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Original Research Article

Cytotoxic effects of *Saccharomyces cerevisiae* TC6 and *Lactobacillus brevis* TBRC 3003 isolated from Thai fermented foods

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Abstract

Purpose: *To determine the cytotoxic effect, anti-colony formation effect and antimigratory effect of Saccharomyces cerevisiae TC6 isolated from Thai water kefir, and Lactobacillus brevis TBRC 3003 isolated from picked cabbage.*

Methods: *Crude microbial extracts were obtained from whole cultures (cells and broths) using ethyl acetate as extracting solvent, and the dried extracts were redissolved in ethanol before use. Cytotoxic, antiproliferative and antimigratory effects of the two microbial extracts on MCF-7, HepG2, and HeLa were tested using 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetra zolium bromide (MTT), clonogenic formation and wound healing assays.*

Results: *Lb. brevis TBRC 3003 showed the highest cytotoxicity toward HepG2 cells (IC_{50} of 669.72 µg/mL), while S. cerevisiae TC6 showed the highest cytotoxicity against MCF-7 (IC_{50} of 691.49 µg/mL) and HeLa (IC_{50} of 379.16 µg/mL) based on MTT assay. Anti-colony formation test showed that S. cerevisiae TC6 was most the most effective in inhibiting colony formation of HepG2 (IC_{50} of 311.12 µg/mL) and HeLa (IC_{50} of 494.64 µg/mL), while Lb. brevis TBRC 3003 was the most potent in inhibiting colony formation of MCF-7 (IC_{50} of 267.88 µg/mL).*

Conclusion: *Both microbes can potentially be implemented in functional foods as bio-therapeutics with chemopreventive properties against breast, liver and cervical cancers.*

Keywords: *Cytotoxicity, Cancer, Lactobacillus brevis, Saccharomyces cerevisiae, Thai fermented foods*

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INTRODUCTION

Over the past decade, evidence has shown the essential functions of gut microbiota in human well-being and mental health [1-3]. In the human gastrointestinal tract, beneficial microbes are defined as 'probiotics'. Probiotics mostly belong to the group of microorganisms' known as lactic acid bacteria that are ubiquitous in fermented foods, yogurt, and fermented milk [4]. Probiotic

strains mainly comprise members of the genera *Bifidobacterium, Lactobacillus, Lactococus, Streptococcus*, and *Enterococcus*. In addition, some strains of *Bacillus* and *Saccharomyces* are widely used as probiotics [5]. Certain probiotics are shown to exhibit anticancer properties. Crude extracts of Bacillus anthracis and Streptomyces sp. SRF1 exerted cytotoxicity toward MCF-7 cells (breast cancer) [6,7], while the supernatant of *Lactobacillus acidophilus* 36YL isolated from human vagina, exhibited cytotoxicity against HT-29, HeLa and MCF-7 [8]. Probiotic bacteria have now emerged as alternative treatment to human ailments and are extensively studied as bio-therapeutics. Cancer is the primary cause of death in Thailand, with 60,000 fatalities in a year from liver and bladder, lung, colon, breast and cervical cancer. In the next 21 years, 24 million Thai citizens are predicted to be diagnosed with cancer [9]. Chemotherapy is one of the most effective treatments for cancer patients; however, many chemotherapeutic drugs exert high cell toxicity leading to unpleasant side effects to the patients [10]. Some patients develop drug resistance after long-term use, rendering chemotherapeutic drugs no longer effective. These adverse effects of cancer chemotherapy have initiated the discovery of novel anticancer compounds or alternative treatments. Microbes have recently established their candidacy as an alternative anticancer treatment through generation of various bioactive compounds such as enzymatic antioxidants, non-enzymatic antioxidants, metabolites, immune toxins, proteins for therapeutic purposes [11].

Fermentation is a low-cost process for obtaining probiotics-enriched functional foods. However, knowledge on cytotoxic effects of microbes isolated from Thai fermented foods on cancer cells remains scarce. *Saccharomyces cerevisiae* TC6, isolated from Thai water kefir in Nakhon Ratchasima Province, Thailand, [12] showed probiotic properties (preliminary work), while *Lactobacillus brevis* TBRC 3003 isolated from pickled cabbage (*Brassica* sp.) at the Thailand Bioresource Research Center (TBRC*)*, Pathum Thani, Thailand also showed probiotic potential from preliminary assessment tests. Thus, here, cytotoxic effects, anti-colony formation and antimigratory properties of two microbes, *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 were evaluated.

EXPERIMENTAL

Microbial sources

S. cerevisiae TC6 (NCBI accession no. LC336452.1) was isolated from Thai water kefir from Nakhon Ratchasima Province, Thailand [12. *Lb. brevis* TBRC 3003 originated from pickled cabbage (*Brassica* sp.) was purchased from TBRC. Both microbes were kept in 20% glycerol stocks at -80 °C.

Culture of microbial strains

Lb. brevis TBRC 3003 was cultivated in de Man, Rogosa and Sharpe (MRS) broth pH 6.8 (Difco, Detroit, MI, USA) and anaerobically cultured at 37 °C for 24 h. *S. cerevisiae* TC6 was grown in Yeast Peptone Dextrose (YPD) agar pH 7.0 (20 g/L glucose, 20 g/L peptone and10 g/L yeast) and aerobically cultured for 24 h at 30 °C. The standard culture of each strain was prepared by inoculation of 10 µL of a culture in a frozen stock (- 80 °C) into 10 mL MRS or YPD broth and incubated for 24 h at 37 °C for bacterium and at 30 °C for yeast. The strains were then subsequently sub-cultured in 10 mL broth for 24 h before cultivation in a 500 mL flask in the next step.

Crude microbial extraction

Overnight microbial cultures (1% v/v) were inoculated in corresponding broths (100 mL) in 500 mL flasks at 37 °C at 200 rpm for 2 days. Negative controls were broths without microbial inoculations. Crude microbial extracts were obtained from whole cultures (cells and broths). Then, 100 mL ethyl acetate (ETAC) was added to the cultures for crude microbial extraction at 37 °C, 200 rpm for 6 h and the ETAC layer as whole cell metabolite extract was separated and dried using a rotatory evaporator. Dried crude microbial extracts were reconstituted in 95% ethanol and stored at -20 °C until required for use.

Cancer cell lines

MCF-7 (breast cancer), HeLa (cervical cancer) and HepG2 (liver cancer) cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). These cancer cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% of fetal bovine serum containing 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37 °C under 5% CO_2 in an incubator. Cancer cell cultures were renewed with DMEM every 2-3 days until 80% confluency was reached. Cultured cell lines were washed with phosphate-buffered saline (PBS), pH 7.2 before trypsinization with 0.25% Trypsin-EDTA. DMEM were added to the cell lines and cell colonies were counted using an inverted microscope (NIB-9000, Xenon, China).

Cytotoxicity assay

Cytotoxicity was measured using 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetra zolium bromide (MTT)(Sigma, USA) assay described by [13]. MCF-7, HeLa and HepG2 cells (5×10^3 cells/ml) were transferred into 96-well plates and incubated at 37 ºC under 5% CO_2 for 72 h. Crude microbial extracts in ethanol (400, 600, 800 and 1,000 µg/mL) were added to the wells and incubated for 72 h followed by addition of MTT (5 mg/mL) dissolved in PBS buffer (pH 7.2) and incubated for 4 h. The purple color appeared if cells were alive. Doxorubicin was used as a positive control. The A590 nm value was recorded using a microplate reader (M965+, Mastertech, Taiwan). Cytotoxicity of crude microbial extracts against cancer cells was measured. Percentage cytotoxicity of ≤ 50% represented non-cytotoxic effects, while percentage cytotoxicity of > 50% represented cytotoxic effects. Cell morphology was also observed using an inverted microscope (NIB-100, Xenon, China). The IC_{50} values were calculated by linear approximation regression of the percentage cytotoxicity versus the microbial extract concentration.

Clonogenic assay

The clonogenic assay was used to evaluate anti-colony formation of crude microbial extract [14]. Cancer cells (500 cells/well) were pipetted into 6-well plates and treated with various concentrations of crude microbial extracts (400, 600, 800 and 1,000 µg/mL) for 24 h. Subsequently, the cells were washed with PBS buffer, resuspended in fresh DMEM and grown for 24 days. After that, cells were washed with PBS buffer three times, fixed with 100% methanol and stained with 0.5% crystal violet for 1 h. The stained colonies were captured using a digital camera (Nikon D50).

Wound healing assay

Cancer cells (2.5×10^5 cell/well) in DMEM supplemented with 5% FBS were transferred into 24-well plates for 24 h. A vertical wound was made using a sterile 200 µL pipette tip on a cell monolayer. Next, the cells were incubated in DMEM (5% FBS) in the absence (untreated cells or control) or presence of crude microbial extracts (400, 600, 800 and 1,000 µg/mL) for 48 h. Cell migration was captured at 12, 24, 36 and 48 h using an inverted microscope (NIB-9000, Xenon, China). Relative closure of the wound (% of control) was obtained as the average distance (width) between edges of the wound at 12, 24, 36 and 48 h, divided by the original wound width (at 0 h) that was defined as 100%. All experiments were performed in triplicate.

Statistical analysis

Data were collected in triplicate, with results represented as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS software (demo version). Statistically significant differences were assumed at $p < 0.05$.

RESULTS

Cytotoxicity of microbial extracts

Highest antiproliferation on every cancer cell was observed at maximum dose (1,000 µg/mL) of both microbial extracts. Both microbial extracts were least cytotoxic toward MCF-7 cells based on the lowest cytotoxicity (%) from 1000 µg/mL *Lb. brevis* TBRC 3003 extract of 66.02% and from 1000 µg/mL *S. cerevisiae* TC6 extract of 71.30% (Figure 1). *S. cerevisiae* TC6 appeared more effective towards MCF-7 cells than *Lb. brevis* TBRC 3003. Likewise, *S. cerevisiae* TC6 showed higher cytotoxicity (%) towards HeLa (90.92% at 1000 µg/mL) cells at every concentration than *Lb. brevis* TBRC 3003 extracts (Figure 1). However, *Lb. brevis* TBRC 3003 extract showed higher cytotoxicity (%) towards HepG2 (95.69% at 1000 µg/mL) at every concentration than *S. cerevisiae* TC6 extract (Figure 1).

These results concurred with the calculated IC_{50} values. The lowest half maximal inhibitory concentration (IC_{50}) value of 669.72 µg/mL towards HepG2 cells was found in *Lb. brevis* TBRC 3003 extract, while the lowest IC_{50} values of 691.49 µg/mL and 379.16 µg/mL towards MCF-7 and HeLa cells, respectively were found in *S. cerevisiae* TC6 extract (Table 1). This suggested that *S. cerevisiae* TC6 extracts were more cytotoxic against MCF-7 and HeLa cells, while *Lb. brevis* TBRC 3003 extracts were more cytotoxic against HepG2 cells. Doxorubicin, one of the most extensively used commercial anticancer drugs, was a positive control and it showed the lowest IC_{50} values against all cancer cell lines at 0.6-9.1 µg/mL. Negative controls (culture broth extract without microbes) showed no cytotoxicity on any cancer cells.

Apoptosis in cancer cells

Changes in cell morphology induced by *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 extract treatments (1000 µg/mL for 72 h) were

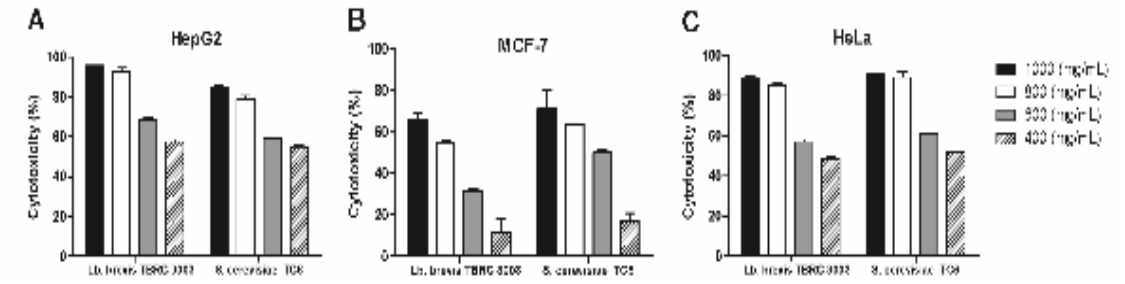

Figure 1: Cytotoxicity (%) of various concentrations of microbial extracts on cancer cells for 72 h exposure. **A:** HepG2 cells. **B:** MCF-7 cells. **C:** HeLa cells

Table 1: IC_{50} (µg/mL) of crude microbial extracts against cancer cells

Variable	IC_{50} (µg/mL) of crude microbial extracts for cytotoxicity		
	HepG2	MCF-7	HeLa
S. cerevisiae TC6	$715.97 \pm 0.78^{c,C}$	$691.49 \pm 2.07^{b,B}$	$379.16 \pm 2.09^{b,A}$
Lb. brevis TBRC 3003	$669.72 \pm 0.47^{b,B}$	$787.79 \pm 20.95^{c,C}$	$429.87 \pm 1.21^{c,A}$
Doxorubicin	$9.10 \pm 0.22^{a,C}$	$7.04 \pm 0.80^{a,B}$	$0.61 \pm 0.04^{a,A}$

Lower-case and upper-case letter superscripts represent significant differences ($p < 0.05$) in columns and rows, respectively

examined under a phase contrast microscope for preliminary characterization of apoptotic cells. As a result, cells became circular and shrunken, while cell adhesion disintegrated (Figure 2) when compared to non-treated cancer cells. This indicated that both microbial extracts were able to induce apoptosis resulting in cancer cell death as observed using apoptotic bodies (Figure 2).

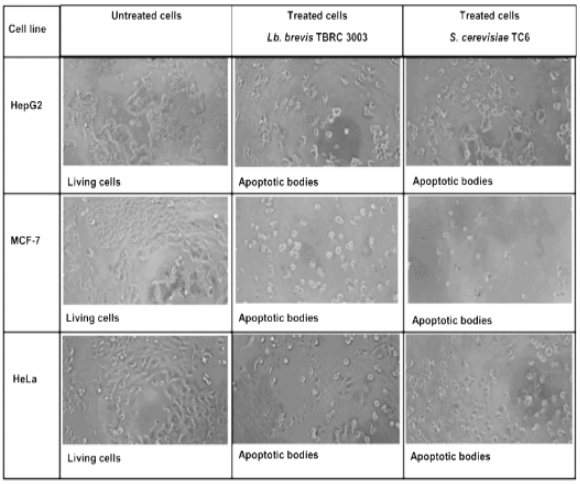

Figure 2: Morphology of cancer cells untreated/treated with microbial extracts (1,000 µg/mL) at 72 h

Anti-colony formation

In addition to cytotoxicity, the antiproliferative property of the microbial extracts on the long-term viability of cancer cells was conducted using a clonogenic assay. As a result, microbial extracts from *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 produced dose-dependent decrease in clonogenic formation of HepG2, MCF-7 and HeLa cells (Table 2, Figure 3) with IC_{50} values lower than those found for cytotoxicity (Table 3), except for HeLa cells.

The results suggest that *S. cerevisiae* TC6 was more effective in inhibiting cell regrowth (IC_{50} of 311.12 µg/mL towards HepG2 cells and 494.64 µg/mL towards HeLa cells) than *Lb. brevis* TBRC 3003 in all cells (IC_{50} of 504.66 µg/mL towards HepG2 cells and 927.60 µg/mL towards HeLa cells), except for MCF-7 cells (Table 3).

Antimigratory effects

Next, the antimigratory effects of *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 on cancer cells were determined. Results demonstrated that both strains exerted antimigratory properties in a dose-dependent manner (Table 4). However, HeLa cells were resistant to antimigratory effects caused by microbial extracts as observed by the lowest relative closure of the wound (% of control) from 1,000 µg/mL microbial extracts (Table 4). The microbial crude extracts were able to induce apoptosis as detected by apoptotic characteristics (Figure 2) and also confer antimigratory effects as observed by cell migration inhibition (Figures 4-6).

Table 2: Colony formation of crude microbial extracts on cancer cells

Cancer cell	Concentration (µg/mL) of crude microbial extract	Colony formation (% of control)	
		Microbial strains	
		S. cerevisiae TC6	*Lb. brevis* TBRC 3003
HepG2	0	100.00 ± 0.00 ^{e,A}	100.00 ± 0.00 ^{e,A}
	400	49.63 ± 1.58 ^{d,A}	62.69 ± 1.05 ^{d,B}
	600	34.33 ± 1.05 ^{c,B}	28.73 ± 1.58 ^{c,A}
	800	27.24 ± 0.52 ^{b,A}	23.14 ± 1.05 ^{b,A}
	1000	10.44 ± 1.05 ^{a,A}	9.71 ± 1.05 ^{a,A}
MCF-7	0	100.00 ± 0.00 ^{e,A}	100.00 ± 0.00 ^{e,A}
	400	35.01 ± 0.21^{d,B}	23.67 ± 0.33 ^{d,A}
	600	2.59 ± 0.21^{c,A}	20.09 ± 0.656 ^{c,B}
	800	0.84 ± 0.00 ^{b,A}	15.83 ± 0.87 ^{b,B}
	1000	0.00 ± 0.00 ^{a,A}	2.97 ± 0.54 ^{a,B}
HeLa	0	100.00 ± 0.00 ^{c,A}	100.00 ± 0.00 ^{e,A}
	400	90.60 ± 0.79 ^{b,A}	91.92 ± 0.39 ^{d,A}
	600	0.00 ± 0.00 ^{a,A}	68.39 ± 0.38 ^{c,B}
	800	0.00 ± 0.00 ^{a,A}	61.29 ± 1.37 ^{b,B}
	1000	0.00 ± 0.00 ^{a,A}	39.69 ± 0.78 ^{a,B}

Lower-case and upper-case letters represent significant differences ($p < 0.05$) in columns and rows, respectively

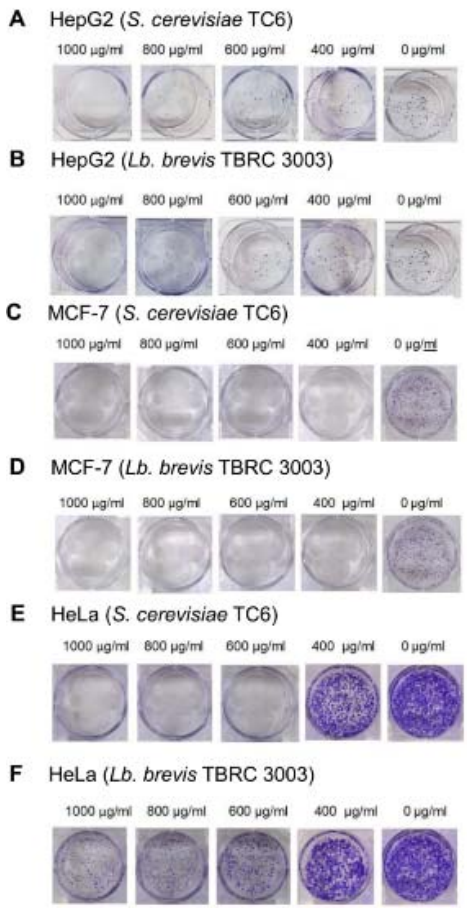

Figure 3: Clongenic formation assays. **A:** HepG2 cells treated with *S. cerevisiae* TC6 extract. **B:** HepG2 cells treated with *Lb. brevis* TBRC 3003 extract. **C:** MCF-7 cells treated with *S. cerevisiae* TC6 extract. **D:** MCF-7 cells treated with *Lb. brevis* TBRC 3003 extract. **E:** HeLa cells treated with *S. cerevisiae* TC6 extract. **F:** HeLa cells treated with *Lb. brevis* TBRC 3003 extract

Table 3: IC_{50} (µg/mL) of microbial extracts for colony formation of cancer cells

Cell line	IC_{50} (µg/mL) of microbial extract for colony formation	
	S. cerevisiae TC6	*Lb. brevis* TBRC 3003
HepG2	311.12 ± 0.20 ^{a,A}	504.66 ± 0.06 ^{b,B}
MCF-7	398.02 ± 0.31 ^{b,B}	267.88 ± 0.10 ^{a,A}
HeLa	494.64 ± 0.12 ^{c,A}	927.60 ± 0.21 ^{c,B}

Lower-case and upper-case letters represent significant differences ($p < 0.05$) in columns and rows, respectively

DISCUSSION

Cytotoxicity of *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 extracts were investigated on HepG2, MCF-7 and HeLa cells. Although several microbial strains isolated from Thai fermented foods have been reported, detailed analyses of their cytotoxicities remain scarce. Results revealed that *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 extracts were anti-carcinogenic against HepG2, MCF-7 and HeLa cancer cells through antiproliferation, induction of apoptosis and antimigratory properties. However, data regarding the cytotoxicity and antiproliferative effects of *S. cerevisiae* and *Lb. brevis* are limited, and further investigations at the cellular level are required with regard to gene expression mechanisms.

This is the first report demonstrating the cytotoxicity of the probiotics *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 isolated from Thai fermented foods on cancer cells; HepG2, MCF-7 and HeLa cells in a dose-dependent manner.

Table 4: Relative closure of wound (% of control) on cancer cells by crude microbial extracts

Cell line	Microbial strain	Conc. (µg/mL) of crude extract	Relative closure of the wound (% of control)				
			0 h	12 h	24 h	36 h	48 h
HepG2	*S. cerevisiae* TC6	0	100.00±0.00^{a,A}	82.96±0.30^{d,B}	70.17±0.05^{d,C}	54.40±0.13^{d,D}	0.00±0.00^{e,E}
		400	100.00±0.00^{a,A}	87.40±0.71^{c,B}	75.30±0.92^{c,C}	57.79±1.62^{c,D}	52.59±0.84^{d,E}
		600	100.00±0.00^{a,A}	87.08±1.22^{c,B}	83.14±1.70^{b,C}	59.18±0.33^{c,D}	58.00±0.33^{c,D}
		800	100.00±0.00^{a,A}	92.52±0.01^{a,A}	97.82±0.43^{a,B}	83.31±0.02^{b,C}	82.02±1.79^{b,C}
		1000	100.00±0.00^{a,A}	99.16±0.38^{b,A}	97.48±0.39^{a,B}	94.17±0.02^{a,C}	91.80±0.56^{a,D}
	Lb. brevis TBRC 3003	0	100.00±0.00^{a,A}	87.55±0.77^{b,B}	76.90±0.52^{c,C}	50.35±0.30^{d,D}	0.00±0.00^{e,E}
		400	100.00±0.00^{a,A}	86.68±1.52^{b,B}	82.96±0.43^{b,C}	69.32±1.36^{c,D}	62.01±0.32^{d,E}
		600	100.00±0.00^{a,A}	89.00±0.27^{b,B}	83.33±0.74^{b,C}	70.33±0.42^{c,D}	68.93±0.42^{c,E}
		800	100.00±0.00^{a,A}	96.36±0.22^{a,B}	97.14±0.07^{a,B}	91.03±0.55^{b,C}	89.29±1.87^{b,C}
		1000	100.00±0.00^{a,A}	97.58±1.27^{a,B}	97.64±0.19^{a,B}	93.36±0.35^{a,C}	91.82±0.34^{a,C}
MCF-7	*S. cerevisiae* TC6	0	100.00±0.00^{a,A}	76.06±0.50^{c,B}	67.23±0.54^{e,C}	51.01±0.14^{e,D}	0.00±0.00^{e,E}
		400	100.00±0.00^{a,A}	80.42±0.04^{b,B}	72.07±0.08^{d,C}	65.63±0.04^{d,D}	51.43±0.13^{d,E}
		600	100.00±0.00^{a,A}	80.55±0.07^{b,B}	72.78±0.14^{c,C}	68.26±0.14^{c,D}	53.40±0.04^{c,E}
		800	100.00±0.00^{a,A}	99.31±0.14^{a,B}	92.29±0.04^{b,C}	92.26±0.09^{b,C}	92.31±0.00^{b,C}
		1000	100.00±0.00^{a,A}	99.90±0.50^{a,AB}	99.89±0.05^{a,AB}	99.83±0.05^{a,B}	99.81±0.07^{a,B}
	Lb. brevis TBRC3003	0	100.00±0.00^{a,A}	66.23±0.83^{d,B}	42.75±0.33^{e,C}	43.05±0.83^{e,D}	0.00±0.00^{e,E}
		400	100.00±0.00^{a,A}	85.87±0.08^{c,B}	80.82±0.00^{d,C}	65.00±0.07^{d,D}	53.34±0.17^{d,E}
		600	100.00±0.00^{a,A}	91.88±0.09^{b,B}	85.93±0.00^{c,C}	66.66±0.05^{c,C}	60.67±0.09^{c,C}
		800	100.00±0.00^{a,A}	99.06±0.08^{a,B}	88.06±0.04^{b,C}	88.06±0.04^{b,C}	88.06±0.04^{b,C}
		1000	100.00±0.00^{a,A}	99.90±0.04^{a,A}	99.90±0.04^{a,A}	99.90±0.04^{a,A}	99.90±0.00^{a,A}
HeLa	*S. cerevisiae* TC6	0	100.00±0.00^{a,A}	50.50±5.23^{d,B}	42.40±2.68^{d,C}	0.00±0.00^{e,D}	0.00±0.00^{e,D}
		400	100.00±0.00^{a,A}	79.00±0.14^{c,B}	60.30±0.14^{c,C}	41.65±0.70^{d,D}	35.45±0.07^{d,E}
		600	100.00±0.00^{a,A}	79.40±0.25^{c,B}	68.40±0.28^{b,C}	61.90±0.28^{c,D}	49.75±0.21^{c,E}
		800	100.00±0.00^{a,A}	90.85±0.35^{b,B}	89.25±0.35^{a,C}	76.00±0.28^{b,D}	82.40±0.28^{b,E}
		1000	100.00±0.00^{a,A}	97.10±0.14^{a,B}	89.90±0.14^{a,C}	89.80±0.14^{a,C}	89.80±0.14^{a,C}
	Lb. brevis TBRC 3003	0	100.00±0.00^{a,A}	53.40±2.54^{d,B}	43.55±2.05^{d,C}	0.00±0.00^{e,D}	0.00±0.00^{d,D}
		400	100.00±0.00^{a,A}	75.25±0.35^{c,B}	58.55±0.21^{c,C}	41.80±0.14^{d,D}	37.10±1.41^{c,E}
		600	100.00±0.00^{a,A}	77.20±1.55^{c,B}	75.25±1.48^{b,B}	58.20±1.13^{c,C}	56.50±1.13^{bc,C}
		800	100.00±0.00^{a,A}	78.35±7.00^{b,B}	77.15±0.07^{b,C}	74.75±1.16^{ab,D}	64.65±0.07^{b,E}
		1000	100.00±0.00^{a,A}	89.90±0.56^{a,A}	89.30±0.00^{a,A}	89.70±0.56^{a,A}	89.70±0.57^{a,A}

Lower-case and upper-case letters represent significant differences ($p < 0.05$) in columns and rows, respectively within each microbial extract and each cancer cell type

Our findings supported the previous studies showing that *Lb. brevis* originated from regional dairy products exerted cytotoxicity and apoptotic effects toward MCF-7 at high concentrations (4-60 mg/mL for 48 h exposure) [15]. The cytotoxicity of *Lb. brevis* supernatant was both time and concentration-dependent, and showed promise in cancer remedy and prevention. HeLa cells were also more likely to regrow and show resistance to microbial extracts in the long term than other cancer cells. To sum up, lower concentrations of microbial extracts exerted antiproliferative effect for a longer time period when compared to short-term cytotoxicity.

Candidate molecules that play a major part in the cytotoxicity of extracted bacterial metabolites include bioactive peptides that bind to procarcinogenic compounds or non-protein molecules such as short-chain fatty acids (butyrate and propionate) [16] or exopolysaccharide [17]. Previously, stimulation of apoptosis of HT-29 human colon adenocarcinoma cell line was triggered by cell-bound exopolysaccharide extract from *Lb. brevis* isolated from 'Tarkhine', indigenous food of Iran [17]. Apoptotic effects of *Lb. brevis* extract were dependent on dose and time of exposure to HT-29 cells [17]. Previous reports showed that heat-killed non-pathogenic yeast *Saccharomyces cerevisiae* induced apoptosis of MCF-7 *in vitro* in a dose- and time-dependent manner as a caspase-independent mechanism [18].

Microbes with bioactive compounds responsible for their anticancer effects were *B. silvestris*

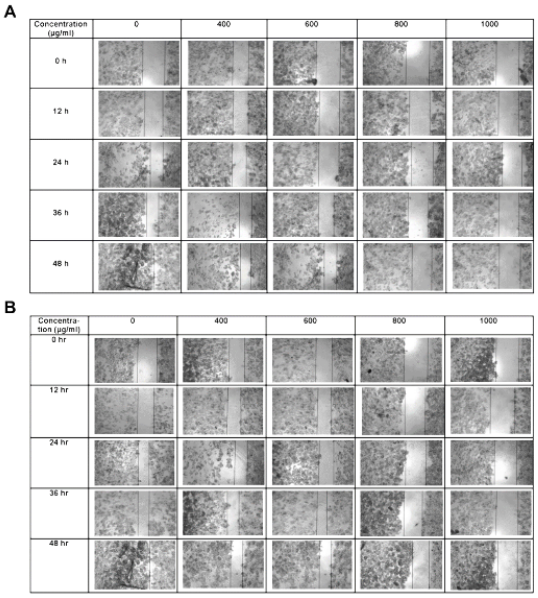

Figure 4: Wound healing assay for HepG2 cells. **A:** Treatment with *S. cerevisiae* TC6 extract. **B:** Treatment with *Lb. brevis* TBRC 3003 extract

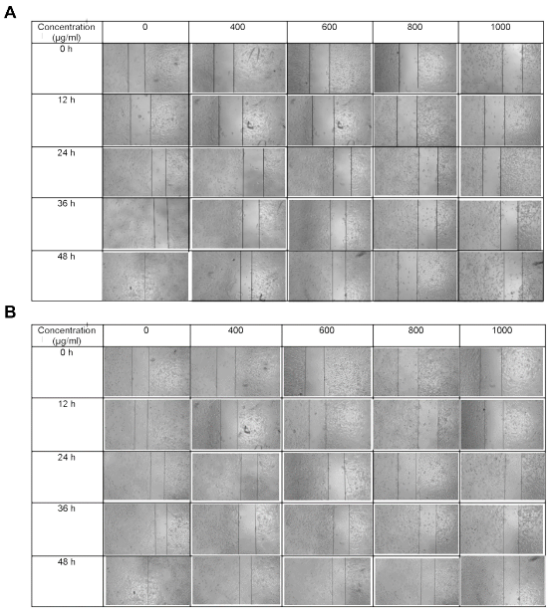

Figure 5: Wound healing assay for MCF-7 cells. **A:** Treatment with *S. cerevisiae* TC6 extract. **B:** Treatment with *Lb. brevis* TBRC 3003 extract

(halobacillin peptide against MCF-7), *Lactococcus lactis* (nisin A peptide against MCF-7 and HepG2), *Ent. cloacae* (L-asparaginases peptide against MCF-7), *B. subtilis* (epsilon-poly-L-lysin against HeLa and HepG2), *Enterococcus* spp. (halobacillin peptide against HeLa) and *B. amyloliquefaciens* (exopolysaccharide against MCF-7) [19]. These bioactive compounds may contribute to the cytotoxic property of the microbial extracts. Compared to previous findings, *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 showed lower cytotoxicity. Phonnok *et al* isolated microbes from various environments and found IC_{50} values in the range of 21-438 µg/mL for the microbial extracts against MCF-7, HepG2, HeLa and U937 cells [11].

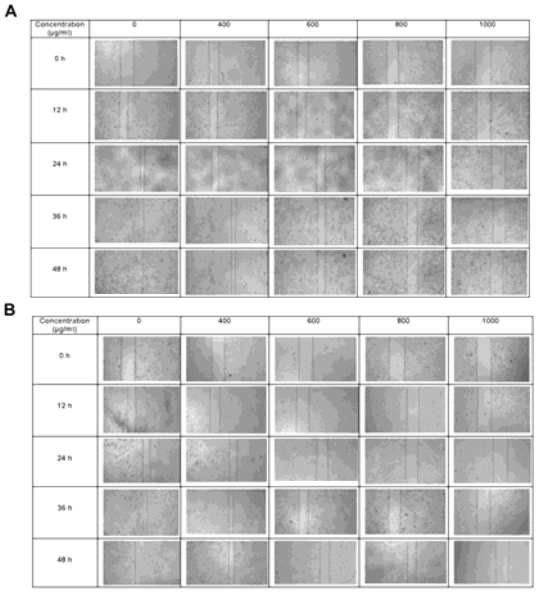

Figure 6: Wound healing assay for HeLa cells. **A:** Treatment with *S. cerevisiae* TC6 extract. **B:** Treatment with *Lb. brevis* TBRC 3003 extract

The most cytotoxic microbial extracts with the lowest IC_{50} (21-79 µg/mL) were derived from *Candida tropicalis, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Bacillus* sp., respectively [11].

However, bioactive compounds in crude microbial extracts still remain to be elucidated. Microbial extracts with maximal cytotoxicity may be achieved by determining the optimal solvent for extraction and the most active microbial fractions from different solvents used during the extraction process. These findings suggest that *S. cerevisiae* TC6 and *Lb. brevis* TBRC 3003 possess potential applications as starter cultures for developing fermented foods that can prevent breast cancer, liver cancer, and cervical cancer.

CONCLUSION

This is the first report on the cytotoxicity of microbes isolated from the Thai fermented foods, *Lb. brevis* TBRC 3003 and *S. cerevisiae* TC6. Crude extracts of both microbial strains exhibit potentials for application as anticancer biotherapeutic reagents. Thus, these microbes can possibly be used in biological systems for

the production of various valuable molecules that improve the quality and functionality of foods with chemopreventive benefits.

DECLARATIONS

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Conflict of interest

The authors declare no conflicts of interest.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by them. Vijitra Luang-In designed, conducted the experiments, analyzed data and wrote the manuscript. Worachot Saengha and Thippiya Karirat conducted the experiments. Benjaporn Buranrat designed the experiments. Sutisa Nudmamud-Thanoi, Nyuk Ling Ma and Arjan Narbad revised the draft of the manuscript. All the authors read and approved the manuscript for publication.

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