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Diversity of cultivable lactic acid bacteria and bacilli in traditional fermented foods in Vietnam

GIANG PHAN THI HANG^{1,2}, MARKÉTA HUSÁKOVÁ¹, PETR KAŠTÁNEK³,
PETRA PATAKOVA^{1*} 

¹Department of Biotechnology, University of Chemistry and Technology, Prague, Czech Republic

²Vinmec International Hospital, Vinmec Healthcare System, Hanoi, Vietnam

³EcoFuel Laboratories, Prague, Czech Republic

*Corresponding author: patakovp@vscht.cz

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Abstract: Lactic acid bacteria (LAB) are commonly used in many European and Asian traditional fermented foods such as yogurt, cheese, sourdough, meat, fruit, vegetables, cereal products, sour spring rolls, fish sauce, sour shrimp, chao, ruou nep, tofu. They not only improve the flavour and texture of fermented products but also they inhibit the development of spoilage bacteria as antimicrobial agents. In this study, thirty-five traditional Vietnamese fermented products were collected for isolation, identification, and characterisation of LAB and bacilli. There were fifty-three species of LAB isolated from samples such as *Lactococcus lactis*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Liquorilactobacillus nagelli*, *Companilactobacillus farciminis*, *Levilactobacillus brevis*, *Lactiplantibacillus pentosus*, *Lactococcus garvieae*, *Lactilactobacillus sakei*, and twenty-one species of bacilli such as *Priestia megaterium*, *Bacillus cereus*, *Bacillus pumilus*, *Metabacillus indicus*. This study aimed to provide information about the occurrence of LAB and bacilli in traditional fermented foods in Vietnam and their brief characterisation.

Keywords: isolation of bacteria; food fermentation; lactic acid; *Bacillus*

In Vietnam, almost all traditional fermented foods are made from non-dairy indigenous materials like vegetables, cereal, meat, fish, and shrimp. Traditional Vietnamese fermented foods are divided into two types: long and short shelf-life products. Long life fermented foods are usually products with high salt concentrations like nuoc mam or mam nem (fish sauces) and tom chua (sour fermented shrimp). They have a shelf life of three months to two years at ambient temperature because a high concentration of salt (up to 25% w/w)

inhibits pathogenic contamination. Their fermentation process lasts from one to eighteen months. On the other hand, short life fermented products, such as fermented vegetables – e.g. cabbage, eggplant or bamboo, and fermented rice (ruou nep) usually have a sour taste and their fermentation time ranges from three to five days. They are intended for quick consumption and should be stored in a cool place, at a temperature range of 2–8 °C, to slow down the fermentation progress and prevent contamination. Several herbs and

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spices can be added during the fermentation process to enhance the taste, such as onion, garlic, ginger, pawpaw, or pepper. Vietnamese traditional fermented foods and beverages may share the way of fermentation but they differ in used raw materials like mam tom (shrimp paste), mam ruoc (sea water shrimp paste), mam tep (small shrimp paste), so it may be difficult to distinguish them for people outside Vietnam (Thanh and Viet Anh 2016). The traditional Vietnamese fermented products used for the isolation of microorganisms in this study are characterised in Table 1.

The traditional fermented foods are produced on a small scale in households, the fermentation processes are carried out spontaneously in most cases, and the fermentation time mainly depends on ambient temperature, so that product quality and their specifications vary and are not defined (La Anh 2015). Some households may use the old batch as starter for the new batch, then the fermentation as well as the shelf life are shorter. Lactic acid bacteria (LAB) are considered the predominant bacteria in fermented products having a crucial role in the fermentation process (Doan et al. 2013). LAB are irreplaceable as starter cultures in fermented foods and increase their shelf life by the production of lactic acid (Rhee et al. 2011). There are few studies describing the lactic acid bacteria (LAB) isolation from Vietnamese fermented foods (Inasu et al. 2005; Doan et al. 2012; Ong et al. 2020). Further, many other non-LAB microorganisms occur in products, both functional and non-functional ones. Functional microorganisms convert chemical components of initial raw materials in fermentation processes into new components that have probiotic properties, antimicrobial or antioxidant effects, or result in peptide production (Tamang et al. 2016). Namely bacteria from the genus *Bacillus* are found frequently in fermented foods and beverages (Tamang 2015). In the food fermentation process, bacilli produce enzymes necessary for the raw material decomposition, such as amylases and proteases (Ramos et al. 2015), extracellular polysaccharides and polypeptides, and lipopeptides with antimicrobial activity (Li et al. 2023) and contribute to aroma formation (Noguchi et al. 2004; Uchida et al. 2004). However, the role of *Bacillus* in food fermentation is not always appreciated.

The main objective of this study was to isolate LAB and bacilli that contribute to the typical character of traditional Vietnamese fermented foods. Theoretically, the knowledge could be used for the development of new fermented products produced by controlled fermentation.

Table 1. Characterisation of used traditional Vietnamese fermented products

Product name	Main component	Process description	Fermentation parameters (temperature, time, and others)	Shelf life
Rice products				
Ruou nep cam, ruou nep	sticky rice	steamed rice inoculated with banh men*, alcohol fermentation	3–5 days, ambient temperature	3–4 days, ambient temperature; 1 week, 2–8 °C
Ruou gao nep	sticky rice	soaked sticky rice ground into a fine powder, cooked to turn into sugar fluid, then cooled and fermented for 14 days, vinegar is then distilled to obtain wine	20–30 days	3–5 years, ambient temperature, avoid sunlight
Bong ruou (dam bong)	rice distilled stillage	steamed rice inoculated with banh men, added water and distilled to obtain alcohol, the residue (stillage) is boiled with water and fermented	2–3 weeks	1–2 months

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Table 1. To be continued

Product name	Main component	Process description	Fermentation parameters (temperature, time, and others)	Shelf life
Me chua, (com me)	sour rice fermented paste	fermentation of overcooked wet rice	7–10 days	8 months
Dam gao	rice vinegar	roasted rice incubated with sugar, water, and alcohol for about 2 weeks to obtain vinegar	2 weeks	12–16 months
Soybean products				
Tuong	glutinous rice, soybean, salt	steamed glutinous rice brewed as starter culture with rice-hydrolysing mould, and ground roasted soybean	approximate 3 months, hot weather, avoid humid and wet	18–24 months
Chao	tofu	tofu with salt, ground red chilli peppers, alcohol fermentation	10–14 days, ambient temperature	4–5 months, room temperature
Vegetable and fruit products				
Dua muoi	mustard leaf	mustard leaf with salt and sugar water, lactic acid fermentation	3–5 days, ambient temperature	3–4 days after fermentation
Bap cai muoi	cabbage	cabbage with salt and sugar water, lactic acid fermentation	2–3 days, ambient temperature	3–4 days after fermentation
Ca muoi	eggplant, salt	eggplant with salt and sugar water, lactic acid fermentation	2–3 days, ambient temperature	3–4 days after fermentation
Mang chua	bamboo	bamboo soaked in rice water and salt, then boiled and incubated with salt, sugar water, garlic, chilli	7–10 days	3–5 days
Dua chuoat muoi	cucumber	cucumber incubated with salt and sugar water, garlic, chilli	3–4 days, ambient temperature	6–12 months
Kieu muoi	scallion (onion type), salt	scallion incubated with salt and sugar water, garlic, chilli	3–4 days, ambient temperature	3–4 days, room temperature; 3–4 weeks, 2–8 °C
Sung muoi	fig, salt, spices	fig incubated with salt and sugar water, garlic, chilli	2–3 days	5–7 days, ambient temperature; 2–3 weeks, 2–8 °C
Hanh muoi	onion, salt	onion incubated with salt and sugar water, vinegar, garlic, chilli	3–4 days	5–7 days, ambient temperature; 2–3 weeks, 2–8 °C

Table 1. To be continued

Product name	Main component	Process description	Fermentation parameters (temperature, time, and others)	Shelf life
Meat products				
Nem chua	raw pork lean, rind, roasted sticky rice powder, garlic, chilli	boiled and sliced pork rind, roasted sticky rice powder and spices (garlic, pepper) incubated at room temperature	2–3 days	3–5 days
Thit muoi	pork, salt	boiled pork incubated with salty water and spice for 24 h	2–3 days	5–7 days after open, 3 months, 2–8 °C
Nem Bui	pork, rice	boiled, sliced pork and lean incubate with salt, roasted rice powder, spices	1–2 days	2–3 days, 2–8 °C
Fish products				
Mam nem	anchovy dipping paste	ground anchovy with salt	20–30 days under sunlight	12 months
Nuoc mam (fish sauce)	fish, salt	sea water fish incubated with salt	12–18 months	18–24 months
Ca thính	fish, rice	fish incubated with salt, roasted corn powder	4–10 days depends on the weather condition	6–8 months
Shrimp products				
Mam tom	shrimp paste	ground shrimp hydrolysed with salt, wine fermentation under sunlight	6 months	2 years
Tom chua	shrimp, rice, salt, spices	fresh or brackish water shrimp incubated with roasted sticky rice powder, salt, spices	4–6 weeks, room temperature in anaerobic condition	12 months
Mam tep	shrimp paste	ground small shrimp hydrolysis with salt, roasted ground rice, wine fermentation at ambient temperature	10–15 incubation under sunlight, continue incubate 1 month at ambient temperature or 15–18 days under sunlight	6–12 months, 2–8 °C
Mam ruoc	shrimp paste	ground small sea water shrimp hydrolysed with salt, wine, sugar fermentation under sunlight	6–9 months	12 months

*Banh men is the traditional fermentation starter composed of an undefined mixture of yeast, bacteria and fungi (Thanh et al. 2008)

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MATERIAL AND METHODS

Sample collection

The collected samples were Vietnamese traditional fermented meat, vegetables, fish, and shrimp purchased in markets in Hanoi, Vietnam in the 2022–2023 period. Microbiological analysis of the samples was performed within 24 h, or exceptionally within 48 h of purchase. Until then, the samples were stored at the recommended temperature (i.e. at ambient temperature or in a refrigerator at 7 °C).

Media for inoculation

For initial isolation of bacteria from the samples, the following culture media were used: de Man-Rogosa-Sharpe agar (MRSA; Merck, Germany), Tryptic Soy Agar (TSA; Merck, Germany), and Triple Sugar Iron Agar (TSI; Merck, Germany).

Bacteria isolation

Liquids from fermented samples (e.g. dua muoi, ca muoi, ruou nep) were diluted using a decimal dilution scheme and sterile 0.9% NaCl in water as diluent. For the inoculation of agar plates, 10^{-2} , 10^{-3} , and 10^{-4} dilutions of the original sample were used (e.g. the liquid was diluted 100 times for 10^{-2} dilution). Solid samples (e.g. nem chua, thit chua, ca thinh) were ground and diluted with PBS buffer (containing in g·L⁻¹: 1.15 Na₂HPO₄, 0.2 KH₂PO₄, 8.0 NaCl, 0.2 KCl, 0.1 CaCl₂, 0.1 MgCl₂, pH 7.4) in a similar way like liquid samples to concentrations of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} . For inoculation, 0.1 mL of each diluted sample was placed on MRSA in Petri dishes and spread out evenly on the agar. The inoculated dishes were incubated at 30 °C for 24–48 h.

For further work, separate colonies grown on the agar and exhibiting special characteristics of LAB, i.e. circular, opalescent or milky white, colourless, transparent, convex, entire or lobate margin, and smooth appearance, were selected. The individual strains were selected and purified by the streak plate method using MRSA and TSA medium.

Identification of bacteria

Identifying the microorganisms was performed by their molecular fingerprint with the 4800 Plus MALDI-TOF MS/TOFTM analyser [BD Bruker MALDI Biotyper (MBT), USA] (MALDI-TOF – matrix-assisted laser ionisation – time of flight; MS – mass spectrometry). The data files from the 4800 Plus MALDI-TOF/TOFTM analyser system were analysed on Data Explorer soft-

ware (version 4.9) to convert to text files. The text files were then entered into the Bionumerics system for further analysis. Accuracy and specificity of bacterial identification correspond to the calculated score. Normally, a score of 2.0 or higher indicates correct identification at the species level, and a score between 1.7 and 1.99 indicates identification at the genus level (BD Bruker MALDI Biotyper Instruction).

Biochemical tests

Chemical and biological tests were performed with the isolates for further determination of their typical characteristics:

Catalase test. 1–2 drops of H₂O₂ was applied directly onto the surface of LAB colonies that have been cultured on MRSA plates at 37 °C for 24–48 h. If gas bubbles emerged, the test was positive and vice versa.

Oxidase test. A colony was taken using a glass rod, and rubbed on a piece of paper. Further a drop of oxidase reagent (N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride) was added to the colony. The results were observed within the first 10 s. The positive reaction gave a blue violet colour.

Indole test. Bacteria were inoculated in MRS broth and kept at 35–37 °C for 18–24 h. Then 200 µL was transferred to Eppendorf tube. Further, 3–5 drops of Kovacs' indole reagent were added into the tube. The positive reaction showed a red ring above the culture solution.

Urease test. Typical colonies were inoculated on urease agar base medium at 35–37 °C for 18–24 h. The result was positive when the environment turned pink-purple, and the result was negative when the medium did not change the colour.

Determination of glucose fermentation. The bacteria were inoculated on TSI plates and cultivated at 37 °C for 24 h. If the bacteria can ferment glucose without fermenting lactose, the colour of the agar will change from red to yellow, if the bacteria can ferment lactose, the inclined medium will turn yellow.

RESULTS AND DISCUSSION

From thirty-five Vietnamese traditional fermented products (Table 1), colonies of different shapes and sizes were isolated. However, typical colonies obtained after 24–48 h growth at 37 °C were white, smooth and circular. After transferring to the streak plates to purify and isolate the pure cultures, 74 isolates were identified using the MALDI-TOF system (Table 2). Forty-three isolates (i.e. 57% of all isolates) were identified with high probability at a species level (the MALDI score above 2)

Table 2. Bacteria identification using the MALDI-TOF system

No.	Sample	Microorganism	Score
Rice products			
1	ruou nep cam	<i>Priestia megaterium</i>	2.24
2		<i>Weissella paramesenteroides</i>	2.16
3		<i>Pediococcus pentosaceus</i>	2.12
4	me chua	<i>Bacillus cereus</i>	2.31
5	ruou nep	<i>Bacillus cereus</i>	2.41
6		<i>Limosilactobacillus fermentum</i>	1.88
7	ruou gao nep	no LAB or <i>Bacillus</i>	–
8	dam gao	<i>Lactiplantibacillus plantarum</i>	2.41
9		<i>Pediococcus pentosaceus</i>	2.06
Soybean products			
10	chao thuan phat	<i>Lysinibacillus xylanilyticus</i>	1.93
11	chao mon dai binh duong	<i>Lysinibacillus boronitolerans</i>	1.83
12	tuong ban	<i>Bacillus atrophaeus</i>	1.74
13		<i>Bacillus amyloliquefaciens</i>	1.85
14	tuong nam dan	no LAB or <i>Bacillus</i>	–
Vegetable and fruit products			
15	dua muoi	<i>Bacillus cereus</i>	1.90
16		<i>Bacillus pumilus</i>	1.96
17		<i>Metabacillus indicus</i>	1.98
18		<i>Lactiplantibacillus plantarum</i>	1.94
19	bap cai muoi	<i>Lactococcus lactis</i>	1.85
20		<i>Lactiplantibacillus plantarum</i>	2.09
21		<i>Limosilactobacillus fermentum</i>	2.03
22	ca muoi	<i>Limosilactobacillus fermentum</i>	2.04
23	bong ruou (dam bong) (beet alcohol)	<i>Bacillus cereus</i>	2.15
24	mang chua	<i>Limosilactobacillus fermentum</i>	2.18
25		<i>Liquorilactobacillus nagelli</i>	2.37
26	dua chuot muoi	<i>Limosilactobacillus fermentum</i>	1.86
27		<i>Weissella cibaria</i>	2.26
28		<i>Pediococcus pentosaceus</i>	2.07
29	kieu muoi	<i>Limosilactobacillus fermentum</i>	1.99
30		<i>Lactiplantibacillus plantarum</i>	2.41
31		<i>Lactiplantibacillus pentosus</i>	2.23
32	sung muoi	<i>Limosilactobacillus fermentum</i>	1.84
33		<i>Weissella confusa</i>	1.80
34		<i>Weissella paramesenteroides</i>	1.97
35	hanh muoi	<i>Lactiplantibacillus plantarum</i>	2.04
36	bap cai muoi	<i>Limosilactobacillus fermentum</i>	1.79
37		<i>Lactiplantibacillus plantarum</i>	2.22
38		<i>Weissella confusa</i>	1.98
39		<i>Limosilactobacillus fermentum</i>	1.93
40	bap cai muoi	<i>Lactiplantibacillus plantarum</i>	2.24
41		<i>Weissella cibaria</i>	2.09

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Table 2. To be continued

No.	Sample	Microorganism	Score
42	bap cai muoi	<i>Leuconostoc lactis</i>	2.12
43		<i>Weissella cibaria</i>	2.11
Meat products			
44	bap cai muoi	no LAB or <i>Bacillus</i>	–
45	bap cai muoi	<i>Limosilactobacillus fermentum</i>	1.79
46		<i>Lactiplantibacillus plantarum</i>	1.70
47		<i>Lactiplantibacillus pentosus</i>	1.94
48		<i>Lactiplantibacillus plantarum</i>	2.08
49	nem chua	<i>Companilactobacillus farciminis</i>	1.85
50		<i>Lactococcus lactis</i>	2.32
51		<i>Levilactobacillus brevis</i>	1.85
52		<i>Pediococcus pentosaceus</i>	2.09
53		<i>Weissella cibaria</i>	2.11
54	thit chua anh xuan (đat to) (pork, rice)	<i>Lactococcus garvieae</i>	2.12
55		<i>Lactococcus lactics</i>	2.17
56		<i>Lactilactobacillus sakei</i>	2.18
57		<i>Weissella cibaria</i>	1.83
58		<i>Pediococcus pentosaceus</i>	2.10
59	thit chua truong food (pork, rice)	<i>Lactiplantibacillus plantarum</i>	2.09
60		<i>Pediococcus pentosaceus</i>	2.15
61	thit muoi	<i>Weissella cibaria</i>	1.97
62	nem bui	<i>Lactiplantibacillus pentosus</i>	1.70
63		<i>Weissella confusa</i>	1.70
Fish products			
64	mam nem	<i>Bacillus amyloliquefaciens</i>	1.83
65		<i>Bacillus pumilus</i>	1.93
66		<i>Bacillus altitudinis</i>	1.89
67		<i>Bacillus subtilis</i>	2.12
68		<i>Bacillus vallismortis</i>	1.89
69	nuoc mam	no LAB or <i>Bacillus</i>	–
70	ca thinh	<i>Lactiplantibacillus pentosus</i>	2.57
71		<i>Limosilactobacillus fermentum</i>	2.11
72		<i>Companilactobacillus farciminis</i>	1.89
73		<i>Lactococcus garvieae</i>	2.01
Shrimp products			
74	mam tom	<i>Bacillus altitudinis</i>	2.01
75	tom chua	<i>Bacillus cereus</i>	2.20
76		<i>Priestia megaterium</i>	2.06
77		<i>Bacillus subtilis</i>	2.10
78	mam tep	no LAB or <i>Bacillus</i>	–
79	mam ruoc	<i>Bacillus pumilus</i>	2.05

Bold – bacteria identified with high probability on a species level (the calculated score above 2.00); description of the main ingredients of the samples can be found in Table 1, or in the case of local specialties it is given after the name; LAB – lactic acid bacteria; MALDI-TOF – matrix-assisted laser ionisation – time of flight

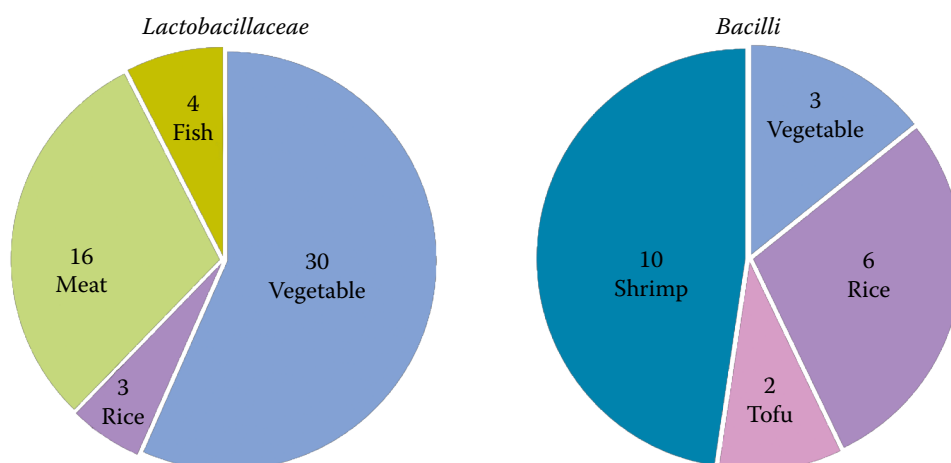


Figure 1. Distribution of bacterial species in different fermented foods

while the rest was identified at a genus level. The most frequently isolated species were *Limosilactobacillus fermentum* (isolated 10 times), *Lactiplantibacillus plantarum* (isolated 9 times), *Weissella cibaria* (isolated 6 times), and *Pediococcus pentosaceus* (isolated 5 times). On the other hand, 24 species were isolated only once. The most commonly isolated species belong to the species that are often isolated from fermented foods and strains with probiotic properties can be found among them (Jiang et al. 2021; Fidanza et al. 2021; Lacerda et al. 2022; Kang et al. 2023). The species names of the families *Lactobacillaceae* and *Bacilli* are given according to novel taxonomy (Gupta et al. 2020; Zheng et al. 2020).

In our research, LAB are predominant bacteria compared to *Bacillus* species in products with sour taste and short fermentation. *Bacillus* species can survive in salty fermented products, which agrees with the results published by Thanh and Viet Anh (2016). Distribution of LAB and bacilli in different samples is shown in Figure 1. Almost all LAB in this research occur in fermented vegetables, meat, and fish with sour taste and in products with short production period (from 3 to 5 days). Otherwise, bacilli occurred in shrimp sauces, alcohol fermented rice, and in many kinds of highly salty sauces (e.g. in mam nem, soybean sprouts, sour shrimp) and in rice alcohol, in which LAB are rarely found. Long fermentation (from 1 to 18 months) is typical of these products. Formation of spores by bacilli may facilitate their survival in the hostile environment of some fermented products (high concentration of salt or low pH). The presence of LAB or *Bacillus* may also depend on the raw materials, fermentation stage, ambient temperature, and season. In summer with higher ambient temperature, normally the microorganisms grow faster. In the fermented cabbage samples

(samples 36–47 in Table 2), the occurrence of LAB was different. Besides some common LAB like *Limosilactobacillus fermentum* or *Lactiplantibacillus plantarum*, the samples contained also different LAB species, or it was not possible to isolate any LAB from them. This probably depends on the traditional fermentation method of each household because some of them can use the sour solution of the previous batch as starter culture for the new batch, which can reduce the time of fermentation. This leads to the altered fermentation duration as well as modified quality of fermented products while the use of starter culture may affect the growth rate of bacteria. The distribution of LAB and bacilli in our research was lower compared to the research of Doan et al. (2012); it may be caused by the differences in used materials, spices, fermentation conditions and time of sample collection. Some isolated species may have significant positive effects on human health like the production of B₁₂ vitamin by *Priestia megaterium* (Biedendieck et al. 2021), bacteriocin production by *Liquorilactobacillus nagelli* (Yetiman and Ortakci 2023) or significance for food preservation like antagonistic activity against *Listeria innocua* found in *Bacillus atrophaeus* (Guo et al. 2016).

The individual separated 74 bacterial species, identified by MALDI-TOF, were used for further characterisation. They were stained according to Gram (all of them as positive) and the selected biochemical tests were performed (Table 3).

LAB exhibited the typical characteristics in biochemical tests: Gram positive, catalase negative, oxidase negative, indole test negative, urease negative, glucose and lactose fermentations positive. On the other hand, *Bacillus* species differed from species to species. The common characteristics of *Bacillus* include

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Table 3. Characterisation of bacterial isolates

No.	Microorganism	Catalase	Oxidase	Indole	Urease	Glucose	Lactose
Rice products							
1	<i>Priestia megaterium</i>	+	+	–	–	+	+
2	<i>Weissella paramesenteroides</i>	–	–	–	–	+	+
3	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
4	<i>Bacillus cereus</i>	+	+	–	–	+	+
5	<i>Bacillus cereus</i>	+	+	–	–	+	+
6	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
8	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
9	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
Soybean products							
10	<i>Lysinibacillus xylanilyticus</i>	+	+	–	–	–	–
11	<i>Lysinibacillus boronitolerans</i>	+	+	–	+	–	–
12	<i>Bacillus atrophaeus</i>	+	+	–	–	+	–
13	<i>Bacillus amyloliquefaciens</i>	+	+	–	–	+	+
Vegetable and fruit products							
15	<i>Bacillus cereus</i>	+	+	–	–	+	+
16	<i>Bacillus pumilus</i>	+	+	–	–	–	–
17	<i>Metabacillus indicus</i>	+	+	–	–	–	–
18	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
19	<i>Lactococcus lactis</i>	–	–	–	–	+	+
20	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
21	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
22	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
23	<i>Bacillus cereus</i>	+	+	–	–	+	+
24	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
25	<i>Liquorilactobacillus nagelli</i>	–	–	–	–	+	+
26	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
27	<i>Weissella cibaria</i>	–	–	–	–	+	+
28	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
29	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
30	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
31	<i>Lactiplantibacillus pentosus</i>	–	–	–	–	+	+
32	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
33	<i>Weissella confusa</i>	–	–	–	–	+	+
34	<i>Weissella paramesenteroides</i>	–	–	–	–	+	+
35	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
36	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
37	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
38	<i>Weissella confusa</i>	–	–	–	–	+	+
39	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
40	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+

Table 3. To be continued

No.	Microorganism	Catalase	Oxidase	Indole	Urease	Glucose	Lactose
41	<i>Weissella cibaria</i>	–	–	–	–	+	+
42	<i>Leuconostoc lactis</i>	–	–	–	–	+	+
43	<i>Weissella cibaria</i>	–	–	–	–	+	+
45	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
46	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
47	<i>Lactiplantibacillus pentosus</i>	–	–	–	–	+	+
Meat products							
48	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
49	<i>Companilactobacillus farciminis</i>	–	–	–	–	+	+
50	<i>Lactococcus lactis</i>	–	–	–	–	+	+
51	<i>Levilactobacillus brevis</i>	–	–	–	–	+	+
52	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
53	<i>Weissella cibaria</i>	–	–	–	–	+	+
54	<i>Lactococcus garvieae</i>	–	–	–	–	+	+
55	<i>Lactococcus lactis</i>	–	–	–	–	+	+
56	<i>Lactilactobacillus sakei</i>	–	–	–	–	+	+
57	<i>Weissella cibaria</i>	–	–	–	–	+	+
58	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
59	<i>Lactiplantibacillus plantarum</i>	–	–	–	–	+	+
60	<i>Pediococcus pentosaceus</i>	–	–	–	–	+	+
61	<i>Weissella cibaria</i>	–	–	–	–	+	+
62	<i>Lactiplantibacillus pentosus</i>	–	–	–	–	+	+
63	<i>Weissella confusa</i>	–	–	–	–	+	+
Fish products							
64	<i>Bacillus amyloliquefaciens</i>	+	+	–	–	+	+
65	<i>Bacillus pumilus</i>	+	+	–	–	–	–
66	<i>Bacillus altitudinis</i>	+	+	–	–	–	–
67	<i>Bacillus subtilis</i>	+	+	–	–	+	–
68	<i>Bacillus vallismortis</i>	+	+	–	–	+	–
70	<i>Lactiplantibacillus pentosus</i>	–	–	–	–	+	+
71	<i>Limosilactobacillus fermentum</i>	–	–	–	–	+	+
72	<i>Companilactobacillus farciminis</i>	–	–	–	–	+	+
73	<i>Lactococcus garvieae</i>	–	–	–	–	+	+
Shrimp products							
74	<i>Bacillus altitudinis</i>	+	+	–	–	–	–
75	<i>Bacillus cereus</i>	+	+	–	–	+	+
76	<i>Priestia megaterium</i>	+	+	–	–	+	+
77	<i>Bacillus subtilis</i>	+	+	–	–	+	–
79	<i>Bacillus pumilus</i>	+	+	–	–	–	–

Samples for which no bacterial isolates were obtained were excluded from the table

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the following results: Gram positive, catalase and oxidase positive, indole and urease negative. They differ in the way of carbohydrate fermentation. Some species ferment both glucose and lactose like *Priestia megaterium*, but others ferment glucose while lactose is not fermented by them, for example *Bacillus subtilis*. Some species are both glucose and lactose fermentation negative like *Bacillus pumilus*. Various fermentative pathways resulting in mixtures of species-specific acids and alcohols were described in different bacilli (Schilling et al. 2007; Suarez et al. 2012; Han et al. 2017). Large-scale production of commercial fermented products such as tuong Nam Dan, ruou gao nep, mam tep and especially nuoc mam includes sterilisation, and fine tuning of the flavour using both specific production steps and addition of flavourings to establish standard parameters of the product. However, these steps may lead to a reduction in the diversity of the microbial population (Ostermeyer et al. 2009).

CONCLUSION

Fifty-three LAB species were isolated from collected samples. *Bacillus* occurred less frequently than LAB in this study. We isolated twenty-one *Bacillus* species from samples mainly from fermented products under high salt concentration. All isolated microorganisms were chemically and biologically tested for further identification and functional characteristics. Many traditional fermented foods and beverages in Vietnam are produced naturally at a small scale in households under traditional experience and conditions that lead to the biodiversity of microorganisms. However, with the change in development that some fermented foods and beverages are shifting from traditional methods to commercial technologies to reduce the production time, together with the climate change with higher temperatures, the sterilisation in advanced technologies to meet food safety conditions leading to the diversity of microorganisms may decline. Scientific data on the functional properties of fermentative microorganisms should be collected widely to make the most use of beneficial bacteria for health as well as limit harmful bacteria for food safety purposes.

REFERENCES

- Biedendieck R., Knuuti T., Moore S.J., Jahn D. (2021): The 'beauty in the beast' – The multiple uses of *Priestia megaterium* in biotechnology. *Applied Microbiology and Biotechnology*, 105: 5719–5737.
- Doan N.T., Van Hoorde K., Cnockaert M., De Brandt E., Aerts M., Le Thanh B., Vandamme P. (2012): Validation of MALDI-TOF MS for rapid classification and identification of lactic acid bacteria, with a focus on isolates from traditional fermented foods in Northern Vietnam. *Letters in Applied Microbiology*, 55: 265–273.
- Doan N.T.L., Van Hoorde K., Cnockaert M., De Brandt E., Aerts M., Le Thanh B., Vandamme P. (2013): A description of the lactic acid bacteria microbiota associated with the production of traditional fermented vegetables in Vietnam. *International Journal of Food Microbiology*, 163: 19–27.
- Fidanza M., Panigrahi P., Kollmann T.R. (2021): *Lactiplantibacillus plantarum* – Nomad and ideal probiotic. *Frontiers in Microbiology*, 12: 1–13.
- Guo Y., Huang E., Yang X., Zhang L., Yousef A.E., Zhong J. (2016): Isolation and characterization of a *Bacillus atrophaeus* strain and its potential use in food preservation. *Food Control*, 60: 511–518.
- Gupta R.S., Patel S., Saini N., Chen S. (2020): Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: Description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the *Subtilis* and *Cereus* clades of species. *International Journal of Systematic and Evolutionary Microbiology*, 70: 5753–5798.
- Han L.L., Shao H.H., Liu Y.C., Liu G., Xie C.Y., Cheng X.J., Wang H.Y., Tan X.M., Feng H. (2017): Transcriptome profiling analysis reveals metabolic changes across various growth phases in *Bacillus pumilus* BA06. *BMC Microbiology*, 17: 156.
- Inasu Y., Bari M. L., Kawasaki S., Kawamoto S. (2005): Bacteria in traditional fermented vegetables produced in northern part of Vietnam. *Japanese Journal of Food Microbiology*, 22: 103–111.
- Jiang S., Cai L., Lv L., Li L. (2021): *Pediococcus pentosaceus*, a future additive or probiotic candidate. *Microbial Cell Factories*, 20: 45.
- Kang C.E., Park Y. J., Kim J.H., Lee N.-K., Paik H.-D. (2023): Probiotic *Weissella cibaria* displays antibacterial and anti-biofilm effect against cavity-causing *Streptococcus mutans*. *Microbial Pathogenesis*, 180: 106151.
- La Anh N. (2015): Health-promoting microbes in traditional Vietnamese fermented foods: A review. *Food Science and Human Wellness*, 4: 147–161.
- Lacerda D.C., Trindade da Costa P.C., Pontes P.B., Carneiro dos Santos L.A., Cruz Neto J.P.R., Silva Luis C.C., de Sousa Brito V.P., de Brito Alves J.L. (2022): Potential role of *Limosilactobacillus fermentum* as a probiotic with anti-diabetic properties: A review. *World Journal of Diabetes*, 13: 717–728.

- Li Z., Zheng M., Zheng J., Gänzle M.G. (2023): *Bacillus* species in food fermentations: An underappreciated group of organisms for safe use in food fermentations. *Current Opinion in Food Science*, 50: 101007.
- Noguchi H., Uchino M., Shida O., Takano K., Nakamura L.K., Komagata K. (2004): *Bacillus vietnamensis* sp. nov., a moderately halotolerant, aerobic, endospore-forming bacterium isolated from Vietnamese fish sauce. *International Journal of Systematic and Evolutionary Microbiology*, 54: 2117–2120.
- Ong B.N., Lam T.D., Le T.L., Nguyen T. C., Thi B.H.T., Phan T.M. (2020): Isolation, identification and evaluation of Lactic acid synthesis of bacteria in traditional fermented products in Vietnam. *IOP Conference Series: Materials Science and Engineering*, 991: 012059.
- Ostermeyer U., Meyer K., Schubring R. (2009): Production and composition of Asian fish sauces (Herstellung und Zusammensetzung von asiatischen Fischsaucen). *Informationen aus der Fischereiforschung*, 56: 1–18. (in German)
- Ramos C.L., Sousa E.S.O., Ribeiro J., Almeida T.M.M., Santos C.C.A.A., Abegg M.A., Schwan R.F. (2015): Microbiological and chemical characteristics of tarubá, an indigenous beverage produced from solid cassava fermentation. *Food Microbiology*, 49: 182–188.
- Rhee S.J., Lee J.E., Lee C.H. (2011): Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories*, 10: S5.
- Schilling O., Frick O., Herzberg C., Ehrenreich A., Heinze E., Wittmann C., Stülke J. (2007): Transcriptional and metabolic responses of *Bacillus subtilis* to the availability of organic acids: Transcription regulation is important but not sufficient to account for metabolic adaptation. *Applied and Environmental Microbiology*, 73: 499–507.
- Suarez C.A.G., Montano I.D.C., Nucci E.R., Iemma M.R.C., Giordano R.L.C., Giordano R.C. (2012): Assessment of the metabolism of different strains of *Bacillus megaterium*. *Brazilian Archives of Biology and Technology*, 55: 485–490.
- Tamang J. P. (2015): Naturally fermented ethnic soybean foods of India. *Journal of Ethnic Foods*, 2: 8–17.
- Tamang J.P., Shin D.H., Jung S.J., Chae S.W. (2016): Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology*, 7: 1–13.
- Thanh V.N., Viet Anh N.T. (2016): Ethnic fermented foods and beverages of Vietnam. In: Tamang J.P. (ed.): *Ethnic Fermented Foods and Alcoholic Beverages of Asia*. New Delhi, Springer International Publishing: 383–409.
- Thanh V.N., Mai L.T., Tuan D.A. (2008): Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. *International Journal of Food Microbiology*, 128: 268–273.
- Uchida H., Kondo D., Yamashita S., Tanaka T., Tran L.H., Nagano H., Uwajima T. (2004): Purification and properties of a protease produced by *Bacillus subtilis* CN2 isolated from a Vietnamese fish sauce. *World Journal of Microbiology and Biotechnology*, 20: 579–582.
- Yetiman A.E., Ortakci F. (2023): Genomic, probiotic, and metabolic potentials of *Liquorilactobacillus nagelii* AGA58, a novel bacteriocinogenic motile strain isolated from lactic acid-fermented shalgam. *Journal of Bioscience and Bioengineering*, 135: 34–43.
- Zheng J., Wittouck S., Salvetti E., Franz C.M.A.P., Harris H.M.B., Mattarelli P., O'Toole P.W., Pot B., Vandamme P., Walter J. (2020): A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70: 2782–2858.

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