Screening and identification of bacteriocin-producing lactic acid bacteria from Thai fermented meat products

Watcharacha Krongkeha[1*]

Abstract

Bacteriocins are peptides that inhibit food pathogens and are produced by both gram-positive and gram-negative bacteria. The objective of this study was to screen, isolate, and identify bacteriocin-producing lactic acid bacteria (LAB) from Thai fermented meat products. A total of 91 isolates were obtained and screened for bacteriocin production. Eight isolates exhibited remarkably wide zones of inhibition based on the agar well diffusion technique against foodborne pathogens, including *Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus*. These isolates are gram-positive bacteria and rod-shaped, except for NF-11, which is round-shaped. The biocatalytic activity of these eight isolates was sensitive to proteolytic enzymes. The results of nucleotide sequences of the 16S rRNA genes revealed that PS-5, PS-7, PS-8 and PJ-5 isolated from Pla-som and Pla-jom were identified as *Lactiplantibacillus pentosus*. MU-9, SK-1 and SK-6 isolated from Mum-moo and Sai-krok were identified as *Lactiplantibacillus plantarum*, while NF-11 isolated from Nham-pla was identified as *Pediococcus pentosaceus*. These selected LAB have the potential to be developed as starter cultures in the fermented food industry.

Keywords: lactic acid bacteria, bacteriocin, fermented meat product, foodborne pathogen

[1] Faculty of Science and Technology, Rajamangala University of Technology Rattanakosin

[*] Correspondence author. E-mail: watcharacha.kro@rmutr.ac.th

Received: February 7, 2024; Revised: June 18, 2024; Accepted: July 5, 2024

Introduction

Nowadays, the food industry is highly concerned with producing safe food for consumers by avoiding the addition of residual and toxic chemicals. Instead, they utilize inhibitors produced by beneficial microorganisms. Thai fermented food products, particularly meat-type fermented foods like fermented pork, sausages, fermented fish, and sour fish, are highly popular among consumers. In the past, it was discovered that the fermentation process of these foods occurred naturally through lactic acid bacteria (LAB) presented on the raw materials. Subsequently, further research focused on studying and selecting potential LAB strains as starter cultures for the production of fermented food products in Thailand (Tangwatcharin, Nithisantawakhup, & Sorapukdee, 2020; Loyda, Wichaporn, Jaranrattanasri, Tochampa, & Singanusong, 2023).

LAB are gram-positive, nonspore-forming, catalase-negative, facultative and fastidious bacteria. LAB possess generally regarded as safe (GRAS) and qualified presumption of safety (QPS) status (Barcenilla, Ducic, López, Prieto, & Álvarez-Ordónez, 2022). LAB play a crucial role in fermentation resulting in texture and flavor improvement as well as the preservation and shelf life extension of food products. Several strains have been developed as probiotic cultures in the fermented food industry. These cultures not only enhance the nutritional value of food products but also contribute to their pleasant flavor and texture. Moreover, LAB play a vital role in inhibiting spoilage and food-borne pathogenic bacteria by secreting inhibitory compounds such as diacetyl, organic acids, bacteriocins and bacteriocin-like inhibitory substances (Mei, Ma, & Xie, 2019; Kaveh, Hashemi, Abedi, Amiri, & Conte, 2023).

Bacteriocins are defined as antimicrobial peptides or proteins synthesized by bacterial ribosomes, with bacteriostatic or bactericidal effects against closely related bacterial strains of both gram-negative and gram-positive bacteria. Several bacteriocin-producing LAB have been reported including strains from the genera *Lactiplantibacillus* (formerly *Lactobacillus*), *Lactococcus, Enterococcus,* and *Pediococcus* but LAB used in food fermentation are from the *Lactobacillus* and *Bifidobacterium* genera (Mokoena, Omatola, & Olaniran, 2021). It is also well-known that bacteriocins produced by LAB are able to inhibit the growth of foodborne pathogens, such as *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* (Xia et al., 2023; Xin et al., 2023). Currently, the commercial bacteriocins used in the food industry include nisin, pediocin and enterocin (Darbandi et al., 2022). Nonetheless, discovering novel bacteriocins remains a challenge for scientists worldwide. It will be possible to employ them as food preservatives on a greater scale if they are discovered.

Thai fermented food has a long history of preservation. Different strains of LAB are used in the fermentation process for each type of Thai food. This results in the possibility of obtaining strains that have the potential to secrete different types of bacteriocins (Woraprayote et al., 2023). However, due to drug resistance identified in some pathogenic bacteria and their limited effectiveness against particular strains, the use of commercial bacteriocins in the food sector is still restricted. Thus, the objectives of this study were to isolate and identify bacteriocin-producing LAB from Thai fermented meat products. These isolated LAB could be considered for selection as starter cultures, enhancing the quality and safety of fermented foods in the food industry.

Methodology

Isolation of LAB capable of producing bacteriocin

Bacteriocin-producing LAB in fermented food product samples were isolated by the spread plate technique using De Man, Rogosa and Sharpe (MRS) agar containing 0.50% calcium carbonate ($CaCO_3$) (Krongkeha, 2022). Briefly, twenty-five grams of the samples were mixed thoroughly with 225 mL of 0.85% sodium chloride (NaCl) solution and homogenized using a stomacher (BagMixer 400, Interscience, Saint-Nom-la-Bretèche, France). Homogenates were diluted serially and spread onto MRS agar medium containing 0.50% calcium carbonate. The plates were then incubated under anaerobic condition at 35 °C for 48 h in an anaerobic jar. Colonies with clear zones were randomly selected due to the production of organic acids, which solubilized $CaCO_3$. The selected colonies were then stored in 20% glycerol at -20 °C for further study.

Screening of antibacterial agent-producing LAB

LAB capable of producing antibacterial agents were selected using the agar well diffusion method. The bacterial cultures were grown in MRS broth and incubated under anaerobic condition at 35 °C for 48 h. The bacterial cells were centrifuged at 12,000 rpm for 10 min. The cell-free supernatant (CFS) was neutralized by adjusting to pH 7.0 with 1 M NaOH and then filtered through a 0.45-micrometer membrane filter. The agar well diffusion technique was performed as described by Ohmomo et al. (1998) with some modifications. The overnight cultures of foodborne pathogens including *Listeria monocytogenes* TISTR 1327, *Escherichia coli* TISTR 887, and *Staphylococcus aureus* TISTR 517 obtained from stock cultures of Thailand Institute of Scientific and Technological Research (TISTR) were inoculated into 100 ml of TSA soft agar (0.75% agar) to the final concentration of 10^7 CFU/ml. Then, 15 ml of the mixture was dispensed into a sterile Petri dish. The plate was then allowed to solidify at room temperature for 30 min. Agar wells were punched by using the sterilized 6 mm cork borer. Then, 70 µl of CFS was added into each well in duplicate and incubated at 37 °C for 24 h. The

diameters of the inhibition zone were measured, with streptomycin at the concentration of 10 µl/ml used as a positive control (Todorov et al., 2010).

Biocatalytic properties of bacteriocin produced by LAB

The effect of various enzymes on bacteriocin was tested by treating cell-free supernatant (CFS) with proteolytic and other types of enzymes including catalase, lipase, trypsin, chymotrypsin, and proteinase K (Sigma, St. Louis, USA) at the final concentration of 1 mg/ml. All samples were adjusted to pH 7 except that treated with pepsin, which was adjusted to pH 3 and filtered through a membrane filter (0.45 µm). After 3 h of incubation, enzyme activity was stopped by boiling for 5 min. Then the sample was subjected to test biocatalytic activity against *L. monocytogenes* by agar well diffusion method (Pilasombut et al., 2005; Hwanhlem, Chobert, & H-Kittikun, 2014). The activity was represented in arbitrary units per milliliter (AU/ml) which was calculated as the ratio of the area of inhibition to the volume of CFS added to each well (H-Kittikun et al., 2015)

Identification of LAB strains by 16S rRNA gene sequencing.

Genomic DNA of selected isolates was extracted using the method of Satomi, Kimura, Mizoi, Sato, & Fujii (1997). The selected LAB were cultured in MRS broth and incubated under anaerobic condition at 35 °C for 18 h. Bacterial cells were harvested by centrifugation at 10,000 rpm for 15 min at 4 °C. Polymerase chain reaction (PCR) was performed using primers 27F and 1492R, resulting in a PCR product approximately 1,500 bp in size. The nucleotide sequencing of the 16S rRNA gene was determined using the method of Weisburg, Barns, Pelletier, & Lane (1991). The nucleotide sequence was compared using local alignment search of the GenBank database using the BLAST (Basic Local Alignment Search Tool) version 2.15.0 program of the National Center for Biotechnological Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The alignment of nucleotide sequences was checked using BioEdit version 7.2.5 program (Hall, 1999). Phylogenetic tree was created using the neighbor-joining method with software MEGA version 7 (Kumar, Stecher, & Tamura, 2016). The confidence intervals were estimated by a bootstrap value of 1,000 replicates. Nucleotide sequences of selected LAB were deposited in GenBank.

Statistical Analysis

All experiments were carried out in triplicates. The data were subjected to one-way analysis of variance (ANOVA) with Tukey's post hoc analysis using SPSS version 17.0 from IBM differences were reported at a significance level of $p<0.05$.

Results and discussion

Isolation of LAB capable of producing bacteriocin

Total LAB counts of fermented food products were in the range of 7.10-8.92 log CFU/ml (Table 1). A total of 91 isolates of LAB were randomly selected by the appearance of colonies with clear zones grown on MRS agar containing calcium carbonate (0.5%). The clear zone formed from lactic acid produced by LAB reacting with calcium carbonate in the culture medium. Therefore, it can be used as an indicator for selecting LAB (Onda, Yanagida, Tsuji, Ogino, & Shinohara, 1999). All isolates were found to be gram-positive bacteria. They showed no catalase and oxidase activity, indicating that the isolated bacteria were tentatively classified as LAB (Table 2). These results are compatible with previous reports. Hwanhlem et al. (2011) found that the numbers of LAB in Pla-som was about 7.70 log CFU/g. In addition, Nanasombat, Phunpruch, & Jaichalad (2012) reported that Pla-jom contained high numbers of LAB (7 log CFU/ml). However, the total LAB counts of Nham-moo was about 5 log CFU/g (Pakwan et al., 2020), which differed from the present study, suggesting a product-specific microflora.

Table 1 Total LAB counts of samples.

Thai fermented food		total LAB counts (log CFU/g)
Thai name	type of meat and main ingredient	
Pla-som	fish: salt: cooked rice: garlic	7.93
Pla-jom	fish: salt: roasted ground rice: garlic	7.10
Sai-krok	minced pork: salt: cooked rice: garlic	8.32
Nham-moo	lean minced pork: salt: cooked rice: garlic	8.37
Nham-pla	lean minced fish: salt: cooked rice: garlic	8.92
Mum-moo	minced pork and liver: salt: roasted rice: garlic	7.26

Table 2 Number of LAB isolates and their indicating physiological characteristics.

samples	number of isolate	isolate code	clear zone	gram stain	oxidase	catalase
Pla-som	20	PS1-20	+	+	-	-
Pla-jom	16	PJ1-16	+	+	-	-
Sai-krok	15	SK1-15	+	+	-	-
Nham-moo	12	NM1-12	+	+	-	-
Nham-pla	18	NF1-18	+	+	-	-
Mum-moo	10	MU1-10	+	+	-	-

+, positive; -, negative.

Screening of bacteriocin-producing LAB

LAB that can produce bacteriocin were selected by the agar well diffusion method. The 91 isolates were tested against foodborne pathogens including *Listeria monocytogenes* TISTR 1327, *Escherichia coli* TISTR 887, and *Staphylococcus aureus* TISTR 517. Streptomycin was used as a positive control. It was found that 8 LAB isolates showed bacteriocin activity against *Listeria monocytogenes* TISTR 1327, *Escherichia coli* TISTR 887, and *Staphylococcus aureus* TISTR 517 (Table 3). Only 5 isolates (PS-5, PS-7, SK-1, NF-11 and MU-9) exhibited strong inhibitory activity against all foodborne pathogens. The highest bacteriocin activity against *L. monocytogenes* TISTR 1327 and *S. aureus* TISTR 517 was observed in PS-7 isolated from Pla-som with 10.42±0.56 and 8.13±0.26 mm diameter, respectively. PJ-5 isolate obtained from Pla-jom showed the strongest bacteriocin activity against *E. coli* TISTR 887 with 8.96±0.10 mm diameter compared to the standard streptomycin at the concentration of 10 µl/ml with 15.06±0.04 mm inhibition zone diameter. All LAB isolates were selected and tested for biocatalytic properties of bacteriocin.

Table 3 Diameter of inhibition zone (mm) for bacteriocin-producing LAB isolates against test organisms.

LAB isolate	sample source	diameter of inhibition zone (mm)		
		L. monocytogenes TISTR 1327	*E. coli* TISTR 887	*S. aureus* TISTR 517
PS-5	Pla-som	9.83±0.03$^{d\ 1/}$	8.67±0.13^{e}	7.88±0.14^{c}
PS-7	Pla-som	10.42±0.56^{b}	8.92±0.13^{d}	8.13±0.26^{b}
PS-8	Pla-som	9.92±1.10^{d}	7.75±0.27^{g}	-
PJ-5	Pla-jom	9.54±0.10^{f}	8.96±0.10^{c}	-
SK-1	Sai-krok	8.80±0.21^{h}	6.67±0.13^{h}	7.13±0.14^{e}
SK-6	Sai-krok	10.08±0.13^{c}	8.46±0.46^{f}	-
NF-11	Nham-pla	9.21±0.19^{g}	8.92±0.13^{d}	7.38±0.14^{d}
MU-9	Mum-moo	9.67±0.30^{e}	9.79±0.19^{b}	7.42±0.13^{d}
streptomycin		18.12±0.03^{a}	15.06±0.04^{a}	20.05±0.07^{a}

-, negative

1/ each value represents the mean ± standard deviation of triplicates followed by different letters in the column are significantly different ($p<0.05$).

Biocatalytic properties of bacteriocin produced by LAB

Protease sensitivity is a key criterion for the characterization of an inhibitory substance as bacteriocins (Saraiva et al., 2014). The biocatalytic activity against indicator bacterial strains of CFS produced by eight selected LAB isolates was completely destroyed by all proteolytic enzymes including trypsin, chymotrypsin, and proteinase K at the concentration of 1 mg/ml when incubated at 37°C for 2 h (Table 4). These results indicated that the substances produced by selected LAB isolates were protein, which was sensitive to the proteolytic enzymes. Catalase and lipase had no effect on the bacteriocin. CFS could rules out the possible role of hydrogen peroxide in bringing about the inhibition of sensitive bacteria (Huang et al., 2024; Thuy et al., 2024).

Table 4 Effect of enzymes on antibacterial activity of bacteriocin-producing LAB isolates against indicator bacteria.

LAB isolate	Inhibitory activity (AU/ml)				
	catalase	lipase	tyrypsin	chymotrypsin	proteinase K
PS-5	1,600	1,600	0	0	0
PS-7	3,200	3,200	0	0	0
PS-8	1,600	1,600	0	0	0
PJ-5	1,600	1,600	0	0	0
SK-1	1,200	1,200	0	0	0
SK-6	3,200	3,200	0	0	0
NF-11	1,200	1,200	0	0	0
MU-9	1,600	1,600	0	0	0

Identification of bacteriocin-producing LAB

Isolates PS-5, PS-7, PS-8, PJ-5, SK-1, SK-6, and MU-9 were gram-positive rods with cell arrangement as single and pairs, while NF-11 was gram-positive cocci arranged in pairs and tetrads (Figure 1). Based on the observed cell morphology, it is tentatively inferred that these isolates likely belong to the group of LAB, specifically within the genus *Lactiplantibacillus* (formerly *Lactobacillus*). In addition, some isolates may belong to the genus *Pediococcus*. Various study on LAB isolated from Thai fermented meat products supports this inference, reporting predominant occurrences of bacteriocin-producing LAB from the genera *Lactococcus* (Noonpakdee,

Santivarangkna, Jumriangrit, Sonomoto, & Panyim, 2003), *Weissella* (Woraprayote et al., 2015), *Lactiplantibacillus* (formerly *Lactobacillus*) (Mettametha, Siripoke, & Swetwiwathana, 2011) and *Pediococcus* (Surachart, Kantachote, Deachamag, & Wonglapsuwan, 2021). The genomes of bacteria in the genus *Pediococcus* isolated from fermented foods were analyzed and found that many strains of bacteria have the potential to produce bacteriocins. Specifically, *Pediococcus pentosaceus* was identified as a producer of both class II and class III bacteriocins, while *Pediococcus acidilactici* was found to produce class III bacteriocins (Surachart, Kantachote, Deachamag, & Wonglapsuwan, 2021).

Genotypic characterization of 8 LAB isolates, namely PS-5, PS-7, PS-8, PJ-5, SK-1, SK-6, NF-11 and MU-9, revealed 16S rRNA gene sequences of 1515, 1503, 1529, 1513, 1521, 1498, 1520 and 1526 bp, respectively (Table 5). Phylogenetic relationships were conducted using the maximum composite likelihood method with *E. coli* ATCC 11775^T as the outgroup. Based on 16S rRNA gene sequence and phylogenetic analyses shown in (Table 5) and (Figure 2), seven isolates were identified as belonging to the genus *Lentilactobacillus*. Isolates PJ-5, PS-5, PS-7 and PS-8 (accession number: PP475799, PP475800, PP475801 and PP475802) were identified as closely related to *Lactiplantibacillus pentosus* (*formerly Lactobacillus pentosus*) at 99% similarity when compared to *Lactiplantibacillus pentosus* 124-2^T. The isolates MU-9, SK-1 and SK-6 (accession number: PP475797, PP475803, and PP475804) belonged to *Lactiplantibacillus plantarum* (*formerly Lactobacillus plantarum*), exhibiting 99% similarity to *Lactiplantibacillus plantarum* JCM 1149^T. Vangpikul, & Itsaranuwat (2021) also reported that *Lactiplantibacillus plantarum* was isolated from Thai traditional pickles. *Lactiplantibacillus plantarum* was found to be the dominant species in fermented foods and is capable of producing bacteriocin (Wang et al., 2018; Parlindungan, Lugli, Ventura, Van Sinderen, & Mahony, 2021). Isolate NF-11 (accession number: PP475798) was classified as *Pediococcus pentosaceus*. showing 100% similarity to *Pediococcus pentosaceus* DSM 20336^T (Figure 3). This is the first report of *Pediococcus pentosaceus* being isolated from nham-plaa, a traditional Thai fermented minced fish tightly wrapped with banana leaf. *Pediococcus pentosaceus* is considered as a bacteriocinogenic strain, which can effectively inhibit a wide range of pathogens (Jiang, Cai, Lv, & Li, 2021).

Table 5 Similarity of 16S rRNA gene sequences of eight LAB isolates compared with other related bacteria from NCBI nucleotide sequence database.

LAB isolate	length of sequence (bp)	identification result	nucleotide sequence comparison			
			closest relative	length of sequence (bp)	sequence homology	NCBI accession no.
PS-5	1515	*Lactiplantibacillus pentosus*	*Lactiplantibacillus pentosus* 124-2^T	1519	99	NR029133
PS-7	1503	*Lactiplantibacillus pentosus*	*Lactiplantibacillus pentosus* 124-2^T	1519	99	NR029133
PS-8	1529	*Lactiplantibacillus pentosus*	*Lactiplantibacillus pentosus* 124-2^T	1519	99	NR029133
PJ-5	1513	*Lactiplantibacillus pentosus*	*Lactiplantibacillus pentosus* 124-2^T	1519	99	NR029133
SK-1	1521	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum* JCM 1149^T	1519	99	NR115605
SK-6	1498	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum* JCM 1149^T	1519	99	NR115605
NF-11	1520	*Pediococcus pentosaceus*	*Pediococcus pentosaceus* DSM 20336^T	1521	100	KX886792
MU-9	1526	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum* JCM 1149^T	1519	99	NR115605

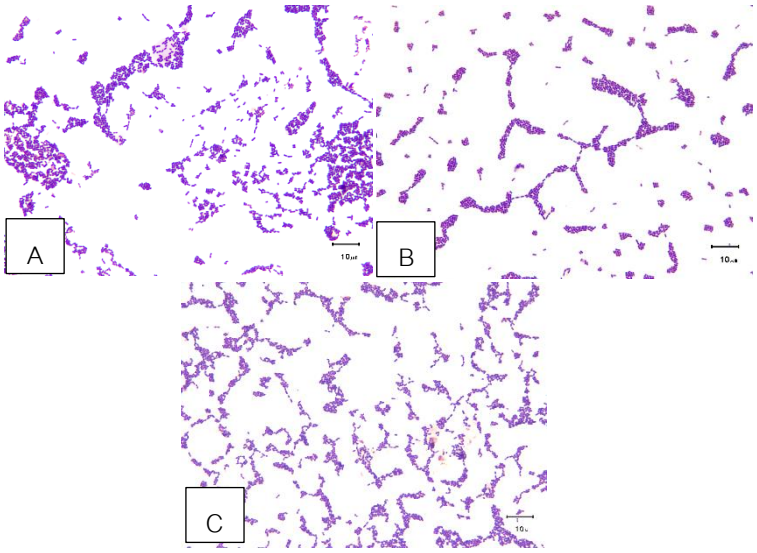

Figure 1 Cell morphology of some LAB isolated from Thai fermented foods; PS-8 (A), SK-1 (B) and NF-11 (C).

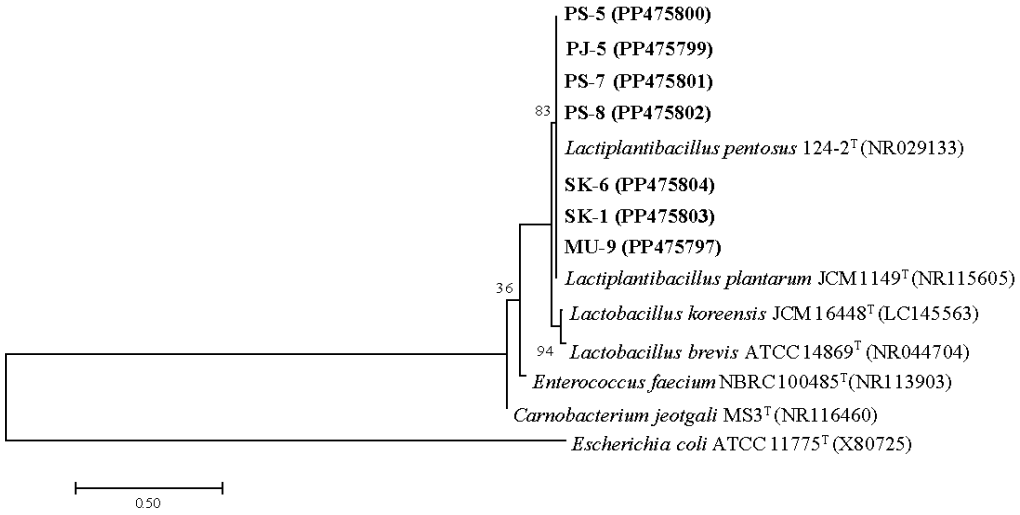

Figure 2 Phylogenetic tree of isolates PS-5, PS-7, PS-8, PJ-5, SK-1, SK-6, MU-9 and related taxa from NCBI database. Scale bar, 0.50 represents substitution per nucleotide position. *Escherichia coli* ATCC 11775 (X80725) is presented as an outgroup sequence.

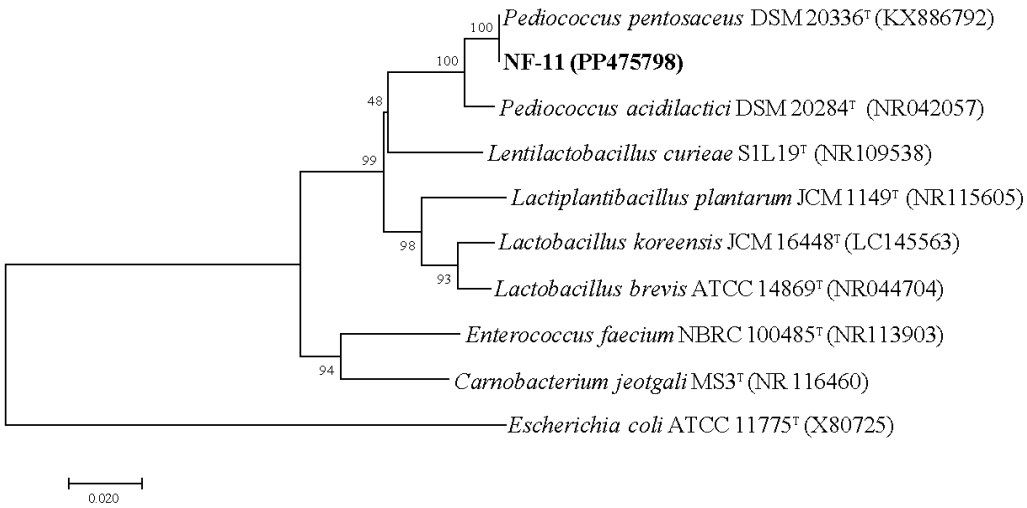

Figure 3 Phylogenetic tree of isolate NF-11 and related taxa from NCBI database. Scale bar, 0.020 represents substitution per nucleotide position. *Escherichia coli* ATCC 11775 (X80725) is presented as outgroup sequence.

Conclusions

LAB isolated from fermented products are a rich source of bioactive compounds like bacteriocin. The studies revealed that several screened LAB isolates showed high inhibitory activity against test organisms such as *Listeria monocytogenes, Escherichia coli,* and *Staphylococcus aureus* which are considered as foodborne bacteria. The biocatalytic activities of CFS were inactivated by proteolytic enzymes. Based on nucleotide sequences of the 16S rRNA genes, selected LAB isolates were identified as *Lactiplantibacillus pentosus, Lactiplantibacillus plantarum,* and *Pediococcus pentosaceus*. The obtained isolates may be used as a starter culture to produce fermented products and also be used as biopreservative agents in food processing and preservation. Further studies on the purification and characterization of bacteriocin produced from isolated LAB, as well as its mode of action, would pave the way for the application of bacteriocin in fermented food products.

Acknowledgement

This work was financially supported by the revenue budget of Rajamangala University of Technology Rattanakosin (C13/2564). The authors would like to thank Rajamangala University of Technology Rattanakosin for all of the supports.

References

Barcenilla, C., Ducic, M., López, M., Prieto, M., & Álvarez-Ordónez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: a systematic review. *Meat Science, 183,* 108661.

Darbandi, A., Asadi, A., Ari, M. M., Ohadi, E.,Talebi, M., Zadeh, M. H., Emamie, A. D., Ghanavati, R., & Kakanj, M. (2022). Bacteriocins: Properties and potential use as antimicrobials. *Journal of Clinical Laboratory Analysis, 36*(1), e24093.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95-98.

H-Kittikun, H., Biscola, V., El-Ghaish, S., Jaffres, E., Dousset, X., Pillot, G., Haertlé, T., Chobert, J. M., & Hwanhlem, N. (2015). Bacteriocin-producing *Enterococcus faecalis* KT2W2G isolated from mangrove forests in southern Thailand: Purification, characterization and safety evaluation. *Food Control, 54,* 126-134.

Huang, F., Zhao, Y., Hou, Y., Yang, Y., Yue, B., & Zhang, X. (2024). Unraveling the antimicrobial potential of *Lactiplantibacillus plantarum* strains TE0907 and TE1809 sourced from *Bufo gargarizans*: advancing the frontier of probiotic-based therapeutics. *Frontiers in Microbiology, 15,* 1347830.

Hwanhlem, N., Buradaleng, S., Wattanachant, S., Benjakul, S., Tani, A., & Maneerat, S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. *Food Control, 22*(3-4), 401-407.

Hwanhlem, N., Chobert, J. M., & H-Kittikun, A. (2014). Bacteriocin-producing lactic acid bacteria

isolated from mangrove forests in southern Thailand as potential bio-control agents in food: Isolation, screening and optimization. *Food Control, 41,* 202-211.

Jiang, S., Cai, L., Lv, L., & Li, L. (2021). *Pediococcus pentosaceus*, a future additive or probiotic candidate. *Microbial Cell Factories, 20,* 45.

Kaveh, S., Hashemi, S. M. B., Abedi, E., Amiri, M. J., & Conte, F. L. (2023). Bio-preservation of meat and fermented meat products by lactic acid bacteria strains and their antibacterial metabolites. *Sustainability, 15*(13), 10154.

Krongkeha, W. (2022). Isolation and identification of GABA-producing lactic acid bacteria from fermented foods. *RMUTSB Academic Journal*, *10*(1), 66-77. (in Thai)

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution, 33*(7), 1870-1874.

Loyda, C., Wichaporn, J., Jaranrattanasri, A., Tochampa, W., & Singanusong, R. (2023). Physicochemical properties, amino acid composition and volatile components of fermented fish (Pla-ra) accelerated by starter cultures. *Trends in Sciences, 20*(5), 6576.

Mei, J., Ma, X., & Xie, J. (2019). Review on natural preservatives for extending fish shelf life. *Foods, 8*(10), 490.

Mettametha, N., Siripoke, S., & Swetwiwathana, A. (2011). Isolation and identification of bacteriocin-producing lactic acid bacteria from minced fermented fish (Nham-pla). *Srinakharinwirot Science Journal, 27*(1), 107-127. (in Thai)

Mokoena, M. P., Omatola, C. A., & Olaniran, A. O. (2021). Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. *Molecules, 26,* 7055.

Nanasombat, S., Phunpruch, S., & Jaichalad, T. (2012). Screening and identification of lactic acid bacteria from raw seafoods and Thai fermented seafood products for their potential use as starter cultures. *Songklanakarin Journal of Science and Technology, 34*(3), 255-262.

Noonpakdee, W., Santivarangkna, C., Jumriangrit, P., Sonomoto, K., & Panyim, S. (2003). Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. *International Journal of Food Microbiology, 81*(2), 137-145.

Ohmomo, S., Kobayashi, M., Yajima, M., Suyanandana, P., Sudka, P., & Bomchai, P. (1999). Screening of thermophilic lactic acid bacteria producing bacteriocins in the Tropics. *Japan Agricultural Research Quarterly, 33,* 125-131.

Onda, T., Yanagida, F., Tsuji, M., Ogino, S., & Shinohara, T. (1999). Isolation and characterization of the lactic acid bacterial strain GM005 producing an antibacterial substance from *Miso*-paste product. *Food Science and Technology Research, 5*(3), 247-250.

Pakwan, C., Chitov, T., Chantawannakul, P., Manasam, M., Bovonsombut, S., & Disayathanoowat, T. (2020). Bacterial compositions of indigenous Lanna (Northern Thai) fermented foods and their potential functional properties. *PLoS ONE, 15*(11), e0242560.

Parlindungan, E., Lugli, G. A., Ventura, M., Van Sinderen, D., & Mahony, J. (2021). Lactic acid bacteria diversity and characterization of probiotic candidates in fermented meats. *Foods, 10,* 1519.

Pilasombut, K., Wajjwalku, W., Nitisinprasert, S., Swetwiwathana, A., Zendo, T., Nakayama, J., Sonomoto, K., & Sakpuaram, T. (2005). Screening and characterization of bacteriocin producing lactic acid bacteria isolated from chicken intestine. *Kasetsart Journal (Natural Science), 39,* 612-621.

Saraiva, M. A. F., Nes, I. F., Baracat-Pereira, M. C., de Queiroz, M. V., Mantovani, H. C., & de Moraes, C. A. (2014). Purification and characterization of a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* PD6.9. *Journal of Microbiology and Antimicrobials, 6*(5), 79-87.

Satomi, M., Kimura, B., Mizoi, M., Sato, T., & Fujii, T. (1997). *Tetragenococcus muriaticus* sp. nov., a new moderately halophiliclactic acid bacterium isolated from fermented squid liver sauce. *International Journal of Systematic and Evolutionary Microbiology, 47*, 832-836.

Surachat, K., Kantachote, D., Deachamag, P., & Wonglapsuwan, M. (2021). Genomic insight into *Pediococcus acidilactici* HN9, a potential probiotic strain isolated from the traditional Thai-style fermented beef nhang. *Microorganisms, 9*(1), 50.

Tangwatcharin, P., Nithisantawakhup, J., & Sorapukdee, S. (2020). Microbiological and physicochemical qualities of Moo Som (Traditional Thai fermented meat) inoculated with lactic acid bacteria starter. *Walailak Journal of Science and Technology, 17(*8), 788-800.

Thuy, T. T. D., Lu, H. F., Bregente, C. J. B., Huang, F. C. A., Tu, P. C., & Kao, C. Y. (2024). Characterization of the broad-spectrum antibacterial activity of bacteriocin-like inhibitory substance-producing probiotics isolated from fermented foods. *BMC Microbiology, 24,* 85.

Todorov, S. D., Wachsman, M., Tomé, E., Dousset, X., Destro, M. T., Dicks, L. M. T., de Melo Franco, B. D. G., Vaz-Velho, M., & Drider, D. (2010). Characterisation of an antiviral pediocin-like bacteriocin produced by *Enterococcus faecium*. *Food Microbiology, 27*(7), 869-879.

Vangpikul, S., & Itsaranuwat, P. (2021). Isolation and selection of lactic acid bacteria with potential probiotics from traditional Thai local pickle products. *RMUTSB Academic Journal, 9*(2), 150-163. (in Thai)

Wang, Y., Qin, Y., Xie, Q., Zhang, Y., Hu, J., & Li, P. (2018). Purification and characterization of plantaricin LPL-1, a novel class IIa bacteriocin produced by *Lactobacillus plantarum* LPL-1 isolated from fermented fish. *Frontiers in Microbiology, 9,* 2276.

Weisburg. W. G., Barns, M. S., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173*(2), 697-703.

Woraprayote, W., Janyaphisan, T., Adunphatcharaphon, S., Sonhom, N., Showpanish, K., Rumjuankiat, K., Visessanguan, W., Elliott, C. T., & Awanwee, P. (2023). Bacteriocinogenic lactic acid bacteria from Thai fermented foods: potential food applications. *Food Bioscience, 52,* 102385.

Woraprayote, W., Pumpuang, L., Tosukhowong, A., Roytrakul, S., Perez, R. H., Zendo, T., Sonomoto, K., Benjakul, S., & Visessanguan, W. (2015). Two putatively novel bacteriocins active against Gram-negative food borne pathogens produced by *Weissella hellenica* BCC 7293. *Food Control, 55,* 176-184.

Xia, T., Teng, K., Liu, Y., Guo, Y., Huang, F., Tahir, M., Wang, T., & Zhong, J. A. (2023). Novel two-

component bacteriocin, acidicin P, and its key residues for inhibiting *Listeria monocytogenes* by targeting the cell membrane. *Microbiology Spectrum, 11*(4), e0521022.

Xin, W. G., Wu, G., Ying, J. P., Xiang, Y. Z., Jiang, Y. H., Deng, X. Y., Lin, L. B., & Zhang, Q. L. (2023). Antibacterial activity and mechanism of action of bacteriocin LFX01 against *Staphylococcus aureus* and *Escherichia coli* and its application on pork model. *Meat Science, 196,* 109045.