Microbial lipases: Potential biocatalysts for the future industry

R. K. Saxena*, P. K. Ghosh, Rani Gupta*, W. Sheba Davidson*, Sapna Bradoo* and Ruchi Gulati***

*Department of Microbiology, University of Delhi, South Campus, Benito Juarez Road, New Delhi 110 021, India
**Department of Biotechnology, CGO Complex, Lodhi Road, New Delhi 110 003, India

Today, lipases stand amongst the most important biocatalysts carrying out novel reactions in both aqueous and nonaqueous media. This is primarily due to their ability to utilize a wide spectrum of substrates, high stability towards extremes of temperature, pH and organic solvents, and chemo-, regio- and enantioselectivity. More recently, the determination of their three-dimensional structure has thrown light into their unique structure–function relationship. Among lipases of plant, both animal and microbial origin, it is the microbial lipases that find immense application. This is because microbes can be easily cultivated and their lipases can catalyse a wide variety of hydrolytic and synthetic reactions. 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.

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. In the above scenario, enzymes such as proteases and amylases have dominated the world market owing to their hydrolytic reactions for proteins and carbohydrates. However, with the realization of the biocatalytic potential of microbial lipases in both aqueous and nonaqueous 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.

It is in the last decade that 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. Lipases (triacylglycerol acylhydrolases) belong to the class of serine hydrolases and therefore do not require any cofactor. The natural substrates of lipases are triacylglycerols, having very low solubility in water. Under natural conditions, they catalyse the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved (Figure 1). Under certain experimental conditions, such as in the absence of water, they are capable of reversing the reaction. The reverse reaction leads to esterification and formation of glycerides from fatty acids and glycerol. The occurrence of the lipase reaction at an interface between the substrate and the aqueous phase causes difficulties in the assay and kinetic analysis of the reaction[3]. The usual industrial lipases are special classes of esterase enzymes that act on fats and oils, and hydrolyse them initially into the substituted glycerides and fatty acids, and finally on total hydrolysis into glycerol and fatty acids[4–6].

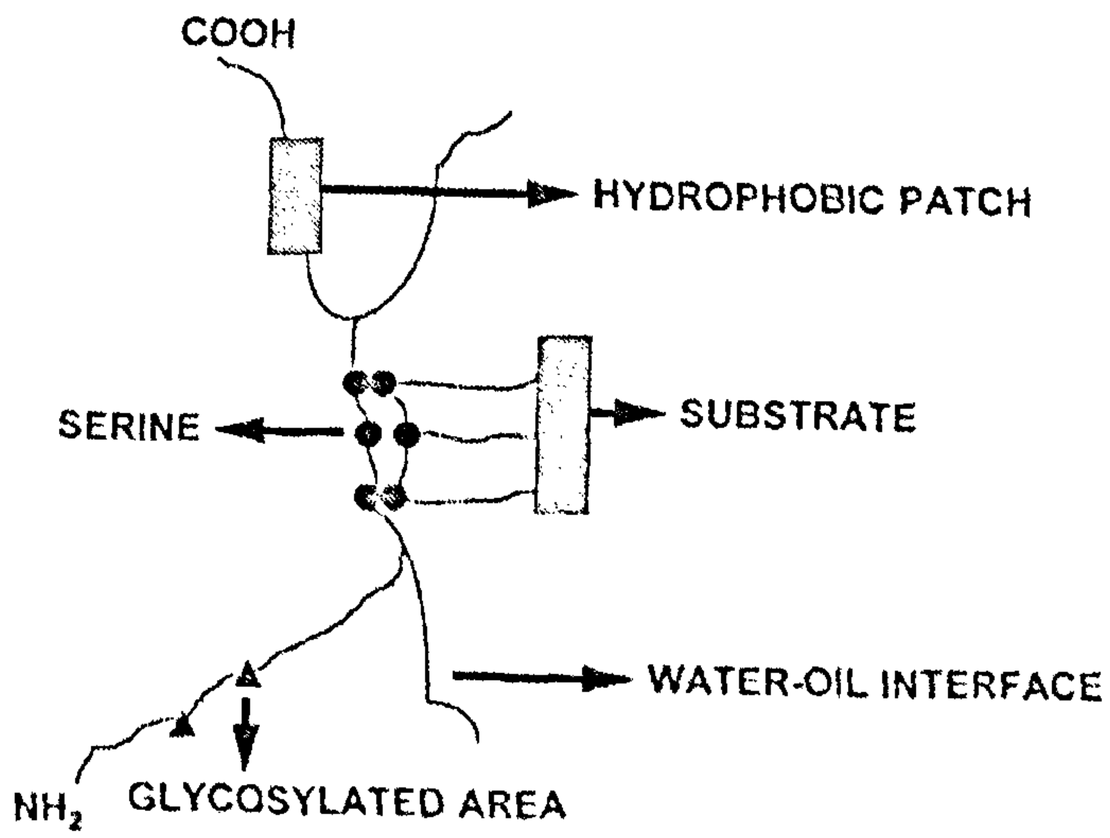

Figure 1. Diagrammatic representation of a lipase molecule showing its main features. Substrate can be any triglyceride.

*For correspondence. (e-mail: micro@dusc.ernet.in)

In nature, the lipases available from various sources have considerable variation in their reaction specificities: this property is generally referred as enzyme specificity. Thus, from the fatty acid side, some lipases have affinity for short-chain fatty acids (acetic, butyric, capric, caproic, caprylic, etc.), some have preference for unsaturated fatty acids (oleic, linoleic, linolenic, etc.), while many others are nonspecific and randomly split the fatty acids from the triglycerides. From the glycerol side of the triglycerides, the lipases often show positional specificity and attack the fatty acids at 1 or 3 carbon position of glycerol or at both the positions but not the fatty acid at the 2 position of the glycerol molecule. However, through random acyl migration, the 2-fatty acid monoglyceride undergoes rearrangement pushing the fatty acid to the 1 or 3 position of the glycerol molecule; as acyl migration is a slow process and as the available lipases do not act on glycerol 2-mono fatty acid esters, the hydrolysis slows down and awaits the acyl migration to complete for enabling the lipase to attack the glyceride at the 1 and/or the 3 position. Interestingly, lipases function at the oil–water interface (Figure 2). The amount of oil available at the interface determines the activity of the lipases[7]. This interface area can be increased substantially to its saturation limit by the use of emulsifier as well as by agitation. The saturation limit depends on the ingredients used as well as the physical conditions deployed. Thus, the activities of lipases can be pronouncedly increased by use of emulsifying agents as well as by methods that increase the size of the emulsion micelles[8,9].

Lipases are not involved in any anabolic processes. Since this enzyme acts at the oil–water interface, it can be used as a catalyst for the preparation of industrially important compounds[3,10–14]. Lipases catalyse the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol and fatty acids, and under certain conditions the reverse reaction leads to esterification and formation of glycerides from glycerol and fatty acids. As lipases act on ester bonds, they have been used in fat splitting, interesterification (transesterification), development of different flavours in cheese, improving pallatability of beef fat for making dog food, etc. A current application involves using lipases in water-deficient organic solvents for synthesizing different value-added esters from organic acids and alcohols. Lipases which are stable and work at alkaline pH, say 8 to 11, which are usually the suitable wash conditions for enzymated-detergent powders and liquids, have also been found, and these hold good potential for use in the detergent industry[15–18].

Research has been carried out on plant lipases[19–26], animal lipases[27–35], and microbial lipases, particularly bacterial and fungal[36–40]. Although pancreatic lipases have been traditionally used for various purposes, it is now well established that microbial lipases are preferred for commercial applications due to their multifold properties, easy extraction procedures, and unlimited supply[3].

Bacterial lipases

A relatively smaller number of bacterial lipases have been well studied compared to plant and fungal lipases[10,41–44]. Bacterial lipases are glycoproteins, but some extracellular bacterial lipases are lipoproteins. Winkler *et al.*[45] reported that enzyme production in most of the bacteria is affected by certain polysaccharides. Most of the bacterial lipases reported so far are constitutive and are nonspecific in their substrate specificity, and a few bacterial lipases are thermostable[3].

Among bacteria, *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., and *Chromobacterium* sp.[14] have been exploited for the production of lipases. Staphylococcal lipases are lipoprotein in nature[42]. Lipases purified from *S. aureus* and *S. hyicus* show molecular weights ranging between 34–46 kDa. They are stimulated by Ca^{++} and inhibited by EDTA. The optimum pH varies between 7.5 and 9.0. 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[42]. Putative active site residues around His 269 and Ser 369 of the *S. hyicus* lipase are highly conserved in the two *S. aureus* lipases and in several eukaryotic lipases[46].

Lipases from different species of *Psuedonomas* were purified by acidification of the culture supernatant, ammonium sulphate precipitation[47], sepharose CL-6B chromatography[41], and isoelectric focussing using CHAPS[47]. The purified lipase of *P. fragi*, *P. fluorescens*, and *P. aeruginosa* were monomeric with molecular weight of 33 kDa, 45 kDa, and 29 kDa, respectively[41,42,47]. The lipase was inhibited by Zn^{++}, Fe^{++}, and Al^{+++} and activated by Ca^{++} (ref. 48). The lipase gene of *P. fragi* has been cloned and sequenced[49].

Fungal lipases

Fungal lipases have been studied since 1950s, and Lawrence[49], and Brockerhoff and Jensen[8] have presented

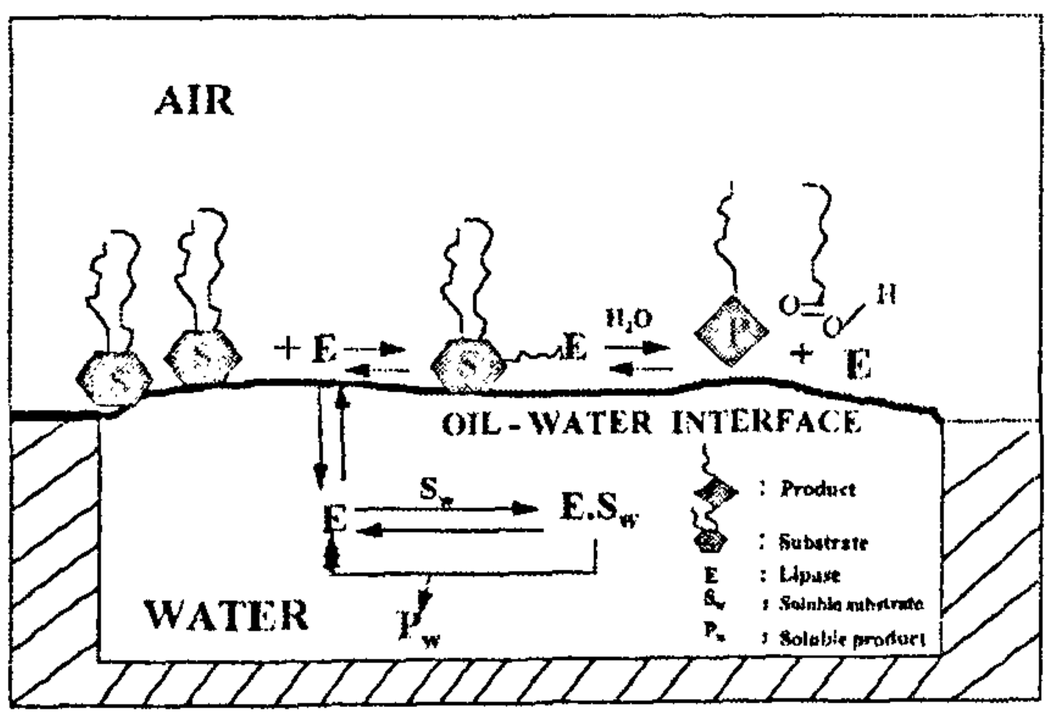

Figure 2. Lipolytic reaction at the oil–water interface.

comprehensive reviews. These lipases[51] 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*[14].

Taxonomic distribution of fungal lipases

Fungal lipases which degrade lipids from palm oil were investigated by Turner (cited by Lazer and Schroder[50], and listed by Sztajer and Zboinska[52]). Among Mucorales, the lipolytic enzymes of the moulds *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[50]. The thermophilic *M. pusillus* is well known as a producer of thermostable extracellular lipase. From a lipase-producing strain of *M. miehei*, two isoenzymes with slightly different isoelectric points but a high degree of antigenic identity could be isolated[53]. A lipase of *M. miehei*, immobilized on a resin (Lipozyme TM) has been commercialized by Novo Industries.

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. *R. japonicus* lipase has been used to produce hard butter suitable for chocolate manufacture by interesterification of palm oil with methyl stearate[54]. The lipases (40 to 45 kDa) of various *Rhizopus* species show maximum activity towards medium-chain fatty acids (C_8–C_{10}). In case of *R. delemar*, extracellular[55] and intracellular[56] lipase isoenzymes have been isolated.

Lipase producers within the order Entomophthorales include *Entomophthora apiculata*, *E. coronata*, *E. thaxteriana*, *E. virulenta*, *Basidiobolus* spp. and *Conidiobolus* spp. The genera *Pichia*, *Hansenula*, and *Saccharomyces* are also reported to produce lipase[43]. Two kinds of cell-bound lipases were purified from *Saccharomyces lipolytica*[50]. Lipases are reported from *Candida curvata*, *C. tropicalis*, *C. valida*, and *C. pellioculosa*[50] and are nonspecific towards the different ester bonds in triglycerides, with the exception of *C. deformans*[50].

The imperfect fungus *Geotrichum candidum* is responsible for acid formation in dairy products by lipolyzing fat. The *G. candidum* lipase features specificity towards fatty acids with a *cis* double bond at C_9, hence is applied for the structural analysis of triglycerides[57].

The intracellular and extracellular lipases of *Aspergillus niger* are 1,3-(regio)-specific[58]. *A. oryzae* was reported to be an efficient host for the heterologous expression of the lipase from *Rhizopus miehei* and *Humicola lanuginosa*[50]. The lipase of *Penicillium roqueforti* is responsible for the flavour of Blue cheese[59]. Lipolytic activity has also been detected in *P. camemberti*, the white surface mould of Brie and Camembert cheese. Lipases with specificity for butyric acid have been isolated from strains of the *Penicillium* species such as *P. cyclopium*[60], *P. verrucosum* var. *cyclopium*, and *P. crustosum*[50]. The *P. cyclopium* lipase has a much higher activity towards di- and monoglycerides than triglycerides. The lipase of *H. lanuginosa* DSM 3819 is suitable as a detergent additive because of its thermostability, high activity at alkaline pH, and stability towards anionic surfactants[61]. *H. lanuginosa* lipases show a high degree of hydrolytic activity with coconut oil and oils having a high content of lauric acid. The two lipases differ in their positional specificity[62].

Lipase production

Lipids are insoluble in water and need to be broken down extracellularly into their more polar components to facilitate absorption if they are to function as nutrients for the cell. Therefore majority of the lipases are secreted extracellularly.

Optimization of growth parameters

A number of reports exist on influences of various environmental factors such as temperature, pH, nitrogen, carbon and lipid sources, agitation, and dissolved oxygen concentration on lipase production[65,67–68]. Lipase production is generally stimulated by lipids[69,70]. The lipase activity steadily increases to a peak and declines. 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. High production of lipase in case of *P. fragi* occurs in peptone-supplemented medium, although different peptones vary in their effectiveness[36,72,73]. Though *Pseudomonas* sp. grow in a basal medium with ammonium sulphate, glucose, citrate or pyruvate, it requires an organic nitrogen source for lipase production[73]. A mixture of arginine, lysine and glutamic acid in medium was observed to be effective for lipase production[73]. A strain of *Penicillium roqueforti* produces maximum amount of lipase when grown in 0.5% casitone and 1% proflobroth[59]. Growth and lipase production by *Micrococcus* sp. were unaffected by peptone of 0.5% to 2%, but lipase production by *Pseudomonas* sp, *A. wentii*, *M. hiemalis*, *R. nigricans*, and *M. racemosus* were stimulated by peptone[74–77]. Soybean meal extract in *Rhizopus oligosporus* culture medium supported good growth and lipase production[68]. Physiological regulation of lipase activity by thermotolerant strain of *P. aeruginosa* EF_2 under various conditions in batch,

fed-batch, and continuous cultures support the contention that nitrate generally stimulates production of lipase[78].

Milk is a good medium for growth of psychrotrophic bacteria and for lipase production which was found to be susceptible to catabolite repression by glucose[74–80]. While glucose is essential for production of lipase by *P. fragi*[73], *A. wentii*[74], *M. hiemalis*[75], *R. nigricans*[76], and *M. racemosus*[77]. *P. aeruginosa* EF_2 (ref. 78) showed no such requirement[81]. Lipase activity per milligram dry weight of mycelium was much higher on lactose, mannose, xylose, fructose, dextrin, and rhamnose in case of *Talaromyces emersonii*[67]. Mannitol, galactose, sucrose[74], fructose, lactose, maltose, raffinose or ribose produced less amount of lipase[76] caused decreased growth with corresponding reduction in lipase activity in *M. racemosus*[77]. Polysaccharides such as glycogen, hyaluronate, laminarin, gum arabic, and pectin stimulated production of lipase in *Serratia marcescens*[47] and *Saccharomycopsis lipolytica*[82]. This might probably be due to the detachment of lipase from the oil surface. A similar mechanism may explain the stimulating effect of lecithin on lipase production, as investigated in *R. japonicus*[64].

Triglyceride is important for lipase production as it can act both as an inducer and inhibitor. Among the triglycerides, olive oil was observed to be effective in inducing lipase[75]. Salts of unsaturated fatty acids inhibited lipase production by *P. fragi*[83], whereas tributyrin and trioctanoin had no effect on lipase production by *P. fragi* and *M. freudenreichii*[84]. Butter oil, corn oil or olive oil inhibited lipase production by *P. roqueforti*[59], *Saccharomycopsis* sp., *B. licheniformis*, *M. caseolyticus* and *Staphylococcus* sp.[86]. Triglycerides such as olive oil, groundnut oil and cotton seed oil, and fatty acids such as oleic acid, linoleic acid and linolenic acid stimulated lipase production by *P. mephitica*[85]. Lipids are considered not to be true inducers[36,72]. *A. wentii* showed reduced growth and lipase production when the synthetic and natural lipids were added to the growth medium[74]. Emulsification of culture media containing oil by gum acacia supported good growth and lipase production in *R. oligosporus*[68]. Triolein, olive oil, tributyrin, and oleic acid butylester were able to induce lipase in immobilized protoplasts, whereas Tween 80 enhanced lipase activity[87].

The initial pH of the growth medium is also important for lipase production. Maximum activity was observed at pH > 7.0 for *P. fragi*[88] and at pH 9.0 for *P. aeruginosa*[81] wherein development of acidity in media reduced lipase activity[88]. In contrast, maximum growth at acidic pH (4.0–7.0) was reported for *S. lipolytica*[86], *M. caseolyticus*[86], *B. licheniformis*, *A. wentii*[74], *M. hiemalis*[75], *R. nigricans*., *Mucor racemosus*[76,77], *R. oligosporus*[68] and *P. aeruginosa* EF_2 (ref. 78).

It has been observed that increasing the temperature above 8°C resulted in a depressing effect on lipase production by *P. fluorescens*[89] and *P. fragi*[88]. However, the rapid inactivation of the *P. fluorescens* lipase by subtilisin at 20°C (ref. 90) indicated that bacterial lipases could potentially be inactivated by simultaneously secreted proteinases and that this effect is likely to be greater in cultures grown at relatively higher temperatures (30–40°C) (ref. 36). Oso[67] determined 45°C to be the best temperature for lipase production by *T. emersonii.* Temperatures in the range of 22–35°C were however observed to be optimum for maximum lipase production for *A. wentii*[74], *M. heimalis*[75], *R. nigricans*[76], *M. racemosus*[77] *R. oligosporus*[68], and *P. aeruginosa*[78].

Aeration has variable effect on lipase production by different organisms. The degree of aeration appears to be critical in some cases since shallow layer cultures (moderate aeration) produced much more lipase than shake cultures (high aeration)[72,89]. Vigorous aeration greatly reduced lipase production by *R. oligosporus*[68], *P. fragi*[91], *P. aeruginosa*[81], and *M. racemosus* resulted in increased lipase production in static culture conditions[77]. However, high aeration was needed for high lipase activity by *P. mephitica* var. *lipolytica*[85], *A. wentii*[74] and *M. hiemalis*[75]. Changing the ratio of surface area to volume and hence, aeration of cultures of *P. fragi* had no effect on the quantity of lipase produced per cell; but increasing aeration by shaking resulted in both increased growth and lipase production, followed by a rapid decrease of lipase activity as shaking continued[51]. Oso[67] reported that stationary conditions in *T. emersonii* favoured maximum lipase production. Row and Gilmour[92] observed that lipase synthesis, by two strains of *P. fluorescens* (psychrotroph), stimulated in milk medium at 7°C, was immediately preceded by a decrease in O_2 tension which resulted in earlier production of lipase. The influence of the concentration of O_2 on lipase productivity by *R. delemar* has been studied in different fermenters. Giuseppin[93] suggested that oxygen is the limiting factor in shake-flask cultures. He also reported that low oxygen concentration negatively affects the metabolism of *R. delemar*, which explains that low oxygen concentration is a useful tool to scale down fermentation processes in cases where a transient or local oxygen limitation occurs.

Lipase assay

Lipases are known to hydrolyse triglycerides and give rise to free fatty acids and glycerol. Therefore, the assay methods involve spectrophotometry or titrimetry[94,95–100], radiolabelling assay[101,102], fluorimetry[103], surface tension method[104], and estimation of free fatty acids by high performance liquid chromatography (HPLC)[42]. Tributyrin plate assay and titrimetry are the most commonly used methods for screening of lipase producers and estimation of lipase activity[105–109], respectively.

Lipase purification

Lipases have been purified from animal, plant, fungal and bacterial sources by different methods[110–122] involving ammonium sulphate precipitation, gel filtration, and ion exchange chromatography. In recent years, affinity chromatographic techniques have come into use as this technique decreases the number of steps necessary for lipase purification as well as increases specificity. Currently, reversed-micellar[112,123–126] and two-phase systems[127–131], membrane processes[132], and immuno-purification[133–134] are being used for purification of lipases.

Lipase properties

pH optima

The lipases that have been studied show profound stability around pH 6.0–7.5 with considerable stability at acidic pH up to 4 and at alkaline pH up to 8. Extracellular lipase of *A. niger*, *Chromobacterium viscosum* and *Rhizopus* sp. are active at acidic pH (refs 38, 63, 140). An alkaline lipase active at pH 11.0 has been isolated from *P. nitroreducens*[65].

Temperature optima and thermal stability

The pancreatic lipases loose activity on storage at temperatures above 40°C, but some microbial lipases are more resistant to heat inactivation. While lipases of *A. niger*[63], *R. japonicus*[64], and *C. viscosum*[38] are stable at 50°C, lipases of thermotolerant *H. lanuginosa* and *P.* sp. *nitroreducens* are stable at 60°C and 70°C (ref. 141), respectively. *C. gigantea* lipase had half life for inactivation of 35.7, 46.4 and 22.9 min. at 45°C, 50°C and 55°C (ref. 12), similar to lipases of *R. japonicus*[142]. In our laboratory, we observed that purified lipase from *A. terreus*[143] retained 100% of its activity at 60°C after 24 h. But, the maximum activities of *C. gigantea* and other lipases from mesophiles were at 30–35°C (ref. 144). Thermophilic bacterial lipases obtained from Icelandic hot spring showed higher lipase activity at 40 to 60°C (ref. 145).

Activation and inactivation of the enzyme

Cofactors are not required for the expression of lipase activity. Divalent cations, such as calcium, generally stimulate the activity. It has been postulated that this is based on the formulation of calcium salts of long-chain fatty acids[3,14]. The lipase activity is inhibited drastically by Co^{++}, Ni^{++}, Hg^{++}, and Sn^{++}; and is slightly inhibited by Zn^{++}, Mg^{++}, EDTA, and SDS[146]. In *H. lanuginosa* S-38, sulphhydryl-reducing agents, like dithiothreitol, did not alter the enzyme activity, but did render it more susceptible to heat inactivation. Inactivation is accelerated by the addition of urea. Reducing compounds (cysteine, 2-mercaptoethanol), chelating agents, (EDTA, *o*-phenanthroline), and thiol group inhibitors (*p*-chloro mercuric benzoate, monoiodoacetate) did not show a detectable effect on lipase in *M. pusillus*, suggesting that lipase is not a metallo-enzyme and it does not require either free -SH group or an intact S–S bridge for its activity. Spontaneous and cyclic AMP-induced lipase formation is greatly enhanced in *Serratia marcescens* SM-6 on exposure to glycogen, hyaluronate, pectin B, and gum arabic[47].

Substrate specificity

Specificity of lipases is controlled by the molecular properties of the enzyme, structure of the substrate, and factors affecting binding of the enzyme to the substrate[147]. Substrate specificity of lipases is often crucial to their application for analytical and industrial purposes. Specificity is shown both with respect to either fatty acyl or alcohol parts of their substrates[147]. Many microbes produce two or more extracellular lipases with different fatty acid specificities. Tributyrin is hydrolysed slowly by some microbial lipases[146,149]. In contrast, *M. miehei* lipase preferentially releases butyric acid from milk fat especially at low pH (ref 150). *Geotrichum candidum* produces a lipase, which shows pronounced specificity for the hydrolysis of esters of a particular type of long-chain fatty acid. Substrate specificity of this lipase has been summarized by Jensen[151], Jensen and Pitas[152], and Macrae[153]. Lipases show both regio- and stereospecificity with respect to the alcohol moeity of their substrates[154]. Lipases can be divided into two groups on the basis of the regiospecificity exhibited acylglycerol substrates[3].

Lipases in the first group catalyse the complete breakdown of triacylglycerol to glycerol and free fatty acids together with diacylglycerols and monoacylglycerol as intermediates in the reaction. These intermediates do not accumulate since they are hydrolysed more rapidly than the triacylglycerol. Examples of the first group of lipases include lipase from *C. cylindracea*[155].

The second group of lipases release fatty acids regiospecifically from the outer 1 and 3 positions of acylglycerols. These lipases hydrolyse triacylglycerol to give free fatty acids, 1,2-diacylglycerols[2,3], and 2-monoacylglycerol. Many extracellular microbial lipases, such as those from *A. niger*[58] and *R. arrhizus*[156], show 1,3-(regio)-specificity. Lipases excreted by *R. japonicus*, *M. miehei*, *H. lanuginosa*, *C. viscosum*, and *P. fluorescens* are also 1,3-(regio)-specific[3]. Till date, there are no authentic reports of lipases which catalyse the release of fatty acids selectively from the central 2-position of

acylglycerols[3], except for a report of Asahara *et al.*[157]. Partial stereospecificity in the hydrolysis of triacyl glycerols has been observed in *R. arrhizus*, *R. delemar*, *C. cylindracea*, and *P. aeruginosa*. Owing to this property, these enzymes can be used to isolate optically pure esters and alcohols[158,159].

Production of an extracellular microbial lipase possessing pronounced stereospecificity in the hydrolysis of triacylglycerols, would be of considerable commercial interest. Most lipases attack triglycerides as readily as partially esterified glycerides, but an enzyme from a specific *P. cyclopium* strain has been shown to attack monoglycerides most rapidly followed by di- and triglycerides, respectively, and it has been described as a partial glycerol ester hydrolase[160]. Several kinds of microbial lipases have already been introduced commercially (Table 1) and exploited for their potential to catalyse a large number of hydrolytic and synthetic reactions in both aqueous and organic media as mentioned below.

Lipases in organic synthesis

For synthetic chemists, the application of lipases as catalysts in organic synthesis is of much advantage. These enzymes can show stereo- and regiospecificity, and tolerate organic solvents in the incubation mixture. Both the activity of the enzyme and the identity of the product depend upon the solvents used, which may vary from aqueous buffer systems through biphasic emulsions and microemulsions, to organic solvents[161–168]. For synthetic purposes, crude enzyme preparations are often convenient. Because the catalyst is always an expensive factor in a chemical reaction, strategies for enzyme recycling are being developed[169]. Synthetic strategies involving microbial lipases can be used to prepare molecules of high positional and configurational purity. Lipases can be used to create biologically active analogues of naturally occurring messenger molecules as antagonists or inhibitors in biological systems[170,171].

Lipase catalyses the hydrolysis of triglycerides at the water–lipid interface. Under given experimental conditions, the amount of water in the reaction mixture will determine the direction of the lipase-catalysed reaction. While in absence of water or its presence in trace quantities esterification and transesterification are favoured; in presence of excess water hydrolysis occurs[8].

Bioconversions in aqueous media

A typical lipase-catalysed reaction in aqueous media is ester hydrolysis. This enzymic conversion can be used for the synthesis of triglycerides as shown for the preparation of platelet-activating factor[172,173]. Another application of the hydrolytic specificity of lipases is the partial hydrolysis of triglycerides to di- and monoglycerides in the food industry, where di- and monoglycerides serve as biocompatible emulsifiers and food additives. These and other applications of lipases in industry and research have been discussed in the review by Iwai and Tsujisaka[174].

Bioconversions in organic media

The synthetic potential of lipases in organic solvents has been widely recognized and is well documented, particularly on the basis of activity of lipases in organic solvents containing low water content[175]. The main application of lipases in organic chemistry is the resolution of enantiomeric compounds, making use of the enantioselectivity of these enzymes[176,177]. The use of organic solvents for lipase-catalysed resolutions has four main advantages in comparison with water as the solvent:

Table 1. Properties of some microbial lipases

Organism	Specificity	Molecular weight (kDa)	Isoelectric point	pH optimum	Temp. optimum (°C)	Specific activity (U mg^{-1})
Chromobacterium viscosum	unspecific	30	7.3	6.5–7.0	70	22.75
Pseudomonas sp.	regio 1,3	32	4.5	7.8	47	7.80
P. fluorescens	regio 1,3	32	4.5	7.0	50–55	3.05
Candida cylindracea	unspecific	120	4.2	7.2	45	53.22
C. curvata	18 : 1 > 16.0 = 14.0	195	—	5.0–8.0	60	4
C. deformans	regio 1,3	207	—	7.0	80	19
Aspergillus niger	regio 1,3	38	4.3	5.6	25	9.02
Geotrichum candidum	*cis*-∇^9-unsaturated fatty acids	55	4.3	6.6	40	14.20
Humicola lanuginosa	unspecific	27.5	—	8.0	60	5.16
Mucor miehei	regio 1,3	—	—	8.0	40	3.25
Penicillium cyclopium						
Lipase A	unspecific	27	4.9	7.5	35	0.09 (A+B)
Lipase B	unspecific	36	4.1	5.8	40	—
Rhizopus arrhizus	regio 1,3	43	6.3	8.0	—	16.08
R. delemar	regio 1,3	41.3	4.2	5.6	35	2.20

(i) racemic mixtures of alcohols or acids need not be esterified before resolution into enantiomers, (ii) these enzymes are more stable in organic solvents than in water, (iii) lipases used need not be immobilized for recovery, owing to their insolubility in organic solvents; they can be collected by filtration in their active state, and (iv) substrates and products may be unstable in aqueous solution. In this case, reaction in organic solvents is essential for formation and isolation of the products. The three main areas of lipase-catalaysed reactions in organic solvents are:

(i) Resolution of racemic alcohols: Racemic alcohols in nonaqueous media can be resolved in a biphasic system, using lipase from *C. cylindracea*. Transesterification reactions were carried out in diethyl ether and heptane by Kirchner *et al.*[178], using porcine pancreatic lipase as the catalyst. Transesterification was also used to prepare useful synthons, such as optically active monoesters of 3-hydroxylglutarate and related compounds[179]. Lipases have been used for the production of pure, biologically active *S*-enantiomer of sulcatol[180]; and for transesterification of cyanohydrin compounds, α-substituted cyclohexanol, and epoxy esters[181].

(ii) Resolution of racemic acids by asymmetric esterification: The use of the stereoselectivity of lipases for the resolution of racemic acid mixtures in immiscible biphasic systems has been demonstrated. Lipase from *C. cylindracea* has been applied to the resolution of 2-bromopropionic acids and 2-chloropropionic acids, which are the starting materials for the synthesis of phenoxy-propionic herbicides[178]. *P. fluorescens* lipase was used for asymmetric ring opening of substituted prochiral glutaric anhydrides of dicarboxylic acids[182]. Porcine pancreatic lipase was used by Gutman and Brando[183] for stereo-selective lactomizations and polycondensations from prochiral hydroxy diesters. Macrocyclic lactones, such as (–) pyrenophorin, can be synthesized stereoselectively by *Pseudomonas* sp. lipase from (w-1)-hydroxyalkanoic esters in anhydrous isooctane at 65°C (ref. 184).

(iii) Regioselective acylations: Lipase-catalysed acylations are not limited to simple alcohols. Lipases also show high regioselective acylations with certain steroids, sugars and sugar derivatives – such as monoacylated sugars[185–187]. The regioselective acylation of hydroxy groups in glycals, which are versatile chiral intermediates, is catalysed by lipases[188]. Another example of the high regiospecificity of lipases is acylation reactions in anhydrous organic solvents for synthesis of hydroxy steroids[189]. Lipases have been deployed for catalysing three major types of reactions, namely transesterification (also called inter-esterification), hydrolysis, and esterification (Figure 3).

As the net energy resulting from transesterification reactions is zero, these reactions can be easily carried out. However, the other two types of reactions are also gaining significant industrial importance. In particular, esterification reactions are industrially important in the synthesis of value-added esters used in the c industry; the reaction is carried out by controll water content in the reaction mixture.

When lipases are incubated with triglycerides, lysis and resynthesis result in acyl migration b glyceride molecules. By controlling the quantity o in the reaction system, it is possible to restr hydrolysis. The transesterification reactions can made to dominate[190]. If a nonspecific lipase is us rearranged triglycerides become more or less the s obtained by chemical transesterification using alkoxides as catalysts. However, by the use of 1,3-s lipases, a mixture of triglycerides is obtained whic obtained by chemical methods.

Lipase-catalysed *trans*-esterification reactions 1 1,3-specific are utilized for making cocoa butter tutes, using cheaper mid-fraction of palm oil and acid. While the mid-fraction of palm oil contai dipalmitoyl-2-monoolein (POP) as the major trigl

Transesterification Reactions

(a) Acidolysis

$$R_1-\underset{\underset{O}{\|}}{C}-OR_2 + R_3-\underset{\underset{O}{\|}}{C}-OH \rightarrow R_3-\underset{\underset{O}{\|}}{C}-OR_2 + R_1-\underset{\underset{O}{\|}}{C}-$$

(b) Alcoholysis

$$R_1-\underset{\underset{O}{\|}}{C}-OR_2 + R_3-OH \rightarrow R_1-\underset{\underset{O}{\|}}{C}-OR_3 + R_2-OH$$

(c) Ester Exchange

$$R_1-\underset{\underset{O}{\|}}{C}-OR_2 + R_3-\underset{\underset{O}{\|}}{C}-OR_4 \rightarrow R_1-\underset{\underset{O}{\|}}{C}-OR_4 + R_3-\underset{\underset{O}{\|}}{C}$$

(d) Aminolysis

$$R_1-\underset{\underset{O}{\|}}{C}-OR_2 + R_3-NH_2 \rightarrow R_1-\underset{\underset{O}{\|}}{C}-NHR_3 + R_2-OH$$

Hydrolysis

$$R_1-\underset{\underset{O}{\|}}{C}-OR_2 + H_2O \rightarrow R_1-\underset{\underset{O}{\|}}{C}-OH + R_2-OH$$

Ester Synthesis

$$R_1-\underset{\underset{O}{\|}}{C}-OH_2 + OH-R_2 \rightarrow R_1-\underset{\underset{O}{\|}}{C}-OR_2-H_2O$$

Figure 3. Various lipase-mediated reactions.

1-(3)-palmitoyl-3-(1)-stearoyl-2-monoolein (POS) and 1-(3)-distearyl-2-monoolein (TOS) are the main constituents of cocoa butter. Therefore, by transesterification between POP and stearic acid or POP and tristearin, valuable equivalents of cocoa butter have been made[190–192].

The mid-fraction of palm oil is obtained by double-stage fractionation of the oils from *n*-hexane[193]. The trans-esterification of the mid-fraction is carried out at 37°C for 20 h using one part of the mid-fraction, 0.7 parts of stearic acid dissolved in water-saturated *n*-hexane (2.8 to 3 parts), and 0.1 parts of celite-immobilized lipase (*Rhizopus arrhizus* lipase from Sigma). The composition of the trans-esterified product approaches that of the cocoa butter[193]. Sunflower and safflower seed oils have also been used for the production of cocoa butter substitutes in Japan[194].

There is a large volume of literature on hydrolysis of fats and oils by lipases used either in the pure form[195,196] or in the immobilized form[197–206] or in the cell-bound form[201,202]. The hydrolysis is carried out using the conventional emulsion systems. The enzymatic hydrolysis has not however replaced the conventional colgate emery process. The saving in energy costs is perhaps not adequate enough to attract adoption of lipase-catalysed fat-splitting process over the conventional chemical process. However, some companies have reported use of lipases, for example use of lipase from *Candida cylindracea* for the splitting of oils, and the use of resulting fatty acids for the production of soaps[203]. It has been claimed that the enzymatic method yielded soaps with better colour and odour, and resulted in an overall cost saving. Oils containing highly unsaturated or conjugated fatty acids are considered particularly amenable to enzymatic-hydrolysis processes[204,205]. Currently, the focus of researches are on investigating the continuous hydrolysis of oils into fatty acids, using membrane bioreactors[203–206] or hollow fibre reactors[207]. The enzymatic method of fat splitting will gain industrial importance with increasing high energy cost or with requirements for large-scale production of special grades of susceptible fatty acids.

Manufacture of esters, through lipase-catalysed synthesis as well as alcoholysis is gaining industrial importance. Several high-purity esters have been manufactured for use in the cosmetic industry. Many of the esters, derived by using these reactions, resemble naturally occurring waxes of commercial importance[208–210]. While the esters produced from short-chain fatty acids have applications as flavour constituents in the food industry[211–213], methyl- or ethyl esters produced from long-chain acids have a potential application for diesel fuels[214]. Esters derived from long-chain fatty acids and long-chain fatty alcohols are referred to as waxes which have uses as lubricants or in the cosmetic industry; kilogram-scale synthesis of such esters has been described by Olivecrona and Bengtsson[215]. Lipase-catalysed ester synthesis requires the maintenance of low concentration of water. The available literature indicates a variation in water concentration from 0.75% to 4% (w/v) in different types of ester synthesis[207]. This condition is satisfied by using nonaqueous solvents like hexane.

Certain lipases also selectively esterify alcohols. Thus, *Rhizopus arrhizus* lipase of Novo Nordisk converts selectively geraniol into its acetate ester, when a mixture of geraniol and nerol is subjected to esterification in hexane. Thus, a reaction between 0.1 M each of geraniol and nerol mixed with acetic acid in hexane for 2 days produced 80% of the total esters as geranyl acetate, and the remaining 20% as nerol acetate. Laboratory-scale studies on an enzyme dose of 1% w/v for the synthesis of geranyl butyrate gave a conversion of almost 100% after 1.5 days at 30°C when 0.1 M geraniol and 0.1 M butyric acid were used as reactants in hexane[216].

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[217–220] (Table 2).

Lipases in dairy industry

Lipases are used extensively in the dairy industry for the hydrolysis of milk fat. Current applications include

Table 2. Important areas of industrial applications of microbial lipases

Industry	Effect	Product
Bakery	Flavour improvement and shelf-life prolongation	Bakery products
Beverages	Improved aroma	Beverages
Chemical	Enantioselectivity	Chiral building blocks and chemicals
Cleaning	Synthesis Hydrolysis	Chemicals Removal of cleaning agents like surfactants
Cosmetics	Synthesis	Emulsifiers, moisturising agents
Diary	Hydrolysis of milk fat Cheese ripening Modification of butter fat	Flavour agents Cheese Butter
Fats and oils	*Trans*-esterification Hydrolysis	Cocoa butter, margarine Fatty acids, glycerol, mono- and diglycerides
Food dressing	Quality improvement	Mayonnaise, dressings and whippings
Health food	*Trans*-esterification	Health food
Leather	Hydrolysis	Leather products
Meat and fish	Flavour development and fat removal	Meat and fish products
Paper	Hydrolysis	Paper products
Pharmaceuticals	*Trans*-esterification Hydrolysis	Speciality lipids Digestive aids

flavour enhancement of cheese, acceleration of cheese ripening, manufacture of cheese-like products, and lipolysis of butter fat, and cream[221]. While the addition of lipases primarily releases short-chain (C_4 and C_6) fatty acids that leads to the development of sharp, tangy flavour, the release of medium-chain (C_{12} and C_{14}) fatty acids tends to impart a soapy taste to the product. In addition, the free fatty acids take part in simple chemical reactions where they initiate the synthesis of other flavour ingredients such as aceto-acetate, β-keto acids, methyl ketones, flavour esters, and lactones[222].

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[221]. 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 detergents

The usage of enzymes in washing powders still remains the single biggest market for industrial enzymes[223]. The world-wide trend towards lower laundering temperatures has led to much higher demand for household detergent formulations. Recent intensive screening programmes, followed by genetic manipulations, have resulted in the introduction of several suitable preparations, for example, Novo Nordisk's Lipolase (*Humicola* lipase expressed in *Aspergillus oryzae*[224]).

Lipases in oleochemical industry

The scope for application of lipases in the oleochemical industry is enormous as it saves energy and minimizes thermal degradation during hydrolysis, glycerolysis, and alcoholysis[223–225]. Miyoshi Oil and Fat Co., Japan, reported commercial use of *Candida cylindracea* lipase in production of soap[226]. The introduction of the new generation of cheap and very thermostable enzymes can change the economic balance in favour of lipase use[3].

The current trend in the oleochemical industry is a movement away from using organic solvents and emulsifiers[227,228]. The various reactions involving hydrolysis, alcoholysis, and glycerolysis have been carried out directly on mixed substrates, using a range of immobilized lipases[229]. 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[230].

Lipases in synthesis of triglycerides

The commercial value of fats depends on the fatty acid composition within their structure. A typical example of a high-value asymmetric triglyceride mixture is cocoa butter. The potential of 1,3-regiospecific lipases for the manufacture of cocoa-butter substitutes was clearly recognized by Unilever[231] and Fuji Oil[232]. Comprehensive reviews on this technology, including the analysis of the product composition, are available[3,229]. In principle, the same approach is applicable to the synthesis of many other structured triglycerides[233] possessing valuable dietic or nutritional properties, for example, human milk fat. This triglyceride and functionally similar fats are readily obtained by acidolysis of palm oil fractions which are rich in 2-palmitoyl glyceride with unsaturated fatty acid(s)[234]. Acidolysis, catalysed by 1,3-specific lipases, is used in the preparation of nutritionally important products which generally contain medium-chain fatty acids[235]. Lipases are being investigated extensively with regard to the modification of oils rich in high-value polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acids. Substantial enrichment in the polyunsaturated fatty acid content of mono-glyceride fraction has been achieved by lipase-catalysed alcoholysis or hydrolysis[236].

Lipases in synthesis of surfactants

Polyglycerol and carbohydrate fatty acid esters are widely used as industrial detergents and as emulsifiers in a great variety of food formulations (low-fat spreads, sauces, ice-creams, mayonnaises). Enzymic synthesis of functionally similar surfactants has been carried out at moderate temperature (60–80°C) with excellent regioselectivity. Adelhorst *et al.*[237] have carried solvent-free esterification of simple alkyl-glycosides using molten fatty acids and immobilized *Candida antarctica* lipase. Fregapane *et al.*[238] obtained mono- and diesters of monosaccharides in high yields, using sugar acetals as starting materials. Lipase from *A. terreus* synthesizes a biosurfactant by transesterification between natural oils and sugar alcohols[239]. Lipases may also replace phospholipases in the production of lysophospholipids. *Mucor miehei* lipase has been used for the transesterification of phospholipid in a range of primary- and secondary alcohols[240]. Lipases may also be useful in the synthesis of a whole range of amphoteric bio-degradable surfactants, namely amino acid-based esters and amides[241,242].

Lipases in synthesis of ingredients for personal care products

Unichem International has recently launched the production of isopropyl myristate, isopropyl palmitate, and 2-ethylhexyl palmitate[243] for use as emollient in personal care products like skin and sun-tan creams, and bath oils. Wax esters have similar application in personal care products and are being manufactured enzymatically, using *C. cylindracea* lipase, in a batch bioreactor[224].

Lipases in pharmaceuticals and agrochemicals

The utility of lipases in the preparation of chiral synthons is well recognized and documented. Several processes have recently been commercialized which have been described by Sainz-Diaz *et al.*[244] and Davis *et al.*[245]. 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[178]. Another impressive example of the commercial application of lipases in the resolution of racemic mixtures is the hydrolysis of epoxyester alcohols[246]. 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 β-blockers and a wide range of other products. 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[247].

Lipases have applications as industrial catalysts for the resolution of racemic alcohols in the preparation of some prostaglandins, steroids, and carbocyclic nucleoside analogues. Regioselective modification of polyfunctional organic compounds is yet another rapidly expanding area of lipase application, particularly in the field of AIDS treatment[248]. Lipases from *A. carneus* and *A. terreus* show chemo- and regiospecificity in the hydrolysis of peracetates of pharmaceutically important polyphenolic compounds[249,250]. Lipases are also useful in the synthesis of the artificial sweetner sucralose by regioselective hydrolysis of octa-acetylsucrose[251].

Lipases in polymer synthesis

The stereoselectivity of lipase is useful for synthesis of optically active polymers[252]. These polymers are asymmetric reagents, and are used as absorbents. In the field of liquid crystals, suitable monomers can be prepared by lipase-catalysed transesterification of alcohols[253], which with racemic alcohols may be accompanied by resolution[254]. The use of chiral glycidyltosylates for the preparation of ferroelectric liquid crystals[246] has also been reported. Thus, this enzyme has diversified commercial use, both in terms of scale and processes. Lipases have been employed successfully in the food industry as well as in hightech production of fine chemicals and pharmaceuticals. Furthermore, this enzyme has potentials in newer fields, for example lipases have successfully been used in paper manufacturing – apparently, the treatment of pulp with lipase leads to a higher quality product and reduced cleaning requirement. Similarly, the enzyme has also been used in association with a microbial cocktail for the treatment of fat-rich effluents from an ice-cream plant. This could also be utilized in waste processing of many food industries[255].

Current status of lipase research in India

Researches on microbial lipases in India date back to late seventies when a few reports on screening and production of lipase from a few fungi and bacteria appeared. The initial emphasis on screening exercises was followed by process optimization for maximum lipase production. Scientists at the National Diary Research Institute, Karnal[74,76,77] investigated physico-chemical conditions of lipases produced by *M. racemosus*, *A. wentii*, and *P. chrysogenum*. In 1981, one group highlighted the lipolytic activity of thermophilic fungi of paddy straw compost[256]. Systematic screening strategies were employed by Bhaduria[257]. This study reported *A. niger*, *A. flavus*, *A. fumigatus* and *Penicillium glaucum* as the potential lipase producers isolated from the kernels of chironji and walnut.

Owing to the industrial applications of lipases, the Department of Biotechnology, New Delhi, promoted research activities in this important area and consequently the momentum of research on lipases picked up in India. We have carried out large-scale process optimization for lipase production using *A. terreus*, *A. carneus* and *B. stearothermophilus*[37,143,239]. Chakrabarty *et al.*[258] utilized extracellular microbial lipases for transesterification reactions for producing valuable transformed edible oils which cannot be obtained by chemical interesterification methods[258]. Chand *et al.*[259] carried out fat splitting using castor-bean lipase. Lipases from *H. lanuginosa* and *Y. lipolytica* have also been reported for the synthesis of geranyl esters[260]. Kundu *et al.*[121] isolated and characterized an extracellular lipase from the conidia of *N. crassa*, with an apparent molecular weight of 54 kDa and 27 kDa, determined by gel filtration and SDS-PAGE, suggesting thereby the presence of two identical subunits.

Since 1988, extensive work on various aspects of lipase research, starting from production and purification to characterization and industrial applications, has been carried out on various fungi and bacteria in South Campus, Delhi University[4,37,112,143,239,249,250]. Novel thermo-

stable and alkaline lipases from *A. terreus* and *A. carneus* are being developed for the production of biosurfactants, glycerides, and pharmaceutically important compounds. These lipases show regio- and chemoselective cleavage of polyphenolic compounds in a novel manner. Lipase from a strain of *B. stearothermophilus* shows remarkable activity even at 100°C. Besides this, a rapid zymogram for lipase activity in polyacrylamide gels was developed which is of immense use to investigators in this field[112].

The ability of lipases to show increased stability and selectivity in organic solvents has been exploited by various researchers: Parmar *et al.*[261], Gupta[262] at Indian Institue of Technology, Delhi, Qazi and his group[263], and SPIC Science Foundation. Biotransformations on polyacetoxy arylmethyl ketones, benzylphenylketone peracetates, esters of polyacetoxy aromatic acids, and peracetylated benzopyranones, using commercial lipases have been carried out by Parmar *et al.*[261]. Qazi and his group[263] have exploited the enantioselective behaviour of microbial lipases for the resolution of racemic drugs. Gupta and his group[262] have investigated enzyme activity in organic solvents. They noted enhancement of enzyme activity in aqueous-organic solvent mixtures. Sridhar *et al.*[264] employed lipase-catalysed ester interchanges for the modification of selected Indian vegetable oils into cocoa butter substitutes and high oleic oils. Satyanarayana and Johri[256], Sharath and Kamat[266], and scientists at SPIC Anna University Bioprocess Laboratory have started work on the production of novel microbial lipases, which is yet to be exploited at commercial level. Scientists at the Central Leather Research Institute, Madras, and at the Central Food Technology Research Institute, Mysore, are using microbial lipases in the treatment of leather, and the production of flavour esters, respectively.

The work being carried out in Indian laboratories has made considerable progress. Novel lipases with properties of chemo-, regio- and enantioselectivity have been isolated, which may be eligible for exploitation at commercial level for industrial applications in course of time. But, some of the indigenously developed technologies for the production of lipases are already in the commercial production stage. Furthermore, comparison of some of the lipases produced by microorganisms indigenously is at par or even better than the well-known commercially available imported lipases. Thus, utilizing these lipases will greatly boost many biotechnology-based industries with the ushering of the 21st century.

Conclusion

Lipases are amongst the most important biocatalysts that carry out novel reactions in both aqueous and nonaqueous media. Lipases have the remarkable ability to carry out a wide variety of chemo-, regio- and enantioselective transformations. Thus lipases are the tools of choice for organic chemists. 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. 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.

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. However, complete understanding of the lipase molecule requires greater input of research effort.

1. Gerhartz, W., *Enzymes in Industry: Production and Application*, VCH, Weinheim, 1990.
2. Priest, F. G., *Encyclopedia of Microbiology*, Academic Press, New York, 1992, vol. 2.
3. Macrae, A. R. and Hammond, R. C., *Biotechnol. Gen. Eng. Rev.*, 1985, **3**, 193–219.
4. Ghosh, P. K., Saxena, R. K., Gupta, R., Yadav, R. P. and Davidson, S., *Sci. Prog.*, 1996, **79**, 119–157.
5. Iwai, W. and Mieko, J., *Yushi*, 1989, **32**, 84–91.
6. Bjorkling, F., Godtfredsen, S. E. and Kirk, O., *Trends Biotechnol.*, 1991, **9**, 360–363.
7. Verger, R. *Trends Biotechnol.*, 1997, **15**, 32–38.
8. Brockerhoff, H. and Jensen, R. (eds), *Lipolytic Enzymes*, Academic Press, New York, 1974, pp. 1–340.
9. Borgstrom, B. and Brockman, H. L. (eds), *Lipases*, Elsevier, New York, 1984, pp. 1–420.
10. Sugiura, M., in *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier Science Publishers, Amsterdam, 1984, pp. 505–523.
11. Stein, I. V. and Arends, I. M., *Biotechnologia*, 1989, **5**, 133–136.
12. Tombs, M. P., in *Biotechnology in Food Industry* (ed. Tombs, M. P.), Prentice Hall, New Jersey, 1991, pp. 127–146.
13. Woolley, P. and Petersen, S. B. (eds), *Lipases, their Structure, Biochemistry and Application*, Cambridge University Press, UK, 1994.
14. Godfredson, S. E., in *Microbial Enzymes and Biotechnology* (eds Fogarty, W. M. and Kelly, E. T.), Elsevier Applied Sciences, The Netherlands, 1990, pp. 255–273.
15. Iwai, M., Okumura, S. and Tsujisaka, Y., *Agric. Biol. Chem.*, 1980, **44**, 2731–2732.
16. Nishio, T., Takanashi, K., Yoshimoto, T., Kodess, Y., Satio, Y. and Inada, Y., *Biotechnol. Lett.*, 1987, **9**, 187–190.
17. Langrand, G., Randot, N., Triantaphylides, C. and Baratii, *J. Biotechnol. Lett.*, 1990, **12**, 581–586.
18. Gillis, A., *J. Am. Oil Chem. Soc.*, 1988, **65**, 849–852.
19. Martin, H. F. and Peers, F. G., *Biochem. J.*, 1953, **55**, 523–529.
20. Ory, R. L. S., Angelo, A. J. and Altschul, A. M., *J. Lipid Res.*, 1962, **3**, 99–105.
21. Hirayama, O. and Matsuda, H., *Nippon Nogei Kagaku Kaishi*, 1975, **49**, 569–576.
22. Theimer, R. R. and Rosnitschek, I., *Planta*, 1978, **139**, 249–256.

23. Huang, A. H. C., in *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier, Amsterdam, 1984, pp. 419–442.
24. Mohankumar, C., Arumugham, C. and Kaleysaraj R., *J. Am. Oil Chem. Soc.*, 1990, **67**, 665–669.
25. Thomas, P. P., Gopalkrishnan, N. and Damodaran, A. D., *Oleagineus*, 1991, **46**, 245–246.
26. Mukherjee, K. D. and Hills, H. J., in *Lipases, their Structure, Biochemistry and Application*, Cambridge University Press, UK, 1994, pp. 49–75.
27. Volhard, F., *Z. Klin. Meds.* 1901, **42**, 414–429.
28. Hull, M. and Keaton, R. W., *J. Biol. Chem.*, 1917, **32**, 127–140.
29. Barrowman, J. A. and Darnton, S. J., *Gastroenterology*, 1970, **59**, 13–21.
30. Hamosh, M., in *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier, Amsterdam, 1984, pp. 49–81.
31. Moreau, H., Gargouri, Y., Lecat, D., Junien, J. L. and Verger, R., *Biochim. Biophys. Acta*, 1988, **960**, 286–293.
32. Winkler, F. K., D'Arcy, A. and Hunsiker, W., *Nature*, 1990, **343**, 771–774.
33. Carriere, F., Moreau, H., Raphel, V., Laugier, R., Benicourt, C., Junien, J. L. and Verger, R., *Eur. J. Biochem.*, 1991, **202**, 75–83.
34. Carriere, F., Raphel, V., Moreau, H., Bernadac, A., Devaux, M. A., Grimaud, R., Barrowman, J. A., Benicourt, C., Junien, J. L., Laugier, R. and Verger, R., *Gastroenterology*, 1992, **102**, 1535–1545.
35. Winkler, F. K. and Gubernator, K., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambridge University Press, UK, 1994, pp. 139–157.
36. Lawrence, R. C., Fryer, T. F. and Reiter, B., *J. Gen. Microbiol.*, 1967, **48**, 401–418.
37. Yadav, R. P., Saxena, R. K., Gupta, R. and Davidson, S., *Folia Microbiol.*, 1998, **43**, 373–378.
38. Yamaguchi, T., Muroya, N., Isobe, M. and Sugiura, M., *Agric. Biol. Chem.*, 1973, **37**, 999–1005.
39. Yamaguchi, S., Mase, T. and Takeuchi, K., *Gene*, 1991, **103**, 61–67.
40. Suzuki, M., Yamamoto, H. and Mizugaki, M., *J. Biochem.*, 1986, **100**, 1207–1213.
41. Sztajer, H., Borkowski, J. and Sobiech, K., *Biotechnol. Appl. Biochem.*, 1991, **13**, 65–71.
42. Brune, A. K. and Gotz, F., in *Microbial Degradation of Natural Products* (ed. Winkelmann, G.), VCH, Weinheim, 1992, pp. 243–263.
43. Stead, D., *Dairy Res.*, 1986, **53**, 481–505.
44. Petersen, S. B. and Drablos, F., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambridge University Press, UK, 1994, pp. 23–48.
45. Winkler, K. W., Ulrich, K., and Stuckmann, M., *J. Bacteriol.*, 1979, **138**, 663–670.
46. van O'ort, M. G., Deveer, A. M. Th. J., Dijkman, R., Tjeenk, M. L., Verheij, H. M., Haas, G. H. de, Wenzig, E. and Gotz, F., *Biochemistry*, 1989, **28**, 9278–9285.
47. Nishio, T., Chikano, T. and Kamimura, M., *Agric. Biol. Chem.*, 1987, **51**, 181–187.
48. Kugumiya, W., Otani, Y., Hashimoto, Y. and Takagi, Y., *Biochim. Biophys. Res. Commun.*, 1986, **141**, 185–190.
49. Lawrence, R. C., *Dairy Sci. Abstr.*, 1967, **29**, 1–8.
50. Lazar, G. and Schroder, F. R., in *Microbial Degradation of Natural Products* (ed. Winkelmann, G.), VCH, Weinheim, 1992, pp. 267–291.
51. Lawson, D. M., Brzozowski, A. M., Dodson, G. G., Hubbard, R. E., Huge-Jensen, B., Boel, E. and Derewenda, Z. S., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambridge University Press, UK, 1994, pp. 77–94.
52. Sztajer, H. and Zboinska, C., *Acta Biotechnol.*, 1988, **8**, 169–175.
53. Huge-Jensen, B., Galluzzo, D. R. and Jensen, R. G., *Lipids*, 1987, **22**, 559–565.
54. Matsuo, T., Sawamura, N., Hashimoto, Y. and Hashida, W., European Patent Application EP 0 035 883, 1981.
55. Iwai, M., Tsujisaka, Y. and Fukumoto, J., *Agric. Biol. Chem.*, 1974, **38**, 1241–1247.
56. Tahoun, M. K., *Grasasy Aceites*, 1986, **37**, 191–193.
57. Litchfield, C., *Analysis of Triglycerides*, Academic Press, New York, 1972.
58. Okumura, S., Iwai, M. and Tsujisaka, Y., *Agric. Biol. Chem.*, 1976, **40**, 655–660.
59. Eitenmiller, R. R., Vakil, J. R. and Shahani, K. M., *Food Sci.*, 1970, **35**, 130–133.
60. Iwai, M., Okumura, S. and Tsujisaka, Y., *Agric. Biol. Chem.*, 1975, **39**, 1063–1070.
61. Huge, J. B. and Gormsen, E., EP Patent 258068, 1987.
62. Ibrahim, C. O., Hayashi, M. and Nagai, S., *Agric. Biol. Chem.*, 1987, **57**, 37–45.
63. Fukumoto, J., Iwai, M. and Tenjisaka, Y., *J. Gen. Appl. Microbiol.*, 1963, **9**, 353–361.
64. Aisaka, K. and Terada, O., *Agric. Biol. Chem.*, 1980, **44**, 799–805.
65. Watanabe, N., Ota, Y., Minoda, Y. and Yamada, K., *Agric. Biol. Chem.*, 1977, **41**, 1353–1358.
66. Tsujisaka, Y., Iwai, W., Mieko, J. and Tominaga, Y., *Agric. Biol. Chem.*, 1973, **37**, 1457–1464.
67. Oso, B. A., *Can. J. Bot.*, 1978, **56**, 1840–1843.
68. Nahas, E., *J. Gen. Microbiol.*, 1988, **134**, 227–233.
69. Omar, I. C., Hayashi, M. and Nagai, S., *Agric. Biol. Chem.*, 1987, **51**, 37–45.
70. Suzuki, I. W. and Takahiro, J., *Appl. Microbiol. Biotechnol.*, 1988, **27**, 417–422.
71. Haas, M. J. Cichowicz, D. J. and Bailey, D. G., *Lipids*, 1992, **27**, 571–576.
72. Nashif, S. A. and Nelson, F. E., *J. Dairy Sci.*, 1953, **36**, 471–480.
73. Alford, J. A. and Pierce, D. A., *J. Bacteriol.*, 1963, **86**, 24–29.
74. Chander, H., Batish, V. K., Sannabhadt, S. S. and Srinivasan, R. A., *J. Food Sci.*, 1980, **45**, 598–600.
75. Akhtar, M. W., Mirza, A. Q. and Chughtai, I. D., *Appl. Environ. Microbiol.*, 1980, **40**, 257–263.
76. Chander, H., Batish, V. K., Ghodekar, D. R. and Srinivasan, R. A., *J. Dairy Sci.*, 1981, **64**, 193–196.
77. Chopra, A. K., Chander, H., Batish, V. K. and Ranganathan, B., *J. Food Protec.*, 1981, **44**, 661–664.
78. Gilbert, J. E., Cornish, A. and Jones, C. W., *J. Gen. Microbiol.*, 1991, **137**, 2223–2229.
79. Fox, P. F. and Stepaniak, L., *J. Dairy Res.*, 1983, **50**, 77–81.
80. Griffiths, M. W. and Phillips, J. D., *J. Food Protec.*, 1984, **47**, 697–702.
81. Nadkarni, S. R., *Enzymologia*, 1971, **40**, 286–301.
82. Ruschen, S. and Winkler, V. K., *FEMS Microbiol. Lett.*, 1982, **14**, 117–121.
83. Smith, J. L. and Alford, J. A., *Appl. Microbiol.*, 1966, **14**, 699–705.
84. Lawrence, R. C., Fryer, T. F. and Reiter, B., *Nature*, 1967, **213**, 1264–1265.
85. Kosugi, Y. and Kamibayshi, I., *Biotechnol. Lett.*, 1971, **10**, 199–204.
86. Jonsson, U. and Snygg, B. G., *J. Appl. Bacteriol.*, 1974, **37**, 571–581.
87. Johri, B. N., Alurralde, J. D. and Klein, J., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 367–371.
88. Nashif, S. A. and Nelson, F. E., *J. Dairy Sci.*, 1953, **36**, 459–470.
89. Alford, J. A. and Elliott, L. E., *Food Res.*, 1960, **25**, 296–303.
90. Anderson, R. E., Hedlund, C. B. and Jonsson, U., *Dairy Sci.*, 1979, **62**, 361–367.
91. Lu, J. Y. and Liska, B. J., *Appl. Microbiol.*, 1969, **18**, 108–114.
92. Row, M. T. and Gilmour, A., *Milchwissenschaft*, 1982, **37**, 597–600.
93. Giuseppin, M. L. F., *Appl. Microbiol. Biotech.*, 1984, **20**, 161–165.
94. Jensen, R.G., *Lipids*, 1983, **18**, 650–657.
95. Nachlas, M. M. and Blackburn, R., *J. Biol. Chem.*, 1958, **230**, 1051.

96. Lowry, R. R. and Tinsley, T., *J. Am. Oil Chem. Soc.*, 1976, **53**, 470–575.
97. Kwon, D. Y. and Rhee, J. S., *J. Am. Oil Chem. Soc.*, 1986, **63**, 89–92.
98. Renard, G., Grimaud, J., Elzant, A., Pina, M. and Graiulle, J., *Lipids*, 1987, **22**, 539–541.
99. von Tigerstrom, R. G. and Stelmaschuk, S., *Can. J. Microbiol.*, 1989, **35**, 511–514.
100. Rogel, A. M., Stone, W. L. and Adebonojo, F. O., *Lipids*, 1989, **24**, 518–525.
101. Cabot, M. C., Welsh, C. J., Zhang, Z. C. and Chabbott, H., in *Methods in Enzymology* (eds Conn, P. M. and Means, A. R.), Academic Press, Orlando, 1987, pp. 301–313.
102. Huang, J., Roheim, P. S., Sloop, C. H. and Wong, L., *Anal. Biochem.*, 1989, **179**, 413–417.
103. Kouker, G. and Jaeger, K. E., *Appl. Environ. Microbiol.*, 1987, **53**, 211–213.
104. Verger, R., *Methods Enzymol.*, 1989, **64**, 340–392.
105. Linfield, W. M., O'Brien, D. J., Seratas, P. and Basauskas, R. A., *J. Am. Oil Chem. Soc.*, 1984, **61**, 1067–1071.
106. Naka, Y. and Nakamura, T., *Biosci. Biotechnol. Biochem.*, 1992, **56**, 1066–1070.
107. Akhtar, M. W., Mirza, A. Q., Nawazish, M. N. and Chughtai, M. I. D., *Can. J. Microbiol.*, 1983, **29**, 664–669.
108. Mirza, A. Q., Akhtar, M. W., Nawazish, M. N. and Chughtai, M. I. D., *Can. J. Microbiol.*, 1982, **28**, 618–622.
109. Moskowitz, G. J. R., Cassaigne, J. R., West, T. S. and Feldman, L. I., *J. Agric. Food Chem.*, 1977, **25**, 1146–1150.
110. Desnuelle, P., in *The Enzymes III* (ed. Boyer, P. D.), Academic Press, New York, 1972, pp. 575–616.
111. Verger, R., in *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier, Amsterdam, 1984, pp. 83–150.
112. Yadav, R. P., Saxena, R. K., Gupta, R. and Davidson, S., *Biotechniques*, 1998, **24**, 754–756.
113. Torossian, K. and Bell, A. W., *Biotechnol. Appl. Biochem.*, 1991, **13**, 205–211.
114. Menge, U., Hecht, H. J., Schomburg, D. and Schmid, R. D., in *Lipases: Structure, Mechanism and Genetic Engineering* (eds Alberghina, L., Schmid, R. D. and Verger, R.), VCH, Weinheim, 1990, GBF monographs **16**, pp. 59–62.
115. Charton, E., Davies, C., Sidebottom, C. M., Sutton, J. L., Dunn, P. P. J., Slabas, A. R. and Macrae, A. R., in *Lipases: Structure, Mechanism and Genetic Engineering* (eds Alberghina, L., Schmid, R. D. and Verger, R.), VCH, Weinheim, 1991, GBF monographs **16**, pp. 335–338.
116. Nagaoka, K. and Yamada, Y., *Agric. Biol. Chem.*, 1973, **37**, 2791–2796.
117. Ishihara, H., Okuyama, H., Ikezawa, H. and Tejima, S., *Biochim. Biophys. Acta*, 1975, **338**, 413–422.
118. Isobe, K., Akiba, T. and Yamaguchi, S., *Agric. Biol. Chem.*, 1988, **52**, 41–47.
119. Isobe, K. and Nokihara, K., in *Lipases: Structure, Mechanism and Genetic Engineering* (eds Alberghina, L., Schmid, R. D. and Verger, R.), VCH, Weinheim, 1991, GBF monographs **16**, pp. 345–348.
120. Tahoun, M. K. and Ali, H. A., *Enzyme Microb. Technol.*, 1986, **8**, 429–432.
121. Kundu, M., Basu, J., Guchhait, M. and Chakrabarti, P., *J. Gen. Microbiol.*, 1987, **133**, 149–153.
122. Lang, S., Katsiwela, E., Kleppe, F. and Wagner, F., in *Lipases: Structure, Mechanism and Genetic Engineering* (eds Alberghina, L., Schmid, R. D. and Verger, R.), VCH, Wehinheim, 1991, GBF monographs **16**, pp. 361–364.
123. Dekker, M., Van't Riet, K., Weifers, S. R., Baltussen, J. W. A., Laane, C. and Bijsterbosch, B. H., *Chem. Eng. J.*, 1986, **33**, B27–B33.
124. Goklen, K. E. and Hatton, T. A., *Sep. Sci. Technol.*, 1987, **22**, 831–841.
125. Rahaman, R. S., Chee, J. Y., Cabral, J. M. S. and Hatton, T. A., *Biotechnol. Prog.*, 1988, **4**, 218–224.
126. Vicente, M. L. C., Aires-Barros, M. R. and Cabral, J. M. S., *Biotechnol. Techniques*, 1990, **4**, 137–142.
127. Hustedt, H., Kroner, K. H. and Kula, M. R., in *Partitioning in Aqueous Two-Phase Systems: Theory, Methods, Uses and Applications to Biotechnology* (eds Walter, H., Brooks, D. E. and Fisher, D.), Academic Press, Orlando, 1985, pp. 529–587.
128. Menge, U. and Schmid, R. D., in Proceedings of 15th Scandinavian Symposium on Lipids held from 11–15 June, Skorping, Denmark, 1989, pp. 305–316.
129. Albertsson, P. A., *Partitioning of Cells and Macromolecules*, J. Wiley, New York, 1986, 3rd edn.
130. Dunhaupt, A., Lang, S. and Wagner, F., in *Lipases: Structure, Mechanism and Genetic Engineering* (eds Alberghina, L., Schmid, R. D. and Verger, R.), VCH, Weinheim, 1991, GBF monographs **16**, pp. 389–392.
131. Walter, H., Brooks, D. E. and Fisher, D., in *Partitioning in Aqueous Two-phase System: Theory, Methods, Uses and Applications to Biotechnology* (eds Walter, H., Brooks, D. E. and Fisher, D.), Academic Press, London, 1985.
132. Sztajer, H. and Bryjak, M., *Bioprocess Eng.*, 1989, **4**, 257–259.
133. Harlow, E. and Lane, D., *Antibodies*, Cold Spring Harbor Publications, Cold Spring Harbor, New York, 1988.
134. Hill, C. R., Thompson, L. G. and Kenney, A. C., in *Protein Purification Methods* (eds Harris, E. L. V. and Angal, S.), IRL Press, New York, 1989, pp. 282–292.
135. Iwai, M., Tsujisaka, Y. and Fukumoto, J., *J. Gen. Appl. Microbiol.*, 1964, **10**, 87–93.
136. Tsujisaka, Y., Okumura, S. and Iwai, M., *Biochim. Biophys. Acta*, 1977, **489**, 415–422.
137. Seino, H., Uchibori, T., Nishitani, T. and Inamasu, S., *J. Am. Oil Chem. Soc.*, 1984, **61**, 1761–1765.
138. Hoq, M. M., Yamane, T., Shimizu, S., Funada, T. and Ishida, S., *J. Am. Oil Chem. Soc.*, 1984, **61**, 776–781.
139. Blain, J. A., Akhtar, M. W. and Patterson, J. D. E., *Pak. J. Biochem.*, 1976, **10**, 41–53.
140. Laboureur, P. and Labrousse, M., *Bull. Soc. Chim. Biol.*, 1966, **48**, 747–769.
141. Liu, W. H., Beppu, T. and Arima, K., *Agric. Biol. Chem.*, 1973, **37**, 157–163.
142. Suzuki, M., Yamamoto, H. and Mizugaki, M., *J. Biochem.*, 1986, **100**, 1207–1213.
143. Yadav, R. P., Saxena, R. K., Gupta, R. and Davidson, S. *Biotechnol. Appl. Biochem.*, 1998, in press.
144. Christakopoulos, P., Tzia, C., Kekos, D. and Maous, B. J., *Appl. Microbiol. Biotech.*, 1992, **38**, 194–197.
145. Sigurgisladottir, S., Konraosdottir, M., Jonsson, A., Kristjansson, J. K. and Matthiasson, E., *Biotechnol. Lett.*, 1993, **15**, 361–366.
146. Patkar, S. and Bjorkling, F., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambrdige University Press, UK, 1994, pp. 207–224.
147. Jensen, R. G., deJong, F. A. and Clark, R. N., *Lipids*, 1983, **18**, 239–252.
148. Liu, W. H., Beppu, T. and Arima, K., *Agric. Biol. Chem.*, 1973, **37**, 1344–1355.
149. Sugiura, M. and Isobe, M., *Chem. Pharm. Bull.*, 1975, **23**, 1226–1230.
150. Moskowitz, G. J., Cassaigne, R., West, I. R., Shen, T. and Feldman, L. I., *J. Agric. Food Chem.*, 1977, **25**, 1146–1150.
151. Jensen, R. G., *Lipids*, 1974, **9**, 143–157.
152. Jensen, R. G. and Pitas, R. E., in *Lipids* (eds Paoletti, R., Porcellati, G. and Jacini, G.), Raven Press, New York, 1976, pp. 141–146.
153. Macrae, A. R., in *Microbial Enzymes and Technology* (ed. Fogarty, W. M.), Applied Science, London, 1983, pp. 225–250.
154. Chapman, D., *Introduction to Lipids*, McGraw-Hill, London, 1969.

155. Benzonana, G. and Esposito, S., *Biochim. Biophys. Acta*, 1965, **105**, 121–136.
156. Semeriva, M., Benzonana, G. and Desnvelle, P., *Bull. Soc. Chim. Biol.*, 1967, **49**, 71–81.
157. Asahara, T., Matori, M., Ikemoto, M. and Yasuhide, O., *Biosci. Biotech. Biochem.*, 1993, **57**, 390–394.
158. Lavayre, J., Verrier, J. and Baratti, J., *Biotechnol. Bioeng.*, 1982, **24**, 2175–2187.
159. Cambou, B. and Klibanov, A. M., *J. Am. Chem. Soc.*, 1984, **106**, 2687–2692.
160. Okumura, S., Iwai, M. and Tsujisaka, Y., *J. Biochem.*, 1980, **87**, 205–211.
161. Yamane, T., *J. Am. Oil Chem. Soc.*, 1987, **64**, 1657–1662.
162. Zaks, A. and Klibanov, A. M., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 3192–3196.
163. Jones, J. B., *Tetrahedron*, 1986, **42**, 3351–3403.
164. Yamad, H. and Shimizu, S., *Angew. Chem. Int. Ed. Engl.*, 1988, **27**, 622–642.
165. Chen, C. S. and Sih, C. J., *Angew. Chem.*, 1989, **101**, 711–724.
166. Kilbanov, A. M., *Trends Biochem. Sci.*, 1989, **14**, 141–144.
167. Kilbanov, A. M., *Acc. Chem. Res.*, 1990, **23**, 114–120.
168. Zaks, A., *Biocatalysts for Industry*, 1990, **8**, 161–180.
169. Takahashi, K., Tamaura, Y., Kodera, Y., Mihoma, T., Saito, Y. and Inada, Y., *Biochem. Biophys. Res. Commun.*, 1987, **142**, 291–296.
170. Snyder, F., *Am. J. Physiol.*, 1990, **107**, 697–708.
171. Workman, P., *Cancer Cells*, 1991, **4**, 926–932.
172. Eibl, H., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4074–4077.
173. Eibl, H. and Engel, J., in *The Series: Progress in Experimental Tumour Research* (eds Eibl, H., Hilgard, P. and Lunger, C.), Karger Verlag, Basel, 1992, pp. 1–5.
174. Iwai, M. and Tsujisaka, Y., *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier Science Publishers, Amsterdam, 1984, pp. 443–470.
175. Goldberg, M., Thomas, D. and Legoy, M. D., *Eur. J. Biochem.*, 1990, **190**, 603–609.
176. Martin, V. S., Woodard, S. S., Katsuki, T., Yamada, Y., Ikeda, M. and Sharpless, K. B., *J. Am. Chem. Soc.*, 1981, **103**, 6240–6327.
177. Chen, C. S., Fujimoto, Y., Girdaukas, G. and Sih, C. J., *J. Am. Chem. Soc.*, 1981, **104**, 7294–7299.
178. Kirchner, G., Scollar, M. P. and Kilbanov, A. M., *J. Am. Chem. Soc.*, 1985, **107**, 7072–7076.
179. Theisen, P. D. and Heathcock, C. H., *J. Org. Chem.*, 1988, **63**, 2374–2378.
180. Stokes, T. M. and Ochlschlager, A. C., *Tetrahedron Lett.*, 1987, **28**, 2091–2094.
181. Langrand, G., Secchi, M., Buono, G., Baratti, J. and Triantaphylides, C., *Tetrahedron Lett.*, 1985, **26**, 1857–1860.
182. Yamamoto, K., Nishioka, T., Oda, J. and Yamamoto, Y., *Tetrahedron Lett.*, 1988, **29**, 1717–1720.
183. Gutman, A. L. and Braudo, T., *J. Org. Chem.*, 1989, **54**, 5645–5646.
184. Ngooi, T. K., Scilimati, A., Gur, Z. and Sih, C. J., *J. Org. Chem.*, 1989, **54**, 911–914.
185. Sweers, H. M. and Wong, C. H., *J. Am. Chem. Soc.*, 1986, **108**, 6421–6422.
186. Therisod, M. and Klibanov, A. M., *J. Am. Chem. Soc.*, 1986, **108**, 5638–5640.
187. Therisod, M. and Klibanov, A. M., *J. Am. Chem. Soc.*, 1987, **109**, 3977–3981.
188. Holla, W., *Angew. Chem. Int. Ed. Engl.*, 1989, **28**, 220–221.
189. Riva, S., Bovara, R., Ottolina, G., Secundo, F. and Carrea, G., *J. Org. Chem.*, 1989, **54**, 3161–3164.
190. Macrae, A. R., *J. Am. Oil Chem. Soc.*, 1983, **60**, 291–294.
191. Coleman, M. H. and Macrae, A. R., Pat. UK No. 577, 933, 1980.
192. Matsno, T., Sawamura, N., Hashimoto, Y. and Hashida, W., Pat. UK, Appl. No. 2, 035; 359A, 1980.
193. Tanaka, Y., Irinatsu, Noguchi, A. and Kobayashi, T., Euro Pat. No. 237307, 1984.
194. Kurashige, J., Matsuzaki, N. and Makabe, K., *J. Dispersion Sci. Technol.*, 1989, **10**, 531.
195. Kosugi, Y. and Suzuki, H., *J. Ferment. Technol.*, 1993, **61**, 287–290.
196. Linfield, W. M., Brien, D. J. O., Serota, S. and Barauskas, R. A., *J. Am. Oil Chem. Soc.*, 1984, **61**, 1067–1070.
197. Lieberman, R. B. and Oelis, D. F., *Biotechnol. Bioeng.*, 1975, **17**, 1401–1404.
198. Kilora, A., Shahani, K. M. and Wagner, F. W., *Biotechnol. Bioeng.*, 1977, **19**, 1703–1706.
199. Lavayre, J. and Bratti, J., *Biotechnol. Bioeng.*, 1982, **24**, 1007–1010.
200. Kimura, Y, Tanaka, A., Sonomota, K., Nihira, T. and Fukui, S., *Eur. J. Appl. Microbial. Biotechnol.*, 1983, **17**, 107–110.
201. Kosugi, Y. and Suzuki, H., *J. Ferment. Technol.*, 1973, **51**, 895–899.
202. Bell, G., Todd, J. R., Blain, J. A., Patterson, J. D. E. and Shaw, C. E. L., *Biotechnol. Bioeng.*, 1981, **23**, 1703–1706.
203. Reetz, M. T., Zonta, A. and Simplekamp, J., *Biotechnol. Bioeng.*, 1996, **49**, 527–534.
204. Sonntag, N. O. V., *J. Am. Oil Chem. Soc.*, 1979, **56**, 726–729.
205. Stansby, M. E., *J. Am. Oil Chem. Soc.*, 1979, **56**, 793–797.
206. Pront, W., Burgt, M. V. D., Boswinkel, G. and Riet, K. V., *J. Am. Oil Chem. Soc.*, 1991, **68**, 852–856.
207. Malcata, F. X., Reyes, H. R., Garcia, H. S., Hill, C. G. and Amundson, C. H., *J. Am. Oil Chem. Soc.*, 1990, **67**, 890–910.
208. Mukherjee, K. D. and Kiewitt, I., *J. Agri. Food Chem.*, 1988, **36**, 133.
209. Trani, M., Ergan, F. and Andre, G., *J. Am. Oil Chem. Soc.*, 1991, **68**, 20–22.
210. Gillies, B., Yamazaki, H. and Armstrong, D. W., *Biotechnol. Lett.*, 1988, **10**, 54–56.
211. Welsh, F. W. and Williams, R. E., *J. Food Sci.*, 1989, **54**, 1565–1569.
212. Mittelbach, M., J., *Am. Oil Chem. Soc.*, 1990, **67**, 168–171.
213. Bloomer, S., Adlercreutz, P. and Mathiasson, B., *J. Am. Oil Chem. Soc.*, 1992, **69**, 966–973.
214. *NOVO's Handbook of Practical Biotechnology* (ed. Boyce, C. O. L.), NOVO Industries, NOVO Alle. 2880, Bagsvaerd, Denmark, 1986.
215. Olivecrona, T. and Bengtsson, G., in *Lipases* (eds Borgstrom, B. and Brockman, H. L.), Elsevier, Amsterdam, 1984, pp. 205–262.
216. Nielsen, T., *Fat Sci. Tech.*, 1985, **87**, 15–17.
217. John, V. T. and Abraham, G., in *Biocatalysts for Industry* (ed. Dordick, J. S.), Plenum Press, New York, 1990, vol. 10, pp. 193–197.
218. Kotting, J. and Eibl, H., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambridge University Press, UK, 1994, pp. 289–313.
219. Villeneuve, P. and Foglia, T. A., *Inform*, 1997, **8**, 640–650.
220. Godfrey, T. and Hawkins, D., *Eur. Food Drink Rev.*, 1991, 103–107.
221. Falch, E. A., *Biotechnol. Adv.*, 1991, **9**, 643–658.
222. Vulfson, E. N., in *Lipases: their Structure, Biochemistry and Application* (eds Woolley, P. and Petersen, S. B.), Cambridge University Press, UK, 1994, pp. 271–288.
223. Arbige, M. V. and Pitcher, W. H., *Trends Biotechnol.*, 1989, **7**, 330–335.
224. Hoq, M. M., Yamane, T., Shimizu, S., Funada, T. and Ishida, S., *J. Am. Oil Chem. Soc.*, 1985, **62**, 1016–1021.
225. Buhler, M. and Wandrey, C., 1987, **35**, 511–514.
226. McNeill, G. P. and Yamane, T., *J. Am. Oil Chem. Soc.*, 1991, **68**, 6–10.
227. Ergan, F., Trani, M. and Andre, G., *Biotechnol. Lett.*, 1988, **10**, 629–634.
228. Sonntag, N. O. V., *J. Am. Oil Chem. Soc.*, 1984, **61**, 229–232.

229. Coleman, M. H. and Macrae, A. R., UK Patent No.1 577 933 (Unilever Limited), 1980.
230. Matsuo, T., Sawamura, N., Hashimoto, Y., Hashida, W., AU Patent No. 544682, 1981.
231. King, D. M. and Padlye, F. B., Eur. Patent No. 0 209 327 (Unilever plc), 1990.
232. Jandacek, R., Whiteside, J. A., Holcombe, B. N., Volpenheim, R. A. and Taulbee, J. D., *Am. J. Clin. Nutr.*, 1987, **45**, 940–945.
233. Soumanou, M. M., Bornschener, U. T., Menge, U. and Schmid, R., *J. Am. Oil Chem. Soc.*, 1997, **74**, 427–433.
234. Kotting, J., Eibl, H. and Fehrenback, F. J., *Chem. Phys. Lipids*, 1988, **47**, 117–122.
235. Eibl, H. and Unger, C., *Can. Treat. Rev.*, 1990, **17**, 233–242.
236. Fregapane, G., Sarney, D. B. and Vulfson, E. N., *Enzyme Microb. Technol.*, 1991, **13**, 796–800.
237. Adelhorst, K., Bjorkling, F., Godtfredsen, S. E. and Kirk, O., *Synthesis*, 1990, **1**, 112–115.
238. Fregapane, G., Saney, D. B. and Vulfson, E. N., *Enzyme Microb. Technol.*, 1991, **13**, 796–800.
239. Yadav, R. P., Saxena, R. K., Gupta, R. and Davidson, S., *J. Sci. Ind. Res.*, 1997, **56**, 479–248 .
240. Montet, D., Servat, F., Pina, M., Graille, J., Galzy, P., Arnaud, A., Ledon, H. and Marcou, L., *J. Am. Oil Chem. Soc.*, 1990, **67**, 771–774.
241. Hills, M. J., Kiewitt, I. and Mukherjee, K. D., *Biochim. Biophys. Acta*, 1990, **1042**, 237–240.
242. Kloosterman, M., Elferink, V. H. M., Jack van Lersel, J., Roskan, J. H., Meijer, E. M., Hulshof, L. A. and Sheldon, R. A., *Trends Biotechnol.*, 1988, **6**, 251–256.
243. Young, J. W. and Bratzler, R. L., in Proceedings of Chiral-90 Symposium, Spring Innovation Ltd., Manchester, 1990, pp. 23–28.
244. Sainz-Daiz, C. J., Wohlfahrt, G., Nogoceke, E., Hernandez-Laguna, A., Smeyers, Y. G. and Menge, Q., *Theochemistry*, 1997, **390**, 225–237.
245. Davis, H. G., Green, R. H., Kelly, D. R. and Roberts, S. M., in *Biotransformations in Preparative Organic Synthesis*, Academic Press, London, 1990.
246. Margolin, A. L., in *Chemical Aspects of Enzyme Biotechnology* (eds Baldwin, T. O., Rauschel, F. M. and Scott, A. I.), Plenum Press, New York, 1990, pp. 197–202.
247. Bornemann, S., Cassells, J. M., Combes, C .L., Dordick, J. S. and Hacking, A. J., *Biochem. Biophys. Acta*, 1989, **1265**, 250–253.
248. Margolin, A. L., Crenne, J. Y. and Klibanov, A. M., *Tetrahedron Lett.*, 1989, **28**, 1607–1610.
249. Parmar, V. S., Pati, H. N., Yadav, R. P., Kumar, A., Bisht, K. S., Gupta, R., Davidson, S., Poonam and Saxena, R. K., *Biocatal. Biotransform.*, 1998, **16**, 17–25.
250. Parmar, V. S., Kumar, A., Poonam, Pati, H. N., Saxena, R. K., Davidson, S. and Gupta, R., *Biochim. Biophys. Acta*, 1998, in press.
251. Bornemann, S., Casselle, J. M., Combs, C. L., Dordick, J. S. and Hacking, A. J., UK Patent No. 2 224 504, 1989.
252. Margolin, A. L., Crenne, J. Y. and Klibanov, A. M., *Tetrahedron Lett.*, 1987, **28**,1607–1610.
253. Pavel, K. and Ritter, H., *Makromol. Chem.*, 1992, **193**, 323–328.
254. Margolin, A. L., Fitzpatrick, P. A., Dublin, P. L. and Klibanov, A. M., *J. Am. Chem. Soc.*, 1991, **113**, 4693–4694.
255. West, S., *Food Proc.*, 1987, **56**, 35–39.
256. Satyanarayana, T. and Johri, B. N., *Curr. Sci.*, 1981, **50**, 680–682.
257. Bhaduria, R., *Sci. Cult.*, 1989, **55**, 173–175.
258. Chakrabarty, M. M., Chaudhary, S. G., Khatoon, S. and Chatterjee, A., in Proceedings of World Conference in Biotechnology for the Fats and Oils Industry, Hamburg, 1987, pp. 10–12.
259. Chand, S., Srinivasulu, C. and Kahapatra, S. N., *Oils Oilseeds J.*, 1974, April–May, 17–19.
260. Chand, S. and Kaur, J., in National Conference on Bioprocessing and Recombinant-DNA Technology, Lucknow, 18 March, 1998, p. 18.
261. Parmar, V. S., Prasad, A. K., Sharma, N. K., Bisht, K. S., Sinha, R. and Taneja, P., *Pure Appl. Chem.*, 1992, **64**, 1135–1139.
262. Gupta, M. N., *Eur. J. Biochem.*, 1992, **203**, 25–32.
263. Qazi, G. N., in 38th Annual Meeting of the Association of Microbiologists of India, Delhi, 1997, p. 232.
264. Sridhar, R., Lakshminarayana, G. and Kaimal, T. N. B., *J. Am. Oil Chem. Soc.*, 1991, **68**, 726–730.
265. Sidhu, A., Sharma, R., Soni, S. K. and Gupta, J. K., in 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 1996, p. 81.
266. Sharan, E. and Kamat, M. Y., in 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 1996, p. 82.

ACKNOWLEDGEMENTS. We thank Ms Rekha Kohli for her technical help in preparation of this manuscript. Financial assistance from the Department of Biotechnology, Government of India, New Delhi, is acknowledged.