Characterization of Partially Purified Extracellular Lipase Fractions from *Pseudomonas fragi* CRDA 037

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ABSTRACT: The purification of extracellular lipases from the culture medium of *Pseudomonas fragi* CRDA 037 was obtained by ammonium sulfate precipitation, followed by ion-exchange chromatography and then by size exclusion chromatography, and re-size exclusion chromatography, which resulted in two enzymatic fractions, FIVa′ and FIVb′. The fractions FIVa′ and FIVb′ had specific activities of 105.5 and 121.6 U/mg, respectively, with purification folds of 169.3 and 195.2, respectively, using triacetin as a substrate. The two purified fractions showed optimal activities at pH 9.5 and 10.0, respectively, at 80°C. Three bands were found in fraction FIVa′ and two bands in fraction FIVb′ by native polyacrylamide gel electrophoresis; these results indicated that homogeneity of the purified fractions was not achieved. The enzyme efficiency values, calculated as the ratio of V_{max} to K_m value for fractions FIVa′ and FIVb′, were 72.16×10^{-2} and 38.15×10^{-2}, respectively. The lipase activity of fraction FIVa′ was more specific for the hydrolysis of fatty acid esters with fatty acid chain lengths of C12 to C18, whereas that of fraction FIVb′ showed a relatively broader range of specificity. The lipase activity of fraction FIVa′ showed higher specificity toward triacetin, tristearin, and tripalmitin as the substrate, whereas that of fraction FIVb′ exhibited higher affinity toward triacetin, trimyristin, and triolein. The effect of selected salts and detergents on the lipase activity of the purified fractions was also investigated. The lipase activity of the purified lipase fractions was completely inhibited by 10 mM of $FeCl_2$, $FeCl_3$, and Ellman's reagent. However, 10 mM of $CaCl_2$ and EDTA activated the two purified lipase fractions by 20 to 50%.

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Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are enzymes that catalyze the hydrolysis of fatty acid ester bonds in triacylglycerols (1). Lipases are produced by many microorganisms, including fungi and bacteria (2). Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, making them very attractive tools for industrial applications (3). Lipases can be used in the pharmaceutical and agrochemical industries for the synthesis of structured triacylglycerols and for the production of health products (4). Lipases are already being used as additives in the food industry for the development and enhancement of food flavors (5).

Pseudomonas fragi is an aerobic, gram-negative, psychrotrophic bacterium. *Pseudomonas fragi* can grow at temperatures of 7°C or below (6) and has been isolated from milk and other dairy products, dairy utensils, meat, water, and soil (7). *Pseudomonas fragi* was first associated with the spoilage of milk through the development of a fruity off-flavor (8). Under controlled conditions, it can produce flavor-active metabolites, including free fatty acids (FFA) and fatty acid esters, that contribute to the development of fruity aroma (9–10).

This study was part of ongoing research in our laboratory aimed at the development of lipases with specificity toward the hydrolysis of butter fat and edible oils to produce precursors for the production of natural flavors by other enzymes such as esterases (11) and lipoxygenases (12). The specific objectives of this study were to carry out the purification and characterization of the extracellular lipase fractions from *P. fragi* in terms of pH, temperature, kinetic parameters, stability, and substrate specificity, using triacylglycerols, butter fat, and edible oils as substrates.

MATERIALS AND METHODS

Biomass production and partial purification of extracellular lipase. The biomass production of *P. fragi,* as well as the extraction of crude extracellular lipase (FI), was performed as described by Schuepp *et al*. (13). The partially purified enzymatic extract (FII) was obtained by ammonium sulfate precipitation at 20 to 40% of saturation (13).

Purification. FII was first subjected to ion exchange chromatography (IEC) on a Source 15 Q, HR 10/10 column (Amersham Pharmacia Biotech, Uppsala, Sweden) using the fast-protein liquid chromatography system (Amersham Pharmacia Biotech). After the column was equilibrated with two column volumes of Ammediol-HCl buffer (0.02 M, pH 9.0), the enzyme suspension (1 mL containing 50 mg of protein) was injected. Gradient elution consisted of eluant A [Ammediol-HCl buffer (0.02 M, pH 9.0)], and eluant B, the same as

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A, but with 1 M NaCl. Gradient elution from 100% buffer A to 100% buffer B was carried out in 17.5 min at a flow rate of 2 mL/min. The eluted protein fractions were monitored at 280 nm; the fractions were collected and dialyzed against sodium phosphate buffer (1 mM, pH 8.0) and lyophilized.

The purified fraction (FIIId), obtained from IEC, was further purified by size-exclusion chromatography (SEC) on a preparative Superdex 75, HR 16/50 column (Amersham Pharmacia Biotech). The column was equilibrated with two column volumes of sodium phosphate buffer (0.05 M, pH 8.0), and a 1 mL sample of enzyme suspension (50 mg protein) was injected. The flow rate was 1 mL/min and the eluted protein fractions were monitored at 280 nm and collected. The fractions were dialyzed against sodium phosphate buffer (1 mM, pH 8.0) and lyophilized. The purified fractions (FIVa and FIVb) obtained from SEC were rechromatographed under the same conditions. The eluted purified enzymatic fractions (FIVa′ and FIVb′) were collected and lyophilized.

Protein determination. The protein content of the enzymatic fractions was determined according to the method described by Hartree (14). Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was used as a standard for calibration.

Lipase assay. The FFA from the lipase-catalyzed hydrolysis of triacetin were titrated with NaOH (0.05 M) using a DL 53 automatic titrater (Metler Toledo, AG, Gréifensee, Switzerland) according to the procedure described by Schuepp *et al.* (13), with the minor modification of adding 50 µL of Tween-20. The specific activity of lipase was defined as µmol of FFA per mg protein per min.

Gel electrophoresis. Native polyacrylamide gel electrophoresis (PAGE) was carried out using a Phastsystem unit (Amersham Pharmacia Biotech). Native-PAGE minigels of 12.5% polyacrylamide were precast as outlined in the Pharmacia manual (15) and run on the Phastsystem using PhastGel native buffer strips (Amersham Pharmacia Biotech). Low-molecular-weight standards (Amersham Pharmacia Biotech), ranging from 14.4 to 94.0 kDa, were run in tandem with the sample fractions. The minigels were then transferred to the development compartment of the Phastsystem unit for silver staining (16).

Determination of pH and temperature optima. The effect of pH on the enzymatic activity of the lipase fractions was investigated using a wide range of buffers, including citrate-phosphate (pH 3.0 to 7.0), sodium phosphate (pH 6.0 to 8.0), Ammediol-HCl (pH 8.0 to 10.0), phosphate-NaOH (pH 11.0 to 11.5), and hydroxide-chloride (pH 12.0). The optimal temperature for lipase activity was determined by incubating the reaction mixture with triacetin as substrate at different temperatures ranging from 25 to 90°C, at intervals of 5°C.

Effect of inhibitors and activators on lipase activity. The effect of certain inhibitors and activators, including $FeCl_2$, $FeCl_3$, $CaCl_2$, $MgCl_2$, sodium deoxycholate, diisopropyl fluorophosphate (DIFP), *N*-bromosuccinimide (NBS), Ellman's reagent and EDTA, on the activity of the purified lipase fractions was investigated at two concentrations (1 and 10 mM). The lipase activity was assayed by the titrimetric method (35°C, pH 9.0) using the substrate triacetin in the presence and absence of the salts and detergents.

Substrate specificity. The specificity of the lipase activity of the purified fractions was studied using a wide range of triacylglycerols, including triacetin, tributyrin, tricaproin, tricaprylin, trimyristin, tripalmitin, tristearin, and triolein. The substrate solution was prepared by sonicating 100 µmol of each substrate and 100 µL of Tween-20 in 10 mL Ammediol-HCl buffer (0.05 M, pH 9.0) for 2 min using an Ultrasonic Processor 2020 XL sonicator (Heat Systems, Farmingdale, NY). The lipase assay was performed by titrimetric method (35°C, pH 9.0).

The lipase activity of the purified enzyme extracts was also investigated using butter fat and olive, canola, and fish oils. The reaction mixture, containing 200 mg of oil and 100 µL of Tween-20 in 10 mL of Ammediol-HCl buffer (0.05 M, pH 9.0), was homogenized by sonication. The enzyme assay was conducted by the titrimetric method (35°C, pH 9.0).

Analysis of FFA by gas chromatography (GC). The hydrolytic activity of the purified lipase fractions toward butter fat and olive, canola and fish oils was determined by GC analysis of the FFA. The FFA were extracted with 20 mL hexane and concentrated to 6 mL using a gentle stream of nitrogen gas. The extracted FFA were then methylated and subjected to GC analysis according to the method of Badings and De Jong (17) using a Varian model 3400 gas chromatograph (Varian Associates, Sunnyvale, CA). The analysis was performed on an Omegawax 320 capillary column (30 m × 0.32 mm; Supelco, Oakville, Ontario, Canada). The flow rates for the carrier gas (helium), hydrogen, and air were 1.8, 30.0, and 300.0 mL/min, respectively. The temperatures of the injector and the detector were 200 and 230°C, respectively. After an isothermal period of 5 min, the column temperature was increased from 40 to 200°C at a rate of 8°C/min.

RESULTS AND DISCUSSION

Purification of extracellular lipase. Purification of FII by IEC resulted in two major fractions, FIIIa and FIIId, and two minor ones, fractions FIIIb and FIIIc (Fig. 1). Fraction FIIId exhibited the highest total and specific lipase activities and a 11.8-fold increase in purification with respect to the crude fraction FI (Table 1). On further purification by SEC (Fig. 2), fraction FIIId was resolved into one major peak (FIVa) with three shoulder peaks (FIVb, FIVc, and FIVd); however, fractions FIVa and FIVb contained the highest activity (Table 1). Reapplication of fractions FIVa and FIVb on the SEC column resulted in two purified fractions, FIVa′ and FIVb′, with an approximate twofold increase in specific activity (Table 1). The increase in specific activity during the purification process could be attributed to the removal of inhibitory materials such as proteases and cellular debris that could either decrease or mask the lipase activity (18). A lipase fraction from *P. fluorescens* (19) was obtained with a 10-fold increase in purification. In addition, two other lipase fractions from *P. aeruginosa* (20,21) were reported with an overall purification

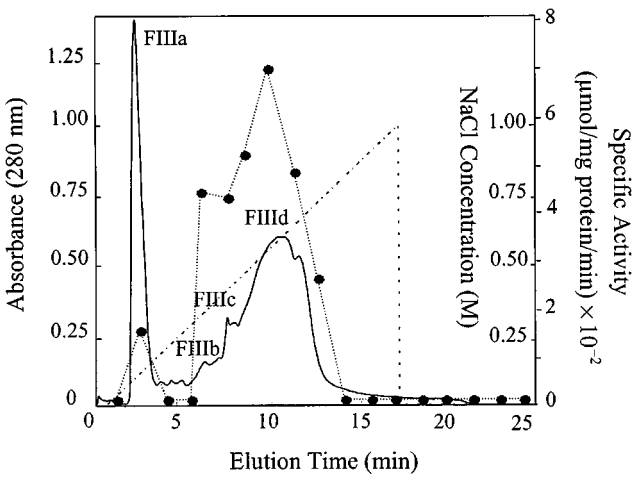

FIG. 1. Purification profile of partially purified lipase extract (FII) by ion-exchange chromatography (IEC) on Source 15 Q: absorbance at 280 nm (—), specific activity using triacetin as substrate (●…●), and NaCl gradient (- — - — -).

factor of 1265 and 60. Lipase fractions from *P. tolaasii* (22), *P. putida* (23), and *P. aeruginosa* 3SK (24) were obtained possessing 1000-, 21-, and 5-fold increases in purification, respectively.

Electrophoretic analysis. The native electropherogram of fractions FIVa′ and FIVb′ (Fig. 3) shows the presence of three protein bands with estimated molecular weights of 15.2, 25.8, and 38.5 kDa in fraction FIVa′ and major and minor bands with estimated molecular weight of 15.2 and 25.8 kDa for fraction FIVb′. These findings suggest that the molecular weight bands of 15.2 and 25.8 kDa, visualized in all the purified fractions, correspond to the lipase enzyme since the majority of lipases from *Pseudomonas* species (25–29) have reported molecular weights between 25 to 65 kDa. In addition, the electropherogram (Fig. 3) shows the presence of high-molecular-weight dark smears in all lanes containing the lipase fractions; these findings suggest the occurrence of high-molecular-weight protein aggregation in the enzymatic fractions during gel electrophoresis. Stöcklein *et al.* (30) reported that during native-PAGE of the enzymatic extract from *Penicillium expansum*, the purified lipase migrated as a di- and tetramer with estimated molecular weights of 52 and 100 kDa, respectively.

Effect of pH and temperature on lipase activity. Fractions FIVa′ and FIVb′ exhibited the highest activity in the pH range of 9.5 to 10.0 (Fig. 4); however, the decrease in the lipase activity of the purified fractions at pH values less than 7.0 could be due to incomplete ionization of FFA (1). Most *Pseudomonas* species have been reported to exhibit maximal lipase activity in the pH range of 7.0 to 9.0, including enzymes from *P. tolaasii* (22), *P. aeruginosa* EF2 (23), and *P. putida* (24). Schuepp *et al.* (13) reported similar findings for the lipase activity of the FII from *P. fragi* CRDA 037, which had a pH optimum of 9.0. The lipase extract from *P. fragi* 22.39B (25) exhibited optimal activity in the pH range of 9.0 to 9.5, whereas that from *P. pseudoalcaligenes* F-111 (29) showed its highest activity in the pH range of 6.0 to 10.0.

The lipase activity of the resize-exclusion chromatography (re-SEC) purified fractions FIVa′ and FIVb′ increased with a concomitant increase in temperature, reaching a maximum at 80°C (Fig. 5). A small increase in enzyme activity at 40°C was also observed for lipase activity in all enzymatic fractions. Schuepp *et al.* reported an optimal temperature of 35°C for the lipase activity of the FII from *P. fragi* CRDA 037 (13). Lipases from *Pseudomonas* species, including *P. fragi* 22.39B (25), *Pseudomonas* sp. f-B-24 (26), *Pseudomonas* sp. KWI-56 (27), and *P. mephitica* var. *lipolytica* (31), exhibited maximal activity at high temperatures ranging from 60 to 80°C. In addition, lipases from *P. aeruginosa* EF2 (23) and *P. fluorescens* AK102 (32) showed the highest enzymatic activity at 55°C.

Kinetic parameters. Kinetic parameters were obtained for the purified lipase fractions using triacetin as substrate (Table 1). Although triacetin is rather an uncommon substrate to

TABLE 1
Purification Scheme of the Extracellular Lipase from *Pseudomonas fragi* CRDA 037

Fraction		Total protein (mg)	Specific activity[f]	Total activity[g]	Recovery (%)	Purification (fold)	K_m (mM)	V_{max}[h]	Enzyme efficiency ($\times 10^{-2}$)[i]
Crude	FI[a]	1559.0	0.62	9.39	100.0	1.0	—[j]	—	—
PP	FII[b]	608.0	2.60	15.82	168.5	4.3	8.06	0.087	1.08
IEC	FIIId[c]	243.9	7.12	17.37	185.0	11.8	8.46	0.167	1.97
SEC	FIVa[d]	30.9	49.63	17.24	183.6	79.7	5.90	1.053	17.84
	FIVb[d]	19.8	73.14	14.51	154.5	117.4	3.74	1.339	35.76
Re-SEC	FIVa′[e]	3.0	105.50	3.17	33.7	169.3	3.85	2.781	72.16
	FIVb′[e]	4.6	121.60	5.64	60.1	195.2	5.49	2.093	38.15

[a]Crude extracellular lipase produced by *P. fragi*.
[b]Partially purified (PP) extracellular lipase fraction produced by ammonium sulfate precipitation at 20–40% of saturation.
[c]Ion exchange chromatography (IEC) of FIId.
[d]Size exclusion chromatography (SEC) of fraction FIIId, obtained by IEC.
[e]Resize-exclusion chromatography (re-SEC) of fractions FIVa and FIVb, obtained from SEC.
[f]Specific activity is expressed as μmol free fatty acid (FFA) per min per mg protein $\times 10^{-2}$, using triacetin as substrate.
[g]Total activity is expressed as μmol FFA per min.
[h]V_{max} is calculated as μmol FFA per mg protein per min.
[i]Enzyme efficiency is calculated as V_{max}/K_m.
[j]Not determined.

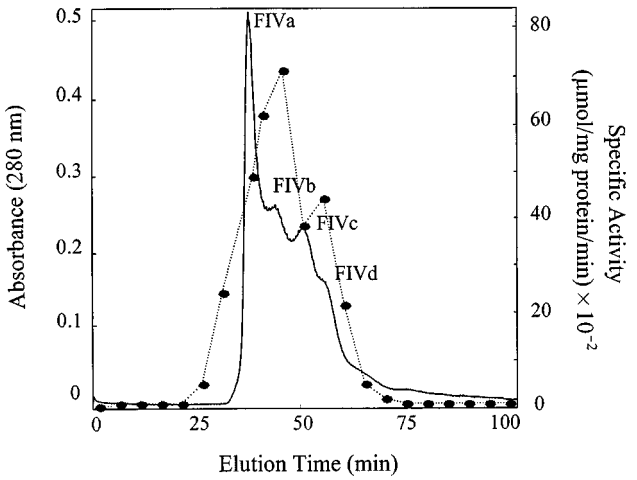

FIG. 2. Purification profile of a lipase fraction (FIIId) obtained from IEC by size-exclusion chromatography (SEC) on Superdex 75 (Amercham Pharmacia Biotech, Uppsala, Sweden) at 280 nm (—), and specific activity using triacetin as substrate (●…●).

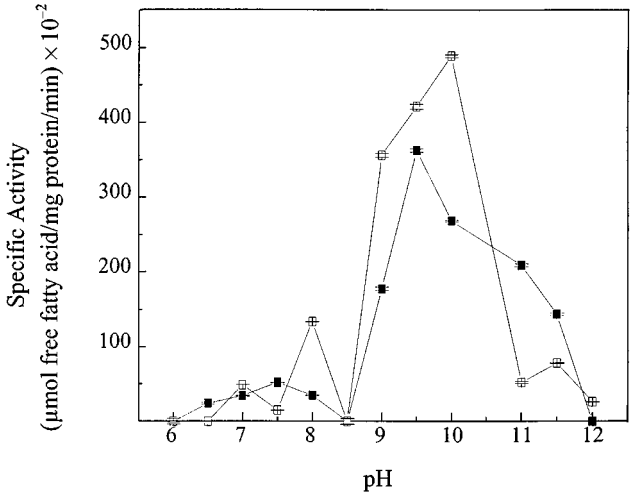

FIG. 4. Effect of pH on the activity of the purified fractions FIVa′ (■) and FIVb′ (□) obtained from re-SEC. See Figure 3 for abbreviations.

assay for lipase activity, it is widely used for the investigation of lipolytic activity due to its high degree of solubility. The purification of the extracellular lipase fractions FIVa and FIVb from *P. fragi* CRDA 037 by IEC and SEC resulted in a decrease in the K_m values. These findings suggest that the affinity of lipase activity toward the substrate was enhanced as a result of purification. However, the K_m value of the re-SEC-purified fraction FIVb′ was higher than that of the SEC-purified fraction, FIVb, suggesting that the former fraction could possess specificity toward a substrate other than triacetin. Schuepp *et al.* (13) reported that the partially purified lipase extract from *P. fragi* CRDA 037 had a K_m value of 7.1 mM. The purified lipase fraction from *Lactobacillus plantarum* exhibited a K_m value of 2.31 mM using tributyrin as substrate (33), while those from *P. fragi* (34) and *P. fluorescens* (35) showed K_m values of 0.9 and 3.65 mM, respectively.

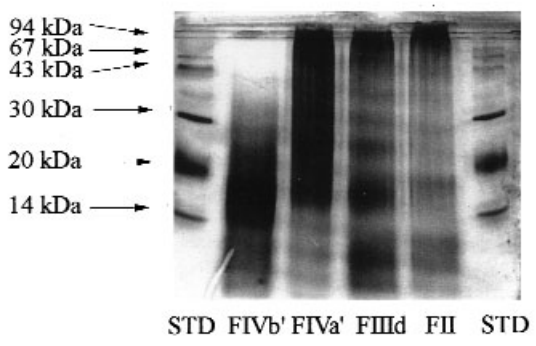

FIG. 3. Electrophoretic profile of the lipase fractions from *P. fragi* CRDA 037 on native polyacrylamide gel. STD, standard protein markers; FII, purified lipase fraction obtained by FIIId; (FIVa′ and FIVb′), purified lipase fractions obtained by resize-exclusion chromatography (re-SEC). See Figures 1 and 2 for other abbreviations.

The V_{max} value for the IEC purified fraction FIIId (0.167 μmol/mg protein/min) was twice as high as that (0.087 μmol/mg protein/min) obtained for the partially purified fraction FII. In addition, further purification of the IEC-purified fraction FIIId resulted in the two SEC fractions FIVa and FIVb, whose V_{max} values were approximately 10 times higher than those obtained for fraction FIIId; further re-SEC of the two purified fractions produced fractions FIVa′ and FIVb′, possessing approximately a twofold increase in lipase activity.

Effect of inhibitors and activators on lipase activity. The enzymatic activity of the purified lipase fractions was strongly inhibited by 10 mM $FeCl_2$ and $FeCl_3$ (Table 2). The presence of 1 mM $FeCl_2$ activated the lipase fractions,

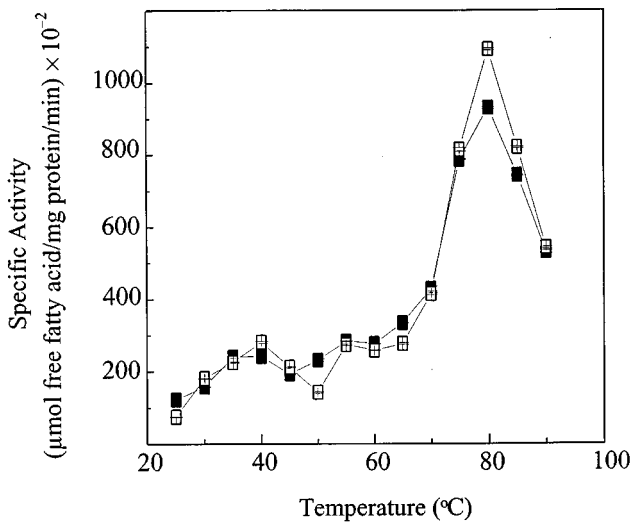

FIG. 5. Effect of temperature on the activity of the purified fractions FIVa′ (■) and FIVb′ (□) obtained from re-SEC. See Figure 3 for abbreviations.

TABLE 2
Effect of Salts and Detergents on the Activity of the Purified Lipase Fractions, FIVa′ and FIVb′, from *Pseudomonas fragi* CRDA 037

Substance	Concentration (mM)	Relative activity (%)[a] FIVa′[b]	FIVb′[c]
Blank	0.0	100.0 (0.723)[d]	100.0 (0.347)[d]
$FeCl_2$	1.0	136.4 (0.859)[d]	106.5 (0.971)[d]
	10.0	0.0	4.4 (1.814)[d]
$FeCl_3$	1.0	72.9 (0.365)[d]	51.7 (0.481)[d]
	10.0	0.0	0.0
$CaCl_2$	1.0	131.6 (0.600)[d]	96.0 (0.605)[d]
	10.0	149.0 (0.321)[d]	118.8 (0.856)[d]
$MgCl_2$	1.0	119.8 (0.517)[d]	56.4 (0.568)[d]
	10.0	153.4 (0.370)[d]	99.2 (1.566)[d]
Sodium deoxycholate	1.0	54.3 (0.889)[d]	58.8 (0.295)[d]
	10.0	56.4 (0.545)[d]	65.0 (0.391)[d]
Diisopropyl fluorophosphate	1.0	67.0 (0.509)[d]	64.8 (0.456)[d]
	10.0	16.1 (0.485)[d]	0.0
N-Bromosuccinimide	1.0	90.7 (0.363)[d]	77.6 (0.685)[d]
	10.0	20.6 (5.474)[d]	0.0
Ellman's reagent	1.0	90.9 (0.642)[d]	99.5 (0.204)[d]
	10.0	0.0	0.0
EDTA	1.0	73.1 (0.398)[d]	53.2 (0.239)[d]
	10.0	136.8 (1.360)[d]	152.9 (0.105)[d]

[a]The relative activity is expressed as a percentage of the activity obtained with a metal salt or a chemical reagent to that obtained without any metal salt or a chemical reagent. See Table 1 for abbreviations.
[b]Enzyme fraction obtained by re-SEC of fraction FIVa on Superdex 75 (Amersham Pharmacia Biotech, Uppsala, Sweden).
[c]Enzyme fraction obtained by re-SEC of fraction FIVb on Superdex 75.
[d]Relative SD calculated as (SD/mean) × 100.

whereas 1 mM $FeCl_3$ slightly inhibited the enzymatic activity of fractions FIVa′ and FIVb′ (22.1 and 48.3%, respectively). In contrast, the hydrolytic activity of the purified lipase fraction FIVa′ increased with the addition of 1 and 10 mM of $CaCl_2$ (132 and 149%, respectively); fraction FIVb′ was only activated by 18.8% using 10 mM of $CaCl_2$. In addition, the enzyme activity of fraction FIVa′ increased by 20 and 53% in the presence of 1 and 10 mM of $MgCl_2$, respectively, whereas that of fraction FIVb′ decreased by 43.6 and 0.8%, respectively. The literature (13,23,25–27,29,33,36) reported that the lipase activity was strongly inhibited by both iron ions present in $FeCl_2$ and $FeCl_3$.

Sodium deoxycholate and DIFP at concentrations of 1 mM caused partial inhibition of the enzymatic activity in both fractions FIVa′ and FIVb′ with residual activity ranging from 54.3 to 58.8% (Table 2). An increase in the concentration of sodium deoxycholate to 10 mM did not produce a further decrease in activity, whereas in the the presence of 10 mM of DIFP, the lipolytic activity of fractions FIVa′ and FIVb′ decreased by 83.9 and 100%, respectively. The literature indicated that lipase activity from *P. fragi* was inhibited by 37.5 and 100%, using DIFP (37) and and sodium deoxycholate (13), respectively.

A decrease of 80 and 100% in the hydrolytic activity of the purified lipase fractions FIVa′ and FIVb′, respectively, was observed using 10 mM NBS. However, total inhibition of lipase activity in both fractions was obtained using 10 mM Ellman's reagent. The trend of these results is in agreement with those reported in the literature (13,38).

The addition of 1 mM of EDTA to the reaction mixture resulted in a decrease in the enzymatic activity of the lipase fractions FIVa′ and FIVb′ by 26.9 and 56.8%, respectively, while the presence of 10 mM EDTA produced a 36.8 and 52.9% increase, respectively. These findings suggest that at high EDTA concentrations, other enzymes present in the extract using the same substrate as lipase were also inhibited by EDTA, which in turn promoted lipase activity. The literature (22,26,39,40) indicated that extracellular lipase activity was strongly inhibited by EDTA. However, the lipase extract from *P. aeruginosa* EF2 (23) showed an increase in activity in the presence of EDTA.

Substrate specificity. The activity of the purified lipase fractions toward triacylglycerols (C2 to C18:1) was investigated (Fig. 6). Fraction FIVa′ showed the highest activity toward triacetin and a broad selectivity favoring long-chain fatty acid triacylglycerols. Fraction FIVb′ exhibited similar specificity, but discriminated strongly against tristearin. Schuepp *et al.* (13) reported that the FII from *P. fragi* CRDA 037 showed the highest activity toward trimyristin, followed by triacetin, and then by tributyrin. Nishio *et al.* (41) reported

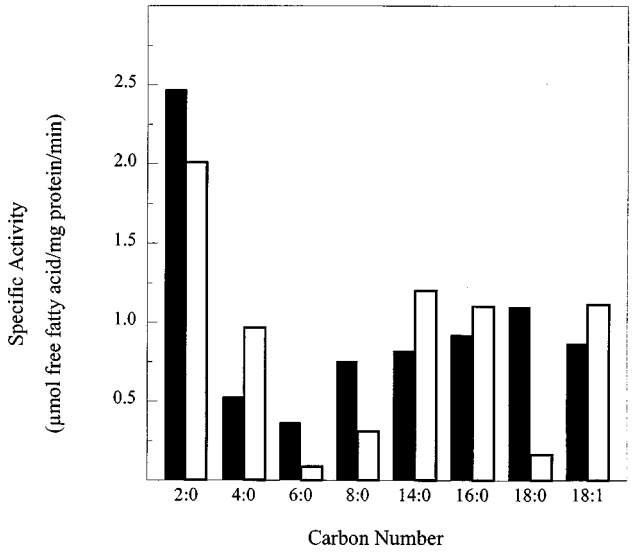

FIG. 6. Substrate specificity of the purified lipase fractions FIVa′ (■) and FIVb′ (□), obtained from re-SEC, toward triacylglycerols. See Figure 3 for abbreviations.

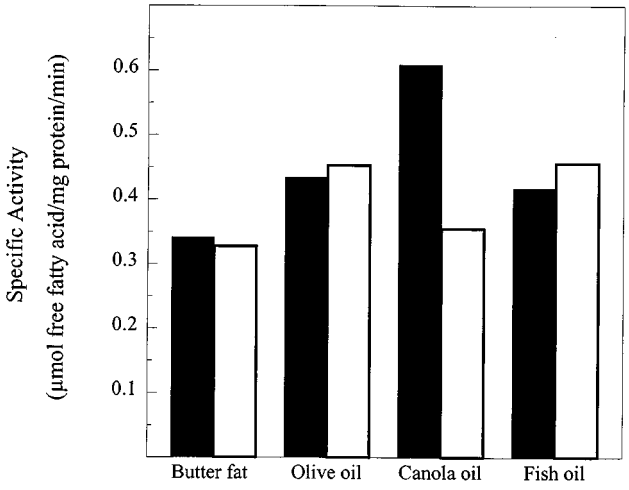

FIG. 7. Specificity of the purified lipase fractions FIVa′ (■) and FIVb′ (□), obtained from resize-exclusion chromatography, toward butter fat and the three edible oils. See Figure 3 for abbreviations.

that the purified extracellular lipase fraction from *P. fragi* 22.39B demonstrated the highest affinity toward tributyrin, followed by tricaprylin. However, the lipase activity from *P. aeruginosa* (42) and *Bacillus subtilis* 168 (43) extracts was able to degrade both emulsified long-chain fatty acid esters as well as soluble monomeric esters.

The specificity of the purified lipase fractions, FIVa′ and FIVb′, toward butter fat and a number of edible oils, including olive, canola and fish oils, was also investigated. Figure 7 shows that the lipolytic activity in fraction FIVa′ exhibited highest specificity toward canola oil, whereas that of fraction FIVb′ showed the highest affinity toward fish and olive oils. However, the lipase activities in both fractions exhibited similar affinity for butter fat. Lipase activity in the enzymatic extracts from *Pseudomonas* sp. f-B-24 (26), *Pseudomonas* sp. KWI-56 (27), *P. pseudoalcaligenes* F-111 (29), and *P. expansum* (30) was also reported to be capable of hydrolyzing olive and fish oils.

GC analysis (Table 3) demonstrated that both purified fractions, FIVa′ and FIVb′, showed a lipolytic activity capable of hydrolyzing butter fat and the edible oils. The results showed that the lipase activity in fraction FIVa′ exhibited a selected specificity toward saturated fatty acids of chain lengths of C12:0 to C18:0 as well as the fatty acid C20:4; however, the lipolytic activity in fraction FIVb′ exhibited a broader range of fatty acid specificity. These results suggest that the two purified enzymatic fractions FIVa′ and FIVb′ possess different lipases with unique substrate specificities.

TABLE 3
Gas Chromatography Analysis of FFA Produced by the Hydrolysis of Butter Fat and Edible Oils Using the Purified Lipase Fractions FIVa′ and FIVb′

Substrate	Enzyme fraction	Relative percentage of FFA[a]												
		C8	C10	C12	C14	C14:1	C16	C16:1	C18	C18:1	C18:3	C20:0	C20:4	NI[b]
Butter fat														
	FIVa′	—[c]	—	27.21	—	—	—	—	23.03	—	—	—	26.78	22.98
	FIVb′	14.60	16.04	8.46	10.97	14.04	5.60	5.79	4.53	11.59	—	—	—	8.39
Olive oil														
	FIVa′	—	—	23.48	—	—	—	—	121.54	—	—	—	33.08	21.91
	FIVb′	14.61	15.67	8.36	10.93	13.99	5.61	5.99	4.67	11.96	—	—	—	8.22
Canola oil														
	FIVa′	—	—	—	20.34	—	19.52	—	16.97	—	—	—	27.61	15.56
	FIVb′	—	5.91	10.36	4.42	6.40	14.35	6.51	—	22.81	8.31	4.56	9.89	6.49
Fish oil														
	FIVa′	—	—	—	22.70	—	21.12	—	16.66	—	—	—	25.69	13.83
	FIVb′	—	—	17.65	17.32	12.08	13.64	—	39.31	—	—	—	—	—

[a]The relative percentage of an individual FFA is calculated as a percentage of its peak area compared to the total peak area of total fatty acids. See Table 1 for abbreviations.
[b]Peak not identified.
[c]Not detected.

The purification of the extracellular lipase extract from *P. fragi* CRDA 037 resulted in two enzymatic fractions, but did not obtain homogeneous purified proteins. The lipase activities in both purified fractions exhibited certain similarities in terms of their properties thereby indicating that they are alkaline lipases and very thermostable enzymes. In addition, the enzymatic activities in the purified fractions were capable of hydrolyzing short- and long-chain fatty acid esters of triacylglycerols as well as butter fat and selected edible oils; however, the hydrolytic activities of the two purified fractions showed a certain selective substrate specificity.

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