DRUG FROM THE DARK: POTENTIAL OF CAVE MICROORGANISMS IN DRUG DISCOVERY
CHEEPTHAM’S LABORATORY

- Current Students (MSc, Honours, Directed, and CUEF programs)
  - Baylee Out, Alison McClean, Raniyah Alnadhi, Hayfaa Bibi Zafiirah Golapkhan.

- Former Students (MSc, Honours, Directed, and CUEF programs)
COLLABORATORS

Dr. Ken Wagner: TRU
Dr. Kingsley Donkor: TRU
Koent Watson: TRU
Dr. Prenilla Naidu: RIH
Dr. Julian Davies: UBC
Dr. Gerry Wright: McMaster
Dr. Jianping Xu: McMaster
Dr. Edith-Blondel-Hill: UBC/KGH
Dr. Pieter Dorrestein: UCSD
Dr. Arjun Banskota: NRC, Halifax
Sarah Boyle: Mount Revelstoke and Glacier National Parks

12/30/2013
Funds and Permit (Cave Microbiology Projects)

- TRU Research Innovation Fund
- TRU Faculty of Science
- SfAM Student into Work Grant
- Ministry of Natural Resource Operations (Wells Gray Provincial Park Use Permit #102172)
CAVE EXPLORATION

- Phil Whitfield and the BC Speleological Federation members
  - Lorna and Adrian Duncan
  - David Wall
- Greg Horne: Jasper National Park of Canada
- Nicholaus Vieira
- Christian Stenner
- Jesse Martin
- Frank Ritcey: Kamloops Naturalist Group
- Rob Countless
ANTIBIOTIC RESISTANCE SURVEILLANCE PROJECT

• Thanks to:
  • Drs. Ken Wagner, Gerry James, Raymond Maung, Gwen Stephens (former RIH), Lei Ang (former RIH), Blondel-Hill (KGH) and Lisa Steele. Sue Whitehead and staff at RIH Pathology Department-Microbiology Lab. Wendy Cummer, Linda Koo and Haleh Bahrami from LifeLabs Kamloops/Burnaby. Carolynne Fardy of TRU Microbiology Lab.

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  • Dr. Ken Wagner and his wife, Charlotte Wagner.
  • Kamloops Pathology Associates (2013)
  • Interior Health (2011-2012).
  • Raj Parmar, bioMérieux Canada Inc.
  • SfAM Student Into Work Grant
CAVES AS EXTREME HABITATS

- Limited to completely no light
- Limited nutrients (organic matters)/Low-biomass
- Stable temperature (often low all year long)
- High in minerals (both concentration and type)
- High/low humidity (often high 90-100%)
- Close to almost-close system
  - Low to no exchange to outside environment
- Accessibility
CAVE STUDY AT TRU

WHY DO WE STUDY CAVE MICROBIOLOGY?
WHAT HAVE BEEN DONE?
WHAT DID WE FIND? AND WHERE ARE WE GOING?
WHY IS IT IMPORTANT TO STUDY CAVE MICROBIAL DIVERSITY?

• Poorly understood extreme environments
• Microbial contribution to cave ecology
  • Cave formation/degradation/conservation
  • Biomineralization and bioprecipitation (secondary mineral deposits/speleothems)
• Astrobiology
  • Such environments: similar to earlier environments on Earth
  • Clues to earlier microbial life on Earth and other Planets
• Applications in Science in particular biotechnology
• Microbial evolution
WHY IS THE SEARCH FOR NOVEL COMPOUNDS FROM MICROBES STILL ALIVE AND WELL IN 2013?

Clatworthy et al. 2007
RESEARCH QUESTIONS

• Can new drugs be found in the volcanic caves?
• What are volcanic cave microbial communities?
• What are the roles of cave microbes in cave formation/cave degradation?
• What are unique metabolic systems in cave microbes that allow them to survive and thrive in such conditions?
RESEARCH APPROACHES

- Culturable based method: conventional and low nutrient isolation
  - Different isolation media and favourable conditions
- Nonculturable based method (metagenomic study)
- Chemotaxonomic study and phylogenetic analysis
  - 16S rRNA sequencing
- Screening for antimicrobial agents
  - Target panel: a number of multi-drug resistant microorganisms
- SEM of cave samples
- Chemical analyses of cave samples
  - Atomic Adsorption Spectrometry/CE analyses/Maldi-TOF
HELMCKEN FALLS CAVE, WELLS GRAY PROVINCIAL PARK, BC

Source: http://www.infoplease.com/atlas/region/britishcolumbia.html

Helmcken Falls Spring 2009
SAMPLE COLLECTION AT THE HELMCKEN FALLS CAVE
Cave contour mapping was compiled in ArcView 3.2 from bearings, distances and slopes taken from Helmcken Falls cave (Kent Watson).

Approximate cave height (5m), length (72m), and width (20m).
CAVE ACTINOMYCETES ISOLATION

Enumeration of actinomycetes, bacteria, and fungi isolated from cave samples

Hickey Tresner Agar  Bennett’s Agar  SCNA agar  Actinomycete agar
UMPGA-based phylogenetic tree of the cave bacteria

The 27F/1492R primers were used to amplify the 1400bp bacterial 16S rRNA gene. Sequences generated in this study (1,000 to 1,400 bp long) were submitted to BLAST and RDB to identify the closest relatives.
<table>
<thead>
<tr>
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<th><strong>UV exposure</strong></th>
<th><strong>Without UV</strong></th>
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<tbody>
<tr>
<td>1</td>
<td><em>M. luteus</em></td>
<td>6 (1.5%)</td>
</tr>
<tr>
<td>2</td>
<td>MRSA</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td>3</td>
<td><em>M. smegmatis</em></td>
<td>21 (5.25%)</td>
</tr>
<tr>
<td>4</td>
<td><em>E. coli</em></td>
<td>7 (1.75%)</td>
</tr>
<tr>
<td>5</td>
<td>ESBL <em>E. coli</em></td>
<td>1 (0.25%)</td>
</tr>
<tr>
<td>6</td>
<td><em>A. baumannii</em></td>
<td>3 (0.75%)</td>
</tr>
<tr>
<td>7</td>
<td><em>P. aeruginosa</em></td>
<td>19 (5.97%)*</td>
</tr>
<tr>
<td>8</td>
<td><em>K. pneumoniae</em></td>
<td>87 (27.35%)*</td>
</tr>
<tr>
<td>9</td>
<td><em>C. albicans</em></td>
<td>33 (8.25%)</td>
</tr>
</tbody>
</table>

Note: Plug assay was conducted for this preliminary screen. Four hundred cave actinomycetes were isolated. *Only 318 isolates tested.
CHEMICAL ANALYSES OF CAVE SAMPLES

The most abundant elements found in our 15 samples tested
The second most abundant elements in our 15 samples tested.
The least abundant elements found in our 15 samples tested.
“LIMESTONE AND BASALT ARE OFTEN RICH IN REDUCED SULFUR, IRON, AND MANGANESE”
SEM OF A POPCORN SAMPLE
SEM OF A FLOOR ROCK SAMPLE
SEM OF A CEILING ROCK SAMPLE
EXAMPLES OF MICROBIAL MATS FROM A LAVA CAVE IN HAWAII
(NORTHUP ET. AL. 2011)
Cure from the cave: volcanic cave actinomycetes and their potential in drug discovery

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The effects of UV light on the antimicrobial activities of cave actinomycetes

Devon Rule and Naowarat Cheeptham*
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Abstract: The goal of this study was to determine whether actinomycetes isolated from a volcanic cave in western Canada could produce novel antimicrobial compounds against six multidrug-resistant pathogens when exposed to UV light. One hundred and seventy-six actinomycete strains isolated from Helmcken Falls Cave, Wells Gray Provincial Park, BC, were screened against six pathogens using the “plug assay” in UV light and no light conditions. Of the 176 strains tested, 100 or 57% of the cave actinomycete strains had antimicrobial activities against the pathogens in 124 different instances: 35 instances when exposed to UV and no light, 30 when exposed to UV light, and 59 instances when exposed to no light. The metabolites of six actinomycete strains also lost their antimicrobial activities when exposed to UV light. While the metabolites produced by these strains have yet to be determined, exposure to lighted environments may either deactivate or enhance the antimicrobial activities of cave actinomycete strains. This study represents a confirmation that cave actinomycetes are potential sources of novel antimicrobial compounds and also is the first report of the enhancement of antimicrobial activities of some cave bacteria by exposure to UV light. Further investigation of the role of UV light with respect to activation/deactivation of antimicrobial activities of cave actinomycetes is required.
SUMMARY

• The Helmcken cave microbiome possesses a great diversity of microbes with the potential for studies of novel microbial interactions and the isolation of new types of antimicrobial agents.
RASPBERRY RISING: TUPPER CAVE SYSTEM

Nicholaus Vieira & Christian Stenner

Interesting link: crazycaver.com
RASPBERRY RISING: TUPPER CAVE SYSTEM

Christian with soil sample collection  After a month of incubation
Bacterial matt on the cave wall
MEDIA COVERAGE

Ice Cave Explorers: Mapping the Underworld

http://globalnews.ca/ice-caves

http://globalnews.ca/video/868738/full-story-mapping-the-underworld

By Global 16x9
The Search for New Antibiotics

http://www.youtube.com/watch?v=B3_ujD53aL8
PSEUDOGYMNOMASCUS SPP. IN NAKIMU CAVE

SARAH BOYLE: MOUNT REVELSTOKE AND GLACIER NATIONAL PARKS

CAVE CONSERVATION

AWARENESS IS THE KEY!
SENSITIVE CAVE RESOURCES

• Mineral resources
• Paleontological and archaeological resources
• Air and Water
• Sediments
• Biota
• Microbiota
POLYCHROME HALL, ALTAMIRA CAVE, SPAIN
POZO ALFREDO MINE, RIOTINTO, SPAIN

Prof. Cesareo Saiz-Jimenez
One of Canada’s most exceptional concentrations of cave mineral deposits - *helictites* (eccentric stalactites) in the Chamber of Candles, Candlestick Cave, VI

Phil Whitfield

Cave pearls, formed as calcite builds in layers on small pebbles constantly agitated by dripping water. A relatively rare deposit in Canadian caves, these are in Castleguard Cave.
Bear skeleton, Wormhole Cave, VI. (Photo courtesy of Martin Davis)

Human skull and femur, Renaissance Cave, BC.
A MICROBIOLOGIST’S PERSPECTIVE

• Human visitation
  • introduces new organic matter and exotic microorganisms into caves, which may harm native microbial populations (Northup, 2009).
  • can cause the direct damage inflicted on the cave.
  • leaves behind skin cells, bacteria and fungi from hair and skin, hair, and occasionally vomit, feces, urine, or mud and dirt from other caves.
  • One of the major impacts on oligotrophic (low-nutrient) caves is the enrichment of new organic carbon from outside.
  • Scientists: collecting samples such as drilling of rocks and speleothems (Spötl and Mattey, 2012).
Recently, it was stated that “with today’s leisure tourism, the frequency of visits to many caves and other subterranean sites should be looked upon as a potential risk for the conservation of cultural heritage. Archaeologists, environmentalists and microbiologists agree on the beneficial effect of closing subterranean sites for their conservation” (Saiz-Jimenez et al. 2011).
Adequate cave management, including a sound scientific management of the microclimate, environment and visits, is the best way to help preservation.
• “The degree to which individuals impact cave resources ultimately hinges on their personal awareness, patience and respect for the cave environment, and these factors can be influenced by informed peer pressure, group culture and societal standards. Within the organized caving community, value is placed on cave mapping, resource inventory and specialized studies as elements of exploration – “recreation in the service of science””.
At the beginning of the hike to collect cave soil and rock samples in Fall 2010
Thank you for your attention. Any questions?

Thanks to my husband, Joe Dobson, and my son, Ryder Cheeptham Dobson, for always believing in me and for all the support.