# PROMICON

### Key message

The application of synthetic phototrophic microbial consortia holds promise for sustainable bioenergy production. Nevertheless, strategies for the efficient construction and regulation of such consortia remain challenging. Applying tools of genetic engineering and light regulation, this study successfully constructed a synthetic community of phototrophs, enabling the production of biohydrogen and fatty acids through nitrogen and carbon dioxide fixation. This approach demonstrates a promising strategy for sustainable bioenergy production through the integration of genetic engineering and light regulation.



## Background

Phototrophic microbial communities are commonly found in lightexposed environments. Such light-driven consortia contribute substantially to the global primary production of organic compounds by fixing carbon dioxide and/or nitrogen gas. With humankind facing ever-growing energy demands and environmental problems, such synthetic phototrophic consortia may provide a promising alternative to current energy generation methods. These consortia can efficiently convert  $CO_2$  and  $N_2$  gases together with water and solar energy into bioenergy products.

However, when attempting to create a synthetic microbial consortium outside the specific environmental conditions of its natural habitat, one strain may outcompete the others and dominate the community. This imbalance can compromise the stability of the consortium. Therefore, developing effective strategies to maintain strain equilibrium and regulate the overall functionality of the consortium remains a significant challenge.

## ENGINEERING A PHOTOAUTOTROPHIC MICROBIAL COCULTURE TOWARD ENHANCED BIOHYDROGEN PRODUCTION

## Objective

This study set out to construct and successfully regulate a phototrophic community, enabling  $H_2$  and fatty acid production through carbon and nitrogen fixation. It cocultivated a community of phototrophs using *Rhodopseudomonas palustris* (*R. palustris*) with either the wild type of *Synechocystis sp.* PCC 6803, or an engineered strain, *Synechocystis\_acs* (an acetate overproducing strain). Various light regulation strategies, including constant illumination, circadian light-dark illumination, and circadian light-infrared illumination, were employed. These strategies facilitated trophic dependence through carbon and nitrogen assimilation and allowed for the regulation of coculture growth. The coculture enabled biohydrogen production in a light-based system feeding on  $CO_2$  and  $N_2$ , highlighting the potential of controlling a phototrophic community.

#### Results

- Elemental balance confirmed carbon capture and nitrogen fixation into the consortium.
- The strategy of circadian illumination effectively limited oxygen levels in the system, ensuring the activity of the nitrogenase in *R. palustris*, despite oxygenic photosynthesis happening in *Synechocystis*.
- When infrared light was introduced into the circadian illumination, the production of H<sub>2</sub> (9.70 μmol mg-1) and fatty acids (especially C16 and C18) was significantly enhanced.
- Proteomic analysis indicated acetate exchange and lightdependent regulation of metabolic activities.
- Infrared illumination significantly stimulated the expression of proteins coding for nitrogen fixation, carbohydrate metabolism, and transporters in *R. palustris*, while constant white light led to the most upregulation of photosynthesis-related proteins in *Synechocystis\_acs*.

#### Source

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