Current Research in Biotechnology 11 (2026) 100361

Contents lists available at ScienceDirect

Current Research in Biotechnology

journal homepage: www.elsevier.com/locate/crbiot

Tailored bacterial co-cultures improve *Tisochrysis lutea* growth and nutrient profiles under xenic conditions: a new pathway to improve microalgal production

Tamára F. Santos [a,1], Beatriz Simões [a,1], Veronica Rossetto [a], Hugo Pereira [b], Inês B. Maia [a], Marta Oliveira [a], Aschwin Engelen [a], João Navalho [b,c], João Varela [a,b,*]

[a] CCMAR – Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal
[b] GreenColab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, 8005-139, Portugal
[c] Necton S.A., Portuguese Company of Marine Cultures, 8700-152 Olhão, Portugal

ARTICLE INFO

Keywords:
Microbial consortia
Mutualistic interactions
Microalgal microbiomes
Vitamin B_{12} production
Co-culture strategies

ABSTRACT

The marine haptophyte *Tisochrysis lutea* is a valuable source of high-value compounds, including polyunsaturated fatty acids like docosahexaenoic acid, and pigments (e.g., fucoxanthin). However, high production costs and variability remain major challenges for its large-scale application in aquaculture, pharmaceuticals, and biotechnology industries. Therefore, strategies to enhance biomass production and quality are actively explored. In natural environments, *T. lutea* establishes mutualistic interactions with bacteria to obtain essential nutrients such as vitamin B_{12}, yet the role of bacteria in industrial cultures remains poorly understood. In this study, 145 bacterial strains were isolated and taxonomically identified from industrial *T. lutea* cultures, with members of the class Gammaproteobacteria and Actinomycetia being the most prevalent. Forty isolates were screened individually in co-culture with *T. lutea* revealing strain-specific effects on growth and biochemical composition. Seven beneficial strains were used to design 21 tailored bacterial blends. Several consortia enhanced biomass production (up to 74 %) and increased key bioactive compounds, particularly methylcobalamin (up to 300 %). These findings demonstrate the potential of tailored bacterial consortia to enhance *T. lutea* productivity and nutritional quality under production-relevant xenic conditions, enabling strategic microbiome modulation for specific industrial goals.

1. Background

Microalgae are a highly diverse group of eukaryotic microorganisms known for their ability to produce bioactive compounds, including lipids, proteins and pigments, with a range of a000pplications in biotechnology and industries such as food, feed and pharmaceuticals (Ahamed et al., 2015; Chua and Schenk, 2017; Hamidi et al., 2020).

Among the microalgal species cultivated at an industrial scale, *Tisochrysis lutea* is a golden-brown marine haptophyte commonly produced during spring and summer (Gangadhar et al., 2020; Gonçalves de Oliveira-Júnior et al., 2020; Pereira et al., 2021). This species is particularly rich in polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA), and exhibits morphological traits that enhance its suitability for aquaculture and feed, including its small size and absence of a rigid cell wall (da Costa et al., 2017; Nalder et al., 2015). Additionally, its biomass is a significant source of carotenoids, particularly fucoxanthin, a bioactive pigment with antioxidant, anti-microbial, anti-cancer and anti-inflammatory properties, making it valuable for the pharmaceutical, cosmetic and food industries (Bigagli et al., 2021; Gangadhar et al., 2020; Gao et al., 2021; Pereira et al., 2021).

Despite its biotechnological potential, large-scale production remains limited by high production costs, variability in biomass composition, sensitivity to environmental conditions and challenges in optimizing cultivation efficiency (Chua and Schenk, 2017; Hamidi et al., 2020; Tong et al., 2023). To address such issues, different optimization strategies have been explored, focusing on the modulation of culture

* Corresponding author at: CCMAR – Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal.
E-mail address: jvarela@ualg.pt (J. Varela).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.crbiot.2025.100361
Received 15 September 2025; Received in revised form 28 November 2025; Accepted 8 December 2025
Available online 13 December 2025

conditions aiming at improved growth and biomass composition (Gao et al., 2020). For instance, changes in the culture medium salinity have been linked to variations in microalgae biomass productivity and production of high-value products, such as carotenoids and fatty acids (Macías-de la Rosa et al., 2024). In that sense, the co-cultivation of microalgae with other microorganisms has also been recently explored as a valid alternative for the optimization of industrial scale processes towards biomass and biocompounds production (Esteves et al., 2025; Pathom-aree et al., 2024). In nature, microalgae coexist with various microorganisms, including bacteria, fungi, and other algae, forming complex ecological networks (Ahamed et al., 2015; Miller et al., 2024; Natrah et al., 2014; Seymour et al., 2017). While some interactions can negatively affect the organisms involved, others are mutually beneficial and can be crucial for their survival and development (Berthold et al., 2019; Lian et al., 2018; Yao et al., 2019). The nature of these interactions varies depending on the species, and environmental conditions, among other factors. However, mutualistic interactions appear to occur more frequently than antagonistic ones (González-González and de-Bashan, 2021; Lian et al., 2021; Yao et al., 2019). Even though mutualism is a known fact in natural ecosystems, bacteria in industrial microalgae cultures are often perceived as undesired contaminants due to their potential to compete for nutrients or cause culture collapse, despite their potential beneficial effects, which are frequently overlooked (Ahamed et al., 2015; Berthold et al., 2019; Fulbright et al., 2018; Lian et al., 2021).

Emerging evidence suggests that specific bacteria can improve microalgal production by enhancing growth and biomass quality while simultaneously reducing costs and overcoming cultivation challenges, such as nutrient limitation or culture instability (Berthold et al., 2019; Borges Lopes et al., 2025; González-González and De-Bashan, 2021; Le Chevanton et al., 2013; Padmaperuma et al., 2018). Reports on the co-culturing the plant growth-promoting bacteria *Azospirillum brasilense* and *Bacillus pumilus* with *Chlorella sorokiniana* resulted in higher growth rates, lipid, carbohydrate and chlorophyll yields (Amavizca et al., 2017). Similarly, an artificially designed bacterial consortium containing *Flavobacterium* sp., *Hyphomonas* sp., *Rhizobium* sp., and *Sphingomonas* sp. improved growth and lipid content of *Chlorella vulgaris* (Cho et al., 2015). Even though co-cultivation studies with *T. lutea* are limited, one study showed that the cultivation of this species in xenic conditions improved in comparison to axenic ones, highlighting the importance of alga-bacteria interactions (Nef et al., 2022). Nevertheless, most studies focus on the introduction of external bacterial strains selected from literature reports, analyzing their effects in axenic microalgal cultures, while the potential of naturally occurring bacteriomes in microalgal systems remains largely untapped.

While interactions with bacteria have been increasingly recognized as influential in microalgal productivity, little is known about their role in *T. lutea* cultures, particularly in industrial settings. This microalga is vitamin B_{12}-dependent, relying on this cofactor for key metabolic pathways, such as methionine synthesis and carbon cycling (Croft et al., 2005; Nef et al., 2022). However, marine environments typically contain low levels of this micronutrient (between 0.01 and 4.7 pM; Bannon et al., 2025), often insufficient to sustain its growth (Nef et al., 2022, 2019). Nef et al., (2019) reported maximal growth rate when *T. lutea* was supplemented with around 30 pM of cobalamin, while *T. lutea* in cobalamin-free culture medium demonstrated a five-fold lower growth rate. Since only a restricted group of prokaryotes can synthesize vitamin B_{12}, it is likely that *T. lutea* naturally establishes mutualistic interactions with surrounding bacteria to fulfill its growth requirements (Croft et al., 2005; Nef et al., 2022).

Although extensive research has been conducted on optimizing the production of this microalga, very few studies have explored its interactions with bacterial communities, particularly under xenic conditions. In this context, the present study aimed to isolate and identify naturally occurring bacteria from *T. lutea* cultures grown in industrial photobioreactors and investigate their impact, either as individual isolates or as tailored bacterial combinations, on the growth and biochemical composition of the microalgal biomass. By working with non-axenic cultures, this study reflects realistic, on-site cultivation conditions and seeks to improve *T. lutea* productivity and the yield of valuable compounds through targeted manipulation of its associated bacterial community.

2. Materials and methods

Isolation and identification of bacteria from T. lutea cultures

Bacteria were isolated from industrial *T. lutea* cultures cultivated at Necton S.A. (Olhão, Portugal). Sampling was conducted weekly across six independent 19 m^3 tubular photobioreactors (T-PBR) over six complete production cycles, covering the process from inoculation until biomass harvesting. Samples were collected between June and October 2020, covering the typical high-yield cultivation period for *T. lutea* under southern Portuguese climate conditions.

Collected samples were inoculated into non-selective heterotrophic media, and serial dilutions of the liquid suspension were prepared using sterile seawater. Subsequently, 100 μL of each dilution was plated onto solid plate count agar (PCA, 23.5 g L^{-1}, Liofilchem) and marine agar (MA, 52.35 g L^{-1}, US Biological Life Sciences) followed by incubation at 27 °C in the dark for 24–72 h.

Colonies were selected and isolated through successive cross-streaking on new plates under the same incubation conditions. Bacterial DNA from the isolated colonies was extracted using the Quick-DNA Miniprep Kit (Zymo Research Corporation) according to the manufacturer's instructions. DNA quality was assessed after electrophoresis on 1.5 % agarose gel (NZYtech) under 100 V for 30 min.

The isolated bacteria were identified through sequencing of the 16S rRNA gene sequencing, with amplification through polymerase chain reaction (PCR) using the primers F27 (5′-AGA GTT TGA TCG TGG CTC AG-3′) and R1492 (5′-TAC GGY TAC CTT GTT ACG ACT-3′) (Weisburg et al., 1991). PCR amplification was performed in 20 μL reactions using standard reagents concentrations: 5 μL of template DNA, 5.95 μL of molecular biology-grade H_2O (NZYtech), 4 μL of 5 × Colorless GoTaq®flexi buffer (Promega), 1.6 μL of $MgCl_2$ (25 mM; Promega), 1.25 μL of dNTP mix (10 mM; Promega), 1 μL of each primer, and 0.20 μL of GoTaq® G2 Flexi DNA Polymerase (5U μL^{-1}; Promega).

The amplification reaction was performed in a 2720 Thermal Cycler (Applied Biosystems, USA) under the following conditions: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation (95 °C, for 30 s), annealing (50 °C, for 30 s) and elongation (72 °C, for 1.5 min), with a final extension at 72 °C for 10 min.

The amplified DNA was sequenced with an Applied Biosystems 3130XL DNA sequencer (Life Technologies BV, Porto, Portugal). Sequences (forward and reverse) were processed using CLC Genomics Workbench 21 (Qiagen, Denmark) and identification was done using the BLASTn tool of the National Center for Biotechnology Information (NCBI).

Tisochrysis lutea cultures

The inoculum of *T. lutea* was provided by Necton S.A. (Olhão, Portugal). Cultures were maintained under standardized conditions throughout the study, unless otherwise stated.

Scale-up was performed in sterile Erlenmeyer flasks (50–2000 mL) containing sterilized seawater supplemented with Nutribloom® Plus (NB^+, Necton S.A.) with nitrate adjusted to 8 mM, the standard concentration used for this species.

Cultures were maintained through regular subculturing and grown in an incubator shaker (IIS-4075R, JEIO TECH) at 24 °C under constant light (100 μmol m^{-2} s^{-1}) and agitation (140 rpm). Evaporation was compensated with sterile distilled water and added as needed. All materials were autoclaved at 120 °C for 20 min, and culture media were

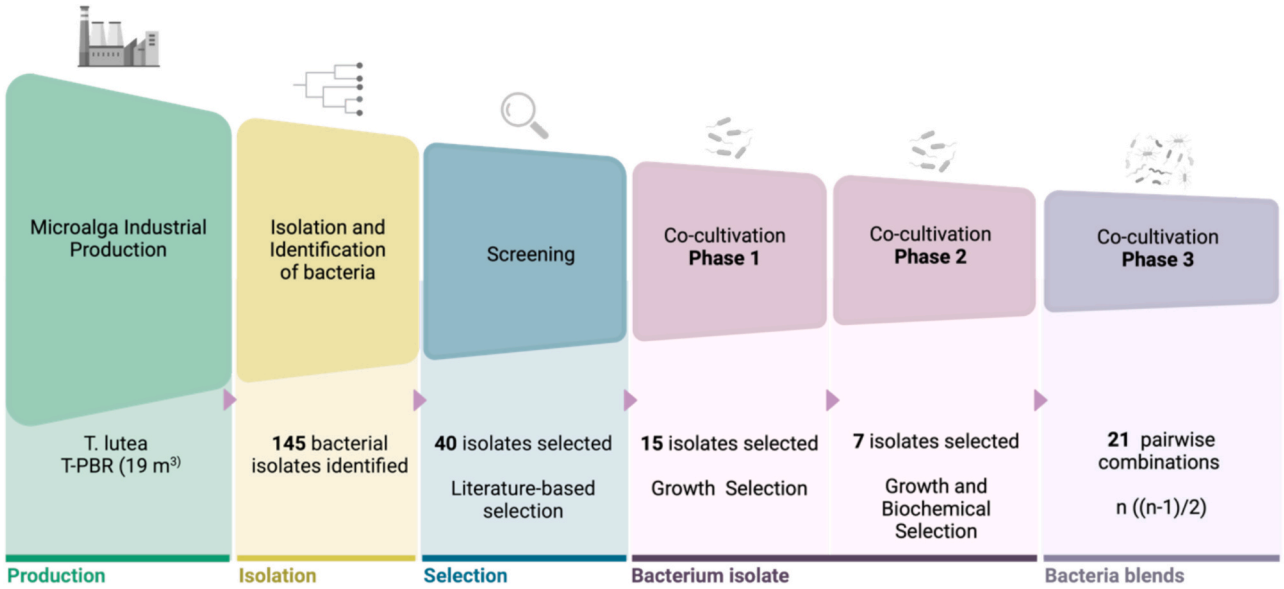

Fig. 1. Bacterial selection process for *Tisochrysis lutea* co-culture trials. Schematic representation of the bacterial selection process conducted in three sequential phases. From 145 identified isolates, 40 were selected for Phase 1 and individually co-cultured with *T. lutea* for 7 days. Based on *T. lutea* growth performance and biochemical potential, 15 isolates progressed to Phase 2 and were tested for 8 days. Seven isolates were then selected for Phase 3, which were combined into 21 pairwise bacterial consortia and tested to assess potential synergistic effects. Created in https://BioRender.com.

filtered through 0.20 μm polyethersulfone filters (Filtropur S, Sarstedt) to ensure sterility. Additionally, natural seawater was filtered to remove solids, and salinity was adjusted to 33 ppt.

Bacterial isolates preparation

A literature review was conducted to define the criteria for bacterial selection, prioritizing isolates with documented beneficial effects on plants or algae cultures, including those known to promote growth or produce bioactive compounds (Supplementary material, Table S1). On the other hand, strains reported as pathogenic to humans and/or fish were excluded from the study. All isolates were initially screened for their ability to grow in liquid plate count broth (PCB) medium to ensure their compatibility with co-culture conditions. Strains that failed to grow under these conditions were excluded from further experiments.

Prior to the cultivation trials, the selected bacteria were grown heterotrophically, inoculated into a sterilized PCB medium, previously prepared with tryptone (5 g L^{-1}, Biolife), glucose (1 g L^{-1}, Scharlau) and yeast extract (2.5 g L^{-1}, Biolife). To further enhance bacterial growth, the medium was supplemented with additional glucose (15 g L^{-1}, Scharlau).

Liquid cultures were incubated at 30 °C in the dark for 48 h under constant agitation (180 rpm), followed by a new 24 h subculturing step under the same culture conditions. Bacterial growth was monitored through turbidity measurements at 600 nm using a BioTek Synergy Neo2 multi-mode reader (BioTek Instruments Inc., USA).

Co-culture assays

The co-cultivation experiments were conducted phototrophically in three sequential phases to evaluate the effects of bacterial isolates on *T. lutea* growth and biochemical composition, following the same experimental conditions. Strains were selected for subsequent phases based on their ability to enhance microalgal growth and/or influence biochemical parameters, including fatty acid profiles, pigment, protein, and vitamin B_{12} contents. Fig. 1 presents a schematic overview of the entire selection process.

A total of 40 bacterial isolates were selected from the 145 initially identified strains and individually tested in Phase 1. Selection was based on taxonomic classification, cultivation characteristics, and potential functional relevance. An overview of the selection process and the rationale for including or excluding specific isolates is detailed in Supplementary material (Table S1). Based on growth performance and reported biochemical potential, 15 isolates were selected for Phase 2, aiming at confirming the performance of the selected bacterial isolates in xenic *T. lutea* cultures and thereby proving consistent beneficial effects in naturally changing microbiomes. After further refinement, 7 isolates were chosen for Phase 3, where they were tested as pairwise bacterial consortia to assess potential synergistic effects.

For all experimental phases, an inoculum of *T. lutea* (10^6 cells mL^{-1}, optical density (OD) of 0.2 at 680 nm) in the exponential growth phase was transferred to 100 mL Erlenmeyer flasks containing sterile seawater supplemented with NB^+ with a final nitrate concentration of 8 mM. Simultaneously, bacterial cultures were transferred to 50 mL tubes and centrifuged at 3000 *g* for 2 min, and bacterial pellets were resuspended in sterile seawater before inoculation into the microalgae cultures. The final bacteria concentration was adjusted to an OD of 0.05 at 600 nm (OD_{600}). Throughout the experiments, OD was used as a rapid method to evaluate the growth performance of the co-cultures, where OD_{600} was used to monitor bacterial growth, while OD_{680} to assess microalgal biomass, following standard practice for each organism. In addition, since the presence of bacteria and other microorganisms in microalgae cultures can impact dry weight measurements (Griffiths et al., 2011) cell counts were performed to determine *T. lutea* cellular concentration and further evaluate growth performance. Co-cultures were incubated in a shaker (IIS-4075R, JEIO TECH) at 24 °C under continuous light (100 μmol m^{-2} s^{-1}) and agitation (140 rpm).

Phase 1 lasted 7 days, with sampling on days 0 and 7 while Phases 2 and 3 were conducted over 8 and 11 days, respectively, with sampling every 48 h. Microalgal biomass production was assessed by measuring OD_{680} and performing cells counts, whereas bacterial presence and proliferation were monitored by OD_{600} and colony-forming unit (CFU) counts. Once *T. lutea* reached the stationary growth phase, biomass was harvested by centrifugation (7000*g*, 5 min, Beckman Coulter Avanti J-25 High-Performance centrifuge), freeze-dried, and stored at −20 °C for biochemical analysis.

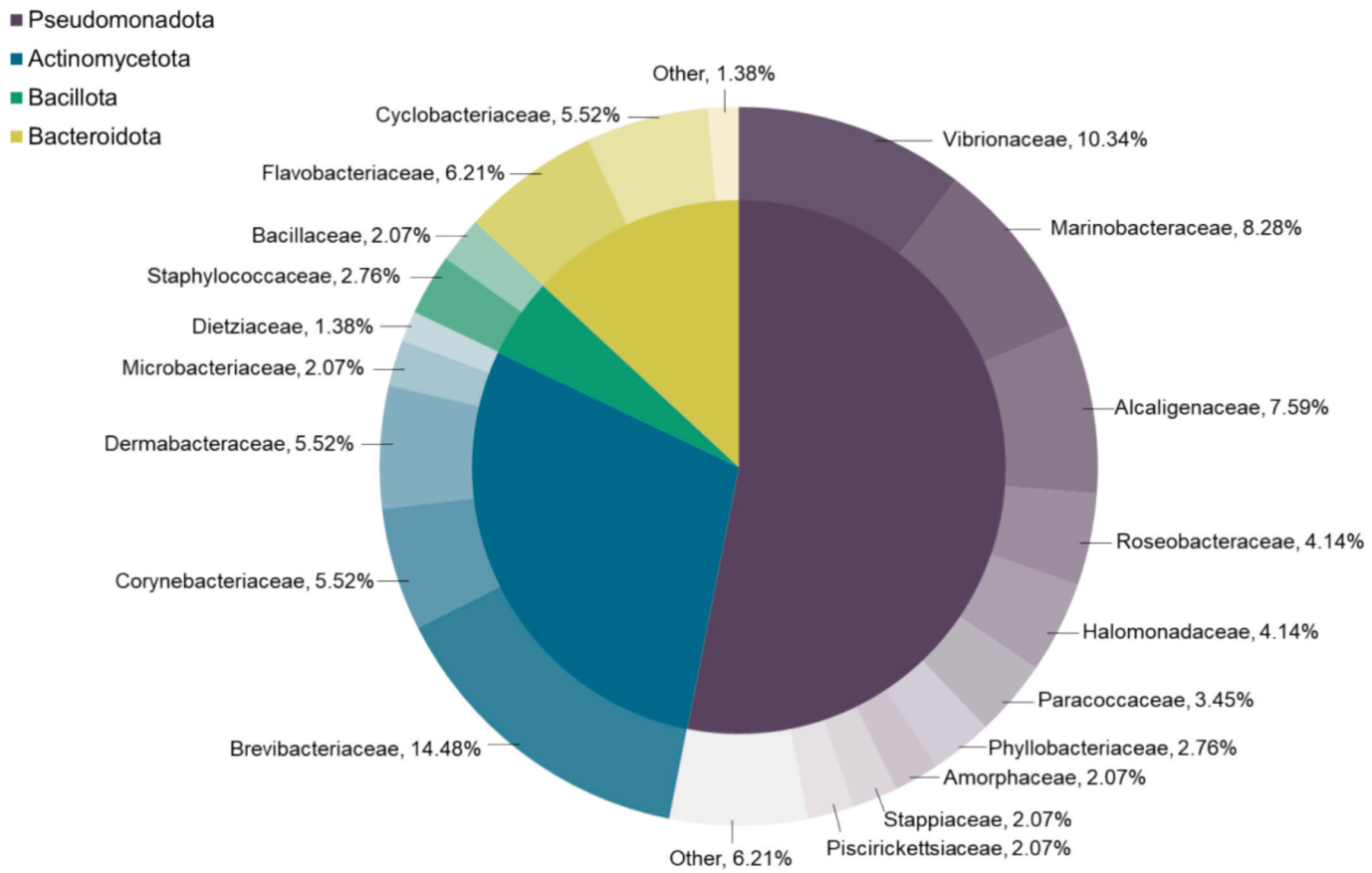

Fig. 2. Relative abundance and taxonomic distribution of the 145 culturable bacterial isolates associated with *Tisochrysis lutea*, classified at the phylum and family levels. Families with a relative abundance below 1 % were grouped under the category "Other". *T. lutea* was produced in industrial tubular photobioreactors (T-PBR) at Necton facilities between June and October (2020), using Nutribloom® Plus (NB+) as the culture medium. Bacterial isolates were obtained using Marine Agar and PCA.

Biochemical analysis of the produced biomass

Fatty acid methyl esters profile

The fatty acid methyl esters (FAME) profile was determined following a modified Lepage and Roy procedure (Lepage and Roy, 1984), as described by Pereira et al. (2012). Approximately 10 mg of dried biomass was mixed with 1.5 mL of a methanol: acetyl chloride (20:1 v/v) derivatization mixture. After derivatization, a hexane: water mixture (4:1 v/v) was added to the samples, followed by centrifugation to separate the hexane fraction containing the extracted FAMEs. The organic phase was dried using anhydrous sodium sulfate, concentrated under gentle nitrogen flow, and filtered before analysis by gas chromatography-mass spectrometry (GC–MS; Pereira et al., 2012). Each FAME was quantified using calibration curves, generated with a Supelco® 37 Component FAME Mix (Merck). For compounds not included in the standard mix, quantification was performed using the calibration curve of the structurally closest FAME (Pereira et al., 2012).

Protein content

Total protein content was estimated by measuring the total nitrogen content of the dry biomass (1 to 2 mg) using an Elementar Analyzer model Vario III, according to the manufacturer's instructions. The total nitrogen (%) was converted to total protein content using a conversion factor of 4.59, as previously established for the closely related species *Isochrysis galbana* (Lourenço et al., 2004).

Pigment profile

The pigment profile of *T. lutea* was assessed following an extraction protocol using methanol with 0.03 % of butylated hydroxytoluene (BHT), based on the methodology described by Schüler et al. (2020). Approximately 5 mg of dried biomass was mixed with the extraction solution and disrupted using glass beads in a mixer mill (Retsch MM400) at 20 Hz for 3 cycles of 1 min, with 30 s breaks on ice. The mixture was then centrifuged (12,000*g* for 6 min), and the supernatant, containing the extracted pigments, was collected in an amber vial until the pellet was colorless. The extract was concentrated under gentle nitrogen flow, resuspended in HPLC-grade methanol (High-Performance Liquid Chromatography grade), and filtered (0.22 μm) before analysis.

The pigment profile was determined using HPLC (Chromaster, Hitachi, VWR) equipped with a diode array detector (5430 DAD, Hitachi, VWR) and a Purospher® STAR RP-18 column (250 × 2.1 mm, 5 μm, Merck), operated at 27 °C with a flow rate of 1 mL min^{-1} for a total runtime of 40 min per sample. The injection volume was 50 μL. Pigment identification was performed by comparison with standards at 450 nm, and concentrations were determined using standard calibration curves (Couso et al., 2012; Schüler et al., 2020).

Vitamin B_{12} content

The concentration of vitamin B_{12}, in the form of methylcobalamin (MeCbl) was determined by an external laboratory (GreenColab – Associação Oceano Azul, Portugal). The samples were purified using Amberlit® resin, and analysis was performed with HPLC (Chromaster, Hitachi, VWR) with a diode array detector (5430 DAD, Hitachi, VWR) and a C18 column (150 x 4.6 mm, 5 μm), using the wavelength of 230 nm. The injection volume was set to 50 μL, and MeCbl concentration was determined by comparison with the calibration curve prepared from standard solutions. Limit of detection (LOD) and limit of quantification (LOQ) were determined at 0.082 μg 100 g^{-1} and 0.25 μg 100 g^{-1},

respectively.

Statistical analysis

Experiments for Phases 1 and 2 were performed in duplicate and analytical triplicates. Statistical analyses were conducted using GraphPad Prism® software (8.0.2). Statistical differences in growth and biochemical composition of co-cultures with bacterial isolates were analyzed using non-parametric Kruskal-Wallis test with Dunn's multiple comparison test, in relation to the control. Bacterial concentration data were analyzed using an unpaired Mann-Whitney test. For bacterial consortia experiments (Phase 3), statistical differences were assessed using non-parametric Kruskal-Wallis test with Dunn's multiple comparison test.

Results and discussion

Isolation and identification of naturally occurring bacteria in T. lutea cultures

A total of 145 culturable bacterial isolates, belonging to 27 families and 37 genera, were identified from *T. lutea* cultures (Supplementary material; Table S2). Among these, 101 and 44 isolates were classified at the species and genus levels, respectively. Brevibacteriaceae (14.5 %), belonging to the class Actinomycetia, was the most abundant bacterial family (Fig. 2). Families within the class Gammaproteobacteria, including Vibrionaceae (10.3 %) and Marinobacteraceae (8.3 %) were the second most abundant taxa followed by Alcaligenaceae (7.5 %) from the class Betaproteobacteria.

While these findings provide insight into the culturable bacteriome associated with *T. lutea*, it is important to acknowledge that culture-dependent methods capture only a small fraction of the microbial diversity (Hinsu et al., 2021). Despite this limitation, culture-based studies remain crucial, as they enable the isolation of relevant bacteria for experimental and biotechnological applications. Additionally, the relative dominance of specific groups observed here may partly reflect cultivation conditions rather than true ecological prevalence. As this study focused on generating culturable isolates for co-culture assays, mechanistic drivers of these patterns were not explored.

The cultivable bacterial diversity observed from *T. lutea* cultures suggests a complex and dynamic system shaped by environmental conditions and microalgal exudates, which selectively promote the growth of specific bacterial groups (Fuentes et al., 2016). Previous studies have shown that Gammaproteobacteria are commonly associated with microalgal cultures (Lian et al., 2018). Similarly, Alpha- and Gammaproteobacteria were the predominant bacterial groups in cultures of *I. galbana*, *Thalassiosira pseudonana*, and *Nannochloropsis oceanica* (Ling et al., 2020). Our results align with these findings, as Gammaproteobacteria represented the dominant taxon in *T. lutea* cultures (29.66 %).

Gammaproteobacteria are known for their metabolic versatility, enabling them to degrade organic matter, cycle nutrients, and produce bioactive compounds that can modulate algal growth (Seymour et al., 2017). Additionally, many Gammaproteobacteria establish symbiotic relationships with microalgae, supplying essential micronutrients (e.g., vitamin B_{12}) in exchange for fixed carbon (Ramanan et al., 2016). This mutualistic association may contribute to their dominance in *T. lutea* cultures. However, not all Gammaproteobacteria are beneficial, as certain Vibrionaceae bacteria have been linked to negative effects on microalgae, including growth inhibition and cell lysis (Fuentes et al., 2016).

Interestingly, Actinomycetes (28.97 %) were also highly abundant, surpassing Alphaproteobacteria (15.86 %). This differs from previous reports, where Actinomycetes were either underrepresented or absent in microalgal microbiomes (Ling et al., 2020). Actinomycetes prevalence here may reflect the rich media used for bacterial isolation, which supports the growth of Actinomycetes capable of metabolizing complex substrates. Additionally, dark incubation and extended cultivation times may have favored slower-growing taxa. Although commonly associated with oligotrophic environments, many Actinomycetes also grow well on enriched media under laboratory conditions (Barka et al., 2016). These bacteria are also known to produce secondary metabolites such as antibiotics and growth-promoting compounds that could play a role in modulating microbial competition or protecting *T. lutea* from pathogens (Farda et al., 2022).

In contrast, families such as Aurantimonadaceae, Halieaceae, Hyphomonadaceae, Idiomarinaceae, and Pseudomonadaceae were present in low abundance, each accounting for approximately 0.69 % of the total bacterial population. Although low-abundance taxa are often overlooked, they may function as keystone species, influencing community stability and nutrient cycling despite their small population sizes (Jousset et al., 2017). However, it is important to note that the relative abundance presented here reflects the diversity of culturable isolates selected for identification, not the in-situ abundance in the source

Table 1
Identification of the 40 bacterial isolates selected for the co-culture trials with *Tisochrysis lutea*, according to BLASTn searches against the NCBI nt database.

Strain Code	Probable ID	Query Cover (%)	Identity (%)	GenBank Accession code
7	*Marinobacter nauticus*	100	100	MT325887.1
8	*Marinobacter* sp.	97	93.85	KR004809.1
14	*Roseovarius aquimarinus*	100	99.38	OR654359.1
17	*Corynebacterium glyciniphilum*	100	99.67	MK414897.1
19	*Pseudomonas* sp.	100	99.62	MT187898.1
22	*Halomonas alkaliphila*	100	99.74	CP024811.1
33	*Pseudoalteromonas* sp.	100	99.84	MN889178.1
45	*Sulfitobacter* sp.	92	95.53	MZ262822.1
47	*Arenibacter* sp.	100	99.28	MN784288.1
60	*Halomonas titanicae*	100	99.71	CP054580.1
69	*Hyphomonas* sp.	100	97.96	MK029430.1
73	*Marinobacter* sp.	100	99.06	CP045367.1
74	*Brevibacterium* sp.	100	94.37	LC133762.1
75	*Cyclobacterium* sp.	100	92.4	KY770661.1
78	*Microbacterium* sp.	100	98.84	MH712125.1
80	*Roseovarius* sp.	100	100	MH650971.1
81	*Priestia flexa*	100	99.72	MW110779.1
84	*Haliea salexigens*	99	100	OR654371.1
86	*Brevibacterium* sp.	100	100	ON810589.1
88	*Flagellimonas marinaquae*	100	99.29	AP027268.1
95	*Methylophaga muralis*	99	98.21	KX279370.1
96	*Marinobacter adhaerens*	99	97.74	MT507042.1
99	*Methylophaga* sp.	99	97.32	OQ055141.1
103	*Cyclobacterium marinum*	88	95.28	LT601135.2
105	*Brevibacterium* sp.	96	96.95	MF431766.1
106	*Nitratireductor* sp.	100	99.84	MT457434.1
107	*Nitratireductor* sp.	100	100	MT457434.1
108	*Microbacterium* sp.	100	90.91	MK967164.1
109	*Feifantangia zhejiangensis*	100	99.84	NR_148332.1
114	*Cyclobacterium* sp.	94	92.55	KY770661.1
116	*Alcaligenes faecalis*	100	93.16	MH029146.1
123	*Tritonibacter mobilis*	93	98.33	MK493584.1
131	*Brevibacterium* sp.	100	100	MT433875.1
132	*Pseudidiomarina maritima*	100	99.59	KT986182.1
133	*Microbacterium* sp.	100	99.83	KU560428.1
138	*Cyclobacterium marinum*	100	100	LT601135.2
147	*Cyclobacterium* sp.	100	93.55	LT630358.2
149	*Marinobacter adhaerens*	100	95.09	MT507043.1
156	*Marinobacter* sp.	100	99.83	MT457438.1
157	*Nitratireductor* sp.	100	100	MT457434.1

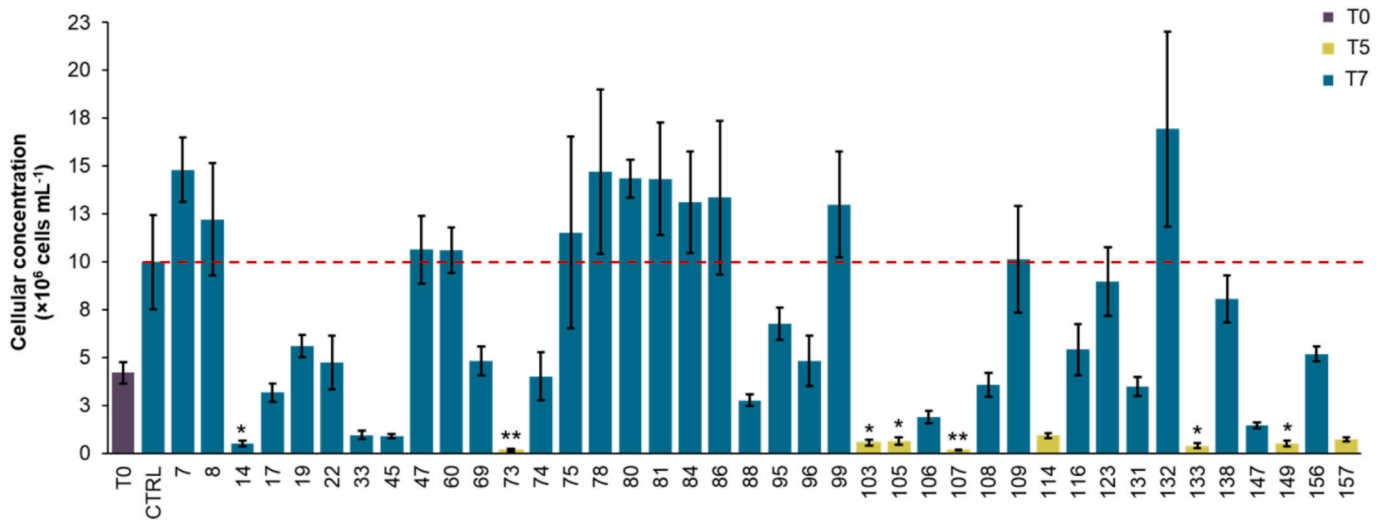

Fig. 3. Cellular concentration of *Tisochrysis lutea* ($\times 10^6$ cells mL^{-1}) immediately after inoculation (T_0) and at the end of Phase 1 (days 5 and 7), in control (CTRL) and co-culture conditions with bacterial isolates (7–157). Bars represent mean values ± SD (n = 2). Statistical differences were assessed using non-parametric Kruskal-Wallis test with Dunn's multiple comparison test, compared to the control; *: $p < 0.05$, **: $p < 0.01$).

microbiome. Isolates were chosen based on colony morphology and taxonomy to capture broad diversity, which may have led to an under-representation of dominant taxa and an overrepresentation of rarer phenotypes. Overall, the bacterial diversity observed in *T. lutea* cultures suggests complex ecological interactions that may influence the physiology and productivity of this microalga.

Co-culture with bacterial isolates

Forty bacterial isolates were selected based on taxonomic and functional criteria and tested in co-culture with xenic *T. lutea* (Table 1). Importantly, all co-cultivation assays were performed under xenic conditions, i.e., the natural microbiome associated with *T. lutea* was preserved prior to the addition of the bacterial strains to the culture. As such, the outcomes likely reflect both direct interactions, between the inoculated bacteria and the microalga, as well as indirect effects mediated by the pre-existing microbial community. Given this xenic panorama, these patterns represent functional outcomes under mixed-community conditions and cannot be attributed mechanistically to individual strains.

A significant decrease in algal cell concentration was observed in 7 out of 40 co-cultures, corresponding to 17.5 % of the total tested bacteria, with 8 cultures collapsing by day 5 (Fig. 3). Some bacterial strains, such as *Nitratireductor* spp. and *Marinobacter* spp., have been previously identified as promoters of microalgal growth (Fuentes et al., 2016; Liu et al., 2020; Seymour et al., 2017). However, in this study, all tested *Nitratireductor* and *Marinobacter* isolates had no effect on *T. lutea* growth, emphasizing the strain-specific nature of these interactions and their dependence on the culture conditions (Ahamed et al., 2015; Tait et al., 2019). Conversely, co-cultures with *Roseovarius* sp. (80) increased *T. lutea* cell concentration by 31 % compared to control at Day 7. *Roseovarius* belongs to the phylum Pseudomonadota, class Alphaproteobacteria, being closely related to *Roseobacter*, a well-known genus in algae-bacteria interactions (Ramanan et al., 2016).

Based on the growth results of Phase 1 (individual isolates), 15 bacterial strains exhibiting enhanced or neutral effects on *T. lutea* growth were selected for further research: *Marinobacter nauticus* (7), *Marinobacter* sp. (8), *Arenibacter* sp. (47), *H. titanicae* (60), *Cyclobacterium* sp. (75), *Microbacterium* sp. (78), *Roseovarius* sp. (80), *P. flexa* (81), *H. salexigens (84), Brevibacterium* sp. (86), *Methylophaga* sp. (99), *F. zhejiangensis (109) and P. maritima* (132). Additionally, the two strains *Halomonas alkaliphila* (22) and *Alcaligenes faecalis* (116) were also included, as both have been reported to be involved in B_{12} synthesize (Nef et al., 2022; Prabaningtyas et al., 2021). Although individual bacteria do not always supply this micronutrient to microalgae, when in co-culture with other bacteria can foster new mutualistic interactions that promote B_{12} co-production or exchange (Nef et al., 2022; Wienhausen et al., 2024). Even through these isolates did not show significant growth-promoting effects in the initial screening, they may still contribute positively to *T. lutea* when combined with other bacteria This group of 15 isolates constituted the basis for the second experimental phase.

Isolates such as *Marinobacter nauticus* (7), *Marinobacter* sp. (8), and *P. maritima* (132), maintained the performance observed in the first phase, with similar cell concentrations across both assays. In contrast, *Priestia flexa* (81), which had previously matched the performance of the control, led to decreased algal growth in Phase 2 (Fig. 4A).

H. salexigens (84) demonstrated strong potential to enhance *T. lutea* growth. It yielded the highest final algal cell concentration (2.51×10^7 cells mL^{-1}), exceeding the control (1.59×10^7 cells mL^{-1}) by over 58 %, thereby confirming its consistent growth-promoting effect across trials. *H. salexigens* belongs to the phylum Pseudomonadota, class Gammaproteobacteria, a group known for its ecological versatility and roles in nutrient cycling. Its positive impact on algal growth may reflect strain-specific traits, possibly including the biosynthesis of micronutrients such as vitamin B_{12}, a function previously described for related genera (Nef et al., 2019; Ramanan et al., 2016). Also, this isolate and *Roseovarius* sp. (80) showed a significantly higher bacterial abundance in co-culture (Fig. 4B), which may suggest a mutualistic interaction, potentially involving nutrient exchange or metabolic support. *Haliea* were recently described following the amendment of the family Idiomarinaceae and the identification of *H. salexigens* as a novel species (Liu et al., 2019; Urios et al., 2008; Wu et al., 2009). Because of their recent classification, little is known about their ecological role or interaction with microalgae. However, the isolation of *Haliea alexandrii* from the microbiome of *Alexandrium catenella* hints that members of this genus may be naturally associated with dinoflagellates and other microalgae (Yang et al., 2020).

Regarding the biochemical profile of the biomass (Table 2), co-cultures containing *Microbacterium* sp. (78), *Roseovarius* sp. (80), *P. flexa* (81), *Methylophaga* sp. (99), and *A. faecalis* (116) showed a higher total protein content compared to the control. This aligns with previous findings where *Microbacterium* has been described as a symbiotic, growth-promoting bacterium in *C. vulgaris*, particularly under

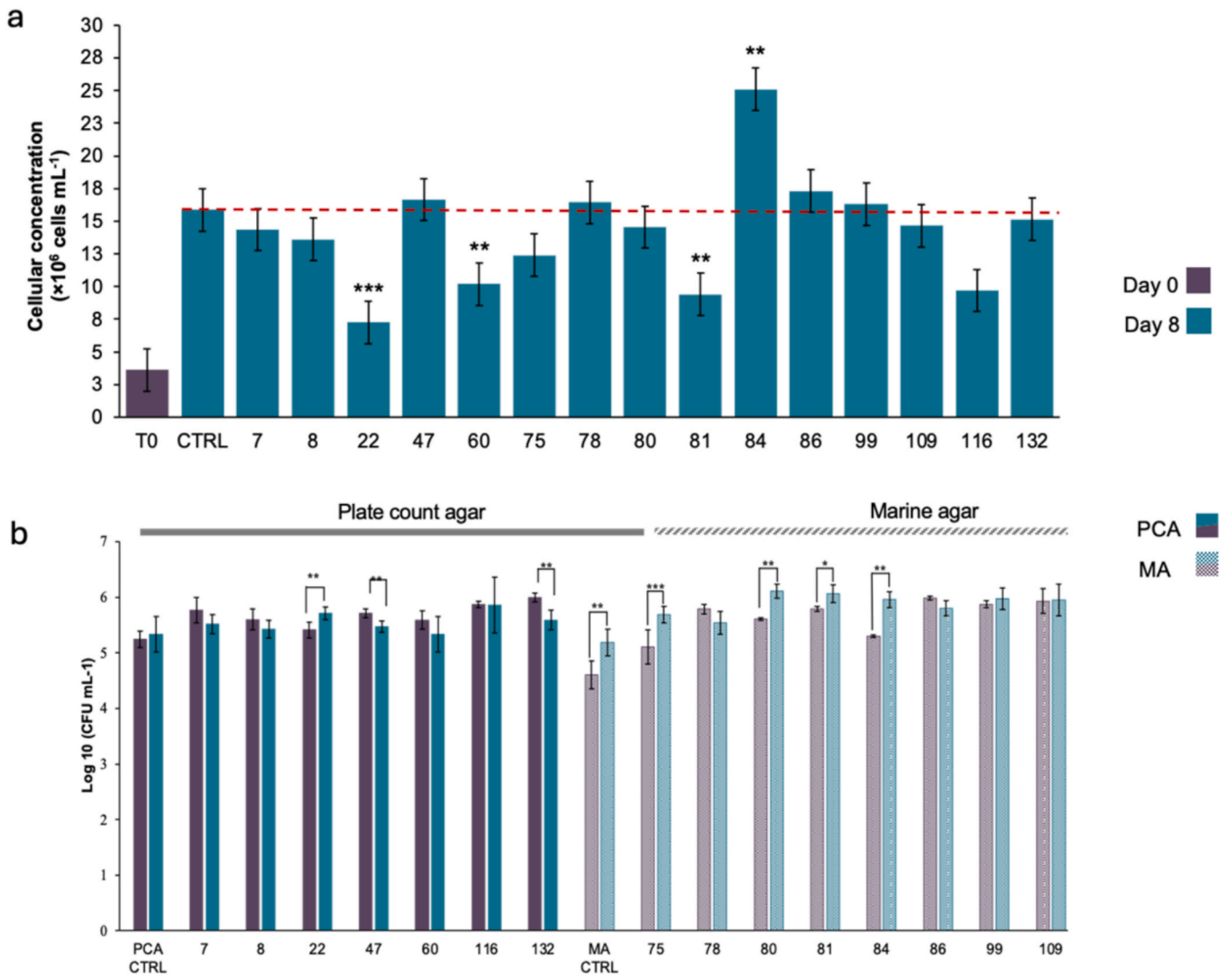

Fig. 4. (A) Cellular concentration ($\times 10^6$ cells mL^{-1}) of *Tisochrysis lutea* at inoculation (Day 0) and after 8 days (Day 8) of Phase 2 of culture with selected bacterial isolates or without added bacteria (CTRL). Bars represent mean values ± SD. Values marked with one or more asterisks (*) represent significant differences when compared to the CTRL (Kruskal-Wallis test with Dunn's multiple comparison test, **: $p < 0.01$, ***: $p < 0.001$). (B) Evolution of bacterial abundance (Log_{10} CFU mL^{-1}) in *T. lutea* cultures during Phase 2. Counts were performed on marine agar (MA; patterned bars) or plate count agar (PCA; solid bars) at Day 0 and Day 8. Bars represent mean values ± SD. Values marked with one or more asterisks (*) indicate statistically significant differences between Day 0 and Day 8 for each treatment (unpaired Mann-Whitney test; **: $p < 0.01$, ***: $p < 0.001$).

nitrogen-regulated conditions (Kim et al., 2015). A similar mutualistic interaction was observed with *Chlamydomonas reinhardtii*, where *Microbacterium* supported long-term algal viability and hydrogen production under aerobic conditions (Fakhimi et al., 2024).

In terms of MeCbl, co-cultures with several strains, including *M. nauticus* (7), *Marinobacter* sp. (8), *H. alkaliphila* (22), *Microbacterium* sp. (78), *Roseovarius* sp. (80), *P. flexa* (81), *Feifantangia zhejiangensis* (109), *A. faecalis* (116), and *P. maritima* (132), showed higher absolute concentrations (ranging from 8.39 to 14.75 μg g^{-1}) than the control (8.12 μg g^{-1}). The highest levels were recorded in cultures with *H. alkaliphila* (22), *A. faecalis* (116), *Roseovarius* sp. (80), and *P. flexa* (81). In line with this, *H. alkaliphila*, *A. faecalis*, and *Roseovarius* have been reported as vitamin B_{12}-producing bacteria (Nef et al., 2022; Prabaningtyas et al., 2021). As for *P. flexa*, a strain of the same genus, namely *Priestia megaterium*, has often been described as a natural cobalamin producer (Biedendieck et al., 2021).

No significant differences among co-cultivations were detected in the total concentrations of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (Table 2), except for significantly lower SFA content in co-cultures with *P. maritima* (132). However, analysis of the fatty acid profile (Supplementary material; Table S3) revealed a significant reduction in C20:4(*n*-6) in the co-culture with *Microbacterium* sp. (78) and lower levels of C16:0 and C22:0 in cultures with *P. maritima* (132). In similar studies, co-cultures of *I. galbana* with *Marinobacter* sp. had also presented significant decrease in the concentration of C22:0, when compared to the axenic microalga or with pure bacterial culture. In addition, lower absolute values of C16:0 and C20:4 (n-6) were also observed in the same cultures, possibly suggesting that the co-culture of microalgae with bacteria can be associated with a decrease in production of specific fatty acids (Griffiths et al., 2011).

In the pigment profile, all co-cultures presented lower fucoxanthin and lutein content in comparison to the control, where *H. titanicae* (60) and *Cyclobacterium* sp. (75) presented a significantly lower content of fucoxanthin, and *Marinobacter* sp. (8), *H. alkaliphila* (22), *H. titanicae* (60), *P. flexa* (81), and *Methylophaga* sp. (99) presented a significantly lower lutein content (Table 2). Although the exact cause cannot be determined from our dataset, similar reductions in lutein and β-carotene have been reported in other microalgae under changes in light acclimation, oxidative balance or microbial interaction, suggesting that the patterns observed here may reflect a comparable physiological

Table 2
Biochemical composition of *Tisochrysis lutea* biomass collected from the culture inoculum start (Day 0) and end (Day 8) of Phase 2 in cultures inoculated with 15 selected bacterial isolates (7 to 132) or without added bacteria (CTRL). The table includes concentrations of protein, fucoxanthin, lutein, β-carotene (all in mg g^{-1}), saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (mg g^{-1}), total fatty acids (Total FA, mg g^{-1}), and vitamin B_{12} (methylcobalamin (MeCbl, μg g^{-1}). Total FA values correspond to the sum of the individual fatty acid fractions (SFA + MUFA +PUFA). All values are expressed as mean ± SD ($n = 2$), except for isolate 80 ($n = 1$). Values marked with one or more asterisks (*) indicate statistically significant differences from the CTRL (*Kruskal-Wallis test with Dunn's multiple comparison test*, *: $p < 0.05$, **: $p < 0.01$).

	Protein (mg g^{-1})			Fucoxanthin (mg g^{-1})			Lutein (mg g^{-1})			β-Carotene (mg g^{-1})			SFA (mg g^{-1})			MUFA (mg g^{-1})			PUFA (mg g^{-1})			Total FA (mg g^{-1})			MeCbl (μg g^{-1})
Inoculum	206.92	±	5.07	5.75	±	1.14	0.14	±	0.02**	3.52	±	1.18	11.98	±	0.33	7.20	±	2.53*	33.20	±	1.77	52.39	±	4.63	6.15
CTRL	239.03	±	1.71	7.87	±	2.22	2.04	±	0.14	3.17	±	0.43	37.06	±	9.83	37.68	±	7.61	63.39	±	17.13	138.13	±	34.57	8.12
7	240.01	±	0.14	4.31	±	0.65	1.54	±	0.29	2.22	±	0.43	26.78	±	0.29	26.35	±	0.35	42.33	±	2.34	95.46	±	1.69	9.42
8	244.89	±	0.69	4.57	±	0.17	1.57	±	0.06*	2.18	±	0.07	29.54	±	1.39	30.00	±	1.39	48.90	±	4.58	108.44	±	7.36	10.19
22	230.81	±	7.75	3.97	±	0.35	1.33	±	0.09*	1.89	±	0.14	23.24	±	1.95	21.42	±	2.77	34.11	±	5.21	78.77	±	9.93	12.76
47	256.41	±	0.47	4.18	±	0.24	1.43	±	0.12	2.01	±	0.27	21.55	±	7.56	19.31	±	7.47	32.56	±	14.01	73.42	±	29.03	5.96
60	246.52	±	13.39	3.52	±	0.08*	1.22	±	0.08**	1.65	±	0.09	22.30	±	1.56	20.66	±	3.14	32.59	±	2.27	75.55	±	6.97	6.27
75	243.61	±	32.78	3.63	±	0.43*	1.27	±	0.24	1.62	±	0.27	24.29	±	0.02	23.05	±	0.95	34.07	±	0.70	81.41	±	1.67	5.90
78	261.09	±	1.52	3.83	±	0.55	1.25	±	0.17	1.74	±	0.30	17.67	±	0.24	17.80	±	0.59	28.57	±	1.97	64.04	±	2.80	8.39
80	270.86			5.94			0.93			1.94			7.26			6.41			9.60			23.26			11.46
81	269.87	±	10.21	5.06	±	0.06	0.92	±	0.04*	1.61	±	0.06	15.16	±	3.03	12.44	±	2.45	21.40	±	4.54	49.00	±	10.01	11.50
84	243.21	±	9.42	4.43	±	0.59	1.31	±	0.22	1.87	±	0.36	13.82	±	0.90	12.63	±	1.28	20.28	±	1.30	46.73	±	3.47	7.29
86	235.67	±	23.19	4.70	±	0.03	1.45	±	0.18	2.06	±	0.28	14.78	±	0.18	13.84	±	0.54	20.78	±	0.74	49.40	±	1.46	7.20
99	270.53	±	4.13	5.73	±	0.31	1.04	±	0.07*	2.16	±	0.06	12.11	±	0.74	10.55	±	0.58	17.90	±	1.74	40.56	±	3.06	7.22
109	239.04	±	38.26	3.90	±	0.65	1.29	±	0.10	1.72	±	0.10	12.45	±	0.14	11.57	±	0.74	16.50	±	0.36	40.52	±	1.24	9.29
116	284.29	±	55.44	4.21	±	3.43	1.25			1.24	±	1.13	12.36	±	1.12	10.54	±	2.40	12.82	±	11.33	35.72	±	14.84	14.75
132	226.16	±	1.37	4.10	±	0.07	1.32	±	0.07	1.96	±	0.15	11.27	±	0.01*	10.20	±	0.03	14.75	±	1.10	36.21	±	1.12*	8.61

adjustment (Bonnefond et al., 2022; Fu et al., 2016; Haghjou et al., 2009).

Based on the growth, biochemical, and taxonomic data, seven strains were selected for the design of tailor-made bacterial blends: *M. nauticus* (7), *Marinobacter* sp. (8), *Microbacterium* sp. (78), *Roseovarius* sp. (80), *H. salexigens* (84), *A. faecalis* (116) and *P. maritima* (132). With the selected bacteria phylogenetically related to taxa known to promote microalgal growth and metabolite production, members of the *Marinobacter* and *Halomonas* genera and the *Roseobacter* clade are often associated with beneficial effects on microalgae, as many of these bacteria are also linked to the production of compounds and micronutrients essential for microalgae development and survival (Nef et al., 2022; Seymour et al., 2017). *Marinobacter* spp. are known to produce siderophores that chelate iron, enhancing algal growth (Amin et al., 2009). Similarly, the co-cultivation of *Halomonas aquamarina* with *N. oceanica* was shown to improve the growth and biochemical traits of the microalga (Subasankari et al., 2020).

A. faecalis is commonly found in soil, water, and aquatic systems and is generally considered non-pathogenic (Batt, 2014). Its presence in algal-bacterial consortia is well documented, particularly in bioremediation and wastewater treatment (Tamer et al., 2006). Moreover, *A. faecalis* is a recognized vitamin B_{12}-producing strain (Prabaningtyas et al., 2021; Zhang et al., 2016), which is consistent with the high MeCbl levels observed in our study.

Priestia sp. formerly classified as *Bacillus* (Gupta et al., 2020) has been shown to enhance biomass productivity and cell density in *Chrysotila roscoffensis* and *Phaeodactylum tricornutum*, with minimal effects on pigments or fatty acid content (Fang et al., 2024). Moreover, *Priestia* was reported as a bioflocculating bacterium (Shahadat et al., 2023), which could assist in harvesting algal biomass by lowering the volume that needs to be collected by centrifugation or other energy-intensive procedures. Finally, *F. zhejiangensis* was proposed as a new genus and species, and its potential for biotechnological applications remains largely unexplored (Zheng et al., 2015).

Co-culture with the designed bacterial combinations

Building on the findings from Phase 2 with the seven isolates identified, a total of 21 bacterial blends were generated. When co-cultured with *T. lutea*, none of these blends led to a decrease in biomass production. In fact, six blends were associated with improvements in growth (43–74 %) compared to the control, including the following combinations: *M. nauticus* (7) + *P. maritima* (132); *Marinobacter* sp. (8) + *Microbacterium* sp. (78); *Marinobacter* sp. (8) + *A. faecalis* (116); *Microbacterium* sp. (78) + *H. salexigens* (84); *Roseovarius* sp. (80) + *P. maritima* (132); *H. salexigens* (84) + *P. maritima* (132), with *H. salexigens* (84) + *P. maritima* (132) displaying significantly higher biomass production in comparison to the control (Fig. 5).

All bacterial strains that had shown a trend towards growth promotion of *T. lutea* in their previous individual co-cultivation trials were also present in these combinations, except for *A. faecalis* (116). Prior work by (Cho et al., 2015) had already demonstrated that certain bacteria (*Flavobacterium*, *Hyphomonas, Rhizobium* and *Sphingomonas*) were more effective when co-cultured as a consortium with *C. vulgaris* than when tested individually. The contrasting results observed for *A. faecalis* in isolate versus blend experiments are consistent with the possibility that bacterial behavior may vary depending on their surrounding microbial context. However, further studies are needed to evaluate context-dependent bacterial behavior to support these findings.

Interestingly, while the control cultures of *T. lutea* reached the stationary phase by day 8, cultures supplemented with bacterial blends only entered this phase by day 11. No significant differences in cell concentration were observed on day 8, but several blends showed marked improvements by day 11 (Fig. 5). The control reached a maximum cell concentration of 1.83×10^7 cells mL^{-1} at day 8, whereas by day 11, cultures with the combinations *Marinobacter* sp. (8) +

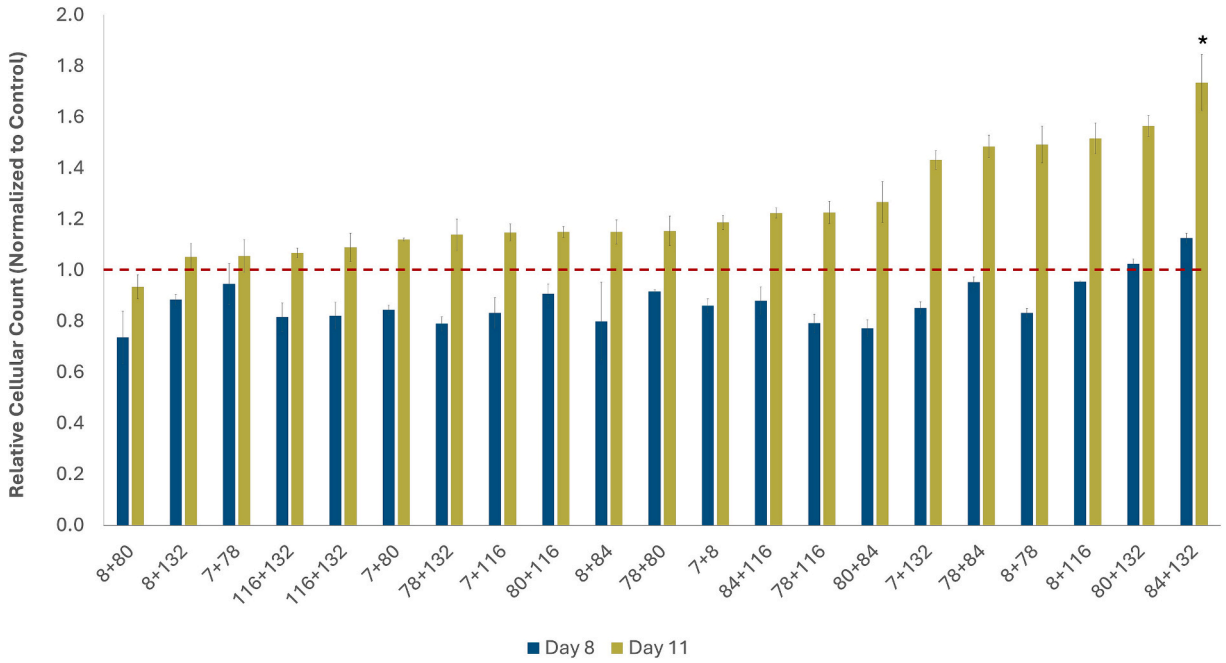

Fig. 5. Normalized cellular counts for Tisochrysis lutea in co-culture with designed bacterial mixes (from 7/8 to 116/132) on Day 8 and Day 11 of Phase 3, relative to the control without bacterial isolates. Bar graph shows the mean normalized cellular counts for each treatment group at two-time points: Day 8 (blue bars) and Day 11 (green bars). Values are normalized to the corresponding control group (CTRL) at each time point; a value of 1.0 indicates no difference from the control. The dashed horizontal line represents the control reference (CTRL = 1.0). Bars represent the mean ± standard deviation (n = 2). Values marked with one (*) indicate statistically significant differences from the CTRL (*Kruskal-Wallis test with Dunn's multiple comparison test*, *: $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Microbacterium sp. (78), *Marinobacter* sp. (8) + *A. faecalis* (116), *Microbacterium* sp. (78) + *H. salexigens* (84), *Roseovarius* sp. (80) + *P. maritima* (132), and *H. salexigens* (84) + *P. maritima* (132) reached 1.91×10^7, 1.94×10^7, 1.90×10^7, 2.00×10^7, and 2.22×10^7 cells mL^{-1}, respectively.

These results suggest that specific bacterial combinations may extend the exponential growth phase, potentially by contributing to nutrient recycling, delaying stress responses, or modulating metabolic demand, thereby sustaining algal growth over a longer period (Astafyeva et al., 2022; Natrah et al., 2014; Sauvage et al., 2022). Nonetheless, further studies are needed to identify the underlying mechanisms of these effects.

The nine best performing bacterial blends, based on growth performance, strain representation, and preliminary indications of functional interest, such as vitamin B_{12} production, were selected for biochemical characterization. As such, the biochemical results discussed below refer exclusively to these nine blends. After 11 days of co-culture there were no significant changes in protein content, even in combinations that included *Microbacterium* sp. (78; Table 3). While all samples showed higher protein content relative to day 0 (199.90 ± 3.53 mg g^{-1}) no combination exceeded the control (248.16 ± 1.03 mg g^{-1}).

Carotenoids analysis showed that co-cultures with bacterial blends generally resulted in lower lutein, α-carotene and β-carotene contents (Table 3). This reduction may reflect physiological adjustments to bacterial presence, possibly linked to resource reallocation or stress mitigation mechanisms (Berthold et al., 2019; Cho et al., 2015). However, fucoxanthin content remained stable across all treatments, including the control, which is relevant, given its commercial value and bioactive and health-promoting properties (Gao et al., 2020; Mohamadnia et al., 2021). Similarly, neoxanthin and violaxanthin levels were unaffected. Although decreases in other pigments were observed, these findings suggest that the core pigment profile was at least partially preserved despite bacterial augmentation. The lower fucoxanthin concentrations obtained, when compared to values reported in the literature, may reflect the moderate light intensity and nutrient-replete conditions used in this study, which favor biomass growth over pigment induction (Mohamadnia et al., 2021; Pereira et al., 2021). Maximal fucoxanthin production typically requires specific stress conditions or targeted enrichment strategies that were not applied in this study. In addition, several microorganisms have been associated with the production of carotenoids (Asker, 2018; Raita et al., 2023). Since *T. lutea* cultures were xenic prior to inoculation with the designed bacterial blends, it is possible that the detection of astaxanthin in the samples may have originated with the naturally occurring microbiome rather than from *T. lutea* pigment biosynthesis.

Regarding fatty acids, the complete profile for all tested bacterial blends is provided in Supplementary material (Supplementary material; Table S4). Eicosapentaenoic (EPA; C20:5n-3) and DHA (C22:6n-3) acids, two PUFAs of high relevance to aquaculture, remained unchanged. Likewise, total SFA, MUFA, and PUFA contents did not differ significantly among treatments, confirming the results observed in Phase 2, where co-culture effects were restricted to specific fatty acids rather than overall lipid fractions.

Several blends, including *Microbacterium* sp. (78) + *A. faecalis* (116), *Marinobacter* sp. (8) + *A. faecalis* (116), *Marinobacter* sp. (8) + *Microbacterium* sp. (78), and *Microbacterium* sp. (78) + *H. salexigens* (84), were linked to higher concentrations of hexadecadienoic acid (C16:2). Co-cultures combining *A. faecalis* (116) with either *Marinobacter* sp. (8) or *Microbacterium* sp. (78) also promoted higher levels of docosapentaenoic acid (C22:5; DPA), a long-chain omega-3 PUFA with recognized anti-inflammatory and cardioprotective roles, compared to control cultures without bacterial addition. Although typically less abundant than EPA or DHA in microalgae, elevated DPA levels suggest a favorable biochemical shift that may enhance the nutritional value of algal biomass for aquafeed applications (Weylandt, 2016). Also, the latter combination (78 + 116) was associated with increased concentrations of α-linolenic acid (C18:3n-3), a valuable precursor of long-chain PUFAs such as EPA, DPA, and DHA, which are essential for the survival and development of fish and other marine species, particularly during early life stages (Anderson and Ma, 2009; Nalder et al., 2015).

Table 3
Protein and carotenoid composition of *Tisochrysis lutea* biomass collected at Day 0 (inoculum) and Day 11 of Phase 3. Results from cultures inoculated with designed bacterial blends (7/132 to 84/132) or without added bacteria (CTRL). The table includes concentrations of protein, fucoxanthin, lutein, α-carotene, β-carotene, neoxanthin, violaxanthin, astaxanthin, and zeaxanthin (all in mg g^{-1}). Values are presented as means ± standard deviation (n = 2). Statistically significant differences were assessed from the CTRL (*Kruskal-Wallis test with Dunn's multiple comparison test; $p > 0.05$*).

	Protein (mg g^{-1})			Fucoxanthin (mg g^{-1})			Lutein (mg g^{-1})			α-Carotene (mg g^{-1})			β-Carotene (mg g^{-1})			Neoxanthin (mg g^{-1})			Violaxanthin (mg g^{-1})			Putative astaxanthin† (mg g^{-1})			Zeaxanthin (mg g^{-1})		
Inoculum	199.90	±	3.53	7.26	±	2.05	0.42	±	0.22	0.02	±	0.00	3.56	±	0.49	0.41	±	0.20	1.42	±	0.20	0.09	±	0.03	0.01	±	0.00
CTRL	248.16	±	1.03	9.20	±	2.46	1.32	±	0.37	0.04	±	0.01	6.41	±	1.87	0.41	±	0.11	2.25	±	0.61	0.06	±	0.02	0.04	±	0.01
7 + 132	268.45	±	6.17	7.13	±	0.35	0.64	±	0.07	0.02	±	0.00	3.57	±	0.34	0.29	±	0.01	1.48	±	0.07	0.03	±	0.00	0.02	±	0.00
8 + 78	258.74	±	16.33	7.96	±	0.04	0.64	±	0.02	0.02	±	0.00	3.38	±	0.23	0.34	±	0.03	1.72	±	0.03	0.03	±	0.00	0.02	±	0.00
8 + 116	254.27	±	2.40	6.99	±	0.18	0.64	±	0.00	0.02	±	0.01	3.40	±	0.88	0.27	±	0.02	1.55	±	0.01	0.03	±	0.00	0.03	±	0.01
78 + 84	280.53	±	1.92	7.21	±	0.61	0.60	±	0.06	0.02	±	0.00	3.75	±	0.76	0.30	±	0.02	1.71	±	0.01	0.06	±	0.03	0.03	±	0.00
78 + 116	278.87	±	7.98	6.12	±	1.71	0.44	±	0.17	0.02	±	0.01	2.98	±	1.63	0.28	±	0.11	1.37	±	0.57	0.10	±	0.08	0.03	±	0.00
80 + 84	278.41	±	11.52	6.43	±	0.04	0.55	±	0.06	0.02	±	0.00	3.06	±	0.12	0.28	±	0.01	1.39	±	0.00	0.03	±	0.00	0.02	±	0.00
80 + 132	279.72	±	5.27	7.60	±	0.55	0.69	±	0.10	0.02	±	0.00	2.68	±	0.25	0.32	±	0.01	1.66	±	0.09	0.04	±	0.00	0.02	±	0.00
84 + 116	252.35	±	4.98	6.58	±	0.16	0.46	±	0.01	0.02	±	0.00	2.30	±	0.39	0.29	±	0.02	1.48	±	0.01	0.04	±	0.01	0.03	±	0.00
84 + 132	249.99	±	20.54	7.01	±	0.40	0.63	±	0.10	0.02	±	0.00	2.60	±	0.51	0.31	±	0.04	1.49	±	0.27	0.04	±	0.01	0.02	±	0.00

†Putative identification based on retention time and absorption spectrum.

Fig. 6. Methylcobalamin concentration (µg g^{-1}) of Tisochrysis lutea from the inoculum (day 0) and end of the experiment (day 11) without (CTRL) or in co-culture with the designed bacterial mixes (7/132 to 84/132). Values represent concentration means ± SD (n = 2). Statistically significant differences were assessed from the CTRL (*Kruskal-Wallis test with Dunn's multiple comparison test; $p > 0.05$*).

Regarding the production of MeCbl (Fig. 6), three of the designed bacterial blends obtained higher amounts: *Microbacterium* sp. (78) + *A. faecalis* (116), *Roseovarius* sp. (80) + *H. salexigens* (84) and *H. salexigens* (84) + *P. maritima* (132). Among these, the combination 80 + 84 achieved the highest vitamin B_{12} concentration (20.46 ± 0.63 µg g^{-1}), showing nearly a three-fold increase compared to the control (6.76 ± 0.13 µg g^{-1}), followed by 78 + 116 blend (18.08 ± 1.04 µg g^{-1}). The lowest concentration was detected in 8 + 78 (5.90 ± 0.22 µg g^{-1}), below the T_0 baseline (6.26 ± 0.07 µg g^{-1}).

Interestingly, the blends outperformed the best-producing individual isolates (e.g., 116: 14.75 µg g^{-1}; 22: 12.76 µg g^{-1}), suggesting that cooperative metabolic interactions enhanced vitamin B_{12} accumulation beyond the levels achieved by single strains.

These results provide evidence that introducing selected bacterial blends into xenic *T. lutea* cultures may translate into meaningful gains under production relevant conditions. Using production values reported for *T. lutea* cultivated in industrial 15 m^3 tubular photobioreactors (T-PBR; Nazemi et al., 2021) the growth improvements obtained with the 80 + 84 blend would correspond to a potential 1.5-fold increase in biomass yield, going from 161 kg to 240 kg, together with a marked enrichment in vitamin B_{12} content. Such enhancements could improve process efficiency and biomass quality in commercial settings. In addition, recent studies indicate that tailored microbial consortia may reduce nutrient inputs and support more sustainable PBR operation (Maglie et al., 2021; Palacios et al., 2022; Thurn et al., 2022).

Although the production pipeline would need to incorporate bacterial fermentation, the high value of *T. lutea* indicates that the benefits could outweigh the associated costs. A techno-economic assessment would be a valuable next step to evaluate the feasibility of implementing these consortia at scale. From an applied perspective, vitamin B_{12}-enriched algal biomass is particularly relevant for aquaculture feed formulations, as many animals, especially fish and crustaceans, lack the ability to synthesize this essential nutrient or to efficiently extract it from plant-based diets (Watanabe et al., 2014). Vitamin B_{12} plays a central role in cellular metabolism, particularly in methyl group transfer reactions and DNA synthesis, and is critical for neurological function and energy production in vertebrates (Moravcová et al., 2025).

Consequently, microalgae fortified with MeCbl provide a sustainable source of B_{12} while delivering added nutritional and functional benefits, particularly relevant for antibiotic-free plant-based feeds.

In sum, the results obtained in this work support bacteria functional potential, with several combinations yielding higher MeCbl concentrations. However, improved vitamin B_{12} content did not always correspond to enhanced algal growth. For instance, the 78 + 116 and 80 + 84 blends, despite being the top vitamin B_{12} producers, had no measurable impact on *T. lutea* performance. This suggests that MeCbl accumulation alone may not be sufficient to promote growth, potentially due to strain-specific differences in nutrient exchange, metabolic compatibility, or the timing of micronutrient release (Sultana et al., 2023).

By contrast, three of the five B_{12}-enhancing combinations, *Microbacterium* sp. and *H. salexigens* (78 + 84), *Roseovarius* sp. and *P. maritima* (80 + 132) and *H. salexigens* and *P. maritima* (84 + 132), also led to significantly higher cell densities and delayed stationary phase, indicating a dual effect on growth and micronutrient enrichment. These results agree with previous co-culture assays involving *Roseovarius* sp. (80) and *H. salexigens* (84), which were present in the top-performing blends as well. In these cases, the positive correlation between growth and B_{12} concentration reinforces the hypothesis that micronutrient provisioning can contribute to algal productivity (Nef et al., 2022, 2019).

As all co-cultures were conducted using xenic *T. lutea* cultures, the observed outcomes likely reflect a combination of direct microalgal-bacterial interactions and secondary effects mediated by the native microbiota. Together, these results highlight the potential to functionally tailor bacterial consortia for specific goals, whether to improve algal productivity or enhance MeCbl and selected fatty acid content in *T. lutea*. A summary of the overall performance of the nine selected bacterial combinations is provided in Supplementary material (Fig. S1), highlighting their specific contributions to *T. lutea* growth and biomass composition.

These improvements are particularly relevant for aquafeed applications, as long-chain n-3 fatty acids such as DPA, and precursors like α-linolenic acid (C18:3n-3), contribute to fish growth, development, and immune function (Anderson and Ma, 2009; Nalder et al., 2015). Additionally, vitamin B_{12} is crucial for metabolic and neurological processes but must be obtained through diet, as fish cannot synthesize it endogenously (Moravcová et al., 2025).

Conclusions

The culturable bacterial community associated with industrial *T. lutea* cultures was diverse, and co-culture trials revealed both antagonistic and beneficial interactions. Notably, blends such as *Microbacterium* sp. (78) + *H. salexigens* (84) and *Roseovarius* sp. (80) + *P. maritima* (132) enhanced biomass yield, MeCbl and DPA levels. These findings support the use of tailored bacterial consortia to improve growth and biochemical traits in non-axenic cultures. The demonstrated increases in MeCbl indicate a feasible basis for targeted production, and scale-up experiments are already in progress to evaluate their robustness and industrial applicability in sustainable microalgae-based bioprocesses.

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.

Acknowledgements

This work was supported by the "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026), within the WP5 *Algae Vertical*, funded by the *Next Generation EU* European Fund and the Portuguese *Recovery and Resilience Plan (PRR)*, under the "Agendas for Business Innovation" incentive line (Scheme C5 – Capitalization and Business Innovation). Additional support was provided by the Portuguese Foundation for Science and Technology (FCT) through projects UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020) and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020). This study was further funded by FCT through the PhD grant SFRH/BD/140143/2018 (Tamára Santos). The authors also acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431 *(SIMBA – Sustainable Innovation of Microbiome Applications in Food Systems)*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crbiot.2025.100361.

References

Ahamed, S.A.K., Kim, J.-J., Choi, T.-O., Choi, T.-J., 2015. Growth Promotion of Pavlova viridis by Bacteria Isolated from the Microalga. J. Life Sci. 25, 568–576. https://doi.org/10.5352/JLS.2015.25.5.568.

Amavizca, E., Bashan, Y., Ryu, C.M., Farag, M.A., Bebout, B.M., De-Bashan, L.E., 2017. Enhanced performance of the microalga Chlorella sorokiniana remotely induced by the plant growth-promoting bacteria Azospirillum brasilense and Bacillus pumilus. Sci. Rep. 7. https://doi.org/10.1038/srep41310.

Amin, S.A., Green, D.H., Hart, M.C., Küpper, F.C., Sunda, W.G., Carrano, C.J., 2009. Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism. Proc. Natl. Acad. Sci. 106, 17071–17076. https://doi.org/10.1073/pnas.0905512106.

Anderson, B.M., Ma, D.W., 2009. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis. 8, 33. https://doi.org/10.1186/1476-511X-8-33.

Asker, D., 2018. High throughput screening and profiling of high-value carotenoids from a wide diversity of bacteria in surface seawater. Food Chem. 261, 103–111. https://doi.org/10.1016/j.foodchem.2018.03.109.

Astafyeva, Y., Gurschke, M., Qi, M., Bergmann, L., Indenbirken, D., de Grahl, I., Katzowitsch, E., Reumann, S., Hanelt, D., Alawi, M., Streit, W.R., Krohn, I., 2022. Microalgae and bacteria interaction—evidence for division of diligence in the Alga Microbiota. Microbiol. Spectr. 10. https://doi.org/10.1128/spectrum.00633-22.

Bannon, C., White, P.L., Rowland, E., More, K.J., Gleason, A., Roberts, M., Devred, E., Beazley, L., LaRoche, J., Bertrand, E.M., 2025. Seasonal patterns in B-vitamins and cobalamin co-limitation in the Northwest Atlantic. Limnol. Oceanogr. 70, 3370–3385. https://doi.org/10.1002/lno.70204.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol. Mol. Biol. Rev. 80, 1–43. https://doi.org/10.1128/MMBR.00019-15.

Batt, C.A., 2014. Alcaligenes, in: Encyclopedia of Food Microbiology. Elsevier, pp. 38–41. https://doi.org/10.1016/B978-0-12-384730-0.00006-9.

Berthold, D.E., Shetty, K.G., Jayachandran, K., Laughinghouse, H.D., Gantar, M., 2019. Enhancing algal biomass and lipid production through bacterial co-culture. Biomass Bioenergy 122, 280–289. https://doi.org/10.1016/j.biombioe.2019.01.033.

Biedendieck, R., Knuuti, T., Moore, S.J., Jahn, D., 2021. The "beauty in the beast"—the multiple uses of Priestia megaterium in biotechnology. Appl. Microbiol. Biotechnol. 105, 5719–5737. https://doi.org/10.1007/s00253-021-11424-6.

Bigagli, E., D'Ambrosio, M., Cinci, L., Niccolai, A., Biondi, N., Rodolfi, L., Dos Santos Nascimiento, L.B., Tredici, M.R., Luceri, C., 2021. A comparative in vitro evaluation of the anti-inflammatory effects of a Tisochrysis lutea extract and fucoxanthin. Mar. Drugs 19, 334. https://doi.org/10.3390/md19060334.

Bonnefond, H., Lie, Y., Lacour, T., Saint-Jean, B., Carrier, G., Pruvost, E., Talec, A., Bernard, O., Sciandra, A., 2022. Dynamical Darwinian selection of a more productive strain of Tisochrysis lutea. Algal Res. 65, 102743. https://doi.org/10.1016/j.algal.2022.102743.

Borges Lopes, L.D., Soares, R.C., de Freitas, R.M., Amarante, D.O., de Carvalho, F.C.T., Cavalcante, K.M., dos, S.P, de Sousa, O.V, de Menezes, F.G.R, 2025. Co-culture of microalgae and bacteria for the production of bioactive compounds. Ann Microbiol 75, 16. https://doi.org/10.1186/s13213-025-01804-y.

Cho, D.-H., Ramanan, R., Heo, J., Lee, J., Kim, B.-H., Oh, H.-M., Kim, H.-S., 2015. Enhancing microalgal biomass productivity by engineering a microalgal–bacterial community. Bioresour. Technol. 175, 578–585. https://doi.org/10.1016/j.biortech.2014.10.159.

Chua, E.T., Schenk, P.M., 2017. A biorefinery for Nannochloropsis: Induction, harvesting, and extraction of EPA-rich oil and high-value protein. Bioresour. Technol. 244, 1416–1424. https://doi.org/10.1016/j.biortech.2017.05.124.

Couso, I., Vila, M., Vigara, J., Cordero, B.F., Vargas, M.Á., Rodríguez, H., León, R., 2012. Synthesis of carotenoids and regulation of the carotenoid biosynthesis pathway in response to high light stress in the unicellular microalga *Chlamydomonas reinhardtii*. Eur. J. Phycol. 47, 223–232. https://doi.org/10.1080/09670262.2012.692816.

Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., Smith, A.G., 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438, 90–93. https://doi.org/10.1038/nature04056.

da Costa, F., Le Grand, F., Quéré, C., Bougaran, G., Cadoret, J.P., Robert, R., Soudant, P., 2017. Effects of growth phase and nitrogen limitation on biochemical composition of two strains of Tisochrysis lutea. Algal Res. 27, 177–189. https://doi.org/10.1016/j.algal.2017.09.003.

Esteves, M.V., Marques, D.M.C., de Almeida, J.D., Faria, N.T., Ferreira, F.C., 2025. Marine microalgae–microorganism co-cultures: an insight into Nannochloropsis sp., use and biotechnological applications. Foods 14, 1522. https://doi.org/10.3390/foods14091522.

Fakhimi, N., Torres, M.J., Fernández, E., Galván, A., Dubini, A., González-Ballester, D., 2024. Chlamydomonas reinhardtii and Microbacterium forte sp. nov., a mutualistic association that favors sustainable hydrogen production. Sci. Total Environ. 913, 169559. https://doi.org/10.1016/j.scitotenv.2023.169559.

Fang, J., Sun, Y., Zhu, Z., Li, J., Cheng, P., Agathos, S.N., Fu, Y., Zhang, L., Zhou, C., Han, J., 2024. Algae-bacteria consortia promotes the cell growth of marine microalgae Phaeodactylum tricornutum and Chrysotila roscoffensis. Algal Res. 80, 103535. https://doi.org/10.1016/j.algal.2024.103535.

Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., Pellegrini, M., 2022. Actinomycetes from caves: an overview of their diversity, biotechnological properties, and insights for their use in soil environments. Microorganisms 10, 453. https://doi.org/10.3390/microorganisms10020453.

Fu, K., Li, J., Wang, Y., Liu, J., Yan, H., Shi, L., Zhou, L., 2016. An innovative method for rapid identification and detection of vibrio alginolyticus in different infection models. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00651.

Fuentes, J., Garbayo, I., Cuaresma, M., Montero, Z., González-del-Valle, M., Vílchez, C., 2016. Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. Mar. Drugs 14, 100. https://doi.org/10.3390/md14050100.

Fulbright, S.P., Robbins-Pianka, A., Berg-Lyons, D., Knight, R., Reardon, K.F., Chisholm, S.T., 2018. Bacterial community changes in an industrial algae production system. Algal Res. 31, 147–156. https://doi.org/10.1016/j.algal.2017.09.010.

Gangadhar, K.N., Rodrigues, M.J., Pereira, H., Gaspar, H., Malcata, F.X., Barreira, L., Varela, J., 2020. Anti-hepatocellular carcinoma (HepG2) activities of monoterpene hydroxy lactones isolated from the marine Microalga Tisochrysis Lutea. Mar. Drugs 18, 567. https://doi.org/10.3390/md18110567.

Gao, F., Sá, M., Teles (Cabanelas, I.I., Wijffels, R.H., Barbosa, M.J., 2021. Production and monitoring of biomass and fucoxanthin with brown microalgae under outdoor conditions. Biotechnol Bioeng 118, 1355–1365. https://doi.org/10.1002/bit.27657.

Gao, F., Teles (Cabanelas), I.I., Ferrer-Ledo, N., Wijffels, R.H., Barbosa, M.J., 2020. Production and high throughput quantification of fucoxanthin and lipids in Tisochrysis lutea using single-cell fluorescence. Bioresour. Technol. 318, 124104. https://doi.org/10.1016/j.biortech.2020.124104.

Gonçalves de Oliveira-Júnior, R., Grougnet, R., Bodet, P.-E., Bonnet, A., Nicolau, E., Jebali, A., Rumin, J., Picot, L., 2020. Updated pigment composition of Tisochrysis lutea and purification of fucoxanthin using centrifugal partition chromatography coupled to flash chromatography for the chemosensitization of melanoma cells. Algal Res. 51, 102035. https://doi.org/10.1016/j.algal.2020.102035.

González-González, L.M., de-Bashan, L.E., 2021. Toward the enhancement of microalgal metabolite production through microalgae–bacteria consortia. Biology (Basel) 10, 282. https://doi.org/10.3390/biology10040282.

Griffiths, M.J., Garcin, C., van Hille, R.P., Harrison, S.T.L., 2011. Interference by pigment in the estimation of microalgal biomass concentration by optical density. J. Microbiol. Methods 85, 119–123. https://doi.org/10.1016/j.mimet.2011.02.005.

Gupta, R.S., Patel, S., Saini, N., Chen, S., 2020. Robust demarcation of 17 distinct bacillus species clades, proposed as novel Bacillaceae genera, by phylogenomics and comparative genomic analyses: description of Robertmurraya kyonggiensis sp. nov. and proposal for an emended genus bacillus limiting it only to the members of the Subtilis and Cereus clades of species. Int. J. Syst. Evol. Microbiol. 70, 5753–5798. https://doi.org/10.1099/ijsem.0.004475.

Haghjou, M.M., Shariati, M., Smirnoff, N., 2009. The effect of acute high light and low temperature stresses on the ascorbate–glutathione cycle and superoxide dismutase activity in two Dunaliella salina strains. Physiol. Plant. 135, 272–280. https://doi.org/10.1111/j.1399-3054.2008.01193.x.

Hamidi, M., Kozani, Safarzadeh, Safarzadeh Kozani, Pouya, Pierre Pooria, G, Michaud, P, Delattre, C, 2020. Marine bacteria versus microalgae: Who is the best for biotechnological production of bioactive compounds with antioxidant properties and other biological applications? Mar. Drugs. https://doi.org/10.3390/md18010028.

Hinsu, A., Dumadiya, A., Joshi, A., Kotadiya, R., Andharia, K., Koringa, P., Kothari, R., 2021. To culture or not to culture: a snapshot of culture-dependent and culture-independent bacterial diversity from peanut rhizosphere. PeerJ 9, e12035. https://doi.org/10.7717/peerj.12035.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G.A., Youssef, N.H., Zhang, X., Wei, Z., Hol, W.H.G., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. ISME J. 11, 853–862. https://doi.org/10.1038/ismej.2016.174.

Kim, H.-J., Choi, Y.-K., Jeon, H.J., Bhatia, S.K., Kim, Y.-H., Kim, Y.-G., Choi, K.Y., Kim, H. J., Lee, S.H., Lee, Y.K., Yang, Y.-H., 2015. Growth promotion of Chlorella vulgaris by modification of nitrogen source composition with symbiotic bacteria, Microbacterium sp. HJ1. Biomass Bioenergy 74, 213–219. https://doi.org/10.1016/j.biombioe.2015.01.012.

Le Chevanton, M., Garnier, M., Bougaran, G., Schreiber, N., Lukomska, E., Bérard, J.-B., Fouilland, E., Bernard, O., Cadoret, J.-P., 2013. Screening and selection of growth-promoting bacteria for Dunaliella cultures. Algal Res. 2, 212–222. https://doi.org/10.1016/j.algal.2013.05.003.

Lepage, G., Roy, C.C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification Supplementary key words triglyceride gas-liquid chromatography. J. Lipid Res. 25, 1391–1396.

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

Lian, J., Wijffels, R.H., Smidt, H., Sipkema, D., 2018. The effect of the algal microbiome on industrial production of microalgae, Microbial Biotechnology. John Wiley and Sons Ltd. https://doi.org/10.1111/1751-7915.13296

Ling, T., Zhang, Y.-F., Cao, J.-Y., Xu, J.-L., Kong, Z.-Y., Zhang, L., Liao, K., Zhou, C.-X., Yan, X.-J., 2020. Analysis of bacterial community diversity within seven bait-microalgae. Algal Res. 51, 102033. https://doi.org/10.1016/j.algal.2020.102033.

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

Liu, Y., Lai, Q., Shao, Z., Zhu, H.-H., 2019. Pseudidiomarina maritima Wu et al. 2009 is a later heterotypic synonym of Pseudidiomarina tainanensis Jean et al. 2009 and emended description of the species. Int J Syst Evol Microbiol 69, 3765–3768. https://doi.org/10.1099/ijsem.0.003678.

Lourenço, S.O., Barbarino, E., Lavín, P.L., Lanfer Marquez, U.M., Aidar, E., 2004. Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors. Eur. J. Phycol. 39, 17–32. https://doi.org/10.1080/0967026032000157156.

Macías-de la Rosa, A., López-Rosales, L., Contreras-Gómez, A., Sánchez-Mirón, A., García-Camacho, F., Cerón-García, M. del C., 2024. Salinity as an abiotic stressor for eliciting bioactive compounds in marine microalgae. Toxins (Basel) 16, 425. https://doi.org/10.3390/toxins16100425.

Maglie, M., Baldisserotto, C., Guerrini, A., Sabia, A., Ferroni, L., Pancaldi, S., 2021. A co-cultivation process of Nannochloropsis oculata and Tisochrysis lutea induces morpho-physiological and biochemical variations potentially useful for biotechnological purposes. J. Appl. Phycol. 33, 2817–2832. https://doi.org/10.1007/s10811-021-02511-2.

Miller, I.R., Bui, H., Wood, J.B., Fields, M.W., Gerlach, R., 2024. Understanding phycosomal dynamics to improve industrial microalgae cultivation. Trends Biotechnol. 42, 680–698. https://doi.org/10.1016/j.tibtech.2023.12.003.

Mohamadnia, S., Tavakoli, O., Faramarzi, M.A., 2021. Enhancing production of fucoxanthin by the optimization of culture media of the microalga Tisochrysis lutea. Aquaculture 533, 736074. https://doi.org/10.1016/j.aquaculture.2020.736074.

Moravcová, M., Siatka, T., Krčmová, L.K., Matoušová, K., Mladěnka, P., 2025. Biological properties of vitamin B 12. Nutr. Res. Rev. 38, 338–370. https://doi.org/10.1017/S0954422424000210.

Nalder, T.D., Miller, M.R., Packer, M.A., 2015. Changes in lipid class content and composition of Isochrysis sp. (T-Iso) grown in batch culture. Aquac. Int. 23, 1293–1312. https://doi.org/10.1007/s10499-015-9884-9.

Natrah, F.M.I., Bossier, P., Sorgeloos, P., Yusoff, F.M., Defoirdt, T., 2014. Significance of microalgal–bacterial interactions for aquaculture. Rev. Aquac. 6, 48–61. https://doi.org/10.1111/raq.12024.

Nazemi, F., Karimi, K., Denayer, J.F.M., Shafiei, M., 2021. Techno-economic aspects of different process approaches based on brown macroalgae feedstock: a step toward commercialization of seaweed-based biorefineries. Algal Res. 58, 102366. https://doi.org/10.1016/j.algal.2021.102366.

Nef, C., Dittami, S., Kaas, R., Briand, E., Noël, C., Mairet, F., Garnier, M., 2022. Sharing vitamin B12 between bacteria and microalgae does not systematically occur: case study of the haptophyte Tisochrysis lutea. Microorganisms 10, 1337. https://doi.org/10.3390/microorganisms10071337.

Nef, C., Jung, S., Mairet, F., Kaas, R., Grizeau, D., Garnier, M., 2019. How haptophytes microalgae mitigate vitamin B12 limitation. Sci. Rep. 9, 8417. https://doi.org/10.1038/s41598-019-44797-w.

Padmaperuma, G., Kapoore, R.V., Gilmour, D.J., Vaidyanathan, S., 2018. Microbial consortia: a critical look at microalgae co-cultures for enhanced biomanufacturing. Crit. Rev. Biotechnol. 38, 690–703. https://doi.org/10.1080/07388551.2017.1390728.

Palacios, O.A., López, B.R., de-Bashan, L.E., 2022. Microalga Growth-Promoting Bacteria (MGPB): a formal term proposed for beneficial bacteria involved in microalgal–bacterial interactions. Algal Res. 61, 102585. https://doi.org/10.1016/j.algal.2021.102585.

Pathom-aree, W., Sattayawat, P., Inwongwan, S., Cheirsilp, B., Liewtrakula, N., Maneechote, W., Rangseekaew, P., Ahmad, F., Mehmood, M.A., Gao, F., Srinuanpan, S., 2024. Microalgae growth-promoting bacteria for cultivation strategies: recent updates and progress. Microbiol. Res. 286, 127813. https://doi.org/10.1016/j.micres.2024.127813.

Pereira, H., Barreira, L., Figueiredo, F., Custódio, L., Vizetto-Duarte, C., Polo, C., Rešek, E., Engelen, A., Varela, J., 2012. Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. Mar. Drugs 10, 1920–1935. https://doi.org/10.3390/md10091920.

Pereira, H., Sá, M., Maia, I., Rodrigues, A., Teles, I., Wijffels, R.H., Navalho, J., Barbosa, M., 2021. Fucoxanthin production from Tisochrysis lutea and Phaeodactylum tricornutum at industrial scale. Algal Res. 56, 102322. https://doi.org/10.1016/j.algal.2021.102322.

Prabaningtyas, S., Ardyati, T., Suharjono, S., Retnaningdyah, C., 2021. Exploration of vitamin B12-producing bacteria from Indonesia Eutrophic Lake: a new strategy to improve microalgae biomass production. Biodiversitas 22. https://doi.org/10.13057/biodiv/d221047.

Raita, S., Feldmane, L., Kusnere, Z., Spalvins, K., Kuzmika, I., Berzina, I., Mika, T., 2023. Microbial carotenoids production: strains, conditions, and yield affecting factors. Environ. Clim. Technol. 27, 1027–1048. https://doi.org/10.2478/rtuect-2023-0075.

Ramanan, R., Kim, B.H., Cho, D.H., Oh, H.M., Kim, H.S., 2016. Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol. Adv. 34, 14–29. https://doi.org/10.1016/j.biotechadv.2015.12.003.

Sauvage, J., Wikfors, G.H., Dixon, M.S., Kapareiko, D., Sabbe, K., Li, X., Joyce, A., 2022. Bacterial exudates as growth-promoting agents for the cultivation of commercially relevant marine microalgal strains. J. World Aquac. Soc. 53, 1101–1119. https://doi.org/10.1111/jwas.12910.

Schüler, L.M., Santos, T., Pereira, H., Duarte, P., Katkam, N.G., Florindo, C., Schulze, P.S. C., Barreira, L., Varela, J.C.S., 2020. Improved production of lutein and β-carotene by thermal and light intensity upshifts in the marine microalga *Tetraselmis* sp. CTP4. Algal Res. 45, 101732. https://doi.org/10.1016/j.algal.2019.101732.

Seymour, J.R., Amin, S.A., Raina, J.B., Stocker, R., 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. Nat. Microbiol. 2, 17065.

Shahadat, H., Ahmad Shukri, Z.N., Rohisyamuddin, O., Khor, W.H., Khatoon, H., Zahidul, I., MIinhaz, T.M., Kamaruzzan, A.S., AhmadIderis, A.R., Kasan, N.A., 2023. Isolation and characterisation of bioflocculant-producing bacteria from mud crab (Scylla sp.) aquaculture ponds. J. Sustain. Sci. Manag. 18, 98–115. https://doi.org/10.46754/jssm.2023.08.007.

Subasankari, K., Thanappan, V., Jeyapragash, D., Anantharaman, P., Sarangi, R.K., 2020. Growth promoting studies on co-culturing *Nannochloropsis oceanica* with *Halomonas aquamarina* actively enhance the algal biomass and lipid production. Biocatal. Agric. Biotechnol. 29, 101790. https://doi.org/10.1016/j.bcab.2020.101790.

Sultana, S., Bruns, S., Wilkes, H., Simon, M., Wienhausen, G., 2023. Vitamin B12 is not shared by all marine prototrophic bacteria with their environment. ISME J. 17, 836–845. https://doi.org/10.1038/s41396-023-01391-3.

Tait, K., White, D.A., Kimmance, S.A., Tarran, G., Rooks, P., Jones, M., Llewellyn, C.A., 2019. Characterisation of bacteria from the cultures of a Chlorella strain isolated from textile wastewater and their growth enhancing effects on the axenic cultures of Chlorella vulgaris in low nutrient media. Algal Res. 44, 101666. https://doi.org/10.1016/j.algal.2019.101666.

Tamer, E., Amin, M.A., Ossama, E.T., Bo, M., Benoit, G., 2006. Biological treatment of industrial wastes in a photobioreactor. Water Sci. Technol. 53, 117–125. https://doi.org/10.2166/wst.2006.344.

Thurn, A.-L., Stock, A., Gerwald, S., Weuster-Botz, D., 2022. Simultaneous photoautotrophic production of DHA and EPA by Tisochrysis lutea and Microchloropsis salina in co-culture. Bioresour Bioprocess 9, 130. https://doi.org/10.1186/s40643-022-00612-5.

Tong, C.Y., Honda, K., Derek, C.J.C., 2023. A review on microalgal-bacterial co-culture: the multifaceted role of beneficial bacteria towards enhancement of microalgal metabolite production. Environ. Res. 228, 115872. https://doi.org/10.1016/j.envres.2023.115872.

Urios, L., Intertaglia, L., Lesongeur, F., Lebaron, P., 2008. Haliea salexigens gen. nov., sp. nov., a member of the Gammaproteobacteria from the Mediterranean Sea. Int. J. Syst. Evol. Microbiol. 58, 1233–1237. https://doi.org/10.1099/ijs.0.65470-0.

Watanabe, F., Yabuta, Y., Bito, T., Teng, F., 2014. Vitamin B12-containing plant food sources for vegetarians. Nutrients 6, 1861–1873. https://doi.org/10.3390/nu6051861.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Weylandt, K.-H., 2016. Docosapentaenoic acid derived metabolites and mediators – the new world of lipid mediator medicine in a nutshell. Eur. J. Pharmacol. 785, 108–115. https://doi.org/10.1016/j.ejphar.2015.11.002.

Wienhausen, G., Moraru, C., Bruns, S., Tran, D.Q., Sultana, S., Wilkes, H., Dlugosch, L., Azam, F., Simon, M., 2024. Ligand cross-feeding resolves bacterial vitamin B12 auxotrophies. Nature 629, 886–892. https://doi.org/10.1038/s41586-024-07396-y.

Wu, Y.-H., Shen, Y.-Q., Xu, X.-W., Wang, C.-S., Oren, A., Wu, M., 2009. Pseudidiomarina donghaiensis sp. nov. and Pseudidiomarina maritima sp. nov., isolated from the East China Sea. Int. J. Syst. Evol. Microbiol. 59, 1321–1325. https://doi.org/10.1099/ijs.0.005702-0.

Yang, Q., Jiang, Z., Zhou, X., Zhang, R., Xie, Z., Zhang, S., Wu, Y., Ge, Y., Zhang, X., 2020. Haliea alexandrii sp. nov., isolated from phycosphere microbiota of the toxin-producing dinoflagellate Alexandrium catenella. Int. J. Syst. Evol. Microbiol. 70, 1133–1138. https://doi.org/10.1099/ijsem.0.003890.

Yao, S., Lyu, S., An, Y., Lu, J., Gjermansen, C., Schramm, A., 2019. Microalgae-bacteria symbiosis in microalgal growth and biofuel production: a review. J. Appl. Microbiol. 126, 359–368. https://doi.org/10.1111/jam.14095.

Zhang, Y.-B., Zhou, J., Xu, Q.-M., Cheng, J.-S., Luo, Y.-L., Yuan, Y.-J., 2016. Exogenous cofactors for the improvement of bioremoval and biotransformation of sulfamethoxazole by Alcaligenes faecalis. Sci. Total Environ. 565, 547–556. https://doi.org/10.1016/j.scitotenv.2016.05.063.

Zheng, G., Chen, Z.-G., Jiang, R.-J., Yang, Z.-J., 2015. Feifantangia zhejiangensis gen. nov., sp. nov., a marine bacterium isolated from seawater of the East China Sea. Antonie Van Leeuwenhoek 108, 1441–1447. https://doi.org/10.1007/s10482-015-0598-9.