Development of Quantitative Community Proteomics Using Metabolic Stable Isotope Labeling to Characterize a Natural Microbial Community from Acid Mine Drainage

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OVERVIEW

Research Objectives:

- Metabolic labeling of multiple bacterial strains in a natural community from acid mine drainage.
- Quantitative proteomics measurements among different community samples.
- Characterization of protein abundance changes and strain population dynamics.

Results:

- A biofilm grown in a laboratory bioreactor was metabolically labeled with nitrogen-15.
- The ^{15}N-labeled biofilm sample served as a reference for comparison to unlabeled field samples by shotgun proteomics.
- A similar protein expression profile was found between bioreactor samples and field samples.
- Quantitative proteomics results showed changes in strain composition of the community.

INTRODUCTION

From single-species cultures grown in laboratory to multiple-species cultures harvested from field

- Community genomics: the sequencing of the genomes of multiple symbiotic species in a community
- Community proteomics: the characterization of the proteomes of the species in the community
- Proteogenomics: the integration of whole community genomics with proteomics

Motivations for studying microbial communities from natural environments

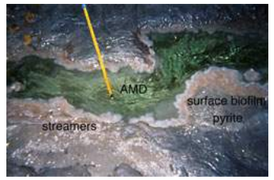
- Interactions between different microbial strains (e.g. division of labor, competition, etc.)
- Interactions between natural environments and microbial communities.
- Characterization of microbes whose growth condition has not been established.

Challenges in developing quantitative proteomics for analyzing field samples of natural communities

- First, *in vitro* stable isotope labeling methods may not provide satisfactory reproducibility for community proteomes, since they are downstream in sample processing. Also, field samples of natural microbial communities cannot be labeled metabolically with stable isotopes.
- Second, a community copes with perturbations by simultaneously adjusting the cell numbers of its constituent species and the expression level of proteins per cell in individual species. It is a challenge to deconvolute these two variable factors from measured protein abundance ratios between samples of equal total cell number.

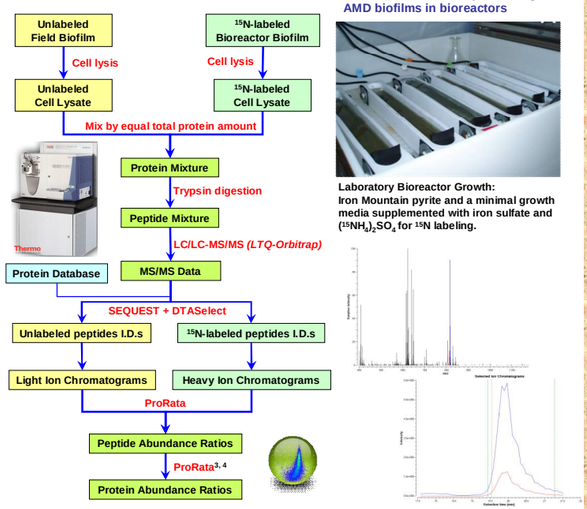
Community Genomics → Qualitative Community Proteomics → Quantitative Community Proteomics

EXPERIMENTAL



A Natural Microbial Community in Acid Mine Drainage[1-3]

- Location: the Richmond Mine in Iron Mountain near the city of Redding in northern California
- Environment: temperature = 35 ~ 57°C; pH = 0.5 ~ 0.9; high metal concentration
- Energy source: pyrite (FeS_2) oxidation $FeS_2 + 14\,Fe^{3+} + 8\,H_2O \rightarrow 15\,Fe^{2+} + 2\,SO_4^{2-} + 16H^+$
- Nitrogen source: N_2 fixation
- Carbon source: CO_2 fixation



Metabolic stable isotope labeling of AMD biofilms in bioreactors

Laboratory Bioreactor Growth:
Iron Mountain pyrite and a minimal growth media supplemented with iron sulfate and $(^{15}NH_4)_2SO_4$ for ^{15}N labeling.

RESULTS AND DISCUSSION

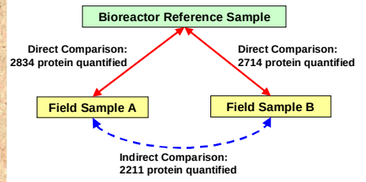
Protein Identification Results of the ^{15}N-labeled Bioreactor Sample

The bioreactor samples provided protein identifications from every major strain in the AMD community, and enabled relative protein quantification with field samples.

RUN1 Species	Proteins	Spectra	% of Total
5way_CG_LeptoII	999	5038	51.16%
fer1	246	693	7.04%
fer2	274	870	8.84%
gpl	248	1874	19.03%
UBA_LeptoII	817	446	4.53%
UBA_LeptoIII	175	797	8.09%
Unass_arch	75	113	1.15%
Unass_bact	22	16	0.16%
Totals	2856	9847	

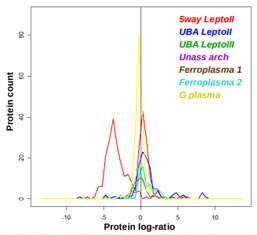
RUN2 Species	Proteins	Spectra	% of Total
5way_CG_LeptoII	983	5026	51.13%
fer1	238	687	6.99%
fer2	263	862	8.77%
gpl	242	1847	18.79%
UBA_LeptoII	798	436	4.44%
UBA_LeptoIII	184	866	8.81%
Unass_arch	67	96	0.98%
Unass_bact	23	9	0.09%
Totals	2798	9829	

Quantitative Proteomics to Compare Field Samples by Common Reference Experimental Design



This common reference experimental design has at least three advantages over a more straightforward design of directly comparing the two field samples with *in vitro* labeling methods.

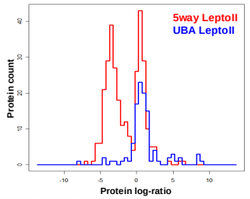
1. Metabolic labeling provides more reproducible and precise quantification results.
2. Systematic biases are canceled out by taking the ratio of two measured ratios.
3. Additional types of field samples can be readily added to the comparison by comparing them to the common reference sample.



Overview of Protein Quantification Results Between Two Field Samples

- Proteins were quantified from all major strains in the AMD community.
- Excellent quantification precision and reproducibility between the duplicates were achieved, due to the metabolic labeling method, the high performance of Orbitrap-LTQ-MS, and the quantification algorithm ProRata.

Strain population Change Inferred by Quantitative Proteomics Results



The AMD microbial community comprises a dominant species, *Leptospirillum II*, and seven other minor species. By assuming the average protein abundance ratio to be 1:1 in a species, we estimated the cell number changes for every species between the two biofilms and normalized the measured protein abundance ratios from a species by its cell number change. There were two *Leptospirillum II* strains in the GS2 biofilm. We found that the cell number for one of the two *Leptospirillum II* strain was reduced by ~16-fold, while that of the other strain remained the same. This raises an interesting question of why only one strain has a great reduced population size.

REFERENCES

1. Baker, B.J. and Banfield, J.F. (2003) *FEMS Microbiology Rev.*, 44, 139-152
2. Ram, R.J., VerBerkmoes, N.C., Thelen, M. P., Tyson, G.W., Baker, B.J. Blake, R.C. II, Shah, M., Hettich, R.L. and Banfield, J.F. (2005) *Science*, 308, 1915-1920.
3. Lo. I, Denef, V.J., VerBerkmoes, N.C., Shah, M.B., Goltsman, D., DiBartolo, G., Tyson, G.W., Allen, E.E., Ram, R.J., Detter, J.C., Richardson, P., Thelen, M.P., Hettich, R.L., Banfield, J.F. (2007) *Nature*, 446, 537-541.
4. Pan, C., G. Kora, D.L. Tabb, D.A. Pelletier, W.H. McDonald, G.B. Hurst, R.L. Hettich, and N.F. Samatova, (2006) *Analytical Chemistry*, 78, 7110-7120
5. Pan, C., G. Kora, W.H. McDonald, D.L. Tabb, N.C. VerBerkmoes, G.B. Hurst, D.A. Pelletier, N.F. Samatova, and R.L. Hettich, (2006) *Analytical Chemistry*, 78, 7121-7131

CONCLUSIONS

- The microbial community found in acid mine drainage can be cultured in laboratory bioreactors, which enables metabolic labeling with nitrogen-15 for quantitative proteomics measurements.
- The proteome of the bioreactor sample was similar to those of field samples.
- Using a ^{15}N-labeled bioreactor sample as the common reference, the proteomes of two field samples can be quantified in remarkable depth (> 2500 proteins) with high quantification reproducibility and accuracy
- Environmental perturbation can lead not only to abundance changes of proteins, but also to population changes of the constituent strains in a community

ACKNOWLEDGEMENTS

- Funding was provided by the DOE Genomics:GTL Program (Office of Science), Department of Energy
- The data analysis work was supported by the "Exploratory Data Intensive Computing for Complex Biological Systems" project from U.S. Department of Energy (Office of Advanced Scientific Computing Research, Office of Science)
- Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725