Vol.9, No.2 pages 35-52 Food Agricultural Sciences and Technology (FAST) Research Article

Lactic acid bacteria profiles associated to Thai traditional fermented foods

Md Faridunnabi Nayem[1], Arin Buranasajja[1], Hongfah Boonsuksom[1], Noppasorn Suknuntee[1], Ailada Kongjui[2], Chanchai Boonla[2], Nattida Chotechuang[1*]

[1] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok, 10330, Thailand*

[2] *Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Patumwan, Bangkok, 10330, Thailand*

[*] *Corresponding author: nattida.c@chula.ac.th*

Received: 7th April 2023, **Revised**: 22nd May 2023, **Accept**: 26th May 2023

Abstract - Probiotics are health promoting gut microbiota which has been used in commercial products to extend its benefits for supporting the gut function and immunity. Besides these, probiotics contribute to reducing blood cholesterol, diabetics, coronary heart disease, and allergic symptoms as well as improving mood along with cognitive activities, reducing skin aging, and promoting longevity. Probiotics aid in promoting longevity as a function of antioxidant activity which allows it to be a potential source of supplement. These factors open an interesting prospect of screening well known probiotics community containing higher antioxidant activity from various Thai local fermented foods. This study aimed to screen targeted lactic acid bacteria (LAB) from 15 plant and animal based Thai local fermented foods by $CaCO_3$ containing MRS agar and analyzed its antioxidant and probiotic properties. It was found that all 11 isolates considered as potential LAB as it had shown clear zone on $CaCO_3$ containing MRS agar, exhibited over 70% inhibition of DPPH in both supernatant and pellet. But only one isolate from plant based fermented food showed potential probiotic properties, including acid-bile tolerance, hydrophobicity, and antimicrobial activity, and it was the one that had the highest antioxidant

Citation: Nayem, M.F. Buranasajja, A., Boonsuksom, H., Suknuntee, N., Kongjui, A. Boonla, C., & Chotechuang, N. (2023). Lactic acid bacteria profiles associated to thai traditional fermented foods. *Food Agricultural Sciences and Technology, 9*(2), 35-52.

activity. This isolated strain might be further analyzed and developed as a potential longevity promoting supplement.

Keywords: Probiotic, antioxidant activity, acid-bile tolerance, hydrophobicity, DPPH

1. Introduction

Fermentation is an ancient and economical method of processing and storing of food, that improves the shelf life of perishable fresh foods, maintains stability and safety of consumable foods, prevents microbial infestation, and enhances digestibility and mineral bioavailability (Nout, 2013; Tamang *et al.*, 2016; Terefe, 2016). The functional properties of fermented foods are dependent on the salt concentration during fermentation and fermentation process. The evident effect of salt in fermented food is to suppress the growth of pathogens and regeneration of health promoting bacteria (Park *et al.*, 2014). Additionally, these health-promoting microbes are considered as potential probiotics; some of them are scientifically proven health benefits include lowering the risk of type II diabetics, showing anti-aging and anti-obesity effect (Cui *et al.*, 2015; Kim *et al.*, 2011). These health benefits are mainly assisted by the metabolic activities of the microbes that are generated during fermentation (Das *et al.*, 2020).

The general meaning of probiotic is "good for life". Probiotics are defined according to WHO as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO, 2006). Probiotics are considered as helpful against cancer, different allergic effect, diabetics, coronary heart disease, aging, lactose intolerance etc. They are also effective in treating stress, anxiety, depression and stimulating mood and cognitive activities (Sivamaruthi *et al.*, 2019). Above 10^{14} CFU/ml microorganisms reside in human gastrointestinal tract, out of these *Lactobacilli and Bifidobacterium* are considered as conventional probiotics and have shown considerable antioxidant properties (Kim *et al.*, 2020).

Lactic acid bacteria (LAB) are one of the major sources of probiotics because of its availability in most consumable fermented foods. *Lactobacillus* spp is one of the most popular isolated genera of LAB where probiotics have been isolated. Besides this, other LABs are *Leuconostoc, Lactococcus, Pediococcus, Enterococcus, and Streptococcus* (Hill *et al.*, 2014). In the past decades, *Lactobacillus* spp. are the most widely appreciated and used probiotics which have been used for treating human health complications (Singh *et al.*, 2013). In general, *Lactobacillus* spp. are gram positive, catalase negative and non-spore forming (McCoy & Gilliland, 2007). This species had been isolated in past decades most extensively considering its enhancement of antioxidant, anti-aging, and longevity (Woo *et al.*, 2014). It was recorded that health promoting activities of probiotics had been found to be different in species to species, even different in same species because of difference in strains. *Lactobacillus helveticus* KLDS1.8701, *Lactobacillus fermentum* JX306, *Lactobacillus plantarum* FLPL05, *Lactobacillus mucosae* LMU1001, *Lactobacillus plantarum* LPL0902 & LPL0302 etc. are the potential candidates for enhancing of antioxidant activity in the animal model which was confirmed prior *in vitro* analysis of antioxidant properties

like DPPH, H_2O_2 resistance and reducing ability measurement (Li *et al.,* 2018; Zhang *et al.,* 2020; Yu *et al.,* 2016). On the other hand, strains like *Lactobacillus fermentum* MBC2 (Schifano *et al.,* 2019), *Lactobacillus fermentum* JDFM216 (Park *et al.,* 2017), *Lactobacillus fermentum* JX306 (Zhang *et al.,* 2020), *Lactobacillus rhamnosus* GG (ATCC 53103) (Yun *et al.,* 2022), *Lactobacillus fermentum* U21 (Marsova *et al.,* 2020), *Lactobacillus gasseri* SBT2055 (Nakagawa *et al.,* 2016) etc. have been used for prolonging life span, growth, and healing liver, kidney, brain, spleen damages by promoting antioxidant activity *in vivo*. Besides this *Lactobacillus plantarum* HY7714 delays skin aging (Lee *et al.,* 2015). As it was reported that, numerous strains of *Lactobacillus* spp. had been testified to possess antiaging effects possibly for their free radical scavenging activity and promoting effect of antioxidant enzymes (Zhao *et al.*, 2017). Most importantly food safety of LAB had been authorized as safe by conducting clinical trials (Saarela *et al.*, 2000). Worldwide mostly LAB had been isolated from dairy based products and available in the market as an ingredient of dairy based products. Nowadays, a few non-dairy based LAB rich products are available in the global market like juice of apple, tomato, pineapple, and orange with *Lactobacillus sanfranciscensis* (Zhu *et al.*, 2020); sohiong juice enriched with *Lactobacillus plantarum* (Vivek *et al.*, 2020) and juice of pomegranate with *Lactobacillus plantarum* ATCC 14917 (Mantzourani *et al.*, 2019). A few studies also reported the isolation of LAB from non-dairy products mustard (Lin *et al.*, 2021), dry fermented sausage (Mora *et al.*, 2015), and fermented vegetables (Xiao *et al.*, 2020).

From past studies, it is evident that fermented foods could be the major source of probiotics. So far, every literature has discussed isolation of LAB from single source and no comparison of LAB strains has been performed. Thailand has more variety of fermented foods across different provinces which could be a promising source of LAB. However, more than 10 different fermented food samples from different sources have not yet been explored and compared. Considering these factors, this study was designed to provide a summary of antioxidant activity and potential probiotic profiles of LAB isolated from different traditional fermented food sources to establish a potential source of target LAB probiotics with significant antioxidant activity.

2. Materials and methods

2.1 Sample collection

Fermented foods were categorized into two groups: plant and animal based fermented foods. These foods were collected from different provinces of Thailand from local market.

Table 1. Food sources and profiles of isolates characterization

Source	Fermented foods	Code	Salt concentration (%)	Province	Gram staining (No. of isolate)		$CaCO_3$ positive (No. of isolate)	DPPH inhibition (No. of isolate)		Probiotic property (No. of isolate)		
					Gram positive	Gram negative		Supernatant	Pellet	Hydrophobicity	Acid-bile tolerance	Antimicrobial activity
Plant	Phak kard dong A (Fermented green cabbage)	**NYC8**[1]	8.0-16.0	Nakornsawan	4	1	1	1	1	1	1	1
	Bai miang A (Fermented tea leaf)	**NYN4**[1]	0.0 -5.0	Nan	2	2	0	N/A	N/A	N/A	N/A	N/A
	Bai miang B (Fermented tea leaf)	**NYN5**[1]	0.0 -5.0	Nan	5	0	0	N/A	N/A	N/A	N/A	N/A
	Bai miang C (Fermented tea leaf)	**NYN6**[1]	0.0 -5.0	Nan	5	1	0	N/A	N/A	N/A	N/A	N/A
	Nam hed (Fermented Mushroom)	**NYNE1**[1]	1.0 - 1.5	Nakhon Ratchasima	3	1	0	N/A	N/A	N/A	N/A	N/A
	Phak kard dong B (Fermented green cabbage)	**NYNE7**[1]	8.0-16.0	Udon	6	2	0	N/A	N/A	N/A	N/A	N/A
	Nho mai dong (Fermented Bamboo)	**NYW3**[1]	8.0-16.0	Kanchanaburi	5	2	0	N/A	N/A	N/A	N/A	N/A
	Phak sian dong (Fermented wild spider flower)	**NYW4**[1]	8.0-16.0	Kanchanaburi	3	2	1	1	1	1	0	N/A

Table 1. Food sources and profiles of isolates characterization (cont.)

Source	Fermented foods	Code	Salt concentration (%)	Province	Gram staining (No. of isolate)		$CaCO_3$ positive (No. of isolate)	DPPH inhibition (No. of isolate)		Probiotic property (No. of isolate)		
					Gram positive	Gram negative		Supernatant	Pellet	Hydrophobicity	Acid-bile tolerance	Antimicrobial activity
Animal	Plasom A (Sour Fish) Banana leaf wrapper	**NYNE2**[2]	2.0-2.5	Udon	4	2	1	1	1	1	0	N/A
	Plasom B (Sour Fish) Plastic wrapper	**NYNE3**[2]	2.0-2.5	Udon	8	5	0	N/A	N/A	N/A	N/A	N/A
	Kung jom A (Fermented shrimp)	**NYNS1**[3]	5.0-10.0	Udon	2	3	2	2	2	0	0	N/A
	Kung jom B (Fermented shrimp)	**NYNS2**[3]	5.0-10.0	Udon	2	2	2	2	2	1	0	N/A
	Mum (Beef sausage)	**NYNE4**[4]	2.0-2.5	Udon	8	2	0	N/A	N/A	N/A	N/A	N/A
	Pla kem (Salted fish)	**NYW1**[5]	12.0-25.0	Kanchanaburi	8	2	1	1	1	1	N/A	N/A
	Pla ra (Salted fish)	**NYW2**[6]	12.0-25.0	Kanchanaburi	4	2	1	1	1	1	N/A	N/A

1. Voraputhaporn (2004).
2. Reungsang *et al.* (2006).
3. Hwanhlem *et al.* (2010).
4. Sumon (2009)
5. Runglerdkriangkrai *et al.* (2015)
6. Promchote (2017)

Table 1 represented the selected fermented foods (Phak kard dong, Bai miang, Nam hed, Nho mai dong, Phak sian dong, Plasom, Mum, Pla kem, Pla ra) and their collection sources. The collected foods were used initially for isolation of potential lactic acid bacteria and stored in refrigerator for short time at 4°C in case of necessity.

2.2 Isolation of lactic acid bacteria from Thai local fermented foods

Isolation of lactic acid bacteria was done considering Bautista-Gallego *et al.* (2013) method with some modifications. Briefly, one gram of the food samples was homogenized in 0.1% peptone by adjusting the volume to 10 ml and serially diluted with 0.1% peptone and then spread on MRS agar. Afterwards, the plates were incubated at 37^{0}C for 48 h. The colonies from extensive growth plates were picked by following Harrison-disc method (Harrigan & McCance, 1976). Then the gram staining of isolated bacteria was done considering Tripathi and Sapra (2020) method. The gram positive (+) rod shaped colonies were re-streaked on MRS agar containing 0.3% $CaCO_3$ for purification considering Wu *et al.* (2012) method. The colonies that created clear zone in the MRS agar plate were selected and stored in MRS broth containing 50% glycerol at -20^{0}C for further analysis.

2.3 Determination of *in vitro* antioxidant activity of isolated bacteria

2.3.1 Preparation of sample

Isolated bacterial samples were cultured in MRS broth at 37^{0}C for 18 h prior to further analysis. After incubation, intact cells and fermented supernatant were separated by centrifugation at 6000g for 10 min at 4^{0}C. Isotonic saline (0.9%) was used for washing the sample three times and sample was resuspended in equal volume of isotonic saline. The cell pellet concentration was adjusted to 10^8 CFU/ml.

2.3.2 DPPH free radical scavenging activity

Antioxidant activity was determined as mentioned in Tang *et al.* (2017). Briefly, 1 ml of fermented supernatant or intact cells was added to the 0.2 mM ethanolic DPPH of same volume. The solution was mixed thoroughly and kept in the dark environment for 30 min at ambient temperature. Control sample contained 1 ml of deionized water with same amount of ethanolic DPPH solution (0.2 mM). Blank group was the 1 ml of ethanol rather than DPPH solution. After incubation, the mixed solution was centrifuged for 10 min and the supernatant was measured the absorbance at 517 nm.

Scavenging inhibition activity

$$(\%) = [1-(A_{sample} - A_{blank}) / A_{control}] \times 100$$

2.4 Determination of potential probiotics

2.4.1 Determination of hydrophobicity

Hydrophobicity of isolates was analyzed as described in Khullar *et al.,* 2022 method. Initially, fresh culture at 10^7-10^8 CFU / ml was centrifuged at 6000g for 5 min to separate cell pellet. Cell pellet was allowed to wash twice by phosphate buffer (PBS) and resuspended it in the same buffer. The absorbance of aqueous phase

was measured at 600 nm. Equal volume of organic solvent (xylene) was added in the cell suspension and mixed vigorously for 2 mins. Cell suspension was incubated at laboratory temperature for 1 hour. After phase separation, the absorbance of aqueous phase was measured at 600 nm.

$$\text{Cell surface hydrophobicity (\%)} = (A_0 - A_1) / A_0 \times 100$$

A_0 = Absorption before mixing with hydrocarbons

A_1 = Absorption after mixing with hydrocarbons

2.4.2 Determination of in-vitro gastric acid and bile salt tolerance

Probiotics must have the potential to survive at gastrointestinal condition. Thus, probiotic properties of isolated bacterial samples were analysed under simulated gastrointestinal condition *in vitro* considering Gbassi *et al.* (2009) method with minor modifications.

One or two colonies of isolates were put in MRS broth and allowed it to incubate at 37°C for 24 hours. 1 ml of fresh culture was mixed with 20 ml PBS buffer and incubated at 37°C for 20 minutes in shaking incubator (100 rpm). After incubation, cell viability was counted by placing the samples on MRS plate by following spread plate method. In the same culture, 9 ml of simulated gastric fluid (SGF: mixing of NaCl 9 g/l and pepsin 3 g/l; adjusted pH at 1.8) was mixed properly and allowed to incubate at 37°C for 2 hours at shaking condition. Cell viability was checked every hour. After 2 hours, 9 ml of simulated intestinal fluid (SIF: mixing of NaCl 9 g/l, pancreatin 10 g/l, bile 3 g/l, and lipase 0.03 g/l; adjusted pH near 6.8) was added in the same culture, mixed vigorously, and allowed to incubate at 37°C for 3 hours at shaking condition. Cell viability was checked every hour.

2.4.3 Antimicrobial activity

The isolates that showed hydrophobicity and acid-bile tolerance were selected to analysis antimicrobial activity. Antimicrobial activity was performed by agar well diffusion method considering Khullar *et al.* (2022). At first, *Escherichia coli* ATCC25922 was spread on nutrient agar by cotton swap to create the lawn prior to making well. After drying of the plate, 100l of fresh MRS culture of isolates was dropped in three different wells and let it dry in lab temperature. After drying, all the plates are incubated at 37°C for 24 hours to see the clear zone. After incubation, the antimicrobial activity of the colonies was measured by measuring the diameter of clear zone in each plate.

2.5 Identification of lactic acid bacteria

Identification of *lactobacillus* spp. was initialized by DNA extraction considering Dashti *et al.* (2009) method with minor modifications. Briefly, two colonies of freshly incubated lactic acid bacteria was placed in test tube containing 200 µl of autoclaved distilled water and put it on freezer at -20° C. After removing from freezer, it was boiled at 100 ° C for 1 min in water bath. Then it was centrifuged at 1000 rpm for 5 mins and separated the supernatant for further analysis.

Identification of lactic acid bacteria was done as mentioned in Öz *et al.* (2017). Briefly, 30 µl of reaction mixture was prepared that was consisted of template DNA, Taq DNA polymerase, dNTPs, and

PCR buffer. Identification of isolates could be done by the selection of 16S rDNA coding region sequence and PCR amplification. Primers was used for amplification of 16S-rRNA gene are forward primer 27F (5'-AGA GTT TGA TCC TGGCTC AG-3') (Yi *et al.*, 2019) and reverse primer 1492R (5'-GGTTACCTT-GTTACGACTT-3') (Tajabadi *et al.,* 2012). PCR products were analyzed by agarose gel electrophoresis and scanned in gel documentation system. PCR amplifications were sequenced, and sequenced result was evaluated by Bioedit program and compared with GenBank database sequences.

2.6 Statistical analysis

All the experiments were performed three times and experimental data were analyzed by windows SPSS (version 28) software. All the data were expressed as mean ± Standard deviation. Duncan multiple range test ($p < 0.05$) was performed to determine significant differences among the samples.

3. Results and discussion

3.1 Isolation of lactic acid bacteria from Thai local fermented foods

Fermented foods were collected from different parts (central, north, north-east and west) of Thailand (Table 1). These collected foods from different provinces can be categorized into two groups: plant and animal-based food. These fermented foods can be further divided into lactic acid fermented foods and complex fermented foods (protein and cellulose hydrolysate); based on fermentation process takes place in it. Lactic acid fermentation naturally performed in Phak kard dong (fermented green cabbage), Phak sian dong (fermented wild spider flower), Nho mai dong (fermented bamboo), Plasom (sour fish); in contrast complex fermentation performed in Bai miang (fermented tea leaf), Kung jom (fermented shrimp), Mum (fermented beef sausage), Pla kem (salted fish) and Pla ra (salted fish). Complex fermented foods also contain a little amount LAB that aided aroma generation (Wu *et al.*, 2020) which made this food group a novel source of LAB probiotic.

In this study, preliminary screening was done by MRS agar which contains some compound that limited the growth of other bacteria except lactic acid bacteria. This screening method was designed to isolate facultative anaerobic bacteria to get simple cultivation strains. Consequently, screening was done by the inspection of cell morphology considering gram staining method. Only gram-positive isolate was selected for the next analysis. Maximum number of gram-positive isolates were found in animal based fermented foods that includes Plasom B, Pla kem A, Mum and followed by Plasom A, Pla ra, Kung jom A, and Kung jom B. Whereas in the plant- based fermented foods, maximum number of gram-positive isolates were found in Phak kard dong B and followed by Nho mai dong, Bai miang B, Bai miang C, Phak kard dong A, Phak sian dong, Nam hed, and Bai miang A as shown in Table-1. Most of the isolates from all fermented foods contained more than 50% of gram-positive rod shaped which was further streaked on MRS agar plate containing 0.3% $CaCO_3$ to screen the potential LAB. Colony generating lactic acid was tested by observing clear zone from the reaction of the acid and $CaCO_3$. (Wu *et al.,* 2012).

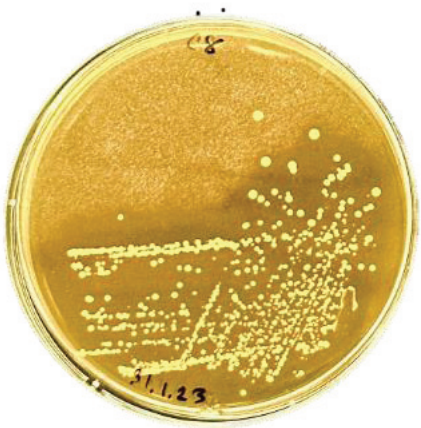

Figure 1. Growth of isolate on MRS agar containing 0.3% $CaCO_3$

The clear zone was observed (Figure 1) in nine isolate colonies (NYC8, NYNS 1 B, NYNS 1 S, NYW2, NYNS2B, NYNS2S, NYNE2B, NYW1Y, and NYW4) that could be specified as LAB isolates.

Mostly LAB in fermented foods including Phak kard dong, Phak sian dong, and Plasom etc imparts sour taste in the food. LAB growth and survival depends on the lactic acid fermentation in fermented foods which is affected by several factors (Hofvendahl & Hahn-Hägerdal, 2000). In the complex fermentations, some microbes, like *Bacillus spp,* yeast, and mold, could produce some enzymes such as cellulase which could retard the growth of LAB in those fermented ecosystems. In this study, LAB could be found in Plasom A (wrapped with banana leave) but not in Plasom B (wrapped with plastic film) as shown in (Table 1). This demonstrated that packaging could be a key factor associated with LAB growth, particularly, effect from their oxygen barrier properties. The anaerobic condition is expected to initiate the growth of LAB compared to aerobic condition. However, the rapid growth of LAB at anaerobic condition could cause faster pH drops and this low pH environment could limit the growth of LAB in that environment (Adamberg *et al.*, 2003) as observed in Plasom B wrapped in plastic film. Besides these factors, fermentation time could also have noticeable effect on the viable count of LAB in fermented foods. Undefined fermentation time could narrow the probability of LAB presence in LAB containing food (Li *et al.*, 2022).

In this study, daily consumable fermented foods were collected randomly from the local market. Some fermented foods (Bai miang, Plasom) in this study contained gram positive isolates but no clear zone was observed in any MRS agar containing 0.3% $CaCO_3$ which used for screening LAB. Thus, the number of LAB isolates obtained from these sources was lower than our expectation. This observation demonstrated that fermented food as a good source of potential probiotic, key factors such as fermentation stage, manufacturing process and storage condition should be criteria for selection.

3.2 In vitro antioxidant activity of isolated bacteria

DPPH inhibition capacity of the $CaCO_3$ positive isolates were further analyzed to screen the LAB that possessed antioxidant activity. After analysis of DPPH inhibition capacity, all isolates were found to show more than 70% antioxidant property (Figure 2).

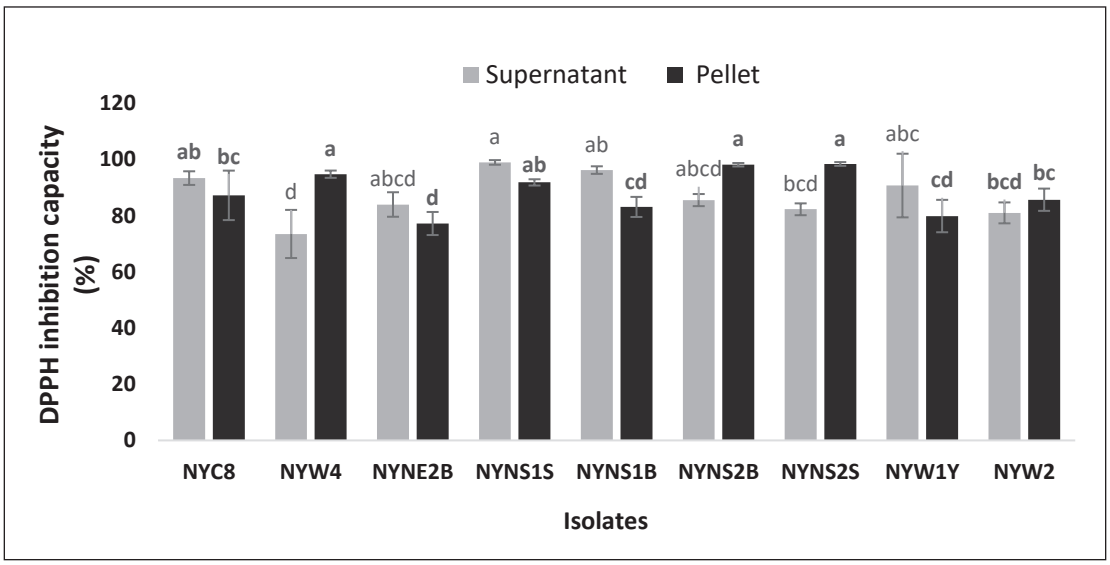

Figure 2. DPPH inhibition capacity of targeted isolates

Antioxidant activity of fermented supernatant was found higher about 99% in NYNS1S isolate followed by NYNS1B, NYC8, NYW1Y, NYNS2B, NYNWE2B, NYNS2S, NYW2 and NYW4 isolates. The antioxidant activity of fermented supernatant comes from the extracellular polysaccharides e.g., pyruvate which non-enzymatically scavenge some sort of free radicles (Hofvendahl, & Hahn-Hägerdal, 2000). On the other hand, NYNS2B isolate showed highest antioxidant activity in pellet followed by NYNS2S, NYNS1S, NYW4, NYC8, NYNS1B, NYW2, NYW1Y, and NYNE2B. In the pellet, antioxidant activity is generated by many enzymatic and non-enzymatic factors. Non enzymatic antioxidant activity initiates by the presence of Mn^{2+}, -SH group of amino acid side chain (Horsburgh *et al.*, 2002) and the surface-active compounds that includes polysaccharides, protein, and lipoteichoic acid (Li *et al.*, 2012; Yi *et al.*, 2009). Whereas superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) enzymes act as the main major enzymatic defense system which contributes antioxidant activity. Overall antioxidant activity was found higher in animal based fermented foods compared to the plant based fermented foods. In general, animal-based foods are composed of high amount of lipids which undergoes peroxidation and generates cytotoxic compounds that include hydroperoxides, hydroxy alkanals malondialdehyde etc. (Kanner & Lapidot, 2001). The screened isolates showed an antioxidant defense system to survive the cytotoxic environment which elevated its antioxidant property.

This antioxidant property of isolates from Thai fermented food in this study showed relatively higher antioxidant property compared to those in previous studies (Tang *et al.*, 2017; Li *et al.*, 2018; Liu & Pan., 2010). This could be due to several factors including manufacturing processes such as salt content, antimicrobial activity from raw materials and fermented temperature. These factors could cause stress conditions which affected the bacterial strains to adapt and express the defense mechanism, for example increasing the cell wall thickness and exopolysaccharide to protect the bacterial cell from stress environments. Particularly, there are some reports which demonstrated that temperature could be a key factor associated with this defense mechanism. The higher temperature could cause stress and minimize the growth of LAB but stimulate the generation of exopolysaccharide to neutralize the stress by different mechanisms (Ortega-Morales *et al.,* 2001; Hill *et al.,* 1994; Robijn *et al.,* 1995). The generation of exopolysaccharide in LAB contributed to antioxidant activity (Nguyen *et al.,* 2020). Thus, based on this information, fermented foods of Thailand that located in the tropical zone as temperatures ranging in 30°-37°C, could be a potential source of LAB with antioxidant activity.

3.3 Potential probiotic properties

Probiotics must pass through the digestion tract prior providing health benefits to their host. Acidic condition of stomach provides a natural barrier to screen out pathogenic microorganisms but not probiotics. After reaching the intestine, probiotic starts accumulating to the epithelial cell of intestine and stimulates immunity system and most importantly inhibits the growth of harmful bacteria (Nikoskelainen *et al.*, 2003). Probiotics maintain sound health of gastro-intestinal (GI) tract by stimulating gut microbiota with synthesis and releasing antimicrobial compounds. These functioning manners of probiotics are interlinked with the health promoting effects (Parvez *et al.*, 2006). Thus, analysis of probiotic properties of the isolates were initialized to establish a potential candidate of probiotics.

Hydrophobicity is considered as one of the major probiotic properties which reflects the adhesion of probiotic candidates with the epithelial cell wall of intestinal mucosa. This adhesion is necessary to exhibit health promoting effects and stimulation of immune systems. Potential probiotic strain must have hydrophobicity of 40% to maintain adhesion and interaction with the epithelial cell wall of intestinal mucosa (Del Re *et al.,* 2000). Conventionally, cell surface hydrophobicity can be analyzed using xylene as an organic solvent as it is independent of electrostatic interaction (Martins *et al.*, 2009). From this study, maximum hydrophobicity was observed in NYC8 followed by NYNS2B, NYW2, NYW4, NYNE2B, and NYW1Y respectively as shown in Table 2.

Table 2. Potential probiotic properties of targeted isolates

Fermented Foods	Isolates	Hydrophobicity (%)	Acid tolerance (CFU/ml)	Bile tolerance (CFU/ml)	Antimicrobial activity (Diameter, m)
Phak kard dong A (Fermented green cabbage)	NYC 8	92.56	2.49×10^{5}	1.84×10^{4}	2.12×10^{-2}
Kung jom A (Fermented shrimp)	NYNS1S	-79.08	-	-	N/A
Kung jom A (Fermented shrimp)	NYNS1B	-43.34	-	-	N/A
Kung jom B (Fermented shrimp)	NYNS2B	48.83	-	-	N/A
Kung jom B (Fermented shrimp)	NYNS2S	-52.33	-	-	N/A
Plasom A (Sour Fish) Banana leaf wrapper	NYNE2B	4.50	-	-	N/A
Pla kem A (Salted fish)	NYW1Y	1.61	-	-	N/A
Pla ra (Salted fish)	NYW2	31.85	-	-	N/A
Phak sian dong (wild spider flower)	NYW4	24.04	-	-	N/A

The hydrophobicity of NYC8 and NYNS2B strains were 92.56% and 48.83% which exceeded the least requirement of potential probiotic (40%). Results of NYC8 and NYNS2B reflected better adhesion with the mucosa cell wall and survivability in the gastrointestinal tract (Kos *et al.,* 2003). Hydrophobicity of LAB comes from their surface properties that is contributed by (lipo-)teichoic acids, polysaccharide interaction with protein and. S-layer protein (Deepika *et al.*, 2009).

After hydrophobicity analysis, all the nine isolates were mixed with PBS before introducing acid-bile solution to reduce the cell deformation, homogenous mixing and maintaining the pH. It was found that only NYC8 isolate out of nine isolates showed the resistance in both simulated gastric and intestine environment. Initially the concentration of NYC8 was 2.45×10^{6} CFU/ml (Table 2) which was reduced to 2.49×10^{5} after two hours of gastric environment and after three hours of bile treatment, it came down to 1.84×10^{4} CFU/ml. Bile tolerance of NYC8 isolate could be attributed to bile tolerant protein synthesis adaptation over the time against oxidative damage (Ruiz

et al., 2013; Russo *et al.*, 2012). The overall viability of NYC8 isolate was observed more than 50 % which made it potential candidates of probiotics.

Antimicrobial activity of probiotic is another essential probiotic property which reflects the potentiality of a probiotic bacteria to inhibit the growth of deleterious pathogenic bacteria in the intestine. In this study, antimicrobial property was initially screened by testing on *E. coli ATCC25922*. Only NYC8 showed inhibitory action on *E. coli* by creating clear zone of 2.12×10^{-2} m on MRS agar plate. Since the inhibition capacity of LAB is associated with the generation of antimicrobial substances that includes bacteriocins, organic acid, and hydrogen peroxide (Fei *et al.,* 2018; Servin, 2004), the inhibitory activity of this LAB isolates is required further investigation.

In the present study, NYC8 showed clear zone on MRS agar plate, antioxidant activity and all the primary potential probiotic properties. Therefore, this targeted isolate was further identified through sequencing analysis and its identity was found as *Lactobacillus spp*. This was only potential strain could be further developed to be probiotic strain which had antioxidant activity. Interestingly, the strain was obtained from plant-based fermented food (Phak-kard Dong A), some compositions and conditions of this fermented food associated to viability/availability of this potential stain and its property should be further investigated.

4. Conclusion

According to the information obtained in this study, Thai traditional fermented foods could be the source of LAB with significant antioxidant activity which could be developed as potential probiotic stains. This observation also demonstrated fermented food as a good source of potential probiotic, key factors such as fermentation stage, manufacturing process and storage condition should be criteria for source of isolate selection.

The isolate NYC8 could be the potential probiotic candidate which had antioxidant activity; therefore, this isolate could be further developed as probiotic strain to promote function of gastrointestinal tract and health benefits. Further study *in vivo* should be investigated to understand the antioxidant mechanism in the living organism. The enzymatic defense system such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) should also be elucidated.

5. Acknowledgement

The authors are grateful to The Development of Food and Food Additive from Innovative Microbial Fermentation Research Group, Chulalongkorn University in providing funds for this research.

6. References

Adamberg, K., Kask, S., Laht, T. M., & Paalme, T. (2003). The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. *International journal of food microbiology*, *85*(1-2), 171-183.

Bautista-Gallego, J., Arroyo-López, F. N., Rantsiou, K., Jiménez-Díaz, R., Garrido-Fernández, A., & Cocolin, L. (2013). Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. *Food Research International, 50*(1), 135-142.

Cui, M., Kim, H. Y., Lee, K. H., Jeong, J. K., Hwang, J. H., Yeo, K. Y., Ryu, B. H., Choi, J. H., & Park, K. Y. (2015). Antiobesity effects of kimchi in diet-induced obese mice. *Journal of Ethnic Foods*, *2*(3), 137-144.

Das, G., Paramithiotis, S., Sundaram Sivamaruthi, B., Wijaya, C. H., Suharta, S., Sanlier, N., Shin, H. S., & Patra, J. K. (2020). Traditional fermented foods with anti-aging effect: A concentric review. *Food Research International*, *134*,109269.

Deepika, G., Green, R. J., Frazier, R. A., & Charalampopoulos, D. (2009). Effect of growth time on the surface and adhesion properties of Lactobacillus rhamnosus GG. *Journal of Applied Microbiology*, *107*(4), 1230-1240.

Del Re, B., Sgorbati, B., Miglioli, M., & Palenzona, D. (2000). Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in Applied Microbiology, 31*(6), 438-442.

Fei, Y., Li, L., Zheng, Y., Liu, D., Zhou, Q., & Fu, L. (2018). Characterization of *Lactobacillus amylolyticus* L6 as potential probiotics based on genome sequence and corresponding phenotypes. *LWT, 90*, 460-468.

FAO UN & WHO. (2006). Probiotics in food: Health and nutritional properties and guidelines for evaluation. *FAO Food and Nutrition Paper, 85*.

Harrigan, W. F., & McCance, M. E. (1976). *Laboratory methods in food and dairy microbiology*. Academic Press Inc.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). The international scientific association for probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*(8), 506-514.

Hofvendahl, K., & Hahn-Hägerdal, B. (2000). Factors affecting the fermentative lactic acid production from renewable resources1. *Enzyme and Microbial Technology*, *26* (2-4), 87-107.

Horsburgh, M. J., Wharton, S. J., Karavolos, M., & Foster, S. J. (2002). Manganese: Elemental defence for a life with oxygen. *Trends in Microbiology*, *10*(11), 496-501.

Ho, S. T., Hsieh, Y. T., Wang, S. Y., & Chen, M. J. (2019). Improving effect of a probiotic mixture on memory and learning abilities in d-galactose-treated aging mice. *Journal of Dairy Science*, *102*(3), 1901-1909.

Hwanhlem, N., Watthanasakphuban, N., Riebroy, S., Benjakul, S., H-Kittikun, A., & Maneerat, S. (2010). Probiotic lactic acid bacteria from Kung-Som: isolation, screening, inhibition of pathogenic bacteria. *International Journal of Food Science & Technology*, *45*(3), 594-601.

Kanner, J., & Lapidot, T. (2001). The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biology and Medicine*, *31*(11), 1388-1395.

Kim, H., Kim, J. S., Kim, Y., Jeong, Y., Kim, J. E., Paek, N. S., & Kang, C. H. (2020). Antioxidant and probiotic properties of *Lactobacilli* and *Bifidobacteria* of human origins. *Biotechnology and Bioprocess Engineering*, *25*(3), 421-430.

Kim, B., Park, K. Y., Kim, H. Y., Ahn, S. C., & Cho, E. J. (2011). Anti-aging effects and mechanisms of kimchi during fermentation under stress-induced premature senescence cellular system. Food Science and Biotechnologre senescence cellular system. *Food Science and Biotechnology, 20*(3), 643-649.

Khullar, G., Det-udom, R., Prombutar, P., & Prakitchaiwattana, C. (2022). Probiogenomic analysis and safety assessment of Bacillus isolates using Omics approach in combination with In-vitro. *LWT*, *159*, 113216.

Lee, D. E., Huh, C. S., Ra, J., Choi, I. D., Jeong, J. W., Kim, S. H., ... & Ahn, Y. T. (2015). Clinical evidence of effects of Lactobacillus plantarum HY7714 on skin aging: a randomized, double blind, placebo-controlled study. *Journal of Microbiology and Biotechnology*, *25*(12), 2160-2168.

Li, B., Evivie, S. E., Lu, J., Jiao, Y., Wang, C., Li, Z., ... & Huo, G. (2018). Lactobacillus helveticus KLDS1. 8701 alleviates d-galactose-induced aging by regulating Nrf-2 and gut microbiota in mice. *Food & function*, *9*(12), 6586-6598.

Li, M., He, Z., He, L., Li, C., Tao, H., Ye, C., Liu, L., Zeng, X., & Ran, G. (2022). Effect of fermentation parameters on the anthocyanin content, sensory properties, and physicochemical parameters of potato blueberry yogurt. *Fermentation*, *8*(10), 489.

Li, S. Y., Zhao, Y. J., Zhang, L., Zhang, X., Huang, L., Li, D., Niu, C., Yang, Z., & Wang, Q. (2012). Antioxidant activity of lactobacillus plantarum strains isolated from traditional Chinese fermented foods. *Food Chemistry, 135*(3), 1914-1919.

Liu, C. F., & Pan, T. M. (2010). In vitro effects of lactic acid bacteria on cancer cell viability and antioxidant activity. *Journal of Food and Drug Analysis*, *18*(2), 8.

Lin, S. W., Tsai, Y. S., Chen, Y. L., Wang, M. F., Chen, C. C., Lin, W. H., & Fang, T. J. (2021). *Lactobacillus plantarum* GKM3 Promotes Longevity, Memory Retention, and Reduces Brain Oxidation Stress in SAMP8 Mice. *Nutrients*, *13*(8), 2860.

Mantzourani, I., Terpou, A., Alexopoulos, A., Kimbaris, A., Bezirtzoglou, E., Koutinas, A. A., & Plessas, S. (2019). Production of a potentially synbiotic pomegranate beverage by fermentation with *Lactobacillus plantarum* ATCC 14917 adsorbed on a prebiotic carrier. *Applied Biochemistry and Biotechnology*, *188*(4), 1096-1107.

Marsova, M., Poluektova, E., Odorskaya, M., Ambaryan, A., Revishchin, A., Pavlova, G., & Danilenko, V. (2020). Protective effects of Lactobacillus fermentum U-21 against paraquat-induced oxidative stress in Caenorhabditis elegans and mouse models. *World Journal of Microbiology and Biotechnology*, *36*, 1-10.

Martins, F. S., Silva, A. A., Vieira, A. T., Barbosa, F. H., Arantes, R. M., Teixeira, M. M., & Nicoli, J. R. (2009). Comparative study of Bifidobacterium animalis, Escherichia coli, Lactobacillus casei and Saccharomyces boulardii probiotic properties. *Archives of Microbiology*, *191*, 623-630.

McCoy, S., & Gilliland, S. E. (2007). Isolation and characterization of Lactobacillus species having potential for use as probiotic cultures for dogs. *Journal of Food Science*, *72*(3), 94-97.

Mora, L., Escudero, E., Aristoy, M. C., & Toldrá, F. (2015). A peptidomic approach to study the contribution of added casein proteins to the peptide profile in Spanish dry-fermented sausages. *International Journal of Food Microbiology*, *212*, 41-48.

Nakagawa, H., Shiozaki, T., Kobatake, E., Hosoya, T., Moriya, T., Sakai, F., & Miyazaki, T. (2016). Effects and mechanisms of prolongevity induced by Lactobacillus gasseri SBT2055 in Caenorhabditis elegans. *Aging Cell*, *15*(2), 227-236.

Nguyen, P. T., Nguyen, T. T., Bui, D. C., Hong, P. T., Hoang, Q. K., & Nguyen, H. T. (2020). Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications. *AIMS Microbiology*, *6*(4), 451-469.

Nikoskelainen, S., Ouwehand, A. C., Bylund, G., Salminen, S., & Lilius, E. M. (2003). Immune enhancement in rainbow trout (Oncorhynchus mykiss) by potential probiotic bacteria (Lactobacillus rhamnosus). *Fish & Shellfish Immunology*, *15*(5), 443-452.

Park, M. R., Ryu, S., Maburutse, B. E., Oh, N. S., Kim, S. H., Oh, S., ... & Kim, Y. (2018). Probiotic Lactobacillus fermentum strain JDFM216 stimulates the longevity and immune response of Caenorhabditis elegans through a nuclear hormone receptor. *Scientific Reports*, *8*(1), 7441.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171-1185.

Promchote, P.T. (2017). Chemical compositions and antioxidant properties of Pla-ra Thai indigenous fermented fish product. *Journal of Science and Technology Ubon Ratchathani University*, *19*(2), 159-172.

Reungsang, A., Tungwongchai R. & Chaiyachet, O. (2006). Quality indices of Plaa-som produced in the Northeastern part of Thailand. *KU Science Journal*, *24* (1-3), 20-36.

Ruiz, L., Margolles, A., & S´anchez, B. (2013). Bile resistance mechanisms in Lactobacillus and Bifidobacterium. *Frontiers in Microbiology, 4*, 396.

Runglerdkriangkrai, J., Hinsui, J. Maneerote, J. (2015). *Fishery product science and technology*. Kasetsart University Press.

Russo, P., de la Luz Mohedano, M., Capozzi, V., de Palencia, P. F., L´opez, P., Spano, G., & Fiocco, D. (2012). Comparative proteomic analysis of Lactobacillus plantarum WCFS1 and ΔctsR mutant strains under physiological and heat stress conditions. *International Journal of Molecular Sciences, 13*(9), 10680-10696.

Schifano, E., Zinno, P., Guantario, B., Roselli, M., Marcoccia, S., Devirgiliis, C., & Uccelletti, D. (2019). The foodborne strain Lactobacillus fermentum MBC2 triggers pept-1-dependent pro-longevity effects in Caenorhabditis elegans. *Microorganisms*, *7*(2), 45.

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews, 28*(4), 405-440.

Singh, V. P., Sharma, J., Babu, S., Rizwanulla, & Singla, A. (2013). Role of probiotics in health and disease: a review. *JPMA. The Journal of the Pakistan Medical Association*, *63*(2), 253-257.

Sivamaruthi, B. S., Prasanth, M. I., Kesika, P., & Chaiyasut, C. (2019). Probiotics in human mental health and diseases-A minireview. *Tropical Journal of Pharmaceutical Research*, *18*(4), 889-895.

Sumon, V. (2009). Mum (Beef): Process of meat product. *Kasetsart Livestock Magazine, 35* (139), 69-70.

Tang, W., Xing, Z., Li, C., Wang, J., & Wang, Y. (2017). Molecular mechanisms and in vitro antioxidant effects of Lactobacillus plantarum MA2. *Food Chemistry*, *221*, 1642-1649.

Tripathi, N., & Sapra, A. (2020). *Gram staining*. StatPearls Publishing.

van Niel, E. W., Hofvendahl, K., & Hahn-Hägerdal, B. (2002). Formation and conversion of oxygen metabolites by Lactococcus lactis subsp. lactis ATCC 19435 under different growth conditions. *Applied and Environmental Microbiology*, *68*(9), 4350-4356.

Vivek, K., Mishra, S., & Pradhan, R. C. (2020). Characterization of spray dried probiotic Sohiong fruit powder with *Lactobacillus plantarum*. *LWT*, *117*, 108699.

Voraputhapor, W. (2004). Process of vegetables and fruits products. *J. Office of Academic Service, Khon Kaen University, 12* (2).

Woo, J. Y., W. Gu, K. A. Kim, S. E. Jang, M. J. Han, & D. H. Kim. (2014). *Lactobacillus pentosus var. plantarum* C29 ameliorates memory impairment and inflammaging in a D-galactose-induced accelerated aging mouse model. *Anaerobe, 27*, 22-26.

Wu, C., Li, T., Qi, J., Jiang, T., Xu, H., & Lei, H. (2020). Effects of lactic acid fermentation-based biotransformation on phenolic profiles, antioxidant capacity and flavor volatiles of apple juice. *LWT*, *122*, 109064.

Wu, Y. Y., Liu, F. J., Li, L. H., Yang, X. Q., Deng, J. C., & Chen, S. J. (2012). Isolation and identification of nitrite-degrading lactic acid bacteria from salted fish. *Advanced Materials Research, 393*, 828-834.

Xiao, M., Huang, T., Huang, C., Hardie, J., Peng, Z., Xie, M., & Xiong, T. (2020). The microbial communities and flavour compounds of Jiangxi yancai, Sichuan paocai and Dongbei suancai: Three major types of traditional Chinese fermented vegetables. *LWT*, *121*, 108865.

Yi, Z. J., Fu, Y. R., Li, F. M., Gao, K. S., & Zhang, X. G. (2009). Effect of LTA isolated from bifidobacteria on D-galactose-induced aging. *Experimental Gerontology*, *44*(12),760-765.

Yu, X., Li, S., Yang, D., Qiu, L., Wu, Y., Wang, D., & Wei, H. (2016). A novel strain of Lactobacillus mucosae isolated from a Gaotian villager improves in vitro and in vivo antioxidant as well as biological properties in d-galactose-induced aging mice. *Journal of Dairy Science*, *99*(2), 903-914.

Yun, B., Ryu, S., Kang, M., Lee, J., Yoo, J., Kim, Y., & Oh, S. (2022). Probiotic Lacticaseibacillus rhamnosus GG increased longevity and resistance against foodborne pathogens in Caenorhabditis elegans by regulating microRNA miR-34. *Frontiers in Cellular and Infection Microbiology*, *11*, 1404.

Zhao, J., Tian, F., Zhao, N., Zhai, Q., Zhang, H., & Chen, W. (2017). Effects of probiotics on d-galactose-induced oxidative stress in plasma: A meta-analysis of animal models. *Journal of Functional Foods*, *39*, 44-49.

Zhang, D. I., Li, C., Shi, R., Zhao, F., & Yang, Z. (2020). JX306 Restrain D-galactose-induced Oxidative Stress of Mice through its Antioxidant Activity. *Polish Journal of Microbiology*, *69*(2), 205-215.

Zhu, W., Lyu, F., Naumovski, N., Ajlouni, S., & Ranadheera, C. S. (2020). Functional efficacy of probiotic Lactobacillus sanfranciscensis in apple, orange and tomato juices with special reference to storage stability and in vitro gastrointestinal survival. *Beverages*, *6*(1), 13.