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Review

Phytohormones and Effects on Growth and Metabolites of Microalgae: A Review

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Received: 13 March 2018; Accepted: 4 April 2018; Published: 9 April 2018

Abstract: Microalgae cultivation is booming in agriculture, aquaculture, and bioenergy sectors. A wide range of bioactive compounds with attractive properties can be produced with microalgae, including pigments, vitamins, proteins, carbohydrates, and lipids. The biofuel yields from microalgae can exceed the yields obtained with energy crops by 10–100 times. Therefore, such cultivation is promising for the regulation of the biosynthesis of microalagae with phytohormones, which can enhance the production of high-valued bioproducts. This review reports the effect of auxins, abscisic acid, cytokinins, gibberellins, and ethylene on microalgal growth and metabolites, as well as the crosstalk of different phytohormones. The use of phytohormones is also promising because it can also reduce the inputs necessary to grow the selected microalgae and maximize the yields.

Keywords: microalgae; phytohormones; auxins; gibberellins; cytokinins; abscissic acid; bioproducts

1. Introduction

Phytohormones are chemical messengers involved in a broad spectrum of physiological and biochemical processes of higher plants at very low concentrations. Conventionally, phytohormones consist of five classes—auxins, abscisic acid, cytokinins, gibberellins, and ethylene—as well as their precursors and synthesized analogs. In the 19th century, Charles Darwin firstly suggested that certain chemical compounds are capable of stimulating the growth of crops [1]. Since then, tremendous work has been done to study the stimulatory impact of phytohormones on quantity and quality of crops and vegetables.

On the other hand, microalgae can be used as bio-fertilizer or feed food in agriculture and aquaculture. Bioactive compounds with attractive properties are abundant in microalgae, including pigments, carbohydrates, vitamins, proteins, carbohydrates, and lipids, of which the potential economic substances in public health, the food industry, and the medical field are astaxanthin, β-carotene and chlorophylls, polysaccharides, and polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [2,3]. Especially, theoretical biofuel yields of some oleaginous microalgae exceed those of land crops by 10–100 times [4]. Microalgae have been regarded as a potential feedstock for biofuels, as endeavors to tackle global warming and energy crisis.

Therefore, the regulation of the biosynthesis of microalgae with phytohormones supplemented for the harvest of more high-valued products is promising. However, phytohormones are versatile and enigmatic. The functions of these chemicals remain fragmentary and depend on their concentrations, their localization in the tissues and organs of plants, and the crosstalk of different groups of

hormones [5]. In this review, we summarize and analyze the functions of phytohormones, especially their effects on the growth and biosynthesis of microalgae. The interactive effects of different phytohormones are also discussed (Table 1). In order to better understand effects of phytohormones on growth and bioproducts of microalgae, the background of every group of phytohormones including its basic information and physiological roles in plants and microalgae is also provided.

2. Auxins

Auxins are commonly investigated phytohormones and have functions in meditating cell division and cell expansion to facilitate plant growth [6]. Auxins have direct effects on the mitosis, the transition of cells from dormancy to active status, stimulates breathing and tolerance to adverse environmental factors, and positively influences biosynthetic processes [7]. It also controls a spectrum of processes such as the growth of cells, the formation of vascular tissue, and the development of roots [8]. The composition and concentrations of auxins physiologically vary with species, different growth phases, and the conditions of culture. The dominating substances of auxins in microalgae are indole-3-acetic acid (IAA) and indole-3-butanoic acid (IBA) [9]. Various concentrations of IAA or its derivatives are identified in microalgae extracts and supernatants, implementing both stimulatory and inhibitory effects on the growth and metabolism of higher plants and microalgae. In 1937, IAA was first announced and given the name "auxin" [10]. IAA (Figure 1) is commonly synthesized in chloroplasts, and further forms conjugate with glucose, oligosaccharides, aspartic acid, nucleic acids, and proteins [5].

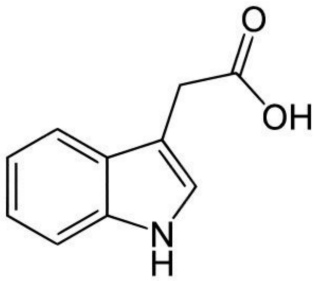


Figure 1. The structure of indole-3-acetic acid (IAA).

Tremendous evidence has been revealed about the effects of auxins on physiological and biochemical processes of higher plants. It has been found that exogenous IAA is able to accelerate the development of parthenocarpic fruit and induce drought tolerance [11]. In the apical and intercalary zones of the thallus of the red alga *Grateloupia dichotoma*, IAA had an augmentation of cell division and elongation but an unfavorable impact on branching [12]. The biological roles of auxins in algae and higher plants are similar [13]. IAA biosynthesis genes from terrestrial plants have homologs in the genome of multicellular brown algae *Ectocarpus siliculosus*. It seems that IAA plays a crucial role in transmitting cell–cell positional information and in inducing a signaling pathway similar with that of territorial plants [14].

IAA, indole-3-butyric acid (IBA), indole-3-propionic acid (IPK), and indole-3-acetamide (IAM) have been uncovered in 46 species of microalgae affiliated with the families *Cyanophyta* and *Chlorophyta* [9]. Auxins play a versatile role in microalgal growth and metabolism, and even very small concentrations of auxins can stimulate growth, biomass production, and the biosynthesis of valuable biomolecules [15]. In IAA, indole-3-*n*-propionic acid (IPA), and IBA at a 50 ppm concentration supplemented with the growth medium of *Chlorella vulgaris*, it was found that cell counts per unit volume were increased by 11–19 times after 26 days of growth [16]. IAA caused the maximum growth of *Scenedesmus obliquus* at 10^{-5} M with an enhancement of 1.9-fold compared to the control [17]. In addition, 1-naphthaleneacetic acid (NAA) significantly increased the biomass yield of *Chlorella pyrenoidosa* by 2.2-fold [18]. It is hypothesized that auxins may promote growth by stimulating

the activity of photosynthesis by boosting the contents of chlorophylls [19] and by activating cellular redox systems [20].

With a spectacular stimulatory effect on growth, the potential for auxins to produce valuable chemicals such as pigments, fatty acids, and polysaccharides is deemed to be worth investigating for viable and scalable industrious applications. The stimulatory effect of auxins on lipid content and lipid productivity of *C. pyrenoidosa* and *Scenedesmus quadricauda* was revealed [21], in which a concentration of 60 mg/L IPA exhibited a maximum growth rate with a threefold increase, and lipid production increased fourfold. It was revealed that IBA, a natural auxin, more proficiently promoted biomass (28.5% increased) and lipid productivity (33.5% increased) than NAA and 2,4-dichlorophenoxyacetic acid (2,4-D), two synthetic auxins [22]. Meanwhile, both 2,4-D and IBA effectively enhanced the accumulation of α-linolenic acid (ALA) with a productivity of 2.12 $g/m^2/day$ [22]. In the microalgae *S. obliquus*, poly-unsaturated fatty acid content reached up to 56% at 10^{-5} M IAA, while the highest carbohydrate content and the highest protein content (34%) were achieved at 10^{-8} M [17]. Similar evidence was found in *C. vulgaris* [23], in which IAA exhibited the highest accumulation of lipids with enriched palmitic (C16:0) and stearic (C18:0) acids. Auxins can also enhance, in addition to growth and lipids, the biosynthesis of pigments, monosaccharides, and soluble proteins in *C. vulgaris* [20]. Increases of 213–273% for chlorophyll a and b content and of 164–258% for carotenoid content of *C. pyrenoidosa* were also found with seven precursors and analogs of auxins supplemented [24].

However, some findings imply that the effects of auxins on growth and metabolism are dose-dependent. For example, the biosynthesis of chlorophyll-a, carotenoids, and fatty acids in *S. quadricauda* was induced by auxins, and the quantities of lipids and their profiles depended on auxin type and the specific concentrations [15]. Furthermore, all phytohormones tested had both stimulatory and inhibitory effects on *S. quadricauda* cell size, which was also dose-dependent. Consistent with this, IAA and 2,4-D exhibited a "low-dosage stimulation and high-dosage inhibition" impact on the growth and production of fatty acid methyl esters (FAMEs) of a freshwater microalgae *Scenedesmus* sp. [19].

Importantly, auxins are able to induce algal tolerance to biotic and abiotic stresses [25]. It was found that the addition of phytohormones enhanced the growth of microalgae compared to those without exogenous phytohormones at low concentrations of ammonium regardless of pH levels [26]. At the same time, exogenous phytohormones contributed to the tolerance to high-ammonium stress and counteracted the drop of algal productivities. More results showed that auxins (IAA, IBA, NAA, and PAA) had a protective effect upon the growth and metabolism of *C. vulgaris* exposed to the stress of heavy metals (Cd, Cu, and Pb), which could imply that auxins activate enzymatic (ascorbate peroxidase, catalase, and superoxide dismutase) and non-enzymatic (ascorbate and glutathione) antioxidant systems, and consequently restrain the accumulation of lipid peroxidation and hydrogen peroxide [20]. Evidence that auxins combined with stress treatments can further stimulate productivities of biomoleculars of microalgae has been accumulating. IAA significantly increased microalgal growth, particularly when the nitrogen source was reduced to 40% [27]. A stepwise strategy was also conducted, in which *Dunaliella tertiolecta* cells were cultured with an addition of 2,4-D at the first step, boosting cell division and increasing biomass productivity by 40%. At the second step, the salt stress remarkably raised lipid content from 24 to 70% [28].

Table 1. Typical cases of effects of phytohormones on microalgae.

	Specific	Species	Targets Promoted	Reference
Auxins	Auxins	*Scenedesmus quadricauda*	Cell size, growth, biomass, chlorophyll-a, carotenoids, fatty acids	[15]
	IAA	*Nannochloropsis oculata*	Cell density, division, chlorophyll-a	[29]
	IAA	*Nannochloropsis oceanica*	Growth, lipid, PUFA, EPA	[30]
	IAA	*Scenedesmus obliquus*	Growth, fatty acid, protein, carbohydrate	[17]
	Auxins	*Chlorella vulgaris*	Pigments, soluble proteins, monosaccharides	[20]
	IAA	*Dunaliella salina*	Growth, β-carotene	[31]
ABA	ABA	*Dunaliella salina*	Growth, β-carotene	[31]
	ABA	*Chlorella pyrenoidosa*	Lipid	[26]
	ABA	*Chlorella sp.*	Lipid	[32]
	ABA	*Chlorella saccharophila*	Lipid, TAG	[33]
	ABA	*Haematococcus pluvialis*	Carotenoids	[34]
	ABA	*Dunaliella salina*	β-carotene	[35]
CKs	Kinetin, Zeatin	*Acutodesmus obliquus*	Biomass, lipid, carbohydrate	[36]
	CK	*Chlorella protothecoides*	Biomass, lipid, ALA	[22]
	Kinetin	*Dunaliella salina*	Growth, β-carotene	[31]
	CKs	*Chlorella vulgaris*	Cell divisions, pigments	[37]
	CK	*Gracilaria caudata*	Pigments, proteins	[38]
GAs	GA3	*Chlorella pyrenoldosa*	Growth, lipid, UFAs	[39]
	GA	*Nannochloropsis oculata*	Cell diameter, lipid	[29]
	GA	*Chlorella ellipsoidea*	Growth, lipid	[40]
	GA3	*Chlorella vulgaris*	Cell number, pigment, protein, monosaccharide	[41]
	GA3	*Microcystis aeruginosa*	Dry weight, cell number, chlorophyll-a, phycocyanin, protein	[42]
	GA3	*Chlamydomonas reinhardtii*	Cell number and size, dry weight, chlorophylls, biodiesel, proteins	[27]
Ethylene and its precusors	Ethephon	*Chlorella vulgaris*	SFAs, a-tocopherol, c-aminobutyric acid, asparagine, proline	[43]
	Ethylene	*Haematococcus pluvialis*	Astaxanthin	[44]
	ACC	*Haematococcus pluvialis*	Astaxanthin	[45]
Combinations	NAA + Zeatin	*Chlorella sorokiniana*	Biomas, chlorophyll-a, lipid	[46]
	Auxins + BL	*Chlorella vulgaris*	Chlorophylls, proteins, monosaccharides	[47]
	CKs + BR	*Chlorella vulgaris*	Cell number, chlorophylls, proteins, monosaccharides	[48]
	IBA + NAA	*Scenedesmus sp., Chlorella sorokiniana*	Lipid	[49]
	IAA + DAH	*Scenedesmu obliquusi, Ourococcus multisporus, Chlorella vulgaris*	Growth, PUFAs	[50]
	GA + Kinetin	*Scenedesmus quadricauda*	Chlorophyl, protein	[51]

3. Abscisic Acid

In 1961, an extracted compound from the peel of mature cotton bolls, now known as abscising, was found to induce defoliation [52]. Later, an isolated compound from the leaves of birch and maple trees, now known as dormin, was revealed to trigger a resting state in the trees' buds [53]. In 1967, abscisin and dormin were combined with the unique name of abscisic acid (ABA) [54]. Structural formula of ABA, a sesquiterpene (C15), is presented in Figure 2. ABA was firstly identified by gas chromatography and mass spectrometry (GC-MS) in brown and green algae [55] and is synthesized in plant tissues with free and bound forms. The bound form results from the interaction of an ester of abscisic acid and D-glucose [56]. In the chloroplasts of green leaves that have stopped growing, ABA is synthesized and transported to other parts of the plant, suppressing growth and inducing a transition to a state of rest [5]. It has also been found that increased ABA content in *Nicotiana plumbaginifolia* results in delayed seed germination [57]. Accumulating evidence permits the conclusion that ABA (1) induces phylloptosis, closure of the stomata, and ageing, (2) maintains bud and seed dormancy, (3) hinders the syntheses of nucleotides and proteins, and (4) inhibits plant growth [58].

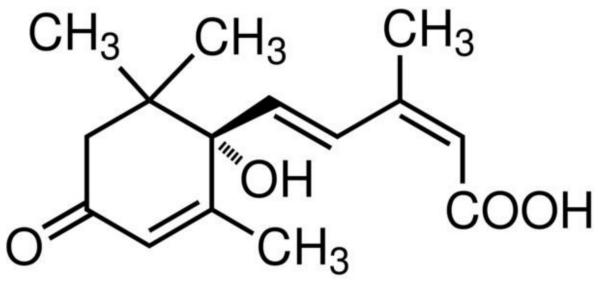


Figure 2. The structure of abscisic acid.

In microalgae *Haematococcus pluvialis*, ABA suppressed the process of growth and triggered cell transition from the active phase to the resting phase [34]. The decreased growth was also found in two marine diatoms, *Coscinodiscus granii* [59] and *Nannochloropsis oceanica* [60]. However, controversial evidence has been found. Endogenous ABA was absent in the earlier growth phase of *Nannochloropsis oculata* but appeared at the beginning of the second fast growth phase [29]. It was further discovered that ABA could enlarge cell diameter, enhance cell division, but scarify the accumulation of lipids at 0.25 mg/L after 14 days of cultivation.

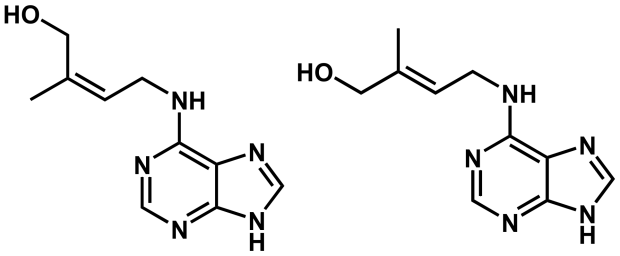
ABA can alleviate detrimental effects caused by conditions that are stressful for higher plants and microalgae [61]. The concentration fluctuation of endogenous ABA is considered a signal for the gene expression of proteins such as dehydrins, which is sensitive to cold, drought, and salinity stress [62]. It has also been stated that overexpressing the key regulatory gene involved in ABA biosynthesis can increase ABA concentration in plants and the higher ABA level boosts stress tolerance [63]. Similarly, it has been shown that ABA improves resistance to temperature and salinity stresses by controlling the uptake of water and ions in higher plants [64].

When it comes to algae, ABA has been reported to mitigate stresses for cyanobacteria (salt stress) [65] and algae (salt, osmotic, oxidative, drought, and nutrient stresses) [60,66]. Therefore, it has been proposed that the cultivation of microalgae can lead to more high-valued bioproducts with the combination of exogenous ABA and stress. One study showed that high salinity stress activates ABA metabolism in halotolerant microalgae *Dunaliella salina*, and then an interrelationship between endogenous ABA levels and β-carotene production was discovered [35]. Another example revealed that exogenous ABA enhances stress tolerance to dehydration for a green alga *H. pluvialis* by accumulating carotenoids, and the accumulated carotenoids further serve as a protective agent against oxidative stress damage [34]. In a microalgae *S. quadricauda*, culture supplemented with 2 μM, ABA increased biomass production 2.1-fold in nitrogen-limited conditions after 48 h of cultivation, so this may be a potential strategy for efficient microalgal cultivation for biofuel production [67]. Meanwhile,

lipid accumulation of microalgae with only ABA supplemented has also been revealed [32,33]. It was shown that 1.0 mg/L ABA imposed an augmentation of lipid productivity of *C. vulgaris* ZF strain and FACHB-31 by 123% and 44%, respectively [32].

4. Cytokinins

Cytokinins (CKs) are derivatives of purine basic nitrogens of adenine and adenosine, discovered in the middle of the 20th century [68]. In plant tissues, zeatin is a predominant form of CKs and may exist in a *cis*- or *trans*-configuration as shown in Figure 3, of which *trans*-zeatin serves as the most active and widespread isoform of CKs [69,70]. Isoprenoid CKs isopentenyladenine was first identified in *Chara globularis* by liquid chromatography and mass spectrometry (LC-MS) [71]. However, the *cis*-zeatin type is the predominant cytokinin in microalgae [72,73], inconsistent with *trans*-zeatin dominating in plants. Isopentenyladenine-type cytokinins are present in moderate levels, while low concentrations of *trans*-zeatin and lower concentrations of dihydrozeatintype cytokinins have been identified. In addition, ribotides are common conjugates combined with the cytokinin free bases [73].



cis-zeatin *trans*-zeatin

Figure 3. The structures of *cis*-zeatin and *trans*-zeatin.

Cytokinins have functions many physiological processes in plants, including stimulation of cell division, differentiation and growth, the promotion of biogenesis and chloroplast differentiation, the regulation of seed dormancy and germination, impediment of the leaf senescence, the formation of shoots from calluses in a culture, and inhibition of the root apical meristem. Moreover, CKs facilitate the assimilation of nutrition [74], the formation of nitrogen-fixing nodules [75], tolerances to adverse environments [76], and the improvement of the quantity and quality of crop plants [77]. It has also been uncovered that CKs play a key role in biological processes of microalgae including the induction of cell division, the stimulation of growth processes, and the augmentation of photosynthetic activity. Under adverse conditions, microalgal CKs have a protective effect on physiological activities, especially photosynthesis [72].

Inorganic nitrogen sources, such as ammonia, are a predominant regulator of gene expression of adenosine phosphate-isopentenyltransferase (IPT), a key enzyme involved in cytokinin biosynthesis, and this nitrogen-dependent synthesis of cytokinins occurs not only in roots but also in leaves. Consequently, the local synthesis of cytokinins is crucial as a response to acquisition and distribution of nutrition [69]. CK biosynthesis of microalgae appears to be similarly nitrogen-dependent, as RNA, specific proteins, and polypeptides are increased if nitrogen assimilation is enhanced through the activation of glutamatdehydrogenase [37]. Importantly, the content and composition of microalgal CKs is significantly influenced by lighting regimes and the availability of carbon sources [72,78]. It has been found that cytokinins are present in low concentrations in dark periods and in relatively high concentrations in light periods. Cytokinins appear to be quickly consumed in the log phase of growth, as variable levels of free bases and ribosides have been detected. Furthermore, excessive cytokinins are likely to be stored as *O*-glucosides, since low but growing levels of *O*-glucosides have been found in all cultures exposed to lights. The biosynthesis of ribotides appears also to be light-dependent, with a high ribotide transient peak detected in the illuminated period [79].

Therefore, CKs play a crucial role in photosynthesis. There are more examples of CK promoting the accumulation of photosynthetic pigments in *C. vulgaris* [37] and *D. salina* [31], leading to a boost in growth. However, there is contradictory evidence that synthetic cytokinins do not affect chlorophyll content but improve the growth of *Chlamydomonas reinhardtii* [80], while CKs have a stimulatory effect on pigments of *Gracilaria caudata* but do not affect growth [38]. Furthermore, CKs are found to stimulate synthesis of a variety of biochemicals, especially fatty acids. The highest biomass productivity of *Acutodesmus obliquus* was achieved with exogenous 0.1 mg/L zeatin in the early log phase. Meanwhile, when kinetin was added at a middle log phase and zeatin was added at an initial log phase, lipid productivity increased by 64.95% and 63.06%, respectively [36]. In one study, BAP exhibited a 1.26-fold boost of biomass yield, and both BAP and kinetin contributed to a significant increase in the production of α-linolenic acid (ALA) in *C. pyrenoidosa* [18]. A similar study revealed that kinetin exhibited an increase in the biosynthesis of ALA by 26.5% at a 1 ppm concentration in a culture of *Chlorella protothecoides*. It also appeared that the stimulatory effect of optimum concentrations of CKs on biomass was greater than that on lipids [22].

5. Gibberellins

Gibberellins (gibberellic acid (GA)) were discovered in studies on fungal ascomycetes *Gibberella fujikuroi* on rice plants [81], which were later isolated and named gibberellins A and B [82] and subsequently detected in extracts of the marine algae genera *Fucus* L. [83]. The majority of gibberellins are acids and denoted by GA (gibberellic acid) followed by a number corresponding to the order in which the gibberellin was discovered. GA1, GA3, GA4, GA5, GA6, and GA7 (see Figure 4) are the most active forms [84], and the rest are their precursors. In addition, the number of gibberellins in plants can reach up to 136, while only a minority of them is biologically active. In a model plant, *Arabidopsis*, increased concentrations of GAs are correlative with a longer hypocotyl, activated stem elongation, earlier flowering, and inhibited seed dormancy [85,86].

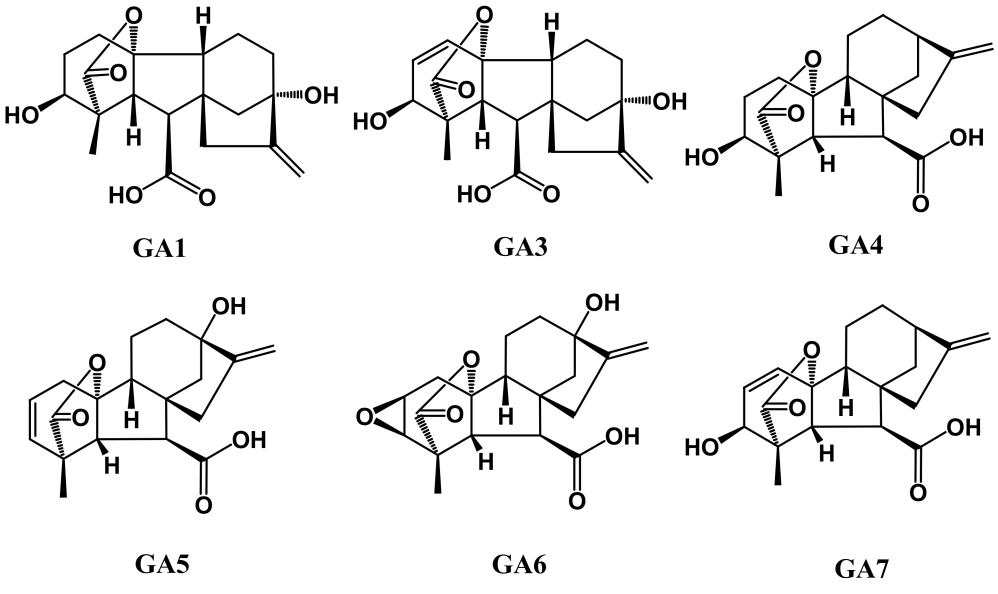


Figure 4. Structures of gibberellic acid (GA).

The LC-MS technique facilitated the discovery of 19 GA forms in *Ch. reinhardtii*, and GAs have been found in 31 microalgal species. It has also been indicated that the concentration of GAs in slowly growing microalgal cultures was higher than that of a rapidly growing culture [87]. The functions of microalgal gibberellins are physiologically similar to those of higher plants. Externally applied gibberellins shorten the lag phase considerably, activate cell division and growth in the log phase,

stimulate the accumulation of pigments and proteins, and reduce the toxicity of heavy metals in a microalgal culture. It has also been stated that gibberellins are mainly involved in cell elongation and expansion but not in cell division [72].

Accumulating evidence indicates that gibberellins impose an influence on microalgae growth and metabolism by regulating uptakes and utilizations of nutrition, especially by meditating carbon metabolism [39,42,88]. One example revealed that GA3 had a stimulatory effect on the growth of *Microcystis aeruginosa* by enhancing the uptake of nitrogen and the capability to utilize carbohydrates. Cellular pigments were consequently accumulated, and the content of proteins and microcystin was then increased [42]. Another study indicated that the growth and lipid accumulation of *C. pyrenoidosa* was enhanced by GA3 by augmenting the esterase activity and mediating intracellular distributions of carbon. Both maximum lipid content (292.3 mg/g) and lipid productivity (17.1 mg/L/day) were obtained with 20 mg/L GA3, and higher levels of GA3 exhibited a boost of unsaturated fatty acids content by 1.6 times [39]. Moreover, 4 mg/mL gibberellins increased biomass, lipid, and DHA production of *Aurantiochytrium* sp. by 14.4%, 43.6%, and 79.1%, respectively. It was supposed that gibberellins accelerate the rate of utilization of glucose, while the metabolisms of glycolysis and the TCA cycle are inhibited [88].

It has also been postulated that GAs especially alleviate adverse effects caused by heavy metals [25,41]. With 10^{-5} M GA3 supplementation, *C. vulgari* cells have been found to bioaccumulate and bioconcentrate toxic metals from an algal culture medium. Moreover, GA3 had a protective effect on *C. vulgaris* against the stress of Pb and Cd with the accumulations of cell counts and proteins, photosynthetic pigments, and monosaccharide. It was hypothesized that GA3 plays a positive role in growth and enhances the adaptive ability against adverse environments [41].

6. Ethylene

Ethylene (CH_2=CH_2) is a gaseous plant hormone that regulates a spectrum of physiological processes such as the growth and development of plants, aging [89] and tolerances to environmental factors from biotic (pathogen invasion) and abiotic stresses including drought, high salinity, and cold [90]. The effect of ethylene on plants was first revealed on etiolated sprouts of pea, suppressing growth of the stem [91]. In the 1920s, ethylene was found to accelerate fruit ripening [92]. Importantly, ethylene is known to trigger a "triple response": an exaggeration of growing of the apical hook, combined with the depression of hypocotyl and root extension, which is thought to protect the apical meristems of shoots and roots from damage [93].

However, the data of ethylene's effects on algae is scarce. Ethylene was recently detected in a filamentous charophyte *Spirogyra pratensis* and found to regulate cell development, suggesting that the ethylene hormone system of *S. pratensis* is homologous to that of plants [94]. Accumulating evidence shows that the light regime and the composition of culture medium have a determined impact on the formation of ethylene [95–97]. It was found that the concentration of ethylene increased threefold when glucose and methionine were added into the medium. By contrast, the level of ethylene dropped with serine supplemented in a nitrogen-free medium. Furthermore, the absence of light had an inhibitory effect on the formation of ethylene in a strain of *Hapalosiphon*, while the presence of glucose in the culture contributed to the production of ethylene in the dark [95]. Aminocyclopropane-1-carboxylic acid (ACC) is considered an intermediate precursor of ethylene. ACC oxidase catalyzes the last step of ethylene biosynthesis in *H. pluvialis*, the activity of which is inhibited by salicylhydroxamic acid (SA), heavy metals, and darkness [96], and the synergistic effect of ACC and light intensity on ET production has also been confirmed [45]. Culture samples of macroalgae *Ulva intestinalis* have exhibited a significant rise in ethylene concentration when low-light conditions were altered to become high-light conditions [97].

Ethylene has long acted as a growth inhibitor, but evidence is accumulating that it can also promote growth and biosynthesis, mainly determined by dosage [44,45,89]. For instance, 0.05 mL/L ethylene was able to remarkably increase and quicken the astaxanthin accumulation of *H. pluvialis*,

while 0.1 mL/L ethylene inhibited it [44]. Moreover, the effect of ACC is dose-dependent [45], and the effect of ethylene is also influenced by the tissue and plant species of interest, as well as by endogenous and environmental signals [89]. Further, the addition of ethephon (an ethylene releaser) in *C. vulgaris* culture leads to higher levels of proline and saturated fatty acids, but lower levels of citrate and unsaturated fatty acids [43].

7. The Crosstalk for Different Phytohormones

Besides these five groups of phytohormones described above, there are other important hormone-like and growth regulation substances such as brassinosteroids, jasmonic acid, polyamines, salicylates, and signal peptides [39], which involve a spectrum of physiological activities in the growth of microalgae. For example, brassinosteroids play roles in cell division, elongation, and differentiation of the vascular system, and jasmonic acid can enhance the tolerances of microalgae to adverse environments [22]. Similarly, salicylates are able to trigger defensive responses [32]. Overall, a variety of physiological and biochemical processes is regulated by phytohormones. However, it should be kept in mind that the majority of the results available are derived from studies where phytohormones are supplemented externally and then may alter the concentrations of other hormones and compounds, interacting with various other signal transduction routes. One study revealed that ethylene, auxin, and GA responses can be attributed to the functions of DELLA proteins (a family of proteins of transcriptional regulators), and it was further suggested that DELLA proteins serve as coordinators of multiple phytohormone signal inputs, mediating the growth of plants [98]. Another research showed that exogenous brassinolide increased the concentration of ABA of *C. vulgaris* in response to heat stress [99].

Phytohormones also work together on the response to stresses. In the unicellular oleaginous microalga *N. oceanica*, the transcriptions of ABA and CKs were up- and downregulated, respectively, upon nitrogen starvation. Meanwhile, the endogenous ABA and CKs appeared to interact antagonistically in response to nitrogen deficiency. Consistent with this, exogenous CKs tend to activate cell cycle progression, while exogenous ABA serve as both a growth inhibitor and a stimuli to stress tolerance. These findings suggest that ABA and CKs play a sophisticated interactive role in the orchestration of cellular biosynthesis to tackle adverse environmental factors [60].

Importantly, the combination of phytohormones is widely used to enhance algal cultivation for valued products. GA stimulated the accumulation of proteins and chlorophylls during the exponential phase of growth, and, when it was combined with kinetin, production increased twofold [15]. It was also found that kinetin + 2,4-D can favor the growth of *H. pluvialis* and *D. salina* [100], and GA + NAA can boost the growth of *Skeletonema costatum* [101]. In another study on *Chlorella sorokiniana*, two combined treatments of NAA + GA3 and NAA + zeatin stimulated biomass productivity compared to the control by 138% and 136%, respectively. Moreover, NAA + 2-phenylacetic acid and NAA + zeatin + GA3 exhibited higher yields of biomass and chlorophyll a. It was further supposed that the combinations from different phytohormone families led to more biomass productivity than those from the same families [46]. Significant increases in algal growth and in the levels of biochemical parameters, including chlorophylls, proteins, and monosaccharides were found with the combined treatments of brassinolide (BL) + IAA [47] and BL + *trans*-zeatin [48]. Similar to this, combined phytohormones have been observed to stimulate biomass and lipid yields of two freshwater microalgae, implying that proton pumps and an anti-oxidative mechanism were activated by the stimulants [49,102].

8. Conclusions

Land plants evolved more than 450 million years ago from a lineage of freshwater charophyte green algae [103]. To a large degree, the biological roles of phytohormones of microalgae and higher plants are similar. On one hand, phytohormones mediate microalgal activities: (a) the effect of phytohormones on microalgae is dose-dependent; (b) phytohormones, more or less, enhance the

adaptive ability of microalgae against biotic or abiotic stresses; (c) phytohormones work interactively in the orchestration of cellular biosynthesis through a complex signal network. On the other hand, a bulk of evidence has been provided in this review that phytohormones enable microalgae to accumulate more biomass or high-valued bioproducts, which will facilitate the scale-up cultivation of microalgae, contributing especially to the industrialization of biodiesel from microalgal lipid. Nevertheless, the scalable and viable microalgal production still requires more investigations and studies, and the manipulation of phytohormone on microalgae has a great potential to become a pillar of the "Green Revolution" for not only producing useful biochemicals but also alleviating global warming and energy crisis.

Acknowledgments: The staff of China-EU Institute for Clean and Renewable Energy at Huazhong University of Science and Technology should be acknowledged, together with the staff of the laboratory of Yunjun Yan at College of Life Science and Technology, Huazhong University of Science and Technology. This work was financially supported by the National Natural Science Foundation of China (No. J1103514), the National High Technology Research and Development Program of China (2011AA02A204 and 2013AA065805), the Innovation Foundation of Shenzhen Government (JCYJ20120831111657864), the Natural Science Foundation of Hubei Province (No. 2015CFA085), and the Fundamental Research Funds for HUST (No. 2014NY007).

Author Contributions: Xingfeng Han and Yunjun Yan conceived and designed the framework of this review; Xingfeng Han and Huiru Zeng did literature research; Xingfeng Han analyzed the data and wrote the paper; Huiru Zeng and Pietro Bartocci modified the paper format; Pietro Bartocci, Francesco Fantozzi, and Yunjun Yan contributed to manuscript revision.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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