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REVIEW ARTICLE

Yeast lipases: enzyme purification, biochemical properties and gene cloning

Jyoti Vakhlu*
Department of Biotechnology
University of Jammu
Jammu-180006 (J&K)
India
Tel: 094191-17624
E-mail: jyotivakhlu@yahoomail.com

Avneet Kour
Department of Biotechnology
University of Jammu
Jammu-180006(J&K)
India
E-mail: avneetneeti@rediffmail.com

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Lipases are placed only after proteases and carbohydrases in world enzyme market and share about 5% of enzyme market. They occur in plants, animals and microorganisms and are accordingly classified as plant, animal and microbial lipases. Wherever they exist, they function to catalyze hydrolysis of triglycerides to glycerol and fatty acid. Like carbohydrases and proteases, lipases of microbial origin enjoy greater industrial importance as they are more stable (compared to plant and animal lipases) and can be obtained in bulk at low cost. Majority of yeast lipases are extracelluar, monomericglycoproteins with molecular weight ranging between ~33 to ~65 kD. More than 50% reported lipases producing yeast, produce it in the forms of various isozymes. These lipase isozymes are in turn produced by various lipase encoding genes. Among many lipase producing yeasts *Candida rugosa* is most frequently used yeast as the source of lipase commercially. This review is aimed at compiling the information on properties of various yeast lipases and genes encoding them.

Lipases (EC 3.1.1.3) are a class of hydrolases that are primarily responsible for the hydrolysis of acylglycerides. They are ubiquitous and indispensable for the bioconversion of lipids (triacylglycerol) in nature. In addition to their biological significance, lipases hold tremendous potential for exploitation in biotechnology. They posses the unique feature of acting at the aqueous and non – aqueous interface which distinguishes them from esterases (Verger, 1997; Schmidt and Verger, 1998). The concept of lipase interfacial activity evolved from restriction of their catalytic activity to interface between lipid and water. The catalytic activity of lipases depends largely on the aggregated state of substrates. Experimental evidences suggest that the activation involves unmasking and structuring of enzyme-active-site, through conformational changes, that require presence of oil-in water droplets. Recent studies on the structure of several lipases have provided some clues for understanding their hydrolytic activity, interfacial activation and stereoselectivity of lipases (Kazlauskas and Bornscheuer, 1998). Enzymes such as proteases and carbohydrases have been used industrially for a number of years and corner the largest share of the world wide enzyme market. Whilst lipases at present account for less than 5% of the market, this share has the potential to increase dramatically via a wide range of different applications.

The lipases catalyze wide range of reactions, including hydrolysis, inter-esterification, alcholysis, acidolysis, esterification and aminolysis. They catalyse the hydrolysis of fatty acid ester bond in the triacylglycerol (TAG) and release free fatty acids (ffa) (Sheldon, 1993). The reaction is reversible; the direction of the reaction depends upon the water content available in the reaction. In low water media lipases catalyse esterification, transesterification and interesterification. Biochemical and molecular characterization of a number of lipases of different sources has brought to light great deal of heterogeneity in them with regard to specificity, amino acid sequence and catalytic properties. Based on the inhibition of their enzyme activity by chemical modification, lipases were initially classified as serine hydrolases. Serine present attheir active site has been shown to be enclosed in the highly conserved domain and represents the only common feature shared by all determined lipases sequenced so far (Antonian, 1988).

*Corresponding author

Although lipases can be produced easily on a large scale by growing microorganisms in a fermentor, yet their use was, till recently confined largely to oleo-chemistry and dairy based industry. However the last quarter of the 20th century has witnessed unprecedented use of lipases in biotechnology, manufacture of pharmaceuticals and pesticides, single cell protein production, biosensor preparation and in waste managementetc(Torossianet al. 1991; Gandhi, 1997; Yadavet al. 1998, Pandey et al. 1999; Jaeger et al. 1999; Saxena et al. 1999).Lipases have become an integral part of the modern food industry and are used in the preparation of a variety of products including fruit juices, baked food, vegetable fermentation and dairy enrichment. They are also used in leather industry for processing hides and skins (bating) and for treatment of activated sludge and other aerobic waste products where they remove the thin layer of the fats and by so doing provide for oxygen transport. The lipid digesting preparation is employed in sewage disposal plants in USA under the trade name lipase M-Y (Meito Sangyo Co., Nagoya Japan). Lipases may also assist in the regular performance of anaerobic digesters. Nearly 1000 tonnes of lipase are used annually in detergent industry, primarily as lipid stain digesters. They also are used as flavour development agents in the preparation of cheese, butter and margarine. These hydrolases are endowed with substrate specificity that surpasses any known enzyme. This property confers to them the potential that is literally boundless. The growing interest in lipases is reflected by publication of an average of 1000 research papers per year (Pandey et al. 1999), on different aspects of these enzymes.

Some of the common sources of lipases are tabulated in Table 1. Pancreatic lipase of porcine origin is one of the earliest recognized and is still the best known lipase. Plant lipases are not used commercially; the animal and microbial lipases are used extensively. The most important source of animal lipase is the pancreas of cattle, sheep, hogs and pigs. The disadvantage with pancreatic(/animal) lipases is that they cannot be used in the processing of vegetarian or kosher food. Also, that these extracts contain components which have undesirable effect. The pig pancreatic extract contains trypsin, which produces bitter tasting amino acids. They are also likely to contain residual animal viruses, hormones, etc.

Microbes are major source of the 100 or so enzymes produced industrially for reasons mentioned above. Yeast has been used in food and other industries since ages. They have earned acceptability since long and are considered natural. Yeasts are also considered to be easy to handle and grow, in comparison to bacteria (Kademi et al. 2003).

Among microbial lipases extensive reviews have been written on bacterial lipases (Jaeger et al. 1999; Arpigny and Jaeger, 1999). Yeast lipases have received a raw deal despite the fact that *Candida rugosa* is the most frequently used organism for lipase synthesis. Benjamin and Pandey (1998) have written a review exclusive on *Candida rugosa* lipase. The information on numerous other yeast lipases is scattered. This communication is aimed at organizing the literature available on other yeast lipases. The areas reviewed are application, protein purification, and biochemical properties of yeast lipases and characterization of genes encoding these enzymes.

YEAST LIPASES

Sources and application

Lipases produced by various yeasts have been tabulated in Table 2.

The lipase produced by *Candida rugosa* is fast becoming one of the most industrially used enzymes. This is because of its use in avariety of processes due to its high activity, both in hydrolysis as well as synthesis (Redondo et al. 1995). A Japanese company has used the *Candida rugosa* lipase for production of fatty acids from castor bean long back in 1985 (Macrae and Hammond, 1985). Pandey et al. (1999) investigated the production of flavour in concentrated milk and creams by using microbial lipases. Organolephtically each lipase develops a characteristic flavour. The *Candida rugosa* lipase was rated the most suitable lipase in this case. *Candida antarctica* AY30 immoblised lipase has been used for esterification of functional phenols for synthesis of lipophillic antioxidants subsequently used in sunflower oil (Pandey et al. 1999). Uppenberg and co workers (1994) developed *Candida antarctica* lipase into recombinant enzyme used for detergent formulation. The extra-cellular lipase produced by the asporogenic *Candida cylindracea* ATCC 14830 (CCL/CRL) hydrolyses triglycerides without specificity, both in attacked position of the glycerol molecule and in the nature of fatty acid released. This relaxed specificity vis-à-vis other lipases makes CCL/CRL particularly useful for industrial application (Lotti et al. 1993).

In detergent industry, lipases find use as lipid stain digesters. Lipases from *Candida cylindracea* and *Candida lypolytica* (now *Yarrowia lipolytica*) are choice enzymes for the purpose (Pierce et al. 1990; Batenburg et al. 1991).Polyglycerol and carbohydrate fatty acid esters are widely used as industrial detergents and as emulsifiers in variety of food formulations (low fat spreads, ice creams, mayonnaise). Enzymatic synthesis of functionally similar surfactants has been carried out at moderate temperature (60ºC – 80ºC) with excellent regioselectivity. Recently, Unichem International has launched production of isopropyl myristate, isopropyl palmitate and 2-ethylpalmitate for use of emollient in personal care products. Presently these compounds are being manufactured enzymatically using *C. cylindracea* lipase in batch bioreactor.

A promising new field is the use of microbial lipase as biosensors. Biosensors can be chemical or electronic in nature. An important analytical use of lipases is

Table 1. Common mammalian, fungal and bacterial sources of lipases.

Source	Name
Mammalian	Human Pancreatic Lipase Horse Pancreatic Lipase Pig Pancreatic Lipase Guinea Pig Pancreatic Lipase
Fungal	*Rhizomucormeihei* *Pencilliumcamberti* *Humicolalanuginosa* *Rhizopusoryzae* *Aspergillus niger* *Candida rugosa** *Candida antarctica* Lipase A* *Candida antarctica* Lipase B * *Geotrichiumcandidum**
Bacterial	*Chromobacteriumviscosum* *Pseudomonas cepacia* *Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Pseudomonas fragi* *Bacillus thermocatenulatus* *Staphylococcus hyicus* *Staphylococcus aereus* *Staphylococcus epidermidis*

* indicates yeast lipases. (http://www.au-kbc.org/beta/bioproj2/sources.htm)

determination of lipids for clinical purpose (Pandey et al. 1999). The basic concept is to utilize a lipase to generate glycerol from triacylglycerol and quantify the released glycerol or alternatively the non-esterified fatty acid by chemical and enzymatic method. This principal enables physicians precisely to diagnose patients with cardiovascular complaints. Non-specific lipases, especially of *Candida rugosa* with high specific activity has been selected to allow rapid liberation of glycerol *Candida rugosa* lipase biosensor, which optically conjugates to biorecognition group in DNA, has been developed as probe by Pittner et al (1995,cf. Pandey et al. 1995).

The application of lipases in organic synthesis is tremendous. Stereoselectivity of lipases for resolution of racemic acid mixture in immiscible biphasic system has been demonstrated. Efficient kinetic resolution processes are in vogue for the synthesis of Niknomycin-B, non-steroid anti-inflammatory drugs Naproxen, ibuprofen, suprofen and ketoprofen, the potential antiviral agent lamividine (that can also be used against HIV) and enantiospecific synthesis of antitumour agents alkaloids, antibiotics and vitamins (Pandey et al. 1999). Hernaiz et al. (1997) have isolated two iso-forms, labelled A and B from *Candida rugosa* that are stereoselective.

Preparation of optically active amines that are intermediate in preparation of pharmaceuticals and pesticides have been described by Smidt and his coworker (1996).This involved reacting stereospecific N-acylamines with lipase preferably from *C. antarctica*. In an attempt to determine substrate specificity of lipases, alkyl esters of 2 aryl- propionic acid, a class of non-steroid anti- inflammatory drugs were hydrolysed with *Candida rugosa* lipase. All transformations were found to be highly selective. Lipases are also used for enantiospecific catalysis. The stereo selective enatio-discrimination of *Candida rugosa* lipase yielded optically pure propionic acid derivative in S-form. The S-form was then converted to corresponding R form, which was effective against the insect pest Tetramuchus (Pandey et al. 1999).

Triglycerides, steryl esters, resin acids, free fatty acids and sterols which are lipophylic extractives (/extracts) of wood (commonly referred to as pitch or wood resin) have negative impact on paper machine run ability and quality of paper. Kontkanen and his group (2004) in their study tested 19 commercial lipase preparations able to show degradation of steryl esters. They found lipase preparations of *Pseudomonas sp. Chromobacteriumviscosum and Candida rugosa* were shown to have highest steryl esterase activity. All the three enzymes were able to hydrolyse steryl esters totally to completion in presence of a surfactant (thesit). Preliminary characterization of enzymatic activity revealed that the lipase preparation of *Pseudomonas* sp. could be the most potential industrial enzyme but among yeast *Candida*

rugosa lipase (CRL) ruled the roost (Kontkanen et al. 2004). To introduce polymer to cellulosic material a new approach was developed by Gustavsson et al. (2004) using ability of a cellulose binding module of *Candida antarctica* lipase B conjugate to catalyze ring opening polymerization of epsilon-caprolactone in close proximity to cellulose fiber surface. Wang et al. (2003) demonstrated effective biocatalysis also by *Candida antarctica* Lipase (CAL B) in resolution of several 1-or 2-hydroxyalkanephosphonates. The enaniomers of phosphogabob and fosfomycin were prepared using CALB-mediated resolution as key step Table 3 enumurates some selected yeast lipases which are already being produced commercially (Kazlauskas and Bornscheuer, 1998).

PROTEIN PURIFICATION AND BIOCHEMICAL PROPERTIES

Like many other microbial lipases, yeast lipases are also purified by two major purification techniques i) precipitation techniques (with salts, alcohols etc.) and, ii) chromatographic techniques (ionic, hydrophobic interaction, affinity and molecular sieving). Table 3 summarizes some of the properties of reported yeast lipases.

Candida *Spp.*

There are several reports on the multiple forms of lipases produced by the microorganism. This multiplicity has been ascribed to post–transcriptional processing, existence of different genes, deglycosylation etc. Among yeasts *Candida aldicans, Candida Antarctica, Candida rugosa, Geotrichum asteroids, Geotrichumcandidium, Trichosporonfermentans, Saccharomycopsis lipolytica, Yarrowia lipolytica* (formally *Candidaparalipolytica*),etc. are reported to produce multiple lipase forms.

Various strains of *Candida rugosa / cylindracea* (L.1754, ATCC 14830,DSMZ 2031) are known to produce lipase. Purification and characterization has been reported for a number of them. Two distinct lipases from *Candida rugosa* (*C. cylindrcea* L.1754) were identified and separated by high-resolution anion exchange column mono Q after ethanol extraction of crude lipase. Lipase I eluted at 0.05 MNaCl whereas lipase II eluted at 0.15 MNaCl from this column. The less anionic nature of lipase I was confirmed by native polyacryamide gel electrophoresis and isoelectricfocusing. Both the proteins have apparent molecular weight of 58 kD on SDS-PAGE. The isoelectric focusing point of lipase I and II are ~5.8 and ~5.6 respectively (Veeraragavan and Gibbs, 1989). The same year Shaw and Chang (1989) demonstrated presence of three distinct lipase forms A, B and C in *Candida rugosa*lipase supplied by Sigma Co. All the three forms exhibit significant lipolytic activity. All the three lipases bind to DEAE-Sepharose pre-equilibrated with 0.1 M phosphate buffer pH 6.8 but lipase A bound weakly to this column and could be eluted with 0.2 M - 0.25 MNaCl. Lipase B and C were co-eluted with 0.3 M - 0.6 MNaCl. Shaw and Chang (1989) proposed that lipase A and C correspond to lipase II and I reported by Veeraragavan and Gibbs (1989). They further proposed that lipase B was not discovered by these workers probably because they transformed it into lipase I by ethanol treatment of crude lipase powder used. The apparent molecular weights of lipase A, B and C was found to be ~362, ~200 and ~143 KD. The results from SDS-PAGE indicate that lipase A and C are composed of subunits of the same molecular weight *i.e.* 62 KD. It is quite possible that lipases A, B and C are hexamer, trimer and dimer of the same subunit. Lipase A has higher optimal reaction temperature and better thermal stability compared to that of lipase C. The optimal pH for lipase A and C are 7.0 and 5.0 respectively. Lipase A and C exhibit higher lipolytic activity on esters with intermediate acyl chain length, Not much is known about, characteristics of lipase B and mechanism of its transformation from lipase I reported by Veeraragavan and Gibbs (1989).

Five years latter Chang and his group (Chang et al. 1994) reported that PAGE pattern of lipolytic enzymes obtained from three commercial samples (different manufacturers) of *Candidarugosa* lipase differed. They studied the effect of culture conditions on the production of lipase from *Candida rugosa* (ATCC 14830) and proposed that culture conditions not only influence the production of lipase but also changed the pattern of formation of multiple forms of lipase. They showed presence of tween 20 and 80 in culture media for *Candidarugosa* resulted in production of various forms of lipase showing different substrate specificities and thermal stabilities. The results suggest that the specificity and stability of lipase preparation can be modulated by culture condition. Chang et al. (1994) proposed that multiple patterns of lipases could result from change in gene expression, variable percentage of covalently linked carbohydrates, partial proteolysis and posttranscriptional modifications. Hitherto five genes belonging to lipase gene family, have been isolated from *Candidarugosa,* which suggest that some of the multiple lipase forms that Chang and his group have identified, are the result of change in gene expression.

Lotti and her group in the University of Milan, Italy have been studying various aspects of lipase production by *Candida rugosa/cylindracea* for about a decade. They (Lotti et al. 2001) undertook a flow cytometric study to evaluate growth-production process of *Candida rugosa* cells of different culture media. The yeast follows a complex pattern of lipase production depending on the presence of multiple lipase encoding genes whose expression is modulated by Carbon source. The C-source employed during fermentation can act as a repressor *e.g.* glucose, sorbitol. Neutral substrates may be successfully employed in two-step fermentation, where the first step of biomass growth is followed by the induction of lipase gene expression. Most of the lipases including those from *Candidarugosa* are glycosylated in conformity with rest of the lipases *Candidarugosa* lipases are widely used in

Table 2.Reported lipase producing yeasts, their cellular localization and number of lipase isozymes produced by each yeast.

Source	Cellular - location	*Isoforms*	Reference
Arxulaadeninivorans	Extra-cellular	*1**	Boer et al. 2005
Candida albicans	Extra-cellular	10*	Hube et al. 2000
Candida antarctica	Extra-cellular	2*	Høegh et al. 1995 Rotticci et al. 2001
Candida ernobii	Extra-cellular	1	Pignede et al. 2000a Pignede et al. 2000b
Candida parapsilosis CBS 604	Cell bound	1*	Neugnot et al. 2002
Candida rugosa/Cylindracea ATCC 14380 DMS 2031 L 1754	 Extra-cellular Extra-cellular Extra-cellular	 5* 3 2	 Brocca et al. 1995 Benjamin and Pandey, 2001 Veeraragavan and Gibbs, 1989
Candida curvata	-	-	Lazar and Schroder, 1992
Candida tropicalis	-	-	Lazar and Schroder, 1992
Candida deformans CBS 2071	Extra-cellular	3*	Bigey et al. 2003
Geotrichum asteroids FKMF 144	Extra-cellular	2	Kazanina et al. 1981
Geotrichumcandidum ATCC 34614 NRCC205002 NRRL Y-552 NRRL Y-533 CMICC 335426 ATCC 66592	Extra-cellular	 2* 2* 2* 2* 2 2	 Shimada et al. 1990 Bertolini et al. 1994 Bertolini et al. 1994 Bertolini et al. 1994 Charton et al .1992 Jacobsen and Poulsen, 1992
Geotrichum sp. FO401B	Extra-cellular	2	Ota et al. 2000
Kurtzmanomyces sp. I-11	Extra-cellular	1	Kakugawaet al. 2002
Kluyveromyces lactis	Extra-cellular	1*	Oishi et al.1999
Saccharomysiscerevisiae	-	-	Oishi et al.1999
Saccharomysifibuligera	-	-	Pandey et al.1999
Trichosporonasteroides	Extra-cellular	1	Dharmsthiti and Ammaranond, 1997
Trichosporoncutaneum	Extra-cellular	-	Chen et al.1993
Trichosporonfermentans WU-C12	Extra-cellular	2*	Chen et al. 1993 Chen et al. 1994 Arai et al. 1997
Yarrowia lipolytica	Extra-cellular and cell bound	2, 1*	Ota et al. 1982 Destain et al. 1997 Pignede et al. 2000a Pignede et al. 2000b

* bookmarks the isozymes, wherein the genes encoding them have been identified, isolated and cloned.

biotransformations on account of their thermal stability and substrate specificity. The percentage and nature of carbohydrate attached seem to be important in structure interaction of glycoprotein with oil water interface, thermal stability, hydrophobic hydrophilic interaction, and over all catalytic activity of lipases.

Elimination of non-covalently bound sugar produces diminution in enzymatic activity in hydrolysis of tributyrin, in synthesis of heptyl oleate and in thermal stability. The equilibrium of this enzyme with lactose or with dextran produces partial reactivation of the biocatalyst.

Benjamin and Pandey (2001) isolated and characterized three distinct forms of lipases from *Candida rugosa* (DM - 2031), produced in solid-state fermentation. Three distinct forms of extra-cellular lipase (lipA, lipB, and LipC) were isolated by ammonium sulphate precipitation, dialysis, ultra filtration and gel filtration using Sephadex-200. The purification was 43-fold with specific activity 64.35 mg/ml. SDS- PAGE of purified lipase revealed three distinct bands indicating the existence of three iso-forms with apparent molecular weight of 64, 62 and 60 kD. All the three forms have optimal activity at 35-40ºC and pH 7-8. Ag^{++} and Hg^{++} strongly inhibit the activity of all the iso –forms whereas Ca^{++} and Mg^{++} enhance the lipase activity. The activity of all the three forms was completely inhibited by serine protease inhibitors namely, -dichloroisocoumarin and pefabloc. Phenylmethanesulphonyl fluoride inhibited their activity partially.

Lopez et al. (2004) compared three pure isoenzymes from *Candida rugosa* (CRL: Lip 1, Lip 2 and Lip 3) in terms of their selectivity and reactivity in both aqueous and organic media. This analysis indicated that Lip 1 and Lip 3 have similar stability, lower than that of lip2. In aqueous media however Lip 3 was the most active enzyme so far as hydrolysis of P-niotrophenyl ester is concerned and Lip 1 showed highest activity on the hydrolysis of most assayed triaylglycerides. High difference in the isozyme was found in hydrolysis of triacylglycerides. Short, medium and long acyl chain triacylglycerides was preferred substrate for Lip 3, Lip 1 and Lip 2 respectively. In organic media Lip 1 and Lip 3 provided excellent results in terms of enantioselectivity in the resolution of ibuprofen (value over 0.90) and conversion, whereas initial esterification rate was higher for Lip 3, Lip 2 resulted in lower values of conversion, enantiomeric access and enantioselectivity. The performance of pure isozyme in enantioselectivity and esterification of substrate was compared with different CRL crude preparation with known isozymatic content and the different results could not be explained on their isoenzymatic profile.

As CRLs can be hardly separatedfrom each other and characterized as pure proteins, detailedinformation is available for only some of them. CRL1 over expressedin *Pichiapastoris* was found to exert highest activity on medium-chainsubstrates (C8-C10) in the hydrolysis of both triglyceridesand methyl-esters, whereas recombinantCRL4 and CRL2 overexpressed in *Pichiapastoris* acted preferentiallyon long-chain molecules (C16 - C18).A third isoform, CRL3, is characterized by itssignificant activity on short-chain soluble substrates and by its ability to hydrolyse cholesterol estersof long-chain fatty acids. Cholesterol esteraseactivity was also demonstrated more recently in CRL2 (Brocca et al. 2003).

Geotrichum

Couple of species of genus *Geotrichum* are known to produce lipases most of themextra-cellular. Tsujisaka et al. (1973) purified lipase of *Geotrichumcandidum*Link by means of ammonium sulphate fractionation, DEAE - Sephadex chromatography and gel filtration of sephadex G-100 and G-200. The purified enzyme was subsequently crystallized. The crystallized preparation was found to be homogenous electrophoretically as well as centrifugally. The molecular weight and pI value of the enzyme were estimated to be ~55 kD and ~4.33. The crystalline preparation contained about 7% carbohydrate and a very small amount of lipids. This lipase was active on olive oil at pH 5.6 and 7.0 at 40ºC. This enzyme maintained its stability in the pH range of 4.2 - 9.8 for 24 hrs. The enzyme is stable at temperature below 55ºC for 15 min.

Two forms of lipases have been isolated from *Geotrichumcandidum* ATCC 34614 by combining of ethanol precipitation and chromatography on Sephacryl HR anion exchange and polybuffer exchange 94. The molecular weight of enzymes has been estimated to be ~56 KD. The optimum pH value and isoelectric focusing point of the iso-forms is 6.8 and 6.0 and 4.46, 4.56 for lipase I and II respectively. The enzymes are found to remain stable in the pH range 6.0 - 8.0. Monovalent ions had little effect on activity of both the enzyme whereas divalent ion at concentration above 50mM inhibited the activity in concentration dependent manner. At concentration less than 10 mM ionic detergent sodium dodecyl sulphate completely inhibited lipase activity (Veeraragavan et al. 1990).

Like species of genus *Candida, Geotrichumcandidum* is also known to produce various forms of lipase. Purification and characterization of different lipase iso-forms produced by various strains of *Geotrichumcandidum* is difficult on account of overlapping in their physical and biochemical properties. At times the heterogeneity is result of the difference in glycosylation. The reports that appeared recently on substrate selectivity of apparently purified lipase iso-forms are contradictory.

Shimada et al. (1990) reported that there is no significant difference in the substrate selectivity of two lipase iso-forms of *Geotrichumcandidum* ATCC 34614, although the two forms have slightly different stability and biochemical properties. In contrast, the lipase iso-forms (termed lipase A and B) from strain *Geotrichumcandidum* CMICC 335426 have shown to have markedly different substrate specificity (Sidebottom et al. 1990) These investigators also isolated

Table 3.Selected examples of yeast that are used for production of lipases commercially with their commercial names and list of suppliers.

Name	Commercial name	Commercial source
Candida cylindracea (CRL)	ChiroCLEC-CR Lipase AY Lipase MY, Lipase OF-360 Chirazyme® L-3	Atlus Biologics Amano Meito Sangyo Boehringer Mannheim Sigma
Geotricumcandidum (GCL)	Chirazyme®L-8 SP 524, Lipolase®	Boehringer Mannheim Nova-nordisk
Candida antartica A (CAL –A)	Chirazyme®L-5, SP526,	Boehringer Mannheim Nova-nordisk
Candida antartica A (CAL –B)	Chirazyme®L-2 SP 525 or Novozym 435b	Boehringer Mannheim Nova-nordisk Sigma
Yarrowia lipolytica (earlier *Candida lypolytica*, CLL)	Lipase L	Amano

two isozymes from stain ATCC 34614 which showed selectivity similar to those of the two iso-forms isolated from the strain CMICC 335426. Moreover comparison of specificity profiles and partial peptide sequences revealed that lipase A is similar to lipase II and lipase B is similar to lipase I. The contradictory results about the substrate selectivity of various isozymes may be on account the different growth conditions employed by different investigators. Growth conditions are known to alter relative yields of lipases produced by *Geotrichumcandidum*. On the other hand, the presence of multiple, differentially expressed lipase genes coding for products having similar chromatographic properties cannot be ruled out. Two lypolitic enzymes were isolated from the culture fluid of yet other species of *Geotrichum,Geotrichumasteroides* by Kazanina et al. (1981). Lipase produced by *Trichosporonheteromorphum* ATCC 20001 were examined in media containing soyabean oil and residues but at present the strain ATCC 20001 has been reidentified as *Geotrichumklebahnii*. Using high-performance liquid chromatography Jacobsen and Poulsen (1992) isolated two lipolytic proteins with MW ~61 and ~57 kD from Sephadex G-100 fraction of extracellular lipase from *Geotrichumcandidum* ATCC 66592 were separated. Purified lipases were immunologically identical, but differed substrate specificity.

Trichosporon

*Trichosporon*is yet another yeast studied in detail for lipase production. So far four species of the genus are reported to produce lipase. Chen et al. (1992) isolated *Trichosporonfermentans* WU–C12 from soil that showed maximum lipase production (128 U/ml) after four days of growth at 30ºC. Of the various C sources tested for their effect on growth and lipase production, use of tung oil followed by olive oil (3% W/V) resulted in highest lipase productivity 146 U/ml and 126 U/ml respectively. On the contrary, when cultured in media containing glucose as C source *Trichosporonfermentans* WU-C12 produced only 34 U/ml lipase. Addition of glucose to corn steep –olive oil medium reduced the lipase production in *Trichosporon* unlike *Yarrowia lipolytica* (formally *Candida paralypolytica, Saccharomycopsis lipolytica*), *and Geotrichumcandidum*. These results suggest that the lipase produced by*Trichosporonfermentans* WU-C12 is constitutive*Trichosporonfermentans* WU-C12 is reported to produce two types of extra cellular and three types of intracellular lipases (Chen et al. 1993). Chen et al. (1993) further subjected *Trichosporonfermentans* WU-C12 to UV rays, which resulted in the production of higher quantities of extra cellular lipase than the parental strain. They proposed that enhancement of lipase production in mutant could be because of improvement in utilization of wide range of *n*- alkanes, permeability of lipase and lipase productivity per cell. The effect of surfactants on lipase production by *Trichosporonfermentans* WU-C12 was investigated. Addition of surfactants resulted in 2-3 times increase in extra cellular lipase activity. It was also observed that the total extra and intracellular activity was affected by the combination of surfactants and petroleum products used as Carbon source (Chen et al. 1994). These workers purified two types of extra-cellular lipases, lipase I and lipase II from this strain of *Trichosporon* by combining acetone precipitation with successive chromatography on butyl-toyopearl 650 MToyopearl HW-55 and Q - Sepharose FF. The molecular weight of lipase I was ~53 kD as estimated by SDS-PAGE and ~160 KD by gel filtration

while that of lipase II was ~55 kD as estimated by SDS-PAGE and ~60 KD by gel filtration. Both the lipases are stable in the pH range of 4.0 - 8.0 for 24 hrs at 30ºC. Both lipase II and I seemingly are thermo stable. Lipase I was stable at 40ºC for 30 min at pH 5.5 and lipase II under same conditions remainsstable up to 50ºC. As far as their substrate specificity is concerned both lipases hydrolyse 1, 2, 3 positions of triolein and cleave all the three ester bonds, irrespective of their position in triglyceride.

*Trichosporonfermentans*WU-C12 lipase (TFL I) is almost identical to *Geotrichumcandidum* lipase I and II which suggests similarity in their three dimensional structure. *G. Candidum* ATCC 34614 produces 2 or four kinds of monomeric lipases whereas *Tichosporonfermentans* WU-C12 lipase I am trimer with molecular mass of 160 KD as determined as by gel filtration. Difference in the number of subunits in mature lipases in both the genus lead to different substrate specificity. In addition difference of three amino acid residues and /or glycosylation might have an effect on unit structure of TFL GCL, II and I.

Another lipase producing species of *Trichosporon* is Trichosporonasteroids that was isolated from raw milk Dharmsthiti and Ammaranond (1997). The lipase was purified to homogeneity (eight fold) using various techniques including ammonium sulphate fractionation and gel filtration on Sephadex G200. The molecular weight of this lipase was estimated to be ~37 KD with temperature and pH optima of 60ºC and 5.0 respectively. It maintains stability over wide range of pH 3.0 to 10.0 and at temperature below 70ºC. It is not a metallo-1`protein as metal ion chelators do not inhibit the enzyme activity. The recovery after purification was noticeably low which could be due to spontaneous aggregation of the enzyme that leads to reduced enzyme activity. Gel filtration pattern of the enzyme indicated a molecular mass of 200 kD, which is in contrast to the mass estimated by SDS PAGE. This difference is perhaps caused by aggregation of lipase molecules.

Yarrowia lipolytica and other yeasts

Several enzymes are secreted by *Yarrowia lipolytica* (earlier *Saccharomycopsis lipolytica, Candida lipolytica, Candida paralipolytica*) and lipase and esterase activities have been detected and analyzed in variousstudies (Sugiura et al. 1976; Ota et al 1982; Ota and Yamada, 1966). Lipase secretion was first reported in 1948 by Petersand Nelson (Pignede et al. 2000a). An extracellular and two cell-bound types of activitycorresponding to lipase I (39 kDa) and lipase II (44 kDa) weredescribed by Ota and coworkers (Sugiura et al. 1976; Ota et al. 1982). Cell bound enzymes were purified from the organism with total recovery of 8% by chromatography on CM -Sepharose CL-6B, DEAE - Seharose CL-6B and Sephadex G-100columns. The purified lipase requires oleic acid for the hydrolysis of triglycerides and hydrolyses tricaprylin at largest initial rate. Under experimental conditions the pH optima for hydrolysis of olive oil as substrate were about 8.0. Both enzymes remain stable for 20 min below 37ºC and at pH ranging between 4.5-8.0 for 22 hrs at 5ºC (Ota et al. 1982). The extracellularlipase required oleic acid as a stabilizer-activator, whereasthe cell-bound lipases did not and differed in several propertiesfrom the extracellular enzyme (Ota et al. 1984). Productionof the extracellular and cell-bound enzymes were reported to dependon the Carbon and Nitrogen composition of the medium. Extracellularlipase was only detected in cultures grown with an organic nitrogensource, and lipase levels were shown to be modulatedby cell morphology. In minimal medium supplemented with *N*-acetylglucosamineor citrate buffer, both of which promote dimorphic growth, higherlevels of cell-bound lipases were detected. However, no clearrelationship was established between the dimorphic state and lipaseproduction (Novotny et al. 1994). Like many others Ota et al. (1982) have demonstrated that media engineering can lead to increase in production of extra-cellular enzymes. Lipase II and I were purified nearly 69 and 58 fold respectively. Lipase I had optimum pH of 8.2 for hydrolysis of olive oil as substrate and 7.5 for tributyrin hydrolysis. Lipase II has pH optima 8.0 for olive oil, 7.5 for tributyrin and 7.0 for triolein hydrolysis. Ota et al. (1982) concluded that there is no significant difference in the enzymological properties of lipase II and I. They have proposed that one enzyme is modified protein of the other, and the modification is caused by enzymatic reaction or binding of some components. Strangely enough the extra-cellular lipase as well as the two purified cell bound lipases require oleic acid as a the lipase activator for hydrolysis of exogenous triglycerides but the cell bound lipase located on living yeast cell appeared not to have this requirement. N-terminal amino acid sequence of purified the 39 kDa extracellular lipase (called lipase A) was determined by Kuno and Ota, 1996. Destain and coworkers isolated *Yarrowia lipolytica*strains overproducing an extracellular lipase. The secreted lipase wasshown to have an apparent molecular mass of 38.5 kD, giving threeisofocusing (pIs) of 5.0, 5.2, and 5.4. The sequenceof the first 49 aa of the N-terminus was determined andfound to be identical to that of lipase A (Destain et al. 1997). This sequence is similarto that of cell-bound lipase I; however, the extracellular lipaseand lipase I are considered to differ in amino acid composition (Kuno and Ota, 1996).

Kluyveromyces lactis is yet another yeast that produces extra cellular phospholipase B (PBL) (Oishi et al. 1999). The enzyme purified to homogeneity from culture medium is highly glycosylated with apparent molecular weight of 160-250 kD. The enzyme has two pH optima, pH 2.0 and pH 7.5. The enzyme was reported to have at acidic pH the enzyme hydrolyses all phospholipids substrates with out metal ion .On the other hand at alkaline pH it showed specificity for phosphatidylcholine and lysophosphatidylcholine and required Ca^{++}, Fe^{+++} or Al^{+++} for activity. The alkaline activity gets increased over 20 fold in presence of Al^{+++} compared to the presence of Ca^{++}.

Table 4.Overview of various biochemical properties (molecular weight, optimum pH and temperature and pI values etc.) of selected lipases produced by yeasts.

Yeast	MW in kD	PH optima	Stable pH range	Tº optima	Reference
Arxulaadeninivorans	50	7.5	-	30ºC	Boer et al. 2005
Candida albicans ATCC 36082	38	-	-	-	Fu et al. 1997 Hube et al. 2000
Candida antarctica	33	-	-		Høegh et al. 1995 Rotticci et al. 2001
Candida cylindracea L1754	L1- 58 L2- 58	-	5.8 - 6.8	-	Veeraragavan and Gibbs, 1989
Candida. Cylindracea *Commercial preparation*	LA-62 LB- 62 LC-62	7.0 5.0	-	-	Shaw and Chang, 1989
Candida. rugosa ATCC 14380	60	5.0	-	-	Lotti et al. 1993
Candida. rugosa DMS 2031	Lip A-64 LipB- 62 LipC- 60	7.8 7.8 7.8	-	35 - 40ºC	Benjamin and Pandey, 2001
Geotrichumcandidum link	55	5.6 - 7.0	4.2 - 9.8	40ºC	Tsujisaka et al. 1973
Geotrichumcandidum ATCC 34614	Lip 1 56 Lip2 56	6.8 6.0	6.8 - 8.0	-	Veeraragavan et al. 1990
Kluyveromyces lactis	160-250	2.0 - 7.5	-	-	Oishi et al. 1999
Kurtzmanomyces sp. I-11	49	1.9 - 7.2	below 7.1	75ºC	Kakugawa et al. 2002
Saccharomyces cerevisiae	63				Oishi et al. 1999
Yrrowia .lipolytica (formally *Saccharomycopsis lipolytica, Candida lipolytica)*	Lip 1-39 Lip-44	8.2 8.0	4.5 - 8.0	37ºC	Ota et al. 1982
Trichosporonfermentans *WU-C12*	Lip 1-53 Lip2- 55	5.5	4.0 - 8.0	40ºC	Arai et al. 1997
Trichosporonasteroides	37	5.0	3.0 - 10.0	50ºC	Dharmsthiti and Ammaranond, 1997

a: 146 hrs of culture.

An extracellular lipase produced by the glycolipid-producing yeast *Kurtzmanomyces* sp. I-11 was purified by ammonium sulfate precipitation and column chromatographies on DEAE-Sephadex A-25, SP-Sephadex C-50, and Sephadex G-100 (Kakugawaet al. 2002). Its molecular mass was estimated to be approximately 49 kDa. By SDS-PAGE and optimum temperature for the activity was 75ºC. The activity was very stable at temperatures below 70ºC, the enzyme was in pH range of 1.9 - 7.2 and stable at pH below 7.1. N-terminal sequence of the Kurtzmanomyces lipase was found to be similar to that of lipase A from *Candida antarctica,* though the pH profiles of the two lipases were significantly different (Kakugawa et al. 2002). Overview of biochemical properties of lipases and their isozymes has been given in Table 4.

GENE CLONING

In view of wide application of lipases referred to above and, difficulties in purifying their various isozymes due to their overlapping biochemical properties and obtaining these enzymes in bulk, attempts have been made to directly

clone genes encoding them. It started with the primary objective to unravel the peptide structure of the enzyme, which is required to understand the molecular mechanism underlying the catalytic reaction and the relationship between the structure and function of lipase. Lipases gene of number of yeasts have been cloned and expressed, hyper expressed and bioengineered.

Candida

Lotti et al. (1993) reported that lipases of *Candida cylindracea* are encoded by multiple genome sequences, now on the basis of DNA-DNA homology at least seven are presumed to be present. Brocca et al. (1995) isolated and cloned 5 different forms of lipase from the species. The five-lipase genes comprising lipase gene family of *Candida rugosa* exhibit 80-88% pair wise identity, in nucleotide sequence. All genes encoding these isozymes are located on the same chromosome, which suggests their origin through gene duplication. Expression of cloned genes of genus candida is demanding due to strong codon bias in *candida*spp. as universal leucine sequence codon CUG is used for serine (Ohama et al. 1993). CUG is used with high frequency (3% of the codon) in LIPI of *Candida cylindracea* including those corresponding to catalytic site. All the lipase sequences show distinct pattern for several restriction sites and not minor variation as would be expected in case of alleles. Sequence analysis performed on 5 fully sequenced genes demonstrated that the highest identity between any two-lipase genes is 88%, a value less than usually observed in allelic variants of the same gene. The available sequences of CRL correspond to isozymes derived from the strain ATCC 14830. The inconsistencies in catalytic properties reported from different laboratories are due to presence of different lipase proteins in *Candida cylindracea* whose composition and ratio may be different in different commercial lipase preparations, possibly depending on the strain and media components used. Presence of multiple isoforms of lipases in *Candida* spp. is well established (Chang et al. 1994). Lotti and Alberghina (2003) indicated that CRL- encoding genes are subjected to regulation at the level of transcription depending on the composition of culture medium and the physiology of culture growth. This complex family of enzymes provide *Candidarugosa* cells with large and versatile pool of catalysts to suit the composition and environment of the culture media. However significance of lipases for the growth of *Candida rugosa* growth far from clear at present. The heterologous expression of cloned genes in host cells is desirable in the case of CRLs not only because of the well known advantages it offers in making large amount of purified enzymes available but also because it seems to be the only feasible approach to obtain pure CRL isoforms without the need of any sophisticated purification techniques required to separate similar proteins. Compared to commercial lipase preparation the recombinant Lip I showed a comparable activity towards triacylglycerides different in acyl group chain length and various methyl-esters differing in acyl group chain length (Brocca et al. 1998). Secretion of 200 ug lipase ml (-1) culture was achieved in sorbitol - based medium, in batch cultivation of *Pichiapastoris* expressing *Candida rugosa* lipase I (CRLI) (Passolunghi et al. 2003). These workers found that the large amount of recombinant protein was retained intra-cellularly throughout the fermentation pointing to transport step as a major bottleneck. To monitor the product localization and amount a translational fusion product with green fluorescent protein (GFP) was constructed that was expressed and translated by this group.

A lipase encoding gene was isolated from the pathogenic yeast *Candida albicans* (ATCC 36082) by transforming the lipase minus strain of S*accharomyces cerevisiae* with genomic library of *Candida albicans* and screening for lipolytic activity (Fu et al. 1997). Two identical clones exhibiting lipolytic activity were identified. Their nucleotide sequence analysis identified an ORF encoding a protein of 351 amino acid residues. The sequence contained the Gly-X-Ser-X-Gly- motif found in all prokaryotic and eukaryotic lipases, suggesting a similar activity for encoded the proteins. Southern analysis, using this lipase as probe, has suggested that lipase gene may be present in *Candida glabrata*, *Yarrowia lipolytica* (formally *C. parapsilosis)*, *Candida tropicalis*, *Candidakrusci* but not in,*Candida pseudotropicalis* or S*accharomyces cerevisiae*. Northern blot analysis has shown that expression of lip I transcript is detected only when *Candida albicans* was grown in media containing tween 80, other tweens or triglycerides as the sole carbon source. Whereas Sabouraud Dextrose broth or yeast/peptone/dextrose and carbohydrate supplementation inhibited LIPI expression. Hube et al. (2000) have cloned and characterized 10 lipase genes in *Candida albicans*. The ORF of all the reported *Candida albicans* lipase genes are 1281 - 1416 bp long and encode highly similar proteins having up to 80% identical amino acid sequence. Each deduced lipase sequence has conserved lipase motif, 4 conserved putative N-glycosylation sites and similar hydrophobicity profiles. Lip 3 – Lip 6 is expressed in all media at the times of growth when tested by Northern blot and PCR analysis. Lip 1, 2, 4, 5, 6, and 8 were expressed in media having tween 40 as the sole carbon source. These genes are also expressed in media deficient in lipids. Transcripts of most lipase genes were detected during yeast to hyphal transition. Lip 5, 6, 8, and 9 were found to express during experimental infection of mice. These data reveal lipid independent, highly flexible in-vitro and in-vivo expression of large number of Lip genes, possibly reflecting broad lipolytic activity, which may contribute to the persistence and virulence of *Candida albicans* in human tissue. Stehr et al. (2004) investigated expression pattern of multigene family of *Candida albicans* using reverse transcription polymerase chain reaction in experimental infections and in samples of patients suffering from oral candidosis. The finding illustrate that individual lipase genes are differentially regulated in a mouse model system. This study indicated that the lipase gene expression profile depended on stage of infection rather than on organ localization. The temporal regulation of lipase gene

expression was also detected in an experimental model of oral candidosis.

Candida deformans CBS 2071 extra-cellular lipase has been cloned by complementation in *accharomycecerevisiae* with the genomic library of *Candida deformans* (Bigey et al. 2003). Three members of the lipase gene family CdLIP1, CdLIP2 and CdLIP3 were cloned and characterized. The MADLI-TOF data suggests that it is LIPI that produces the extra-cellular lipase. Each deduced lipase sequence has a Glu-His-Ser-Leu-Gly- (Gly-/Ala)-Ala conserved motif, eight cysteine residues and encodes a N-terminal sequence. These lipases were very similar to lipases from the related yeast *Yarrowia lipolytica.* Significant homologies were found with some other yeasts and fungi.

Neugnot et al. (2002) cloned and the over expressed the genecoding lipase from *Candida parapsilosis* CBS 604. Two ORFs (*CpLIP1* and *CpLIP2*) wereisolated and the deduced 465-amino-acid protein sequences containedthe consensus motif (G-X-S-X-G) which is conserved among lipolyticenzymes. Only one of the two deduced proteins (CpLIP2) containedpeptide sequences obtained from the purified lipase/acyltransferase.Homology investigations showed that CpLIP2 has similaritiesprincipally with 11 lipases produced by *Candida albicans* (42 - 61%)and the lipase A from *Candida antarctica* (31%) but not withthe other lipases sequenced so far. Both *CpLIP1* and *CpLIP2* were expressed in *Saccharomyces cerevisiae* , but only *CpLIP2* coded for an active protein. The substrate specificity and thecatalytic behavior of purified recombinant CpLIP2, with or withouta C-terminal histidine tag, were not changed compared to thoseof the native lipase.

Two lipases lipase A and B have been cloned and expressed by an extremophile yeast *Candida antarctica*by Høegh et al. (1995) and have been expressed in *Aspergillus oryzae*. *C. antarctica* lipase A has also been cloned and expressed in *Pichiapastoris* under AOX 1 promoter by Rotticci-Mulder and his group (2001).

Geotrichum

The yeast *Geotrichumcandidum* produces isozymes in culture medium that show close physical and biochemical properties but are different as for as substrate specificities are concerned. Shimada et al. (1989) isolated the lipase encoding cDNA clone of *Geotrichumcandidum* ATCC 34614 (Geo) from the Geo. cDNA library by colony hybridization. The library was probed by ^{32}P - labeledoligonucleotides corresponding to a partial amino acid sequence of this enzyme. The nucleotide sequence of this cDNA determined by dideoxy chain termination method. The deduced amino acid sequence from cDNA coincided with the sequence of the protein. The cloned cDNA coded a protein of 554 amino acids and a hydrophobic signal sequence of 19 amino acids. *Geotrichum* lipase contains the Gly-X-Ser-X-Gly sequence, which is believed to form part of the interfacial lipid recognition site. Use of this cDNA as probe during Southern DNA analysis of *Geotrichumcandidum* genome indicated existence of two genes on the chromosome of the yeast. Following these leads two forms of lipase (lipase I and II) were actually isolated and purified from this yeast. The two lipases isoenzymes have similar amino acid composition but different terminal sequences not found in the primary structure of lipases I deduced from the cDNA sequence. This confirmed the presence of two different lipase genes and ruled out the possibility of the formation of observed multiple forms by proteolytic digestion (Sugihara et al. 1990). The same year Shimada et al. (1990) cloned and sequenced the cDNA for lipase II of *Geotrichumcandidum*. They found the overall length of the two lipases is same, the overall identity being 84%. Homology search has indicated that the *Geotrichumcandidum* lipase and *Candida cylindracea* lipase are homologous enzymes and are members of the cholinesterase family. Three years latter, Nagao et al. (1993) using the previously isolated lipase I and II cDNAs as probe isolated two chromosomal lipase genes lip 1 & 2, encoding lipase I and II. Both the genes were reported to contain contained 5'- and 3'- flanking regions in addition to coding region. Both the coding regions contained no introns and exhibit 86% homology in nucleotide sequence. Vernet et al. (1993) cloned, and sequenced the gene encoding lipase II of *Geotrichumcandidum* strain ATCC 34614 (GCL II) after amplified using the polymerase chain reaction. The intron less lipase gene was expressed and secreted from *Saccharomyces cerevisiae* at approximately 5 mg/liter of culture. Recombinant GCL II was purified by immunoaffinity chromatography and characterized using a combination of substrates and independent analytical methods. Bertolini et al. (1994) have reported that lipase iso-forms may be present in other *Geotrichum*strains as well, but their purification and isolation is difficult on account of their overlapping physical and biochemical properties. Bertolini and his group (1994) have located lipase genes present in four strains (ATCC 34614, NRCC 205002, NRRLY-552 and NRRLY -553) of *Geotrichumcandidum* by molecular cloning by polymerase chain reaction. Each strain contains two lipase genes that are closely related to lipase I and II of *Geotrichumcandidum* ATCC 34614, so far investigated. Each lipase – gene family exihibits sequence variation (polymorphism), which is confirmed by southern analysis as well. Only two genes are found in each of the strain investigated and the biochemical studies also suggest the existence of lipase with at least two different substrates specificities. Most of the amino acid substitutions are located on the protein surface; some are present in structural features possibly involved in determining substrate specificity. The conflicting reports about existence of more than two lipase isoforms probably originate from differential expression of these genes in different culture conditions, heterogenesis in posttranslational modification and different efficiency of

Table 5.Yeasts that harbour lipases gene families along with the number of lipases genes contributing towards each family.

Name	Number of lipase encoding genes	Reference
Candida albicans	10	Hube et al. 2000
Candida antarctica	2	Høegh et al. 1995 Rotticci et al. 2001
Candida rugosa ATCC 14380	5	Lotti et al. 1993
Candida deformans CBS 2071	3	Bigey et al. 2003
Geotrichum candidum ATCC 34614 NRCC 205002 NRRL Y-552 NRRL Y-533	 2 2 2 2	 Shimada et al. 1990 Bertolini et al. 1994 Bertolini et al. 1994 Bertolini et al. 1994
Trichosporonfermentans WU-C12	2	Arai et al. 1997
Yarrowia lipolytica	3	Pignede et al. 2000a

purification. Catoni et al. (1997) studied *Geotrichumcandidum* ATCC 335426 lipases A and B in *P. pastoris*. The sequences encoding mature lipases were fused in frame with the µ-factor signal sequence and expressed under control of the AOX 1 promoter. Depending on the clones, the extracellular production of lipases A and B varied from 1 U/ml to 23 U/ml and 1 U/ml to 50 U/ml, respectively. Optimization studies of recombinant lipase B production increased the activity to 200 U/ml of culture medium.

Trichosporon

Arai et al. (1997) isolated a cDNA clone encoding extra-cellular lipase of*Trichosporonfermentans* WU-C12 (TFL I) by using 0.8 kb fragment amplified by PCR with synthetic nucleotide corresponding to partial amino acid of TFL I. On characterization it emerged the cDNA clone contains 1689 bases long ORF, which encodes 563 amino acids from the ATG initiation codon. Two potential sites for N-glycosylation are found in this enzyme. Complete data search indicated high homology between TFL and *Geotrichumcandidm* lipase (GC ATCC 34614). Mature lipase I (TFL I) of*Trichosporonfermentans* WU-C12 Is 84 % and 99.5% homologus to GCL I and II respectively at amino acid level and nucleotide sequence identities are 86.1% and 99.9% respectively (Arai et al. 1997). There are differences of only 3 amino acid residues at position 23-25 between TFL GCL II and I. The deduced amino acid sequence of mature TFL I share 41% and 42.3% overall identity with those of lipase III and IV from *Candida cylindracea* earlier cloned by Lotti's group.

Yarrowia lipolytica and other yeasts

Several lipases have been detected in *Yarrowia lipolytica*, including intracellular, membrane-bound, and extracellular enzyme. Three lipase encoding genes have been isolated from *Yarrowia lipolytica*, *lip1* code for 486-aa and lip3 which codes for 498-aa lipase. Lip1 and lip3 are reported to be intracellular but lip2 which has been isolated by Pignede et al. 2000a and Pignede et al. 2000b, belongs to the class of extra-cellular lipases. PCR and Southern analysis show that unlike some yeasts, like *Candida rugosa* and *G. candidum* in which several highly homologous genes are present *Y. lipolytica* has a single gene encoding extracellular lipase. This is also supported by the low residual extracellular lipase activity(less than o.5% U/ml) (Pignede et al. 2000a and Pignede et al. 2000b).

The phospholipase encoding cDNA of *Kluyveromyces lactis* was identified by combination of several procedures and sequence was designated as KILB. KIPLB encods a protein consisting of 640 amino acids. The deduced amino acid sequence showed 66.7% similarity with the *Trichosporondelbrueckii*PLB.The amino acid sequence contained the lipase consensus sequence (G-X-S-X-G) and the catalytic aspartic acid motif.

The lipase-encoding *Arxulaadeninivorans,* ALIP1 gene was isolated using fragments of lipase isolates obtained by trypsin digestion for the definition of oligonucleotide primers in a PCR screening approach (Boer et al. 2005). The gene harbours an ORF of 1347 bp encoding a 420 amino acid protein of some 50 kDa preceded by an N-terminal 28 prepro-secretion sequence. The deduced amino acid sequence was found to be similar to the lipases from *Candida albicans* and *Candida parapsilosis* (34-38% identity) and more distantly related to other lipases. The

sequence contains the consensus pentapeptide motif (-Gly-X-Ser-X-Gly-) that forms a part of the interfacial lipid recognition site in lipases. The expression of the gene is regulated by carbon source. In media supplemented with Tween 20, induction of the ALIP1 gene and accumulation of the encoded lipase in the medium is observed, thus demonstrating gene regulation by lipophilic compounds. The enzyme is dimericwith pH optimum at pH 7.5 and a temperature optimum at 30ºC.

CONCLUDING REMARKS

Like most other organisms lipases are ubiquitous in yeasts. Lipases are amongst the most important biocatalysts that carry out novel reactions in aqueous and non - aqueous media. They show wide variety of chemio-, regio- and enantioselective transformations. The ease in handling them, their broad substrate tolerance, high stability under varied temperature and solvents, high enantioselectivity and easy availability account for their wide spread popularity. As a result, today they have earned immense application in different areas of industrial microbiology and biotechnology where they display amazing versatility in catalytic behaviour. Yeast lipases are quite relevant to biotechnology industries and attract special attention, as yeast products have been consumed by man since ages and are considered safe and natural. Yeast lipases by and large are extracellular, monomericglycoproteins though there are some reports like in *Yarrowia lipolytica* wherein lipase two out of three isozymes present are cell bound as well as intacellular in nature. The molecular weight of various yeast lipases reported is between ~33 to ~65 kD. Nucleotide sequence homology study of various cloned lipases from yeasts suggest that they lipase isozymes are produced by different genes. Post transcriptional and post translational modifications do also contribute towards production of various isoenzymes. In two widely studied lipase producing yeasts *Candida rugosa*nad*Geotrichumcandidum* the lipase genes have been located on the same chromosome. These results have led to the presumption, that the various lipase encoding genes have perhaps resulted from gene duplication. More than 50% of yeasts produce lipases isozymes encoded by different genes constituting the lipase gene families. Like many other organisms including mammals presence of lipase gene families is quite prevalent in yeasts and at present *Candida albicans* leads with 11 lipase encoding genes. Members of various lipase gene families have been tabulated in Table 5.

FUTURE PROSPECTS

Compared to enormous potential lipases in general and yeast lipases in particular, their actual use in biotechnology industry at present is almost negligible. Hydrolysis of fats and oils is still being carried out by the conventional emulsion system and not with the use of lipases. The technology has not advanced to provide substitutes of the conventional chemical process. However one yeast enzyme, CCL is being used for splitting oil and use of fatty acids produced for production of soaps (Reetze and Schimossek, 1996; Saxena et al. 1999). Introduction of new generation thermostable yeast lipases can tilt economic balance in favour of lipases. Novel lipases can also be used for the synthesis of whole range of amphoteric biodegradable surfactants namely amino acid based ester amides. The prospects of industrial application of lipases are bright. However it calls for discovering novel lipases by extensive screening and transformation of the known ones by genetic manipulations. Greater understanding of all aspects of lipase production and upstream, downstream processing of factors effecting lipase activity and stability. The mechanism by which these factors influence the three-dimensional structure of lipases and their ability to catalyse. Factors such as immobilization and the nature of organic solvents used also affect the catalytic efficiency of lipase. Thorough understanding of the factors can help in the development of tailor made lipases for specific application and in long run open up new vistas.

Biotech industry can be classified into three groups, which are listed below.

1. Industries producing high volume, and low value products *e.g.* ethanol, high fructose syrup.

2. Those making high volume and moderate value commodities *e.g.* organic acids, amino acids, food products polymers.

3. Industries manufacturing low volume, high value products *e.g.* pharmaceuticals, vitamins, enzymes etc.

Whatever be the volume or value of the product of industry most of these are used for consumption. It is not necessary to emphasize that use of yeasts in manufacture of consumable items is safe. In fact yeast has been used since ages in manufacture of food products like cheese, yogurt, curd, bread etc. and are considered natural.

Dozen of lipases including yeast lipases are now commercially available nevertheless those employed in large - scale industrial process and products are still limited to few cases. This is mainly due to high price/ low availability or non-optimal operational features of naturally available enzymes. Perspectives in the use of lipases as industrial catalysts strongly rely on the production of recombinant enzymes with biochemical and catalytic features improved by protein engineering methods.

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