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Review

An insight into plant lipase research – challenges encountered

Sonali Seth, Debamitra Chakravorty, Vikash Kumar Dubey, Sanjukta Patra *

Department of Biotechnology, Indian Institute of Technology Guwahati, Assam 781 039, India

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ABSTRACT

Lipases from bacterial, fungal, and animal sources have been purified to homogeneity with very few of them being contributed from plants. Plant lipases are mostly found in energy reserve tissues, for example, oilseeds. They act as biocatalysts which are attractive due to their high substrate specificity, low production cost and easy pharmacological acceptance due to their eukaryotic origin. Hence plant lipases represent better potential for commercial applications in organic synthesis, food, detergent and pharmacological industries. However, low expression, uneconomical fold purity and the plethora of difficulties related to their recombinant expression has been limiting their commercial applicability and posing challenges to many researchers. This article focuses on comprehensive approaches that have been reported to date to address these challenges.

Contents

Introduction

The present enzyme industry is a result of modern biotechnology boom. The world market for enzymes is expected to reach $7 billion by 2013 [1]. A major fraction of the enzyme industry is represented by lipases (EC 3.1.1.3). Lipases are versatile because they can perform both hydrolysis and synthesis reactions which are chemo-selective, regio-selective and enantio-selective. In addition, in water restricted environments, lipases can perform esterification and transesterification reactions [2]. Despite the extensive range of microbial lipases, the use of these enzymes on an industrial scale is still limited due to their high production costs as they need to be grown in fermenters and further downstream processing and product formulation adds to cost. The technological load to implement a microbe based lipase product stands high. Further, they also have acceptability issues. This promotes the search for other sources of the enzyme [3]. Lipases have been isolated from

* Corresponding author. Tel.: +91 361 2582213; fax: +91 361 2582249.
E-mail address: sanjukta@iitg.ernet.in (S. Patra).

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Table 1
Uniprot ID and properties of characterized plant lipases.

Species	Uniprot ID of sequence	Mol. wt. kDa	Opt. pH	Opt. temp. °C	Substrate	Organic solvent	Inhibitor	Co-factor	Application	References
Glycine max	K7N361	NA	8	24	Olive oil	NA	EDTA Hg^{2+}	Ca^{2+} Mg^{2+}	NA	[10]
Hordeum vulgare var. distichum	F2ECV3	Not characterized							Synthesis of flavour esters	[11]
Jatropha curcas	B6V3M3 D2CGD5	NA	7.5	37	Palm oil	NA	Fe^{3+}	Ca^{2+} Mg^{2+}	Biodiesel production	[12]
Oryza sativa	A2XRD4 Q6ZIA4	9.4	11	80	Trioein	NA	CHAPS, digitonin, SDS, NP-40, Trition X-100 Mg^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}	Potassium acetate, sodium acetate, NaCl	Hydrolysis	[13,14]
Phaseolus vulgaris	Q7X9E8	NA	7.0	NA	Triacetin	NA	Ca^{2+}	Tween-20	NA	[15]
Ricinus communis	E7BKQ9 Q5VKJ7	62	9	NA	Triricinolein	Benzene, hexane, isooctane	Deoxycholate, octylglucoside, Triton X-lG0	Na^{+} K^{+}	Esterfication reactions for production of triacylglycerosl	[16,17]
Sorghum bicolour	C5YDR3	Not characterized							Fermentation	[18]
Triticum aestivum	Q8L5T0	143	8	37	NA	NA	diethylpyrocarbonate and dicyclohexylcarbodiimide	NA	Chiral syntheses; enantioselectivity; kinetic resolution; synthesis of ethyl esters	[19]
Zea mays	B6SZ60 B6TYC3	65	NA	NA	Triolein	NA	$CaCl_2$, EDTA, phosphate	NaCl	Synthesis of ethyl esters as a flavouring agent	[20,21]
Brassica napus	K4IYE1	34	7.0	37	NA	NA	Fe^{3+}, Fe^{2+}, Zn^{2+}, Hg^{2+} Cu^{2+}	Ca^{2+} Bi^{3+}	NA	[22]
Cucumis melo subsp. melo	E5GBX1	NA	6	31–40	NA	di-isopropyl ether, n-heptane, cyclohexane, cyclohexanol	NA	Na^{+} Ca^{2+}	NA	[23]
Elaeis guineensis var. tenera	G8FGP5 K4NZ15	NA	4.5	30	NA	NA	Sodium cyanide, resorcinol, cholesterol, lecithin and glycylglycine	Phenol, L-cysteine and EDTA	NA	[12,24]

various sources: bacterial (45%), fungal (21%), animal (18%), plant (11%), and algal (3%) [4]. While looking for advantages like low cost, specific applications, easy acceptability and their direct application as biocatalyst with partial purification, plants can be a novel source. Lipases in plant tissues mainly include non-specific lipid acylhydrolases, phospholipases A1, A2, B, C, D, monoacylglycerol, and triacylglycerol lipases. TAG[1] lipases are mainly concentrated in seeds as energy reservoirs [5,6]. They are stable in pH range of 4.0 to 9.0, temperature range of 25 °C to 60 °C and have varied molecular weight from 19 to 270 kDa [7]. Recently seed lipases have been the focus of attention as biocatalysts. In fact, lipases present in the crude extract from plant sources can directly catalyze hydrolysis or synthesis reactions of lipids which is one of the advantages [8]. They also have very specific applications which have been taken up in a later part of the review. However, there is a different side of the coin also. Very few plant lipases have been explored due to the complications of laborious purification steps [9]. This is due to their low expression in the reported tissue and the loss of activity when traditional purification strategies are employed. Until date only twenty-nine plant lipase sequences have been reported in UniProtKB. For the IDs Q9ZTW1, Q1H5B7, F4JT30, I1GX39, D7M611, F1AM71, F1AM70, Q672R1, A5B6N6, K3Y1Q2, K4B7D9, M1A9J4, L0AUJ8, B9IAL4, G7L902, G7KSR9, J3MG33, I1QLL3, A9QVW1, Q7F959 only temperature and pH optimisation has been carried out. We have tried to include the rest of the Uniprot IDs in Table 1 along with their till date research update. This reflects a lot is yet to be done in plant lipase research. This paper focuses on the potential application of plant lipases, challenges encountered in plant lipase research with their intended solutions to make them available for industrial uprise.

Despite the limited research on plant lipases till date, they have been explored for several specific applications. To endorse the research significance some exclusive applications of plant lipases have been discussed here. It can be said that these lipases can be explored from food industry to pharmaceutical industry and organic synthesis.

Food industry

The advantage of using plant lipases in food industries as compared to other sources of lipase is their acceptability in comparison to microbial lipases. Plant lipases have improved stability in solvent catalyzed reactions such as interesterification. Additional importance of plant lipases are in their low cost of production and downstream processing. In this context, *Carica papya* lipase (CPL) has been used successfully by Mangos et al. in the synthesis of low-calorie short and long-chain triacylglycerols (TAG) for use in infant formulae [25]. Papaya lipase has also been used to produce structured triacylglycerols using interesterification reactions of ethyl esters with tripalmitin [26]. The unavailability and high cost of human milk fat can be compromised through the synthesis of human-resembling milk fat by carrying out transesterification of tripalmitin with fatty acids of rapeseed oil using papaya latex [27]. Recently, human milk fat substitute has been synthesized using CPL self-immobilized in papaya latex as a biocatalyst and used as a low-cost alternative to commercial lipases [28]. Other

[1] *Abbreviations used:* TAG, triacylglycerol acylhydrolase; HMFS, human milk fat substitutes; CPL, *Carica papaya* lipase; sn-BSP, 1-butyroyl-2-stearoyl-3-palmitoyl-sn-glycerol; TG, triacylglycerol; GA3, gibberellic acid; ABA, abscisic acid; BR, brassinosteroids; PMSF, phenyl methane sulfonyl fluoride; FA, fatty acid.