Application of Proteomics and Lipid studies in Environmental Biotechnology

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Microbial metal reduction

Figure 1: *Desulfovibrio vulgaris* Biofilm on metal surface  (Matthew Fields, Montana state university)

Figure 2: *Shewanella oneidensis* CN32 cells on the surface hematite  (Alice Dohnalkova, Pacific Northwest National Laboratory)

\[
\text{U(VI+)} \xrightarrow{\text{Microbial metal reduction}} \text{U(IV+)}
\]

(Soluble in water)  (insoluble mineral uraninite)
Sulfate and Metal reduction: Biofouling

- Biomagnification of mercury – Methyl mercury formation.
- Biocorrosion
- Biofouling of crude oil
Radioactive and heavy metal waste management

Mining

Stored legacy waste

Medical

Research

Power Plants

Figure credits
www.greatbasinminewatch.org
www.fas.org/irp/imint/doe_hanford_fff_01c.jpg
http://nc.sierraclub.org/images/NuclearPowerPlant.jpg
Desulfovibrio vulgaris Hildenborough

- Sulfate reducing bacterium
- Anaerobic organism
- Found in heavy metal and nuclear waste sites
- Genome was sequenced in 2003

Using Environmental Microbes

Understand the affect of environmental factors on bioremediation potential of *D. vulgaris*.

Apply this knowledge to accelerate the bio-containment of heavy metal waste or limit adverse impact of such organisms in the environment.

**Physiologically Relevant environmental stresses**

- Salt
- pH
- Oxygen
- Nitrate
- Heavy metals

Exposure to Air
Genome sequence indicates the presence of 3480 genes
Functional genomics pipeline

Transcripts

Proteins/ lipids

Metabolites

Biomass production

Analysis
Why proteomics?

Proof of expression/Change

Differential Expression:

Activation State

Localization:

Post translational modifications:

- Secretion
- Membrane Interaction

- Phosphorylation
- Glycosylation
iTRAQ Peptide Labeling Strategy

**iTRAQ Tag**

- **Reporte**r
- **Balance**
- **Peptide**

<table>
<thead>
<tr>
<th>Mass</th>
<th>Reporter</th>
<th>Balance</th>
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<tr>
<td>114</td>
<td>$^{13}\text{C}$</td>
<td>$^{13}\text{C},^{18}\text{O}$</td>
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<tr>
<td>115</td>
<td>$^{13}\text{C}_2$</td>
<td>$^{18}\text{O}$</td>
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<tr>
<td>116</td>
<td>$^{13}\text{C}_2,^{15}\text{N}$</td>
<td>$^{13}\text{C}$</td>
</tr>
<tr>
<td>117</td>
<td>$^{13}\text{C}_3,^{15}\text{N}$</td>
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Denature and reduce

Separation by liquid chromatography

Detection by MS/MS

Tandem LC-MS Proteomics

Long SCX Separation

Salt → SCX Column → Fractions
ACN → RP Trap → RP Column

Quantitation

T0 control

Peptide Sequence

mass/charge
2-D Liquid Chromatography

Strong Cation Exchange (SCX) Separation

Salt  →  SCX Column  →  Fractions

UV detection

Absorbance

minutes

Reverse Phase (RP) Separation

Acetonitrile  →  RP Trap  →  RP Column

Individual fractions

Comparative Proteomics

Total Ion Chromatogram

Quantitation

Peptide Sequence

Sequence: I G S T A D N L J

mass/charge
Oxidative response mechanisms in *D. vulgaris*

Dolla et al J Biotechnol 126:87-100
Rodionov Genome Biol 5:R90.
iTRAQ analysis of 0.1% $O_2$ exposure in *D. vulgaris*

Growth (OD)

Time (hours)

$t = 0$

$t = 5$ hours

Nitrogen

$1000$ppm $O_2$ (0.1% $O_2$)
Use of internal replicates

Graph showing the relationship between Log (V1 Replicate 1) / T0 and Log (V1 Replicate 2) / T0.
Comparison of Stressed and Control
Use of error to determine significant changers
Both transcript and protein data pointed to PerR

DVU2826: hypothetical protein
*DVU2247: alkyl hydroperoxide reductase C
*DVU2318: ruberythrin, putative
DVU2121: response regulator
*DVU3093: rubredoxin-like protein
DVU0267: hypothetical protein
DVU0024: conserved hypothetical protein
DVU2681: hypothetical protein
*DVU0772: hypothetical protein
*DVU3094: ruberythrin
DVU0264: Transmembrane complex, ferredoxin, 2 [4Fe-4S]
DVU0259: DNA-binding response regulator
Response to low O₂

0.1% O₂

H₂O₂ + Fe²⁺ → OH⁻ + HO⁻ + Fe³⁺

CydAB

Redox enzymes

SodB

O₂⁻

H₂O₂

H₂O

H₂O₂

Kat

O₂ + H₂O

H₂O

RoO

H₂O

Rub

Ngr

PerR

Rdl

Hmc

Cyt C₃

Hyd

H⁺ + e⁻

H₂

Increasing

Observed

Not seen

Some interesting hypothesis to follow up on

- *D. vulgaris* adapted to occupy sub-oxic environment?

- Per regulon in *D. vulgaris* provides additional mitigation over and above SOR and SOD based activity

- The *D. vulgaris* Per regulon may contain additional members than initially predicted

- And can be experimentally tested
Importance of Cell wall in environmental stresses

- The cell wall presents the first line of defense for microorganisms
  - Salt stress/ cold stress/ heat/ solvent
  - Optimal homeoviscococity is determined by the distribution of fatty acids in the cell wall and readjusted as the environment changes
Fatty Acid distribution

Schematic of Cell wall (Gram negative)

- O-specific side chain
- Porin
- Braun’s lipoprotein
- Lipopolysaccharide
- Outer membrane
- Periplasmic space and peptidoglycan
- Plasma membrane

Fatty Acid Structure

- Carboxyl group
- Hydrocarbon chain
- Saturated fatty acids
- Mixture of saturated and unsaturated fatty acids
FAME analysis for PLFA quantification

PLFA extraction → Methyl Esterification → GCMS detection and quantification

Note: The Phospholipid Fatty acid (PLFA) distribution of a microbe is unique
Salt stress in *D. vulgaris*

Control : No stress
Stress : 250 mM NaCl
Integrating Functional Genomics

Proteomics

Transcriptomics

Metabolomics

Lactate→Pyruvate→Acetyl-CoA
Acetyl-P→Acetate

0 5 10 15 20 25 30
Time [min]

Asp  Phe  Glu  Pro  Ile  Leu  Lys  Arg  Val  His  Met  Asp

ESPP2

Environmental Stress Pathway Project

DOE GENOMICS-GTL
ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT
U.S. DEPARTMENT OF ENERGY
Uptake of Osmolyte is a primary mechanism of stress response

But we were missing some important aspects of cell wide data
Relative change of the 8 major types PLFA after stress
Changes in PLFA to increase fluidity are documented for many bacteria in both Salt and Cold stress.

Increase in branched PLFA also reduces packing and increases fluidity of the membrane.
Salt stress in *D. vulgaris*

Mukhopadhyay et al. J Bacteriology (2006) 4068-4078; 188
PLFA Studies

• Not only is a change in PLFA profile a significant marker of stress response

• PLFA studies can help ID microbes in an ecosystem and provide a excellent HT method to assess the biotechnological potential of a microbial community for a particular function
Environmental Stress factors

- Soil Stressors:
  - SO₄
  - Organics (Humic Acids)
  - Ionic Strength
  - O₂
  - NO₃
  - pH
  - Eh

- Vadose Zone:

- Groundwater Stressors:
  - pH
  - Eh
  - Temperature
  - Flow Rate
  - Oxygen (aerobic vs. anaerobic)

- Aquifer Key:
  - Microbe Survival
  - Migration

- Contaminant Plume:
  - LNAPLs
  - DNAPLs
Summary

• Systems biology approaches allow us to obtain cell wide data that can be used to build models for how an organism interacts with its environment.

• These models provide the foundation to build bio-remedial approaches in the environment.
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ESPP (vimss.lbl.gov)

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