Original Article

Antioxidant activities and Probiotic Properties of *Lactobacillus paracasei* MSMC37-3

Porntipha Vitheejongjaroen, MSc[1,2], Ulisa Pachekrepapol, PhD[3], Narathip Puttarat, PhD[2], Surasak Yooyongsatit, PhD[4], Cheng Nilbu-Nga, MD[5], Malai Taweechotipatr, PhD[2,4]

[1] Biomedical sciences Program, Srinakharinwirot University, Bangkok, Thailand
[2] Center of Excellence in Probiotics, Strategic Wisdom and Research Institute, Srinakharinwirot University, Bangkok, Thailand
[3] Division of Food Science and Nutrition, Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Nakhon Nayok, Thailand
[4] Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand
[5] Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand

Background: The natural antioxidants from biological resources have gained much attention. There are reports of some strains of *Lactobacillus* that may be a good natural source for antioxidants, thereby helping the human body from oxidative damages.

Objective: To investigate the antioxidant activities and probiotic properties of strains isolated from healthy infants.

Materials and Methods: Probiotic isolates were selected for in vitro antioxidant activities of probiotics using hydroxyl and DPPH radical scavenging, and intracellular free radical. The selected probiotic was identified by 16S rRNA gene sequencing and determined for probiotic properties including resistance to simulated gastrointestinal conditions, adhesion property, hemolysis activity, antimicrobial and antibiotic activity.

Results: Among probiotic isolates, viable cells, cell free supernatant and intracellular cell free extract of MSMC37-3 exhibited antioxidant capacities. MSMC37-3 isolate was identified as *Lactobacillus paracasei* on basis of 16S rRNA gene sequencing. *L. paracasei* MSMC37-3 showed more than 95% survival rate in simulated gastrointestinal conditions, adherent to intestinal cells and did not show hemolytic activity. *L. paracasei* MSMC37-3 exhibited antimicrobial activity against 9 indicator bacteria. *L. paracasei* MSMC37-3 was susceptible to eight antibiotics.

Conclusion: These results indicated that *L. paracasei* MSMC37-3 is a potential probiotic strain for application in the development of healthy products.

Keywords: Probiotics; *Lactobacillus*; Free radical; Antioxidant; Oxidative stress; Probiotic properties

J Med Assoc Thai 2022;105(Suppl.1): S107-14
Website: http://www.jmatonline.com

Excessive reactive oxygen species (ROS) cause oxidative stress and lead to various diseases, including metabolic syndrome, cancer, neurodegenerative diseases and aging[1]. Hydroxyl radical (HO), hydrogen peroxide (H_2O_2), and superoxide radicals (O_2) are ROS generated by cells during respiration in the mitochondria, and cell-mediated immune functions. Hydrogen peroxide is highly reactive causing macromolecules oxidation and enormous biological damage[2].

Correspondence to:

Taweechotipatr M.

Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand.

Phone: +66-2-6495393, **Fax:** +66-2-6495394

Email: malai@g.swu.ac.th

How to cite this article:

Vitheejongjaroen P, Pachekrepapol U, Puttarat N, Yooyongsatit S, Nilbu-Nga C, Taweechotipatr M. Antioxidant activities and Probiotic Properties of *Lactobacillus paracasei* MSMC37-3. J Med Assoc Thai 2022;105 (Suppl.1): S107-14.

doi.org/10.35755/jmedassocthai.2022.S01.00112

Normally, the human body has a repair system by creating antioxidants to protect them from oxidative stress[3]. Some strains of probiotics are able to synthesize compounds that act as antioxidants and play an important role in various biological activities[4]. Therefore, probiotics may be an important source of antioxidants that can balance and protect cells from the oxidative stress conditions.

Probiotic strains, mainly lactobacilli and bifidobacteria, are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[5,6]. Most probiotic are found in the gastrointestinal tract (GIT) and have a beneficial effect on the balance of the host's intestinal microflora[7]. Probiotics should possess acid and bile tolerant properties to survive through the GIT. Colonization of probiotics in GIT prevents the growth of harmful bacteria by organic acids and antimicrobial compounds produced by probiotics[8]. Probiotics have good specific properties and are used as an ingredient in food products. In addition, probiotics have various benefits to human health including improvement of the immune system, anti-cancer property and modification of lactose intolerance and metabolic function[9]. Antioxidant activity, one of the important characteristics of probiotics, results from

production of various antioxidant enzymes and compounds[10]. Beneficial health effects of probiotics isolated from humans are species-specific interactions, so probiotics can be used effectively and safely for human consumption[11]. Various types of bifidobacteria and lactobacilli isolated from human origin exhibited antioxidant activities and high stability and safety in gastrointestinal conditions[12]. Twenty lactobacilli isolated during fermentation of agave exhibited hydroxyl radical and 2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) radical scavenging capacity, and showed great potential of probiotics[13]. Additionally, *Lactobacillus plantarum* Y44 significantly inhibited intracellular ROS production in intestinal cells and increased the cell viability of intestinal cells injured by H_2O_2[14]. In oxidized oil induced oxidative stress mice, *L. plantarum* AR501 enhanced the antioxidant enzymes and decreased the lipid peroxidation[15]. In randomized clinical trial, probiotic supplementation improved antioxidant markers in healthy volunteers[16], type 2 diabetes patients[17] and rheumatoid arthritis patients[18]. Probiotics have proved to be beneficial by improving antioxidant status and preventing oxidative disorders in the human body.

At present, few studies have compared antioxidant capabilities of viable cells, cell free supernatant and intracellular cell free extract of probiotic isolated from human. Some probiotic strains have shown antioxidant capacity in chemical assay but do not reduce free radical in cells. In addition, the probiotic properties vary depending on the species. Probiotics that have adverse effects must not be used in food products even though they have specific disease-related properties. The objectives of this study were to assess the in vitro antioxidant activities of probiotics using two different methods and intracellular free radical, and to evaluate probiotic properties including resistance to simulated gastrointestinal conditions, adhesion property, hemolysis activity, antimicrobial assessment and antibiotic susceptibility. These results may be useful for the application of probiotics in health products.

Materials and Methods

Selection and preparation of probiotics

Probiotics were isolated from healthy human infants (ethical approval number SWUEC37/2551). Cultures were used from frozen stocks and sub-cultured three times on de Man, Rogosa, Sharpe (MRS) agar (HiMedia Lab, India) at 37°C for 24 to 48 h under anaerobic conditions (10% O_2; 10% CO_2, 80% N_2) before the experiment.

For preparation of viable cells, probiotic isolate was adjusted to approximately 10^9 colony forming units (CFU)/mL in phosphate buffered saline (PBS; pH 7.2) for later use. For preparation of cell free supernatant (CFS), probiotics viable cells were inoculated at 10^9 CFU/mL to MRS broth and incubated for 24 to 48 h at 37°C under anaerobic conditions. The supernatants were collected after centrifuged at 4,000 x g for 5 min at 4°C and filtered through a sterilized 0.22 um pore size filter. The intracellular cell-free extract (ICFE) was prepared by incubating the cell pellets of probiotic isolate with 1 mg/mL lysozyme at 37°C for 30 min, followed by ultrasonic disruption in five 1-min intervals in an ice bath (power 25 to 30%, Sonoplus HD 2070, Germany). The sample was centrifuged at 8,000 x g for 10 min at 4°C, and the resulting supernatant was obtained.

Antioxidant activities of probiotic

Determination of hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of probiotic isolates were determined by the method of Vitheejongjaroen et al (2021)[19]. The reaction mixture contained 1.0 mL of 0.5 mM $FeSO_4$, 0.5 mL of 0.435 mM brilliant green (Sigma, USA) and 0.5 mL of different samples (viable cells, CFS and ICFE), followed by the addition of 0.75 mL of 3.0% w/v H_2O_2 to initiate the reaction. The mixture was incubated at room temperature for 20 min. The absorbance was measured at 624 nm. The brilliant green as a blank control and 1% (w/v) ascorbic acid (Sigma, USA) was used as a positive control. The hydroxyl radicals scavenging activity was calculated using the following formula:

scavenging activity (%) = $((A_s - A_c) / (A_b - A_c)) \times 100$

Where A_s was the absorbance of samples, A_b was the absorbance of the blank control, and A_c was the same amount of water and sample mixture.

Determination of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging

The hydrogen donating ability of the three samples (viable cells, CFS, ICFE) of probiotic isolates were investigated using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) stable radical assay with slight modification[20]. The samples (50 µL) was mixed thoroughly with 100 µL of 0.2 mM DPPH solution (DPPH; Sigma, USA) and allowed to react at room temperature. After 30 min, the mixture solutions were measured spectrophotometrically at 517 nm. Ascorbic acid solution (1%) was used as a positive control[21].

Investigation of antioxidant activities of probiotic isolates on human colon carcinoma cell line

The human colon carcinoma (Caco-2) cell line (ATCC HTB-37) was obtained from American Type Culture Collection (ATCC) and maintained at 37°C with 90% humidity atmosphere and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin. The culture medium was replaced every 48 h for 48 h prior to experimental treatment.

Generation of intracellular ROS in the Caco-2 cells was evaluated by measuring the oxidation level of 2′, 7′-dichlorofluorescein diacetate (DCFH DA; Sigma, USA) according to the method of Hou et al (2019)[22] with slightly modifications. The Caco-2 cells were seeded in black 96-well microplate at a density of $2.4x10^4$ cells/well (100 µL/well), incubated overnight, and treated with the probiotic samples for 24 h. The control cells were exposed to deionized water.

After adding hydrogen peroxide (H_2O_2; Merck, Germany) to the final concentration of 500 µM, the cells were incubated for further 2 h. Subsequently, the cells were incubated with 25 µM DCFH-DA solution at 37°C for 30 min, the medium was removed and washed once with PBS. The intracellular ROS in cells was detected by measuring fluorescence intensities at excitation and emission wavelengths of 485 and 535 nm.

Genotypic identification of probiotic isolates

For the detection of 16S rRNA gene sequence, the bacterial universal primers 20F (5'-AGTTTGATCCTG GCTC-3') and 1530R (5'-AAGGAGGTGATCCAGCC-3') was used. Amplification reactions were performed in a total volume of 100 µL of PCR Master Mix (5 U Taq DNA polymerase, 2 mM dNTP, 25 mM $MgCl_2$, 10X Taq Buffer, deionized H_2O, 0.25 mM forward primer, 0.25 mM reverse primer) and 300 µL of colony probiotics in milliQ water. The PCR product was run and purified using a QIA quick PCR purification kit (QIAGEN, USA). The sequence was analyzed by U2Bio in Korea and submitted to a search for similarity in the National Center for Biotechnology Information (NCBI) GenBank database and EzTaxon bioinformatics software.

Measurement of general probiotic properties
Resistance to simulated gastrointestinal conditions

Probiotic strain was evaluated under stress conditions. MRS broth was adjusted with 1 N hydrochloric acid (HCL) to either pH 3.0 or 4.0. Bile salt conditions were prepared by dissolving powdered bovine bile (Sigma, USA) in MRS broth to a final concentration of 0.3% (w/v) or 0.8% (w/v). The strain culture was inoculated at $1x10^9$ CFU/mL in conditions and incubated at 37°C for 3 h under anaerobic condition. MRS broth was used as negative control. The number of bacterial colony-forming units was enumerated by spread plate counts on MRS agar incubated at 37°C for 24 to 48 h.

In vitro adherence assay

Adhesion of the probiotic strain was measured using minor modifying the protocol described by Dimitrov et al (2014)[23]. Briefly, Caco-2 cells monolayers were prepared and seeded at a concentration of $1x10^5$ cells/well in 24-well standard tissue culture plates. After cultured for 14±1 days, the cells were washed twice with PBS (pH 7.2) and re-suspended with antibiotics-free medium. Subsequently, overnight culture of probiotic strain (10^9 CFU/mL in DMEM) was added and incubated for 1 h. The cells lysed by Triton® X-100 (Merck, Germany) for 10 min after washing twice with PBS to remove non-adherent bacteria. The lysed cells with adhering bacteria were plated on MRS agar and incubated at 37°C for 48 h under anaerobic conditions. *L. rhamnosus* GG (LMG 18243) was used as a positive control. Adhesion of bacteria to Caco-2 cells were calculated as the percentage, compared with initial bacterial number added in each well.

Hemolytic activity

For hemolytic activity, the probiotic strain was streaked on brain heart infusion agar (BHI) supplemented with 5% (v/v) defibrinated sheep blood. The overnight culture was then sub-culture on sheep blood agar plate and incubated at 37°C for 48 h under anaerobic conditions. A clear zone and a green zone surrounding the colony indicated complete hemolysis (β-hemolysis) and partial hemolysis (β-hemolysis) of red blood cells, respectively. Probiotic strain without changes in the agar indicated the absence of a hemolytic reaction (γ-hemolysis).

Antimicrobial assessment

Antimicrobial activity of probiotic strain against indicator strains was evaluated by the agar well-diffusion method[24]. Ten indicator strains including *Staphylococcus aureus* ATCC 6538, methicillin resistant *S. aureus* DSMT 20654, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Proteus mirabilis* DMST 8212, *Shigella dysenteriae* DMST 15111, *Vibrio cholerae* DMST 2873, and *Pseudomonas aeruginosa* DMST 5870 were adjusted to the concentration of $1x10^8$ CFU/mL in PBS. Each indicator strain was swabbed thrice on Mueller Hinton agar plates (MH) and incubated at 37°C under aerobic condition. The agar well, 4 mm in diameter was cut with cork borer and added with 25 µL of probiotic culture supernatant to each well. MRS broth was used as a negative control. The diameters of inhibition zones were measured and recorded in millimeter (mm) after 24 h incubation at 37°C.

Antibiotic susceptibility testing

Antibiotic susceptibility of the probiotic strain was evaluated using the standard disk diffusion method[25]. Briefly, the probiotic strain (10^8 CFU/mL) was swabbed on MH agar surfaces. Then, ten antibiotic discs including ampicillin (AMP; 10 µg), chloramphenicol (CHL; 30 µg), erythromycin (ERY; 15 µg), gentamycin (GEN; 10 µg), nalidixic acid (NAL; 30 µg), penicillin G (PEN; 10 U), tetracycline (TET; 30 µg), vancomycin (VAN; 30 µg), bacitracin (B; 10 µg), streptomycin (S; 10 µg) (Bio-Rad Lab, USA) were placed onto plate and incubated at 37°C under anaerobic condition. After 24 to 48 h, the diameter of the inhibition zone was measured to determine the antibiotic susceptibility (CLSI, 2010).

Statistical analysis

Statistical differences were evaluated by one-way analysis of variance (ANOVA), followed by a Dunnett's test using GraphPad Prism Software version 5.01 (GraphPad Software, USA). The differences were considered significant with p-values of less than 0.05.

Results

Antioxidant activities of probiotics

The antioxidant capacities of probiotic isolates were tested using viable cells, CFS and ICFE. Among probiotic isolates, MSMC37-3 was found to have antioxidant activities

by reducing two radical scavenging (Table 1). The hydroxyl free radical scavenging results of MSMC37-3 isolate were 10.01±1.77%, 15.11±1.66% and 35.34±1.23% for viable cells, CFS and ICFE, respectively. In the DPPH radical scavenging activity, viable cells and CFS showed the value of 13.57±1.90% and 21.74±2.82%, respectively. While ICFE showed scavenging activity with values ranging from 30.73±2.89%. The radicals scavenging activity of ICFE exhibited the highest scavenging capability ($p<0.05$). Therefore, MSMC37-3 isolate was selected for further cell antioxidant assays.

The intracellular ROS analysis was applied in the present study to quantitatively evaluate the antioxidant activity of viable cells, CFS and ICFE of MSMC37-3 isolate in Caco-2 cells as shown in Table 1. The MSMC37-3 isolate inhibited free radical in Caco-2 cells. The cellular antioxidant activity of MSMC37-3 were 22.56±1.39%, 44.90±2.28%, and 51.23±3.82% for viable cells, CFS and ICFE, respectively. The ICFE and CFS showed significantly higher antioxidant activity than the viable cells ($p=0.0003$; $p=0.0001$).

Genotypic identification of probiotics

Comparative analysis of 16S rRNA gene sequences confirmed the results from the cluster analysis and revealed that MSMC37-3 probiotic isolate was 99% relatively similar to *Lactobacillus paracasei* JCM 1171^T. The national center for biotechnology information (NCBI) accession numbers for the 16S rRNA gene sequences of *L. paracasei* MSMC37-3 was deposited under MW147140.1.

General probiotic properties

The simulated gastrointestinal tolerance of *L. paracasei* MSMC37-3 was detected (Figure 1). *L. paracasei* MSMC37-3 had more than 95% survival rate at pH 3.0 conditions and was able to grow at 96.13% at pH 4. The survival rates of *L. paracasei* MSMC37-3 against 0.3% and 0.8% bile salts were 100.69% and 99.71%, respectively. The results were not significantly different ($p>0.05$). The adhesion capacity of the strains to Caco-2 cells showed adhesion rates of 3.77% (Figure 1). Adhesion capacity of *L. paracasei* MSMC37-3 was higher than LGG but not significant. For hemolytic activity, *L. paracasei* MSMC37-3 did not exhibit hemolytic activity (γ-hemolysis). Therefore, *L. paracasei* MSMC37-3 was considered safe for this activity. *L. paracasei* MSMC37-3 exhibited inhibitory activity against bacterial indicator strains as shown in Table 2. The *L. paracasei* MSMC37-3 showed a moderate zone of inhibition against three indicator strains, methicillin resistant *S. aureus*, *M. luteus* and *P. mirabilis*, while weak zone of inhibition was found for six indicator strains including *S. aureus*, *B. subtilis*, *E. coli*, *S. dysenteriae*, *V. cholerae*, and *P. aeruginosa*.

The different scores reflected the different degrees of inhibition expressed in mm. The zone of inhibition ranged from 6.50 mm to 12.00 mm. The antibiotic susceptibility results of *L. paracasei* MSMC37-3 against 10 tested antibiotics are shown in Table 3. *L. paracasei* MSMC37-3 was sensitive to 8 antibiotics including ampicillin, chloramphenicol, penicillin, erythromycin, tetracycline, bacitracin and streptomycin, whereas it was resistant to gentamycin, nalidixic acid, and vancomycin.

Discussion

Most organisms have defense and repair system against the oxidative stress by creating the antioxidants and non-enzymatic antioxidants. The results of the present study demonstrated that the viable cells, CFS and ICFE of *L. paracasei* MSMC37-3 exhibited hydroxyl radicals and DPPH scavenging ability. These results indicated that all three component types of probiotic *L. paracasei* MSMC37-3 exhibited antioxidant properties. Similar results were reported by Yang et al (2020)[21] showed that effect of intact cells of *L. brevis* KU15151 scavenged free radical. The scavenging capacity of CFS indicates that the extracellular metabolite contains free radical-scavenging components.

Table 1. Antioxidant activity of viable cells, cell free supernatant (CFS) and intracellular cell free extract (ICFE) of MSMC37-3 in hydroxyl radicals, DPPH scavenging assays and cellular antioxidant activity on Caco-2 cells exposed to H_2O_2

Treatments	Hydroxyl free radical scavenging activity (%)	DPPH radical scavenging activity (%)	Cellular antioxidant activity (%)
MSMC37-3			
Viable cells	10.01±1.77***	13.57±1.90***	22.56±1.39***
CFS	15.11±1.66***	21.74±2.82***	44.90±2.28***
ICFE	35.34±1.23***	30.73±2.89***	51.23±3.82***
Ascorbic acid	23.39±0.23***	82.35±0.45***	73.55±0.80***
Negative control	3.79±0.43	1.65±0.14	6.98±1.47

Asterisks denote significantly different from MRS broth as negative control
*** $p<0.001$.
Each value is the mean±SD of three independent readings.

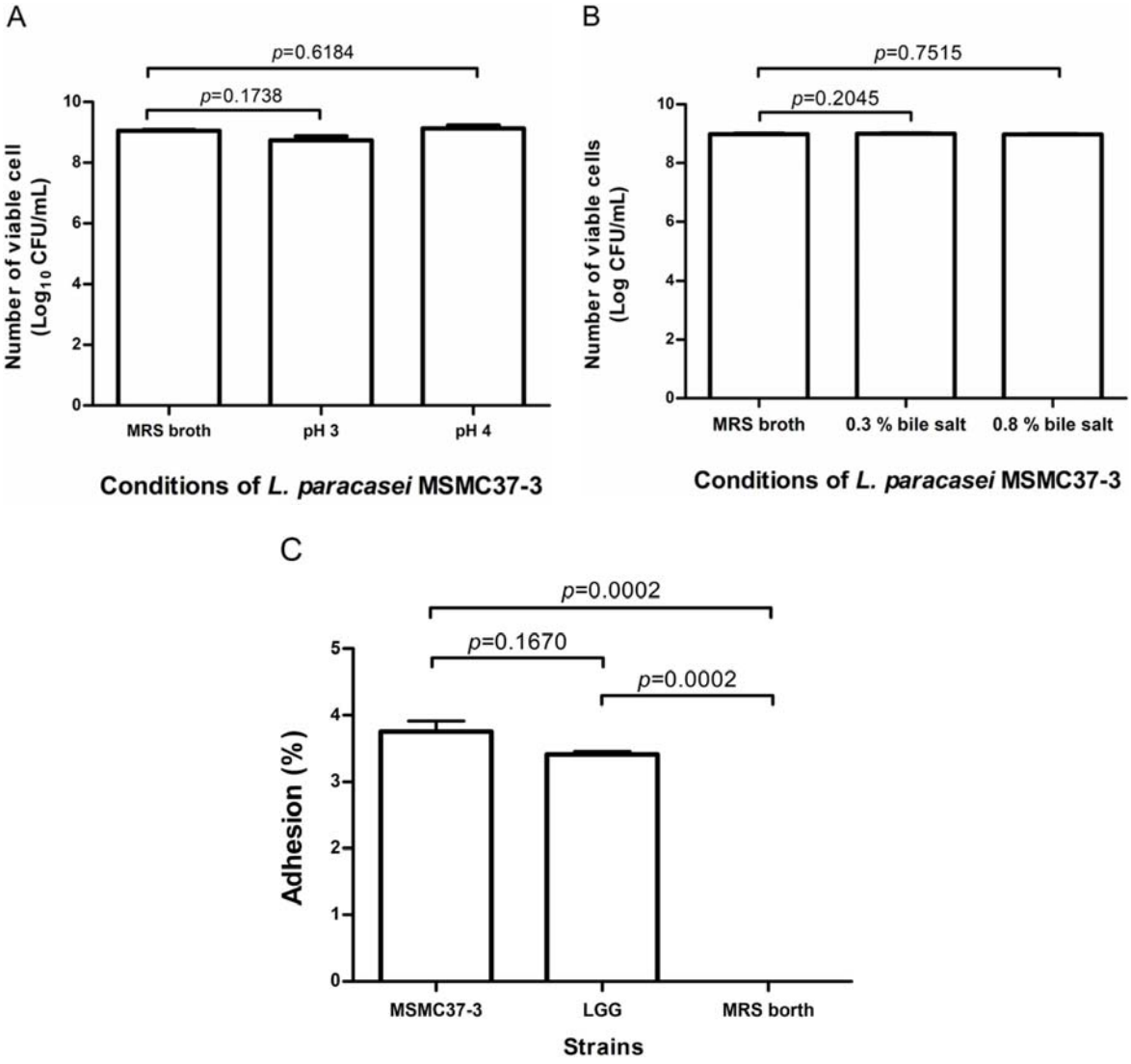

Figure 1. Low pH (A) and bile salt (B) tolerance and adhesion capacity to Caco-2 cells (C) of *L. paracasei* MSMC37-3.

The intracellular cell-free extracts from probiotics were found to have the ability to chelate metal ions, free radical scavenging abilities, and decreasing activity[26]. However, there was a wide range of scavenging activity indicating that antioxidative properties are strain specific[27]. Therefore, some strains of probiotics that are high in antioxidants survive better under oxidative stress than others.

Some strains of probiotics have been reported to be able to scavenge free radicals and protect the host from injury from oxidative stress. In the present study, *L. paracasei* MSMC37-3 was found to inhibit free radicals, protect oxidative cell damage induced by H_2O_2. The free radical reduction mechanism of *Lactobacillus* treatment enhances antioxidant enzymes and expression of gene, thereby reducing oxidative stress[28]. Probiotics also protect cells from apoptosis that damages cells and the mechanism involves inhibition of mitochondria and lipid peroxidation apoptosis pathways[29]. Our results indicated that *L. paracasei* MSMC37-3 may contain bioactive compounds or mechanisms to prevent oxidative stress.

The most common lactic acid bacteria (LAB) strains such as *Lactobacillus* are considered probiotics. *Lactobacillus* plays an important role in maintaining the colonic microbial ecosystem in the human gastrointestinal tract. *Lactobacillus* isolated from human origin was more effective than LAB isolated from other sources[30]. Most strains isolated from human origin are resistant to stress condition and are able to adhere to intestinal cells in the GIT

Table 2. Antimicrobial activity of *L. paracasei* MSMC37-3 against clinically significant pathogens

Zone of inhibition (mm)	
S. aureus	8.00±1.50 (W)
MRSA	10.25±0.75 (M)
S. pyogenase	0.00±0.00 (N)
M. luteus	11.75±0.25 (M)
B. subtilis	7.50±0.50 (W)
E. coli	6.50±0.75 (W)
P. mirabilis	12.00±1.50 (M)
Shigella dysenteriae	6.50±0.75 (W)
V. cholerae	8.50±1.50 (W)
P. aeruginosa	9.50±0.25 (W)

The different scores reflect the different degree of growth inhibition expressed in mm (n=3, mean ± SD), diameter of well was designed at 4.0 mm.
N = no inhibition; W = weak zone of inhibition between 4 and 10 mm; M = moderate zone of inhibition between 10.1 and 20 mm; S = strong zone of inhibition ≥20.1 mm

Table 3. Antibiotic susceptibility of *L. paracasei* MSMC37-3 by disc diffusion assay

Inhibition zone (mm)	
Ampicillin	30.75±0.25 (S)
Chloramphenicol	28.50±1.75 (S)
Gentamycin	8.25±0.25 (R)
Nalidixic acid	10.50±1.50 (R)
Penicillin	30.50±1.50 (S)
Erythromycin	32.75±1.75 (S)
Tetracycline	31.75±1.00 (S)
Vancomycin	8.25±0.75 (R)
Bacitracin	12.25±0.50 (S)
Streptomycin	12.25±0.50 (S)

Antibiotic susceptibility was interpreted based on the diameter of inhibition zone (mm).
R = resistant; S = susceptible

as the gastrointestinal tract is the habitat of probiotics. This study, MSMC37-3 isolated from healthy human infants was identified as *L. paracasei*. Previous studies have found that *L. fermentum* and *L. plantarum* are the most common species in samples isolated from Iranian infant feces[30].

Survivability of probiotics in gastrointestinal conditions is a major criterion of generally recognized as safe (GRAS) to confer health benefits[31]. The tolerance of *L. paracasei* MSMC37-3 to low pH and bile salts demonstrated ability to survive more than 95%. Many lactobacilli are resistant to stress condition and stabilize due to their cytoplasmic buffering capacity[32]. Moreover, ability of probiotics to survive under the gastrointestinal conditions depends not only on the species but also on the strain[33].

The present study indicated that viable *L. paracasei* MSMC37-3 was able to colonize the gastrointestinal tract. The surface molecules of each probiotic strain affect adhesion and colonization ability. In addition, the passive forces, electrostatic interactions, hydrophobic, steric forces also contribute to their ability to bind to the intestinal epithelial cells[34].

Hemolytic activity is one of virulence factors causing damages to red blood cells. Probiotics with hemolytic activity must not be used in the health products[35]. Probiotic with complete hemolysis (β-hemolysis) and partial hemolysis (α-hemolysis) were not selected. In the present study, *L. paracasei* MSMC37-3 did not exhibit hemolytic activity (γ-hemolysis). Therefore, *L. paracasei* MSMC37-3 is safe to be used in the health products.

Antagonistic activity of *L. paracasei* MSMC37-3 against bacterial indicator strains may be due to the organic acids, antimicrobial substance and other compounds that work in acidic environments[36]. Xanthopoulos et al (2000)[37] reported that *L. paracasei* and *L. acidophilus* isolated from infant feces inhibited various pathogenic bacteria.

According to previous studies, most lactobacilli are sensitive to antibiotics that inhibit protein synthesis. In this study, *L. paracasei* MSMC37-3 was resistant to gentamycin, nalidixic acid, and vancomycin. These results are in agreement with Cizeikiene and Jagelaviciute (2021)[38] who found that six *Lactobacillus* strains were resistant to gentamycin. Moreover, many *Lactobacillus* spp. are resistant to nalidixic acid as a gram-negative spectrum antibiotic[39]. Lactobacilli that are resistant to vancomycin may be intrinsic drug resistance because d-alanine residue at the terminal end in peptidoglycan was replaced with d-lactate or d-serine, preventing vancomycin binding[40]. In addition, antibiotic resistance appears to be intrinsic factor and the degree of resistance depends on the genus or species[38].

The *L. paracasei* MSMC37-3 exhibited the antioxidant capacity and reduced intracellular free radical in intestinal cells. Therefore, it may be an option for treating gastrointestinal (GI) disease such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), colitis, colon cancer and oxidative stress in GIT. However, the antioxidant capacity of *L. paracasei* MSMC37-3 in other cells has yet to explored. Previous studies have shown that oral administration of probiotics can improve antioxidant status in humans. Kleniewska et al (2016)[16] exhibited a significant increase of antioxidant enzyme including superoxide dismutase (SOD), glutathione peroxidase (GPx) catalase (CAT) activity after *L. casei* combined with inulin administration in healthy volunteers. Ejtahed et al (2012)[17] showed that probiotic consumption improved antioxidant status of blood in patients with type 2 diabetes.

Conclusion

The results of this study indicated that viable cells, cell free supernatant and intracellular cell-free extract of *Lactobacillus paracasei* MSMC37-3 exhibited antioxidant capacities on hydroxyl radicals, DPPH scavenging assays and cellular antioxidant activity on Caco-2 cells exposed to H_2O_2. It can also be concluded that *L. paracasei* MSMC37-3 has great probiotic potential, which could be further studied in animal and clinical trials and applied in the nutraceutical products and functional foods or as alternative medicine in the future.

What is already known on this topic?

Some strains of probiotic *Lactobacillus* are known to have antioxidants capacity and free radical scavenging activity to prevent the cells from oxidative damages.

What this study adds?

Lactobacillus paracasei MSMC37-3 from healthy infant exhibited the hydroxyl and DPPH free radical scavenging activity and reduced the free radical in Caco-2 intestinal cell line. Moreover, *L. paracasei* MSMC37-3 tolerated in stress condition of simulated GI conditions, adherent to intestinal cells, inhibited some pathogenic and opportunistic bacteria, and did not show hemolytic activity.

Acknowledgements

This study was funded by Research and Researchers for Industries Program, Thailand Science Research and Innovation (grant number PHD62I0002) and the National Research Council of Thailand and supported by the Faculty of Medicine and HRH Princess Maha Chakri Sirindhorn Medical Center (grant number 066/2552 and 174/2559) and Center of Excellence in Probiotics, Strategic Wisdom and Research Institute, Srinakharinwirot University.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol Sci 2017;38:592-607.
2. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018;13:757-72.
3. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
4. Feng T, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. Gut Microbes 2020;12:1801944.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506-14.
6. Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Rome, Italy: FAO/WHO; 2002.
7. Pieniz S, Andreazza R, Anghinoni T, Camargo F, Brandelli A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. Food Control 2014;37:251-6.
8. Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. Int J Food Microbiol 2006;106:1-24.
9. Guarner F, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, et al. World Gastroenterology Organisation Global Guidelines: probiotics and prebiotics October 2011. J Clin Gastroenterol 2012;46:468-81.
10. Spyropoulos BG, Misiakos EP, Fotiadis C, Stoidis CN. Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. Dig Dis Sci 2011;56:285-94.
11. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek 2002;82:279-89.
12. Kim H, Kim JS, Kim Y, Jeong Y, JE, Kim JE, Paek NS, et al. Antioxidant and probiotic properties of lactobacilli and bifidobacteria of human origins. Biotechnol Bioproc 2020;25:421-30.
13. Hernandez-Delgado NC, Torres-Maravilla E, Mayorga-Reyes L, Martin R, Langella P, Perez-Pasten-Borja R, et al. Antioxidant and anti-inflammatory properties of probiotic candidate strains isolated during fermentation of agave (Agave angustifolia Haw). Microorganisms 2021;9:1063.
14. Mu G, Gao Y, Tuo Y, Li H, Zhang Y, Qian F, et al. Assessing and comparing antioxidant activities of lactobacilli strains by using different chemical and cellular antioxidant methods. J Dairy Sci 2018;101: 10792-806.
15. Lin X, Xia Y, Wang G, Yang Y, Xiong Z, Lv F, et al. Lactic acid bacteria with antioxidant activities alleviating oxidized oil induced hepatic injury in mice. Front Microbiol 2018;9:2684.
16. Kleniewska P, Hoffmann A, Pniewska E, Pawliczak R. The influence of probiotic *Lactobacillus casei* in combination with prebiotic inulin on the antioxidant capacity of human plasma. Oxid Med Cell Longev 2016;2016:1340903.
17. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012;28:539-43.
18. Vaghef-Mehrabany E, Homayouni-Rad A, Alipour B, Sharif SK, Vaghef-Mehrabany L, Alipour-Ajiry S. Effects

of probiotic supplementation on oxidative stress indices in women with rheumatoid arthritis: a randomized double-blind clinical trial. J Am Coll Nutr 2016;35:291-9.
19. Vitheejongjaroen P, Kanthawang P, Loison F, Jaisin Y, Pachekrepapol U, Taweechotipatr M. Antioxidant activity of *Bifidobacterium animalis* MSMC83 and its application in set-style probiotic yoghurt. Food Biosci 2021;43:101259.
20. Xing J, Wang G, Zhang Q, Liu X, Gu Z, Zhang H, et al. Determining antioxidant activities of lactobacilli cell-free supernatants by cellular antioxidant assay: a comparison with traditional methods. PLoS One 2015;10:e0119058.
21. Yang SJ, Kim KT, Kim TY, Paik HD. Probiotic properties and antioxidant activities of *Pediococcus pentosaceus* SC28 and *Levilactobacillus brevis* KU15151 in fermented black gamju. Foods 2020;9:1154.
22. Hou Y, Li X, Liu X, Zhang Y, Zhang W, Man C, et al. Transcriptomic responses of Caco-2 cells to *Lactobacillus rhamnosus* GG and *Lactobacillus plantarum* J26 against oxidative stress. J Dairy Sci 2019;102:7684-96.
23. Dimitrov Z, Gotova I, Chorbadjiyska E. In vitro characterization of the adhesive factors of selected probiotics to Caco-2 epithelium cell line. Biotechnol Biotechnol Equip 2014;28:1079-83.
24. Prabhurajeshwar C, Chandrakanth K. Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. Clin Nutr Exp 209;23:97-115.
25. AlKalbani NS, Turner MS, Ayyash MM. Isolation, identification, and potential probiotic characterization of isolated lactic acid bacteria and in vitro investigation of the cytotoxicity, antioxidant, and antidiabetic activities in fermented sausage. Microb Cell Fact 2019;18:188.
26. Lin MY, Yen CL. Antioxidative ability of lactic acid bacteria. J Agric Food Chem 1999;47:1460-6.
27. Bengoa AA, Zavala L, Carasi P, Trejo SA, Bronsoms S, Serradell M, et al. Simulated gastrointestinal conditions increase adhesion ability of *Lactobacillus paracasei* strains isolated from kefir to Caco-2 cells and mucin. Food Res Int 2018;103:462-7.
28. Huang L, Duan C, Zhao Y, Gao L, Niu C, Xu J, et al. Reduction of aflatoxin B1 toxicity by *Lactobacillus plantarum* C88: A potential probiotic strain isolated from Chinese Traditional Fermented Food "Tofu". PLoS One 2017;12:e0170109.
29. Nowak A, Paliwoda A, Blasiak J. Anti-proliferative, pro-apoptotic and anti-oxidative activity of *Lactobacillus* and *Bifidobacterium* strains: A review of mechanisms and therapeutic perspectives. Crit Rev Food Sci Nutr 2019;59:3456-67.
30. Davoodabadi A, Soltan Dallal MM, Lashani E, Tajabadi Ebrahimi M. Antimicrobial activity of *Lactobacillus* spp. isolated from fecal flora of healthy breast-fed infants against diarrheagenic *Escherichia coli*. Jundishapur J Microbiol 2015;8:e27852.
31. Choi SS, Kang BY, Chung MJ, Kim SD, Park SH, Kim JS, et al. Safety assessment of potential lactic acid bacteria *Bifidobacterium longum* SPM1205 isolated from healthy Koreans. J Microbiol 2005;43:493-8.
32. Krulwich TA, Sachs G, Padan E. Molecular aspects of bacterial pH sensing and homeostasis. Nat Rev Microbiol 2011;9:330-43.
33. Cizeikiene D, Jagelaviciute J. Investigation of antibacterial activity and probiotic properties of strains belonging to *Lactobacillus* and *Bifidobacterium* genera for their potential application in functional food and feed products. Probiotics Antimicrob Proteins 2021;13:1387-403.
34. Tallon R, Arias S, Bressollier P, Urdaci MC. Strain- and matrix-dependent adhesion of *Lactobacillus plantarum* is mediated by proteinaceous bacterial compounds. J Appl Microbiol 2007;102:442-51.
35. Yasmin I, Saeed M, Khan WA, Khaliq A, Chughtai MFJ, Iqbal R, et al. In vitro probiotic potential and safety evaluation (hemolytic, cytotoxic activity) of *Bifidobacterium* strains isolated from raw camel milk. Microorganisms 2020;8:354.
36. De Keersmaecker SC, Verhoeven TL, Desair J, Marchal K, Vanderleyden J, Nagy I. Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium* is due to accumulation of lactic acid. FEMS Microbiol Lett 2006;259:89-96.
37. Xanthopoulos V, Litopoulou-Tzanetaki E, Tzanetakis N. Characterization of *Lactobacillus* isolates from infant faeces as dietary adjuncts. Food Microbiol 2000;17:205-15.
38. Montassier E, Valdes-Mas R, Batard E, Zmora N, Dori-Bachash M, Suez J, et al. Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. Nat Microbiol 2021;6:1043-54.
39. Khemariya P, Singh S, Nath G, Gulati AK. Isolation, identification and antibiotic susceptibility of nis^{+} *Lactococcus lactis* from dairy and non-dairy sources. Czech J Food Sci 2013;31: 323-31.
40. Deghorain M, Goffin P, Fontaine L, Mainardi JL, Daniel R, Errington J, et al. Selectivity for D-lactate incorporation into the peptidoglycan precursors of *Lactobacillus plantarum*: role of Aad, a VanX-like D-alanyl-D-alanine dipeptidase. J Bacteriol 2007;189: 4332-7.