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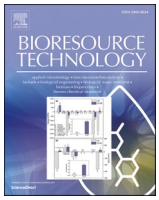

Review

Unconventional high-value products from microalgae: A review

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HIGHLIGHTS

- Production of untraditional high-value products from microalgae are reviewed.
- Modification of biochemical pathways in microalgae for novel products are proposed.
- Based on the current analysis, the market for microalgae-based valuables will increase.

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ABSTRACT

Microalgae have gained significant importance in biotechnology development, providing valuable goods and services in multiple applications. Although there is a rising market for most of these applications, the incorporation and introduction of microalgae into new venues will extend in the near future. These advances are due to the vast biodiversity of microalgal species, recent genetic engineering tools, and culture techniques. There are three main possible approaches for novel algal compounds from: (1) recently isolated yet less known microalgae; (2) selectively stressed conditions; and (3) enzymatically adjusted compounds from conventional molecules. All these approaches can be combined in a specific manner. This review discusses the opportunities, potential and limitations of introducing novel microalgae-based products, and how the recent technologies can be deployed to make these products financially viable. To give an outlook to the future, an analysis of the developments and predicted future market that further enlarge the promise of cultivating microalgae for commercial purposes are considered.

1. Introduction

Microalgae, which is often used for both the prokaryotic blue-green bacteria, or cyanobacteria (kingdom Monera and division Cyanophyta), and the eukaryotic diatoms and microalgae (kingdom Protista), are photosynthetic unicellular organisms, commonly living in freshwater and marine aquatic environments, with cell diameter ranging between one to hundreds of microns. Their cells contain a vast array of chemical compounds referred as secondary metabolites. These metabolites are biosynthesized by different complicated pathways within the cells, and their production and accumulation are affected by many factors such as biotic and abiotic environmental conditions (Andrade et al., 2021). Algal secondary metabolites can be of a high value or can be used as a green feedstock for many important products (Tang et al., 2020).

The advantages of using microalgae as templates to produce these high-value products over other organisms that microalgae can grow photoautotrophically via photosynthesis, heterotrophically and therefore can be cultivated in fermenters with organic sugars, or mixotrophically combining light and sugars as a source of energy (Verma et al., 2020), with some algal species that can tolerate extreme environments (Sydney et al. 2019), and living in ecologically diversified niches (Hanschen and Starkenburg, 2020). Microalgae also have unique characteristics that make them excellent systems for genetic modification to synthesize and accumulate non-native products, such as *Dunaliella salina, Chlorella*, *Chlamydomonas* and *Phaeodactylum* sp. since their genomes have been fully sequenced and available at the GeneBank. In the last few years, microalgal culture, harvesting and extraction biotechnologies increased rapidly. Today, microalgae are already grown

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commercially to produce health food supplements (Nutraceuticals), pharmaceuticals, cosmetics, lubricants, feed for aquaculture hatcheries, in agriculture and in many other applications in life (Levasseur et al., 2020). In addition to their already established use, their contents are studied extensively for biofuel production. The living cells can be used in bioremediation of agricultural wastes, wastewater treatment, and flue gas adsorption. Examples of current algae-based products are: proteins, polyunsaturated fatty acids (omega 3, 6 and 9) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), chlorophylls, antioxidants, tocopherols, collagen, astaxanthin, lutein, β-carotene, phycobiliproteins, polysaccharides, glycoproteins, hyaluronic acid, triacylglycerides (TAGs) and other lipids for third generation of biodiesel, ethanol, butanol and other chemicals such as iodine, active enzymes, and stable isotope chemicals.

In spite of the tremendous number of microalgae species in nature that was estimated between hundreds of thousands and several million extant species (Norton et al., 1996), where ~73,000 species have been isolated and published or are being processed for names (Guiry, 2012), only very few strains are used in industry. Among these few algal strains are *Spirulina* and *Chlorella* that lead the microalgae market worldwide in nutrition and health related algae-based food (Koyande et al., 2019). However, there are many more beneficial species yet to explore. The main reason why the majority of strains have not achieved large-scale production is usually related to the lack of strain robustness, relatively slow growth rate, low productivity, or the lack of added value from the algal source, which add up to the phibitory high price (Borowitzka and Vonshak, 2017). Another reason is that the commercial production of microalgae-derived products is still based on traditional technologies.

Nevertheless, scientists and biotechnologies are continuously looking to isolate novel algal strains that naturally contain high amounts of interesting chemicals, and optimizing their growth conditions. Additionally, the advanced methodologies in genetic engineering offer promising possibilities for increasing the productivity of microalgae. Recent attempts are focused on the development of targeted gene-editing tools (e.g., CRISPR/Cas9 technology), and inventing highly efficient throughput screening methods for fast and robust strain improvement approaches (Lin et al., 2019). At present, there are more than 15,000 annotated and fully sequenced genes of algal enzymes, which are available in the GenBank (http://www.ncbi.nlm.nih.gov/gene/). More algal genome studies will facilitate the access to functional and vital genes, including industrial and other biotechnological genes, for future applications.

Several reviews have demonstrated the production of traditional high-value materials from microalgae (e.g., Chew et al., 2017; Levasseur et al., 2020 and references therein). However, and from a different perspective, this paper examines the feasibility of producing new, exciting unconventional high-value products from microalgae. It also deliberates the potential to increase the productivities of products via suggested synthetic pathways, and then their commercialization through optimal cultivation conditions and controlling processes, using the experience gained from successful commercial-scale algal facilities.

2. Food supplements and nutraceuticals

Scientists and producers are taking a keen interest in developing new methods to increase the algal biomass yield, especially of species rich in nutraceutical substances that can be utilized directly as whole cells added to human foods and beverages, as tablets or extracts which can be used as health food ingredients.

2.1. Fucoxanthin

Fucoxanthin is an important pigment and one of the main carotenoids in the chloroplasts of Phaeophyta (macroalgae) and marine diatoms (microalgae), which absorbs light basically in the green-yellow region of the visible spectrum, peaking at 510–525 nm, giving them a brown or brownish-green color. Fucoxanthin is synthesized in the low light/dark phase of the xanthophyll cycle, and plays a key role in the light harvesting and photoprotection processes (Büchel, 2020).

Many studies have demonstrated that consuming fucoxanthin can support important biological functions as well as it has medicinal effects on health conditions. It has been showed that fucoxanthin can help in curing from many chronic medical conditions such as heart disease, type 2 diabetes, high cholesterol, hypertension, obesity, osteoporosis, metabolic syndrome, liver disease, cancer, eyes and bones heath, as it serves as an antioxidant and scavenger for reactive oxygen species (ROS), inflammation-associated disorders and is anti-bacterial carotenoid (Bae et al., 2020). Recently, fucoxanthin was employed in body weight management products (Yanmei and Qinghua, 2016), such as fücoTHIN® from Garden of Life and Solaray Fucoxanthin Formula from Solaray®. Moreover, this valuable pigment has also showed promising results in skin prevention from photoaging in UVB-irradiated hairless mice (Rodríguez-Luna et al., 2018). Fucoxanthin Nutraceuticals are approved by the Food Standards Australia and New Zealand (FSAN), and currently are being investigated by the United State Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). Moreover, fucoxanthin has been shown to be safe for animals (Yi et al., 2015), therefore, it can be used in many feed products.

The current price for pure fucoxanthin ranges from USD 40,000 to 80,000 per kilogram (Joel, 2016), depending on quality and other criteria. In 2020, the global fucoxanthin market was valued at approximately USD 600 million, growing at a compound annual growth rate (CAGR) of 6% during 2018–2025 (Global Fucoxanthin Market, 2020).

Usually, fucoxanthin is extracted from macroalgae such as *Undaria pinnatifida, Eisenia bicyclis, Cystoseira abies-marina, Himanthalia elongata* and *Sargassum muticum*. While growing macroalgae in ponds for fucoxanthin is a time-consuming process and commercially unprofitable, diatoms such as *Halamphora coffeaeformis, Phaeodactylum tricornutum* and *Odontella aurita* can contain 2–4% of dry weight, which is 100 to 200-fold more fucoxanthin per biomass weight than in macroalgae (Popovich et al., 2020; Xia et al., 2018). Another interesting source of fucoxanthin is the green microalga *Isochrysis galbana* T-Iso (also named as *Tisochrysis lutea*) with highest fucoxanthin content up to ~2% of dry weight (Gao et al., 2021). As a general optimization strategy, low light of 10–50 μmol photons m^{-2} s^{-1}, nitrogen-replete culture medium, a salinity between 25 and 31 ppt, temperature between 21 and 23 °C, and bubbling instead of agitation (Gómez-Loredo et al., 2016) are favorable conditions to increase the yield of fucoxanthin in microalgae.

The first commercial report on the production of microalgal fucoxanthin was in 2018 by Algatechnologies Ltd., a microalgal biotechnology company located in Israel, which introduced a registered patent of natural 3% fucoxanthin oleoresin with the trademark Fucovital® from *P. tricornutum* grown in a closed, fully controlled tubular photobioreactor exposed to sunlight.

2.2. Dihomo-γ-linolenic acid (DGLA)

The market demand for the long chain polyunsaturated fatty acids (LC-PUFAs) is in increase because of their various health-related effects (Saini and Keum, 2018). It was found that the 20-carbon omega-6 LC-PUFA dihomo-γ-linolenic acid (DGLA, 20:3 n-6) offers potential for the treatment of inflammatory disorders including asthma, atopic eczema and psoriasis, atherosclerosis and arthritis, suppressing cancerogenesis, supports the immune system, as it is important in infant formulas for development of the nervous system and preventing depression in adults (Tateishi and Kawashima, 2019, Patent # US10342773B2), and being safe as food (US FDA, GRAS Notice 000041, 2001). Since DGLA is the immediate metabolic precursor of arachidonic acid (ARA, 20:4 n-6), it is extremely an uncommon PUFA, constitutes only trace amounts in the fatty acid profile of native living species. Therefore, and due to its health benefits in human nutrition, there is a need to develop sustainable production of this valuable fatty acid.

The size of the global market of LC-PUFAs including DGLA from algae was estimated at US$2.49 billion in 2019, growing at a CAGR of 7.7% (Algae oil market, 2020).

There is a potential to produce DGLA from the oleaginous fungus strain *Mortierella alpina* S14, which is a Δ5 desaturase-impaired mutant of the ARA-producing wild-type strain 1S-4 (Kawashima et al., 2000). However, more efficient and stable expression systems for the enzymes that are involved in PUFA synthesis and lipid conversion are needed. In addition, developing molecular tools such as the gene-silencing or targeted gene-disruption are required to improve the proliferation of *M. alpina* for large-scale production of PUFA (Kikukawa et al., 2018). For a microalgal source of DGLA, the Δ5 desaturase mutant (P127) of the green oleaginous freshwater microalgal species *Lobosphaera* (formerly *Parietochloris*) *incisa* can be a good candidate since it produces significant amount of DGLA (up to 12.3% of dry weight), and stable under different conditions (Umidjon et al., 2016, US Patent 9315837). To optimize the conditions for enhancing DGLA in the latter algal mutant strain for large-scale production, a 2-stage process of achieving dense cultures under optimal conditions to reach 2 g L^{-1} biomass, and then change the culture conditions to moderately high light and nitrogen starvation (Abu-Ghosh et al., 2015) for accumulating DGLA.

2.3. Fucosterol

Sterols, also known as steroid alcohols, are essential lipid compounds found in the membranes of all eukaryotic cells and have multiple important functions such as controlling the membrane permeability and fluidity. In photosynthetic organisms, phytosterols are plant-derived sterols that are structurally and functionally reminiscent of cholesterol in animals. Phytosterols have specific roles in cell proliferation, cell signaling, and modulating activities of membrane-bound enzymes (Valitova et al., 2016). Nowadays, one of the most important contributions to human wellness involves the combination of health effects resulting from sterol intake, particularly from algae. Fucosterol (24-ethylidene cholesterol), is a phytosterol of marine algae, which have been studied for a variety of health benefits due to its properties as antioxidant, cholesterol-lowering, anti-Alzheimer's, immunomodulatory and anti-inflammatory, anti-diabetes, anti-cancer, as well as its therapeutic activity for some human ailments such as live injury (Abdul et al., 2016).

While fucosterols are characteristic metabolites of brown macroalgae, e.g., *Sargassum carpophyllum* and *Pelvetia siliquosa*, however prasinophyte microalgae such as *Giraudyopsis, Chrysowaernella, Chrysomeris,* and *Chrysoderma* sp. provide an alternative source (Luo et al., 2015). The functionalities of fucosterol extracted from microalgae are still undetermined and thus more research is needed in this regard.

2.4. Ubiquinone coenzyme Q_{10} (CoQ_{10}) and ubiquinol ($CoQH_2$)

The Ubiquinone 2,3-dimethoxy-5-methyl-6-multiprenyl-1,4- benzoquinone ($C_{59}H_{90}O_4$, MW 863.34 g mol^{-1}), also known as Co-EnzymeQ or shortly "CoQ_{10}", consists of ten subunits in humans (Raizner, 2019), and is important for almost all living cells. CoQ_{10} plays a vital role in producing cell energy in the mitochondria through the electron transport chain and is a special lipid-soluble antioxidant in the lipoproteins and plasma membranes (Sachinidis et al., 2021). Usually, deficiency in CoQ_{10} is diagnosed by heterogeneous clinical symptoms with different seriousness levels that are based on the patient's age (Salviati et al., 2017). On the other hand, CoQ_{10} has been used in the treatment of a variety of human health disorders, such as kidney diseases, cardiovascular diseases, diabetes, the metabolic syndromes, inflammation, neurodegenerative diseases, early-stage Huntington disease, inhibition of LDL oxidation, and a supplement of well-health conditions like human fertility and aging in women and men (Shukla and Dubey, 2018). Coenzyme Q_{10} is considered the third most consumed nutraceutical (Arenas-Jal et al., 2020), and its manufacturing is not strictly required a safety or a purity certificate before releasing to the market (Sood and Keenaghan, 2020). The acceptable daily intake (ADI) of CoQ_{10} for humans is 1200 mg/day/person, calculated from the no-observed-adverse-effect level (NOAEL) by Hidaka et al. (2009).

The reduced form of CoQ_{10} is ubiquinol (2,3-dimethoxy-5-methyl-6-mul tiprenyl-1,4-hydroquinone, $C_{59}H_{92}O_4$, MW 865.36 g mol^{-1}), or shortly "$CoQH_2$", which is most abundant form in the relaxed human cells, therefore, is an essential plasma antioxidant (Mancini et al., 2011). Moreover, $CoQH_2$ protects from oxidative DNA degeneration, lipid peroxidation and other harmful molecules (Chen et al., 2019).

For several years already, efforts have been made to cover the growing demands of the nutraceutical and pharmaceutical industries for CoQ_{10} and $CoQH_2$, mainly by chemical or semi-chemical synthesis. However, most of these synthesis processes are very expensive and non-environmentally friendly, and the final product, in most cases, lacks stereo-selectivity due to the mixture of CoQ_{10} optical isomers and lack scalability (Choi et al., 2005). Hence, the concentration of CoQ_{10} in wild organisms is low, new technologies are required to develop a commercially cost-efficient, genetically non-transformed microalgal systems to produce these valuable UV protectors naturally. Dürr (2016, US Patent No. 9376660 B2) invented a method to naturally increase CoQ_{10} and $CoQH_2$ yield with a constant high quality. According to the method, induction of the oxidative stress in microalgae to produce more CoQ_{10} is achieved by incubation the cells with trivalent iron in the form of Fe^{3+} (33 to 66 μg g^{-1} dry mass) added to the culture medium. In his experiments, the author used the following cyanobacterial/algal cultures: *Spirulina maxima, Chlorella kessleri Spirulina vulgaris*, and *Spirulina fusiformis*. The biggest CoQ_{10} yield was obtained from *Spirulina vulgaris* with an improvement of 24-fold, from 4.1 μg/g CoQ_{10} (without addition of Fe^{3+}) to 94 μg/g CoQ_{10} (after adding Fe^{3+} with a gradually increasing of light intensity from initially 20 to 120 μmol photons m^{-2} s^{-1}), and the QH_2/Q_{10} ratio was improved 4.5-fold as well. Moreover, by this method the growth phase was shorter and, therefore, the overall cultivation time was also shortened.

The algal mass or their oil can be added directly to the food, drinks, and vegetarian animal food, without the need to extract the active compounds, to reduce the cost. However, further research on the pharmacokinetics and safety of CoQ_{10} on humans is needed when it is consumed directly from the whole algal cells.

3. Biopharmaceuticals and theranostics

The secondary bioactive metabolites that are extracted from microalgae have been profoundly used in diagnosis and therapeutics (theranostics). However, due to their low yields from plant sources and the use of plants for food, governments and researchers have switched their attention to microalgae as production platform for bioactive compounds. Moreover, it is less difficult to generate mutant cell lines of microalgae than plants to increase the yield of the value-added chemicals so that their price goes down and, therefore, they become more available for clinical research. Nowadays, there are various pharmaceutical products being obtained from microalgae, which are Generally Recognized As Safe (GRAS). Here is a list of novel and exciting microalgae pharmaceuticals and therapeutics, with the possibilities and technologies to increase their production:

3.1. Cannabinoids

The extracts of *Cannabis sativa* plant are collectively called "phytocannabinoids", which are terpenophenolic compounds that have been used for millennia in medicine, and for recreational purposes. The two most abundant and widely recognized as the main bioactive constituent substances are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the main pharmacologically active compound, which is a partial agonist of CB1 and CB2 cannabinoid receptors in the brain (Russo, 2018). CBD is an isomer of THC, which is the main active compound of

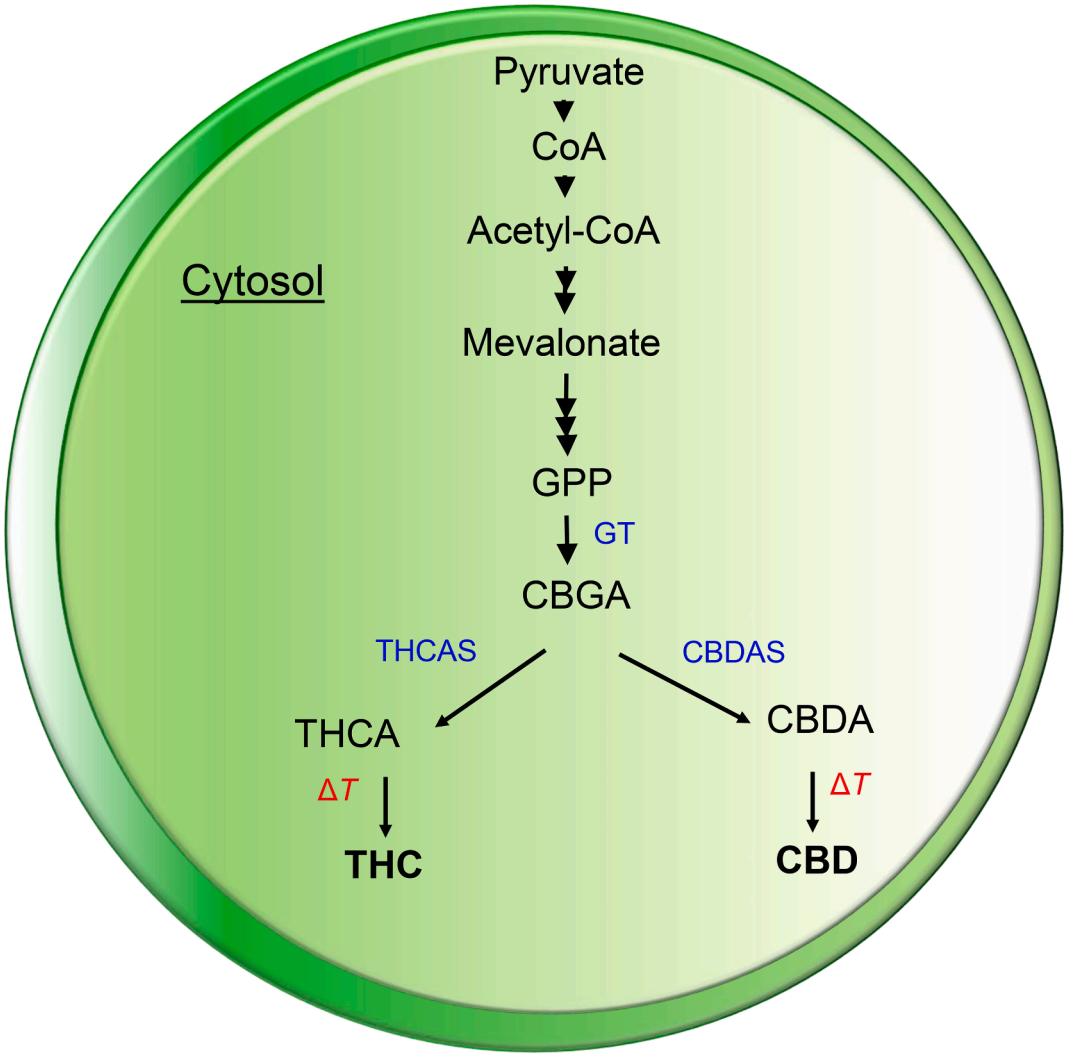

Fig. 1. Suggested biosynthetic pathway for cannabinoids Tetrahydrocannabinolic acid (THCA) and Cannabidiol (CBD) in oleaginous microalgae. Precursor molecules: GPP, geranyl diphosphate; CBGA, cannabigerolic acid; THCA, tetrahydrocannabinolic acid; and CBDA, cannabidiolic acid. Catalytic key enzymes: GT, geranyltransferase; THCAS, tetrahydrocannabinolic acid synthase; and CBDAS, cannabidiolic-acid synthase.

the non-psychoactive cannabis and is the second most abundant in the psychoactive hemp varieties (Clark and Merlin, 2013). However, many other phytocannabinoids such as cannabichromene (CBC), cannabidivarin (CBDV), and cannabigerol (CBG) are still under investigation.

A one gram of CBD power with a certificate of analysis (COA) could cost from USD 60 to USD 200 (Nickus, 2020). The global market of legal cannabis-derived products is expanding rapidly, and the value of the current worldwide demand for cannabis was an estimated at USD 344.4 billion (Global Cannabis Report, 2019). Therefore, there is a need for biotechnology techniques to produce these trace, valuable cannabinoids to meet their demand, and to enable more studies for understanding the full potential contribution of these cannabinoids in medicine. The first step in any successful procedure that leads to the production of cannabinoids in economically viable method is the identification of a suitable microorganism with desirable phenotyping traits including high growth rate, and the ability to tolerate the accumulation of end-product. In addition, the platform organisms that will be used in cannabinoid production must have a biological system that naturally provides cellular supplies of the cannabinoid precursors. Then looking for a biosynthetic pathway in the cells which can be modified with the possibility of coordinating the expression of all required genes encoding the catalytic enzymes needed for the production of the desired cannabinoid from specific starter molecules. Recently, Luo et al. (2019) succeeded to engineer a pathway in the yeast *Saccharomyces cerevisiae* to produce THC and CBD by fermentation, from the simple sugar galactose, after a few attempts by other groups to mutate microbes for this purpose such as Tan et al. (2018).

Up-to-date, no report has shown a complete biosynthesis of cannabinoids or their precursors in recombinant microalgae, and how an industrial process should be designed to facilitate economic production in large scale bioreactor. As for, we suggest the following:

There are two independent pathways for cannabinoid biosynthesis in *C. sativa*: (i) the plastidial methylerythritol phosphate that leads to for the biosynthesis of olivetolic acid (OLA), a major intermediate in this pathway, by condensation condensing of one molecule of hexanoyl-CoA molecule with three molecules of malonyl-CoA molecules (Gülck and Møller, 2020), and (ii) the cytosolic mevalonate pathway. The oleaginous microalgae can accumulate high amounts of fatty acids in their cells (>20% of total biomass), which make them good candidates for cannabinoid-engineered pathways, since the biosynthesis of cannabinoids begins with fatty acids (Thomas et al., 2020). Examples of oleaginous microalgae are *Chlorella* sp., *Nannochloropsis* sp., *Phaeodactylum tricornutum, Tetraselmis* sp. and others. Initially, the fatty acid hexanoic acid is converted to coenzyme A (CoA) form by an acyl activating enzyme (Stout et al. 2012). CoA is then acetylated to acetyl-CoA through β-oxidation, by breaking down of fatty acids, and then it enters

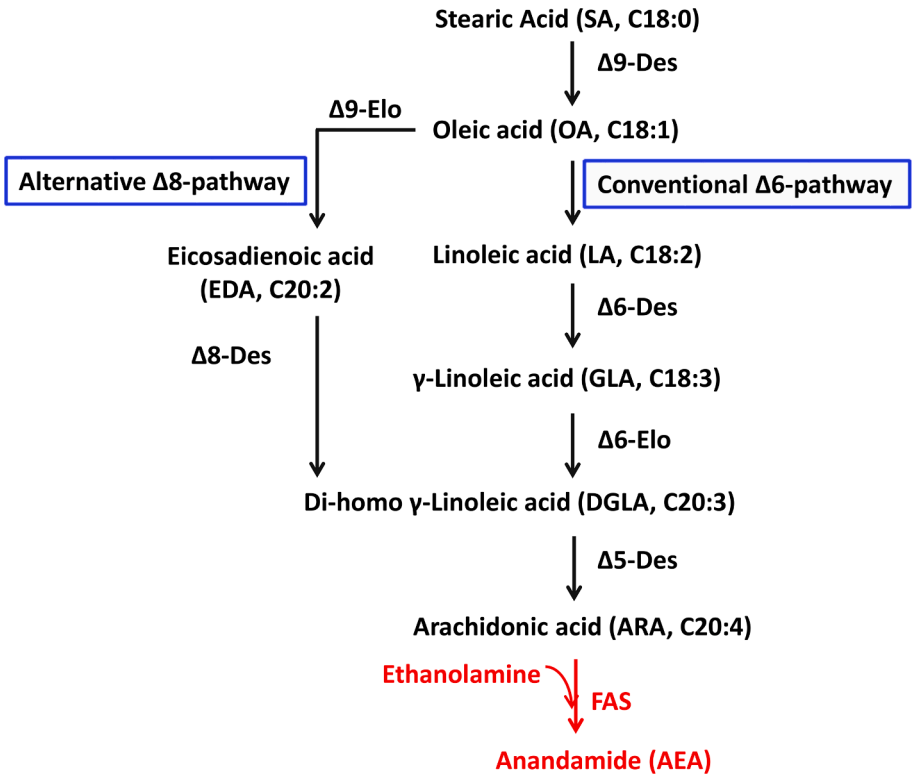

Fig. 2. Suggested biosynthetic pathway for anandamide (AEA) production in the green, freshwater microalga *Lobosphaera incisa*. Fatty Acid Synthase (FAS) catalyzes the condensation of arachidonic acid (ARA) and ethanolamine to form anandamide. Elo, Elongase; Des, Desaturase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the mevalonate pathway (Shi and Tu, 2015). The aim here is to modify the native mevalonate pathway in microalgae to produce the major cannabinoids by providing a high flow of geranyl pyrophosphate (GPP), a main precursor molecule for cannabinoids synthesis, in the cytosol. However, GPP is toxic to cells at high concentrations (Sarria et al., 2014), which creates a significant challenge during synthesis. To avoid autotoxicity, the functional interaction of such intermediates is a selection parameter. Therefore, it is suggested that GPP should be immediately converted to cannabigerolic acid (CBGA), a key monoterpene precursor of THC and CDBs, by introducing the genes of the catalyzing enzyme geranyltransferase in the microalgae genome. Another two key enzymes are required in the pathway leading to CBDs synthesis are Tetrahydrocannabinolic acid synthase and THCAS and Cannabidiolic-acid synthase (CBDAS), as suggested in Fig. 1. This step can be achieved by the efficient methodology of precise genome editing in the nucleus exploiting Homologous Recombination (HR) in synchronized cell cultures developed by Angstenberger et al. (2020).

It is noteworthy to mention here that the growth conditions influence enzyme production and activation in the algal cells, such as medium composition, growth temperature, light intensity, and oxygen/carbon dioxide availability.

3.2. Anandamide (cannabimimetic substances)

Anandamide is N-arachidonoylethanolamine (AEA), a neurotransmitter that is an endocannabinoid in animal tissues (Devane et al., 1992), which is generated in animal cells from its precursor, N-arachidonoyl phosphatidylethanolamine (NAPE), through hydrolysis by a phospholipase D (Giuffrida et al., 1999). Many studies have shown that the endocannabinoid system plays a role in various physiological activities, such as energy metabolism, cardiovascular regulation, emotion, reproduction, and has a role in pathophysiological conditions (Pacher et al., 2006). Nowadays physicists modulate the activity of the endocannabinoid system to treat many physiological and pathophysiological conditions such as inflammation and pain, neuroprotection, memory processing, motor coordination, smoking cessation, control of appetite, pre-and postnatal development, fibrotic skin diseases, and reproduction (Kirkham, 2005; Smita et al., 2007; Ashton and Moore, 2011; Woodhams et al., 2017; Correia-Sá et al., 2020), which also can act as a behavioral reinforcer and reward drugs of abuse (Parsons and Hurd 2015).

AEA can also be generated by non-oxidative reaction of the polyunsaturated fatty acid arachidonic acid (ARA) and the substrate ethanolamine of other fatty acids, and is catalyzed by anandamide synthase (Deutsch and Chin, 1993). The oleaginous freshwater green microalga *Lobosphaera bisecta* is considered the richest plant source of the ARA (up to 47% of total fatty acids, and 54% of biomass (Gao et al., 2020a, 2020b). Nitrogen starvation and excessive irradiances are deleterious for the accumulation of ARA in this alga (Solovchenko et al., 2008), and, therefore, this microalgal species could be a good platform for genetic engineering for anandamide production in the presence of the substrate ethanolamine (see suggested modified pathway in Fig. 2).

3.3. β-Glucans

Many cytotoxic anti-cancer chemotherapeutic drugs are originally purified from plants, such as vincristine, etoposide, and paclitaxel, while other plants with immunomodulatory activities have been commercialized and can be purchased over the counter. In the recent years, a herbal ingredient with a large molecule that is responsible for many immunomodulation functions was discovered and named β-D-glucan or simply called "β-glucan" (Mélida et al., 2018). β-Glucans (known as chrysolaminarins) consist of glucose polymers, which are linked together by a 1 → 3 linear β-glycosidic chain, and are branched by species-specific glycosidic chains, e.g., the side branches of β-glucans in bacteria are 1 → 4, whereas in fungi the side branches are 1 → 6.

The current data of β-glucans pharmacological dynamics of that these molecules are potent immunomodulators with boosting effects on the innate immune response by triggering gut microflora (Del Cornò et al., 2020). Other reports showed that β-glucans can also enhance the acquired immunity by increasing the phenotypic and functional maturation of monocyte-derived dendritic cells with a considerable production of interferon-γ, IL-12 and IL-10 (Song et al., 2020), which can be used in cancer immunotherapy. β-Glucans also function as scavengers reacting with reactive oxygen species (ROS) and other free radicals in the gut, thereby exerting antioxidative activity (Carballo et al., 2018).

Although β-glucans are abundant inside the cell wall of bacteria and some fungi strains; however, they are mostly contaminated with endotoxins that affect their safety and potential benefits, which increases the cost of their purification process. Therefore, microalgae-derived β-glucans can be a replacement for these bioresources. The β-glucans extracted from the freshwater alga *Euglena gracilis* are linked by 1 → 3 glycosidic bonds without branching and are stored in the pyrenoids is referred as "paramylon" according to Bacic et al. (2009), which can be more than 80% of the alga dry weight (Sun et al., 2018). Paramylon can highly stimulate porcine leukocytes *in vitro* as it activates human lymphocytes (Russo et al., 2017). In 2019, *E. gracilis* was ascribed the qualified presumption of safety (QPS) status. Toxicity studies regarding consumption of *E. gracilis* have shown no safety concerns, up to the highest dose tested of 3300 mg according to EFSA Biohaz Panel (2019) and 5000 mg according to Symonds et al. (2019), of *E. gracilis* per kg body weight, which is considered as the no observed adverse effect level (NOAEL). The cultivation of *E. gracilis* in large-scale for commercialization purposes is meant for the "5Fs of Biomass", a term that is used to refer to the production of food, fiber, feed, fertilizer, and fuel from biomass.

E. gracilis can grow at low pH of ~3.5, which could be a tactic strategy to prevent contamination during outdoor cultivation (Kim et al., 2020). *E. gracilis* can also grow heterotrophically, with a higher proliferation rate than photoautotrophically. However, the heterotrophic growth is more subjected to contamination mainly by *S. cerevisiae* and *B. subtilis*, and other contaminants, even at low pH, which might require more expensive control conditions such as the use of antibiotics and highly monitored closed growing systems, resulting in the increase of overall production cost. Moreover, heterotrophic large-scale grown cells may alter nutritional content. Recently, the Japanese startup Euglena Co. started its first production of *E. gracilis* in 2019, with an annual maximum production of 160 tons, using various, unrevealed techniques and strategies to maximize the productivity and minimize contamination. Adaptation evolution can be one of the cultivation solutions, combined with low-cost quality control.

On the other hand, new breed cell lines of *E. gracilis* can be generated by efficient methods such as the use of heavy ion beams (Yamada et al., 2016a), and performing selective cell sorting) by fluorescence activated cell sorting (Yamada et al., 2016b) to obtains mutants with preferred phenotypic traits, such as tolerance to high temperature of 32 °C. Cells generated by this technology were identified as natural mutants, which are subject to safety regulation regarding the use of genetically modifies organisms. Recently, a commercial scale, yet more accurate, quantification of β-glucan via single-cell analysis in *E. gracilis* was invented, using high-throughput broadband raman flow cytometry at a throughput of >1000 cells·s^{-1} (Hiramatsu et al., 2020).

3.4. Hoshinolactam and dolastatins

Hoshinolactam is an aromatic molecule belongs to the chemical class lactam. It was naturally isolated in 2017 from the filamentous cyanobacterium *Oscillatoria* sp. (Ogawa et al., 2017). Hoshinolactam has shown antiprotozoal and antitrypanosomal activity, especially against the parasite *Trypanosoma brucei* (the concentration needed to obtain 50% inhibition [IC_{50}] = 3.9 nM) without significant cytotoxicity to the host cells with multidrug resistance-5 (IC_{50} > 25 μM), which makes its activity equivalent to that of the antibiotics (Ogawa et al., 2017).

Dolastatins are a family of peptides that have medicinal properties, which are mainly isolated from the cyanobacteria *Symploca hydnoides* and *Lyngbya majuscule* (Mondal et al., 2020). Among all, dolastatin 10 (D10) is the most effective antiprotozoal metabolite discovered so far with IC_{50} of 0.1 nM (Fennell et al., 2003). It was shown that D10 is 100-fold more potent than other natural drugs such as vinblastine, of the same cell line due to its ability to destabilize microtubule synthesis, and therefore it depolymerizes cell microtubules (Kumar et al., 2017). Moreover, D10 was introduced in clinical trials as an antitumor compound although it did not progress beyond phase II due to its high cytotoxicity. However, the dolastatin-10 based antibody-drug conjugates (ADC) polatuzumab vedotin and brentuximab that contain monomethyl auristatin E (MMAE) with less toxicity are FDA-approved agents for the treatment of several lymphomas (Carroll et al., 2020). With the progressing advanced synthesis methods, highly stereoselective synthesis of D10 was invented (Wang et al., 2017). Dolastatin 15 (D15) is another antimitotic agent that arrests cell proliferation in many cell types at low nanomolar range (Cragg et al., 2005). D15 has attracted the attention for its potent therapeutic effects, which is still undergoing clinical examination.

Nevertheless, the bottleneck in extracting hoshinolactam and dolastatins from wild marine cyanobacteria is their low content percent per mass weight (6.5 mg of hoshinolactam per 2 kg wet weight [Ogawa et al., 2017], and 24 mg dolastatins per 5 g dry weight [Luesch et al., 2001]), which makes their price high or inconvenient, ranging between USD 30,000 and 60,000 per gram (Chemical Book, dolastatin 10 manufacturers). Therefore, to make their production economically feasible, which in turn will help to proceed more clinical tests, controlled mutagenesis for manipulation of target pathway, and efficient cultivation bioprocess are needed to improve overall productivity. The current attempts in this regard are that endophytic microbes, including a *Bacillus* (bacterium) species, with a required plasmid to recombine to other endophytic fungal sources to yield more D10 on fermentation (Newman, 2018; Newman and Cragg, 2020).

3.5. Recombinant therapeutic proteins "molecular farming"

Therapeutic proteins, including human growth hormones, serum amyloids, vaccines, angiotensin I-Converting Enzyme Inhibitory (ACEI) peptides and others have attracted many producers for their medicinal value. Antibody-based therapy, however, is a form of immunotherapy that uses monoclonal antibodies, which is one of hotspots and fast-growing areas of drug research and development. Such therapy has been recently recognized as highly effective at treating several serious human diseases such as cancers and acute infections (Theobald, 2020). Currently, therapeutic antibodies are produced by mammalian cells in facilities that cost hundreds of millions of dollars, using expensive culturing and purifying techniques. Therefore, the price of antibody-based therapeutics is one of a few most expensive medicines on the market nowadays. After several studies, it has been shown that various plant species have the capacity to produce recombinant pharmaceutical and therapeutic proteins and peptides, i.e., "molecular farming", which earned attention and support from the community of plant engineering and biotechnology. In 2012, the FDA approved the first recombinant plant-derived drug for human use, taliglucerase alfa (Elelyso™), which was an important breakthrough. Monoclonal antibodies that are already produced in transgenic plants are:

(1). Avicidin, recognizes colorectal and prostate cancers' antigen, (2). T84.66, recognizes epithelial cancers (carcinomas), (3). CaroRx, recognizes the adhesion protein of the human tooth-decay bacterium *Streptococcus mutans* (4). PIPP, recognizes the hormone human chorionic gonadotropin (hCG), and (5). scFv, recognizes the malignant B lymphocytes in the murine B-cell lymphoma 38C13.

However, because of the technical limitations of plants such as their lower yields compared to the traditional epithelial mammalian cell

lines, the prerequisite conventional affinity-based purification schemes for plant recombinant proteins, the massive investment in the infrastructures, and the unfavorable regulatory uncertainty has led the common pharmaceutical industry to be off the plant-based proteins (Buyel, 2018). Therefore, microalgae would be an ideal system for the expression of therapeutic recombinant proteins since transgenic microalgae can be made in a much short time, they propagate fast, and the cost of produced proteins is much less compared to any other eukaryotic cell-system. In addition, producing recombinant proteins in microalgae is possible to control via the growth conditions, as there are various promoters that are regulated by factors such as irradiance or nutrient concentration in the growth medium. For instance, the promoter of nitrate reductase is induced by nitrate availability or concentration, which is an essential molecule in protein synthesis.

The green microalga *Chlamydomonas reinhardtii* is a leading unicellular model and one of the best candidates to produce recombinant proteins since the genome of this alga was annotated and now is readily available (Merchant et al., 2007), and with the high metabolic flexibility. *C. reinhardtii* is often used in studying many biological aspects of microalgae and plants such as evolution, biochemistry and physiology, algae-based biofuel production, and mutant generation. The first effort for "molecular farming" in *C. reinhardtii* was the production of antibodies to the herpes simplex virus (HSV) by Mayfield et al. (2003).

On the downside, using *C. reinhardtii* as a platform in molecular farming has some challenges. *C. reinhardtii* is a freshwater microalga that is easy to get contaminated by other algal species or microbes, it contains low oil percent, grows slow in open ponds, is sensitive to high solar irradiance, has gene expression malfunctions due to its internal RNA silencing mechanism, has poor expression rate of heterologous proteins, and low number of transgenes that regulate protein expression (Butler et al., 2020).

The halophyte microalgae *Dunaliella* is another algal species that can be used as a factory to produce recombinant proteins. As of molecular farming, *Dunaliella* acquires numerous advantages. It grows autotrophically and is able to grow in different salinity levels from low 0.05 M NaCl to extremely high 5.5 NaCl (Chen and Jiang, 2009), minimizing the risk of contamination, and therefore is suitable for cultivation in open photobioreactors with low cost. Moreover, *Dunaliella* can beneficially utilize cheap carbon sources like acetate (Feng et al., 2020), lacks the rigid cellulosic cell wall (Borowitzka and Siva, 2007), which makes it suitable for easy genetic manipulation (Song et al., 2019). Because it is unicellular, *Dunaliella* and does produce chimera tissues and, therefore, no regeneration difficulties exist during gene manipulation, which is the case in plants (Ahmad and Mukhtar, 2017). Moreover, *Dunaliella* can steer the recombinant proteins outside the cells and protect them from degradation by proteases (Georgianna et al., 2013). Another unique feature of *Dunaliella* is that it can be exploited for a simultaneous production and extraction of products by a procedure so-called "milking" of microalgae, maintaining the biomass for recycling in the production path (Kleinegris et al., 2010). The later method is very efficient in the recombinant protein production process.

3.6. Anti-viral substances

The use of active materials from natural resources in the manufacturing of medicines is ancient and a well-established practice. Numerous studies have reported that algae and cyanobacteria are excellent source of antiviral products. For example, The A1 and A2 compounds extracted from the microalga *Cochlodinium polykrikoides* showed activity against A and B influenza viruses and herpes simplex virus-1 (HSV-1) (Hasui et al., 1995). The polysaccharide fractions isolated from the red macroalga *Griffithsia* sp. that naturally inhabits the waters off New Zealand, showed high protective activity against HIV-1-induced cytopathicity in T-lymphoblastoid cells (Mori et al., 2005). Other red macroalgae such as *Kappaphycus alvarezii* and *Hypnea musciformis* (carrageenophytes) have cell walls that contain sulphated polysaccharides with similar antiviral properties of the microalga *Porphyridium* (Bauer et al., 2021). In another study by Silva et al. (2015), seven active compounds were extracted by ethyl acetate from the cyanobacterium *Leptolyngbya* sp. and the microalgae *Chlorellaceae* inhibited 80% of seasonal influenza A and B viruses in Madin-Darby Canine Kidney (MDCK)-infected cells. Since then, several reports have been published regarding the antiviral potential of algae-derived polysaccharides. Márquez-Escobar et al. (2020) used Algevir technology, a system to express proteins by viral vectors in microalgae, to produce an antigenic protein called ZK in the microalga *Schizochytrium* sp. as a vaccine candidate against Zika virus.

The outbreak of Coronavirus disease in December 2019 (COVID-2019) is a potentially lethal and highly contagious disease, which was declared as pandemic in 2020 by the World Health Organization (WHO). Continuous supportive evidence suggests that extreme reactive inflammation, oxidation, and an increase in cytokine level are very likely to contribute to the severe COVID-2019 pathology. It was suggested that carrageenan, along with the sulfated polysaccharides and exopolysaccharides extracted from *Porphyridium* sp. can combat against COVID-19 (Hans et al., 2021; Sami et al., 2021). Ulvan, mauran, laminarin, fucoidan, and galactofucan sulfate are all ionic sulfated polysaccharides and exopolysaccharides extracted from marine algae and cyanobacteria, which possess various unique structures and functionalities, with no substitutes from terrestrial organisms (Cunha and Grenha, 2016). These bioactive metabolites, along with the other specialized antioxidant pigments in the algal crude, were shown to have biomedical applications in wound dressing, tissue engineering, anti-aging and moisturizing creams, stimuli-responsive drug delivery, and cancer bioimaging and therapy (Manivasagan et al., 2016; Demay et al., 2021). The stability of the sulfated polysaccharides is pH-independent (Kim and Venkatesan, 2015). Algal polysaccharides have an excellent oral safety profile in humans and animals, and they are GRAS according to FDA specifications (FDA Gras Notice, 2014; Lean et al., 2015).

Although the high potential of the sulfated polysaccharides, however, many of their theranostic applications are still unexploited. It is noteworthy that a precise design and novel polysaccharide synthesis in microalgae, with customized properties for specific applications is an interesting topic with many benefits, including a lower production cost. However, as till to date only a very few studies have been carried out in this area. Future investigations in this regard by means of new chemical methods and pipeline database of species-specific structural and functional variabilities of the marine polymers will lead to more possibilities for novel ones.

3.7. Sunscreen pigments and UV protection

Microalgae contain natural pigments that can be used in sun care products and other applications. The multifunctional mycosporine-like amino acids (MAAs) are secondary metabolites extracted from microalgae and cyanobacteria inhabiting marine ecosystems with high sunlight exposure, particularly high in ultraviolet radiation (UVR), to protect them from harmful light energy (Rosic, 2019). MAAs have shown high efficiency in absorbing UVR radiation within the range 310 and 360 nm, having a high molar absorptivity (ξ) from 2.8×10^4 to 5.0×10^4 $M^{-1} \cdot cm^{-1}$ (Sun et al., 2020), therefore competing with commercial filters. They consist of a repetition of either a cyclohexenimine or cyclohexenone chromophore and a nitrogen substituent (amino group), with generally low molecular weight (<400 Da), and they are water-soluble. The difference between MAAs types is the ring and substituent that determine their particular absorption spectra.

The first formula containing liposomes of the two MAAs shinorine and porphyra-334 was developed by Schmid et al. (2006) and now is globally commercialized under the brand name of Helioguard®365, which shows protective abilities against UV-A-induced cell-viability loss and DNA damage. Moreover, MAAs have gained special attention due to their applications in non-biological materials such as fabrics, plastics,

varnishes, and paints to protect them against UVR that lowers their quality and durability (Kageyama and Waditee-Sirisattha, 2019). Hence, they can be introduced into many cosmetical and pharmaceutical applications, they may hold a promising future. With the availability of MAAs mainly from the cyanobacterial species *Aphanizomenon* (Stefano et al., 2007), *Chlorogloeopsis* spp. (O'connor et al., 2011, Patent no. WO2011158041), *N. commune* (Matsui et al., 2011) and *Lyngbya purpurem* (Chandra et al., 2020), there is a feasibility to produce them at commercial scale by optimizing production rate, or by the genetic manipulation.

Another natural sun-protection material that is often encountered is scytonemin. This pigment is a distinctive dimeric indole alkaloid located in the mucilaginous sheath surrounding the cells of numerous cyanobacterial species that produce extracellular polysaccharides, which colors the cells into yellowish-brown. Scytonemin provides sunscreen protection by absorbing light spectrum around 315–400 nm (Kang et al., 2020), and is mainly extracted from *Scytonema* sp. and *Nostoc* sp. (Sinha et al., 2021).

4. Eco-friendly products

4.1. Endotoxin, as pesticide-free alternatives for biological control of pets

Pesticides including herbicides, insecticides, and fungicides are chemicals applied in agriculture to control pathogens and pests, and to develop crop yields that have dramatically increased in the last few decades. However, using these synthetic pesticides results in several environmental problems. The insecticide residues are toxic to wildlife, and they induce harmful interaction changes on non-target pests. There is an accumulative evidence that these chemicals are also harmful to humans and animals as they pass from one organism to another when consumed, which have been related to the increase of some diseases such as type 2 diabetes, Parkinson's disease, obesity, neurotoxicity, endocrine disruption, and specific types of cancers (Rani et al., 2020). Other serious environmental threat is the contamination of ground water (reviewed in Yadav and Sharma, 2019). Therefore, there is a need to develop green, target-specific pesticides, which are environmentally friendly and biodegradable.

The biological pesticides or "biopesticides" are based on the act of pathogenic microorganisms that are specific to a target pest, which are ecologically sound. These living organisms release endotoxins and degrading enzymes that can lyse the cell wall of intrusive organisms, or even can introduce viral infections in pests. These include biofungicides (Trichoderma), bioherbicides (Phytopthora), and bioinsecticides (*Bacillus sphaericus* and *Bacillus thuringiensis*). Biopesticides can be an excellent alternative to synthetic pesticides as they are significantly less harmful to the environment, safe for human and animal health, and the potential benefits to agriculture are considerable (Butu et al., 2020). However, since the cost of biopesticides production via the fermentation of microorganisms is higher than the cost of synthetic pesticides, therefore the latter is preferred to use by farmers. Also, because the nature of the biopesticides as living organisms or metabolites of them, farmers should be highly aware of the use of each product.

Microalgae, mainly cyanobacteria, are considered as one of the most effective biological agents to control fungal pathogen and soil-borne diseases (Righini and Roberti, 2019), and they increase the defense mechanisms in plants (Renuka et al., 2018). The first report of algal isolated active compounds with biopesticidal effects, referred as "chlorellins" from the microalga *Chlorella,* was published by Pratt et al. (1945). Later, the activity of chlorellins in inhibiting the growth of pathogenic bacteria was confirmed by Gupta et al. (2013). One of the most active materials that showed affinity against fungi and yeasts is cryptophycin 1, which is a depsipeptide, and is mainly extracted from the cyanobacterium *Nostoc* sp. (strain ATCC 53789). This product has antimitotic and antiproliferative activities. It blocks the cell cycle of mitosis metaphase, more effectively against *Cryptococcus* (Fidor et al., 2019) and *Candida maltose* SBUG 700 (Bui et al., 2007), and other yeast species. Additionally, allelochemical compounds produced by microalgae are effective in weed control (Latif et al., 2017). For example, Triketones, which are exceptionally important classes of herbicides, were originally developed from the chemical group triketones.

4.2. Biofertilizers and biostimulants

Microalgae are exploited in agriculture as natural biofertilizers to improve soil characteristics since they contain phytohormones and high amounts of micronutrients and macronutrients necessary for plant growth, health, and development (Guo et al., 2020). Recent studies show that microalgae biofertilizers increase nutrient uptake in plants with better growth and crop yields (Renuka et al., 2018). Biofertilizers are sustainable, organic alternative to synthetic fertilizers, cost-effective, and most importantly are environmentally friendly (Abinandan et al., 2019). In addition, there is a growing evidence that microalgae can improve soil health, reduce the occurrence of erosion, can be used in crust formation, agricultural treatment, wastewater treatment for irrigation and metals removal from the soil and nitrogen recovery (Castro et al., 2020). Microalgal extract concentrations can also be used as plant biostimulants for seed germination (Stirk and van Staden, 2020). Biofertilizers can also solve the problem of the leftover crude of defatted or residual biomass after extracting the high-value chemicals from it, through fermentation, which contributes to the reduce in the production cost.

Mutants of cyanobacteria have been also generated to increase their resistant to harsh conditions or to increase their efficiency for stimulating plant growth. For example, mutants of *Anabaena variabilis* were created to resist the herbicides and to increase rice productivities (Singh and Prasad, 2012).

The price of biofertilizers is between USD 300 to USD 1200 per ton (as secondary products), and the market estimation in 2020 was at USD 2.3 billion (Biofertilizers Market, 2021). The forecast predicts its growth through, as several initiatives and favorable regulations issued by governmental agencies will further accelerate the increase of global biofertilizers market during the next ten years.

4.3. Bio-diols

Diols are petroleum-derived chemical molecules containing two hydroxyl groups (−OH), which have many applications in industry. Diols have been widely used as solvents in cosmetics and pharmaceuticals, and serve as intermediates in the manufacturing of specialty chemicals such as fabrication of polymers and production of biosurfactants. The most common diol is ethylene glycol. The synthesis of bio-based linear diols from plants with improved carbon footprint, high performance and being thermal stable has received significant interest due to the reduction in fossil resource, and for being environmentally friendly (Zia et al., 2016). While the bioproduction of certain important diols like 1,3-propanediol has been recently commercialized (Burgo et al., 2020), however, it is still highly costly due to the lack of natural biosynthesis pathways in living organisms. Recent developments in synthetic biology have enabled the construct of completely new *de novo* pathways to non-natural molecules from renewable sources. Benisvy-Aharonovich et al. (2020) reported an efficient method to produce the intermediate material levulinic acid in the green microalga *Chlorella ohadii* (80% of dry weight) by a hydrothermal hydrolysis process using HCl, and then immediately converted it into 1,3-propanediol in water using the yeast *Saccharomyces cerevisiae* as a catalyst.

5. Research needs and perspectives

Systematic screening of the vast numbers of unexamined algal species for active compounds of high nutritional, cosmetic, and pharmaceutical value. This can be performed by means of the recently

Table 1
List of unconventional high-value products with their percent of accumulation in microalgal dry weight.

Product	Potential microalgal species	Content (% of dry weight)	(Ref.)
Nutraceuticals			
Fucoxanthin	*Halamphora coffeaeformis*	3.8	Popovich et al., 2020
	Phaeodactylum tricornutum, Odontella auritacan	2	Xia et al., 2018
	Isochrysis galbana T-Iso	1.7	Gao et al., 2021
DGLA	Δ5 desaturase mutant of *Lobosphaera incisa* (P127)	12	Umidjon et al., 2016
Fucosterol	*Olisthodiscus luteus*	31	Marshall et al., 2002
CoQ_{10}	*Spirulina vulgaris*	0.01	Dürr, 2016
	Spirulina fusiformis, Spirulina maxima	0.008	Dürr, 2016
	Chlorella kessleri	0.003	Dürr, 2016
Biopharmaceuticals			
Cannabinoids	Suggested mutants of: oleaginous microalgae such as	*30–40	–
	Chlamydomonas reinhardtii, Chlorella sp.,		–
	Nannochloropsis sp.,		–
	Tetraselmis sp.,		–
	Phaeodactylum tricornutum,		–
Anandamide	Suggested mutants of: *Lobosphaera bisecta*	**50–60	–
Paramylon	*Euglena gracilis*	80	Sun et al., 2018
Hoshinolactam	*Oscillatoria* sp.	3	Ogawa et al., 2017
Dolastatins	*Symploca* sp.	0.5	Luesch et al., 2001
MAAs	*Nostoc commune*	0.4	Matsui et al., 2011
	Aphanizomenon	0.9–1.1	Scoglio et al., 2008
	Chlorogloeopsis sp.	1–3	O'connor et al., 2011
	Lyngbya purpurem	1.7	Chandra et al., 2020
Scytonemin	*Nostoc commune*	0.6	Matsui et al., 2012
	Scytonema sp.	0.4	Rastogi et al., 2014
Eco-Friendly			
Chlorellins (endotoxin)	*Chlorella* sp.	Whole cell extract	Gupta et al., 2013
Cryptophycin 1 (endotoxin)	*Nostoc* sp. (ATCC 53789)	0.05	Polyzois et al., 2020
Bio-diols	*Chlorella ohadii*	80	Benisvy-Aharonovich et al., 2020

DGLA, dihomo-γ-linolenic acid; CoQ10, ubiquinone coenzyme Q10; MAAs, the multifunctional mycosporine-like amino acids.
*Evaluated based on polyunsaturated fatty acids content in the oleaginous microalgae candidates.
**Evaluated based on arachidonic acid content in green microalga *Lobosphaera bisecta*. Content may increase under nitrogen starvation of the alga.

developed instruments and advanced technologies to identify new microalgal isolates from extreme environments, which naturally contain high amounts of interesting chemicals.

Enhance the yield of target products, organoleptic traits, or nutritional content, via controlled DNA manipulation, random mutagenesis, or genome shuffling (through cell mating), or by using the new molecular biotechnology and nanotechnology tools, without compromising on cell growth or compound's potential Following-up studies regarding mutant culture sustainability, productivity and growth rate are necessary to ensure reliability.

Improve microalgae cultivation bioprocess by optimizing culture media, stress conditions, developing growth systems, and fine-tuning downstream processing to increase biomass or the yield of interest so that the cost, and eventually the price, of the final product decreases.

Advance technologies to reuse extracted algal biomass to produce bioenergy, biofertilizers or animal feed, thus reducing the cost of the high-value product.

6. Conclusions

Microalgae present a suitable green source for many valuable products and are considered as alternatives to other organisms or synthetic methods. In this review, the production of unconventional microalgae-based molecules such as fucoxanthin, cannabinoids, and Eco-friendly products such as plant biostimulants were discussed (and summarized in Table 1), which can be scaled up for commercial purposes. The study of genetic and biochemical pathways of microalgae allows generation of robust phenotypes with new traits for sustainable production of novel exotic materials, which in combination with the new modern cultivating technologies can be produced in much affordable prices.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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