การคัดแยกและการตรวจสอบความสามารถในการยับยั้งจุลินทรีย์ของแบคทีเรียกรดแลคติกที่แยกได้จากผลิตภัณฑ์ปลาหมัก ปลาส้ม

Isolation and Determination of Antimicrobial Activity of Lactic Acid Bacteria from Fermented Fish Product, Pla-Som

Piyorot Hongsachart

Faculty of Applied Science and Engineering, Khon Khaen University, Nong Khai Campus, Nong Khai, Thailand, 43000

E-mail: anise521@yahoo.com, piyorot@kku.ac.th

บทคัดย่อ

การคัดแยกแบคทีเรียกรดแลคติกจากตัวอย่างปลาส้มที่เก็บจากตลาดพื้นบ้านในเขตพื้นที่จังหวัดหนองคาย แยกได้แบคทีเรียกรดแลคติกทั้งหมด 37 ไอโซเลท แบ่งเป็นแบคทีเรียรูปร่างกลม 8 ไอโซเลท และรูปร่างแท่ง 29 ไอโซเลท นำเชื้อที่แยกได้มาทดสอบการต่อต้านการเจริญของแบคทีเรียก่อโรคจำนวน 11 ชนิด ด้วยวิธีดิสดิฟฟิวชัน (disc diffusion method) พบว่าแบคทีเรียกรดแลคติกไอโซเลทหมายเลข L8-16 ให้ผลในการต่อต้านเชื้อแบคทีเรียทั้งแกรมลบและแกรมบวก โดยแสดงการยับยั้งอย่างมาก ต่อเชื้อ *Shigella* sp., *Pseudomonas* sp. และ *Pseudomonas aeruginosa* แสดงการยับยั้งระดับปานกลางต่อเชื้อ *Bacillus megaterium*, *Bacillus cereus*, *Escherichia coli* และ *Salmonella* Typhi ในขณะที่แบคทีเรียกรดแลคติกไอโซเลทหมายเลข L7-1 แสดงการยับยั้งอย่างมากต่อเชื้อ *S.* Typhi และ *P. aeruginosa* และแสดงการยับยั้งระดับปานกลางต่อเชื้อ *Staphylcoccus aureus*, *B. subtilis*, *E. coli* และ *Klebsiella pneumoniae* แบคทีเรียกรดแลคติกที่แยกได้ส่วนใหญ่สามารถยับยั้งแบคทีเรียแกรมลบได้ดีกว่าแบคทีเรียแกรมบวกด้วยขนาดของบริเวณยับยั้งระดับกลาง (11-12 มม.) และขนาดบริเวณยับยั้งระดับมาก (13-15 มม.) ยกเว้นไอโซเลท L8-14 ที่สามารถยับยั้ง *B. cereus* ที่เป็นแบคทีเรียแกรมบวกได้ดีกว่าแบคทีเรียกรดแลคติกชนิดอื่น จากข้อมูลเหล่านี้ แสดงให้เห็นว่าแบคทีเรียกรดแลคติกที่คัดแยกใหม่มีศักยภาพในการยับยั้งการเจริญของแบคทีเรียก่อโรค ซึ่งจะนำไปศึกษาเพื่อพัฒนาเป็นสารกันเสียชีวภาพ (biopreservative) ต่อไป

ABSTRACT

Isolation of lactic acid bacteria (LAB) from Pla-Som samples collected from local markets in the area of Nong Khai province found a total of 37 LAB isolates including 8 cocci and 29 rods. Isolated LAB were subsequently determined antibacterial activities against 11 pathogenic bacteria using the disc diffusion method. It was found that the LAB isolate namely, L8-16 was effective against both Gram-negative and Gram-positive bacteria. It exhibited strong inhibition activity against *Shigella* sp., *Pseudomonas* sp. *P. aeruginosa* and moderate inhibition activity against *Bacillus megaterium*, *Bacillus cereus*, *Escherichia coli* and *Salmonella* Typhi. In addition, the isolate L7-1 also showed strong inhibition activity against *S.* Typhi and *P. aeruginosa* and moderate inhibition activity against *Staphylococcus aureus*, *B. subtilis*, *E. coli* and *Klebsiella pneumoniae*. Overall, the most of LAB isolates inhibited Gram negative bacteria more than Gram-positive bacteria with moderate (11-12 mm) and strong (13-15 mm) inhibition zones. There was only isolate L8-14 showed strong inhibition against *B. cereus*. Based on these results, isolated LAB would be considered to be potential biopreservatives in Pla-Som fermentation.

คำสำคัญ: ความสามารถในการต้านจุลินทรีย์ ผลิตภัณฑ์ปลาหมัก แบคทีเรียกรดแลคติก

Keywords: Antimicrobial activity, Fermented fish product, Lactic acid bacteria

1. Introduction

Pla-Som is a Thai fermented fish product which typically consists of freshwater fish mixed with cooked rice or steamed sticky rice, garlic and salt. The mixture is then left in a lid covered jar or wrapped tightly in plastic bag and allowed to ferment at room temperature until a sour taste is developed (Chadong et al., 2015). It is mostly produced in northeastern, northern and some area parts in central of Thailand. Generally, the production of Pla-Som was based on spontaneous fermentation due to the development of the microflora naturally present in the ingredients. Lactic acid bacteria (LAB) have been found to be the dominant microorganisms in Pla-Som and fermented fish products (Østergaard et al., 1998). The primary role of LAB is organic acids production, especially lactic acid, thereby causing a decrease in pH. These acids also contribute to the sour- taste, aroma and texture of fermented products (Kopermsub and Yunchalard, 2010). The combination of low pH and organic acid is one of important factors to inhibit pathogenic and spoilage bacteria (Alakomi et al., 2000). In addition, salt and garlic are likely to have a pronounced influence on the microbial growth and the

rate of fermentation (Paludan-Müller et al., 1999; Paludan-Müller et al., 2002). However, some of LAB produce antagonistic substances, called bacteriocins, which able to against undesired microorganisms. The bacteriocins produced by LAB are considered to be Generally Recognized as Safe (GRAS) additives (Parada et al., 2007). They are useful to control the growth of undesirable bacteria in foods and accepted by consumer more than chemical preservative as antibiotics (Stoyanova et al., 2007). Hence, bacteriocins produced by LAB have attracted increasing attention. Therefore, Pla-Som is produced and widely consumed in area of Nong Khai province should be investigated to find potential LAB. Thus, this study aimed to isolate and determine antibacterial activity of LAB from Pla-Som collected from areas of Nong Khai province, northeast region of Thailand. Then these LAB will be used to develop Pla-Som with greater quality and safety products.

2. Research Methodology

2.1 Collection of samples

Ten samples of Pla-Som were collected randomly from local markets in the area of amphur Muang, amphur Phonphisai and amphur Rattanawapi, Nong Khai province. The sample codes were assigned with L1 to L10. They were stored at 4°C until analysis.

2.2 Isolation of lactic acid bacteria (LAB)

LAB were isolated from Pla-Som according to Sornlang et al. (2011) with slight modification. Pla-Som samples were divided into 10 g and mixed with 90 ml of 0.85% normal saline and homogenized using a blender. Serial tenfold dilution from the homogenate were prepared and 0.1 ml appropriate dilutions were spread on the MRS (de Man, Rogosa and Sharpe) agar with 0.004% (w/w) bromocresol green and incubated at 37°C for 24 hrs. Colonies with a yellow clear zone around them were picked using tooth pick and inoculated in MRS agar slants. The LAB isolates were purified by re-streaking on MRS agar plates until only a single type of colony was presented. Working cultures were kept on MRS agar plates at 4°C and sub-cultured every 4 weeks.

2.3 Morphological and biochemical tests

The LAB isolated colonies were determined for Gram staining, cell morphology and catalase test with 3% H_2O_2. Gas production was detected via Durham tube (Østergaard et al., 1998).

2.4 NaCl tolerance test

For the detection of NaCl tolerance of LAB, Individual LAB was cultured in MRS broth with different concentration of NaCl (0, 2.5, 5 and 7 %w/v). After sterilization, each test tube was inoculated with 10% (v/v) fresh overnight culture of LAB and incubated at 37°C for 24 hrs. After incubation their growths were

determined by observing culture medium turbidity.

2.5 Determination of acid content and pH value

The acid content and pH value of sample were determined according to Jittrepotch et al. (2015). Ten percent (w/v) of LAB cultured in MRS overnight was inoculated into MRS broth with different concentration of NaCl (0, 2.5, 5 and 7 %w/v) and incubated at 37°C for 72 hrs. Supernatant of LAB samples were collected at 24, 48 and 72 hrs. The acid content was performed through titration with 0.8 N NaOH using phenolphthalein as indicator. The total acidity was calculated as lactic acid and expressed as percentage (w/v). The pH of each LAB supernatant was recorded using a digital pH meter.

2.6 Screening for antimicrobial activity

The disc diffusion method was used to determine the antimicrobial activity of the LAB isolates as described by Bauer et al. (1966). The eleven pure cultures of spoilage and pathogenic bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium, Bacillus cereus*, *Esherichia coli*, *Serratia marcescens*, *Salmonella* Typhi, *Shigella* sp., *Pseudomonas* sp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were inoculated on Nutrient Agar (NA) and transferred to Nutrient Broth (NB). After 24 hrs, a sterile cotton swab was dipped in each culture suspension and swabbed on MRS plate. Then plates were allowed for excess surface moisture to be absorbed. The sterile paper blank discs were placed on the agar plate. Each disc was added with culture free supernatant that was obtained by centrifugation from 3 days and 5 days incubation of the LAB isolates. After incubation at 37°C for 24 hrs, antimicrobial activity was recorded as inhibition zone diameters (mm) around the paper disc.

3. Results

3.1 Isolation and characterization

A total of 37 LAB isolates were isolated from Pla-Som with different fish materials collected from different local markets. All of the LAB isolates changed a color from green to yellow on MRS agar with 0.004% bromocresol green, catalase-negative and Gram positive. These were 8 cocci and 29 rods in shape. Among these 37 isolates were non-producing gas homofermentative LAB and 4 isolates were producing gas heterofermentative. For sodium chloride tolerance, all of the LAB isolates were able to grow in MRS broth with sodium chloride concentration lower than 2.5% (w/v). Whereas there were 34 and 7 isolates able to grow at 5% and 7% (w/v), respectively. Characteristics of 37 LAB isolates are shown in table1.

Table 1 Characteristics of 37 LAB isolates from fermented fish product, Pla-Som

No.	isolates no.	Cell shape	Gas production	Concentration of NaCl (%w/v)			
				0.0	2.5	5.0	7.0
1	L1-3	cocci	No	+	+	+	-
2	L1-15	cocci	No	+	+	-	-
3	L1-24	cocci	No	+	+	-	-
4	L2-5	rod	No	+	+	+	+
5	L3-3	rod	No	+	+	+	-
6	L3-6	rod	No	+	+	+	-
7	L3-8	rod	No	+	+	+	-
8	L3-10	rod	No	+	+	+	-
9	L3-12	rod	No	+	+	+	-
10	L3-21	rod	No	+	+	+	-
11	L4-6	rod	No	+	+	+	+
12	L4-13	rod	No	+	+	+	-
13	L5-4	rod	No	+	+	+	-
14	L5-6	rod	No	+	+	+	-
15	L6-4	cocci	No	+	+	+	-
16	L6-7	cocci	Yes	+	+	-	-
17	L6-8	rod	No	+	+	+	-
18	L7-1	rod	No	+	+	+	-
19	L8-2	cocci	No	+	+	+	-
20	L8-3	cocci	No	+	+	+	-
21	L8-4	rod	No	+	+	+	-
22	L8-5	rod	No	+	+	+	-
23	L8-7	rod	No	+	+	+	-
24	L8-9	rod	No	+	+	+	-
25	L8-10	rod	No	+	+	+	-
26	L8-14	rod	No	+	+	+	+
27	L8-16	rod	No	+	+	+	-
28	L8-17	rod	No	+	+	+	-
29	L8-20	rod	No	+	+	+	-
30	L8-23	rod	No	+	+	+	-
31	L8-24	rod	No	+	+	+	+
32	L9-13	cocci	No	+	+	+	-
33	L10-4	rod	Yes	+	+	+	-
34	L10-8	rod	Yes	+	+	+	-

Table 1 Characteristics of 37 LAB isolates from fermented fish product, Pla-Som (continue)

No.	isolates no.	Cell shape	Gas production	Concentration of NaCl (%w/v)			
				0.0	2.5	5.0	7.0
35	L10-11	rod	No	+	+	+	+
36	L10-12	rod	No	+	+	+	+
37	L10-20	rod	Yes	+	+	+	+

Legend: (+) growth, (-) no growth

3.2 Acid content and pH value

The organic acids produced by LAB isolates in MRS broth were detected by titrimetric methods as total acidity equivalent to lactic acid. The result revealed that organic acid content were in the range of 0.72-0.60 %w/v and pH were 4.2-4.0, respectively.

3.3 Antimicrobial activity

All of LAB isolates were screened for antimicrobial activity against pathogenic bacteria using disc diffusion assay. The LAB supernatants were tested with 4 Gram-positive bacteria and 7 Gram-negative bacteria. The antimicrobial activity of the LAB isolates is shown in table 2. The results revealed that the LAB isolates were able to inhibit Gram-negative bacteria more than Gram positive bacteria. The inhibitory activity varied among the LAB isolates as shown in Figure 1. The degree of inhibition was designated as the largest diameter zones (13-15 mm), the medium diameter zones (11-12 mm), the smallest diameter zones (7-10 mm) and no inhibition. Most of LAB supernatants showed weak and moderate inhibition against Gram positive and Gram negative bacteria. There were 15 LAB supernatants that showed strongly inhibition against Gram negative bacteria. There was only L8-14 LAB supernatant showed strongly inhibition against Gram positive bacteria, *B.cereus.*

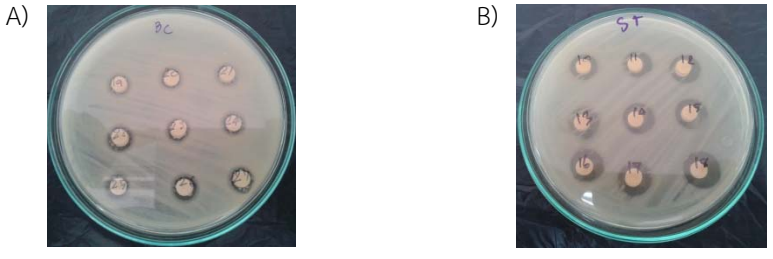

Figure 1 Inhibition zone of the LAB isolates against some Gram positive and Gram negative bacteria using the disc diffusion assay. A) *B. cereus* B) *S.* Typhi

Table 2 Antimicrobial activity of the LAB isolates in terms of inhibition zone using the disc diffusion assay

No.	LAB no.	Inhibition zone (mm)*										
		Gram-positive				Gram-negative						
		S. aureus	*B. subtilis*	*B. megaterium*	*B. cereus*	*E. coli*	*S. marcescens*	*S.* Typhi	*Shigella sp.*	*Pseudomonas sp.*	*P. aeruginosa*	*K. pneumoniae*
1	L1-3	+	+	-	+	+	++	+	+	++	+	+
2	L1-15	+	+	-	+	+	+	+	+	+	++	-
3	L1-24	+	+	-	+	-	+	+	+	+	++	-
4	L2-5	+	+	-	+	-	++	+	+	++	++	-
5	L3-3	+	+	-	-	-	++	-	+	+	++	-
6	L3-6	+	++	++	+	-	+	-	+	-	++	-
7	L3-8	+	+	+	+	-	+	+	++	++	++	-
8	L3-10	+	+	+	+	-	+	-	++	-	++	+
9	L3-12	++	+	+	+	-	+	-	+	+	++	+
10	L3-21	-	+	++	+	-	+	+	++	++	++	+
11	L4-6	++	+	+	+	-	+	+	+++	+	+	+
12	L4-13	++	+	+	+	-	+	++	++	+	+	-
13	L5-4	+	+	+	-	-	+	+	+++	+	+	-
14	L5-6	+	+	+	-	-	+	++	++	+	+	-
15	L6-4	+	+	+	+	-	+	++	++	+	+++	+
16	L6-7	+	+	++	-	-	++	++	++	-	+++	+

Table 2 Antimicrobial activity of the LAB isolates in terms of inhibition zone using the disc diffusion assay (continue)

No.	LAB no.	Inhibition zone (mm)*										
		Gram-positive				Gram-negative						
		S. aureus	*B. subtilis*	*B. megaterium*	*B. cereus*	*E. coli*	*S. marcescens*	*S.* Typhi	*Shigella sp.*	*Pseudomonas sp.*	*P. aeruginosa*	*K. pneumoniae*
17	L6-8	++	+	+	+	-	++	+	+	++	++	++
18	L7-1	++	++	+	+	++	+	+++	+	+	+++	++
19	L8-2	+	+	+	+	-	+	++	-	-	+++	++
20	L8-3	+	+	+	+	-	+	++	-	-	+++	+
21	L8-4	+	-	-	+	-	+	++	++	+	++	+
22	L8-5	+	-	-	+	-	+	++	+	++	++	+
23	L8-7	+	-	-	+	-	+	++	+	++	+	+
24	L8-9	+	-	-	+	-	+	++	++	++	+	+
25	L8-10	+	-	-	++	-	+	++	+	+	+++	+
26	L8-14	++	+	+	+++	+	+	++	++	++	++	+
27	L8-16	+	+	++	++	++	+	++	+++	+++	+++	+
28	L8-17	++	+	+	+	+	+	++	++	+	++	+
29	L8-20	++	+	+	+	-	+	+++	++	-	+	+
30	L8-23	+	++	+	+	-	+	+++	++	+++	+	+
31	L8-24	+	++	+	+	-	+	+	++	-	+++	-
32	L9-13	+	+	++	-	-	+	+	++	-	-	+

Table 2 Antimicrobial activity of the LAB isolates in terms of inhibition zone using the disc diffusion assay (continue)

No.	LAB no.	Inhibition zone (mm)*										
		Gram-positive				Gram-negative						
		S. aureus	*B. subtilis*	*B. megaterium*	*B. cereus*	*E. coli*	*S. marcescens*	*S.* Typhi	*Shigella sp.*	*Pseudomonas sp.*	*P. aeruginosa*	*K. pneumoniae*
33	L10-4	+	++	++	+	-	+	+	++	+++	-	+
34	L10-8	+	+	++	+	-	+	+	+	-	-	+
35	L10-11	+	+	++	+	+	+	++	+	++	-	+
36	L10-12	+	+	+	+	-	+	++	++	+++	-	++
37	L10-20	-	+	+	-	-	+	+++	++	++	++	+

Legend: *The diameter of the inhibition zone, +,7.0-10 mm; ++,11-12 mm; +++,13-15 mm; (-) no inhibition zone

The isolate L8-16 showed strong inhibition activity against *Shigella* sp., *Pseudomonas* sp. and *P. aeruginosa* with 13-15 mm of inhibition zone and moderate inhibition against *B. megaterium*, *B. cereus*, *E. coli* and *S.* Typhi with 11-12 mm of inhibition zone. The isolate L7-1 showed strong inhibition activity against *S.* Typhi and *P. aeruginosa* and moderate inhibition activity against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae*. The isolate L8-23 showed strong inhibition activity against *S.* Typhi and *Pseudomonas* sp. and moderate inhibition activity against *B. subtilis* and *Shigella* sp. The culture supernatants of isolates, L6-4, L6-7, L8-2, L8-3, L8-10 and L8-24 showed strong inhibition activity against *P. aeruginosa*. Isolate L4-6 and L5-4 showed strong inhibition activity against *Shigella sp*. Isolate L10-4 and L10-12 showed strong inhibition activity against *Pseudomonas* sp. Isolate L10-20 showed strong inhibition activity against *S.* Typhi.

4. Discussion

This study attempted to isolate and determine antibacterial activity of LAB from 10 Pla-Som samples collected from local markets area in Nong Khai province. Thirty seven isolates were confirmed to be LAB by cell morphology, Gram staining and catalase negative reaction. Most of LAB isolates were rods, 29 isolates (78.4%) and 8 isolates (21.6%) were cocci. The finding of this study is related to the previous studies that reported LAB isolates from Pla-Som having rods 79-81% and cocci 19-21% of total LAB isolates (Kopermsub et al.2006; Hwanhlem et al. 2011). In addition, these data was in accordance with González et al. (2000) reported that found most of LAB isolates from freshwater fish were rod-shaped cells (95.2%) more than cocci cells (4.7%).

Most of LAB isolates were able to grow in MRS broth containing 5% (w/v) sodium chloride, but only seven isolates were able to grow at the highest concentration 7% (w/v) sodium chloride. Their sodium chloride tolerances are important to grow in fermented condition which salt was used to inhibit the growth of pathogenic and spoilage bacteria in the fermenting production (Paludan-Müller et al., 2002; Cai et al., 1997; Mohd Adnan et al., 2007). Moreover, Noordiana et al. (2013) reported that the LAB medium supplemented with NaCl significantly increased the antibacterial activity.

The homofermentative lactic acid bacteria were major group in Pla-Som that produced organic acids especially lactic acid. These acids give the sour taste and cause a decrease in pH value. In this study, the pH value and total acidity of all LAB isolates were not different from each other. But their inhibition activity against pathogenic bacteria was significantly different as presented in table 2. The LAB supernatant exhibited

inhibition activity in different level between weak to strong depend on the LAB isolates. There were 22 LAB isolates from Pla-Som which exhibited antibacterial effect against at least three pathogenic bacteria with moderate to strong inhibition activity. The isolate L8-16 had the broadest spectrum of inhibition as it inhibited the growth of 7 pathogenic bacteria with a range of moderate to strong inhibition zone diameters. L7-1 and L8-14 also had broad spectrum of inhibition as they inhibited the growth of 6 pathogenic bacteria. Several researchers reported that LAB produced variety of antimicrobial substances against both Gram positive and Gram negative bacteria (Moreno et al., 2002; Mallesha et al., 2010). However, antimicrobial activity results, it was found that *P. aeruginosa* is most sensitive to LAB isolate supernatants followed by *S.* Typhi and *Shigella* sp. with strong inhibition. The pathogenic bacteria such as *S. aureus, B. subtilis*, *B. megaterium* and *B. cereus* were inhibited by several LAB supernatants with moderate inhibition. These results indicated that most of LAB supernatants were able to inhibit Gram negative bacteria more than Gram positive bacteria. This finding is contrary to previous studies which reported that bacteriocins extracted from LAB could inhibit Gram positive more than Gram negative bacteria (Moreno et al., 2002; Savadogo et al., 2004). Because of the outer membranes of Gram positive bacteria have lipoteichoic acid that could be interacted with bacteriocins (Savadogo et al., 2004; Wang G., 2000). But the Gram negative bacteria have outer membrane which covers the cytoplasmic membrane and peptidoglycan layer. This present study, LAB supernatants were not neutralized to pH 7.0 before antibacterial activity test. Therefore, it suggested the low pH value, organic acid and some inhibiting substances or bacteriocins from the LAB isolates may effect to Gram negative bacteria. The presence of inhibition is probably due to organic acids that effect on the permeability barrier properties of Gram negative bacteria outer membrane and cause of outer membrane injury (Alakomi et al, 2000). It may lead to penetration of inhibiting substances into cell membranes that result in membrane leakage. The Gram positive bacteria namely, *S. aureus, B. subtilis, B. megaterium* and *B. cereus* showed less sensitive to inhibiting substances from LAB supernatants compared with Gram negative bacteria. The reason for this observed may due to influence of environmental pH that result in inhibition of electrostatics interactions between positive charges on the bacteriocins and the negative charges of phospholipid molecules. It could reduce the inhibition activity against Gram positive.

5. Conclusion

The most of LAB isolated from Pla-Som collected in area of Nong Khai province have antimicrobial activity. Their antimicrobial activity demonstrates the production of bioactive substances that could be used to inhibit growth of spoilage and pathogenic bacteria contaminated in fermented fish products. The results provided desirable LAB isolates such as L7-1, L8-14, L8-16, L8-23 and L8-24. These isolates had good antimicrobial activity and high tolerance to sodium chloride. They can be used as starters in order to improve Pla-Som quality and safe to consume.

Acknowledgments

Author would like to thank Dr.Wichai Soemphol for helpful suggestions in this research and thank Miss Konkanok Chaisen for technical supports. This work was provided all facilities by Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus.

References

Adnan, A.F.M. and Tan, I.K.P. (2007). Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresour. Technol. 98: 1380-1385.

Alakomi, H.L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K. and Helander, I.M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl. Environ. Microbiol. 66(5): 2001-2005.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. J. Clin. Path. 45(4): 493-496.

Cai, Y., Ohmomo, S., Ogawa, M. and Kumai, S. (1997). Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage. J. Appl. Microbiol. 83: 307-313.

Chadong, K., Yunchalard, S. and Piyatheerawong, W. (2015) Physicochemical characteriatics and protein degradation during fermentation of Plaa-som, A traditional fermented fish product of North-Eastern Thailand. Indian. J. Tradit. Knowle. 14(2): 221-225.

González, C.J., Encinas, J.P., García-López, M. L.and Otero, A. (2000). Characterization and identification of lactic acid bacteria from freshwater fishes. Food Microbiol. 17: 383-391.

Hwanhlem, N., Buradaleng, S., Wattanachant, S. and Benjakul, S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. Food Control. 22: 401-407.

Jittrepotch, N., Rojsuntornkitti, K. and Kongbangkerd, T. (2015). Physico-chemical and sensory properties of Plaa-som, a Thai fermented fish product prepared by using low sodium chloride substitutes. Int. Food Res. J. 22(2): 721-730.

Kopermsub, P., Vichitphan, S. and Yunchalard, S. (2006). Lactic acid bacteria isolated from Plaa-som, a Thai fermented fish product. Thai J. Biotechnol. 7(1): 32-39.

Kophermsub, P. and Yunchalard, S. (2010). Identification of lactic acid bacteria associated with production of plaa-som, a traditional fermented fish product of Thailand. Int. J. Food Microbiol. 138: 200-204.

Moreno, M.R.F., Leisner, J.J., Tee, L.K., Ley, C., Radu, S., Rusul, G., Yancanneyt, M. and Vuyst, L. D. (2002). Microbial analysis of Malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. J. Appl. Microbiol. 92: 147-157.

Noordiana, N., Fatimah, A.B. and Mun, A.S. (2013). Antibacterial agents produced by lactic acid bacteria isolated from threadfin salamon and grass shrimp. Int. Food Res. J. 20(1): 117-124.

Østergaard, A., Embarek, P.K.B., Wedell-Neergaard, C., Huss, H.H. and Gram, L. (1998). Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products. Food Microbiol. 15: 223-233.

Paludan-Müller, C., Huss, H.H. and Gram, L. (1999). Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. Int. J. Food Microbiol. 46: 219-229.

Paludan-Müller, C., Madsen, M., Sophanodora, P., Gram, L. and Møller, P.L. (2002). Fermentation and microflora of plaa-som, a Thai fermented fish product prepared with different salt concentrations. Int. J. Food Microbiol. 73: 61-70.

Parada, J.L., Caron, C.R., Medeiros, A.B.P. and Soccol, C.R. (2007). Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Braz. Arch. Biol. technol. 50(3): 521-542.

Savadogo, A., Ouattara, C.A.T., Bassole, I.H.N., and Traore, A.S. (2004). Antimicrial activities of lactic acid bacterial strains isolated from Burkina Faso fermented milk. Pak. J. Nutr. 3(3): 174-179.

Shylaja, M.R., Selvakumar, D. and Jagannath, J.H. (2010). Isolation and identification of lactic acid bacteria from raw and fermented products and their antibacterial activity. Rec. Res. Sci. Tech. 2(6): 42-46.

Sornplang, P., Leelavatcharamas, V., Sukon, P. and Yowarach, S. (2011). Antibiotic resistance of lactic acid bacteria isolated from a fermented fish product, Pla-chom. Res. J. Microbiol. 6(12): 898-903.

Stoyanova, L.C., Egorov, N.S., Fedoroba, G.B., Katrukha, G.S. and Netrusov, A.I. (2007). Comparison of the properties of bacteriocins formed by *Lactococcus lactis* subsp. *lactis* strains of diverse origin. Appl. Biochem. Microbiol. 43(6): 604-610.

Wang, G. (2010). Antimicrobial peptides: Discovery, Design and Novel Therapeutic Strategies. CABI. pp. 116-119.

❑❑❑❑❑