Forest Rohwer

Grew up on a cattle ranch in Idaho

BAs in Chemistry, Biology & History

PhD in Molecular Immunology

Professor of Biology at SDSU

~200 peer-reviewed pubs in areas of environmental virology, coral reefs, Arctic ecosystems, human health, immunology, bioinformatics, etc...

mainly known for developing metagenomics

run a large lab with ~20 people

12-year old daughter at Roosevelt Middle School
What are phage?
## Abundance of organisms in 1 ml of seawater

<table>
<thead>
<tr>
<th>Organism</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses/Phage</td>
<td>10,000,000</td>
</tr>
<tr>
<td>Heterotrophic Bacteria</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Photosynthetic Bacteria</td>
<td>100,000</td>
</tr>
<tr>
<td>Protozoa</td>
<td>4,000</td>
</tr>
<tr>
<td>Algae</td>
<td>3,000</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>&lt;&lt;1</td>
</tr>
</tbody>
</table>

No Fish or Whales...
Viruses, mostly phage, are the most abundant & diverse life forms on the planet.

10 viruses per cell in all ecosystems

~10^{31} viruses on the planet
Phage are viruses that kill Bacteria... there are also viruses that infect Archaea & Eukaryotes.

Lytic versus lysogenic behavior

Microbes = Archaea & Bacteria
About 10 virus-like particles (VLPs) per microbial cell in all ecosystems
- most are phage that kill Bacteria

Capture microbes and VLPs on 0.02 Anodisc
↓
Stain with SYBR-Gold
↓
Count with epifluorescent microscope
Humans & Coral Reef Decline

Coral diseases are positively correlated with human populations

Coral disease are not correlated with percent coral cover, suggesting opportunistic pathogens

Humans do lots of things that might negatively impact corals, including fishing, dump of sewage, runoff, etc...

Is the decline just "death by a million cuts" or is there an underlying mechanism?
Healthy reef

Shark Shunt

CCA (coralline crustose algae)

microbes

turfs

macros

grazing

predation

doc
Humans kill the sharks & groupers

Williams et al. (2011) J Mar Biol
Fished reef

DDAM - DOC, disease, algae, microbes - positive feedback that increases space for algae

- lots more microbes including potential pathogens

DDAM

- DOC, disease, algae, microbes
- positive feedback that increases space for algae
Global survey of microbial dynamics on coral reefs

- Diver-deployable Niskins
  - Count microbes & measure DOC

- Quibits for metagenomes
  - Sequencing

- Benthic surveys & fish counts

Over 200 sites from central Pacific (Line Islands & USA protectorates), Sri Lanka & Caribbean
Healthy reef

These microbes are starving...

Fished reef

These microbes are fat & happy!

Increased microbial loads & opportunistic diseases (e.g., virulence factors)
Phage & protists control microbial abundances

- typically about 10 phage:cell -
Viral & microbial abundances do not linearly scale on coral reefs

Viral predation pressure is decoupled from mass action driven interactions

Viral predation pressure is decoupled from mass action driven interactions
Lytic versus temperate life cycles for viruses

Lysogen is a cell containing a prophage/provirus

Most bacterial pathogens are lysogens

Prophage carry virulence factors
Phage communities are more virulent at higher cell abundances found on degraded coral reefs (Nature 2016)

Hypothesis: Coral disease is an emergent property
How Phage Kill a Person

Mike Furlan
CFer researcher

initiated & supported this work for 10 years

15 peer-reviewed pubs

worked out virome methods for CF sputum

killed by complications from CF in 2015
Mutations in CFTR mess with mucus structure

Inefficient mucus clearance in CF airways encourages polymicrobial infections

phage, bacteria & fungi colonization
Isolation of viral & microbial communities from sputum

Homogenization and filtration

CsCl density gradient centrifugation

QC using epifluorescence microscopy & 16S/18S PCR

Microbiomes
(plus lots of human DNA)

Viromes

Bioinformatics & Stats

This is a year of your life that you will never get back...

Next Gen Sequencer
Microbial phenotypes encoded by CF phage mucoidy

antibiotic resistance

virulence factors (e.g., exotoxins)
Case study using -omic approaches

Background: CF patient with a Climax community of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. History of mild to major exacerbations 1-2X a year that responded to antibiotic treatments. Lung function recovered after treatments as measured by FEV1.

Days 0-7: Start of a major exacerbation that reduces lung function by half within one week. Lung function does not recover after aggressive antibiotic treatment. Clinical lab cultures *Pseudomonas aeruginosa* & *Stenotrophomonas maltophilia*. In home oxygen is prescribed.

Day 8 & 9: Two samples collected for metatranscriptomics & metabolomics.

Day 9: Patient sent to emergency room and then ICU. Treated with steroids.

Day 10: Patient released from ICU.

Day 11: Patient sent back to ICU; does not respond to steroids second time. Chest X-rays show rapid fluid accumulation.

Day 12: Patient intubated. Lavage is blood and lymph.

Day 13: Patient continues rapid decline, transplant is ruled out & life support is removed.
What happened?

Metatranscriptomes (i.e., the RNAs) were confusing at first...

1) Taxonomy (i.e., 16S rRNAs) showed that *E. coli* STEC B2F1 was extremely abundant.

2) Functional analyses (i.e., the expressed genes) were only from *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Taxonomy</th>
<th>Frequency</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli STEC B2F1</td>
<td>Bacteria &gt; Proteobacteria &gt; Gammaproteobacteria &gt; Enterobacteriales &gt; Enterobacteriaceae &gt; Escherichia &gt; Escherichia coli</td>
<td>2591</td>
<td>93.85</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa PACS2</td>
<td>Bacteria &gt; Proteobacteria &gt; Gammaproteobacteria &gt; Pseudomonadales &gt; Pseudomonadaceae &gt; Pseudomonas &gt; Pseudomonas aeruginosa</td>
<td>2317</td>
<td>4.68</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia K279a</td>
<td>Bacteria &gt; Proteobacteria &gt; Gammaproteobacteria &gt; Xanthomonadales &gt; Xanthomonadaceae &gt; Stenotrophomonas &gt; Stenotrophomonas maltophilia</td>
<td>970</td>
<td>1.47</td>
</tr>
</tbody>
</table>
*E. coli* STEC B2F1 is a Seriously Nasty Attack Bacteria

Produces Shiga toxins (Stxs) - also called verotoxins

Stx2-converting phage 933W, which produces Stx when induced (e.g., antibiotics)

Reported to cause hemolytic-uremic syndrome (HUS)

Stxs also cause attaching and effacing (A/E) lesions

Carry hemolysin-encoding plasmid
Working hypothesis: Patient was infected by *E. coli* STEC B2F1. Antibiotic treatments in the first week killed this pathogen, but initiated a HUS-like response in the lung.

The *E. coli* STEC B2F1 only showed up in the taxonomy because we were detecting legacy 16S rRNAs (confirmed).

Clinical lab never cultured *E. coli* STEC B2F1 because they were not looking for it.

After *E. coli* STEC B2F1 was removed by antibiotic treatment, the patient's normal Climax community was re-establishing. This is why we observed mRNAs from *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. 
One Big Problem...Stx should not kill lung tissue because the cells do not express the receptor Gb3
The interaction of Shiga toxins 1 and 2 (Stx1 and Stx2) with endothelial cells is an important step in the renal coagulation and thrombosis observed in hemolytic uremic syndrome... **bacterial neutral sphingomyelinase (SMase)** rapidly (1 h) sensitized human dermal microvascular endothelial cells (HDMEC) to the cytotoxic action of Stx2. Exposure of endothelial cells to neutral SMase (0.067 U/ml) caused a rapid increase of intracellular ceramide that persisted for hours. Closely following the change in ceramide level was an increase in the expression of globotriaosylceramide (Gb3), the receptor for Stx2... These results describe a rapid response mechanism by which extracellular neutral SMase derived from either bacteria or eukaryotic cells may signal endothelial cells to become sensitive to Shiga toxins. - Obrig et al. (2003) Infection & Immunity

**Pseudomonas**-derived sphingomyelinase C was in patient's metatranscriptome
Metabolomes show that sphingomyelin & ceramide very high in exacerbation samples

Pseudomonas-derived sphingomyelinase C converted human sphingomyelins to ceramide...
The interaction of Shiga toxins 1 and 2 (Stx1 and Stx2) with endothelial cells is an important step in the renal coagulation and thrombosis observed in hemolytic uremic syndrome. Bacterial neutral sphingomyelinase (SMase) rapidly (1 h) sensitized human dermal microvascular endothelial cells (HDMEC) to the cytotoxic action of Stx2. Exposure of endothelial cells to neutral SMase (0.067 U/ml) caused a rapid increase of intracellular ceramide that persisted for hours. Closely following the change in ceramide level was an increase in the expression of globo tria osylceramide (Gb3), the receptor for Stx2. These results describe a rapid response mechanism by which extracellular neutral SMase derived from either bacteria or eukaryotic cells may signal endothelial cells to become sensitive to Shiga toxins. - Obrig et al. (2003) Infection & Immunity
Days 0-7: Start of a major exacerbation that reduces lung function by half within one week. Lung function does not recover after aggressive antibiotic treatment.

Clinical lab cultures *Pseudomonas aeruginosa* and *S. maltophilia*.

Day 9: Patient sent to emergency room and then ICU. Treated with steroids.

Days 10-13: Stops responding to steroids second time.

Chest X-rays show rapid fluid accumulation. Lavage is blood & lymph.

Some time previous to this exacerbation, patient acquired an *E. coli* with the stx-encoding phage. Treatment with antibiotics induce phage & Stx production.

Stx starts inducing massive tissue damage. Steroids temporarily slow down inflammation & antibiotics attenuate Climax community.

Climax community starts to re-establish & expresses spingomylinase. This leads to ceramide to Gb3 production & Stx sensitivity.

Stx induced trauma continues until lungs effectively liquefy.
Trying to get this "-omics" knowledge to the doctors in 24 hours
What STEM skills & knowledge

All of them...seriously

What I do everyday...

(Writing)
Biology
Chemistry/biochemistry
Stats
Math
Engineering
Physics
What can teacher do to support students interested in my field?

Teach the Rules of STEM subjects, not specifics

Quit with the doom & gloom! Humanity is doing very well & the kids need to know...nothing worst than all nature shows ending with some downer. Get them interested/fascinated in the beauty of the natural world. They can be bitter and disillusioned without your help.

Encourage them to get out of the class...seriously, life experience is amazingly important (SCUBA diving, running a boat, fishing, hunting, etc). They need to develop common sense. These skills will also get them into grad school.
What do we do for outreach?

Internships in the lab
   - both students & teachers

Diversity course in Indonesia
   - HBC students learn to dive & do marine biology

Science-art projects