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Microbial Lipases : A Potential Tool for Industrial Applications

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Microbial lipases are currently attracting an enormous attention because of their biotechnological potential. They constitute the most important group of biocatalysts for biotechnological applications. Lipases find use in a variety of biotechnological fields such as food and dairy (cheese ripening, flavour development, EMC technology), detergent, pharmaceutical (naproxen, ibuprofen), agrochemical (insecticide, pesticide) and oleochemical (fat and oil hydrolysis, biosurfactant synthesis) industries. Lipases can be further exploited in many newer areas where they can serve as potential biocatalysts.

Key words: Lipases, industrial applications, enzymes, biocatalysts.

The demand for industrial enzymes, particularly of microbial origin, is ever increasing owing to their applications in a wide variety of processes. Enzyme-mediated reactions are attractive alternatives to tedious and expensive chemical methods. Enzymes find great use in a large number of fields such as food, dairy, pharmaceutical, detergent, textile, and cosmetic industries. With the realization of the biocatalytic potential of microbial lipases in both aqueous and non aqueous media in the last one and a half decades, industrial fronts have shifted towards utilizing this enzyme for a variety of reactions of immense importance.

Lipases have gained importance to a certain extent over proteases and amylases, specially in the area of organic synthesis. The enantioselective and regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesis of cocoa butter substituents, biofuels and for synthesis of personal care products and flavour enhancers[1,2]. Thus, lipases are today the enzymes of choice for organic chemists, pharmacists, biophysicists, biochemical and process engineers, biotechnologists, microbiologists and biochemists.

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Lipases have the remarkable ability to carry out a wide variety of chemo-region and enantioselective transformations. Their general ease of handling, broad substrate tolerance, high stability towards temperatures and solvents, high enantioselectivity and convenient commercial availability have all added to their widespread popularity among organic chemists.

A relatively smaller number of bacterial lipases have been well studied compared to plant and fungal lipases[3,4,5,6]. Bacterial lipases are glycoproteins, but some extracellular bacterial lipases are lipoproteins. Among bacteria, *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., and *Chromobacterium* sp[7] have been exploited for the production of lipases. Fungal lipases[8] are being exploited due to their low cost of extraction, thermal and pH stability, substrate specificity and activity in organic solvents. The chief producers of commercial lipases are *Aspergillus niger*, *Candida cylindracea*, *Humicola lanuginosa*, *Mucor miehei*, *Rhizopus arrhizus*, *R. delemar R. japonicus*, *R. niveus* and *R. oryzae*[7].

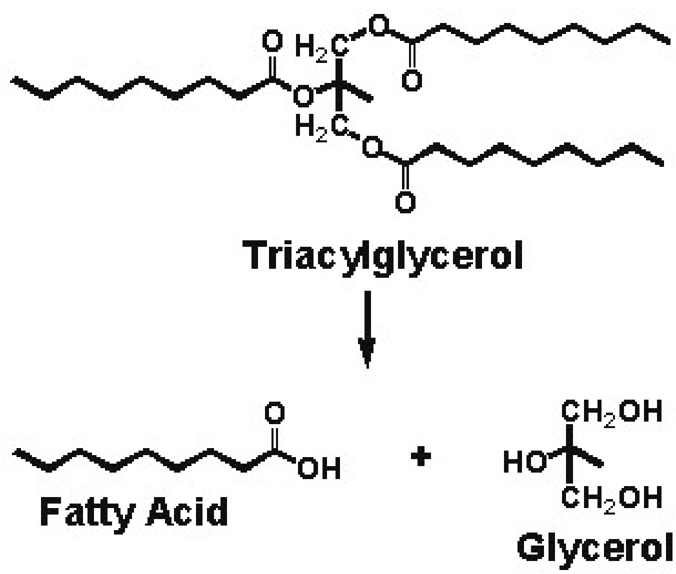

Fig 1. Schematic representation of lipase action

Applications

In the present day industry, lipases have made their potential realized owing to their involvement in various industrial reactions either in aqueous or organic systems, depending on their specificity[9,10,11,12].

Pharmaceutical and Medical applications

Lipases are important in application in pharmaceuticals in transesterificatrion and hydrolysis reaction. They play a prime role in production of specialty lipids and digestive aids[13]. The alteration of temperature during the esterification reaction drastically changes the enantiomeric values and also the stereo preference[14]. Lipases play an important role in modification of monoglycerides for use as emulsifiers in pharmaceutical applications[15]. A preparation of optically active amines that was intermediate in the preparation of pharmaceuticals and pesticides, which involved in reacting stereospecific N-acylamines with lipases, preferably from *Candida antarctica* or *Pseudomonas* sp[16]. Lipases have applications as industrial catalysts for the resolution of racemic alcohols in the preparation of some prostaglandins, steroids, and carbocyclic nucleoside analogues. Lipases from *A.carneus* and *A.terreus* show chemo and regiospecificity in the hydrolysis of per acetates of pharmaceutically important polyphenolic compounds[17,18]. Lipases are also useful in the synthesis of the artificial sweetner sucralose by regioselective hydrolysis of octa-acetylsucrose[19].

The resolution of 2-halopropionic acids, the starting materials for the synthesis of phenoxypropionate herbicides, is a process based on the selective esterification of (*S*)-isomers with butanol, which is catalysed by porcine pancreatic lipase in anhydrous hexane[20]. Another impressive example of the commercial application of lipases in the resolution of racemic mixtures is the hydrolysis of epoxyester alcohols[21]. The reaction products, (*R*)-glycidyl esters and (*R*)-glycidol are readily converted to (*R*) and (*S*)-glycidyltosylates which are attractive intermediates for the preparation of optically active *ß*-blockers and a wide range of other products. In an attempt to determine the substrate specificity for lipases, alkyl esters of 2-arypropionic acids, a class of non-steroidal anti-inflammatory drugs, were hydrolyzed with *Caenorhabditi*s *rugosa* lipase in which all transformations were highly enantioselective[22]. A similar technology has been commercialized to produce 2(*R*), 3(*S*)-methylmethoxyphenyl glycidate, the key intermediate in the manufacture of the optically pure cardiovascular drug Diltiazem[19]. Broad substrate recognition and capability for specific regioselective reactions in a variety of organic solvents makes lipases as an important biocatalyst in biomedical applications[23].

Lipases in food and dairy industry

Lipases have become an integral part of the modern food industry. The use of enzymes to improve the traditional chemical processes of food manufacture has been developed in the past few years. Stead[24] and Coenen *et al.*[25] stated that, though microbial lipases are best utilized for food processing, a few especially psychrotrophic bacteria of *Pseudomonas sp*. and a few moulds of *Rhizopus* sp. and *Mucor* sp. caused havoc with milk and dairy products and soft fruits. Lipase from *Pseudomonas strain P38* is widely used in non-aqueous biotransformation for the synthesis of n-heptane of the flavoring compound butyl caprylate[26]. Immobilized lipases from *C. antarctica* (CAL-B),

*C. cylindracea AY*30, *H. lanuginosa*, *Pseudomonas* sp. and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil Buisman *et al.*[27] .

Lipases are used extensively in the dairy industry for the hydrolysis of milk fat. Current applications include flavour enhancement of cheese, acceleration of cheese ripening,

Table 1. Industrial applications of microbial lipases

Industry	Effect	Product
Bakery	Flavour improvement and shelf life prolongation	Bakery products
Dairy	Hydrolysis of milk fatCheese ripening	Flavour agentsCheese
Fats & oils	Modification of butter fatTransesterification hydrolysis	Butter Cocoa butter, margarine Fatty acids, glycerol
Beverages	Improved aroma	Beverages
Chemical	Enantioselectivity	Chiral building blocks & chemicals
LeatherMeat and Fish	HydrolysisFlavour development & fat removal	Leather productsMeat & fish products
Paper	Hydrolysis	Paper products
Pharmaceuticals	Transestrification hydrolysis	Speciality lipidsDigestive aids
CosmeticsCleaning	SynthesisSynthesis	Emulsifiers, moisturising agentsChemicals
	Hydrolysis	Removal of cleaning agents like surfactants

manufacture of cheese-like products, and lipolysis of butter fat, and cream[28]. More recently, a whole range of microbial lipase preparations have been developed for the cheese manufacturing industry, such as those of *Mucor miehei*, *Aspergillus niger* and *A.oryzae*. A range of cheese of good quality were produced by using individual microbial lipases or mixtures of several preparations[28]. Lipases are widely used for imitation of cheese made from ewe's or goat's milk. Addition of lipases to cow's milk, generates a flavour rather similar to that of ewe's or goat's milk. This is used for producing cheese or the so-called enzyme-modified cheese (EMC). EMC is a cheese that has been incubated in the presence of enzymes at elevated temperatures in order to produce a concentrated flavour for use as an ingredient in other products such as dips, sauces, soups and snacks.

Lipases in oleochemical industry

The current trend in the oleochemical industry is a movement away from using organic solvents and emulsifiers[29,30]. The various reactions involving hydrolysis, alcoholysis, and glycerolysis have been carried out directly on mixed substrates, using a range of immobilized lipases[31]. This has resulted in high productivity as well as in the continuous running of the processes. Enzymatic hydrolysis perhaps offers the greatest hope to successful fat splitting without substantial investment in expensive equipment as well as in expenditure of large amounts of thermal energy[32]. Miyoshi Oil and Fat Co., Japan, reported commercial use of *Candida cylindracea* lipase in production of soap[33]. The introduction of the new generation of cheap and very thermostable enzymes can change the economic balance in favour of lipase use[34].

Environmental and Domestic applications

Bioremediation for waste disposal is a new avenue in lipase biotechnology. Cheng *et al*[35] characterized cold-adapted organo-phosphorus acid anhydrolases for application in the efficient detoxification of pesticide and nerve agents. A bioremediation in fat contaminated cold environment, according to Buchon *et al*[36], cold adapted lipases have great potential in the field of wastewater treatment, active compounds synthesis in cold condition. Further, more efforts are needed in identifying and cloning of novel lipase genes for environmental applications. Suzuki *et al*[37] identified a psychrotrophic strain of the Genus *Acinetobacter strain No.6*, producing an extracellular lipolytic enzyme that efficiently hydrolyzed triglycerides, such as soybean oil during bacterial growth even at 4°C. Godfrey and West[38] reported that about 1000 t of lipases are sold every year in the area of detergents. Lipase is an enzyme, which decomposes fatty stains into more hydrophilic substances that are easier to remove than similar non-hydrolyzed stains[39]. The other common commercial applications for detergents is in dish washing, clearing of drains clogged by lipids in food processing or domestic/ industrial effluent treatment plants[40]. To improve detergency, modern types of heavy duty powder detergents and automatic dish washer detergents usually contain one or more enzymes, such as protease, amylase, cellulase and lipase[41] .

Future perspectives

Today, lipases find immense applications in various areas of industrial microbiology and biotechnology. This statement is well documented by the enormous number of research investigations undertaken in the last one and a half decades. Lipases show immense versatility regarding their catalytic behaviour. Therefore, there is a lot of scope to search for newer lipases with desired selectivity and substrate tolerance. Wide and constant screening of new microorganisms for their lipolytic enzymes will open novel and simpler routes for the synthetic processes. Consequently, this may pave new ways to solve biotechnological and environmental problems. To widen the usage level of lipases, there is an urgent need to understand the mechanisms behind the lipase - catalysed reactions. The unique interfacial activation of lipases has always fascinated enzymologists and recently biophysicists and crystallographers have made progress in understanding the structure-function relationships of these enzymes.

Studies on the mechanisms of production of microbial lipases and the role of lipidic substances used as inducers in lipase production are scanty. Lipases represent an extremely versatile group of bacterial extracellular enzymes that are capable of performing a variety of important reactions, there by presenting a fascinating field for future research.

REFERENCES

1. Gerhartz, W. Enzymes in Industry: Production and Application, VCH, Weinheim. 1990; 33-74.
2. Priest, F.G. Enzymes, extracellular. *In: Encyclopedia of Microbiology* (Lederberg ed) San Diego, CA: Academic Press, 1992; 81-94.
3. Sugiura, M.: Bacterial lipases. *In: Lipases* (Borgstrom B and Brockman HL eds) New York: Elsevier Science Publishers, 1984; 505-523.
4. Sztajer, H., Borkowski, J., Sobiech, K. Purification and some properties of *Pseudomonas fluorescens* lipase. *Biotechnol. Appl. Biochem.,* 1991; **13**: 65-71.
5. Stead, D. Microbial lipases: their characteristics, role in food spoilage and industrial uses. *J. Dairy Res.,* 1986; **53**: 481-505.
6. Petersen, S.B., Drablos, F. *In Lipases: their Structure, Biochemistry and Application* (Woolley P and Petersen S B eds) UK: Cambridge University Press, 1994; 23-48.
7. Godfredson, S. E. In Microbial Enzymes and Biotechnology Fogarty W M and Kelly E T (eds) (The Netherlands: Elsevier Applied Sciences) 1990; 255-273.
8. Lawson, D.M., Brzozowski, A.M., Dodson, G.G., Hubbard, R.E., Huge-Jensen, B., Boel, E., Derewenda, Z.S. *In Lipases: their Structure, Biochemistry and Application* (Woolley P., Petersen SB eds). UK: Cambridge University Press, 1994; 77-94.
9. John, V.T., Abraham, G.: *In: Biocatalysts for Industry* (Dordick JS, ed) New York: Plenum Press, 1990; 193-197.
10. Kotting, J., Eibl, H.: *In Lipases: Their Structure, Biochemistry and Application* (Woolley P., Petersen SB eds). UK: Cambridge University Press 1994; 289-313.
11. Villeneuve, P., Foglia, T.A. Lipase specificities: Potential applications in lipid bioconversions. *Inform.,* 1997; **8**: 640-650.
12. Godfrey, T., Hawkins, D. Enzymatic modification of fats for flavour. *Eur. Food Drink Rev.,* 1991; 103-107.
13. Vulfson, E. N.: Industrial applications of lipases. *In: Lipases* (Wooley P, Petersen SB eds) Great Britain: Cambridge University Press, 1994; 271.
14. Yasufuku, Y., Ueji, S. Improvement five fold of enantioselectivity for lipase catalyzed esterification of a bulky substrate at 57°C in organic solvent. *Biotechnol., Tech;* 1996; **10**: 625-628.
15. Sharma, R., Chisti, Y., Banerjee, U.C. Production, purification, characterization and applications of lipases. *Biotechnol. Adv.,* 2001; **19**: 627-662.
16. Smidt, H., Fischer, A., Fischer, P., Schmid, R.D. Preparation of optically pure chiral amines by lipase catalyzed enantioselective hydrolysis of N-acyl-amines. *Biotechnol. Tech.,* 1996; **10**: 335-338.
17. Parmar, V.S., Kumar, A., Poonam Pati, H.N., Saxena, R.K., Davidson, S., Gupta, R. Potential use of a novel lipase from *Aspergillus carneus* in deacetylation reactions. *Biochem. Biophys. Acta.,* 1998a; **1387**: 325-330.
18. Parmar, V.S., Pati, H.N., Yadav, R.P., Kumar, A., Bisht, K.S., Gupta, R., Davidson, S., Poonam, J., Saxena, R.K. Utility of a novel lipase from *Aspergillus terreus* in deacetylation reactions. *Biocatal. Biotransform.,* 1998 b; **16**: 17-25.
19. Bornemann, S., Casselle, J.M., Combes, C. L., Dordick, J. S., and Hacking A. J. UK Patent No. 2 224 504. *Biochem. Biophys. Act.,;* 1989; **1265**: 250-253.
20. Kirchner, G., Scollar, M.P., Kilbanov, A.M. Resolution of racemic mixtures via lipase catalysis in organic solvents *J. Am. Chem. Soc.,* 1985; **107**: 7072-7076.
21. Margolin, A.L. In *Chemical Aspects of Enzyme Biotechnology* (Baldwin TO, Rauschel F M and Scott AI eds) New York: Plenum press, 1990; 197-202.
22. Botta, M., Cernia, E., Corelli, F., Manetti, F., Soro, S. Probing the substrate specificity for lipases II. Kinetic and modeling studies on the molecular recognition of 2-arylpropionic esters by *Candida rugosa* and *Rhizomucor miehei* lipases. *Biochem. Biophys. Acta.,* 1997; **1337**: 302-310.
23. Margesin, R., Feller, G., Gerday, C., Russel, N.: Cold adapted microorganisms; adaptation strategies and biotechnological potential. *In: The Encyclopedia of Environmental Microbiology,* (John Wiley & Sons eds), New York, 2002; 871-885.
24. Stead, D. Microbial lipases: their characteristics, role in food spoilage and industrial uses. *J. Dairy Res.,* 1986; **53**: 481-505.
25. Coenen, T.M.M., Aughton, P., Verhagan, H. Safety evaluation of lipase derived from *Rhizopus oryzae*: Summary of toxicological data. *Food Chem. Toxicol.,* 1997; **35**: 315-22.
26. Tan, S., Owusu, A.R.K., Knapp, J. Low temperature organic phase biocatalysis using cold-adapted lipase from psychrotrophic *Pseudomonas P38*. *Food Chem.,* 1996; **57**: 415-418.

27. Buisman, G.J.H., Helteren, C.T.W., Kramer, G.F.H., Veldsink, J.W., Derksen, J.T.P., Cuperus, F.P. Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. *Biotechnol. Lett*., 1998; **20**: 131-136.
28. Falch, E.A. Industrial enzymes-developments in production and application. *Biotechnology Advance*., 1991; **9**: 643-658.
29. Ergan, F., Trani, M., Andre, G. Solvent free triglyceride synthesis using Lipozyme TM, IM-20. *Biotechnol. Lett*., 1988;**10**: 629-634.
30. Sonntag, N.O.V. New developments in the Fatty Acid Industry in America. *J. Am .Oil Chem. Soc.,* 1984; 61: 229-232.
31. Coleman, M. H., Macrae, A, R. UK Patent No.1 577 1980; 933 (Unilever Limited).
32. Matsuo, T., Sawamura, N., Hashimoto, Y., Hashida, W. AU Patent No. 544682. 1981.
33. Mc Neill, G.P., Yamane, T. Further improvements in the yield of monoglycerides during enzymatic glycerolysis of fats and oils. *J.Am. Oil Chem. Soc.,* 1991; **68**: 6-10.
34. Macrae, A.R., Hammond, R.C. Present and future applications of lipases. *Biotechnol. Gen. Eng. Rev.,* 1985; 3 193-219.
35. Cheng, T.C., Liu, L., Wang, B., Wu, J., De Frank, J.J., Anderson, D.M., Rastogi, V.K., Hamilton, A.B. Nucleotide sequence of a gene encoding an organophosphorous nerve agent degrading enzyme from *Alteramonas haloplanktis*. *J. Ind. Microbiol. Biotechnol.,* 1997; **18**: 49-55.
36. Buchon, L., Laurent, P., Gounot, A.M., Guespin, M.J.F. Temperature dependence of extracellular enzyme production by Psychotrophic and Psychrophilic bacteria. *Biotechnol. Lett.,* 2000; **22**: 1577-1581.
37. Suzuki, T., Nakayama, T., Kurihara T., Nishino, T., Esaki, N. Cold active lipolytic activity of psychrotrophic *Acinetobacter sp. Strain no.6*. J. *Biosci. Bioeng;* 2001; **92**: 144-148.
38. Godfrey, T., West, S., The application of enzymes in industry. In: *Industrial enzymology*, (Godfrey T, Reichelt J eds). New York: The Nature Press, 1996; 512.
39. Fuji, T., Tatara, T., Minagwa, M. Studies on application of lipolytic enzyme in Detergency I. *J. Am. Oil Chem. Soc.,* 1986; **63**: 796-799.
40. Bailey, J.E., Ollis, D.F. (eds): Applied enzyme catalysis. *In: Biochemical Engineering Fundamentals.* 2nd edn. New York: Mc Graw. Hill, 1986; 157-227.
41. Ito, S., Kobayashi, T., Ara, K., Ozaki, K., Kawai, S., Hatada, Y. Alkaline detergent enzymes from Alkalophiles: Enzymatic Properties, Genetics and Structures. *Extremophiles*. 1998; **2**: 185-190.