109. Characterizing the Pseudomonas Sulfur Regulome

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Project Goals: The Argonne "Environment Sensing and Response" Scientific Focus Area (SFA) program seeks to identify the molecular basis of cellular transport and sensory pathways that mediate the response of terrestrial ecosystems to environmental nutrients. The mechanistic links between and within ecosystems comprised of plants, fungi, and soil bacteria involved in the production of biomass for fuel are currently very poorly defined. The effects of nutrient availability, closely linked to climate, on those mechanistic links, are also inadequately understood. This program will address this knowledge gap by mapping transport and sensor proteins to specific environmental compounds to define their function and biological roles and establish a series of defined connections between the environment and the cell. The knowledge will facilitate the development of system-level models predictive of cellular response to changes in environmental conditions.

In soil communities with sufficient N, P and C inputs available sulfur (S) can become limiting due to competition between community members. Like Phosphorus, the preferred sulfur source is inorganic sulfate, which composes only ~5% of total S present in soil. The remaining sulfur is present as other inorganic species and various organosulfur compounds. Organic sulfur compounds are structurally diverse, with common functional groups including thioesters, thioethers, thiols, sulfate esters and sulfones. Previous studies have shown that the soil sulfur pool is very dynamic, with S rapidly transformed between many inorganic and organic S compounds.

Plants can only take up S as inorganic sulfate or as amino acids methionine and cysteine so there is a requirement for microbial activity to transform complex molecules into plant-available forms. In many cases bacteria can remove a sulfur groups from organic sulfur compounds even when the carbon skeleton cannot be metabolized. In many organisms, elucidation of the transporters that mediate uptake or enzymes associated with sulfur-processing pathways has been limited as bacteria are frequently cultured in media that contains inorganic sulfur at concentrations which fully repress organic sulfur metabolism pathways. Furthermore, even under de-repressed conditions sulfur-metabolizing proteins are often expressed at low levels and are not detected by proteomics-based identification methods. This presents an advantage to transcriptomics-based interrogation of these microbial pathways as RNA-seq is sensitive to small changes in transcript levels. In this study, we characterize the expression profiles associated with bacterial growth on a variety of sulfur compounds to determine the linkage to specific metabolic pathways and transporters. The goal is to develop a modeling approach useful in predicting the Pseudomonas sulfur regulome for a variety of conditions.

Transcriptomics
Transcriptome libraries were generated for P. fluorescens SBW25 cultured on minimal growth media supplemented with 2-Aminoethyl hydrogen sulfate, Cysteine, Glutathione, 4-Nitrophenyl sulfate, Methionine, Methionine Sulfone, α-keto-γ(methylthio)butyricacid, Sodium Sulfate, or Taurine as sole sulfur sources. Pseudomonas was able to utilize all of these sulfur compounds in liquid culture but showed variations in total biomass after 24 hours of growth. 327 genes were found to be significantly differentially regulated in response to sulfur media type, of which 88 genes coded for metabolic enzymes, 457 for transmembrane transporters, and 14 for transcription factors. Functional analysis of differentially regulated transporters indicate that amino acid transporters and transporters that maintain osmotic balance

are associated with the Pseudomonas sulfur regulome. Changes in expression of genes for metabolicenzymes indicate that the net effect of transcriptional regulation of metabolic processes do not favor any particular sulfur metabolic pathway, but instead act to maintain metabolic homeostasis. On sulfur media that was least favorable for growth (2-Aminoethyl hydrogen sulfate and Methionine Sulfone) gene expression was significantly negatively correlated with translated protein sulfur content. On sulfur media that was most favorable for growth (Cysteine, Glutathione, and Sodium Sulfate) gene expression was significantly positively correlated with translated protein sulfur content. This indicates that Pseudomonas actively regulates it proteome to conserve sulfur when bioavailable sulfur is limited. It also suggests that for some organosulfur compounds the bacteria are unable to match S liberation rates with protein synthesis demands resulting in S stress even though growth is permitted on that substrate.

Modeling the Pseudomonas Sulfur Regulome

The Pseudomonas sulfur regulome was modeled as an Artificial Neural Network. In the ANN, gene expression of the significantly differentially expressed transcription factors, enzymes, and transmembrane transporters are predicted as a function of 27 Quantitative Structure-Activity Relationship (QSAR) features of sulfur nutrients. The ANN model accurately predicted gene expression as functions of sulfur media QSAR for transcription factor expression (Pearson's Correlation Coefficient 0.99), enzymes (0.98), and transmembrane transporters (0.96). The ANN was used to predict the transcriptome and biomass for Pseudomonas cultured on 25 additional sulfur media types for which growth data was available and for which the sulfur compound can be described using the set of selected 27 QSAR parameters. Modeled sulfur regulome predicted biomass of Pseudomonas cultured on different sulfur media with an average percent error of 23% (Minimum error 0.8%, maximum error 90%). Accuracy of biomass prediction is dependent upon the similarity of modeled sulfur compound to one of the 9 sulfur compounds used to build the ANN model.

Deconvolution of the Sulfur Regulome

While the ANN has the capacity to predict microbial behavior as a function of nutrient QSAR parameters, the structure of the underlying ANN provides insights into the mechanisms of the Pseudomonas sulfur regulome. The topology of the ANN has three distinct but interlinked subnetworks. Each subnetwork responds to unique QSAR parameters, is driven by the activity of one main transcription factor per subnetwork, and regulated clusters of genes that are specific in their composition of transporters and metabolic functions.

These results indicate that this modeling approach can be used to accurately predict the Pseudomonas sulfur regulome for a variety of conditions. The model can predict the regulome for novel sulfur source with the accuracy of the model for a particular nutrient dependent on the structural similarity of that nutrient to the nutrient used to train the model. This allows for the capacity to understand, predict, and engineer microbial systems to detect and respond to arbitrary environmental compounds.

The submitted manuscript has been created by UChicago Argonne, LLC, Operator of Argonne National Laboratory ("Argonne"). Argonne, a U.S. Department of Energy Office of Science laboratory, is operated under Contract No. DE-AC02-06CH11357.Brookhaven National Laboratory is operated under Contract No. DE-AC02-98CH10886.This contribution originates from the "Environment Sensing and Response" Scientific Focus Area (SFA) program at Argonne National Laboratory. 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).