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Algae-bacteria consortia promote the cell growth of marine microalgae *Phaeodactylum tricornutum* and *Chrysotila roscoffensis*

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Abstract

The commercial exploitation of *Phaeodactylum tricornutum* and *Chrysotila roscoffensis* is hampered by their low biomass productivity. Microalgae-bacteria co-cultivation (MBC), as an emerging approach designed to boost microalgal growth, has recently gained considerable attention. Nevertheless, studies focusing on MBC with these two microalgae remain scarce. In this study, six bacterial strains were isolated from xenic microalgal cultures. Subsequent co-cultivation trials revealed two strains of *Bacillus* spp. and one of *Agrobacterium* sp. were found to promote the growth of axenic *C. roscoffensis* and *P. tricornutum*. Biochemical analysis, encompassing macromolecules, pigments, and fatty acids, indicated that the addition of these beneficial bacteria did not weaken the quality of microalgal biomass, but substantially increased the productivity of bioactive substances. To assess the feasibility of employing these bacteria in large-scale microalgal cultivation, further MBC trials using xenic microalgal cultures were conducted. The results confirmed that the beneficial bacteria were also effective in a non-axenic MBC context.

Keywords: Microalgae-bacteria consortia, Growth-promoting bacteria, *Phaeodactylum tricornutum*, *Chrysotila roscoffensis*, *Bacillus.*

1. Introduction

Microalgae are adept at transforming carbon dioxide and sunlight into commercially valuable substances such as proteins, lipids, polyunsaturated fatty acids (PUFAs), carotenoids, and other bioactive molecules, demonstrating potential applications in functional foods, cosmetics, and pharmaceuticals [1]. Additionally, numerous genetic engineering tools have been developed to enhance the production of these valuable substances in various microalgae species, facilitating the production of both natural and engineered products [2]. Nonetheless, the industrial deployment of microalgae is hampered by their slow growth and low biomass productivity in large-scale operations [3]. As a result, there is an emerging focus on developing technologies, including optimization of cultivation conditions [4], design of highly effective photo-bioreactors [5], and breeding elite strains through genetic engineering or mutagenesis approaches [6], to boost microalgal growth performance.

One seemingly counterproductive strategy used to promote microalgae growth is microalgae-bacteria co-cultivation (MBC). Generally, microalgae and bacteria occupy nearly all ecological niches on Earth, and through co-evolution, have established a variety of interactions [7]. Some bacteria, for example, produce algicidal compounds such as enzymes and allelochemicals that can severely inhibit or destroy microalgal cells [7][8]. Previously, studies on microalgae-bacteria interactions have mainly been focused on the competitive and antagonistic relationships with the purpose of providing guidance for the management of harmful algal blooms [8][11]. Contrary to the original view that bacteria predominantly exert negative effects on microalgae, recent studies have demonstrated that mutualistic interactions between these microorganisms are also universal [12]. Within mutualistic relationships, certain bacteria are found to stimulate microalgal growth by supplying essential growth factors, including vitamins, phytohormones, and macronutrients like nitrogen and phosphate, reciprocally receiving organic carbon and oxygen [7][9][13]. Consequently, MBC, leveraging these symbiotic relationships, is considered a viable strategy to improve microalgal biomass productivity.

The interaction between bacteria and microalgae is species-specific, therefore, identifying growth-promoting bacteria is essential for constructing an effective MBC system for a given microalga. Recent studies have successfully isolated bacteria from xenic microalgal cultures and identified those that positively influence growth in axenic microalgal cultures [12]. To cite an instance, Lian et al. obtained multiple bacterial strains from xenic *Nannochloropsis* sp. and tested the effects of 18 bacteria on axenic *Nannochloropsis*. The results demonstrated that *Maritalea porphyrae* DMSP31 and *Labrenzia aggregata* YP26 significantly enhanced the growth of axenic *Nannochloropsis* [14]. However, it should be noted that the potential applicability of selected bacteria for large-scale microalgal cultivation remains uncertain unless the stimulatory effects are also verified in xenic microalgal cultures. On an industrial scale, where maintaining an axenic status is challenging, a diverse array of bacteria inevitably co-exist with microalgal cells [15]. In such contexts, the stimulatory or inhibitory effects of specific bacteria on axenic microalgal cells are likely to be counteracted by the presence of other co-occurring bacteria. For example, Liu et al. investigated the effects of 18 bacteria on axenic *Nannochloropsis oceanica* KB1. The authors found that eight of these strains, when introduced at a bacterium-to-microalga cell density ratio of 60: 1, significantly reduced the final concentration of KB1. Conversely, this suppressing effect transformed into a growth-promoting effect when the same bacterial strains were inoculated at identical ratios into xenic *N. oceanica* cultures [16]. Therefore, constructing an efficient MBC system should involve two critical steps: identifying beneficial bacteria using axenic microalgal cultures, and verifying their potential applicability with xenic microalgal cultures.

In our previous work, we have developed a method for bacterial removal from microalgae, enabling the axenic culture of *Phaeodactylum tricornutum* and *Chrysotila roscoffensis* [17][18]. *P. tricornutum*, a diatom model organism, is renowned for its prolific production of lipids, PUFAs, and fucoxanthin. *C. roscoffensis*, a coccolithophore of the Haptophyta, is remarkable for thriving under stress and for producing a variety of bioactive compounds, engaging in dual carbon fixation processes—photosynthesis and biocalcification [19]. Both species offer considerable promise for commercialization, yet their associated growth-promoting bacteria remain unidentified. The establishment of these axenic strains has provided us with a unique platform to explore the interactions between microalgae and bacteria, free from external microbial influences, and to potentially identify beneficial bacterial species.

In the present study, we initially isolated six bacterial strains from non-axenic cultures of *P. tricornutum* and *C. roscoffensis*. After that, three strains were tentatively classified as promoters of microalgal growth, inferred from their positive impact on the growth of two axenic microalgal strains. Besides that, we also comprehensively assessed the impacts of these bacteria to the microalgae by examining biomass composition, photosynthetic efficiency, fatty acid (FA) profiles, and pigment content. Due to the arduous task of maintaining axenic conditions during large-scale production, we also conducted trials with non-axenic strains to confirm the facilitating effects of the bacteria in a semi-continuous cultivation system. This research offers insights into microalgal-bacterial dynamics and furnishes critical information for establishing a MBC system aimed at elevating microalgal growth efficiency in mass cultivation.

2. Materials and Methods

2.1 Microalgae strain and general cultivation conditions

Both xenic and axenic strains of *C. roscoffensis* NMBjih026-8 and *P. tricornutum* NMBguh002 were preserved in the Microalgae Collection Center of Ningbo University using antibiotic-free medium. Because all microalgae species in this center have been subcultured using NMB3# medium (each liter containing 100.0 mg KNO_3, 10.0 mg KH_2PO_4, 10.0 mg EDTA-Na_2, 2.5 mg $FeSO_47H_2O$, 250.0 μg $MnSO_4H_2O$, 6.0 μg VB_1, and 0.05 μg VB_{12}) [20] for a long time, NMB3# was adopted as the basal growth medium in this study. For all experiments, microalgae cells (density was represented by the optical density at 750 nm (OD_{750}) ≈ 0.15) were inoculated into fresh medium with an inoculation ratio of 1: 9 (cultures volume: medium volume) and cultivated at 23 ± 1°C with an illumination intensity of 60 μmol photons m^{-2} s^{-1} and a light/dark cycle of 12 h/12 h. The initial pH and salinity were set to 8.30 and 25‰, respectively. Before conducting cultivation trials, the axenic status of microalgae strains was investigated based on SYBI Green I [18]. Additionally, due to the considerable challenges in maintain axenic status under aerated conditions, the cultivation process was conducted without aeration.

2.2 Isolation, identification, and screening of growth-promoting bacteria

For bacteria isolation, 1 mL of xenic microalgae (*C. roscoffensis* and *P. tricornutum*) cultures were diluted 10^6-fold using sterilized NMB3# liquid medium. Then, 200 μL of diluted cultures were plated onto solid 2216E medium (1% agar-containing) and incubated at 23 ± 1°C for 72 h. For each plate, 15 colonies were randomly picked and transferred into liquid 2216E medium, and then placed in a shaker (23 ± 1°C, 200 rpm) for 24 h. Subsequently, the bacteria were serially diluted and streaked onto 2216E agar plates. After two days of incubation, bacteria colonies were picked and transferred into liquid 2216E medium, and cultivated for 24 h for DNA extraction using CTAB method [21]. Universal primers of 27F and 1492R were adopted to amplify the 16S rRNA genes. After sequencing, the taxonomic status of the isolated bacteria was preliminarily determined by performing a Nucleotide BLAST search on NCBI. Then, maximum-likelihood (ML) tree was further constructed from an alignment of 25 16S rRNA sequences to identify the phylogenetic characterizations of six bacteria strains.

The impacts of six bacteria strains on axenic *C. roscoffensis* and *P. tricornutum* were preliminarily evaluated using batch cultivation mode. In brief, 100 μL of bacteria cultures ($OD_{600} \approx 0.25$) and 4 mL of axenic microalgal cultures were co-inoculated into a 100 mL glass conical flask (bottleneck diameter 18 mm, bottom diameter 65 mm, height 106 mm) containing 26 mL of NMB3# medium. During the cultivation period of eight days, the cell density (OD_{750}) was tested every two days by Thermo Fisher Scientific Microplater Reader (Varioskan LUX, Finland), based on which the specific growth rate (μ, in d^{-1}) was further calculated according to the formula (1):

$$\mu\ (d^{-1}) = (\ln N_{t1} - \ln N_{t2}) / t_1 - t_2 \qquad (1)$$

In which t_1 and t_2 are the initial and end time of cultivation period, and N_{t1} and N_{t2} are the corresponding OD_{750} values at those time points. At the final day of cultivation, 10 mL of microalgal cultures collected using pre-weighted Whatman GF/D microfiber filter (pore size 2.7 μm) were washed by 30 mL of ultrapure water and then dried to a constant weight at 65°C for 48 h to calculate the biomass concentration. In this section, the axenic microalgal cultures were taken as control groups.

2.3 Comprehensive analysis of co-cultured bacteria on axenic microalgae

To comprehensively evaluate the effects of co-cultured bacteria on *C. roscoffensis* and *P. tricornutum*, additional cultivation trials with enlarged cultivation volume (300 mL medium in 500 mL glass conical flask) (bottleneck diameter 34 mm, bottom diameter 107 mm, height 187 mm) were further conducted. Throughout the entire cultivation period of 12 days, the photosynthetic parameters such as the maximal photosystem II (PSII) quantum yield (F_v/F_m) and relative electron transfer rate at PSII (ETR) and cell density (OD_{750}) were measured every two days by WATER-PAM (WALZ, Germany) and microplate reader, respectively. The specific growth rate and biomass concentration were determined with the same methods as those described in section 2.2.

Besides, value-added substances including pigments and FAs as well as macronutrients composition of the biomass including proteins, carbohydrates, and lipids were investigated at the 6th and 12th days. As for the extraction and quantification of FAs and pigments, related details were same with our previous report [22]. For proteins, 10 mg of lyophilized microalgae powder were supplemented with 2 mL of extraction solution (composed of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, and 1% Triton X-100) and then subject to homogenization at 12,000 rpm for 15 min (XHF-DY, SCIENTZ, China). Subsequently, an ice bath of 3 h was performed to extract the proteins. Then, the supernatant was collected, diluted and finally mixed with Coomassie brilliant blue reagent, of which the OD_{595} was measured and further employed to determine the protein concentration [23]. An improved phenol-sulfuric acid method reported by Chen et al. (2023) [24] was adopted to determine the carbohydrate content. The lipid was extracted based on a chloroform-methanol method [25]. In brief, extraction solution composed of 5 mL methanol and 1.25 mL chloroform were supplemented into 20 mg (W1) of microalgae powder. After a homogenization at

12,000 rpm for 15 min, the mixture was placed into a shaker for lipid extraction at 200 rpm for 12 h. Then, the supernatant generated by the centrifugation was transferred into a new tube and mixed with 2.5 mL of chloroform and 4.5 mL of sodium chloride (1%). After vortex for 15 min followed by a centrifugation, the lower organic phase was transferred into a pre-weighted tube (W2) and dried with nitrogen. The tube with extracted lipids was weighted (W3), and the lipid content was calculated using equation (2):

$$\text{Lipid content} = (\text{W3}-\text{W2})/\text{W1} \times 100 \qquad (2)$$

2.4 Verification of stimulatory effect based on xenic microalgal strains

In this section, a two-stage cultivation process was performed with a cultivation volume of 300 mL in 500 mL of glass conical flask (bottleneck diameter 34 mm, bottom diameter 107 mm, height 187 mm). At the 1st stage, non-axenic microalgal cultures were inoculated into fresh NMB3# liquid medium with an inoculation ratio of 1: 9 and cultivated for 6 days. The 2nd stage of semi-continuous cultivation started from Day 6. During the subsequent cultivation process, 30% microalgal cultures were replaced by equal volume of 2×NBU3# medium containing 300 μL of bacteria cultures ($OD_{600} \approx 0.25$) every two days. For the control group, the supplemented medium contained 300 μL of filtrates of bacteria cultures collected by GF/F Whatman microfiber filter (pore size 0.7 μm) rather than bacteria cultures. All the other cultivation conditions were the same with in the previous experiments. Cell density was tested every two days during the 1st stage, and every day for the 2nd stage.

2.5 Statistical analysis

All treatments with three or four biological replicates were tested in this study, and the values were displayed as mean ± SD (standard deviation) (n = 3 or 4). Statistical analysis was conducted based on SPSS Statistics 25.0 by one-way ANOVA, and the difference was considered statistically significant when $p < 0.05$.

3. Results and Discussions

3.1 Bacteria isolation and identification

Upon two rounds of purification and isolation, six bacterial strains devoid of miscellaneous sequencing peaks were isolated from xenic microalgal cultures. Their 16S rRNA gene sequences were submitted to the NCBI database, assigned with accession numbers OR394553–3945538. Phylogenetic analysis via a maximum-likelihood (ML) tree revealed that three strains were closely related to *Priestia flexa*, while another one was akin to *Rossellomorea aquimaris* (Fig. 1). Notably, *Priestia* and *Rossellomorea* have been recently reclassified into separate genera from *Bacillus* [26]. For practical purposes, these four strains in our study were designated as Bac1–4 and discussed as representatives of *Bacillus* spp. The remaining two strains were definitively identified as *Agrobacterium tumefaciens* and *Marinobacter adhaerens*, denoted as Agr1 and Mar1, respectively.

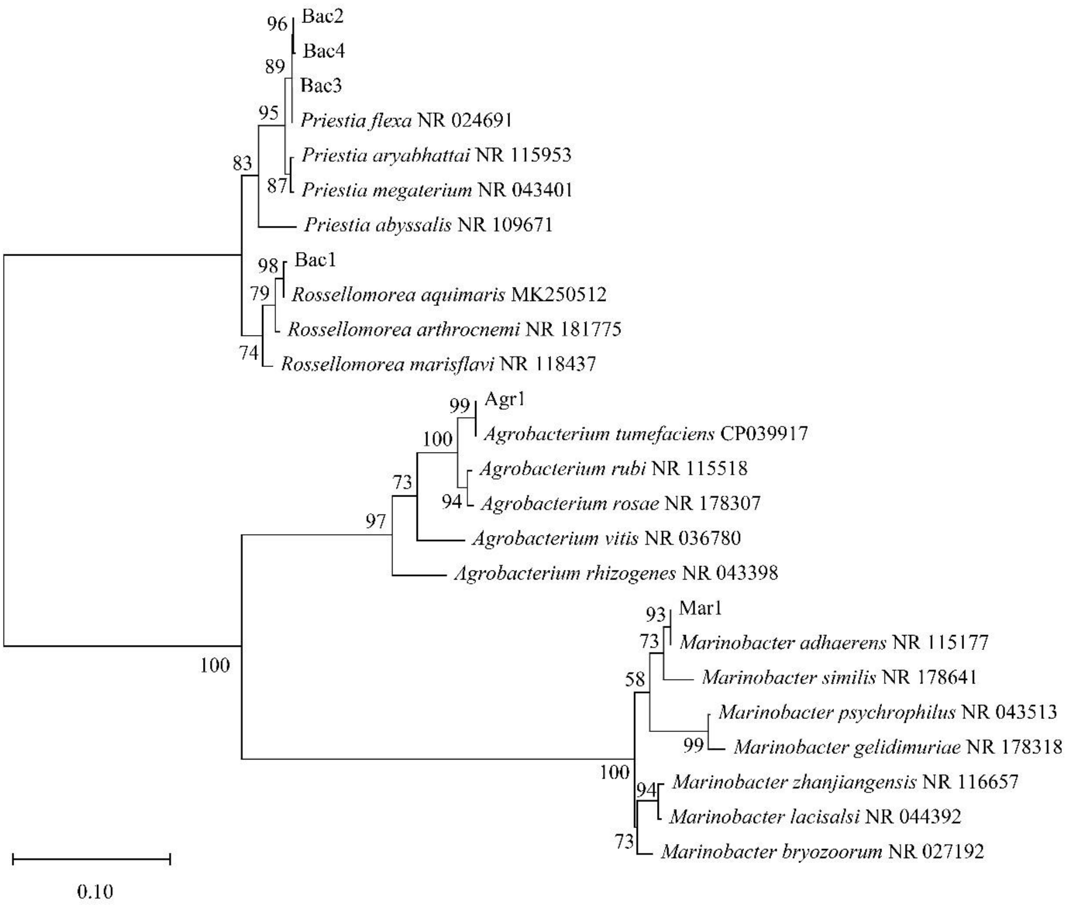

Fig. 1. The maximum-likelihood tree of six bacteria isolated in this study. Phylogenic analysis was performed based on 16S rRNA sequences from 25 bacteria species. The serial numbers after the species names indicate the corresponding GenBank entries.

Natural ecosystems feature a myriad of associations between microalgae and bacteria, ranging from nutrient competition and exchange (*e.g.*, nitrogen, phosphorus, and carbon dioxide) to the metabolism of growth factors (*e.g.*, vitamins and phytohormones), cross-kingdom signaling, algicidal, and antibacterial behavio[9][13]. While some interactions benefit microalgae proliferation, others can be detrimental. To enhance microalgal cultivation efficiency through an MBC strategy, we evaluated the impact of all six bacterial strains on microalgae growth.

3.2 Screening of growth-promoting bacteria

We assessed the influence of co-cultured bacteria on *C. roscoffensis* and *P. tricornutum* using axenic strains in a batch cultivation system. Axenicity was confirmed with a nucleic acid staining technique prior to co-cultivation [18]. In axenic samples, fluorescence signal denoting either *C. roscoffensis* cells in oval shape (Fig. 2a) or *P. tricornutum* cells in fusiform shape (Fig. 2b) was visible. Contrastingly, xenic samples displayed numerous smaller signals indicative of bacterial presence (Fig. 2c, d).

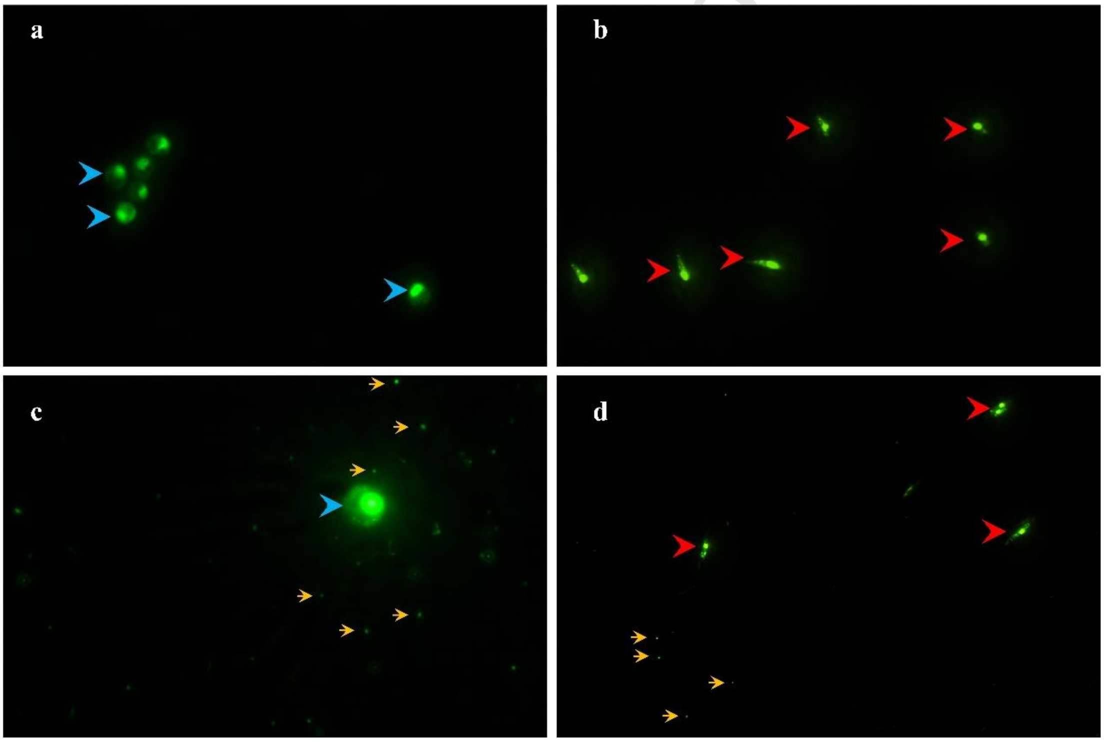

Fig.2. Fluorescence observation of axenic *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* (a, b) and xenic *C. roscoffensis* and *P. tricornutum* (c, d). The blue and red arrow heads indicate the microalga cells, while the yellow arrows represent the bacteria.

After eight days of co-cultivation, enhancement in *C. roscoffensis* growth was noted with two *Bacillus* species (Fig. 3a). Specifically, the axenic *C. roscoffensis* group reached an OD_{750} of 0.163, whereas the MBC groups of axenic *C. roscoffensis* with Bac2 and Bac3 exhibited OD_{750} values of 0.239 and 0.215, respectively—increases of 46.3% and 31.9%. For *P. tricornutum*, the addition of Bac3 and Agr1 led to significantly higher final cell densities (22.8%–25.3%) compared to the axenic control (Fig. 3b). Similar trends were observed for specific growth rates and biomass concentrations (Fig. 3c, d). As a result, Bac2 and Bac3 were preliminarily deemed favorable for *C. roscoffensis* growth, and Bac3 and Agr1 for that of *P. tricornutum*.

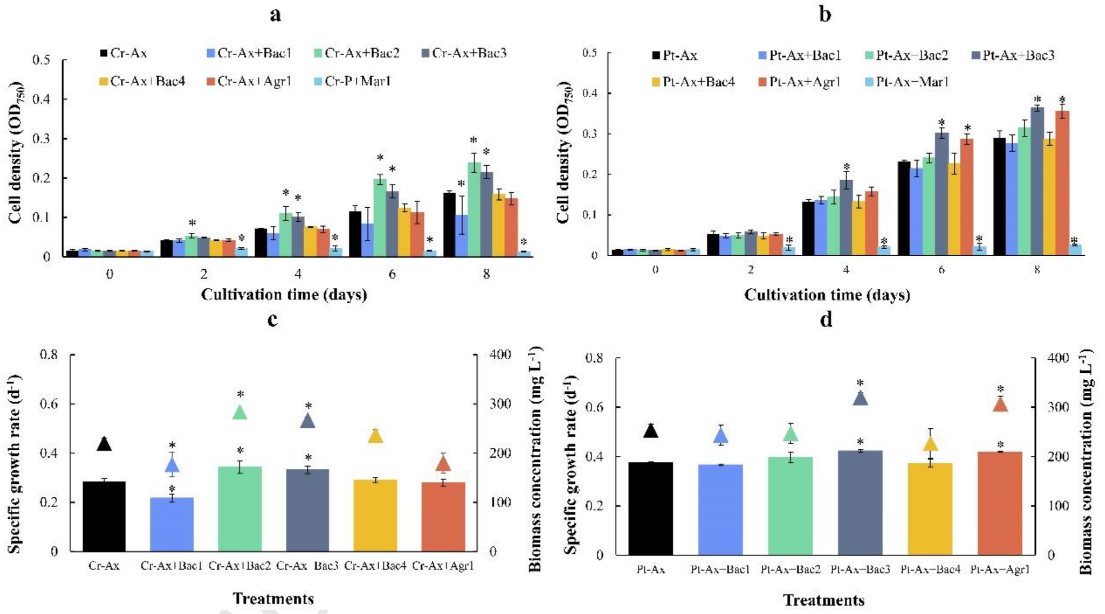

Fig. 3. Effects of different bacteria on *Chrysotila roscoffensis* and *Phaeodactylum tricornutum*. (a, b) indicate the cell density, (c, d) show the biomass concentration (scatter diagram) and specific growth rate (histogram). Results were displayed as mean ± SD (*n* = 3). "*" indicates significant difference with the control group. Cr, Ax, and Pt represent *C. roscoffensis*, axenic strain, and *P. tricornutum*, respectively.

Bacillus is one of the genera most extensively researched for its role in plant growth promotion. Volatile compounds produced by *Bacillus*, including 3-hydroxy-2-butanone and 3,5-ditert-butylphenol, have been documented to positively influence plant cells by altering auxin homeostasis, regulating cell enlargement, and boosting photosynthetic efficiency and chlorophyll content. These activities may bolster plant growth, improve disease resistance, and elevate tolerance to abiotic stress [27][28]. Our findings indicate that *Bacillus* could be a promising candidate for identifying bacteria that promote microalgae growth. *Agrobacterium*, known for its role in genetic transformation, displayed a notable stimulatory effect on *P. tricornutum* growth, yet conversely inhibited *C. roscoffensis*. Microscopic analysis further indicated that the *C. roscoffensis* cells aggregated when they were co-cultivated with *Agrobacterium*. Moreover, Mar1 significantly hindered the growth of both *C. roscoffensis* and *P. tricornutum*. *Marinobacter*, often associated with the phycosphere, is recognized for producing algicidal substances toxic to harmful algal bloom species, like *Karenia mikimotoi* and *Noctiluca scintillans* [29][30]. Our microscopic observations indicated that Mar1 might possess algicidal properties, as the microalgal cells when cultivated with this bacteria appeared intact yet lacked visible organelles.

3.3 The effects of growth-promoting bacteria on microalgal metabolism

To procure ample microalgal biomass for a comprehensive assessment of the effects of growth-promoting bacteria on microalgae, expanded MBC trials were undertaken.

3.3.1 Growth performance and photosynthetic parameters

As illustrated in Fig. 4a–d, co-culturing Bac2 and Bac3 with *C. roscoffensis*, and Bac3 and Agr1 with *P. tricornutum*, led to marked increases in cell density, growth rate, and biomass compared to the axenic controls, corroborating previous batch results.

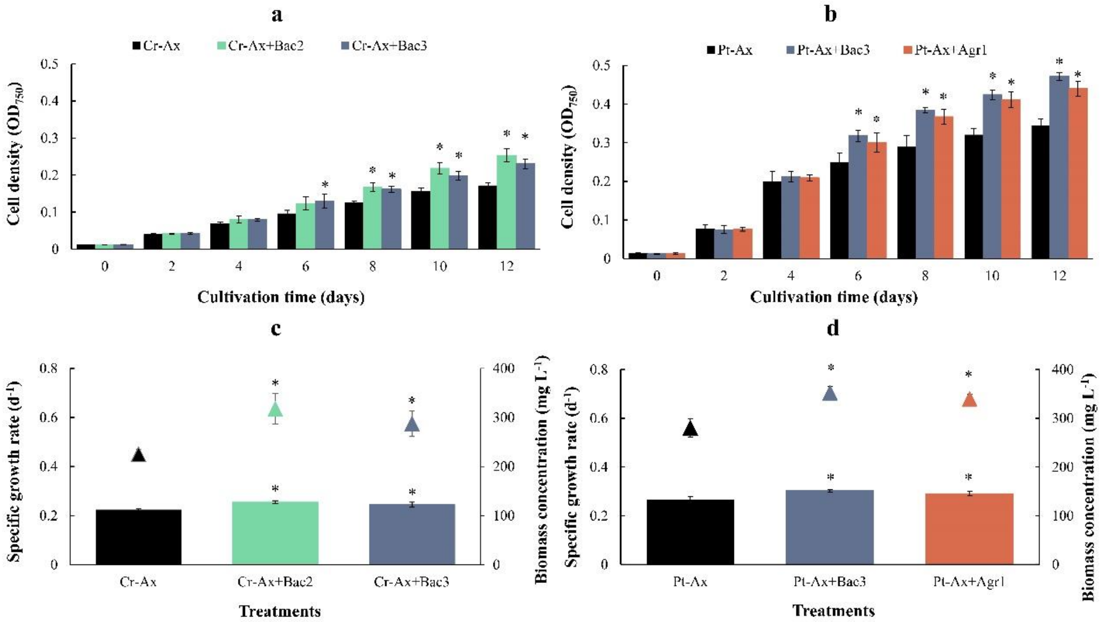

Fig. 4. Performance of *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* when co-cultured with growth-promoting bacteria in an enlarged cultivation volume. (a, b) indicate the cell density, (c, d) show the biomass concentration (scatter diagram) and specific growth rate (histogram). Results were displayed as mean ± SD ($n = 3$). "*" indicates significant difference with the control group. Cr, Ax, and Pt represent *C. roscoffensis*, axenic strain, and *P. tricornutum*, respectively.

Additionally, photophysiological metrics, such as F_v/F_m and ETR—indices of microalgal physiological status—were also evaluated. In this study, F_v/F_m values for MBC groups of axenic *C. roscoffensis* with Bac2 and Bac3 began around 0.4, peaked on Day 4 (around 0.6), and then returned to approximately 0.4, mirroring the trend of the axenic group. Although F_v/F_m remained similar across treatments, MBC ones of axenic *C. roscoffensis* with Bac2 and Bac3 exhibited higher values during the early co-cultivation period (Days 2 and 4), suggesting a beneficial impact of Bac2 and Bac3 on the photosynthetic activity of *C. roscoffensis*. Notably, the F_v/F_m of the MBC treatment of axenic *C. roscoffensis* with Bac2 decreased below the axenic group on Days 8 and 12 (Fig. 5a). The F_v/F_m trends of *P. tricornutum* paralleled those observed in *C. roscoffensis* (Fig. 5a, b). ETR trends in this study mirrored F_v/F_m patterns, with early cultivation spikes followed by later period declines in MBC groups (Fig. 5c, d).

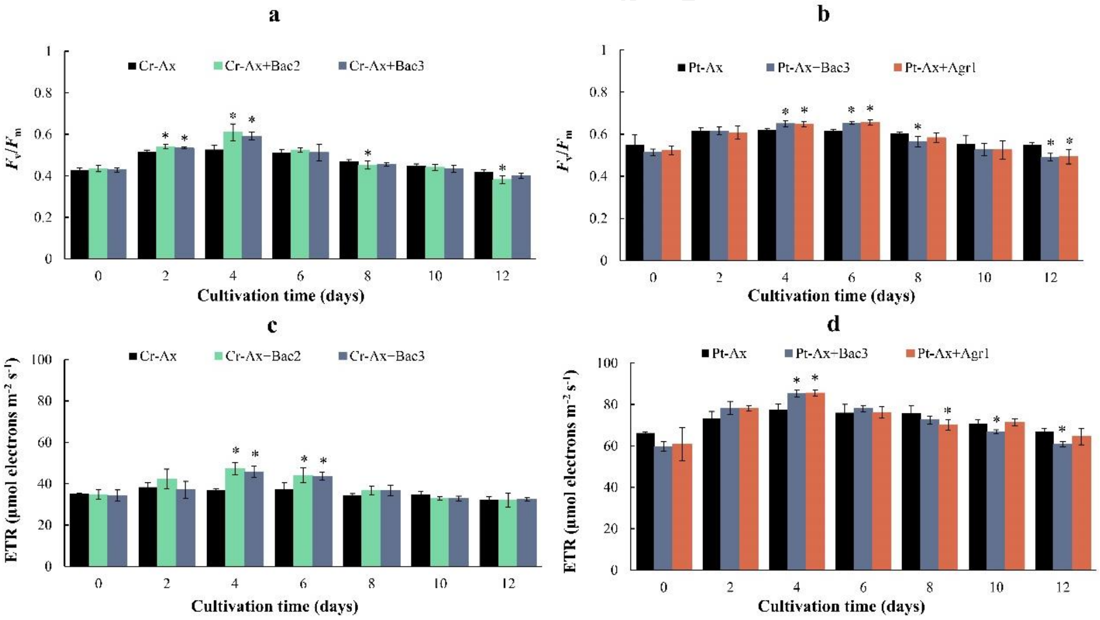

Fig. 5. F_v/F_m (a, b) and ETR (c, d) of *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* when co-cultured with growth-promoting bacteria. Results were displayed as mean ± SD (*n* = 3). "*" indicates significant difference with the control group. Cr, Ax, and Pt represent *C. roscoffensis*, axenic strain, and *P. tricornutum*, respectively.

F_v/F_m and ETR, which are indicators of maximum quantum efficiency of PSII and electron flow during photosynthesis, respectively, can gauge photosynthetic activity and typically decrease under nutrient scarcity [31]. Additionally, an increase in ETR generally points to enhanced biomass accumulation, while a decline suggests a reduction in photosynthetic processes [32]. In comparison to the axenic group, the elevated F_v/F_m and ETR values in the MBC treatments during the early cultivation period suggest that the bacteria improve photosynthetic processes under nutrient-adequate conditions. Conversely, the lower values during later stage suggest that the bacteria exacerbate nutrient depletion in the cultivation system. These observations indicate that the co-cultured bacteria enhance microalgal growth and biomass primarily by promoting photosynthetic processes rather than directly supplying macronutrients like nitrogen and phosphate.

It has been reported that the symbiotic bacteria can stimulate microalgal growth by different cooperative mechanisms including gas exchange and compensation, specific phytohormones, nutrient acquisition promotion and signal exchange [12]. *Bacillus* spp. and *Agrobacterium* spp. reportedly enhance plant growth by supplying macronutrients like nitrogen and phosphorus. For instance, *Bacillus pumilus* can fix atmospheric nitrogen, significantly promoting growth in *Chlorella vulgaris* under nitrogen-deficient conditions [33]. Likewise, *Scenedesmus obliquus* co-cultured with *Bacillus megaterium* demonstrated higher cell density and phosphate removal efficiency compared to monospecific cultures, likely due to bacteria-mediated phosphate release [34]. *Agrobacterium* can convert immobilized phosphorous into available phosphate for plant uptake and is commonly used as a common inoculant to enhance phosphate availability [35]. However, the observed decline in F_v/F_m and ETR in the MBC groups during the later cultivation period indicates that this is not the case for Bac2, Bac3, or Agr1. Additionally, both *Bacillus* and *Agrobacterium* are also recognized as sources of phytohormones, such as auxins, cytokinin, and gibberellin, which are employed in sustainable agriculture to enhance crop growth and quality by stimulating plant physiological and molecular processes [36]. For example, Zhang et al. reported that *Bacillus* (C-1) and *Agrobacterium* (O-7, O-16, C-2, and C-8) enhance biomass accumulation in *Ochromonas* and *Chlorella* by producing indole-3-acetic acid (IAA) [37]. This raises the possibility that the potential IAA secretion capacity may also contribute to the stimulatory effects of Bac2, Bac3, and Agr1, although further research is required.

In addition, due to the non-aerated conditions and the use of NMB3# medium in this study, the growth rates, cell densities, F_v/F_m and ETR values obtained from the axenic control groups were relatively low compared to previous reports [38][39], suggesting that the microalgal cells in the control groups have been under stressful conditions. Considering the fact that the more stressed the control group is, the more pronounced the stimulatory effects of the co-cultured bacteria on the microalgal cells might appear, it should be noted that the beneficial effects displayed in this study may be overestimated more or less.

3.3.2 Composition of microalgae biomass

To assess whether MBC alters the biomass quality of microalgae, the biochemical compositions were further investigated on Day 6 and 12 (Table 1). On Day 6, the protein, lipid, and carbohydrate content in axenic *C. roscoffensis* cultures were determined to be 34.9%, 14.9%, and 16.9%, respectively. By Day 12, protein and carbohydrate levels in axenic *C. roscoffensis* samples decreased to 26.3% and 10.8%, while lipid content rose to 30.8% (Fig. 6a). At both time points, the macromolecular composition of MBC treatments of axenic *C. roscoffensis* with Bac2 and Bac3 closely resembled that of axenic *C. roscoffensis*. The macromolecular profiles of *P. tricornutum* also shifted over time. As the cultivation period extended, protein content in Pt-Ax samples fell from 34.5% to 20.6%, with lipid and carbohydrate content increasing from 13.2% and 10.8% to 22.6% and 15.0%, respectively (Fig. 6b). Similar to *C. roscoffensis*, no significant differences in macronutrient content were observed across *P. tricornutum* treatments. Additionally, consistent with observations from F_v/F_m and ETR, the decreased protein levels and increased lipid levels in both *C. roscoffensis* and *P. tricornutum* during the later cultivation stage further confirmed that the microalgal cells experienced nutrient deficiencies [40].

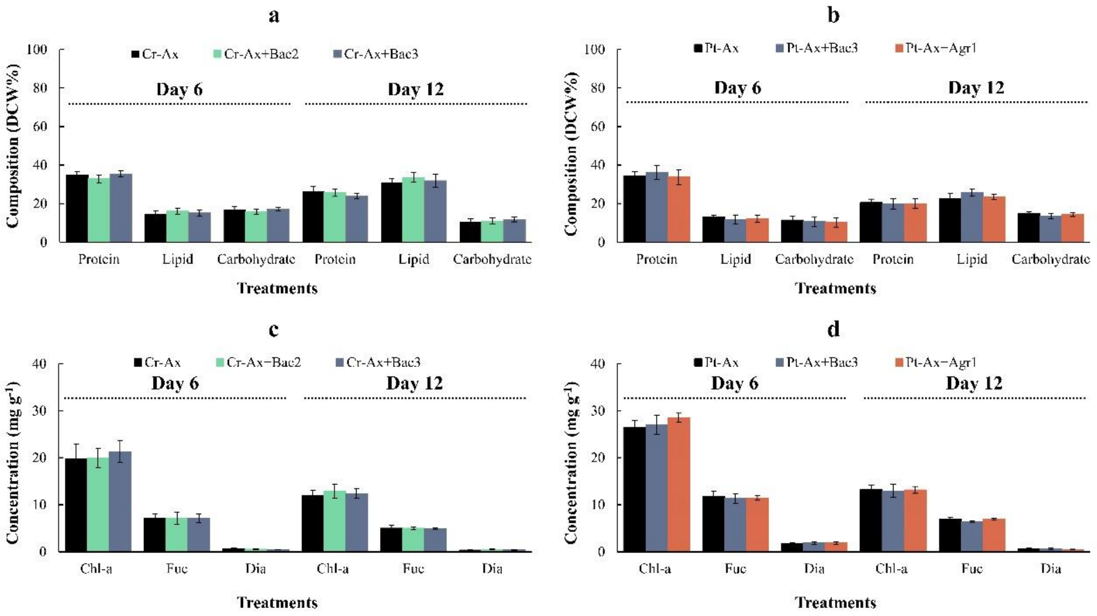

Fig. 6. Macronutrient compositions (a, b) and pigment compositions (c, d) of *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* when co-cultured with growth-promoting bacteria. DCW, dry cell weight. Results were displayed as mean ± SD (*n* = 4). Cr, Ax, and Pt represent *C. roscoffensis*, axenic, and *P. tricornutum*, respectively. Chl-*a*, Fuc, and Dia indicate chlorophyll a, fucoxanthin, and diadinoxanthin, respectively.

Given that both *C. roscoffensis* and *P. tricornutum* can accumulate fucoxanthin [19][22], pigment composition was also examined. On Day 6, all *C. roscoffensis* treatments displayed similar fucoxanthin levels, approximately 7.0 mg g^{-1}, which declined to about 5.0 mg g^{-1} by Day 12. Additionally, chlorophyll a and diadinoxanthin contents remained consistent among all groups, with approximately 20.0 mg g^{-1} chlorophyll a on Day 6 dropping to 12.0 mg g^{-1} on Day 12, and diadinoxanthin ranging from about 0.5 mg g^{-1} on Day 6 to 0.4 mg g^{-1} on Day 12 (Fig. 6c). In *P. tricornutum*, pigment contents, notably higher than those of *C. roscoffensis*, also diminished over time (Fig. 6d). For instance, fucoxanthin levels started around 11.5 mg g^{-1} on Day 6 and fell to 7.0 mg g^{-1} on Day 12. The pigment profiles of Pt-Ax+Bac3 and Pt-Ax+Agr1 paralleled those of Pt-Ax throughout the cultivation. These results suggest that co-cultivated Bac2, Bac3, and Agr1 had minimal impact on the pigmentation of both microalgae species.

Polyunsaturated fatty acids (PUFAs), known for their health benefits [41], were analyzed for variation between the axenic and bacteria-supplemented groups. On Day 6, the three *C. roscoffensis* groups predominantly comprised palmitic acid (PA, C16:0), stearic acid (SA, C18:0), α-linolenic acid (ALA, C18:3), and oleic acid (OA, C18:1), with measurable levels of docosahexaenoic acid (DHA, C22:6) ranging from 6.99–7.49%. By Day 12, ALA, OA, and linoleic acid (LA, C18:2) concentrations rose while PA decreased, resulting in an unsaturated fatty acids (UFAs) ratio of around 80%. These findings accorded with previous research on other *Chrysotila* species, where PA, OA, ALA, and SA were predominant [42][43]. For *P. tricornutum*, eicosapentaenoic acid (EPA, C20:5), varying within the ranges of 36.2%–39.4% on Day 6 and 35.0%–36.7% on Day 12, was definitely the most abundant FA of all three groups (Table 2). Besides that, PA and palmitoleic acid (PLA, C16:1), showing no significant differences among different treatments, also reached considerably high ratios at both two time points.

Table 1 Fatty acid compositions of *C. roscoffensis* under different cultivation modes

TFA%	Day 6	Day 12

	Cr-Ax	Cr-Ax+Bac2	Cr-Ax+Bac3	Cr-Ax	Cr-Ax+Bac2	Cr-Ax+Bac3
C14:0	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.5 ± 0.1^{b}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
C16:0	24.6 ± 1.2^{a}	24.2 ± 3.2^{a}	25.9 ± 1.4^{a}	14.6 ± 1.4^{b}	15.6 ± 0.8^{b}	14.6 ± 0.8^{b}
C16:1	1.8 ± 0.0^{a}	1.8 ± 0.3^{a}	1.8 ± 0.5^{a}	1.4 ± 0.2^{a}	1.8 ± 0.2^{a}	1.3 ± 0.2^{a}
C16:2	3.7 ± 0.5^{a}	3.8 ± 0.7^{a}	3.7 ± 0.7^{a}	0.9 ± 0.1^{b}	0.7 ± 0.1^{b}	1.1 ± 0.2^{b}
C18:0	2.6 ± 0.2^{a}	2.5 ± 0.5^{a}	2.2 ± 0.3^{a}	3.4 ± 1.1^{a}	3.3 ± 0.8^{a}	2.8 ± 0.8^{a}
C18:1	14.5 ± 0.7^{b}	14.3 ± 1.0^{b}	13.4 ± 0.8^{b}	19.9 ± 0.7^{a}	20.0 ± 1.3^{a}	20.9 ± 0.7^{a}
C18:2	7.6 ± 0.8^{b}	8.4 ± 0.9^{b}	7.3 ± 0.8^{b}	13.6 ± 1.0^{a}	12.7 ± 0.4^{a}	13.6 ± 0.8^{a}
C18:3	15.5 ± 0.3^{b}	16.1 ± 1.3^{b}	15.0 ± 1.0^{b}	25.7 ± 2.1^{a}	25.9 ± 2.5^{a}	25.6 ± 0.7^{a}
C18:4	18.8 ± 2.2^{a}	18.6 ± 2.5^{a}	19.5 ± 0.7^{a}	10.2 ± 1.2^{b}	10.2 ± 1.1^{b}	10.6 ± 0.7^{b}
C20:5	3.1 ± 0.3^{a}	2.8 ± 0.5^{a}	3.2 ± 0.3^{a}	1.5 ± 0.1^{b}	1.4 ± 0.1^{b}	1.1 ± 0.1^{b}
C22:6	7.2 ± 0.3^{a}	7.0 ± 0.4^{a}	7.5 ± 0.5^{a}	7.9 ± 0.8^{a}	7.5 ± 0.7^{a}	7.5 ± 1.0^{a}
SFA	27.7 ± 1.0^{a}	27.2 ± 2.6^{a}	28.6 ± 1.1^{a}	18.9 ± 0.2^{b}	19.8 ± 0.1^{b}	18.3 ± 1.3^{b}
MUFA	16.3 ± 0.7^{b}	16.1 ± 0.8^{b}	15.2 ± 0.9^{b}	21.3 ± 0.9^{a}	21.8 ± 1.4^{a}	22.2 ± 0.6^{a}
PUFA	56.0 ± 1.6^{a}	56.7 ± 2.2^{a}	56.2 ± 2.0^{a}	59.8 ± 0.7^{a}	58.4 ± 1.5^{a}	59.5 ± 1.4^{a}

Results were displayed as mean ± SD ($n = 3$). TFA, total fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Different letters of superscripts in the same row mean statistically significant difference between groups ($p < 0.05$). Cr and Ax represent *C. roscoffensis* and axenic strain, respectively.

Table 2 Fatty acid compositions of *P. tricornutum* under different cultivation modes

TFA%	Day 6			Day 12		
	Pt-Ax	Pt-Ax+Bac3	Pt-Ax+Agr1	Pt-Ax	Pt-Ax+Bac3	Pt-Ax+Agr1
C14:0	6.0 ± 1.2^{a}	5.8 ± 0.3^{a}	6.3 ± 1.3^{a}	5.1 ± 0.3^{a}	4.6 ± 0.2^{a}	5.5 ± 0.3^{a}
C16:0	21.3 ± 1.2^{a}	20.0 ± 0.1^{a}	20.2 ± 0.1^{a}	17.9 ± 1.0^{b}	17.2 ± 1.5^{b}	17.6 ± 0.5^{b}
C16:1	27.1 ± 1.6^{b}	26.1 ± 1.0^{b}	27.0 ± 1.3^{b}	33.2 ± 0.5^{a}	33.7 ± 1.9^{a}	34.7 ± 1.1^{a}
C18:0	2.1 ± 0.5^{a}	1.9 ± 0.6^{a}	2.0 ± 0.4^{a}	0.7 ± 0.0^{b}	0.5 ± 0.1^{b}	0.5 ± 0.0^{b}
C18:1	2.7 ± 1.1^{a}	2.1 ± 0.5^{a}	2.0 ± 0.5^{a}	3.7 ± 0.6^{a}	3.9 ± 1.0^{a}	2.7 ± 0.1^{a}
C18:2	2.9 ± 0.3^{a}	2.6 ± 0.5^{ab}	2.8 ± 0.6^{a}	1.8 ± 0.0^{ab}	1.7 ± 0.6^{b}	2.0 ± 0.1^{ab}
C18:3	0.5 ± 0.6^{a}	0.9 ± 0.6^{a}	0.9 ± 0.3^{a}	0.7 ± 0.1^{a}	0.5 ± 0.2^{a}	0.9 ± 0.0^{a}
C20:4	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.3 ± 0.1^{a}
C20:5	36.2 ± 1.9^{b}	39.4 ± 1.5^{a}	37.6 ± 0.5^{ab}	35.8 ± 0.2^{b}	36.7 ± 0.3^{ab}	35.0 ± 1.1^{b}
C22:6	0.8 ± 0.2^{a}	0.8 ± 0.2^{a}	0.8 ± 0.1^{a}	0.7 ± 0.1^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}
SFA	29.4 ± 1.2^{a}	27.7 ± 0.3^{a}	28.5 ± 1.4^{a}	23.7 ± 0.9^{b}	22.3 ± 1.6^{b}	23.6 ± 0.5^{b}
MUFA	29.8 ± 2.6^{b}	28.2 ± 1.3^{b}	29.0 ± 0.9^{b}	36.9 ± 1.1^{a}	37.6 ± 2.1^{a}	37.4 ± 1.0^{a}
PUFA	40.8 ± 1.9^{b}	44.1 ± 1.3^{a}	42.5 ± 0.6^{ab}	39.4 ± 0.3^{b}	40.1 ± 0.6^{b}	39.0 ± 1.1^{b}

Results were displayed as mean ± SD ($n = 3$). TFA, total fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Different letters of superscripts in the same row mean statistically significant difference between groups ($p < 0.05$). Pt and Ax represent *P. tricornutum* and axenic strain, respectively.

In summary, the above data demonstrated that the MBC modes induced no remarkable variations in the macromolecule composition, FAs profile, or pigments contents of two microalgae. Nevertheless, considering the fact that the microalgal cell density and biomass concentration were significantly enhanced by co-cultured bacteria, it can be speculated that the productivities of bioactive substances generated based on the MBC system are still much higher compared to those of axenic control groups. For example, the fucoxanthin productivity of axenic *C. roscoffensis* was calculated as 0.09 mg L^{-1} d^{-1}, while this value substantially increased to 0.13 mg L^{-1} d^{-1} when axenic *C. roscoffensis* was co-cultured with Bac2 (Fig. 3c and 5c).

3.4 Verification of the stimulatory effect under semi-continuous cultivation mode based on xenic microalgae

During mass cultivation, maintaining axenic microalgal cultures in open raceway ponds or closed photobioreactors is exceedingly difficult. This condition can lead to the disruption of beneficial interactions between microalgae and bacteria by other existing bacteria. It is, therefore, crucial to determine whether growth-promoting bacteria retain their beneficial role in non-axenic (xenic) microalgal cultures. Previous batch cultivation results (Fig. 5a–d) showed that microalgae in the MBC system exhibited higher F_v/F_m and ETR values than axenic cultures, suggesting that growth-promoting bacteria may have a more pronounced effect under nutrient-rich conditions. Consequently, non-axenic strains of C. *roscoffensis* and *P. tricornutum* were used to further explore the role of growth-promoting bacteria in large-scale cultivation. To ensure the dominance of beneficial bacteria in the microbial communities of these cultures, 300 μL of bacterial cultures ($OD_{600} \approx 0.25$) were introduced into the fresh

NBU3# medium at each dilution.

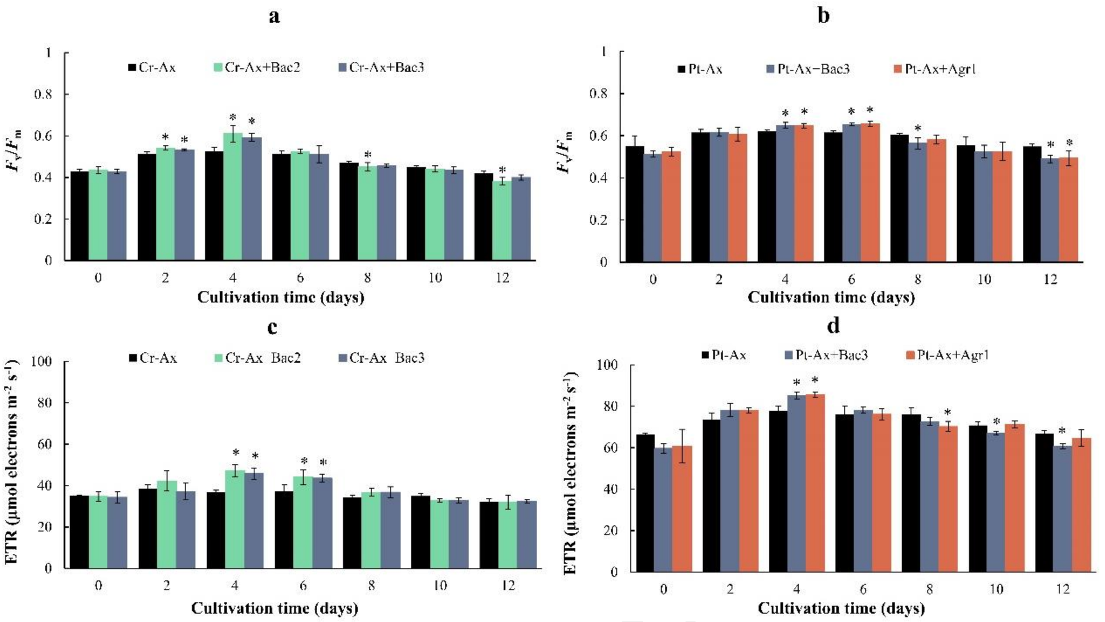

Fig. 6. F_v/F_m (a, b) and ETR (c, d) of *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* when co-cultured with growth-promoting bacteria. Results were displayed as mean ± SD (n = 3). "*" indicates significant difference with the control group. Cr, Ax, and Pt represent *C. roscoffensis*, axenic strain, and *P. tricornutum*, respectively.

In the semi-continuous cultivation phase (Day 6 to Day 14), the OD_{750} of non-axenic *C. roscoffensis* without added beneficial bacteria (referred to as Cr-Xe, where Cr and Xe denote *C. roscoffensis* and non-axenic strain, respectively) ranged from 0.09 to 0.17. In comparison, Cr-Xe cultures supplemented with Bac2 and Bac3 exhibited OD_{750} ranges of 0.11–0.26 and 0.10–0.24, respectively, which were consistently higher than Cr-Xe throughout the period of observation (Fig. 7a). *P. tricornutum* showed a similar trend (Fig. 7b). The non-axenic control group (Pt-Xe) reached a peak OD_{750} of 0.33 on Day 14, whereas Pt-Xe groups co-cultivated with Bac3 and Agr1 attained OD_{750}

values of 0.44 and 0.41, representing increases of approximately 34% and 23%, respectively. These findings confirm that the stimulatory effects of growth-promoting bacteria are effective in non-axenic cultivation systems.

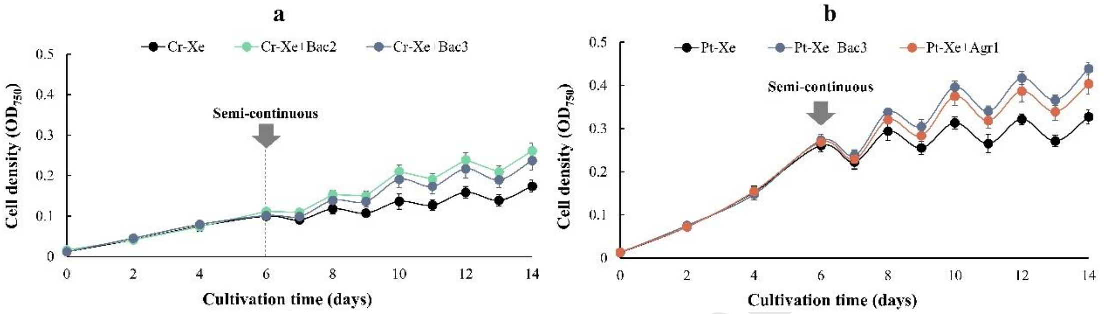

Fig. 7. Cell density of non-axenic *Chrysotila roscoffensis* and *Phaeodactylum tricornutum* co-cultured with growth-promoting bacteria under semi-continuous cultivation mode. Cr, Xe, and Pt represent *C. roscoffensis*, xenic strain, and *P. tricornutum*, respectively. Results were displayed as mean ± SD (*n* = 3).

4. Conclusions

In this study, six bacterial strains were isolated from non-axenic cultures of *C. roscoffensis* and *P. tricornutum*. Of these, three were shown to stimulate the growth of axenic microalgae significantly and were identified as growth-promoting bacteria, while one demonstrated algicidal properties. The impact of growth-promoting bacteria on axenic *C. roscoffensis* and *P. tricornutum* was extensively assessed, revealing that co-cultivation with these beneficial bacteria enhances the photosynthetic process under conditions of nutrient sufficiency, yet has minimal effects on the macromolecular composition, pigment content, and FA profiles. Moreover, the promotion of microalgal growth by these bacteria was confirmed under semi-continuous cultivation with non-axenic microalgae, indicating that the beneficial effects of the bacteria persisted when they predominated in the microbial community. These findings suggest that MBC is a promising approach for improving the productivity of microalgal cultivation.

CRediT authorship contribution statement

Jiaqi Fang: Data curation, Project administration, Writing – original draft. **Yan Sun:** Methodology, Formal analysis. **Zhujun Zhu:** Methodology, Formal analysis. **Jian Li:** Formal analysis, Writing-review & editing. **Pengfei Cheng:** Supervision, Writing – review & editing. **Spiros N. Agathos:** Formal analysis, Writing-review & editing. **Yuanyuan Fu:** Methodology, Formal analysis. **Lin Zhang:** Investigation, Writing – review & editing, Supervision. **Chengxu Zhou:** Writing – review & editing. **Jichang Han:** Funding acquisition, Supervision, Resources, Writing – review & editing.

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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References

[1] J. Ampofo, L. Abbey, Microalgae: Bioactive composition, health benefits, safety and prospects as potential high-value ingredients for the functional food industry, Foods. 11 (2022) 1744, https://doi.org/10.3390/foods11121744.

[2] S.A. Amin, L.R. Hmelo, H.M. Van Tol, B.P. Durham, L.T. Carlson, K.R. Heal, E.V. Armbrust, Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria, Nature. 522 (2015) 98-101, https://doi.org/10.1038/nature14488.

[3] F. Delrue, P.A. Setier, C. Sahut, L. Cournac, A. Roubaud, G. Peltier, A.K. Froment, An economic, sustainability, and energetic model of biodiesel production from microalgae, Bioresour Technol. 111 (2012) 191-200, https://doi.org/10.1016/j.biortech.2012.02.020.

[4] S. Kim, M. Moon, M. Kwak, B. Lee, Y.K. Chang, Statistical optimization of light intensity and CO_2 concentration for lipid production derived from attached cultivation of green microalga *Ettlia* sp., Sci Rep-uk. 2018, 8(1), 15390, https://doi.org/10.1038/s41598-018-33793-1.

[5] C.Y. Tong, C.J.C. Derek, Membrane surface roughness promotes rapid initial cell adhesion and long term microalgal biofilm stability, Environ Res. 2022, 206, 112602, https://doi.org/10.1016/j.envres.2021.112602.

[6] A. Sreenikethanam, S. Raj, J.R. Banu, P. Gugulothu, A.K. Bajhaiya, Genetic

engineering of microalgae for secondary metabolite production: Recent developments, challenges, and future prospects, Front Bioeng Biotech. 2022, 10: 836056, https://doi.org/10.3389/fbioe.2022.836056.

[7] R. Ramanan, B.H. Kim, D.H. Cho, H.M. Oh, H.S. Kim, Algae-bacteria interactions: Evolution, ecology and emerging applications, Biotechnol Adv. 34 (2016) 14-29, https://doi.org/10.1016/j.biotechadv.2015.12.003.

[8] B. Zhang, W. Li, Y. Guo, Z.Q. Zhang, W.X. Shi, F.Y. Cui, P.N.L. Lens, J.H. Tay, Microalgal-bacterial consortia: from interspecies interactions to biotechnological applications, Renew Sust Energ Rev. 118 (2020) 109563, https://doi.org/10.1016/j.rser.2019.109563.

[9] M. Pal, P.J. Yesankar, A. Dwivedi, A. Qureshi, Biotic control of harmful algal blooms (HABs): A brief review, J Environ Manage. 268 (2020) 110687, https://doi.org/10.1016/j.jenvman.2020.110687.

[10] M.B. Cooper, A.G. Smith, Exploring mutualistic interactions between microalgae and bacteria in the omics age, Curr Opin Plant Biol. 26 (2015) 147-153, https://doi.org/10.1016/j.pbi.2015.07.003.

[11] S.P. Fulbright, S. Chisholm, K.F. Reardon, Growth inhibition of *Nannochloropsis* species by *Bacillus pumilus*, Algal Res. 20 (2016) 70-76, https://doi.org/10.1016/j.algal.2016.09.016.

[12] C.Y. Tong, K. Honda, C.J.C. Derek, A review on microalgal-bacterial co-culture: The multifaceted role of beneficial bacteria towards enhancement of microalgal

metabolite production, Environ res. (2023) 115872, https://doi.org/10.1016/j.envres.2023.115872.

[13] E. Cirri, G. Pohnert, Algae-bacteria interactions that balance the planktonic microbiome, New Phytol. 223 (2019) 100-106, https://doi.org/10.1111/nph.15765.

[14] J. Lian, P. Schimmel, S. Sanchez-Garcia, R.H. Wijffels, H. Smidt, D. Sipkema, Different co-occurring bacteria enhance or decrease the growth of the microalga *Nannochloropsis* sp. CCAP211/78, Microb Biotechnol. 14 (2021) 1159-1170, https://doi.org/10.1111/1751-7915.13784.

[15] D.T. Newby, T. J. Mathews, R. C. Pate, M.H. Huesemann, T.W. Lane, B.D. Wahlen, S. Mandal, R.K. Engler, K.P. Feris, J.B. Shurin, Assessing the potential of polyculture to accelerate algal biofuel production, Algal res. 19 (2016) 264-277, https://doi.org/10.1016/j.algal.2016.09.004.

[16] B.L. Liu, E. E. Eltanahy, H.W. Liu, E.T. Chua, S.R. Thomas-Hall, T.J. Wass, K.H. Pan, P.M. Schenk, Growth-promoting bacteria double eicosapentaenoic acid yield in microalgae, Bioresource Technol. 316 (2020) 123916, https://doi.org/10.1016/j.biortech.2020.123916.

[17] J.C. Han, L. Zhang, S. Wang, G.P. Yang, L. Zhao, K.H. Pan, Co-culturing bacteria and microalgae in organic carbon containing medium, J Biol Res-Thessalon. 23 (2016) 1-9, https://doi.org/10.1186/s40709-016-0047-6.

[18] J.J. Liu, Y. Sun, L. Zhang, X.H. Li, Z.C. He, C.X. Zhou, J.C. Han, Screening of antibiotics to obtain axenic cell cultures of a marine microalga *Chrysotila*

roscoffensis, Front Bioeng Biotechnol. 11 (2023) 1218031, https://doi.org/10.3389/fbioe.2023.1218031.

[19] D.H. Zhou, R. Meng, P. Xiao, T. Chang, Y.R. Li, J.C. Han, P.F. Cheng, C.X. Zhou, X.J. Yan, Frequent antibiotic exposure stabilized the associated bacterial community while altering physiological and biochemical characteristics of the coccolithophore *Chrysotila roscoffensis*, Algal Res. 67 (2022) 102807, https://doi.org/10.1016/j.algal.2022.102807.

[20] D.H. Cho, R. Ramanan, B.H. Kim, J. Lee, S. Kim, C. Yoo, H.S. Kim, Novel approach for the development of axenic microalgal cultures from environmental samples, J Phycol. 49 (2013) 802-810, https://doi.org/10.1111/jpy.12091.

[21] K. Minas, N.R. McEwan, C.J. Newbold, K.P. Scott, Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures, FEMS Microbiol Lett. 325 (2011) 162-169, https://doi.org/10.1111/j.1574-6968.2011.02424.x.

[22] Y. Sun, Y. Xin, L.Y. Zhang, Y. Wang, R.L. Liu, X.H. Li, C.X. Zhou, L. Zhang, J.C. Han, Enhancement of violaxanthin accumulation in *Nannochloropsis oceanica* by overexpressing a carotenoid isomerase gene from *Phaeodactylum tricornutum*, Front Microbiol. 13 (2022) 942883, https://doi.org/10.3389/fmicb.2022.942883.

[23] J.J. Sedmak, S.E. Grossberg, A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250, Anal Biochem. 79 (1977) 544-52, https://doi.org/10.1016/0003-2697(77)90428-6.

[24] M. Le Chevanton, M. Garnier, G. Bougaran, N. Schreiber, E. Lukomska, J.B. Berard, J.P. Cadoret, Screening and selection of growth-promoting bacteria for *Dunaliella* cultures, Algal Res. 2 (2013) 212-222, https://doi.org/10.1016/j.algal.2013.05.003.

[25] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can J Biochem Physiol. 37 (1959) 911-7, https://doi.org/10.1139/o59-099.

[26] M.A. Grant, E. Kazamia, P. Cicuta, A.G. Smith, Direct exchange of vitamin B_{12} is demonstrated by modelling the growth dynamics of algal-bacterial cocultures, ISME J. 8 (2014) 1418-1427, https://doi.org/10.1038/ismej.2014.9.

[27] M. Kai, B. Piechulla, Plant growth promotion due to rhizobacterial volatiles -An effect of CO_2? FEBS Lett. 583 (2009) 3473-3477, https://doi.org/10.1016/j.febslet.2009.09.053.

[28] X.M. Liu, H.M. Zhang, The effects of bacterial volatile emissions on plant abiotic stress tolerance, Front Plant Sci. 6 (2015) 774, https://doi.org/10.3389/fpls.2015.00774.

[29] M. Ding, P.K. Gao, D.Z. Xu, E.J. Xing, Y. Li, L. Sun, R.J. Wang, W.L. Zhang, Characterization and algicidal activity of bacteria from the phycosphere of the harmful alga *Karenia mikimotoi*, Braz J Microbiol. 53 (2022) 891-901, https://doi.org/10.1007/s42770-022-00727-z.

[30] T. Keawtawee, K. Fukami, P. Songsangjinda, Use of a *Noctiluca*-killing bacterium *Marinobacter salsuginis* strain BS2 to reduce shrimp mortality caused by

Noctiluca scintillans, Fisheries Sci. 78 (2012) 641-646, https://doi.org/10.1007/s12562-012-0497-1.

[31] A. Carstensen, A. Herdean, S.B. Schmidt, A. Sharma, C. Spetea, M. Pribil, S. Husted, The impacts of phosphorus deficiency on the photosynthetic electron transport chain, Plant Physiol. 177 (2018) 271-284, https://doi.org/10.1104/pp.17.01624.

[32] T. Mogany, V. Bhola, L. Ramanna, F. Bux, Photosynthesis and pigment production: elucidation of the interactive effects of nutrients and light on *Chlamydomonas reinhardtii*, Bioprocess Biosyst Eng. 45 (2022) 187-201, https://doi.org/10.1007/s00449-021-02651-2.

[33] J.P. Hernandez, L.E. Debashan, D.J. Rodriguez, Y. Rodriguez, Y. Bashan, Growth promotion of the freshwater microalga *Chlorella vulgaris* by the nitrogen-fixing, plant growth-promoting bacterium *Bacillus pumilus* from and zone soils, Eur J Soil Biol. 45 (2009) 88-93, https://doi.org/10.1016/j.ejsobi.2008.08.004.

[34] D. Li, R. Liu, X. Cui, M. He, S. Zheng, W. Du, C. Wang, Co-culture of bacteria and microalgae for treatment of high concentration biogas slurry, J Water Process. Eng. 41 (2021) 102014, https://doi.org/10.1016/j.jwpe.2021.102014.

[35] W. Elhaissoufi, C. Ghoulam, A. Barakat, Y. Zeroual, A. Bargaz, Phosphate bacterial solubilization: a key rhizosphere driving force enabling higher P use efficiency and crop productivity, J Adv Res. 38 (2022) 13-28, https://doi.org/10.1016/j.jare.2021.08.014.

[36] J. Poveda, F. González-Andres, *Bacillus* as a source of phytohormones for use in agriculture, Appl Microbiol Biot. 15 (2021) 1-17, https://doi.org/10.1007/s00253-021-11492-8.

[37] B. Zhang, J. Chen, Y. Su, W. Sun, A. Zhang, Utilization of indole-3-acetic acid–secreting bacteria in algal environment to increase biomass accumulation of *Ochromonas* and *Chlorella*, Bioenerg Res. 15 (2022) 242-252, https://doi.org/10.1007/s12155-021-10246-8.

[38] E.B. Pérez, I.C. Pina, L.P. Rodríguez, Kinetic model for growth of *Phaeodactylum tricornutum* in intensive culture photobioreactor, Biochem Eng. 40 (2008) 520-525, https://doi.org/10.1016/j.bej.2008.02.007.

[39] T.B. Hao, Y.F. Yang, S. Balamurugan, D.W. Li, W.D. Yang, H.Y. Li, Enrichment of f/2 medium hyperaccumulates biomass and bioactive compounds in the diatom *Phaeodactylum tricornutum*, Algal res. 47 (2020) 101872, https://doi.org/10.1016/j.algal.2020.101872.

[40] F. Di Caprio, A fattening factor to quantify the accumulation ability of microorganisms under N-starvation, New Biotechnol. 66 (2022) 70-78, https://doi.org/ 10.1016/j.nbt.2021.04.001.

[41] Z.D. Song, G.J. Lye, B.M. Parker, Morphological and biochemical changes in *Phaeodactylum tricornutum* triggered by culture media: Implications for industrial exploitation, Algal Res. 47 (2020) 101822, https://doi.org/10.1016/j.algal.2020.101822.

[42] H. Kanda, R. Hoshino, K. Murakami, Q. Zheng, M. Goto, Lipid extraction from microalgae covered with biomineralized cell walls using liquefied dimethyl ether, Fuel. 262 (2020) 116590, https://doi.org/10.1016/j.fuel.2019.116590.

[43] A.S.P. Moreira, J. Gonçalves, T.A. Conde, D. Couto, T. Melo, I.B. Maia, H. Pereira, J. Silva, M.R. Domingues, C. Nunes, Chrysotila pseudoroscoffensis as a source of high-value polar lipids with antioxidant activity: A lipidomic approach, Algal Res. 66 (2022) 102756, https://doi.org/10.1016/j.algal.2022.102756.

Highlights

- MBC is an efficient strategy to increase microalgal biomass productivity.
- MBC shows no negative effects on the quality of microalgal biomass.
- Effects of bacteria on algae need to be verified in both axenic and xenic systems.