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Isolation of Endophytic Bacteria from Palm Oil Fruits and Characterization of Their Lipases

FANDY DJAFAR[1], TRESNAWATI PURWADARIA[2*], AND ARNOLD PARLINDUNGAN SINURAT[2]

[1]*Faculty of Biotechnology, Universitas Katolik Indonesia Atma Jaya, Jalan Jenderal Sudirman 51, Jakarta 12930, Indonesia*
[2]*Indonesian Research Institute for Animal Production, Departemen Pertanian, POBox 221, Bogor 16002, Indonesia*

Lipases (EC 3.1.1.3) can be produced in palm oil fruits by fruit cells or endophytic microbes. The purpose of this research is to isolate endophytic lipolytic bacteria of palm oil fruit and characterization their lipases. The bacteria were isolated and screened using medium Kouker and Jaeger containing olive oil, minerals, yeast extract, peptone, agar, and rhodamine B as an indicator. Fifteen endophytic bacteria were isolated and identified having the microbial lipase activity. Most of them showed rod shape, positively Gram test, spore formation, and motility except one bacteria strain K which was coccus. The enzyme was produced using submerged culture in the same medium but not containing agar and rhodamine B. Based on data of enzyme activity towards p-nitrophenyl palmitate as a substrate, protein concentration, and specific activity, two bacteria were selected, those were BSWt2(1) and Ink 1.3 isolates. Microscopic and biochemistry analyses show that BSWt2(1) and Ink 1.3 were identified as *Bacillus brevis* and *B. lacterosporus* respectively. Crude lipase from *B. brevis* BSWt2(1) and *B. lacterosporus* Ink 1.3 showed optimum activities at pH 8.0-9.0 and 60°C, and at pH 8.5 and 60-70°C. The enzymes were stable pre-incubated at pH 7.5-9.0 and pH 7.5-8.0 respectively, and they were stable pre-incubated for 2-4 hours at 80°C and for 2-8 hours at 100°C. Based on stability in high temperature, lipase from both isolates were specific and might be applicable for use in waste water treatment in palm oil factories.

Key words: palm oil-fruits, endophytic bacteria, lipase-activity

Lipase (*triacylglycerol acylhydrolase*, EC 3.1.1.3) is a carboxyesterase that hydrolyzes and synthesizes acylglycerol chains (Jaeger *et al.* 1999). The hydrolysis reaction degrades the triglyceride to diglyceride, monoglyceride, glycerol, and free fatty acid (John and Abraham 1991). The lipase often shows positional specificity attacking the glycerol side of the triglycerides, 1 or 3 carbon positioned of glycerol molecules are preferable. The hydrolysis of 2-fatty acid monoglyceride occurs after lipase attacks the glyceride at the 1 and /or the 3 position (Saxena *et al.* 1999) and the 2 acyl moiety migrates to the 1 or 3 position of the glycerol molecule. The hydrolysis reactions occur when lipase meet a substrate a high water content, while in organic solvent conditions trans- and inter-esterification reactions occur.

Lipase is produced by bacteria, fungi, animals, plants, and humans. Lipase in palm oil fruits can be produced by endogeneous microbes including endophytic bacteria. The association between the endophytic bacteria and palm oil may be pathogenic, commensal, or mutual symbiosis. The presence of endophytic bacteria in fruit palm oil tissue produce fatty acids and compounds for the germination process. The fatty acids are extracted in crude palm oil, thus reducing the oil quality (Strobel 2002). Therefore, endophytic bacteria of palm oil fruit may contain a novel bacterium that is useful for lipase production in the biotechnological industry (Strobel and Daisy 2003).

Many kinds of bacteria produce lipase like *Bacillus megaterium*, *B. stearotermophilus*, *Serratia marcescens*, *Aeromonas* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Geobacillus thermoleovorans*, and *Vibrio fischeri*. The enzymes have been well characterized such as their optimum pH and temperature, as well as their molecular weight (Li *et al*. 1995; Lin *et al.* 1996; Pencreac'h and Baratti 1996; Sharma *et al.* 2001; Snellman *et al.* 2002; Abdou 2003; Lee *et al.* 2003; Oh *et al.* 2003; Quintana-Castro *et al*. 2009; Ranjitha *et al*. 2009). Lipolytic molds were observed on *Aspergillus oryzae* and *Rhizopus oryzae*, while *Candida rugrosa* and *C. antartica* were lipolytic yeasts (Toida *et al.* 1995, Benjamin and Pandey 1996, Kose *et al.* 2002, Shiraga *et al.* 2005).

Lipase potentially hydrolyzes palm oil into flavouring compounds and could be used to produce biodiesel by inter-esterification reactions. In palm oil industries, lipase is well known as a biocatalyst in oil extraction and waste treatment (Wenten 2004). The addition of lipase will enhance the metabolisable energy of feed ingredients with high lipid content such as palm kernel meal, palm oil sludge, or crude palm oil. Lipase will be very useful for digesting oil pollutant in waste water management of palm oil or coconut oil factories. The application of lipase in feed and waste management system will be more effective if the microenvironment condition is suitable for the enzyme characteristic including the optimum pH and temperature, as well as pH and temperature stabilities. Therefore research was carried out to isolate and screen the lypolytic endophytic bacteria from palm oil fruits and to identify and to determine the characteristics of the enzymes produced by the bacteria.

MATERIALS AND METHODS

Isolation and Screening of Lipolytic Bacteria. Endophytic bacteria were isolated from palm oil fruits obtained from PT Agricinal, Bengkulu. The medium used for isolation and screening contains 2.5% olive oil (w/v); 0.2% glycerol (w/v); 0.002% rhodamine B (w/v), Merck; 1.5% peptone (w/v); 0.2% KH_2PO_4 (w/v); 0.01% $MgSO_4 \cdot 7H_2O$ (w/v); 0.5% NaCl (w/v); 0.01% biotin (w/v); 0.5% yeast

*Corresponding author, Phone: +62-251-8240752/3; Fax: +62-251-8240754; Email: tpurwadaria@yahoo.co.uk

extract (w/v); 0.2% beef extract (w/v); and 3.0% agar (w/v). The media were adjusted to pH 7.2 with NaOH 0.1 M (Kouker and Jaeger 1987). Isolation of endophytic bacteria was carried out according to Coombs and Franco (2003) using to sterilize the surface of palm oil fruits for 5 min. The sterilized fruits were peeled and the mesocarp was aseptically transferred to a mortar to which was added 0.85% NaCl. The extract (100 µL) of the crushed fruit was incorporated in 10 mL peptone water containing 1.0% peptone (w/v), 0.5% NaCl (w/v), a

Production of Lipase. The production medium composition was similar with the screen medium but not excluding rhodamine B and agar. One percent of inoculum was added to an aliquat of production medium and incubated at 37°C, 150 rpm for 48 h. After that, 0.2 % NaN_3 was added to the culture to eliminate the isolate. The cell free supernatant containing the crude extracellular enzyme was collected after centrifuging the culture at 12 000 x g, 4°C for 30 min. The crude enzyme was kept in 4°C refrigerator for further analyses.

Enzyme Assay and Protein Concentration. Lipase activity was determined by using p-nitrophenyl palmitate (pNPP) (Sigma) as a substrate (Snellman *et al.* 2002). The assay was carried out according to modification of Pencreac'h and Baratti (1996). Substrate was prepared by mixing 1 vol of freshly 16.5 mM pNPP in 2-propanol with 9 vol buffer pH 7.0 containing 0.4% triton X100. Incubation was carried out at 45°C for 30 min. Na_2CO_3 was replaced with 500 µL TCA 0.1 M to stop the reaction. One nKat of enzyme was defined as the amount of lipase which liberates 1 nanomol of p-Nitrophenol (pNP) per second under the given assay conditions. The activity of lipase was determined by subtracting the pNP concentration of the sample and the control. All assays were conducted in triplicate, the mixture of buffer and substrate was used as a blank. Specific activities (nKat mg^{-1}) were determined using the amount of enzyme protein (mg), as quantified by using coomasie blue solution with bovine serum albumin as a standard (Bradford 1976).

Statistical Analysis. Analysis of variance in lipase production was performed by completely randomized design for fifteen kinds of lipolytic bacteria and every treatment was repeated three times. Significant difference between treatments was carried out by Duncan analysis.

Identification of the Best Isolate. Based on three variables, i.e., lipase activity (nKat mL^{-1}), protein concentration (µg mL^{-1}), and specific activity (nKat mg^{-1}), two isolates were selected and identified. The identification was carried out in School of Life Science and Technology, Institut Teknologi Bandung.

Optimum pH and Temperature. The effect of pH difference on pNPP hydrolysis was measured over a pH range from 6.0 to 9.0 by using Mc. Ilvaine buffer 0.1 M (pH 6.0-7.5), K_2HPO_4 buffer 0.1 M (pH 7.5-8.0), and Tris-Cl buffer 0.1 M (pH 8.0-9.0) under standard enzyme assay conditions. The optimum temperature was determined by performing the standard assay at temperatures ranging from 40 to 80°C at optimum pH.

pH and Temperature Stability. The effect of pH on the stability of assayable lipase activity was determined by using the same buffers in the range of pH 6.5 to pH 9.0. After pre-incubation for 30 min at each pH at 25°C, the enzyme activity was determined under optimum assay conditions, at pH 8.0 and at 60°C and 70°C respectively for two selected isolates. Thermal stability was determined by assaying the lipase activity after pre-incubation of the enzyme at pH optimum at 80 and 100°C for 0, 2, 4, and 8 h. Following incubation, the enzyme activity was determined by assay under optimal pH and temperature.

RESULTS

All bacteria isolated from palm oil fruits were screened for lipolytic activity. Fifteen isolates grew well on screen medium at 37°C and showed good lipolytic activity, but they did not grow at 50°C (data were not shown). All isolates showed similar microscopic characteristics such as bacil, Gram positive, and spore forming, except that of K isolate was coccus, Gram positive and non-spore forming (Table 1). All bacils were motile, while the coccus was non-motile.

The fifteen isolates had highly significantly different activity ($P<0.0005$) (Table 2). BSWt 2(1), Ink 1.3, and Ink 1.10 isolates produced the highest assayable activity among the others (0.54, 0.48, 0.48 nKat mL^{-1} respectively). Isolate K produced the highest protein concentration 296 µg mL^{-1}. The highest specific activity was produced by 4.08 nKat mg^{-1}, however, it had significantly lower activity than those from

Table 1 Characterization of endophytic and lipolytic bacteria isolated from palm oil fruit

Isolates	Morphology	Gram staining	Spore	Motility test
BSWt 2(1)	Streptobacil	+	+	Motile
B.A	Streptobacil	+	+	Motile
K	Staphylococcus	+	-	Non-motile
Ink 1.a	Streptobacil	+	+	Motile
Ink 1.c	Bacil	+	+	Motile
Ink 1.3	Duplobacil	+	+	Motile
Ink 1.4	Bacil	+	+	Motile
Ink 1.5	Streptobacil	+	+	Motile
Ink 1.6	Bacil	+	+	Motile
Ink 1.9	Bacil	+	+	Motile
Ink 1.10	Duplobacil	+	+	Motile
Ink 1.11	Bacil	+	+	Motile
Ink 1.12	Streptobacil	+	+	Motile
Ink 2.2	Streptobacil	+	+	Motile
Ink 2.6	Streptobacil	+	+	Motile

Table 2 Activity, protein concentration, and specific activity of enzyme produced by bacterial isolates

Isolates	Activity (nKat mL^{-1})	Protein (μg mL^{-1})	Specific activity (nKat mg^{-1})
BSWt 2(1)	0.54^{a}	225^{abc}	2.44^{bcde}
B.A	0.17^{de}	142^{defg}	1.24^{ef}
K	0.29^{bc}	296^{a}	0.98^{ef}
Ink 1.a	0.16^{e}	104^{fghi}	1.57^{cdef}
Ink 1.c	0.23^{cde}	64^{hi}	4.08^{a}
Ink 1.3	0.48^{a}	173^{cdef}	3.11^{abc}
Ink 1.4	0.16^{ed}	228^{abc}	0.72^{f}
Ink 1.5	0.29^{bc}	188^{bcde}	1.57^{cdef}
Ink 1.6	0.17^{ed}	116^{efghi}	1.47^{cdef}
Ink 1.9	0.35^{b}	252^{ab}	1.50^{cdef}
Ink 1.10	0.48^{a}	198^{bcd}	2.93^{abcd}
Ink 1.11	0.26^{bcd}	199^{bcd}	1.34^{def}
Ink 1.12	0.20^{ed}	85^{ghi}	2.30^{bcdef}
Ink 2.2	0.16^{ed}	50^{i}	3.45^{ab}
Ink 2.6	0.16^{ed}	127^{defgh}	1.29^{def}

Different superscript characters in the same column shows highly significant difference ($P<0.0005$).

Table 3 Characterization of BSWt 2(1) and Ink 1.3 isolates*

Characteristic	Isolate	
	BSWt 2(1)	Ink 1.3
Macroscopic		
Colony edge	Undulate	Lobate
Colony opacity	Opaque	Opaque
Microscopic	Bacil	Bacil
Spore formation	Ellips and subterminal	Ellips and central
Motility	Motile	Motile
Bio-chemical test:		
Protease	+	+
Amylase	−	−
Lipase (Tributirin)	++	−+
Gelatinase	+	+
Nitrate reduction	+	+
Methyl red	+	+
Voges-Proskauer	−	−
Urease	−	−
Indole reaction	−	+
H_2S production	−	−
Catalase	+	+
Growth on citrate	−	−
Glucose fermentation	−+	+
Sucrose fermentation	+	+
Lactose fermentation	−	−
Triple s ugar iron agar	+	+
Conclusion	*Bacillus brevis*	*Bacillus laterosporus*

*Identification was held at Laboratory of Microbiology, School of Biological Science and Technology, Institut Teknologi Bandung. + was positive; −, negative; −+, weakly positive; ++, very strongly positive.

BSWt 2(1) and Ink 1.3. The crude enzymes of the two isolates were selected for feed application. High assayable activity of crude enzymes was more useful than their specific activity. Specific activity was more important, if the enzyme would be purified for enzyme characterization. BSWt 2(1) was chosen for further experiment for high assayable activity and protein concentration, but moderate specific activity, while Ink 1.3 was chosen for high assayable activity and specific activity, but moderate protein concentration. Identification on the macroscopic observation and biochemistry test for BSWt 2(1) and Ink 1.3. showed that both isolates were *Bacillus brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 (Table 3).

Enzyme produced by *B. brevis* BSWt 2(1) was most active at pH 8.0-9.0, while that of *B. laterosporus* Ink 1.3 at pH 8.5 (Fig 1). The measurement of enzyme activity at higher than pH 9.0 was discontinued, because pH higher than 9.0 (9.5) resulted saponification reaction in pNPP that increases the assayable activity. Optimum temperature at phosphate buffer pH 8.0 of lipase produced by *B. brevis* BSWt 2(1) was observed at 60°C, and the activity sharply decreased at 70°C. The optimum temperature for lipase activity produced by *B. laterosporus* Ink 1.3 at Tris-buffer pH 8.5 was at 60-70°C and the activity sharply decreased at 80°C.

The pH stability was observed by the relative activity of lipase pre-incubated for 30 min at different pH towards activity without incubation. The lipases produced by *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 were more

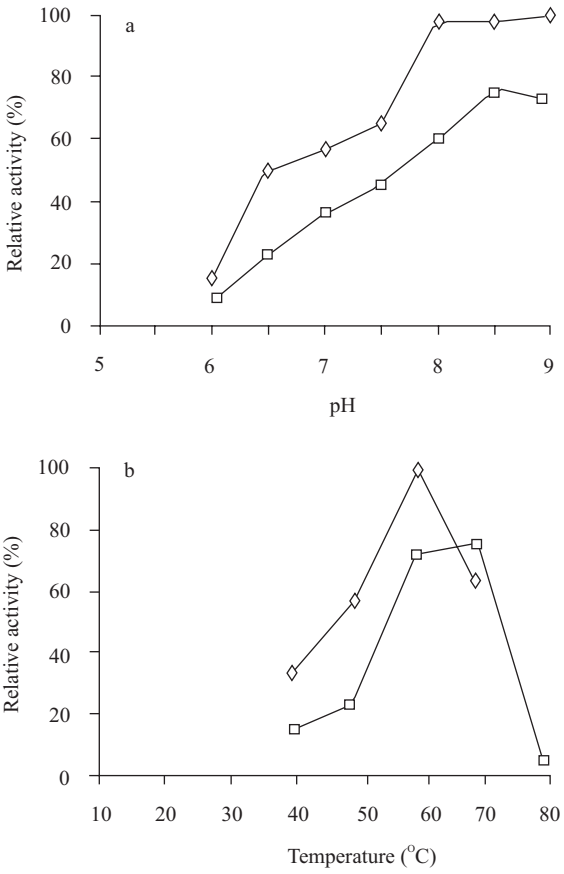

Fig 1 Optimum pH (a) and temperature (b) of lipases produced by *Bacillus brevis* BSWt 2(1) (◊) and *B. laterosporus* Ink 1.3 (□). Buffers used were Mc Ilvaine (pH 6.0-7.5), phosphate (pH 7.5-8.0), and Tris-Cl (pH 8.0-9.0).

Table 4 The effect of pH pre-incubation on lipase specific activity of *Bacillus brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3

Lipase	Kind of buffers	pH	Preincubation (min) 0 (nKat mg^{-1})	Preincubation (min) 30 (nKat mg^{-1})	Relative specific activity (times)*
B. brevis BSWt 2(1)	Mc. Ilvaine	6.5	0.99	0.66	0.66
		7.0	3.34	2.36	0.70
		7.5	4.99	4.74	0.95
	Phosphate	7.5	2.89	2.65	0.92
		8.0	4.25	3.94	0.93
	Tris-Cl	8.0	1.28	1.62	1.27
		8.5	2.26	2.55	1.13
		9.0	2.93	3.28	1.12
B. laterosporus Ink 1.3	Mc Ilvaine	6.5	1.13	0.86	0.76
		7.0	2.55	2.12	0.83
		7.5	5.33	4.00	0.75
	Phosphate	7.5	3.89	4.17	1.08
		8.0	4.43	4.80	1.08
	Tris-Cl	8.0	2.17	0.90	0.42
		8.5	2.16	0.89	0.41
		9.0	5.15	1.92	0.37

*The comparison of specific activity at 30 min towards 0 min pre-incubation time.

stable at pH 7.5-9.0 and at pH 7.5-8.0 respectively (Table 4). Although assayable temperature lipase from *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 were decreasing at 70 and 80°C respectively (Fig 1), temperature pre-incubation at 80°C for 4 h and 100°C for 8 h did not reduce assayable activity without pre-incubation at optimum pH and temperature (pH 8.0 and 60°C for *B. brevis* BSWt 2(1), and pH 8.5 and 70°C for *B. laterosporus* Ink 1.3). Higher pre-incubation temperature resulted higher assayable activity. Lipase produced by *B. brevis* BSWt 2(1) was more stable to high pre-incubation

DISCUSSION

Endophytic bacteria are known as the bio-prospecting microbes from many aspects (Strobel and Daisy 2003). The advantage of isolation endophytic bacteria from palm oil fruit was to collect isolate producing novel lipase activity. Lipase from these isolates can be used for many purposes like bioremediation process around palm oil plantation and as an animal feed additive.

A medium containing olive oil and fluorescent rhodamine B can be used to detect the microbial lipase. The lipase digests olive oil to produce free fatty acids that form complex molecules with rhodamine B (Kouker and Jaeger 1987; Olivia *et al*. 1998). The complex molecules were appeared as an orange fluorescence colony. Two days incubation time is the optimum time to screen highly

Table 5 The effect of temperature pre-incubation at 80 and 100°C on lipase specific activity of *Bacillus brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3

Lipase	Pre-incubation Temperature (°C)	Pre-incubation Time (h)	Specific activity (nKat mg^{-1})	Relative specific activity (times)*
B. brevis BSWt 2(1)	80	0	6.33	1.00
		2	6.49	1.03
		4	7.33	1.16
		8	5.69	0.90
	100	0	4.67	1.00
		2	5.60	1.20
		4	6.09	1.30
		8	5.73	1.23
B. laterosporus Ink 1.3	80	0	4.74	1.00
		2	6.30	1.33
		4	5.43	1.15
		8	3.93	0.83
	100	0	4.74	1.00
		2	7.92	1.67
		4	6.47	1.37
		8	5.66	1.20

*The comparison of specific activity at each pre-incubation time towards 0 h incubation.

lipolytic bacteria qualitatively that produce more obvious orange fluorescence around their colonies, while at one day incubation time, the low- and high-lipolytic bacteria showed a similar orange fluorescence. Fifteen endophytic bacteria, which produced lipase-like assayable activity have been isolated and purified. Fourteen isolates were bacils, and one isolate (K) was a staphylococcus (Table 1).

This result might indicate that the isolated bacteria may have a similar genus (*Bacillus*), or species but different strain. According to Carrim *et al.* (2006) lipolytic endophytic bacteria from the leaf and stem of *Jacaranda decurrens* includes *Corynebacterium aquaticum*, *C. renale*, *Pseudomonas stutzeri* and *Staphylococcus* sp. Lipase and endophytic lipolytic activity were detected in the mesocarp of palm fruit during the germination process (Strobel 2002), while the role of endophytic lipolytic bacteria in *J. decurrens* has not yet been discussed.

The hydrolytic activity of lipase can be measured by a specific substrate such as pNPP. This reaction was terminated by addition of TCA 0.1 M. Many workers used Na_2CO_3 to stop the reaction. However, the addition of Na_2CO_3 results in a saponification reaction that increases the yellow absorbance and which can give a faulty result. Palmitin was chosen from other substrates such as tributirin, stearin or olein, because the highest fatty acid composition in palm oil is palmitate at 43.0% (Sauvant *et al*. 2002). This reaction was also affected by high pH buffer, the saponification reaction between the substrate and buffer (without enzyme addition) occurred at pH ≥9.

Although the bacteria have similar morphology and Gram stain, their lipase activity was significantly different (Table 2). *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 which had been explained in the result were selected for further experiments. Other strain of *B. brevis* produced lipase activity was also reviewed by Sharma *et al*. (2001). The enzymes of our two microbes had different substrate specific activity. Enzyme produced by *B. brevis* BSWt 2(1)

showed more lipase activity in tributirin agar than that from *B. laterosporus* Ink 1.3 (Table 2), while palmitin (pNPP) substrate was more preferred by *B. laterosporus* Ink 1.3 enzyme. The specific activity of enzyme from *B. laterosporus* Ink 1.3 to hydrolize palmitin was higher than that *B. laterosporus* Ink 1.3 (Table 3). Lipases from different strains have certain substrate specificities have been found in *Bacillus* sp. BP-7 and *B. subtilis* BP-7 which have higher hydrolysis activity to methylumbeliferyl (MUF)-butirin than that to MUF-olein, while enzyme from *B. subtilis* prefers to hydrolize MUF-olein (Prim *et al*. 2003).

As mentioned in the result enzymes from *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 had optimum activity at pH 8.0-9.0 and 8.5, respectively (Fig 1). Enzyme from *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 were stable at pH 7.5-9.0 and pH 7.5-8.0 respectively (Table 4). Lipase from *P. pseudoalcaligenes* had an optimum activity at pH 8.0 and had a broad range pH stability from pH 6.0-10.0 (Lin *et al.* 1996). Enzyme from *Candida rugosa* had optimum activity at pH 7.0-8.0 (Benjamin and Pandey 2001). Generally like other enzymes from bacteria, our enzymes have optimum activity under alkaline conditions.

Bacillus brevis BSWt 2(1) and *B. laterosporus* Ink 1.3 lipases have optimum activity at temperatures of 60°C and 60-70°C respectively and can be called thermophilic (Fig 1). Oh *et al. (*2003) reported that the optimum lipase activity from *B. stearothermophilus* was at 60-65°C. This species grows well under thermophilic conditions, therefore it is very possible that its enzyme is also thermophilic. Benjamin and Pandey (2001) reported that the lipase optimum activity from *C. rugosa* (mesophilic yeast) was 35-40ºC, while lipase from *Aspergillus oryzae* (mesophilic mold) has optimal at 30ºC (Toida *et al.* 1995). In our result *B. brevis* BSWt 2(1) and *B. laterosporus* Ink 1.3 grew well at 37ºC (mesophilic) but they did not grow at 50ºC. However, their enzymes have optimum activity at high temperature and are thermophilic. The specificity of the enzymes will be useful for their application in the 60ºC environment.

The relative activity of enzymes from *B. brevis* BSWt2(1) and *B. laterosporus* Ink 1.3 was still high, although incubated at 80 and 100ºC (Table 5). The relative activity of both lipases showed a proportional increase with increased time at two hours incubation. After two hours incubation, relative activity of *B. laterosporus* Ink 1.3 was decreased, while that of *B. brevis* BSWt 2(1) was still increasing. Although enzymes of *B. brevis* BSWt2(1) and *B. laterosporus* Ink 1.3 have optimum temperature at 60ºC and 60-70ºC respectively, or the reduction of assayable activity occurrs at 80°C (Fig 1), pre-incubation at 80 and 100ºC did not reduce the relative activity. After pre-incubation at 80 and 100ºC, the enzymes were assayed at their optimum temperature, pH 8.0 and 60ºC for *B. brevis* BSWt 2(1) and pH 8.5 and 70ºC for *B. laterosporus* Ink 1.3. It is difficult to explain why at high temperature incubation (80 and 100ºC) the enzyme activity was stable, but at high temperature assay reaction the enzyme activities at 80ºC were lower than those at 60ºC. Possible explanation might be due to the active site conformation of enzyme molecules. High temperature pre-

The pasteurization or even sterilization processes before storage of foods will kill the bacteria, whereas heat resistant lipase previously produced by them will remain active (Anderson 1980). The activity of this lipase might change the quality in fatty foods during prolonged chilled or dehydrated storage. Prim *et al.* (2003) reported the characterization from other lipases. Lipase from *B. subtilis* has a good relative activity after fourty days incubation at 4, 20, 30, and 45ºC. The temperature stability incubations of *B. brevis* BSWt2(1) and *B. laterosporus* Ink 1.3 were not carried out at those low temperatures, since we intend to apply the enzyme as feed additive and for waste management around palm oil factories. However, incubation at high temperature already proves the stability of the enzyme. According to Liebeton *et al. (*2001), the stability of lipase was correlated with disulfide bonds. The disulfide bond stabilized the active site conformation of the enzyme to high temperature.

In bioremediation processes, both our lipases can be appropriately used to decrease the glyceride in palm oil waste. Based on the field observation at the palm oil factory, the conditions of the fresh waste from palm oil were 55-80ºC and pH 5.0-6.0. The gastroinstestinal pH from poultry is acid and the body temperature is 40ºC, and the temperature during the pelleting process is 70-85ºC. Based on the characterization results, both lipases had optimum activity at alkaline and thermophilic conditions so were not appropriate as feed supplement, however, pelleting temperature will not reduce the activity of these enzymes. The enzyme will be useful if the hydrolysis is carried out as pre-treatment in the optimum microenvironmental conditions. Thus, the effectiveness from this activity must be correlated with the amount of enzyme added. There was another aspect of lipase activity which was not measured in this experiment, that is the interesterification reaction. The interesterification process was correlated with the biodiesel process. Therefore, more research is suggested to explore the characteristics of both lipases.

Although the application of enzymes produced in this experiment has to be explored. Further results of this study showed that the isolation of endophytic lipolytic bacteria from the mesocarp of palm fruit is important. The isolated bacteria produced specific enzyme activity which is stable at high incubation temperatures.

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