Ken Nealson
Wrigley Professor of Geobiology
USC

*SHEWANELLA* and Genomes to Life !! THE FUTURE!!

WHERE ARE WE GOING?

HOW WILL WE GET THERE?

WHAT ARE THE CHALLENGES AND TRAPS?
Genomes to Life: Shewanella and the future !!

Genomes & Genomics: For sake of this discussion, I include

Genome composition, gene expression, & metabolism

Genomics ➔ Physiology ➔ Ecophysiology

Ecology

Predictable Community Behavior
Successful Manipulation of Natural Communities
Shewanella in the future:

Short Term: Genomic/Proteomic/Metabolic Connections
Linkage of physiology to genomic information

Mid Term: Ecophysiology
Questions regarding regulation of MR-1
How does the cell”work”?
Linkage of laboratory to microcosm and field data

Long Term: Community structure and activities
Genetic variability and use of genomic approaches
Predictable community ecology
The “old view” of *Shewanella oneidensis*

Gamma Purple proteobacteria

MR-1; when Isolated was One of ~10, Now >50!
The “new view” of *Shewanella*

Now MR-1 is again one of 1, although a strain of *S. benthica* is almost finished by a Japanese group (JAMSTEC)
Excitement of the “new view”:

May be able to use this information to dissect specific aspects of both ecology and evolution:

Ecology:
Involved in many different redox processes
Aerobic and anaerobic niches
Metal cycling connected with carbon cycling
Potential for dealing with many toxic metals and radionuclides

Can we understand *Shewanella* well enough to begin to use it?

what it does
how it does it
how it regulates
how it interacts with other organisms

All of this well enough to make predictions that work.
Dangers of the “new view”

1. We forget that it is what it does that counts, rather than what its potential is; clearly it is capable of doing many different things – which will it do, and when?

2. We forget that surface attachment may be vital for expression of some of its functions.

3. We forget that it seldom lives alone

4. We forget that there are many species of this genus, and that they may exhibit fundamental differences.
Starting Cultures

IT’S WHAT IT DOES THAT COUNTS!!

Pure Culture on MnO₂

Five Days Incubation

Breathing Mn oxide!

Form  Serine  Lactate
With this kind of versatility, what will it really do?
This kind of insight helps us frame the questions that we know we need to answer. Need constant feedback from Federation for this!

Start with sets of Conditions:
1. Nutrient limitation (C,P,N,S)
2. Electron donors (hydrogen, formate, lactate, serine)
3. Electron acceptors (O_2, NO_x, metals, etc.)

Process measurement
   Oxygen metabolism
   Nitrate uptake
   Metal reduction
   Growth rate (DNA, RNA, protein synthesis)
   Specific synthesis of cytochromes
Table 1: Molar Growth Yields and Products Excreted by *Shewanella* growing anaerobically with TMAO as electron acceptor

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Growth Yield&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gen. Time (h)</th>
<th>CO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Acetate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Alanine&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NH&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% C Recov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>17.5</td>
<td>12</td>
<td>2.8</td>
<td>0.0</td>
<td>0.11</td>
<td>0.9</td>
<td>104</td>
</tr>
<tr>
<td>Cysteine</td>
<td>17.5</td>
<td>12</td>
<td>2.7</td>
<td>0.0</td>
<td>0.10</td>
<td>1.1</td>
<td>98</td>
</tr>
<tr>
<td>Lactate</td>
<td>11.5</td>
<td>7</td>
<td>2.0</td>
<td>0.42</td>
<td>0.06</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td>Formate</td>
<td>5.0</td>
<td>13</td>
<td>nd</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>a</sup>Molar growth yield as µg dry weight/µmole of substrate oxidized  
<sup>b</sup>Product excreted is expressed µmol/µmole of substrate oxidized
Surface attachment may be crucial to activities:

Studies of effect of attachment on genomic expression

Complex interactions of *Shewanella* with surfaces
  Gene modulation via and during surface attachment
  Importance of attachment for key reactions

Will require close collaboration between physiology and genomics
Fermentative Communities (complex carbohydrates)

- Lactate
- Formate
- Hydrogen
- Amino Acids

Aerobic Organotrophs and Lithotrophs

- Acetate, NH₃, H₂S, Alanine, TMA, DMS, Fe(II)

Shewanella spp. (anaerobic respiraton)

- Nitrate, nitrite
- Sulfite, sulfur
- Thiosulfate, DMSO
- TMAO, Glycine
- Fe(III), Mn(IV)
- Etc.

Acetate, CO₂, NH₃, Alanine

H₂, CO₂-utilizing communities – methanogens, acetogens
Acetate-utilizing methanogenic community

Shewanella does not live alone!!
Shewanella (and probably all other bacteria!)
SELDOM ARE FOUND ALONE!!

Consider natural partners: need environmental data

Do genomics with and without associated organisms

Expression of key activities
    May want to use mixed cultures for remediation

Genomic indicators in response to other cultures
    May lead to insights regarding regulation
    Cell-cell communication
    Metabolite removal or supply
MR-1 is one of many shewanellae
Now see a large diversity of shewanellae:

Get some sense of genomic variability of Shewanella group

Choose several strains for sequencing

Choose with care and some insight

Goal should be to assess the viability of genomic approach for “real world” work
SUMMARY AND CLOSING THOUGHTS:

1. Immediate future is well defined:
   chemostats and nutrient limitation
   definition of cell regulation
   relationship between genome, proteome, and physiology
   metabolome – need fluxes not numbers!

2. Next steps will involve interactions with environment

3. More difficult endeavors will include:
   community interactions
   diversity within the group
   models of community interactions – predictive ecology

4. Perhaps most important single thing now will be a close link
   between molecular scientists and those doing physiology. We
   need to make sure we are asking the right questions!!
SF Team

- Argonne National Lab
  - Carol Giometti
  - Sandra Tollaksen
  - Gyorgy Babnigg

- BIATECH
  - Eugene Kolker
  - Alex Picone
  - Sam Purvine
  - Brian Tjaden
  - Tim Cherny
  - Alex Nesvizhskii (ISB)
  - Andy Keller (ISB)
  - Serg Stoliar (UW)

- Oak Ridge National Lab
  - Liyou Wu
  - Dorothea Thompson
  - Matthew Fields
  - Yongqing Liu
  - Adam Leaphart

- Michigan State University
  - Jim Tiedje
  - James Cole
  - Joel Klappenbach

- PNNL
  - Jim Fredrickson
  - Alex Beliaev
  - Margie Romine
  - Yuri Gorby
  - Dick Smith
  - Mary Lipton

- University of Southern California
  - Ken Nealson
  - Sasha Tsapin