Original Article

JHSMR Journal of Health Science and Medical Research

Phenotypic and Genomic Analysis of Enterococcus thailandicus MEDPSU_PRO_001 Isolated from Chicken Feces, a Potential Probiotic with Aggregation Ability

Thanchanok Muangkaew, B.Sc.[1], Kamonnut Singkhamanan, Ph.D.[1], Monwadee Wonglapsuwan, Ph.D.[2], Chonticha Romyasamit, Ph.D.[3], Sirikan Suwannasin, M.Sc.[1], Ei Phway Thant, M.Sc.[1], Komwit Surachat, Ph.D.[1,4]

[1]Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

[2]Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

[3]Department of Medical Technology, School of Allied Health Sciences, Walailak University, Thasala, Nakhon Si Thammarat 80161, Thailand.

[4]Translational Medicine Research Center, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

Received 30 August 2024 ● Revised 4 September 2024 ● Accepted 4 September 2024 ● Published online 25 February 2025

Abstract:

Objective: This study aimed to explore the genotypic and phenotypic of *Enterococcus thailandicus* MEDPSU_PRO_001, a strain isolated from chicken feces, as a probiotic candidate through comprehensive *in silico* and *in vitro* analysis.

Material and Methods: This study examined the genetic characteristics of *E. thailandicus* MEDPSU_PRO_001 by using Oxford nanopore long-read sequencing sequences to identify significant genetic details associated with probiotic potential and pathogenicity; such as virulence factor genes and antibiotic-resistant genes, and the findings were supported by phenotypic assays.

Results: *E. thailandicus* MEDPSU_PRO_001, with a genome size of 2,771,294 bp, was analyzed for its probiotic potential. No antimicrobial resistance genes were identified, with a cutoff value of 80% identity; although *vanY* (33.91% identity) and *aac(6')-Ii* (72.63% identity) genes were detected. Phenotypic studies confirmed the strain's lack of resistance to vancomycin. Furthermore, genes associated with adherence; including *ebpA*, *ebpB*, *ebpC*, *srtC*, *ecbA*, and *efaA* and EPS cluster, were identified. This highlights their role in the adherence and aggregation of probiotic strains that were

This paper was from The 3rd Annual Health Research International Conference (AHR-iCON, August 29-30, 2024).
Contact: Komwit Surachat, Ph.D.
Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
E-mail: komwit.s@psu.ac.th

J Health Sci Med Res
doi: 10.31584/jhsmr.20251157
www.jhsmr.org

supported by phenotypic assays against probiotic and pathogens. Additionally, the presence of bacteriocin-encoding gene Laps was noted. No hemolysis activity supports the safety of this strain.

Conclusion: This comprehensive genomic analysis of *E. thailandicus* MEDPSU_PRO_001 reveals its beneficial characteristics; such as aggregation ability, as a potential probiotic. This study provides valuable insights into the probiotic nature of this strain, underscoring the safe utilization of *E. thailandicus*.

Keywords: bioinformatics, *Enterococcus thailandicus*, pathogen, probiotic

Introduction

Probiotic organisms, as defined by the World Health Organization (WHO), are live microorganisms that, when administered in sufficient quantities, provide health benefits to the host[1]. These benefits can include antibacterial activities against pathogens, balancing the gut microbiota, regulating the immune system, synthesizing vitamins and essential amino acids, and digesting complex carbohydrates[2].

In addition to these health advantages, probiotic strains must possess fundamental abilities to be defined as probiotics. They should be able to compete, adhere, persist, and endure the challenging conditions of the gastrointestinal tract (GIT), such as bile salts, stomach enzymes, and low pH, by harboring genes related to these properties[3]. Moreover, effective probiotics must be safe for the host[4].

The *Enterococcus* genus, classified within the Firmicutes phylum, has the ability to survive under various environmental conditions[5]. It constitutes a significant portion of the gut microbiota in humans and various animal species, playing a vital role in intestinal health. Even though certain *in vitro* tests, such as evaluating their survival in GIT, adherence, and antimicrobial efficacy, are commonly used for probiotic analysis, relying solely on phenotypic characterization is insufficient for a comprehensive understanding of probiotic microorganisms, according to the European Food Safety Authority (EFSA) guideline[6]. Exploring genomic data from high-throughput DNA sequencing can enhance our understanding of probiotic strains by identifying genes linked to probiotic properties, potential virulence, and additional chemicals and metabolic pathways that improve strain effectiveness.

E. thailandicus was reported as a novel strain by Tanasupawat *et al.* (2008) and reported in prior studies[7]; however, detailed genomic insights and related safety information remain limited. Therefore, this study aimed to characterize the genome of *E. thailandicus* MEDPSU_PRO_001 to validate its potential health benefits as a probiotic strain. The findings from this study could facilitate the safe utilization of *E. thailandicus* in probiotic formulations and contribute to its development in various industries, including food, pharmaceuticals, and biotechnology.

Material and Methods

Bacterial strains

We screened several strains of *Enterococcus* species from different sources and found that *E. thailandicus* MEDPSU_PRO_001, obtained from chicken feces in Songkhla, Thailand, exhibited superior probiotic capabilities, such as tolerance to acidic and bile conditions, enhanced adhesion to intestinal cells, and strong inhibition of pathogens. This strain was then selected for phenotypic and genomic analysis to understand its beneficial traits and probiotic potential. *Staphylococcus aureus* ATCC 29213 was purchased from the American Type Culture

Collection (ATCC) and was grown at 37 °C for 16–18 hours in Trypticase soy agar (TSA) (HIMEDIA, India) and sub-cultured in TSA broth at 37 °C for 6 hours and stored at -80 °C in 15% glycerol.

Assessment of phenotypic safety and probiotic properties

Vancomycin susceptibility test

The antibiotic susceptibility of *E. thailandicus* MEDPSU_PRO_001 was assessed using the broth microdilution method[8]. The bacterial suspension was prepared in BHI broth and adjusted to 0.5 McFarland standard (approximately 10^8 CFU/mL), then further diluted to a 1:100 dilution (10^6 CFU/mL) for initial seeding. Subsequently, 100 µL of this inoculum was mixed with 100 µL of varying concentrations of vancomycin (ranging from 0.5 to 256 µg/mL) in a 96-well plate. Untreated control contained *E. thailandicus* MEDPSU_PRO_001 without vancomycin. The plates were incubated at 37 °C for 16-18 hours. The minimum inhibitory concentration (MIC) was determined. For quality control, *S. aureus* ATCC 29213 was used. Results were interpreted according to the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines.

Hemolysis assay

E. thailandicus MEDPSU_PRO_001 was streaked on Blood agar (HIMEDIA, India) containing 5% blood. After incubation at 37 °C for 48 hours, hemolysis activity was determined[9]. *S. aureus* ATCC 25213, known to exhibit β-hemolysis, was used to validate the hemolysis assay.

Auto-aggregation and co-aggregation

The ability of the isolates to auto-aggregate was assessed using the method outlined by[10]. The auto-aggregation percentage was calculated with the following formula:

$$\text{Auto-aggregation\%} = 1 - (At/A_0) \times 100$$

At means absorbance at time t, while A_0 denotes absorbance at t=0.

Cell suspensions were prepared for co-aggregation using the same method as for the auto-aggregation analysis[10] with slight modification. Equal volumes of the cell suspensions of *E. thailandicus* and the pathogen of all strains, including *E. coli* and *A. baumannii,* were mixed in a cuvette, and absorbance was measured at OD600 using UV VIS Spectrophotometer (Shimadzu, Japan) (designated as A_0). The mixture was then incubated at 37 °C, and the absorbance was measured at 2 hours, 4 hours, and 24 hours (designated as At). The percentage of co-aggregation was calculated using the equation from[11].

$$\text{Co-aggregation\%} = (A_0 - At)/A_0 \times 100$$

Genomic DNA extraction

Genomic DNA of *E. thailandicus* MEDPSU_PRO_001 was extracted with the ZymoBIOMIC™ DNA Miniprep Kit, followed by the manufacturer's instructions. The concentration and purification of extracted gDNA were evaluated using a Nanodrop (Thermo Fisher Scientific, Waltham, MA, United States). DNA purity and quantity were confirmed using agarose gel electrophoresis.

Whole genome sequencing

The gDNA of *E. thailandicus* MEDPSU_PRO_001 was subjected to long-read whole-genome sequencing (WGS) using the Oxford Nanopore Technologies (ONT) system with the MinION Mk1c device (Oxford Nanopore).

Genome assembly, annotation and sequence analysis

FASTQC (version 0.11.9) was used to estimate the read quality, while low-quality reads were removed with FASTp[12] *De novo* assembly process executed with Unicycler

v0.5.0[13]. Genome annotation was performed using Prokka v.1.12[14]. Rapid Annotations with the Subsystems Technology (RAST)[15] were also used to predict functional annotations. The BAGEL4 (http://bagel4.molgenrug.nl/databases.php)[16] and antiSMASH version 7.0[17] tools were utilized to identify the clusters responsible for the biosynthesis of bacteriocin and secondary metabolites, respectively[18].

Comparative genomic analysis

A pangenome study was conducted by using rotary[19] against the available sequences from NCBI database and phylogenetic tree was constructed.

Virulence factor and antibiotic resistance genes

The Virulence Factors Database (VFDB)[20] was used to identify virulence factors. The Comprehensive Antibiotic Resistance Database (CARD)[21], used to identify antibiotic resistance genes with a minimum length of 60% and 80% identity criteria, was used to identify mobile genetic elements[22].

Results

Assessment of phenotypic safety and probiotic properties

Antibiotic susceptibility

The MIC value for vancomycin in *E. thailandicus* MEDPSU_PRO_001 was 1 μg/ml, indicating its susceptibility to the antibiotic (Table 1).

Hemolysis assay

Hemolysis activity was determined by streaking the bacteria on blood agar containing 5% blood. Gamma-hemolysis was shown after 48 hours of incubation (Table 2).

Auto-aggregation and co-aggregation

The results of the auto-aggregation assay showed higher levels of auto-aggregation after 24 hours compared to 2 hours of incubation (Figure 1). The auto-aggregation after 24 hours was 36.98% for *E. thailandicus* and 28.84% for *E. faecalis* 1003. Table 3 represented the results of the co-aggregation of *E. thailandicus* with *E. coli* and *A. baumannii* separately at 37 °C after 2, 4, and 24 hours of incubation, amounting to 34.180% and 30.986%, respectively.

Genome features

The genetic characteristics of *E. thailandicus* MEDPSU_PRO_001 were illustrated with the creation of its genome map (Figure 1 and Table 1). With a GC level of 36.80%, the total genome length of *E. thailandicus* MEDPSU_PRO_001 was 2,771,294 base pairs. A total of 2,496 protein-coding sequences (CDS) were discovered. In addition, there are 221 subsystems, 2593 coding sequences, 68 tRNAs, 18 rRNAs, and a total of RNAs.

Table 1 Vancomycin resistance profile of the *E. thailandicus* PSU_MED_PRO_001

Antibiotic	MIC (μg/ml)	Interpretation
Vancomycin	1	S

Table 2 Blood agar result and interpretation of *E. thailandicus* PSU_MED_PRO_001

Result	Interpretation	Symbol
No change in the medium	Organism dose not hemolyze red blood cell	Gamma-hemolysis

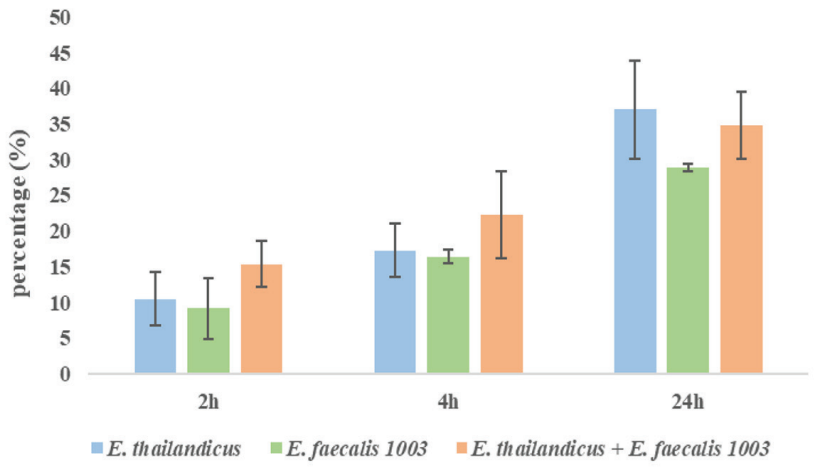

Figure 1 The percentage of auto-aggregation for *E. thailandicus*, *E. faecalis* 1003 and co-aggregation of *E. thailandicus* with *E. faecalis* 1003 at 2, 4 and 24 hours

Table 3 Co-aggregation (%) of *E. thailandicus* PSU_MED_PRO_001 with 2 pathogens during 2, 4, and 24 hours incubation at 37 °C

Bacteria	Time (hour)	Pathogen bacteria	
		E. coli	***A. baumannii***
E. thailandicus	2	6.455	0.805
	4	11.523	8.250
	24	34.180	30.986

Table 4 *E. thailandicus* MEDPSU_PRO_001 genome features

General features	Value
Genome size (bp)	2,771,294
GC content (%)	36.80
rRNA	18
tRNA	68
CDS	2,496
Number of Subsystems	221
Number of Coding Sequences	2,593
Number of RNAs	86

CDS=Coding sequence

Functional characterization

The RAST functional annotation study provides a detailed overview of the genome annotation and subsystem distribution for the bacterial species, as analyzed. The vertical bar chart on the left highlights the subsystem coverage, showing that 73% of the gens have been successfully assigned to specific subsystems, indicating a high level of functional characterization, while 27% remain unclassified, suggesting areas for further investigation. The central pie chart illustrates the distribution of functional categories within the annotated genome, with each segment representing a different subsystem category as shown in Figure 3.

Exopolysaccharide (EPS) production genes

After an extensive analysis of an EPS biosynthesis cluster next to the *epsH* gene, a cluster of 32307 bps was found in the studied strains (Figure 4)[23], which includes crucial elements related to synthesis, modification, regulation, and export systems[24]. The synthesis of EPS is crucial for several functions, including assisting in adhesion to host intestinal cells, preventing the colonization of pathogenic organisms, and acting as a prebiotic[25-27].

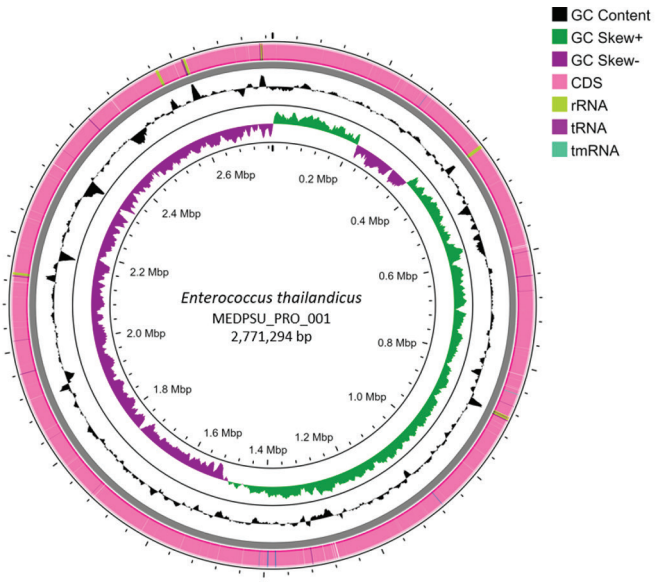

Figure 2 *E. thailandicus* MEDPSU_PRO_001 circular genome map. The highlights essential genomic properties, including GC content and GC skew, illustrated by black line graphs and colored portions. Demonstration with colored bars, the functional genetic elements such as ribosomal RNA (rRNA), transfer RNA (tRNA), coding sequences (CDS), and transfer–messenger RNA (tnrRNA) reveal details about the genetic makeup of *E. thailandicus*

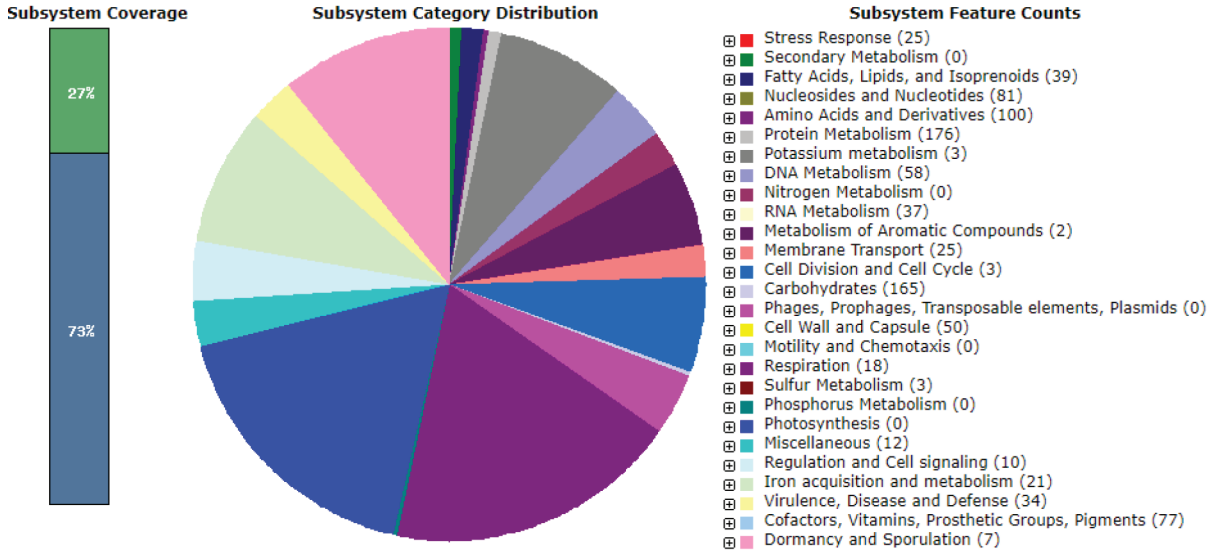

Figure 3 The subsystem coverage and distribution of functional categories within the annotated genome of the bacterial species, as analyzed using RAST

Bacteriocin-encoding genes

Bagel4 analysis revealed several potential bacteriocin-encoding genes in *E. thailandicus* MEDPSU_PRO_001 (Figure 5). It was found that Linear Azole-containing Peptides (LAPs), antibacterial peptides, are a subclass of class I bacteriocins characterized by their inclusion of various heterocyclic rings, such as thiazole and (methyl) oxazole. These rings are formed through an ATP-dependent cyclodehydration process followed by a flavin mononucleotide-dependent dehydrogenation of the amino acid cysteine, serine, and threonine[28]. Additionally, other genes linked to biosynthesis were present in their operons, including modification genes (LapBotD and cyclodehydration enzyme) and ABC transporters[29].

Pan-genome analysis and phylogenetic tree

Pan-genome analysis and phylogenetic tree represent a distribution of gene clusters among 27 different strains. On the left side of the figure, the phylogenetic tree illustrates the evolutionary relationships among the strains *E. thailandicus* MEDPSU_PRO_001 and 26 strain of *E. thailandicus* from The National Center for Biotechnology Information (NCBI), where each branch points (node). The right side of the figure showcases a presence-absence matrix comprising 5955 gene clusters, where each row corresponds to one of the 27 strains and each column represents a distinct gene cluster. In this matrix, light blue indicates a specific gene cluster within a strain, whereas white denotes its absence[30], demonstrating the 1946 core genes and 43 accessory genes (Figure 6).

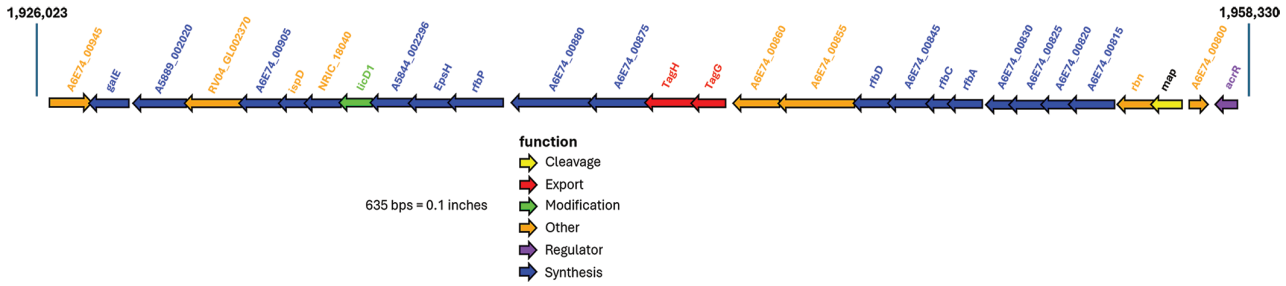

Figure 4 Illustration of EPS gene clusters found in *E. thailandicus* MEDPSU_PRO_001 (Gene functions were defined by using Uniprot)[23]

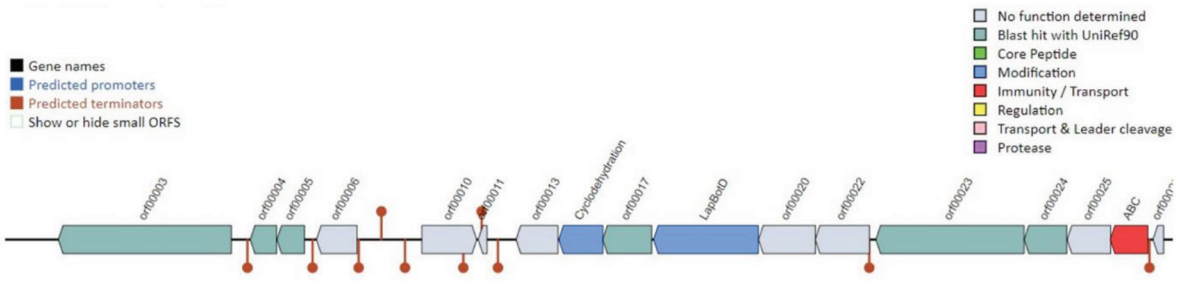

Figure 5 Genomic organization of bacteriocin-related genes in *E. thailandicus* from Begel 4

Figure 6 Phylogenetic tree and presence–absence matrix of 27 strains. Phylogenetic tree illustrating the evolutionary relationships among 27 strains (left) and a presence–absence matrix of 5955 gene clusters (right)

Virulence factor and antibiotic resistance

This study elucidates the virulence factors with their associated functions, providing critical insights into bacterial pathogenicity and the characteristics of probiotics. The key functions of identified genes such as *ebpA*, *ebpB*, *ebpC*, *srtC*, *ecbA*, and *efaA* are categorized into adherence, as shown in Table 5.

Table 5 Lists the virulence factors and genes from the virulence factor database analysis revealed were present in *E. thailandicus*

Function	Virulence factors	Related genes
Adherence	Ebp pili, EcbA, EfaA	*ebpA, ebpB, ebpC, srtC, ecbA, and efaA*

The analysis of antimicrobial resistance genes exhibited the gene *vanY* with 33.91% identity, which is located within the *vanB* cluster and is part of the glycopeptide resistance gene family. Glycopeptide antibiotics, such as vancomycin and teicoplanin, are critical for treating severe infections caused by Gram–positive bacteria, especially those resistant to other antibiotic classes. In addition, *vanY* gene is responsible for modifying the terminal residues of peptidoglycan precursors in the bacterial cell wall. The second gene we identified was *aac(6')–Ii*, the AAC (6') family, which is known for its role in aminoglycoside resistance. The *aac(6')–Ii* gene has a matching region with 72.63% identity as shown in Table 6. There was no mobile genetic element that could transfer to other bacteria or pathogens in our studied strain.

Table 6 Lists of the antibiotic resistance and genes from the comprehensive antibiotic resistance database revealed were present in *E. thailandicus*

Gene	AMR gene family	Drug class	Resistance mechanism	% Identity of matching region
vanY gene in *vanB* cluster	*vanY*, glycopeptide resistance gene cluster	glycopeptide antibiotic	antibiotic target alteration	33.91
AAC(6')–Ii	*AAC(6')*	aminoglycoside antibiotic	antibiotic inactivation	72.63

Discussion

Throughout history, *Enterococcus* species that are commensals in the GI tract have been shown to be safe and useful[10]. However, they developed sometimes as opportunistic pathogens, causing a range of infections, particularly in immunocompromised individuals or those with underlying health conditions[31]. Therefore, the characterization of both phenotype and genotype is important to evaluate the virulence of *Enterococcus* species[32]. This study provides significant insights into the phenotype and genotype of *E. thailandicus* MEDPSU_PRO_001. Antibiotic resistance genes in *Enterococcus* species are frequently associated with plasmids or transposons, increasing the potential for horizontal gene transfer[33]. In the case of *E. thailandicus* MEDPSU_PRO_001, a low level of antimicrobial resistance genes was identified, and no mobile elements were detected, indicating a low risk of gene transfer to other pathogens. Phenotypic testing also demonstrated sensitivity to vancomycin. Vancomycin is normally used as a first-line defense against *Enterococcus*. Therefore, identifying VRE should be addressed, implemented, and prevented in clinical settings because treatment failures may occur if *Enterococcus* is vancomycin-resistant[34]. The discovery of the vanY gene in the vanB cluster of vancomycin-sensitive *E. thailandicus* is intriguing and warrants further investigation. Although vancomycin resistance genes typically suggest potential resistance, our results showed sensitivity, indicating a possible discordance between genotypes and phenotypes. This may be due to an inactive vanY gene, mutations, regulatory factors, or the genetic context of the vanB cluster. Testing different strains and conditions, along with genomic and transcriptomic analyses, is essential to understand why *E. thailandicus* is sensitive to vancomycin despite having resistance genes[35]. These findings are consistent with a previous study demonstrating the sensitivity of *E. thailandicus* TC1 to vancomycin[36]. Additionally, the absence of virulence factors, responsible for the colonization, invasion, and the development of pathogenic alterations in bacterial infections are crucial to develop the probiotic bacteria. Previous investigations revealed that cytohemolysin (cyl), aggregates (as), and gelatinases were the main virulence characteristics of *Enterococcus* species[37,38]. *E. thailandicus* MEDPSU_PRO_001 demonstrated only genes related to adherence. that is important for adhering to the intestinal cells to thrive, colonize, and stay inside the host. Moreover, probiotic *Enterococcus* species from prior studies have been found to include genes related to adherence[39], and research on *E. faecium* also revealed that the presence of *efaA* and *srtC* genes does not necessarily indicate virulence traits[40,41].

Moreover, the exhibition of the EPS gene cluster suggests great adherence and aggregation abilities, supported by their aggregation characteristics. *E. thailandicus* exhibited the ability to co-aggregate with *E. faecalis* 1003, which aids the survival of beneficial bacteria under stress and hostile conditions and enhances the antibacterial activity of co-incubated probiotic bacteria in a micro-environment[27]. The co-aggregation with pathogens in this study was concordant with the previous studies exploring that co-aggregation with pathogens was caused by interactions between lectin and carbohydrates as well as proteinaceous elements on the cell surface and this property serves as a protective barrier to prevent the invasion of harmful bacteria[42]. Additionally, no hemolysin production gene and no hemolysis zone support the safety of our strain in contrast to *E.thailandicus* TC1, a possible opportunistic pathogen by alpha-hemolysin production[36]. The identified bacteriocin-encoding genes, such as Linear Azole-containing Peptides (LAPs), potentially exhibit antibacterial activity against pathogens, although phenotypic assessment of bacteriocin activity was limited in our study. However, this research provides fundamental phenotypic

and genomic characteristics of *E. thailandicus* MEDPSU_PRO_001, supporting its potential application as a probiotic in the health industry.

Conclusion

The study revealed no harmful traits, such as virulence factors or significant antibiotic resistance genes and resistant profile. Additionally, *E. thailandicus* MEDPSU_PRO_001 demonstrated good probiotic properties, including the ability to co-aggregate with both beneficial and pathogenic bacteria and bacteriocin production. Overall, the phenotypic, genomic analysis and safety assessment of *E. thailandicus* MEDPSU_PRO_001 supports its potential as a probiotic candidate for further applications in clinical settings.

Acknowledgement

This research was funded by the National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University, grant number MED6701384Mc.

References

1. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 2024;11:4745-67. doi: 10.3390/ijerph110504745.
2. Zhang YJ, Li S, Gan RY, Zhou T, Xu DP, Li HB. Impacts of gut bacteria on human health and diseases. Int J Mol Sci 2015;16:7493-519. doi: 10.3390/ijms16047493.
3. Ghattargi VC, Gaikwad MA, Meti BS, Nimonkar Y, Dixit K, Prakash O, et al. Comparative genome analysis reveals key genetic factors associated with probiotic property in *Enterococcus faecium* strains. BMC Genomics 2018;19:652. doi: 10.1186/s12864-018-5043-9.
4. Wang X, Yang Y, Huycke MM. Risks associated with enterococci as probiotics. Food Res Int 2020;129:108788. doi: 10.1016/j.foodres.2019.108788.
5. Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. Microbiol Mol Biol Rev 2016;76:685-706. doi: 10.1128/mmbr.00023-12.
6. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). Scientific opinion on establishing food-based dietary guidelines. EFSA Journal 2010;8:1460. doi: 10.2903/j.efsa.2010.1460.
7. Tanasupawat S, Sukontasing S, Lee JS. *Enterococcus thailandicus* sp. nov., isolated from fermented sausage ('mum') in Thailand. Int J Syst Evol Microbiol 2008;58:1630-4. doi: 10.1099/ijs.0.65535-0.
8. Nam Hee K, Hye Won K, Sun Min P, Go Hun S, Tae Jin C, Ha Ry Y, et al. Virulence patterns and prevalence of seven *Enterococcus* species isolated from meats and leafy vegetables in South Korea. Food Control 2020;108:106867. doi: 10.1016/j.foodcont.2019.106867.
9. Kim Y, Choi SI, Jeong Y, Kang CH. Evaluation of Safety and Probiotic Potential of *Enterococcus faecalis* MG5206 and Enterococcus faecium MG5232 Isolated from Kimchi, a Korean Fermented Cabbage. Microorganisms 2022;10:2070.
10. Kunzes A, Anila K, Savitri, Tek Chand B. Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. LWT - Food Sci Technol 2016;66:428-35. doi: 10.1016/j.lwt.2015.10.057.
11. Nagaoka S, Hojo K, Murata S, Mori T, Ohshima T, Maeda N. Interactions between salivary *Bifidobacterium adolescentis* and other oral bacteria: in vitro coaggregation and coadhesion assays. FEMS Microbiology Letters 2008;281:183-9. doi: 10.1111/j.1574-6968.2008.01092.x.
12. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34:i884-90. doi: 10.1093/bioinformatics/bty560.
13. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595.
14. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 2014;30:2068-9. doi: 10.1093/bioinformatics/btu153.
15. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75. doi: 10.1186/1471-2164-9-75.
16. van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP. BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. Nucleic Acids Research 2018;46:W278-81. doi: 10.1093/nar/gky383.

17. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research 2023;51:W46–50. doi: 10.1093/nar/gkad344.
18. Min B, Yoo D, Lee Y, Seo M, Kim H. Complete genomic analysis of *Enterococcus faecium* heat–resistant strain developed by two–step adaptation laboratory evolution method. Front Bioeng Biotechnol 2020;8:828 doi: 10.3389/fbioe.2020.00828.
19. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large–scale prokaryote pan genome analysis. Bioinformatics 2015;31:3691–3.
20. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Research 2018;47:D687–92. doi: 10.1093/nar/gky1080.
21. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 2019;48:D517–25. doi: 10.1093/nar/gkz935.
22. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, Petersen TN. Detection of mobile genetic elements associated with antibiotic resistance in *Salmonella enterica* using a newly developed web tool: MobileElementFinder. J Antimicrob Chemother 2020;76:101–9. doi: 10.1093/jac/dkaa390.
23. Consortium TU. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Research 2022;51:D523–31. doi: 10.1093/nar/gkac1052.
24. Tarique M, Ali AH, Kizhakkayil J, Liu SQ, Oz F, Dertli E, et al. Exopolysaccharides from *enterococcus faecium and streptococcus thermophilus*: bioactivities, gut microbiome effects, and fermented milk rheology. Food Chem X 2024;21:101073. doi: 10.1016/j.fochx.2023.101073.
25. Comerlato CB, Prichula J, Siqueira FM, Ritter AC, Varela APM, Mayer FQ, et al. Genomic analysis of *Enterococcus durans* LAB18S, a potential probiotic strain isolated from cheese. Genet Mol Biol 2022;45:e20210201. doi: 10.1590/1678–4685–gmb–2021–0201.
26. Salazar N, Gueimonde M, de Los Reyes–Gavilán CG, Ruas–Madiedo P. Exopolysaccharides Produced by Lactic Acid Bacteria and Bifidobacteria as Fermentable Substrates by the Intestinal Microbiota. Crit Rev Food Sci Nutr 2016;56:1440–53, doi: 10.1080/10408398.2013.770728.
27. Ayyash M, Stathopoulos C, Abu–Jdayil B, Esposito G, Baig M, Turner MS, et al. Exopolysaccharide produced by potential probiotic *Enterococcus faecium* MS79: Characterization, bioactivities and rheological properties influenced by salt and pH. Lwt 2020;131:109741. doi: 10.1016/j.lwt.2020.109741.
28. Egan K, Field D, Ross RP, Cotter PD, Hill C. In silico Prediction and Exploration of Potential Bacteriocin Gene Clusters Within the Bacterial Genus *Geobacillus*. Front Microbiol 2018;9:2116. doi: 10.3389/fmicb.2018.02116.
29. Teber R, Asakawa S. In Silico Screening of Bacteriocin Gene Clusters within a Set of Marine Bacillota Genomes. Int J Mol Sci 2024;25:2566.
30. Tettelin H, Masignani V, Cieslewicz MJ, Donati C, Medini D, Ward NL, et al. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: Implications for the microbial pan–genome. Proc Natl Acad Sci 2005;102:13950–5. doi: 10.1073/pnas.0506758102.
31. Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of Enterococci. Microbiol Spectr 2019;7. doi: 10.1128/microbiolspec.GPP3–0053–2018.
32. Ramos S, Silva V, Dapkevicius MLE, Igrejas G, Poeta P. Enterococci, from Harmless Bacteria to a Pathogen. Microorganisms 2020;8. doi: 10.3390/microorganisms8081118.
33. Anadón A, Martínez–Larrañaga MR, Aranzazu Martínez M. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regul Toxicol Pharmacol 2006;45:91–95. doi: 10.1016/j.yrtph.2006.02.004.
34. Rengaraj R, Mariappan S, Sekar U, Kamalanadhan A. Detection of vancomycin resistance among *Enterococcus faecalis* and *Staphylococcus aureus*. J Clin Diagn Res 2016;10:Dc04–6. doi: 10.7860/jcdr/2016/17552.7201.
35. Yoo IY, Kwon JA, Lee M, Jung S, Kim JO, Ha SI, et al. Prevalence and molecular characterization of vancomycin variable *enterococcus faecium* isolated from clinical specimens. Ann Lab Med 2024;44:450–4. doi: 10.3343/alm.2023.0430.
36. Wu X, Wu B, Li Y, Jin X, Wang X. Identification and safety assessment of *Enterococcus thailandicus* TC1 isolated from healthy pigs. PLoS One 2021;16e0254081. doi: 10.1371/journal.pone.0254081.
37. Coque TM, Patterson JE, Steckelberg JM, Murray BE. Incidence

of hemolysin, gelatinase, and aggregation substance among enterococci isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. J Infect Dis 1995;171:1223-9. doi: 10.1093/infdis/171.5.1223.

38. Ahsan T, Hemel MMM, Sonia SJ, Shamsuzzaman SM. Distribution of Virulence genes among enterococcus species isolated from patients of urinary tract infection attended in a tertiary care hospital of Bangladesh. Mymensingh Med J 2024;33:107-15.
39. Tarek N, Azmy FA, Khairalla AS, Abdel-Fattah M, Jefri OA, Shaban M, et al. Genome sequencing of *Enterococcus faecium* NT04, an oral microbiota revealed the production of enterocin A/B active against oral pathogens. Heliyon 2023;9.
40. El Jeni R, Ghedira K, El Bour M, Abdelhak S, Benkahla A, Bouhaouala-Zahar B. High-quality genome sequence assembly of R. A73 *Enterococcus faecium* isolated from freshwater fish mucus. BMC microbiology 2020;201-12.
41. Ghattargi VC, Gaikwad MA, Meti BS, Nimonkar YS, Dixit K, Prakash O. Comparative genome analysis reveals key genetic factors associated with probiotic property in *Enterococcus faecium* strains. BMC genomics 2018;19:1-16.
42. Tareb R, Bernardeau M, Gueguen M, Vernoux JP. In vitro characterization of aggregation and adhesion properties of viable and heat-killed forms of two probiotic *Lactobacillus* strains and interaction with foodborne zoonotic bacteria, especially *Campylobacter jejuni*. J Med Microbiol 2013;62:637-49. doi: 10.1099/jmm.0.049965-0.