บทความรับเชิญ

Thai Lactic Acid Bacteria: Diversity and Applications

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Introduction

Lactic acid bacteria (LAB) are widely distributed in many environments and occur naturally in various food products. They are considered to be harmless or even to improve human and animal health (probiotics). LAB have a GRAS status (generally recognized as safe). Lactic acid fermentation occurs during the preparation of a wide variety of foods, made from raw materials of animal and plant origin. In Asian countries, LAB have been isolated and used for fermented foods [1, 2]. *Lactobacillus bulgaricus, Streptococcus thermophilus, S. lactis, S. diacetylactis, S. cremoris,* and *Leuconostoc* sp. are used for fermented milk [3]. There are the strains of *Lactobacillus sakei,* and *Leuconostoc mesenteroides* in *sake; Tetragenococcus halophilus* in miso and soy sauce; *L. platarum, L. brevis,* and *Leu. mesenteroides* in pickles in Japan [1]. In Thailand, lactic acid bacteria refer to a large group of beneficial bacteria that have similar properties and all produce lactic acid as an end product of the food fermentation [4]. They are many genera and species distributed in foods, cane molasses, silage and are also found in the digestive systems.

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Diversity of lactic acid bacteria in Thai fermented foods

Thai traditional fermented foods are produced by natural fermentation in processes which vary from simple to complicated [5]. Fishery products, *nam-pla* (fish sauce), *ka-pi* (shrimp paste) and *bu-du* are produced from fish with a large proportion of salt. Fermented fish with salt and carbohydrate are *pla-ra, pla-som, pla-chao, som-fak,* and *pla-chom.* Meat products, *nham* (fermented pork) is made from minced red pork mixed with pork rind, garlic, pepper, salt, chili, and trace of potassium nitrate. *Sai-krog-prieo* (fermented sausage) and *mam* (fermented beef) or (pork sausage) are produced from shredded pork or beef meat with fat, cooked rice, salt, sugar, pepper, and spices. Plant products, *naw-mai-dong* (fermented bamboo shoot) were fermented by adding the brine into the sliced pieces of bamboo shoot which packed in the jars. *Phak-gard-dong* (pickled green mustard) was fermented with the mixture of green mustard and brine and packed tightly in jar. *Miang* (fermented tea leaves) is produced in the northern part of Thailand. *Khao-mak* (fermented glutinous rice) is produced by loog-pang (mould and yeast) as a starter but LAB are found in this product. LAB also contribute to fermentation of *khanom-jeen* (rice noodle) and soy sauce. A number of fermented foods are home-made but some are produced at commercial factories using natural fermentation [5].

Lactic acid bacteria are perhaps the most widespread and desirable microorganisms in food fermentations. They convert most available carbohydrates to lactic acid, with small amounts of acetic acid, resulting in a lowering of the pH [6]. The homofermentative strains of *Lactobacillus pentosus, L. plantarum, L. sakei, L. farciminis, L. alimentarius/ farciminis, L. acidipiscis, L. pantheris, L. thailandensis, L. camelliae,* and other *Lactobacillus* sp., *Pediococcus pentosaceus, P. acidilactici, P. siamensis, Tetragenococcus halophilus, T. muriaticus, Enterococcus hirae, E. faecalis, E. faecium, E. casseliflavus, E. camelliae, E. thailandicus,* and *Lactococcus garviae* are found in a variety of Thai fermented foods [7-16]. Heterofermentative strains of *L. vaccinostercus, L. fermentum, L. brevis, L. suebicus,* other *Lactobacillus* sp., *Weissella confusa, W. thailandensis,* and *Leuconostoc* sp. are also isolated as shown in Table 1 [8-10, 14, 17]. The strains of *W. cibaria/kimchii, W. confusa, P. pentosaceus, P. acidilactici, L. fermentum, L. brevis, L. farciminis, L. plantarum,* and *L. sakei* were also isolated from fermented sausages [18]. The DL-lactic acid producing *L. pentosus* and *L. plantarum* strains that contained *meso*-diaminopimelic acid in the cell wall are the predominant rod-shaped LAB in fermented Thai foods. *P. pentosaceus* and *T. halophilus* strains are the major coccal bacteria. L-lactic acid producing *T. halophilus, L. farciminis,* and *L. acidipiscis* strains occur in fermented fish with high salts, such as *pla-ra* and soy sauce mash [9, 10, 19]. The strains of other *Lactobacillus* species, *P. acidilactici, Enterococcus, Weissella* and *Leuconostoc* are the minor LAB in fermented Thai foods. The LAB are widely distributed, but they are not generally specific for one kind of fermented products though the concentration of salt influences the flora of them. The predominant LAB in Thailand *(L. pentosus, L. plantarum, L. farciminis, L. acidipiscis, P. pentosaceus* and *T. halophilus*) are clearly different from the *L. acidophilus, L. casei, L. bulgaricus, Lc. lactis,* and *Lc. cremoris* which are used for and found in dairy products [20]. Enterococci, especially *E. faecalis* and *E. faecium* strains are common organisms in the intestinal tract of man and other animals and it is difficult to keep them out of foods [21]. Their presence in fermented foods may indicate inadequate sanitary practices.

Diversity of lactic acid bacteria in molasses, silage, and in human and animal faeces

During the study of LAB in fermented can molasses in Thailand, *L. buchneri, L. casei, L. cellobiosus, L. mali,* and *L. reuteri* strains were isolated [22]. The strains of *L. reuteri* and *L. cellobiosus* produced succinic acid from citric acid. According the citric acid is converted to succinic acid by lactobacilli in fermentation process, the flavor of the fermented products will greatly be influenced [23]. In central and north-eastern Thailand, the silage of sorghum and grass contained lactobacilli ranged from 10^5-10^7 CFU/g wet weight and corn silage contained 10^8 CFU/g. The dominant lactobacilli found were *L. plantarum, L. acidophilus, L. brevis, L. buchneri, L. casei, L. delbrueckii, L. farciminis, L. fermentum, L. fructosus,* and *L. gasseri* [24]. Niamsup, *et al.,* 2003 [25], reported the strains of *L. thermotolerans,* a novel thermotolerant species isolated from chicken faeces. *L. plantarum, L. salivarious, L. saerimneri, L. gasseri, E. raffinosus, E. faecalis, L. amylovorus, L. johnsonii* strains were isolated from human faeces, chicken faeces, and pig faeces [26, 27]. Ta-yuan and Rodtong 2006 [28] reported 5 isolates of *Lactobacillus* and *Carnobacterium* group exhibited the potential for exopolysaccharide EPS production.

New species of lactic acid bacteria in Thailand

The novel homofermentative lactic acid bacteria, *L. acidipiscis from pla-ra, L. thailandensis, L. camelliae, E. camelliae* and *P. siamensis* were isolated from *miang* and *E. thailandicus* from *mam* while the heterofermentative strains, *W. thailandensis* were isolated from *pla-ra* [10-12]. Niamsup, *et al.,* 2003 [25], reported the strains of *L. thermotolerans,* a novel thermotolerant species isolated from chicken faeces however they were reclassified as a later synonym of *L. ingluviei* [29]. Recently, the novel marine lactic acid bacteria were isolated from salted fish and shrimp paste *(ka-pi*), and they were proposed to *be Alkalibacterium thallassium, A. pelagium, A. pultridalgicola,* and *A. kapii.* These bacteria contained D-ornithine in cell wall peptidoglycan and were slightly halophilic and alkaliphilic [30].

Table 1 Diversity of lactic acid bacteria in Thai fermented foods.

Species	Fermented Products
L. pentosus	*Nham*[1], *Sai-krog-prieo*[1], *Pla-chom*[2], *Kung-chom*[2], *Pla-som*[2], *Som-fak*[2], *Phak-gard-dong*[3], *Phak-sian-dong*[3], *Naw-mai-dong*[3], *Hom-dong*[3], *Miang*[3], *Sour durian*[3], *Soy sauce mash*[3]
L. plantarum	*Nham*[1], *Sai-krog-prieo*[1], *Mam*[1], *Kung-chom*[2], *Pla-som*[2], *Som-fak*[2], *Phak-koom-dong*[3], *Phak-nam-dong*[3], *Sauerkraut*[3], *Thua-ngok-dong*[3], *Hom-dong*[3], *Khaomak*[3], *Miang*[3], *Soy sauce mash*[3]
L. brevis	*Sai-krog-prieo*[1], *Mam*[1], *Sour fish spawn*[2], *Phak-sian-dong*[3], *Naw-mai-dong*[3], *Somfak*[2]
L. fermentum	*Sai-krog-prieo*[1], *Pla-som*[2], *Pla-chao*[2], *Phak-gard-dong*[3], *Phak-sian-dong*[3], *Thua-ngok-dong*[3], *Khanom-jeen*[3]
L. sakei	*Nham*[1], *Mam*[1], *Som-fak*[2]
L. farciminis	*Sai-krog-prieo*[1], *Pla-ra*[2], *Pla-chom*[2], *Kung-chom*[2], *Hoi-dong*[2], *Soy sauce mash*[3]
L. alimentarius/farciminis	*Pla-som*[2]
L. acidipiscis sp. nov	*Pla-ra*[2], *Pla-chom*[2], *Soy sauce mash*[3]
L. pantheri, L. suebicus, L. thailandensis sp. nov., *L. camelliae* sp. nov.	*Miang*[3]
Lactobacillus sp.	*Nham*[1], *Phak-koom-dong*[3], *Hom-dong*[3]
P. acidilactici	*Nham*[1], *Mam*[1], *Sai-krog-prieo*[1]
P. siamensis sp. nov	*Miang*[1]
P. pentosaceus	*Nham*[1], *Mam*[1], *Sai-krog-prieo*[1], *Pla-som*[2], *Som-fak*[2], *Phak-sian-dong*[3]
T. halophilus	*Nam-pla*[2], *Ka-pi*[2], *Pla-ra*[2], *Pla-chom*[2], *Kung-chom*[2], *Bu-du*[2], *Tai-pla*[2], *Pla-paeng-daeng*[2], *Hoi-dong*[2], *Soy sauce*[3]
T. muriaticus	*Nam-pla*[2], *Ka-pi*[2]
Lactococcus garviae	*Pla-som*[2]
E. hirae	*Sai-krog-prieo*[1], *Pla-chom*[2], *Pla-som*[2]
E. faecalis	*Pla-ra*[2]
E. casseliflavus, E. camelliae sp. nov	*Miang*[1]
E. thailandicus sp. nov	*Mam*[1]
W. thailandensis sp. nov	*Pla-ra*[2]
W. confusa	*Sai-krog-prieo*[1], *Pla-som*[2], *Satoh-dong*[3]
W. cibaria/W. kimchii	*Sai-krog-prieo*[1]
Leuconostoc sp.	*Nham*[1]

Note: [1]Fermented pork or beef, [2]Fermented fish, [3]Fermented plant materials

Fermentation products of lactic acid bacteria

Some LAB isolated from Thai fermented foods such as *P. pentosaceus* from fermented (pork *nham*), and *Lactobacillus* strains from fermented fish *(pla-som, som-fak,* and *hoi-dong*) produce antimicrobial substances by which *Staphylococcus aureus, Bacillus subtilis, Micrococcus varians, Listeria* spp., *Vibrio* sp., *Aeromonas* sp., *P. pentosaceus,* and *L. pentosus* are inhibited [31-35]. The isolates from fermented sausages, identified as *W. confusa, P. acidilactici,* and *L. plantarum,* could inhibit the growth of *B. cereus* and 2 of them could also inhibit *S. aureus* [18]. The bacteriocin-producing strains of *L. plantarum, L. fermentum, L. reuteri, L. salivarius, Lc. lactis, P. pentosaceus, P. acidilactici, E. faecium, E. faecalis,* and *E. raffinosus* were isolated from Thai fermented food such as *nham,* fermented meat, and *pla-ra,* other sources such as ornament fish, tofu's whey, sugar apple, silage, and chicken intestine [36, 37]. They showed antimicrobial activity with narrow, moderate and broad spectrum against Gram-positive and Gram-negative bacteria (Table 2). The strains identified as *L. plantarum, L. pentosus, W. confusa,* and *E. faecium* and *Weissella* spp. could produce diacetyl and acetoin [18, 38]. *L. plantarum, L. pentosus,* and *Enterococcus* strains showed amylolytic enzyme activity while *L. plantarum* and *L. brevis* strains showed the productivity of γ-aminobutyric acid (GABA). Glutamate decarboxylase encoding genes *(gadB*) of *L. brevis* LSF8-13 was found to contain conserved PLP-binding domain and its recombinant glutamate decarboxylase was expressed in *E. coli* and then purified and characterized [39]. *Lactococcus* strains was optimized for the production of L-lactate from cassava starch [40]. *P. pentosaceus* strains from *nham* produced exopolysaccharides [41]. Fermentation end-products, hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde and bacteriocins are contributed to the antagonistic activities, GABA to blood pressure, and diacetyl is the characteristic aroma and flavor associated with butter, cottage cheese, and butter-milk [42].

Table 2 LAB strains, source, product, and activities

Strain	Source	Product/Antimicrobial
L. plantarum PMU33	*Somfak*	Plantaricin W/Broad
L. fermentum onil2	*Nham*	Peptide 1256da/Narrow
L. reuteri KUB-AC5	Chicken intestine	Peptide KAC5/*E. coli* *Salmonella*
L. salivarius AC21	Chicken intestine	Salivacin K21/Broad
Lc. lactis WNC20	*Nham*	Nisin Z/Nisin type
Lc. lactis N100 & N190	*Nham*	Nisin Z/Nisin type
Lc. lactis BCC strains	Fermented meat	Nizin Z and A/*S. aureus*
Lc. lactis KU-T1	Tofu's whey	Unknown/Narrow
P. acidilactici KUB-L0026	Silage	Peptide KPA260/*B. cereus* *E. coli, Salmonella*
P. pentosaceus TISTR 536	*Nham*	Pediocin PA-1/Class IIa type
P. pentosaceus WNK19	*Nham*	Pediocin PA-1/Class IIa type
P. pentosaceus BCC strains	Fermented meat	Pediocin/*L. monocytogenes* *E. faecalis*
E. faecalis NKR-4-1	*Pla-ra*	Two-peptide lantibiotic/Broad
E. faecalis K-4	Silage	Enterocin SE-K4/Class IIa type
E. faecium NKR-5-3	*Pla-ra*	Peptide A, B, C, D/Broad
E. faecium KU-B5	Sugar apple	Enterocin A, B, X/Class IIa type
E. raffinosus KU822	Ornament fish	Peptide A, B/Moderate
L. brevis LSF8-13	*Somfak*	GABA
P. pentosaceus AP-1 & AP-3	*Nham*	Exopolysaccharide
L. pentosus AP17-1	*Pla-ra*	Diacetyl, acetoin
L. pentosus SR4-2 & SR8-1	Soy sauce mash	Diacetyl, acetoin
W. confusa AP2-1	*Moo-yor*	Diacetyl, acetoin
E. faecium PM3-13, PM3-14, PM4-9	Pasteurized Milk	Diacetyl, acetoin
L. pentosus	*Somfak*	Amylolytic
L. palntarum, Enterocuccus sp.	Fermented rice noodle	Amylolytic
T. muriaticus	Fish sauce	Histamine
L. plantarum, L. salivarius, L. saerimneri	Human intestine	Tumor necrosis factor-α TNF-α

Applications of lactic acid bacteria

Fermented foods are of different varieties depending on the raw material used. Some are consumed raw such as *nham* (fermented pork). The technology of fermentation still remains indigenous relying on the adventitious bacteria. A large scale production of fermented foods is of need improvement for the following reasons: the development of quality such as aroma, flavor, and texture; the ability to fasten the fermentation; and the hygiene. The raw materials, starter culture, process and the quality control of product are important. Diversity of LAB are reported and can be used for application (Table 1). It is important to select bacterial strains which have high acid production and their specific activities (catalase, nitrate reductase, nitrite reductase and so on). Catalase activity of LAB is reported [43]. It catalyzes the conversion of hydrogen peroxide that prevents the occurance defects due to fat rancidity and discolorations of foods. The strains contain catalase, nitrate reductase, and nitrite reductase will be useful in production of fermented pork [44]. The homofermentative, L-(+) lactic acid and diacetyl producing *Lactobacillus* strains were selected for green mustard and yoghurt fermentation in comparison to natural fermentation [33]. The use of LAB as probiotics in inproving animal performance is reported [45]. *L. pentosus* and *E. faecium* strains are designed to assess the effects on the intestinal microecology and the body weight gains in gold fish, *Carassius auratus* [46]. *Lactobacillus* spp. isolated from chicken gastrointestinal tracts that mixed with a formulated shrimp diet as probiotics for feeding black tiger shrimp *Penaeus monodon* leading to higher growth and survival. They demonstrated potential of control *Vibrio harveyi* in the shrimp GI tract and provided highly healthy shrimp protected against such diseases [47]. *P. pentosaceus* TISTR 536 which produced pediocin PA-1 was applied as a starter culture for *nham.* Its pediocin was more stable during the fermentation period than nisin Z and it showed strong antagonism against the contaminant, *Salmonella antum. L. reuteri* KUB-AC5, *E. faecium* NKR-5-3, and *E. faecalis* K-4 could be applied as a probiotics with anti-*Salmonella* activity for broiler chicken, a starter for *pla-ra,* and for silage, respectively [36, 48]. The technology of production of cultures included frozen and liquid form is required for the application in fermentation of fish, meat, and vegetables including the probiotics.

Research activities and workshop on lactic acid bacteria

In Thailand, there are many researchers work on lactic acid bacteria such as in Chiang Mai University, Chulalongkorn University, Mahidol University, Kasetsart University, Khon Kaen University, Prince of Songkla University, Suranaree University of Technology, King Mongkut's University of Technology Thonburi, Srinakharinwirot University, and many

Research Institutes. The National Center for Genetic Engineering and Biotechnology has held a Workshop on the Classification and Applications on Acetic and Lactic acid Bacteria, March 10-11, 1998 and on Probiotics: Research and Opportunities, July 29, 2003, in Bangkok. Thailand-Japan Joint Workshop 2008 on Bioproduction by Efficient Utilization of Thai Bioresources, October 16, 2008, held in Maha Sarakham, Thailand supported by International Center for Biotechnology, Osaka University.

Future trends

LAB are distributed in numerous fermented foods and many sources in Thailand. Those LAB are useful for applications in food fermentation as the starters and biopreservatives, in bioplastic production and for improving animal performance in Thailand. The ancient traditions of using LAB in food and feed, combined with recent knowledge on positive health effects caused by ingestion of probiotics LAB, suggests them as promising alternatives to chemical preservatives. LAB produce a variety of antimicrobial compounds, the pH-reducing products, lactic acid and acetic acid, as well as hydrogen peroxide, formic acid, propionic acid, and diacetyl. Many LAB produce proteinaceous compounds, bacteriocins however the lipolytic LAB produce significant amounts of antimicrobial fatty acids that contribute to the sensory quality of fermented foods and the phenyllactic acid contribute to the antifugal effect in synergy with other compounds including the cyclic dipeptides is still novel in the research field of antifugal LAB [49] Practically, the isolation of starch hydrolyzing LAB strains, producing L-or D-lactic acid is still interesting and the strains are useful for the production of bioplastic.

Conclusion

LAB have been isolated from various food products (fermented milk, fermented fish, meat, vegetables or plant products) and many kinds of sources in Thailand. In fermented foods, they occur naturally with other microorganisms and are responsible for their souring and ripening. Homofermentative strains of *L. pentosus, L. plantarum, L. camelliae, L. thailandensis, L. pantheris, L. farciminis, L. acidipiscis, L. sakei , Lc. garviae, E. camelliae, E. thailandicus, P. acidilactici, P. pentosaceus, P. siamensis, T. halophilus,* and *T. muriaticus* strains occur in a variety of fermented Thai foods. Heterofermentative strains of *L. vaccinostercus, L. fermentum, L. suebicus, L. brevis, W. confusa, W. thailandensis* and *Leuconostoc* sp. were also isolated. There are diverse species found in the same product. Recently, several Thai researchers have tried to use lactic acid bacteria as starters in the productions of fermented pork and vegetables. This should lead to improve food fermentation and hygiene. In addition, the LAB strains isolated from related sources are useful for the industrial applications.

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