**Topical: Quantitative Biology Accelerates Ecosystems Evolution for Sustained Spaceflights**

Cristal Zuniga, Biology Department, San Diego State University, crzuniga@eng.ucsd.edu. **Environmental Microbiology, Computational Biology.**

Michael Betenbaugh, Chemical Engineering Department, Johns Hopkins University, **Microbial Ecology, Chemical Engineering.**

Pavlo Bohutskyi, Pacific Northwest National Laboratory, **Chemical Engineering.**

Daniel Zielinski, Bioengineering Department, University of California San Diego, **Computational Biology, Biomedical Engineering**

#

#

# **Executive Summary**

We will employ quantitative biology to identify the effects of altered gravity and radiation on the biological systems focusing on rational strain engineering strategies. Quantitative biology can provide new insight into intra- and intercellular interactions, untangling complex microbial networks and facilitating direct evolution toward specific phenotypes. Our goal is to enhance microbial partnerships and networks that can provide optimized biomass for food and maximum oxygen for recycling in space environments, providing a novel bio-based system for air during space flight.

# **Quantitative biology of life in space, characterizing metabolic interactions and directing evolution.**

Currently, quantitative biology tools such as genome-scale metabolic models have been developed for organisms under monoculture and in phototrophic communities. These models can be continuously improved and successfully applied to understand and enhance metabolism and evolution in space, facilitating sustained spaceflights. The approach detailed in this proposed project will provide novel methods and advances in quantitative biology that can shed light on metabolism in space as well as development of standardized computational tools for application to bio-based exploration technologies. Specifically, we will undertake to describe: Quantitative biology of life in space, characterizing metabolic interactions and directing evolution. We will take advantage of solar energy and inorganic carbon in spaceflights using smart phototrophic communities. We will perform rational strain engineering towards key phenotypes that produce oxygen and commodities for sustained breathable atmospheres.

The model phototrophic communities suggested in this paper have been broadly characterized on Earth using advanced computational methods and various omic data, such as shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics. We have unraveled community structure, interactions, and community dynamics associated with evolution. We will demonstrate if our microbial communities change their phenotypes under altered gravity and radiation. We will compare the metabolism under monoculture and community conditions, developing new algorithms for strain engineering useful to unravel evolution. Experiments performed in space under reduced gravity and radiation environments will enable cleaner genome-scale analysis that will focus on understanding evolution in space as well as into increasing community resilience more than the 7% obtained on Earth. By optimizing the community interactions in space flight setting, we hope to achieve a 25% increase in community biomass productivity that will speed the development of these systems in non-earth setting.

**Background.** Experimental measurement of metabolic mechanisms is difficult in any environment of the universe because of the low resolution of physiological methods (e.g. growth rates, organism-specific metabolomics). However, quantitative biology enables theoretical calculations to successfully infer the workings of intra-organismal and inter-organismal systems (Zuñiga, Zaramela, & Zengler, 2017). Quantitative biology combines kinetic, metabolic, and statistical modeling approaches with omics data to enhance our knowledge and understanding about metabolic exchange and mass resource allocation (Zaramela et al., 2021; Zuniga, Tibocha-Bonilla, & Betenbaugh, 2021). Model-driven approaches guide effective experimental design and yield new insights into biological function and control.

In addition, most microbes typically live and grow in complex interacting heterogeneous and spatially organized communities or cocultures. A crucial part of these interactions is the exchange of molecules between different organelles, cells, and the environment. Phototrophic communities (smart communities) include photoautotrophs of cyanobacteria or green algae (cyanobionts or photobionts) in a stable symbiosis with a heterotrophic fungus (mycobionts) (Fig. 1), and thrives through division of labor in which organic carbon and oxygen needed for biosynthesis is provided by the photobionts while the fungi provide water, mineral nutrients, protection, some carbon dioxide and production of a vast array of complex metabolites including some readily converted into biofuels, commodities, and dietary supplements (Li et al., 2017). We have shown that these smart communities can produce biomass while secreting organic chemicals (methanol, formaldehyde, ethanol, butanal, and succinate) (Zuñiga et al., 2019, 2020), that are declared as primary feedstock for chemical synthesis by the Environmental Protection Agency of the United States of America (Zuñiga et al., 2020). Utilization of smart phototrophic communities will prolong spaceflights by having a constant supply of added value compounds while producing oxygen from light and waste carbon dioxide. Culturing of smart communities is not limited to liquid phase other bioreactor configurations including solid and mix will be also tested. Here, we propose to perform experiments with smart communities under reduced gravity and radiation in space. Our smart communities are a bio-based tool that has been extensively validated on Earth and they have shown to be simple and stable. Smart communities have shown possible targets of evolution when performing overall fitness tests linking genotype and streamlined genomes with improved growth. We will focus on selecting new strain engineering targets to direct evolution towards optimized oxygen production rates and biomass synthesis.

***Taking advantage of solar energy and inorganic carbon in spaceflights using smart microbial communities.***

**Challenge:** Several reports have shown that cells are sensitive to a gravity field, changing phenotypic traits that include morphology, biochemical pathways, and gene expression under monoculture (Tirumalai et al., 2017). However, those descriptive analysis at low gravity are limited scope since phenotypes under environmental pressure in response to coexistence with a partner have not been identified yet. Additionally, another challenge in long-term spaceflights is to sustainably close the loop of carbon and oxygen on board.

**Rationale:** Smart phototrophic communities are nature’s solar panels, consisting of photosynthetic organisms that capture light energy and heterotrophic organisms that benefit from photosynthetically fixed carbon (Li et al., 2017; Palmqvist, Franklin, & Näsholm, 2017; Polka, Hays, & Silver, 2016; Vera et al., 2010; Zuñiga et al., 2019, 2020). These communities interact symbiotically, exemplifying an exquisite division of labor for the “common good”. Smart communities can release additional oxygen to the environment. Our previous analyses have shown that smart communities actively exchange additional metabolites (e.g. amino acids, vitamins) sustaining the heterotrophs in minimal media devoid of any organic carbon source (Zuñiga et al., 2019). Several smart communities have been developed by our team to study metabolic interactions and to apply this unique platform to harness sunlight and carbon dioxide (photoautotroph) and convert those inputs into complex liquid and gas metabolic precursors and products (fungi or bacteria or humans) (Li et al., 2017; Polka et al., 2016; Zuñiga et al., 2019, 2020).

Two key questions in evolutionary biology address how biological systems become genetically stable without members outcompeting themselves and how communities manage deleterious gene loss to attain streamlined genomes (Morris, Lenski, & Zinser, 2012). Genome-scale metabolic models enable to perform systematic studies related to fitness, maintenance, and resilience to environmental and genetic perturbations. Genome-scale reconstructed networks are organized and systematized knowledge bases that have multiple uses, including conversion into computational models that interpret and predict phenotypic states (Fang, Lloyd, & Palsson, 2020).

**Current state:** Phototrophic communities, which can also include additional partners, thrive in the harshest environments on earth, including extremes of temperatures, light, radiation, salinity, and desiccation. Those conditions are like the ones found in space. Previously, our team constructed the smart phototrophic pair that included the microalgae *Chlorella vulgaris* and *Saccharomyces cereviciae*. We integrated metatranscriptomics, metabolomics and phenotyping with computational modeling to reveal condition-dependent secretion and cross-feeding of metabolites in a synthetic community (Zuñiga et al., 2019). We predicted and validated how microbial interactions between members are highly dynamic and are driven by the availability of organic and inorganic nutrients in solid and liquid culture media. Environmental factors, such as ammonia concentration, influenced community stability by shifting members from collaborating to competing. This smart community can be used as a nutritional supplement that depending on the necessities of the travelers the growth of the fungi or algae can be enriched. Fungi can also serve as an ingredient to perform fermentation (e.g. milk, yoghurts and cheese, fermented vegetables and minimally processed vegetable products). We have also created smart communities composed by the cyanobacterium *Synechococcus elongatus*, engineered to secrete sucrose by over-expression of a proton/sucrose symporter (CscB), in partnership with the bacteria *Escherichia coli* K-12, *Escherichia coli* W, and *Bacillus subtilis* or the oleaginous yeasts *Rhodosporidium toruloides* and *Yarrowia lipolytica* (10.1038/s41467-020-17612-8). Each smart community had different production phenotypes. For example, only the pairs containing *E. coli* were able to produce butanal and formaldehyde.

The genome-scale fitness tests performed by simulating knock-outs of all genes in the models showed that members of microbial communities can efficiently compensate for some metabolic activities, overcoming up to 27% of phenotypic traits lethal to one member, allowing the damaged participant to thrive in the community though they could not in monoculture. Predicted phenotypes can help to elucidate community structure, streamline genomes, and target of evolution during community assembly.

**Gaps:** Since the average ISS missions (expeditions) usually last about six months, the long term supply of oxygen and food is limited by cost and volume. The constant requirement of oxygen synthesis and other metabolites using the carbon dioxide of the cabin will prolongate the resources during spaceflights and directing evolution.

**Recommendations:** a) Characterize growth phenotypes of our smart community in a space flight, testing the effect of low gravity and radiation on growth phenotypes. Suggested experiments will take advantage of the fact that space shuttles produce oxygen through a chemical electrolysis system that combines water and electricity to produce hydrogen and oxygen, storing hydrogen on a spacecraft is dangerous for its flammability, so the hydrogen is vented overboard. To reduce this risk, we recommend pairing current chemical efforts to supply oxygen spaceflights with biological efforts, taking smart communities during space flights that are tailored for several purposes e.g. high production rates of oxygen, synthesis of biofuels, commodities, and/or dietary supplements. b) Determine if genetic targets of evolution of our *Chlorella vulgaris* and *Saccharomyces cerevisiae* smart community are the same on Earth and during space flight. Previously, we predicted the essentiality of 3,496 metabolic genes in mono- and co-culture. Results suggested that metabolic genes exhibit higher level of phenotypic variation, triggering adaptive benefits to an organism. We found that both partners compensate for loss of gene functions in the other partner, contributing to the survival of the community. However, this compensation depends on the community growth stage and biomass composition. The number of essential genes required for growth in co-culture was reduced by 39% (from 194 to 115) for the alga and by 31% (from 106 to 73) for the fungus. Based on model simulations, nine fungal mutants that changed their growth phenotypes under mono- and co-culture were selected for experimental validation. Seven mutants (systematic gene name is shown, followed by synonym in parentheses) with essential genotypes were associated with fructose and mannose metabolism (*YDL055C* (*PSA1*−); *YFL045C* (*SEC53*−); *YER003C* (*PMI40*−)), glutamine metabolism (*YPR035W* (*GLN1*−)), phospholipid biosynthesis (*YBR029C* (*CDS1−*)), riboflavin metabolism (*YBR153W* (*RIB7*−)) and sterol metabolism (*YHR007C* (*ERG11*−)), and two mutants with non-essential genotypes were associated with oxidative phosphorylation (*YAL012W* (*CYS3*−)) and methionine metabolism (*YKL055C* (*OAR1*−)). Six of the seven predicted essential fungal knockouts (KOs) were rescued when grown in co-culture, confirming our predictions. The recovery of essential gene loss by the community sheds light on the lack of cultivability routinely observed from environmental samples.

**References**

Fang, X., Lloyd, C. J., & Palsson, B. O. (2020). Reconstructing organisms in silico: genome-scale models and their emerging applications. *Nature Reviews Microbiology*, *18*(December), 23–26. https://doi.org/10.1038/s41579-020-00440-4

Li, T., Li, C.-T., Butler, K., Hays, S. G., Guarnieri, M. T., Oyler, G. A., & Betenbaugh, M. J. (2017). Mimicking lichens: incorporation of yeast strains together with sucrose-secreting cyanobacteria improves survival, growth, ROS removal, and lipid production in a stable mutualistic co-culture production platform. *Biotechnology for Biofuels*, *10*(1), 55. https://doi.org/10.1186/s13068-017-0736-x

Morris, J. J., Lenski, R. E., & Zinser, E. R. (2012). The black queen hypothesis: evolution of dependencies through adaptive gene loss. *MBio*, *3*(2), e00036-12-e00036-12. https://doi.org/10.1128/mBio.00036-12

Palmqvist, K., Franklin, O., & Näsholm, T. (2017). Symbiosis constraints: Strong mycobiont control limits nutrient response in lichens. *Ecology and Evolution*, *7*(18), 7420–7433. https://doi.org/10.1002/ece3.3257

Polka, J. K., Hays, S. G., & Silver, P. A. (2016). Building spatial Synthetic biology with compartments, scaffolds, and communities. *Cold Spring Harbor Perspectives in Biology*, *8*(8), a024018. https://doi.org/10.1101/cshperspect.a024018

Tirumalai, M. R., Karouia, F., Tran, Q., Stepanov, V. G., Bruce, R. J., Ott, C. M., … Fox, G. E. (2017). The adaptation of *Escherichia coli* cells grown in simulated microgravity for an extended period is both phenotypic and genomic. *Npj Microgravity*, *3*(1), 15. https://doi.org/10.1038/s41526-017-0020-1

Vera, J.-P. de, Möhlmann, D., Butina, F., Lorek, A., Wernecke, R., & Ott, S. (2010). Survival potential and photosynthetic activity of lichens under mars-like conditions: A laboratory study. *Astrobiology*, *10*(2), 215–227. https://doi.org/10.1089/ast.2009.0362

Zaramela, L. S., Moyne, O., Kumar, M., Zuniga, C., Tibocha-Bonilla, J. D., & Zengler, K. (2021). The sum is greater than the parts: exploiting microbial communities to achieve complex functions. *Current Opinion in Biotechnology*, *67*, 149–157. https://doi.org/10.1016/j.copbio.2021.01.013

Zuñiga, C., Li, C.-T., Yu, G., Al-Bassam, M. M., Li, T., Jiang, L., … Zengler, K. (2019). Environmental stimuli drive a transition from cooperation to competition in synthetic phototrophic communities. *Nature Microbiology*, *4*(12), 2184–2191. https://doi.org/10.1038/s41564-019-0567-6

Zuñiga, C., Li, T., Guarnieri, M. T., Jenkins, J. P., Li, C.-T., Bingol, K., … Zengler, K. (2020). Synthetic microbial communities of heterotrophs and phototrophs facilitate sustainable growth. *Nature Communications*, *11*(1), 3803. https://doi.org/10.1038/s41467-020-17612-8

Zuniga, C., Tibocha-Bonilla, J. D., & Betenbaugh, M. J. (2021). Kinetic, metabolic, and statistical analytics: addressing metabolic transport limitations among organelles and microbial communities. *Current Opinion in Biotechnology*, *71*, 91–97. https://doi.org/10.1016/j.copbio.2021.06.024

Zuñiga, C., Zaramela, L., & Zengler, K. (2017). Elucidation of complexity and prediction of interactions in microbial communities. *Microbial Biotechnology*, *10*(6), 1500–1522. https://doi.org/10.1111/1751-7915.12855