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Research Article: Identification of Lactic Acid Bacteria Isolated from Local Commercial *Yahe* (Fermented Caridean-Shrimp) Products

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Abstract

Fermentation was used since ancient times as an easy method of food preservation, which also maintains and/or improves the nutritional and sensory properties of food. A research as aimed at identifying strain of lactic acid bacteria (LAB) from fermented caridean-shrimp, which properties suitable for starter cultures in food fermentation. A total of 18 LAB stains were obtained from ten different samples, in each sample consisted of commercial LAB strain that isolated from ten samples of caridean-shrimp. The LAB strains from ten samples were screened for resistance to biological barriers (acid and bile salts), and the three most promising strains were selected. The three bacteria strains were isolated from samples of caridean-shrimp and were characterized by the API 50 CHL system of identification. Three lactic acid bacteria species were identified and included *Lactobacillus plantarum*, and *Lactobacillus acidophilus*. Strain Y^{11b,2}, Y^{11e,2}, Y^{85,1}, which showed probiotic characteristics reducing cell growth of cancer, could be suitable as a starter culture for food fermentation because of its strong acid production and high acid tolerance. This is the first report to describe bacteria, isolated from caridean-shrimp, *Lactobacillus Plantarum* (Y^{11b,2}, Y^{11e,2}) and *Lactobacillus acidophilus* (Y^{85,1}) which have the probiotic characteristics and the acid tolerance needed for its use as a starter culture in food fermentation.

Keywords: Caridean-shrimp, probiotic, lactic acid bacteria, fermentation.

Introduction

Fermented food products are important source of amino acid and peptides (Rajapakse et al., 2005; Sathivel et al., 2003). Fermented products, especially fermented fishery products are extensively consumed in Southeast Asian countries since the 15th century; they are consumed as staples, side dishes or condiments/ seasonings in daily foods; the product imparts delicacy and high nutritional properties (Faithong et al., 2010). According to Yoshida Y (1998), the widespread consumption of fishery fermented products over a wide geographical area throughout Southeast Asia is due to the simplicity of the processing techniques and uniformity of the final fermented products. Although these products are known with different names in each region, their main function is the same, to provide a salty and umami taste to the food (Mizutani et al., 1992). Fermented shrimp products are among the fishery fermented products with widespread consumption in Southeast Asian countries (Hajeb and Jinap, 2012).

Fermented shrimp products are mainly categorized into sauces, pastes, and lacto-fermented products. Those products are known with diverse names by local population of each nation. While, most of the people in the countries in this region regularly use shrimp sauce and paste in their daily cooking, fermented shrimp products are only produced and consumed by the people of Malaysia, Thailand, Cambodia and the Philippines. Depending on the species of shrimp, the quantity of salt used, and the treatment of raw materials prior to fermentation, different types of products are produced (Mizutani et al., 1992). LAB is widespread in nature. They are commonly found in raw fish and prawn. Mauguin and Novel (1994) reported that *Lactobacillus*, *Lactococcus* and *Carnobacterium* were isolated from seafood. Furthermore, LAB is also found in Thai fermented foods such as pla-jom (fermented fish), kung-jom (fermented shrimp) and hoi-dong (fermented mussel) (Tanasupawat et al., 2000). Tanasupawat et al. (1998) reported that forty-seven strains of LAB (*Lactobacillus* and *Leuconostoc*) were isolated from fermented fish products in Thailand. The Cambodian products prahok (fish paste), kapi (shrimp

paste), and *toeuk trey* (fish sauce) found that gram-positive cocci and rods, such as *Bacillus*, *Clostridium*, *Staphylococcus*, and *Tetragenococcus*, were the major microbial populations (Mony et al., 2014). The isolation and selection of LAB which can be used as starter culture in fermented food present a considerable challenge to standardization and management of quality of the products. Lactic acid bacteria which originating from the products are specially adapted to their ecology during fermentation (Hugas and Monfort, 1997). However, isolation and characterization of LAB from fermented caridean-shrimp has not been documented in Cambodia yet. Thus, the objective of this research is to isolate and identify the lactic acid bacteria from local commercial fermented caridean-shrimp (*yahe*) products from Kampot province of Cambodia.

Material and Methods

Collection of Sample

Sample of from local commercial fermented caridean-shrimp products (Fig. 1) were purchased from local commercial processors in Kampot Province in Cambodia at ten samples. The samples were collected in clean plastic bottles and brought to the Food Bioprocessing Laboratory of Faculty of Agro-Industry, Royal University of Agriculture (RUA) for microbial analysis.

Fig 1. Processed fermented marine shrimp collected from local commercial processors

Isolation of Lactic Acid Bacteria (LAB): Samples were taken from local commercial fermented caridean-shrimp products. The standard dilution plating technique was carried out wherein 0.1ml of the appropriate dilutions of the sample was spread plated on de Man Rogosa and Sharpe (MRS) agar medium with 1% CaCO_3 and incubated at 37°C for 24-48 hrs (Hoque et al., 2010). Colonies were randomly selected based on their morphological appearance and streak plating was then used to

purify the isolates. The individual bacterial colonies were stored in MRS agar stab at 4°C for further analysis.

Isolation of Lactic Acid Bacteria (LAB): Samples were taken from local commercial fermented caridean-shrimp products. The standard pour plating technique was done for enumeration of LAB. Decimal dilutions of the samples was pipetted out in Petri dishes and poured with de Man Rogosa and Sharpe (MRS) agar medium added with 1% CaCO_3 . The petri dishes were incubated at 37°C for 24-48 hrs (Hoque et al., 2010). After the incubation period, typical colonies of LAB were picked up and inoculated on MRS agar stab. The growth was streaked on the same agar medium and the process was repeated until purity of the isolates was obtained as confirmed by microscopic examination. The pure cultures of LAB isolates in MRS agar stab were stored at 4°C for future studies.

Physiological and Biochemical characterization: The purified isolates were further characterized on the basis of their biochemical tests i.e. tolerance at different NaCl concentrations (3%, 5%, 8%, 11%, and 14%); growth at different pHs (3.0, 4.0 and 5.0); and growth at different temperatures (10°C and 45°C). **Identification of Lactic Acid Bacteria:** The ability of selected strains of LAB for fermentation of various carbohydrates was done using API 50 CHL identification kit, a standardized system, consisting of 50 biochemical tests (Hassan Pyar and Peh Kok, 2019). API 50 CHL was used for the identification of *Lactobacillus* species following the manufacturer's instructions (Biomérieux Marcy L'Etoile, France) (Ghanbari et al., 2009). Ten (10) ml of distilled water was dispensed into the incubation box with the strips placed in it and the bacterial cultures were introduced into the API 50 CHL medium (5ml), at concentration of McFarland standard #2. The whole set-up of the system was then incubated at 35°C for 48 h, after which the wells were filled with the bacterial suspensions at the line mark with the addition of mineral oil. Isolated bacterial strains were characterized as to their ability for carbohydrate fermentation. Isolates were identified based on the color change of each API strips reaction after incubation and recorded as + or -. The change in color of the bromocresol purple indicator from purple to yellow indicated positive result while no color change means negative or unable to ferment a specific carbohydrate. For esculin test, the change from purple to darker color or black also indicated positive result. Numerical profiles of strains were identified adding positive values in indicative API 50CH table. Identification of *Lactobacillus* species was done with software database (Biomérieux, France, V 5.1) (Hassan Pyar and Peh Kok, 2019).

Results and Discussion

After proper selection of acid-forming bacteria (Fig 2) and purification (Fig 3), the isolates were subjected for physiological and biochemical tests. Results revealed that Lactic acid bacteria (LAB) isolates are all Gram-positive (+), non-spore forming, either rod- or cocci-shaped, catalase (-), oxidase (-) in Table 1, and acid-tolerant (Table 2). After proper characterization, the selected isolates were tested for their ability to ferment various carbohydrates using the API 50 CHL test kit. These are resumes to be *Lactobacillus plantarum* 2, *Lactobacillus plantarum* 2, and *Lactobacillus acidophilus* 3 (Table 4).

Fig. 2 Plate showing clearing around colonies of acid-forming bacteria on MRS + CaCO₃ agar plate

Fig. 3 Plate showing discrete colonies of subculture LAB isolate

Eighteen (18) bacterial strains were isolated from fermenting caridean-shrimp and seven train after gram staining catalase and oxidase (Table 1), however only three bacterial strains have tolerance to high salt concentration and high temperature (Table

2); and these were characterized by the API 50 CHL System of identification (Table 3). The identity (%) of the isolates is shown in Table 4. Among the isolates studied, two strains were identified as *Lactobacillus plantarum* 2, and one strains as *Lactobacillus acidophilus* 3.

Table 1. The gram stain, catalase, oxidase of lactic acid bacteria isolated from fermenting caridean-shrimp

Code	Gram	Shape	Catalase	Oxidase
Y'11a	+	Coccus	-	-
Y'11b	+	Coccus	-	-
Y'11c	+	Coccus	-	-
Y'11e	+	Coccus	-	-
Y'11f	+	Coccus	-	-
Y'61	+	Coccus	-	-
Y'85	+	Coccus	-	-

Lactobacillus plantarum is commonly found in milk products, meat and a lot of vegetable fermentations including sauerkraut, pickles, brined olives, Korean Kimchi, Nigerian ogi, sourdough, and other fermented plant material, also some cheeses, fermented sausages, and stock fish (Nybohm et al., 2008). According to Amalia et al. (2018) reported that *L. plantarum* was play role in resulting secondary metabolites, which gave umami flavor in shrimp paste. *L. plantarum* has significant antioxidant activities and also helps to maintain intestinal permeability (Bested et al., 2013).

Table 2. Physiological, biochemical characteristics, and phenotypic properties of lactic acid bacteria isolated from fermenting caridean-shrimp

Code	Temperature		pH			NaCl				
	10 °C	45 °C	3	4	5	3%	5%	8%	11%	14%
Y'11b,2	*	*	-	*	**	***	***	**	*	-
Y'11e,2	*	*	-	*	**	***	***	***	*	*
Y'85,1	*	*	-	*	**	***	***	**	*	*

Note: *, weak growth; **, moderate growth; ***, strong growth; -, no growth

Lactobacilli had complex nutrient and requirement as indicator of many assay, such as the amount of amino acids in food fermented products (Banwart, 1989; Jockers, 2013; Fredriksen et al., 2012). *Lactobacillus plantarum* is a widely distributed and versatile lactic acid bacterium. It represents part of the microbiota of many foods and feeds, including dairy, meat, fish, vegetable fermented products, and silage; it is also a natural inhabitant of the human and animal mucosa. *Lactobacillus plantarum*, derived from the environment or used in controlled fermentations, is generally associated with desirable properties in many fermented foods, and is actually added to a variety of them in order to enhance their quality or the associated health benefits (Corsetti and Valmorri, 2011).

Table 3. Phenotypic properties of lactic acid bacteria from fermenting caridean-shrimp using API 50 CHL gallery kit

No		Y'11b,2	Y'11e,2	Y'85,1
1	0	+	+	+
2	GLY	-	-	+
3	ERY	-	-	-
4	DARA	-	-	-
5	LARA	-	-	-
6	RIB	+	+	-
7	DXYL	-	-	-
8	LXYL	-	-	-
9	ADO	-	-	-
10	MDX	-	-	-
11	GAL	-	-	-
12	GLU	+	+	+

No		Y'11b,2	Y'11e,2	Y'85,1
13	FRU	+	+	+
14	MNE	+	+	-
15	SBE	-	-	-
16	RHA	-	-	-
17	DUL	-	-	-
18	INO	-	-	-
19	MAN	-	-	-
20	SOR	-	-	-
21	MDM	-	-	-
22	MDG	-	-	-
23	NAG	+	+	+
24	AMY	-	-	-
25	ARB	-	-	-
26	ESC	+	+	+
27	SAL	-	-	-
28	CEL	+	+	-
29	MAL	+	+	+
30	LAC	-	-	-
31	MEL	-	-	-
32	SAC	-	-	+
33	TRE	-	-	+
34	INU	-	-	-
35	MLZ	-	-	+
36	RAF	-	-	-
37	AMD	-	-	-
38	GLYG	-	-	-
39	XLT	-	-	-
40	GEN	+	+	-
41	TUR	-	-	+
42	LYX	-	-	-
43	TAG	-	-	-
44	DFUC	-	-	-
45	LFUC	-	-	-
46	DARL	-	-	-
46	LARL	-	-	-
48	GNT	-	-	-
49	2KG	-	-	-
50	5KG	-	-	-

Three lactic acid bacteria species were identified and included *Lactobacillus plantarum*, and *Lactobacillus acidophilus*. According to a result study of Bao Le and Seung Hwan Yang (2019) stated that *L. plantarum* has an impact on preventing high cholesterol level and may be used as starter culture for shrimp fermentation. On the other hand, *L. plantarum* is known to grow at low pH. For instance, *Lactobacillus plantarum* can grow at pH value as low as 4.5 (McDonald et al., 1990; Guyot et al., 2000). *Lactobacillus plantarum* is the most common bacterium used in silage inoculants. During the anaerobic conditions of ensilage,

the organisms quickly dominate the microbial population and with 48 hours they begin to produce lactic and acetic acids, further diminishing their competition. *Lactobacillus plantarum* strains produce high levels of heterologous proteins have been found to remain highly competitive. The quality could allow the species to be utilized as an effective biological pretreatment for lignocellulosic biomass (Kim et al., 2019).

Table 4: Identity (%) of isolates from fermenting caridean-shrimp

Code	Species	ID (%)
Y'11b,2	<i>Lactobacillus plantarum</i> 2	97.3
Y'11e,2	<i>Lactobacillus plantarum</i> 2	88.3
Y'85,1	<i>Lactobacillus acidophilus</i> 3	71.6

Surface microflora of *Lactobacillus* spp. *Leuconostoc* spp. and *Pediococcus* spp. can be responsible for the natural fermentation of most fruits and vegetables; however, the application of pure starter culture like *L. plantarum*, *L. rhamnosus*, *L. gasseri*, and *L. acidophilus* gives more successful results due to uniform and predictable quality of the finished products (Egbe et al., 2017). The present study investigated the LAB present in fermentation of caridean-shrimp. Two strains of *Lactobacillus plantarum* and one strain of *Lactobacillus acidophilus* were identified as the main LAB involved in the spontaneous fermentation of caridean-shrimp. This is in agreement with the work of (Lennox and Efiuvwevwere, 2013), wherein these LAB species were involved in the microbial dynamics of fermenting cucumber. Researches that interest on fermenting caridean-shrimp shoot up in the recent years mainly due to their perceived health benefits to consumers. There is therefore an earnest desire among research scientists for new techniques of preserving foods existing in a natural environment. Preservation by biological means such as the application of useful microorganisms and their metabolites has been proven to increase the storage life and improve the safety of foods (Ross et al., 2002) would most likely make this LAB a potential inoculant (El-Ghhaish et al., 2011).

Conclusion and Recommendation

Totally, there are three strains of LAB that can be isolated from fermented caridean-shrimp, two of which are *Lactobacillus plantarum* and one is *Lactobacillus acidophilus*. Lactic Acid Bacteria (LAB) isolated should apply as: Potential anti-pathogenic bacteria in Vitro, Potential bio-control pathogenic bacteria in fermented foods, and quality improvement of fermented products.

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