POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, No 4, pp. 3–8

SYNTHESIS, PROPERTIES, AND APPLICATION OF LIPASE FROM *CANDIDA ANTARCTICA* FOR HIGH YIELD MONOACYLGLYCEROL BIOSYNTHESIS

Marek Adamczak

Chair of Food Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn

Key words: *Candida antarctica*, esterification, lipase, monoacylglycerol

Synthesis of extracellular lipases by *Candida antarctica* was induced by adding fat or oil to the culture medium. The highest lipase activity (9.1 U) was obtained after 72 h of cultivation of yeast in medium supplemented with 2% (v/v) 1,2,3-trioleoyl-*sn*-glycerol. On the basis of SDS-PAGE analysis, *Candida antarctica* synthesized two forms of lipase having molecular weight of 36 and 52 kDa. Lipase from *Candida antarctica* showed the highest activity at pH 8.0 and temperature of 35°C and was stable in this range of temperature and pH. Activity of *Candida antarctica* lipase was higher on substrates having short-chain and medium-chain fatty acids than on these containing long-chain fatty acids. Using *Candida antarctica* lipase immobilized on Celite 545 for the synthesis of monoacylglycerols, 100% of conversion was obtained after esterification of oleic acid with isopropylidene glycerol. After hydrolysis of protected monoacylglycerols with trifluoroacetic acid, pure monoacylglycerols were obtained.

INTRODUCTION

In the last decade, the application of lipases in organic synthesis has become increasingly important [Bornscheuer & Kazlauskas, 1999]. However, what has to be pointed out, beginning of utilization of enzymes in organic media can be found for the first time in 1930s in papers presented by a Polish scientist Ernest Aleksander Sym [1933; 1936]. This fact described by Halling and Kvittingen [1999] started an interesting discussion between the Authors of this paper and Klibanov [Klibanov, 2000; Kvittingen, 2000].

Lipase has been widely used for lipid modification and synthesis of special compounds, *e.g.* pharmaceuticals, polymers, biodiesels, biosurfactants *i.e.* mono- and diacylglycerols (MAG, DAG) [Kazlauskas & Bornscheuer, 1998].

The MAG are used as desirable emulsifiers in the food, pharmaceuticals and cosmetics industries [Bornscheuer, 1995]. They are usually synthesized by chemical glycerolysis but the reaction is carried out at high temperatures (above 200°C) with alkaline catalyst used for commercial production of MAG. The products of the reaction have an undesired darkcolour, burnt taste and molecular distillation has to be used to remove the by-products [Yamane, 1999].

The utilization of lipases to catalyze the glycerolysis of oils and fats increased because of the mild reaction conditions and possibility of obtaining pure MAG. Several approaches have been presented for enzymatic synthesis of MAG, but they are not ideal and appropriate lipase is needed [Akoh *et al*., 1992; Bornscheuer *et al*., 1996; Millqvist Fureby *et al*., 1994; Plou *et al*., 1996; Tuter *et al*., 1999]. On the basis of our earlier results monoacylglycerols were obtained by enzymatic glycerolysis in medium with high substrate concentration with yield of about 45% [Adamczak & Bednarski, 1994].

The aim of the presented work was to obtain and characterize crude lipase synthesized by *Candida antarctica* (CAL) and to verify its ability to catalyze the reaction of monoacylglycerol synthesis.

MATERIALS AND METHODS

All chemicals were purchased from Sigma, Merck, Bio-Rad or Aldrich, at the highest purity available. For the HPLC analysis, HPLC-grade solvents were used.

Synthesis of lipase by *Candida antarctica*. The lipase was synthesized by *Candida antarctica* ATCC 28323 maintained on YPEG medium with agar (2% (w/v) yeast extract, 1% (w/v) peptone, 2% (w/v) glucose). The inoculum was prepared in liquid YPEG medium. The batch cultivation was performed by the submerged shake method in 500 cm^3 Erlenmeyer flasks filled with 100 cm^3 of the cultivation medium. The main component of the cultivation medium was corn steep liquor supplemented with 2% (w/w) of 1,2,3-trioleoyl-*sn*-glycerol or olive oil or soybean oil or oleic acid or waste poultry fat or glucose or saccharose or starch. Fatty acid composition of waste poultry fat was described by Bednarski *et al*. [1994]. The culture medium was sterilized at 121°C for 20 min and cultivation was performed at 30°C in a shaker (G-25, New Brunswick Scientific) at 200 rev/min. Every 24 h, the samples were taken for the determination of biomass growth, extra- and intracellular lipolytic activity and culture liquid acidity.

Author's address for correspondence: Marek Adamczak, Katedra Biotechnologii Żywności, Uniwersytet Warmińsko-Mazurski w Olsztynie, ul. J. Heweliusza 1, 10-718 Olsztyn, tel./fax: (48 89) 523 38 38, e-mail: Marek.Adamczak@uwm.edu.pl

Biomass determination. Biomass concentration was measured by dry cell determination at 105°C after culture liquid centrifugation (3 000 g for 10 min) and cell washing with hexane to remove the remaining fat.

Lipase separation. The lipolytic preparation from *Candida antarctica* (CAL) was obtained by protein precipitation from post-culture medium, adding chilled acetone (-20°C) in proportion 1:1 (v/v).

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis analyses were performed with MiniProtean electrophoresis unit (Bio-Rad Laboratories, Richmond, CA, USA). Solution of crude CAL (50 U and 20 U) was mixed with SDS-sample buffer. After heating at 95°C for 5 min, samples were separated on polyacrylamide gel (12.5%) within stacking gel (4%). The gels were stained for protein detection with Coomassie Brilliant Blue or by a silver staining procedure.

Native polyacrylamide gel electrophoresis. Solution of lipase was mixed with sample buffer. Sample was separated on polyacrylamide gel (7.5%) with a stacking gel (4.5%). For zymogram preparation, proteins were first renaturated by a 12-h incubation in a Triton X-100 solution (0.5% in 0.1 M Tris-HCl buffer, pH 7.5). Next the gel was incubated in a mixture of freshly prepared solution A and B (1:1) for 2 h, where A: 20 mg α-naphtyl acetate dissolved in 5 cm^3 acetone, followed by the addition of 50 cm^3 of 1 M Tris-HCl (pH 7.5); B: 50 mg Fast Red TR salt dissolved in 50 cm^3 of 1 M Tris-HCl (pH 7.5) [Krebsfanger *et al*., 1998].

Lipolytic activity and stability assay of CAL by pH-stat method. The lipolytic activity was determined in a medium (extracellular activity) and in a supernatant after cell disruption by French press (Biotek). A pH-stat titration method with the use of the Titroline alpha (Schott) titration set was used. The substrate used was emulsion composed of 5% (w/v) of olive oil (Sigma), 2% (w/v) of arabic gum, 20 mM $CaCl_2$ and 50 mM NaCl. The emulsion was prepared with homogenizer (Ultraturrax T25, IKA) for 5 min at 24 000 rpm. The reaction was performed at 37°C and pH 8.0, unless the influence of the different parameters on lipolytic activity was to be determined (pH, temperature optimum and stability). Lipolytic activity unit (U) was defined as the amount of enzyme that liberates 1 μmol equivalent of free fatty acid from olive oil in 1 minute under the assay conditions.

The lipolytic preparation was characterized in respect of both its activity and stability in relation to temperature in the range of 5–60°C and acidity of pH 3–10. For determination of temperature stability, solution of CAL was incubated for 1 h in phosphate buffer (pH 8.0) at a temperature from 5 to 60°C. The effect of pH on lipase stability was analyzed after pre-incubation of lipase in 0.1 M buffers (citric buffer, pH 3–6; phosphate buffer, pH 7–8; glycine buffer, pH 9–10) at 4°C for 24 h. Residual activity was measured using pH-stat method.

Activity of CAL towards different substrates. Substrate specificity towards sunflower oil, soybean oil, olive oil, poultry fat, milk fat, tributyrin, triolein was determined by pH-stat assay.

Photometric assay of CAL activity. Lipase activity was also measured by the photometric assay with *p*-nitrophenylpamitate (pNPP), *p*-nitrophenylmirystate (pNPM), and p-nitrophenylobutyrate (pNPB) according to Winkler and Stuckmann [1979]. One activity unit (U_s) was defined as the amount of enzyme necessary to release one mmol of *p*-nitrophenol per min under assay conditions (35°C, pH 7.0).

Monoacylglycerol synthesis. Free and immobilized on Celite 545 CAL was used for MAG synthesis. Activated Celite (washed with water, ethanol (95%) and 5% HNO_3 then dried overnight at 80°C) was mixed with the lipase solution and then acetone, chilled to -20°C, was added to obtain immobilized lipase. The esterification of glycerol or 1,2-*O*-isopropylidene-*rac*-glycerol (IPG) by oleic acid was performed (Figure 1). The reactions were performed in solvent free system. The molar ratio of the reaction components was 1:1. The reaction was carried out for 24 h at 35°C. Molecular sieve 4 Å was added to the reaction mixture after 4 h of incubation to remove water formed during the reaction.

The water concentration in the reaction environment was regulated by dehydrating reaction substrates with 4 Å (Sigma) molecular sieves and in CAL by drying over P_2O_5. The water content in the reaction environment was determined by the Karl Fisher method with the application of the Titroline alpha set (Schott).

The acetonide hydrolysis was performed with either hydrochloric acid (50% v/v HCl at 75°C for 1 h) or trifluoroacetic acid (TFA) (90% v/v TFA at room temperature for 10 min). After cleavage, the product was extracted in a *tert*-butyl-methylether and rinsed with water.

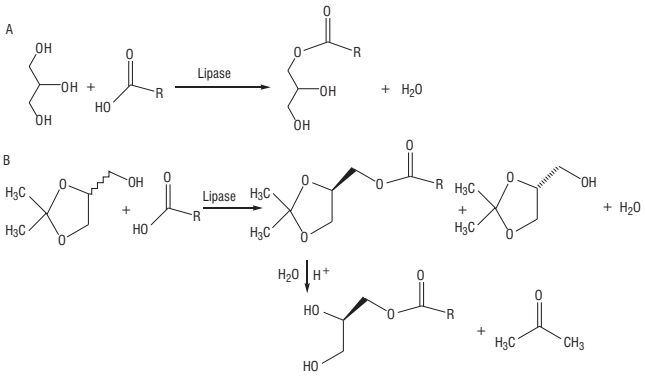


FIGURE 1. Synthesis of monoacylglycerols by (A) esterification of glycerol and free fatty acid, and (B) esterification of 1,2-*O*-isopropylidene-*rac*-glycerol and further hydrolysis of ester.

Extraction and analysis of reaction mixture. The lipase was removed from the reaction mixture by centrifugation at 3 000 H×g for 10 min. The reaction products were extracted with petroleum ether and both thin layer chromatography (TLC) and the high-performance liquid chromatography (HPLC) was used to monitor the progress of the reaction. The sample of reaction mixture was spotted on the silica gel 60 plates developed with hexane:diethyl ether:acetic acid (80:20:2 v/v). The bands were visualized under ultraviolet

light after spraying the plates with 2,7-dichlorofluorescein.

The HP 1050 system (Hewlett Packard) with the SUPELCOSIL™ LC-18 (25 × 4.6, 5 mm) column connected to the reflactive index detector and diode array UV detector (206 nm) was used. The mobile phase was acetone: acetonitrile 50:50 (v/v). The column was heated to reach 45°C, and the solvent flow rate was programmed according to the flow gradient: 0.5 cm^3/min for 7 min, increased to 3 cm^3/min within 3 min and held for 6 min before decreasing the solvent flow rate to 0.5 cm^3/min within 4 min.

Data are presented on the graphs as the mean value with error bars representing the standard deviations.

RESULTS AND DISCUSSION

Synthesis and properties of CAL

Lipases are common enzymes playing an important role in lipids metabolism and can be obtained from mammalian, bacterial, fungal and plant sources. Screening for the lipases is being expanded from the conventional animals and plants to microbial sources. Microbial lipases are very diverse in their enzymatic properties, which makes them useful for specific application [Jaeger & Reetz, 1998]. Microbial lipases are mostly extracellular and can be constitutive or inducible enzymes [Hemachander *et al*., 2001; Ito *et al*., 2001; Lin *et al*., 2001; Pratt *et al*., 200]. Induction of lipase biosynthesis can be performed in the medium supplemented with lipase substrate, products of the reaction catalyzed by lipases or in the presence of substrate analogues [Adamczak & Bednarski, 1996; Dalmau *et al*., 2000; Jaeger *et al*., 1994; Maia *et al*., 2001].

The highest value of extracellular lipolytic activity was obtained after cultivation of *Candida antarctica* in the medium supplemented with 2% 1,2,3-trioleoyl-*sn*-glycerol. The extracellular lipolytic activity after 72 h of culturing was 9.1 U (Figure 2). The lipolytic activity of *Candida antarctica* in the medium supplemented with other lipid substrates was between 1.3–2.3 U.

The lowest values of lipolytic activity were obtained in the medium supplemented with carbohydrates and the lipolytic activity was not detected in the medium supplemented with saccharose and starch (data not published). Factors controlling lipase synthesis and transport have been investigated only in a few cases, *e.g.* the production of lipase by *Pseudomonas* sp. has been shown to be strongly induced by triacylglycerols and detergents and not repressed by glucose or glycerol [Gilbert *et al*., 1991]. Dalmau *et al*. [2000] indicated the request for lipidic substrates and the inhibition effect of glucose in the production of lipase by *Candida rugosa*.

Hoshino *et al*. [1991] analyzed lipolytic activity of *Aspergillus oryzae* cell homogenate and found that 70.7% of the lipase activity was present in cell wall, 13.0% in nuclear, 11.1% in microsome, 2.6% in mitochondria, and 2.4% in cytosol.

Candida antarctica synthesized lipases which are mostly secreted into the medium and as little as 0–8% of the lipases activity was detected in the cell mainly at the end of the lag-phase (Figure 2).

Candida antarctica produces two different lipases, named A and B [Hoegh *et al*., 1995]. *Candida antarctica* lipase B is one of the most powerful catalysts very active towards a broad range of esters, amides, and thiols [Anderson

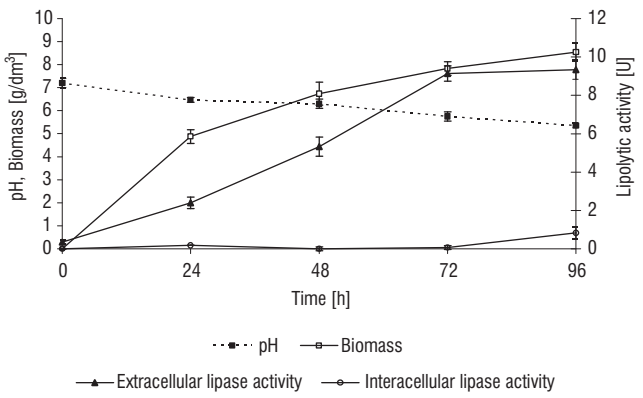


FIGURE 2. Time course of lipase synthesis by *Candida antarctica* in medium supplemented with triolein.

et al., 1998]. The CAL separated by precipitation with acetone also showed two bands by electrophoresis analysis (Figure 3). SDS-PAGE analysis showed two protein bands with molecular weight of about 36 and 52 kDa (Figure 3, 2–4). These two bands correspond to CAL-B (36 kDa) and CAL-A (52 kDa). According to the information from Swiss Prot (P41365), precursor of CAL-B has a molecular weight of 35 517 Da and according to Weber *et al*. [1995] its weight is 33 KDa, while the molecular weight of CAL-A was reported to be 45 kDa [Patkar *et al*., 1993]. Analysis by native gel electrophoresis and activity staining confirms the presence of two isoenzymes of CAL in crude preparation (Figure 3, 1).

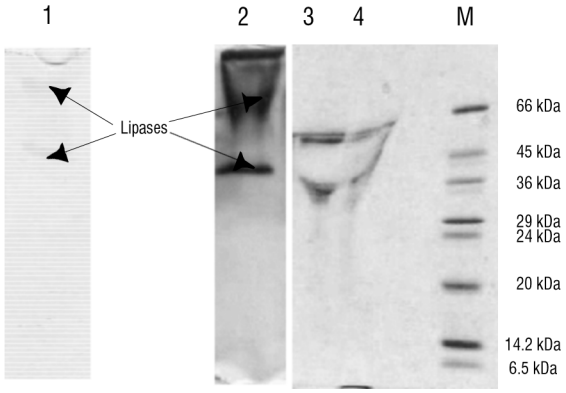


FIGURE 3. Native PAGE (left) and SDS-PAGE (right) analysis of CAL. M – molecular weight standard (bovine serum albumin, 66 kDa; chicken egg ovalbumin, 45 kDa; rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; bovine erythrocytes carbonic anhydrase, 29 kDa; bovine pancreas trypsinogen, 24 kDa; soybean trypsin inhibitor, 20 kDa; bovine milk α-lactalbumin, 14.2 kDa; bovine lung aprotinin). 1 – activity staining of CAL; 2 – silver staining of CLA; 3, 4 – Commassie Brilliant Blue staining of CAL, 50 U and 20 U, respectively.

From the supernatant, after 96 h of cultivation by precipitation with acetone concentrated enzyme preparation was obtained with the activity of 50 U (224 U/mg of protein).

Lipase from *Candida antarctica* showed the highest activity at pH 8.0 and the enzyme was stable at pH range of 7–9 (Figure 4). The optimum temperature for the hydrolytic activity of CAL was found to be 35°C (Figure 5). Hydrolytic activity of CAL decreased sharply at temperatures above 45°C after 1 h of storage (Figure 5).

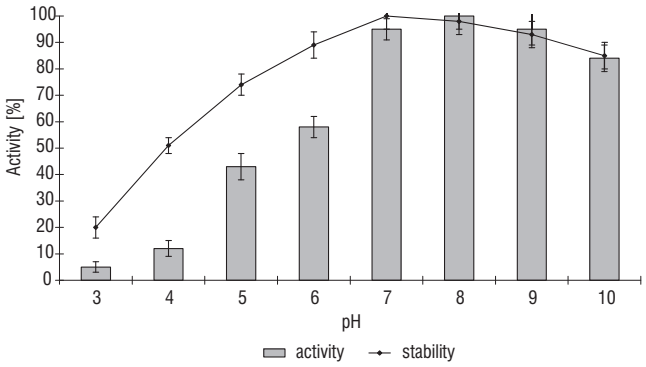


FIGURE 4. Effect of pH on hydrolytic activity and stability of the lipase from *Candida antarctica*.

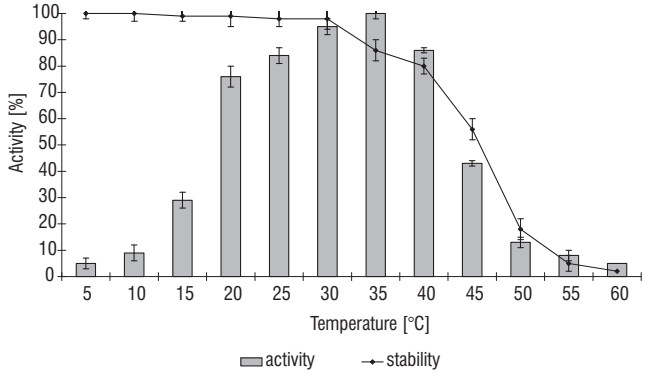


FIGURE 5. Effect of temperature on hydrolytic activity and stability of lipase from *Candida antarctica*.

The CAL-B shows little or no interfacial activation and considering, the shape of the binding site proposed by Pleiss *et al.* [1998] only fatty acid having maximum 13 carbons can bind completely inside the binding pocket. CAL showed high activity towards tributyrin (629.7 U) and smaller activity towards other triacylglycerols (from 14.9 to 147.5 U) (Figure 6). It was also confirmed by photometric assay. CAL shows higher activity towards pNPB than pNPP, 285.5 U_s and 25 U_s, respectively. Based on this observation, CAL can be classified as esterase.

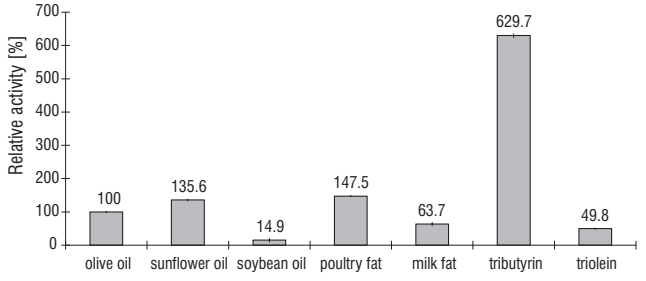
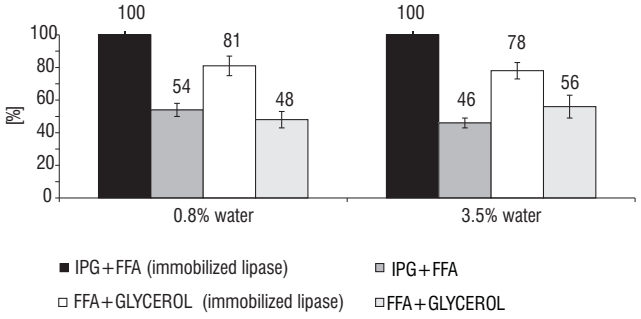


FIGURE 6. Activity of CAL towards different substrates.

Monoacylglycerols synthesis

So far, one paper only has been published describing the use of commercial lipase from *Candida antarctica* (SP 382, Novo Nordisk, Inc.) for the synthesis of MAG by esterification of IPG with free fatty acids or fatty acids methyl esters with yield from 27 to 50% (w/v) [Akoh, 1993].

In the presented experiment, the water contents in the reaction environment were 0.8 and 3.5% (w/w) and were not significantly influenced by the yield of MAG synthesis (Figure 7). After 24 h of the reaction of oleic acid with IPG catalyzed by immobilized CAL, 1,2-isopropylidene-3--oleoyl-*sn*-glycerols or 2,3-isopropylidene-1-oleoyl-*sn*--glycerol with 100% conversion were obtained. Free lipase was less effective than immobilized CAL probably because of ability of Celite to absorb water, formed during esterification as proposed also by Bellot *et al*. [2001]. As expected, in the reaction of glycerol esterification with fatty acid, a mixture of mono- (81–48%), di- (9–27%) and triacylglycerols (10–25%) was obtained.



FIRGURE 7. Conversion to protected MAG and synthesis yield of MAG by lipase from *Candida antarctica*.

The yield of the MAG synthesis decreased by approximately 45–60% when the immobilized preparations were reused. More advantageous results after reuse of immobilized CAL were obtained for the water content in the reaction mixture reaching 0.8%.

The application of TFA for the acetonide complexes hydrolysis proved to be the most efficient and the fastest method. Using this method, 100% of MAG was obtained in all experiments. Using a solution of HCl, a mixture of MAG (36±8% w/v), DAG (52±10% w/v) and TAG (12±5% w/v) was obtained. It was caused by acyl migration occurring in HCl solution. Although a following reaction of IPG with fatty acid cleavage of acetonide is necessary, this method of MAG synthesis is very attractive because of the high yield of MAG, which can be obtained.

CONCLUSIONS

The results obtained in this paper encourage further research into utilization of CAL in different reaction. Further work will be also directed into continuous method of MAG synthesis using different fatty acids or fatty acid vinyl esters (FAVE), in the medium with or without the presence of an organic solvent.

ACKNOWLEDGEMENTS

Author thanks Prof. Dr hab. Włodzimierz Bednarski (University of Warmia and Mazury in Olsztyn) for helpful comments concerning the manuscript.

This work was financially supported by the State Committee for Scientific Research, Poland (grant PBZ-KBN/021/ /P06/99).

REFERENCES

1. Adamczak M., Bednarski W., Application of enzymatic glycerolysis for production of monoglycerides from waste fats. Pol. J. Food Nutr. Sci., 1994, 3/4, 129–133.
2. Adamczak M., Bednarski W., Some factors affecting lipase production by yeasts and filamentous fungi. Biotechnol. Lett., 1996, 18, 1155–1160.
3. Akoh C.C., Lipase-catalyzed synthesis of partial glyceride. Biotechnol. Lett., 1993, 15, 949–954.
4. Akoh C.C., Cooper C., Nwosu C., Lipase G-catalyzed synthesis of monoglycerides in organic solvent and analysis by HPLC. J. Am. Oil Chem. Soc., 1992, 69, 257–260.
5. Anderson E.M., Larsson K.M., Kirk O., One biocatalyst – many applications: the use of *Candida antarctica* B-lipase in organic synthesis. Biocatal. Biotrans., 1998, 16, 181–204.
6. Bednarski W., Adamczak M., Kowalewska-Piontas J., Zadernowski R., Biotechnological methods for the up-grading and modification of animal waste fats. Acta Biotechnol., 1994, 14, 387–393.
7. Bellot J.C., Choisnard L., Castillo E., Marty A., Combining solvent engineering and thermodynamic modeling to enhance selectivity during monoglyceride synthesis by lipase-catalyzed esterification. Enzyme Microb. Technol., 2001, 28, 362–369.
8. Bornscheuer U., Gaziola L., Schmid R.D., Application of vinyl esters for the lipase-catalyzed high-yield synthesis of monoacylglycerols, 1996, *In*: Enzyme Engineering XIII (eds. S. Dordick, A.J. Russell), The New York Academy of Science, New York, pp. 757–761.
9. Bornscheuer U.T., Lipase-catalyzed synthesis of monoacylglycerols. Enzyme Microb. Technol., 1995, 17, 578–586.
10. Bornscheuer U.T., Recent advances in the lipase--catalyzed biotransformation of fats and oils. Recent Res. Devel. Oil Chem., 1999, 3, 93–106.
11. Bornscheuer U.T., 2000, Enzymes in lipid modification. Wiley-VCH, Weinheim, pp. 424.
12. Bornscheuer U.T., Kazlauskas R.J., 1999, Hydrolases in organic synthesis: regio- and stereoselective biotransformation. Wiley-VCH, Weinheim, pp. 336.
13. Dalmau E., Montesinos J.L., Lotti M., Casas C., Effect of different carbon sources on lipase production by *Candida rugosa*. Enzyme Microb. Technol., 2000, 26, 657–663.
14. Gilbert E.J., Drozd J.W., Jones C.W., Physiological regulation and optimization of lipase activity in *Pseudomonas aeruginosa* EF2. J. Gen. Microbiol., 1991, 137, 2215–2221.
15. Halling P.J., Kvittingen L., Why did biocatalysis in organic media not take off in the 1930s? TIBTECH, 1999, 17, 343–344.
16. Hemachander C., Bose N., Puvanakrishnan R., Whole cell immobilization of *Ralstonia pickettii* for lipase production. Proc. Biochem., 2001, 36, 629–633.
17. Hoegh I., Patkar S., Halikier T., Hansen M.T., Two lipases from *Candida antarctica* cloning and expression in *Aspergillus oryzae*. Can. J. Bot., 1995, 73 (Suppl. 1), S869–S875.
18. Hoshino T., Ogura T., Okano K., Inoue N., Shinano H., Inductive conditions of lipase synthesis by *Aspergillus oryzae* and its localization in a cell. Nippon Suisan Gakkaishi, 1991, 57, 511–515.
19. Ito T., Kikuta H., Nagamori E., Honda H., Ogino H., Ishikawa H., Kobayashi T., Lipase production in two-step fed-batch culture of organic solvent-tolerant *Pseudomonas aeruginosa* LST-03. J. Biosci. Bioeng., 2001, 91, 245–250.
20. Jaeger K.-E., Reetz M.T., Microbial lipase form versatile tools for biotechnology. TIBTECH, 1998, 16, 396–403.
21. Jaeger K.-E., Ransac S., Dijkstra B.W., Colson C., van Heuvel M., Misset O., Eggert T., Eipper A., Reetz M.T., Bacterial lipase. FEMS Microb. Rev., 1994, 15, 29–63.
22. Kazlauskas R.J., Bornscheuer U.T., Biotransformations with lipases, 1998, *In*: Biotechnology (eds. H.-J. Rehm, G. Reed, A. Puhler, P. Stadler), Wiley-VCH, Weinheim, pp. 37–191.
23. Klibanov A.M., Answering the question: "Why did biocatalysis in organic media not take off in 1930s". TIBTECH, 2000, 18, 85–86.
24. Krebsfanger N., Zocher F., Altenbucher J., Bornscheuer U.T., Characterization and enantioselectivity of a recombinant esterase from *Pseudomonas fluorescens*. Enzyme Microb. Technol., 1998, 22, 641–646.
25. Kvittingen L., Response from Kvittingen. TIBTECH, 2000, 18, 86.
26. Lin Y.-C., Wu J.-Y., Chen T.-L., Production of *Acinetobacter radioresistens* lipase with repeated batch culture in presence of nonwoven fabric. Biotechnol. Bioeng., 2001, 76, 214–218.
27. Maia M.M.D., Heasley A., Camargo de Morais M.M., Melo E.H.M., Morais Jr. M.A., Ledingham W.M., Lima Filho J.L., Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation. Bioresource Technol., 2001, 76, 23–27.
28. Millqvist Fureby A., Adlercreutz P., Mattiasson B., Lipase-catalyzed alkoholysis of triglycerides for the preparation of 2-monoglycerides. Enzyme Microb. Technol., 1994, 16, 1042–1047.
29. Patkar S.A., Bjorking F., Zundel M., Schulein M., Svendsen A., Heldt-Hansen H.P., Gormsen E., Purification of two lipases from *Candida antarctica* and their inhibition by various inhibitors. Indian J. Chem., 1993, 32B, 76–80.
30. Pleiss J., Fisher M., Schmid R.D., Anatomy of lipase binding sites: the scissile fatty acid binding site. Chem. Physic. Lipids, 1998, 93, 67–80.
31. Plou F.J., Barandiaran M., Caivo M.V., Ballesteros A., Pastor E., High-yield production of mono- and di--oleylglycerol by lipase-catalyzed hydrolysis of triolein. Enzyme Microb. Technol., 1996, 18, 66-71.
32. Pratt J., Cooley J.D., Purdy C.W., Straus D.C., Lipase activity from strains of *Pasteurella multocida*. Curr. Microbiol., 2000, 40, 306–309.
33. Sym E.A., Über die esterasewirkung III. Biochemi. Z., 1933, 258, 304–324.
34. Sym E.A., Eine methode der enzymatischen estersynthesen. Enzymologia, 1936, 1, 156–160.
35. Tuter M., Babali B., Kose O., Dural S., Ayse Aksoy H., Solvent-free glycerolysis of palm and palm kernel oils

catalyzed by a 1,3-specific lipase and fatty acid composition of glycerolysis products. Biotechnol. Lett., 1999, 21, 245–248.
36. Weber H.K., Stecher H., Faber K., Some properties of commercially available crude lipase preparations, 1995, *In*: Preparative biotransformation (ed. M.F. Roberts) Wiley VCH, New York, pp. 5:2.1.
37. Winkler U.K., Stuckmann M., Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J. Bacteriol., 1979, 138, 663–670.
38. Yamane T., Monoacylglycerols, 1999, *In*: Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation (eds. M.C. Flickinger, S.W. Drew), John Wiley&Sons, Inc., pp. 1810–1818.

Received July 2002. Revision received October 2002 and accepted June 2003.

OTRZYMYWANIE, WŁAŚCIWOŚCI I ZASTOSOWANIE LIPAZ Z *CANDIDA ANTARCTICA* DO WYDAJNEJ SYNTEZY MONOACYLOGLICEROLI

Marek Adamczak

Katedra Biotechnologii Żywności, Uniwersytet Warmińsko-Mazurski w Olsztynie, Olsztyn

Celem niniejszej pracy była synteza i charakterystyka surowego preparatu lipaz z *Candida antarctica* (CAL) oraz sprawdzenie jego zdolności do katalizowania reakcji syntezy monoacylogliceroli.

Obecność w pożywce tłuszczu lub oleju indukowała drożdże *Candida antarctica* do syntezy zewnątrzkomórkowych lipaz. Najwyższą aktywność lipaz (9.1 U) uzyskano po 72 godz. hodowli drożdży w pożywce z 2% (v/v) dodatkiem 1,2,3-trioleinoilo--*sn*-glicerolu (rys. 2). Na podstawie analizy SDS-PAGE wykazano, że drożdże *Candida antarctica* syntetyzowały dwie lipazy o masach cząsteczkowych 36 i 52 kDa (rys. 3). Lipazy z *Candida antarctica* charakteryzowały się najwyższą aktywnością i stabilnością aktywności w pH 8.0 i temperaturze 35°C (rys. 4, 5). Aktywność lipaz z *Candida antarctica* była wyższa w reakcjach z substratami zbudowanymi z krótkołańcuchowych i średniołańcuchowych kwasów tłuszczowych, niż z estrami długołańcuchowych kwasów tłuszczowych (rys. 6). Stosując lipazy z *Candida antarctica* immobilizowane na Celite 545 do syntezy monoacylogliceroli uzyskano 100% wydajność estryfikacji kwasu oleinowego z izopropylodieno glicerolem (rys. 7). Po hydrolizie kompleksów acetonoidowych w roztworze kwasu trifluorooctowego uzyskano czyste monoacyloglicerole.

Wyniki uzyskane w prezentowanej pracy zachęcają do prowadzenia dalszych badań nad wykorzystaniem CAL. Następne prace będą dotyczyły opracowania ciągłych metod syntezy MAG z wykorzystaniem różnych kwasów tłuszczowych lub estrów winylowych kwasów tłuszczowych (FAVE), w reakcjach prowadzonych w obecności rozpuszczalników organicznych lub bez ich udziału.