**Discovery of Cytotoxic Natural Products from Marine Parasite Microbiomes**

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**Natural Products Drug Discovery**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Organic product is extracted</th>
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<tr>
<td>Bacteria</td>
<td>Crude extract</td>
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<tr>
<td>Parasite</td>
<td>Initial bioactivity screening</td>
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<tr>
<td>Structure Elucidation</td>
<td>Analysis of bioactive compound</td>
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**Overview**

Marine organisms produce a diverse array of secondary metabolites, many of which have therapeutic potential. Some properties of these secondary metabolites include antibiotic, antifungal, antiviral, cytotoxic, and anticoagulant properties. Through harvesting the bacteria found in parasitic microorganisms and extracting these secondary metabolites, cytotoxic compounds can be isolated and identified. These compounds can then be further developed as potential drug candidates. This research focuses on creating a natural product extract library from marine bacterial isolates and analyzing the extracts for cytotoxicity against cancer cells. Through collaboration with our partners at Wayne State University, we can identify bioactive compounds from organic extracts of marine bacteria.

**Creation of a Microbial Chemical Extract Library**

Creation of a Microbial Chemical Extract Library

**Culturing and Harvesting:**

Pure cultures of bacteria were scaled up to liquid cultures by graduate students in the DISCO lab. The compounds the bacteria produce are then chemically extracted.

**Extraction and Fractionation of Bacterial Natural Products**

- **Primary Filtration:** removes natural products from bacterial extract
- **Secreted organic compounds are removed from the liquid media using hydrophobic resin.**
- **Organic compounds are extracted from XAD-16 resin / cells with organic solvents and dried.**

- **Removal of Nuisance Compounds:**
  - Following primary extraction, crude extracts are fractionated using solid phase extraction (SPE) cartridges.
  - Polaris and media components are removed to enhance natural product concentration.
  - Non-polar compounds that are often “false positives” remain bound to the column.

**Anti-Cancer Activity Screening of Bacterial Extracts**

Collaborators at WSU screened fractionated extracts for anti-cancer activity against UMSCC23, a laryngeal squamous cell carcinoma.

- **UMSCC23 viability after 48h exposure to 100μg/ml of DSK224 and DSK243**

**Acknowledgements**

- NC Department of Marine Fisheries
- North Carolina Biotechnology Center
- UNCW Center for Innovation and Commercialization
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- UNCW Center for Marine Science
- Marine Biotechnology in North Carolina (Marbionc)

**References**


**Next Steps for Lead Strains**

- Recent bioassay identified two toxic bacterial strains isolated from scallop gills
- 16S rRNA sequencing identified these as *Gordonia bronchialis* (DSK 224) and *Gordonia hongkongensis* (DSK 243)

**LCMS Chromatograms of Bacterial Chemical Extracts**

- The HLB parent fractions of active strains were further fractionated using a C-18 reversed phase cartridge. A mobile phase gradient was used to separate fractions by relative polarity.
- The C-18 fractions were then sent to WSU for bioassay testing to identify which fractions contain bioactive compounds.

**C-18 SPE of Parent Fractions**

- Bioassay Testing of C-18 Fractions & Future Steps

**Anti-Cancer Drugs**

- **UNCW’s Drug DISCOvery Group**

**Scallop Trematodes**

Bacterial isolates from scallop (*Argopeten irradians*) gills infected with trematode parasites have been shown to produce a variety of natural products that may have therapeutic potential against antibiotic-resistant pathogens or cancer cells.

**Gar Fish Isopods**

Bacterial isolates from ectoparasitic isopods of gar fish have been chemically extracted and analyzed for production of potentially bioactive natural products.