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Review Article

Are Algae the Future Source of Enzymes?

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HIGHLIGHTS

- Various proteins and enzymes are produced during algal photosynthesis.
- Algae use phosphoglycolate phosphatase and glycolate oxidase as metabolizing enzymes.
- Algae possess the ability to produce commercial enzymes.
- Out of the 10,000 algae species, only a few are cultivated on an industrial scale.
- Algal wastes can be manipulated and recycled for production of various enzymes.

ABSTRACT

Various proteins and enzymes produced during algal photosynthesis can be used in economic development and environment management, such as in wastewater treatment, production of fine chemicals, and biodiesel production. This mini-review presents various enzymes isolated from algae and suggests that algae, given their unique properties, could be explored for large-scale production of enzymes as future biocatalyst factories.

Introduction

Nearly two billion years ago, oxygen-producing cyanobacteria and green algae made Earth's atmosphere suitable for the evolution of aerobic metabolism and complex life (Castresana and Saraste, 1995). Various proteins and enzymes are produced during algal photosynthesis, such as proteins associated with light harvesting, carboxylating enzymes, glycolate-metabolizing proteins, and enzymes involved in protection from reactive oxygen species. Most cyanobacteria and red algae involve phycobilin light-harvesting pigment-protein complexes that occur to the exclusion of chlorophyll-based complexes. The bifunctional enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase is applied to catalyse the reactions of carbon fixation and photorespiration by carboxylation or oxygenation of ribulose 1,5-bisphosphate (Marcus and Gurevitz, 2000). Algae also use phosphoglycolate phosphatase and glycolate oxidase or glycolate dehydrogenase as metabolizing enzymes. Superoxide dismutases, ascorbate peroxidase, and glutathione peroxidases are the key applied enzymes for the removal of active oxygen species generated predominantly by algal photosynthetic processes (Raven et al., 1999). In addition, algae use several enzymes to produce starch, sucrose, and glycogen-including carbonic anhydrase, phosphoglycerate kinase, dehydrogenase, fructose-1,6-biphosphatase, sucrose phosphorylase, sucrose phosphate synthase, sucrose-phosphate hydrolase, glycogen synthase, and starch synthase (González-Fernández and Ballesteros, 2012). Some studies reported the ability of cyanobacteria to produce numerous biological catalysts—for example, amylase, cellulase, lipase, urease, lactamase, L-asparaginase, L-glutaminase, arylsulphatase, and chitinase (Chakdar et al., 2012). Recently, investigations on marine algae have shown that they develop the production of a broad spectrum of bioactive peptides and

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Table 1. Characteristics of some enzymes isolated from algae.

Enzyme	Microalgae	Km (mM)	MW (Da)	Optimal pH	Optimal Temperature (°C)	Inhibitor	Reference
5′-Adenylylsulfate reductase	*Enteromorpha intestinalis*	-	-	-	25	-	Gao et al. 2000
Carbonic anhydrase	*Anabaena flos-aquae*	15.6	-	-	-	Diamox	Ingle & Golman 1975
Carbonic anhydrase	*Chlamydomonas reinhardtii*	-	76000	-	-	azide	Sültemeyer 1998
Δ_4-Desaturase	*Pavlova* sp.	-	-	-	-	-	Pereira et al. 2004
Glucose-6-phosphate dehydrogenase	*Anacystis nidulans*	0.37	-	6.7	25	ATP	Grossman & Mc Gowan 1975
Glycolate dehydrogenas	*Chlamydomonas* sp.	220	-	8.5	25	N-ethylmaleimide	Nelson & Tolbert 1970
Glycolic acid oxidase	*Anabaena flos-aquae and Oscillatoria* sp.	-	-	8.0	28	sodium hydroxyethanesulfonate	Grodzinski & Colman 1970
Hydrogenase	*Chlamyabmonas moewusii*	-	-	7.0	-	Carbon monoxide	Ward 1970
Hydroperoxide lyase	*Oscillatoria* sp.	0.007	56000	6.4	30	Quercetin	Andrianarison et al. 1989
Isocitrate lyase	*Polytomella caeca*	-	-	-	-	-	Haigh & Beevers 1964
Laccase	*Tetracystis aeria*	0.051	212000	8.0	-	-	Otto et al. 2010
β-Lactamase	*Coccochloris elabens*	-	-	-	-	-	Kushner & Breuil 1977
Lipoxygenase	*Oscillatoria* sp.	-	124000	8.8	25	Esculetin	Beneytout et al. 1989
Nitrogenase	*Anabaena cylindrica*	-	220000	-	-	-	Haystead et al. 1970
phosphoenolpyruvate carboxylase	*Anabaena flos-aquae*	-	-	-	-	-	Colman et al. 1976
D-Ribulose-1, 5-diphosphate carboxylase	*Agmenellum quadruplicatum*	-	456000	-	-	-	Tabita et al. 1974; Tabita et al. 1976
Ribulose bisphosphate carboxylase	*Cylindrotheca* sp.	0.0087	560000	-	-	-	Read & Tabita 1994
Ribonucleotide reductase	*Scenedesmus obliquus*	-	-	6.5	30	dATP	Feller & Follmann 1976
Superoxide dismutase	*Euglena* sp.	-	-	8.5	25	Cyanide	Asada et al. 1977

proteins required for human nutrition supplementation and pharmaceuticals (Samarakoon and Jeon, 2012). Wide ranges of algal modifications on natural compounds have been reported. These have been carried out via various mechanisms, such as hydroxylation, reduction, side-chain degradation, and isomerization (Faramarzi et al., 2008; Arabi et al., 2010). Moreover, the ease of culturing and simple growing conditions led to the application of algae in removing some organic contamination from aquatic ecosystems (Forootanfar et al., 2013).

Isolated enzymes from algae

A brief overview of the number of scholarly publications associated with algae-isolated enzymes in the last decade shows rising interest in using algae as a potential source

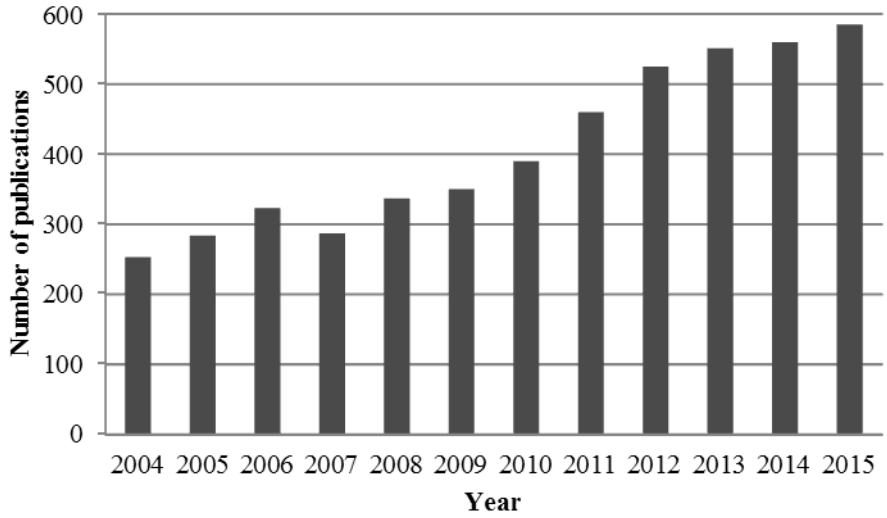

Figure 1. Number of publications related to algal enzymes, based on data obtained from Scopus (accessed 03 Agust, 2016).

of enzymes (Fig. 1). Increasing industrial application of biocatalysts that can modify industrial process conditions lead to considerable efforts in investigating the large-scale production of enzymes. Although enzymes are currently used in a wide range of biotechnological and industrial applications, the present enzyme content is still not adequate to meet all demands (Mogharabi and Faramarzi, 2014). While enzymes isolated from plant and animal sources are less stable than microbial enzymes, algae appear to be a good alternative source of enzymes as they can be cultivated in large quantities within a short time by fermentation and because they are susceptible to gene manipulation. Table 1 shows the characteristics of some isolated enzymes from algae. Superoxide dismutase, an essential component in the biological defence against oxygen toxicity, was isolated from red and blue-green algae, such as *Porphyridium cruentum* and *Plectonema boryanum* (Asada et al., 1975; Misra and Fridovich, 1977). Carbonic anhydrase activity was reported in four species of blue-green algae, including *Anabaena flos-aquae*, *Anacystis nidulans*, *Coccochloris peniocystis*, and *Oscillatoria* sp. (Ingle and Colman, 1975). Cyanobacterial species and some unicellular green algae are able to use urea as a nitrogen source, and conduct urease and urea amidolyase activity. Urease activity is dependent on the nickel concentration observed in the cyanobacterium *Anabaena cylindrica* (Mackerras and Smith, 1986). Although laccases are usually found in higher plants, fungi, and insects (Forootanfar et al., 2011), the production of an extracellular laccase-like enzyme was reported in the coccoid green soil alga *Tetracystis aeria* (Otto et al., 2010). The partial purification of glucose 6-phosphate dehydrogenase has been reported from *Anabaena flos-aquae* and *Anacystis nidulans* by ammonium sulphate fractionation and exclusion gel chromatography (Grossman and Mc Gowan, 1975). Andrianarison et al. (1989) isolated hydroperoxide lyase, which converts the conjugated diene 13-hydroperoxide of linoleic acid to 13-oxotrideca-9,1-dienoic acid from the blue-green algae *Oscillatoria* sp.

Large-scale production of algal enzymes

In recent years, interest in a variety of renewable biofuels has increased due to the instability of petroleum fuel costs and environmental hazards associated with an increasing atmospheric carbon dioxide level. There are currently wide global research efforts focused on increasing and modifying the accumulation of alcohols, hydrocarbons, lipids, polysaccharides, and other energy-storage compounds in algae. Significant advances in algae genomics that have been achieved during the last decade suggest algae to be a potential low-cost host for the production of enzymes, recombinant proteins, and novel metabolites. Several nuclear genomes have been sequenced, including *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Cyanidioschyzon merolae*, *Ostreococcus lucimarinus*, *Ostreococcus tauri*, and *Micromonas pusilla* (Radakovits et al., 2010). During the last decade, DNA transformation procedures for several algae spices have progressed, and organellar transformation systems for the green alga *C. reinhardtii* have led to interesting studies on gene function and expression. The first attempts that expressed foreign enzymes in the chloroplast of the single-celled eukaryotic alga *C. reinhardtii* involved the bacterial

neomycin phosphotransferase and β-glucuronidase genes. Blowers et al. (1989) reported the bacterial neomycin phosphotransferase structural gene fused to the chloroplast promoter for the large subunit gene of ribulose-l,5-biphosphate carboxylase. Kumar et al. (2004) reported the first successful transfer the T-DNA of *Agrobacterium tumefaciens* carrying the genes coding for β-glucuronidase and hygromycin phosphotransferase to the nuclear genome of the green alga *C. reinhardtii*. The expression of xylanase from the nuclear genome of the *C. reinhardtii* by linking the gene directly to an antibiotic resistance gene via the foot-and-mouth disease virus's self-cleaving 2A sequence was investigated by Rasala et al. (2012).

Nowadays, cyanobacteria and algae are viewed as increasingly attractive cell factories for producing renewable biofuels and bioactive chemicals due to their ability to capture solar energy and their relatively simple genetic background for genetic manipulation (Ribeiro et al., 2015). Algae possess the advantages of low-cost production, without the need to use fresh water and high-value arable land. Moreover, they show potential as a future source of enzymes (Chen et al., 2015). Among the 10,000 algae species, only a few thousand strains are kept in collections and a few hundred are explored for chemical content; fewer still are cultivated on an industrial scale (Borowitzka, 2013). Algae, as an extremely diverse group of photosynthetic organisms, have a wide range applications—from human and animal nutrition to cosmetics and the production of high-value molecules such as fatty acids, pigments, and stable isotope biomolecules (Hajimahmoodi et al., 2010; Milledge, 2011). Moreover, algae possess the ability to produce commercial enzymes with potential industrial applications. At the current rate of consumption, if the petroleum-driven transport of the USA is replaced by biodiesel, nearly 0.53 billion m^3 of biodiesel is needed annually. In addition, more than 66,000 kt/y of oil reach biomass is required to replace a mere 5% fuel demand of the USA in the field of transportation. Moreover, to replace all transport fuels in Europe by biodiesel from microalgae, 9.25 million ha would be needed (Chisti, 2008). Modified strains could not only produce enzymes and algal compounds but also express specific genes that cannot be expressed in yeast. The success of the commercial large-scale production of algal enzymes depends on many factors. One of the most important is the development of cost-effective algal culturing techniques. The high cost of algal culture techniques is due to the relatively slow growth rate of algae and the need for light (Norsker et al., 2011). The increasing industrial applications of enzymes describe an ongoing attempt to investigate new enzyme sources as well as improve older ones. However, over the last few decades, great advances have been achieved in our understanding of the biology of algae and in the engineering requirements of large-scale culture systems. Despite the success of open systems, many of the new algae and algal products must be grown free of potential contaminants, such as heavy metals and microorganisms. Closed photobioreactors have attracted much interest as they exercise better control of the cultivation conditions than open systems do (Shriwastav and Bose, 2015).

Conclusion

In recent decades, the increasing availability of biocatalysts and developments in related biochemical knowledge have led to the investigation of new sources for enzyme production. Algae have unique properties and could be explored for the large-scale production of enzymes as future biocatalyst factories.

Competing Interests

All the authors confirmed that there is no conflict of interest in this study.

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