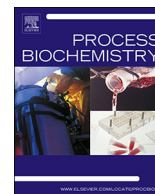




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Review

Novel lipases discovery specifically from marine organisms for industrial production and practical applications

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ABSTRACT

Lipases are acknowledged as relevant biocatalysts for numerous important applications in food, detergents and pharmaceutical processing, ester and peptide synthesis, transesterifications, biosurfactant production, and in the resolution of racemic mixtures to produce optically active compounds. Lipases are produced by organisms of microbial, plant, and animal origin. Microbial lipases have attracted far more interest from researchers and industries than lipases from other sources, due both to their specific features and ease of production on large scale. Notwithstanding current achievements, there is still a quest for lipases with improved/novel catalytic features and improved stability, namely in harsh environments. Marine organisms can be an adequate source for such lipases as marine enzymes have proved useful for both process improvement and for the development of new manufacturing procedures/new products. The production of lipases is influenced by the composition of the culture medium, e.g. carbon and nitrogen sources, as well as by physical chemical parameters such as temperature and pH. In this review relevant, types of lipases from marine organisms are identified, their role is described and the novel features of these enzymes are discussed.

1. Introduction

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are serine hydrolases that catalyze the hydrolysis of triacylglycerols (TAGs) into glycerol and fatty acids, the synthesis of esters from various alcohols and fatty acids, and transesterification and aminolysis reactions [1–5].

Triacylglycerols are predominantly uncharged insoluble lipids, safe for those esterified with short-chain fatty that are sparingly water-soluble [6,7], that are usually hydrolyzed by TAG-lipases [8–11]. Both lipases and esterases (carboxylesterases, E.C. 3.1.1.1) promote the hydrolysis of carboxylic esters, yet lipases are most active on long-chain fatty acids, namely those comprising acyl chains of more than ten carbon atoms [12,13]. Lipases constitute the third largest enzyme group, after proteases and carbohydrases, based on their market value [2]. Lipases are useful enzymes, with applications in medicine, pharmaceuticals, cosmetics, leather, fine chemicals, detergents, paper manufacture and feed and food industries and in wastewater treatment [6,14–17]. Lipases often have multiple roles, as in the food industry, where they are involved in the improvement of the quality of bread

through changes in the flour lipids; in the enhancement of flavor of butter, cheese, and margarine; and in the synthesis of structured lipids for baby foods. Furthermore, lipases are useful in increasing the titer of polyunsaturated fatty acids in vegetable oils and in the improvement of the digestibility of natural lipids (Table 1) [3,6]. Plenty of microorganisms have been identified as lipase producers, yet the genera *Rhizopus*, *Candida* and *Rhizomucor*, among fungi, and the genera *Pseudomonas* and *Chromobacterium*, among bacteria, are the most common sources for the production of commercial lipases [12,18].

Marine microorganisms are known to be involved in the degradation of organic matter through specific pathways, and often rely on extracellular enzymes to produce low molecular weight products that can be used as substrates. Production of enzymes by marine microorganism under controlled, commonly used laboratory conditions can be challenging as these conditions often differ from those in natural habitats (Fig. 1).

A multi-parameter optimization is often required, as reported in the production of an extracellular lipase by the marine fungus *Aspergillus sydowii* [19]. Many marine microorganisms are extremophiles that live

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Table 1
Relevant applications, function and mechanisms of marine lipases.

Application	Function	Mechanism	References
Dairy Industry	To improve flavor and speed up the ripening of cheese	Modification of lipids by releasing fatty acids from triglycerides (flavor improvement). Increased accumulation of FFA through lipolysis (accelerated ripening)	[5,24]
Bakery	flavor enhancement, prolonged shelf-life, improved texture and softness of baked goods	Esterification with release of short chain fatty acids	[25,26]
Production of biodiesel	Chemical modification of vegetable oils to yield compounds compatible with most engines	Transesterification of vegetable oils with methanol/ethanol	[4,27]
Functional foods and nutraceuticals	Improve the nutritional features of vegetables and fish oils through the incorporation of EPA and DHA	Ester synthesis through acidolysis	[28]
Household	Additives in detergents for washing at low temperatures. Removal of fatty stains	Hydrolysis	[29]
Fats and oils	Adding value to low-cost oils and fats	Lipases involved in altering the properties of lipids by hydrolysis, esterification, and transesterification; increased melting points	[30]
Meat and fish	Flavor and nutritional improvement	Decrease of fat content through hydrolysis	[26]
Pharmaceutical industry and medical applications	Production of pure enantiomers through resolution of racemic mixtures	Regio-, chemo- and enantioselective nature of lipases, that allow the resolution of racemates, With no need for cofactors. Lipases are often compatible with organic solvents and thermostable, easing process design and productivity by allowing operation at high substrate/product concentrations	[4,31,32]

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FFA: free fatty acids.

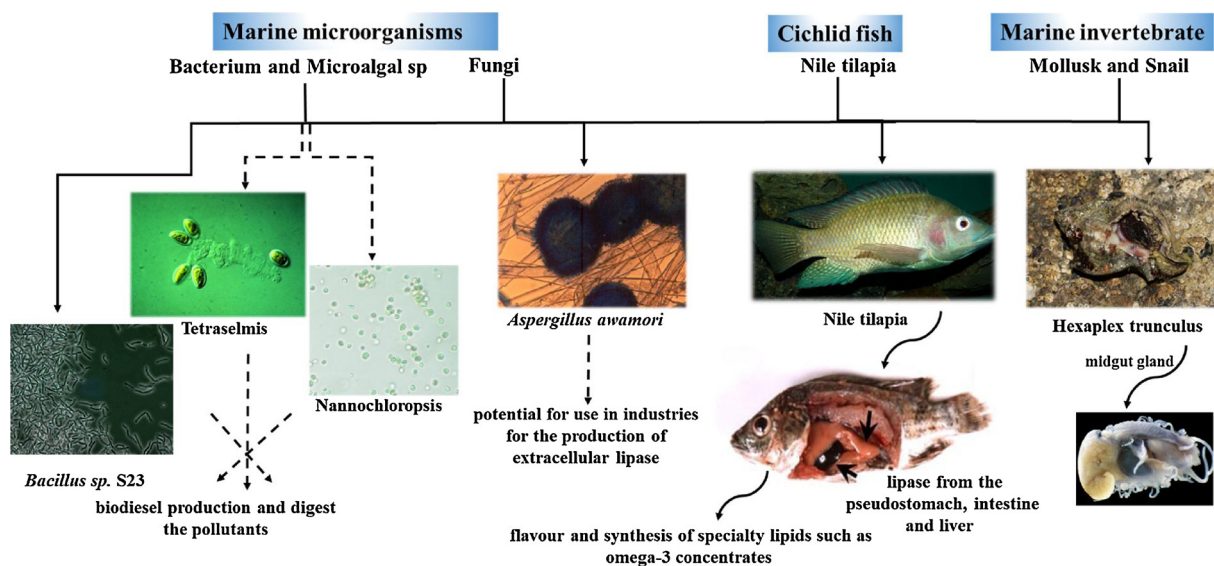


Fig. 1. Production of lipase by marine organisms with unique features for application in industry, medicine and biotechnology. The lipase digestive enzymes of *Hexaplex trunculus* is involved in the absorption of nutrients, food storage and excretion. Marine microalgae such as *Tetraselmis* and *Nannochloropsis* have been acknowledged as producers of lipase and their biomass as a potential sustainable source of biofuel, as lipase activity can enhance the yield of biodiesel.

in harsh environments such as deep sea hydrothermal vents, polar seas or extreme saline bodies. These microorganisms produce extremozymes that remain active under assorted conditions, often too severe for most enzymes. For instance, microorganisms isolated from cold environments have been reported to produce psychrophilic lipases [5,20], such as the lipase from *Janibacter* sp. strain HTCC2649, active on mono- and diacylglycerols and stable in the presence of several metal ions [21]. Enzymes from marine microorganisms have been considered potentially useful for industrial applications in the sectors of food, textile, cleaning, environmental, and pharmaceuticals (Table 2) [6,22,23].

2. Synthesis of lipase by marine fungi

The estimated number of species of marine fungi is about 10,000, although the current total of identified species is still only in excess of 1100 (<http://www.marinefungi.org/>, assessed on February 23, 2018), roughly doubling the number of species identified in 2010 [33]. Part of these species inhabit deep seas where they endure extreme environmental conditions [34–36]. It is likely that their metabolites, including

enzymes, are active and stable under extreme conditions, e.g. temperature, pH, pressure and salinity [34–37].

2.1. Marine filamentous fungi as lipase producers

Lipases are produced by almost all living organisms as fat-degrading enzymes [36]. Filamentous fungi are recognized as the best source of extracellular lipases for industrial scale production, namely species from genera *Rhizopus*, *Mucor*, *Geotrichum*, *Scopulariopsis*, *Trichoderma*, *Endosmosis*, *Penicillium* and *Aspergillus* [4,65]. Research on the isolation of lipases from marine fungi is still relatively recent, but it has already produced some promising results. For example, a marine fungus isolated from the Arabian Sea in the Indian coastline and classified as *Aspergillus awamori* BTMFW032, was found to produce an extracellular lipase that could decrease up to 92% of the fat and oil content of the polluted effluents with oil [2]. Potumarthi and co-workers screened marine soil samples collected near an oil extraction platform in the Arabian Sea for lipase producing microorganisms. The identification of lipase producers was performed by incubation in selective solid media.

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