Extensive Interdependencies and Unexpected Roles for B Vitamins in Microbial Communities

Aaron T. Wright[1*] (Aaron.Wright@pnnl.gov), Margaret F. Romine, Premchendar Nandhikonda[1], Yukari Maezato[1], Lindsey N. Anderson[1], Stephen R. Lindemann[1], Mary S. Lipton[1], Young-Mo Kim[1], Thomas O. Metz[1], Chengdong Xu[1], Dmitry Rodionov[2], Irina Rodionova[2], and **Janet K. Jansson**[1]

[1]Pacific Northwest National Laboratory, Richland, WA; [2]Sanford-Burnham Medical Research Institute, La Jolla, CA

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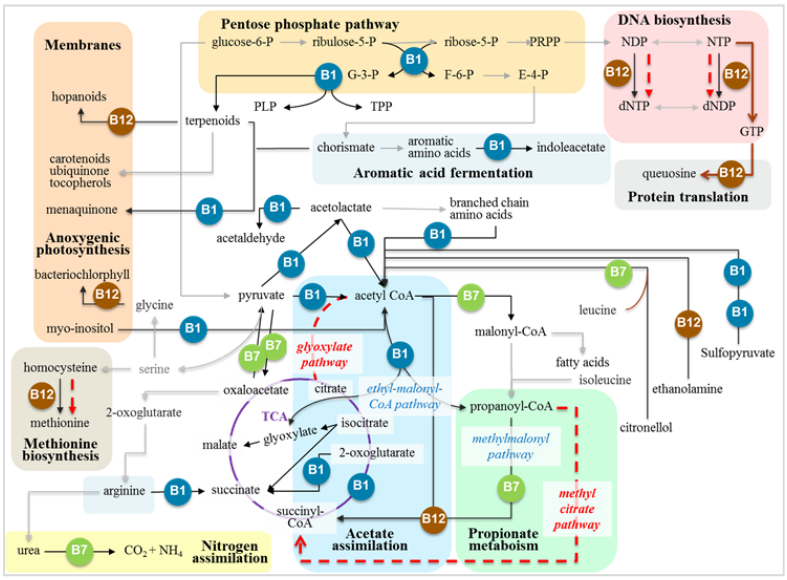
Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph consortia whose member genome sequences have been defined. Our project leverages unique capabilities including multi-omics measurements, advanced functional imaging, taxonomic profiling and metabolic and regulatory network modeling to elucidate underlying reaction mechanisms within complex microbial communities. Our research plan supports DOE goals to achieve a predictive understanding of microbially-mediated carbon and energy transformation.

Individual members of complex microbial communities can interact by exchanging metabolites and signaling compounds. However, the interdependency of species interactions in communities has only begun to be explored. To address this challenge we have focused our studies of microbial interactions on model, tractable autotroph-heterotroph consortia comprised of one cyanobacterium and associated heterotrophs (UCCs, Unicyanobacterial Consortia). These model consortia are valuable resources because they are stable and tractable, and have undergone extensive genome reconstruction and characterization. Our hypothesis was that ***specific members of the community would be dependent on other members for essential metabolites that they could not synthesize themselves***. This could be predicted from the genome information by determining whether each member possessed the ability to synthesize specific required nutrients, or not. Our genome reconstruction work suggested that extensive B vitamin interdependencies could exist among community members (see Figure below), and that these dependencies could govern community-coordinated carbon and energy cycling. Additionally, the requirement for vitamin exchange to support growth and possible regulatory roles in our model UCC suggests a new hypothesis to test. Namely, ***that the phototrophic producers could modulate vitamin availability to control member abundances and coordinate overall community metabolism***.

To test these hypotheses, we performed a combination of chemical probe profiling, regulon analysis, metabolomics, imaging, and microbial genetics experiments. We have previously developed and employed chemical profiling probes as B vitamin mimics to identify the function and specificity of a wide range of experimentally unidentified and/or predicted membrane-embedded B vitamin transporters, and also to directly characterize intracellular enzyme-cofactor associations in living microbial systems.[1] Here, we developed an activity based probe (ABP) to mimic vitamin B_{12}, which is produced by only four members of a UCC model community that contains >18 species. The probe was tested for its ability to capture B_{12}-binding proteins



expressed by *Halomonas* sp. HL-48, an isolate from the consortium that is capable of both synthesizing and salvaging B_{12}. Proteomic analysis revealed that the probe captured a total of 45 proteins. Components of all three expected B_{12}-dependent enzymes were among those detected. A review of the remaining proteins, suggested that B_{12} acts as an allosteric regulator of enzyme activity, a finding that has not previously been observed in any organism. Three of the captured proteins are involved in porphyrin biosynthesis; one at the branch point between heme and B_{12} biosynthesis, another associated with cobalt insertion into B_{12}, and the last likely involved in salvage of the B_{12} precursor, cobinamide. Allosteric effects at these positions could result in redirection of metabolism between biosynthesis of heme versus B_{12} as well as between B_{12} biosynthesis versus salvage. An additional 17 proteins could be linked to methionine recycling or C1 metabolism via folate. Folate is required for biosynthesis of proteins and nucleotides and thus required in high amounts during cell division. We also identified a new B_{12}-mediated regulator, designated PhrR, and performed regulon analysis to identify likely DNA promoters controlled by PhrR. Significantly, the genes under control of PhrR encode functions associated with light damage repair, chemotaxis, and biosynthesis of folate and ubiquinone. Metabolomic analysis of wild type and a PhrR knockout mutant validated a role for PhrR and B_{12} in controlling folate levels.

We then translated our B_{12} probe analyses and regulon analysis to determine that the five phototrophic species in the model community: *Rhodobacteriaceae*, *Algoriphagus*, and *Halomonas* species, utilize B_{12} as a light sensing ligand for transcriptional control of genes required for biosynthesis of light harvesting apparatus. These genes include those encoding production of bacteriochlorophyll, carotenoids, folate, and queousine as well as enzymes involved in DNA damage repair. We are now evaluating a new hypothesis that ***vitamin interdependency is important for establishing community stability***. We are currently investigating this hypothesis by testing mechanisms of vitamin exchange and evaluating the impact of vitamin supplementation on community population dynamics and metabolism.

References

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This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), as part of BER's Genomic Science Program (GSP). This contribution originates from the GSP Foundational Scientific Focus Area (FSFA) at the Pacific Northwest National Laboratory. Proteomic analyses were performed in the William R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by OBER and located at PNNL.