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Biotechnological Applications for Microalgal Enzymes

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Abstract

Enzymes are crucial components of biological reactions and are used to scale and optimise many industrial processes. Many research are currently focusing their emphasis on more renewable and environmentally sustainable sources for the synthesis of these enzymes, due to the growing commercial demand for novel and more efficient enzymes to assist further improve these processes. Microalgae are particularly promising in this regard since they can be grown in photobioreactors, allowing for the generation of large amounts of biomass at a low cost. The rising number of publications in this area, particularly in the utilisation of microalgae as a source of new enzymes, reflects this. Varied microalgal enzymes with various industrial uses (e.g., lipids and biofuel production, healthcare, and bioremediation) have been investigated to date, and modifying enzymatic sequences implicated in lipid and carotenoid production has yielded encouraging results. However, in many situations, the entire biochemical pathways/systems leading to the synthesis of potentially essential bioactive chemicals are still unknown (e.g., for the synthesis of polyketides). Nonetheless, current breakthroughs in microalgal genomics and transcriptome techniques are making it easier to find sequences encoding targeted enzymes, boosting the chances of their identification, heterologous production, and characterization. This review examines the current state of the art in marine and freshwater microalgal enzymes with potential biotechnological uses, as well as the field's future prospects.

***Keywords:** Microalgae; Enzymes; Marine biotechnology; -Omics Technologies; Heterologous Expression; Homologous Expression*

Introduction

Around 71 percent of the Earth's surface is covered by water, with salt water accounting for 96.5 percent of it. Water encompasses (and frequently participates in) every chemical process that is biologically relevant due to its molecular structure and chemical characteristics. Enzymes play a crucial part in such reactions: They are

biologically active organic macromolecules that catalyse processes. Enzymes are widely employed to improve, scale, and optimise industrial production in a variety of industries (including food processing, detergent, medicines, biofuel, and paper manufacture) due to their substrate specificity. Hydrolases, for example, are enzymes that catalyse the hydrolysis of chemical bonds and have uses in a variety of industries. Cellulases for biofuel production, amylases for syrup manufacture, papain, phytases, and galactosidases for food processing and pharmaceutical uses are examples of industrially relevant hydrolases [7]. Microalgae are photosynthetic unicellular organisms that may be mass-cultivated in photobioreactors under controlled conditions with relatively little amounts of micro- and macronutrients, making them ideal for this market. Microalgae are still used in a variety of biotechnological processes. This tendency is plainly obvious when searching the existing literature in the PubMed database. When looking at the entire 20-year period between "1999–2018," it is obvious that, starting in 2012, the number of articles containing both "microalgae" and "biotechnology" has increased rapidly, peaking in the years 2015–2016. The literature on microalgal biotechnological applications is dominated by four main research areas: (1) direct use of microalgal cells for bioremediation and as food supplements; (2) extraction of bioactives for various applications (e.g., cosmeceutical, nutraceutical, and pharmaceutical applications, and biofuel production); (3) use of microalgae as platforms for heterologous expression or endogenous gene editing and overexpression; and (4) use of microalgae as platforms for hetero Due to the high costs of enzyme extraction and characterisation, as well as the rarity of annotated microalgal genomes, the latter field appears to be less well-studied than the others.

Microalgae-derived enzymes

Triglycerides (TAGs) and poly-unsaturated fatty acids (PUFA) are the most investigated from a biotechnological standpoint, particularly for the manufacture of biodiesel and nutraceuticals. Following acid- or base-catalyzed transesterification processes, TAGs, esters produced from glycerol and three chained fatty acids (FA), which are normally stored in cytosol-located lipid droplets [19], can be utilised to produce biodiesel [20]. PUFAs, on the other hand, have been shown to provide health benefits, particularly omega-3 fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The acyl-CoA diacylglycerol acyltransferase (DGAT), which is engaged in the last step in the TAG biosynthetic pathway, is the most studied enzyme involved in lipid synthesis. The acyl-CoA diacylglycerol acyltransferases type 1, 2, and 3 (DGATs 1-2-3) enzymes are involved in the acyl-CoA-dependent synthesis of TAGs from their precursor sn-1,2-diacylglycerol (DAG) [25]. Each DGAT isoenzyme contributes differently to the fatty acid profile of TAG in different species. Guo et al. found a gene encoding DGAT1 in the green alga Chlorella ellipsoidea, and an experiment involving overexpression of DGAT1 was then carried out in the oleaginous microalga Nannochloropsis oceanica. The first DGAT2 sequence was discovered in the green alga Ostreococcus tauri, and many investigations using DGAT2 overexpression were carried out. In the diatoms Phaeodactylum tricornutum [30] and Thalassiosira pseudonana, as well as the oleaginous microalgae Neochloris oleoabundans and N. oceanica, DGAT2 overexpression increased TAG synthesis. Different DGAT2 isoforms (NoDGAT2A, 2C, and 2D) have been discovered in N. oceanica, as well as various combinations of overexpression and underexpression, have been investigated. Different fatty acid synthesis profiles resulted from these combinations, with some suited for nutritional applications and others for biofuel reasons [34]. Even though the green alga Chlamydomonas reinhardtii is a

typical biofuel feedstock, it showed no apparent trends after overexpression of various DGAT2 isoforms, with increasing TAG levels in some investigations [35], but not in others [36]. Cui and colleagues [37] recently identified a dual-function wax ester synthase (WS)/DGAT enzyme in P. tricornutum, whose overexpression resulted in an accumulation of TAGs and wax esters.

Temperature

Because just a few eukaryotes are well suited to high temperatures, temperature has a significant impact on biota distribution. In both oxy and anoxic settings, it regulates the dispersion of phototrophic organisms [14]. The temperature of the fluids in SHVs ranges from 10 to 119 °C, with temperatures reaching 95.8 °C in the sediments. The temperature of the fluids in DHVs can reach 400 degrees Celsius, while seawater seeping deep into the earth's crust can produce fluids with a temperature of 1200 degrees Celsius [20]. Low temperature (up to 50 °C), medium temperature (up to 200 °C), and high temperature (up to 400 °C and higher) are the most common classifications for venting fluids [21]. Despite the harsh conditions, it has been discovered that the majority of the biota linked with vents lives at temperatures between 10 and 25 °C. Shallow and deep Mediterranean HVs are equally represented, with temperatures exceeding the boiling point of water (see Vulcano and Panarea Islands), but never exceeding 135 °C, owing to their proximity to 1200 m. Unlike DHVs, which rely solely on chemosynthesis for primary production, SHVs rely on photosynthesis as well, thanks to the availability of light radiation. The SHVs are model sites for studying global environmental changes such as ocean warming and acidification due to the cohabitation of these two metabolisms, high CO2 concentrations, and better accessibility. Other genes, including as glucose-6-phosphate dehydrogenase (G6PD), 6-desaturase, 6-phosphogluconate dehydrogenase (6PGD), glycerol-3-phosphate acyltransferase (GPAT1-GPAT2), and acetyl-CoA synthetase 2, have been targeted in order to boost high-value added lipid production (ACS2). Increased lipid content was observed when these enzymes were overexpressed. Two patents for desaturases have been submitted in specific. One is a Nannochloropsis spp. 6-desaturase enzyme that transforms linoleic acid to -linolenic acid (GLA) and -linolenic acid (ALA) to stearidnoic acid (Patent Code: CN101289659A, 2010). The other is a 6-desaturase enzyme found in Arctic chlamydomonas sp. ArF0006, which converts oleic acid to linoleic acid. Gene disruption has been used in the past to boost lipid production and/or modify lipid profiles. In C. reinhardtii, CRISPR/Cas9 ribonucleoproteins were used to knock out the PLA2 gene, microRNA silencing of the stearoyl-ACP desaturase (which generates oleic acid by adding a double-bond to a lipid chain) in C. reinhardtii [45], and meganuclease and TALE nuclease genome alteration in P. tricornutum This method involved changing the expression of seven genes that could affect lipid content (UDP-glucose pyrophosphorylase, glycerol-3-phosphate dehydrogenase, and enoyl-ACP reductase), acyl chain length (long chain acyl-CoA elongase and a putative palmitoyl-protein thioesterase), and fatty acid saturation (-3 fatty acid desaturase**.** Unlike DHVs, which rely solely on chemosynthesis for primary production, SHVs rely on photosynthesis as well, thanks to the availability of light radiation. The SHVs are model sites for studying global environmental changes such as ocean warming and acidification due to the cohabitation of these two metabolisms, high CO2 concentrations, and better accessibility. SHV hydrothermal fluids are typically high in CO2 (95–98 percent of total fluid composition), present as gas bubbling, with little sulphide and methane coemission. The active emissions of these CO2-dominated fluids change the carbonate chemistry of the

saltwater around the HVs, leading in pH values lower than ambient seawater (acidification), having consequences for the entire ecosystem. Furthermore, HVs have a broad variety of inorganic chemical compounds generated by hydrothermal activity, which contributes to their mineralogical richness and complexity. Minerals like these could operate as inorganic surfaces that encourage the synthesis of organic molecules, but they could also create chemical gradients that encourage the interaction of electron donors and acceptors. Because HVs expose entire communities to a lifetime of increased CO2 levels, they serve as natural laboratories for measuring the effects of ocean acidification on the structure of marine ecosystems. The chemical characterization of extreme compounds is the most difficult phase, but it is also the most important for understanding their properties and determining the best application for them. The significant difficulty of determining EPS necessitates the creation of new purification protocols, microscopic examination, and sensitive spectroscopy approaches. Furthermore, carboxylesterases, lipases, and cellulases have been demonstrated to be particularly appealing enzymes for a variety of chemical sectors, including food, laundry, and pharmaceuticals. The major issue in enzyme research is finding compounds with new enzymatic activity and increased stability, and deep hydrothermal vents, as recently reviewed by Jin et al., provide an excellent paradigm for this type of research.

Conclusion

The importance of marine hydrothermal vent systems as ecological and bioprospecting models is highlighted in this paper, which focuses on the Mediterranean region. We demonstrated here that most studies focus on shallow systems, whereas knowledge of deep systems is more fragmented, both in terms of bacterial diversity and isolation of new physiologically active compounds. In terms of microbial communities, archaeal populations are understudied in both shallow and deep settings. Other inventive techniques to filling the existing gaps in the topic could also be beneficial. This is the case with new generation robotics and census actions based on Citizen Science and Local Ecological Knowledge, which are appropriate tools for involving not only researchers but also the general public. By following these tactics, it will be feasible to continue exploring the seafloor and find additional shallow places and hydrothermal emissions that are easier to reach for divers.

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