Genomic Characterization and Hydrolysis Enzyme of *Piscibacillus salipiscarius* RBU1-1^T and *Allobacillus salarius* SKP4-8^T Isolated from Fermented Fish Products

Sirilak Namwong[1*], Apichai Chaimongkol[2], Engkarat Kingkaew[2], Wongsakorn Phongsopitanun[3], Nitcha Chamroensaksri[4] and Somboon Tanasupawat[3**]

ABSTRACT

Two halophilic bacterial strains, *Piscibacillus salipiscarius* RBU1-1^T and *Allobacillus salarius* SKP4-8^T, were isolated from traditional Thai fermented foods, namely pla-ra from Ratchaburi, Thailand, and ka-pi from Samut Sakhon, Thailand, respectively. Comparative genomics confirmed their taxonomic distinctiveness, with RBU1-1^T representing a novel species and SKP4-8^T showing clear genomic separation from its closest relatives. The draft genomes were 2.99 Mb (36.7 mol% G+C) for RBU1-1^T and 2.59 Mb (38.8 mol% G+C) for SKP4-8^T. Both strains encoded hydrolytic enzymes and secondary metabolite pathways, with SKP4-8^T displaying higher protease activity and RBU1-1^T harboring ectoine biosynthetic and stress-response genes. RBU1-1^T encoded the biosynthesis pathways for ectoine, tryptophan, and thiamine, while SKP4-8^T encoded thiamine, riboflavin, coenzyme A, and menaquinone, indicating their potential contributions to postbiotic and nutritional value. Safety assessment revealed the absence of biogenic amine, antibiotic biosynthesis, and major virulence genes in both strains. Although antimicrobial resistance-associated genes were detected, no complete clusters were identified, suggesting low phenotypic risk. Computational pathogenicity prediction indicated a low probability for RBU1-1^T (0.242) and a higher probability for SKP4-8^T (0.945), though these require cautious interpretation. Overall, genomic evidence and enzymatic activity support RBU1-1^T and SKP4-8^T as safe, functional starter culture candidates, with RBU1-1^T showing strong potential through postbiotic biosynthesis and SKP4-8^T through proteolytic activity.

Keywords: *Allobacillus salarius*, Fermented fish product, Halophilic bacteria, *Piscibacillus salipiscarius*

INTRODUCTION

Pla-ra and ka-pi are traditional Thai fermented products preserved with high salt concentrations, prepared from whole fish and small shrimp, respectively (Sangjindavong *et al*., 2008). During fermentation, both native and microbial enzymes—mainly proteases and lipases—participate in the breakdown of proteins and lipids, generating free amino acids and fatty acids that enhance the overall flavor profile (Hajep and Jinap, 2012). Lipases, belonging to the serine hydrolase

[1]Department of Food Innovation and Professional Chef, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand
[2]Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[3]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand
[4]National Biobank of Thailand (NBT), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand
[*]Corresponding author. E-mail address: sirilak.na@ssru.ac.th
[**]Co-corresponding author. E-mail address: Somboon.T@chula.ac.th
Received 29 August 2025 / Accepted 20 March 2026

superfamily, catalyze lipid degradation, while proteases cleave peptide bonds (Bachovchin and Cravatt, 2012). However, enzyme activity is often reduced under extreme saline and alkaline conditions (Daroonpunt *et al*., 2016).

Several moderately halophilic, rod-shaped bacterial species isolated from traditional Thai fermented products such as pla-ra and ka-pi have been reported to synthesize various hydrolytic enzymes, especially lipases and proteases. Representative taxa include *Allobacillus salarius* (Yiamsombut *et al*., 2022), *Piscibacillus salipiscarius* (Tanasupawat *et al*., 2007), *Gracilibacillus thailandensis* (Chamroensaksri *et al*., 2010), *Oceanobacillus kapialis* (Namwong *et al*., 2009), and *Virgibacillus kapii* (Daroonpunt *et al*., 2016). Despite increasing research interest, the enzymatic profiles and genomic characteristics of microorganisms associated with Thai fermented products such as pla-ra and ka-pi remain insufficiently characterized.

The present study aims at characterizing the genome sequences of strains RBU1-1^T and SKP4-8^T, with a focus on identifying and annotating genes encoding proteases and serine hydrolases. Furthermore, the hydrolytic enzyme activities of these strains were evaluated to elucidate their possible functional roles within the fermentation environment.

MATERIALS AND METHODS

Genome sequencing and function annotation

Strains RBU1-1^T and SKP4-8^T were isolated from pla-ra from Ratchaburi, Thailand, and ka-pi from Samut Sakhon, Thailand, respectively. Whole-genome sequencing of strains RBU1-1^T and SKP4-8^T was conducted by Center for Omics and Bioinformatics, Graduate School of Frontier Sciences, University of Tokyo and the World Data Center for Microorganisms (WDCM) under the Global Catalogue of Microorganisms (GCM) 2.0 project (Yiamsombut *et al*., 2022), respectively. Genomic DNA was isolated from cultured cells using the DNeasy UltraClean Microbial Kit (Qiagen). Libraries were prepared using the QIAseq FX DNA Library Kit (Qiagen), and sequencing was performed on an Illumina MiSeq system with the MiSeq Reagent Kit v3, producing 300 bp paired-end reads over 600 cycles. Low-quality sequences were removed and adapters trimmed using Trimmomatic (https://astrobiomike.github.io/genomics/de_novo_assembly#trimmomatic). The remaining high-quality reads were assembled *de novo* into contigs using SPAdes v3.12 (Bankevich *et al*., 2012). The draft genome sequences of strain RBU1-1^T (accession number: BBCD00000000) and strain SKP4-8^T (accession number: VMHE00000000) have been submitted to GenBank. Functional annotation of the genomes was carried out using a combination of DFAST (Tanizawa *et al*., 2018), RAST (Aziz *et al*., 2008), Prokka v1.12 (Seemann, 2014), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Genome visualization, including circular maps and phylogenomic relationships, was produced using TYGS and Proksee tools, respectively (Grant *et al*., 2023). Comparative genomic analyses were conducted using several bioinformatic tools. The average nucleotide identity (ANI) between the studied strains and closely related type strains was obtained using both the ANIb and ANIm algorithms available in JSpeciesWS (Richter *et al*., 2016). The digital DNA–DNA hybridization (dDDH) estimates were derived from the Genome-to-Genome Distance Calculator (GGDC v2.1) using the BLAST+ algorithm (Meier-Kolthoff *et al*., 2013), and formula 2 (identities/HSP length) was applied, as this approach is considered most suitable for draft genome assemblies.

To predict functional and safety-related features, antimicrobial resistance genes were screened using the Comprehensive Antibiotic Resistance Database (CARD) (Alcock *et al*., 2020) and ResFinder version 4.7.2 (Bortolaia *et al*., 2020). Pathogenic potential was inferred using PathogenFinder (Cosentino *et al*., 2013). Additionally, the metabolic pathways and functional gene categories were annotated through the Kyoto Encyclopedia of Genes and Genomes (KEGG) resource (https://www.kegg.jp) as described by Kanehisa *et al*. (2016).

Serine hydrolase activity assay

Bacterial isolates that exhibited lipolytic activity on agar screening plates were further cultivated in JCM no. 377 broth using a rotary shaker set at 200 rpm and incubated at 37 °C for 48 h. Serine hydrolase activity was quantified using *p*-nitrophenyl palmitate (*p*-NPP) as the substrate, according to the procedure described by Kemp *et al*. (2013) with minor modifications. The reaction mixture contained 1 mL of *p*-NPP solution (0.001 g of *p*-NPP dissolved in 1 mL isopropanol and then diluted with 18 volumes of 50 mM phosphate buffer, pH 7.0, supplemented with 0.5% w/v Triton X-100 and 5% w/v NaCl) or 1.5 mL of blank solution (consisting of 0.01 g gum arabic, 0.02 g sodium deoxycholate, 50 µL Triton X-100, and 9 mL of 50 mM phosphate buffer, pH 7.0). Each reaction was initiated by adding 0.5 mL of crude enzyme extract and incubated at 37 °C for 60 min. The reaction was terminated with 1 mL of 0.01 N NaOH, and absorbance was recorded at 410 nm. All assays were performed in triplicate. One unit (U) of lipase activity was defined as the enzyme quantity required to release 1 ng of *p*-nitrophenol per minute under the specified assay conditions (Pliego *et al*., 2015). All statistical tests were conducted using two-sided t-tests at the 95% confidence level ($\alpha = 0.05$) (Beath and Jones, 2018).

Protease assays

Strains RBU1-1^T and SKP4-8^T were grown in modified JCM no. 377 broth under shaking conditions (200 rpm) at 37 °C for 24 h. After incubation, cultures were centrifuged at 10,000 rpm for 20 min at 4 °C, and the resulting supernatant was collected for protease activity measurements following the procedure of Hiraga *et al*. (2005) with minor adjustments. For the enzyme assays, 0.5 mL of the crude extract was combined with 1.5 mL of 1.33% (w/v) Hammarsten casein prepared in 50 mM Tris-HCl buffer (pH 7.5) supplemented with 5% (w/v) NaCl. The reaction mixture was incubated at 37 °C for 60 min, and the reaction was stopped by adding 2 mL of 0.44 M trichloroacetic acid (TCA). After centrifugation at 2,500 rpm for 10 min, 0.5 mL of the clarified supernatant was mixed with 2.5 mL of 0.44 M sodium carbonate and 0.5 mL of Folin–Ciocalteu reagent. The mixture was then incubated for 20 min at 37 °C, and absorbance was read at 660 nm. All assays were performed in triplicate. One unit (U) of protease activity was defined as the enzyme quantity required to release 1 µg of tyrosine equivalent per minute under the assay conditions. A control (blank) reaction was prepared in parallel, with enzyme added only after TCA addition. Total soluble protein in the supernatant was quantified according to the Lowry method (Lowry *et al*., 1951). All statistical tests were conducted using two-tailed t-tests at the 95% confidence level (Beath and Jones, 2018).

Hemolysis activity

Strains RBU1-1^T and SKP4-8^T were inoculated on JCM no.377 agar medium supplemented with 2% (w/v) NaCl and 5% (v/v) sheep blood and incubated at 37 °C for 24 h. After incubation, hemolytic activity was evaluated by observing the appearance of a clear zone, a greenish zone, or no visible zone surrounding the colonies, corresponding to β-hemolysis, α-hemolysis, and γ-hemolysis (non-hemolytic), respectively.

Vancomycin susceptibility

Susceptibility to vancomycin was evaluated by the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (Humphries *et al*., 2021). A vancomycin disk containing 30 µg was used in the assay. The bacterial cell density of each culture was adjusted to McFarland standard No. 1, and the suspension was uniformly spread onto Mueller–Hinton (MH) agar (Merck, Darmstadt, Germany) using a sterile cotton swab. After allowing the inoculated plates to stand at room temperature for 10 min, they were incubated under optimal growth conditions for each strain. The diameters of the inhibition zones were then measured and interpreted according to the breakpoint criteria reported by Humphries *et al*. (2021).

RESULTS AND DISCUSSION

Genome sequencing and functional annotation

The polyphasic taxonomic profiles of *Piscibacillus salipiscarius* RBU1-1^T and *Allobacillus salarius* SKP4-8^T (Table 1), isolated from traditional fermented fish (pla-ra) and shrimp paste, were previously characterized by Tanasupawat *et al*. (2007) and Yiamsombut *et al*. (2022). Phylogenomic analysis based on complete protein sequences (Figure 1) confirmed that strain RBU1-1^T (accession no. BBCD00000000) and strain SKP4-8^T (accession no. VMHE00000000) are members of the genera *Piscibacillus* and *Allobacillus*, respectively.

Piscibacillus. Similarly, strain SKP4-8^T shared ANIb and ANIm values of 93.18% and 93.91%, respectively, with *Allobacillus saliphilus* KCTC 33906^T, and the corresponding dDDH value was 53.2% (confidence interval: 50.5–55.9%). These results, which are below the species demarcation thresholds, support the taxonomic independence of strain SKP4-8^T from its nearest phylogenetic relative.

Comparative genome analysis indicated that strain RBU1-1^T exhibited average nucleotide identity (ANIb and ANIm) values of 76.63% and 83.20%, respectively, and a digital DNA–DNA hybridization (dDDH) estimate of 20.3% (confidence interval: 18.1–22.7) when compared with its closest

Table 1. Characteristics of strains RBU1-1^T, SKP4-8^T and other closely related species.

Characteristic	1	2
Cell size (mm)	0.4–0.56×1.5–4.0	0.25–0.3×0.8–3.5
Oxidase	+	+
Spore shape	Oval	Oval
NaCl range (optimum) (%, w/v)	2–30 (10–20)	1–6 (5–10)
Temperature range (°C)	15–48 (37)	25–45 (37)
Acid production from:		
Galactose	+	-
D-Glucose	+	+
D-Fructose	+	-
Nitrate reduction	-	+
Hydrolysis of:		
Starch	+	+
Casein	+	
Gelatin	+	+
Tween 80	-	+
Peptidoglycan type/variant	A1*c*/*meso*-DAP	*meso*-DAP
Fatty acid profile	anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$
Polar lipids	PG, DPG	PG, DPG
DNA G+C content (mol%)	36.7	38.8

Note: PG* = phosphotidylglycerol; DPG** = diphosphatidylglyceral; + = positive reaction; w = weak reaction; - = negative reaction.

Taxa: 1, *Piscibacillus salipiscarius* JCM 13188^T (Tanasupawat *et al*., 2007); 2, *Allobacillus salarius* KCTC 33905^T (Yiamsombut *et al*., 2022).

relative, *Piscibacillus halophilus* DSM 21633^T (accession no. FOES00000000). These values are substantially below the generally accepted boundaries for species-level assignment 95–96% for ANI and 70% for dDDH (Richter and Rosselló-Móra, 2009), confirming that strain RBU1-1^T represents a distinct species within the genus

The draft genomes of strains RBU1-1^T and SKP4-8^T were estimated at 2.99 Mb with a G+C content of 36.7 mol%, and 2.59 Mb with a G+C content of 38.8 mol%, respectively (Figure 2). Hydrolytic genes and secondary metabolite clusters were predicted using the DFAST server (Tanizawa *et al*., 2018). Both strains encode intracellular and extracellular hydrolytic enzymes (Table 2). Intracellular enzymes are involved in lipid-associated processes such as lipid remodeling (Wei *et al*., 2018), maintenance of lipid homeostasis (Flammersfeld *et al*., 2020), ester bond hydrolysis by lipolytic enzymes (Johan *et al*., 2021), and fatty acid metabolism (Schmidt *et al*., 2022). Additional hydrolytic enzymes identified in these strains are listed in Table 2. With respect to secondary metabolites, both strains were predicted to produce β-lactones.

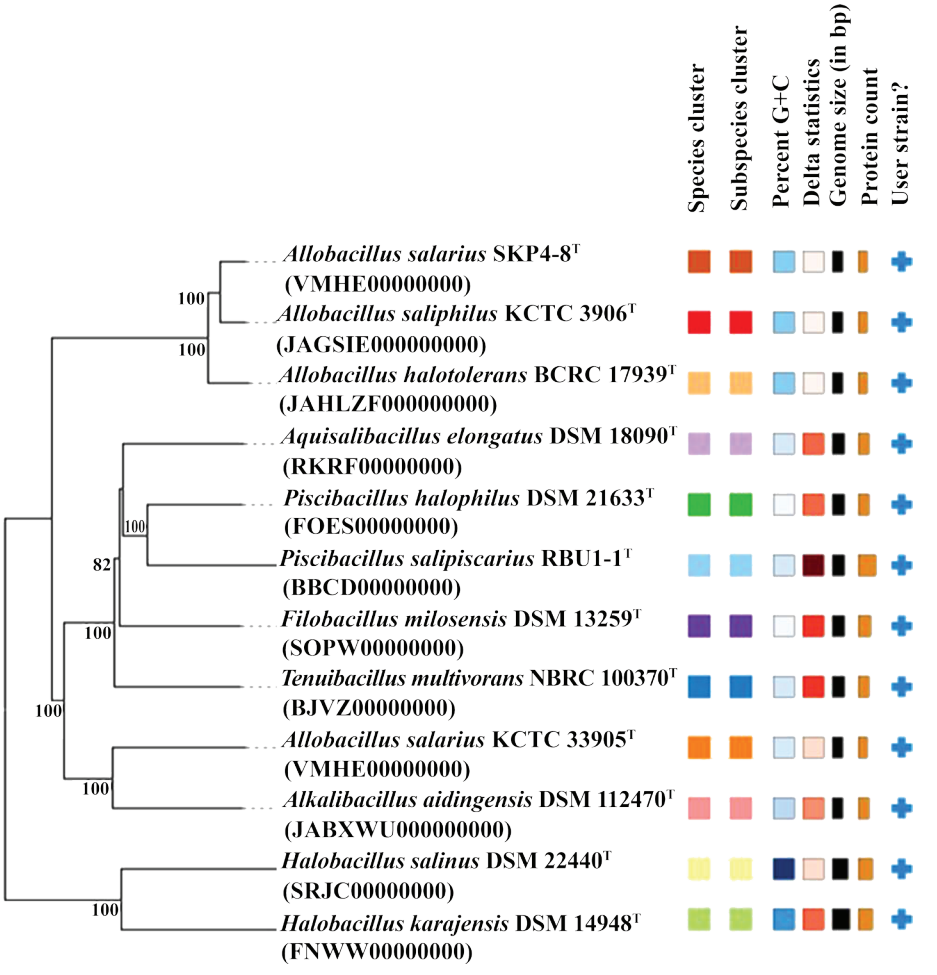


Figure 1. Phylogenomic tree illustrating the relationships among strains RBU1-1^T, SKP4-8^T, and their phylogenetically related taxa, as generated using the TYGS platform. Bootstrap support values based on the Genome BLAST Distance Phylogeny (GBDP) method are shown above the branches and were calculated from 100 replicates, yielding an average support of 90.6%. The tree was midpoint rooted.

Table 2. Gene function annotation and protease gene clusters of strains RBU1-1^T and SKP4-8^T.

Family	Number of genes		Name	Properties
	RBU1-1^T	SKP4-8^T		
Extracellular enzyme	1	1	Serine hydrolase	Hydrolysis of protein and lipid
	-	1	Serine peptidase (S8)	Protein-digesting enzymes such as subtilisin
	-	1	Prolyl oligopeptidase (S9)	
	-	1	Neutral Zn metallopeptidase	
	-	1	Zn metallopeptidase	
	-	1	Trypsin-like Peptidase	
	1	-	Bacillopeptidase F	Proteolytic enzyme such as a serine endopeptidase
Intracellular enzyme	1	1	Prepilin peptidase	Biogenesis of type IV pili
	-	1	Tripeptidase	Hydrolysis of a tripeptide
	1	1	Pyroglutamyl-peptidase I	Removing N-terminal pyroglutamyl groups from peptides
	1	1	M23 family peptidase	A well-investigated family of peptidoglycan hydrolases
	-	1	Protein-glutamate O-methylesterase *CheB*	Regulation of bacterial signalling
	-	1	2',3'-Cyclic-nucleotide 2'-phosphodiesterase	Nucleotide metabolism
	1	3	Metallophosphoesterase	Lipid remodeling
	2	1	Patatin-like phospholipases	Lipid homeostasis
	1	1	Carboxylesterase	Lipolytic enzymes for cleavage of ester linkages
	5	5	Thioesterase	Fatty acid metabolism
	1	4	Glycerophosphodiester phosphodiesterase	Cleavage of phosphoric diester bonds
	1	1	Cyclic-di-AMP phosphodiesterase GdpP	Hydrolysis of c-di-AMP
	2	2	CAAX proteases and bacteriocin-processing	Regulation of intramembrane proteolysis (RIP)
	1	1	ATP-dependent zinc metalloprotease	Regulation of proteolysis within intracellular membrane
	1	-	S1 family (Serine protease *HtrC*)	Prevention of severe cellular malfunctions under physiological and stress conditions
	1	-	Rhomboid family intramembrane serine protease	Turnover of misfolded or degraded membrane proteins
	1	-	L-2,4-Diaminobutyric acid acetyltransferase (*ectA*)	Protective agent (ectoine) against stress conditions
	1		L-2,4-Diaminobutyric acid transaminase (*ectB*)	
	1		Ectoine synthase (*ectC*)	
	1	1	Betalactone	Antibacterial agent

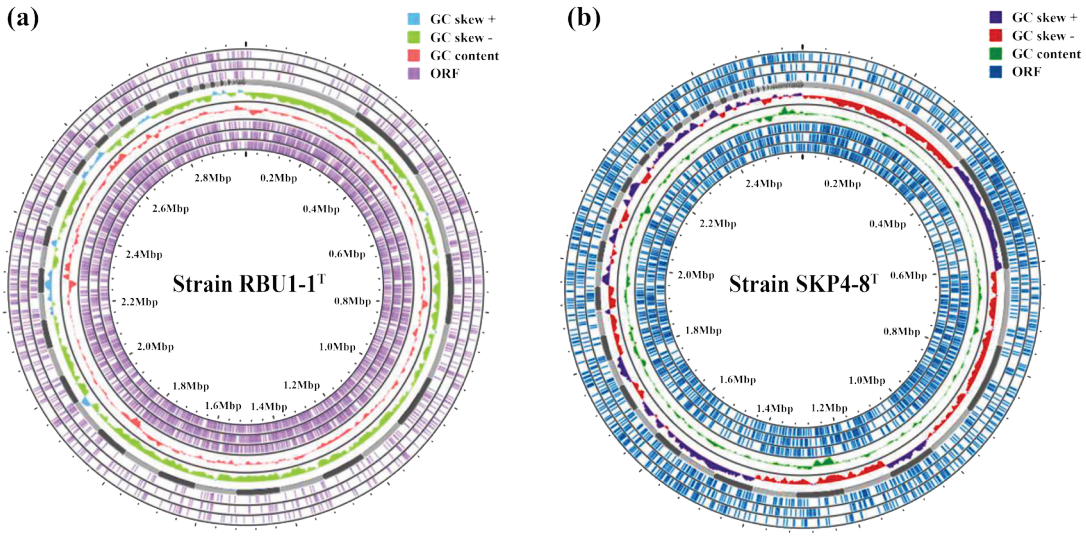


Figure 2. Circular genomic maps of strain RBU1-1^T (a) and SKP4-8^T (b).

Although antiSMASH analysis predicted the presence of a β-lactone biosynthetic gene cluster in both strains, closer examination indicated that these clusters are highly fragmented and exhibit low similarity to known functional β-lactone pathways (e.g., fengycin) (Figure 3). The absence of essential core biosynthetic genes suggests that these clusters are likely incomplete and non-functional, thereby minimizing potential safety concerns regarding antibiotic production.

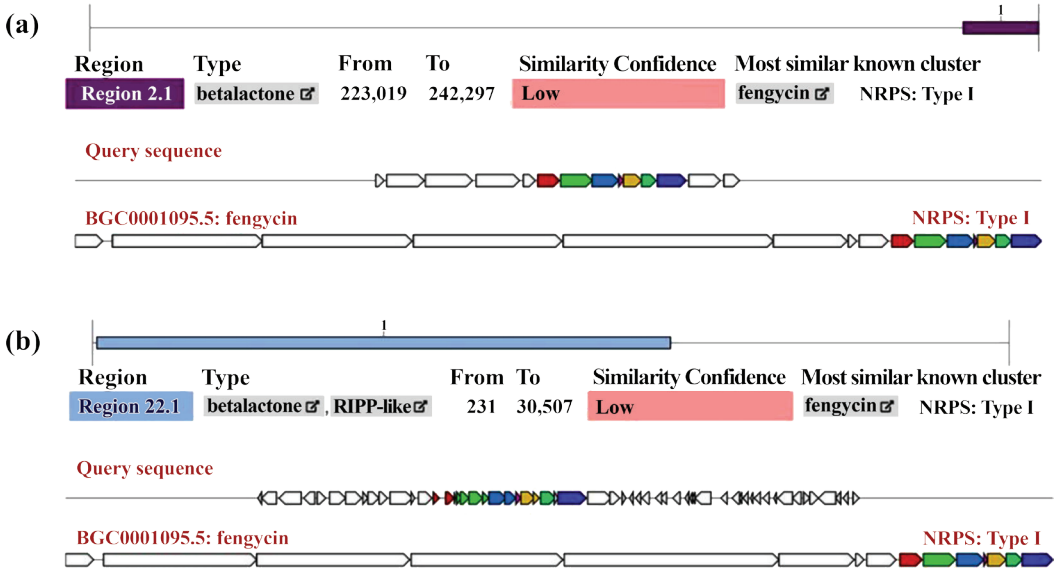


Figure 3. AntiSMASH analysis of predicted β-lactone biosynthetic gene clusters in strains SKP4-8^T (a) and RBU1-1^T (b). The sequence alignments show comparisons of the identified genomic regions and the closest known reference cluster, fengycin.

Strain SKP4-8^T harbored intracellular enzymes including protein-glutamate O-methylesterase (*CheB*) and 2′,3′-cyclic-nucleotide 2′-phosphodiesterase, which are involved in bacterial signaling regulation and nucleotide metabolism (Djordjevic *et al*., 1998). In contrast, strain RBU1-1^T encoded stress-response mechanisms, notably the S1 family serine protease (*HtrC*) and ectoine, a protective compatible solute implicated in cellular stability under physiological and stress conditions (Kang *et al*., 2022).

Comparative genomic analysis of strain RBU1-1^T and strain SKP4-8^T revealed a high degree of similarity, with 1,787 orthologous clusters shared between the two strains, representing their conserved core genome (Figure 4). Strain RBU1-1^T contained 58 unique clusters, while strain SKP4-8^T possessed 21 unique clusters, indicating the presence of strain-specific genomic features. Overall, RBU1-1^T harbored 1,845 clusters and SKP4-8^T contained 1,808 clusters, reflecting comparable genome sizes and a close genetic relationship. The unique clusters identified in each strain may represent specialized metabolic capacities or adaptive traits.

For RBU1-1^T, these may include pathways associated with the biosynthesis of beneficial metabolites (e.g., ectoine, tryptophan, and thiamine), stress tolerance, and other traits that strengthen its technological suitability as a starter culture. In contrast, the unique clusters of SKP4-8^T may encode niche-specific adaptations, potentially contributing to traits such as flavor formation, substrate utilization, or enhanced resilience under environmental stress conditions. Overall, the predominance of shared clusters highlights the close genomic relatedness of the two strains, while the limited number of unique clusters suggests strain-specific attributes that may provide functional diversity and complementary roles in fermentation processes.

Functional annotation of the predicted protein sequences was carried out using the BlastKOALA web-based platform. In strain RBU1-1^T, a total of 4,814 coding sequences were assigned to five major KEGG functional categories: Genetic Information Processing (197 genes), Environmental Information Processing (138 genes), Signaling and Cellular Processes (138 genes), Metabolism of Genetic Information (130 genes), and Carbohydrate Metabolism (108 genes). For strain SKP4-8^T, 2,717 annotated genes were distributed across the same KEGG pathway groups, including Genetic Information Processing (217 genes), Signaling and Cellular Processes (167 genes), Metabolism of Genetic Information (157 genes),

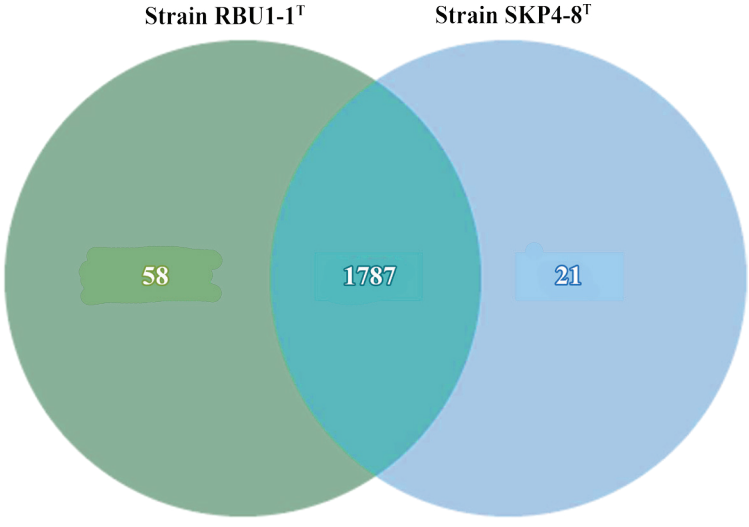


Figure 4. Comparative Venn diagram showing shared and strain-specific proteins between *Piscibacillus salipiscarius* RBU1-1^T and *Allobacillus salarius* SKP4-8^T based on orthologous clustering.

Environmental Information Processing (131 genes), and Carbohydrate Metabolism (98 genes). These profiles indicate that both genomes possess broad metabolic versatility and encode multiple systems related to cellular regulation and stress response.

For the *in silico* safety assessment and evaluation of potentially beneficial traits, KEGG pathway analysis revealed that strain RBU1-1^T harbors the genetic capacity to synthesize ectoine, tryptophan, and thiamine (Table 3). The detection of ectoine biosynthetic genes in strain RBU1-1^T is significant, as ectoine is a postbiotic with proven therapeutic benefits. Clinical studies confirm its safety and effectiveness in improving skin health, alleviating eye and respiratory allergies, reducing pharyngitis, and easing mucositis in chemotherapy patients (Xingrong *et al*., 2025). These findings suggest that RBU1-1^T can produce functional postbiotic metabolites that may enhance the quality and value of fermented foods. For strain SKP4-8^T, genes involved in the biosynthesis of thiamine, riboflavin, coenzyme A, and menaquinone were identified (Table 3). Menaquinone has been reported to exert multiple physiological benefits, including protective roles against osteoporosis, cardiovascular disorders, inflammatory conditions, cancer, Alzheimer's disease, diabetes, and peripheral neuropathy (Jadhav *et al*., 2022). These biosynthetic functions are relevant to food fermentation, as they contribute to stress tolerance, nutritional enhancement, and potential flavor compound formation. Importantly, strain RBU1-1^T and SKP4-8^T lack the genetic machinery required for the production of undesirable biogenic amines such as cadaverine, putrescine, histamine, and tyramine, which are commonly associated with food safety concerns. Likewise, no antibiotic biosynthesis genes were detected, thereby minimizing the risk of antimicrobial compound production during fermentation and preventing potential adverse effects associated with antibiotic contamination. Therefore, these strains can be considered biogenic amine non-producers and pose no safety concern in this aspect.

While toxin-related genes such as the magnesium and cobalt exporter (*tlyC*) and hemolysin III (*hlyIII*) were annotated in both strains, these are not indicative of pathogenicity. The *tlyC* gene plays an essential role in metal ion transport, while *hlyIII* has been found in many commercially used fermentation strains without compromising their safety (Kingkaew *et al*., 2025). Analysis using the VirulenceFinder program and DFAST annotation further confirmed the absence of major virulence determinants in both RBU1-1^T and SKP4-8^T. Common virulence-associated genes, including *esp* (enterococcal surface protein), *ace* (collagen adhesion), *sprE* (serine protease), *gelE* (gelatinase), and *cylL* (cytolysin), were absent from the genomes. Based on the CARD antibiotic resistance gene detection database, strain SKP4-8^T showed a total of 167 hits (3 strict and 164 loose), with an identity range of 19.87–100.0% (Supplementary File), while strain RBU1-1^T showed 184 hits (2 strict and 182 loose), with an identity range of 20.19–86.5% (Supplementary File). Most of these hits were classified as loose matches with low sequence identity and were mainly associated with putative transporters, efflux-related proteins, regulatory proteins, and other conserved cellular functions. Such low-identity hits, particularly those in the range of approximately 19–20%, are generally interpreted as distant homologs or conserved domains rather than evidence of functional antimicrobial resistance determinants. Although these analyses detected resistance-related genes, no complete gene clusters required for functional antibiotic resistance were identified, suggesting that the strains are unlikely to exhibit active resistance phenotypes.

Among the resistance-related genes identified, *vanY* and *vanT* were associated with vancomycin tolerance. This feature is commonly found in bacteria that produce peptidoglycan precursors ending with D-lactate rather than the usual D-alanine at the terminal position. The *vanY* gene encodes a D-Ala–D-Ala carboxypeptidase, contributing to this intrinsic mechanism of resistance. Additionally, genes implicated in resistance to cationic antimicrobial peptides were also present in the genomes. Nevertheless, the presence of antimicrobial resistance (AMR) genes at the genomic level does not necessarily indicate that the strains express phenotypic resistance (Kingkaew *et al*., 2023). This interpretation was supported by the antibiotic susceptibility assay, in which both strains were

Table 3. The beneficially compound synthesis-associated genes from strain RBU1-1^T and SKP4-8^T genome.

Strain (s)	Metabolic pathway	Gene symbol (s)	Gene product	Locus tag
RBU1-1^T	Ectoine biosynthesis (Map ID: 00260)	*ectA*	L-2,4-Diaminobutyric acid acetyltransferase [EC:2.3.1.178]	fig|6666666.1460533.peg.1674
		ectB	Diaminobutyrate-2-oxoglutarate transaminase [EC:2.6.1.76]	fig|6666666.1460533.peg.1672
				fig|6666666.1460533.peg.1673
		ectC	L-Ectoine synthase [EC:4.2.1.108]	fig|6666666.1460533.peg.1671
	Thiamine biosynthesis (Map ID: 00730)	*thiE*	Thiamine-phosphate pyrophosphorylase [EC:2.5.1.3]	fig|6666666.1460533.peg.3487
		phoA, phoB	Alkaline phosphatase [EC:3.1.3.1]	fig|6666666.1460533.peg.3914
				fig|6666666.1460533.peg.3915
				fig|6666666.1460533.peg.3916
		rsgA, engC	Thiamine phosphate phosphatase [EC:3.6.1.- 3.1.3.100]	fig|6666666.1460533.peg.347
	Tryptophan biosynthesis (Map ID: 00400)	*trpD*	Anthranilate phosphoribosyltransferase [EC:2.4.2.18]	fig|6666666.1460533.peg.917
		trpC	Indole-3-glycerol phosphate synthase [EC:4.1.1.48]	fig|6666666.1460533.peg.918
		trpE	Anthranilate synthase component I [EC:4.1.3.27]	fig|6666666.1460533.peg.914
		trpG	Anthranilate synthase component II [EC:4.1.3.27]	fig|6666666.1460533.peg.916
		trpA	Tryptophan synthase alpha chain [EC:4.2.1.20]	fig|6666666.1460533.peg.923
		trpB	Tryptophan synthase beta chain [EC:4.2.1.20]	fig|6666666.1460533.peg.922
		trpF	Phosphoribosylanthranilate isomerase [EC:5.3.1.24]	fig|6666666.1460533.peg.920
SKP4-8^T	Thiamine biosynthesis (Map ID: 00730)	*thiE*	Thiamine-phosphate pyrophosphorylase [EC:2.5.1.3]	fig|6666666.1460534.peg.2035
		rsgA, engC	Thiamine phosphate phosphatase [EC:3.6.1.- 3.1.3.100]	fig|6666666.1460534.peg.454

Table 3. Conted

Strain (s)	Metabolic pathway	Gene symbol (s)	Gene product	Locus tag
SKP4-8^T	Riboflavin biosynthesis (Map ID: 00740)	*ribE, RIB5*	Riboflavin synthase [EC:2.5.1.9]	fig|6666666.1460534.peg.2476
		ribH, RIB4	6,7-Dimethyl-8-ribityllumazine synthase [EC:2.5.1.78]	fig|6666666.1460534.peg.2478
		ribD	diaminohydroxyphosphoribosylaminopyrimidine deaminase 5-Amino-6-(5-phosphoribosylamino) uracil reductase [EC:3.5.4.26 1.1.1.193]	fig|6666666.1460534.peg.2475
		ribF	Riboflavin kinase / FMN adenylyltransferase [EC:2.7.1.26 2.7.7.2]	fig|6666666.1460534.peg.557
		ribBA	3,4-Dihydroxy 2-butanone 4-phosphate synthase / GTP cyclohydrolase II [EC:4.1.99.12 3.5.4.25]	fig|6666666.1460534.peg.2477
		ycsE, yitU, ywtE	5-Amino-6-(5-phospho-D-ribitylamino)uracil phosphatase [EC:3.1.3.104]	fig|6666666.1460534.peg.1429
	Coenzyme A biosynthesis (Map ID: 00770)	*coaE*	Dephospho-CoA kinase [EC:2.7.1.24]	fig|6666666.1460534.peg.836
		coaD, kdtB	Pantetheine-phosphate adenylyltransferase [EC:2.7.7.3]	fig|6666666.1460534.peg.400
		coaX	Type III pantothenate kinase [EC:2.7.1.33]	fig|6666666.1460534.peg.2489
		coaBC, dfp	Phosphopantothenoylcysteine decarboxylase / Phosphopantothenate---cysteine ligase [EC:4.1.1.36 6.3.2.5]	fig|6666666.1460534.peg.448
	Menaquinone biosynthesis (Map ID: 00130)	*menB*	Naphthoate synthase [EC:4.1.3.36]	fig|6666666.1460534.peg.2191
		menE	o-Succinylbenzoate---CoA ligase [EC:6.2.1.26]	fig|6666666.1460534.peg.2196
		menA	1,4-Dihydroxy-2-naphthoate polyprenyltransferase [EC:2.5.1.74]	fig|6666666.1460534.peg.2081 fig|6666666.1460534.peg.2185
		menC	o-Succinylbenzoate synthase [EC:4.2.1.113]	fig|6666666.1460534.peg.2560
		menD	2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene -1-carboxylate synthase [EC:2.2.1.9]	fig|6666666.1460534.peg.2189
		menF	Menaquinone-specific isochorismate synthase [EC:5.4.4.2]	fig|6666666.1460534.peg.2188
		ubiE	demethylmenaquinone methyltransferase / 2-methoxy -6-Polyprenyl-1,4-benzoquinol methylase [EC:2.1.1.163 2.1.1.201]	fig|6666666.1460534.peg.85
		menH	2-Succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase [EC:4.2.99.20]	fig|6666666.1460534.peg.2190
		menI, DHNAT	1,4-Dihydroxy-2-naphthoyl-CoA hydrolase [EC:3.1.2.28]	fig|6666666.1460534.peg.2184

susceptible to vancomycin under the tested conditions. Importantly, both isolates originated from traditional Thai fermented foods that have been consumed safely for generations, further supporting their overall safety and suitability as potential starter cultures.

These findings suggest that both RBU1-1^T and SKP4-8^T possess genomic and metabolic traits that are beneficial for application in fermented fish products. The identified biosynthetic pathways for amino acids, vitamins (including thiamine and riboflavin), and compatible solutes like ectoine can contribute to improved flavor development, enhanced nutritional value, and increased stress tolerance during fermentation. Moreover, their non-pathogenic genomic profiles and absence of undesirable biogenic amine biosynthesis pathways support their potential as safe and functional starter cultures for fermented fish production, consistent with the traditional safety record of Thai fermented foods.

In addition, computational pathogenicity prediction assigned strain RBU1-1^T a low probability of being a human pathogen (0.242), whereas strain SKP4-8^T was predicted to have a higher probability (0.945). As these predictions were derived solely from *in silico* analyses, they should be interpreted with caution. The elevated PathogenFinder score observed for strain SKP4-8^T is likely attributable to conserved genes that share sequence similarity with homologs present in pathogenic bacteria. Such genes are often involved in basic cellular processes, including metabolism, transport, and regulation, and do not necessarily represent virulence determinants. Importantly, no known virulence gene clusters were detected in the genome of strain SKP4-8^T, supporting the interpretation that the high score does not indicate actual pathogenic potential (Cosentino *et al*., 2013). Nevertheless, both strains exhibited gamma-hemolytic activity (i.e., non-hemolysis) on sheep blood agar (Figure 5), and no major pathogenicity-related genes were detected in their genomes. Furthermore, both strains were isolated from safe, non-hazardous sources, and no experimental evidence of pathogenic behavior has been observed to date. To further assess the safety of strains SKP4-8^T and RBU1-1^T, antibiotic susceptibility was examined using a disk diffusion assay. Both strains were susceptible to vancomycin (inhibition zone, 37±0.02 mm), indicating the absence of phenotypic resistance under the tested conditions. Although *vanY*, *vanT*, and other resistance-related genes were detected in the genomes, their presence did not translate into an antimicrobial-resistant phenotype. This finding supports the view that genomic detection of AMR-related genes alone is insufficient

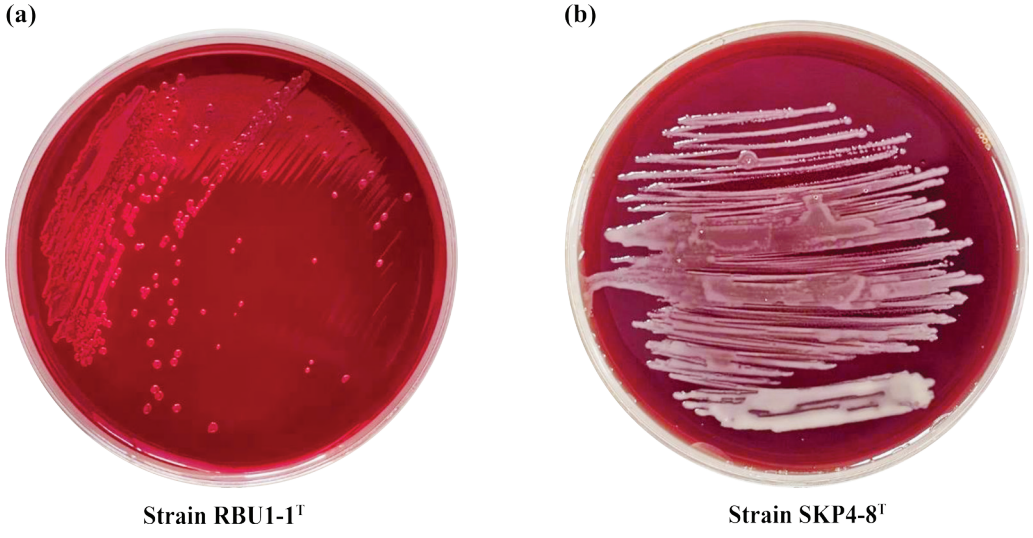


Figure 5. Hemolytic activity of strain RBU1-1^T (a) and SKP4-8^T (b) on blood agar plates.

to infer functional resistance (Cholsaktrakool *et al*., 2025). Additionally, *in vivo* studies, including acute or subchronic oral toxicity and systemic translocation assessments in animal models, would provide direct evidence to support the genomic safety findings. Taken together, the genomic evidence, the absence of virulence factors, the lack of harmful metabolite pathways, and the safe origins of these strains strongly support the conclusion that strains RBU1-1^T and SKP4-8^T are safe and technologically suitable for application as starter cultures in traditional Thai fermented foods. Among them, strain RBU1-1^T appears to have particularly strong potential, while ongoing studies will further expand our understanding of the safe application of both strains in food fermentation.

Hydrolytic enzyme activities

Strains RBU1-1^T and SKP4-8^T were identified as halophilic organisms, capable of growth across NaCl concentrations of 1–30% (w/v) and 1–16% (w/v), respectively (Yin *et al*., 2015). The occurrence of the *HtrC* gene, encoding a serine protease, together with the ectoine biosynthetic genes *ectA* (L-2,4-diaminobutyric acid acetyltransferase), *ectB* (L-2,4-diaminobutyric acid transaminase), and *ectC* (ectoine synthase), in strain RBU1-1^T, likely contributes to its ability to tolerate a wider range of salinity conditions. In contrast, strain SKP4-8^T grows optimally within a narrower salt concentration range of 1–16% NaCl (w/v). The production of hydrolytic enzymes by both strains is consistent with the salt-dependent activity profiles reported for other moderately halophilic bacteria, such as *Virgibacillus* sp. SK37, which exhibits proteolytic activity under high-salinity conditions (Sinsuwan *et al*., 2015). Initial screening on skim milk agar plates [containing 1% (w/v) skim milk and 5% (w/v) NaCl] demonstrated clear zones of casein hydrolysis surrounding colonies of both isolates cultivated on modified JCM no. 377 medium. Subsequent quantitative assays of proteolytic activity revealed maximum enzyme levels of 0.52 U·mL^{-1} for strain RBU1-1^T and 2.78 U·mL^{-1} for strain SKP4-8^T (Figure 6). These values were determined using extracellular enzyme extracts in 50 mM Tris-HCl buffer (pH 7.0) supplemented with 5% (w/v) NaCl and 1.33% (w/v) Hammarsten casein. The independent t-test ($p = 0.0002$) revealed a significant difference between mean protease activity between strain SKP4-8^T and strain RBU1-1^T.

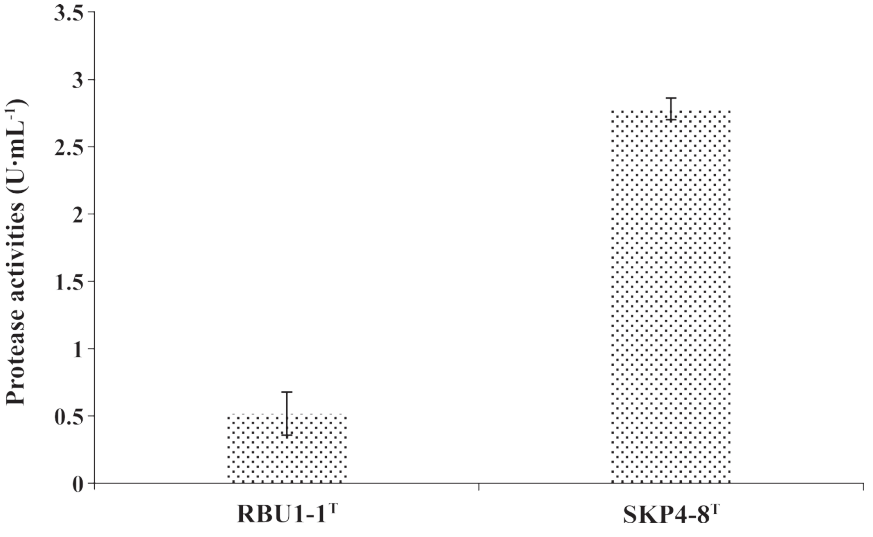


Figure 6. Protease activity of strains RBU1-1^T and SKP4-8^T cultivated in modified JCM medium supplemented with 5% NaCl (w/v) for 2 days at 37 °C.

Note: The independent t-test ($p<0.05$) revealed a significant difference in mean protease activity between the two strains.

Genomic analysis revealed distinct differences in protease gene content. Strain SKP4-8^T carried five protease genes, including serine peptidase (S8), prolyl oligopeptidase (S9), neutral Zn metallopeptidase (neprilysin), Zn metallopeptidase, and a trypsin-like peptidase. In contrast, strain RBU1-1^T contained only one protease gene, encoding bacillopeptidase F. These genomic profiles are consistent with the observed higher protease activity in strain SKP4-8^T, suggesting a positive correlation between protease gene diversity and enzymatic function.

In addition, both strains encoded a serine hydrolase gene. Enzyme activity assays using *p*-nitrophenyl palmitate (*p*-NPP) as the substrate demonstrated serine hydrolase activities of 0.72 U·mL^{-1} for SKP4-8^T and 0.51 U·mL^{-1} for RBU1-1^T at 2.5% (w/v) NaCl (Figure 7). An independent t-test showed no significant difference in serine hydrolase activity between strains RBU1-1^T and SKP4-8^T ($p = 0.5486$). Together, these results indicate that the proteolytic and lipolytic enzymes of both strains remain active under saline conditions, consistent with the salt-tolerant enzymatic profiles characteristic of halophilic bacteria (Sinsuwan *et al*., 2015).

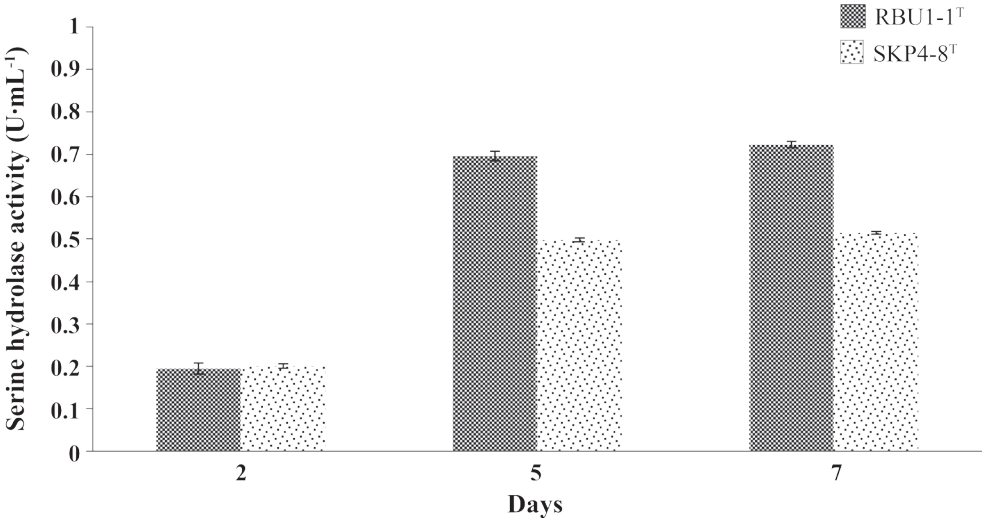


Figure 7. Serine hydrolase activity of strains RBU1-1^T and SKP4-8^T cultivated in modified JCM medium supplemented with 5% NaCl (w/v) for 7 days at 37 °C.
Note: Non-significant difference was detected (independent t-test, $p \geq 0.05$).

CONCLUSIONS

Genomic and functional analyses of *P. salipiscarius* RBU1-1^T and *A. salarius* SKP4-8^T, isolated from Thai fermented foods, confirmed their taxonomic distinctiveness and technological potential. Strain RBU1-1^T represents a novel species, whereas strain SKP4-8^T also showed clear genomic divergence from its closest relatives. Both strains encode hydrolytic enzymes and secondary metabolite pathways, with RBU1-1^T harboring ectoine biosynthetic and stress-response genes and SKP4-8^T exhibiting higher protease activity. Their predicted ability to synthesize functional metabolites, including ectoine, tryptophan, and thiamine in RBU1-1^T, and thiamine, riboflavin, coenzyme A, and menaquinone in SKP4-8^T, supports their potential postbiotic and nutritional value.

Safety assessment revealed the absence of biogenic amine biosynthesis genes, antibiotic biosynthesis pathways, and major virulence determinants. Although some antimicrobial resistance-associated genes were detected, no

complete resistance gene clusters were identified, indicating a low likelihood of functional resistance. This interpretation was supported by phenotypic assays, in which both strains exhibited gamma-hemolysis and remained susceptible to vancomycin under the tested conditions.

Overall, the genomic and functional traits of both strains support their potential application in fermented fish products. Their hydrolytic enzymes may enhance protein degradation and flavor development, while the biosynthesis of vitamins and postbiotic metabolites may improve the nutritional quality and stability of fermentation. Collectively, these findings support strains RBU1-1^T and SKP4-8^T as safe, halophilic, and promising starter culture candidates for traditional Thai fermented foods, particularly fermented fish products.

ACKNOWLEDGEMENTS

This research was supported by the Grant for International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund (CUGRP-61-01-33-01), Chulalongkorn University. We thank the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University for facilities.

LITERATURE CITED

Alcock, B.P., A.R. Raphenya, T.T.Y. Lau, *et al*. 2020. CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. **Nucleic Acids Research** 48(1): 517–525. DOI: 10.1093/nar/gkz935.

Aziz, R.K., D. Bartels, A.A. Best, *et al*. 2008. The RAST server: rapid annotations using subsystems technology. **BMC Genomics** 9(1): 1–15. DOI: 10.1186/1471-2164-9-75.

Bachovchin, D.A. and B.F. Cravatt. 2012. The pharmacological landscape and therapeutic potential of serine hydrolases. **Nature Reviews Drug Discovery** 11(1): 52–68. DOI: 10.1038/nrd3620.

Bankevich, A., S. Nurk, D. Antipov, *et al*. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. **Journal of Computational Biology** 19(5): 455–477. DOI: 10.1089/cmb.2012.0021.

Beath, A. and M.P. Jones. 2018. Guided by the research design: Choosing the right statistical test. **The Medical Journal of Australia** 208(4): 163–165. DOI: 10.5694/mja17.00422.

Bortolaia, V., R.S. Kaas, E. Ruppe, *et al*. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. **Journal of Antimicrobial Chemotherapy** 75(12): 3491–3500. DOI: 10.1093/jac/dkaa345.

Chamroensaksri, N., S. Tanasupawat, A. Akaracharanya, W. Visessanguan, T. Kudo and T. Itoh. 2010. *Gracilibacillus thailandensis* sp. nov., from fermented fish (*pla-ra*). **International Journal of Systematic and Evolutionary Microbiology** 60(4): 944–948. DOI: 10.1099/ijs.0.011981-0.

Cosentino, S., M. Voldby Larsen, F. Møller Aarestrup and O. Lund. 2013. PathogenFinder: distinguishing friend from foe using bacterial whole-genome sequence data. **PLoS ONE** 8(10): e77302. DOI: 10.1371/journal.pone.0077302.

Cholsaktrakool, P., K. Kawang and N. Sangpiromapichai, *et al*. 2025. Inference of antimicrobial resistance (AMR) from a whole genome database outperforming AMR gene detection. **iScience** 28(8): 112962. DOI: 10.1016/j.isci.2025.112962.

Daroonpunt, R., M. Uchino, Y. Tsujii, M. Kazami, D. Oka and S. Tanasupawat. 2016. Chemical and physical properties of Thai traditional shrimp paste (*ka-pi*). **Journal of Applied Pharmaceutical Science** 6(4): 58–62. DOI: 10.7324/JAPS.2016.60509.

Djordjevic, S., P.N. Goudreau, Q. Xu, A.M. Stock and A.H. West. 1998. Structural basis for methylesterase *CheB* regulation by a phosphorylation-activated domain. **Proceedings of the National Academy of Sciences of the United States of America** 95(4): 1381–1386. DOI: 10.1073/pnas.95.4.1381.

Flammersfeld, A., A. Panyot, Y. Yamaryo-Botté, P. Aurass, J.M. Przyborski, A. Flieger, C. Botté and G. Pradel. 2020. A patatin-like phospholipase functions during gametocyte induction in the malaria parasite *Plasmodium falciparum*. **Cellular Microbiology** 22(3): e13146. DOI: 10.1111/cmi.13146.

Grant, J.R., E. Enns, E. Marinier, A. Mandal, E.K. Herman, C.Y. Chen, M. Graham, G. Van Domselaar and P. Stothard. 2023. Proksee: in-depth characterization and visualization of bacterial genomes. **Nucleic Acids Research** 51(1): 484–492. DOI: 10.1093/nar/gkad326.

Hiraga, K., Y. Nishikata, S. Namwong, S. Tanasupawat, K. Takada and K. Oda. 2005. Purification and characterization of serine proteinase from a halophilic bacterium, *Filobacillus* sp. RF2-5. **Bioscience, Biotechnology and Biochemistry** 69(1): 38–44. DOI: 10.1271/bbb.69.38.

Hajep, P. and S. Jinap. 2012. Fermented shrimp paste products as source of umami in Southeast Asia. **Journal of Nutrition and Food Sciences** S10: 006. DOI: 10.4172/2155-9600.S10-006.

Humphries, R., A.M. Bobenchik, J.A. Hindler and A.N. Schuetz. 2021. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. **Journal of Clinical Microbiology** 59(12): 1110–1128.

Jadhav, N., S. Ajgaonkar, P. Saha, *et al*. 2022. Molecular pathways and roles for vitamin K_2-7 as a health-beneficial nutraceutical: challenges and opportunities. **Frontiers in Pharmacology** 13: 896920. DOI: 10.3389/fphar.2022.896920.

Johan, U.U.R., R.N.Z.R. Abd, N.H.A. Kamarudin and M.S.M. Ali. 2021. An integrated overview of bacterial carboxylesterase: structure, function and biocatalytic applications. **Colloids and Surfaces B: Biointerfaces** 205: 111882. DOI: 10.1016/j.colsurfb.2021.111882.

Kanehisa, M., Y. Sato and K. Morishima. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. **Journal of Molecular Biology** 428(4): 726–731. DOI: 10.1016/j.jmb.2015.11.006.

Kang, J.Y., B. Lee, J.A. Kim, M.-S. Kim and C.H. Kim. 2022. Identification and characterization of an ectoine biosynthesis gene cluster from *Aestuariispira ectoiniformans* sp. nov., isolated from seawater. **Microbiological Research** 254: 126898. DOI: 10.1016/j.micres.2021.126898.

Kemp, L.E., M. Rusch, A. Adibekian, *et al*. 2013. Characterization of a serine hydrolase targeted by acyl-protein thioesterase inhibitors in *Toxoplasma gondii*. **Journal of Biological Chemistry** 288(38): 27002–27018. DOI: 10.1074/jbc.M113.460709.

Kingkaew, E., W. Woraprayote, A. Booncharoen, K. Niwasabutra, T. Janyaphisan, R.-K. Vilaichone, Y. Yamaoka, W. Visessanguan and S. Tanasupawat. 2023. Functional genome analysis and anti-*Helicobacter pylori* activity of a novel bacteriocinogenic *Lactococcus* sp. NH2-7C from Thai fermented pork (*nham*). **Scientific Reports** 13(1): 20362. DOI: 10.1038/s41598-023-47687-4.

Kingkaew, E., W. Woraprayote, J. Sitdhipol, R.K. Vilaichone, W. Visessanguan and S. Tanasupawat. 2025. Genomic assessment, metabolic profile mapping, and anti-*Helicobacter pylori* activity of *Lactococcus lactis* SK2-659 from Thai fermented green mustard (*pak-kad-dong*). **Probiotics and Antimicrobial Proteins** 17: 1–17. DOI: 10.1007/s12602-025-10505-6.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. **Journal of Biological Chemistry** 193(1): 265–275. PMID: 14907713.

Meier-Kolthoff, J.P., A.F. Auch, H.P. Klenk and M. Göker. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. **BMC Bioinformatics** 14: 1–14. DOI: 10.1186/1471-2105-14-60.

Namwong, S., S. Tanasupawat, K.C. Lee and J.S. Lee. 2009. *Oceanobacillus kapialis* sp. nov., from fermented shrimp paste in Thailand. **International Journal of Systematic and Evolutionary Microbiology** 59(9): 2254–2259. DOI: 10.1099/ijs.0.007161-0.

Pliego, J., J. Mateos, J. Rodriguez, F. Valero, M. Baeza, R. Femat, R. Camacho, G. Sandoval and E. Herrera-López. 2015. Monitoring lipase/esterase activity by stopped flow in a sequential injection analysis system using *p*-nitrophenyl butyrate. **Sensors** 15(2): 2798–2811. DOI: 10.3390/s150202798.

Richter, M., R. Rosselló-Móra, F.O. Glöckner and J. Peplies. 2016. JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. **Bioinformatics** 32: 929–931. DOI: 10.1093/bioinformatics/btv681.

Sangjindavong, M., P. Chuapoehuk, J. Runglerdkriangkrai, W. Klaypradit and D. Vareevanich. 2008. Fermented fish product (*pla-ra*) from marine fish and preservation. **Kasetsart Journal - Natural Science** 42: 129–136.

Schmidt, M., T. Proctor, R. Diao and L. Freddolino. 2022. *Escherichia coli* YigI is a conserved gammaproteobacterial acyl-CoA thioesterase permitting metabolism of unusual fatty acid substrates. **Journal of Bacteriology** 204(8): e0001422. DOI: 10.1128/jb.00014-22.

Seemann, T. 2014. Prokka: rapid prokaryotic genome annotation. **Bioinformatics** 30: 2068–2069. DOI: 10.1093/bioinformatics/btu153.

Sinsuwan, S., A. Jangchud, S. Rodtong, S. Roytrakul and J. Yongsawatdigul. 2015. Statistical optimization of the production of NaCl-tolerant proteases by a moderate halophile, *Virgibacillus* sp. SK37. PMID: 27904342. **Food Technology and Biotechnology** 53(2): 136–145.

Tanasupawat, S., S. Namwong, T. Kudo and T. Itoh. 2007. *Piscibacillus salipiscarius* gen. nov., sp. nov., a moderately halophilic bacterium from fermented fish (*pla-ra*) in Thailand. **International Journal of Systematic and Evolutionary Microbiology** 57(Pt 7): 1413–1417. DOI: 10.1099/ijs.0.64945-0.

Tanizawa, Y., T. Fujisawa and Y. Nakamura. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. **Bioinformatics** 34(6): 1037–1039. DOI: 10.1093/bioinformatics/btx713.

Wei, T., M. Quareshy, Y.Z. Zhang, D.J. Scanlan and Y. Chen. 2018. Manganese is essential for PlcP metallophosphoesterase activity involved in lipid remodeling in abundant marine heterotrophic bacteria. **Applied and Environmental Microbiology** 84(15): e01109–18. DOI: 10.1128/AEM.01109-18.

Xingrong, L., B.M.T. Gorish, M.A. Qaria, A. Hussain, W.I.Y. Abdelmula and D. Zhu. 2025. Unlocking ectoine's postbiotic therapeutic promise: mechanisms, applications, and future directions. **Probiotics and Antimicrobial Proteins** 17: 1–26. DOI: 10.1007/s12602-025-10506-5.

Yiamsombut, S., P. Kanchanasin, W. Phongsopitanun, N. Kuncharoen, A. Savarajara, W. Shi, L. Wu, J. Ma and S. Tanasupawat. 2022. *Allobacillus salarius* sp. nov. and *Allobacillus saliphilus* sp. nov., isolated from shrimp paste (*ka-pi*) in Thailand. **Archives of Microbiology** 204(1): 71. DOI: 10.1007/s00203-021-02694-9.

Yin, J., J.C. Chen, Q. Wu and G.Q. Chen. 2015. Halophiles, coming stars for industrial biotechnology. **Biotechnology Advances** 33(7): 1433–1442. DOI: 10.1016/j.biotechadv.2014.10.008.