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Biotechnological Approach of Microbial Lipase: A Review

[1]D. Sharma, [2]B. Sharma and [3]A.K. Shukla
[1]Centre for Biodiversity, Rajiv Gandhi University, Rono Hills,
Itanagar-791 112, Arunachal Pradesh, India
[2]Department of Forestry, North Eastern Regional Institute of Science and Technology,
Nirjuli-791109, (Itanagar), Arunachal Pradesh, India
[3]Department of Botany, Rajiv Gandhi University, Rono Hills,
Itanagar-791 112, Arunachal Pradesh, India

Abstract: Lipases (Triacylglycerol acylhydrolases E.C.3.1.1.3) are ubiquitous enzymes which catalyze the hydrolysis of triacylglycerols to glycerols and free fatty acids. Lipolytic reactions occur at the lipid-water interface, where lipolytic substrates usually form equilibrium between monomeric, micellar and emulsified states. Due to their high enantioselective and regioselective nature, lipases have been utilized for the resolution of chiral drugs (Flurbiprofen, Naproxen, Ibuprofen and Suprofen) fat modification, fragrance development in dairy product and for the synthesis of personal care product and cosmetics. In addition to these, lipases find use in variety of biotechnological fields such as cheese ripening, detergent, biosurfactant, bioremediation, polymer synthesis, agrochemicals and perfumery and in paper and pulp industry. Lipases are also being used for the development of biosensors for the qualitative determination of triacylglycerols. Nowadays, novel lipases are being developed by site directed mutagenesis and recombinant DNA technology to improve their selectivity and stability.

Key words: Lipase, bacteria, fungi, enzyme, biotechnology

INTRODUCTION

The field of industrial enzymes is now experiencing major R and D initiatives, resulting in both the development of a number of new products and in improvement in the process and performance of several existing products. Today nearly 4000 enzymes are known and out of these, about 200 are commercially used. According to a report from Business Communications Company, Inc. the global market for industrial enzymes was estimated at $2000 millions in 2004 (Fig. 1). Volume growth of industrial enzymes is between 4% and 5% AAGR (average annual growth rate), which is accompanied by decreasing prices, due to the increase in the number of smaller players competing in the market. As a result, the market is expected to rise at an AAGR of a little over 3% over the next 4 years and the total industrial enzyme market in 2009 is expected to reach nearly $2352 millions (Rajan, 2004). At least 75 percentages of all industrial enzymes including lipases is hydrolytic in action. It became generally accepted that lipases remain enzymatically active in organic solvent, making them ideal tools for organic chemists (Zaks and Klibanov, 1984). Several studies has been carried out to analyze the activities of microbial enzymes under different chemical, physical and environmental conditions (Shukla and Mishra, 1996, 1997; Shukla *et al*., 1989, 1996; Shukla, 2000; Salis *et al*., 2005; Shah *et al*., 2007; Sharma and Shukla, 2007, 2008a; Colin *et al*., 2010). Currently, a number of research publications have extensively discussed developments in the area of enzymes from thermophilic microbes. Adaptation of extremophiles to hot environments (Danson *et al*., 1992; Stetter, 1999), production of heat-stable enzymes from thermophiles and hyperthermophiles (Huber and Stetter, 1998; Niehaus *et al*., 1999; Sharma and Shukla, 2008a, b), structure and function relationships of thermozymes (heat-tolerant enzymes) (Zeikus *et al*., 1998a, b) and biotechnological and industrial applications of thermostable enzymes (Cowan, 1996; Holst *et al*., 1997; Hough and Danson, 1999; Eichler, 2001) are among the topics that have been studied.

Lipases catalyze the hydrolysis of triacylglycerols to glycerols and free fatty acids at the lipid-water interface, where lipolytic substrates usually from equilibrium between monomeric, micellar

Corresponding Author: D. Sharma, Centre for Biodiversity, Rajiv Gandhi University, Rono Hills, Itanagar-791 112, Arunachal Pradesh, India Tel: +91 360 2002998, +919862070025

and emulsified states (Martinelle *et al*., 1995). Two criteria have been used to classify a lipolytic enzyme as a "true" lipase (EC.3.1.1.3): (a) it should be activated by the presence of an interface, that is, its activity should increase as soon as the triglycerides form an emulsion. This phenomenon was termed as "interfacial activation" (Sarda and Desnuelle, 1958). (b) It should contain a surface loop of a protein covering the active site of the enzyme which is called as "lid" and moving away on contact with the interface (Brzozowski *et al*., 1991; Derewenda *et al*., 1992). Hydrolysis of glycerolesters with an acyl chain length of <10 carbon atoms with tributyrylglycerol (tributyrin) as a standard substrate usually indicates the presence of an esterases (Jensen, 1983).

The enormous interest in lipases is reflected by the excellent work on molecular biology including three dimensional structures and biotechnological application of lipases ranging from bacteria to fungi (Alberghina and Lotti, 1998; Talon *et al*., 1995; Kazlauskas and Bornscheuer, 1998; Ortaggi and Jaeger, 1998; Rubin and Dennis, 1997; Schmid *et al*., 1994).

LIPASE PRODUCING MICROORGANISMS

Lipase production from a variety of bacteria, fungi and actinomycetes has been reported by several workers (Sztajer *et al*., 1988, Rapp and Backhaus 1992; Kulkarni and Gadre 2002). Lipase producers have been isolated mainly from soil, or spoiled food material that contains vegetable oil. Bacterial lipases are glycoproteins, but some extracellular bacterial lipases (e.g, Staphylococcal lipase) are lipoproteins in nature (Brune and Gotz, 1992). Most of the bacterial lipases reported so far are constitutive and are non specific in their substrate specificity (Macrae and Hammond, 1985). Among bacteria, *Achromobacter* sp., etc., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp. *Chromobacterium* sp. have been studied for the production of lipases (Godt fredson, 1990). Lipases with novel properties have also been discovered from several extremophilic bacteria isolated from antarctic ocean (Feller *et al*., 1990), hot springs (Gowland *et al*., 1987; Lee *et al*., 1999), compost heaps (Gowland *et al*., 1987; Rathi *et al*., 2000) and highly salty or sugary environments (Elwan *et al*., 1985; Ghanem *et al*., 2000). Psychrophilic and thermophilic organisms, as well as organisms having different oxygen demand (aerobic, microaerophilic and anaerobic) are reported to produce lipases. Lipases with specificity for butyric acid have been isolated from species of *Penicillium* such as *P. cyclopium*, *P. verrucosum* var. *cyclopium* and *P. crustosum* (Lazar and Schroder, 1992). Among Mucorales, the lipolytic enzymes of the moulds *Mucor griseocyanus*, *Mucor hiemalis*, *M. miehei*, *M. lipolyticus*, *M. pusillus*, *Rhizopus japonicus*, *R. arrhizus*, *R. delemar*. *R. nigricans*, *R. nodosus*, *R. microsporus* and *R. chinesis* have been studied in great detail (Lazar and Schroder, 1992; Coca and Dustet, 2006). The thermophilic *M. pusillus* is well known as a producer of thermostable extracellular lipase. Due to the 1, 3-(regio)-specificity of *Rhizopus*, lipases that are especially suited for the conversion of triglycerides to their corresponding monoglycerides and interesterification reactions of fats and oils, have food and pharmaceutical applications. Diversity of lipase producing bacteria, actinomycetes and fungi are listed in Table 1 and 2.

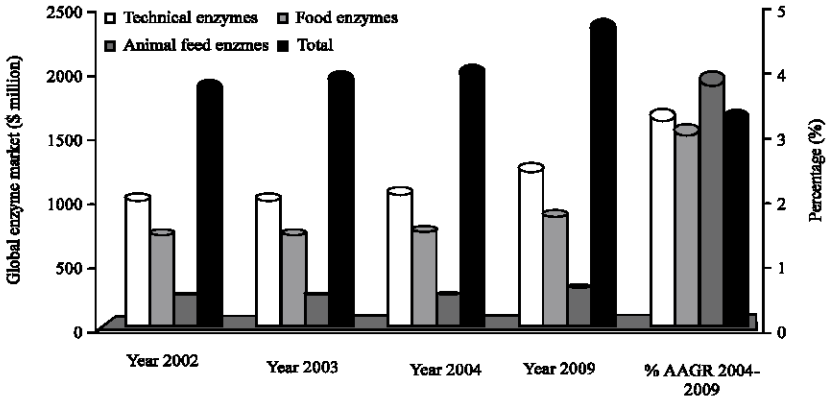

Fig. 1: Global enzyme markets by application sectors, by 2009 ($ millions)

Table 1: Lipase producing Bacteria and actinomycetes.*Indicates recombinant strain

Achromobacter lipolyticum,
Acinetobacter baumannii, *A. calcoaceticus* 69-V, *A. Radioresistens*,
Aeromonas hydrophila MCC-2*, *A. sobria LP004*
Anerovibrio lipolytica
Bacillus acidocaldarius, *B. alcalophilus*, *B.*
atrophaeus SB-2, *B. stearothermophilus* SB-1, *B. licheniformis* SB-3, *B. circulans*, *B. pumilus*, *B. subtilis*, *B. thermocatenulatus**, *B. thermoleovorans ID-1**
Brevibacterium linens
Brochothrix thermosphacta
Burkholderia cepacia, *B. glumae*, *B.pseudomallei*
Campylobacter jejuni, *C. coli*, *C. lari*
Chromobacterium viscosum, *C. viscosum pararipoliticum*
Escherichia coli
Flavobacterium odoratum
Lactobacillus casei-subsp-*casei* LLG, *L. casei* subsp. *pseudoplantarum* LE2, *L. plantarum* 2739, *L. casei* 2756, *L. fermentum* DT41, *L. acidophilus* A2, *L. sanfranciscensis*
Lactococcus helveticus
Leuconostoc citrovorum
Moraxella TA144*
Mycobacterium rubrum
Pediococcus pentosaceus SV61
Propionibacterium arabinosum ATCC 4965, *P. shermanii* ATCC 6915, *P. acnes*,
P. freudenreichii subsp. freudenreichii.
Proteus vulgaris K80
Pseudomonas aeruginosa, *P. alcaligenes*, *P.cepacia**, *P. fragi **, *P. fluorenscens*, *P. glumae*, *P. mendocina* 3121-1, *P. mephitica var. lipolytica*, *P. plantarii*, *P. pseudoalcaligenes*, *P.putida* 3SK, *P. stutzeri*, *P. solanacearum*, *P.tolaasii*, *P. wisconsinensis*
Selenomonas lipolytica
Serratia liquefaciens, *S. marcescens* 345
Staphylococcus aureus, *S. epidermidis*, *S. haemolyticus*, *S. hyicus**, *S. carnosus**, *S. warneri and S. xylosus*
Streptococcus cremoris, *S. diacetilactis S.faecalis*, *S. lactis*, *S. thermophilus*
*Streptomyces exfoliates** M11, *S. cinnamomeus*, *S. parvulus*, *S. clavuligerus*, *S. coelicolor*, *S. rimosus*
Thermoactinomyces vulgaris
Thermosyntropha lipolytica gen. nov., sp. nov.
Vibrio cholerae El
Xanthomonas campestris pathovar *sesami*

FACTORS INFLUENCING THE ACTIVITY OF LIPASE ENZYME

A number of reports exist on influences of various environmental parameters such as temperature, pH, carbon and lipid sources, nitrogen, agitation and dissolved oxygen concentration on lipase production (Watanabe *et al*., 1977; Omar *et al*., 1987; Suzuki and Takahiro, 1988; Nahas and de Assis 1988). Lipase production is usually coordinated with and dependant on the availability of triglycerides. Besides this, free fatty acids, hydrolysable esters, bile salts and glycerol also stimulate lipase production.

Bacterial lipase: Normally, bacterial lipases have neutral or alkaline pH optima with the exception of *P. fluorescens* SIK W1 lipase, which has an acidic optimum at pH 4.8 (Andersson *et al*., 1979). Bacterial lipases possess stability over a wide range, from pH 4 to 11 (Khyami-Horani, 1996; Dong *et al*., 1999; Gupta *et al*., 2004). However, lipases from *Bacillus stearothermophilus* SB-1, *B. atrophaeus* SB-2 and *B. licheniformis* SB-3 are active over a broad pH range i.e, pH 3-12 (Bradoo *et al*., 1999). Some lipases from *Baccilus thermoleovorans* CCR11 were found to be most active at pH 9.0-10.0 and stable in a broad range of pH values 5.0-11.0, retaining more than 80% of activity after 26h at 30°C (Castro-Ochoa *et al*., 2005). Lipases purified from *S. aureus* and *S. hyicus* show optimum pH varies between 7.5 and 9.0, respectively. There enzyme activities are stimulated by Ca^{++} and inhibited by EDTA. Bacterial lipases generally have temperature optima in the range 30-60°C (Dharmsthiti *et al*., 1998; Litthauer *et al*., 2002). However, reports exist on bacterial lipases with optima in both lower and higher ranges (Oh *et al*., 1999; Sunna *et al*., 2002). Thermal stability data are available only for species of *Bacillus*, *Chromobacterium*, *Pseudomonas* and *Staphylococcus*. The thermostability of the enzyme from *Bacillus* sp. was enhanced by the addition of stabilizers such as ethylene glycol, sorbitol, glycerol, with the enzyme retaining activity at even after 150 min of incubation at 70°C (Nawani and Kaur, 2000). A few

Table 2: Lipase producing yeasts and fungi. * Indicates recombinant strain

Actinomucor taiwanensis
Alternaria alternata
Aspergillus awamori, *A. carneus*, *A. flavus*, *A. flavipes*, *A. foetidus*, *A. fumigatus*, *A. japonicus*, *A. niger*, *A. oryzae*, *A. repens*, *A. saitoi*, *A. sydowi*, *A. tamari*, *A. wentii*
Basidiobolus
Botryosphaeria
Botrytis cinerea
Byssochlamys fulva
Candida antarctica, *C. albicans*, *C. curvata*, *C. cylindracea*, *C. deformans*, *C. entomophila*, *C. lipolytica*, *C. parapsilosis*, *C. paralipolytica*, *C. rugosa*, *Candida rugosa**
Conidiobolus
Cryptococcus sp.
Cunninghamella echinulata.
Fusarium culmorum, *F. heterosporum**, *F. moniliforme*, *F. oxysporum*, *F. oxysporum f. sp. Vasinfectum*, *F. solani*, *Microdochium nivale (syn. Fusarium nivale).*
Fusidium sp. BX-1.
Galactomyces geotrichum
Geotrichum asteroids, *G. candidum*, *G. candidum**
Humicola insolens DSM 1800, *H. lanuginosa**
Mucor mucedo, *M. phillipovi*, *M. circinelloides*, *M. pusillus*, *M. fragilis*, *M. racemosus*, *M. javanicus*, *M. hiemalis*, *M. miehei**
Neurospora crassa
Oospora fragrans, *Oospora*
*Penicillium camembertii**, *P. caseicolum*, *P. candidum*, *P. chrysogenum*, *P. citrinum*, *P. cyclopium* M1, *P. expansum*, *P. funiculosum*, *P. restrictum*, *P. roqueforti*, *P. simplicissimum*, *P. solitum*, *P. verrucosum var. cyclopium*,
Pichia burtonii
Pythium ultimum
Rhizomucor miehei
Rhizopus arrhizus, *R. boreas*, *R. chinensis*, *R. circinans*, *R. cohnii sp.*, *R. delemar*, *R. fusiformis*, *R. javanicus*, *R. japonicus*, *R. microsporus*, *R. nigricans*, *R. niveus**, *R. oligosporus*, *R. oryzae*, *R. rhizopodiformis*, *R. stolonifer*, *R. thermosus*, *R. usamii*
Rhodotorula rubra, *R. glutinis*
Saccharomycopsis lipolytica
*Schizosaccharomyces pombe**
Sporotrichum (Chrysosporium) thermophile Apinis
Sterigmatomyces sp.
Syncephalastrum racemosum
Thermomyces lanuginosus
Trichosporon asteroids, *T. fermentans* WU-C12
Trichothecium roseum
Ustilago maydis
Yarrowia lipolytica

Pseudomonas lipases have been reported which are stable at 100°C or even beyond to 150°C with a half-life of a few seconds (Rathi *et al*., 2001). A highly thermotolerant lipase has been reported from *B. stearothermophilus*, with a half-life of 15-25 min at 100°C (Bradoo *et al*., 1999). An overview of biochemical properties of bacterial lipases and their isozymes have been given in Table 3.

Fungal lipase: The fungal lipase have optimum pH range from 4-8 (Arai *et al*., 1997; Ota *et al*., 1982). However, some fungi like, trichosporonasteroides can grow at both acedic and alkaline pH (Dharmsthiti and Ammaranond, 1997). Most of the fungi has temperature optimum between 25-30°C for maximum lipase activity except for some thermophilic fungi which have temperature optimum of 45-75°C (Dharmsthiti and Ammaranond, 1997; Kakugawa *et al*., 2002; Vakhlu and Kour, 2006). Production of the extracellular and cell bound enzymes were reported to depend on the Carbon and Nitrogen composition of the medium (Vakhlu and Kour, 2006). Like many others Ota *et al*. (1982) have demonstrated that media engineering can lead to increase in production of extracellular enzymes. The enzyme purified from culture medium incubated with *Kluyveromyces lactis* was reported to be highly glycosylated with apparent molecular weight of 160-250 kD. The enzyme had two pH optima, pH 2.0 and pH 7.5 and the enzyme was reported to have hydrolyses all phospholipids substrates with out metal ion at acedic pH (Oishi *et al*., 1999). On the other hand at alkaline pH it showed specificity for phosphatidylcholine and lysophosphatidylcholine and required Ca^{++}, Fe^{+++} or Al^{+++} for activity. The alkaline activity gets increased over 20 fold in presence of Al^{+++} compared to the presence of Ca^{++}. An extracellular lipase produced by the glycolipidproducing yeast *Kurtzmanomyces* sp. I-11 was purified by ammonium sulfate precipitation and column chromatographies on DEAE-Sephadex A-25, SP-Sephadex C-50 and Sephadex G-100 (Kakugawa *et al*., 2002). Its optimum temperature for the activity was 75°C and found very stable

Table 3: Overview of various biochemical properties of bacterial lipases

Source	Molecular weight	pH, temperature stability	Substrate specificity	Reference
Acinetobacter calcoaceticus	30.5 kDa	Stable at pH 8.0 and temperature 40°C	Enzyme hydrolyzes tri -, di-, mono-acylglycerols	Brune and Gotz (1992)
Acinetobacter sp. RAG-1	33 kDa	Active at temperatures up to 70°C	Hydrolyzes wide range of *pnp* esters, but preference for medium-length acyl chains (C6, C8)	Snellman *et al.* (2002)
Alcaligenes sp.	n.s.	65% residual activity at 60°C after 10 min	Enzyme hydrolyzes natural fats and oils	Brune and Gotz (1992)
Bacillus sp.	22 kDa	Stable over pH 5.0-11.5, stable at 65°C for 30 min at pH 5.6	Tricaprylin, tricaprin, 1,3-regiospecific lipase	Sugihara *et al.* (1991)
Bacillus sp.	45 kDa	Stable for 12 h at 60°C	Triolein hydrolyzed at all positions; broad fatty acid specificity	Nawani and Kaur (2000)
Bacillus sp. strain398	50kDa	Stable over pH 4-11, stable up to 60°C, 50% residual activity at 65°C after 30min	Tricaprylin among triacylglycerides; *pnp* caproate among *pnp* esters	Kim *et al.* (1994)
Bacillus strain A30-1 (ATCC 53841)	65kDa	90-95% residual activity after 15 h at pH 5.0-10.5, half-life of 8 h at 75°C	High activity on tricaprin and trilaurin among various triacyl-glycerides; corn, olive, cottonseed, coconut,soyabean, wheatgerm oil among other oils	Wang *et al.* (1995)
Bacillus sp. THLO27	69 kDa	Stable over pH 6.0-8.0, 80% residual activity after 1 h at 75°C	Preference for C4-C12 fatty acid; 1,3-regiospecific	Dharmsthiti and Luchai (1999)
B. alcalophilus	n.s.	Stable at pH 10.0-10.5, 80% activity at pH 11.0 after 1 h; stable at 60°C for 1 h, 70% residual activity at 75°C	n.s.	Ghanem *et al.* (2000)
B. licheniformis strain H1	n.s.	Stable at alkaline pH 9-11, 65% residual activity at pH 12 after 30 min at 4°C, retained 100% activity after 15 min at 70°C	n.s.	Khyami-Horani (1996)
B. pumilus B26 (recombinant lipase)	n.s.	Stable at pH 8.5 and temperature 35°C	Hydrolyzes various long triacylglycerols (C14-C18) and triolein (C18:1)	Kim *et al.* (2002)
B. subtilis 168	19 kDa	Stable at pH 12; 100% activity after 30 min. at 40°C	Preference for C8 fatty acid; 1,3-regiospecific	Lesuisse *et al.* (1993)
B. thermo-catenulatus	n.s.	Stable at pH 9-11 for 12 h at 30°C, 48.5% residual activity at 60°C for 30 min	Tributyrin, *pnp* caprate	Schmidt-Dannert *et al.* (1996)
B. thermo-oleovorans ID-1	34kDa	Stable at pH 7.5, half-life at 70°C 30 min	Broad	Lee *et al.* (1999)
Burkholderia sp. lipase	30 kDa	Stable at pH 6.0-12.0, half-life of more than 12 h at 90-100°C	High rate of hydrolysis towards mustard oil, linseed oil, neem oil and almond oil, preference for long chain (>C12) triacylglycerides)	Rathi *et al.* 2000, 2001; Bradoo *et al.* (2002)
Pseudomonas sp. KWI-56	33kDa	Stable at pH 4-10; stable up to 60°C at pH 7.0 for 24 h	Triacylglycerides (C10-C14), whale wax	Brune and Gotz (1992)
Pseudomonas sp. (PSL)	30 kDa	Stable at pH 6-12 after 4 h at 40°C; stable at 25-50°C for 30 min	n.s.	Dong *et al.* (1999)
Pseudomonas sp. strain	n.s.	Stable at pH 8.0, 70% decrease in activity after 5 min at 60°C	Highest activity for *pnp* KB 700A (recombinant lipase) caprate, 20-fold higher activity towards 1(3) position than 2 position	Rashid *et al.* (2001)
P. aeruginosa EF2	29 kDa	Stable at pH 9.0, half-life at 45°C 6 H, at 70°C 2.1 min	Preference for C18 fatty acid; 1,3-regiospecific	Gilbert *et al.* (1991)
P. aeruginosa LP 602	n.s.	90% residual activity at pH 8 after 5 h; 50% residual activity at 55°C after 2 h	High activity towards melted butter, castor, coconut oil	Dharmsthiti and Kuhasuntisuk (1998)
P. cepacia DSM 50181	n.s.	Stable over pH 2.0-12.0,	n.s.	Dunhaupt *et al.* (1992
P. fluorescens AK 102	33 kDa	pH 4.0-10.0, stable below 50 °C for 1 h; 100%	Broad	Kojima *et al.* (1994)
P. fluorescens MC50	55 kDa	Stable over pH 6.0-9.0	Triacylglycerols	Brune and Gotz (1992)
P. fluorescens NS2W	n.s.	Stable over pH 3-11 with more than 70% residual activity; stable up to 60°C with more than 70% residual activity for at least 2 h	n.s.	Kulkarni and Gadre (2002)

Table 3: Continued

Source	Molecular weight	pH, temperature stability	Substrate specificity	Reference
P. fragi 22.39B	33 kDa	Stable up to 51°C at pH 9.0 for 24 h; stable over pH 6.5-10.5 at 30°C for 24 h	Triacylglycerols, methyl oleate, Tween, Span, 1,3-regiospecific	Brune and Gotz (1992)
P. luteola	n.s.	Half-life of 84 min. at pH 12.25; half-life of 116 min at 65°C	Preference for medium-chain saturated and unsaturated fatty acids	Litthauer *et al.* (2002)
P. mendocina 3121-1	62kDa	Different for different substrates	Hydrolyzes *pnp* butyrate, Tween-80, olive oil	Surinenaite *et al.* (2002)
P. multocida	n.s.	n.s.	Tweens specific for Tween-40	Pratt *et al.* (2000)
P. pseudoalcaligenes F-111	32 kDa	Stable over pH 6.0-10.0, stable up to 70°C	High activity towards linseed, soybean oil, preference for C12, C14 *pnp* esters	Lin *et al.* (1996)
Serratia marcescens	52 kDa	70% activity after 24 h at pH 8, high activity at 5°C, 15% activity at 80°C	Michelis-Menten constant 1.35 mM on tributyrin	Abdou (2003)
Staphylococcus aureus	46 kDa	n.s.	Preference for short chain triacylglycerides and pnp esters (caprate)	Paiva *et al.* (2000)
S. hyicus	46 kDa	n.s.	Preference for phospholipids, neutral lipids, *pnp* esters irrespective of chain length	Simons *et al.* (1996)
S. haemolyticus	45kDa	Stable at pH 5-11 for 24 h; stable at 50°C in presence of Ca^{2+}	High activity on tributyrin, tripropionin, trimyristin, *pnp* caprylate	Oh *et al.* (1999)

*ns: not shown

Table 4: Overview of various biochemical properties of selected lipases produced by fungi

Yeast	MW in kD	pH optima	Stable pH range	T°C optima	Reference
Arxulaadeninivorans	50	7.5	-	30°C	Boer *et al.* (2005)
Candida albicans ATCC 36082	38	-	-	-	Fu *et al.* (1997) Hube *et al.* (2000)
Candida curvata	195	5.0-8.0	-	60°C	Lazar and Schroder (1992)
Candida cylindracea L1754	L1- 58 L2- 58	-	5.8 - 6.8	-	Veeraragavan and Gibbs (1989)
Candida Cylindracea					
Commercial preparation	LA-62	7.0			
	LB- 62				
	LC-62	5.0	-	-	Shaw and Chang (1989)
Candida. rugosa ATCC 14380	60	5.0	-	-	Lotti *et al.* (1993)
Candida. rugosa DMS 2031	Lip A-64	7.8		35 - 40°C	Benjamin and Pandey (2001)
	LipB- 62	7.8			
	LipC- 60	7.8			
Geotrichum candidum link	55	5.6 - 7.0	4.2 - 9.8	40°C	Tsujisaka *et al.* (1973)
Geotrichum candidum	Lip 1 56				
ATCC 34614	Lip2 56	6.0	6.8 - 8.0	-	Veeraragavan *et al.* (1990)
Humicola lanuginosa	27.5	8.0	-	60°C	
Kluyveromyces lactis	160-250	2.0 - 7.5	-	-	Oishi *et al.* (1999)
Kurtzmanomyces sp. I-11	49	1.9 - 7.2	below 7.1	75°C	Kakugawa *et al.* (2002)
Mucor michei	-	8.0	-	40°C	Lazar and Schroder (1992)
Penicillium cyclopium					
Lipase A	27	7.5	-	35°C	
Lipase B	36	5.8	-	40°C	Iwai *et al.* (1975)
Rhizopus arrhizus	43	8.0	-	-	Lazar and Schroder (1992)
Rhizopus delemar	41.3	5.6	-	35°C	Lazar and Schroder (1992)
Saccharomyces cerevisiae	63	-	-	-	Oishi *et al.* (1999)
Yrrowia lipolytica	Lip 1-39	8.2	4.5-8.0	37°C	Ota *et al.* (1982)
	Lip-44	8.0			
Trichosporon fermentans WU-C12	Lip 1-53	5.5	4.0 - 8.0	40°C	Arai *et al.* (1997)
	Lip2- 55				
Trichosporon asteroides	37	5.0	3.0 - 10.0	50°C	Dharmsthiti and Ammaranond (1997)

at temperatures below 70°C. The enzyme had pH range of 1.9- 7.2 and stable at pH below 7.1. N-terminal sequence of the *Kurtzmanomyces* lipase was found to be similar to that of lipase A from *Candida antarctica*, though the pH profiles of the two lipases were significantly different (Kakugawa *et al.*, 2002). Lipase from *Geotrichum candidum* is active on olive oil at pH 5.6 and 7.0 at 40°C. This enzyme maintained its stability in the pH range of 4.2- 9.8 for 24 h and at temperature below 55°C for 15 min (Tsujisaka *et al.*, 1973). Chen *et al.* (1992) purified two

types of extra-cellular lipases, lipase I and lipase II from *Trichosporonfermentans* WU-C12. Both lipase II and I seemingly were thermo stable. Lipase I was stable at 40°C for 30 min at pH 5.5 and lipase II under same conditions remains stable up to 50°C. The lipase enzyme extracted from *Aspergillus niger* exerts its maximum activity between pH 4.5~5.5 at 25°C and was stable from pH 3 to 10.5 at 30°C for 24 h. The lipase activity was found to be strongly inhabited by metal ions such as Zn^{2+}, Fe^{2+}, Hg^{2+} and the percentage inhibition being 98, 89 and 26%, respectively (Sugihara *et al*., 1988). The biochemical properties of fungal lipases and their isozymes are shown in Table 4.

Enzyme purification: Purification of the enzyme is essential in industries such as fine chemicals, pharmaceuticals and cosmetics and also for understanding the 3-D structure and the structure-function relationships of proteins (Aires-Barros *et al*., 1994; Saxena *et al*., 2003). For industrial purposes, the purification strategies employed should be inexpensive, rapid, high-yielding and amenable to large-scale operations. Various purification strategies used for lipases have been reviewed several times highlighting clearly the importance of designing optimal purification schemes for various microbial lipases (Palekar *et al*., 2000; Saxena *et al*., 2003). The quality of purified products varies with the order of the purification steps. This aspect has been evaluated through different purification protocols pursued by various investigators (Chuang *et al*., 1997; Hong and Chang, 1998; Sugihara *et al*., 1988; Lotrakul and Dharmsthiti, 1997; Dharmsthiti and Luchai, 1999; Labuschagne *et al*., 1997; Gilbert *et al*., 1991; Lee and Rhee, 1993; Kumura *et al*., 1993; Koblitz and Pastore, 2006). Pre-purification steps involve concentration of the culture supernatant containing the enzyme by ultrafiltration, ammonium sulfate precipitation or extraction with organic solvents. Precipitation often gives a high average yield although with limited purification; and such enzyme preparations are used in detergent formulations (Aires-Barros *et al*., 1994). However, for certain applications, such as synthetic reactions in pharmaceutical industry, further purification is needed. Since lipases are known to be hydrophobic in nature, having large hydrophobic surfaces around the active site, the purification of lipases may best be achieved by opting for affinity chromatography, such as hydrophobic interaction chromatography. The use of hydrophobic interaction chromatography has increased tremendously in the past few years (Kordel *et al*., 1991; Hong and Chang, 1998; Imamura and Kitaura, 2000). Affinity methods can be applied at an early stage, but as the hydrophobic matrices are expensive, alternatively ion exchange and gel filtration are usually preferred after the precipitation step (Schmidt-Dannert *et al*., 1994, 1996; Jose and Kurup, 1999; Ghanem *et al*., 2000; Imamura and Kitaura, 2000; Litthauer *et al*., 2002; Snellman *et al*., 2002; Abdou, 2003). Cultivation and purification schemes for various bacterial and fungal lipases are listed in Table 5 and 6.

RELATIVE MOLECULAR WEIGHT OF MICROBIAL LIPASE

Lipases are reported to be monomeric proteins having molecular weights in the range of 16,000-670,000 Daltons. The most interesting feature observed with many lipases is the formation of high molecular weight aggregates. Relative molecular weight of some of the common lipase producing bacteria abd fungi are listed in Table 3 and 4.

Bacteria: Aggregate formation has been reported in lipases of both Gram-positive and Gram-negative bacteria (Table 5). Among Gram-positive bacteria, aggregation has been reported with crude as well as purified *Staphylococcus aureus* lipase (Kotting *et al*., 1983). The aggregation was also observed with purified lipase produced by *Bacillus subtilis*, *Bacillus thermocatenulatus* and *Bacillus* sp. THL027 (Lesuisse *et al*., 1993; Sugihara *et al*., 1991; Imamura and Kitaura, 2000; Schmidt-Dannert *et al*., 1996). In Gram-negative bacteria, it has been well documentedfor the members of genus *Pseudomonas*. Aggregates were reported either of pure *Pseudomonas* lipases (Fox and Stepaniack, 1983; Gilbert *et al*., 1991) or of the lipases associated with lipophilic molecules (Lu and Liska, 1969; Stuer *et al*., 1986; Kordel *et al*., 1991). The *Pseudomonas aeruginosa* PAC 1R was shown to form lipase purification lipopolysaccharide aggregates (Stuer *et al*., 1986). During purification of lipases, such aggregates were dissociated by treatment with detergents like Triton X-100 or CHAPS (Chartrain *et al*., 1993; Stuer *et al*., 1986) or solvents like isopropanol (Dunhaupt *et al*., 1992). Lipases purified from *Staphylococcus aureus* and *S. hyicus* show molecular weights ranging between 34-46 kDa. The lipase genes from *S. hyicus* and *S. aureus* have been cloned, sequenced and compared with other lipases. This revealed two conserved domains separated by 100 amino acids which are likely to form active site (Brune and Gotz,1992). Putative active site residues around His 269 and Ser 369 of *S. hyicus* lipase were found to be highly conserved in two *S. aureus* lipase and in several eukaryotic lipases (Petersen and Drablos, 1994).

Table 5: Cultivation and lipase purification from bacteria

Organism	C T(°C)	C Time(h)	Assay method	Purification scheme	Purification fold	Reference
*Aeromonashydrophila*MCC-2*	37		Titrimetry usingtributyrin as substrate → Sephadex G-100	Culture supernatant → 25-50% $(NH_4)2SO_4$ ppt →DEAE Sepharose CL-6B	33	Chuang *et al.* (1997)
Aeromonassobria	37	48	Spectrophotometriy using *p*NPP as substrate	Culture broth → ultrafiltration → Phenyl sepharose	9.5	Lotrakul and Dharmsthiti (1997)
Acinetobacter radioresistens CMC-1	30	Till Statio nary phase	Photometry using *p* NPP OR Titrimetry using olive oil	Culture supernatant→ 30-80% $(NH_4)2SO_4$ ppt→ Mono Q→ Phenyl sepharose CL 4B	44	Hong and Chang (1998)
Bacillus sp.	28	80	Titrimetry using olive oil as substrate	Culture supernatant → $(NH_4)2SO_4$ ppt → Acrinol treatment→ EAESephadexA-50→ Toyopearl HW-55F→ Butyl toyopearl 650 M	7762	Sugihara *et al.* (1991)
Bacillus sp. THL027	65	48	Titrimetry using olive oil as substrate	Crude enzyme → Ultrafiltration → Sephadex G-100	2.8	Dharmsthiti and Luchai (1999)
Bacillus sp. H 257	50	12	Enzymatic estimation of glycerol Monolauroylglycerol as substrate	Cell free extract → acetone ppt→ octyl sepharose CL 4B→ Q sepharose→ superose 12	3028	Imamura and Kitaura (2000)
Bacillus Thermoleovorans ID-1	65	3.5	Spectrophotometry using *p*NPB as substrate	Culture supernatant→ 30-80 % $(NH_4)2SO_4$ ppt → DEAE Sephacel→ Sephacryl S-200	223	Lee *et al.* (1999)
*Bacillus thermocatenulatus**	30	5	PhotometryUsing *p* NPP and pH-stat using tributyrin or triolein	Cell breakage→ Butyl sepharose→ ultrafiltration→ TSK G3000	125	Schmidt-Dannert *et al.* (1996)
Chromobacterium viscosum	26	96		-Culture supernatant→ Barium aceteate treatment→ Palmitoyl cellulose → Concentration at 50 °C→ Acetone ppt→ Sephadex G- 75	63.15	Horiuti and Imamura (1977)
Flavobacterium odorum	30	24	Spectrophotometry using *p*NPP and colorimetry using olive oil	Culture supernatant → ppt in three phase partitioning using t-butanol and $(NH_4)2SO_4$ → Super Q→ Hydroxylapatite	14	Labuschagne *et al.* (1997)
Lactobacillus casei sub sp. *Pseudoplantarum*	30	16	Titrimetiry using tributyrin as substrate	Cell pellet→ French press→ crude extract→ Sephadex G25 → Mono Q HR → Superose 12 → Mono QHR	54.39	Lee and Lee (1989)
Pseudomona saeruginosa	30	17	Titrimetry using olive oil and photometry using *p*NPP	Culture supernatant→ ultrafiltration→ CHAPS solublization→ IEF	1264.6	Shabtai and Daya-Mishne (1992)
Pseudomonas aeruginosa EF2	37	Continuou sculture	Titrimetry using olive oil	Culture supernatant→ concentrated supernatant→ Mono Q → FPLC Superose 6	31	Gilbert *et al.* (1991)
Pseudomonas cepacia	30	72	PHstat method using olive oil Orphotometry using *p*NPP	Culture supernatant → cross flow filtration→ isopropanol treatment I → is oporpanol tereatment II→ liquid/liquid extraction → phenyl sepharose	400	Dunhaupt *et al.* (1992)
Pseudomonas fragi	-	-	Titrimetry using lard or tributyrin	Culture supernatant→ ultrafiltration→ $(NH_4)2SO_4$ pptn→ DEAE Sephadex	103	Mencher and Alfora (1967)
Pseudomonas fragi	25	96-120	Titrimetry using coconut oil	Centrifuged _seudoa broth→ $(NH_4)2SO_4$ pptn→ Dialysis → Acetone fractionation → Sephadex G-200 → DEAE cellulose	99.79	Lu and Liska (1969)
Pseudomonas	30	16	pH stat method using	Crude extract →	20.9	Lee and Rhee (1993)

Table 5: Contienued

Organism	C T(°C)	C Time(h)	Assaymethod	Purification scheme	Purification fold	Reference
putida 3SK			olive oil	ultrafiltration → DEAE Sephadex A- 50 → Sephadex G-100		
Pseudomonas fluorescens AK102.	30	72	Titrimetry using olive oil	Crude enzyme→ (NH4) 2SO4 pptn→ DEAE Toyopearl 650 M→ Phenyl Toyopearl 650M	6.1	Kojima *et al.* (1994)
Pseudomonas fluorescens	25	-	Titrymetry using olive oil	Culture supernatant→ concentrate → (NH4)2SO4 pptn→ DEAE-cellulose→ Octyl sepharose CL-4B	3390	Sztajer *et al.* (1991a, b)
Pseudomonas fluorescens No. 33	17	168	Photometry- UHT milk as substrate and phenol red as colour reagent	Photometry- UHT milk x Hcl pptn (pH 4.8) → Octylsepharose CL-4B→ DEAE Toyopearl 650 S→ Toyppearl HW-50S	4270	Kumura *et al* (1993)
Pseudomonas glumae	-	-	Photometry using *p*NPP OR Titrimetry on olive oil	Crude enzyme pptn form Unilever→ DEAE- triacryl-Mphenyl sepharose→ alcohol-acetone precipitation→ DEAE cellulose	7.2	Taipa *et al.,* (1995)
Pseudomonas tolassi	30	36	Photometry using β-naphthyl caprylate	Cell-free supernatant→ ultrafiltration→ DEAE - cellulose DE 52→ Sephadex G-150	9	Baral and Fox (1997)
Propionibacterium acnes	37	144-168	Titrimetry using triolein	Culture supernatant → ultrafiltration→ Sephadex G100→ CM sephadex C-50.	-	Ingham *et al.* (1981)

(-) indicates no data available, * indicates product of cloned gene. CT= Cultivation temperature; C time= Cultivation time

Table 6: Cultivation and purification of lipase enzyme from fungi

Species	CT	C. time (h)	Assay method	Purification Scheme	Purifi-cation fold	Reference
Aspergillus niger	30°C	-	Spectrophotometrey method using pNPP	Culture supernatant→ 25% $(NH_4)2SO_4$pptn→ Butryl Toyopers pptn→ Sephadex G-75	100	Sugihara *et al.* (1988)
Aspergillus wantii	30°C	3 days	-	-	-	Harish *et al.* (1980)
Fusarium oxysporium	30°C	48 h	Spectrophotometrey method using pNPP	-	-	Prazeres *et al.* (2006)
Fusarium solani FS1	25°C	72 h	Spectrophotometrey method using pNPP	-	-	Maia *et al.* (1999)
Mucor griseocyanus 55.1.1	30°C	8 days	Spectrophotometrey method using pNPP	-	-	Coca and Dustet (2006)
Rhizopus sp.	30°C	120 h	Spectrophotometrey method using pNPL	Cultural supernatent→ pptn using 70% $(NH_4)SO_2$→ Sephacryl S200	9.7	Koblitz and Pastore (2006)

(-) indicates no data available CT: Cultivation temperature; C time: Cultivation time

Fungus: An extracellular and two cell-bound types of activity corresponding to lipase I (39 kDa) and lipase II (44 kDa) were described in fungi by Ota and coworkers (Ota *et al.*, 1982). Destain and coworkers isolated *Yarrowia lipolytica* strains over producing an extracellular lipase. The secreted lipase was shown to have an apparent molecular mass of 38.5 kD, giving three isofocusing (pIs) of 5.0, 5.2 and 5.4. The sequence of the first 49 amino acids of the N terminus was determined and found to be identical to that of lipase A (Destain *et al.*, 1997). An extracellular lipase produced by the glycolipid producing yeast *Kurtzmanomyces* sp. I-11 showed molecular mass approximately at 49 kDa. Kakugawa *et al.* (2002) have reported *Rhizopus* lipase consisting molecular weight of 37.5 kD. The molecular weight and pI value of the lipase enzyme produced by *Geotrichum candidum* were estimated to be ~55 kD and ~4.33, respectively. The crystalline preparation contained about 7% carbohydrate and a very small amount of lipids (Tsujisaka *et al.*, 1973). Chen *et al.* (1992) described the lipase properties of *Trichosporon fermentans* WU-C12. These workers purified two types of extra-cellular lipases, lipase I and

Table 7: Industrial applications of microbial lipases

Industry	Action	Product or application
Detergents	Hydrolysis of fats	Removal of oil stains from fabrics
Dairy foods	Hydrolysis of milk fat, cheese ripening, modification of butter fat	Development of flavoring agents in milk, cheese and butter
Bakery foods	Flavor improvement	Shelf-life prolongation
Beverages	Improved aroma	Beverages
Food dressings	Quality improvement	Mayonnaise,dressings and whippings
Health foods	Transesterification	Health foods
Meat and fish	Flavor development	Meat and fish products; fat removal
Fats and oils	Transesterification; hydrolysis	Cocoa butter, margarine, fatty acids, glycerol, mono- and diglycerides
Chemicals	Enantioselectivity, synthesis	Chiral building blocks, chemicals
Pharmaceuticals	Transesterification, hydrolysis	Specialty lipids, digestive aids
Cosmetics	Synthesis	Emulsifiers, moisturizers
Leather	Hydrolysis	Leather products
Paper	Hydrolysis	Paper with improved quality
Cleaning	Hydrolysis	Removal of fats

lipase II from this strain of *Trichosporon* by combining acetone precipitation with successive chromatography using butyl-toyopearl 650 Mtoyopearl HW-55 and Q-Sepharose FF. The molecular weight of lipase I was ~53 kD as estimated by SDS-PAGE and ~160 KD by gel filtration while that of lipase II was ~55 kD as estimated by SDS-PAGE and ~60 kD by gel filtration. Both the lipases are stable in the pH range of 4.0-8.0 for 24 h at 30°C. Both lipase II and I seemingly are thermo stable. Lipase I was stable at 40°C for 30 min at pH 5.5 and lipase II under same conditions remains stable up to 50°C. Isoelectric focusing of the purified enzyme from *Aspergillus niger* yielded a single peak of protein at pH 4.1. The molecular weight of the enzyme was estimated to be 35 kD from SDS-PAGE and Sephadex G-100 colume chromatography (Sugihara *et al*., 1988). An overview of biochemical properties of lipases and their isozymes has been given in Table 6.

INDUSTRIAL APPLICATION OF MICROBIAL LIPASES

Lipases found promising application in chemical processing, dairy industry, agrochemical industry, paper industry, oleochemical industry, cosmetics, pharmaceuticals, synthesis of surfactants, detergent industry, polymer synthesis and personal care products (Table 7). Many lipases are active in organic solvents where they catalyze a number of reactions that includes esterification, transesterification, regioselective acylation of glycols and menthols and synthesis of peptides and other chemicals (Rao and Divakar, 2001; Ducret *et al*., 1998; Zhang *et al*., 2001; Therisod and Klibanov, 1987; Weber *et al*., 1995; Shin *et al*., 1997).

Nowadays commercial use of lipase is a billion dollar business that comprises a wide variety of different applications. About 1000 tons of lipase is sold every year in the field of detergent (Azim *et al*., 2001). Lipases are used for removing the pitch from the pulp in paper industry (Kennedy and White, 1984). They are also used in flavour development for dairy products and beverages and synthetic chemistry (Boland *et a l*., 1991; El-Khattabi *et al*., 1999; Schmid and Verger, 1998; Stecher and Faber, 1997).

Lipases in food industry: Lipases have become an integral part of the modern food industry (Theil, 1995). It is desirable for the production of flavours in cheese and for interesterification of fats and oils. It also accelerates the ripening of cheese and lipolysis of butter, fats and cream. The addition of lipases releases the short chain (C4 and C6) fatty acids which gives the sharp, tangy flavour while the release of medium chain fatty acid (C 12 and C14) gives the soapy taste to the product. Cocoa butter is a high value fat that contains palmitic acid and stearic acid that has a melting point of 37°C (Vulfson, 1994). Lipases are also used for the conversion of triacylglycerols to diacylglycerols and monoacylglycerols; and then these products gives rise to non-esterified fatty acids and fatty acid propan-2-yl esters. Lipases are also used as emulsifiers in food, pharmaceuticals and cosmetics industries (McGee, 2000; Fleming, 1991). Lipases are used for the production of maltose-and lactose-like sugar fatty acid esters.

Some method utilizes the immobilized *Rhizomucor miehei* lipase for transesterification reaction that replaces the palmitic acid in palm oil with stearic acid. Immobilized lipases from *Candida antarctica* (CAL-B), *C. cylindracea AY*30, *Humicola lanuginosa*, *Pseudomonas* sp. and *Geotrichum candidum* are being used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants that can be used in sunflower oil (Xu *et al*., 1995; Jaeger and Reetz, 1998). Immobilized lipase from *Staphylococcus warneri* and *S. xylolus* was used for the development of flavour ester (Talon *et al*., 1995). *Aspergillus* lipases were highly selective for the short chain acids and alcohols while *Candida rugosa*

lipase was selective for propionic acid, butanol, pentanol and hexanol (Shin *et al*., 1997). Lipases from *Mucor miehei* and *Candida antarctica* was immobilized and used for the synthesis of short chain flavour thioester. An industrially feasible method was developed for the production of glycerol-glycolipid from a mixture containing glycosidases and fatty acid by using P*enicillium camembertii* lipase.

Lipases in the resolution of chiral compounds: In all biological system chirality is predominant and has been preserved since the beginning of evolutionary time. Chirality refers to the spatial configuration of the molecules, such as D- and L-aminoacids. By definition a chiral molecule is one which lack reflectional symmetry or handness. When these enantiomers are present in equimolecular amounts within a mixture, the resultant mixture is termed racemic. The first successful attempt to resolve the enantiomers from the racemic mixture was performed by Louis Pasteur, in which he manually resolved a racemic mixture of sodium ammonium tartarate into its individual enantiomers.

Because of their excellent capabilities for specific regioselectivity the enzyme specially the multifaceted lipases (glycerol ester hydrolases:EC 3.11.3) have now emerged as an important biocatalysts for the future industrial applications. Lipases are indispensable for the bioconversion of lipids (tryacylglycerols). The natural lipases have excellent regioselectivity and therefore are used in biomedical applications. There are reports on the application of microbial lipases to produce compounds in high enantiomeric excess. The lipases are used in organic chemistry for the resolution of enantiomeric compounds. Lipases have been used for the production of pure biologically active S-enantiomer of Sulcatol transesterification of cyanohydrin compounds and epoxyesters. Lipases from *Candida cylindracea* has been applied to the resolution of 2-Bromopropionic acids and 2-Chloropropionic acids which are starting materials for the synthesis of phenoxy propionic herbicides. *Candida rugosa* lipases were however highly S-specific but *Aspergillus niger* lipases were highly enantiospecific E-100.

Lipase catalysed enantioselective esterification in the derivation of model compounds: Members of the lipase family have been found to be particularly suitable for such applications and lipases from *Candida cylindracea*, *C. antarctica*, Porcine pancreas lipase and other sources have been used to resolve the enantiomers of flurbiprofen, naproxen, ibuprofen and suprofen (Mustranta, 1992; Mertoli *et al*., 1996). The S-enantiomer of flurbiprofen (2-fluoro-α-methyl- [1, 1-biphenyl] -4-acetic acid) possess most of its antiinflammatory action, but the presence of the R-enantiomer is reported to greatly enhance its gastrointestinal toxicity (Wechter *et al*., 1993). A later study reported that R-flurbiprofen was less potent as an analgesic than S-flurbiprofen but it cause little toxicity as compared to its antipode (Lotsch *et al*., 1995). This makes the resolution of the enantiomers of this drugs particularly desirable. The lipase catalysed enantioselective esterification of racemic flurbiprofen has been reported (Morrone *et al*., 1995). The factors known to profoundly affect the reaction rate and enantioselectivity and solvent hydrophobicity, water content or water activity and the type of lipase (Valivety *et al*., 1992). It has been established that increasing hydrophobicity exponentially increases the rate of lipase catalysed reaction. It would also seem that these lipases (*Candida rugosa*, *Mucor javonicus*) were also affected by the nature of solvent medium. Lipases from *Pseudomonas aeruginosa*, *P. flurescens*, *Bacillus cepacia*, *B. subtilis*, *Chromobacterium viscosum*, *Alcaligenes* sp. and *Serratia marcescens* are typical biocatalyst for synthetic organic reaction. The calcium antagonists Diltiazem, which is used for the treatment of blood pressure, is of considerable importance. The kinetic resolution of (2S, 3S)-configurated methyl p-methoxyphenyl-glycidate which is separated from the desired (2R, 3S) ester by *S. marcescens* lipase is the key step in the synthesis of Diltiazem (Schmid and Verger, 1998; Matsumae *et al*., 1990).

Lipases as Biosensor: The quantitative determination of triacylglycerol is of great importance in clinical diagnosis and in food industry. The lipid sensing device as a biosensor are rather cheaper and less time consuming as compared to the chemical methods for the determination of tryacylglycerols. Biosensor can be of three types (a) chemical (b) biochemical or (c) electronic. Biochemical biosensor utilizes the enzymes or other proteins (antibodies), cells or cell extract immobilized on a suitable matrix linked to a transducer. An analytical biosensor was developed for the determination of lipids for the clinical diagnosis (Masahiko *et al*., 1995). Here, in quantitative determination of lipases are used to generate glycerol from triacylglycerol in the analytical sample and to quantify the released glycerol by enzymatic or chemical methods. This principle enabled the physician to diagnose the patients with cardiovascular complaint. *C. rugusa* lipase biosensor from *Candida rugosa* has been developed as a DNA probe (Benjamin and Pandey, 2001).

Lipase in detergents: Enzymes can reduce the environmental load of detergent products, since they save energy by enabling a lower wash temperature to be used; allow the content of other, often less desirable, chemicals in detergents to be reduced; are biodegradable, leaving no harmful residues; have no negative impact on sewage treatment processes; and do not present a risk to aquatic life (Falch, 1991). Ever since the discovery of lipases it became part and parcel of the detergent industry along with proteases, amylases and cellulases. Lipases, protease, amylase and celluloses were used as they can split fats, proteins, starch and cotton-fluff, respectively. Nowadays they were extensively used in household detergent, industrial cleaner and leather processing. Lipase has also been used to clean clogged drain which is due to the food and non-food material deposition. In 1992, Novo Nordisk introduced the first commercial recombinant lipase 'Lipolase' originating from fungus *Thermomyces lanuginose* which was expressed in *Aspergillus oryzae* (Jaeger and Reetz, 1998). Later in 1995 two product namely 'Lumafast' from *Pseudomonas mendocina* and 'Lipomax' from *Pseudomonas alcaligenes* was introduced (Jaeger and Reetz, 1998). With the prospect of recombinant lipase enzyme the lipase-mediated detergent promises a high tonnage.

Lipases in bioremediation: Oil spills in refinery, shore sand and processing factories could be handled by the use of lipases from different origins (Nakamura *et al*., 1994). It has been also used for the degradation of wastewater contaminants such as olive oil from oil mills. Another important application has been reported for the degradation of polyester waste, removal of biofilm deposits from cooling water systems and also to purify the waste gasses from factories (Anonymous, 1995).

Lipases in cosmetics and perfumery: Lipases have potential application in cosmetics and perfumeries because it shows activities in surfactants and in aroma production (Metzger and Bornscheuer, 2006). Monoacyl glycerols and diacylglycerols are produced by esterification of glycerols and are used as a surfactant in cosmetics and perfume industries.

Lipases in paper making industry: The enzymatic pitch control method using lipase was put into practice in a large-scale paper-making process as a routine operation in the early 1990s and was the first case in the world in which an enzyme was successfully applied in the actual paper-making process (Bajpai, 1999). *Candida rugosa* lipases were used by Nippon Paper Industry, Japan to develop a pitch control method to hydrolyse 90 percentage of wood tryglycerides (Reetz and Jaeger, 1998).

CONCLUSION

Lipases are remarkable biocatalysts for high-value application in oleochemical industry and in the production of fine chemicals. Lipases are capable of catalyzing novel reactions, in both aqueous and non-aqueous media and are capable of regioselective, enantioselective and chemoselective biotransformations. Thus lipases are potential tools for the organic chemists. The widening application of microbial lipases in biotechnology has necessitated the continued research and development of novel lipases with broad substrate tolerance, high enantioselectivity and high stability. The growing demand for lipases has shifted the trend towards prospecting for novel lipases, improving the properties of existing lipases for established technical applications and producing new enzymes tailor-made for entirely new areas of application. This has largely been possible due to outstanding events in the field of molecular enzymology. Rational protein engineering, by way of mutagenesis and directed evolution, has provided a new and valuable tool for improving or adapting enzyme properties to the desired requirements. Quantitative improvement of the lipase gene can be achieved by employing recombinant DNA technology and protein engineering, especially through site directed mutagenesis and directed evolution of the strain. Thus, the modern methods of genetic engineering combined with an increasing knowledge of structure and function are allowing further adaptation to industrial needs and the exploration of novel applications.

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