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Production and Biochemical Characterization of an Extracellular Lipase from *Rhizopus chinensis* CCTCC M201021

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Abstract: The aim of the study was to investigate the extracellular lipase production by the fungus, *Rhizopus chinensis* CCTCC M201021. The biochemical characteristics of this lipase were also identified. The inducing effect of vegetable oils (soybean oil, sunflower oil, sesame oil, rice bran oil and olive oil), fatty acids (palmitic acid, oleic acid, linoleic acid and stearic acid) and surfactants (Tween 80, sodium dodecyl sulphate, gum Arabic and Triton X-100) on lipase production by *Rhizopus chinensis* CCTCC M201021 was investigated. Soybean oil enhanced lipase production by 102% and was the highest among other oils studied. There was no significant effect on lipase production by surfactants at $p<0.05$. The unsaturated fatty acids, oleic and linoleic acid significantly enhanced the lipase production by 147%. The optimum temperature for lipase activity was 30°C and the optimum pH was 6.0. The enzyme had stability in the pH range of 4.0 to 10.0 for 1 h. The enzyme retained stability of 60% of the maximum at 70°C after pre-incubation for 1 h. Zn^{2+} and Cu^{2+} ions depressed the lipase activity but Mg^{2+} and Ca^{2+} ions were found to stimulate the lipase activity. The enzyme had broad substrate specificity towards both p-nitrophenyl esters and triglyceride substrates since it efficiently hydrolyzed long-chain and short-chain fatty acids but greater preference was observed for the long-chain fatty acids. The lipase was able to hydrolyze both the 1, 3-position and the internal position of triolein.

Key words: Enzyme characterization, extracellular lipase, lipase inducers, position specificity, *Rhizopus chinensis* CCTCC M201021

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) catalyze both the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids (Peterson and Daniel, 2006) and therefore are popular biocatalysts which find more and more promising applications in the synthesis of biosurfactants, dairy industry, pharmaceutical processing, nutrition and cosmetics (Sharma *et al*., 2001). Lipase production by microorganisms that can produce lipases with special properties such as thermostability, stability in organic solvents and substrate specificities has also become an attractive research area. Only about 2% of the world's microorganisms have been tested as enzymes sources (Hasan *et al*., 2006). Finding new microorganisms, which are capable of producing enzymes such as lipases need to be investigated, which in turn can contribute to the availability of unique enzymes with interesting properties. Production studies are therefore an important area for finding the proper cultivation medium for the optimum production of a particular enzyme.

Filamentous fungi are an attractive source of industrial enzymes (Li *et al*., 2006) and they can produce lipases both by submerged and solid substrate fermentations. Fungi represent a good source of lipases since they are excreted from the fungal cell into the fermentation media and this makes their extraction easy and affordable (Silva *et al*., 2005).

The strong esterification ability of *Rhizopus chinensis* CCTCC M201021 whole-cell lipase has been successfully applied in our lab to the biosynthesis of ethyl esters of short-chain fatty acids in a non-aqueous phase (Xu *et al*., 2002). The *R. chinensis* CCTCC M201021

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whole cell lipase exhibited the highest esterification ability (100%) among other lipases studied and in addition the extracellular lipase from this strain exhibited esterification activities (88%) which are higher than that of the lipases from *Rhizopus arrhizus*, *Aspergillus niger*, *Candida lipolytica* among other (Xu *et al*., 2002). It was shown that the extracellular lipase from *R. chinensis* CCTCC M201021 could be a potential enzyme for biocatalysis application and it was therefore decided to investigate its biochemical properties and also the effect of lipase inducers on its extracellular lipase production.

Lipase production by various microorganisms has been found to be induced by fatty acids, lipids, especially by vegetable oils such as soybean, sunflower, olive, corn oil and others and also by a variety of surfactants (Espinosa *et al*., 1990; Corzo and Revah, 1999; Domínguez *et al*., 2003; Lima *et al*., 2003). Studies on the mechanisms of production of microbial lipases and the role of lipidic substances used as inducers in lipase production are scarce (Shimada *et al*., 1992). The search for new lipases with greater thermostability and various substrate specificities is continuing although many lipases have already been described (Ghosh *et al*., 1996). In the present study the effects of lipase inducers, such as oils, fatty acids and surfactants on the extracellular lipase production by *R. chinensis* CCTCC M201021 was investigated. The lipase was then partially purified and characterized to determine if the enzyme has use in further industrial research.

MATERIALS AND METHODS

The study presented here was conducted at the Laboratory of Brewing Microbiology and Applied Enzymology, Key Laboratory of Industrial Biotechnology, School of Biotechnology, Ministry of Education, Southern Yangtze University during the period 2006-2007.

Microorganism, medium and culture conditions: *Rhizopus chinensis* CCTCC M201021 (China Centre for Type Culture Collection) was maintained on potato dextrose agar (PDA) slants. The basal medium for fermentation contained 1% (w/v) maltose, 4% (w/v) peptone, 4% (w/v) soybean powder, 0.05% (w/v) magnesium sulphate heptahydrate and 0.2% (w/v) potassium hydrogen phosphate. The initial pH of the medium was adjusted to 5.5. The soybean powder used was a waste product from a soybean oil production plant. Flasks were inoculated with a spore suspension. The inoculum was prepared by washing spores off from slant with 10 mL sterile distilled water. The spore suspension was prepared using 2 mm in diameter sterilized glass beads followed by filtration through cotton wool. Flasks were then inoculated with 400 μL of spore suspension. The inoculum contained an average of 1×10^8 spores mL^{-1}. Growth experiments were performed in triplicate in 250 mL shake-flasks containing 30 mL basal medium which were incubated at 30°C and 150 rpm for 72 h in a reciprocal shaking-bed.

To the basal medium several inducers for lipase production were added. The inducers were soybean oil, sunflower oil, sesame oil, olive oil, rice bran oil, Tween 80, gum Arabic, sodium dodecyl sulphate (SDS) and Triton X-100. In addition various fatty acids (linoleic, oleic, palmitic and stearic acid) were also added as inducers to the basal medium at a concentration of 2% (w/v). Linoleic acid was separately sterilized by mircofiltration using a 0.22 μm pore size filter paper. The surfactants (gum Arabic, sodium dodecyl sulphate (SDS), Tween 80 and Triton X-100) were added at a concentration of 0.2% (w/v) and the vegetable oils at a concentration of 2% (w/v). The concentrations of the various inducers were selected based on previous studies carried out in our laboratory (Data not shown).

All data were subjected to a Duncan multiple media analysis (DMRT) and differences at a significance level of 95% were considered as effects produced by particular treatments. For each treatment, three determinations (n = 3) were performed and the mean values were calculated.

Enzyme assay: Lipase activity was determined by the olive-oil emulsion method (Xu *et al*., 2002). The reaction system consisted of 2 mL of 3% polyvinyl alcohol (PVA)/olive oil (3:1) and 2.5 mL 0.025 mol L^{-1} phosphate buffer (pH 7.5) in a 100 mL flask. Samples collected from shake flask fermentation were centrifuged at 8000 rpm for 10 min before analysis. In this assay 200 μL of supernatant was inoculated into the reaction system, which was then incubated for 15 min at 40°C. The reaction was stopped by the addition of 7.5 mL 95% ethanol. The fatty acids liberated were titrated with standard 0.05 mol L^{-1} NaOH in the presence of 25 μL of 0.2% (w/v) phenolphthalein in ethanol as indicator. One lipase activity unit was defined as the amount of enzyme that produced 1 μmole of fatty acid per minute under the assay conditions described.

Partial purification of the lipase: All purification steps were carried out at 4°C. The cell-free supernatant was prepared by collecting the fermentation broth (obtained from shake-flask fermentation) after 72 h and filtering it through several layers of cheesecloth to remove the mycelium followed by centrifugation at 12,000 x g for

30 min. The supernatant was then stored at 20°C until further use. Solid ammonium sulphate was slowly added to the filtrate under stirring to achieve 40% saturation and stirred for 3 h, which was then followed by centrifugation at 12,000x g for 15 min. The resultant pellet obtained was discarded. The resultant supernatant was mixed with solid ammonium sulphate to reach a saturation of 60% and stored at 4°C for 24 h to allow complete precipitation. The pellet was then collected by centrifugation at 12,000x g for 30 min. The resultant supernatant was filtered through a 0.45 μm membrane filter (Millipore Corporation, Bedford MA, USA) to remove insoluble materials. The supernatant was then dialyzed against 0.05 mol L^{-1} Tris-HCl buffer (pH 7.5) containing 1.2 mol L^{-1} ammonium sulphate for 12 h using a non-protein binding dialysis bag with a molecular weight cut-off of 10 kDa and then loaded onto a Phenyl Sepharose 6 Fast-Flow (GE Healthcare, Shanghai, China) column (1.6×15 cm). The column was run at a flow rate of 1 mL^{-1} min. The binding buffer consisted of 1.2 mol L^{-1} ammonium sulphate in 0.05 mol L^{-1} Tris-HCl buffer (pH 7.5) and elution was achieved with a step-wise gradient of 0.8 mol L^{-1} ammonium sulphate to 0.0 mol L^{-1} ammonium sulphate.

Protein determination: Protein measurements were carried out by the method of Lowry *et al.* (1951), using bovine serum albumin as standard.

Optimum pH and stability: Lipase activity was assayed at different pH values ranging from 4.0 to 10.0 by the titrimetric method (Xu *et al.*, 2002). The following buffers (0.05 mol L^{-1}) were used: Sodium acetate buffer (pH 4.0-5.0), sodium phosphate buffer (pH 6.0-7.0) and Tris-HCl (pH 8.0-10.0). To determine the pH stability the lipase was preincubated in different buffers ranging from 4.0 to 10.0 for 1 h at 40°C. The titration assay was then carried out to determine the lipase stability.

Optimum temperature and thermal stability: The titration assay described was used to determine the temperature optimum of the lipase which was assayed at different temperatures ranging from 20 to 80°C in a 0.025 mol L^{-1} potassium phosphate buffer (pH 7.5) and the thermal stability after pre-incubation for 1 h.

Substrate specificity: Lipase activity on different nitrophenyl esters (Sigma-Aldrich, St. Louis, MO-USA) (p-nitrophenyl, propionate (C_3), caproate (C_6), laurate (C_{12}) and palmitate (C_{16}) was studied by the method of Winkler and Stuckmann (1979). Substrate specificity of the enzyme against triacylglycerols (tributyrin (C_4) and triolein (C_{18}) and vegetable oils (sunflower oil, soybean oil, sesame oil, rice bran oil and olive oil) was determined by preparation of an emulsion by sonication of the PVA/substrate (3: 1). Relative activity was expressed as the percentage of the maximum activity.

Effect of metal ions: The lipase was incubated with various metal ions (Ba^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}) at a concentration of 5 mM in 0.025 mol L^{-1} potassium phosphate buffer (pH 7.5) for 1 h. The titration assay was then carried out to measure the residual activity. Relative activity was expressed as the percentage of the maximum activity.

Effect of organic solvents on lipase stability: The lipase was incubated in the presence of various organic solvents (methanol, ethanol, propanol, isopropanol, dodecanol, acetone, hexane, cyclohexane, heptane and dimethylsulfoxide) at a final concentration of 10.0% (v/v) in 0.05 mol L^{-1} potassium phosphate buffer (pH 7.5) for 1 h. The titration assay was then carried out to determine the lipase stability. Relative activity was expressed as the percentage of the maximum activity.

Determination of the positional specificity of the lipase: The positional specificity of the lipase was determined using High Performance Liquid Chromatography (HPLC) (Waters TM 600) of the hydrolysis products obtained with triolein. The conditions for the HPLC were followed according to Berner and Dieffenbacher (1999). The standards at a concentration of 1.0 mg mL^{-1} (Sigma-Aldrich, St. Louis, MO-USA) were triolein, oleic acid, 1, 3-diolein, 1, 2-diolein, 1-monoolein and 2-monoolein. The reaction mixture contained 1 mL triolein and 4 mL of enzyme sample (40 Units) and was incubated at 30°C and 150 rpm for 8 h. The pH of the reaction medium was kept at 7.0 to prevent acyl chain migration. The reaction products were extracted with 5 mL of solvent solution containing n-hexane/isopropanol in a volume ratio of 90: 10.

RESULTS AND DISCUSSION

Effect of vegetable oils on lipase production: Table 1 shows the influence of inducers on the lipase production (U mL^{-1}). Triglycerides are common lipase inducers and so are widely used to enhance lipase production. The addition of oils such as soybean oil, olive oil and rice bran oil significantly enhanced the lipase production at $p<0.05$ with soybean oil obtaining the highest activity (12.2 U mL^{-1}). Significant differences in lipase production were found when different oils were used. Sunflower oil and sesame oil resulted in production levels comparable

to the control. Rice bran oil and olive oil had similar effects on the lipase production. Soybean oil is cheap and easily available on the market, therefore it has potential as an inducer for lipase production by the strain studied here. The effect of oils on the lipase production by various fungal strains has been studied and it has been shown that the type of oil proved to be of great importance. Significant differences in lipase production by *Lactobacillus delbrueckii* were found when different lipids were used (El-Sawah *et al*., 1995). Lipase production by *Metarhizium anisopliae* was best induced by soybean oil, rice bran oil and olive oil among others (Silva *et al*., 2005).

Effect of fatty acids on lipase production: Significant differences ($p<0.050$) in lipase production were found with different fatty acids used (Table 1). The addition of oleic acid (C18: 1) and linoleic acid (C18: 2) enhanced lipase production significantly ($p<0.050$), which were the highest among the fatty acids studied. Stearic acid and palmitic acid also enhanced lipase production. The unsaturated fatty acids, oleic acid and linoleic acid had a more profound effect on lipase production. El-Sawah *et al*. (1995) and Hama *et al*. (2004) have mentioned the effects of fatty acids on the membrane permeability. Certain fatty acids are incorporated into the membrane and thus increasing the permeability to the enzyme. Hama *et al*. (2004) have shown that the fatty acid added to the culture of *R. oryzae* was found at the highest concentration in the membrane but it had varying effects on the activity. Oleic acid and linoleic acid may therefore have resulted in a facilitated secretion of the lipase from the fungus.

Table 1: Influence of inducers on lipase production by *R. chinensis* CCTCC M201021[A]

Inducer compound	Lipase activity (U^D mL^{-1})
Triglycerides (2% w/v)	
Control (NO, NS, NFA)[B]	6.03±0.28[a]
Soybean oil	12.20±0.49[c]
Sunflower oil	6.92±0.31[a]
Sesame oil	7.00±0.47[a]
Rice bran oil	8.75±0.35[b]
Olive oil	9.33±0.95[b]
Surfactants (0.2% w/v)	
Control[C]	12.20±0.49[d]
Tween 80	9.41±0.59[c]
Sodium dodecyl sulphate	7.93±0.10[b]
Gum Arabic	5.15±0.25[a]
Triton X-100	7.72±0.87[b]
Fatty acids (2% w/v)	
Control (NO, NS, NFA)[B]	6.03±0.28[a]
Palmitic acid (C16: 0)	12.70±0.67[c]
Oleic acid (C18: 1)	14.90±0.21[d]
Linoleic acid (C18: 2)	14.80±0.87[d]
Stearic (C18: 0)	8.83±0.71[b]

[A]Each value represents the average of three replicates, ± stands for S.D. among replicates. [B]NO: No surfactant, NS: any surfactant, NFA: Any fatty acid added. [C]Control refers to the cultures containing soybean oil. Surfactants were added to these cultures. [D]U: One lipase activity unit was defined as the amount of enzyme that produced 1 μmol of fatty acid per minute at pH 7.5 and 40°C. Means followed by same letter in same column are not significantly different according to Duncan multiple range test ($\alpha = 0.05$). Each group of inducers (triglycerides, surfactants and fatty acids) was analyzed individually by DMRT

Effect of surfactants on lipase production: There was no improvement on the production of lipase by *R. chinensis* CCTCC M201021 rather an inhibition. Tween 80, sodium dodecyl sulphate, gum Arabic and Triton X-100 resulted in a reduction in lipase production (Table 1). Domínguez *et al*. (2003) also reported that surfactants did not significantly increase the extracellular lipase production by the yeast, *Yarrowia lipolytica*. Surfactants have often been proposed as a means to increase extracellular lipase production by some microorganisms, due to their potential ability to increase cell wall permeability and/or to release cell bound enzymes (Corzo and Revah, 1999). However, their efficiency is strongly strain-dependent and sometimes they can even inhibit lipase activity (Lin *et al*., 1995).

Partial purification: The ammonium sulfate precipitation at a 40-60% saturation followed by Phenyl Sepharose 6 Fast-Flow (GE Healthcare) chromatography (Fig. 1) resulted in a 35-fold purification of the lipase with a specific activity of 26.5 U mg^{-1}. This sample was used in the investigation of the biochemical characteristics of the lipase from *R. chinensis* CCTCC M201021.

Effects of temperature and pH on lipase activity and stability: The effect of temperature on the activity and stability is shown in Fig. 2. The optimum temperature of

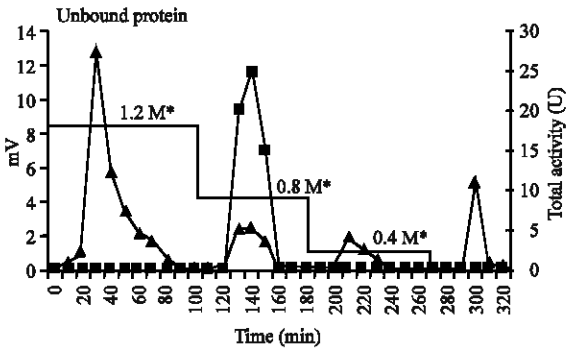

Fig. 1: Elution profile of the lipase on a Phenyl Sepharose 6 Fast Flow column. Total lipase activity (U) (■), mV (detector response at 280 nm) (▲). *Concentrations of ammonium sulphate contained in 0.025 mol L^{-1} Tris-HCl buffer (pH 7.5)

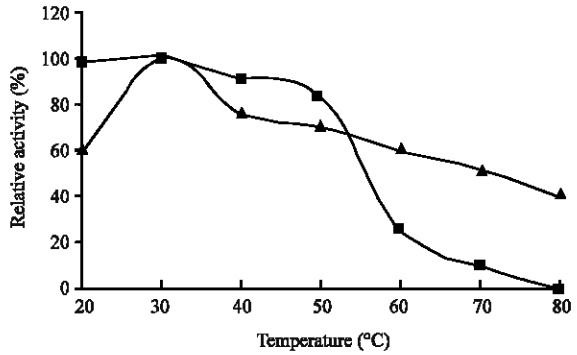

Fig. 2: Effect of temperature on activity (▲) and stability (■) of partially purified *R. chinensis* CCTCC M201021 lipase

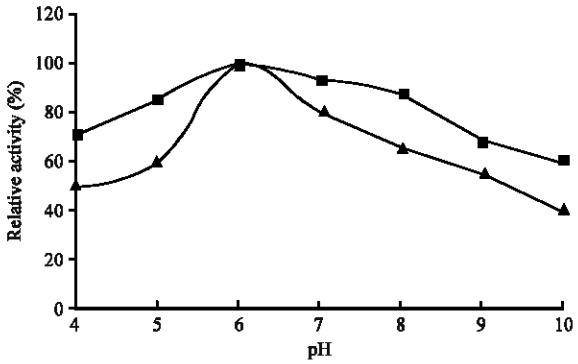

Fig. 3: Effect of pH on activity (▲) and stability (■) of partially purified *R. chinensis* CCTCC M201021 lipase

the lipase was 30°C at pH 7.5. The lipase from *R. homothallicus* from submerged fermentation (Mateos Diaz *et al*., 2006) and from *R. delemar* (Hass *et al*., 1992) also exhibited an optimum temperature of 30°C. At temperatures between 30 and 50°C the lipase had activity over the range 80 and 100%. The enzyme was not active at temperatures beyond 60°C and had 20% activity of the maximum at this temperature. The enzyme was stable up to 70°C and retained 40% of its original activity at 80°C after 1 h incubation at pH 7.5. Due to the temperature stability of *R. chinensis* CCTCC M201021 extracellular lipase; This enzyme can be classified as a thermostable lipase among *Rhizopus* strains. The basis for stability at relatively higher temperatures is still unclear, but it might be linked to the fact that the lipases are highly hydrophobic (Gordillo *et al*., 1995).

Figure 3 shows the pH optimum and pH stabilities of the extracellular lipase. It was found to have stability over a wide pH range. The extracellular lipase was highly stable at pH ranges from pH 4.0-10.0 at 40°C where it exhibited stability of 70% or more of the maximum. The remarkable pH stability makes the enzyme a potential lipase. The optimal pH was observed at 6.0 and showed reasonable activities at higher pH values having 44.3% activity of the maximum at pH 10.0. These findings are consistent with the pH optima of lipases from *R. oryzae* (Razak *et al*., 1997) and *R. rhizopodiformis* (Razak *et al*., 1997). The pH stability range for the lipase from *R. chinensis* CCTCC M201021 is wider than that reported for lipases from *R. oryzae* (Razak *et al*., 1997; Haas *et al*., 1992; Hiol *et al*., 2000) and therefore with its additional thermostability, makes it suitable for a wide range of industrial applications.

Substrate specificity: One of the most important properties of lipases is the substrate specificity towards triacylglycerols (Plow *et al*., 1996). The *R. chinensis* CCTCC M201021 lipase efficiently hydrolyzed a variety of vegetable oils (sunflower oil, soybean oil, sesame oil, rice bran oil and olive oil) at pH 7.5 and 40°C. The relative activity (%) on each of the oils was expressed as that on triolein (Table 2). Among the oils tested rice bran oil was hydrolyzed least effectively (73% relative activity). All other oils resulted in activities of above 80% of that measured against triolein. Sunflower oil (94% relative activity) and sesame oil (94% relative activity) resulted in the highest activities among the oils studied. The lipase was found to have a broad substrate-specificity, which has potential in the biocatalysis industry and has an economic potential application in the oleochemical industry. The lipase was found to have highest activity against triolein (Table 2), which proves that the lipase preferably hydrolyzes longer carbon chains. Tributyrin resulted in an activity of 60% of that of triolein.

Table 2: Substrate specificity of partially purified *R. chinensis* CCTCC M201021 lipase towards triglycerides. The reaction system consisted of 2 mL of 3% polyvinyl alcohol (PVA)/substrate (3: 1) and 2.5 mL 0.025 mol L^{-1} phosphate buffer (pH 7.5) in a 100 mL flask (Xu *et al*., 2002)

Substrate	Relative activity (%)
Sunflower	92
Soybean	90
Sesame	92
Rice bran	72
Olive	82
Triolein	100
Tributyrin	60

Table 3: Substrate specificity of partially purified *R. chinensis* CCTCC M201021lipase towards *p*-nitrophenyl esters

Substrate	Relative activity (%)[a]
p-NP propionate (C_3)	70
p-NP caproate (C_6)	81
p-NP laurate (C_{12})	100
p-NP palmitate (C_{16})	110

Duplicate data. [a]Relative activity was calculated compared to that of p-nitrophenyl laurate

Table 4: Effect of metal ions on activity of partially purified *R. chinensis* CCTCC M201021 lipase

Metal ions (5 mM)	Relative activity (%)[a]
None	100
$BaCl_2$	85
$CaCl_2$	126
$MgCl_2$	103
$ZnCl_2$	36
$CuSO_4$	21.4
$MnCl_2$	71.4

Duplicate data. [a]Relative activity was expressed as the percentage of the maximum activity.

Table 5: Effect of organic solvents on lipase stability. The lipase was incubated for 1 h at 40°C in 50 mM potassium phosphate buffer (pH 7.5) containing 10% (v/v) of various organic solvents. DMSO: Dimethyl sulfoxide

Substrate	Relative activity (%)
None	100
Methanol	82
Ethanol	107
Propanol	100
Isopropanol	120
Dodecanol	92
Acetone	93
Hexane	92
Heptane	108
Cyclohexane	107
DMSO	92

In Table 3 the relative activity of the lipase towards various p-nitrophenyl esters is shown. The lipase hydrolyzed longer chain esters of p-nitrophenyl palmitate (110% relative activity) and laurate (100% relative activity) more efficiently than the shorter ones of p-nitrophenyl caproate (81% relative activity) and propionate (70% relative activity). The short-chain esters were hydrolyzed to above 70% relative activity showing that the enzyme has broad-substrate specificity.

Effect of metal ions on lipase activity: The effect of various metal ions was tested at 5 mM in 0.025 mol L^{-1} potassium phosphate buffer at pH 7.5 and 40°C (Table 4). Zn^{2+} and Cu^{2+} ions depressed the lipase activity but Mg^{2+} and Ca^{2+} ions were found to stimulate the lipase activity especially Ca^{2+} up to 126%. These findings are consistent with those reported for lipase from *Yarrowia lipolytica* (Yu *et al*., 2007). Ba^{2+} and Mn^{2+} retained activity of 85% and 71% respectively after 1 h incubation.

Stability in organic solvents: The lipase had good stability (above 80%) in both water-immiscible organic solvents (cyclohexane, dodecanol, heptane and hexane) and non-water-immiscible organic solvents (acetone, ethanol, isopropanol, methanol and propanol) at a concentration of 10% (v/v) (Table 5). Ethanol, isopropanol, heptane and cyclohexane enhanced the enzyme stability by 7 to 8% at pH 7.5 and 40°C. The stability of the *R. chinensis* CCTCC M201021 lipase in organic solvents makes it an ideal enzyme for biotransformations in organic solvents and other applications such as enzyme precipitation and improvement of the enantioselectivity of the enzyme.

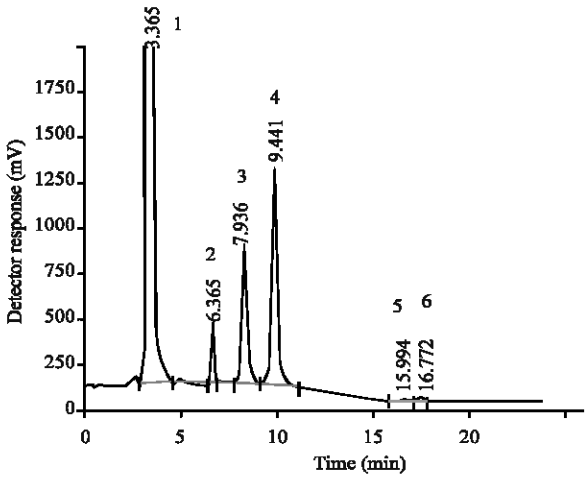

Fig. 4: HPLC analysis of triolein hydrolysis products generated by *R. chinensis* CCTCC M201021 extracellular lipase. Peak 1: triolein; Peak 2: oleic acid; Peak 3: 1,3-diolein; Peak 4: 1,2-diolein; Peak 5: 1-monoolein; Peak 6: 2-monoolein

Positional specificity of the lipase: HPLC analysis of the hydrolysis products produced after reaction with *R. chinensis* CCTCC M201021 extracellular lipase and triolein at 30°C for 8 h showed peaks of oleic acid at 6.365 min, 1, 3-diolein at 7.936 min and 1, 2-diolein at 9.441 min, 1-monoolein at 15.994 min and 2-monoolein at 16.772 min (Fig. 3). From this data it can be concluded that the extracellular lipase from *R. chinensis* CCTCC M201021 is able to hydrolyze both the 1, 3-position and the internal position of triolein. *Rhizopus* lipases described in literature are only able to hydrolyze the 1, 3-position of triolein except the lipase from *R. homothallicus* (Mateos Diaz *et al*., 2006), which was also able to hydrolyze the internal position of triolein.

CONCLUSIONS

In this research, the effect of inducers on the production of extracellular lipase by *R. chinensis* CCTCC M201021 was investigated followed by the partial purification and biochemical characterization of the lipase. Fermentation studies are necessary for each newly isolated strain since each strain can have different responses towards media components and common lipase inducers. The type of oils used during fermentation proved to be important for lipase production by

R. chinensis CCTCC M201021. It is the first time that the attempt has been made to characterize the extracellular lipase from the fungal strain, *R. chinensis* CCTCC M201021. The enzyme's high temperature and pH stability and its good stability in organic solvents make it a potential enzyme in a variety of other industrial applications. The lipase from *R. chinensis* CCTCC M201021 is able to hydrolyze both the 1, 3-position and the internal position of triolein, which is an exception when compared to other *Rhizopus* lipases that are usually 1, 3-position specific. The strain studied here has potentially useful properties, which are useful for further industrial research.

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