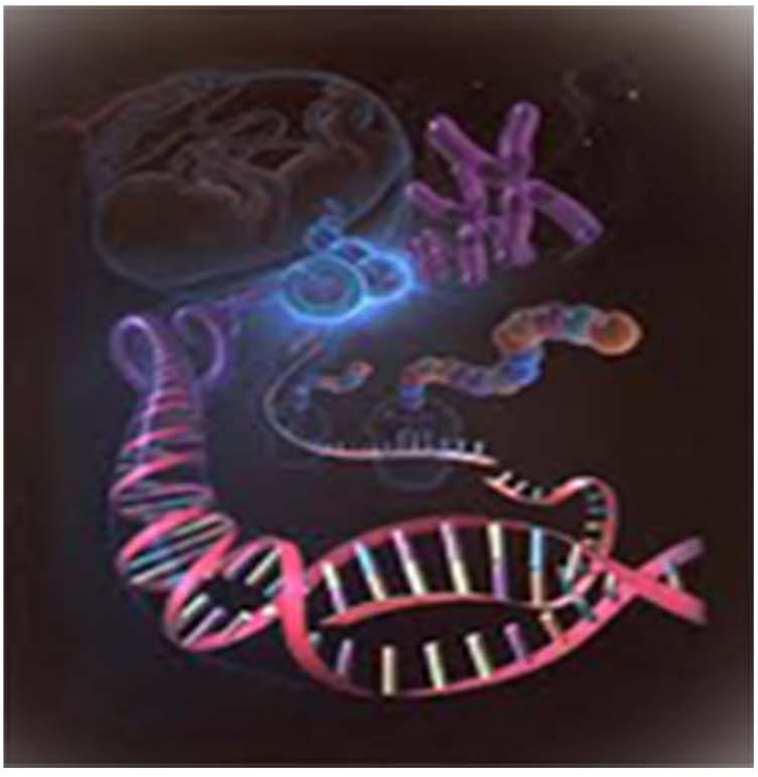
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 4, October 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 4, October 2013

Review Article

A REVIEW ON "MICROBIAL LIPASE-VERSATILE TOOL FOR INDUSTRIAL APPLICATIONS"

Teena Momsia[1]* and Prerna Momsia[1]

*Corresponding Author: **Teena Momsia,** ✉ dr.teena.momsia@gmail.com

Lipases are a class of enzymes which catalyze the hydrolysis of long chain triglycerides. At present, microbial lipases are attaining much awareness with the rapid progress of enzyme technology. Lipases represent the most significant group of biocatalysts for industrial applications. The present review describes various industrial applications of microbial lipases in the food, flavor, medical and pharmaceuticals, fine chemical synthesis, domestic and environmental agrochemicals, use as biosensor, bioremediation and cosmetics and perfumery.

Keywords: Lipases, Biocatalysts, Lipolytic, Industrial applications

INTRODUCTION

Enzymes are considered as nature's catalysts. Most enzymes today (and probably nearly all in the future) are produced by the fermentation of bio-based materials (Louwrier, 1998). Several thousand enzymes possessing different substrate specificities are known, however only comparatively few enzymes have been isolated in a pure form and crystallized, and little has been known about their structure and function. The advent of protein engineering techniques makes their application to important industrial enzymes (Cheetham, 1995).

Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer (Wiseman, 1995).

Lipases (triacylglycerol acylhydrolases 3.1.1.3) are ubiquitous enzymes that catalyze various reactions and display a wide range of potential industrial applications (Soberon-Chavez and Palmeros, 1994). Lipases occur widely in bacteria, yeasts and fungi (Jaegar *et al.,* 2000, Gao *et al.,* 2000, Dalmou *et al.,* 2000). Although lipases have been found in

[1] Life Science Department, Jaipur National University, Jaipur, Rajasthan.

many species of animals and plants, the enzymes from microbial sources (such as bacteria, yeast and fungi) are currently receiving particular attention because of their actual and potential applications in industry mainly in the detergents, oils and fats, dairy and pharmaceutical industries (Sarkar *et al*., 1998; Cardenas *et al*., 2001). Lipolytic enzymes are currently attracting vast attention because of their biotechnological potential. They constitute the most important group of biocatalysts for biotechnological applications (Benjamin and Pandey, 1998).

HISTORICAL BACKGROUND OF LIPASES

The presence of lipases has been observed as early as in 1901 for *Bacillus prodigiosus*, *B. pyocyaneus* and *B. fluorescens* by the microbiologist Sir Eijkman, which represent today's best studied lipase producing bacteria now named *Serratia marcescens*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, respectively. Enzymes hydrolyzing triglycerides have been studied for well over 300 years and the ability of the lipases to catalyze the hydrolysis and also the synthesis of esters has been recognized nearly 70 years ago (Hasan *et al.,* 2006; Hossain *et al.,* 2010). In 1856, Claude Bernard first discovered a lipase in pancreatic juice as an enzyme that hydrolyzed insoluble oil droplets and converted them to soluble products. Lipases have traditionally been obtained from animal pancreas and are used as a digestive aid for human consumption either in crude mixture with other hydrolases (pancreatin) or as a purified grade. Initial interest in microbial lipases was generated because of a shortage of pancreas and difficulties in collecting available material. Lipases differ greatly as regards both their origins (which can be bacterial, fungal, mammalian, etc.) and their properties, and they can catalyze the hydrolysis, or synthesis, of a wide range of different carboxylic esters and liberate organic acids and glycerol. They all show highly specific activity towards glyceridic substrates (Hasan *et al.,* 2006).

INDUSTRIAL USES OF LIPOLYTIC ENZYMES

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential. These are an excellent alternative to many classical organic techniques in the selective transformation of complex molecules and possess many features that favor their use as an excellent biocatalyst. They Impart specificity to a reaction, in which a chemical process is typically more non-specific. In addition, the use of enzyme can decrease side reactions and simplify post-reaction separation problems (Pandey *et al*., 1999). Lipase catalyzed processes are reported to offer cost-effectiveness too, in comparison with traditional downstream processing in which energy consumption and toxic by-products might often pose problem (Jansen *et al.,* 1996). The alkaline thermophilc lipases find application in detergent industry. Many fatty food stains and human sebum contain triglycerides which are hydrolyzed by lipases to produce fatty acids, monoglycerides and diglyceries, which are easier to remove than unhydrolyzed triglycerides (Fuji *et al*., 1986). The optimization of industrially relevant lipase

properties can be achieved by directed evolution. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavor compounds (Jaeger *et al.,* 2002).

Microbial lipases are also more stable than their corresponding plant and animal enzymes and their production is more convenient, safer and can be obtained in bulk at low cost (Vakhlu and Kour, 2006). Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications (Jaeger *et al.,* 1999).

There is growing interest in large scale purification of lipases. The chemo-, regio- and enantio-specific behavior of these enzymes has caused tremendous interest among scientists and industrialists. Lipases from a large number of bacterial, fungal and a few plant and animal sources have been purified to homogeneity. Different strategies are being used for the purification of various fungal lipases (Saxena *et al.,* 2003; Iftikhar *et al.,* 2011).

Several species of bacteria, yeasts and molds produce lipases (Table 1). Taxonomically close strains may produce lipases of diverse types.

The requirement for greater control over microorganisms and enzymes for industrial purposes has led to a greater center of attention on Genetic Engineering and Recombinant DNA technology. This technology,

Table 1: Isolation of Lipases from Various Lipolytic Microorganisms Approximate From Year 2000

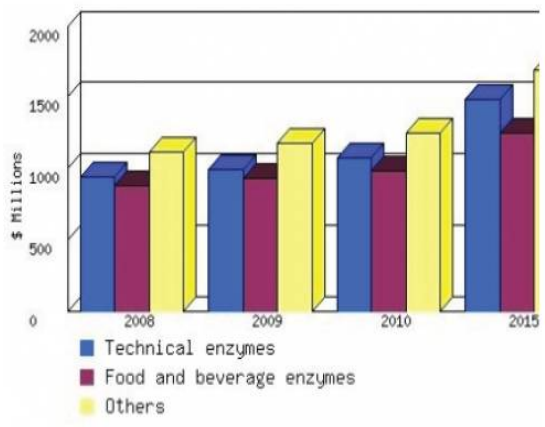
Microorganisms	References
Bacillus sp.	(Imamura *et al.,* 2000)
Bacillus subtilis	(Ruiz *et al.,* 2005; Eggert, 2003)
Staphylococcus xylosus	(Mosbah *et al.,* 2005)
Penicillium cyclopium	(Chahinian *et al.,* 2000)
Aspergillus niger	(Namboodiri *et al.,* 2000)
Trichosporon laibacchii	(Liu *et al.,* 2004)
Rhizopus sp.	(Macedo *et al.,* 2003, Momsia *et al.,* 2012)
Rhizomucor miehei	(Herrgoard *et al.,* 2000)
Candida cylidracae	(Muralidhar *et al.,* 2001)
Acinetobacter sp.	(Snellman *et al.,* 2002)
Fusarium solani	(Knight *et al.,* 2000)

allows genetic modification of microorganisms to produce the desired enzyme under specific conditions and helps either to produce a particular type of enzyme or enhance the quantity of enzyme produced from the single recombinant microorganism. Studies are being conducted on ways to improve or modify protein structure and its function thus finally the enzyme. Biotechnology is therefore, being increasingly viewed as a possible solution against traditional chemicals processes. The production of enzymes from natural sources and their environment friendly characteristics has led the industry to believe that enzymes are indeed a sustainable alternative to chemicals in industrial processes. The factors that aid their increased application is the availability of wide enzyme types, environmental friendly behavior, minimized energy consumption, easily controlled processes, possible modification of enzyme characteristics,

environmentally friendly by-products, minimal greenhouse gas emissions, decreased use of non-renewable sources and proliferation of chemicals in environment. The multi faceted benefits availed through using enzymes is bound to convert into a greener world (Kumar, 2009).

The industrial enzyme market is divided into three application segments: technical enzymes, food enzymes, and other uses (Figure 1). Technical enzymes for detergent, pulp and paper manufacturing, among others are the largest segments, with a 52% share. Growth will parallel the overall market. The confectionery and sweetener segment is the largest sector in food applications and is expected to grow at a healthy AAGR of around 3%. Overall, the enzymes in various food application sectors will be showing healthy growth, with an AAGR of 3.1% (Rajan, 2004; Hasan *et al.,* 2006).

Figure 1: Global Enzyme Markets by Application Sectors ($ millions)



The requirement for greater control over microorganisms and enzymes for industrial purposes has led to a greater center of attention on Genetic Engineering and Recombinant DNA technology. This technology, allows genetic modification of microorganisms to produce the desired enzyme under specific conditions and helps either to produce a particular type of enzyme or enhance the quantity of enzyme produced from the single recombinant microorganism. Studies are being conducted on ways to improve or modify protein structure and its function thus finally the enzyme. Biotechnology is therefore, being increasingly viewed as a possible solution against traditional chemicals processes. The production of enzymes from natural sources and their environment friendly characteristics has led the industry to believe that enzymes are indeed a sustainable alternative to chemicals in industrial processes. The factors that aid their increased application is the availability of wide enzyme types, environmental friendly behavior, minimized energy consumption, easily controlled processes, possible modification of enzyme characteristics, environmentally friendly by-products, minimal greenhouse gas emissions, decreased use of non-renewable sources and proliferation of chemicals in environment. The multi faceted benefits availed through using enzymes is bound to convert into a greener world (Kumar, 2009; Hasan *et al.,* 2010).

APPLICATIONS OF LIPASES

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:

a. Lipases in fat and oleochemical industry: Lipases are part of the family of hydrolases that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification, interesterification, and transesterification reactions in nonaqueous media.

Figure 2: Acidolysis

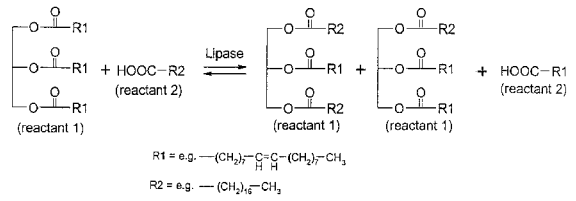


Figure 3: Inter-Esterification

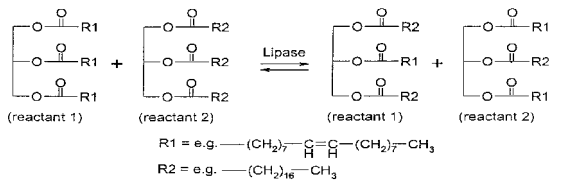
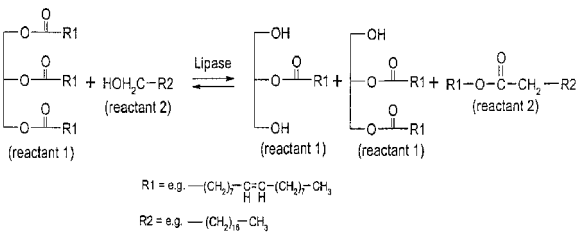


Figure 4: Alcoholysis



This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries (Houde *et al.,* 2004; Hasan *et al.,* 2006; Sekhar, 2012).

Some fats are much more valuable than others because of their structure. Less valuable fats can be converted into more useful species using blending of chemical methods but these tend to give quite random products. Lipase catalyzed transesterification of cheaper oils can be used, for example to produce cocoa butter from palm mid-fraction (Hasan *et al.,* 2006).

The lipase catalyzed transesterification in organic solvents is an emerging industrial application such as production of cocoa butter equivalent, human milk fat substitute "Betapol", pharmaceutically important Polyunsaturated Fatty Acids (PUFA) rich/low calorie lipids, "designers fats or structured lipid" and production of biodiesel from vegetable oils (Jaeger and Reetz, 1998; Nakajima *et al.,* 2000).

Mucor miehei (IM 20) and *Candida antarctica* (SP 382) lipases were used for esterification of free fatty acids in the absence of organic solvent or transesterification of fatty acid methyl esters in hexane with isopropylidene glycerols (Akoh, 1993).

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 (Arbige and Pitcher, 1989; Buhler and Wandrey, 1987; Saxena *et al.,* 1999). Miyoshi Oil and Fat Co., Japan, reported commercial use of *Candida cylindracea* lipase in

production of soap (McNeill and Yamane, 1991; Saxena *et al.,* 1999). The introduction of the new generation of cheap and very thermostable enzymes can change the economic balance in favor of lipase use (Macrae and Hammond, 1985).

Lipolysis is the "constructive" consequences of the ability of lipase to hydrolyze lipids so as to obtain fatty acids and glycerol, both of which have important industrial applications. For instance, fatty acids are used in soap production (Hoq ,1985; Hasan *et al.,* 2006). Glycerides are the major components of depot, or storage, fats in plants and animal cells. Those that are solid at room temperature are known as oils. Lipases catalyze the hydrolysis of fats and oils, and other carboxylic acid esters. In 1983, MacRae *et al.* has reported that oil, which being solid at room temperature in crude condition could be converted into fluid conditions by substitution of about 4-50% of its palmitic acid content. Lipozyme (immobilized *Mucor meihei* lipase) have been used to catalyze glycerolysis of melted tallow to synthesize monoglycerides (Stevenson, 1993, Hasan *et al.,* 2006).

b. Production of biodegradable polymer: Lipases have become one of the most important groups of enzymes for its applications in organic syntheses. Lipases can be used as biocatalyst in the production of useful biodegradable compounds. 1-Butyl oleate was produced by direct esterification of butanol and oleic acid to decrease the viscosity of biodiesel in winter use. Tri-methylol-propane esters were also similarly synthesized as lubricants. Lipases can catalyze ester syntheses and transesterification reactions in organic solvent systems has opened up the possibility of enzyme catalyzed production of biodegradable polyesters. Aromatic polyesters can be synthesized by lipase biocatalysis (Linko, 1998; Hasan *et al.,* 2006).

c. Lipases in detergent industry: The usage of enzymes in washing powders still remains the single biggest market for industrial enzymes (Arbige and Pitcher, 1989; Hasan *et al.,* 2006). The world-wide trend towards lower laundering temperatures has led to much higher demand for household detergent formulations. Recent intensive screening programs, 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* by Hoq *et al.,* 2006).

The most commercially important field of application for hydrolytic lipases is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. The cleaning power of detergents seems to have peaked; all detergents contain similar ingredients and are based on similar detergency mechanisms. To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes, such as protease, amylase, cellulase and lipase (Ito *et al.,* 1998).

Pseudomonas lipase preparations have been used for preparation of washing powder formulations. *Pseudomonas medocina* (Lumafast®) and *Pseudomonas alcaligenes*

(Lipomax®) lipases have been manufactured by Genencor international USA, as detergent additive (Jaeger *et al*. 1994, Reetz and Jaeger 1998). The Novo group has reported a highly alkaline, positionally non-specific lipase, from a strain of *Streptomyces* sp. that was useful in laundry and dish-washing detergents as well as industrial cleaners (Pandey *et al*. 1999). Several lipase-producing organisms and their manufacturing processes are patented for preparation of detergent lipases (Holmes, 1993, Ishida *et al.,* 1995; Lawler and Smith, 2000).

The other common commercial applications for detergents is in dish washing, a bleaching composition (Nakamura and Nasu, 1990), decomposition of lipid contaminants in dry cleaning solvents (Abo, 1990), liquid leather cleaner (Kobayashi, 1989), contact lens cleaning (Bhatia, 1990), clearing of drains clogged by lipids in food processing or domestic/industrial effluent treatment plants (Bailey and Ollis, 1986), degradation of organic wastes on the surface of exhaust pipes, toilet bowls, etc. (Moriguchi *et al.,* 1990), removal of dirt/cattle manure from domestic animals by lipases and cellulases (Abo, 1990), washing, degreasing and water reconditioning by using lipases along with oxidoreductases, which allows for smaller amounts of surfactants and operation at low temperatures (Novak *et al.,* 1990). The lipase constituents causes an increase in detergency and prevents scaling.

d. Lipases in food processing, flavor development and improving quality: Lipases have become an integral part of the modern food industry (Theil, 1995; Sharma *et al.,* 2011). It is desirable for the production of flavors 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 flavor 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 tri-acylglycerol to diacylglycerols and monoacylglycerols; and then these products gives rise to non-esterified fatty acids and fatty acid propan-2-yl esters.

Lipase mediated modifications based on microbial lipases which are regio-specific and fatty acid specific, are of immense importance and could be exploited for retailoring of vegetable oils are likely to occupy a prominent place in oil industry for tailoring structured lipids, since enzymation modifications are specific and can be carried out at moderate reaction conditions and cheap oils could also be upgraded to synthesize nutritionally important structured triacylglycerols like cocoa, butter substitutes, low calories triacylglycerols and oleic acid enriched oils (Gupta *et al.,* 2003; Hasan *et al.,* 2006). Lipases have also been used for addition to food to modify flavor by synthesis of esters of short chain fatty acids and alcohols, which are known flavor and fragrance compounds (Macedo, 2003).

The use of enzymes to improve the traditional chemical processes of food manufacture has been developed in the past

few years. Stead (1986) and Coenen *et al.* (1997) stated that, though microbial lipases are best utilized for food processing, a few, especially psychrotrophic bacteria of *Pseudomonas* sp. and a few moulds of *Rhizopus* sp. and *Mucor* sp. caused havoc with milk and dairy products and soft fruits. Cold active lipase from *Pseudomonas* strain P38 is widely used in non-aqueous biotransformation for the synthesis of n-heptane of the flavoring compound butyl caprylate (Tan *et al.,* 1996). Immobilized lipases from *C. antarctica* (CAL-B), *C. cylindracea AY*30, *H. lanuginosa*, *Pseudomonas* sp. and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil Buisman *et al.* (1998).

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 flavor 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).

e. Medical and pharmaceutical applications of lipases: Lipases are important in application in pharmaceuticals in transesterification and hydrolysis reaction. They play a prime role in production of specialty lipids and digestive aids (Vulfson, 1994). The alteration of temperature during the esterification reaction drastically changes the enantiomeric values and also the stereopreference (Yasufuku *et al*., 1996). Lipases play an important role in modification of monoglycerides for use as emulsifiers in pharmaceutical applications (Sharma *et al*., 2001).

Lipases may be used as digestive aids (Gerhartz, 1990). Lipases are the activators of Tumor Necrosis Factor and therefore can be used in the treatment of malignant tumors (Kato *et al.,* 1989). Although Human Gastric Lipase (HGL) is the most stable acid lipase and constitutes a good candidate tool for enzyme substitution therapy (Ville *et al.,* 2002). Lipases have earlier been used as therapeutics in the treatment of gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. (Mauvernay *et al.,* 1970) Berrobi *et al.* have filed a patent for pharmaceutical preparations that contain hyaluronidase and/or thiomucase enzymes in addition to lipases for use in skin inflammations. Lipase from *Candida rugosa* have been used to synthesize lovastatin, a drug that lower serum cholesterol level. The asymmetric hydrolysis of 3-phenylglycidic acid ester which is a key intermediate in the synthesis of diltiazem hydrochloride, a widely used coronary vasodilator, was carried out with

S. marcescens lipase (Matsumae *et al.,* 1993; Hasan *et al.,* 2006).

f. 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. Unichem International (Spain) has launched the production of isopropyl myristate, isopropyl palmitate and 2-ethylhexylpalmitate for use as an emollient in personal care products such as skin and sun-tan creams, bath oils, etc. Immobilized *Rhizomucor meihei* lipase was used as a biocatalyst. The company claims that the use of the enzyme in place of the conventional acid catalyst gives products of much higher quality, requiring minimum downstream refining (Hasan *et al.,* 2006). Wax esters (esters of fatty acids and fatty alcohols) have similar applications in personal care products and are also being manufactured enzymatically (Croda Universal Ltd.). The company uses *C. cylindracea* lipase in a batch bioreactor. According to the manufacturer, the overall production cost is slightly higher than that of the conventional method, but the cost is justified by the improved quality of the final product (Hasan *et al.,* 2006). Retinoids (Vitamin A and derivatives) are of great commercial potential in cosmetics and pharmaceuticals such as skin care products. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase (Maugard *et al.,* 2002). Lipases have been used in hair waving preparation (Saphir, 1967). Lipases have also been used as a component of topical antiobese creams (August 1972) or as oral administration (Smythe, 1951).

g. Lipases application in Waste/effluent/ sewage treatment/ oil biodegradation: Lipases are utilized in activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed from the surface of aerated tanks to permit oxygen transport (to maintain living conditions for the biomass). This skimmed fat-rich liquid is digested with lipases (Bailey and Ollis, 1986) such as that from *C. rugosa*. Biodegradation of petroleum hydrocarbons in cold environments, including Alpine soils, is a result of indigenous cold-adapted microorganisms able to degrade these contaminants. Seven genotypes involved in the degradation of *n*-alkanes (*P. putida* GPo1 alkB; *Acinetobacter* spp. alkM; *Rhodococcus* spp. alkB1, and *Rhodococcus* spp. alkB2), aromatic hydrocarbons (*P. putida* xylE), and polycyclic aromatic hydrocarbons (*P. putida* ndoB and *Mycobacterium* sp. strain PYR-1 nidA) was determined in 12 oil-contaminated (428-30,644 mg of total petroleum hydrocarbons [TPH]/kg of soil) Margesin *et al.* (1989, 2003) have found that monitoring of soil microbial lipase activity is a valuable indicator of diesel oil biodegradation in freshly contaminated, unfertilized and fertilized soils. Fungal species can be used to degrade oil spills in the coastal environment, which may enhance ecorestoration as well as in the

enzymatic oil processing in industries (Gopinath *et al.,* 1998).

FUTURE PROSPECTS

On the studies of production of microbial lipases and the role of lipidic substances used as inducers in lipase production are scanty. Lipases represent an extremely versatile group of bacterial extracellular enzymes that are capable of performing a variety of important reactions, thereby presenting a fascinating field for future research. The understanding of structure-function relationships will enable researchers to tailor new lipases active at low temperatures for biotechnological applications. Developments in research are expected from interchange of experiences between biochemists, geneticists and biochemical engineers. Wide and constant screening of new microorganisms for their lipolytic enzymes at low temperature will open novel and simpler routes for the synthetic processes. Consequently, this may pave new ways to solve biotechnological and environmental problems.

Lipases are remarkable biocatalysts for high-value application in various industries. Lipases are capable of catalyzing novel reactions. 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, 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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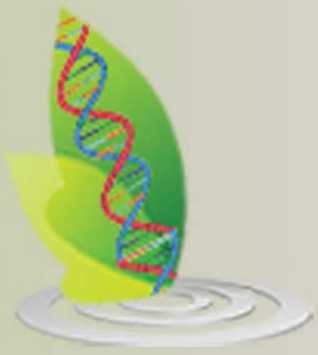

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ISSN 2250-3137
9 772250 313001 04