

## Yeast lipases: enzyme purification, biochemical properties and gene cloning

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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 extracellular, monomeric glycoproteins 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 possess 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, alcoholysis, 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 at their 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 (Antoniani, 1988).

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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 20<sup>th</sup> century has witnessed unprecedented use of lipases in biotechnology, manufacture of pharmaceuticals and pesticides, single cell protein production, biosensor preparation and in waste management etc (Torossian et al. 1991; Gandhi, 1997; Yadav et 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 a variety 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. Organoleptically each lipase develops a characteristic flavour. The *Candida rugosa* lipase was rated the most suitable lipase in this case. *Candida antarctica* AY30 immobilised lipase has been used for esterification of functional phenols for synthesis of lipophilic 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 lipolytica* (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 (60°C – 80°C) 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
<b>Mammalian</b>	Human Pancreatic Lipase Horse Pancreatic Lipase Pig Pancreatic Lipase Guinea Pig Pancreatic Lipase
<b>Fungal</b>	<i>Rhizomucormeihei</i> <i>Pencilliumcamberti</i> <i>Humicolalanuginosa</i> <i>Rhizopusoryzae</i> <i>Aspergillus niger</i> <i>Candida rugosa</i> * <i>Candida antarctica</i> Lipase A* <i>Candida antarctica</i> Lipase B * <i>Geotrichiumcandidum</i> *
<b>Bacterial</b>	<i>Chromobacteriumviscosum</i> <i>Pseudomonas cepacia</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas fragi</i> <i>Bacillus thermocatenuatus</i> <i>Staphylococcus hyicus</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>

\* 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 lamivudine (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*



























