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Review

Purification and Application of Lipases from *Pseudomonas* Species

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Abstract. Lipases are important hydrolytic enzymes that hydrolyze long chain triacylglycerol into diacylglycerol, monoacylglycerol, glycerol and fatty acids. Lipases are found in microorganisms, fungi, plants and animals. Commercially, useful extracellular lipases are isolated from different bacterial species, including *Bacillus, Achromobacter, Alcaligenes, Arthrobacter, Pseudomonas*, *Staphylococcus* and *Chromobacterium* species. Among the *Pseudomonas* species, *Pseudomonas aeruginosa*, *P. cepacia* and *P. fluorescence* are the major producers of lipases. Bacterial lipases have great industrial applications because of their stability, selectivity and broad substrate specificity. Due to their large scale application in industrial sectors, attention is given to isolate *Pseudomonas* lipases. In this review, purification strategies for lipases isolated from *Pseudomonas* species have been focussed.

Keywords: lipases, *Pseudomonas,* industrial applications, purification strategies

Introduction

Lipases are ubiquitous enzymes widely present in many species of animals, plants, bacteria, yeast and fungi (Dong *et al.,* 1999). Lipases hydrolyze long chain triacylglycerol into diacylglycerol, monoacylglycerol, glycerol and fatty acids (Yamamoto and Fujiwara, 1995). Bacterial lipases have great industrial applications because of their stability, selectivity and broad substrate specificity. Lipases can be isolated from gram negative as well as gram positive bacteria.

Microorganisms having potential to produce lipases can be found in various habitats including industrial wastes, vegetable-oil processing factories, dairies, soil contaminated with oil, oil seeds, decaying food, compost heaps, coal tips, and hot springs (Qamsari *et al.,* 2011). Among microorganisms, several species of bacteria, yeast and fungi are potential producers of extracellular lipases (Veerapagu *et al.,* 2013). Additionally, many, if not all, commercially useful extracellular lipases are isolated from different bacterial species, including *Bacillus achromobacter, Alcaligenes, Arthrobacter, Staphylococcus chromobacterium* and *Pseudomonas* (Qamsari *et al.,* 2011; Gupta *et al.,* 2004).

Among the *Pseudomonas* species, *P. aeruginosa*, *P. cepacia* and *P. fluorescence* are the major producers of lipases (Stoyanova *et al.,* 2012). *Pseudomonas* are motile, rod shaped, aerobic, gram negative and non fermentative bacteria. *Pseudomonas* is present in soil, water, animals and plants.

Microbial enzymes have various industrial applications in food, detergent, paper, leather, pharmaceutical and textile industries (Hasan *et al.,* 2006). *Pseudomonas* lipases are widely used in food, pharmaceutical, detergent and textile industries for quality improvements of food items, giving proper shape to pharmaceuticals products, for removal of oil stains from fabrics by hydrolysis of fats and increase fabric absorbance, respectively (Sharma *et al.,* 2001). Due to their large scale application in industrial and health sectors, attention is given to isolate bacterial lipases. In this mini review, lipases from *Pseudomonas* species have been foussed.

Sources of lipases. Lipases are found in living organism including prokaryotes bacteria and archaea and

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eukaryotes fungi, plants and animals (Cai-hong *et al.,* 2008). Microbial lipases are more important as compared to plant and animal lipases because of their easy genetic modification, growth on inexpensive media and higher catalytic activities.

In biotechnological applications, mostly bacterial and fungal lipases are used among microbial lipases. The extracellular bacterial lipases are more important because these enzymes are easily produced (Palekar *et al.,* 2000; Jaeger *et al.,* 1994). The lipase producing bacterial species include *Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Burkholderia, Chromobacterium* and *Pseudomonas*. Of these, lipases obtained from *Pseudomonas* bacteria are extensively used for a variety of biotechnological applications (Beisson *et al.,* 2000; Pandey *et al*., 1999).

Pseudomonas as a lipase producer. Lipases are produced and isolated from both gram positive and gram negative bacteria. Gram negative bacteria contribute to a larger part of bacterial lipases. Genus *Pseudomonas* is the most important gram negative genus, containing at least seven lipase producing species i.e., *P. aeruginosa, P. alcaligenes, P.fragi, P. glumae, P.cepacia, P. fluorescens* and *P. putida* (Kojima *et al.,* 2003).

Purification strategies for lipases. The literature represents different purification strategies for lipases including chromatography, aqueous two-phase systems, Reversed micellar systems, Immuno purification and membrane processes. After production of extracellular lipases microbial cells are removed from the fermented media by centrifugation or filtration. Organic solvents, ammonium sulphate precipitation and ultra filtration are used to concentrate the cell free supernatant. 80% purification protocols involve precipitation, in which 60% using ammonium sulphate, 35% using HCl or ethanol. After precipitation step, chromatographic techniques including affinity chromatography, ion exchange chromatography and gel filtration are used for further purification (Pabai *et al.,* 1995). Precipitation methods give 87% yield as compared with other techniques (Aires-Barros *et al.,* 1994).

Chromatography. Chromatography is generally used for the purification of extracellular lipases, sometimes one type of chromatographic technique is not sufficient to achieve the required level of purity. Therefore, different chromatographic techniques are used to get the required purity. Ion exchange chromatography is one of the most commonly used chromatographic techniques. It is used in 67% of the purification procedures. Mostly diethylaminoethyl (DEAE) group is used as an anion exchange (58%) while carboxymethyl (CM) as a cation exchange (20%). The most frequently employed ion-exchangers are the diethylaminoethyl (DEAE) group in anion exchange (58%) and the carboxymethyl (CM) in cation exchange (20%). Commonly strong ion exchangers on the basis of triethylaminoethyl groups (Veeraragavan *et al.,* 1990) and Q-Sepharose (Menge *et al.,* 1991) are used in lipase purification. Gel filtration is the second common purification technique used in 60% of the purification procedures. Affinity chromatography used in 27% of the purification procedures whereas, hydrophobic interaction chromatography is used in 18% of purification procedures (Farooqui *et al.,* 1994). A procedure used by Chartrain *et al.* (1993) for the purification of lipase from *Pseudomonas aeruginosa* MB5001 consists of three steps. The procedure involves concentration of lipase in crude extracts by using ultra filtration, ion exchange chromatography and gel filtration. Lee and Rhee (1993) purified lipase from *Pseudomonas putida* 3SK by using ion exchange chromatography and gel filtration. Sharon *et al.* (1998) purified an extracellular lipase from *P. aeruginosa* KKA-5 by ammonium sulphate precipitation and chromatography.

Membrane processes. In downstream processing of lipase purification, membrane filtration has been used for removal of microbial cells and concentration of lipases in the supernatant of the spent media. The ultra filtration capillary membranes were studied for the purification of lipase from *Pseudomonas fluorescens* (Sztajer and Bryjak, 1989). Polyacrylonitrile and polysulphone capillary membranes were reported in literature for purification of lipases.

Aqueous two phase systems. The aqueous two phase systems consisting of two incompatible polymers such as dextran or PEG dissolved in water solution or phosphate buffer used for purification of proteins. The separation of proteins in aqueous two phase systems generally depend upon the physical and chemical properties including protein hydrophobicity, charge and size. The separations of proteins in aqueous two phase systems is affected by changing polymers, molecular mass of the polymer, pH and addition of salt to the system. This is an interesting procedure for the purification of lipases as compared to other procedures (Gupta *et al.,* 1999; Albertsson *et al.,* 1990). Literature

reports various examples of lipase purification by using aqueous two phase systems (Table 1). During purification, the hydrophobicity of lipase is broken down by using detergents in aqueous two-phase systems. *Pseudomonas cepacia* lipase was purified by using detergent-based aqueous two-phase systems (Saxena *et al.,* 2003; Terstappen *et al.,* 1992).

Industrial applications of lipases. The microbial enzymes were introduced first into industry by Dr. Jokichi Takamine in the year 1894. Microbial lipases are used in food, paper, textile, pharmaceutical, leather and other industries (Hasan *et al.,* 2006; Underkofler *et al.,* 1957). Presently, about 4000 enzymes are known and nearly 200 are commercially used in various industries. Most commonly, the industrial lipases are of microbial origin. The total enzymes sale was a few million dollars annually until 1960s (Wilke, 1999) however, the demand for lipase is increasing day by day (Bornscheuer *et al.,* 2002). The world enzymes demand is fulfilled by 12 producers and 400 suppliers. Approximately 60% of industrial enzymes are produced in Europe (Balashev *et al.,* 2001). Mostly, 75% commercial enzymes are hydrolases including carbohydrases, proteases, pectinases and lipases. These enzymes catalyse the hydrolysis of natural organic compounds into glucose, peptides, glycerol and fatty acids, respectively (Underkofler *et al.,* 1957). The world commercial enzyme market was about 2 billion dollars in 2004 and reached to about 2.4 billion dollars in 2009. Lipases are about 4% of the world's enzyme market and due to their large scale applications in industrial sectors, special attention is given to lipases (Hasan, *et al.,* 2006). Several products based on bacterial lipases have been launched successfully in the market in the past few years. A number of such products are from *Pseudomonas* spp., such as Lumafast and Lipomax with their major application as detergent enzymes, while ChiroCLEC-PC, Chirazyme L-1 and Amano P, P-30 and PS have tremendous potential in organic synthesis (Jones and Richard, 1952).

Table 1. Purification strategies used for bacterial lipase purification

Pseudomonas spp	Purification strategies	Source
Pseudomonas putida 3SK	Ion exchange chromatography and gel filtration	Lee and Rhee (1993)
Pseudomonas aeruginosa KKA-5	Ammonium sulfate precipitation and chromatography	Sharon *et al.* (1998)
Pseudomonas fluorescens	Capillary membranes	Sztajer and Bryjak (1989)
Pseudomonas cepacia	Detergent-based aqueous two-phase systems	Saxena *et al.* (2003)

Lipases in the food. Lipases have broad applications in food industry and they are widely used in the production of variety of products e.g., fruit juices, baked foods, fermented vegetables, cheese, butter and soups. In 1976, Unilever found a method with a mixed hydrolysis and synthesis reaction to produce a cocoa-butter substitute using an immobilized lipase. This method is based on an immobilized lipase from *Rhizomucor miehei* and now used commercially by Quest-Londs Croklaam. This lipase is responsible for a trans esterification reaction replacing palmitic acid in palm oil with stearic acid to form the stearic-oleic-stearic triglyceride with the desired melting point for use in chocolate (Sharma *et al.,* 2001; Undurraga *et al.,* 2001; Coleman and Macrae, 1980). The esters produced from short-chain fatty acids have applications as flavouring agents in food industry (Gokbulut and Arslanoglu, 2013). Lipases are also used to give special flavour and taste to food by synthesis of fatty acids and alcohols, which are accepted as flavour and aroma compounds (Gandhi, 1997) for e.g., the lipase enzymes used in dairy industry for the lipolysis of butter fat and cream. Addition of lipases to such products primarily releases short- chain (C4 and C6) fatty acids that form sharp flavour but the release of medium-chain (C12 and C14) fatty acids leads to formation of a smooth taste (Saxena *et al.,* 1999). The majority of the commercial lipases are used for flavour development, in dairy products and processing of other foods like meat, vegetables, fruit, baked foods, milk products and beer. Lipases are widely used for the hydrolysis of milk fat in dairy industry. Lipase also enhances cheese ripening and the lipolysis of fat, butter and cream (Sharma *et al.,* 2001).

Lipases in detergents. Lipases are added to detergents used generally in household, industrial laundry and in household dish washers. About 1000 tonnes of lipases are sold annually in this area. Lipases catalyze the breakdown of fatty stains; into hydrophilic parts that are easily removed from similar non-hydrolyzed stains (Joseph *et al.,* 2007). Novo Nordisk, in 1994, introduced

the first commercial lipase called lipolase TM. Genencor International in 1995 produced two bacterial lipases Lumafast™ and Lipomax™ from *Pseudomonas mendocina* and *P. alcaligenes,* respectively (Gerritse *et al.,* 1998; Jaeger and Reetz, 1998). Lipases are suitable additives in detergents, because they have both thermophilic 30- 60 °C and alkalophilic pH 10-11 properties. Moreover, they have the abilities to function in the presence of the various components of washing powder formulations like surfactants and proteases. In addition, they have wide range of substrate specificities to hydrolyze fats of various compositions (Sharma *et al.,* 2001; Jaeger and Reetz, 1998).

Lipases in pulp and paper industry. The main source of paper and pulp industry is wood. Triglycerides and waxes are present in wood, causes serious problems in paper and pulp production. Lipases are used in this industry to remove triglycerides and waxes from pulp, processed for paper making (Table 2). Nippon Paper Industries in Japan introduced a system for the removal of triglycerides from wood based on the lipase of *Candida rugosa* (Sharma *et al.,* 2001; Gandhi, 1997).

Lipases in leather industry. One of the most important applications of the lipases is to remove subcutaneous fat and hair from animal skin in leather industry. The conventional methods for e.g., surfactants and organic solvents used for this purpose are harmful to environment due to the production of dangerous products such as volatile organic compound (Hasan *et al.,* 2006). On the contrary, lipases are safe, efficient and environmental friendly. Additionally, lipases are used along with other hydrolytic enzymes such as: proteases at alkaline pH in leather processing are a new approach currently emerging.

Lipases in environmental management. Bioremediation of waste products of lipid processing factories and restaurants are carried out by both ex situ and in situ (Pandey *et al.,* 1999).

Lipases in diagnosis. Lipases are used in clinical diagnosis of analytical samples to form glycerol from triacylglycerol which is then quantified either by chemical or enzymic methods. Patients with cardiovascular complaints are diagnosed very accurately with this approach. In this protocol glycerol dehydrogenase catalyze the hydrolysis of lipids by oxidation to produced glycerol. The NADH formed, during this reaction is measured by fluorescence spectroscopy (Benjamin and Pandey, 1997).

Lipases in pharmaceutical industry. Lipases are generally used in pharmaceutical industry for the synthesis of anti tumour agents, alkaloids, antibiotics and vitamins (Jaeger and Eggert, 2002). They are also used in anti-inflammatory drugs such as naproxen, ibuprofen ect. (Xin *et al.,* 2001; Chen and Tsai, 2000; Arroyo *et al.,* 1999; Ducret *et al.,* 1998; Xie *et al.,* 1998; El-Sawah *et al.,* 1995) and antihypertensive agents including angiotensin converting enzyme (ACE) inhibitors such as captopril, enalapril, ceranopril, zofenapril, lisinopril, and the calcium channel- blocking drugs such as diltiazem (Sharma *et al.,* 2001).

Table 2. Industrial application of microbial lipases.

Microorganisms	Industrial applications	Source
Rhizomucor miehei	Food industry	Sharma *et al.* (2001)
Pseudomonas mendocina	Detergent industry	Jaeger and Reetz (1998)
Pseudomonas alcaligenes	Detergent industry	Gerritse *et al.* (1998)
Candida rugosa	Paper and pulp industry	Gandhi (1997)

Conclusion

Lipases are an important group of hydrolases which play a key role in transport, digestion and processing of fats oil. Lipases are produced by different biotic agents including bacteria, human being and even viruses. Various purification strategies are discussed in this review for lipases. Due to their activities at different pH and temperature range, the importance of lipases is increasing day by day in several industries, such as food, detergents, chemicals, pharmaceuticals, etc. to replace the conventional catalyst by bio catalyst which is cheap, safe and environment friendly.

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