

Vitamin B₁₂-associated interactions between *Mesorhizobium* sp. TaiHu and *Synechococcus* sp. PCC 7002 revealed by multi-omics analysis

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Abstract

The marine cyanobacterium *Synechococcus* sp. PCC 7002 (Syn7002) is a model organism that lacks the gene cluster required for vitamin B₁₂ biosynthesis, necessitating cooperative interactions with other microbes. In this study, we established a synthetic microbial consortium by co-culturing Syn7002 with a bloom-forming *Microcystis* community, followed by purification, and subsequently investigated the interactions between Syn7002 and the associated microbial community. Electron microscopy revealed numerous rod-shaped bacteria clustered around Syn7002 cells, indicating close spatial associations between species. Metagenomic analysis showed that the early-stage community consisted mainly of Syn7002, *Mesorhizobium* sp. TaiHu (MesTH) and *Pseudomonas* sp. TaiHu (PseTH), although the abundance of PseTH declined after community stabilization. Investigation of vitamin B₁₂ regulation between MesTH and Syn7002 through metatranscriptomic analysis revealed upregulation of nitrogen metabolism-related genes in the microbial community. Transcriptomic data further indicated that vitamin B₁₂ biosynthesis and transport genes were significantly upregulated in MesTH. Combined with vitamin B₁₂-positive control experiments, these results confirm potential vitamin B₁₂ complementarity between the two strains. The results further suggest that MesTH promotes the growth of Syn7002 in the community by providing the small amount of vitamin B₁₂ needed for its growth. These findings provide new insights into vitamin-mediated microbial interactions and reveal additional transcriptional features of the synthetic community.

Impact Statement

This study establishes a synthetic community centred on the model cyanobacterium *Synechococcus* sp. PCC 7002 and describes the association of *Mesorhizobium* sp. TaiHu with vitamin B₁₂, which is linked to Syn7002's growth. Our findings describe microbial interactions associated with vitamin B₁₂ within the community and report changes in the expression of nitrogen-related genes during community formation. These insights advance our understanding of the complex dynamics of vitamin-mediated associations in controlled settings, thereby providing a reference for untangling the multifaceted relationships in natural environments.

DATA AVAILABILITY

Metatranscriptome and metagenome data supporting this study have been deposited in the National Center for Biotechnology Information under accession numbers PRJNA1261236 (BioProject) and PRJNA1264977, respectively.

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Abbreviations: MesTH, *Mesorhizobium* sp. TaiHu; PseTH, *Pseudomonas* sp. TaiHu; SEM, scanning electron microscopic; Syn7002, *Synechococcus* sp. PCC 7002.

All supporting data, code and protocols have been provided within the article or through supplementary data files. A supplementary table is available with the online version of this article.

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INTRODUCTION

As ancient prokaryotic autotrophs, cyanobacteria have a long evolutionary history and have made profound contributions to oxygen production as photosynthetic micro-organisms [1]. Beyond their global ecological role, cyanobacteria also engage in close interactions with surrounding microbial communities in the phycosphere, where they release abundant organic carbon that supports bacterial growth, while bacterial metabolites, in turn, promote cyanobacterial development [2–4]. Cyanobacteria influence microbial community formation through nitrogen fixation and by producing algal toxins that selectively impact associated micro-organisms [5]. In addition, many cyanobacterial metabolites exhibit antiviral, antibacterial and antifungal activities, providing an effective means to characterize the compositional dynamics of algal-associated microbial communities [6]. Under nutrient-sufficient conditions, cyanobacteria may further display antagonistic and competitive traits within microbial communities [7]. Importantly, harmful cyanobacterial blooms are frequently associated with marked shifts in these microbial interactions, often accompanied by increases in the abundance of taxa such as *Proteobacteria*, *Actinobacteria* and *Firmicutes* [8–10]. Understanding the ecological roles and interactions of these micro-organisms is therefore critical for elucidating cyanobacterial bloom dynamics. However, *in situ* approaches, such as metagenomics, face considerable challenges in characterizing interactions between specific microbial groups and algae. These challenges arise from both the complexity of natural environments and methodological limitations, including sequencing depth, sample heterogeneity and DNA extraction efficiency. Microbial co-cultivation in the laboratory offers new perspectives for investigating microbial metabolism [11]. However, the interactions between *Synechococcus* sp. PCC 7002 (Syn7002) and its associated microbial community have not yet been elucidated, and laboratory-based cultivation offers a more controlled and reliable framework to investigate these microbial interactions.

Syn7002 belongs to the phylum of *Cyanobacteria*, has more attention due to its rapid growth and is amenable to genetic engineering [12]. Additionally, as a marine microalga, Syn7002 contributes significantly to the global carbon cycle and functions as a key primary producer through carbon fixation [13]. However, Syn7002 lacks a complete metabolic pathway and requires supplemental vitamin B₁₂ under laboratory conditions [14]. Numerous studies have shown that vitamin B₁₂ is essential for many marine bacteria and unicellular eukaryotes [15, 16]. In natural environments, Syn7002 relies on metabolic complementarity with other micro-organisms within the global ocean microbial community. Metagenomics research provides many genomic insights that facilitate the cultivation of previously unculturable micro-organisms, thereby opening up potential avenues for the utilization of microbial resources [17]. *Mesorhizobium* sp. TaiHu (MesTH), a member of nitrogen-fixing bacteria, has attracted considerable attention for its ability to fix atmospheric nitrogen. During the formation of a stable microbial community in this study, MesTH maintained a relatively high abundance, and we subsequently isolated and purified this strain. This study highlights the necessity of systematic laboratory investigations to elucidate microbial community dynamics, including processes of isolation, purification, and interaction with others.

This study was based on prior laboratory research [14] and employed metagenomic and metatranscriptomic analyses of synthetic microbial communities to investigate the microbial interaction mechanisms during their formation. Successive streak-plate purifications clarified and preserved the inherent stable relationship between Syn7002 and MesTH, while the abundance of *Pseudomonas aeruginosa* sp. TaiHu (PseTH) steadily declined. Notably, Syn7002 also formed a stable community with the epibiotic bacteria of *Microcystis*. The results indicate that MesTH ensures Syn7002 survival by supplying vitamin B₁₂ in the formed microbial community, and electron microscopy additionally revealed a physical association between the two strains. These findings reveal potential metabolic interactions between micro-organisms under controlled laboratory conditions, thereby providing both a basis and a perspective for future research on microbial community interactions in complex field environments.

METHODS

Sampling

Syn7002, stored in our laboratory and purified through three rounds, was cultivated in vitamin B₁₂-supplemented BG11 medium at 28 °C under a light intensity of 70 μmol m⁻² s⁻¹ with shaking at 180 r.p.m. Syn7002 was co-cultured with algal sediment collected from a cyanobacterial bloom in Lake TaiHu, with the culture adjusted to a final volume of 50 ml. After establishing the synthetic community, the salinity gradient was gradually increased, leading to the disappearance of *Microcystis* and the stabilization of a community consisting of Syn7002 and *Microcystis*-associated epibionts. The community was then streak-purified for three successive generations on LB (Luria-Bertani, LB) medium, sampled for metagenomic sequencing to determine its composition and subsequently subjected to an additional three rounds of streak purification followed by metagenomic sequencing. Finally, a synthetic community was constructed using Syn7002 and MesTH to investigate their potential interactions. MesTH was purified using the streak plate method on LB medium, after which it was cultured in LB medium. Both MesTH and Syn7002 were then washed with vitamin B₁₂-free BG11. For the Syn7002+vitamin B₁₂ group, vitamin B₁₂ (4 μg l⁻¹) was supplemented. The Syn7002+MesTH group involved combining 50 ml of the algal suspension with an equal volume of MesTH. For metatranscriptomic sequencing, 50 ml of culture was collected on filters at 0, 1, 5, 10 and 20 days (designated as T0, T1, T2, T3 and T4, respectively). Culture growth was monitored by measuring absorbance every 2 days. The 0.2 μm filter (Millipore, USA) was used

to collect samples and stored at -80°C until used. For the targeted metabolomics assay of B vitamins, bacterial colonies from day 20 post-community stabilization were harvested by centrifugation, and the pellet was collected.

Bacteria isolation and identification

The culture from the stable culture was streaked onto LB medium to isolate a single colony (MesTH and PseTH), and this process was repeated for three generations. The full-length 16S rRNA sequence was amplified using primers 27F (5'-AGAGTTTGATC-CTGGCTCAG-3') and 1492R (5'-GGTACCTTGTTACGACTT-3') after culture at 28°C , 180 r.p.m. We analysed the sequences online using BLAST (<https://www.ncbi.nlm.nih.gov/>) to obtain species information.

DNA extraction and sequencing

Microbial DNA was extracted by the cetyltrimethylammonium bromide kit method (Noblerlyder, China) following the manufacturer's protocol. The purity and integrity of DNA were assessed using a NanoDrop 2000 spectrophotometer and 1% agarose gel electrophoresis, respectively. The sequencing library was constructed using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA) according to the manufacturer's instructions. Paired-end sequencing (2×250 bp) was performed on the Illumina NovaSeq 6000 platform to analyse the micro-organisms in different groups.

RNA extraction and sequencing

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). The RNA quantification was evaluated by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies). The VAHTS Universal V6 RNA-seq Library Prep kit (Vazyme, China) was used for library construction, following the manufacturer's instructions. Paired-end sequencing (2×250 bp) of micro-organisms from different groups was performed on the Illumina NovaSeq 6000 platform by Novogene Biotechnology Co., Ltd. (Beijing, China).

Metagenome assembly and binning

FastQC (version 0.11.9) [18] and Trimmomatic (version 0.39) [19] were used for quality control until the quality was above Q20. Megahit (version 1.2.9) [20] and the Gaussian model approach as described by Ren *et al.* [21] were used to obtain high-quality binning results. Prokka (version 1.14.5) [22] and CheckM (version 1.2.3) [23] were used for bin annotation, contamination and completeness evaluation. Identification of vitamin B₁₂ biosynthetic and transport pathways was performed by aligning the protein sequences of annotated genes against a custom bacterial vitamin B₁₂ database, using alignment thresholds of 60% for both per cent identity and query coverage. The gene names for vitamin B₁₂ were referenced to the KEGG and MetaCyc [24] databases.

Metatranscriptome analysis

Quality control is conducted as part of metagenomic processing (see section Metagenome assembly and binning). SortMeRNA (version 4.3.7) [25] was used to remove rRNA from the data. The STAR software (version 2.7.11b) [26] maps clean reads back to the metagenome, while the featureCounts software (version 2.0.3) [27] is used to quantify a trend, and cluster analyses were performed using the Mfuzz (version 2.66) R package [28]. The log₂(fold change) and adjusted *P*-values (FDR) for all genes were calculated using the DESeq2 package (version 1.0.4) [29].

Targeted metabolomic analysis of B vitamins

For targeted metabolomic analysis of B vitamins, microbial cell pellets were collected by centrifugation. Approximately 40 mg of sample (wet weight) was accurately weighed and subjected to extraction. The metabolites were extracted with 500 µl of 50% methanol aqueous solution by vortexing for 1 min in the dark, followed by ultrasonication in an ice-water bath for 30 s. After centrifugation (13,000 r.p.m., 5 min, 4°C), the supernatant was collected. The residue was re-extracted twice with 100 µl of pure methanol, and all supernatants were combined. The combined extract was dried under a gentle stream of nitrogen, pre-frozen and then lyophilized. The dried material was reconstituted in 200 µl of ultrapure water, vortexed and centrifuged, and the final supernatant was filtered through a 0.22 µm aqueous-phase syringe filter prior to analysis. The extracts were analysed for specific B vitamins, including riboflavin (vitamin B₂), pantothenic acid (vitamin B₅) and cobalamin (vitamin B₁₂), using LC-MS/MS (Agilent 1200 HPLC coupled to an API 4000 mass spectrometer). Data processing and peak integration were conducted using Analyst Software (version 1.6).

SEM analysis

Scanning electron microscopic (SEM) observation of the location of Syn7002 cells and bacteria. The logarithmic growth phase of the Syn7002 culture was centrifuged at 8,000 r.p.m. for 10 min. The sediment was washed twice with PBS, then fixed in 2.5% glutaraldehyde overnight. Subsequently, the sample was dehydrated in a graded ethanol series for 10 min at each step, coated with gold, and then examined by scanning electron microscopy. SEM under the two experimental conditions was carried out at the

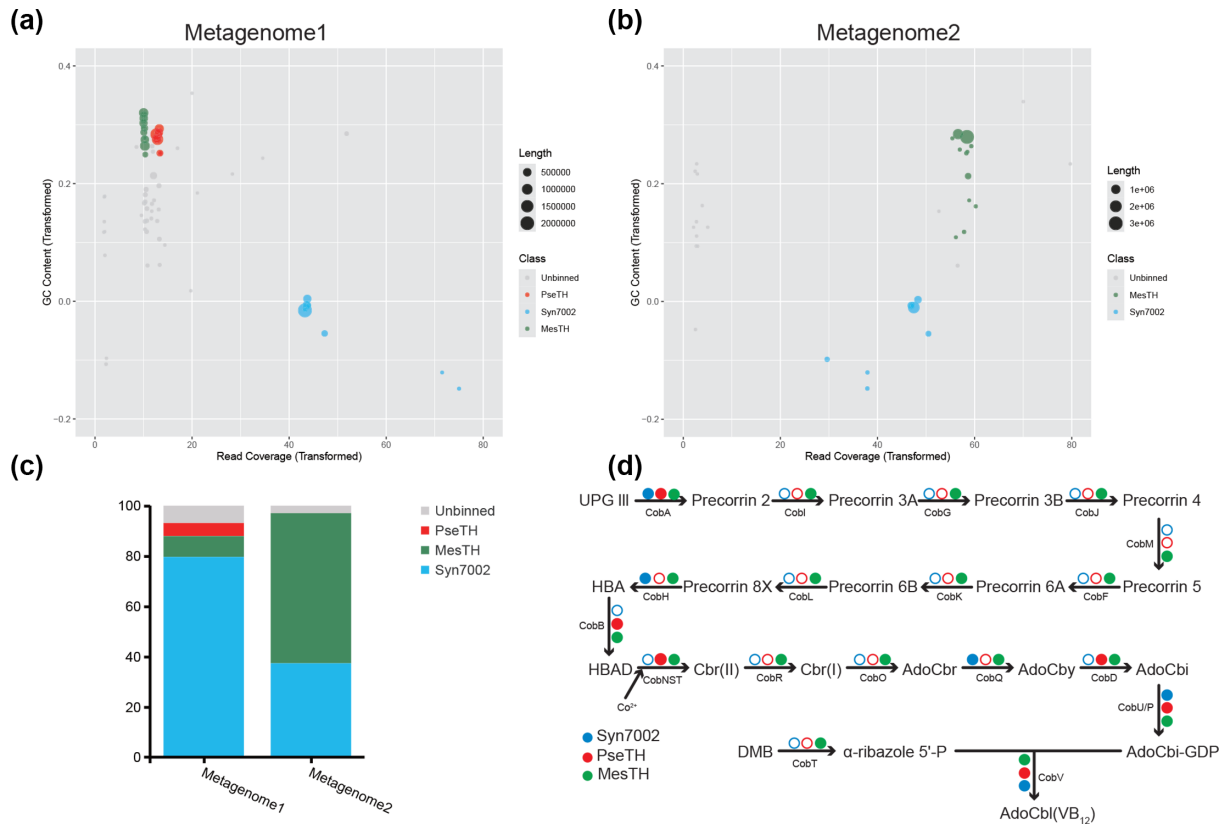


Fig. 1. Dynamic changes in the composition of the synthetic microbial community. (a) Microbial composition derived from metagenomic sequencing of the first three consecutive generations of streak-plate culture and (b) from the second three consecutive generations of streak-plate purification culture. (c) Changes in microbial relative abundance during the assembly of the synthetic community. (d) Schematic representation of the presence or absence of vitamin B₁₂ biosynthesis-related genes in Syn7002, PseTH and MesTH at the genomic level.

Wuhan Institute of Virology, Chinese Academy of Sciences (Wuhan, China). Sample preparation for SEM followed a previously described protocol [30].

RESULTS

Syn7002 and MesTH establish a stable community

Synthetic communities simplify the process of elucidating microbial relationships, while consecutive purifications during co-culture under a gradually increasing salinity gradient minimize weak associations and reduce interference from non-focal species. Metagenomic sequencing of the first three consecutive generations of streak-plate culture showed that Syn7002, PseTH and MesTH constituted the major components of the community (Fig. 1a). As the community progressed through the second set of three consecutive generations of streak-plate purification, the relative abundance of PseTH declined (Fig. 1b). Syn7002 exhibited the highest relative abundance after the first round of purification, whereas MesTH became the dominant component after the second round, indicating that the community reached stability at this stage (Fig. 1c). Genomic analysis further revealed that these three highly abundant microbial species collectively formed a complete vitamin B₁₂ biosynthesis pathway, with MesTH exhibiting a relatively high level of involvement based on both the presence of biosynthetic genes and its relative abundance (Fig. 1d).

Vitamin B₁₂ as a potential link between MesTH and Syn7002

To further investigate the ecological niche and functional role within the synthetic community stably co-cultured with Syn7002, we established a vitamin B₁₂-supplemented group serving as a positive control (Fig. 2a). The results revealed that the presence of MesTH promoted the growth of Syn7002 compared to the group without vitamin B₁₂ supplementation (Fig. 2b, c) and reduced the extinction of Syn7002 caused by vitamin B₁₂ deficiency. Scanning electron microscopy revealed the presence of numerous rod-shaped bacteria clustered around Syn7002 cells within the synthetic community, where MesTH exhibits a rod-shaped morphology, suggesting a potential interaction between Syn7002 and MesTH (Fig. 2d).

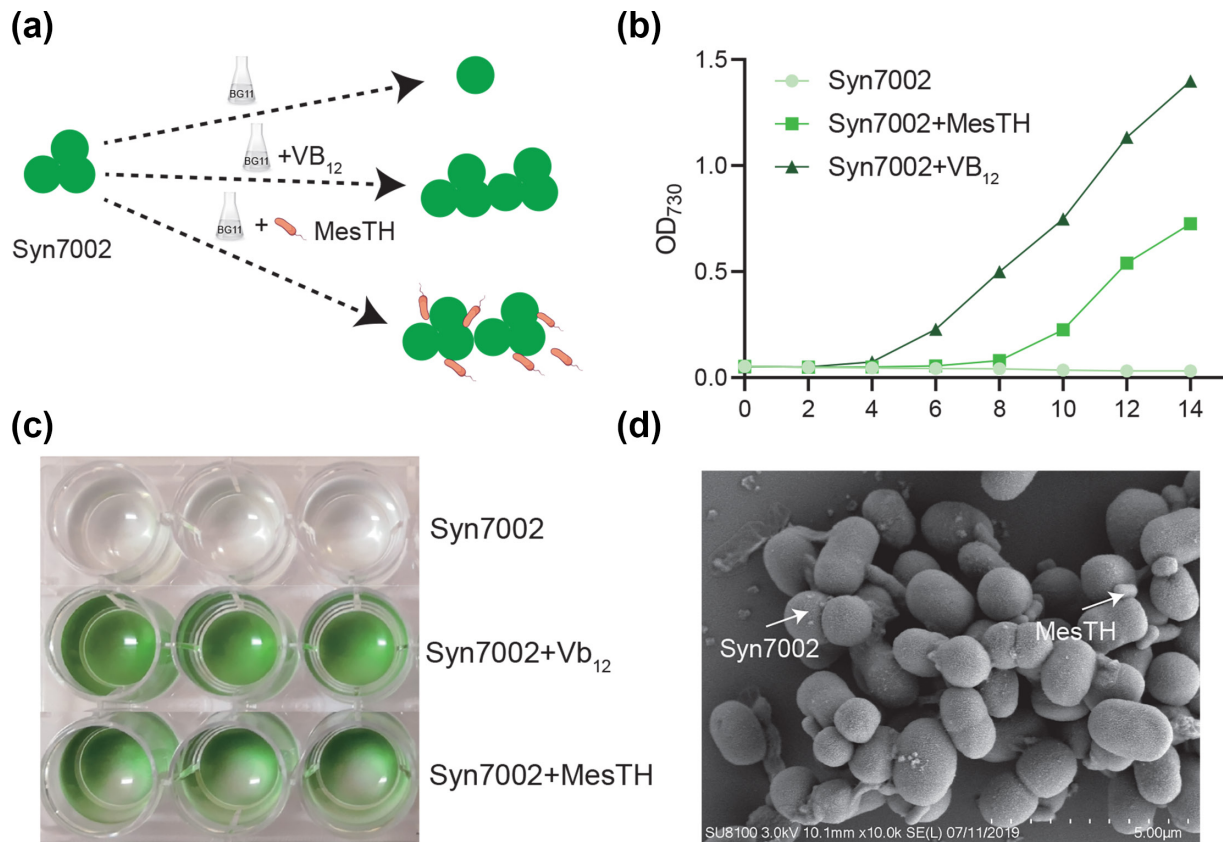


Fig. 2. MesTH and vitamin B₁₂ enhance the growth of Syn7002. (a) The schematic diagram of experimental design. (b, c) Changes in algae growth status and absorbance values under different treatments. (d) Relative position of Syn7002 and MesTH in culture.

Transporters and nitrogen metabolism sustain Syn7002

Metatranscriptomics was employed to further investigate the gene expression patterns within the synthetic community. The expression patterns of the top 200 most variable genes were largely consistent among replicates within each time point (Fig. 3a). The overall expression trend indicated coordinated transcriptional changes in genes related to ABC transporters, nitrogen metabolism and biofilm-associated processes during the early stage of the microbial community (Fig. 3b). Transcriptome trend and cluster analysis of MesTH revealed that some ABC transporters' activity and nitrogen metabolism increased during the middle stage but declined in the later stages of community formation. In Syn7002, ABC transporter activity was elevated during both the middle and late stages (Fig. 3b). In conclusion, the observed transcriptional patterns suggest a potential association between nitrogen metabolism-related gene expression in MesTH and cyanobacteria and biofilm-associated processes during the middle stages of colony development.

Expression of vitamin B₁₂ synthesis and transporter genes

To illustrate the changes in vitamin B₁₂ biosynthesis and transporter genes during the establishment of stable communities, we demonstrated the expression profiles of these genes in both MesTH and Syn7002 via our metatranscriptomic data. Both vitamin B₁₂ biosynthesis and transport-related genes were more highly expressed in MesTH compared to Syn7002 at all stages during community formation, relative to the initial stage of community assembly (Fig. 4). In contrast, Syn7002 exhibited low-level expression of vitamin B₁₂ transporter genes, indicating that this low level of vitamin B₁₂ transport is sufficient to sustain its survival.

Nitrogen metabolism genes in Syn7002 were significantly upregulated

To investigate gene expression changes and identify significant DEGs in the synthetic community composed of Syn7002, we compared each stage of stable microbial community formation to the initial time point. We assigned unannotated genes and pseudogenes a systematic identifier using the genome name plus a serial number. In the early stage following MesTH introduction, genes associated with autotrophy, such as *acsF*, were notably upregulated (Fig. 5). During the mid-stage of community formation,

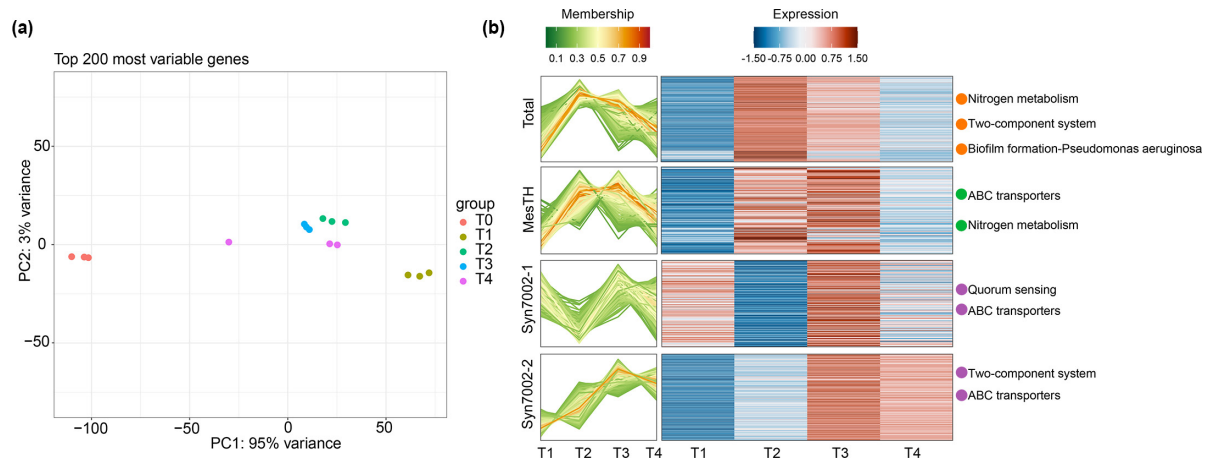


Fig. 3. Integrated transcriptome analysis reveals community-wide gene expression dynamics during synthetic microbiome assembly. Metatranscriptomic samples were collected at five time points (T0–T4), corresponding to days 0, 1, 5, 10 and 20, respectively. (a) Analysis of gene expression levels (based on FPKM values) among samples and groups of the top 200 most variable genes. (b) The analysis focused on gene sets associated with community-related microbial activities. Gene expression and cluster analysis (using FPKM values) were performed for the synthetic microbial community (747 genes), MesTH (365 genes) and two major co-expressed clusters within Syn7002 designated as Syn7002-1 (316 genes) and Syn7002-2 (620 genes).

genes involved in nitrogen metabolism, including *nrtB* and *nitT*, showed significant upregulation. In the later stage, genes related to stress responses (*rpoE*) and biofilm formation (*pilA*) were predominantly upregulated.

MesTH promotes the survival of Syn7002 via vitamin B in the synthetic community

Based on our overall findings, we propose a model in which co-cultivation of natural *Microcystis*-dominated communities with Syn7002 leads to gradual shifts in microbial community composition (Fig. 6). Integration of metatranscriptomic data across community assembly with genomic information from these isolates suggests that Syn7002 and MesTH may be associated through vitamin B₁₂ processes. Notably, targeted metabolomics detected no free vitamin B₁₂ in the pelleted cultures (Table S1, available in the online Supplementary Material), which could indicate its rapid uptake and utilization. Concurrently, genes related to protein synthesis exhibited transcriptional changes during the stages of community formation, which may reflect potential active protein synthetic activity.

DISCUSSION

Syn7002 is a marine cyanobacterium characterized by phototolerance, halotolerance and a high amenability to genetic engineering, but it lacks a complete gene cluster for vitamin B₁₂ biosynthesis. This study employed a microbial community of *Microcystis* from a eutrophic lake to establish an artificial synthetic consortium with Syn7002, aiming to investigate changes in community composition and gene expression patterns during the stabilization of Syn7002 within the consortium. Transcriptomic analysis showed that genes related to vitamin B₁₂ biosynthesis and transport, as well as nitrogen metabolism in MesTH, exhibited altered expression patterns during community formation.

Although many marine micro-organisms require vitamin B₁₂, they lack a complete biosynthetic gene cluster for its production, thereby depending on interactions with other members of the microbial community [31]. In the marine ecosystem, specific prokaryotes including archaea are capable of producing vitamin B₁₂ [32]. Vitamin B₁₂ functions as a key intermediary between micro-organisms and is an essential substrate for methionine biosynthesis, thus playing a critical role in cellular metabolism. Additionally, vitamin B₁₂ enhances the resilience of micro-organisms to low-temperature stress and facilitates the establishment of inter-microbial interactions [33, 34]. Many studies have shown that the genomes of the associated microbial communities encode a complete B₁₂ synthesis pathway, leading to enhanced B₁₂ production and simultaneously promoting algal growth [35–37]. *Microcystis* is a common bloom-forming cyanobacterium that disrupts freshwater ecosystems and is frequently found in eutrophic lakes [38]. Moreover, an indoor study has revealed the presence of associated bacteria within such blooms, including *Ralstonia pickettii* [39]. Li et al. used metagenomics to demonstrate that other micro-organisms within the *Microcystis* community promote the growth of *Microcystis* through vitamin B₁₂ [40]. However, due to the complexity of field environments, current metagenomic research methods are limited. Salinity is an important factor influencing the structure of algae-associated bacterial communities. Previous studies have shown that salinity gradients can significantly affect the richness and composition of epibacterial communities associated with macroalgae [41, 42]. In this study, a gradual increase in salinity during co-cultivation was used to

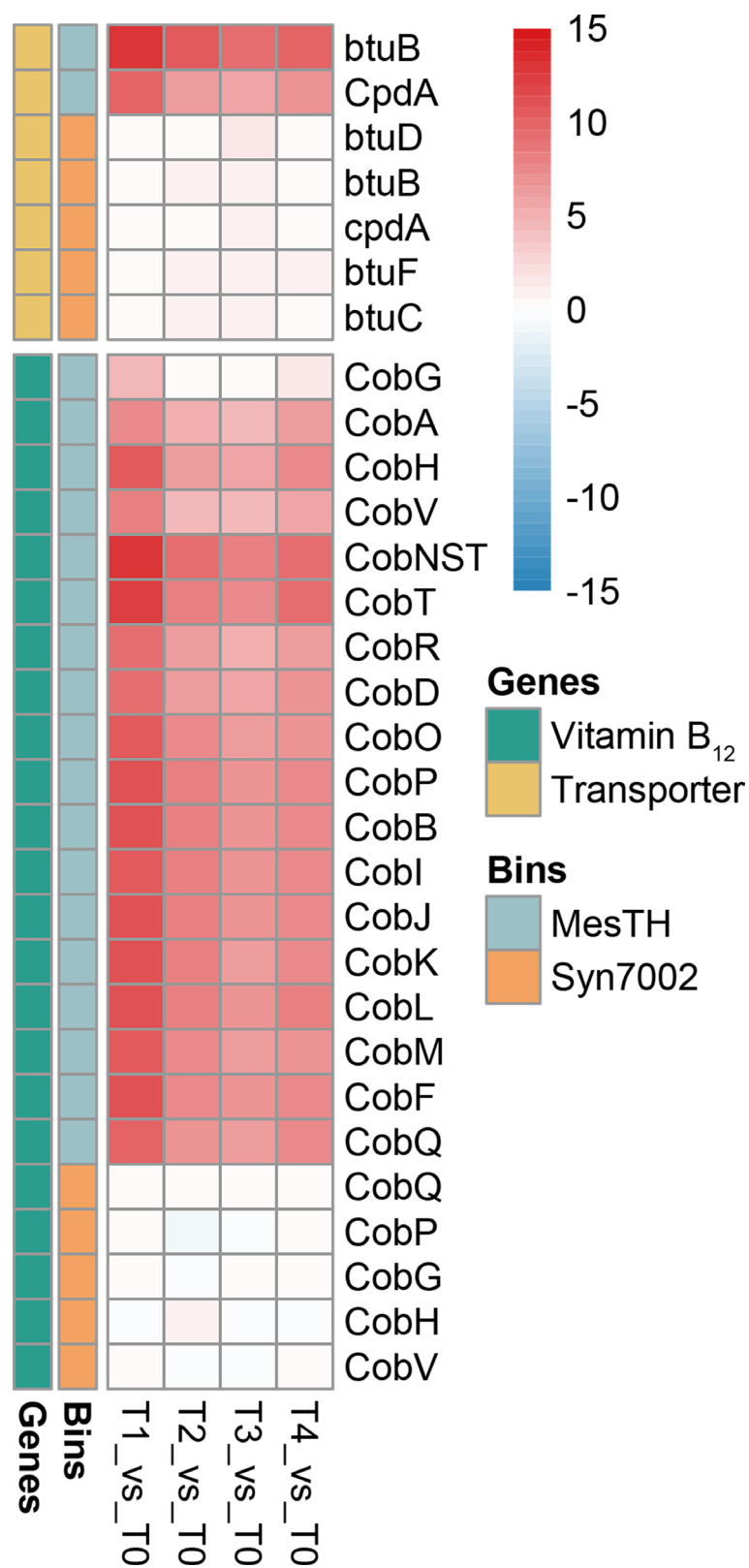


Fig. 4. Expression profiles of vitamin B₁₂ biosynthesis and transport-related genes in MesTH and Syn7002 at different stages of community formation, presented as log₂(fold change) relative to the initial stage. Metatranscriptomic samples were collected at five time points (T0–T4), corresponding to days 0, 1, 5, 10 and 20, respectively.

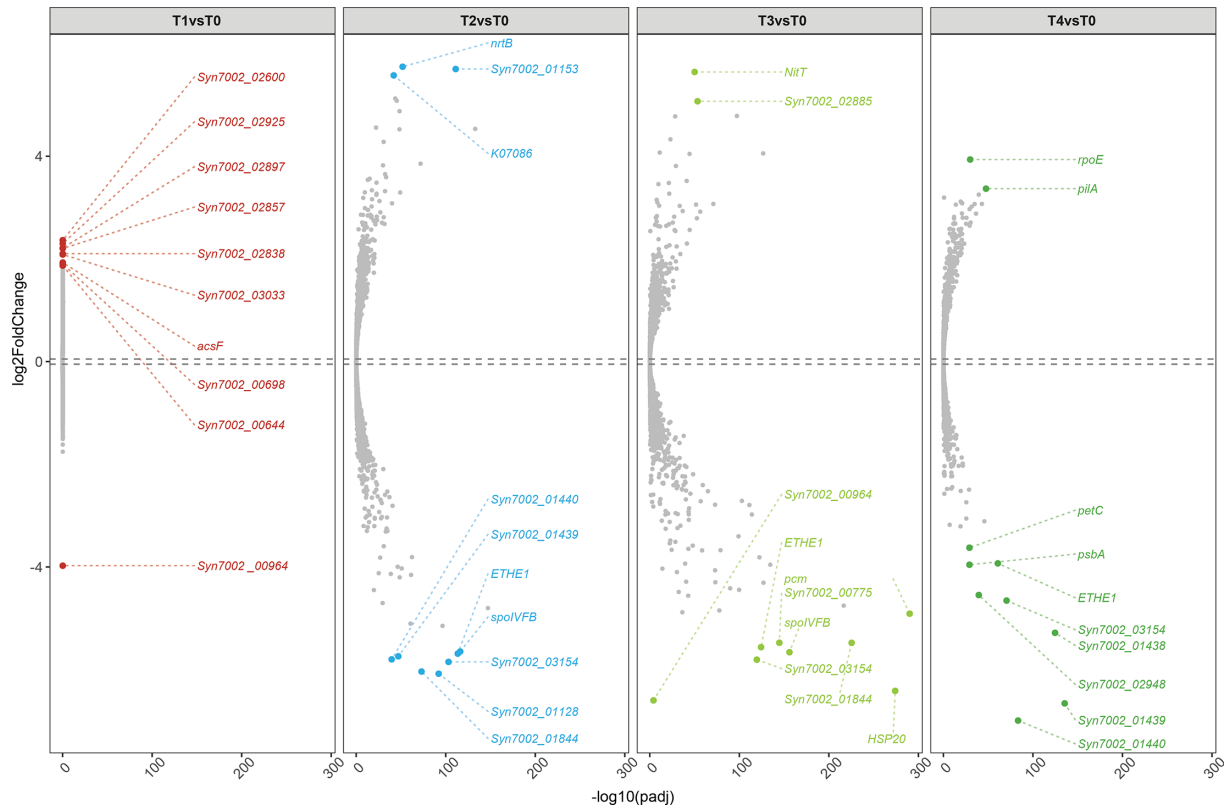


Fig. 5. Genes in Syn7002 that exhibit significant changes during community development. At each time point, differential expression was determined by comparison to the condition without MesTH supplementation. Metatranscriptomic samples were collected at five time points (T0–T4), corresponding to days 0, 1, 5, 10 and 20, respectively. Unannotated features are labelled in the format: genome name followed by a unique number. All genes are shown as grey dots based on their differential expression, with the top 10 genes by absolute $\log_2$ (fold change) highlighted in colour.

manipulate environmental conditions, which may help identify microbial taxa that are able to maintain relatively stable associations with Syn7002 under changing salinity, while reducing the influence of transient or loosely associated community members during synthetic community assembly. The dynamics of bacterial community relationships undergo continuous changes during co-cultivation, with only those micro-organisms that establish stable interactions within the community being ultimately retained. In a long-term indoor experiment with the Syn7002 synthetic colony, the interaction between algae and bacterial communities evolved from antagonism to mutualism, enabling healthy growth without the need for additional nutrients [43].

MesTH and PseTH are common environmental micro-organisms belonging to the *Proteobacteria*. In the early stages, they were present at high abundances along with Syn7002, which is consistent with the long-term indoor study by Zhang *et al.* [43]. Moreover, as the cultivation time in the laboratory increased, the abundance of PseTH initially increased before subsequently decreasing. *Pseudomonas* spp. are common bacterial species that promote algal growth and significantly inhibit the growth of algae-inhibiting bacteria [43]. The reduced abundance of PseTH may be associated with its limited participation in vitamin B₁₂ biosynthetic steps, although this interpretation remains speculative, and other factors such as resource competition or antagonistic interactions cannot be excluded. Long-term observations by Nair *et al.* showed that nitrogen fixation by heterotrophic bacteria supports the stability of algae-associated systems at later stages [13]. In our study, at the stage in which the community stabilized, the abundance of MesTH increased, whereas that of PseTH decreased. Because MesTH possesses the capacity for nitrogen fixation, this shift may be associated with enhanced nitrogen fixation and changes in nitrogen availability, suggesting that nitrogen could be a contributing factor to microbial community stabilization. In addition, these patterns may reflect the activation of key biosynthetic pathways in response to vitamin B₁₂ availability, thereby supporting the synthesis of essential proteins required to maintain cellular functions.

Transcriptomic analysis of the synthetic community showed that genes related to vitamin B₁₂ transport were consistently upregulated during community formation, with a more pronounced activation in MesTH than in Syn7002. This observation indicates pronounced vitamin B₁₂ transport activity within the artificially constructed microbial consortium and may correlate with previous reports demonstrating that trace amounts of vitamin B₁₂ suffice to sustain Syn7002 growth [44]. These findings indicate an association between Syn7002 and MesTH, in which vitamin B₁₂ may play a role in mediating their interaction;

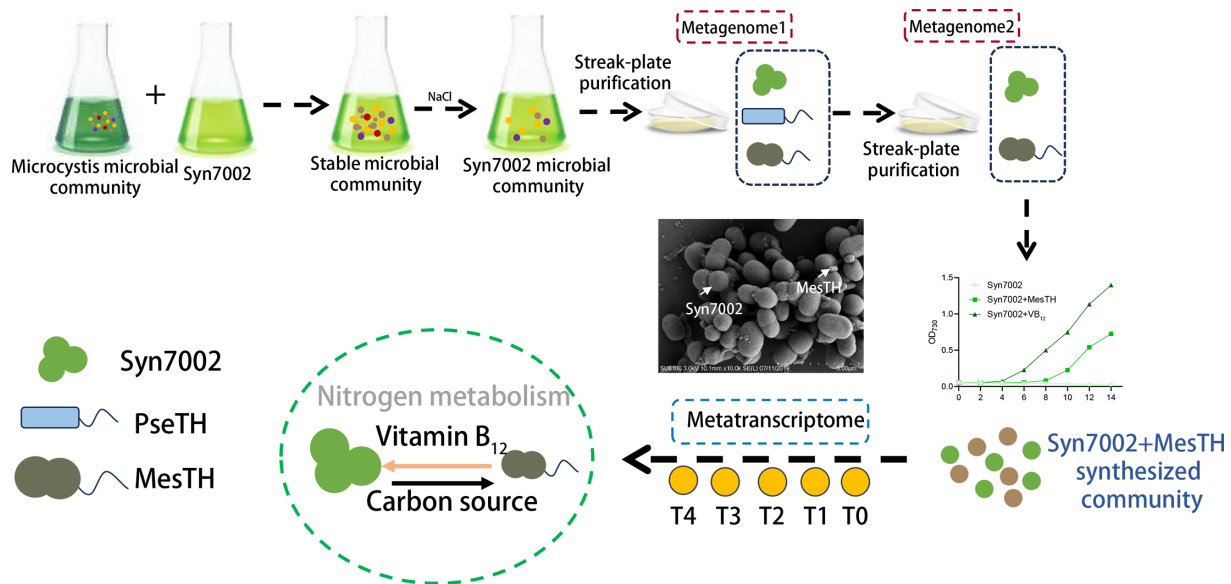


Fig. 6. Schematic of the experimental design and a putative model of microbial interaction mechanisms. Changes in nitrogen metabolism-related gene expression observed in the transcriptomic data are indicated and may reflect potential functional interactions between species. Metatranscriptomic samples were collected at five time points (T0–T4), corresponding to days 0, 1, 5, 10 and 20, respectively.

however, whether vitamin B₁₂ is released into the extracellular environment or transferred through direct intercellular contact remains unclear. In addition, genes involved in nitrogen metabolism and biofilm formation were also upregulated, suggesting additional transcriptional responses associated with co-cultivation. Vitamin B₁₂ is an essential cofactor required for methionine biosynthesis and thus plays a central role in protein synthesis. Vitamin B₁₂ deprivation induces a shift in methionine synthesis from the vitamin B₁₂-dependent methionine synthase (metH) to a vitamin B₁₂-independent isozyme (metE) in *Escherichia coli* [45]. However, Syn7002 contains an incomplete set of genes required for vitamin B₁₂ biosynthesis [14], necessitating cooperative interactions with other micro-organisms in the community to accomplish the synthesis of vitamin B₁₂. Under vitamin B₁₂-limited conditions, expression of the *btuB-cpdA-btuF-btuC* operon, responsible for cobalamin transport, is significantly upregulated [46], consistent with observations in the present study. These results indicate that vitamin B₁₂ deficiency substantially limits the physiological functions of Syn7002. Although targeted metabolomic analysis of vitamin B₁₂ did not detect significant free vitamin B₁₂ in the synthetic microbial community, likely due to rapid utilization by Syn7002 (see Table S1), these metabolites may be used for the synthesis of essential cellular components such as amino acids. Genomic analysis showing that MesTH contains a complete vitamin B₁₂ biosynthesis gene cluster, combined with co-cultivation experiments, suggests that MesTH is associated with the metabolic activity of Syn7002 within the synthetic consortium, potentially through mechanisms involving vitamin B₁₂.

In conclusion, our study demonstrates that vitamin B₁₂ plays crucial roles in the formation and stability of microbial communities within synthetic ecosystems. Vitamin B₁₂ is essential for the growth of Syn7002, while the presence of MesTH supports Syn7002 proliferation. Notably, differential gene expression analysis revealed that nitrogen metabolism-related genes were upregulated during the community formation phase, which may reflect transcriptional responses associated with co-culture conditions rather than direct evidence of functional nitrogen assimilation. While this study provides insights from controlled indoor experiments on microbial interactions, it has certain limitations, including limited verification of nitrogen metabolism changes and lack of validation across other microalgal species. The omics analyses conducted nonetheless provide a descriptive basis to inform future studies on the underlying mechanistic relationships.

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Author contributions

G.L.: investigation, writing—original draft and writing—review and editing; P.B.: investigation, data curation and writing—review and editing. M.R. and Q.L.: data curation and writing—review and editing. T.L.: writing—review and editing, funding acquisition and project administration.

Conflicts of interest

The authors declare that they have no conflict of interest.

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