Tuning C_1-metabolism for efficient utilization of biogas in synthetic photoautotroph-methanotroph binary consortium.

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Project Goals: In nature, microbial communities have developed a highly efficient way to recover energy and capture carbon from both CH_4 and CO_2 through interspecies coupling of methane oxidation to oxygenic photosynthesis. However, in order to successfully utilize mixed culture for biotechnology applications, both fundamental knowledge and technological gaps have to be addressed. The knowledge gap refers to the lack of systematic study for identifying and quantifying the interactions between community members and how the interaction feedbacks affect system dynamics. The technological gap refers to the lack of effective methodology, and fast and low-cost analytical tools to characterize mixed culture systems frequently or in real-time. The overall research objective is to help address those gaps through developing experimental/computational tools to characterize a synthetic photoautotroph-methanotroph binary consortium, to identify and validate interspecies interactions at both systems and cellular levels, and to engineer a model methanotroph-photoautotroph coculture pair for enhanced production of chemicals.

Abstract: Paramount to the successful engineering of a synthetic photoautotroph-methanotroph consortia is a deep understanding of the physiology of native phototrophic and methane-converting bacteria. Such knowledge can then be used to achieve optimal growth and carbon conversion of robust co-cultures of engineered cells. *Methylomicrobium alcaliphilum 20Z^R*, is a methanotrophic bacterium able to utilize a range of C1 compounds whose physiology we are currently seeking to both catalog and optimize. This organism will then be used as a methanotrophic partner for construction of a robust synthetic photoautotroph-methanotroph binary consortium. Through a holistic study of *M. alcaliphilum* physiology, we aim to engineer a tunable system for construction of stable binary consortium for a multitude of applications in industrial biotechnology. Previous examples of this included the construction of a comprehensive metabolic framework of *M. alcaliphilum*[1], allowing us to better understand the movement of carbon and energy through the cell[1]. Two current areas of focus are electron/energy flow during methane oxidation and multistep gene expression from transcription through translation.

The first step of CH_4 utilization by *Methylomicrobium alcaliphilum* employs the membrane bound particulate methane monooxygenase (pMMO) which uses O_2 to oxidize CH_4 to CH_3OH for further processing. Also required by this enzyme is an electron pair, the source of which has been a point of contention among scientists. Our previous, metabolic modeling has led us to assert that electrons moving against their voltage gradient, or "uphill", from cytochrome C through the bc_1 complex are being supplied to pMMO for CH_4 oxidation. To test this, we have measured the electron transfer activity of *M. alcaliphilum* intracytoplasmic membranes (ICMs) by monitoring the oxidation of cytochrome c in the presence of ICMs. Our preliminary results show that in the presence of a proton gradient, cytochrome c is oxidized by ICMs, even when cytochrome c oxidase is completely inhibited by KCN (**Figure 1**). These results seem to suggest that a proton gradient can be used to drive electrons uphill in ICMs. We are currently performing further experiments to implicate the bc_1 complex in this observation.

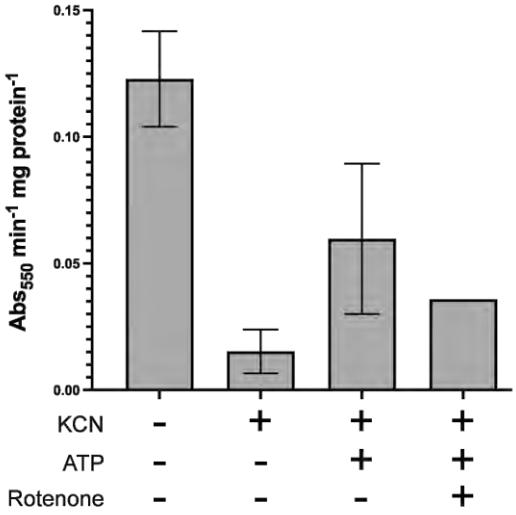

Figure 1. Oxidation of reduced cytochrome c in the presence of isolated *M. alcaliphilum* ICMs. Cytochrome c oxidation was monitored by measuring the decrease in Abs_{550} over time.

In addition to these studies we have set about expanding our ability to control the relative expression of genes within *M. alcaliphilum*. Many regulatory elements exist within bacteria at both the transcription and translational levels. We have currently been investigating the effect that different promoters, ribosome binding sites, and codon usage biases have on gene regulation in *M. alcaliphilum* by measuring the expression of reporter genes containing these elements. Preliminary results have identified representative regulatory elements which can be used in combination with one another to build a tunable gene expression repertoire in *M. alcaliphilum*. The combination of multiple elements will allow for not only turning genes of interest on and off, but fine tuning the expression of genes over a spectrum gene product outputs.

Our research activities will help us to increase carbon conversion efficiency in the methanotrophic partner as well as tune the expression of both endogenous and exogenous genes in relevant pathways. Using our engineered methanotroph in co-culture with optimize the efficiency of biogas conversion to value-added products.

References

1. Akberdin, I. R., Thompson, M., Hamilton, R., Desai, N., Alexander, D., Henard, C. A., … & Kalyuzhnaya, M. G. (2018). Methane utilization in *Methylomicrobium* alcaliphilum *20Z R*: a systems approach. *Scientific Reports*, *8*(1), 2512.

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