VITAL- Viral InhibiTors from ALgae: Generating Extracts for Antiviral Activity Assays

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Background and Significance

The lack of effective treatments for SARS-CoV-2 and other emerging viral pathogens has expanded the search for unique antiviral compounds. Algae from marine and extreme environments may provide novel compounds with novel activity for use in therapeutic drug development. Previously published research indicates that phototrophic organisms synthesize a large range of effective antiviral compounds, often with no or low cytotoxicity. Algae (broadly referring to cyanobacteria, eukaryotic microalgae and seaweeds) synthesize a wide range of bioactive compounds with antiviral potential, such as, nucleosides, polyphenols, sulfoglycolipids, lectins, sulfated polysaccharides, cyclic depsipeptides, β-carbolines, indolocarbazoles, and proteins, some of which have been demonstrated to inhibit virus replication and attachment. Indeed, some of these antiviral compounds are even active specifically against coronaviruses. Significantly, some algal-based compounds have been shown to have broad-spectrum antiviral activity (SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HIV, HCV and Ebola), while also being well tolerated in vivo by rodents. Given the divergent phylogenies and respective biodiversity of biosynthetic pathways among cyanobacteria, eukaryotic microalgae, and marine macroalgae, we expect additional potent antivirals to exist. The antiviral activities of novel compounds synthesized by phototrophs, specifically against SARS-CoV-2, are not well characterized. Indeed, recent evidence indicates that anti-SARS-CoV-2 activity from algal bioproducts is an area in need of further research. Therefore, to develop effective and well-tolerated treatments for SARS-CoV-2 infections, as well as other emerging and evolving microbial pathogens (e.g., Influenza-A viruses), there is a critical need to discover novel compounds exhibiting potent antiviral activity without cytotoxicity.

Materials and Methods

Marine and Extreme Algae

We identified 20 promising genera of algae including representatives from the Cyanophyta, Rhodophyta, Chlorophyta, and Ochrophyta as promising candidates for novel biocompound discovery (Table 1). Biomass was obtained from our extremophile collection, our marine collections derived from Sequim Bay, WA, cultivation in outdoor testbeds in Arizona, or from commercial biomass producers, in the case of Arthrospira and Saccharina.

Extract Generation and Fractionation

Harvested biomass of the fastest growing cultures (to maximize biomass) was freeze-dried, ball-milled, and solvent extracted (1:1 methanol-dichloromethane, v/v) following the method described by Orjala et al., (2012). A portion of the freeze-dried biomass was retained for future reference along with a portion of crude extract dissolved in dimethylsulfoxide (DMSO) at 10 mg/mL. The remaining concentrated extract was fractionated using a solid-phase extraction column packed with Diaion HP-20SS, via elution with a stepwise gradient of deionized water and 2-propanol (IPA) to obtain 8 fractions of different polarity; 1) 0% IPA, 2) 20% IPA, 3) 40% IPA, 4) 70% IPA, 5) 90% IPA, 6) 100% IPA, 7) ethyl acetate, 8) acetone. Extract fractions were dried under nitrogen and measured gravimetrically (Figure 1). Fractions were then dissolved at 10 mg per mL dimethyl sulfoxide (DMSO), if sufficient extract was present or at 500 µg/mL for lower mass fractions. Fractionation reduces the possibility of complex compound interactions and potential interferences with bioactivity. Extract fractions and crude biomass were then stored at -80 °C until further experimentation.
Results and Discussion
Based on an in-depth literature review, we identified 20 promising genera of algae including representatives from the Cyanophyta, Rhodophyta, Chlorophyta, and Ochrophyta as candidates for novel biocompound discovery. Of these, we generated sufficient amounts of crude biomass extracts from 18, and semi-purified fractionated bioextracts of 15 (Table 1). Several of the algae were excluded due to slow growth rates and the inability to generate sufficient biomass within our experimental timeframe (e.g., Griffithsia, Galdieria, Porphyridium). Griffithsia was intended to serve as our positive control species due the previously observed antiviral activity. The slow growth rate of this organism highlights the need for alternative sources of novel biocompounds from algae that are readily cultivatable for mass production of therapeutic compound precursors. Several of the other algal genera included in our protocol have shown previously-evaluated antiviral properties and can serve as alternative positive controls. Specifically, Porphyridium demonstrated anti-HSV activity,15–18 Arthrospira demonstrated anti-HSV, HIV, HCMV, and IFV activities,19–21 and Sargassum demonstrated anti-HIV and HSV activities.22–24 Additionally, one compound in particular, caulerpin, has recently been isolated from Sargassum as well as several genera of Chlorophyta and demonstrated potential anti-SARS-CoV-2 protease activity in molecular docking analyses.2,25 Based on the previously established ability of caulerpin to inhibit another ssRNA+ virus, CHKV,26 and the ability of similar indole-alkaloid compounds to inhibit coronaviruses,27 we consider caulerpin to be an especially promising candidate in the search for anti-SARS-CoV-2 drugs.

The fractionation of the crude extracts by compound polarity indicates the diversity of compounds present in the crude extracts as many of the algal extracts have different relative distributions of compounds (Figure 1).

Table 1. VITAL BioLibrary

<table>
<thead>
<tr>
<th>#</th>
<th>Genus</th>
<th>Phylum (common name)</th>
<th>MCRL BioCompound Library Status</th>
<th>Previously Observed Antiviral Activity (from genus)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Porphyridium cruentum</td>
<td>Rhodophyta (Red microalga)</td>
<td>Living culture and harvested biomass accessioned in VITAL Library</td>
<td>Sulfated polysaccharides show anti-HSV-1/2 activity</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>CCMP675</td>
<td></td>
<td></td>
<td>Sulfogalactolipid shows anti-HSV-1/2 activity</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Exopolysaccharides show anti-VHSV, AFSV, MuLV, MuSV-124, RSV, HSV, VSV, Vaccinia virus activity</td>
<td>18, 19</td>
</tr>
<tr>
<td>2</td>
<td>Griffithsia pacifica</td>
<td>Rhodophyta (Red seaweed)</td>
<td>Living culture in VITAL Library</td>
<td>Griffithsins show anti-SARS activity</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>UTEX2317</td>
<td></td>
<td></td>
<td>Griffithsins show anti-coronavirus activity</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Griffithsins show anti-HIV activity</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>Pyropia sp. Sequim Bay, WA</td>
<td>Rhodophyta (Red seaweed)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Eicosapentaenoic acid as dietary supplement against ISAV Anti-inflammatory increasing levels of IL-10 which may be important in some viral infections (e.g., SARS-CoV-2)</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Galdieria sp. CCME-YP-4A</td>
<td>Rhodophyta (Red microalga)</td>
<td>Living culture in CCME and harvested biomass available in VITAL Library.</td>
<td>Lipophilic extract shows anti-InfV activity Polysaccharides show anti-Inf-B &amp; mumps activity</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>Gelidium sp. Sequim Bay, WA</td>
<td>Rhodophyta (Red microalga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Macrophage stimulation Methanolic extract shows anti-VsV activity Polysaccharides show anti-HSV activity</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>Gracilaria sp. Sequim Bay, WA</td>
<td>Rhodophyta (Red microalga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>Palmeria palmata OSU</td>
<td>Rhodophyta (Red microalga)</td>
<td>Voucher biomass, crude extracts, extracts residuals,</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>No.</td>
<td>Species/Strain</td>
<td>Kingdom/Phylum</td>
<td>Description</td>
<td>Activity</td>
<td>References</td>
</tr>
<tr>
<td>-----</td>
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<tr>
<td>8</td>
<td><em>Arthrospira platensis</em> UTEX3086</td>
<td>Cyanophyta (Blue-green alga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Aqueous, phosphate buffer extracts show anti-HSV activity. Long-term consumption of <em>Spirulina</em> may improve AIDS/HIV clinical endpoints. Lipoprotein protects against InfV via immune activation. C-phycocyanin shows AV activity. Aqueous extract protects against InfV infection in vivo.</td>
<td>30, 32, 33, 41, 42</td>
</tr>
<tr>
<td>9</td>
<td><em>Arthrospira</em> (Commercial Spirulina)</td>
<td>Cyanophyta (Blue-green alga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Cyanobacterium</em> sp. SSL1-turbo</td>
<td>Cyanophyta (Blue-green alga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Ambigol A inhibits HIV RT. Depsipeptides show anti-InfV activity. Malynagamides show anti-HIV activity. Extracts and sulfoglycolipids show anti-HIV activity.</td>
<td>43, 44, 45, 46</td>
</tr>
<tr>
<td>11</td>
<td><em>Anabaena</em> sp. ATCC33081</td>
<td>Cyanophyta (Blue-green alga)</td>
<td>Living culture in CCME and harvested biomass available in VITAL Library.</td>
<td>Sulfoglycolipids inhibit HIV RT. Aqueous and lipophilic extracts show anti-HIV, HSV, RSV activity.</td>
<td>11, 47, 48</td>
</tr>
<tr>
<td>13</td>
<td><em>Ulva</em> sp.2 (tubular) Sequim Bay, WA</td>
<td>Chlorophyta (Green seaweed, green tide)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Polysaccharide shows anti-HSV-1 activity. Carotenoids show anti-inflammatory activity.</td>
<td>55, 56</td>
</tr>
<tr>
<td>14</td>
<td><em>Chlorella sorokiniana</em> DOE1412</td>
<td>Chlorophyta (Green microalga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td><em>Nannochloropsis oceanica</em> CCAP849/10</td>
<td>Ochrophyta-Eustigmatophyta (Golden microalga)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Sulfated polysaccharides show anti-inflammatory activity. Biofactory for HBV antibody.</td>
<td>57, 58, 59</td>
</tr>
<tr>
<td>16</td>
<td><em>Phaeodactylum tricornutum</em> UTEX646</td>
<td>Ochrophyta (Diatom)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Multi-mechanism anti-HIV activity from crude extract. Anti-HSV activity from fucoidan. Sulfoquinovosyldiacylglycerol anti-HSV activity. Palmitic acid shows anti-HIV activity.</td>
<td>60, 61, 62, 63</td>
</tr>
<tr>
<td>17</td>
<td><em>Sargassum</em> sp. Sequim Bay, WA</td>
<td>Ochrophyta (Brown seaweed)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Fucoidan shows anti-SARS-CoV-2 activity. Laminaran inhibits HIV-RT.</td>
<td>64, 65, 66, 67</td>
</tr>
<tr>
<td>18</td>
<td><em>Saccharina latissima</em> SeaGrove, AK</td>
<td>Ochrophyta (Sugar Kelp)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Fucoidan shows anti-SARS-CoV-2 activity. Laminaran inhibits HIV-RT.</td>
<td>68, 69, 70, 71</td>
</tr>
<tr>
<td>19</td>
<td><em>Fucus</em> sp. Sequim Bay, WA</td>
<td>Ochrophyta (Bladderwrack)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Fucoidan protects against HSV infection in vivo and in vitro. Sulfated shows anti-HIV activity.</td>
<td>72, 73, 74, 75</td>
</tr>
<tr>
<td>20</td>
<td><em>Nereocystis luetkeana</em> Sequim Bay, WA</td>
<td>Ochrophyta (Bull Kelp)</td>
<td>Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.</td>
<td>Extract (likely phenolic compound) shows anti-HSV activity.</td>
<td>76, 77, 78, 79</td>
</tr>
</tbody>
</table>
**Figure 1.** Distribution of biocompounds fractionated from algal methanol:dichloromethane extracts as a function of 2-propanol (IPA) concentration in water; 1) 0% IPA, 2) 20% IPA, 3) 40% IPA, 4) 70% IPA, 5) 90% IPA, 6) 100% IPA, 7) ethyl acetate, 8) acetone, for 15 different phylogenetically diverse algae (A through O).

**Conclusions**

In this seed project, we generated a novel bio-library of crude and semi-purified extracts derived from our unique collections of marine and extreme algae. The generation of a unique metabolite collection is expected to directly contribute to the discovery of new targets for the development of medical therapeutics effective against SARS-CoV-2 and other emerging diseases of concern in support of the U.S. DOE National Virtual Biotechnology Laboratory (NVBL) and Office of Science efforts. Discovery and development of natural compounds with therapeutic activity against infectious diseases protects our first responders by reducing risk and improving readiness. Biomolecules from phylogenetically diverse biological sources provide a unique primary means of novel drug development for therapeutic testing of antivirals, they are readily accessible, have a low cost of production, and have potential for holistically improving health outcomes. Furthermore, identified biocompatible algal compounds are a future resource for screening as anticancer, antifungal, antibacterial, and anti-inflammation agents. We present here the first steps in our efforts in establishing a unique natural products pipeline for microalgae and macroalgae at PNNL. The prepared biomass extracts containing compounds with potential antiviral activity will be assayed by our academic collaborators for antiviral activity and cytotoxicity. Knowledge gained from this preliminary testing will inform future efforts focused on developing therapeutic targets for coronaviruses and other emerging pathogens.
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