

Review Article

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Diversity of Lactic Acid Bacteria (LAB) in Fermented Fish Products: A Review

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ABSTRACT

The purpose of this review article on the diversity of Lactic acid bacteria (LAB) in fermented fish products is to empower the readers about the various diversity of lactic acid bacteria in fermented fish products, their role in fish preservation, their origin and molecular approaches for identification of Lactic acid bacteria (LAB) from fermented foods. Fermented fish products are very popular in Southeast Asian countries such as Thailand, Indonesia, Philippines, Malaysia, China, and Northeast parts of India. It is considered a delicacy and is eaten in their day to day life. LAB plays an important role in fish fermentation also it is responsible for the unique characteristics of fermented fish. Certain LAB species also display probiotic activity and have been widely used in the food industry as a result of their potential health benefits. This review provides information on LAB species associated with various fermented fish products of Southeast Asian countries.

Keywords

Lactic acid bacteria,
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Introduction

Lactic acid bacteria (LAB) are a group of gram-positive bacteria that are devoid of cytochromes and preferring anaerobic conditions, they are usually non-motile, non-

sporulating, catalase-negative, oxidase-negative, acid-tolerant and strictly fermentative bacteria that produce lactic acid as a major or sole product of fermentative metabolism. They are either rod-shaped (bacilli) or spherical-shaped (cocci) that share

common metabolic and physiological characteristics. LAB are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acid derivatives, and vitamins. Based on their fermentative metabolism LAB are divided into two distinct groups. The homo-fermentative group that utilizes the Embden-Meyerhof-Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. Hetero-fermentative bacteria produce equimolar amounts of lactate, CO₂, ethanol, or acetate from glucose exploiting the phosphoketolase pathway. The homo-fermentative group consists of *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, etc and the heterofermentative group includes *Leuconostoc*, *Weissella*, etc. (Vasiljevik and Shah, 2008).

Lactic acid bacteria (LAB) are acid-producing (lactic acid) and are acid-tolerant which helps LAB to outcompete other bacteria in a natural fermentation thus inhibiting the growth of spoilage as well as pathogenic microorganisms (Kobayashi *et al.*, 2004). Most fermented foods owe their origin to the fact that the processes used in their production are inhibitory to many microorganisms. As a result, fermented products generally have a longer shelf life than their original substrate and their ultimate spoilage is different (Adams and Mitchell, 2002). Lactic acid bacteria (LAB) have been shown to produce uricase (Handayani *et al.*, 2018), reduce ochratoxin A (Luz *et al.*, 2018), reduce acrylamide formation in bread (Nachi *et al.*, 2018), produce exopolysaccharides (Abid *et al.*, 2018), remove tannins (Shang *et al.*, 2019). Some LAB strains have also been reported to increase folic acid levels in fermented milk (Purwadani *et al.*, 2017).

LAB are amongst the most important groups of microorganisms used in the food industry. The industrial significance of the LAB is

further evidenced by their generally recognized as safe (GRAS) status, because of their ubiquitous appearance in food and their contribution to the healthy and sound microbiota of animal and human mucosal surfaces. Lactic acid bacteria (LAB) include members of the genera *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Aerococcus*, *Carnobacterium*, *Leuconostoc*, *Lactococcus*, and *Pediococcus*. Peripherally *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* also are regarded as LAB; these belong to the order Lactobacillales.

Lactic acid bacteria (LAB) and fermentation of fish

Fish is a perishable commodity and hence fermentation of fish for increasing its shelf life is an old age practice. The fermentation of fish is mainly carried out in Asian countries. The storage life of perishable fish can be increased by acid-fermentation with added carbohydrates and salts. Both freshwater and seawater fish are preserved by this method. Rice, flour, millet, and even syrup or sugar are used as carbohydrate sources. In Southeastern countries, rice is commonly used as a carbohydrate source whereas Millet is used as the main carbohydrate source in Northeastern countries. The organic acids produced from the added carbohydrates in combination with salt control the extent of acid fermentation and keep the quality of the product (Rhee *et al.*, 2011).

During fermentation, the production of lactic acid from LAB decreases the pH of the product thereby decreasing the number of other microbes. The typical acid-forming bacteria rapidly increase in number, becoming the predominant microbes after fermentation has started and reaches their maximum density at the end of fermentation. Also, it contributes to the unique and characteristic

flavor of the fermented fish. LAB plays an important role in food fermentation that causes changes in taste, texture, and smell with improved preservation of the product (Hugas, 1998). Fermented fish products formed the unique acids, alcohols, phenols, and other fermented flavor substances and good taste under the action of microorganisms and enzymes of their metabolic process.

Lactic acid bacteria (LAB) also play an important role as probiotics in many Asian fermented foods. Koreans who travel overseas for several days without fermented foods often experience uncomfortable stomach symptoms and poor digestion. More research is needed to identify the lactic acid bacteria in Asian fermented foods and their physiological functions in the human diet (Rhee *et al.*, 2011)

Origin of Lactic acid bacteria (LAB) in traditional fermented fish products

Fermentation as a method of food preservation is one of the most old age practice in the world. Fermented fish products are reported to be dominated by mostly lactic acid bacteria by many researchers. Lactic acid bacteria (LAB) are the most commonly used microorganisms in fermentation technique although most of the fermentation of traditional products are done using spontaneous fermentation at an anaerobic condition in which microorganism already present in the raw materials becomes the normal flora at the time of fermentation. Studies have demonstrated that lactic acid bacteria are part of the normal intestinal microbiota in fish. Presence of Lactic acid bacteria in the raw materials used for fermentation such as rice, garlic, banana leaves including fish has also been reported (Paludan-Müller *et al.*, 1999). Some information on the presence of *Lactobacillus* spp. in the digestive tract of salmonids have

been already reviewed (Ringø *et al.*, 1995). There are reports that LAB is a part of native microbiota of aquatic animals (Ringo, 2004). Itoi *et al.*, (2008) reported that halotolerant strains of *Lactococcus lactis* isolated from the intestinal tract of the pufferfish *Takifuguniphobles* caught in Shimoda, Shizuoka, Japan. Nair and Surendran (2005) isolated LAB from various samples of fresh and frozen fish and prawn. Among the isolates, *Lactobacillus plantarum* was the dominant species. Thus, in traditional methods the fermentation is initiated with spontaneous growth of fortuitous microorganisms during the production. Some studies also suggested that fermented food characteristics varied with raw material and additional material used that caused the microbial diversity in the food (Kopermsub *et al.*, 2006). Although the use of starter cultures would be an appropriate approach for the control and optimization of the fermentation process.

Role of Lactic acid bacteria (LAB) in food preservation

Presence of Lactic acid bacteria in fermented food has been the major reason for the preservation of food. The inhibitory effect towards spoilage and pathogenic bacteria mainly comes from organic acid. Lactic acid produced by LAB is a useful compound for food preservation because it maintains the acidic conditions of the fermented products, and is lethal to bacteria that cause food spoilage and food poisoning (Kobayashi *et al.*, 2004). During the process of fermentation, LAB could utilize carbohydrate substrates available in the fermenting matrix and produce organic acids, especially lactic acid that not only contributes to the taste, aroma, and texture of the product but also lowers the product's pH that is one of the key factors to ensure the quality and safety of the product. Besides that, strains of certain LAB

species display probiotic activity and have been widely used in food industry as a result of their potential health benefits (Giraffa, 2004).

Molecular approaches for identification of Lactic acid bacteria (LAB) from fermented foods

The correct species identification of LAB is of paramount importance from the technological, ecological, and safety point of view (Temmerman *et al.*, 2004).

Recently, knowledge of the taxonomic diversity and heterogeneity of LAB related to fish fermentation requires cultivable approaches combined with various DNA-based techniques (Vignolo *et al.*, 2012). As a powerful tool, 16S ribosomal RNA gene (16S rDNA) sequence analysis has become a major criterion for the phylogenetic identification of bacteria and is increasingly being adopted for rapid analysis of the LAB communities involved in many kinds of fermented food products (Kopermsub and Yunchalard, 2010; Koyanagi *et al.*, 2011). Among various DNA typing methods, amplified ribosomal DNA restriction analysis (ARDRA) of 16S rDNA has been considered as the prototype of a DNA fingerprinting method mostly used to identify isolates present in many fermented foods (Rodas *et al.*, 2003; Santos *et al.*, 2005; Solieri *et al.*, 2012).

Diversity of lactic acid bacteria (LAB) in fermented fish products around the globe

Many studies have been done on the isolation and characterization of Lactic acid bacteria (LAB) from fermented fish products. Most of the studies have been carried out in Southeast Asian countries like Thailand, Indonesia, China, Malaysia, etc. where fermented fish products are considered a delicacy and are eaten in their day to day life.

Thailand

Some important fermented fish products of Thailand are Plaa-som, Som-fak, pla-ra, pla-chom, kung-chom, and hoi-dorng.

Plaa-som

Plaa-som is a traditional fermented fish product widely consumed in the south of Thailand. It is made from fish, sugar, salt, and roasted rice and is fermented with natural microbial flora. Either the whole fish or fish fillets are fermented with either cooked rice or steamed sticky rice, garlic, and salt until a final acceptable sour-tasting product is obtained. After fermentation, the fish is cooked by either deep frying or roasting and consumed. As a traditional fermented food product, its recipes vary by region throughout the Kingdom of Thailand, depending upon local consumer preferences and ingredient availability (Valyasevi and Rolle, 2002). The production process traditionally relies on a spontaneous fermentation initiated by natural fortuitous microorganisms, mainly lactic acid bacteria (LAB), that are found in the ingredients, on the processing utensils, and in the local atmosphere as natural starters (Khieokhachee *et al.*, 1997; Valyasevi and Rolle, 2002; Visessanguan *et al.*, 2004).

Hwanhlem *et al.*, (2011) isolated Lactic acid bacteria (LAB) from Plasom at various fermentation periods using MRS agar medium containing 0.3% (w/v) CaCO₃ as a preliminary screening medium, 138 isolates which exhibited a clear zone and growth on MRS agar supplemented with CaCO₃ were isolated. However, only 133 isolates were identified as LAB. Calcium carbonate is used as an indicator of acid-producing strains since it gets dissolved when it interacts with acid-forming a clear zone (Onda *et al.*, 2002). Of these 133 isolates, 25 isolates were cocci, 75 isolates were short rods and 33 isolates were

rods. The strain which showed the best lactic acid production and pH reduction ability were identified upto species level by 16S rDNA analysis and were identified as *Streptococcus salivarius* and *Enterococcus faecalis* based on their morphological properties, biochemical tests, and 16S rDNA analysis. These strains were gram-positive, non-spore forming cocci, catalase-negative, and formed off-white colonies.

Kopermsub and Yunchalard (2010) studied the distribution and succession of predominant LAB species during plaa-som fermentation. The isolates were screened and grouped by amplified ribosomal DNA restriction analysis (ARDRA), followed by 16S rDNA sequencing. The predominant LAB species were *Lactococcus garvieae*, *Weissellacibaria*, *Pediococcus pentosaceus*, *Streptococcus bovis*, *Lactobacillus plantarum*, and *Lactobacillus fermentum*. Early stages of the process were dominated by the presence of *Lactococcus garvieae*, *Streptococcus bovis*, and *Weissellacibaria*. At 48 hours into fermentation, *Weissellacibaria*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum* were prevalent, and gave way to a dominance of *Lactobacillus plantarum* that completed the fermentation.

Som-fak

Som-fak is a Thai product composed of minced fish fillet, salt (2–5%), ground boiled rice (2–12%), and minced garlic (4%). The mixture is tightly packed in banana leaves or plastic bags and left to ferment for two to five days at 30°C (Saisithi *et al.*, 1986). Som-fak can be served raw or cooked either as a main course with vegetables or as a snack.

Lactic acid bacteria (LAB) were isolated and characterized from a Thai low-salt fermented fish (SomFak) product (Paludan-Müller *et al.*, 1999). One-hundred and eighty-five LAB were isolated from raw materials and during

fermentation of som-fak, isolates were identified upto species level by phenotypic characterization. At the start of fermentation, *Leuconostoc* spp., *Lactobacillus brevis* and *Lactococcus lactis* were dominant, followed by more acid-tolerant species of *Lactobacillus curvatus*, *Lactobacillus casei*, *Lactobacillus pentosus* and *Lactobacillus plantarum*. At the end of fermentation *Lactobacillus plantarum* was the most dominant microflora.

Pediococcus sp. and *Lactobacillus* sp. have been identified as the dominating LAB genera in commercial samples of som-fak and those prepared in the laboratory (Tanasupawat *et al.*, 1993; Saisithi *et al.*, 1986).

Hoi-dorng

Hoi-dorng is a high salt fermented product of Thailand. Hoi-dorng is produced from sea mussel meat washed in brine (10% NaCl) and water. After drainage, sea salt is added (ratio: 7:1 by weight) and mixed well. The product matures for 4–5 days and is packed in sealed glass jars, it has a shelf-life of 3–6 months (Phithakpol *et al.*, 1995).

Østergaard *et al.*, (1998) isolated and screened for lactic acid bacteria (LAB) capable of inhibiting *Listeria* sp. (*Listeria innocua*) from three Thai fermented fish products (Hoi-dorng, Plaa-som, and Som-fak). The strains isolated were identified to be *Lactobacillus* sp., *Lactobacillus plantarum*, *Carnobacterium piscicola*, and *Lactococcus lactis*. *Lactobacillus* species were the most predominant, accounting for 29 of the 44 strains.

Pla-ra, Pla-chom, Kung-chom, and Hoi-dorng

Traditional fermented fish with salt—nam-pla, budu, tai-pla, pla-ra, pla-chom, kung-chom, and hoi-dorng - are found in different parts of Thailand (Tanasupawat and

Komagata, 1995). Pla-ra is a highly salted fermented fish, whereas pla-chom, kung-chom, and hoi-dong, are the lowly salted products. Lactic acid bacteria (LAB) were isolated and characterized from these four products (Tanasupawat *et al.*, 1998). The isolated bacteria were identified by phenotypic and chemotaxonomic characteristics, including fluorometric DNA-DNA hybridization. *Lactobacillus farciminis*, *Leuconostoc* sp., and other *Lactobacillus* sp. were found in Pla-ra, *Lactobacillus pentosus*, *L. farciminis* and other *Lactobacillus* sp. in Pla-chom, *L. pentosus*, *L. plantarum*, and *L. farciminis* in Kung-chom and *L. farciminis* in Hoi-dong.

Indonesia

Bekasam

Bekasam is a fermented fish product of Indonesia with a sour taste and it is a popular food in Central Java, South Sumatra, and South Kalimantan. Bekasam production involves a spontaneous fermentation process of freshwater fish, supplemented by salt, and rice or fermented cassava (Murtini *et al.*, 1997). In som-fak, the rapid growth of LAB causes pH to decrease below 4.5 in two days is essential to prevent spoilage and to ensure the safety of the product (Østergaard *et al.*, 1998).

Choesri *et al.*, (2013) isolated and characterized LAB isolates from bekasam. Seventy-four isolates were isolated out of which 62 isolates (84%) belonged to LAB based on morphological and biochemical characteristics. Although the isolates were not identified upto species level.

Rusip

Rusip is one of the typical lactic acid fish fermented food originating from Bangka Belitung, Indonesia. It is a traditional

fermented fish products known from Bangka Belitung and found in Lampung and West Kalimantan. Small fish such as anchovy or Bilis fish are used as raw materials in the manufacture of rusip. Fermentation of rusip is usually a spontaneous process involving lactic acid bacteria (LAB) with palm sugar as a source of carbohydrates. Genus of lactic acid bacteria involved in a food fermentation may vary depending on region, type of substrate, and or fermentation stages.

The LAB species encountered in the final end product of rusip with the addition of salt and roasted rice are *Streptococcus* and *Lactobacillus*, whereas in rusip with the addition of salt and brown sugar were *Leuconostoc* and *Streptococcus*. These lactic acid bacteria were found in the final product of rusip originating from manufacturers in Bangka (Dessi, 1999). Kusmarwati *et al.*, (2014) found *Pediococcus* as bacteriocin producing lactic acid bacteria from commercial rusip in Bangka and West Kalimantan. Yuliana *et al.*, (2018) isolated and identified lactic acid bacteria from rusip at different fermentation stages for upto 15 days. The results showed that the lactic acid bacteria contributing during rusip fermentation was *Leuconostoc*, *Streptococcus*, and *Lactococcus*. Based on the fermentation periods, the presence of these bacteria was varying. The genus *Streptococcus* was more common in early fermentation, whereas the genus of *Lactococcus* was more common in mid-fermentation and at the end of fermentation, the most dominant was *Leuconostoc*.

Chao

Chao is a traditional fermented food from Pangkajene and Kepulauan Regency, South Sulawesi, Indonesia. This product is fermented from fish and white rice. Tembang fish (*Sardinella gibbosa*) is one type of fish that is often processed.

Matti *et al.*, (2019) isolated and identified proteolytic lactic acid bacteria (LAB) from chao. Characterization and selection of isolate were performed based on morphological and biochemical characteristics, proteolytic activities, phenotype, and repetitive sequence-polymerase chain reaction (Rep-PCR) amplification. The similarity of isolates was compared based on the 16S rRNA gene sequence and phylogenetic analysis. Sixty isolates of LAB were isolated from chao. Fifteen isolates were halotolerant proteolytic LAB. Further identification confirmed the strains to be *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Pediococcus pentosaceus*, and *Pediococcus acidilactici*.

China

Chouguiyu (Stinky Mandarinfish)

Chouguiyu (stinky mandarinfish), is a traditional fermented fish product of China, famous for its uniquely strong odour and desirable taste. It is made from mandarinfish by spontaneous fermentation under an anaerobic condition with low-salt concentration. It is found in many areas in China, especially the Lakelands where the mandarinfish are cultivated. Among them, the most famous one is Huangshan Chouguiyu that is produced in the Huangshan Mountain area in Anhui province and the product is a note for its uniquely firm but tender texture and special strong odour.

Dai *et al.*, (2013) studied the diversity of predominant species of LAB involved in the traditional fermented fish product, Chouguiyu at different stages of fermentation. Sixty-one isolates of lactic acid bacteria (LAB) were isolated using MRS agar media and characterized from various fermentation periods based on a combination of phenotypic and genotypic approaches including amplified ribosomal DNA restriction analysis

(ARDRA) and 16S rRNA partial gene sequencing analysis. LAB isolates from the Chouguiyu exhibited a big diversity with 8 species belonging to 6 different genera. *Lactobacillus sakei* was the predominant species (63%) during the fermentation. The other lactic acid bacteria (LAB) species identified were *Lactococcus garvieae*, *Lactococcus raffinolactis*, *Lactococcus lactis*, *Vagococcus* sp., *Enterococcus hermannienseis*, *Macrocococcus caseolyticus* and *Streptococcus parauberis*, the latter was recovered from the different fermentation periods, especially at the initial stages of the fermentation.

Traditional Fermented Sea-fish (*Trachinotus ovatus*, *Tanichthys albonubes*, and *Ilishae longata*)

Zhu *et al.*, (2016) isolated and characterized LAB from three kinds of traditional fermented fish (*Trachinotus ovatus*, *Tanichthys albonubes*, and *Ilishae longata*). Three strains of LAB were identified from the three kinds of fermented fish, *Leuconostoc citreum*, *Lactococcus lactis*, and *Lactobacillus pentosus*. All three strains were possessed a strong antibacterial activity and met the standards of the starter cultures.

Philippines

Burong Bangus

Burong Bangus is a traditional fermented fish and rice product of the Philippines in which milkfish, "*Chanos chanos*" or "bangus", is used.

Olympia *et al.*, (1992) analyzed the microflora of "burong bangus" for lactic acid bacteria (LAB) during its different stages of fermentation. The results showed the succession of lactic acid bacteria during fermentation. *Streptococcus* initiated the fermentation process and was persistent up to

the latter part of fermentation, this was followed by *Pediococcus* but comprised only a small percentage of the microflora, and then *Lactobacillus* and *Leuconostoc* appeared and were generally present up to the end of the fermentation, with *Lactobacillus* predominating among the microflora in the final days.

Burong isda

"Burong isda" is a traditional fermented fishery product in the Philippines which is popular in the Central Luzon region, most notably in the province of Pampanga. "Buro" means 'fermented' and "isda" means 'fish'. There are several types of "burong isda" available, the names of which are derived from the type of fish used. It is made from cooked rice and raw filleted fish fermented with salt and *angkak* (red yeast rice) for around a week.

Olympia *et al.*, (1995) isolated and characterized starch-hydrolyzing Lactic Acid Bacteria (LAB) from "Burong Isda" using conventional taxonomic and DNA-DNA reassociation method. Nine strains of lactic acid bacteria that hydrolyzed starch were isolated and it was found that all the isolates belong to *Lactobacillus plantarum*.

Malaysia

Budu

Budu is a traditional fermented seafood product of Malaysia. It is prepared from anchovy fish and salt, then letting the combination of the mixture to ferment for up to 200 days. Tamarind and palm sugar are often added to the mix.

Liasi *et al.*, (2009) isolated lactic acid bacteria (LAB) from the fermented food product, Budu, and their antimicrobial activity, and antibiotic susceptibility of the isolates were

determined. The isolates were identified as genus *Lactobacillus* (*Lactobacillus casei*, *Lactobacillus plantarum*, and *Lactobacillus paracasei*), and the most dominant species was *Lactobacillus paracasei*.

Pekasam

Pekasam is a Malaysian fermented fish product usually made from freshwater fish with ground roasted uncooked rice (Ezzat *et al.*, 2015). Pekasam is most widely consumed in Peninsular Malaysia and used as an additive to improve the taste of foods.

Muryany *et al.*, (2017) isolated and characterized Lactic Acid Bacteria (LAB) from Pekasam using 16S rRNA gene sequence analysis, the isolates were identified as *Lactobacillus plantarum* and *Lactobacillus pentosus*. The isolates also exhibited the potential probiotic properties to be developed as biotherapeutic agents.

India

Ngari, Hentak, and Tungtap

Ngari, tungtap and hentak are traditional fermented fish products of North-East India. Ngari is a fermented fish product of Manipur in North-East India prepared from the fish (*Puntius sophore*). The fish is rubbed with salt, dried in the sun for 3-4 days, pressed tightly in an earthen pot, sealed airtight and then stored at room temperature for 4-6 months (Thapa, 2002). Ngari is eaten as a side-dish withcooked rice. Hentak is a ball-like thick paste prepared by fermentation of a mixture of sun-dried fish (*Esomus danricus*) powder and petioles of aroid plants (*Alocasia macrorrhiza*) in Manipur (Thapa, 2002). Dry fish is crushed to powder, an equal amount of petioles of aroid plants is mixed and a ball-like thick paste is made. The mixture is kept in an earthen pot and is fermented for 7–9 days. Hentak is consumed as curry by the

people of Manipur or as a condiment with boiled rice. Sometimes, it is given to mothers in confinement and patients in convalescence (Sarojnalini and Singh, 1988). Tungtap is a popular fermented fish product, commonly consumed by the Khasi and Jaintia tribes of Meghalaya in North-East state of India (Thapa, 2002). Dry fish (*Puntius* spp. and *Danio* spp.) is mixed with salt, kept in an earthen pot and fermented for 2-6 months. It is consumed as a pickle and a taste enhancer.

Thapa *et al.*, (2004) studied the composition of microorganisms, mainly the lactic acid bacteria of ngari, hentak, and tungtap. Lactic acid bacteria were pre-dominant in all the three products. The isolates were identified as *Enterococcus faecium*, *Lactococcus lactis*, *Lactococcus plantarum*, *Lactobacillus fructosus*, *Lactobacillus amylophilus*, *Lactobacillus coryniformis subsp. Torquens* and *Lactobacillus plantarum*. These LAB species showed high degree of hydrophobicity indicating the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character.

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