From the **War Metaphor** to the **Microbial Planet**

Forum on Microbial Threats 12/12/12

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Yale University
News from Yale University:

*Obamadon gracilis*
War Metaphor – Infectious Disease

- **War** – fight the enemy
- **Weapons** that kill – antibiotics
- **Mined** -- microbial world is tapped out for antibiotics
- **Arms race** -- antibiotic-resistance genes evolved in the hospital
Microbial Planet -- Health

• Cooperation – working together to achieve healthy communities and better health

• Complexity of microbial world
  – Variety of habitats and communities
  – Species richness, temporal, spatial variation
  – Vast diversity of as-yet-unculturable organisms

• Communication
  – Signals – communities coordinated by signals
  – Antibiotics – signals in disguise
  – Resistance genes – backtalk?
The Microbial Planet for Human Health

The “environment” as a reservoir of:

- New antibiotics
- Old resistance genes
- New resistance genes?
The Microbial Planet for Human Health

Dogma:
The soil has been fully mined for antibiotics.

True/False?
Novel antibiotic from ordinary culturable soil bacteria
Antibiotic Discovery -- 1993

Structurally novel antibiotic -- zwittermicin

New class of antibiotics – aminopolyols

Novel biosynthetic features
Zwittermicin A Biosynthetic Gene Cluster

NRPS/PKS Components:
- ORF1: PKS/NRPS
- ORF2: NRPS
- ORF3: NRPS
- ORF4: ACP
- ORF5: Acyl-CoA DH
- ORF6: ZmaR (acetyltransferase)
- ORF7: PKS
- ORF8: 3-OH butyryl-CoA DH
- ORF9: ACP
- ORF10: Acyl-CoA DH
- ORF11: Adenylation Domain (Ser)
- ORF12: NRPS/PKS
- ORF13: Monooxygenase
- ORF14: ABC transporter
- ORF15: FkbH
- ORF16: NRPS
- ORF17: Type II Te
- ORF18: NRPS
- ORF19: NRPS
- ORF20: Ppant Transferase
- ORF21: LacI family
- ORF22: Sensory regulator
- ORF23: Hydrolase
- ORF24: Oxidoreductase
- ORF25: Membrane Protein
- ORF26: Carbamoyltransferase (VioL)
- ORF27: VioB (DAP synthase)
- ORF28: VioK (Amidotransferase)

Modifying Enzymes:
- ORF13: Monooxygenase
- ORF14: ABC transporter
- ORF23: Hydrolase
- ORF24: Oxidoreductase

Resistance:
- ORF6: ZmaR (acetyltransferase)

Regulation:
- ORF21: LacI family
- ORF22: Sensory regulator

Transport:
- ORF14: ABC transporter
- ORF25: Membrane Protein

Chan et al., 2006. PNAS 10:14349-14354
Polyketide Synthesis

LM  M1  M2  M3  M4  M5  M6

AT  ACP  KS  AT  ACP  KS  AT  ACP  KS  AT  ACP  Te

S-CoA  Starter unit: Propionyl-CoA

S-CoA  Extender units (X6): (2S)-Methylmalonyl-CoA

6-Deoxyerythronolide B

Erythromycin A
Novelty of Zwittermicin Synthesis

- Requires ~60 genes
- Non-ribosomal peptide synthetases
- Polyketide synthase
- Previously, four extender units for polyketide synthesis known
- Zwittermicin pathway introduced two new extender units (amino-malonyl-CoA; hydroxy-malonyl-CoA)
Zwittermicin Biosynthesis

Emmert et al., 2004
Zwittermicin is Produced by *Bacillus cereus*

common soil bacterium
readily cultured
$10^4$ zwittermicin-producing *B. cereus* per gm soil
Why was zwittermicin missed for 50 years?

- Source -- isolated from an alfalfa root in Arlington, WI

- Screen – oomycete inhibition
Chromista
  Straminipila
    Oomycetes

Plants

Fungi

Animals

Myxomycota

Plasmodiophoromycota

Bacteria

Archaea
Inhibition of Phytophthora
The Small World Initiative
The Microbial Planet for Human Health

The “environment” as a reservoir of:

• New antibiotics
• Old resistance genes
• Potential resistance genes?
What is the potential pool of antibiotic resistance genes?

- Evolution beyond the hospital
- In culturable bacteria?
- In non-cultur-able bacteria?
- Unrecognizable by sequence?
- Movement to pathogens from whom?
Culturing as a Bottleneck

• The vast majority of bacteria in the environment are not readily culturable in the lab
• In soil, culturable bacteria represent ~1%-01% of community
• Unculturables diverge phylogenetically from culturables
How do we obtain access to the resistance genes in unculturable bacteria?
Metagenome
The collective genomes of an assemblage of organisms

Metagenomics
The genomic analysis of an assemblage of organisms

Functional metagenomics
Identification of genes from metagenome by functional expression

Handelsman et al., 1998
Rondon et al., 2000
Handelsman, 2005
Collect soil

Extract DNA

Digest

Ligate into vector

Transform *E. coli*

Screen transformants
Resistance genes from soil form a new clade of acetyltransferases from soil in Alaska and Wisconsin.

*Environ Micro.* 6(9), 981–989

**Phylogenetic Analysis of AAC(6’) Genes**
Unusual motifs for resistance to kanamycin, amikacin, & sulopenem

kanamicin

amikacin

imipenem

sulopenem
The Microbial Planet for Human Health

The “environment” as a reservoir of:

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Metagenomic Analysis of B-lactam Resistance

Expressed resistance genes -- soil and microbial mat

Ampicillin, cephalosporin -- ~ 10-100 clones per 1 Gbp of DNA

Sulopenem – none or 1 per 1-10 Gigabase of DNA

Only reported resistance determinant in clinical isolates encodes KPC-2 (B-lactamase)
**Sulopenem resistance gene from microbial mat in Puerto Rico in *E. coli***

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Map of imiCG1 subclone

- Ferritin gene – 62% identity to *Clostridium* ferritin
- Iron-binding protein in many bacteria;
- Storage function;
- Possible oxidation activity – Fe$^2+$ to Fe$^{3+}$
Sulopenem resistance gene from microbial mat in Puerto Rico in *E. coli*

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No effect of *E. coli* ferritin, similar activity from other Firmicutes.
**Ferritin clone**

_E. coli_ EPI 300 containing sulopenem resistance gene
left, LB
right, LB amended with 100 uM Fe$^{2+}$
Summary

• Zwittermicin represents a new class of antibiotics and increases known polyketide extender units by 50%
• More opportunity for discovery of natural products from environmental bacteria
• Metagenomics provides access to antibiotic resistance genes in uncultured environmental communities
• Housekeeping genes can offer resistance
• Is the high frequency of these genes in host-associated communities a problem?