Characterization and Lipolytic Activity of *Staphylococcus* Strains Isolated from Thai Fermented Fish Products

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ABSTRACT

Twelve tetracocci isolated from Thai fermented fish products (pla-ra, kung-chom and nam-pla) were screened for their lipolytic activity on agar plates. Most of them exhibited lipolytic activity on the medium supplemented with 1% (v/v) of tributyrin, Tween 20, Tween 40, Tween 60 or Tween 80. The isolates exhibited lipolytic activity ranging from 0.042±0.032 to 9.548±0.969 Units·mL^{-1} and from 0.111±0.023 to 5.939±0.119 Units·mL^{-1} when cultivated in broth supplemented with 1% (v/v) Tween 20 and Tween 80, respectively. In the media containing 1% (v/v) coconut oil, lard or palm oil, the lypolytic activity was 0.042±0.032 to 9.548±0.969 Units·mL^{-1}, 0.548±0.009 to 6.00±0.136 Units·mL^{-1} and 0.83 ±0.020 to 5.25±0.030 Units·mL^{-1}, respectively, when incubated at 37 °C for 24 h. The isolates were identified as members of the genus *Staphylococcus* based on their phenotypic characteristics and 16S rRNA gene sequences. They were closely related to *S. nepalensis* NCTC 13834^T, *S. condimenti* DSM 11674^T, *S. simulans* ATCC 27848^T, *S. hominis* subsp. *novobiosepticus* GTC 1228^T, *S. edaphicus* P5085^T, *S. saprophyticus* subsp. *saprophyticus* ATCC 15305^T and *S. lloydii* ATCC 43959^T, with 99.78-100 % similarity. Isolate SPJ-1 from kung-chom, identified as *S. condimenti*, exhibited the highest lipolytic activity (6.287±0.159, 5.939±0.119 and 5.996±0.136 Units·mL^{-1}) when cultivated in Tween 20, Tween 80 and lard.

Keywords: Bacteria, Fermented fish, Fish sauce, Lipolytic activity, *Staphylococcus*

INTRODUCTION

Lipases are enzymes that catalyze the hydrolysis of fats and oils, releasing free fatty acids (FFAs). They are extensively distributed in plants, animals and microorganisms. Microbial lipases are commercially significant in the food industry and are more widely available than plant or animal lipases. Numerous microorganisms in the genera *Acinetobacter, Bacillus, Burkholderia, Chromobacterium, Enterococcus, Geobacillus, Pseudomonas, Staphylococcus* and *Yersinia* have been reported to produce lipases (Jaeger *et al*., 1994; Hasan *et al*., 2006; Sharma *et al*., 2011; Javed *et al*., 2018).

Staphylococci are widely distributed on the skin and mucous membranes of humans and animals, as well as in soil, water, air, and various foods (Heo *et al*., 2020). Coagulase negative staphylococci (CNS) have been found in seafood, fermented fish and soybean with high salt concentrations

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Received 21 February 2022 / Accepted 6 July 2022

(Tanasupawat *et al*., 1991; 1992; Mauriello *et al*., 2004; Guan *et al*., 2011; Jeong *et al*., 2014). They contribute to the color, aroma and taste of fermented foods (Hugas and Monfort, 1997; Leroy *et al*., 2006; Chauhan *et al*., 2008; Irlinger, 2008; Schleifer and Bell, 2009). Lipases from staphylococci have been applied in the production of flavor esters, detergent formulations, synthesis of biodiesel, dairy products, paper products, cosmetics and pharmaceuticals as well as in the preparation of lipolytic starter cultures for fermented foods such as sausages, ham and fermented meat. In addition, lipases have been used as food additives for modifying flavor by the synthesis of esters of short chain fatty acids (SCFAs), namely acetate (C2), propionate (C3) and butyrate (C4), which are known as flavor and fragrance compounds (Jessen, 1995; Talon and Montel, 1997; Javed *et al*., 2018).

Fermented fish products are commonly consumed in Southeast Asian countries (Chaves-López *et al*., 2014, Giyatmi and Irianto, 2017). In Thailand, there is a wide variety of fermented fish products. Pla-ra (fermented fish) is made from combining freshwater fish with salt (11.5-23.9% NaCl) and roasted rice, and fermenting the mixture for 6-10 months. Kung-chom (fermented shrimp) is made from small shrimp, ground roasted rice, salt (3.2-9.4% NaCl) and garlic, and fermented for 3-5 days. Finally, nam-pla (fish sauce) is made from brackish water, marine, or freshwater fish mixed with salt (22.8-26.2% NaCl) and fermented for 5-18 months (Phithakpol and Kasetsat, 1995).

Many halophilic bacteria present in fermented fish products have been previously isolated, such as *Gracilibacillus thailandensis* and *Piscibacillus salipiscarius* from pla-ra, and *Lentibacillus juripiscarius* from nam-pla (Yiamsombut *et al*., 2022). In addition, several lactic acid bacteria (*Tetragenococcus halophilus, Lactobacillus pentosus, Lb. plantarum* and *Lb. farciminis*) are sometimes present in some low-salt products such as kung-chom (Tanasupawat and Visessanguan, 2014). *Tetragenococcus halophilus* strains were found to utilize fish proteins, and some strains increased oligopeptide content of the finished product (Udomsil *et al*., 2010). During fish fermentation, various volatile compounds are formed that are believed to be responsible for the distinct aroma of fish sauce (Fukami *et al*., 2002). The fermentation of fish with *Staphylococcus* sp. SK1-1-5 showed lipase activity that appeared to yield higher levels of volatile fatty acids (Yongsawatdigul *et al*., 2007). Therefore, the screening of lipase-producing bacteria involved in producing fermented fish is an interesting topic for study. This research deals with the characterization of staphylococcal strains isolated from Thai fermented fish products based on their phenotypic characteristics and 16S rRNA gene sequences, including the determination of their lipolytic activity.

MATERIALS AND METHODS

Source of fermented fish and bacterial isolation

The strains used in this study were isolated from fermented fish products comprising three pla-ra (fermented fish) samples collected from Maha Sarakham Province, three kung-chom (fermented shrimp) samples collected from Surin Province, and five nam-pla (fish sauce) samples collected from Samut Prakan Province, Thailand (Table 1). Each sample was thoroughly mixed by stomacher for 2 min. Isolation of strains was carried out by spread plate technique. In brief, 1 g of the fermented fish sample was 10-fold serially diluted in 9 mL of sterile saline solution (0.85% NaCl). Then, a 0.1-mL volume of each diluted sample was transferred to Tryptic soy agar plate (TSA; Difco), immediately spread with a glass spreader, and then incubated at 37 °C for 24-48 h. After two days, colonies with distinct appearance were purified by streaking on TSA. Pure cultures were stored at -20 °C in nutrient broth (NB; Merck) containing 15% (v/v) glycerol and lyophilized. *Staphylococcus condimenti* $F2^T$ and F8 isolated from soy sauce mash (Tanasupawat *et al*., 1991; Probst *et al*., 1998) were used as reference strains in this study.

Identification of the isolates

Phenotypic characterization

The morphological and cultural characteristics of strains including cell morphology, Gram-staining and colonial appearance were investigated. Biochemical characteristics were assessed by tests for catalase, oxidase, coagulase, nitrate reduction, citrate utilization and hydrolysis of arginine, aesculin and urea as described by Barrow and Feltham (1993); the Methyl Red (MR) and Voges-Proskauer (VP) test were used to detect the production of acid and neutral end products, respectively. Physiological characteristics, namely growth in 6, 8 and 11% (w/v) NaCl, and growth at pH 3.5 and 9.0 were also determined (Barrow and Feltham, 1993). Acid production from carbohydrates was evaluated (Tanasupawat *et al*., 1998).

Analysis of 16S rRNA gene sequences

Genomic DNA of each strain was extracted using DNA extraction kit (Bio-Rad Laboratories, Inc.). Amplification of the 16S rRNA gene was done by polymerase chain reaction (PCR) using the universal primers 27F (5'-AGAGTTTGATC MTGGCTCAG-3') and 1492R (5'-TACGGYTA CCTTGTTACGACTT-3') (Lane, 1991). The PCR conditions were initial denaturation at 94 °C for 3 min, followed by 30 cycles of DNA denaturation at 94 °C for 1 min, primer annealing at 50 °C for 1 min, DNA extension at 72 °C for 2 min, and a final extension at 72 °C for 3 min. The PCR products were sequenced by Macrogen®, Korea. The obtained sequences were blasted against the EzBioCloud database at https://www.ezbiocloud.net/ (Yoon *et al*., 2017) to compare and identify species. The sequences were aligned using the BioEdit program (Thompson *et al*., 1997). A phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using the program MEGA version 6 (Tamura *et al*., 2013). The confidence values of individual branches were determined using the bootstrap analyses based on 1,000 replications (Felsenstein, 1985).

Screening of bacteria for lipolytic activity

Twelve staphylococci were evaluated for lipase production by cultivating them on lipolytic agar medium. The medium was composed of 0.5% (w/v) peptone, 0.3% (w/v) beef extract, 0.01% (w/v) $CaCl_2 \cdot 2H_2O$, 1.5% (w/v) agar and 1% (v/v) of each of five inducers: tributyrin, Tween 20, Tween 40, Tween 60 and Tween 80 (Barrow and Feltham, 1993). After incubation at 37 °C for 24-48 h, the strains showing an opaque zone were chosen for quantitative enzymatic assay. *Staphylococcus condimenti* F-2^T (= DSM 11674^T) and F-8 (= DSM 11675) isolated from soy sauce mash were used as reference strains.

Assay of lipase activity

The isolates were grown in nutrient broth at 37 °C for 24 h. Cell concentrations of cultures were adjusted using McFarland standard solution No. 0.5 to obtain 1.5×10^8 CFU·mL^{-1}. One percent inoculum of each isolate was transferred to 50 mL nutrient broth supplemented with 1% of either Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), coconut oil (caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and linoleic acid) (Boateng *et al*., 2016), palm oil (palmitic acid, oleic acid and linoleic acids) (Sambanthamurthi *et al*., 2000) or lard (palmitic acid, stearic acid, oleic acid and linoleic acid) (Rohman *et al*., 2012) as a substrate, and then incubated on a rotary shaker (180 rpm) for 24 h at 37 °C. The culture supernatant was collected by centrifugation at 10,000 rpm, 4 ºC for 10 min and used as crude enzyme for quantifying lipase activity.

Lipase activity was analyzed by colorimetric method using *p*-nitrophenyl butyrate (*p*-NPB) as a substrate. Fifty microliters of crude enzyme was added to the reaction mixture containing 135 µL of 0.4% Triton X, 0.1% gum arabic in 50 mM Tris-HCl buffer (pH 7), and 15 µL of 30 mg *p*-NPB in 10 mL of isopropyl alcohol. Then the mixture was incubated at 37 °C for 1 h. After incubation, lipase activity was measured at 405 nm in a microplate

reader. The enzymatic activity was calculated and expressed as units per mL (Units·mL^{-1}). One unit of lipase activity was defined as an enzyme releasing 1 µmol fatty acid per min under standard assay conditions (Boonmahome and Mongkolthanaruk, 2013; Phoottosavako *et al*., 2015).

RESULTS AND DISCUSSION

Isolation and identification of the isolates

Twelve tetracocci were isolated from three fermented fish products: pla-ra collected from the market in Maha Sarakham Province, kung-chom (fermented shrimp) from Surin Province and nam-pla (fish sauce) collected from a factory in Samut Prakan Province (Table 1). Cells of the isolates were spherical shaped and formed tetrads or clusters. Colonies were circular, low convex with entire margin, non-pigmented and white in color. They grew at temperatures of 30, 35, 37 and 40 °C; at pH 6.0 and 7.0; and in 0-3% NaCl. Tests for catalase and for nitrate reduction were positive, but tests for coagulase and oxidase were negative. The isolates showed variable characteristics for hydrolysis of urea, arginine and aesculin, for citrate utilization, and for methyl-red and acid production (Table 2).

On the basis of 16S rRNA gene analysis, the 12 isolates were found to be members of the genus *Staphylococcus* (Schleifer and Bell, 2009), as shown in Figure 1. The isolates JPR2-5 and JPR3-3 were closely related to *S. nepalensis* NCTC 13834^T (99.78-99.85 % similarity). Isolates JPR3-11, SKK-1, SPJ-1 and SKJ-1 were closely related to *S. condimenti* DSM 11674^T (99.78-99.85 % similarity). In addition, isolate JPR4-8 was closely related to *S. simulans* ATCC 27848^T (99.78 % similarity), SK554-1 was closely related to *S. hominis* subsp. *novobiosepticus* GTC 1228^T (99.86 % similarity), while isolates SKB200-7 and SP025 were closely related to *S. edaphicus* P5085^T (99.93-100.0 % similarity), isolate SPB40-5 was closely related to *S. saprophyticus* subsp. *saprophyticus* ATCC 15305^T (99.93 % similarity), and BF3-1 was closely related to *S. lloydii* ATCC 43959^T (100.0 % similarity) (Table 1 and Figure 1).

Table 1. Samples, collection location, isolate number, nearest species and 16S rRNA gene sequence similarity (%) of isolates from fermented fish products.

Sample	Province	Isolate number	Nearest species	% Similarity	Length (bp)	Accession number
Pla-ra	Maha Sarakham	JPR2-5	*S nepalensis* NCTC 13834^T	99.85	1340	LC511694
(fermented		JPR3-3	*S. nepalensis* NCTC 13834^T	99.78	1351	LC511695
fish)		JPR3-11	*S. condimenti* DSM 11674^T	99.85	1378	LC511696
		JPR4-8	*S. simulans* ATCC 27848^T	99.78	1376	LC511697
Kung-chom	Surin	SKK-1	*S. condimenti* DSM 11674^T	99.78	1381	LC511698
(fermented		SPJ-1	*S. condimenti* DSM 11674^T	99.85	1360	LC511699
shrimp)		SKJ-1	*S. condimenti* DSM 11674^T	99.85	1374	LC511700
Nam-pla	Samut Prakan	SK554-1	*S. hominis* subsp.	99.86	1392	LC511701
(fish sauce)			*novobiosepticus* GTC 1228^T			
		SKB200-7	*S. edaphicus* P5085^T	100.0	1359	LC511702
		SP025	*S. edaphicus* P5085^T	99.93	1353	LC511703
		SPB40-5	*S. saprophyticus* subsp.	99.93	1384	LC511705
			saprophyticus ATCC 15305^T			
		BF3-1	*S. lloydii* ATCC 43959^T	100.0	1344	LC511704

Table 2. Phenotypic characteristics of 12 isolates from fermented fish products.

Characteristics	Isolate number													
	JPR2-5	JPR3-3	JPR3-11	JPR4-8	SKK-1	SPJ-1	SKJ-1	SK554-1	SKB200-7	SP025	SPB40-5	BF3-1	F2	F8
Growth in 6% NaCl	+	+	+	+	-	-	-	-	-	-	+	-	+	+
8% NaCl	-	+	-	+	-	-	-	-	-	-	+	-	+	+
11% NaCl	-	+	-	-	-	-	-	-	-	-	-	-	+	+
Growth at pH 3.5	-	-	-	+	+	+	+	-	-	+	+	+	-	-
at pH 9	-	-	+	+	+	-	+	-	-	+	+	+	-	-
Arginine hydrolysis	+	+	+	+	+	+	+	-	+	-	+	-	+	+
Citrate utilization	+	-	-	-	+	-	-	-	-	-	+	+	-	-
Methyl red	+	-	+	+	+	+	+	-	+	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrolysis of														
Aesculin	+	+	-	-	-	+	-	-	-	-	-	-	-	-
Urea	+	-	+	-	+	-	+	-	+	-	-	-	+	+
Acid from:														
L-Arabinose	-	+	-	-	-	-	-	+	-	+	-	-	-	-
D-Fructose	-	+	+	-	+	+	+	+	+	+	+	+	+	+
D-Galactose	-	-	+	-	+	+	+	+	-	+	+	+	+	w
D-Glucose	-	+	+	-	+	+	+	+	+	-	+	+	+	+
Lactose	-	+	+	-	+	+	+	+	-	+	+	+	+	w
D-Maltose	-	-	-	-	-	-	-	+	-	+	-	+	-	-
D-Mannitol	-	+	-	-	-	-	+	+	+	+	+	-	+	+
Raffinose	-	-	-	-	-	-	-	+	-	+	-	-	-	-
Rhamnose	-	+	-	-	-	-	-	+	-	+	+	-	-	-
D-Ribose	-	+	+	-	-	+	+	+	-	+	+	-	-	-
Salicin	-	+	+	-	-	+	+	+	-	+	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	+	+	+	+	-	+	+
Sucrose	+	+	+	-	+	+	+	+	+	+	-	+	w	w
D-Trehalose	-	-	+	-	+	-	+	+	+	+	+	+	w	w
D-Xylose	-	-	-	-	-	+	+	+	-	+	-	-	-	+

Note: + = positive reaction; w = weak positive reaction; - = negative reaction

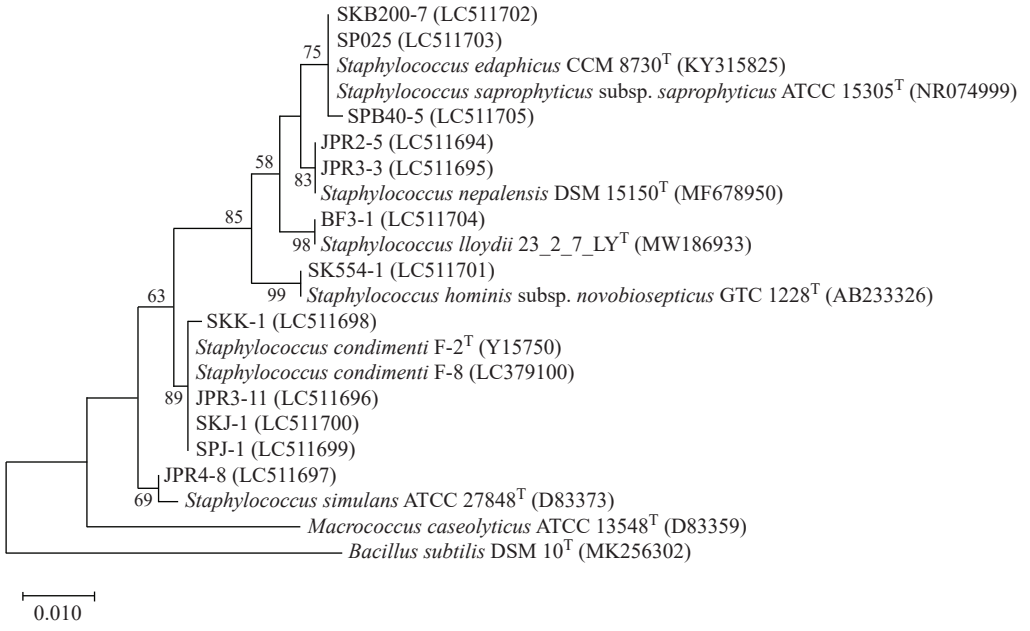

Figure 1. Phylogenetic tree of *Staphylococcus* strains based on 16S rRNA gene sequence. The numbers on the branches indicate percent bootstrap values from 1,000 replicates; only values >50 % are indicated. Bar = 0.01 substitutions per nucleotide position. *Bacillus subtilis* DSM 10^T was used as an outgroup.

Screening of isolates for lipolytic activity

Most of the isolates showed an opaque zone on 1% lipolytic medium with tributyrin, Tween 20, Tween 40 and Tween 80 after incubation for 24 h; however, some isolates exhibited no activity on tributyrin, Tween 40 or Tween 60, as shown in Table 3.

Assay of lipase activity

The isolates showed lipolytic activity in broth with Tween 20 and with Tween 80, ranging from 0.042±0.006 to 6.203±0.017 Units·mL^{-1} and from 0.111±0.023 to 5.939±0.119 Units·mL^{-1}, respectively (Figure 2). The activity of the isolates in broth with coconut oil, palm oil and lard ranged from 0.042±0.032 to 9.548±0.969 Units·mL^{-1}, from 0.83±0.020 to 5.25±0.030 Units·mL^{-1} and from 0.548±0.009 to 6.00±0.136 Units·mL^{-1}, respectively (Figure 3). The isolate SPJ-1, which was closely related to *S. condimenti*, showed lipolytic activity for Tween 20 (6.287±0.159 Units·mL^{-1}), Tween 80 (5.939±0.119 Units·mL^{-1}), palm oil (4.05±0.199 Units·mL^{-1}), coconut oil (7.111±0.119 Units·mL^{-1}) and lard (5.996±0.136 Units·mL^{-1}), respectively (Figures 2 and 3), while isolates JPR3-11 and SKK-1, also closely related to *S. condimenti*, similarly showed high activity in coconut oil (9.111±0.092 Units·mL^{-1} and 9.548±0.969 Units·mL^{-1}, respectively). Isolate JPR4-8, which was closely related to *S. simulans* ATCC 27848^T, showed high activity in coconut oil (6.801±0.311 Units·mL^{-1}). Isolate SK554-1, which was closely related to *S. hominis*, exhibited activity of 5.25±0.030 Units·mL^{-1} in palm oil (Figure 3). The reference strains *Staphylococcus condimenti* F-2^T (= DSM 11674^T) and F-8 (= DSM 11675) exhibited lipolytic activity ranging from 1.513±0.020 to 2.142±0.009 Units·mL^{-1} and from 0.467±0.111 to 1.291±0.009 Units·mL^{-1} when cultivated in broth supplemented with 1% (v/v) Tween 20 and Tween 80, respectively. The lipolytic activity of both strains in coconut oil, lard and palm oil were 2.287±0.077 to 2.674±0.070, 1.525±0.112 to 2.644±0.170 Units·mL^{-1} and 1.95±0.086 to 2.99±0.065 Units·mL^{-1}, respectively. Our results revealed that the isolates from fermented fish products exhibited higher lipolytic activity than either the F-2^T or F-8 strain (Hammes *et al*., 1995).

Table 3. Lipolytic activity on agar plates of isolates from fermented fish products.

Isolate number	Substrate				
	Tributyrin	Tween 20	Tween 40	Tween 60	Tween 80
JPR2-5	-	+	-	+	+
JPR3-3	-	+	-	-	++
JPR3-11	-	+	+	+	++
JPR4-8	+	++	-	+	+++
SKK-1	+	++	+	+	+++
SPJ-1	+	++	+	-	+++
SKJ-1	+	+	+	+	++
SK554-1	+	+	+	-	+
SKB200-7	+	+	+	-	+
SP025	+	+	+	-	+
SPB40-5	+	+	+	-	+
BF3-1	+	+	+	-	+

Note: +++ = strong activity; ++ = moderate activity; + = weak activity; - = no activity

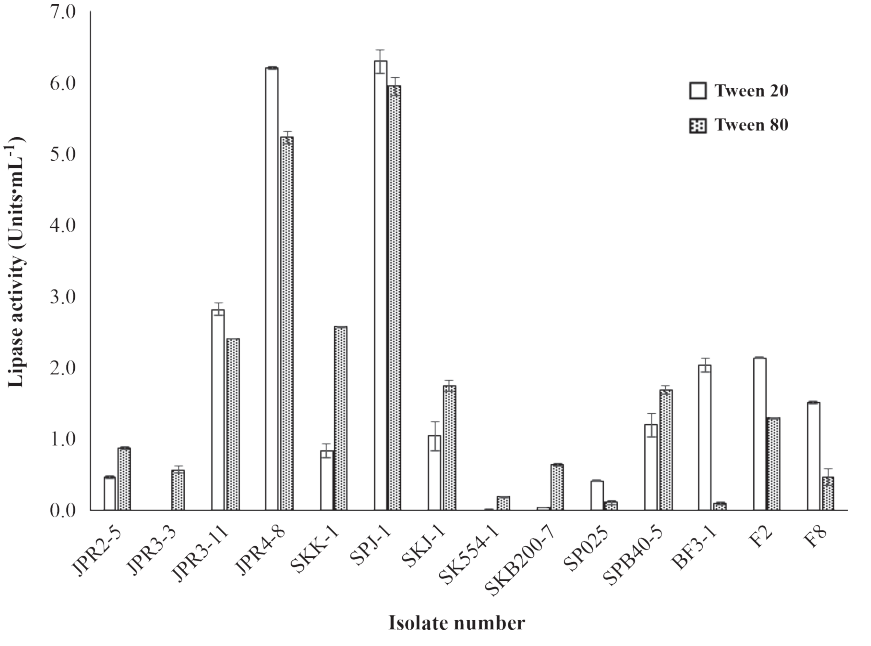

Figure 2. Lipase activity of isolates cultivated in nutrient broth supplemented with 1% (v/v) Tween 20 and Tween 80 at 37 ℃ for 24 h.

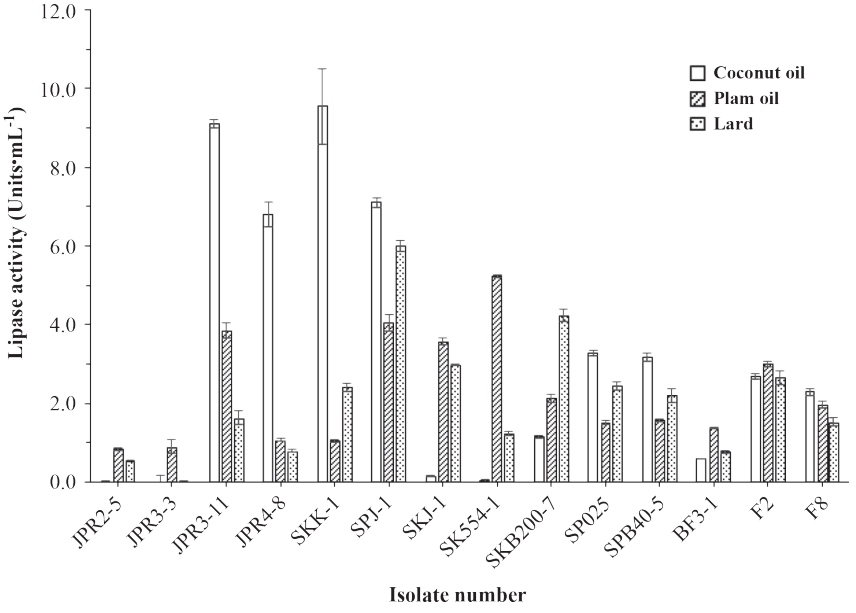

Figure 3. Lipase activity of isolates cultivated in nutrient broth supplemented with 1% (v/v) coconut oil, palm oil and lard at 37 °C for 24 h.

Isolate SPJ-1, which was closely related to *S. condimenti*, showed high lipolytic activity for Tween 20, Tween 80, coconut oil and lard (Figures 2 and 3), while JPR3-11 and SKK-1, also closely related to *S. condimenti*, showed high activity in coconut oil. However, both isolates showed higher lipolytic activity than *Staphylococcus condimenti* $F2^T$ or F8. These results were in accordance with Esakkiraj *et al*. (2010), who reported that vegetable and animal oils such as coconut oil, palm oil and cod liver oil positively increased lipase production by *S. epidermidis* CMST-Pi1. Moreover, Tween 20 and Tween 80 consist of fatty acid acyl esters, namely laurate and oleate (common fatty acids in vegetable and animal oils), respectively (Boekema *et al*., 2007). They were found to induce a lipase operon and stimulate growth of lipase-producing bacteria such as *Staphylococcus aureus*, *S. epidermidis* and *Burkholderia glumae* (Nielsen *et al*., 2016). The high lipase activity in Tween 80 is based on the esters of oleic acid (Rajendran *et al*., 2009). Microbial lipases that are regiospecific and fatty acid-specific could be exploited for retailoring vegetable oils. Inexpensive oils could also be upgraded to synthesize nutritionally important structured triacylglycerols like cocoa butter substitutes (Hasan *et al*., 2006). Given appropriate lipid and inducer sources, isolate SPJ-1 could give high lipase activity to many sources; thus, it should be further studied for optimization and applied in food and feed industries.

Staphylococcal lipases are extracellular, and their production is influenced by several nutritional and physical factors (Ebrahimpour *et al*., 2008). In fermented products, lipases from staphylococci are involved in the development of aromas from fat (Hammes *et al*., 1995). *Staphylococcus piscifermentans* and *S. condimenti* strains from fermented foods were reported to exhibit lipase activity (Hammes *et al*., 1995). *Staphylococcus xylosus* produces a mesophilic thermostable lipase (Talon and Montel, 1997) and is used in lipolytic starter cultures for fermented sausages and ham (Jessen, 1995), while *S. epidermidis* from spoiled frozen marine fish also exhibits lipase activity (Joseph *et al*., 2006).

Lipase is a versatile hydrolytic enzyme that is applied in the treatment of oil- and grease-containing wastewater, and in pre-treatment of solid waste/industrial wastewater for anaerobic treatment (Behera *et al*., 2019). Microbial lipases are applied in detergent, biodiesel, pharmaceutical and leather industries (Hasan *et al*., 2006). *Staphylococcus xylosus* produces a highly thermostable lipase which is acid- and alkaline-resistant, and remains active after 24 h in a broad range of pH (4-11) (Khoramnia *et al*., 2010). *Staphylococcus carnosus* produces more methyl-branched aldehydes, acids and corresponding esters from leucine, isoleucine and valine, while *S. xylosus* produces more 3-methyl-1-butanol (Søndergaard and Stahnke, 2002). *Staphylococcus warneri* M was also found to exhibit extracellular lipase activity (Yokoi *et al*., 2012). *Staphylococcus saprophyticus* strain showed an optimum of lipolytic activity at pH 6 and at 30 °C (Sakinç *et al*., 2007). *Staphylococcus simulans* strain secretes a non-induced lipase, tetrameric protein (Sayari *et al*., 2001). *Staphylococcus arlettae* JPBW-1 isolated from a rock salt mine produced a lipase that was active over a broad range of temperatures (30-90 °C), pH (7.0-12.0) and NaCl (0-20%) (Chauhan and Garlapati, 2013). *Staphylococcus hominis* MTCC 8980 was induced to yield a maximum lipase activity of 1.82 Units·mL^{-1} under optimized conditions (Behera *et al*., 2019).

CONCLUSION

In this study, isolates closely related to *Staphylococcus nepalensis* (two isolates), *S. condimenti* (one), and *S. simulans* (one) were found in pla-ra collected from Maha Sarakham Province. Three isolates closely related to *S. condimenti* were isolated from kung-chom collected from Surin Province. Isolates closely related to *S. edaphicus* (two), *S. hominis* (one), *S. saprophyticus* (one), and *S. lloydii* (one) were found in nam-pla collected from Samut Prakan Province. Isolate SPJ-1, which was closely related to *S. condimenti*, showed high lipolytic activity for Tween 20, Tween 80, palm oil, coconut oil and lard; isolates JPR3-11 and SKK-1 also showed high activity in coconut oil (9.111±0.092 Units·mL^{-1} and 9.548±0.969 Units·mL^{-1}, respectively). Isolate JPR4-8, which was closely related to *S. simulans* ATCC 27848^{T}, showed high activity in coconut oil (6.801±0.311 Units·mL^{-1}), while isolate SK554-1, which was closely related to *S. hominis*, exhibited activity of 5.25±0.030 Units·mL^{-1}. Lipases have been used in the environmental treatment of oil- and grease-containing wastewater, and hold considerable commercial interest for biotechnological applications. Our isolates showed properties that may be beneficial in such applications, and thus warrant further study for lipase characterization and production.

ACKNOWLEDGEMENTS

This study was supported by the Grant for International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund (CUGRP-61-01-33-01), Chulalongkorn University, as well as by Chulalongkorn University. We would like to thank the Pharmaceutical Research Instrument Centre, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing research facilities.

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