Invertebrate/Chemosynthetic Symbioses

I. Introduction
   A. Invertebrate/chemosynthetic symbiosis forms basis of entire ecosystem in the deep sea. Dizzying array of associations - can’t cover all of them
   B. Important: That the vent ecosystem is the only on earth that doesn’t depend on light energy - rather on chemical energy
   C. Another key point - good evidence of symbiosis as a key source of metabolic innovation
   D. Has since been discovered that these symbioses exist elsewhere besides vents - where there are appropriate chemical substrates.

II. Hydrothermal Vents and Discovery of vent ecosystems
   A. Deep sea hydrothermal vents were originally discovered by geologists studying plate tectonics and the sea floor. 1500 - 3500 m
      1. Ocean floors are sites of rift zones – areas of spreading of the earths crust
         a) As crust spreads – soft rock rises to fill gap Show slides
         b) Sites of very hot active volcanism (75% of earths volcanos on ocean floor)
      2. Hydrothermal vents are formed at rift zones by seawater seeping into cracks in rock - getting super heated and returning forcefully to crust surface. Show slide
         a) Process results in dissolving compounds into seawater
            (1) Includes high conc of H₂S, H₂, methane, Fe²⁺ and Mn²⁺.
            (2) Water can be up to 400°
   B. Vent communities discovered in 1977 at Galapagos rift and symbiosis discovered in 1979
      1. Oceanographers discovered a rich abundance of invertebrates around these vents were geologists – so didn’t know what to make of the life at first Show slide of Alvin
         a) Initially some speculation on the nature of ecosystem –
            (1) Thought that inverts could be feeding on freeliving chemosynthetic bacteria
            (2) we’ll talk about the discovery that these invertebrates are harboring symbiotic chemosynthetic bacteria.
      2. These assoc. make up the majority of biomass in these areas.
      3. remarkable new diversity: 97% endemism. 22 new families of orgs discovered
      4. Distribution: They occur in a very limited area - concentrations of O₂ and sulfide have to be within a specific range.
         a) O₂ rapidly oxidizes sulfide so sulfide doesn’t persist in water for long distance
         b) Description of environment see table show slide of smokers
<table>
<thead>
<tr>
<th>Water type</th>
<th>°C</th>
<th>µM O₂</th>
<th>mM H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>2</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>Black Smoker</td>
<td>350</td>
<td>0</td>
<td>up to 12</td>
</tr>
<tr>
<td>White Smoker</td>
<td>2 – 20</td>
<td>Up to 110</td>
<td>0-1</td>
</tr>
</tbody>
</table>

c) In most cases orgs are thiotrophs – using sulfur in their metabolism – we’ll see how ahead
   (1) But sulfide is extremely toxic to aerobic orgs – sets up an apparent paradox
5. Productivity is very high, growth is fast - important implications for instability of their environments
6. Communities can be very unstable in space and time – vents can close, sulfide supply disappears – the ecosystem declines very quickly
   a) Average life span from decades to 200 years

III. Cold seep communities and anoxic sediments
A. Subsequently these symbioses have been discovered in
   1. cold seep areas - vary in depth and location but seeps are low temp (ie not hydrothermal) leakage of
      a) sulfide
      b) methane or other hydrocarbons
   2. margin between oxic and anoxic zone in sediments. Will go into detail about this later
B. These two communities differ profoundly from hydrothermal vents. Are dominated by a very different assemblage of organisms - so community structure different from vents - but common thread is symbiosis.
C. Whereas vent communities restricted to very small areas immediately surrounding vents - cold seeps and anoxic sediments can be much, much bigger
D. Productivity is much lower at these sites – sulfide, methane are in much lower concentrations
E. So communities are slower growing but more stable in time and space

IV. Chemoautotrophic bacteria
A. Will first talk about chemosynthetic bacteria - to gain appreciation for their importance in the picture
B. Thiotrophic and methanotrophic bacteria occur free-living - hints at incredible diversity of metabolic strategies present in prokaryotes - euks are very limited by comparison
   1. Members of the bacteria domain
   2. No symbiotic chemoautotrophic bacteria have been cultured - not a lot is known about them - but molecular phylogenetic studies have been done
   3. Show that they do ally with other chemo and methano bacteria - purple sulfur bacteria - likely that they evolved from these. Show slide
   4. Phylogenetics also show that there seems to be specificity between hosts - different hosts are harboring different symbionts more about this after we describe hosts
C. How do these bacteria pay the bills?
   1. these bac are chemoautotrophs
a) autotroph: 1° producers - use light or chemical energy to manufacture sugars, fats, proteins - reduced organic carbon - required for metabolism
b) chemoautotrophs: derive energy from oxidizing compounds

2. Rely on energy derived from oxidizing reduced compounds - either sulfide or various sulfur compounds or methane

3. Oxidation of these compounds provides energy and reducing power to fuel the Calvin cycle

4. Let’s look first at photosynthesis: can be broken into 2 reactions - light and dark **show slide**
   a) Light reaction is where the light comes in - results in energy and reducing power for dark reaction
   b) so ribulose bisphosphate carboxylase oxygenase (rubisco) involved in assimilation of CO₂ into the 2 3-c sugars

5. Now look at chemosynthesis by thiotrophs - sulfur oxidizers: equivalent of the light reaction is the oxidation of sulfide and other sulfur compounds
   a) sulfide oxidation: **show slide**
      (1) Quick review: oxidation is the removal electrons from a compound
      (2) so H₂S is oxidized to SO₄⁻
      (3) In this process, O₂ acts as oxidizing agent (oxidant) - it is the electron acceptor so it is reduced to H₂O
      (4) involves several very specific enzymes:
   b) the dark reaction - involves same pathways, same enzymes
      (1) Rubisco is present
   c) Important: one way to look for the presence of chemosynthesis is to see if can detect enzyme activities involved in these pathways

6. The methanotrophs do a similar oxidation progression but oxidize reduced organic carbon such as methane:
   a) These bacteria use a whole different set of enzymes particular to methane oxidation pathways - could also look for those

V. Discovery of the symbiosis - what was the original evidence for the presence of a symbiosis?
A. Interesting detective story
B. Tubeworms were found to by mouthless and gutless
   1. Possible that they were taking up dissolved organic material (DOM) – but growth rates too high for this
C. Supporting evidence:
   1. Microscopic evidence
      a) Trophosome tissue of worm stained with DAPI - a stain that specifically stains DNA - filled with 3-5 um bodies
      b) Electron microscopy of tissues of a worm showed bacteria-like bodies
         (1) typical gram negative look
   2. Analyzed tissue for lipopolysaccharide - a characteristic component of bacterial cell walls only
   3. Enzymes:
a) Rubisco - lacking in animals but present in photosynthetic or chemosynthetic bac - suggests autotrophy
b) sulfide metabolism - enzymes present in sulfur oxidizing bacteria

VI. Who are the players? And what is their morphology? **See table at end of lecture**

A. Invertebrates: over 100 sp of hosts described to date in a wide diversity of taxa.
   1. We will discuss three different kinds of annelid worms and several different molluscs **show invert slide**

B. Many of these inverts are mixotrophs: they derive their energy using more than one trophic strategy - they might feed **and** get nutrients from their symbionts.

C. All hosts have to deal with problem of supplying sulfide - necessary for chemosynthesis - and handling of toxic compounds - different ways to walk this line - will go into detail later on this for a few

D. Am going to fill out extensive table to help us make sense of diversity
   1. Annelids
      a) Vestimentiferan worms **slides**
         (1) Phylum Annelida, class Polychaeta, Family Siboglinidae.
             Subfamily Vestimentifera
         (2) first significant invert described at vents
         (3) anatomy - thick body wall
             (a) gutless
             (b) plume - high surface area to volume ratio gas exchange area
             (c) trophosome that houses bacteria - highly vascularized - important in delivery of compounds to bacteria - remote from the seawater
                 (i) will see later that transport of O₂ and sulfide in blood critical to functioning of symbiosis
         (4) bacteria housed intracellularly - in bacteriocytes
         (5) specific example: *Riftia*