Screening of Potential Probiotic Lactic Acid Bacteria with Anticancer Properties

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Background: *Probiotics have shown to reduce cancer recurrence and side effects in colorectal cancer patients.*
Objective: *To isolate the lactic acid bacteria from Thai healthy newborn feces and screen good probiotics with anticancer properties.*
Material and Method: *Lactic acid bacteria were isolated from newborn feces and selected for the cytotoxicity property against human cancer cell lines by MTT assay and probiotics property by acid and bile tolerance tests.*
Results: *Among 200 lactic acid bacteria isolated, 3 and 1 isolates significantly demonstrated strong and moderate anti-proliferative effect against Caco-2 cells, respectively. Likewise, 4 and 5 isolates showed significant strong and moderate inhibitory effect on U937 cells, respectively. Seven candidate strains did not displayed cytotoxic to normal cells (Vero), except MSMC 112-2. All candidates showed good probiotics properties in resistance to acidic condition (pH 2-4) and to 1-4% bile concentrations, except MSMC105-3 showed intolerance at 4% bile concentration. The nucleotide sequence homology showed that MSMC95-4, MSMC104-2, MSMC111-2, MSMC112-2 and MSMC215-1 strains belong to Enterococcus faecalis (99.4%, 98.8%, 99.5%, 98.7 and 98.9%, respectively), MSMC105-3 is Lactobacillus salivarius (99.1%) and MSMC171-1 is Enterococcus faecium (99.3%).*
Conclusion: *The authors have isolated lactic acid bacteria which have anticancer and good probiotics properties.*

Keywords: *Lactic acid bacteria, Probiotics, Anticancer, Caco-2 cells, U937 cells*

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Colorectal cancer (CRC) is a leading cause of death; however it has long precancerous stage that provides an opportunity for chemoprevention to interfere the cancerous development. Prevention by dietary and lifestyle can harness or limit the risk of colorectal cancer development[1]. Probiotics has been shown as a good biotherapeutic in CRC patients to protective and early stage therapeutics[2]. Probiotics are defined by Food and Agricultural Organization of the United Nation and the WHO (FAO/WHO) 2002 as "live microorganisms administered in adequate amounts that confer a beneficial health effect on the host"[3].

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The most common of microorganisms used as probiotics are lactic acid bacteria. The group of lactic acid bacteria (LAB) shows characteristics of gram-positive, non-motile, non-sporulating, non-catalase producing facultative anaerobic bacteria[4]. LAB are commonly found in the composition of animals and humans' gastrointestinal tract (GIT) in variable amounts depending on the animal species, age of the host, or location within the gut[5]. Probiotic are known to be beneficial not only balance of intestinal flora but also for their antimicrobial, antioxidant, anti-inflammatory and anticancer effect[6]. The daily intake of LAB such as *Lactobacillus casei* strain *Shirota* provides a positive effect on the activity of NK cells which seemed to exert a key role in protecting human against cancer[7]. A recent study found antiproliferative effects of the cell-free filtrate and the cell-free lyophilized filtrate of 3 LAB (*Pediococcus pentosaceus*, *Lactobacillus*

plantarum, and *Weissella confusa*) on human colorectal adenocarcinoma cell line Caco-2[8]. Increasing evidence also suggested that LAB including *L. casei*[9], *L. rhamnosus*[10], *L. acidophilus*[11], all have the abilities of inhibiting tumor growth in rodents. The cytoplasmic fraction (CF) of *L. lactis* ssp. *lactis* and *L. brevis* had strong arginine deiminase (ADI) activity which can induce apoptosis of the stomach adenocarcinoma cells and human acute leukemia cells, respectively[12,13]. Health promoting effects of probiotic bacteria are very strain dependent and their positive role on colon cancer may also vary from one strain to another. Therefore there is a need to find new probiotic strains with anticancer properties.

In addition, GIT composes of strong acid and contain bile as an emulsifier, the acid and bile tolerance properties are important for LAB to survive, adhere to intestinal surfaces and colonize in the GIT[14]. This is the first study on isolation the lactic acid bacteria from healthy newborn feces, especially with the anticancer properties in Thailand. The cultured supernatants of the isolated lactic acid bacteria were used to screen the potential isolates that secrete the bioactive compounds that could inhibit growth of the adjacent and distant cancer cell lines; human colon adenocarcinoma cells (Caco-2) and human monocytic leukemic cells (U937), respectively. The candidate strains were subjected to test for acid and bile tolerance properties and species identification for further health application.

Material and Method

Isolation and selection of lactic acid bacteria

The lactic acid bacteria were isolated from 200 Thai healthy newborn feces which obtained from The Department of Obstetrics and Gynecology, HRH Princess Maha Chakri Sirindhorn Medical Center. All subjects gave the informed consent (Human ethical research authorization No. SWUEC37/2551). Fecal swabs obtained from 0-5 day-old newborn were diluted in 0.8% normal saline solution (NSS), serially diluted and applied onto de Man-Rogosa-Sharpe (MRS) agar plates (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37°C for 24-48 h under anaerobic condition in an anaerobic box (Mitsubishi, Japan). Colonies with different morphologies were isolated and sub-cultured on MRS agar plates to obtain the pure colonies. They were presumptively screened for lactic acid bacteria by Gram staining and catalase activity. Only those that were Gram-positive and catalase-negative were selected and maintained as frozen cultures in MRS broth with 20% glycerol at -80°C for further investigation.

Preparation of lactic acid bacteria conditioned media

Lactic acid bacteria conditioned media was prepared as previously described[15]. In brief, lactic acid bacteria cultures were grown anaerobically in MRS broth at 37°C overnight. The overnight cultures were diluted with MRS broth to obtain a final concentration of 10^8 cells/ml and grown anaerobically at 37°C for another 48 h. Conditioned media were prepared by centrifugation of the 48 h-bacterial culture at 4,000 x g for 10 min and filter-sterilized using a 0.22 µm pore size filter (Sigma, USA) and concentrated by speed-vacuum drying (Rotational Vacuum Concentrator RVC 2-18, Germany). The residual pellet was resuspended in 500 µl of serum-free culture media, denoted as lactic acid bacteria-conditioned media and frozen at -20°C until analysis.

Cell line culture

The cell lines used were human colon adenocarcinoma cells (Caco-2), human monocytic leukemic cells (U937) and noncancerous monkey kidney cells (Vero). Vero cells, Caco-2 and U937 were cultured in M199 medium (Gibco-Invitrogen, USA), Dulbecco's Modified Eagle medium (Gibco-Invitrogen, USA) and RPMI 1640 (Gibco-Invitrogen, USA), respectively. For cell culture condition, all cell culture media were supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco-Invitrogen, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco-Invitrogen, USA) and cultured at 37°C at an atmosphere of 5% CO_2-95% air mixture.

MTT assay

MTT assay[16] was used to assess cell proliferation based on the capacity of enzyme succinate dehydrogenase in mitochondria of viable cells to metabolize a colorless tetrazolium salt to a blue formazan. One hundred eighty microliters of Caco-2 cells were seeded in 96-well plates at a final density $5.9x10^3$ cells/ml or $1.2x10^4$ cells/ml for Vero cells and incubated for 24 h at 37°C in an atmosphere of 5% CO_2-95% air mixture. After 24 h incubation, 20 µl of bacterium-conditioned media (10% v/v) was added and incubated for another 24 h. For U937 cells, 90 µl were seeded in 96-well plates at a final density $5x10^4$ cells/ml, then 10 µl of bacterium-conditioned media (10% v/v) was added and incubated for another 24 h. For medium control condition, cell culture medium was added to a final volume of 200 µl/well for Caco-2 cells and Vero cells, or 100 µl/well for U937 cells. Thereafter, the MTT solution (5 mg/ml) was added to the cells and left for 3 h at 37°C in CO_2 incubator.

The blue MTT-formazan crystals were dissolved by adding dimethysulfoxide (DMSO) (Fisher Scientific, India). The OD was measured at 595 nm using a microplate reader (Biotek®, Instrument, Inc., USA). The experiment was done in triplicate wells and three independent experiments. The % cell viability of each wells were calculated from the absorbance of the treated cells compared with the absorbance of the control cells. The % inhibition was calculated by the formula:

% inhibition = % cell viability of MRS broth control - % cell viability of treated cells

Acid tolerance test

MRS broth (pH 6.5) was adjusted with 1 N HCl to pH values 2, 3, and 4 and the unadjusted MRS broth was used as a bacterial media control[17]. Overnight cultures of the candidate probiotic-strains were diluted to 10 ml with the pH-adjusted MRS broth to give an initial bacterial concentration of $1x10^8$ cells/ml and incubated at 37°C for 3 h under anaerobic conditions. The samples were taken and prepared 10-fold serial dilutions (10^{-1}-10^{-5}) with PBS (pH 7.2). Then 100 µl suspension of each serial dilution were spread onto MRS agar plates and incubated anaerobically at 37°C for 24-48 h. The viable bacteria grown on MRS agar were counted and compared with unadjusted MRS media (control) condition. The experiments were done in duplicate.

Bile tolerance test

MRS broth containing 0%, 1%, 2%, 3% and 4% bovine bile (Sigma, USA) were prepared[17]. The candidate lactic acid bacteria overnight culture was inoculated into the bile adjusted-MRS to obtain the initial bacterial concentration of $1x10^8$ cells/ml and incubated at 37°C for 3 h under anaerobic conditions. After incubation, samples were taken and 10-fold serial dilutions of each sample were prepared (10^{-1}-10^{-5}). Viable cell counts were determined by spreading each bacterial dilution onto MRS agar plates. The plates were incubated under anaerobic conditions at 37°C for 24-48 h. The viable bacteria grown on MRS agar were counted and compared with unadjusted MRS media (control) condition. All experiments were done in duplicate.

Identification of lactic acid bacteria

The 16 S rDNA sequence of candidate strains were amplified and analyzed using colony PCR method as described previously[18]. Briefly, the 16S rDNA was amplified using primers 518F (5' CCAGCAGCCGCGGTAATACG 3') 800R (5' TACCAG GGTATCTAATCC 3'). The PCR products were purified using Geneaid Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). The sequence analyses of PCR products were performed by Macrogen, Seoul, Korea. The consensus DNA sequences from forward and reverse sequences were generated using Bioedit® program and used for analysis of sequence similarity through BLAST with the nucleotide sequence deposited in the Ribosomal Database Project (RDP-XI; http://rdp.cme.msu.edu). The strain identity was determined based on the highest scores and a similarity of >98% to 16S ribosomal gene sequences of type strains was used as the criterion for identification.

Statistical analysis

The MTT assays were performed triplicate in three independent experiments. Results were reported as means and standard error of the mean (SEM). Statistical analysis was evaluated by one-way ANOVA followed by Dunnett's test using the GraphPad Prism® version 5.01. Significant differences were considered at $p<0.05$.

Results

Selection of lactic acid bacteria isolates

A total of 200 lactic acid bacteria were isolated from Thai healthy newborn feces as gram-positive and catalase-negative characteristics. The colonies on MRS agar plate varied from small to medium colonies (1-2 mm) with white circular, convex and smooth colonies. Microscopic study showed cell morphology varied from long and slender rods, straight rods to bent rods, and short rods to coccobacilli and cocci. Some isolates exhibited bipolar staining or internal granulations.

Cytotoxic effect on cancer cells

The conditioned media from 3 and 17 lactic acid bacteria isolates significantly demonstrated cytotoxic effect against Caco-2 and U937 cells, respectively compared with the MRS broth (control) (Fig. 1 and Fig. 2). The conditioned media from 3 lactic acid bacteria isolates of MSMC95-4, MSMC112-2 and MSMC215-1 showed strongest inhibitory effect (+++) as shown by the decrement of the Caco-2 cell viability as $39.30\pm8.48\%$ ($p<0.05$), $28.12\pm7.27\%$ ($p<0.01$) and $25.43\pm3.89\%$ ($p<0.01$), respectively (Table 1 and Fig. 1). However, one isolate of MSMC 137-1 showed moderate inhibitory effect (++, 53.99% cell viability) but did not show significantly different with MRS media

control. Similarly, 17 isolates displayed significant growth inhibition effect on leukemia cells (U937) at $p<0.05$, $p<0.01$ and $p<0.001$) compared to MRS media control (Fig. 2). Four lactic acid bacteria isolates; MSMC104-2, MSMC105-3, MSMC111-2 and MSMC171-1 exhibited >50% growth inhibition (+++) as shown in Table 2. Five isolates of MSMC112-2, MSMC137-1, MSMC181-1, MSMC182-1 and MSMC209-1 showed moderate antiproliferative activity (++) while the rest, 8 isolates showed about 20-40% growth inhibition (+) against U937 cells.

Acid and bile tolerance test

The 7 lactic acid bacteria isolates; MSMC95-4, MSMC104-2, MSMC105-3, MSMC111-2, MSMC112-2, MSMC171-1 and MSMC215-1 which showed strong inhibitory effect against cancer cells were further investigated for acid and bile tolerance test. The bacterial cell number remaining after incubation in the adjusted acidic MRS media compared with MRS broth control for 3 h allowed us to roughly separate the isolates according to the acid tolerance properties into 3 groups; the viable counts of MSMC95-4, MSMC104-2 and MSMC171-1 isolates showed highest acid tolerance as the cell number decreased at pH 2, 3 and 4 by about 4, 3 and 1 log values, respectively (Table 3). The viable count of MSMC105-3 and MSMC215-1 decreased at pH 2, 3 and 4 by about 5, 2 and 1 log values while MSMC111-2, MSMC112-2 showed less acid tolerance at pH 2 but at pH 3 and pH 4 showed better tolerance.

According to bile tolerance, all the selected

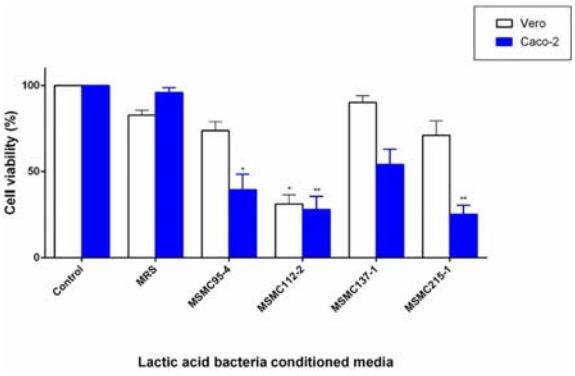

Fig. 1 Growth inhibitory effects of 4 conditioned media of lactic acid bacteria isolates against Caco2 cells. The data expressed as mean ± SEM of three independent experiments. Control, cell culture media control; MRS, bacteria media control. Asterisks denote significantly different from MRS bacteria media control: * $p<0.05$, ** $p<0.01$.

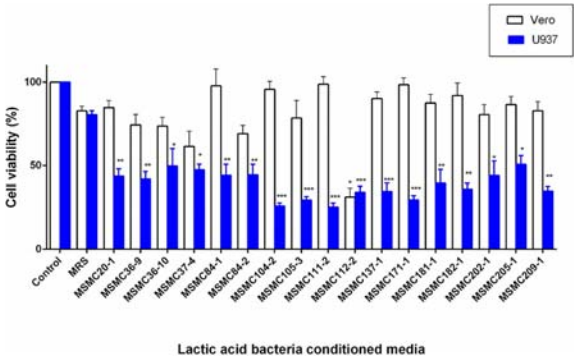

Fig. 2 Growth inhibitory effects of 17 conditioned media of lactic acid bacteria isolates against U937 cells. The data expressed as mean ± SEM of three independent experiments. Control, cell culture media control; MRS, bacteria media control. Asterisks denote significantly different from MRS bacteria media control; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 1. Growth inhibitory effects of 4 lactic acid bacteria isolates against Caco-2 cells and Vero cells. The data were expressed as mean ± SEM of three independent experiments

Lactic acid bacteria isolates	Cell viability (%)		Inhibition (%)	
	Caco-2 cells	Vero cells	Caco-2 cells	Vero cells
MRS broth	96.02±2.63	82.80±2.63	-	-
MSMC95-4	39.30±8.48	73.76±4.55	56.73^{+++}	-
MSMC112-2	28.12±7.27	31.34±5.02	67.91^{+++}	51.46^{+++}
MSMC137-1	53.99±8.42	90.17±3.75	42.04^{++}	-
MSMC215-1	25.43±3.89	71.01±8.05	70.59^{+++}	-

MRS = bacteria media control.
Inhibition (%): - = <20%; $^{+}$ = 20-40%; $^{++}$ = 41-50%; $^{+++}$ = >50%

Table 2. Growth inhibitory effects of 17 lactic acid bacteria isolates against U937 cells and Vero cells. The data were expressed as mean ± SEM of three independent experiments

Lactic acid bacteria isolates	Cell viability (%)		Growth inhibition (%)	
	U937 cells	Vero cells	U937 cells	Vero cells
MRS broth	80.55±2.30	82.80±2.63	-	-
MSMC20-1	43.59±3.88	84.79±4.03	36.97^{+}	-
MSMC36-9	41.90±4.27	74.37±5.52	38.66^{+}	-
MSMC36-10	49.68±9.90	73.60±5.13	30.87^{+}	-
MSMC37-4	47.52±2.87	61.40±8.76	33.03^{+}	21.40^{+}
MSMC84-1	44.16±5.73	97.79±8.89	36.39^{+}	-
MSMC84-2	44.46±5.52	69.03±4.13	36.09^{+}	-
MSMC104-2	26.04±1.77	95.72±3.97	54.51^{+++}	-
MSMC105-3	29.49±2.06	78.47±8.69	51.06^{+++}	-
MSMC111-2	25.34±2.07	98.85±3.90	55.21^{+++}	-
MSMC112-2	34.23±3.47	31.34±5.02	46.32^{++}	51.46^{+++}
MSMC137-1	34.64±4.64	90.17±3.75	45.91^{++}	-
MSMC171-1	30.16±2.29	98.50±2.38	50.93^{+++}	-
MSMC181-1	39.36±7.74	87.56±4.55	41.20^{++}	-
MSMC182-1	36.05±3.22	91.98±7.19	44.50^{++}	-
MSMC202-1	44.06±7.65	80.53±5.78	36.50^{+}	-
MSMC205-1	50.75±4.55	86.52±4.39	29.80^{+}	-
MSMC209-1	35.04±2.41	82.83±5.29	45.51^{++}	-

MRS = bacteria media control
% Growth inhibition: - = <20%; $^{+}$ = 20-40%; $^{++}$ = 41-50%; $^{+++}$ = >50%

Table 3. Survival of the candidate lactic acid bacteria isolates grew in MRS broth at pH 2.0, 3.0, and 4.0 after 3 h incubation

Isolate	MRS (0 h)	MRS (3 h)	pH		
			2	3	4
MSMC95-4	$1.80x10^{8}$	$7.13x10^{8}$	$6.63x10^{4}$	$7.03x10^{5}$	$9.83x10^{7}$
MSMC104-2	$7.00x10^{7}$	$6.23x10^{8}$	$5.40x10^{4}$	$1.67x10^{5}$	$4.97x10^{7}$
MSMC105-3	$7.00x10^{7}$	$1.13x10^{8}$	$9.17x10^{3}$	$9.20x10^{6}$	$6.47x10^{7}$
MSMC171-1	$9.67x10^{7}$	$2.77x10^{8}$	$2.57x10^{4}$	$5.70x10^{6}$	$3.53x10^{7}$
MSMC215-1	$3.33x10^{8}$	$4.43x10^{8}$	$3.67x10^{3}$	$3.87x10^{6}$	$9.33x10^{7}$
MSMC111-2	$2.00x10^{8}$	$1.03x10^{9}$	$1.33x10^{3}$	$1.63x10^{5}$	$5.37x10^{7}$
MSMC112-2	$1.20x10^{8}$	$1.50x10^{8}$	$1.10x10^{2}$	$2.40x10^{5}$	$1.03x10^{8}$

lactic acid bacteria grew well in bile 1%, 2%, 3% and 4% bile tested, except MSMC105-3 that could not grew in 4% bile (Table 4).

Species identification

Genotypic characterization by 16s rDNA sequencing of the selected lactic acid bacteria strains that exhibited the anticancer against human colon adenocarcinoma cells (Caco-2) and human monocytic leukemic cells (U937) were determined. The isolates MSMC95-4, MSMC104-2, MSMC111-2, MSMC112-2 and MSMC215-1 were identified as *Enterococcus faecalis* with 99.4%, 98.8%, 99.5%, 98.7 and 98.9% identity, respectively. MSMC171-1 was identified into *Enterococcus durans* (99.3% identity), *Enterococcus faecium* (99.3% identity) or *Enterococcus* lactis (99.3% identity). MSMC105-3 belongs to *Lactobacillus salivarius* with 99.1% identity (Table 5).

Table 4. Survival of the candidate lactic acid bacteria isolates grew in MRS broth supplemented with 1%, 2%, 3%, and 4% bile after 3 h incubation

Isolate	MRS (0 h)	MRS (3 h)	% Bile concentration			
			1%	2%	3%	4%
MSMC95-4	$8.10x10^8$	$1.04x10^9$	$5.47x10^9$	$3.23x10^9$	$2.40x10^9$	$1.43x10^8$
MSMC104-2	$2.40x10^8$	$3.93x10^8$	$2.40x10^9$	$1.63x10^9$	$1.10x10^9$	$5.33x10^8$
MSMC105-3	$2.80x10^8$	$5.03x10^8$	$1.23x10^9$	$5.67x10^8$	$1.67x10^8$	0
MSMC171-1	$2.10x10^8$	$2.83x10^8$	$3.40x10^9$	$2.27x10^9$	$5.67x10^8$	$1.67x10^8$
MSMC215-1	$3.30x10^8$	$4.33x10^8$	$2.60x10^9$	$2.20x10^9$	$1.33x10^9$	$7.67x10^8$
MSMC111-2	$3.70x10^7$	$1.80x10^8$	$8.67x10^8$	$1.40x10^9$	$2.77x10^9$	$3.47x10^9$
MSMC112-2	$4.40x10^8$	$5.50x10^8$	$1.13x10^9$	$2.23x10^9$	$3.20x10^9$	$4.73x10^9$

Table 5. Genotypic characterization of the candidate lactic acid bacteria isolates

Lactic acid bacteria isolate	Nucleotide sequences of 16S rDNA (RDP-II)	% Homology
MSMC95-4	*Enterococcus faecalis*	99.4% (1,331 base pairs)
MSMC104-2	*Enterococcus faecalis*	98.8% (1,478 base pairs)
MSMC105-3	*Lactobacillus salivarius*	99.1% (1,506 base pairs)
MSMC111-2	*Enterococcus faecalis*	99.5% (1,324 base pairs)
MSMC112-2	*Enterococcus faecalis*	98.7% (1,302 base pairs)
MSMC171-1	*Enterococcus faecium*	99.3% (1,334 base pairs)
	Enterococcus lactis, or *Enterococcus durans*	99.3% (1,334 base pairs)
MSMC215-1	*Enterococcus faecalis*	98.9% (1,347 base pairs)

Discussion

When orally administered, the viable probiotics grow in GIT and produce microbial-produced metabolites that will affect with cell proliferation, differentiation, inflammation and reduce CRC risk[19]. The supernatant of bacterial culture media was commonly used for testing the bioactive compounds. However, MRS media contains sodium acetate which may interfere with the tests' efficacy so the conditioned media was widely used to test any active compounds that microorganisms produced. In addition, the previous studies have shown that some *L. fermentum* strains have more potency in soluble factors produced in the supernatant and not the bacterial pellet itself[20]. In order to evaluate the possibility of inhibitory effect of the MRS broth itself on the cancer cell growth, we firstly evaluated MRS bacterial media control at 5-20% (v/v) to test on both cancer cells growth. The result showed that at 5-10% MRS media control had no growth inhibitory effect on both cancer cells growth but the inhibitory effect of media was observed at 15-20% (data not shown) so we considerately used the MRS media control and *Lactobacillus*-conditioned media at 10% (v/v) concentration to test the cytotoxicity.

Among 200 lactic acid bacteria isolated from Thai healthy newborn fecal samples, four and seventeen isolates could inhibit Caco-2 cells and U937 cells proliferation, respectively (Table 1 and Table 2). Most of the selected isolates showed significant growth inhibition effect on cancer cell lines (Fig. 1 and Fig. 2) but according to the criteria of grading of % growth inhibition, they were recorded as +, ++ and +++ (Table 1 and Table 2). Therefore, we selected only those isolates that exhibited more than 50% growth inhibition (+++) which also showed significant inhibitory effect to Caco-2 and U937 cell lines ($p<0.01$, $p<0.001$) for further study.

The high number of starter lactic acid bacteria ingested allowed the viable lactic acid bacteria to survive and provide beneficial effect in GIT. The pH of gastric acid is 1.5 to 3.5[21]. The candidate lactic acid bacteria could survive in pH 2-4 although reduced in cell number (Table 3). In this experiment, we used the starter lactic acid bacterial cell number as about 10^8 and

the bacteria cell about 10^3- 10^4 still alive at pH 2 and 10^7 at pH 4. In addition they could survive and grow well in the presence of high percent bile (4%) (Table 4). These results showed that they have good properties of probiotics which could enhance the viability of microorganisms in GIT and be able to secrete the bioactive compounds. Therefore, there were 7 candidates (including MSMC112-2) that have anticancer activity and also tolerated well to acid and bile were subjected to genotypic identification and found to be the genera *Enterococcus* and *Lactobacillus* (Table 5). However, *Enterococcus faecalis*, strain MSMC 112-2 which showed significant growth inhibition effect against both cancer cells but also inhibit growth of normal cell, so it is noteworthy for the further use of this strain.

Several evidences showed that lactic acid bacteria displayed anti-proliferative effects against cancer cell lines. Kahouli et al[22] reported that *L. fermentum* NCIMB5221 was potent in suppressing colon cancer cells and promoting normal epithelial colon cell growth by the production of short chain fatty acids (SCFAs). Thirabunyanon et al[23] reported *Pediococcus pentosaceus* FP3, *L. salivarius* FP25, *L. salivarius* FP35, and *Enterococcus faecium* FP51 isolated from newborn feces showed antiproliferation of Caco-2 cell. Lee NK et al[6] reported *Lactococcus lactis* KC24 isolated from kimchi have anticancer activities against gastric carcinoma (AGS), colon carcinoma (HT-29 and LoVo), breast carcinoma (MCF-7), and lung carcinoma (SK-MES-1) cells (>50% cytotoxicity). The recent study from Sevda ER et al[8] reported the effect of cell-free filtrate and cell-free lyophilized filtrate of *Pediococcus pentosaceus*, *Lactobacillus plantarum* and *Weissella confusa* inhibit the growth of colon cancer cell (Caco-2) in a dose-dependent manner, by using MTT assay and *L. plantarum* showed the strongest inhibitory effect. However, Ewaschuk et al[24] reported that *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *Bifidobacterium breve*, *B. newbornis*, *B. longum* and *Streptococcus thermophilus* reduced the viability and induced apoptosis of human colon cancer, HT-29 and Caco-2 cells demonstrated by DNA ladder assay. Nevertheless, another researcher used the concentrated supernatants from *L. plantarum* and found the promotion of cell death in a human promyelocytic cell line (HL60) by inducing necrosis rather than apoptosis at high doses[25]. Therefore, the mechanism of lactic acid bacteria on the cancer cell growth inhibition is still unclear. The health benefit of probiotics is very strain specific. In our study, the isolation of probiotics from Thai healthy newborn feces (GIT) ensure that the selected probiotics isolates would be friendly to Thai or Asian bacteria consortium in GIT. In addition, the candidate strains of lactic acid bacteria exhibited anticancer activity and not toxic to normal cells; however, the further study on the mechanism of action and other properties of these strains are needed to be investigated.

Conclusion

We have identified 6 lactic acid bacteria, belong to genera *Enterococcus* and *Lactobacillus* from Thai healthy newborn; MSMC95-4, MSMC104-2, MSMC105-3, MSMC111-2, MSMC171-1 and MSMC215-1 which exhibited anticancer activity against colon carcinoma cell and leukemia cell lines but were not toxic to normal cells (Vero). Moreover, they had good probiotic characteristics in terms of acid tolerance, and bile tolerance. Therefore, these lactic acid bacteria strains could be used as potential functional probiotics in food or supplements which may help cancer prevention and they are good probiotics in GI tract, in terms of acid and bile tolerance. However, more investigations are needed to understand the mechanisms of the inhibitory effects on cancer cells and other details for application use.

What is already known on this topic?

Lactic acid bacteria are known to have antitumor property both in vitro and in vivo.

What this study adds?

We could isolate the lactic acid bacteria from Thai newborn feces which exhibited the antiproliferative activity against colon carcinoma cell line and leukemia cell lines, remarkably did not exhibit toxicity to normal cells and also tolerated well in high acid and bile condition.

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Potential conflicts of interest

None.

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การคัดกรองโพรไบโอติกแบคทีเรียกรดแลคติกที่มีศักยภาพในการต้านมะเร็ง

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ภูมิหลัง: *โพรไบโอติกมีคุณสมบัติลดการกลับเป็นซ้ำและผลข้างเคียงของมะเร็งลำไส้ใหญ่และไส้ตรงในผู้ป่วยได้*

วัตถุประสงค์: *ผู้นิพนธ์ต้องการคัดแยกเชื้อแบคทีเรียกรดแลคติกจากอุจจาระของทารกไทยแรกคลอดที่มีสุขภาพดี โดยคัดเลือกสายพันธุ์ที่มีคุณสมบัติในการยับยั้งการเจริญเติบโตของเซลล์ไลน์จากเซลล์มะเร็ง*

วัสดุและวิธีการ: *ผู้นิพนธ์ได้ทำการคัดแยกแบคทีเรียกรดแลคติกจากอุจจาระของทารกไทยแรกคลอด โดยคัดเลือกสายพันธุ์ที่มีคุณสมบัติในการยับยั้งการเจริญเติบโตของเซลล์ไลน์จากเซลล์มะเร็งลำไส้ใหญ่และเซลล์มะเร็งเม็ดเลือดขาวด้วยวิธี MTT assay และทดสอบคุณสมบัติการทนกรดและน้ำดี*

ผลการศึกษา: *จากเชื้อแบคทีเรียกรดแลคติกจำนวน 200 สายพันธุ์ที่คัดแยกได้จากอุจจาระของทารกแรกคลอดที่มีสุขภาพดี พบว่าเชื้อแบคทีเรียกรดแลคติกจำนวน 3 สายพันธุ์ และ 1 สายพันธุ์ สามารถยับยั้งการเจริญของเซลล์มะเร็งเซลล์มะเร็งลำไส้ใหญ่ (Caco-2) ได้ดีและค่อนข้างดี อย่างมีนัยสำคัญทางสถิติ ตามลำดับ ในทำนองเดียวกันมีเชื้อแบคทีเรียกรดแลคติกจำนวน 4 และ 5 สายพันธุ์ สามารถยับยั้งการเจริญของเซลล์มะเร็งเม็ดเลือดขาวชนิดโมโนซัยท์ U937 ได้ดี และค่อนข้างดี อย่างมีนัยสำคัญทางสถิติ ตามลำดับ จึงได้คัดเลือกเชื้อ 7 สายพันธุ์ที่ยับยั้งการเจริญของเซลล์มะเร็งทั้ง 2 ชนิดได้ดี และไม่มีผลยับยั้งการเจริญของเซลล์ปกติ (Vero) ยกเว้น 1 สายพันธุ์ คือ MSMC 112-2 ที่มีผลยับยั้งการเจริญของเซลล์ปกติ มาศึกษาถึงความสามารถทนต่อกรดและน้ำดีพบว่าเชื้อทั้ง 7 สายพันธุ์สามารถทนสภาวะกรดที่ระดับ pH 2-4 และทนต่อเกลือน้ำดีที่ระดับความเข้มข้น 1-4% ยกเว้น MSMC105-3 ไม่สามารถเจริญได้ในน้ำดี 4% ผลการพิสูจน์ชนิดของเชื้อ โดยเปรียบเทียบความเหมือนของลำดับนิวคลีโอไทด์ พบว่า เชื้อ MSMC95-4, MSMC104-2, MSMC111-2, MSMC112-2, MSMC215-1 เป็น Enterococcus faecalis (99.4%, 98.8%, 99.5%, 98.7%, 98.9% ตามลำดับ), เชื้อ MSMC105-3 เป็น Lactobacillus salivarius (99.1%), เชื้อ MSMC171-1 เป็น Enterococcus faecium (99.3%)*

สรุป: *ผู้วิจัยได้คัดแยกเชื้อแบคทีเรียกรดแลคติกที่มีคุณสมบัติยับยั้งการเจริญของเซลล์มะเร็งและมีคุณสมบัติโพรไบโอติกที่ดี*