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Lipases in the castor bean seed of Chinese varieties: Activity comparison, purification and characterization

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Abstract The lipase activities in the three main Chinese castor bean varieties [ZiBi 05 (ZB 5), TongBi 06 (TB 6), JinBi 02 (JB 2)] were investigated. Two lipases were found in extracts from the endosperms of all the three varieties. One lipase is located in the lipid bodies, showing higher activity at pH 4.5 and exhibiting the highest activity at day 1 of germination. The other lipase is located in the particulates, showing higher activity at pH 9.5 and particularly active at day 4 of germination. The lipid bodies from ZB 5 show the highest lipase activity at day 1 and pH 4.5 among the three varieties during the 8 d of germination. The acid lipase located in the lipid bodies of ZB 5 of germination 1 d was purified to homogeneity by DEAE-sepharose CL-6B and butyl-sepharose CL-4B chromatography after isolation, delipidation and solubilization, with a purification factor of 16.1 and overall activity recovery of 17.7%. The characterizations (such as optimum pH, pH stability, optimum temperature, thermal stability, effect of metal ions, organic solvents stability, substrate specificity) of the purified acid lipase were conducted in detail. A combination of the characterized properties may make this enzyme a useful biocatalyst for industrial application.

Keywords castor bean, acid lipase, lipid body, particulate, germination

Introduction

Lipases (EC 3.1.1.3) are widely distributed among animals, seeds of plants and microorganisms. They can hydrolyze triacylglycerols into fatty acids and glycerol. The isolation of new lipolytic activity from living organisms has attracted much interest because of their many potential applications in synthetic reactions. The applications of lipases in industry include the synthesis of food ingredients[1], pharmaceuticals, agrochemicals, fragrances, flavors[2], and textiles[3]. Lipases are also used as additives in detergents[4].

Compared with information of mammalian and bacterial lipases, our knowledge about plant lipases is still very limited. Some plant seeds contain a large amount of triacylglycerols. During germination, lipase formation is induced to hydrolyze the triacylglycerols. The plant seeds contain a large amount of lipids but only a small amount of water, at least during the initial phase of germination. The lipases that evolve for catalysis under these conditions can be quite useful in predominantly organic media with only small amounts of water present[5].

The lipolytic activity in castor bean (*Ricinus communis L.*) seed was found in the lipid bodies and glyoxysomal fractions. There have been several attempts to investigate the lipases of castor bean[6−8]. However, to the best of our knowledge, there is no study about the castor beans of Chinese varieties, the growing area and production of which are the second largest in the world. In this paper, we investigate the lipase activity in three main Chinese castor bean varieties during germination. We have also successfully purified and characterized the acid lipase from ZiBi 05 (ZB 5).

1 Materials and methods

1.1 Materials

Castor bean ZB 5, TongBi 06 (TB 6) and JinBi 02 (JB 2) were obtained from the Zibo Academy of Agricultural Sciences (Shandong Province, China), Tongliao Academy of Agricultural Sciences (Inner Mongolia Municipality, China) and Shanxi Academy of Agricultural Sciences (China) respectively. DEAE-sepharose CL-

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6B and Butyl-sepharose CL-4B were purchased from Pharmacia. All reagents and chemicals were analytical grade and purchased from commercial suppliers (Shanghai Chemical Company, China).

1.2 Preparation of extracts

Shoshi and Harry's method was used with some modifications[7]. Castor bean seeds were selected and soaked in running tap water (room temperature, about 20 ℃) for 24 h. The end of this inhibition period was designated as day 1 of germination. Germination was carried out on the moist filter paper in darkness at 30 ℃. Endosperm tissue was carefully removed and the rest of the seedling was discarded. Ten endosperms were washed with distilled water for three times and ground in 10 mL grinding medium containing 0.4 mol/L sucrose, 10 mmol/L KCl, 1 mmol/L EDTA, 2 mmol/L dithiothreitol, 1 mmol/L $MgCl_2$, and 165 mmol/L Tricine-HCl buffer (pH 7.5) for 10 min with a mortar and pestle, and were filtered through a layer of nylon cloth of pore size 20 μm×20 μm. The homogenate was centrifuged at 270 g for 10 min. The pellet and residues on the nylon cloth were combined and rehomogenized with 5 mL of grinding medium for 5 min in the same mortar. The mortar and the pestle were washed with 5 mL grinding medium. After filtration, the homogenate was centrifuged at 270 g for 10 min to remove the cell debris. The two supernatants were combined and fractionated into fat layer, supernatant, and particulate fractions by centrifugation at 10 800 g for 30 min. The fat layer and particulate fractions were suspended in 2 mL of grinding medium, and the supernatant fraction was recentrifugated. The resulting fat layer, particulate fractions and supernatant fractions were individually combined and resuspended in grinding medium for the measurement of lipase activity.

1.3 Analytical procedures

1.3.1 Measurement of hydrolysis activity of lipase

Lipase activity was assayed essentially by the titrimetric method[9]. Olive oil was used as substrate. The assay mixture contained 2 mL of emulsified olive oil, 2 mL of 50 mmol/L of selected buffer, and 0.1 mL of enzyme solution. The reaction was carried at 37 °C for 10 min and stopped by addition of 7.5 mL of 95% ethanol. Released fatty acids were determined by titration with potassium hydrogen phthalate standardized with 0.05 mol/L NaOH. One unit of lipase activity was defined as the amount that liberates one umol fatty acid per minute under the specific condition. A colorimetric method was also used to determine the lipase activity[10−11]. The free fatty acids released were measured as copper soaps, using 1, 5-diphenylcarbazide. Palmitic acid was used as the standard. The absorbance at 550 nm was measured with a spectrophotometer. The 1 mL reaction mixture contained 0.7 mL of selected buffer, 0.1 mL of 2 mmol/L dithiothreitol, 0.1 mL of 25 mmol/L triolein and 0.1 mL of enzyme preparation. The reaction was started by addition of enzyme and stopped by addition of 6 mL of chloroform-*n*-heptane-methanol (4:3:2, v/v) solution.

1.3.2 Estimation of protein and total lipid

Protein was assayed according to a modified Lowry method measuring light absorbance at 750 nm using bovine serum albumin as standard[12]. The lipids were extracted by the method of Bligh and Dyer[13]. The total lipid was determined by drying an aliquot of the chloroform extract and weighing the lipid residue.

1.3.3 SDS-PAGE analysis

SDS-PAGE gels of 12.5% concentration were prepared using the method of Laemmli[14]. Bio-Rad Mini protean slab-gel apparatus was used for running the gels and a lower molecular weight marker was used as the protein standard. The protein bands were visualized with Coomassie blue R-250.

1.4 Purification of acid lipase from the lipid body of ZB 5

All purification procedures were carried out at 4 °C and the lipase activity was assayed by the colorimetric method throughout the purification.

1.4.1 Isolation and delipidation of the lipid body

About 30 g of ZB 5 peeled castor bean endosperms at day 1 of germination were homogenized in 150 mL of grinding medium. The fat layer was resuspended in 10 mL of grinding medium, extracted with 50 mL of diethyl ether for 1 h, and then centrifuged at 2 000 g for 5 min. The top layer of ether was subsequently removed. The extraction process was repeated twice and the residual diethyl ether evaporated under a stream of nitrogen.

1.4.2 Solubilization of acid lipase from lipid body membrane

The protein content of the lipid body membrane preparation was adjusted to 8 mg/mL and was solubilized using 10 mmol/L 3-cholamidopropyl-dimethylammonio-l-propanesulphonate (CHAPS) by gently stirring the preparation for 1 h at a final protein concentration of 4 mg/mL. The suspension was centrifuged at 102 000 g for 1 h and the supernatant, containing solubilized acid lipase, was removed carefully. A further delipidation step was carried out to remove triacylglycerol not extracted by the initial ether and CHAPS extractions, in order to minimize the aggregation state of the solubilized protein 50 mL of 200 mmol/L sodium acetate buffer of pH 4.2 was added to 50 mL of solubilized acid lipase solution to give a resulting pH of about 4.5. The mixture was stirred gently for 3 h, and the pH of the solution was increased

carefully to 7.5 with 1 mol/L NaOH solution. After incubation of the acid lipase solution at pH 7.5 and 4 °C for more than 3 h , the released lipid components were extracted three times using 300 mL diethyl ether and the residual diethyl ether was evaporated under a nitrogen stream[15].

1.4.3 Column chromatography of delipidated and solubilized acid lipase

The delipidated and solubilized acid lipase solution was centrifugated at 10 000 g for 10 min. The supernatant was applied to a 1 cm×20 cm column filled with DEAE Sepharose CL-6B previously equilibrated with 25 mmol/L Tris-HCl buffer containing 4 mmol/L CHAPS and operated at a flow rate of 1 mL/min. The sample was eluted with the same buffer using a NaCl gradient (0–1 mol/L NaCl) and the absorbance was monitored at 280 nm. Fractions (4 mL each) containing the peak of lipase activity eluted from the DEAE Sepharose column were pooled and the concentration of ammonium sulphate was adjusted to 40% saturation at 4 °C with an equal volume of 80% ammonium sulphate stock solution (confected with 25 mmol/L Tris-HCl buffer). Then resulting solution was loaded on to a 1.5 cm×15 cm column packed with butyl-sepharose CL-4B preequilibrated with 25 mmol/L pH 7.5 Tris-HCl buffer containing 40% saturation ammonium sulphate for hydrophobic interaction chromatography. The sample was eluted with the same buffer using an ammonium sulphate gradient (40%–0% saturation) at a flow rate of 1 mL/min and the absorbance was monitored at 280 nm. Fractions (4 mL each) with the peak of lipase activity eluted from the butyl-sepharose column were collected and dialyzed in the 25 mmol/L pH 7.5 Tris-HCl buffer containing 2 mmol/L CHAPS for 48 h with three changes in the buffer at 4 °C. SDS-PAGE was applied to the dialyzed enzyme to evaluate its purity and investigate its properties.

1.5 Characterization of purified acid lipase

1.5.1 Determination of optimum pH and the pH stability

The optimum pH of the enzyme was measured at 37 °C by the titrimetric method. 0.05 mol/L sodium succinate buffer (pH 4.0–6.0) was used. To determine the pH stability of lipase, the enzyme was preincubated in different buffers for 3 h at 30 °C. The residual lipase activity was assayed by the titrimetric method.

1.5.2 Determination of optimum temperature and thermal stability

The optimum temperature of the enzyme was evaluated by measuring the lipase activity at different temperatures (25–60 °C) in 0.05 mol/L pH 4.6 sodium succinate buffer by the titrimetric method. The effect of temperature on lipase stability was determined by measuring the residual activity after 30 min of preincubation at various temperatures (30–90 °C).

1.5.3 Determination of effect of metal ions

To determine the effect of metal ions on lipase activity, enzyme assays were performed in the presence of various metal ions at a final concentration of 1 mmol/L in 0.05 mol/L pH 4.6 sodium succinate buffer by the titrimetric method. The activity assayed in the absence of metal ions served as control.

1.5.4 Stability of enzyme in organic solvents

To investigate the effect of organic solvents on lipase stability, aliquots of the enzyme in 0.05 mol/L pH 4.6 sodium succinate buffer was incubated with equal volumes of organic solvent (at a final concentration of 50%, v/v) in microfuge tubes at 30 °C and shaken at 300 r/min for 2 h. The residual activity was measured with the titrimetric method. The initial activity was assayed immediately after the addition of the solvent[16−17].

1.5.5 Substrate specificity of hydrolysis and esterification

The hydrolyzing activity of enzyme on different major and minor vegetable oils was studied using titrimetry. The esterification activity of enzyme on different fatty acids was investigated by the method of Kiran[18].

2 Results and discussions

2.1 Changes in lipid content and lipase activity of the endosperm during germination

The fresh weight of endosperm tissues of the three varieties increased slowly in the first 2 d of germination and then more rapidly, until at day 6 for ZB 5 and day 7 for JB 2 and TB 6. They are roughly 2, 2.5 and 3 times heavier than those of the dry seeds, respectively (see Fig.1).

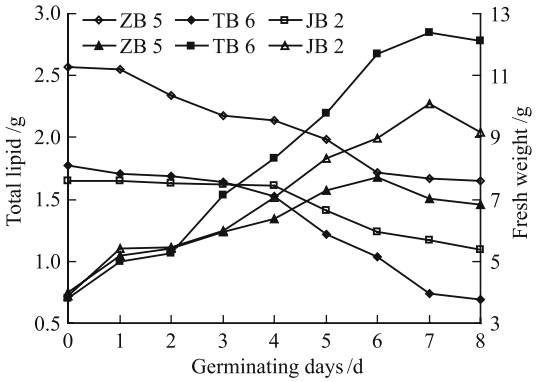

Fig.1 Changes in total lipid and fresh weight during germination (The contents were expressed on ten endosperms basis)

The total lipid of the endosperm of TB 6 remained unchanged for the first 4 d of germination and then fol-

lowed a sharp declination. About 60% of the lipid was consumed after day 8 of germination (see Fig.1). These findings are in good agreement with previous results from Shoshi and Harry[7] and Lin, *et al.*[19] However, the total lipid of ZB 5 and JB 2 changed slowly during 8 d of germination, about 40% of the lipid of these two varieties was consumed after 8 d of seedling growth (see Fig.1).

The experimental results with the isolated crude fat layer and particulate show that two lipases exist in all the three castor bean varieties: One from the fat layer shows higher activity at pH 4.5 which is present in the dry seeds and with the highest activity at day 1 of germination, and the second from the particulate showed higher activity at pH 9.5 which is particularly active at day 4 (see Fig.2).

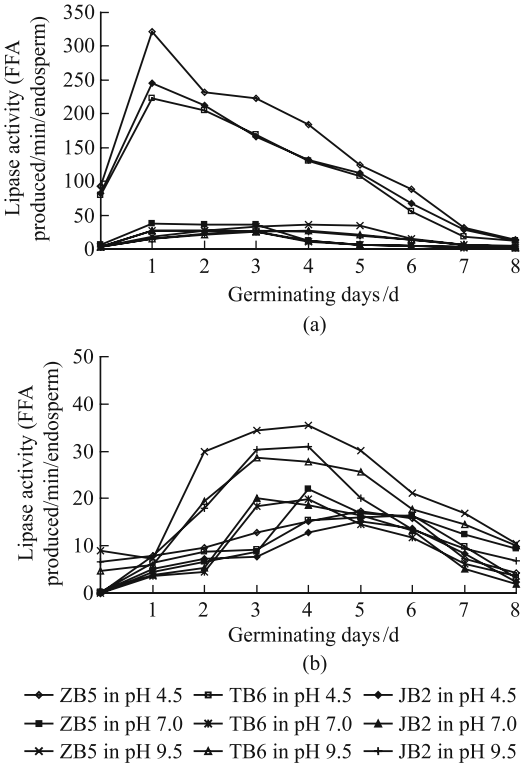

Fig.2 Changes in lipase activity in the fat layers (a) and particulates (b) of castor bean during germination. Buffers were succinate-NaOH (pH 4.5), Tris-HCl (pH 7.0) and glycine-NaOH (pH 9.5) (The enzyme activity was measured by the colorimetric method)

This result supported the observation of Shoshi and Huang and is also similar to the findings of Sanders, Abigor and Victor[20−22]. The fat layer from ZB 5 shows the highest activity at day 1 and pH 4.5, and the particulate from ZB 5 also shows the highest activity at day 4 and pH 9.5. In both the fat layer and the particulate, the lipase activity of JB 2 is lower than that of ZB 5. Lipases in TB 6 has the lowest activity(see Fig.2). To some degree, this changing trend of lipase activity is consistent with the change in total lipid content. Furthermore, the lipase activity of ZB 5 at day 1 and pH 4.5 is about 10 times higher than that of ZB 5 at day 4 and pH 9.5 (see Fig.2). Therefore, we chose the lipase from the lipid body of ZB 5 after germinating 1 day for further purification. To the best of our knowledge, this is the first investigation involving the comparison of the lipase activity among different castor bean varieties under the same condition during germination.

2.2 Purification of the acid lipase from the lipid body of ZB 5

The above experimental results demonstrate that the castor bean acid lipase is located in the lipid body which is comprised of oleosins and phospholipids[23]. In order to purify the acid lipase, the lipid in the membrane or coat of the lipid body must be removed first. Analysis of the published data on the isolation of lipase from lipid-containing seed shows that delipidation could be performed with acetone, hexane, sulfur ester and diethyl ether[24]. In the preliminary experiments, it is found that the diethyl ether shows the best effectiveness (data not shown). Once the lipid contained in the lipid body was removed by extraction with diethyl ether, the acid lipase required solubilization from the membrane or coat of lipid body before further purification. Table 1 shows that only about 28% of protein and 32% of lipase activity were recovered after solubilization with the 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS). This is not in agreement with the results of Cornella[15], in which an activity recovery of 58% was obtained. The lower recovery rate may be described by the low effectiveness of detergent, or the activity loss resulted from detergent, diethyl ether and the fluctuation of pH. Hence, choosing an effective detergent and optimizing operating conditions are necessary in order to obtain a higher activity recovery rate.

The delipidated and solubilized acid lipase is loaded on DEAE-sepharose CL-6B for further purification. Three protein peaks are eluted at about 0.2, 0.27, 0.56 mol/L NaCl and the lipase activity is located between 0.15 mol/L and 0.30 mol/L NaCl. The protein recovered in the fractions showing lipase activity was 21.8% of the sample loaded.

The key step in the purification process is hydrophobic chromatography on Butyl-sepharose CL-4B, resulting in 7-fold purification (see Table 1).

Three protein peaks are eluted at about 28%, 18% and 13% ammonium sulphate saturation, but the lipase activity is located only in the second peak. Hydrophobic chromatography is not as specific as affinity chromatography techniques, but in this case produces a good purification result. The acid lipase of castor bean is a me-

Table 1 Protein recovery and specific activity of the acid lipase from ZB 5 during the purification procedure (Total lipase activity refers to the amount which was taken on to the next stage of protein purification)

Steps	Total protein/mg	Total activity*/(unit·mg^{-1})	Specific activity/(unit·mg^{-1})	Purification/fold	Activity recovery/%
Delipidated Preparation	280.0	5 268.0	20.1	1.00	100.0
Solubilized Preparation	76.7	1 794.8	23.4	1.17	31.9
DEAE-sepharose Cl-6B	25.6	1 226.9	48.0	2.10	21.8
Butyl-sepharose CL-4B	3.1	995.3	324.2	16.10	17.7

Note:*Measured with the colorimetric method.

mbrane-bound protein. The parts of the protein molecules which normally interact with membrane lipids can also interact with the hydrophobic gel material.

The whole purification result is summarized in Table 1, the acid lipase is purified 16.1-fold and the activity recovery is 17.7%. The purification effect is to some extent improved compared with the method of Cornella because of using the key hydrophobic chromatography[15]. In addition to the activity measurements and protein determinations, the purification process was also monitored by SDS-PAGE. After the final hydrophobic interaction chromatography only one polypeptide band was observed with a molecular mass of 60 kDa .

2.3 Purified acid lipase characterization

Lipases are known to be diversified in their catalytic properties[25−26]. Therefore it is important to characterize them.

2.3.1 Optimum pH and pH stability

The purified acid lipase exhibits maximum lipolytic activity at pH 4.6. It shows more than 90% of its activity between pH 4.0 and 4.6. When pH exceeds 4.6, its activity decreases very rapidly. At pH 6.0 it only retains about 9% of its activity (see Fig.3).

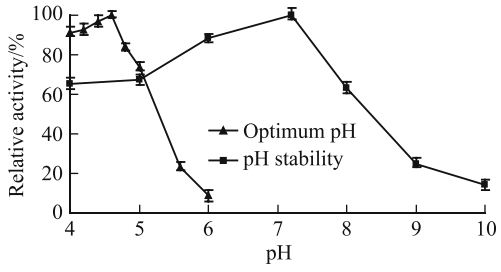

Fig.3 Optimum pH and pH stability of the acid lipase from ZB 5 (Lipase activity was measured with the titrimetric method using olive oil as the substrate at 37 °C. Buffers were 50 mmol/L succinate-NaOH (pH 4.0–6.0), Tris-HCl (pH 7.0–8.0) and glycine-NaOH (pH 9.0–10.0))

The purified acid lipase is most active at pH 4.0–8.0. It retains 65.1% of residual activity (see Fig.3) at pH 4.0. Low pH toleration for lipase activity has seldom been reported. The enzyme is stable among pH 4.0–8.0, while the highest stability is observed at pH 4.0–7.0 (see Fig.3). The remarkable stability of the castor bean lipase in this range justifies it to be a potential acidic lipase.

2.3.2 Optimum temperature and thermal stability

The optimum temperature for the hydrolysis of olive oil is 35 °C and the enzyme is active in temperature range of 25–45 °C. It retains more than 50% of its activity at 45 °C. However when the temperature surpasses 45 °C, the lipolytic activity decreased rapidly and only showed about 10% of its activity at 60 °C (see Fig.4).

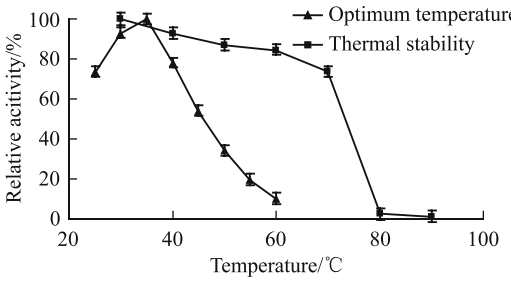

Fig.4 Optimum temperature and thermal stability of the acid lipase from ZB 5 (Lipase activity was measured with the titrimetric method using olive oil as the substrate at pH 4.6)

The purified enzyme was stable between 30 °C and 70 °C. The enzyme retained 73.5% of its activity at 70 °C. Its activity was close to zero at 80 °C (see Fig.4). The high thermal stability of this acid lipase foreshows its potential applications in the industry.

2.3.3 Effect of metal ions

As reported from studies on other lipases, a concentration as low as 1 mmol/L of some metal ions can affect enzyme activity[26−27]. Our results indicated that the purified acid lipase was almost unaffected by Ca^{2+}, Mg^{2+}, Fe^{3+} and Sn^{2+}. The enzyme was inhibited in the presence of Zn^{2+}, Co^{2+}, Pb^{2+} and Cu^{+}. Cu^{+} was the strongest inhibitor, inhibiting about 55.8% of its activity. Mn^{2+}, Na^{+}, K^{+}, Al^{3+} and Li^{+} activated the enzyme to some degree (see Table 2).

Table 2 Effects of different metal salt on the activity of the acid lipase from ZB 5

Salt	Concentration/(mmol·L^{-1})	Relative activity	Salt	Concentration/(mmol·L^{-1})	Relative activity
None	1	100.0±2.3	$FeCl_3$	1	78.8±1.9
$ZnSO_4$	1	69.2±3.5	$CoCl_2$	1	67.3±2.2
$CaCl_2$	1	98.1±2.7	$SnCl_2$	1	103.8±1.1
$MgCl_2$	1	102.0±1.3	$AlCl_3$	1	121.2±3.5
$MnSO_4$	1	128.8±4.4	LiCl	1	113.5±0.9
NaCl	1	115.4±1.8	$PbCl_2$	1	69.2±1.3
KCl	1	113.5±4.2	CuCl	1	44.2±4.1

Note: Lipase activity was measured with the titrimetric method in the presence of 1 mmol/L of different metal salt at 37 °C. All measurements were repeated three times.

2.3.4 Organic solvents stability

Lipases are known for their ability to work in aqueous as well as organic solvents. The stability of the purified acid lipase was thus investigated in various polar and nonpolar organic solvents by mixing enzyme aliquots with the solvents at 1:1 ratio for 2 h at 30 °C. The enzyme was inhibited by methanol, ethanol, propan-2-ol and butanol while remained active in all the other organic solvents (see Table 3).

Table 3 Stability of the acid lipase from ZB 5 in organic solvents

Solvents	Residual activity/%	
	After 60 min	After 120 min
Control (no solvent)	100.0±2.1	100.0±2.7
Methanol	28.6±1.3	14.2±3.2
Ethanol	35.7±4.1	0.0±2.8
Propan-2-ol	21.4±3.4	17.9±1.2
Butanol	19.6±2.2	3.6±3.7
Ethylacetate	99.9±3.3	99.8±2.7
Diethyl ether	99.9±5.3	99.7±4.7
Benzene	100.0±2.9	99.3±3.8
Toluene	94.6±4.2	91.1±2.9
Xylene	98.2±1.5	98.1±2.1
Cyclohexane	96.4±2.3	96.3±3.3
Hexane	100.0±2.9	100.0±2.2
Petroleum ether	99.9±1.7	97.4±4.3
Isooctane	100.0±1.5	100.0±2.8
Dimethyl carbonate	99.8±3.7	99.8±2.5
Diethyl carbonate	99.9±3.1	98.0±3.8

Note: Aliquots of the enzyme in 0.05 mol/L pH 4.6 sodium succinate buffer was incubated with equal volumes of organic solvent (at a final concentration of 50%, v/v) in microfuge tubes at 30 °C for 2 h and shaken at 300 r/min. The residual activity was measured with the titrimetric method. All measurements were repeated three times.

In previous studies on the use of lipase for transesterification and synthesis of esters, the reactions have been allowed to occur in media containing water immiscible organic solvents and a small amount of water because the enzymes are less susceptible to denaturation in such systems[28]. Since castor bean acid lipase retains more than 90% of its activity and exhibits fair stability in the presence of diethyl ether, hexane, isooctane, dimethyl carbonate and diethyl carbonate, the enzyme might be useful for transesterification and ester synthesis in organic solvent systems.

2.3.5 Substrate specificity

The purified acid lipase hydrolyzed the vegetable oils efficiently with hydrolysis ratio reaching above 70% at 37 °C except for the sunflower oil where the hydrolysis ratio was 46.1% (see Fig.5).

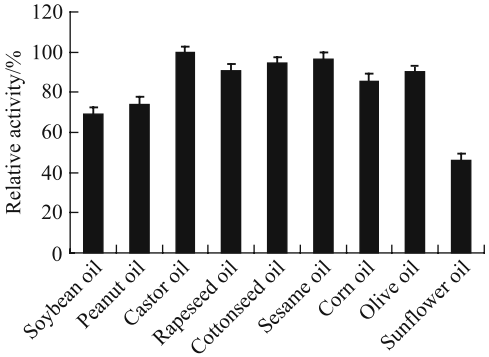

Fig.5 Hydrolysis of the acid lipase from ZB 5 on different vegetable oils (Oils emulsified with 3% polyethylene alcohol (1:3, v/v) were used as the substrates for the titrimetry)

In the oleochemical industry, enzymatic hydrolysis of oils and fats is an energy saving process compared with the conventional high-temperature and high-pressure processes, and unsaturated fatty acids can be produced without oxidation[29]. For the enzyme to be used for this purpose, the hydrolysis of oils and fats should be

done to a higher extent. Moreover, vegetable oils are currently used for the production of biodiesel in Europe and North America[30]. The acid lipase from castor bean may be suitable for this purpose because it is active and stable in various organic solvents, and has high degree of hydrolysis of vegetable oils.

The esterification specificity of the acid lipase for different substrates was also investigated. The results showed that the esterification activity for saturated fatty acid increased along with the chain-length. The esterification activity of unsaturated fatty acid was less than that of the saturated fatty acid with same chain-length. As far as the short chain alcohols were concerned, the longer the chain, the higher the esterification activity was (see Fig.6).

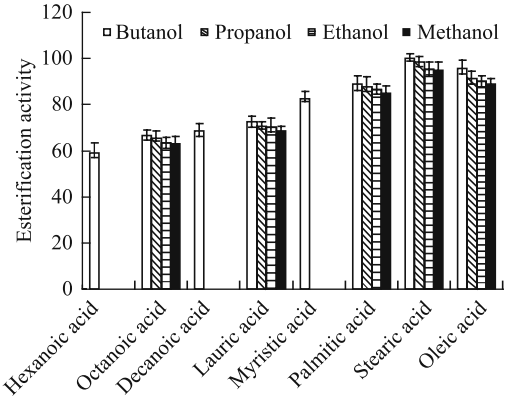

Fig.6 Effect of substrate on the esterification of the acid lipase from ZB 5 (A stock solution containing 0.16 mol/L fatty acid and 0.33 mol/L alcohol was used. The reaction was carried out at 37 °C for 6 h with shaking at 250 r/min, the residual fatty acid was titrated with 0.02 mol/L NaOH)

3 Conclusions

In this work, we investigated the lipase in the three main Chinese castor bean varieties. We found that there are two lipases in all the three castor bean varieties, one located in the lipid bodies, with a higher activity at pH 4.5, the other located in the particulate, with a higher activity at pH 9.5. The lipid bodies from the ZB 5 of germination 1 d showed the highest lipase activity among the three castor bean varieties during the 8 d of germination.

Lipase lied in the lipid bodies of ZB 5 of germination 1 d was simply purified to homogeneity by a key hydrophobic chromatography with a higher purification fold and activity recovery, which were better than the previous studies.

The purified acid lipase possessed a number of functional properties to its application as an industrial catalyst. Investigation is now in progress to test its effectiveness of producing fine chemicals in non-aqueous media in our lab.

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