Designing a stable, functional consortium between a phototroph and a heterotroph

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Natural biological systems often exist in a consortium, containing multiple organisms that coexist and interact with each other[1]. These systems are complex and interesting, though the mechanisms of interaction between members and the influence(s) the partners have on each other are poorly understood. In this context, the combination of a heterotroph with a phototroph within a coculture is particularly interesting. Although most heterotrophs are susceptible to oxidative stress generated by the phototroph in a coculture environment, previous work has shown phototroph-heterotroph cocultures to be functional, and resistant to perturbations and stable over time. Synthetic consortia have been shown to improve growth of cyanobacteria as compared to pure culture[2]. *Vibrio natriegens* is an exceptional heterotrophic bacterium, known for its rapid growth, versatility in genetic engineering, and resilience to various environmental conditions. In their natural aquatic habitats, *Vibrio* species often coexist with phototrophic organisms, particularly cyanobacteria[3]. *Cyanothece* sp. ATCC 51142, a unicellular marine cyanobacterium, thrives under carbon and nitrogen-fixing conditions, demonstrating strong growth in these environments. Both these organisms are well studied at the systems level and are genetically amenable. We aim to establish a stable, sustainable and functional coculture system involving *V. natriegens* and *Cyanothece* 51142, with the goal of understanding the mechanism of interaction between a photoautotroph and a heterotroph.

In our initial coculture experiments using a transgenic *V. natriegens* strain expressing a fluorescent reporter and *Cyanothece* 51142, we observed that both strains could coexist while promoting mutual growth. We tested various parameters, such as salt concentrations and inoculum ratios, to identify the optimal growth conditions necessary for coculture. We hypothesize that, unlike many heterotrophs, *V. natriegens* is tolerant to oxidative stress, and the elevated oxygen levels produced by the cyanobacterium in the coculture may enhance its growth and metabolism. RNA-seq analysis, ^{13}C metabolic analysis, and coculture modeling are currently underway to investigate differential gene expression and metabolic regulation in both organisms, including oxidative stress responses under coculture conditions compared to pure cultures. The insights gained will be applied to manipulate interactions between the two organisms, enabling the use of photosynthetically fixed carbon and nitrogen as feedstocks for nitrogen-rich product formation by the heterotroph.

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1. Takemura, A. F., Chien, D. M. & Polz, M. F. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. *Front. Microbiol.* **5**, (2014).
2. Hays, S. G., Yan, L. L. W., Silver, P. A. & Ducat, D. C. Synthetic photosynthetic consortia define interactions leading to robustness and photoproduction. *J Biol Eng* **11**, 4 (2017).
3. Lima, M. *et al.* The new chassis in the flask: advances in Vibrio natriegens biotechnology research. *Biotechnology Advances* (In Press).