Research Article

Antibiotic Resistance of Lactic Acid Bacteria Isolated from Cambodian Fish Paste Product

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Abstract
Fish paste product is considered an important food in Cambodia. However, the status of antimicrobial susceptibility of microbes in this product are a concern. This study aimed to isolate lactic acid bacteria (LAB) from Cambodian fish paste and to investigate their resistant property of antibiotics. Fifteen LABs were isolated with cell forms of 14 as cocci and 1 as rods. Isolates of the bacteria were identified as *Staphylococcus piscifermentans* (14 strains) and *Lactobacillus plantarum* (1 strain). Using the disk diffusion method, the resistance was investigated of the 15 LAB isolate strains to eight clinically crucial antibiotics: penicillin (Pen), ampicillin (Amp), erythromycin (Ery), tetracycline (Tet), vancomycin (Van), streptomycin (Str), sulfamethoxazole-trimethoprim (Sul) and metronidazole (Met). It was found that all 15 LAB isolates were resistant to Met. One isolate strain was resistant to Pen, Amp, Tet, Str and Sul. Furthermore, 7 and 2 isolate strains were resistant to Tet and Van, respectively. All 15 isolate strains were sensitive to Str and Ery. The LAB isolate strains were sensitive to Pen, Amp, Sul (14 strains), Tet (6 strains) and Van (13 strains). These results showed that 14 of the LAB isolate strains were sensitive to 5 antibiotics (Pen, Amp, Ery, Str and Sul) and could be considered as strains for utilization as starter culture for fish fermentation. Additionally, these finding will be conduct to assess the antibiotic resistance incidences of LABs in Cambodian fermented foods.

Keywords: Fish paste, Lactic acid bacteria, *Staphylococcus piscifermentans*, *Lactobacillus plantarum*, Antibiotics resistance

1 Introduction

Fish paste is considered an important food in the daily diet of Cambodians and can be eaten raw or cook depending on consumer preference [1], [2]. Fish paste processors can be classified into small-, medium- and large-scale operators, with the small-scale fish paste processors being largely rice farmers. The average annual production of fish paste by the medium-scale processors averages 50–1,000 t, while for the large-scale processors it is more than 1,000 t. Freshwater fish catches from Tonle Sap Lake or the Mekong River are usually used as raw material for fish paste products but some producers use fish from aquaculture farms [3]. Fish paste is marketed in two types: bony or boneless. In boneless fish paste processing, the bones are removed after discarding the head and scales. The fish are soaked in water at room temperature for 24 h and then sun-dried for 2 h to extract moisture. Then, the fish are soaked in a saline solution and stored in

tightly sealed jars in the sun for 1–2 months [4].

Lactic acid bacteria (LAB) are types of bacteria that produce lactic acid in fermented foods. LAB produce organic acid and this decreases pH in the products. Furthermore, LAB contribute to the aroma, taste and texture of fermented foods [5]. Nowadays, LAB are an important component in processing fermented foods by large-scale processors and they are considered safe for human consumption [6]. Nonetheless, some strains of LAB produce bacteriocins that have antibiotic resistance [7].

The misuse and overuse of antibiotic therapy has resulted in antibiotic-resistant bacteria in the environment and this reduces the effectiveness of the antibiotics. Additionally, some strains of LAB could modify the chemical structure and produce proteins that affect the molecular activity of antibiotics [8], [9]. Aquaculture has fast developed and encountered many kinds of bacterial diseases. Farmers are intensively used veterinary drugs in aquaculture. Farmers mixed antibiotics with feed in order to improve health of fish and cure diseases. The misuse of antibiotics in fish feed is occurred antibiotic resistance bacteria [10]. Fish farm and surrounding environment usually appear antibiotic resistance. Furthermore, antibiotic resistance can be spread to human by water chain or food [11]. Therefore, antibiotic resistance of LAB should be evaluated.

Cambodian fish paste has been a popular condiment for over a century, but there have been no reports of antibiotic resistance of LAB for this product. Hence, this research study aimed to isolate LAB and investigate their antibiotic resistance to eight clinically important antibiotics: penicillin (Pen), ampicillin (Amp), erythromycin (Ery), tetracycline (Tet), vancomycin (Van), streptomycin (Str), sulfamethoxazole-trimethoprim (Sul) and metronidazole (Met) obtained from fish paste products collected in four provinces in Cambodia. The results from the study should be vital in the safety assessment of LAB strains in Cambodian fish paste product. The benefit of safety assessment of LAB strains that be found on fish paste products will be conducive to control fermented fish products in Cambodia.

2 Materials and Methods

2.1 *Sample collection*

Eleven fish paste samples were randomly collected from Phnom Penh city (3 samples), Kondal province (3 samples), Kompong Chnange province (3 samples) and Kompot province (2 samples). All the samples had used freshwater fish as raw material. Each sample was carefully placed into sterile plastic box and transported to Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus in one day. All samples were uniquely coded and stored at 4 °C.

2.2 *Isolation of lactic acid bacteria (LAB)*

2.2.1 Sample preparation

Each fish paste sample (25 g) was added to 225 mL of 0.1% peptone water (HiMedia, India). The serial decimal dilutions were prepared in 0.1% peptone water. Dilution solutions were spread on separate sterile Petri dishes containing De Man, Rogosa and Sharpe (MRS) agar (Merck, Germany) containing 0.5% $CaCO_3$ and were incubated at 35 °C for 24–48 h. The bacterial colonies that presented in the clear zone on the plate were collected and counted [12].

2.2.2 Catalase properties and Gram-stain analysis

To isolate the LAB, the bacterial colonies that produced a clear zone on an agar plate were picked and cultured in tubes containing MRS agar (1.5% agar). The catalase activity and Gram-stain were determined. Each isolated bacteria colony was tested for catalase by adding a drop of 3% hydrogen peroxide (H_2O_2) solution (Siribuncha, Thailand) on the cells. A bubble showed the presence of catalase in the cells. The bacterial colonies were identified based on Gram-staining using a microscope (Primo Star, ZEISS, Germany) [12].

2.2.3 pH and total acidity (TA) of lactic acid production

The selected LAB isolates were cultured at 37 °C for 24 h in 40 mL MRS broth (Merck, Germany). The cell-free solution was obtained by centrifuging the culture media at 8,000×g for 10 min at 4 °C. The pH of the cultured broth was directly measured using a pH electrode meter (Sartorius, Germany). A sample (20 mL) of supernatant was taken for TA testing. The solution was titrated with standardized 0.1 N NaOH (QRëC, New Zealand). The TA value was expressed as %(w/v) lactic acid [13].

2.3 *Identification of lactic acid bacteria*

The pure isolates of LAB were performed by Gibthai Co., Ltd (Thailand). The isolates of LAB were identified based on 16S rDNA sequencing, using the PCR primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3, 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3 and the sequencing primers 785F 5' (GGA TTA GAT ACC CTG GTA) 3', 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3. The PCR amplification was conducted in a 30 µL reaction mixture by utilizing an EF-Taq (SolGent, Korea). The 20 ng of genomic DNA was used as the template. Taq DNA polymerase was activated at 95 °C for 2 min, 35 cycles of 95 °C for 1min, 55 °C, and 72 °C for 1 min each, and the final step at 72 °C for 10 min. The multiscreen filter plate (Millipore Corp., Bedford, MA, USA) was carried out to purify the amplification products. A PRISM BigDye Terminator v3.1 Cycle sequencing Kit was performed as sequencing reaction. The extension products–containing DNA samples were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated for 5 min at 95 °C, then placed on ice for 5 min. Next, the mixture was analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA) [14].

2.4 *Antibiotic resistance*

The disk diffusion method of Bauer [15] was carried out to test for antimicrobial susceptibility. The 8 antibiotics (Pen, Amp, Ery, Tet, Van, Str, Sul and Met) were bought from HiMedia Laboratories Pvt. Limited (HiMedia, India). These antibiotics are usually used to treat animals or humans [16]. The pure LAB isolate species were further cultured in MRS broth (Merck, Germany) using an inoculated concentration of approximately 7 Log CFU/mL and incubated for 24 h at 35 °C. Then, culture broth was swabbed on Mueller Hinton agar (HiMedia, India) plates using a sterile cotton swab to seed the antibiotic disks. The agar plates were incubated at 35 °C for 48 h. A ruler was used to measure the diameters (in millimeters) of the antibiotic inhibition zones. In the microbiology laboratory usually reports the result of the activity of drug against the organism in three categories, such as resistance, intermediate (moderate susceptible) and susceptible (sensitive) [17]. The method of Charteris *et al.* [18] was used to evaluate antimicrobial susceptibility. Resistance (R), moderate susceptible (MS) and sensitive (S) were evaluated by the diameter of the zone of inhibition for each strain. Resistance (R) had inhibition zone diameter (clear zone) less than or equal to 8 mm for Str, Sul and Met, less than or equal to 13 mm for Ery, less than or equal to 14 mm for Tet and Van and less than or equal to 19 mm for Amp and Pen. Moderate susceptible (MS) had inhibition zone diameter from 9 to 18 mm for Str, Sul and Met, 13 to 15 mm for Amp, 14 to 18 mm for Ery, 15 to 18 mm for Tet, 15 to 16 mm for Van and 20 to 27 mm for Pen. Sensitive (S) had inhibition zone diameter more than or equal to 19 mm for Ery, Str, Sul and Met, more than or equal to 16 mm for Amp, more than or equal to 17 mm for Van, more than or equal to 19 mm for Tet and more than or equal to 28 mm for Pen.

2.5 *Statistical analysis*

Analysis of data on the pH and total acidity production in culture broth was performed using a completely randomized design (CRD). A value of $p < 0.05$ was used to define statistical significance using the SPSS program version 21.0 (SPSS Inc., Chicago, IL, USA).

3 *Results and Discussion*

Table 1 lists the characteristics of the 15 bacterial isolates from the fermented fish products. LAB were completely isolated from fish paste product based on the selective medium of MRS agar, which contained 0.5% (w/v) $CaCO_3$. It was found that 98 isolates appeared with a clear zone encircling the bacterial colonies due to reaction to the colonies' production. Of the 98 colonies, 15 were selected because of their pH and total acidity production. Of these strains, the cell form was 14 as cocci and 1 as rods.

The identification of the LAB bacteria isolated from the fish paste products and their pH and total acidity (%) in MRS broth are presented in Table 2. The 15 isolates were identified as *S. piscifermentans* (14 isolates) and *L. plantarum* (1 isolate) with the sequences revealing 99% homology (Figures 1 and 2). The values in the MRS broth for the pH and lactic acid percentage of *S. piscifermentans* were in the ranges 4.46–4.93 and 0.88–1.13%(w/v), respectively. The values in MRS broth for the pH and lactic acid

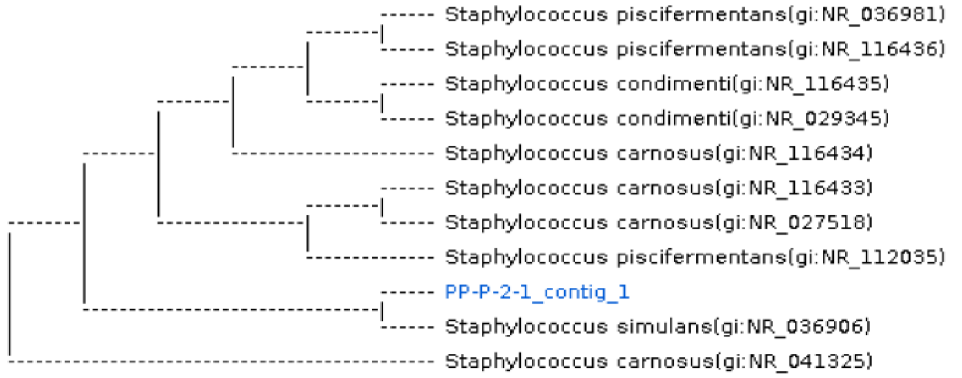

Figure 1: Phylogenetic tree of *S. piscifermentans* form fish paste product.

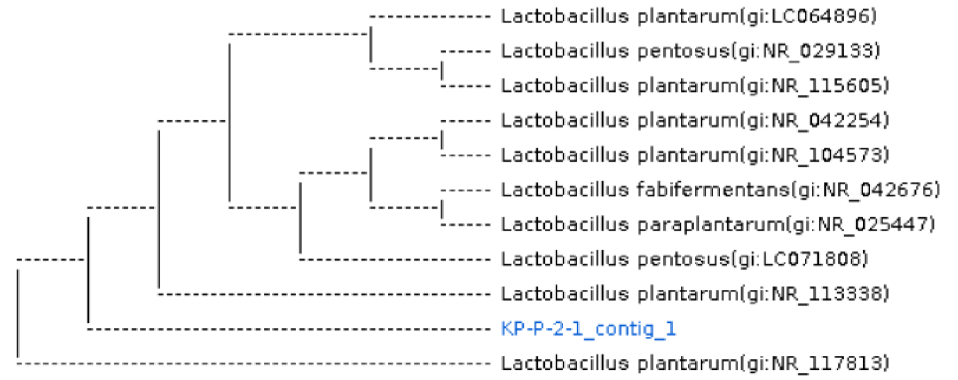

Figure 2: Phylogenetic tree of *L. plantarum* from fish paste product.

Table 1: Characteristics of 15 bacterial isolates from fermented fish products

Bacteria Isolate Strain	Cell Form	Gram-staining	Catalase
PP-P-1-1	Cocci	+	+
PP-P-1-2	Cocci	+	+
PP-P-2-1	Cocci	+	+
PP-P-3-1	Cocci	+	+
PP-P-3-2	Cocci	+	+
KDL-P-1-1	Cocci	+	+
KDL-P-2-1	Cocci	+	+
KDL-P-3-1	Cocci	+	+
KPC-P-1-1	Cocci	+	+
KPC-P-2-1	Cocci	+	+
KPC-P-3-1	Cocci	+	+
KPC-P-3-2	Cocci	+	+
KP-P-1-1	Cocci	+	+
KP-P-1-2	Cocci	+	+
KP-P-2-1	Rods	+	-

Table 2: The identification of LAB isolated from fish paste product and its production of pH and total acidity (%) in MRS broth

Strain ID	Strain Species	% Identity	Parameters	
			pH^{ns}	Total Acidity %(w/v)^{ns}
PP-P-1-1	*S. piscifermentans*	99	(4.92±0.09)	(0.90±0.04)
PP-P-1-2	*S. piscifermentans*	99	(4.94±0.02)	(0.93±0.04)
PP-P-2-1	*S. piscifermentans*	99	(4.46±0.15)	(0.95±0.03)
PP-P-3-1	*S. piscifermentans*	99	(4.89±0.00)	(0.97±0.04)
PP-P-3-2	*S. piscifermentans*	99	(4.93±0.03)	(0.88±0.03)
KDL-P-1-1	*S. piscifermentans*	99	(4.92±0.01)	(0.98±0.04)
KDL-P-2-1	*S. piscifermentans*	99	(4.51±0.05)	(1.11±0.17)
KDL-P-3-1	*S. piscifermentans*	99	(4.63±0.04)	(0.90±0.05)
KPC-P-1-1	*S. piscifermentans*	99	(4.91±0.00)	(1.02±0.09)
KPC-P-2-1	*S. piscifermentans*	99	(4.86±0.08)	(1.01±0.02)
KPC-P-3-1	*S. piscifermentans*	99	(4.51±0.03)	(1.13±0.04)
KPC-P-3-2	*S. piscifermentans*	99	(4.51±0.01)	(1.07±0.23)
KP-P-1-1	*S. piscifermentans*	99	(4.47±0.01)	(0.94±0.00)
KP-P-1-2	*S. piscifermentans*	99	(4.48±0.00)	(0.82±0.01)
KP-P-2-1	*L. plantarum*	99	(4.61±0.02)	(1.57±0.17)

The value in the parentheses show the mean ± the standard deviation (mean±SD). ^{ns}Non-significant different.

percentage of *L. plantarum* were 4.61 and 1.57% (w/v), respectively. In this study, most of LAB isolates were *S. piscifermentans* (93.33%), which was not unexpected as it is known that *S. piscifermentans* can be found in fermented foods, such as fish, sausages and meat [19]. According to Tanasupawat *et al.* [20], *S. piscifermentans* was isolated from fermented fish in Thailand and it could grow under conditions with a high salt content. Chandravanshi and Majumdar [21] reported that 10 strains of LAB from traditional fermented fish products of Northeast India were identified as *S. piscifermentans* and on the basis of its resistance to most antibiotics, it was non-hemolytic and non-pathogenic. Additionally, *S. piscifermentans* contributed to improve red color and flavor as well as decreased pH in fermented fish products. Furthermore, Hajar and Hamid [19] reported that *S. piscifermentans* had been isolated from Malaysian fermented shrimp *cincaluk*, and it inhibited the growth of some pathogenic bacteria, such as *Salmonella typhimurium*, *Bacillus subtilis*, *Escherichia coli* and *S. aureus*. Singh *et al.* [22] revealed that *S. piscifermentans* isolated from fermented fish was a non-toxin and also had high probiotic, antimicrobial and anticancer activities which could be useful for human health as natural therapeutic agents. In addition, *S. piscifermentans* was used to co-inoculate with salt-tolerant yeasts to inhibit biogenic amines in soy sauce products. The result showed that it could decrease contents of biogenic amines 31.51% in laboratory-scale experiments [23]. Therefore, *S. piscifermentans* is a non-pathogenic Gram-positive, catalase positive and *Staphylococcus* species that can be utilized as part of starter cultures in combination with *S. condimenti* and *S. carnosus* for processing fermented foods. *S. piscifermentans* is essential in starter culture to avoid the growth of harmful bacteria and thus decrease the risk of food poisoning, while playing a role as a food preservative.

L. plantarum was reported as the most common LAB isolated from fermented food products [24]. *L. plantarum* was isolated from the fish paste product in the current study. According to Kopermsub and Yunchalard [25], *L. plantarum* was isolated from a traditional fermented fish products (*plaa-som*) of Thailand. It was advantageous for the future advancement of beneficial LAB starters to develop a more tractable *plaa-som* product.

Li *et al.* [26] revealed that *L. plantarum* strains isolated from Chinese fermented foods could be treated as possible antioxidants in foods. Furthermore, Kanno *et al.* [27] reported that *L. plantarum* isolated from fermented Japanese foods (*narezushi*) had DPPH and superoxide radical scavenging capacity. In addition, *L. plantarum* was utilized as starter culture to reduce cyanide toxicity, improve taste and enhance the microbial safety of products [28]. Furthermore, various LAB were isolated from fish paste products, such as *Streptococcus bovis*, *L. garvieae*, *Pediococcus pentosaceus*, *Weissella cibaria* and *L. fermentum* [25], *S. salivarius* and *Enterococcus faecalis*, *Lactococcus* sp. [29] and *Leuconostoc mesenteroids*, *S. faecalis*, *P. halophilus*, *Tretragenococcus* sp. and *Ln. durionis* [30]. Bover-Cid and Holzapfel [31] and Fadda *et al.* [32] stated that LAB assisted in food fermentation, as *L. lactic*, *L. plantarum* and *S. thermophilus* could produce biogenic amines, such as tyramine and was resistant to some kind of antibiotics.

The inhibition zone diameters (clear zone) of the 8 antibiotics in the 15 LAB isolate strains are shown in Table 3. The 15 LAB isolate strains were further cultured in MRS broth. The zone diameters in all 15 LAB isolate strains of the eight antibiotics (Pen, Amp, Ery, Tet, Van, Str, Sul and Met) were in the ranged 0–39 mm, 0–42.33 mm, 23.67–31.33 mm, 0–41.00 mm, 0–36.33 mm, 0–26.33 mm, 13.33–24.33 mm and 0–29.67 mm, respectively. The *L. plantarum* strain was resistant to 7 antibiotics (Pen, Amp, Tet, Van, Str, Sul and Met) but not to Ery. In addition, 7 strains and 1 strain of *S. piscifermentans* were resistant to Tet and Van, respectively. All 14 *S. piscifermentans* and *L. plantarum* strains were strongly sensitive to Ery. Additionally, *S. piscifermentans* strains were sensitive to Pen, Amp, Str, Sul (14 strains), Tet (7 strains) and Van (13 strains). In this study, most of LAB isolate strains were sensitive to antibiotics (93.33%), with the exception of one strain that was resistant to antibiotics (6.67%).

Figure 3 showed the percentage of antibiotic resistance (R), moderately susceptible (MS) and sensitive (S) in the 15 isolate strains of LAB. There were 6.67% (R) and 93.33% (S) for penicillin (Pen), 6.67% (R) and 93.33% (S) for ampicillin (Amp), 100% (S) for erythromycin (Ery), 53.33% (R), 6.67% (MS) and 40.00% (S) for tetracycline (Tet), 13.33% (R) and 86.67% (S) for vancomycin (Van), 40.00% (MS) and 60.00% (S) for streptomycin (Str), 6.67% (R) and 93.33% (S) for sulfamethoxazole-trimethoprim (Sul) and 100% (R) for metronidazole (Met).

Table 3: Zone diameters (clear zone) of antibiotic used on 15 LAB strains

Strain ID	Strain's Name	Antibiotics (mm)							
		Pen (10µg)	Amp (10µg)	Ery (15µg)	Tet (30µg)	Van (30µg)	Str (10µg)	Sul (25µg)	Met (50µg)
PP-P-1-1	*S. piscifermentans*	(31.33±3.06)S	(37.67±3.21)S	(26.00±1.00)S	(35.00±2.00)S	(23.33±2.08)S	(24.33±3.06)S	(28.00±1.00)S	(0.00±0.00)R
PP-P-1-2	*S. piscifermentans*	(35.00±2.00)S	(36.67±1.53)S	(27.00±1.73)S	(35.67±1.15)S	(24.00±1.00)S	(19.33±2.08)S	(28.00±2.00)S	(0.00±0.00)R
PP-P-2-1	*S. piscifermentans*	(35.00±2.65)S	(42.33±1.53)S	(31.33±1.53)S	(41.00±1.00)S	(27.67±1.53)S	(21.33±1.53)S	(29.67±0.58)S	(0.00±0.00)R
PP-P-3-1	*S. piscifermentans*	(31.00±2.00)S	(37.00±2.00)S	(27.67±1.53)S	(36.33±2.31)S	(25.67±1.53)S	(20.00±0.00)S	(26.00±2.65)S	(0.00±0.00)R
PP-P-3-2	*S. piscifermentans*	(31.67±4.51)S	(36.67±5.51)S	(27.00±1.00)S	(35.00±5.00)S	(26.00±1.00)S	(21.00±3.61)S	(27.67±2.08)S	(0.00±0.00)R
KDL-P-1-1	*S. piscifermentans*	(35.00±1.00)S	(36.67±2.08)S	(25.33±0.58)S	(13.00±3.61)R	(22.33±2.31)S	(13.33±1.53)MS	(24.33±1.53)S	(0.00±0.00)R
KDL-P-2-1	*S. piscifermentans*	(36.00±1.00)S	(36.33±0.58)S	(23.67±2.52)S	(12.67±0.58)R	(21.67±1.53)S	(19.00±1.73)S	(25.33±1.53)S	(0.00±0.00)R
KDL-P-3-1	*S. piscifermentans*	(36.00±1.73)S	(36.67±2.08)S	(25.67±0.58)S	(14.00±1.00)R	(22.33±2.52)S	(19.67±1.53)S	(26.67±2.08)S	(0.00±0.00)R
KPC-P-1-1	*S. piscifermentans*	(35.00±2.00)S	(38.33±1.15)S	(26.33±2.52)S	(36.00±1.00)S	(21.33±1.53)S	(18.00±1.00)MS	(25.33±1.15)S	(0.00±0.00)R
KPC-P-2-1	*S. piscifermentans*	(39.00±2.00)S	(41.33±1.53)S	(26.67±1.53)S	(13.00±1.00)R	(23.00±1.73)S	(24.00±4.58)S	(26.67±0.58)S	(0.00±0.00)R
KPC-P-3-1	*S. piscifermentans*	(34.33±1.15)S	(38.00±1.00)S	(25.00±2.00)S	(12.33±0.58)R	(21.33±0.58)S	(19.33±0.58)S	(25.00±1.00)S	(0.00±0.00)R
KPC-P-3-2	*S. piscifermentans*	(33.33±3.06)S	(38.33±3.06)S	(26.33±3.06)S	(12.00±2.65)R	(26.33±1.53)S	(16.67±4.04)MS	(27.00±2.00)S	(0.00±0.00)R
KP-P-1-1	*S. piscifermentans*	(31.33±3.79S	(34.67±1.53)S	(26.67±3.51)S	(16.67±3.51)MS	(0.00±0.00)R	(17.67±1.53)MS	(21.33±3.21)S	(0.00±0.00)R
KP-P-1-2	*S. piscifermentans*	(36.33±2.08)S	(40.00±1.00)S	(25.33±3.06)S	(12.00±1.00)R	(22.33±2.31)S	(17.67±0.58)MS	(25.67±1.53)S	(0.00±0.00)R
KP-P-2-1	*L. plantarum*	(0.00±0.00)R	(0.00±0.00)R	(25.67±1.53)S	(0.00±0.00)R	(0.00±0.00)R	(14.00±1.00)MS	(0.00±0.00)R	(0.00±0.00)R

The values in the parentheses show the mean ± the standard deviation (mean±SD).
Pen: Penicillin, Amp: Ampicillin, Ery: Erythromycin, Tet: Tetracycline, Van: Vancomycin, Str: Streptomycin, Sul: Sulfamethoxazole-trimethoprim, and Met: Metronidazole. The values in parentheses with R= Resistant, MS= Moderate susceptible, and S= Sensitive.
All antibiotics were tested in triplicate.

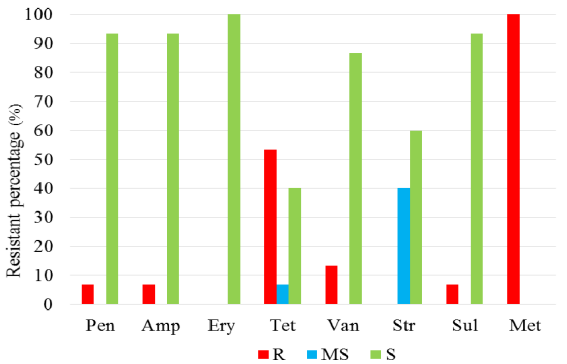

Figure 3: Antibiotic resistance (R), moderately susceptible (MS) and sensitive (S) percentage in 15 isolates of LAB to the antibiotics penicillin (Pen), ampicillin (Amp), erythromycin (Ery), tetracycline (Tet), vancomycin (Van), streptomycin (Str), sulfamethoxazole-trimethoprim (Sul) and metronidazole (Met).

In Cambodia, farmers receive incentives to use antimicrobials as probiotics and prebiotics and in vaccines and feed additives. Biocides and disinfectants are routinely used in food processing. A study showed that the use of antibiotics is widespread and knowledge is limited among veterinarians, animal feed retailers and commercial farmers [33]. Farmers used pre-mixed feed containing antibiotics, while some used antibiotics as feed supplement for growth promotion. Fisheries administration recently identified 34 chemicals and antibiotics used in aquaculture [33]. These included: chloramphenicol, dimetridazole, metronidazole, nitrofuran (including furazolidone), ronidazole, ipronidazole, nitroimidazoles and fluoroquinolones. A recent survey of 91 family-owned swine farms in or around Phnom Penh listed several antibiotics used or kept at home. These included: gentamicin, spectinomycin, streptomycin, thiamphenicol, enrofloxacin, luncomycin, tylosin, amoxicillin, ampicillin, penicillin G, colistin, sulfonamides, trimethoprim and oxytetracycline [34]. The same study reported on the knowledge and practices of farmers in using antimicrobials as a prophylaxis and medicine and in animal feeds. Based on a study of 110 fish farmers, 41% of farmers in Battambang and 37% in Kandal used antibiotics to treat their fish when the stocks were infected with diseases. Herreros *et al.* [35] revealed that most of the LAB strains isolated from Spanish goats' milk cheese, were resistant to vancomycin, cefotoxin, teicoplanin, trimethoprim and oxacillin. Temmerman *et al.* [36] revealed that 187 LAB isolate strains were resistant to vacomycin (65%), penicillin G (23%), tetracycline (26%), chloramphenicol (11%), erythromycin (16%) and kanamycin (79%). Sornplang *et al.* [37] reported that 10 LAB isolate strains from the *pla-chom* product of Thailand were resistant to Van, Str, Sul and Met.

Furthermore, 7 strains and 6 strains were highly resistant to Tet and Pen, respectively, while all 10 LAB strains were highly sensitive to Ery. The results of the current study were consistent with reports from Thailand, Spain, Malaysia, India and China [38]. Most researchers investigated antibiotics associated with *L. plantarum*, but in the current study investigated a strain of *S. piscifermentans* which was isolated from Cambodian fish paste products.

4 Conclusions

In this study, *S. piscifermentans* (14 isolates) and *L. plantarum* (1 isolate) were isolated from fish paste products and identified as lactic acid bacteria (LAB). Furthermore, the 14 isolate strains of *S. piscifermentans* were sensitive to 5 antibiotics (ampicillin, erythromycin, penicillin, streptomycin and sulfamethoxazole-trimethoprim). Due to the many beneficial and special properties of *S. piscifermentans*, it could serve well in food processing and in the development of starter cultures in fish fermentation in the future.

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