Review Article

A short review on extracellular lipase producing microorganisms

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Abstract

Objective: Among 4000 known enzymes, 200 are in commercial use. Among all commercial enzymes, 150 industrial enzymes (including lipases) catalyze hydrolysis of their substrate therefore they are hydrolytic in action. Lipases are the third largest group of industrial enzyme comes after amylases and proteases. Based on origin, lipases can be classified into three classes: animal, plant and microbial. Microbial lipases are mostly applied lipases due to their stability and chemical properties. Microbial lipases are secreted by numerous bacteria, yeasts, actinomycetes and moulds. Microbial lipases might be extracellular, intracellular and membrane bound. Extracellular secretion of lipases has been well documented for a variety of fungi and bacteria. Microbial lipases not only catalyze hydrolytic reactions but also reverse reaction (esterification) and transesterification, therefore demand of lipases are increasing day by day in various industries such as pharmaceutical, dairy, detergents, textile, fat, medical, cosmetic and food. Lipolytic microorganisms have been isolated from different habitats such as edible oil extraction factories, diesel and edible oil contaminated soil, industrial wastes, dairies, etc. **Conclusion:** In the present time, the biotechnological industries are more concentrating towards microbial lipases. To fulfill the current industrial demand of lipases, we have to identify and isolate the novel microbial sources of lipase. Therefore, the present review is focused on lipolytic microorganisms and their habitat.

Keywords: Lipase, fungi, bacteria, extracellular, hydrolysis

Introduction

Lipases are ubiquitous enzymes of great physiological importance and industrial potential. The catalytic action of lipases is to hydrolyze triacylglycerols into free fatty acids and glycerol.

$$\text{Triacylglycerol} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Free fatty acids}$$

In comparison to esterases, lipases show their catalytic action only after adsorption to a water-oil interface (Salah et al., 2007). In eukaryotes, lipases take part in different stages of metabolism of lipids such as digestion and absorption of fat and metabolism of lipoproteins. In plants, lipases are located in energy storage tissues. How the interaction of lipases and lipids occur at the interface is still not fully clear and is a matter of deep research (Balashev et al., 2001). Microbes are always preferred sources of lipases than those derived from plants and animals because (1) wide variety of catalytic activity; (ii) higher yield; (iii) easy genetic manipulation of microbes to produce the enzyme of desired property; (iv) Fast growth of microorganisms on cheap media; (v) regular supply of enzyme without depending on season; (vi) production of microbial lipase is more easy than plants and animals (Ray, 2012). By keeping the above benefits of microbial lipases and their industrial significance in mind, we have to always search novel sources of lipase from natural samples.

Sources of lipases

Lipases are found in both prokaryotes (bacteria and archaea) as well as in eukaryotes (animals, plants and fungi) (Cai-hong et al., 2008). There is no commercial use of plant lipases while lipases of the animal and microbial origin are utilized widely. The major source of animal lipase is the pancreas of sheep, pigs, cattle and hogs. The main problem with pancreatic lipases is that they cannot be utilized in the food industry for the processing of vegetarian food. Also, the contaminants found in pancreatic lipase preparations have unwanted effect. Lipase extract from pig pancreas contain trypsin which is a bitter tasting amino acid. Therefore, due to above mentioned reasons, microbes are

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the preferred choice for the production of 100 or so industrial enzymes (Kademi et al., 2003). Although numerous microorganisms are recognized as potent producers of extracellular lipases including fungi, yeast and bacteria but fungal lipases are being used for diverse applications in biotechnological industries (Singh and Mukhopadhyay, 2012; Sharma and Kanwar, 2014).

Bacterial lipases

Among bacterial lipases, those obtained from *Bacillus* sp. show attractive features that make them potent candidates for various applications in biotechnology based industries. *Bacillus subtilis* (Devi et al., 2012), *B. pumilus* (Sangeetha et al., 2007; Saranya et al., 2014), *B. licheniformis* (Chavan et al., 2012), *B. coagulans* (Lianghua and Liming, 2005), *B. stearothermophilus* (Abada, 2008), *B. amyloliquefaciens* (Selvamohan et al., 2012) and *B. alcalophilus* (Ghanem et al., 2000) are the most common bacterial lipases. In addition, *Pseudomonas* sp., *P. aeruginosa* (Borkar et al., 2009), *Burkholderia multivorans* (Gupta et al., 2007), *B. cepacia* (Padilha et al., 2012), *Staphylococcus caseolyticus* (Volpato et al., 2008), *S. pasteuri* (Aruna and Khan, 2014) have also been reported as bacterial lipase producers.

Fungal lipases

Fungal lipases have been studied since 1950s. They are capable of digesting fat and is characterized by their capability to hydrolyze fat over a broad range of pH and temperatures. These lipasesare utilized due to their cost effective extraction process, specificity to substrate and tolerance to different temperatures, pH, high concentration of organic solvents and metal ions. Filamentous fungi are preferred sources of lipases among the rest of the lipase producing microorganisms. The main producers of commercial lipases mainly are *Rhizopus* sp. (Martinez-Ruiz et al., 2008), *Pencillium* sp. (Gutarra et al., 2009), *Aspergillus* sp. (Abrunhosa et al., 2013), *Mucor* sp. (Hiol et al., 1999), *Candida rugosa* (Rajendran et al., 2008), *Acremonium alcalophilum* (Pereira et al., 2013), *Humicola lanuginosa* (Martinelle et al., 1995), *Cunninghamella verticillata* (Gopinath et al., 2002) and *Geotrichum candidum* (Burkert et al., 2005), *Trichoderma* sp. (Coradi et al., 2013). Lipases from *Aspergillus, Rhizopus* and *Geotrichum candidum* strains are attractive catalysts for lipid modification (Schuster et al., 2002).

The benefits of using filamentous fungi as industrial producers of extracellular lipases in comparison with the rest of lipase producing microorganisms are as follows: (i) capability to use broad range of lignocellulosic biomass residues as source of nutrients; (ii) ability to produce lipases extracellularly in the fermentation broth; (iii) separation of fungal mycelium is very simple from fermentation broth by vacuum filtration in comparison with yeast and bacterial biomasses and (iv) capability to produce lipases both in SSF and SmF (Toscano et al., 2011).

A. niger is one of the most imperative microorganisms exploited in biotechnology. It is known for its ability to secret numerous extracellular enzymes which are considered GRAS (Generally Regarded As Safe) (Rai et al., 2014).

Production of lipase by fungi varies and depends on the type of strain, ingredients of the production medium, sources of carbon and nitrogen and culture conditions such as temperature, pH, aeration Lipase produced by *Mucor* sp. is known for its thermostability and resistance to high alkaline conditions (Cihangir and Sarikaya, 2004). A list of the potent fungi for production of lipase in both solid state and submerged fermentation is presented in Table 1.

Yeast lipases

Lipase producing yeasts belong to seven different genera which include *Kluyveromyces, Candida, Pichia, Saccharomyces, Zygosaccharomyces, Torulaspora, Pseudozyma* and *Lachancea* (Romo-Sanchez et al., 2010).

Habitat of lipase producing microorganisms

Lipase producers have been isolated from diverse habitats such as vegetable oil processing factories, industrial effluents, soil contaminated with edible and diesel oil, dairies, decaying food and oilseeds, coal tips, hot springs and compost heaps (Veerapagu et al., 2013). Soil is a natural complex microhabitat for all living and nonliving components. A large number of plants and microorganisms reside in soil, which makes soil the world's largest reservoir of biological diversity. Soil is a principal habitat of several types of microorganisms (Bhavani et al., 2012). The diversity of such microorganisms depends on several physio-chemical properties related to the climate and type of soil viz. pH, texture, solar radiation, temperature, aeration, mineral composition and water contents. Soil contaminated with oils also possesses a diversity of microorganisms producing enzymes. These microorganisms are being exploited for their potential as lipase producers (Pandey et al., 2015). Extensive work on lipase producing microbes is available with main emphasis on cloning and expression of lipase genes, structural characterization, kinetic parameters, and enzyme action (Sharma et al., 2001). In contrast, comparatively little work has been done on screening of lipolytic microbes from edible oil contaminated soil. Therefore, soil samples from various oil mills were used for the isolation and screening of lipolytic bacteria by Veerapagu et al. (2013). Similarly, many investigators suggested the oily soil samples as the source for the isolation of lipolytic fungi (Narasimhan and

Table 1. Fungi cited in the literatures as potent lipase producers

Genus	Species	References
Rhizopus	*R. arrhizus*	Rajendran and Thangavelu, 2009
	R. chinensis	Wang et al., 2008
	R. oryzae	Minning et al., 1998
	R. homothallicus	Diaz et al., 2006
	R. oligosporus	Iftikhar et al., 2010
	R. japonicus	Paranjothi and Sivakumar, 2016
Penicillium	*P. citrinum*	D'Annibale et al., 2006
	P. restrictum	Azeredo et al., 2007
	P. simplicissimum	Gutarra et al., 2009
	P. verrucosum	Pinheiro et al., 2008
	P. aurantiogriseum	Pandey et al., 2015
	P. wortmanii	Costa and Peralta, 1999
Trichoderma	*T. harzianum*	Coradi et al., 2013
	T. viride	Kashmiri et al., 2006
Geotrichum	*Geotrichum sp.*	Burkert et al., 2004
Aspergillus	*A. carneus*	Kaushik et al., 2006
	A. oryzae	Zhou et al., 2012
	A. fumigatus	Shangguan et al., 2011
	A. sydowii	Bindiya and Ramana, 2012
	A. ibericus	Abrunhosa et al., 2013
	A. japonicus	Souza et al., 2014
	A. carbonarius	Dobrev et al., 2015
	A. nidulans	Niaz et al., 2014
	A. awamori	Basheer et al., 2011
	Aspergillus sp.	Cihangir and Sarikaya, 2004
	A. brasiliensis	Reshma and Shanmugam, 2013
	A. terreus	Sethi et al., 2016
	A. tamarii	Das et al., 2016
	A. niger	Toscano et al., 2011
Colletotrichum	*C. gloesporioides*	Sande et al., 2015
Cercospora	*C. kikuchii*	Costa-Silva et al., 2014
Eremothecium	*E. Ashbyii*	Kalindhi and Vijayalakshmi, 2015
Antrodia	*A. cinnamomea*	Shu et al., 2006
Candida	*C. utilis*	Rehman et al., 2014
	C. cylindracea	Brozzoli et al., 2009
	C. zeylanoides	Canak et al., 2015
	C. guilliermondii	Oliveira et al., 2014
Ophiostoma	*O. piceae*	Vaquero et al., 2015
Pseudozyma	*P. hubeiensis*	Bussamara et al., 2010

Valentin, 2015; Pandey et al., 2015). Sharma et al. (2016) reported screening of lipolytic fungal strains from soil samples collected from five different oil mills of Newai town. Potent lipolytic fungal strain was further identified as *A. niger*.

However, Different type of samples and varied sites were used by workers for isolation of lipolytic fungi. Bindiya and

Ramana (2012) reported isolation and identification of 66 lipolytic fungi from marine sediment samples, whereas Narasimhan and Valentin (2015) used the samples from sun flower oil refinery for screening of fungal isolates. Xia et al. (2011) have reported isolation of lipolytic fungi from soil samples collected from meat processing industry. Fan et al. (2013) reported isolation of lipolytic filamentous fungi from the samples collected from soyabaen bran and dairy industry sites and soil samples from potato field were selected by Niyonzima and More (2013) for isolation.

Conclusion

Lipases are most important enzymes among the other hydrolytic enzymes used in biotechnological industries. Microbes are preferred sources of lipases. We need to isolate the novel microbial sources of lipases in order to meet the demand of industries. Therefore, we must have knowledge about lipolytic microorganisms and their habitat.

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