A jacalin-related lectin domain-containing lipase from chestnut (Castanea crenata): Purification, characterization, and protein identification

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ABSTRACT

A novel lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) was discovered from Korean chestnut (Castanea crenata). The lipase was isolated and purified by ammonium sulfate precipitation and a fast protein liquid chromatography system equipped with HiTrap DEAE-Sepharose Fast Flow, HiTrap Q-Sepharose Fast Flow, and HiPrep Sephacryl S-100 Hi-Resolution columns. The purified C. crenata lipase showed a 15.8% yield, purification fold number of 465.8, and specific activity against triolein of 88.5 mU/mg. The enzyme exhibited hydrolytic activity toward tributyrin, trilaurin, and triolein, and was maximally active at pH 8.0 and 35 °C, with triolein used as the substrate. The activation energy (Ea) and deactivation energy (Ed) of triolein hydrolysis were 38.41 and 83.35 kJ/mol, respectively. In the enzyme kinetic study, Vmax, Km, and kcat were 110.58 mU/mg, 0.11 mM, and 0.221 min⁻¹, respectively. The relatively low Km value indicated that the lipase has high affinity for its substrate. Moreover, Mg²⁺ and Ca²⁺ increased the lipase activity to 115.4% and 108.3%, respectively. The results of peptide fingerprinting revealed that the C. crenata lipase with a molecular weight of 33.3 kDa was structurally similar to the mannose-binding lectin of the jacalin-related lectin domain superfamily, implying that it has potential as a therapeutic agent for use in the biomedical industry.

1. Introduction

Lipases (triacylglycerol [TAG] hydrolases, EC 3.1.1.3) are versatile enzymes that catalyze either the hydrolysis or synthesis of a broad range of esters, depending on the reaction conditions (Chandra et al., 2020). These enzymes are environmentally friendly biocatalysts that have a remarkable capacity to facilitate biochemical processes in industry due to their unique characteristics (e.g., substrate selectivity, regio-selectivity, and chemo-selectivity); unlike conventional chemical catalysts, they do not require high temperature or produce unwanted by-products (Huang et al., 2020). Hence, recent studies have focused on discovering novel lipases with unique properties from natural sources.

Lipases are widely distributed throughout the animal and plant kingdoms, as well as in molds and bacteria. Most lipases used for biotechnological purposes were discovered from proteobacteria (e.g., Pseudomonas punonensis) (Sonkar and Singh, 2020), ascomycete fungi (e.g., Candida rugosa) (Patel et al., 2015), and zygomycete fungi (e.g., Rhizomucor miehei) (Phuah et al., 2012). These lipases are used in the food industry for hydrolyzing milk fats, enhancing the flavor of cheese, reducing bitterness, and preventing rancidity. In addition, lipases can be utilized as catalysts in the detergent, pharmaceutical, agrochemical, and leather industries (Vishnoi et al., 2020).

Although various microbial lipases have been discovered, the high costs of production, downstream processing, and fermenter operation limit the use of these enzymes in large-scale production. This has prompted efforts to identify new enzymes from other sources. Plant lipases, as potential alternative biocatalysts found mostly in energy resources such as oilseeds, are cost-effective, highly substrate-specific, and acceptable due to their eukaryotic origin (Seth et al., 2014). Hence, plant lipases can serve as a novel source of biocatalysts for various industries. To identify novel lipases from plant sources, we analyzed the lipase activity of various domestic agricultural products, such as

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chestnut (Castanea crenata), cabbage (Brassica rapa) seeds, brown rice (Oryza sativa), and Jerusalem artichoke (Helianthus tuberosus). A preliminary screening test showed that crude extract of C. crenata had the highest lipase activity among the domestic agricultural products analyzed. In addition, chestnuts are used widely as part of a healthy diet because of its high antioxidant and mineral contents (Sacchetti et al., 2008). Therefore, C. crenata is a promising plant source for discovery of novel lipases.

In this study, a novel lipase was purified from C. crenata and its enzymatic properties, including optimum conditions, kinetic parameters, and substrate specificity, were characterized. Furthermore, the lectin-related structure of the C. crenata lipase was identified by peptide mass fingerprinting based on bioinformatic analysis.

### Table 1

| Purification method | Total protein (mg) | Total activity (mU) | Specific activity (mU/mg) | Yield (%) | Purification fold |
|--------------------|--------------------|--------------------|--------------------------|-----------|-----------------|
| Crude extract      | 3840               | 729.6              | 0.19                     | 100.0     | 1.0             |
| Ammonium sulfate fractionation (30–40%) | 1050 | 535.5 | 0.51 | 73.4 | 2.7 |
| HiTrap DEAE Sepharose FF (20–30%, pH 8.0) | 52.7 | 306.7 | 5.82 | 42.0 | 30.6 |
| HiTrap Q Sepharose FF (30–40%, pH 8.0) | 4.3 | 188.4 | 43.81 | 25.8 | 230.6 |
| HiPrep Sephacryl S-100 HR (pH 8.0) | 1.3 | 115.1 | 88.50 | 15.8 | 465.8 |

Fig. 1. Sequential chromatography for purification of lipase from C. crenata. (A) Anion-exchange chromatography with HiTrap DEAE-Sepharose FF and (B) HiTrap Q-Sepharose FF. (C) Size exclusion chromatography with HiPrep Sephacryl S-100 HR.
sample was collected into a bottle and stored at 4 °C for subsequent experiments.

2.3. Purification of lipase

The crude protein sample was brought to 30–40% ammonium sulfate saturation. The precipitated proteins were collected by centrifugation at 10,000×g for 30 min at 4 °C, and the precipitate was dialyzed for 18 h.
against 50 mM Tris-HCl (pH 8.0) buffer in dialysis cassettes (Thermo Fisher, Waltham, MA, USA) with a molecular weight cutoff of 10 kDa. The dialyzed solution was concentrated by centrifugation with an Amicon Ultra-15 Centrifugal Filter Unit (Merck Millipore, Darmstadt, Germany) at 3000 g for 15 min at 4 °C and then filtered through 0.45-μm syringe filters. The enzyme concentration was determined by Bradford protein assay (Wrolstad et al., 2005).

The crude protein was then further purified using the ÄKTA Go Protein Purification System (Cytiva, Uppsala, Sweden) equipped with an ultraviolet (UV) detector measuring the absorbance at 280 nm. The filtered crude protein was applied to the HiTrap DEAE-Sepharose Fast Flow column equilibrated with 50 mM Tris-HCl buffer (pH 8.0; buffer A). Elution was performed using a step gradient with buffer A containing 1.0 M NaCl (buffer B) at a flow rate of 5.0 mL/min. The samples were fractionated with buffer B (10% increments), and the lipase activities of the fractions were evaluated. The fractions with the highest lipase activity were collected, desalted, concentrated by ultrafiltration, and loaded onto a HiTrap Q-Sepharose Fast Flow column equilibrated with buffer A. The samples were then fractionated again with buffer B (10% increments), and the fractions with the highest lipase activities were collected, desalted, concentrated by ultrafiltration. Then, the pooled fraction was loaded onto a HiPrep Sephacryl S-100 HR equilibrated with buffer A containing 150 mM NaCl at a flow rate of 0.4 mL/min.

2.4. Lipase assay

2.4.1. Plate assay

Lipase activity was determined based on the color change of pH indicators induced by the fatty acids released by lipolytic activity. Agar medium was prepared by adding 2.0% (w/v) agar to 50 mM Tris-HCl (pH 8.0) buffer. After autoclaving, it was cooled to 60 °C, and rhodamine B solution and 1.0% (v/v) TAGs (tributyrin, tricaprin, tricaprin, trilaurin, tripalmitin, or triolein) were added. Mixtures containing the different TAGs were poured into several plates, and an enzymatic reaction was initiated by adding 50 μL of the enzyme solution to each plate.

2.4.2. Spectrophotometric assay

The substrate solution was prepared by dissolving p-NPP (24.0 mg) into distilled water (29.0 mL) containing SDS (5.6 mg) and Triton X-100 (3.1 mL). A p-NPP stock solution with a final concentration of 2 mM was heated at 65 °C with continuous stirring until the p-NPP had completely dissolved. Then, the solution was cooled to room temperature and stored at 4 °C. The enzyme solution (110 μL) in Tris-HCl buffer (pH 8.0) was loaded into each well of the microplate and pre-incubated at 35 °C for 5 min. The substrate solution (90 μL) was added to initiate hydrolysis, and the amount of p-nitrophenol (p-NP) produced during the reaction was quantified by monitoring the absorbance at 410 nm with a Multiskan FC Microplate Photometer (Thermo Fisher). One unit of activity was defined as the amount of enzyme liberating 1 μmol of p-NP per minute.

2.4.3. Colorimetric assay

The reaction was performed in a two-phase reactor (5 mL) system containing lipase in 50 mM Tris-HCl buffer (pH 8.0) and 10% (v/v) trilein dissolved in IOT. The concentration of liberated oleic acid was determined using modified cupric acetate assay, as described by Kwon et al. (2015). The activity was defined by the slope of the sigmoidal curve at the inflection point and calculated with SigmaPlot 12.5 software.

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the Hoefer SE 250 mini-gel system (GE Healthcare, Chicago, Ill, USA) with a 12% resolving gel and 5% stacking gel, according to the method of Laemmli (1970). Samples were prepared by mixing purified enzyme with 4 × sample buffer containing 3.2 mL of 10% SDS, 1.6 mL of glycerol, 0.4 mL of 1% bromophenol blue, and 0.8 mL of 2-mercaptoethanol. The sample was boiled for 3 min at 100 °C before being loaded onto the gel. A PM2510 ExcelBand enhanced 3-color regular range protein marker (SMOBIO, Hsinchu, Taiwan) was used as a broad range (9–180 kDa) protein standard to estimate the molecular weight of the enzyme. Electrophoretic separation was carried out at 120 V and 20 mA for 90 min, and the migrated proteins were stained with Coomassie Brilliant Blue R-250.

2.6. Molecular weight determination

The molecular weight of the C. crenata lipase was determined by a fast protein liquid chromatography system equipped with the HiPrep Sephacryl S-100 HR column. The column was pre-equilibrated with 50 mM Tris-HCl (pH 8.0) containing 150 mM NaCl at a flow rate of 0.4 mL/min. A calibration curve (retention volume versus molecular weight of the globular protein) was plotted using the following proteins with known molecular weight: bovine serum albumin (66.5 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (29.0 kDa), and ribonuclease A (13.7 kDa). The void volume was determined using blue dextran (2,000 kDa).
2.7. Effects of pH and temperature on lipase activity

To determine the optimum pH of the lipase, hydrolytic activity against triolein was evaluated in 100 mM of Britton–Robinson buffer over a pH range of 4.0–11.0. The pH stability of the enzyme was evaluated by measuring the activity after pre-incubating the enzyme for 24 h at 4 °C over a pH range of 4.0–11.0. The optimum temperature for lipase activity and thermostability of the enzyme were determined by measuring hydrolytic activity toward triolein at various temperatures (20–80 °C), without or with pre-incubation for 5, 10, 15, 30, 60, or 90 min. Then, the thermal deactivation energy ($E_d$) and activation energy ($E_a$) of the lipase-catalyzed hydrolysis were determined by the Arrhenius equation:

$$\ln k_d = -\frac{E_d}{R} \left(\frac{1}{T}\right) + \ln A$$

$$\ln k_a = -\frac{E_a}{R} \left(\frac{1}{T}\right) + \ln A$$

Fig. 4. Effect of pH on C. crenata lipase. (A) The activity at various pH values and (B) pH stability of the lipase. Values with different letters in each column differ significantly from each other, as determined by Duncan’s multiple range test ($p < 0.05$).
where $R = 8.314 \text{ JK}^{-1}\text{mol}^{-1}$, $k_d$ is the thermal deactivation constant (min$^{-1}$) derived from thermostability evaluation, and $k_a$ is the relative enzyme activity (%) derived from the optimum temperature evaluation.

The relative enzyme activity was calculated by the following equation:

\[
\text{Relative enzyme activity} (\%) = \frac{A_k}{A_o}
\]

where $A_k$ represents the enzyme activity at each temperature or pH, and $A_o$ represents the enzyme activity at optimum temperature and pH.

2.8. Effects of metal ions and surfactants on lipase activity

To evaluate the effect of metal ions and surfactants on lipase activity, the lipase assay against triolein described in section 2.4.2 was performed, with 20 mM of metal ions (LiCl, MgCl$_2$, CaCl$_2$, FeCl$_3$) and 1% (w/v) surfactants (Triton X-100, SDS, and Tween-20).

**Fig. 5.** Effect of temperature on C. crenata lipase. (A) Activity at various temperatures and (B) Arrhenius plot for determination of the activation energy ($E_a$). Values with different letters in each column differ significantly from each other, as determined by Duncan’s multiple range test ($p < 0.05$).
2.9. Enzyme kinetics

The Michaelis–Menten constant ($K_m$), maximum velocity ($V_{max}$), turnover number ($k_{cat}$), and catalytic efficiency ($k_{cat}/K_m$) were determined from the Hanes–Woolf plot derived from linear transformation of the Michaelis–Menten equation (E. Y. Kim et al., 2021). Enzyme reaction was initiated by adding 90 μL of p-NPP stock solution (0.05–1.00 mM) to 110 μL of the enzyme solution (0.45 mg/mL) preincubated at pH 8.0 and 35 °C.

2.10. Protein identification

Peptide mass fingerprinting of the purified lipase from *C. crenata* was performed using a Dionex Ultimate 3000 HPLC system (Thermo Fisher).
equipped with an Acquity UPLC BEH 130 C18 column (1.7 μm, 2.1 x 50 mm; Waters, Milford, MA, USA) and TripleTOF 5600+ System (AB Sciex, Framingham, MA, USA) at 50 °C. For sample preparation, the enzyme fraction in SDS-PAGE was separated and destained with acetonitrile solvent for 15 min. One microgram of trypsin was added to the in-gel piece, and the gel was incubated for 18 h at 37 °C. The hydrolyzed peptide was collected through dehydration of the gel with SpeedVac (Thermo Fisher).

Fig. 7. Kinetic parameters of C. crenata lipase. (A) Michaelis-Menten and (B) Hanes-Woolf plots for determination of the kinetic parameters with various p-NPP concentrations (0.02–0.45 mM).

Table 3
Comparison of the kinetic parameters of C. crenata lipase with those of previously reported lipases.

| Lipase source          | Substrate | $K_{m}$ (mM) | $k_{cat}$ (min⁻¹) | $k_{cat}/K_{m}$ (mM⁻¹·min⁻¹) | Reference                   |
|------------------------|-----------|--------------|-------------------|-----------------------------|-----------------------------|
| Bacillus subtilis      | p-NPA     | 0.97         | 0.052             | 0.054                       | Acharya et al. (2004)       |
| Candida rugosa         | p-NPP     | 1.51         | 0.23              | 0.16                        | Patel et al. (2015)         |
| Castanea crenata       | p-NPP     | 0.11         | 0.221             | 1.94                        | This study                  |
| Cordyceps militaris   | p-NPP     | 0.07         | 0.29              | 4.11                        | Park et al. (2019)          |
| Cellulomonas flavigena | p-NPP     | 1.33         | 433.00            | 325.56                      | Prajapati et al. (2014)     |
| Candida antarctica    | p-NPB     | 0.41         | 305.00            | 743.90                      | Qian and Lutz (2005)        |
| Pseudomonas putrefaciens | p-NPP   | 0.16         | 208.76            | 1073.12                     | Sonkar and Singh (2020)     |
| Bacillus thermoamylovorans BHK67 | p-NPP | 7.72 | 13,636.20 | 1766.35 | Sharma et al. (2018) |
The swelled Sep-Pak C18 cartridge with 100% acetonitrile was equilibrated by desalting with distilled water. The bound peptide was eluted with 3 mL of 80% acetonitrile, dried with SpeedVac, and stored at -20°C. It was then dissolved in 15 μL of distilled water with 0.1% formic acid and injected into a liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) system. Distilled water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used for elution of the sample with a flow rate of 0.3 mL/min.

2.11. Secondary structure analysis and homology modeling

The sequences of the C. crenata lipase and three selected jacalin-related lectins from the Protein Data Bank (PDB) were subjected to multiple sequence alignment using Clustal W. Secondary structure prediction of the aligned sequences was performed using ESPript 3.0. To obtain a 3D model of the C. crenata lipase, SWISS-MODEL (https://swissmodel.expasy.org) was searched for the best template. PDB ID: 1ZGR was identified as a suitable template, and homology modeling was conducted based on it. Then, the fitting of the generated 3D model was validated using the Verify 3D tool (https://saves.mbi.ucla.edu/). After modeling, the 3D model was analyzed and visualized using PyMol software (version 2.5.2).

2.12. Statistical analysis

Statistical analysis was conducted using SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Experiments were performed in triplicate. Differences between mean values were assessed using Duncan’s multiple range test. P values < 0.05 were considered to indicate statistical significance.

3. Results and discussion

3.1. Extraction and purification

The crude enzyme solution was prepared by protein fractionation of the crude extract using ammonium sulfate, and further purified by anion-exchange and size-exclusion chromatography. The C. crenata lipase purification process is summarized in Table 1.

During the initial purification step, a 30–40% fraction of the ammonium sulfate precipitate was purified 2.7-fold with a specific activity of 0.51 mU/mg. This fraction was loaded onto a HiTrap DEAE-Sepharose Fast Flow column, and the fraction with 20–30% of buffer B showed the highest lipolytic activity, with 30.6-fold purification and specific activity of 5.82 mU/mg (Fig. 1A). The fraction containing the target enzyme was loaded onto a HiTrap Q-Sepharose Fast Flow column; the lipolytic activity was highest with a 30–40% buffer B fraction, with 230.6-fold purification and specific activity of 43.81 mU/mg (Fig. 1B). Anion-exchange chromatography of the fractionated C. crenata lipase indicated that the protein had a net negative charge at pH 8.0.

The fraction obtained from anion-exchange chromatography was applied to a size-exclusion chromatography system equipped with a HiPrep Sephacryl S-100 HR column. As shown in Fig. 1C, the enzyme solution exhibited a single peak with 465.5-fold purification and specific activity of 88.5 mU/mg. A total yield of 15.8% was achieved via the overall purification procedure. The purification and yield of the C. crenata lipase obtained in this study were higher than those for other lipases reported in previous studies. For instance, a lipase from a castor bean (Ricinus communis L. cv Hale) was purified 10.8-fold with a yield of 8.1% (Maeshima and Beever, 1985). In addition, oil palm (Elaeis guineensis) mesocarp produced a lipase that was purified 3.0-fold with a yield of 8.7% (Abigor et al., 1985), and lipase from Cellulomonas flavigena was purified 21.0-fold with a yield of 1.7% (Prajapati et al., 2014). These results suggest that C. crenata lipase can be obtained with higher purity than other lipases.

3.2. Molecular weight determination

SDS-PAGE was performed to infer the molecular mass of the lipase and verify its purity. A single band with an approximate molecular weight of 31.0 kDa, corresponding to the purified lipase, was observed (Fig. 2A). The molecular weight of the lipase was also determined by size-exclusion chromatography with the HiPrep Sephacryl S-100 HR column. The ratio of elution volume to void volume of the C. crenata lipase was 1.54. From the calibration curve of molecular weight versus the ratio of elution and void volume, the molecular weight of the C. crenata lipase was determined as 33.0 kDa (Fig. 2B).

3.3. Substrate specificity

To evaluate the substrate specificity of the C. crenata lipase, the hydrolytic activity of the lipase against various TAGs (tributyrin, trioctanoin, trilaurin, tripalmitin, and trilinolein) was examined by rhodamine B plate assay. For TAGs with chain lengths of 12–18, a change in color from orange to yellow was observed in regions of active lipases from C. crenata and Pseudomonas fluorescens (a positive control). For TAGs with chain lengths < 12, transparent areas in regions of active lipases were observed when hydrolyzed by the lipases. The results of the rhodamine B plate assay revealed that the purified lipase had hydrolytic activity against short-chain TAG (tributyrin), medium-chain TAG (trilaurin), and long-chain TAG (trilinolein) (Fig. 3). The wide range of substrate specificity could allow use of the C. crenata lipase for the production of various structured TAGs.

3.4. Effect of pH and temperature on lipase activity

The optimum pH of the C. crenata lipase was determined by evaluating lipolytic activity against trilinolein at various pH values (from 4.0 to 11.0) (Fig. 4A). The purified lipase exhibited higher lipolytic activity at pH values of 6.0–8.5; the optimum pH was 8.0 similar to the lipase from P. lipolyticum sp. nov. (Ryu et al., 2006). The pH stability of the purified lipase was evaluated based on the activity after incubation at various pH values for 24 h (Fig. 4B). The lipase retained more than 80% of its original activity at pH 7.0–9.0. However, at pH 6.0 and 10.0, the lipase showed no activity against trilinolein. These results could be due to structural unfolding and deformation of the lipase at high and low pH (Rabban et al., 2015).

The effect of temperature on lipase activity was evaluated at temperatures of 20–60°C using trilinolein as a substrate (Fig. 5A). The purified lipase exhibited increasing lipolytic activity until 35°C, showed a slight decrease from 35°C to 45°C, and then completely lost its activity at 50°C. The optimum temperature was 35°C, similar to previously reported lipases such as lipases from Pseudomonas putida DSM 9914 (Sonkar and Singh, 2020) and Thermomyces lanuginosus (Sibali et al., 2020). The Ea of the lipase-catalyzed hydrolysis, as determined by the Arrhenius equation, was 83.35 kJ/mol (Fig. 5B).

To evaluate the thermostability of the C. crenata lipase, thermal deactivation profiles were determined at various temperatures (4–70°C) (Fig. 6A). The purified enzyme retained > 60% of its original activity when incubated at temperatures lower than 50°C for 60 min. The
enzyme activity was reduced drastically (to 19%) when the enzyme was incubated at 70 °C for 20 min. The results indicated that the C. crenata lipase is thermally stable to 50 °C but loses an immense amount of its lipolytic activity at 70 °C.

The $k_d$ for the C. crenata lipase was determined from the slope of the linear regression analysis of log relative activity (%) against incubation temperature (K). The $E_d$ of the lipase, which followed first-order reaction kinetics, was determined from the slope of the Arrhenius plot, i.e., $\ln k_d$ versus the reciprocal of absolute temperature (Fig. 6B). As shown in Table 2, the $E_d$ of the C. crenata lipase was higher than that reported for other lipases, which indicated that more energy is required to denature the enzyme. In other words, the C. crenata lipase has relatively high

Fig. 8. Multiple sequence alignment of C. crenata lipase and jacalin-related mannose binding lectins. The amino acid sequence of C. crenata lipase was separated into two domains (CCL_A and CCL_B).
thermostability, which is crucial for industrial application of a biocatalyst.

3.5. Kinetic parameters

The kinetic parameters of the C. crenata lipase (V_{max}, K_{m}, and k_{cat}) were determined from Michaelis–Menten (Fig. 7A) and Hanes–Woolf (Fig. 7B) plots, with n-PNP as the substrate. The V_{max}, K_{m}, and k_{cat} values were 100.58 mU/mg, 0.11 mM, and 0.221 min^{-1}, respectively. The k_{cat}/K_{m} of the lipase was 1.94 mM^{-1} min^{-1}. Table 3 compares the kinetic parameters with those of other lipases. It should be acknowledged that the K_{m} value of the C. crenata lipase is lower than that of other lipases, such as Candida rugosa (1.51 mM) (Patel et al., 2015) and C. flavigna (1.33 mM) (Prajapati et al., 2014). Because a lower K_{m} requires a lower concentration of substrate to achieve V_{max}, the C. crenata lipase has relatively high affinity for its substrate. Thus, the lipase maintains optimum activity even at a low substrate concentration.

3.6. Effects of metal ions and surfactants on lipase activity

The effects of metal ions on the activity of the C. crenata lipase were investigated using 20 mM of Li^{+}, Mg^{2+}, Ca^{2+}, and Fe^{2+}. Metal ions can influence enzyme activity by interacting with the enzyme or substrate, which either stabilizes or destabilizes the structural conformation of the enzyme. A previous study suggested that metal ions hinder the interaction between the amino acid side chains of the lipase, thereby inducing changes within the active sites (Ebrahimipour et al., 2011). As shown in Table 4, the hydrolytic activity of the C. crenata lipase decreased to 50.7% and 74.1% of its original activity when treated with Li^{+} and Fe^{2+}, respectively. Meanwhile, Mg^{2+} and Ca^{2+} caused a slight increase in activity, to 115.4% and 108.3% of its original activity, respectively. There are only a few enzymes on which Mg^{2+} and Ca^{2+} have a positive effect in terms of activity, including lipases from Acinetobacter calcoaceticus (Wang et al., 2012) and Bacillus flexus (Niyonzima and More, 2014). Likewise, the increase of C. crenata lipase activity induced by Mg^{2+} and Ca^{2+} could be attributed to stabilization of the enzyme tertiary structure via binding with the lipase (M. H. Kim et al., 2000).

Surfactants such as Triton X-100, SDS, and Tween 20 were also selected as additives, to study their effects on lipase activity. The addition of Triton X-100 decreased enzyme activity to 88.1% of its original activity. In the presence of the anionic surfactant SDS and non-ionic surfactant Tween 20, the relative activity decreased to 39.3% and 19.7%, respectively. A similar effect of these surfactants on lipases was observed with Triton X-100 (Schmidt-Dannert et al., 1994). Another previous study proposed that SDS induced conformational changes in the active site of the lipase, which resulted in partially reversible unfolding and subsequent inactivation (Castro-Ochoa et al., 2005).

3.7. Protein identification and secondary structure prediction

Peptide mass fingerprinting analysis was performed with LC-ESI-MS/MS to identify the C. crenata lipase. After ionizing the peptide fragments produced through trypsin-treated proteolysis, each peptide fragment was identified by comparative analysis of each peak from the predicted sequence (Fig. 52). Four representative charged peptides with [M+2H]^{2+} ion of m/z 947.93,[M+3H]^{3+} ion of m/z 964.82,[M+2H]^{2+} ion of m/z 654.36, and [M+2H]^{2+} ion of m/z 856.89 (Fig. 53) were observed in the LC/MS spectra of each fragmented peptide (Table 51). The amino acid sequences obtained from peptide mass fingerprinting were identified by comparison with the Fagaceae sequence library of The National Center for Biotechnology Information (NCBI) database. The C. crenata lipase showed 97.4% sequence coverage with agglutinin from Castanea crenata Sieb. et Zucc. (Shirasawa et al., 2021), and has a molecular weight of 33.3 kDa (Fig. S4). This result indicated that the enzyme is structurally similar to a lectin that recognizes and binds reversibly to a specific mono- or oligosaccharide, and is abundant in plants (Mishra et al., 2019).

The amino acid sequence of the C. crenata lipase has low similarity with those of previously reported typical lipases, but showed high sequence similarity with jacalin-related mannose-binding lectins. The majority of known plant jacalin-related lectins consist of a single jacalin-related domain with a threefold symmetric beta-prism and three four-stranded beta-sheets. Multiple sequence alignment of C. crenata lipase and jacalin-related mannose binding lectins (jacalin, artocarpin, and mornigaM) and predicted secondary structure showed that the C. crenata lipase this protein is composed of three conserved motifs (Fig. 8). Gg loop, recognition loop, and binding loop are found at the top of beta-prism, the GXXXD motif in binding loop is a common feature of jacalin-related mannose-binding lectins (Kangawa et al., 2014).

The C. crenata lipase is considered to be a holocellulase comprised of two jacalin-related domains, based on the molecular weight and homology modeling (Fig. S5). It has been demonstrated that jacalin-related lectins are responsible for relocating the protein toward the site of pathogen attack, most likely by binding to oligosaccharide signatures typical of the infection process (Esch and Schaffarth, 2017). In addition, they are involved in resistance to abiotic and biotic stresses (Jiang et al., 2006). Interestingly, a lipase possessing lectin domains occasionally has lipolytic activity toward a TAG substrate. For instance, ricin (a lectin isolated from seeds of castor bean, Ricinus communis) composed of the two chains RTA and RTB, which play pivotal roles in ribosome inactivation and cell penetration, respectively, is considered a promising agent for cancer treatment; it was maximally active on p-NP decanoate due to the serine hydrolyase catalytic triad (RTA-Ser-221, RTA-His-40, and RTB-Asp-94) (Lombard et al., 2001; Morlon-Guyot et al., 2003). In the same manner, if we exploit both lipolytic activity and binding ability to particular receptors, the C. crenata lipase could have diverse applications in biomedical science, as well as agricultural life science, because some lectins are not toxic to humans and others are not strong hemagglutinins (Tsaneva and Van Damme, 2020).

4. Conclusions

In this study, a novel lipase was purified from C. crenata, and its catalytic properties and structural characteristics were assessed. The C. crenata lipase showed a 15.8% yield with 465.8-fold purification, and had a specific activity of 88.5 mU/mg against triolein at its optimum temperature and pH (35°C and 8.0, respectively). In enzyme kinetic analysis, the C. crenata lipase exhibited remarkable catalytic performance, i.e., high substrate affinity with a relatively low K_{m} value (0.248 mM). Interestingly, protein identification through LC-ESI-MS/MS revealed that the C. crenata lipase is structurally closely related to the jacalin-related mannose-binding lectin. Although a number of studies have examined the potential applications of lipases and lectins, little is known about lipases containing lectin domains, except ricin. We believe that exploiting both the lipolytic activity and carbohydrate-binding properties of the enzyme could lead to applications in both food and biomedical science.

CRediT authorship contribution statement

Jun Heo: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. Chang Woo Kwon: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. Juno Lee: Data curation. Haena Park: Data curation. Hyunjong Yu: Data curation. Pahn-Shick Chang: Supervision, Project administration, Funding acquisition, Writing – review & editing. All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfst.2020.10.033.

References

Ahigor, D.R., Opote, F.I., Opoku, A.R., Osagie, A.U., 2015. Partial purification and some properties of the lipase present in oil palm (Elaeis guineensis) mesocarp. J. Sci. Food Agric. 36 (7), 599–606. https://doi.org/10.1016/j.jsfa.2015.07.011.

Achariya, P., Rajakumar, E., Sankaranarayanan, R., Rao, N.M., 2004. Structural basis of selection and thermostability of laboratory evolved Bacillus subtilis lipase. J. Mol. Biol. 341 (5), 1271–1281. https://doi.org/10.1016/j.jmb.2004.06.059.

Bhuniar, H., Shaheen, U., Kadam, T., 2016. Characterization of a thermostable alkaline lipase from Bacillus sonorensis sp. Enzym. Res. 2016, 4170684. https://doi.org/10.1155/2016/4170684.

Castro-Ochoa, L.D., Rodríguez-Gómez, C., Valerio-Alfaro, G., Oliart Ros, R., 2005. Screening, purification and characterization of the thermoalkalophilic lipase produced by Bacillus thermovesiculosus CCR11. Enzym. Microb. Technol. 37 (6), 648–654. https://doi.org/10.1016/j.enzmictec.2005.06.003.

Chandra, P., Enepaa, Singh, R., Arora, P.K., 2020. Microbial lipases and their industrial applications: a comprehensive review. Microb. Cell Factories 19 (1), 169. https://doi.org/10.1186/s13239-020-01428-8.

Ebrabhimpour, A., Rahman, R.N.Z.R.A., Basri, M., Salleh, A.B., 2011. High level expression and characterization of a novel thermostable, organic solvent tolerant, 1,3-regioselective lipase from Gluconobacter sp. strain ARM. Bioren. Technol. 102 (13), 6972–6981. https://doi.org/10.1016/j.biotechbioeng.2011.03.083.

Esch, L., Schaffrath, U., 2017. An update on jacinilike lectins and their role in plant defense. Int. J. Mol. Sci. 18 (7), 1592. https://doi.org/10.3390/ijms18071592.

Ferreira, M.M., Santiago, F.L.B., Silva, N.A.G.d., Lutz, J.H.H., Fernandez-Lafuente, R., Mendes, A.A., Hirata, D.B., 2018. Different strategies to immobilize lipase from Geotrichum candidum: kinetic and thermodynamic studies. Process Biochem. 67, 55–63. https://doi.org/10.1016/j.procbio.2017.01.028.

Huang, Z., Cao, Z., Guo, Z., Chen, L., Wang, Z., Sui, X., Jiang, L., 2020. Lipase catalysis of partial hydrolysis of palm oil catalyzed by Bacillus flaxus XJU-1 and its detergent compatibility. J. Biol. Chem. 278 (19), 17006–17011. https://doi.org/10.1074/jbc.F119.108277.

Niyonzima, F.N., More, S., 2014. Biochemical properties of the alkaline lipase of Bacillus flaxus XJU-1 and its detergent compatibility. Biochimie 69 (9), 1108–1117. https://doi.org/10.1016/j.bioch.2014.12.007.

Phau, E.T., Lai, O.-M., Chuong, T.S.-Y., Tan, C.-P., Lo, S.-K., 2012. Kinetic study on partial hydrolysis of palm oil catalyzed by Rhizomucor miehei lipase. J. Mol. Catal. B Enzym. 78, 91–97. https://doi.org/10.1016/j.molcatb.2012.03.009.

Pragajee, V., Patel, V., Harel, T., Kiveti, U., Patek, K., 2014. Kinetic and thermodynamic characterization of lipase produced by Cellulosamum flaviguma UNP3. J. Basic Microbiol. 54 (4), 976–983. https://doi.org/10.1002/jobm.201300065.

Qian, Z., Lutz, S., 2005. Improving the catalytic activity of Candida antarctica lipase B by circular permutation. J. Am. Chem. Soc. 127 (39), 13466–13467. https://doi.org/10.1021/ja050932b.

Rabbani, G., Ahmad, E., Khan, M.V., Ashraf, M.T., Bhat, R., Khan, R.H., 2015. Impact of structural stability of cold adapted Candida antarctica lipase B (CalB) in relation to pH, chemical and thermal denaturation. RSC Adv. 5 (26), 20115–20131. https://doi.org/10.1039/C5RA17093H.

Ryu, H.S., Kim, H.K., Choi, W.C., Kim, M.H., Park, S.Y., Han, N.S., Oh, T.K., Lee, J.K., 2006. New cold-adapted lipase from Photobacterium lipolyticum sp. nov. that is closely related to filamentous fungal lipases. Appl. Microbiol. Biotechnol. 70 (3), 321–326. https://doi.org/10.1007/s00253-005-0058-y.

Sacchetti, G., Neri, L., Dimirgi, M., Mastrocola, D., 2008. Chemical composition and functional properties of three sweet chestnut (Castanea sativa Mill.) ecotypes from Italy. In: IV International Chestnut Symposium, vol. 844, pp. 41–46 (Beijing, China).

Schmidt-Dannert, C., Szajer, H., Stocklein, W., Menge, U., Schmid, R.D., 1994. Screening, purification and properties of a thermostable lipase from Bacillus thermocatulans. Biochim. Biophys. Acta. Lipid. Metabol. 1214 (1), 43–53. https://doi.org/10.1016/S0005-2760(93)80008-3.

Seth, S., Chakravorty, D., Dubey, V.K., Patra, S., 2014. An insight into plant lipase research – challenges encountered. Protein Expr. Purif. 95, 13–21. https://doi.org/10.1016/j.pep.2013.11.006.

Sharma, A., Meena, K.R., Kanwar, S.S., 2018. Molecular characterization and bioinformatics studies of a lipase from Bacillus thermoleovorans BTK67. Int. J. Biol. Macromol. 107, 2131–2140. https://doi.org/10.1016/j.ijbiomac.2017.10.092.

Shirinwala, K., Nishio, S., Terakami, S., Botta, R., Maruzioni, D.T., Irobe, S., 2021. Chromosome-level genome assembly of Japanese chestnut (Castanea crenata Sieb. et Zucc.) reveals conserved chromosome segments in woody rosids. DNA Res. 28 (5) https://doi.org/10.1093/dnares/diaa016.

Silhal, D., Salic, A., Tusek, A.J., Soklak, K., Zelcic, B., Tran, N.N., Hessel, V., Tisima, M., 2020. Sustainable production of lipase from Thermomyces lanuginosus: process optimization and enzyme characterization. Ind. Eng. Chem. Res. 59 (48), 21144–21154. https://doi.org/10.1021/acs.iecr.0c03429.

Sonkar, K., Singh, D.P., 2026. Biochemical characterization and thermodynamic study of lipase from psychrotolerant Pseudomonas panumiana. Bioact. Agric. Biotechnol. 28, 101686 https://doi.org/10.1016/j.bjbc.2020.101686.

Tsaneva, M., Von Damme, E.J.M., 2020. 130 years of plant lipid research. Glycocon. J. 37 (7), 533–551. https://doi.org/10.1016/j.jbc.2019.12.099942.

Vishnoi, N., Dixit, S., Mishra, J., 2020. Microbial lipases and their versatile applications. In: Arora, N.K., Mishra, J., Mishra, V. (Eds.), Microbial Enzymes: Roles and Applications in Industries. Springer Singapore, Singapore, pp. 207–230.

Wang, H., Zhong, S., Ma, H., Zhang, J., Qi, W., 2012. Screening and characterization of a novel alkaline lipase from Acinetobacter calcoaceticus 1-7 isolated from Bohai Bay in
China for detergent formulation. Braz. J. Microbiol. 43 (1), 148–156. https://doi.org/10.1590/S1517-83822012000100016.

Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M., Sporns, P., 2005. Strategies for enzyme activity measurements. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M., Sporns, P., 2005. Handbook of Food Analytical Chemistry. John Wiley & Sons, Hoboken, New Jersey, pp. 329–348.

Yadav, R.P., Saxena, R.K., Gupta, R., Davdison, W.S., 1998. Purification and characterization of a regiospecific lipase from Aspergillus terreus. Biotechnol. Appl. Biochem. 28 (3), 243–249. https://doi.org/10.1111/j.1470-8744.1998.tb00536.x.