Bacterial Enhancement of Microalgal Metabolites

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1. Introduction

Microalgae are photosynthetic microorganisms with a high potential to produce a wide variety of industrial-interest metabolites such as proteins, lipids, carbohydrates, and pigments. Their rapid growth rates and the possibility to be cultivated on nonarable land constitute an advantage against plant-based sources [1].

Overproduction of microalgal metabolites has been optimized by several mechanisms, from the alteration of culture conditions (nutrient concentrations, light intensity, carbon source, salinity, and temperature) to metabolic engineering [2][3][4][5]. Another way to stimulate metabolite production is through the application of chemical triggers, such as phytohormones and analogs regulating microalgal metabolism, or of chemicals that can regulate biosynthetic pathways, induce oxidative stress responses, or act directly as metabolic precursors [6][7][8]. Since these growth-promoting factors are produced by bacteria, microalgae–bacteria consortia have been explored as an alternative route to enhancing microalgae growth and metabolite production [9][10].

Microalgae and bacteria have coexisted since the early stages of evolution. From a biotechnological aspect, the symbiotic interactions cover a wide range, mostly mutualistic, commensalistic, or parasitic [11]. Mutualisms are positive interactions among different species that improve the fitness of the involved partners and are based on the exchange of resources and services [12][13]. A mutualistic microalga–bacteria consortium is based on the exchange of metabolites, mostly the bacterial uptake of extracellular organic carbon released from the algal photosynthesis; in return, the bacterial growth can be stimulated by (1) the removal of oxygen and the generation of carbon dioxide, (2) the supply of nutrients, vitamins, and trace elements for microalgal growth, and (3) the production of growth-promoting factors as well as chelators and phytohormones [14].

2. Enhancement of Biomass and Metabolite Production

The noteworthy increase in microalgae biomass production as a result of co-cultivation with bacteria has driven the study of these mutualistic interactions to boost the production of several microalgae of commercial interest (Table

1).

Table 1. Microalgal growth promotion of some artificial microalgae–bacteria consortia.

Bacteria	Microalgae	Growth Promotion Effect	Culture Medium	Reference
Azospirillum brasilense Cd	*Chlorella sorokiniana* UTEX 2714	11% increase in cell density (g dw/L)	N8 medium	[15]
Azospirillum brasilense Cd	*A. protothecoides* UTEX 2341	90% increase in cell density (g dw/L)	N8-NH_4	
Brevundimonas sp.	*Chlorella ellipsoidea* UTEX 247	50-fold increase in cell density (cel/mL), longer exponential phase	Modified BBM	[16]
Pelagibaca bermudensis KCTC 13073BP	*Tetraselmis striata* KCTC1432BP	2-fold increase in biomass productivity (mg/L/d)	O3 medium	[17]
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 2714	16 and 11% increase in cell density (cel/mL) and growth rate, respectively	Synthetic growth medium (SGM)	[18]
Azospirillum brasilense Cd	*Chlorella sorokiniana* UTEX 2805	40 and 35% increase in cell density (cel/mL) and growth rate, respectively		
Bacillus pumilus ES4	*Chlorella vulgaris* UTEX 2714	1.5-fold increase in cell density (cel/mL)	N-free SGM	[19]
Escherichia coli ATCC 25922	*Chlorella minutissima* UTEX 2341	3.5-fold biomass productivity (mg/L/d)	N8-NH_4, 1% Glucose	[20]
		3.4-fold biomass productivity (mg/L/d)	N8-NH_4, 1% Glycerol	
		7.2-fold biomass productivity (mg/L/d)	N8-NH_4, 1% Acetate	
Rhizobium sp. 10II	*Ankistrodesmus* sp. SP2-15	29% increase in dry weight (mg/L)	BG11 medium	[21]
Stenotrophomona smaltophilia	*Chlorella vulgaris*	22, 20, and 18% increase in biomass (g/L), growth rate and productivity (mg/L/d), respectively	BG11 medium	[22]

Bacteria	Microalgae	Growth Promotion Effect	Culture Medium	Reference
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 395	62% increase in cell size	Synthetic wastewater	[23]
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 2714	3-fold increase in cell density		
Azospirillum brasilense Cd	*Chlorella sorokiniana* UTEX 1602	2.2-fold increase in cell density		
Rhizobium sp.	*Botryococcus braunii*	55% increase in optical density	Modified Jaworski medium	[24]
Muricauda sp.	*Dunaliella* sp.	7% increase in cell biovolume	Modified Walne's medium	[25]
Dinoroseobacter shibae	*Thalassiosira pseudonana*	35% increase in cell density	SW+ medium	[26]
Phaeodactylum tricornutum	*Stappia* sp.	72% increase in cell density	F/2 medium	[27]
Alteromonas sp.	*Isochrysis galbana*	52% increase in cell density	Zobell Marine Broth [30]	[28]
Labrenzia sp.	*Isochrysis galbana*	71% increase in cell density		

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[18][23][29]. In
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bacteria were ubiquitously present in a wide variety of wastewater effluents. These isolated bacteria promoted cell growth of three different algae strains (*C. reinhardtii*, *C. vulgaris,* and *Euglena gracilis*), enhancing microalgal growth up to 2.8-fold. On the other hand, the bacteria *Pelagibaca bermudensis* isolated from the phycosphere of *Tetraselmis striata* enhanced two-fold the biomass productivity of this microalga [17], while population growth was 0.5–3 times higher in *Chlorella ellipsoidea,* with eight bacterial strains isolated from a long-term culture of *C. ellipsoidea* [16]. Growth-promoting bacteria were also found by Lee et al. [31] in the phycosphere of *Haematococcus pluvialis*. The authors reported an increase in *H. pluvialis* growth at all growth stages, due to high auxin production by co-cultivation with the isolated strain *Achromobacter* sp. CBA4603. Similarly, the co-cultivation of four bacterial strains (*Flavobacterium*, *Hyphomonas*, *Rhizobium,* and *Sphingomonas*) isolated from *C. vulgaris* increased the microalgal population by more than 100% when compared to axenic cultures [32]. Likewise, the growth-promoting effect of the marine bacterium *Flavobacterium* sp. was evaluated in three marine microalgae (*Chaetoceros gracilis*, *Isochrysis galbana*, and *Pavlova lutheri*). The results revealed that the bacterium enhanced the specific growth rate and maximal density of *C. gracilis* and kept longer the exponential growth phase of *I. galbana* and *P. lutheri* [33].

The cultivation of microalgae with growth-promoting bacteria results not just in the enhancement of biomass production but also in the increment of the intracellular levels of lipids, carbohydrates, pigments, and proteins (Table 2). Most of the recent studies on artificial microalgal–bacterial consortia are focused on lipid content, due to

the increasing interest in biofuels and biodiesel production. The studies show that this kind of microbial association improves both lipid productivity and lipid quality for biodiesel production. For instance, lipid accumulation in the microalga *C. reinhardtii* was significantly improved by co-cultivation with *Azotobacter chroococcum* under nitrogen starvation [34]. The authors reported an increase of 2.4 times in lipid content and 5.9 times in lipid production with the co-culture and up to 19.4 times the lipid productivity compared with the axenic microalga. This increment was explained by an increase in the levels of expression of genes that positively regulated lipid metabolism, while the expression levels of genes that negatively regulated lipid metabolism decreased. Similarly, Leyva et al. [35] found that the activity of acetyl-CoA carboxylase (ACCase) is enhanced by the co-cultivation of *C. vulgaris* with the MGPB *A. brasilense* under autotrophic and heterotrophic conditions. However, although higher levels of lipids were found in co-cultures (up to a five-fold increase under autotrophic conditions), the authors did not find a direct link with the increase on ACCase activity. Likewise, the total content of C16 and C18, which are the main fatty acids present in biodiesel composition, can increase in symbiotic co-cultures. Xue et al. [34] reported more than 80% content of C16 and C18 in the fatty acids produced by the microalga *C. vulgaris* when cultivated with *Stenotrophomonas maltophilia* as well as an increase of up to 5% when compared to axenic cultures. Similar results were reported by de-Bashan et al. [23] with the cultivation of three different *Chlorella* strains with *A. brasilense* immobilized in alginate beads. Immobilization has been found to maintain the close physical proximity of the two microorganisms to facilitate interaction and avoid external interference from bacterial contaminants [36]. In all the co-cultures, the concentration and variety of fatty acids increased, reaching up to eight different fatty acids in microalgae co-immobilized with the MGPB in comparison to four to five in microalgae-only immobilized cells.

Table 2. Metabolite production enhancement of some artificial microalgae–bacteria consortia.

Bacteria	Microalgae	Metabolite Production Enhanced	Culture Medium	Reference
Escherichia coli ATCC 25922	*Chlorella minutissima* UTEX 2341	6.2-fold lipid productivity (mg/L/d)		[20]
		18.8-fold starch productivity (mg/L/d)	N8-NH_4, 1% Glucose	
		1.8-fold lipid content (%)		
		5.4-fold starch content (%)		
		3.1-fold lipid productivity (mg/L/d)	N8-NH_4, 1% Glycerol	
		9.9-fold starch productivity		

Bacteria	Microalgae	Metabolite Production Enhanced	Culture Medium	Reference
		(mg/L/d)		
		2.9-fold starch content (%)		
		8.2-fold lipid productivity (mg/L/d)	N8-NH_4, 1% Acetate	
		27.1-fold starch productivity (mg/L/d)		
		3.7-fold starch content (%)		
		2.4-fold lipid content (%)		
Azotobacter chroococcum No 1.0233	*Chlamydomonas reinhardtii* cc849	5.9-fold lipid production (mg/L)	N-free TAP medium	[34]
		19.4-fold lipid productivity (mg/L/d)		
Stenotrophomona smaltophilia	*Chlorella vulgaris*	Lipid increase by 8–34%	BG11	[22]
Phaeodactylum tricornutum	*Stappia* sp.	172% increase in fucoxanthin	F/2 medium	[27]
		144% increase in chlorophylls		
Phaeodactylum tricornutum	*Marinobacter* sp.	50% increase in total lipids	F/2 medium	[37]
Rhizobium sp. 10II	*Ankistrodesmus* sp. SP2-15	39% increase in chlorophyll a	BG11	[21]
Methylococcus capsulatus	*Chlorella sorokiniana*	42% increase in carbohydrates	Industrial wastewater with synthetic biogas as methane source	[38]
Methylococcus capsulatus	*Chlorella sorokiniana*	15% increase in lipid content		
Azospirillum brasilense Cd	*Chlorella sorokiniana* UTEX 1602	1.6-fold chlorophyll a (µg/g cells)	Synthetic Wastewater	[23]

Bacteria	Microalgae	Metabolite Production Enhanced	Culture Medium	Reference
		1.6-fold chlorophyll b (µg/g cells)		
		1.7-fold lutein (µg/g cells)		
		2.5-fold violaxanthin (µg/g cells)		
		5.5-fold lipid content (µg/g dw)		
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 395	1.6-fold chlorophyll a (µg/g cells)		
		1.8-fold chlorophyll b (µg/g cells)		
		1.8-fold lipid content (µg/g dw)		
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 2714	2.8-fold chlorophyll a (µg/g cells)		
		2.5-fold chlorophyll b (µg/g cells)		
		2.3-fold lutein (µg/g cells)		
		1.5-fold violaxanthin (µg/g cells)		
		3.9-fold lipid content (µg/g dw)		
Azospirillum brasilense Cd	*Chlorella vulgaris* UTEX 2714	1.4-fold chlorophyll a (µg/g cells)	Synthetic Wastewater	[39]
		2.8-fold chlorophyll b (µg/g cells)		
		2.9-fold ß-carotene (µg/g cells)		
		2.5-fold lutein (µg/g		

Bacteria	Microalgae	Metabolite Production Enhanced	Culture Medium	Reference
		cells)		
		2.3-fold violaxanthin (µg/g cells)		
Phyllobacterium myrsinacearum	*Chlorella vulgaris* UTEX 2714	1.8-fold chlorophyll b (µg/g cells)	Synthetic Wastewater	[40]
		1.8-fold ß-carotene (µg/g cells)		
		2-fold lutein (µg/g cells)		
Azospirillum brasilense Cd	*Chlorella sorokiniana* UTEX 2714	2.2-fold violaxanthin (µg/g cells)	N8 medium	[15]
		3-fold chlorophyll a (µg/mg dw)		
		5-fold chlorophyll b (µg/mg dw)		
		2.5-fold soluble protein (%)		
Azospirillum brasilense Cd	*A. protothecoides* UTEX 2341	40–60% increase in soluble protein	N8-NH_4	[18][29]

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Similarly, microalgal pigment production can be significantly enhanced by co-cultivation with bacteria. Gonzalez-Bashan et al. [40] found that the production of chlorophyll, ß-carotene, lutein, and violaxanthin increased significantly in the microalga *C. vulgaris* when grown with *Phyllobacterium myrsinacearum* co-immobilized in alginate beads. A similar study was carried out with *A. brasilense*, enhancing even more the pigment production of *C. vulgaris*. The co-immobilization of the microorganisms resulted in increments of up to 35% in chlorophyll a, 176% in chlorophyll b, 186% in ß-carotene, 152% in lutein, and 129% in violaxanthin [39]. Likewise, a significant increase in these four pigments was observed in the microalga *C. sorokiniana* when co-immobilized in alginate beads with *A. brasilense* [23]. The use of growth-promoting bacteria to enhance pigment production has great commercial potential, considering the increasing consumer demand for natural products, including the replacement of commonly used synthetic pigments for pigments derived from natural sources [42].

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