Systems Biology Guided by Global Isotope Metabolomics

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Project Goals: The goal of our ENIGMA research is to employ global metabolomics on microbial systems to better understand how they function, what molecular species they consume/produce and identifying metabolic pathways that are effected by various stressors. Examples of stressors include metal contamination and nitrate stress. Here we apply numerous bioinformatic tools to process raw liquid chromatography mass spectrometry data obtained from cellular extracts to identify statistically significant dysregulated metabolites and the pathways they are involved in. We also utilize an autonomous approach to validate metabolite identities through data dependent tandem mass spectral acquisition. Additionally, we employ a systems biology approach to cross reference metabolic data with genomic and proteomic data to look for changes that occur systems-wide. For pathways that are difficult to identify, our goal is to elucidate them with additional cell growth experimental protocols using stable isotope labeled substrates in both stress and non-stress conditions. Global isotope metabolomics is employed to identify these pathways in an unbiased manner. We have used these methods to elucidate nitrate assimilation pathways in *Psuedomonas* strains RCH2, N2A2 and N2E2.[1] We are currently performing similar experiments in a dual labeled system with *Bacillus cereus* ATCC 14579 to identify altered assimilation pathways using fully labeled ^{13}C lactate and ^{15}N nitrate under metal stress, and sulfur metabolomics using *Desulfovibrio vulgaris* Hildenburough (DvH) with ^{34}S labeled sulfate.

Abstract. Bioinformatics has become essential part of analyzing large global metabolomics datasets. The XCMS Online[2] platform significantly decreases the time required to process raw liquid chromatography mass spectrometry (LC-MS) data for retention time alignment, feature detection and statistical analysis of dysregulated features. In a typical metabolomics workflow, the accurate mass of each feature is matched with potential candidates from a database of metabolites. Data dependent tandem mass spectra are often used to validate these identities, which can be done using an autonomous workflow[3] or by manual interpretation of pooled quality control samples. This is followed by analysis of the metabolic pathways they are involved in to determine how they are affecting the whole system as a whole. We have recently developed a streamlined method to easily identify these aberrant pathways directly from the raw metabolomic data using a predictive pathway analysis algorithm[4] integrated into XCMS Online, thereby significantly reducing pathway analysis time. Dysregulated pathways can be further understood with respect to upstream gene and protein expression processes by correlating genomic and

proteomic data, also in an automated approach[5]. Resulting overlaps can be easily visualized using the newly developed Pathway Cloud Plot, where the statistical significance (*p*-value) of the perturbed pathways are plotted versus the percent overlap of the identified dysregulated metabolites in the total identified metabolites of a given pathway. Additional information about the pathway size is indicated by the radius of the bubble. This novel cloud plot allows for an easy visual interpretation of perturbed metabolic pathways in the entire system. *Desulfovibrio vulgaris* Hildenborough was subjected to both nitrate stress and exposure to mercuric chloride and preliminary results indicate alterations in nitrate assimilation and sulfate reduction processes respectfully. Further analysis would be useful to gain more insight into how these processes are changing the system.

In some instances, metabolic pathways can be obscured by multiple enzymatic reactions that utilize the same substrates and/or yield the same end products. To elucidate these pathways, global isotope metabolomics can be employed using substrates that contain stable isotopes. Labeled starting materials are used to culture microbial strains, allowing them to be metabolized into the system until it reaches a steady state. Cell cultures must be quickly pelleted by centrifugation and flash frozen with liquid nitrogen to maintain the metabolic profile of the system without applying additional stress to prevent alterations from fast-acting enzymatic and signaling processes. The global isotope metabolome is tracked throughout a biological system by performing LC-MS on metabolite extracts and by isotope pattern ratio analysis of metabolite features between the labeled and unlabeled samples and between the stressed and unstressed samples. This comparative analysis provides information on energy consumption, biosynthesis and salvage processes that are not easily identified without looking at how the stable isotope is incorporated into the system. The major advantage of this approach is that it is unbiased and therefore able to detect novel processes that could not be achieved by looking at a targeted set of metabolites.[1] Here we demonstrate isotope analysis on *Bacillus cereus* exposed to a metal mixture using ^{13}C-lactate and ^{15}N-nitrate and preliminary results on DvH cultured with ^{34}S-sulfate in the presence of mercuric chloride.

References

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