynthesis of natural antioxidant ergothioneine in Saccharomyces cerevisiae required only two genes from Grifola frondosa. Microb Cell Fact 19:1–10
38. Behbahani BA et al (2018) Oliveria decumbens essential oil: chemical compositions and antimicrobial activity against the growth of some clinical and standard strains causing infection. Microb Pathog 114:449–452
39. Falah F et al (2021) In vitro screening of phytochemicals, antioxidant, antimicrobial, and cytotoxic activity of Echinops setifer extract. Biocatal Agric Biotechnol 35:102102
40. Mehrnia MA et al (2021) Sclerorhachis platyrachis essential oil: antioxidant power, total phenolic and flavonoid content and its antimicrobial activity on some Gram-positive and Gram-negative bacteria "in vitro." Journal of food science and technology (Iran) 18(112):189–198
41. Zanganeh H et al (2021) Evaluation of the chemical and antibacterial properties of Citrus paradise essential oil and its application in Lallemantia iberica seed mucilage edible coating to improve the physicochemical, microbiological and sensory properties of lamb during refrigerated storage. J Food Meas Charact 15(6):5556–5571
42. Unlu M et al (2010) Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food Chem Toxicol 48(11):3274–3280
43. Ribeiro-Santos R et al (2017) Biological activities and major components determination in essential oils intended for a biodegradable food packaging. Ind Crops Prod 97:201–210
44. Pereira RP et al (2021) In vitro assessment of probiotic potential of Saccharomyces cerevisiae DABRP5 isolated from Bollo batter, a traditional Goan fermented food. Probiot Antimicro Prot 13:796–808
45. Abdollahzadeh E et al (2016) Prevalence and molecular characterization of Listeria spp. and Listeria monocytogenes isolated from fish, shrimp, and cooked ready-to-eat (RTE) aquatic products in Iran. LWT 73:205–21
46. Abdollahzadeh E et al (2016) Antimicrobial resistance of Listeria monocytogenes isolated from seafood and humans in Iran. Microb Pathog 100:70–74
47. Kim YJ et al (2021) Anti-biofilm effect of the cell-free supernatant of probiotic Saccharomyces cerevisiae against Listeria monocytogenes. Food Control 121:107667
48. Li R et al (2021) Listeria decontamination of chicken meat with beer brewed with bacteriocin producing Saccharomyces boulardii. LWT 152:112323

49. Koo OK, Kim SM, Kang SH (2015) Antimicrobial potential of Leuconostoc species against E. coli O157: H7 in ground meat. J Korean Soc Appl Biol Chem 58(6):831–838
50. Hamad G, Botros W, Hafez E (2017) Combination of probiotic filtrates as antibacterial agent against selected some pathogenic bacteria in milk and cheese. Int J Dairy Sci 12:368–376
51. Gálvez A et al (2007) Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 120(1–2):51–70
52. Sasikumar K et al (2017) An exopolysaccharide (EPS) from a Lactobacillus plantarum BR2 with potential benefits for making functional foods. Biores Technol 241:1152–1156
53. Bhat B, Bajaj BK (2018) Hypocholesterolemic and bioactive potential of exopolysaccharide from a probiotic Enterococcus faecium K1 isolated from kalarei. Biores Technol 254:264–267
54. Shamekhi S et al (2020) Apoptotic effect of Saccharomyces cerevisiae on human colon cancer SW480 cells by regulation of Akt/NF-κB signaling pathway. Probiot Antimicro Prot 12:311–319
55. Sima P, Richter J, Vetvicka V (2019) Glucans as new anticancer agents. Anticancer Res 39(7):3373–3378
56. Fortin O et al (2018) Cancer chemopreventive, antiproliferative, and superoxide anion scavenging properties of Kluyveromyces marxianus and Saccharomyces cerevisiae var. boulardii cell wall components. Nutr Cancer 70(1):83–96
57. Nami Y et al (2015) The prophylactic effect of probiotic Enterococcus lactis IW5 against different human cancer cells. Front Microbiol 6:1317
58. Plewka D et al (2018) Nuclear factor-kappa B as potential therapeutic target in human colon cancer. J Cancer Res Ther 14(3):516–520
59. Koerber MI et al (2016) NFκB-associated pathways in progression of chemoresistance to 5-fluorouracil in an in vitro model of colonic carcinoma. Anticancer Res 36(4):1631–1639
60. Davoudi Z et al (2014) Molecular target therapy of AKT and NF-kB signaling pathways and multidrug resistance by specific cell penetrating inhibitor peptides in HL-60 cells. Asian Pac J Cancer Prev 15(10):4353–4358
61. Brown K et al (1993) Mutual regulation of the transcriptional activator NF-kappa B and its inhibitor, I kappa B-alpha. Proc Natl Acad Sci 90(6):2532–2536
62. Karin M et al (2002) NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2(4):301–310
63. Altonsy MO, Andrews SC, Tuohy KM (2010) Differential induction of apoptosis in human colonic carcinoma cells (Caco-2) by Atopobium, and commensal, probiotic and enteropathogenic bacteria: mediation by the mitochondrial pathway. Int J Food Microbiol 137(2–3):190–203
64. Hu T et al (2016) Mechanisms of drug resistance in colon cancer and its therapeutic strategies. World J Gastroenterol 22(30):6876