Insights into the metagenomic and metabolomic compositions of the bacterial communities in Thai traditional fermented foods as well as the relationships between food nutrition and food microbiomes

Pongrawee Nimnoi Equal first author, 1 , **Neelawan Pongsilp** Corresp. Equal first author, 2

1 Department of Science and Bioinnovation / Faculty of Liberal Arts and Science, Kasetsart University, Nakhon Pathom, Thailand
2 Department of Microbiology / Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand

Corresponding Author: Neelawan Pongsilp
Email address: pongsilp_n@su.ac.th

Five Thai traditional fermented foods, including Khao-Mak (sweet fermented sticky rice), Pak-Kard-Dong (sour salt-fermented mustard green), Nor-Mai-Dong (sour salt-fermented bamboo sprouts), Moo-Som (sour salt-fermented pork), and Pla-Som (sour salt-fermented fish), were analyzed for their food nutrition and bacterial community structures. Sour salt-fermented bamboo sprouts possessed the highest unique Amplicon Sequence Variables (ASVs), which was 3,476, as well as the highest bacterial diversity and richness, while in contrast, sweet fermented sticky rice possessed the lowest such features. The phylum *Firmicutes* accounted for the largest proportions in both sour salt-fermented meats and sweet fermented sticky rice whereas the *Proteobacteria* occupied the largest proportions in both sour salt-fermented vegetables. The bacterial community structures of both sour salt-fermented meats were similar based on the class composition while the dominant genera compositions were totally different among all foods. Gene functions, enzymes, and metabolic pathways annotated from the bacterial communities in all foods were those involved in growth metabolisms, genetic information processing, environmental information processing, and cellular signaling. Sour salt-fermented bamboo sprouts had the highest numbers of unique annotated genes, enzymes, and metabolic pathways.

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Abstract

Five Thai traditional fermented foods, including Khao-Mak (sweet fermented sticky rice), Pak-Kard-Dong (sour salt-fermented mustard green), Nor-Mai-Dong (sour salt-fermented bamboo sprouts), Moo-Som (sour salt-fermented pork), and Pla-Som (sour salt-fermented fish), were analyzed for their food nutrition and bacterial community structures. Sour salt-fermented bamboo sprouts possessed the highest unique Amplicon Sequence Variables (ASVs), which was 3,476, as well as the highest bacterial diversity and richness, while in contrast, sweet fermented sticky rice possessed the lowest such features. The phylum *Firmicutes* accounted for the largest proportions in both sour salt-fermented meats and sweet fermented sticky rice whereas the *Proteobacteria* occupied the largest proportions in both sour salt-fermented vegetables. The bacterial community structures of both sour salt-fermented meats were similar based on the class composition while the dominant genera compositions were totally different among all foods. Gene functions, enzymes, and metabolic pathways annotated from the bacterial communities in all foods were those involved in growth metabolisms, genetic information processing, environmental information processing, and cellular signaling. Sour salt-fermented bamboo sprouts had the highest numbers of unique annotated genes, enzymes, and metabolic pathways.

Introduction

Fermented foods are defined as foods made through desired microbial growth and enzymatic conversions of raw food ingredients (*Marco et al., 2021*). Traditional fermented foods are prepared and consumed by the regional populations around the world since ancient times and they are recognized for many health benefits. Numerous beneficial microorganisms impart unique properties to fermented foods through their metabolic activities, therefore the analyses of microbial diversity and abundance in fermented foods will facilitate understanding of the roles of microbial taxa in establishing organoleptic properties and offering health benefits (*Deka et al., 2021*). During the fermentation process, microbial community converts raw food ingredients into products which improve particular organoleptic properties, shelf life, and nutraceutical quality of final fermented foods (*Xing et al., 2023; Zhang et al., 2023*).

Thailand is a humid tropical country with diverse ecosystems, thus producing a wide range of agricultural commodities which are raw materials for many Thai traditional fermented foods. Here are examples of the popular ones which were employed in this study. Khao-Mak is sweet fermented sticky rice with low alcohol content. For its preparation, steam-cooked sticky rice is mixed with flour balls containing natural starter microorganisms, and kept in a closed container for 2-3 days. Enzymes produced by starter microorganisms hydrolyze starch in sticky rice into sugar in which some parts are subsequently fermented to alcohol and organic acids (*Rittisorn et al., 2024*). Pak-Kard-Dong is sour salt-fermented mustard green prepared by soaking mustard green in brine for a day and then submerging it under fermented water containing soluble salt, soluble sugar, and rice washing water, in a closed container for 4-5 days. Nor-Mai-Dong is sour salt-fermented bamboo sprouts prepared by soaking peeled bamboo sprouts in brine for a day, and then submerging them under water in a closed container for 7 days. Moo-Som is sour salt-fermented pork whose ingredients are pork, salt, cooked rice, garlic, and pepper. All ingredients are crushed together to form a paste which is then tightly wrapped in a plastic bag for 2-3 days. Pla-Som is sour salt-fermented fish whose ingredients are fish, salt, cooked rice, garlic, and pepper. Fish is soaked in rice washing water containing soluble salt and then soaked in brine. All ingredients are crushed together to form a paste which is then tightly wrapped in a plastic bag for 2-3 days. The spontaneous fermentation of fermented foods engenders the proliferation of numerous microorganisms. Among them, the lactic acid bacteria (LAB) mainly contribute the accumulation of lactic acid, which inhibits the growth of harmful microorganisms by lowering pH and also provides palatable flavors (*Du et al. 2022; Lv et al., 2021*). Up to date, many genera have been reported as the LAB (*Abedi & Hashemi, 2020; Hu et al., 2023; Wang et al., 2021a*). They collectively belong to the phylum *Firmucutes*, class *Bacilli*, orders *Bacillales* and *Lactobacillales*, and families *Bacillaceae*, *Enterococcaceae*, *Lactobacillaceae*, and *Streptococcaceae* according to the National Center for Biotechnology Information (NCBI) Taxonomy database (https://www.ncbi.nlm.nih.gov/taxonomy/). Different microbial communities engender different complex biochemical and physical reactions, establishing diverse and distinct organoleptic properties of final fermented foods (*Alkema et al., 2016*). Thus, the bacterial diversity and community merit the evaluation of their roles in establishing and controlling organoleptic properties of fermented foods.

The recent advances in next-generation sequencing (NGS) technologies have provided the information on microbial diversity and communities in fermented foods, although there is still a lack of clarity on food nutrition, specific bacterial taxa, gene functionality, enzymes, and metabolic pathways which are compared among various types of food fermentation. Therefore, the objectives of this study were to characterize the nutritional compositions as well as the bacterial diversity and communities of five Thai traditional fermented foods including Khao-Mak (sweet fermented sticky rice), Pak-Kard-Dong (sour salt-fermented mustard green), Nor-Mai-Dong (sour salt-fermented bamboo sprouts), Moo-Som (sour salt-fermented pork), and Pla-Som (sour salt-fermented fish). The food nutrition parameters, including calories, carbohydrate, fat, moisture, protein, total sugar, and sodium contents, were determined. The bacterial diversity and communities were analyzed using an Illumina NGS platform. Metagenomic and metabolomic analyses were employed to annotate the gene functions, enzymes, and metabolic pathways. The correlations between food nutrition parameters and bacterial taxa were also evaluated. To the best of our knowledge, this is the first report investigating the following aspects of Thai traditional fermented foods: 1) the food nutrition facts; 2) the bacterial diversity and communities; 3) the annotated gene functions, enzymes, and metabolic pathways; and 4) the influences of food nutrition parameters on abundance of bacterial taxa. This ultimate data would be beneficial for quality control of the bacterial succession in the fermentation processes.

Materials and methods

Thai traditional fermented foods

Five Thai traditional fermented foods, including Khao-Mak (sweet fermented sticky rice; sample A), Pak-Kard-Dong (sour salt-fermented mustard green; sample B), Nor-Mai-Dong (sour salt-fermented bamboo sprouts; sample C), Moo-Som (sour salt-fermented pork; sample D), and Pla-Som (sour salt-fermented fish; sample E), were purchased from Boon Mueang fresh market in Mueang district, Lopburi province of Thailand (14°48′13.662″N 100°36′38.636″E) on 21 January 2024. Food samples were placed in sterile plastic bags, immediately stored in ice boxes, and delivered to the laboratory within 24 h after purchase.

Food nutritional analyses

All food samples (A-E) were determined for their nutrition facts. Calories and carbohydrate contents were analyzed using the In-house method TE-CH-169 based on the method of analysis for nutrition labeling (*Sullivan & Carpenter, 1993*). Fat content was analyzed using the Association of Official Analytical Chemists (AOAC) method 922.06 (*AOAC, 2023*). Moisture content was analyzed using the AOAC methods 925.45A (for samples A, B, and C) and 950.46B (for samples D and E) (*AOAC, 2023*). Protein, total sugar, and sodium contents were determined by the In-house method TE-CH-042 based on the AOAC method 981.10, the In-house method TE-CH-164 based on the AOAC method 977.20, and the In-house method TE-CH-134 based on the AOAC method 984.27, respectively (*AOAC, 2023*).

Food DNA extraction, Illumina NGS, data processing, and bioinformatic analyses

To explore the bacterial communities in five Thai traditional fermented foods through an Illumina NGS, total bacterial DNA was extracted from food samples by using a DNeasy mericon food kit (Qiagen, Inc., Valencia, CA, USA). DNA samples were extracted from nine fractions of each food sample, then three of them were pooled as one replicate. Therefore, three replicates were prepared for each food. The protocols for amplification of the V4 variable region of the 16S rRNA, construction of DNA library, and Illumina sequencing were followed as described in the previous article (*Pongsilp & Nimnoi, 2024*). Data of raw sequence reads was converted to fastq files. The fastq files were consecutively processed using the FLASH software version 1.2.11 (https://ccb.jhu.edu/software/FLASH/) (*Magoč and Salzberg, 2011*), the FASTP software version 0.20.1 (https://github.com/OpenGene/fastp) (*Chen et al., 2018*), the VSEARCH software version 2.21.1 (https://github.com/torognes/vsearch/releases) (*Rognes et al., 2016*), and the QIIME2 software version 2021.4 (https://forum.qiime2.org/) (*Bolyen et al., 2019*) to obtain the effective tags. The effective tags whose sequence abundance was less than five, were excluded to select the final Amplicon Sequence Variables (ASVs). The Classify-sklearn moduler in the QIIME2 software version 2021.4 was employed to compare the ASVs with the sequences available in the SILVA SSU rRNA database (https://www.arb-silva.de/) (*Quast et al., 2013*) for species annotation of each ASV. The ASV data has been deposited into the Sequence Read Archive of the NCBI under the BioProject ID: PRJNA1180770.

Statistical analyses

The analyses for statistical processing of Illumina sequencing data were as follows: 1) the computations of the parameters relative to alpha diversity, which included community diversity (Shannon-Weaver and Simpson’s indices), community richness (Chao1 index and number of observed species), sequencing depth index (Good’s coverage), and dominance as well as beta diversity for quantifying sample variation in species complexity; 2) the Principal Coordinate

Analysis (PCoA) and Non-Metric Multi-Dimensional Scaling (NMDS); 3) the analysis of similarity (ANOSIM); 4) the Unweighted-Pair Group Method with Arithmetic Mean (UPGMA) clustering; 5) the Linear Discriminant Analysis (LDA) Effect Size (LEfSe) algorithm version 1.1.0 (https://huttenhower.sph.harvard.edu/lefse/) (*Segata et al., 2011*); and 6) the Canonical Correlation Analysis (CCA) in the PAST software version 4.03 (https://palaeo-electronica.org/2001_1/past/issue1_01.htm) (*Hammer et al., 2001*). The details of these analyses were described in the previous article (*Pongsilp & Nimnoi, 2024*).

Metagenome data analyses

The PICRUSt2 software version 2.5.0 (https://github.com/picrust/picrust2) (*Douglas et al., 2020*) was employed to analyze the metagenome data for annotation of genes, enzymes, and metabolic pathways by using the information in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) and MetaCyC (https://metacyc.org/) databases. Heat map charts was created to illustrate the abundance and distribution. The Principal Component Analysis (PCA) in the R software version 4.3.2 (https://www.R-project.org/) (*R Core Team, 2023*) was performed to display the clusterings of the annotated genes, enzymes, and metabolic pathways among different samples. The associations of food nutrition facts with bacterial communities were analyzed based on Spearman's correlation coefficients. Statistical significance was indicated by p-value (very significant if p-value was <0.01 and significant if p-value was <0.05). The food nutrition facts, the alpha and beta diversity indices as well as the top ten most abundant bacterial classes were included in the Analysis of Variance (ANOVA) using the Tukey's test. Between-group analysis, ANOVA, and Spearman's correlation coefficients were computed by the SPSS software version 19.0 (IBM Corp., Chicago, IL, USA). All data analyses were performed with three replicate samples.

Results

Food nutrition facts

All except one of the food nutrition parameters, including calories, carbohydrate, fat, moisture, protein, and sodium contents, were significantly different ($p<0.05$) among five Thai traditional fermented foods while total sugar was only detected in sample A (sweet fermented sticky rice) (Table 1). Glucose was almost the sole (>98.3%) sugar in sample A while maltose was presented at a very low concentration (<1.7%). Sample A contained the significantly highest calories, carbohydrate, and total sugar contents and the significantly lowest moisture and sodium contents. Sample B (sour salt-fermented mustard green) exhibited the significantly highest moisture content and the significantly lowest calories, fat, and protein contents. Sample C (sour salt-fermented bamboo sprouts) had moderate contents of all food nutrition parameters. Sample D (sour salt-fermented pork) exhibited the significantly highest fat content and the significantly lowest carbohydrate content. Both significantly highest protein and sodium contents were obtained from sample E (sour salt-fermented fish).

Sequence analysis and indices of bacterial diversity and richness

Totals of 1,516,672 qualified tags and 4,324 ASVs were obtained from all samples, with a mean Good's coverage of 99.90±0.17. The Venn diagram (Figure 1) illustrates the numbers of common, overlapping, and unique ASVs among all foods. Five Thai traditional fermented foods shared ten common ASVs. Samples C (sour salt-fermented bamboo sprouts) showed the highest unique ASVs, followed by samples B, D, E, and A, respectively. Sour salt-fermented vegetables (samples B and C) shared the highest common ASVs. Sample A (sweet fermented sticky rice) was obviously distinct as it shared either none or few common ASVs with the other samples.

The parameters relative to alpha diversity were evaluated (Table 2). Higher Shannon-Weaver and Simpson's indices represent greater bacterial diversity. Shannon-Weaver and Simpson's indices were significantly ranked ($p<0.05$), from highest to lowest, as follows: 1) sample C; 2) samples B and E; 3) sample D; and 4) sample A. Bacterial richness indices (Chao1 index and number of observed species) were significantly ranked, ($p<0.05$), from highest to lowest, as follows: 1) sample C; 2) sample B; 3) samples D and E; and 4) sample A. These results imply that the bacterial communities of sour salt-fermented vegetables had higher richness than sour salt-fermented meats. Sweet fermented sticky rice possessed the bacterial community with the lowest diversity and richness. On the contrary, the significantly highest dominance was obtained from sample A, followed by samples D, B, E, and C, respectively.

Illumina NGS output and bacterial community structures

Among the top ten most abundant phyla present in five Thai traditional fermented foods, the *Firmicutes* was mostly abundant, ranging between 8.79 and 89.81%, followed by the *Proteobacteria* (7.97-60.75%), *Cyanobacteria* (0.21-28.88%), *Halobacterota* (0.00-24.07%), *Bacteroidota* (0.00-17.00%), *Actinobacteriota* (0.08-3.59%), *Fusobacteriota* (0.00-2.89%), *Deinococcota* (0.00-1.39%), *Nanohaloarchaeota* (0.00-0.91%), and *Campylobacterota* (0.00-0.75%). Their relative abundance (Figure 2) obviously shows that the *Firmicutes* accounted for the largest proportions in samples D, E, and A, in descending order. The *Proteobacteria* occupied the largest proportions in samples B and C in which its percentage in sample B was much higher than in sample C. The proportions of *Cyanobacteria* in sample B and *Halobacterota* in sample C were remarkably larger than those in the other samples.

The ASV percentages of the top ten most abundant bacterial classes present in five Thai traditional fermented foods were computed (Table 3). The four classes present in all samples were the *Bacilli*, alpha-*Proteobacteria*, gamma-*Proteobacteria*, and *Cyanobacteriia*. The *Bacilli* exhibited the largest proportions in samples A, D, and E. The alpha-*Proteobacteria* and gamma-*Proteobacteria* occupied the largest proportions in samples B and C, respectively. Sample C was the only one whose the bacterial community structure consisted of all of the top ten most abundant classes. The *Halobacteria, Deinococci,* and *Nanosalinia* were present only in sample C and could be considered as biomarkers of sample C. The bacterial community structures of samples D and E were similar as they consisted of the same seven classes and the *Bacilli* was the sole major contributor.

The finer evolution of the unique bacterial community characteristics in five Thai traditional fermented foods was explained by a biomarker analysis. The LDA scores displayed in the output of a biomarker analysis, which indicated significant differences in intra-group variation, are shown in Supplementary Figure S1. The genera of biomarkers were totally different among all foods. The *Limosilactobacillus* was significantly more abundant in sample A. The abundance of the *Sphingomonas*, *Methylobacterium-Methylorubrum*, and *Pediococcus* was significantly greater in sample B. The *Flectobacillus*, *Lactobacillus*, *Janthinobacterium*, *Halorubrum*, *Haloplanus*, and *Halobellus* exhibited their significantly more abundance in sample C. The significantly more abundance of the *Lactococcus* was displayed in sample D. The more abundant community in sample E consisted of the *Companilactobacillus*, *Proteus*, *Lactiplantibacillus*, *Vibrio*, *Leuconostoc*, and *Levilactobacillus*.

The bacterial genera's distribution and abundance in five Thai traditional fermented foods were derived from a heat map analysis. The colors in a heat map chart signify the abundance levels (Figure 3). A color range from deep blue to dark red denotes the ascending levels of relative abundance. The compositions of dominant genera in each food were remarkably unique. The *Limosilactobacillus* was the only dominant genus in sample A. The dominant genera in sample B included the *Bacillus, Pediococcus, Enterococcus, Methylobacterium-Methylorubrum, Sphingomonas,* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. The dominant genera in sample C were the *Lactobacillus, Deinococcus, Halapricum, Halomicroarcula, Natronomonas, Haloplanus, Janthinobacterium, Halobellus, Flectobacillus*, and *Halorubrum*. Sample D was dominated by the *Lactococcus* and *Macrococcus*. Sample E was dominantly occupied by the *Levilactobacillus, Salinivibrio, Latilactobacillus, Shewanella, Leuconostoc, Fusobacterium, Proteus,* and *Psychrilyobacter*.

The ANOSIM method indicated that the variation of inter-group bacterial community structure was larger than that of the inner-group (R=1). The pairwise dissimilarity coefficients between sample pairs were quantified and are illustrated in a dissimilarity heat map (Figure 4a). The colors and dissimilarity coefficient values in a dissimilarity heat map signify the dissimilarity levels of bacterial community compositions between sample pairs. A color range from red to yellow and low-to-high dissimilarity coefficient values denote the ascending levels of dissimilarity. Among sample pairs, the most similar bacterial community compositions were obtained from a sample pair D and E (a dissimilarity coefficient value of 0.124) whereas the most dissimilarity was noticed from a sample pair A and C (a dissimilarity coefficient value of 0.686). These results were additionally confirmed by the clustering analysis for determining the similarity among all samples, which was calculated by the UPGMA method (Figure 4b). Samples D and E harbored the most similar bacterial communities. Both PCoA and NMDS analyses also provided the consistent results with those of the pairwise dissimilarity and clustering analyses. The ordinations of samples by PCoA and NMDS are illustrated in Figure 5. Even though the bacterial communities were different among all samples, that of samples D and E were plotted adjacent to each other and alienated from the remaining samples, implying the most similar bacterial communities. On the contrary, the farthest distance was derived from a simple pair A and C, representing the most dissimilarity between their bacterial communities.

Effects of food nutrition parameters on bacterial community structures

The effects of food nutrition parameters on bacterial community structures in five Thai traditional fermented foods were evaluated (Supplementary Table S1). The results indicate that the bacterial diversity and richness, the classes gamma-*Proteobacteria, Bacteroidia,* and

Actinobacteria were very significantly ($p<0.01$) positively associated with moisture content while very significantly negatively associated with calories and total sugar contents. The bacterial diversity was significantly ($p<0.05$) negatively associated with fat and protein contents. The bacterial richness was significantly ($p<0.05$) negatively associated with fat content. The *Bacilli* and alpha-*Proteobacteria* had very significantly ($p<0.01$) positive and negative associations, respectively, with fat and protein contents. The *Cyanobacteriia* was very significantly negatively associated with fat, protein, and sodium contents. The *Deinococci* was very significantly positively associated with moisture content while very significantly negatively associated with calories, fat, and protein contents.

To definitively reveal the relationships between food nutrition parameters and bacterial communities, the CCA diagram was constructed (Supplementary Figure S2). The results show that moisture content influenced the bacterial diversity and richness as well as the classes *Halobacteria, Bacteroidia, Deinococci,* and *Nanosalinia*. Calories, carbohydrate, and total sugar contents were directly related to the alpha-*Proteobacteria,* gamma-*Proteobacteria,* and *Cyanobacteriia.* Protein, fat, and sodium contents were directly related to the *Bacilli* and *Fusobacteriia.* In order to comprehend the effects of food types on dominant bacterial taxa, ternary plots were depicted to astutely distinguish the relative abundance of the top ten most abundant classes (Figure 6). As depicted in Figure 6a, the *Bacilli* was most closely associated with sample A. The alpha-*Proteobacteria* and *Cyanobacteriia* were most closely associated with sample B whereas the *Halobacteria* and *Bacteroidia* were most closely associated with sample C. The *Actinobacteria* was also closely associated to sample C, although its relative abundance was lowest. The gamma-*Proteobacteria* was dominant across samples B and C. As depicted in Figure 6b, the *Bacilli* was dominant across samples A, D, and E whereas the gamma-*Proteobacteria* was most closely associated with sample E, although its relative abundance was very low.

Gene functions, enzymes, and metabolic pathways annotated from bacterial communities

Gene functions, enzymes, and metabolic pathways were annotated from the metagenome data of the bacterial communities in five Thai traditional fermented foods against the KEGG and MetaCyC databases using the PICRUSt2 software. The Venn diagrams (Supplementary Figure S3) illustrate the numbers of common, overlapping, and unique annotated genes, enzymes, and metabolic pathways among all foods. As depicted in Supplementary Figure S3a, a total of 4,448 genes were annotated across all foods in which 2,636 were common. Sample C had the highest number of unique annotated genes (403) whereas sample A had no any unique annotated gene. For enzyme annotation (Supplementary Figure S3b), there were a total of 1,288 annotated enzymes across all foods in which 889 were common. Sample C also had the highest number of unique annotated enzymes (61) whereas samples A and D had no any unique annotated enzyme. For metabolic pathway annotation (Supplementary Figure S3c), there were a total of 63 annotated metabolic pathways across all foods in which 28 were common. Sample C also possessed the highest number of unique annotated metabolic pathways (20) whereas samples A, D, and E had no any unique annotated metabolic pathway. Seven annotated metabolic pathways were overlapped within sour salt-fermented vegetables (samples B and C), while in contrast, none was overlapped within sour salt-fermented meats (samples D and E). There was no overlapping annotated enzyme and metabolic pathway between sample A and either of the remaining samples.

The data of the annotated genes and their relative abundance is displayed in a heat map chart (Figure 7), in which the colors signify the abundance levels. The color shades ranging from deep blue into yellow, brown, and red symbolize the lowest to highest abundance levels. Gene functions were annotated using the KEGG database (https://www.genome.jp/kegg/). The more abundant genes in sample A were a ligase gene (*gshA*) (K01919), genes involved in amino acid transport systems (K02029, K02030, and K03293), and transposase genes (K07491 and K07496). The more abundant genes in sample B were a glutathione *S*-transferase gene (*gst*) (K00799) and a serine/threonine kinase gene (K08884). The more abundant genes in sample C included genes coding for a reductase (K00059), an outer membrane receptor protein (K02014), and a chemotaxis protein (K03406). The more abundant genes in sample D included genes involved in an ATP-binding cassette (ABC) transport system (K01990, K01992, and K02003), a protease gene (K07052), and a regulatory gene (*spxA*) (K16509). The sole most abundant gene in sample E was a sugar permease gene (*bglF*) (K02757).

Enzyme annotation is illustrated in a heat map chart (Figure 8). The color symbolization is same as described above in Figure 7. Enzymes were annotated using the KEGG Enzyme database (https://www.genome.jp/kegg/annotation/enzyme.html). The more abundant enzymes in sample A included a ligase (EC:6.3.2.2), a carbamoyl-phosphate synthase (EC:6.3.5.5), and a carboxylase (EC:6.4.1.2). The more abundant enzymes in sample B were a glutathione *S*-transferase (EC:2.5.1.18) and a histidine kinase (EC:2.7.13.3). The more abundant enzymes in sample C were a cytochrome *c* oxidase (EC:1.9.3.1) and a peptidylprolyl isomerase (EC:5.2.1.8). The more abundant enzymes in sample D included a DNA methyltransferase (EC:2.1.1.72) and a beta-glucosidase (EC:3.2.1.86). The sole most abundant enzyme in sample E was an RNA helicase (EC:3.6.4.13).

Metabolic pathway analysis was elucidated and is displayed in a heat map chart (Figure 9). The color symbolization is same as described above in Figure 7. Metabolic pathways were annotated against the MetaCyC database (https://metacyc.org/). Sample A exhibited high abundance of the pentose phosphate (PP) pathways (NONOXIPENT-PWY and PENTOSE-P-PWY) and nucleotide biosynthesis (PWY-7228). Samples B and C were similar regarding that the sole most abundant pathway was aerobic respiration (PWY-3781), though its abundance was strikingly higher in sample C. The more abundant pathways in sample D could be categorized into three groups including 1) glycolysis and organic acid fermentation (ANAEROFRUCAT-PWY, ANAGLYCOLYSIS-PWY, GLYCOLYSIS, and PWY-5484); 2) sucrose degradation (PWY-621); and 3) nucleotide biosynthesis (PWY-7220 and PWY-7222). The more abundant pathways in sample E could be categorized into two groups including 1) glycolysis and organic acid fermentation (ANAEROFRUCAT-PWY, GLYCOLYSIS, PWY-5100, PWY-5484); and 2) sucrose degradation (PWY-621). Despite being more abundant in samples D and E, the abundance of pathways in glycolysis, organic acid fermentation, and sucrose degradation was strikingly lower in sample E than in sample D.

Furthermore, ordinations of samples by PCA based on the genes, enzymes, and metabolic pathways annotated from the bacterial community compositions of five Thai traditional fermented foods are depicted in Figure 10 (a, b, and c, respectively). All three clustering plots consistently attest that the bacterial community compositions of all foods were completely alienated from each other, implying their unique patterns. Sour salt-fermented meats (samples D and E) were the closest pair whereas sour salt-fermented vegetables (samples B and C) and sweet fermented sticky rice (sample A) possessed their own distinct patterns.

Discussion

This study illustrates the variations in food nutrition as well as bacterial diversity and community among five Thai traditional fermented foods. The results exhibit that sample A (sweet fermented sticky rice) had the significant highest calories and carbohydrate contents and it was the only fermented food in which total sugar was detected. This was possibly related to high starch contents in rice grain which are typically up to 80-90% (*Alhambra et al., 2019*). Sweet fermented sticky rice also had mildly alcoholic and sour flavors. In general, bacterial and fungal enzymes, such as alpha-amylase and glucoamylase, hydrolyze starch in sticky rice to sugar which is a main product and the fermentation process also yields other organic compounds such as alcohol and lactic acid (*Mongkontanawat & Lertnimitmongkol, 2015; Rittisorn et al., 2024*). Sample D (sour salt-fermented pork) had the significantly highest fat content. This might due to the original fat content which accounts for 6.3% of pork muscle tissues (*Yi et al., 2023*). Sample E (sour salt-fermented fish) had the significantly highest protein and sodium contents. Protein detected in sample E might be the remainder of the original protein content as fishes contain protein contents varying from below 10% to over 20% (*Alp-Erbay & Yesilsu, 2021*).

The results exhibit that samples C (sour salt-fermented bamboo sprouts) and A (sweet fermented sticky rice) had the significantly highest and significantly lowest bacterial diversity and richness, respectively. The dynamic succession of the bacterial community and richness of these kinds of fermented foods has been previously studied using high-throughput sequencing analysis. The bacterial community in sour salt-fermented bamboo shoots was significantly altered during the fermentation time and also correlated with the production of off-odor compounds. The environmental factors affecting the bacterial distribution included salt concentration as well as fermentation time and temperature (*Hu et al., 2023*). The fermentation of bamboo shoots caused changes in several aspects including food nutrition facts, acidity, toxicity, and organoleptic properties (*Hu et al., 2023; Singhal, Satya & Naik, 2021*). Rice varieties affected the bacterial diversity in a Chinese traditional rice-based fermented food (*Cai et al., 2021*). Ethnic tribes of food-producers affected the bacterial diversity in Indian traditional rice-based fermented beverages (*Yumnam, Hazarika & Sharma, 2024*).

This study reports the dominance of bacterial phyla and classes in five Thai traditional fermented foods. The phylum *Firmicutes* constituted the largest proportions in samples D, E, and A, in descending order. The *Proteobacteria* was mostly dominant in samples B and C in which its percentage in sample B was much higher than in sample C. The *Cyanobacteria* and *Halobacterota* were the second dominant phyla in samples B and C, respectively. These results are similar to those of previous reports. The *Firmicutes* (*Firmicuteota*) was mostly dominant in all five samples (>50%) collected from different points along the production line of a Chinese sticky rice fermented sweet dumplings (*Suo et al., 2023*) and Indian traditional fermented pork fat (*De Mandal et al., 2018*). The *Proteobacteria* and *Firmicutes* established the core microbiotas of Chinese salt fermented mustard green (*Sarengaowa et al., 2024; Wang et al., 2022a*), Taiwanese sour salt-fermented mustard pickle (*Chien et al., 2023*), and Chinese sour salt-fermented bamboo shoots (*Hu et al., 2023*).

A heat map analysis was performed to identify the bacterial genera and their relative abundance in five Thai traditional fermented foods. The *Limosilactobacillus* (formerly *Lactobacillus*) was the sole dominant genus with extreme relative abundance in sample A (sweet fermented sticky rice). The bacterial community in sweet fermented sticky rice has not been reported elsewhere, though the *Lactobacillus* was one of the dominant members in Chinese sticky rice fermented wines and sweet dumplings (*Liang et al., 2020; Suo et al., 2023; Zhao et al., 2022; Zou et al., 2023*). The dominant genera in sample B (sour salt-fermented mustard green) included the *Bacillus, Pediococcus, Enterococcus, Methylobacterium-Methylorubrum, Sphingomonas,* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. While the other studies have found that the LAB (*Lactobacillus, Pediococcus*, and *Weissella*), *Cobetia*, and *Halomonas* were dominant in Chinese and Thai fermented mustard green and their bacterial communities were region-dependent (*Sarengaowa et al., 2024; Yongsawas et al., 2022*). The dominant genera in sample C (sour salt-fermented bamboo sprouts) were the *Lactobacillus, Deinococcus, Halapricum, Halomicroarcula, Natronomonas, Haloplanus, Janthinobacterium, Halobellus, Flectobacillus*, and *Halorubrum.* While the LAB (*Lactobacillus, Lactococcus*, and *Weissella*) were dominant in Chinese sour salt-fermented bamboo shoots (*Hu et al., 2023*). The *Lactobacillus* was the sole dominant genus with extreme relative abundance (91.64%) at the day 3 of the fermentation of Indian fermented bamboo shoots (*Deka et al., 2021*). Sample D (sour salt-fermented pork) contained only two dominant genera including the *Lactococcus* and *Macrococcus*. While four LAB genera (the *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Weissella*) established the core bacteriota (>90%) during the fermentation of the other Thai traditional fermented pork (Nham) (*Santiyanont et al., 2019*). Chinese sour salt-fermented pork contained two dominant LAB (the *Lactobacillus* and *Weissella*) (*Lv et al., 2021*). The *Lactobacillus* contributed almost all of the bacterial communities (96.4-99.9%) in three kinds of Korean salt fermented pork sausages (*Kim et al., 2022*). The *Clostridium* accounted for the vast majority (72.48%) at the day 3 of the fermentation of Indian pork fat (*Deka et al., 2021*). Sample E (sour salt-fermented fish) had the dominant community comprising of the *Levilactobacillus, Salinivibrio, Latilactobacillus, Shewanella, Leuconostoc, Fusobacterium, Proteus,* and *Psychrilyobacter*. The *Turicibacter*, *Pseudonocardia*, *Ancylobacter*, *Gallicola*, and *Leucobacter* exhibited the most abundance (in descending order) in the other kind of Thai traditional salt fermented fish (Pla-Ra) (*Phuwapraisirisan et al., 2024*). Difference in bacterial community was found between different stages in the salt fermentation of hilsa fish (*Tenualosa ilisha*). The *Enterobacter* was mostly abundant in an initial stage (37%), then its abundance was dramatically decreased in a ripe stage (4%). The *Cohnella* and *Bacillus* became the two most abundant genera in a ripe stage, contributing to 11% and 10%, respectively (*Sarkar et al., 2024*). Overall, our current study shows that the LAB were the major bacteria in five Thai traditional fermented foods. The dominant genera in five Thai traditional fermented foods, including the *Bacillus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Latilactobacillus*, *Leuconostoc*, *Levilactobacillus*, *Limosilactobacillus*, and *Pediococcus*, have been identified as the LAB (*Abedi & Hashemi, 2020; Wang et al., 2021a*). The LAB have regularly been recognized as the critical fermentative bacteria in all kinds of fermented foods. Their metabolisms cause conversions of carbohydrate to lactic acid and impart particular organoleptic properties (*Sionek et al., 2023*). The LAB ensure the safety of fermented foods by producing organic acids, mainly lactic acid, and a variety of antimicrobial agents which exclude pathogenic microorganisms and also promote the nutritional value by producing a variety of health-beneficial compounds (*Ayed et al., 2024*). Differences in bacterial composition among fermented foods might be due to various factors including fermentation process, temperature and time, equipment, geographical origin and type of raw material, water activity, pH value, nutrient availability, humidity, and environmental contamination during storage and sale (*Chien et al., 2023; Kim et al. 2022; Zhu et al., 2018*).

In this study, the correlations between food nutrition parameters and bacterial communities were evaluated. Our results attest that moisture content was the factor determining the bacterial diversity and richness as well as the abundance of the gamma-*Proteobacteria*, *Bacteroidia, Actinobacteria*, *Deinococci,* and *Nanosalinia*. Fat content was directly related to the abundance of the *Bacilli*. Both protein and sodium contents directly promoted the abundance of the *Bacilli* and *Fusobacteriia*. The correlations between food nutrition parameters and specific bacterial taxa in the other fermented foods have been reported. The *Bacillus, Enterococcus*, *Lactobacillus, Nocardiopsis*, *Pediococcus,* and *Weissella* were significantly correlated with various volatile compounds (VCs) in Chinese sweet fermented sticky rice wines (*Zou et al., 2023*). The LAB (*Lactobacillus, Lactococcus*, *Leuconostoc,* and *Pediococcus*) were correlated with ethanol in a Chinese fermented sticky rice wine (*Jiang et al., 2020*). The bacterial community structures had positive correlations with color and texture of Chinese traditional salt fermented mustard green (*Sarengaowa et al., 2024*). The phylum *Firmicutes* as well as the genera *Enterobacter* and *Lactococcus* were conducive to the production of flavor compounds while the phyla *Cyanobacteria* and *Proteobacteria* affected the texture formation of Chinese traditional sour salt-fermented bamboo shoots (*Long et al., 2023*). The LAB (*Lactobacillus* and *Weissella*) were positively correlated with most of VCs and free amino acids (FAAs) in Chinese sour salt-fermented pork (*Lv et al., 2021*). The microbial community structure of Chinese traditional salt fermented pork was affected by pH value, water activity, NaCl, and total volatile basic nitrogen (TVB-N) (*Wang et al., 2021b*). The *Bacillus*, *Gallicola*, *Proteiniclasticum*, and *Pseudonocardia* were positively correlated with sweet, cheesy, soy sauce-like, and fish sauce-like aromas of the other kind of Thai traditional salt fermented fish (Pla-Ra) (*Phuwapraisirisan et al., 2024*). The LAB (*Lactobacillus*, *Lactococcus*, and *Leuconostoc*), *Brochothrix*, and *Providencia* played a key role in the production of esters which were major flavor compounds while the *Providencia*, *Vagococcus*, and *Weissella* were alcohol producers in Chinese traditional salt and chili fermented fish (*Yin et al., 2024*). The LAB (*Lactococcus* and *Latilactobacillus*) exerted the highest influences on the production of most volatile flavor compounds in a Chinese salt and sugar fermented tilapia fish surimi (*Li et al., 2024*).

The comprehensive and quantitative analyses were performed to compare the abundance of genes, enzymes, and metabolic pathways annotated from the bacterial communities in five Thai traditional fermented foods. Genes, enzymes, and metabolic pathways were indirectly annotated from the obtained bacterial 16S rRNA gene sequence data using the PICRUSt2 software. Even though the annotation can be achieved by utilizing the genome data available on the KEGG and MetaCyC databases, the confinement had been earlier stated. The existing reference genome data on the databases have a tendency to influence the annotation, although this constraint has been diminished as more high-qualified genome data is assessable (*Douglas et al., 2020; Wright & Langille, 2025*). The annotation is also insufficient to distinguish gene functions which are specific to strains (*Douglas et al., 2020*). Our results unveil that the abundant genes, enzymes, and metabolic pathways of all foods were those involved in growth metabolisms, genetic information processing, environmental information processing, and cellular signaling. The PP pathways were mostly abundant in sweet fermented sticky rice (sample A) and their abundance was strikingly higher than in the other foods. The PP pathways are crucial to carbon-balancing processes, adaptation to oxidative stress as well as biosyntheses of amino acids and nucleotides (*Stincone et al., 2015*). During the fermentation process, the PP pathways are vital to provide nicotinamide adenine dinucleotide phosphate (NADPH) for various metabolisms including the production of alcohol and organic acids (*Laëtitia et al., 2014; Masi et al., 2021*), as evidenced by the enrichment of the PP pathways in the fermentation of rice bran (*Chen & Li, 2023*). The other abundant genes, enzymes, and metabolic pathways in sample A included those essential for amino acid metabolism, genetic information processing, cellular signaling as well as biosyntheses of amino acids, fatty acids and nucleotides. Similarly, carbohydrate, sugar, and amino acid metabolisms were the most influential pathways in Chinese fermented rice wines (*Jiang et al., 2020; Zhao et al., 2022*). The most abundant genes and enzymes in sour salt-fermented mustard green (sample B) were those of a glutathione *S*-transferase, which is responsible for cellular detoxification, oxidative stress response, and basal metabolism (*Lienkamp et al., 2021*), a serine/threonine kinase, which modulates cellular signaling, physiology, proliferation, virulence, and antibiotic persistence (*Nagarajan, Lenoir & Grangeasse, 2022*), and a histidine kinase, which is connected to cellular signaling, pathogenicity, virulence, persistence, biofilm development, and antibiotic resistance (*Ahsan et al., 2024*). The sole most abundant pathway in sample B was aerobic respiration for providing energy to cells. As reported in the other study, the pathways in biosyntheses of amino acids and secondary metabolites as well as carbon metabolism, environmental information processing, and cellular signaling were annotated in Chinese salt fermented mustard green, though their abundance was significantly different among regional samples (*Sarengaowa et al., 2024*). The most abundant genes in sour salt-fermented bamboo sprouts (sample C) function in amino acid metabolism, fatty acid biosynthesis, genetic information processing, and cellular signaling. The most abundant enzymes in sample C were a cytochrome *c* oxidase, which is crucial to aerobic respiration (*Hederstedt, 2022*), and a peptidylprolyl isomerase, which catalyzes protein folding (*Anchal, Kaushik & Goel, 2021*). As same as sample B, the sole most abundant pathway in sample C was aerobic respiration. The major metabolic pathways in the fermentation of Chinese bamboo shoots were gluconeogenesis, the tricarboxylic acid (TCA) cycle, the PP pathways, carbon fixation as well as metabolisms of vitamin, coenzyme A (CoA), sugar, amino sugar, nucleotide sugar, nucleotide, and methane (*Hu et al., 2023*). The more abundant genes in sour salt-fermented pork (sample D) were those involved in genetic information processing and cellular signaling as well as a protease gene whose abundance was strikingly higher in sample D than in the other foods. This was possibly related to high protein content in pork with an average of 27.6 g/100 g (*Drewnowski, 2024*). The more abundant enzymes in sample D included a DNA methyltransferase for regulating a wide range of cellular processes (*Gao et al., 2023*) and a beta-glucosidase for saccharide hydrolysis. The more abundant pathways in sample D play roles in glycolysis, organic acid fermentation, sucrose degradation, and nucleotide biosynthesis. Energy, carbohydrate, and amino acid metabolisms were functional in Indian traditional fermented pork fat (*De Mandal et al., 2018*). The sole most abundant gene in sour salt-fermented fish (sample E) was a sugar permease gene (*bglF*) which plays roles in environmental information processing and cellular signaling. The sole most abundant enzyme in sample E was an RNA helicase for

RNA metabolism. The major functional metabolic pathways in sample E were those involved in glycolysis, organic acid fermentation, and sucrose degradation. The metabolic pathways were dynamic over time during the fermentation of a Chinese traditional fish sauce in which carbohydrate, amino acid, and nucleotide metabolisms gradually dominated whereas energy metabolism gradually abated (*Wang et al., 2022b*).

The annotated genes, enzymes, and metabolic pathways in fermented foods reflect the influences of food-related factors (e.g. raw materials, food nutrition facts, starter microorganisms, and fermentation environments) on bacterial diversity and richness. Thus, the comprehensive and quantitative study on the bacterial diversity and communities of various kinds of fermented foods will facilitate understanding of the functional roles of microbiomes which contribute to the product quality and successful fermentation.

Conclusion

The present study investigated the bacterial diversity and compositions in five Thai traditional fermented foods. Almost food nutrition parameters (calories, carbohydrate, fat, moisture, protein, total sugar, and sodium contents) significantly varied across all foods while total sugar was only detected in sweet fermented sticky rice. Sour salt-fermented bamboo sprouts and sweet fermented sticky rice had the significantly highest and significantly lowest bacterial diversity and richness, respectively. The most abundant phyla present in all foods were the *Firmicutes, Proteobacteria*, *Cyanobacteria*, and *Actinobacteriota* whose percentages were arranged in descending order, though the dominant bacterial taxa and their abundance were variable among all foods. The bacterial community structures of both sour salt-fermented meats (pork and fish) were proximate to each other while that of sweet fermented sticky rice and sour salt-fermented bamboo sprouts were mostly alienated from each other. The effects of food nutrition parameters on bacterial communities were statistically identified. Moisture and calories contents exerted very significantly positive and negative impacts, respectively, on the bacterial diversity and richness as well as the classes gamma-*Proteobacteria, Bacteroidia, Actinobacteria*, and *Deinococci*. Total sugar was very significantly negatively associated with the bacterial diversity and richness as well as the gamma-*Proteobacteria, Bacteroidia,* and *Actinobacteria*. Fat and protein contents had very significantly positive associations with the *Bacilli* and very significantly negative associations with the alpha-*Proteobacteria*, *Cyanobacteriia*, and *Deinococci*. Sodium content very significantly negatively affected the *Cyanobacteriia*. This study unveils the bacterial microbiomes and keystone taxa in five Thai traditional fermented foods and also profiles the differences in metagenomic and metabolomic compositions for understanding the bacterial succession in the fermentation processes.

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Figure 1

Venn diagram presenting the numbers of common, overlapping, and unique ASVs among five Thai traditional fermented foods.

Sample codes (A-E) are as those in Table 1 footnote.

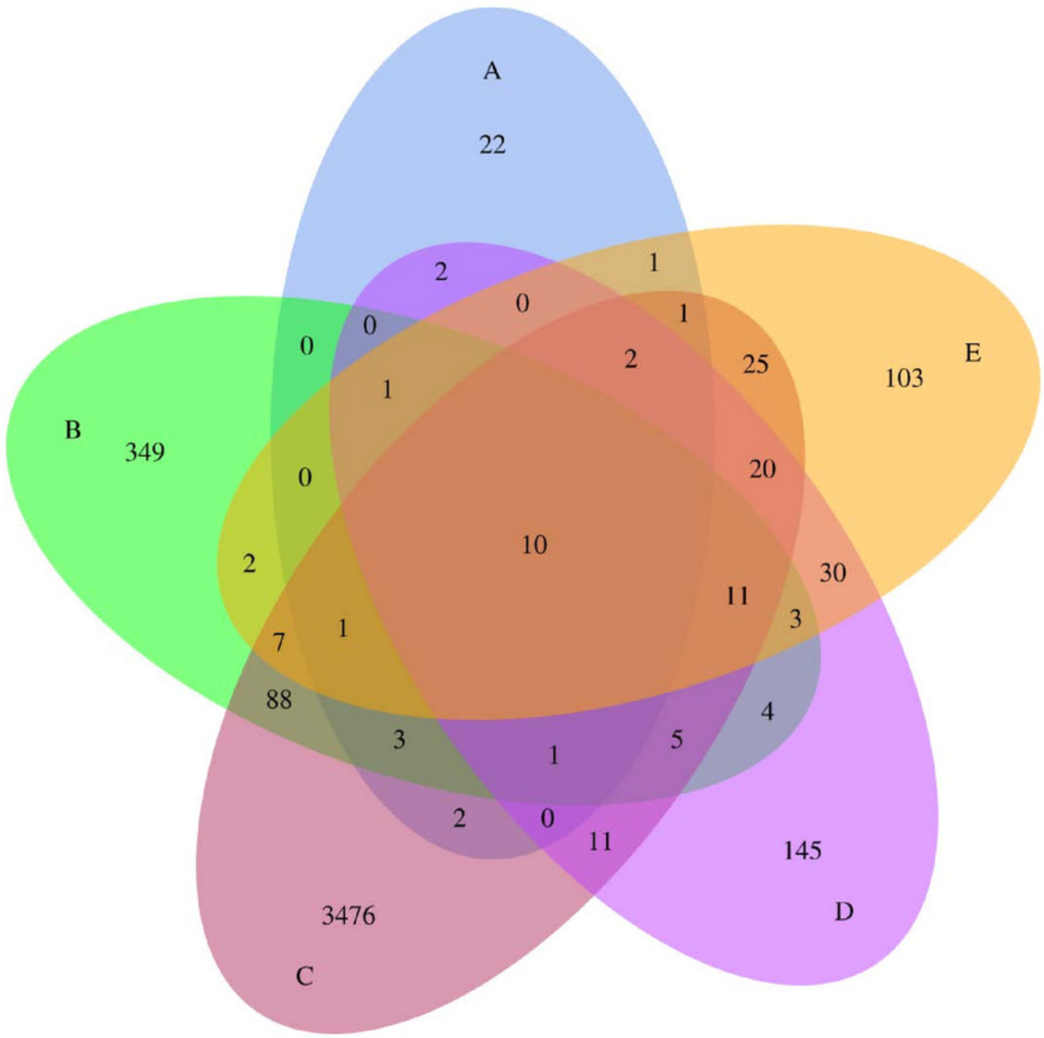

A
22
B
349
C
3476
D
145
E
103
2
1
0
1
0
2
25
0
1
20
0
10
2
30
11
7
1
3
88
4
3
5
1
2
0
11

Figure 2

Relative abundance of the top ten most abundant phyla present in five Thai traditional fermented foods .

Sample codes (A-E) are as those in Table 1 footnote.

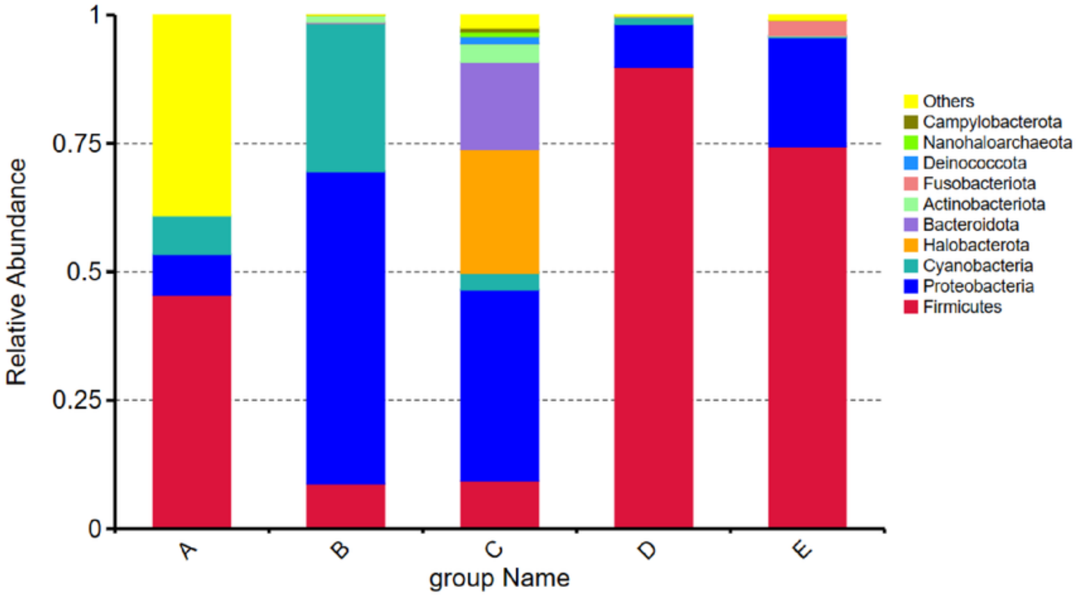

1
0.75
0.5
0.25
0
Relative Abundance
A
B
C
D
E
group Name
Others
Campylobacterota
Nanohaloarchaeota
Deinococcota
Fusobacteriota
Actinobacteriota
Bacteroidota
Halobacterota
Cyanobacteria
Proteobacteria
Firmicutes

Figure 3

Heat map chart displaying the bacterial genera’s distribution and abundance in five Thai traditional fermented foods.

Sample codes (A-E) are as those in Table 1 footnote.

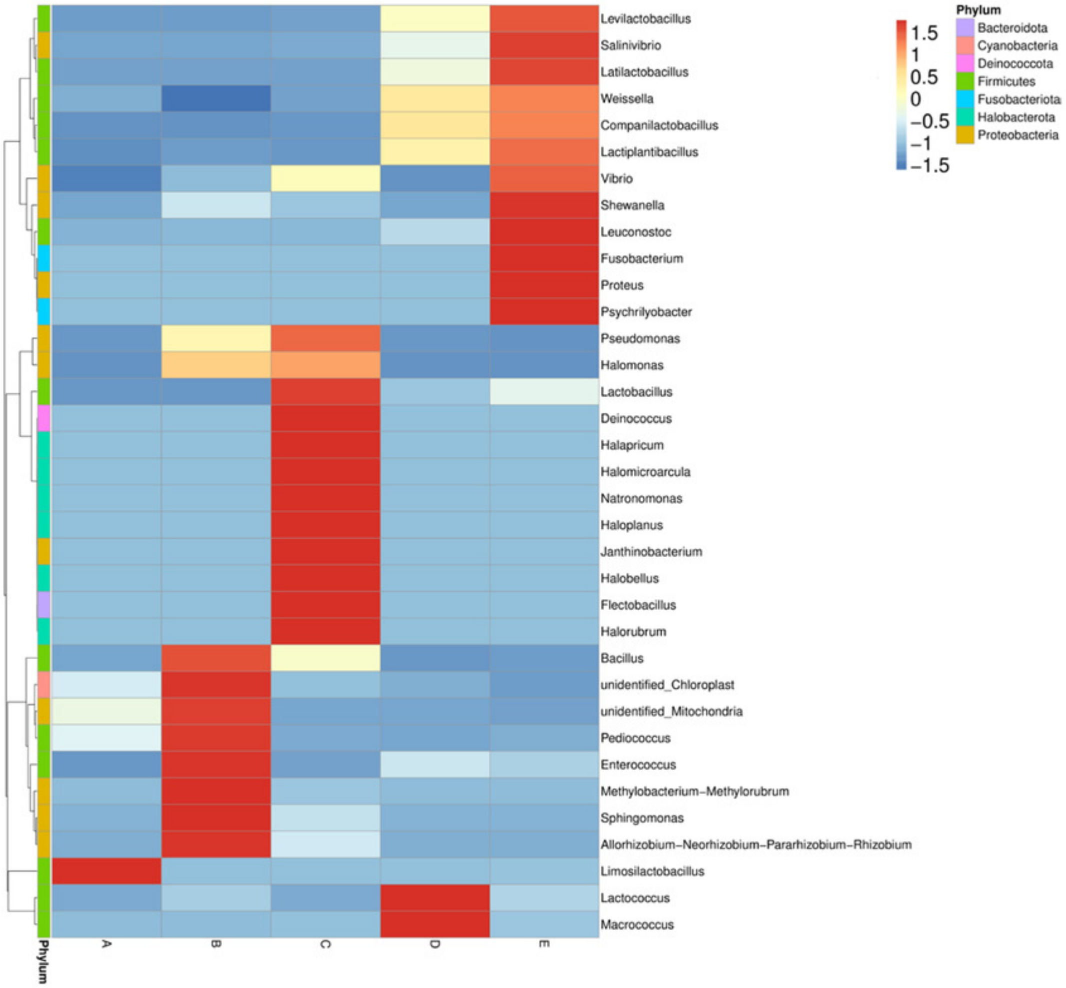

Levilactobacillus
Salinivibrio
Latilactobacillus
Weissella
Companilactobacillus
Lactiplantibacillus
Vibrio
Shewanella
Leuconostoc
Fusobacterium
Proteus
Psychrilyobacter
Pseudomonas
Halomonas
Lactobacillus
Deinococcus
Halapricum
Halomicroarcula
Natronomonas
Haloplanus
Janthinobacterium
Halobellus
Flectobacillus
Halorubrum
Bacillus
unidentified_Chloroplast
unidentified_Mitochondria
Pediococcus
Enterococcus
Methylobacterium-Methylorubrum
Sphingomonas
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Limosilactobacillus
Lactococcus
Macrococcus
Phylum
A
B
C
D
E
1.5
1
0.5
0
−0.5
−1
−1.5
Phylum
Bacteroidota
Cyanobacteria
Deinococcota
Firmicutes
Fusobacteriota
Halobacterota
Proteobacteria

Figure 4

Dissimilarity heat map and UPGMA dendrogram of the bacterial communities in five Thai traditional fermented foods.

(A) Dissimilarity heat map presenting dissimilarity coefficient values of sample pairs. (B) UPGMA dendrogram presenting phyla relative abundance. Sample codes (A-E) are as those in Table 1 footnote.

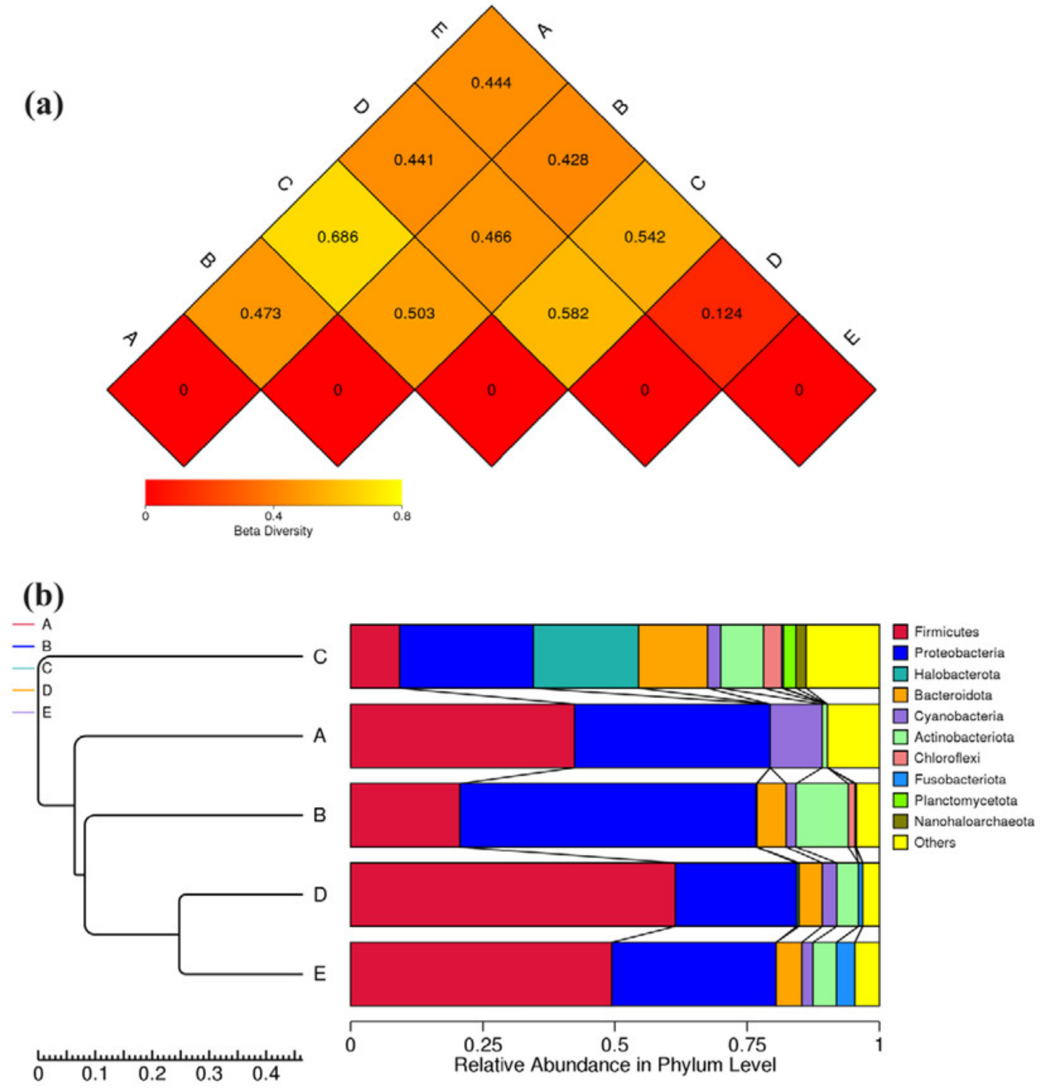

(a)
E
D
C
B
A
A
B
C
D
E
0.444
0.441
0.428
0.686
0.466
0.542
0.473
0.503
0.582
0.124
0
0
0
0
0
0
0.4
0.8
Beta Diversity
(b)
A
B
C
D
E
C
A
B
D
E
0
0.1
0.2
0.3
0.4
0
0.25
0.5
0.75
1
Relative Abundance in Phylum Level
Firmicutes
Proteobacteria
Halobacterota
Bacteroidota
Cyanobacteria
Actinobacteriota
Chloroflexi
Fusobacteriota
Planctomycetota
Nanohaloarchaeota
Others

Figure 5

PCoA and NMDS ordinations of the bacterial composition similarity among five Thai traditional fermented foods.

Sample codes (A-E) are as those in Table 1 footnote. Numbers 1-3 represent sample replicates.

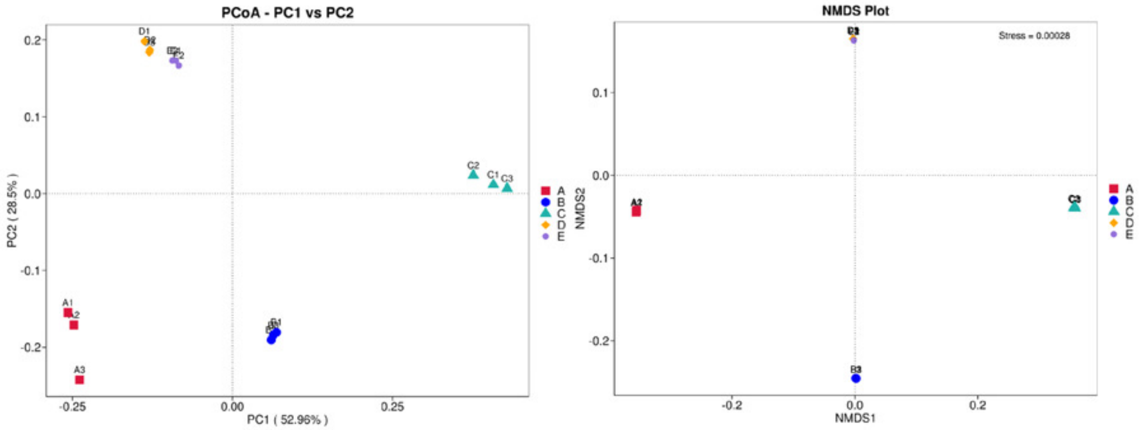
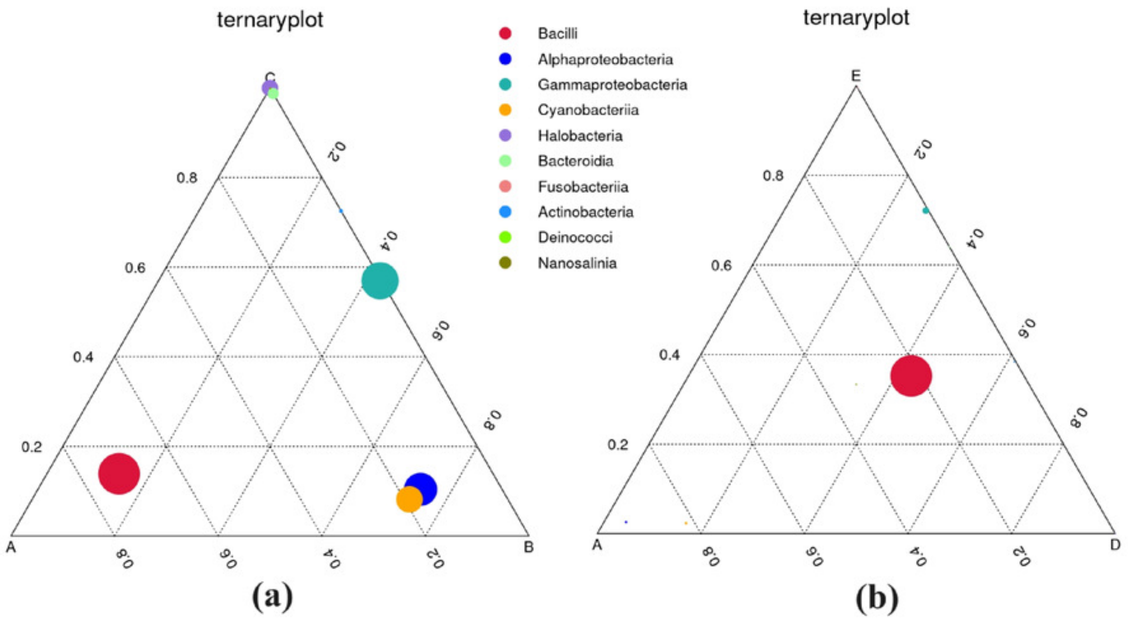


Figure 6

Ternary plots comparing the bacterial classes and their relative abundance in three out of five Thai traditional fermented foods.

(A) Comparison among plant-derived foods (samples A, B, and C). (B) Comparison among sweet fermented sticky rice and sour salt-fermented meats (samples A, D, and E) Color and size of each circle symbolize the bacterial class and its relative abundance, respectively. Sample codes (A-E) are as those in Table 1 footnote.



(a) (b)

Figure 7

Heat map chart displaying the genes (KEGG Orthology no.) annotated from the bacterial communities in five Thai traditional fermented foods and their relative abundance.

Sample codes (A-E) are as those in Table 1 footnote.

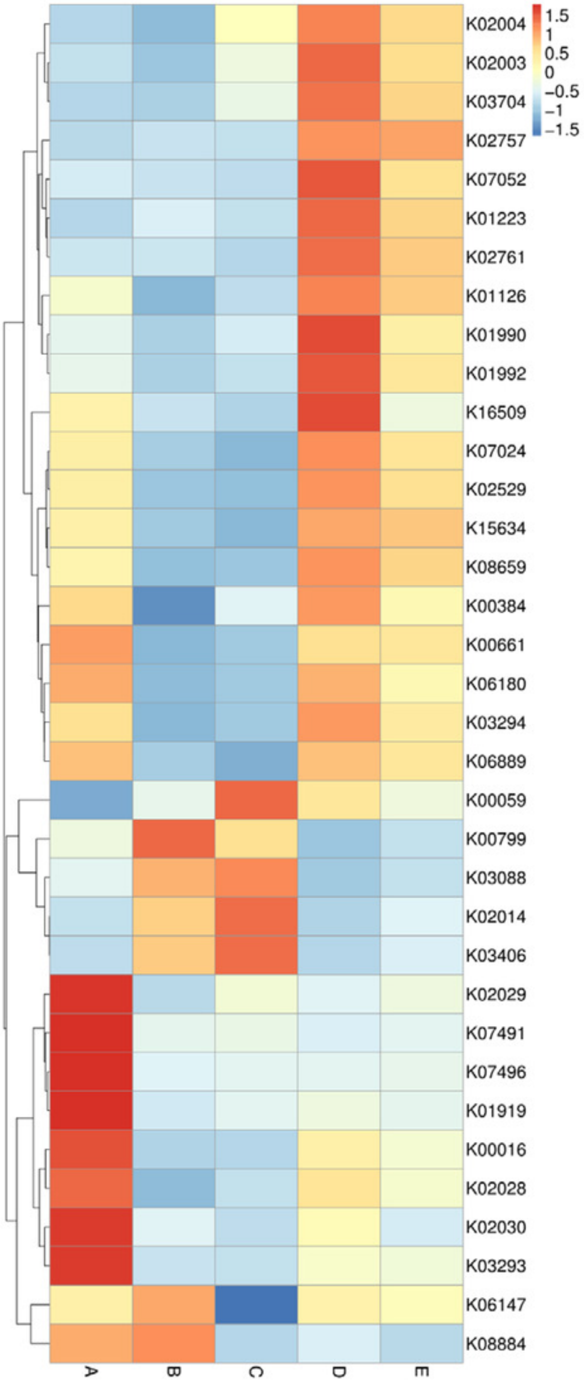

1.5
1
0.5
0
−0.5
−1
−1.5
K02004
K02003
K03704
K02757
K07052
K01223
K02761
K01126
K01990
K01992
K16509
K07024
K02529
K15634
K08659
K00384
K00661
K06180
K03294
K06889
K00059
K00799
K03088
K02014
K03406
K02029
K07491
K07496
K01919
K00016
K02028
K02030
K03293
K06147
K08884
A
B
C
D
E

Figure 8

Heat map chart displaying the enzymes (KEGG Enzyme commission no.) annotated from the bacterial communities in five Thai traditional fermented foods and their relative abundance.

Sample codes (A-E) are as those in Table 1 footnote.

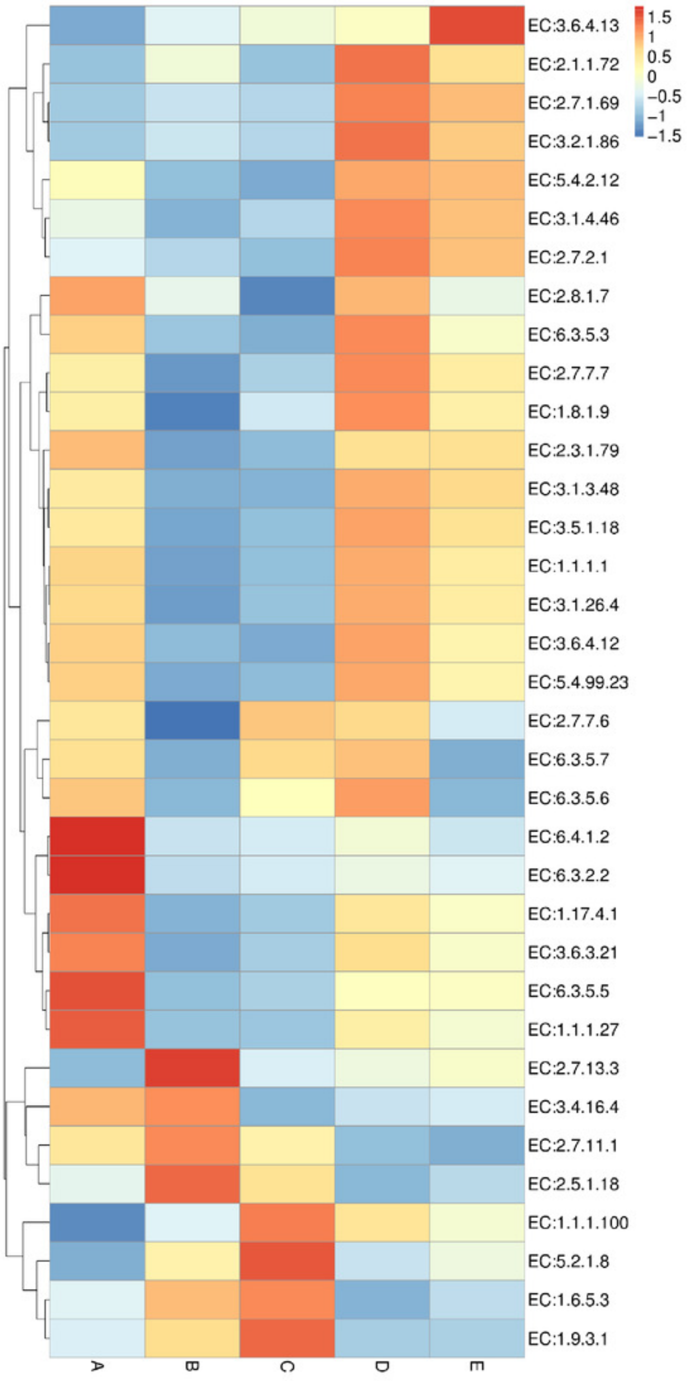

EC:3.6.4.13
EC:2.1.1.72
EC:2.7.1.69
EC:3.2.1.86
EC:5.4.2.12
EC:3.1.4.46
EC:2.7.2.1
EC:2.8.1.7
EC:6.3.5.3
EC:2.7.7.7
EC:1.8.1.9
EC:2.3.1.79
EC:3.1.3.48
EC:3.5.1.18
EC:1.1.1.1
EC:3.1.26.4
EC:3.6.4.12
EC:5.4.99.23
EC:2.7.7.6
EC:6.3.5.7
EC:6.3.5.6
EC:6.4.1.2
EC:6.3.2.2
EC:1.17.4.1
EC:3.6.3.21
EC:6.3.5.5
EC:1.1.1.27
EC:2.7.13.3
EC:3.4.16.4
EC:2.7.11.1
EC:2.5.1.18
EC:1.1.1.100
EC:5.2.1.8
EC:1.6.5.3
EC:1.9.3.1
A
B
C
D
E
1.5
1
0.5
0
−0.5
−1
−1.5

Figure 9

Heat map chart displaying the metabolic pathways (BioCyc ID) annotated from the bacterial communities in five Thai traditional fermented foods and their relative abundance.

S ample codes (A-E) are as those in Table 1 footnote.

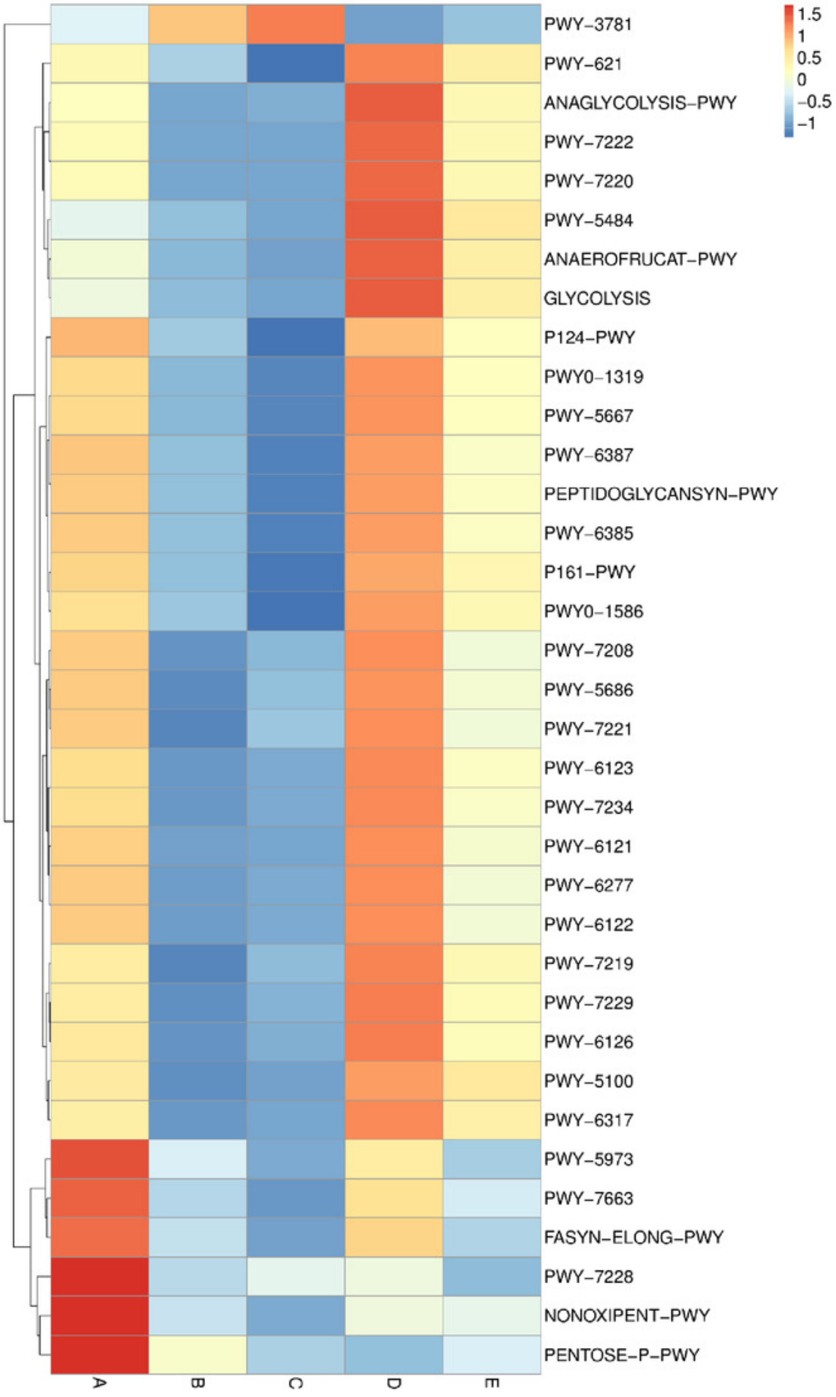

1.5
1
0.5
0
−0.5
−1
PWY−3781
PWY−621
ANAGLYCOLYSIS−PWY
PWY−7222
PWY−7220
PWY−5484
ANAEROFRUCAT−PWY
GLYCOLYSIS
P124−PWY
PWY0−1319
PWY−5667
PWY−6387
PEPTIDOGLYCANSYN−PWY
PWY−6385
P161−PWY
PWY0−1586
PWY−7208
PWY−5686
PWY−7221
PWY−6123
PWY−7234
PWY−6121
PWY−6277
PWY−6122
PWY−7219
PWY−7229
PWY−6126
PWY−5100
PWY−6317
PWY−5973
PWY−7663
FASYN−ELONG−PWY
PWY−7228
NONOXIPENT−PWY
PENTOSE−P−PWY
A
B
C
D
E

Figure 10

Principal Component Analysis (PCA) plots based on the bacterial community compositions of five Thai traditional fermented foods.

(A) Clustering of the annotated genes. (B) Clustering of the annotated enzymes. (C) Clustering of the annotated metabolic pathways Sample codes (A-E) are as those in Table 1 footnote. Numbers 1-3 represent sample replicates.

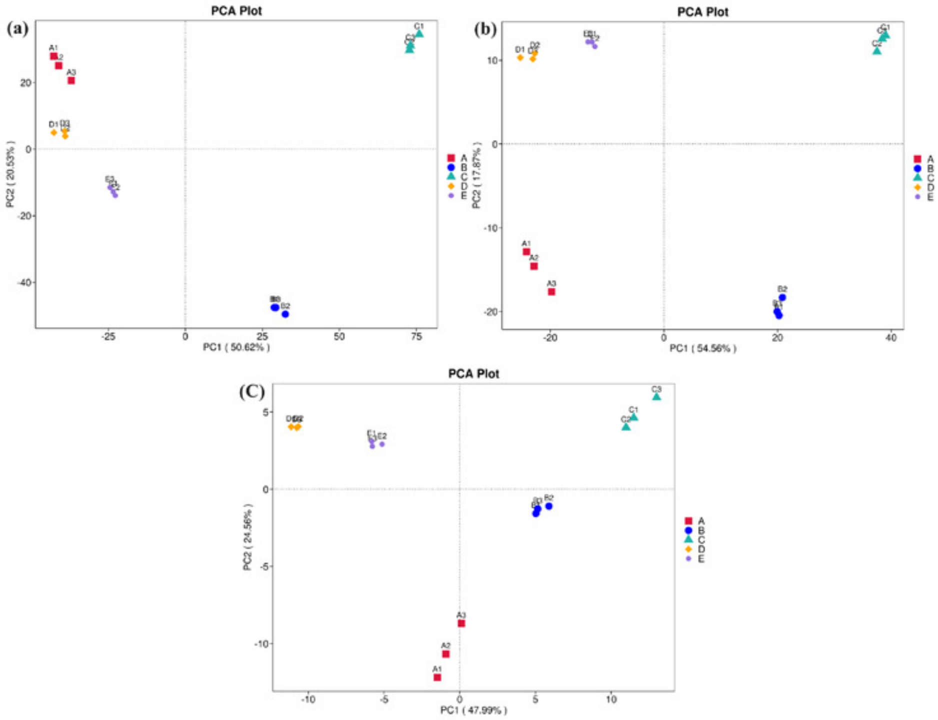


Table 1(on next page)

Food nutrition parameters in five Thai traditional fermented foods.

*All values are the means from three replicates ± SD. **Values with the same letter within a row are not significantly different according to the Tukey's test. Sample codes: A, sweet fermented sticky rice (Khao-Mak); B, sour salt-fermented mustard green (Pak-Kard-Dong); C, sour salt-fermented bamboo sprouts (Nor-Mai-Dong); D, sour salt-fermented pork (Moo-Som); and E, sour salt-fermented fish (Pla-Som).

Food nutrition parameter	Fermented food sample*				
	A	B	C	D	E
Calories (Kcal/100 g)	181.05±0.02e **	21.62±0.05a	26.32±0.01b	102.61±0.33c	113.47±0.02d
Carbohydrate (g/100 g)	40.21±0.02e	3.68±0.01b	4.45±0.02c	3.56±0.06a	7.33±0.05d
Fat (g/100 g)	0.35±0.00c	0.14±0.00a	0.25±0.00b	2.92±0.02e	2.03±0.03d
Moisture (g/100 g)	55.08±0.09a	92.06±0.03e	81.41±0.02d	73.46±0.03c	68.98±0.03b
Protein (g/100 g)	4.26±0.01c	1.33±0.01a	1.67±0.01b	15.61±0.15d	16.51±0.12e
Total sugar (g/100 g)	29.08±0.25b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Sodium (mg/kg)	69.36±0.69a	7,817.96±1.31c	1,828.56±1.71b	14,054.48±1.50d	15,738.20±2.23e

Table 2(on next page)

Bacterial diversity and richness indices of five Thai traditional fermented foods .

*All values are the means from three replicates ± SD. **Values with the same letter within a row are not significantly different according to the Tukey’s test. Sample codes (A-E) are as those in Table 1 footnote.

Index	Fermented food sample*				
	A	B	C	D	E
Shannon-Weaver	1.88±0.19a**	4.12±0.06c	7.57±0.30d	3.51±0.02b	4.15±0.06c
Simpson's	0.65±0.05a	0.89±0.01c	0.96±0.00d	0.83±0.00b	0.89±0.00c
Chao1	32.08±5.34a	285.50±54.62c	1879.46±166.01d	129.71±22.66b	150.59±17.85b
Number of observed species	31.00±4.35a	261.33±43.87c	1769.33±150.44d	128.00±20.88b	143.66±5.71b
Dominance	0.34±0.05e	0.13±0.01c	0.03±0.00a	0.16±0.00d	0.10±0.00b

Table 3(on next page)

Percentages of the top ten most abundant bacterial classes present in five Thai traditional fermented foods.

*All values are the means from three replicates ± SD. **Values with the same letter within a row are not significantly different according to the Tukey’s test. Sample codes (A-E) are as those in Table 1 footnote.

Bacterial class	Fermented food sample*				
	A	B	C	D	E
Bacilli	45.50±5.79b**	8.77±0.81a	8.74±0.43a	89.79±0.97d	73.56±1.66c
alpha-*Proteobacteria*	7.82±3.98d	36.73±3.09e	5.17±0.10c	0.35±0.20b	0.21±0.02a
gamma-*Proteobacteria*	0.15±0.02a	24.02±0.60d	31.99±2.12e	7.98±0.70b	21.03±1.19c
Cyanobacteriia	7.53±3.61d	28.88±3.94e	3.23±0.15c	1.47±0.60b	0.21±0.01a
Halobacteria	0.00±0.00a	0.00±0.00a	24.07±5.08b	0.00±0.00a	0.00±0.00a
Bacteroidia	0.00±0.00a	0.20±0.07c	15.79±2.31e	0.53±0.01d	0.09±0.01b
Fusobacteriia	0.00±0.00a	0.00±0.00a	0.03±0.00c	0.01±0.00b	2.89±0.56d
Actinobacteria	0.00±0.00a	1.29±0.3d	3.41±0.1e	0.12±0.0c	0.07±0.01b
Deinococci	0.00±0.00a	0.00±0.00a	1.39±0.07b	0.00±0.00a	0.00±0.00a
Nanosalinia	0.00±0.00a	0.00±0.00a	0.91±0.26b	0.00±0.00a	0.00±0.00a