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Lipase biodiversity

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Abstract

Industries prefer biocatalysts rather than chemical catalyst. Lipase a biocatalyst is a versatile enzyme that not only hydrolyzes the esters of long chain aliphatic acids form glycerol at oil or water interface but also involved in hydrolysis, transesterification, alcoholysis, and aminolysis. Lipases are widely distributed in microorganisms, plants and animals. Among them microbial lipases are preferred because of easily obtainable. Lipases are used in many fields like food, dairy, detergent, pharmaceutical, agrochemical and oleochemical industries. Based on the data compiled it reveals that the contribution of bacterial lipases is 45%, fungal 21%, animal 18%, plants 11% and algae 3%. This article provides information about comparative account of bacterial, fungal, plant and animal origin lipases along with their biochemical profiles. It also focuses on the need in search of algal lipases.

Keywords: Enzyme, lipase, microorganisms, plants and animals

Introduction

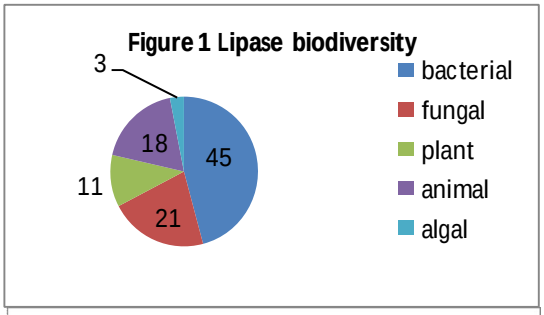
Lipases are the special kind of esterases belong to subclass 1 of hydrolytic enzyme class 3 and have been assigned sub-sub class 3.1.1 due to their specificity for carboxylic acid ester bonds. Naturally, lipase acts on glycerides as they possess a chiral alcohol moiety. They are also useful for the resolution or asymmetrization of ester bearing a chiral alcohol moiety (Aravindan, 2007). The biological function of lipase is to catalyze the hydrolysis of triacylglycerols to give free fatty acid, diacylglycerols, mono-acylglycerols and glycerol. The lipases available from various sources have considerable variation in their reaction specificities. Some lipases have affinity for short chain fatty acid (acetic, butyric, capric acid or decanoic acid etc.) while others have preference for unsaturated fatty acids (oleic, linolenic acid etc.) and many other are non specific and randomly split the fatty acids from the triglycerides. Lipase possesses the unique features of interface between an aqueous and non aqueous phase, provide a new understanding of a rapidly moving field. In the present article a data on lipase of bacterial, fungal, plant and animal origin has been compiled. However, more emphasis has been made towards algal origin which is ephemeral.

Sources of lipase

Lipase occurs widely in nature; however microbial lipases are commercially significant because of low production cost, greater stability and wider availability than other sources. Few review articles were published (Pahoja & Sethar 2002; Gupta & Rathi 2004; Aravindan *et al.,* 2007) on lipase sources along with its industrial applications. Fig.1 illustrates the biodiversity of lipases with biological origin and Fig.2 shows the number of characterized and uncharacterized lipases. The characterization is based on pH, temperature and molecular weight of the enzyme.

Bacterial lipases

Many bacterial lipases are well studied compared to plants and animals. Bacterial lipase is a glycoprotein but some extracellular bacterial lipases are lipoprotein. The organisms are normally grown on nutrient medium containing carbon (oil, sugar and mixed carbon sources), nitrogen, phosphorus sources and mineral salts whereas the production of lipases mostly depends on inducer such as triglycerides, bile salts and glycerol. Lipases from *Pseudomonas* were probably the first studied and have preponderant role in industries, later on *Achromobacter sp., Alcaligones sp., Pseudomonas sp., Staphylococcus sp.,* and *Chromobacterium sp.,* have been exploited for production of lipases. Lang *et al.* (1996) derived crystal structure of the lipases form *Chromobacterium viscosum*. The sources and properties of bacterial lipases are given in Table 1(a). These lipases are characterized for pH, temperature, Pi and molecular weight from both gram positive and gram negative bacteria. It is evident from the Table 1(a) that, pH range is between 4.0 to 10.0; temperature range is between 27 to 80^{o}C;



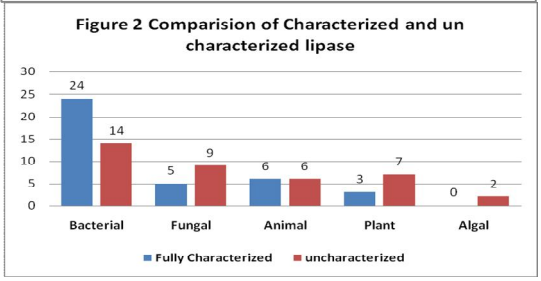


Table 1(a). Biochemical profile of bacterial lipases

Source	pH	Temp.	MW	Reference
Acinetobacter calcoaceticus	8.0	35	30.5	Brune & Gotz (1992)
A. calcoaceticus	5.0	47	23	Dharmsthiti *et al.* (1998)
Acinetobacter sp. CR9	8.0	40	--	Kasana *et al.* (2008)
Acinetobacter sp. RAG-1	9.0	62	33	Snellman *et al.* (2002)
Alcalingenes sp.	9.0	55	--	Brune & Gotz (1992)
Bacillus alcalophilus	10	60	--	Ghanem *et al.* (2000)
B. coagulans BTS-3	8.5	55	31	Kumar *et al.* (2005)
B. licheniformis strain H1	10	55	--	Khyami-Horani (1996)
B. megaterium AKG-1	7.0	55	--	Sekhon et.al. (2004)
B. pumilus B26	8.5	35	--	Kim *et al.* (2002)
B. stearothermophilus	7.4	68	43	Kim *et al.* (2000)
B. subtilis 168	9.9	35	19	Lesuisse *et al.* (1993)
B. thermocatenulatus	8.5	70	--	Schmidt *et al.* (1996)
B. thermoleovorans CCR11	9.5	60	11	Lelie *et al.* (2005)
B. thermoleovorans ID-1	7.5	75	34	Lee *et al.* (1999)
Bacillus sp LBN 4	7.0	50	--	Bora & Kalita, (2009)
Bacillus sp.	5.6	30	22	Sugihara *et al.* (1991)
Bacillus sp. strain 398	8.2	65	50	Kim *et al.* (1994)
Bacillus sp. strain A30-1	7.2	60	65	Wang *et al.* (1995)
Bacillus sp. THLO 27	7.0	70	69	Dharmsthiti *et al.* (1999)
Burkholdria cepacia	7.5	38.5	--	Gupta *et al.* (2001)
Pseudomonas aeruginosa	9.3	50	30	Lidija *et al.* (2009)
P. aeruginosa EF2	9.0	50	29	Gilbert *et al.* (1991)
P. cepacia	5.0	60	--	Dunhaupt *et al.* (1991)
P. fluorescens	9.0	52.5	33	Kojima *et al.* (1994)
P. fluorescens MC50	8.5	35	55	Brune & Gotz (1992)
P. fragi	8.2	65	55	Brune & Gotz (1992)
Pseudomonas sp KWI-56	6.2	52.5	33	Brune & Gotz (1992)
Pseudomonas sp.	8.0	52.5	30	Dong *et al.* (1999)
Pseudomonas sp. strain KB 700A	8.2	35	--	Rashid *et al.* (2001)
Pyrococcus furiosus	7.0	30	28	Chandrayan *et al.* (2008)
Rhodotorula pilimanae lipase I	4.0	47.5	176	Muderhwa *et al.* (1986)
Rhodotorula pilimanae lipaseII	7.0	47.5	21.4	Muderhwa *et al.* (1986)
Salinivibrio sp. strain SA-2	7.7	50	--	Mohammad et al. (2008)
Staphylococcus epidermidis	6.0	40	--	Joseph *et al.* (2006)
S. haemolyticus	8.5	28	45	Byung *et al.* (2000)
Thermobifida fusca	8.0	60	29	Chen et al. (2008)
Thermophilic *Bacillus sp* J33.	--	60	45	Nawani & Kaur (2004)

Temp. = Temperature in ^{0}C, MW = Molecular weight in kDa.

whereas, molecular weight varies from 11 to 176 kDa.

Fungal lipases

Fungal lipases have benefits over bacterial lipases due to their low cost of extraction, thermal and pH stability, substrate specificity and activity in organic solvents. Lipase producers are widespread in the fungal kingdom. The chief producers of lipases are *Aspergillus sp., Candida sp., Mucor sp., Rhizopus sp.,* have been studied in great details. The thermophilic *Mucor pusillus* is well known as a producer of thermostable extracellular lipase and from *M. miehei* two isoenzymes with slightly different isoelectric points could be isolated. The sources and biochemical properties of fungal lipases are given in Table 1(b). Fungal lipases are characterized for pH, temperature and molecular weight. It is interesting to note that, pH range is between 4.0 to 11.0, temperature range is between 25 to 60^{o}C whereas, molecular weight varies from 27 to 120 kDa.

Plant lipases

In plants mostly lipases are present in the form of food reserve tissues of growing seedlings or especially in those which contains large amount of triacylglycerols. Lipase activity in plant seeds increases during germination because the triacylglycerols are converted to soluble sugars by the action of lipase which is then transported to the growing tissues to supply structural carbon and energy to provide support for the growth of young plants. The sources and properties of plant lipases are given in Table 1(c). Plant lipases are characterized for pH, temperature and molecular weight. It is interesting to note that, pH range is in between 4.0 to 8.0, temperature range is in between 25 to 60^{o}C, whereas, molecular weight varies from 40 to 143 kDa. This data indicates that relatively plant lipases are slightly different from bacterial and fungal lipases.

Animal lipases

Animals are also rich sources of lipases but due to the availability of microbial lipases they are rarely studied, but still they have been isolated from many insects, fishes, mammals. Animal lipase plays an important role in digestion of lipids in biological system (Walton & Cowey 1984). Fats required special digestive action before absorption because the end products must be carried in water medium (blood and lymph) in which fats are not soluble. Although little actual fat digestion occurs in the stomach, gastric lipase does digest already emulsified fats such as in egg yolk and cream. The detail biochemical profile of animal lipase is given in Table 1(d).

Properties of lipases

Types of reaction catalyzed by lipase

Lipases are stable and rugged enzyme that act on lipids as well on wide variety of natural and artificial reactant since it has ability to catalyze diversified reaction, few of them are explained below:

Acidolysis: It is the process of reacting an acid with an ester. Acidolysis between Triolein and short chain fatty acid by lipase is carried in organic solvents (Tsuzuki, 2005), *Aspergillus oryzae, Rhizomuco miehei* and *Candida cylindracea* lipases were used. In his experiments he observed ten kinds of lipases as biocatalysts for the incorporation of short chain fatty acids

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Table 1(b). Biochemical profile of fungal lipases

Source	pH	Temp.	MW	Reference
Aspergillus carneus	9.0	37	27	Saxena *et al.* (2003)
A. niger	5.2	47.5	--	Namboodiri *et al.* (2000)
A. niger NCIM1207	8.5	50	--	Mhetras *et al.* (2008)
Aureobasidium pullulans	7.8	35	--	Kudanga *et al.* (2006)
Candida cylindracea	7.2	45	120	Ghosh *et al.* (1996)
C. rugosa	7.0	30	117	Pernas *et al.* (2001)
Fusarium solani	7.25	25	--	Poulsen *et al.* (2005)
Neurospora crassa	7.0	30	54	Kundu *et al.* (1987)
Penicillum nitroaeducens	11.0	33.5	--	Ghosh *et al.* (1996)
Pichia burtonii	6.5	45	51	Sharma *et al.* (2001)
Rhizomucor miehei	8.0	40	--	Herrgard *et al.* (2000)
Rhizopus homothallicus	--	50	29.5	Diaza *et al.* (2006)
R. oryzae	7.5	35	32	Sharma *et al.* (2001)
Thermoactinomyces sp.	8.0	60	--	Khudary *et al.* (2003)

Temp. = Temperature in ^{0}C, MW = Molecular weight in kDa

Table 1(c). Biochemical profile of plant lipases

Source	pH	Temp.	MW	Reference
Caesalpinia bonducella seeds	6.8	60	--	Pahoja *et al.* (2001)
Carissa carandas fruit	5.0	30	--	Mala *et al.* (1995)
Castor bean	9.0	25	62	Maeshima *et al.* (1985)
Cucumis melo	5.0	40	--	Akhtar *et al.* (1978)
Hibiscus cannabinus	5.0	40	--	Akhtar *et al.* (1979)
Lycopersicon esculentum	8	25	--	Matsui *et al.* (2004)
Moringa olifera	5.0	40	--	Dahot *et al.* (1987)
Rice bran	7.8	38.5	40	Aizono *et al.* (1973)
Triticum aestivum	8.0	37	143	Kapranchikov et al. (2004)
Lycopersicon esculentum	8	25	--	Matsui *et al.* (2004)

Temp. = Temperature in ^{0}C, MW = Molecular weight in kDa.

Table 1(d). Biochemical profile of animal lipases

Source	pH	Temp.	MW	Reference
Chicken Adipose	5.2	--	86	Marit *et al.* (1997)
Cyprinion macrostomus	7.5	37	51	Deuerlu et al (2002)
Homo sapiens	7.5	37	---	Jocken *et al.* (2008)
Homo sapiens gastric	4.5	37	10	Carriere *et al.* (2000)
Homo sapiens pancreatic	7.0	37	50	Carriere *et al*. (2000)
Oncorhynchus mykiss tourt	7.0	15	42	Harmon *et al.* (1991)
Rat adipose tissues	--	--	85	Belfrage *et al.* (1977)
Rattus norvegicus	8.6	--	180	Jensen *et al.* (1981)
Rohu (*Labea rohita*)	7.0	37	--	Nayak *et al.* (2004)
Scorpio maurus	9.0	37	50	Zouari *et al.* (2005)
Sus scrofa	8.0	40	240	David *et al.* (1998)
trout adipose	--	--	48	Sheridan *et al.* (1989)

Temp. = Temperature in ^{0}C, MW = Molecular weight in kDa.

(acetic, propionic and butyric acids) into triolein in order to produce one kind of reduced calorie structured lipids.

Transesterification: It is hydrolysis of triglycerides in the presence of alcohol to form methylester and glycerol. A new enzymatic route for methylesters production from soybean oil was suggested by Xu *et al.* (2003) and found that Novozyme 435 (immobilized *Candida antarctica* lipase) gave the highest methyl ester yield of 92% under optimum conditions and transeterification of 30%, based on oil weight molar ratio of methylacetate and oil of 12:1, temperature of 40^{0}C and reaction time of 10 hours.

Esterification: A reaction of an alcohol with an acid to produce an ester and water is called as esterification. Unal (1998) investigated the effect of molecular sieves on the esterification reaction between lauric acid and geraniol in isooctane catalyzed by immobilized lipase.

Intertransesterification: It is one of the most important processes for modifying the physicochemical characteristic of oils and fats. During transesterification, fatty acids are exchanged within and among triacylglycerols until thermodynamic equilibrium are reached. Most vegetable oils are unspecific in chemical composition. To widen their use, vegetable oils are modified chemically by interesterification. Usmani *et al.* (2010) worked on lipase catalysed interesterfication for the production of oleochemicals from non-traditional oils.

Aminolysis

Aminolysis is the conversion of amines and alcohols into amides and esters or it is any chemical reaction in which a molecule is split into two parts by reacting with a molecule of ammonia or an amine. Couturies (2009) described the lipase catalyze chemoselective aminolysis of various aminoalcohols with fatty acids, they used *Candida antarctica* lipase and developed a solvent free enzymatic process for the production of fatty alcohol amides. The aminolysis of linoleylethyl esters with several aminoalcohols from C2 to C6 ethanolamine, aminopropanol, 3-amino-1,2-propanediol, 2-amino-1, 3 propanediol, 4-amino-1-butanol etc. are widely used aminoalcohols.

Lipase mediated conversion of natural oils: Amro *et al.* (2009) suggested that *Pseudomonas aeruginosa* has the ability to degrade castor oil. Rao (2008) worked on production of biodiesel using vegetable oil and ethyl acetate as acyl acceptor.

Hydrolysis: Triglycerides are composed of three glycerol and three fatty acids. Lipase hydrolysis or degrades triglycerides into its component parts of fatty acids and glycerol. Hills and Beevers (1987) suggested that lipase hydrolyzes triacylglycerol to glycerol (Fig.3) and fatty acids which are converted to sugars and support growth of young plants.

Alcoholysis: Hadzir *et al.* (2001) studied the reaction between Triolein and oleyl alcohol catalyzed by Lipozyme and Novozyme to produce wax esters. The best conditions tested to produce wax ester were incubation time of 5 hours, temperature of 50^{0}C for Lipozyme and 60^{0}C for Novozyme. Ghosh *et al.* (1996) investigated lipase catalyzed alcoholysis of soy phospholipids to simultaneously make lysophospholipids and fatty acid esters. Alcoholysis was carried out by stirring a mixture of soy phospholipids fatty acids and alcohols in equimolar

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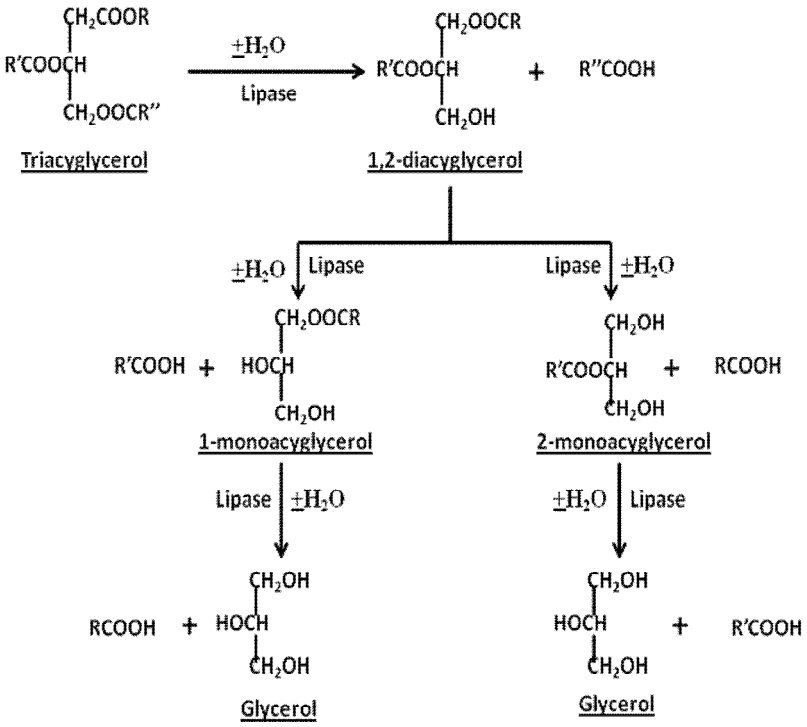
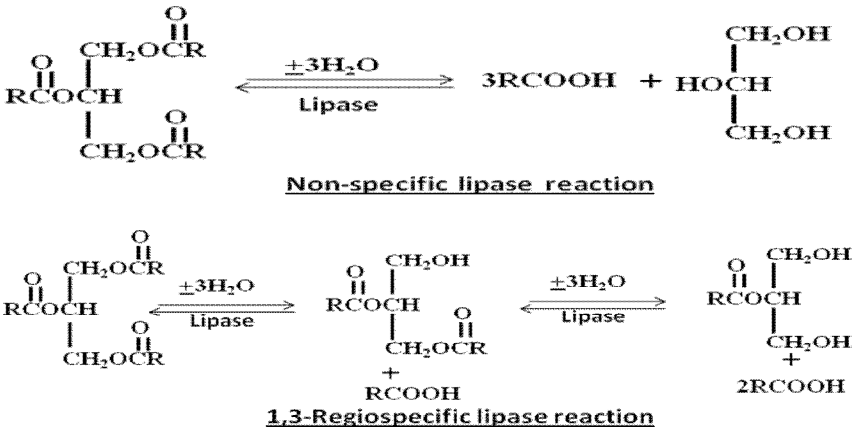
Fig.3. Reaction mechanism of lipase



proportions with esters and 10% of *Mucor miehei* lipase, in the presence of hexane as solvent at 55^{0}C for 24 hours.

Fig. 4. Non-specificity and 1, 3-Regiospecificity of lipase



Substrate specificity of lipases

Lipases are often crucial to their application in industries and laboratories. Different lipases appear too specific in splitting various fatty acids. According to Tsujisaka, et al., (1977) the specificity of lipase is controlled by the molecular properties of enzyme, structure of substrate and factors affecting binding of enzyme to the substrate. Lipase act on substrate in specific and non-specific manner (Fig.4), resulting in complete or hydrolysis of triglycerides into free fatty acids and glycerol or along with triglycerides, monoacylglycerides and diacylglycerides, fatty acids and glycerol's are also formed (Aravindan *et al.*, 2007). Specificity is shown in both the way with respect to either fatty acyl or alcohol part of their substrates (Macrae & Hammond, 1985). Lipase also showed both regiospecificity and stereopecificity with respect to alcohol moiety of their substrate (Ghosh, 1989). Lipase can be divided into two groups on basis of the regiospecificity exhibited on acyl glycerol substrate (Macrae & Hammond, 1985). First group lipase catalyses the complete breakdown of triacylglycerol to glycerol and free fatty acids together with diacylglycerols and monoacylglycerols as intermediates in the reaction, example of such lipase are isolated from *Candida cylindraceal* (Kugumiya, 1986). Second type of lipase release fatty acids regiospecifically from outer 1 and 3 positions of acylglycerols, such lipases hydrolyze triacylglycerol to give free fatty acids, 1,2-diacylglycerol, 2, 3 and 2-monoacylglycerols, example *Aspergillus niger* (Lawson *et al.*, 1994). Asahara (1993) worked on lipase from *Geotrichum sp.* FO401B and reported that it catalyzes the hydrolysis of triacylglycerides and release fatty acids selectively from the central position of acylglycerols.

Steriospecificity is the ability of lipase to discriminate between the enantiomers of a raceminic pair. Steriospecificity is difficult to achieve by chemical methods (IUPAC Commission). Lipases have been used to perform stereospecific reactions to yield optically pure aliphatic and aromatic esters, alcohols, acids and lactones (Welsh *et al.* 2002). These reactions may be important as one isomer of certain compound may have desirable attributes than the other. For example the (R)-isomer of aspartame has a sweet taste while the (S)-isomer has a bitter taste attribute.

Substrates for lipases

Lipase catalyze various reactions since it has ability to act on wide range of substrates they may be artificial and natural, Table 2 summaries commonly used substrate for lipase assays.

Stability in organic solvents

Stability in organic solvents is desirable in synthesis reaction. From the available information it is concluded that lipases are generally stable in organic solvents with few exceptions of stimulation or inhibition (Gupta & Rathi, 2004). Eventually high stimulation is noted in the presence of acetone, isopropanol and ethanol but was unaffected by methanol (Sharma *et al.*, 2009). Stability of lipases in different solvents is described in Table 3. Lescic *et al.* (2001) and Karadzic *et al.* (2006) worked

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Table 2. list of commonly used substrate for lipase assays

Organism	Substrate	Reference
Pseudomonas aeruginosa	Soybean oil	Shabtai *et al.* (1992)
P. aeruginosa	butyl butyrate	Gaur *et al.* (2008)
Pseudomonas sp.	4-nitrophenyl myristate	Amada *et al.* (2000)
Pseudomonas sp.	Castor, coconut, corn, groundnut, linseed, neem, soybean, sunflower oils	Rathi, *et al.* (2000)
Candida rugosa	soybean oil	Sharma *et al.* (2001)
Candida rugosa	Triacetin	Pernas *et al.* (2001)
Candida rugosa	4-nitrophenyl decanoate	Chang *et al.* (2006)
Candida rugosa	4-nitrophenyl laurate	Chang *et al.* (2006)
Penicillium candidum	4-nitrophenyl palmitate & tributyrin	Ruiz *et al.* (2001)
P. aurantiogriseum	4-nitrophenyl caprate	Lima *et al.* (2004)
Streptomyces rimosus	Tween 20 and tween 60	Lescic *et al.* (2001)
Aspergillus niger	Tributyrin	van Heerden *et al.* (2002)
A. carneus	4-nitrophenyl myristate	Saxena *et al.* (2003)
Bacillus megaterium	4-nitrophenyl butyrate	Ruiz.*et al.* (2002)
B. coagulans	4-nitrophenyl palmitate	Kumar *et al.* (2005)
B. coagulans	4-nitrophenyl formate	Kumar *et al.* (2005)
Bacillus sp.	4-nitrophenyl caprylate	Sunna *et al.* (2002)
Burkholderia multivorans	4-nitrophenyl palmitate	Gupta *et al.* (2005)
Psychrobacter sp.	olive oil	Zhang *et al.* (2007)
Aureobasidium pullulans	peanut oil	Liu *et al.* (2008)

Table 3. Effect of solvents on lipases

Microorganism	Solvents	Residual activity
*Streptomyces rimosu**, (Lescic *et al.*, 2001)	1,4-dioxane	100% stable
	Acetone	80%
	Acetonitrile	92%
	Dimethyl sulfoxide	63%
	Ethanol	91%
	N,N-dimethyl formamide	41%
	Tertrahydrofuran	No activity
*Pseudomonas aeruginosa***, (Karadzic *et al.*, 2006)	Acetone	95%
	Butanol	10%
	Chloroform	130%
	Dimethylformamide	120%
	Ethanol	90%
	Hexane	100%
	Isopropanol	65%
	Methanol	80%

**18 h at 50% v/v; ** at 25% v/v, temperature 30^{0}C*

independently on effect of various solvents, time, temperature and concentration on *Streptomyces rimosu,* and *Pseudomonas aeruginosa* respectively. Residual activity ranges from 41% to 100% in *Streptomyces ramous*, whereas, it was widely ranged from 10% to 100% and enhancement found in Dimethylformamide (120%) and Chloroform (130%) in *P. aeruginosa.* The effect of various solvents on lipases is given in Table 3.

Cellular localization of lipase

Mostly the prokaryotes secrete lipase extracellularly, however, eukaryotes synthesis cytosolic origin. Neugnot *et al.* (2001) reported cell bound lipases in *Candida parapsilosis* CBS 604. The cellular localization lipases in different organisms are given in Table 4.

pH and temperature kinetics

Lipases are active over broad pH and temperature range and they have molecular weight ranging from 94 to 840 kDa From available literature it can be interpreted that generally lipases have neutral pH optima but the pH and temperature optima of lipases depends on the habitat of its sources. Lipases possess stability over a wide range from pH 4 to 11 and temperature optima in the range from 10 to 96^{0}C. All organisms contain enzymes but they do not usually catalyze their substrate spontaneously, although certain physical treatments or conditions have been shown to activate the enzyme for example milk contain lipase but it unable to lipolysis milk fat spontaneously, the enzyme can be activated by prevailing some physical and chemical treatments such as heat, light, irradiation or chemicals impair the lipase activity (Chandan & Shahani, 1964).

Effect of metal ions

The activity of lipase may be inhibited or stimulated by cofactors. Divalent cations such as calcium often stimulated enzyme activity due to the formation of calcium salt of long chain fatty acids (Macrae & Hammond, 1985). Calcium stimulated lipases have been reported in the case of *Acinetobacter sp.* RAG-1 (Snellman *et al.*, 2002). In contrast, the lipase from *P. seruginosa* 10145 (Finkelstein *et al.,* 1970) is inhibited by the presence of calcium ions. Further lipases activity is inhibited drastically by heavy metals like Ca^{+2}. Ni^{+2}, Hg^{+2} and Sn^{+2} and slightly inhibited by Zn^{+2} and Mg^{+2} (Patkar & Bjorkling, 1994). *P. aeruginosa* KKA-5 lipase hydrolyze castor oil in the presence of various metal chlorides, $CaCl_2$, $AlCl_3$ (group IIIB), $CrCl_3$ (group VIA) and $MgCl_2$ (group IIA) displays enhanced hydrolysis capability. When Cr^{+3} were used, hydrolysis of castor oil was four times faster than that of calcium, and 1.6 times faster with regards to Al^{+3}. The chlorides of group VIII and alkali metals had no effect on hydrolysis (Sharon *et al.* 1998). The presence of chloride salts of Mg^{+2}, Cu^{+2}, Ca^{+2}, Hg^{+2} and Fe^{+2} resulted in a profound increase in the hydrolytic activity of the purified lipase. Interestingly, Hg^{+2} ions resulted in a maximal increase in lipase activity but Co^{+2} ions showed an antagonistic effect. The EDTA at a concentration of 150 mM markedly inhibited the activity of lipase. However, reconstitution of EDTA-quenched lipase with Hg^{+2}, Mn^{+2} or NH^{+4} ions resulted in the restoration of the enzyme activity (Ghazi *et al.*) Lipase activity was enhanced in the presence of K^+, Ca^{+2} and Mg^{+2} ions, but inhibited by Hg^{+2} ions (Sharma et al, 2009). The addition of Mg^{+2} did not significantly stimulate lipase production. While many other metal ions including Ca^{+2}, Mn^{+2}, Ba^{+2},

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Table 4 Cellular Localization of lipase

Organisms	Localization	Reference
Homo sapiens	Cyt	Waterman *et al*. (1998)
Rattus norvegicus	Cyt	Groener *et al*. (1981)
Sus scrofa	Cyt	Guibe-Jampel et al.(1987)
Homo sapiens, Rattus norvegicus, Sus scrofa and Mus musculus	ER	Dolinsky *et al*. (2004)
Aspergilus niger	Ext	Mhetras et al. (2008)
Bacillus megaterium	Ext	Ruiz et al. (2002)
Penicillium candidum	Ext	Ruiz et al. (2001)
Pseudomonas aeruginosa YS-7	Ext	Shabtai et al. (1992)
Psychrobacter sp. 7195	Ext	Zhang et al. (2007)
Streptomyces rimosus	Ext	Lescic et al. (2001)
Rhizopus oryzae	Ext	Sharma et al. (2001)
Yarrowia lipolytica	Ext	Yu et al.(2007)
Pseudomonas fluorescens	Int	Duong et al. (1994)
Pseudomonas aeruginosa	Int	Wohlfarth et al. (1988)
Candida parapsilosis CBS 604	CB	Neugnot et al. (2001)

Cyt= Cytosol, Ext= Extracellular, Int= Intraellular, CB= Cell bound, ER= endoplasmic reticulum.

Zn^{+2}, metal ions, including Ca^{+2}, Mn^{+2}, Ba^{+2}, Zn^{+2}, Fe^{+2}, and Cu^{+2} exerted inhibitory effects. However, lipase production was decreased slightly, to approximately 5%, with the addition of K^{+} and 30% decrease was observed in lipase production by S5 in an absence of potassium ions. The absence of magnesium ions (Mg^{+2}) in the basal medium was also shown to stimulate lipase production. An alkaline earth metal ion, Na^{+}, was found to stimulate the production of S5 lipase (Raja *et al.* 2006). The lipase activity in presence of a metal ions was compared with control including no metal ion whose activity was taken as 100% and the relative activities at 1mM of Cu^{+2}, Hg^{+2}, Pb^{+2}, Co^{+2}, Cd^{+2} and Li^{+} were 0.44, 24.4, 36.2, 49.1, 64.2, 90.0 and 98.2% respectively. Strong inhibition was observed with heavy metals such as Cu^{+2}, Hg^{+2}, Pb^{+2}, $Co2^{+}$ and Cd^{+2} in the *Todarodes pacificus* (Park *et al.*, 2007).

Effect of light and irradiation

A very limited amount of work has been done to study the effect of light and irradiation upon lipase, especially on the milk lipases but there are no reports on microbial, animal or plant lipases. Exposure of milk to bright sunlight, diffused daylight, mercury vapor lamp or ultraviolet light reduces the lipases activity 40 to 80% depending upon the length of exposure (Kay, 1946; Kannan, 1951). While Standhouders and Mulder (1958) observed that milk lipases was most sensitive to the blue rays of visible light. Tsugo and Hayashi (1962) reported that the lipase activity of milk was reduced by 70% at the irradiation dose of 6.6 X 10^{4}r, Sahani and Chandan (1964) observed that only 1 - 38% activity of the purified enzyme was reduced when irradiated by as high as two and four megarads, respectively.

Lipase assay: Many procedures are available for assaying or characterizing hydrolytic activity of lipases (Chandan & Shahani (1964) and Pinsirodom & Parkin (2001)). Consequently, most lipase assays have been developed on the basis of measuring liberated fatty acids either specifically or nonspecifically. Alternatively the use of chromogenic or fluorogenic model substrates affords the option to spectrophotometry to directly and continuously follow the course of lipase reactions.

The titrimetric method is most common method for nonspecific measurement of fatty acids. In this method, native substrates i.e., triacyglycerols are hydrolyzed to yield fatty acids. Subsamples are withdrawn from reactive mixture at predetermined intervals, and reactivity is quenched by the addition of ethanol. The amount of fatty acids released during the reaction is determined by direct titration with NaOH to a thymolphthalein end point. The quantity of fatty acid released in unit time is measured by the quantity of NaOH required to neutralize the mixture. The milliequivalent of alkali consumed is taken as a

Table 5. List of industrial applications of lipases

Industry	Action	Product of application
Dairy food	Hydrolysis of milk, fat, cheese ripening, modification of butter fat	Development of flavoring agent in milk cheese and butter
Bakery food	Flavor improvement	Shelf life propagation
Beverages	Improved aroma	Alcoholic beverages e.g, sake wine
Food dressings	Quality improvement	Maysoine dressing and whippings
Health food	Transesterification	Health food
Meat and fish	Flavor development	Meat and fish product fat removal
Fats and oils	Transesterification and hydrolysis	Cocoa butter, margarine fatty acids, glycerol mono and diglycerides
Laundry	Reducing biodegradable strains	Cleaning cloths
Cosmetics	Esterification	Skin and sun-tan creams, bath oil etc
Surfactants	Replaces phosphoilpases in the production of lysophospholipids	Polyglycerol and carbohydrates fatty acid esters used as industrial detergents and as emulsifiers in food formulation such as sauces & ice creams.
Agrochemicals	Esterification	Herbicides such as phenoxypropionate
Pharmaceuticals	Hydrolysis of expoyester alcohols	Produce various intermediates used in manufacture of medicine.
Fuel industries	Transesterification	Biodiesel production
Pollution control	Hydrolysis and transesterification of oils and grease	To remove hard stains, and hydrolyze oil and greases.

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measure of the activity of enzyme; this method is called as pH stat method (Chandan & Shahani,1964).

Colorimetirc method conceptually assays for lipase activity using the colorimetric method are similar to titrimetry in that liberated fatty acids are being measured; however, the colorimetric methods is more specific for fatty acids. It involves the uses of chromogenic substrates, α-naphthyl acetate, for example, which upon hydrolysis yield or can be readily converted into a colored product, for colorimetric estimation. The color intensity is directly proportional to the lipase activity.

Spectrophotometric method in this method clearing of a fat emulsion as a function or the lipase concentration is measured spectrophotometrically but nowadays artificial or synthetic substrates are used such as p-nitrophenyl acyl substrates. The basis of this procedure is that lipases possess general esterolytic activity towards a variety of native and non-native carboyl ester substrates. This method quantifies the level of p-nitrophenol released following the hydrolysis of p-nitrophenyl laurate substrate by lipase. Activity of lipase can be calculated by comparing samples A_{410} values to those of the standard curve prepared with p-nitrophenol (Pinsirodom & Parkin 2001).

Another method used extensively involves the quantitative extraction of free fatty acids from lipolyzed substrate either chromotographically or by extraction with chloroform, either or other organic solvents and titration with an alkali (Chandan & Shahani,1964). Qualitative detection for lypolytic activity can be determined by using agar plates containing substrates for lipases with or without an indicator the most widely used methods are tributyrin agar plate, calcium-triolein agar plate and Rhodamine B agar plate. The first method uses tributyrin, a four carbon chain triglyceride, homogenously emulsified in liquid agar media before pouring. Lipase producing microorganisms can be identified by a formation of clear zones around their colonies due to hydrolysis of tributyrin by the enzyme. However, the disadvantages of this method are that esterases can also hydrolyze tributyrin and give the same result. Calcium-triolein medium containing olive oil and calcium chloride can be used more specifically to detect for lipase activity. Lipase producing microorganisms are identified by a formation of white pellet of calcium oleate around the oil droplets in areas where colonies grow. Nonetheless, this method is sometimes difficult to detect for the microorganisms which produce low amount of lipases. The more sensitive method that uses medium containing rhodamine B as an indicator uses olive oil as a substrate for lipases reaction. Lipases activity can be visualized from fluorescence of the product; rhodomine B forms a fluorescent complex with free fatty acids, under UV light at 350 nm (Thongekkaew, 2006).

Recommendation and future prospects

Lipase has wide range of industrial application (Table 5). Lipase is one of the versatile groups of biocatalyst and carries out novel reaction in both aqueous and non aqueous media. Lipase would be isolated from various sources and they have wide range of stability in varying conditions. Lipase has high range of specificity towards many substrates; some lipases are non specific while some are specific since they carry out wide range of reactions such as chemo, region and enantio-selective transformation. Lipase has high potential regarding their catalytic behavior. Lipases have vast applications in various fields such as food, dairy detergent pharmaceutical, and agrochemical and oleochemical industries. It is a tool of choice for researchers but still it shares only about 3% of total market of all enzymes in world, so it is necessary to widen the usage of lipases. There is an urgent need to understand the mechanisms of action and still there is a lot of scope to search for new sources of lipases. On the basis of the present data it is reported here 38 bacteria, 14 fungi, 2 algae, 10 plants and 12 animals' lipases.

Concluding remarks

Algae are found to be major sources of lipids and fats and many lipase inhibitors are isolated from many fresh and marine water algae (Bitou *et al.*, 1999), so hypothetically it may contain lipases. *Chlorella pyrenoidosa* is found to be one of the algae for lipase (Wolfersberger & Pieringer, 1974). Considering the enormous data on bacterial, plant and animal origin lipase, based on their characterization, the algal group is relatively neglected. Therefore, more emphasize has to be given for characterization of algal lipases and hence further work is needed in these aspects.

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References

1. Jayaprakash A and Ebenezer P (2010) Investigation on extracellular lipase production by *Aspergillus japonicus* isolated from the paper nest of *Ropalidia marginata*. *Indian J. Sci.Technol*. 3 (2), 113-117. Domain site: http://www.indjst.org
2. Aizono Y, Funatsu M, Sugano M, Hayashi K and Fujiki Y (1973) Enzymatic properties of rice bran lipase. *Agri. Biol. Chem.* 37, 2031-2036.
3. Akhtar M and Kausar (1978) A study on garma (*Cucumis melo*) seed lipase. *Pak. J. Biochem.* 11, 6-11.
4. Amada K, Haruki M, Imanaka T, Morikawa M and Kanaya S (2000) Overproduction in *Escherichia coli*, purification and characterization of a family I.3 lipase from *Pseudomonas sp.* MIS38. *Biochim. Biophys.* Acta. 1478, 201-210.

Indian Journal of Science and Technology Vol. 4 No. 8 (Aug 2011) ISSN: 0974- 6846

5. Amro A Amara and Soheir R Salem (2009) Degradation of castor oil and lipase productionby *P. aeruginosa. Am. Eurasian J. Agri. & Environ. Sci.* 5(4), 556-563.
6. Aravindan R, Anbumathi P and Viruthagiri T (2007) Lipase application in food industry. *Indian J. Biotechnol.* 6, 141-158.
7. Asahara T, Matori M, Ikemoto M and Ota Y (1993) Production of 2 types of lipases with opposite positional specificity by *Geotrichum* sp. FO401B. *Biosci. Biotechnol. Biochem.* 57, 390-394.
8. Belfrage P, Jergil B, Stralfors P and Tornqvist H (1997) Hormonesensitive lipase of rat adipose tissue; identification and some properties of the enzyme protein. *FEBS Lett.* 75, 259-264.
9. Bhardwaj K, Raju A and Rajasekharan R (2001) Identification, purification, and characterization of a thermally stable lipase from rice bran. A new member of the (phospho) lipase family. *Plant Physiol.* 127, 1728-1738.
10. Bitou N, Ninomiya M, Tsujita T and Okuda H (1999) Screening of lipase inhibitors from marine algae. *Lipids.* 34, 441-445.
11. Bora L and Kalita M C (2007) Production and optimization of thermostable lipase from a thermophilic *Bacillus sp* LBN 4. *The Internet J. Microbiol.* 4-1.
12. Brune A K and Gotz F (1992) Degradation of lipids by bacterial lipases. In: Microbial degradation of natural products. Winkelman G edn. VCH, Weinhein. pp:243-266.
13. Carriere F, Renou C, Lopez V, De Caro J, Ferrato F and Lengsfeld H (2000) The specific activities of human digestive lipases measured from the *in vivo* and *in vitro* lipolysis of test meals. *Gastroenterol.* 119, 949-960.
14. Cauturies L, Taupin D and Yvergnaux F (2009) Lipase-catalyzed chemoselective aminolysis of various amino alcohols with fatty acids. *J. Molecular Catalysis B: Enzymatic.* 56(1), 29-33.
15. Chandan RC and Shahani KM (1964) Milk Lipases. A review. *J. Dairy Sci.* 47, 471-480.
16. Chandrayan SK, Dhaunta N and Guptasarma P (2008) Expression, purification, refolding and characterization of a putative lysophospholipase from *Pyrococcus furiosus*: retention of structure and lipase/esterase activity in the presence of water-miscible organic solvents at high temperatures. *Protein Expr. Purif.* 59, 327-333.
17. Chang SW, Lee GC and Shaw JF (2006) Codon optimization of *Candida rugosa* lip1 gene for improving expression in *Pichia pastoris* and biochemical characterization of the purified recombinant LIP1 lipase. *J. Agric. Food Chem.* 54, 815-822.
18. Chen S, Tong X, Woodard RW, Du G, Wu J and Chen J (2008) Identification and characterization of bacterial cutinase. *J. Biol. Chem.* 283, 25854-25862.
19. Cherif S, Fendri A, Miled N, Trabelsi H, Mejdoub H and Gargouri Y (2007) Crab digestive lipase acting at high temperature: purification and biochemical characterization. *Biochimie.* 89, 1012-1018.
20. Cote A and Shareck F (2008) Cloning, purification and characterization of two lipases from *Streptomyces coelicolor* A3(2). *Enzyme Microb. Technol.* 42, 381-388.
21. Dahot MU and Memon AR (1987) Properties of *Moringa oleifera* seed lipase. *Pak. J. Sci. Ind. Res.* 30, 832-835.
22. David L, Guo XJ, Villard C, Moulin A and Puigserver A (1998) Purification and molecular cloning of porcine intestinal glycerol-ester hydrolase-evidence for its identity with carboxylesterase*. Eur. J. Biochem*. 257, 142-148.
23. Deuerlu N and Akpinar MA (2002) Partial purification of intestinal triglyceride lipase from *Cyprinion macrostomus* Heckel, 1843 and effect of pH on enzyme activity. *Turk. J. Biol*. 26, 133-143.
24. Dharmsthiti S, Pratuangdejkul J, Theeragool G and Luchai S (1998) Lipase activity and gene cloning of *Acinetobacter calcoaceticus* LP009. *J. Gen. Appl. Microbiol*. 44, 139-145.
25. Dharmsthiti, S and Luchai S (1999) Production, purification and characterization of thermophilic lipase from *Bacillus sp*. THL027. *FEMS Microbiol. Lett*. 179, 241-246.
26. Diaz JCM, Rodr´ıguez JA, Roussos S and Cordova J (2006) Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid state fermentation than liquid fermentation procedures. *Enzyme & Microbial Technol*. 39, 1042-1050.
27. Dolinsky VW, Gilham D, Alam M, Vance DE and Lehner R (2004) Triacylglycerol hydrolase: role in intracellular lipid metabolism. *Cell. Mol. Life Sci*. 61(13), 1633-1651.
28. Dong H, Gao S, Han S and Cao S (1999) Purification and characterization of a *Pseudomonas sp*. lipase and its properties in non-aqueous media. *Appl. Microbiol. Biotechnol*. 30, 251-256.
29. Dunhaupt A, Lang S and Wagner F (1991) Properties and partial purification of a *Pseudomonas cepacia* lipase. *GBF monographs*. 16, 389-392.
30. Duong F, Soscia C, Lazdunski A and Murgier M (1994) The *Pseudomonas fluorescens* lipase has a C-terminal secretion signal and is secreted by a three-component bacterial ABC-exporter system. *Mol. Microbiol.* 11, 1117-1126.
31. Eastmond PJ (2004) Cloning and characterization of the acid lipase from castor beans. *J. Biol. Chem.* 279, 45540-45545.

Indian Journal of Science and Technology Vol. 4 No. 8 (Aug 2011) ISSN: 0974- 6846

32. Gatti-Lafranconi P, Caldarazzo SM, Villa A, Alberghina L and Lotti M (2008) Unscrambling thermal stability and temperature adaptation in evolved variants of a cold-active lipase. *FEBS Lett.* 582, 2313-2318.
33. Gaur R, Gupta GN, Vamsikrishnan M and Khare SK (2008) Protein-coated microcrystals of *Pseudomonas aeruginosa* PseA lipase. *Appl. Biochem. Biotechnol.* 151, 160-166.
34. Ghanem EH, Al-Sayeed HA and Saleh KM (2000) An alkalophilic thermostable lipase produced by a new isolate of *Bacillus alcalophilus*. *World J. Microbiol Biotechnol.* 16, 459-464.
35. Ghazi IA, Srivastava M, Kaushal RK, Paul D, Joshi GK and Kanwar SS. Purification, characterization and restoration of (chelated) lipase activity of a Gram-negative bacterial isolate BTG199. (http://www.osmania.ac.in/MicroBiology/12p01.htm).
36. Ghosh M and Bhattacharyya D K (1997). Enzymatic alcoholysis reaction of soy phospholipids. *J. Am. Oil Chemists' Soc.* 74(5), 597-599.
37. Ghosh PK, Saxena RK, Gupta R, Yadav RP and Davidson S (1996) Microbial lipases: Production and applications. *Sci. Prog.* 79, 119-157.
38. Gilbert EJ, Drozd JW and Jones CW (1991) Physiological regulation and optimization of lipase activity in *Pseudomonas aeruginosa* EF2. *J. Gen.Microbiol.* 137, 2215-2221.
39. Groener JEM and Knauer TE (1981) Evidence for the existence of only one triacylglycerol lipase of rat liver active at alkaline pH. *Biochim. Biophys.* Acta. 665, 306-316.
40. Guibe-Jampel E, Rousseau G and Salaun J (1987) Enantioselective hydrolysis of racemic diesters by porcine pancreatic lipase. *J. Chem. Soc. Chem. Commun.* 1987, 1080-1081.
41. Gupta N, Rathi P, Singh R, Goswami VK and Gupta R (2005) Single-step purification of lipase from *Burkholderia multivorans* using polypropylene matrix. *Appl. Microbiol. Biotechnol.* 67, 648-653.
42. Gupta R, Gupta N and Rathi P (2004) Bacterial lipase: an overview of production, purification and biochemical properties. *Appl. Microbiol. Biotechnol.* 64, 763-781.
43. Hadzir NH, Basri M, Rahman MBA, Razak CNA, Rahman RNZA and Saleh AB (2001) Enzymatic alcoholysis of triolein to produce wax esters. *J. Chem. Technol. Biotechnol.* 76, 511-515.
44. Harmon JS, Michelsen KG and Sheridan MA (1991) Purification and characterization of hepatic triacylglycerol lipase isolated from rainbow trout, *Oncorhynchus mykiss .J. Fish Physiol. Biochem.* 9, 361-368.
45. Herrgard S, Gibas CJ and Subramanian S (2000) Role of electrostatic network of residues in the enzymatic action of *Rhizomucor miehei* lipase family. *Biochem.* 39, 2921-2930.
46. Hills MJ and Beevers H (1987) An antibody to the castor bean glyoxysomal lipase (62 kD) also binds to a 62 kD protein in extracts from many young oilseed plants. *Plant Physiol.* 85(4),1084-1088.
47. Hills MJ and Mukherjee KD (1990) Triacylglycerol lipase from rape (*Brassica napus* L.) suitable for biotechnological purposes. *Appl. Biochem. Biotechnol.* 26,1-10.
48. Jaeger KE, Kharazmi A and Hoiby N (1991) Extracellular lipase of *Pseudomonas aeruginosa*: biochemical characterization and effect on human neutrophil and monocyte function in vitro. *Microb. Pathog.* 10, 173-182.
49. Jensen GL and Bensadoun A (1981) Purification, stabilization, and characterization of rat hepatic triglyceride lipase. *Anal. Biochem.* 113, 246-252.
50. Jocken JW, Smit E, Gossens GH, Essers YP, Van Baak MA, Mensink M, Saris WH and Blaak EE(2008) Adipose triglyceride lipase (ATGL) expression in human skeletal muscle is type I (oxidation) fiber specific. *Histochem. Cell Biol.*129, 535-538.
51. Joseph B, Ramteke PW and Kumar A (2006) Studies on the enhanced production of extracellular lipase by *Staphylococcus epidermidis. J. Gen. Appl. Microbiol.* 52, 315-320.
52. Kannan A and Basu KP (1951) Studies on the tributyrinase activity of the milk of cows, buffaloes, goats and sheep as influenced by the stage of lactation, season and some methods of processing. *Indian J. Dairy Sci.* 4, 63.
53. Kapranchikov VS, Zherebtsov NA and Popova TN (2004) Purification and characterization of lipase from wheat (*Triticum aestivum L.*) germ. *Appl. Biochem. Microbiol.* 40, 84-88.
54. Karadzic I, Masui A, Zivkovic LI and Fujiwara NJ (2006) Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. *Biosci. Bioeng.* 102, 82-89.
55. Kasana RC, Kaur B and Yadav SK (2008) Isolation and identification of a psychrotrophic *Acinetobacter sp.* CR9 and characterization of its alkaline lipase. *J. Basic Microbiol.* 48,207-212.
56. Kay HD (1946) A light sensitive enzyme in cow milk. *Nature.*157, 511.
57. Khudary RA, Hashwa F and Mroueh M (2004) A novel olive oil degrading thermo *Actinomyces species* with a high extremely thermostable lipase activity. *Engg. in Life Sci.* 4, 78-82.
58. Khyami-Horani H (1996) Thermotolerant strain of *Bacillus licheniformis* producing lipase. *World J. Microbiol. Biotechnol.* 12,399-401.
59. Kim EK, Sung MH, Kim HM and Oh TK (1994) Occurrence of thermostable lipase in thermophilic *Bacillus sp.* strain 398. *Biosci. Biotechnol. Biochem.* 58, 961-962.

Indian Journal of Science and Technology Vol. 4 No. 8 (Aug 2011) ISSN: 0974- 6846

60. Kim MH, Kim HK, Lee JK, Park SY and Oh TK (2000) Thermostable lipase of *Bacillus stearothermophilus* high-level production, purification, and calcium-dependent thermostability. *Biosci. Biotechnol. Biochem.* 64, 280-286.
61. Kim HK, Choi HJ, Kim MH, Sohn CB and Oh TK (2002) Expression and characterization of Ca(2+)-independent lipase from *Bacillus pumilus* B26. *Biochim Biophys.* Acta. 1583, 205-212.
62. Kojima Y, Yokoe M and Mase T (1994) Purification and characterization of an alkaline lipase from *Pseudomonas fluorescens* AK 102. *Biosci. Biotechnol. Biochem*. 58, 1564-1568.
63. Kudanga T, Mwenje E, Mandivenga F and Read J (2007) Esterases and putative lipases from tropical isolates of *Aureobasidium pullulans*. *J. Basic Microbiol.* 47,138-147.
64. Kugimiya W, Ootani Y and Hashimoto Y (1989) Cloning and expression of *Rhizopus* lipase gene. *Jpn. Kokai Tokkyo Koho JP.* 01080290 A2 Heisei, 11 pp. (Japan) CA 112:932-38.
65. Kumar S, Kikon K, Upadhyay A, Shamsher S and Gupta R (2005) Production, purification, and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. *Protein Exp. & Purification.* 41, 38-44.
66. Kundu M, Basu J, Guchhait M and Chakrabarti P (1987) Isolation and characterization of an extracellular lipase from the conidia of *Neurospora crassa. J. Gen. Microbiol.* 133, 149-153.
67. Lang S, Dunhaupt A and Wagner F (1991) Properties and partial purification of a *Pseudomonas cepacia* lipase. *GBF Monographs.* 16, 389-392.
68. Lawson DM, Brzozowski AM, Dodson GG, Hubbard RE, Huge-Jensen B, Boel E and Derewenda ZS (1994) In: Lipase- their biochemistry, structure and application. Woolley P & Petersen S. (eds.), Cambridge University Press, Cambridge, UK. pp: 77-94.
69. Lee OW, Koh YS, Kim KJ, Kim BC, Choi HJ, Kim DS, Suhartono MT and Pyun YR (1999) Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. *FEMS Microbiol. Lett.* 179,393-400.
70. Lelie DC, Citlali RG, Gerardo VA and Rosamaría OR (2005) Screening, purification and characterization of the thermoalkalophilic lipase produced by *Bacillus thermoleovorans* CCR11. *Enzyme & Microbial Technol.* 37,648-654.
71. Lescic I, Vukelic B, Majeric-Elenkov M, Saenger W and Abramic M (2001) Substrate specificity and effects of water-miscible solvents on the activity and stability of extracellular lipase from *Streptomyces rimosus*. *Enzyme Microb. Technol.* 29, 548-553.
72. Lesuisse E, Schanck K and Colson C (1993) Purification and preliminary characterization of the extracellular lipase of *Bacillus subtilis* 168, an extremely basic pH-tolerant enzyme. *Eur. J. Biochem.* 216,155-160.
73. Lidija T, Izrael Z, Gordana GC, Kristina RG, Miroslav MV and Ivanka MK (2009) Enzymatic characterization of 30 kDa lipase from *Pseudomonas aeruginosa* ATCC 27853. *J. Basic Microbiol.* 49,452 - 462.
74. Lima VMG, Krieger N, Mitchell DA and Fontana JD (2004) Activity and stability of a crude lipase from *Penicillium aurantiogriseum* in aqueous media and organic solvents. *Biochem. Eng. J.* 18, 65-71.
75. Liu Z, Chi Z, Wang L and Li J (2008) Production, purification and characterization of an extracellular lipase from *Aureobasidium pullulans* HN2.3 with potential application for the hydrolysis of edible oils. *Biochem. Eng. J.* 40, 445-451.
76. Macrae AR and Hammond RC (1985) Present and future application of lipases. *Biotech. Genet. Eng. Rev.* 3, 193-219.
77. Maeshima M and Beevers H (1985) Purification and properties of glyoxysomal lipase form castor bean. *Plant Physiol.* 79, 489-493.
78. Mala V, Dahot M U (1968) Lipase activity of *Carissa carandus* fruit. *Sci. Intl.* (Lahore). 7(2), 161-164.
79. Marit WA, Eva D and Cecilia H (1997) Partial purification and Identification of hormone-sensitive lipase from chicken adipose tissue. *Biochem. Biophys. Res. Commun*. 236,94-99.
80. Matsui K, Fukutomi S, Ishii M and Kajiwara T (2004) A tomato lipase homologous to DAD1 (LeLID1) is induced in post-germinative growing stage and encodes a triacylglycerol lipase. *FEBS Lett.* 569, 195-200.
81. McCrae AR, Roehl EL and Brand HM (1990) Bio-ester - Bio-esters. Seifen-Öle-Fette-Wachse. *SOeFW-Journal.* 116, 201-205.
82. Mhetras NC, Bastawde KB and Gokhale DV (2008) Purification and characterization of acidic lipase from *Asperigillus niger* NCIM 1207. *Bioresour. Technol.* 100, 1486-1490.
83. Mohammad AA, Ensieh S, Khosro K, Mahbube K and Saied N (2008) Production of an extracellular thermohalophilic lipase from a moderately halophilic bacterium, *Salinivibrio sp.* strain SA-2. *J. Basic Microbiol.* 48,160-167.
84. Moreau H, Gargouri Y, Lecat D, Junien J L and Verger R (1988) Purification, characterization and kinetic properties of the rabbit gastric lipase. *Biochim. Biophys.* Acta 960, 286-293.
85. Muderhwa JM, Ratomahenina R, Pina M, Graille J and Galzy P (1986) Purification and properties of the lipases from *Rhodotorula pilimanae. Appl. Microbiol. Biotechnol.* 23, 348-354.
86. Namboodiri VMH and Chattopadhayaya R (2000) Purification and biochemical characterization of a novel thermostable lipase from *Asperigillus niger*. *Lipids*. 35, 495-502.

Indian Journal of Science and Technology Vol. 4 No. 8 (Aug 2011) ISSN: 0974- 6846

87. Nawani N and Kaur JJ (2004) Purification, characterization and thermostability of lipase from a thermophilic *Bacillus sp.* J33. *Mol. & Cellular Biochem.* 206,91-96.
88. Nayak J, Nair RGV, Mathew S and Ammu K (2004) A study on the intestinal lipase of Indian Major Carp *Labeo rohita. Asian Fisheries Sci.* 17,333-340.
89. Neugnot V, Moulin G, Dubreng E and Bigey F (2002). The lipase acyltransferase from *Candida parapsilosis*: Molecular cloning and characterization of purified recombinant enzymes. *Eur. J. Biochem.* 269(6),1734-1745.
90. Oh CB, Kim HK, Lee JK, Kang SC and Oh TK (1999) *Staphylococcus haemolyticus* lipase: biochemical properties, substrate specificity and gene cloning. *FEMS Microbiol. Lett.* 179, 385-392.
91. Pahoja VM and Sethar MA (2002) A review of enzymatic properties of lipase in plants, animals and microorganisms. *Pak. J. Appl. Sci.* 2, 474-484.
92. Park J, Cho SY and Choi SK (2007) Purification and characterization of hepatic lipase form *Todarodes pacificus*. *BMB reports.* 254-258.
93. Patkar S and Bjorkling F (1994) In: Lipases: their structure, biochemistry and application. Woolley P & Petersen (eds.). pp:77.
94. Pernas MA, Lopez C, Rua ML and Hermoso J (2001) Influence of the conformational flexibility on the kinetics and dimerization process of two *Candida rugosa* lipase isoenzymes. *FEBS Lett.* 501, 87-91.
95. Pinsirodom P and Parkin KL (2001) Lipase assays. *Central protocol in C3.1.1-C31.13*.
96. Poulsen KR, Snabe T, Petersen EI, Fojan P, Neves-Petersen MT, Wimmer R and Petersen SB (2005) Quantization of pH: evidence for acidic activity of triglyceride lipases. *Biochem.* 44, 11574-11580.
97. Sharma R, Chisti Y and Banerjee UC (2001) Production, purification, characterization, and applications of lipases. *Biotechnol. Adv.* 19, 627-662.
98. Rao BVSK (2008) Importance of microbial sources in the production of biodiesel. Lipid Science and Technology Division, Indian Institute of Chemical Technology.
99. Rashid N, Shimada Y, Ezaki S, Atomi H and Imanaka T (2001) Low temperature lipase from psychrotrophic *Pseudomonas sp.* Strain KB700A. *Appl. Environ. Microbiol.* 67,4064-4069.
100. Rathi P, Bradoo S, Saxena R K and Gupta R (2000) A hyper-thermostable, alkaline lipase from *Pseudomonas sp.* with the property of thermal activation. *Biotechnol. Lett.* 22, 495-498.
101. Romero CM, Baigori MD and Pera LM (2007) Catalytic properties of mycelium-bound lipases from *Aspergillus niger* MYA 135. *Appl. Microbiol. Biotechnol.* 76, 861-866.
102. Ruiz B, Farres A, Langley E, Masso F and Sanchez S (2001) Purification and characterization of an extracellular lipase from *Penicillium candidum. Lipids.* 36, 283-289.
103. Ruiz C, Blanco A, Pastor F I and Diaz P (2002) Analysis of *Bacillus megaterium* lipolytic system and cloning of LipA, a novel subfamily I.4 bacterial lipase. *FEMS Microbiol. Lett.* 217, 263-267.
104. Salameh MA and Wiegel J (2007) Purification and characterization of two highly thermophilic alkaline lipases from *Thermosyntropha lipolytica. Appl. Environ. Microbiol.* 73, 7725-7731.
105. Saxena RK, Davidson WS, Sheoram A and Girri B (2003) Purification and characterization of an alkaline lipase from *Aspergillus carneus*. *Process Biochem.* 9, 239-247.
106. Schmidt-Dannert C, Sztajer H, Stocklein W, Menge U and Schmid RD (1994) Screening purification and properties of a thermophilic lipase from *Bacillus thermocatenulatus. Biochim. Biophys.* Acta. 1214, 43-53.
107. Sekhon A, Dahiya N, Tiwari RP and Hoondal GS (2005) Properties of a thermostable extracellular lipase from *Bacillus megaterium* AKG-1. *J. Basic Microbiol.* 45, 147-154.
108. Shabtai Y and Daya-Mishne N (1992) Production, purification, and properties of a lipase from a bacterium (*Pseudomonas aeruginosa* YS-7) capable of growing in water-restricted environments. *Appl. Environ. Microbiol.* 58, 174-180.
109. Sharma A, Bardhan D and Patel R (2009) Optimization of physical parameters for lipase production from *Arthrobacter sp.* BGCC#490. *Indian J. Biochem. Biophys.* 46, 178-183.
110. Sharma R, Chisti Y and Banerjee UC (2001) Production, purification, characterization, and applications of lipases. *Biotechnol.* 19, 627-662.
111. Sharon C, Nakazato M, Ogawa HI and Kato Y (1998) Lipase-induced hydrolysis of castor oil: effect of various metals. *J. Industrial Microbiol. Biotechnol.* 21, 292-295.
112. Sheridan MA and Eilertson CD (1994) Effects of somatostatin-25 on lipid mobilization from rainbow trout, *Oncorhynchus mykiss*, liver and adipose tissue incubated in vitro. Comparison with somatostatin-14. *J. Comparative Physiol. B: Biochem., Systemic, & Environ. Physiol.* 164,256-260.
113. Snellman EA and Colwell RR (2004) *Acinetobacter lipases*: molecular biology, biochemical properties and biotechnological potential. *J. Industrial Microbiol. Biotechnol.* 31, 391-400.
114. Standhouders J and Mulder H (1958) Some observations on milk lipase III. The destructive effects of light on milk lipase activity. *Netherlands Milk Dairy J.* 13, 122.
115. Sugihara A, Tani T and Tominago Y (1991) Purification and characterization of novel thermostable lipase from *Bacillus sp*. *J. Biochem.* 109, 211-216.

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116. Sunna A, Hunter L, Hutton CA and Bergquist PL (2002) Biochemical characterization of a recombinant thermoalkalophilic lipase and assessment of its substrate enantioselectivity. *Enzyme Microb. Technol.* 31, 472-476.
117. Suzuki M, Yamamoto H and Mizugaki M (1986) Purification and general properties of a metal-insensitive lipase from *Rhizopus japonicus* NR 400. *J. Biochem.* 100, 1207-1213.
118. Suzuki T, Honda Y and Mukasa Y (2004) Purification and characterization of lipase in buckwheat seed. *J. Agric. Food Chem.* 52, 7407-7411.
119. Suzuki T, Honda Y and Mukasa Y (2004) Purification and characterization of lipase in buckwheat seed. *J. Agric. Food Chem.* 52, 7407-7411.
120. Thonjekkaew J (2006) Molecular cloning and functional expression of a noval extracellular lipase from the thermotolerant yeast, *Candida thermophila*. Department of biotechnology, Mahidol University (http://mulinet10.li.mahidol.ac.th/e-thesis/4536484.pdf).
121. Tsugo T and Hayashi T (1962) The effect of Irradiation on Lipase and Xanthine-Oxydase activities in milk. *Jap. Jr. Zootech. Sci.* 33, 125.
122. Tsujisaka Y, Okumura S and Iwai M (1977) Glyceride synthesis by four kinds of microbial lipase. *Biochim. Biophys.* Acta. 489, 415-422.
123. Tsuzuki W (2005) Acidolysis between Triolein and short chain fatty acid by lipase in organic solvents. *Biosci. Biotechnol. Biochem.* 69(7), 1256-1261.
124. Unal MU (1998) Study on the lipase catalyzed esterification in organic solvent. *Tr. J. of Agri. & Forestry* 22, 573-578.
125. Usmani GA and Patil HV (2010) lipase catalysed interesterification for the production and oleochemicals from non-Traditional oils. *Rasayan J. Chem.* 3(2), 354-358.
126. Van Bennekum AM, Fisher EA, Blaner WS and Harrison EH (2000) Hydrolysis of retinyl esters by pancreatic triglyceride lipase. *Biochem.* 39, 4900-4906.
127. Van Heerden E, Litthauer D and Verger R (2002) Biochemical characterization and kinetic properties of a purified lipase from *Aspergillus niger* in bulk phase and monomolecular films. *Enzyme Microb. Technol.* 30, 902-909.
128. Walton MJ, Cowey CB and Adron JW (1984) The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.* 52(1),115-122.
129. Wang Y, Srivastava KC, Shen GJ and Wang HY (1995) Thermostable alkaline lipase from a newly isolated thermophilic *Bacillus,* strain A30-1 (ATCC 53841). *J. Ferment. Bioeng.* 79, 433-438.
130. Waterman IJ, Emmison N and Dutta-Roy AK (1998) Characterisation of triacylglycerol hydrolase activities in human placenta. *Biochim. Biophys.* Acta. 1394, 169-174.
131. Welsh FW and Williams RE (2002) Lipase-mediated production of ethylbutyrate and butyl butyrate in non aqueous systems. *Enzyme & Microbial Technol.* 12(10) 743-748.
132. Wohlfarth S and Winkler UK (1988) Chromosomal mapping and cloning of the lipase gene of *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 134, 433-440.
133. Wolfersberger MG and Pieringer RA (1974) Metabolism of sulfoquinovosyl diglyceride in *Chlorella pyrenoidosa* by sulfoquinovosyl monoglyceride: fatty acyl CoA acyltransferase and sulfoquinovosyl g1yceride:fatty acyl ester hydrolase pathways. *J. Lipid Res.*15, 1-10.
134. Xu Y, Du W, Liu D and Zeng J (2003) A novel enzymatic route for biodiesel production from renewable oils in a solvent free medium. *Biotechnol. Lett.* 25, 1239-1241.
135. Yamada M and Fujita T (2007) New procedure for the measurement of pancreatic lipase activity in human serum using a thioester substrate. *Clin. Chim. Acta.* 383, 85-90.
136. Yu M, Qin S and Tan T (2007) Purification and characterization of the extracellular lipase Lip2 from *Yarrowia lipolytica*. *Process Biochem.* 42, 384-391.
137. Zaliha RN, Rahman RA, Baharum SN, Salleh AB and Basri M (2006) S5 Lipase: an organic solvent tolerant enzyme. *The J. Microbiol*. 44, 583-590.
138. Zhang J, Lin S and Zeng R (2007) Cloning, expression, and characterization of a cold-adapted lipase gene from an antarctic deep-sea psychrotrophic bacterium, *Psychrobacter sp.* 7195. *J. Microbiol. Biotechnol.* 17, 604-610.
139. Zouari N, Miled N, Cherif S, Mejdoub H and Gargouri Y (2005) Purification and characterization of a novel lipase from the digestive glands of a primitive animal: The scorpion. *Biochim. Biophys. Acta.* 1726, 67-74.