Proteomics Approaches in Applied microbiology

Hundreds of microbes have been sequenced
• These sequences encode many valuable biological phenomena
  – Global elemental cycling
  – Novel pathways to convert cellulose to simple sugars
  – Novel pathways to convert sugars to bio-fuels
  – Pathways to degrade pesticides
  – Create engineered plants
  – Antibiotic resistance
    • etc

http://genome.jgi-psf.org/mic_home.html

Other reasons to study microbial physiology.

- Biofouling
- Activation of toxic metals
- Pathogenesis
- Virulence and crop disease

Heavy Metal Waste

- Radioactive and heavy metal waste is a problem
  - Expected cleanup costs in US alone total $300 billion with current techniques
  - Metal reducing bacteria can precipitate heavy metals, and thus halt their movement through the environment. Therefore, bioremediation is of interest.
- *Desulfovibrio vulgaris* has the ability to reduce several heavy metals including uranium, chromium, and iron.

**Desulfovibrio vulgaris Hildenborough**

- Sulfate reducing bacterium
- Anaerobic organism
- Genome sequence available

**Goal:**

Develop better cellular models to understand bioremediation potential

**Transfer of Cellular Information**

- DNA
- mRNA
- Proteins
- Metabolites
A high throughput method to analyze proteins

In 2002 John Fenn and Koichi Tanaka won the Nobel prize.

“for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules”

Today almost all proteomics tools rely on mass spectrometry
Mass Spectrometry

Ion Sources make ions from sample molecules

E.g: Electrospray ionization:

- **Pressure = 1 atm**
- **Inner tube diam. = 100 um**
- **Sample Inlet Nozzle (Lower Voltage)**
- **Sample in solution**
- **N₂ gas**
- **High voltage applied to metal sheath (~4 kV)**
- **Charged droplets**
- **Partial vacuum**
- **MH⁺**
- **MH₂⁺**
- **MH₃⁺**
Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

- Operate under high vacuum (keeps ions from bumping into gas molecules)
- Actually measure mass-to-charge ratio of ions (m/z)
- Key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds exist: for bioanalysis, quadrupole, time-of-flight and ion traps are most used.

Peptide parent ions can be fragmented for MS/MS data

Peptides produce unique ion series that can be matched to theoretically predicted ion series for a given sequence

Peptide sequences are used for protein identification.
Transfer of Cellular Information

DNA → mRNA → Proteins → Metabolites → Proteomics

A Vast Dynamic Range
Not All Proteins Are Created Equal

- Size
- Abundance
- Physical properties
- Associated with membrane

Protein Digestion

\[
\text{~4000 proteins} \times \frac{5-10 \text{ peptides}}{\text{protein}} = \text{~10,000 unique peptides (at least)}
\]
2-D Liquid Chromatography

Strong Cation Exchange (SCX) Separation

Reverse Phase (RP) Separation


Proteomics

Proteomics

- Most high throughput proteomics requires availability of a genome sequence
- Predicted list of proteins and corresponding amino acid sequences are used to create theoretical databases which are used to analyze MS and MSMS spectra
- However, de-novo sequencing of MS data can allow identification of proteins from unsequenced bacteria.

Protein Activity is Dependent On

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Activation State</th>
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- Differential Expression:

<table>
<thead>
<tr>
<th>Localization:</th>
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<tbody>
<tr>
<td>Secretion</td>
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<tr>
<td>Membrane Interaction</td>
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<tr>
<th>Post translational modifications:</th>
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<tr>
<td>Phosphorylation</td>
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<tr>
<td>Glycosylation</td>
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</table>
Artemisinin

Pathways for isoprenoid precursor biosynthesis

Glycolysis

G6P → FDP

DHAP → G3P

PEP → PYR

AcCoA → MEV → IPP

Monoterpenes

Sesquiterpenes

Diterpenes

Mevalonate pathway

TCA Cycle

DXP pathway

Artemisia annua
Artemisinin metabolic pathways

Engineering *E. coli*’s native pathway improves production

Three-fold improvement in production
Recruiting the mevalonate pathway from yeast

The yeast mevalonate pathway improves yields ~90-fold
Optimizing the bottom part of the mevalonate pathway

\[
\text{MevT} \quad \text{atoB} \quad \text{HMGS} \quad \text{tHMGR} \quad \text{Mevalonate}
\]

\[
\text{MBIS} \quad \text{MK} \quad \text{PMK} \quad \text{MPD} \quad \text{idi} \quad \text{ispA} \quad \text{FPP}
\]


Experimental Design

- Control 1
- Control 2
- Stress/mutation
- Engineered strain

Growth vs. Time (hours)
iTRAQ Peptide Labeling Strategy

<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>$^{12}\text{C}$, $^{18}\text{O}$</td>
</tr>
<tr>
<td>115</td>
<td>$^{13}\text{C}_2$</td>
<td>$^{18}\text{O}$</td>
</tr>
<tr>
<td>116</td>
<td>$^{13}\text{C}_2$, $^{15}\text{N}$</td>
<td>$^{12}\text{C}$</td>
</tr>
<tr>
<td>117</td>
<td>$^{13}\text{C}_3$, $^{15}\text{N}$</td>
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Quantitative Proteomics

Sequence: IGS TADNLJ
Nitrate Exposure

- Few changes in nitrate reduction pathway
- Increases in salt stress mechanisms
- Increases in oxidative stress machinery

Model for NaCl Stress

Mukhopadhayay, A, Redding, AM, et al., J. Bacteriology, 2006; 188(11):4068-78
Summary

• Proteomics is an important component of cellular study
• Relative quantification of proteins increases our understanding of cellular pathways
• Understanding cellular pathways enables better cellular engineering

Transfer of Cellular Information
**Summary**

- Cell wide studies are required to observe non-obvious effects on pathways distant from the engineered system/stress.

- Integration across multiple experiments is required to develop testable hypothesis
  - Cell wide studies at various levels
  - Comparative analysis of multiple stress

- Omics studies provide the starting point for further analysis.