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# ANTIVIRAL ACTIVITY OF CULTURED BLUE-GREEN ALGAE (CYANOPHYTA)[1]

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ABSTRACT

*Lipophilic and hydrophilic extracts from approximately 600 strains of cultured cyanophytes, representing some 300 species, were examined for antiviral activity against three pathogenic viruses. Approximately 10% of the cultures produced substances that caused significant reduction in cytopathic effect normally associated with viral infection. The screening program identified the order Chroococcales as commonly producing antiviral agents.*

*Key index words: antiviral; cyanobacteria; cyanophyte; herpes virus; HIV-1; HSV-2; human immunodeficiency virus; natural products; pharmaceutical; respiratory syncytial virus*

Pharmaceutical drug discovery has, for most of the past 40 years, depended heavily on the process of empirically screening large numbers of pure organic compounds or crude natural product extracts to provide new leads. Random screening will continue to play an important role in the drug discovery process for the foreseeable future.

Historically, actinomycetes have been the most prolific producers of new bioactive metabolites. They also have been, not surprisingly, the most closely examined group of organisms and, at the present time, yield known compounds at a rate in excess of 95% of all active leads discovered in primary screening (Bradner 1980).

New sources of cultivable microorganisms for screening are thus of paramount importance. The blue-green algae, or cyanobacteria, constitute such a group of microbes. They can be cultured in the laboratory with relative ease to provide a consistent source of biologically active secondary metabolites.

Cyanophytes are widely distributed in nature and include a great range of physiological and morphological types. Relatively little systematic screening for antiviral activity has taken place and has included only a few families of cyanophytes (Rinehart et al. 1981). As part of a systematic examination of the blue-green algae as potential sources of useful pharmaceutical or agrichemical agents, we began in 1987 to screen extracts of cultured cyanophytes for antiviral activity. Our goals were to examine the distribution of antiviral compounds among the blue-green algae and to determine whether particular geographic or physical sites are especially likely to yield active leads.

More recently, the U.S. National Cancer Institute (NCI) has begun a comprehensive preclinical drug development program for acquired immunodeficiency syndrome (AIDS). As part of this agenda, the NCI is conducting a broad screening program to evaluate synthetic and natural products for potential anti-human immunodeficiency virus (HIV) activity. In part, this screening program has focused on source materials that have not previously been surveyed, including field collections of novel plant materials and marine organisms and culture collections of unusual microbes including fungi, anaerobic bacteria, and the cyanobacteria or blue-green algae (Boyd 1988).

## MATERIALS AND METHODS

Cultures, classification, media, and culture conditions have been described previously (Patterson et al. 1991). Extracts tested for anti-HIV activity were prepared for the U.S. NCI as part of a NCI-funded program to acquire extracts for anticancer and anti-HIV screening. A list of the strains tested is available upon request from the corresponding author.

*Preparation of extracts.* Two methods were used to prepare extracts for testing. In method I, lyophilized cells were extracted

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TABLE 1. *Performance of antiviral screens.*

| Test virus | Algal strains | | Extracts | | | |
|---|---|---|---|---|---|---|
| | | | Hydrophilic | | Lipophilic | |
| | Tested | Positive (%) | Tested | Positive (%) | Tested | Positive (%) |
| HSV-2 | 529 | 54 (10.2) | 529 | 2 (0.4) | 529 | 54 (10.2) |
| RSV | 529 | 13 (2.5) | 529 | 7 (1.3) | 529 | 6 (1.1) |
| HIV-1 | 694 | 72 (10.3) | 635 | 31 (4.9) | 584 | 47 (8) |

with 3:7 ethanol:water (hydrophilic extract), followed by 1:1 dichloromethane:2-propanol (lipophilic extract), as previously described (Patterson et al. 1991). Extracts prepared by method I were tested for anti-herpes simplex virus type 2 (HSV-2) and anti-respiratory syncytial virus (RSV) activity.

In method II, lyophilized cells were extracted with 1:1 dichloromethane:methanol, followed by water. The extraction was carried out by blending the cell mass with 40 mL solvent per gram of lyophilized algal mass in a Waring-type blender for a period of 60 s. The homogenate was allowed to steep overnight at 4° C. After separation by filtration on Whatman #1 paper, the cell mass was allowed to air-dry for a period of approximately 4 h. The cell mass was then blended with water and allowed to stand at 4° C for another overnight extraction. The marc was separated from the extract by centrifugation at 10,000 × *g* for 10 min. The lipophilic extract was reduced to dryness *in vacuo*. The hydrophilic extract was lyophilized. The extracts were at no time exposed to temperatures in excess of 40° C. Extracts were stored at −20° C until tested. Extracts prepared by method II were tested for anti-HIV activity. Method II was selected by the NCI to conform with methods used in other antiviral and anticancer screening programs performed at the NCI.

*Viruses.* Recent clinical isolates of HSV-2 and RSV were obtained from Dr. Nyven Marchette, Department of Tropical Medicine, University of Hawaii.

Viral stock cultures were prepared in monolayers of either mink lung cells (ATCC CCL 64) for HSV-2 or human epithelial carcinoma cells (HEp-2, ATCC CCL 23) for RSV and stored at −70° C until used. Cells were grown in Eagle's minimal essential medium with Earle's salts (EMEM), supplemented with 5–10% heat-inactivated fetal bovine serum (FBS), and maintained in EMEM supplemented with 2% heat-inactived FBS (HSV-2) or 2% heat-inactived chicken serum (RSV).

HIV-1 was maintained as previously described (Weislow et al. 1989).

*Antiviral assays.* Activity of crude algal extracts against HSV-2 and RSV was initially determined qualitatively in a screening assay based on a microscopic examination of cells and visual estimation of cytopathic effect (CPE). Cell monolayers were grown in 24-well plates (Costar, Cambridge, MA). The growth medium was removed by aspiration, and the monolayer was infected with 0.1 mL of virus suspension containing approximately 100 $TCID_{50}$. Following a 1-h incubation at 37°C in a 5% carbon dioxide atmosphere, 1 mL of appropriate maintenance medium was added to each well. Duplicate wells were treated with algal extract or vehicle (solvent) control in amounts not exceeding 10 μL per well. Wells were observed daily under an inverted microscope, and the degree of cytopathic effect was estimated visually.

Those wells estimated to exhibit at least 25% less viral CPE than the vehicle control following 72 h incubation were further assayed to quantify the percentage yield reduction in the amount of virus produced. HEp-2 or mink lung cells were grown as described above. Serial 10-fold dilutions were prepared from the positive wells. After removing the growth medium from the monolayers, 0.1 mL of the serial dilutions was inoculated in triplicate. Following a 1-h incubation at 37° C in a 5% carbon dioxide atmosphere, 1 mL of 1% methyl cellulose (Sigma, St. Louis) overlay medium was added to all wells, and incubation was resumed for 5 days. The cell sheets were stained with napthol blue-black, and the plaques were counted. Percentage of yield reduction was calculated. The minimum inhibitory concentration was chosen as the lowest concentration of extract that produced a 90% or greater reduction in virus yield.

Antiviral activity against HIV-1 was determined in a microculture cytoprotection assay as previously described (Weislow et al. 1989) but with some modification. Briefly, CEM-SS t-lymphoblastoid cells (Nara et al. 1987) in RPMI-1640, containing 10% FBS and 50 $\mu g \cdot mL^{-1}$ of gentamycin, were infected with HIV-$1_{rf}$ at 37° C for 45–60 min, washed, and then distributed to 96 well microculture dishes ($5 \times 10^5$ cells per 100 μL per well) containing dilutions of test agents (100 μL per well). Following incubation of the test plates for 6 days at 37° C in air-$CO_2$, 50 μL of a mixture of 1 $mg \cdot mL^{-1}$ of XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide) and 0.01–0.02 mM *N*-methylphenazonium methosulfate was added to each well. The trays were then reincubated for an additional 4 h to allow for XTT formazan production; their plastic covers were then replaced with adhesive plate sealers (Dynatech, Alexandria, VA), the contents of each plate were mixed, and optical densities were determined with a V-max photometer (Molecular Devices, Inc., Menlo Park, CA) at a test wavelength of 450 nm and reference wavelength of 650 nm. Uninfected cells or cells protected by test agents produced the soluble orange XTT formazon and high optical densities (ODs). Cells not protected by drugs were killed by the virus and/or did not proliferate; they produced less XTT formazan and, thus, lower optical densities. Data were typically expressed as percentage of XTT formazan from untreated control cells, as determined by the following equation: % of untreated control XTT formazan = (test OD/control cell OD) × 100.

## RESULTS

Collection sites and criteria for selection of organisms were detailed previously (Patterson et al. 1991). In brief, cyanophytes were selected to provide a broad representation of the families of the blue-green algae as well as a survey of possible source materials for isolation of cyanophyte strains.

Algal cultures were incubated for periods ranging from 6 to 49 days, with a mean incubation period of 22 days. Yield of cell mass ranged from 0.04 to 1.71 $g \cdot L^{-1}$, with a mean value of 0.27 $g \cdot L^{-1}$. Mean yield of extract was 13.3% of cell dry weight for lipophilic extracts and 21.4% for hydrophilic extracts.

*Selection of test organisms.* The three viruses used for screening are all pathogenic in humans. They represent very different classes of virus and may well detect antiviral agents having very different mechanisms of action. HSV-2 (Herpesviridae) is responsible for genital herpes simplex infection, one of the most common sexually transmitted diseases in the United States. HSV-2 is a representative of double-stranded DNA viruses in which the capsid is assembled in the nucleus and enveloped by budding through the nuclear membrane. Only cellular enzymes are involved in the DNA synthesis. RSV (Paramyxoviridae) is commonly responsible for acute respiratory distress, including bronchiolitis and pneumonia, especially among infants and young

TABLE 2. *HIV-1 positives.*

| Genus | $EC_{50}$ ($\mu g \cdot mL^{-1}$) |
|---|---|
| *Aphanocapsa* | <0.05 |
| *Scytonema* | 3 |
| *Aphanocapsa* | 4 |
| *Hydrocoleus* | 4 |
| *Anabaena* | 5 |
| *Chroococcidiopsis* | 5 |
| *Rivularia* | 5 |
| *Gloeocapsa* | 7 |
| *Oscillatoria* | 7 |
| *Nostoc* | 10 |
| *Microcoleus* | 12 |
| *Phormidium* | 12 |
| *Dermocarpa* | 13 |
| *Plectonema* | 16 |
| *Anabaena* | 19 |
| *Phormidium* | 20 |
| *Synechococcus* | 20 |
| *Apyhanothece* | 25 |
| *Plectonema* | 27 |
| *Anabaena* | 29 |
| *Aphanothece* | 35 |
| *Aphanothece* | 38 |
| *Oscillatoria* | 39 |
| *Chroococcus* | 43 |
| *Lyngbya* | 44 |
| *Gloeocapsa* | 45 |
| *Chroococcus* | 47 |
| *Anabaena* | 50 |
| *Myxosarcina* | 52 |
| *Gloeocapsa* | 58 |
| *Microcystis* | 59 |
| *Pseudanabaena* | 61 |
| *Oscillatoria* | 63 |
| *Synechococcus* | 67 |
| *Lyngbya* | 70 |
| *Scytonema* | 71 |
| *Nostoc* | 72 |
| *Synechococcus* | 81 |
| *Phormidium* | 84 |
| *Coccochloris* | 85 |
| *Lyngbya* | 101 |
| *Synechocystis* | 108 |
| *Nostoc* | 114 |
| *Synechocystis* | 120 |
| *Oscillatoria* | 128 |
| *Nostoc* | 130 |
| *Phormidium* | 152 |
| *Lyngbya* | 154 |
| *Phormidium* | 155 |
| *Chroococcidiopsis* | 157 |
| *Chroococcidiopsis* | 198 |
| *Scytonema* | 200 |
| *Nostoc* | 207 |
| *Calothrix* | 220 |
| *Anabaena* | 242 |
| *Synechococcus* | 250 |
| *Anabaena* | 255 |
| *Cylindrospermum* | 263 |
| *Oscillatoria* | 315 |
| *Oscillatoria* | 319 |
| *Symploca* | 320 |
| *Tolypothrix* | 322 |
| *Aphanocapsa* | 325 |
| *Gloeocapsa* | 345 |
| *Nostoc* | 380 |
| *Microcystis* | 385 |
| *Anabaena* | 409 |
| *Synechocystis* | 417 |
| *Oscillatoria* | 475 |
| *Phormidium* | 728 |
| *Synechocystis* | 800 |
| *Lyngbya* | 1238 |

children. RSV is a representative of single-stranded RNA viruses in which the capsid is assembled in the cytoplasm and enveloped by budding through the plasma membrane. The virus must provide its own enzyme (RNA-dependent RNA polymerase) to synthesize viral RNA. HIV-1 (Retroviridae) is implicated as a causative agent of AIDS. HIV-1 is a retrovirus, a double-stranded RNA virus in which the capsid is assembled in the cytoplasm, following synthesis in the nucleus using DNA produced by the viral enzyme RNA-dependent DNA polymerase (reverse transcriptase). As with other RNA viruses, envelopment occurs by budding through the plasma membrane.

*Screening for antiviral activity.* During the initial screening phase, lipophilic and hydrophilic extracts were tested for their ability to inhibit cytopathic effects normally induced by viral infection. Extracts prepared from 694 strains representing 334 species of cyanophyte were examined for anti-HIV-1 activity; 529 strains were examined for anti-HSV-2 and anti-RSV activity. Not all extracts were obtained in sufficient quantity to allow testing in all systems. Approximately 10% exhibited antiviral activity when tested against HSV-2 or HIV-1, whereas approximately 2% showed activity against RSV. Antiviral activity was more commonly associated with lipophilic extracts than with hydrophilic extracts (Table 1).

Extracts prepared from 72 cyanophyte strains were scored positive in the anti-HIV-1 primary screen (Table 2). An additional 131 strains exhibited weak or trace amounts of antiviral activity in the HIV-1 assay system. As a number of the leads generated by this screening program are being actively investigated by the U.S. NCI, further identification of the producer strains is not possible at the present time.

*Confirmation of antiviral activity.* Antiviral activity against HSV-2 and RSV was confirmed by a plaque-forming assay. Extracts scored positive in the primary screen were tested for ability to reduce the number of plaque-forming units in infected cells. Extracts were scored as positive in this confirmation assay if the number of plaques formed was reduced by 90% or more at a dose less than or equal to $\frac{1}{10}$ of the cytotoxic dose for uninfected host cells. Fifty-four of the 529 strains tested for anti-HSV-2 activity were confirmed in this manner (Table 3); 13 of the 529 strains tested for anti-RSV activity were con-

TABLE 3. *HSV-2 positives confirmed by plaque assay. A = 30% aqueous ethanol; L = 1:1 isopropanol: dichloromethane. Rdxn = % reduction in virus titer: 100 − (mean titer of test cultures / mean titer of virus control cultures × 100).*

| Genus/species | Isolate | Extract | Antiviral MIC (μg/mL) | % Rdxn |
|---|---|---|---|---|
| *Anabaena flos-aquae* (Lyngbye) de Brebisson | UTEX 1444 | L | 2.1 | 92 |
| *Nostoc piscinale* Kutzing ex Bornet & Flahault | ATCC 29107 | L | 2.3 | 99 |
| *Synechococcus elongatus* Nageli | MWQ 6 | L | 2.4 | 90 |
| *Oscillatoria hamelii* Fremy | DT-54-1 | L | 2.8 | 96 |
| *Tolypothrix tenuis* (Kützing) J. Schmidt | GB-9-15 | L | 2.9 | 91 |
| *Synechococcus elongatus* Nageli | RAL 167-02 | L | 3.4 | 96 |
| *Phormidium angustissimum* W. & G. S. West | RAL 169-01 | L | 3.6 | 99 |
| *Stigonema minutum* (Agardh) Hassal ex Bornet & Flahault | DU-9-1 | L | 3.7 | 96 |
| *Nostoc linckia* Strom | FR-1-3 | L | 4.9 | 100 |
| | | A | 54 | 100 |
| *Scytonema javanicum* (Kützing) Bornet ex Bornet & Flahault | GB-9-9 | L | 5.0 | 99 |
| *Microcoleus vaginatus* (Vaucher) Gomont | ATCC 27930 | L | 5.0 | 93 |
| *Nostoc sphaericum* Vaucher ex Bornet & Flahault | EX-5-1 | L | 5.0 | 93 |
| *Synechococcus elongatus* Nageli | RAL 158-02 | L | 5.2 | 100 |
| | | A | 15 | 100 |
| *Phormidium orientale* G. S. West | EA-14-1 | L | 5.2 | 97 |
| *Lyngbya* sp. | EG-11-1 | L | 5.3 | 94 |
| *Gloeocapsa alpicola* (Lyngbye) Bornet | ATCC 27191 | L | 6.3 | 91 |
| *Hapalosiphon fontinalis* (Agardh) Bornet | EX-12-1 | L | 7.1 | 98 |
| *Fischerella ambigua* (Nageli) Gomont | EX-8-1 | L | 7.1 | 96 |
| *Synechocystis pevalekii* Ercegovic | ATCC 29110 | L | 7.2 | 100 |
| *Synechococcus elongatus* Nageli | MWQ 8-14E | L | 7.6 | 91 |
| *Nostoc hatei* Dixit | FR-4-1 | L | 7.7 | 97 |
| *Pseudanabaena catenata* Lauterborn | MWQ 8-11A | L | 11 | 98 |
| *Scytonema bohneri* Schmidle | GB-9-7 | L | 12 | 100 |
| *Aphanocapsa montana* Cramer | RAL PBA-FR | L | 12 | 95 |
| *Chroococcus pallidus* Nageli | GJ-16-1 | L | 14 | 100 |
| *Pseudanabaena catenata* Lauterborn | ATCC 29207 | L | 18 | 99 |
| *Phormidium* sp. | EU-27-1 | L | 18 | 93 |
| *Aphanothece clathrata* W. & G. S. West | GN-1-3 | L | 20 | 100 |
| *Nostoc* sp. | VAC 2 | L | 22 | 90 |
| *Gloeotrichia echinulata* (J. E. Smith) P. Richter | EU-11-1 | L | 24 | 96 |
| *Anabaena catenula* (Kutzing) Bornet & Flahault | UTEX B375 | L | 24 | 91 |
| *Aphanocapsa roeseana* de Bary | ATCC 27185 | L | 29 | 98 |
| *Nostoc calcicola* Brebisson ex Bornet & Flahault | FX-1-13 | L | 29 | 94 |
| *Nostoc linckia* (Roth) Bornet ex Bornet & Flahault | AY-11-1 | L | 30 | 100 |
| *Calothrix fusca* (Kutzing) Bornet & Flahault | EU-10-1 | L | 30 | 100 |
| *Chroococcus dispersus* (Keissler) Lemmerman | GR-1-1 | L | 30 | 100 |
| *Dichothrix baueriana* (Grun.) Bornet & Flahault | GO-25-2 | L | 33 | 99 |
| *Lyngbya lagerheimii* (Mobius) Gomont | ATCC 29117 | L | 39 | 98 |
| *Dichothrix baueriana* (Grun.) Bornet & Flahault | GO-29-2 | L | 41 | 100 |
| *Oscillatoria amphibia* Agardh ex Gomont | DP-16-1 | L | 42 | 100 |
| *Phormidium retzii* (Agardh) Gomont | EA-12-1 | L | 44 | 97 |
| *Myxosarcina spectabilis* Geitler | EU-23-1 | L | 48 | 98 |
| *Aphanocapsa koordersi* Strom | ATCC 27187 | L | 53 | 100 |
| *Calothrix gracilis* Fritsch | AZ-7-10 | L | 59 | 94 |
| *Synechocystis pevalekii* Ercegovic | RAL 248-01 | L | 64 | 100 |
| *Oscillatoria quadripunctilata* Bruhl & Biswas | FE-8-1 | L | 65 | 99 |
| *Microchaete violacea* Fremy | DO-2-1 | L | 78 | 100 |
| *Oscillatoria pseudogeminata* G. Schmid | DV-33-1 | L | 88 | 97 |
| *Chroococcus minimus* (Keissler) Lemmerman | FW-1-5 | L | 89 | 90 |
| *Phormidium cebennense* Gomont | BV-15-1 | L | 96 | 98 |
| *Synechococcus cedrorum* Sauvageau | RAL 259-02 | L | 101 | 98 |
| *Gloeothece samoensis* Wille | GS-19-1 | L | 113 | 99 |
| *Hapalosiphon intricatus* W. & G. S. West | FG-49-10 | L | 116 | 91 |
| *Phormidium africanum* Lemmerman | EB-24-1 | L | 140 | 93 |

firmed (Table 4). Two strains (EX-5-1 and RAL 158-02) exhibited activity against both HSV-2 and RSV.

*Reproducibility.* Nine algal strains were mass-cultured to provide material for chemical analysis. Seven of the strains (CN-2-1, DN-7-1, EU-11-1, EX-5-1, GO-25-2, RAL PBA-FR, and RAL 169-01) have proven to consistently produce antiviral activity. Two strains (RAL 158-02 and RAL 167-02) did not consistently exhibit antiviral activity.

*Characterization of candidate producer cultures.* In an

TABLE 4. *RSV positives confirmed by plaque assay. A = 30% aqueous ethanol; L = 1:1 isopropanol: dichloromethane. Rdxn = % reduction in virus titer: 100 − (mean titer of test cultures / mean titer of virus control cultures × 100).*

| Genus/species | Isolate | Extract | Antiviral MIC (μg/mL) | % Rdxn |
|---|---|---|---|---|
| *Aphanocapsa elachista* W. & G. S. West | ATCC 27175 | A | 1.2 | 93 |
| *Nostoc sphaericum* Vaucher ex Bornet & Flahault | EX-5-1 | L | 5 | 100 |
| *Anabaena doliolum* Bharadwaja | DT-10-1 | A | <10 | 97 |
| *Chroococcus dispersus* (Keissler) Lemmerman | GJ-75-1 | A | 18 | 97 |
| *Synechococcus elongatus* Nageli | RAL 158-02 | A | 29 | 91 |
| *Calothrix membranacea* Schmidle | DT-15-1 | L | 50 | 100 |
| *Nostoc punctiforme* (Kützing) Hariot | UTEX B1629 | L | 60 | 93 |
| *Calothrix castelli* (Massal.) Bornet & Flahault | BX-6-1 | L | 92 | 100 |
| *Synechococcus elongatus* Nageli | GQ-2-5 | A | 100 | 100 |
| *Chroococcus minutus* (Kutzing) Nageli | ATCC 29115 | A | 100 | 90 |
| *Synechocystis pevalekii* Ercegovic | RAL 250-01 | A | 164 | 97 |
| *Phormidium foveolarum* (Mont.) Gomont | ATCC 29121 | L | 271 | 100 |
| *Nostoc muscorum* Agardh ex Bornet & Flahault | ATCC 29151 | L | 445 | 99 |

attempt to determine the extent and distribution of antiviral agents among the blue-green algae, we related the presence of apparent antiviral activity to the taxonomy of the producing strains, the substrate material from which the strain was collected in the field, and the conditions used in cultivation of the producer strain. The order Chroococcales proved to be the most prolific producer of antiviral compounds (Table 5). The substratum from which the organism was collected showed little correlation with the presence of antiviral compounds (Table 6). We were unable to relate the presence of antiviral activity to any conditions of cultivation, including culture medium, incubation period, aeration rate, or illumination intensity.

*Compounds identified by screening.* Of the HSV-2 and RSV leads identified by screening, only two have been subjected to bioassay-directed purification to identify the active agents. Indolocarbazoles, commonly found as antibiotics in *Streptomyces* or *Nocardia* fermentations, were responsible for anti-HSV-2 activity associated with *Nostoc sphaericum* strain EX-5-1 (Knübel et al. 1990). A group of β-carbolines were responsible for the anti-HSV-2 activity associated with *Dichothrix baueriana* strain GO-25-2 (Larsen et al., unpubl.).

Bioassay-directed purification identified a group of related sulfoquinovosyldiacylglycerols (sulfolipids) to be the compounds responsible for anti-HIV-1 activity in two strains, *Phormidium tenue* strain CN-2-1 and *Lyngbya lagerheimii* strain DN-7-1 (strain DN-7-1 has since been made part of the culture collection of algae at the University of Texas as UTEX 2574) (Gustafson et al. 1989). These sulfolipids have been selected by the NCI for preclinical investigation, including pharmacology, toxicology, and formulation development. Recently, the structure and activity of one of the sulfolipids have been confirmed by chemical synthesis and bioassay (Gordon and Danishefsky 1992).

Chemical analysis of selected extracts indicated that many of the anti-HIV-1 leads identified by the screening program are due to the presence of sulfolipids or polysaccharides in the algal extracts. Dereplication analyses utilizing anion exchange and thin-layer chromatographies to identify antiviral ac-

TABLE 5. *Taxonomic grouping of candidate organisms.*

| Order / Family | HSV-2 | | RSV | | HIV-1 | |
|---|---|---|---|---|---|---|
| | Tested | Active (%) | Tested | Active (%) | Tested | Active (%) |
| Chroococcales | 108 | 17 (15.7) | 108 | 6 (5.5) | 141 | 23 (16.3) |
| Chamaesiphonales | 8 | 0 (0) | 8 | 0 (0) | 13 | 4 (30.7) |
| Pleurocapsales | 7 | 1 (14.3) | 7 | 0 (0) | 15 | 1 (6.6) |
| Nostocales | | | | | | |
| Oscillatoriaceae | 164 | 13 (7.9) | 164 | 1 (0.6) | 184 | 21 (11.4) |
| Nostocaceae | 108 | 10 (9.3) | 108 | 4 (3.7) | 115 | 15 (13.0) |
| Microchaetaceae | 22 | 1 (8.3) | 12 | 0 (0) | 18 | 0 (0) |
| Scytonemataceae | 45 | 3 (6.6) | 45 | 0 (0) | 98 | 6 (6.1) |
| Rivulariaceae | 39 | 5 (12.8) | 39 | 2 (5.1) | 69 | 2 (2.8) |
| Stigonematales | | | | | | |
| Stigonemataceae | 23 | 4 (17.4) | 23 | 0 (0) | 32 | 0 (0) |
| Mastigocladaceae | 9 | 0 (0) | 9 | 0 (0) | 9 | 0 (0) |
| Unidentified | 6 | 0 (0) | 6 | 0 (0) | 0 | 0 (0) |
| Total | 529 | 54 (10.2) | 529 | 13 (2.5) | 694 | 72 (10.3) |

TABLE 6. *Characterization of source material.*

| Substrate | HSV-2 | | RSV | | HIV-1 | |
|---|---|---|---|---|---|---|
| | Tested | Active (%) | Tested | Active (%) | Tested | Active (%) |
| Soil | 184 | 17 (9.2) | 184 | 1 (0.5) | 254 | 27 (10.6) |
| Rock | 38 | 4 (10.5) | 38 | 5 (13.1) | 85 | 5 (5.8) |
| Freshwater | 134 | 15 (11.2) | 134 | 2 (1.5) | 147 | 14 (9.5) |
| Marine | 72 | 7 (9.7) | 72 | 2 (2.8) | 88 | 14 (15.9) |
| Plant matter | 16 | 4 (25) | 16 | 0 (0) | 15 | 1 (6.6) |
| Unknown | 85 | 7 (8.2) | 85 | 3 (3.5) | 105 | 11 (10.4) |

tivity associated with sulfolipids or polysaccharides are ongoing.

## DISCUSSION

A variety of macroalgae have been examined for antiviral activity (see e.g. Blunden et al. 1981, Caccamese et al. 1981, Munro et al. 1989). In addition, bioassay-directed fractionation of marine macrophytes has led to isolation of polysaccharides that inhibit viral growth (Ehresmann et al. 1977) or purified viral reverse transcriptase (Nakashima et al. 1987a, b).

In the present study, we have surveyed the blue-green algae for the presence of antiviral agents and have discovered that the occurrence of antiviral compounds is distributed widely among cyanophytes. Certain taxonomic groups, notably the order Chroococcales, are more likely to display antiviral activity. This observation is in marked contrast with the results of our screening to detect anticancer compounds, which identified the families Scytonemataceae and Stigonemataceae as prolific producers of cytotoxic agents (Patterson et al. 1991).

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Blunden, G., Barwell, C. J., Fidgen, K. J. & Jewers, K. 1981. A survey of some British marine algae for anti-influenza virus activity. *Bot. Mar.* 24:267–72.

Boyd, M. R. 1988. Strategies for the identification of new agents for the treatment of AIDS: a national program to facilitate the discovery and preclinical development of new drug candidates for clinical evaluation. *In* DeVita, V. T., Jr., Hellman, S. & Rosenberg, S. A. [Eds.] *AIDS Etiology, Diagnosis, Treatment, and Prevention.* Lippincott, Philadelphia, pp. 305–17.

Bradner, W. T. 1980. Approaches to new drugs: fermentation approaches to new drug development. *In* Crooke, S. T. & Prestayko, A. W. [Eds.] *Cancer and Chemotherapy.* Academic Press, New York, pp. 313–25.

Caccamese, S., Assolina, R., Furnari, G., Cormaci, M. & Grasso, S. 1981. Antimicrobial and antiviral activities of some marine algae from eastern Sicily. *Bot. Mar.* 24:365–7.

Ehresmann, D. W., Deig, E. F., Hatch, M. T., DiSalvo, L. H. & Vedros, N. A. 1977. Antiviral substances from California marine algae. *J. Phycol.* 13:37–40.

Gordon, D. M. & Danishefsky, S. J. 1992. Synthesis of a cyanobacterial sulfolipid: confirmation of its structure, stereochemistry, and anti-HIV-1 activity. *J. Am. Chem. Soc.* 114: 659–63.

Gräfe, U., Dornberger, K. & Fleck, W. F. 1989. Approaches to new microbial metabolites with nonclassical modes of action. *In* Bushell, M. E. & Gräfe, U. [Eds.] *Progress in Industrial Microbiology,* Vol. 27. Elsevier, Amsterdam, pp. 113–38.

Gustafson, K. R., Cardellina II, J. H., Fuller, R. W., Weislow, O. S., Kiser, R. F., Snader, K. M., Patterson, G. M. L. & Boyd, M. R. 1989. AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). *J. Nat. Cancer Inst.* 81:1254–8.

Knübel, G., Larsen, L. K., Moore, R. E., Levine, I. A. & Patterson, G. M. L. 1990. Cytotoxic, antiviral indolocarbazoles from a blue-green alga belonging to the Nostocaceae. *J. Antibiotics* 43:1236–9.

Munro, M. H. G., Blunt, J. W., Barns, G., Battershill, C. N., Lake, R. J. & Perry, N. B. 1989. Biological activity in New Zealand marine organisms. *Pure Appl. Chem.* 61:529–34.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M. & Yamamoto, N. 1987a. Antiretroviral activity in a marine red alga: reverse transcriptase inhibition by an aqueous extract of *Schizymenia pacifica. J. Cancer Res. Clin. Oncol.* 113: 413–6.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M. & Yamamoto, N. 1987b. Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrob. Agents Chemother.* 31: 1524–8.

Nara, P. L., Hatch, W. C. & Dunlop, N. M. 1987. Simple, rapid, quantitative, syncytium forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *AIDS Res. Hum. Retroviruses* 3: 283–302.

Patterson, G. M. L., Baldwin, C. L., Bolis, C. M., Caplan, F. R., Karuso, H., Larsen, L. K., Levine, I. A., Moore, R. E., Nelson, C. S., Tschappat, K. D., Tuang, G. D., Furusawa, E., Furusawa, S., Norton, T. R. & Raybourne, R. B. 1991. Antineoplastic activity of cultured blue-green algae (Cyanophyta). *J. Phycol.* 27:530–6.

Rinehart, K. L., Jr., Shaw, P. D., Shield, L. S., Gloer, J. B., Harbour, G. C., Koker, M. E. S., Samain, D., Schwartz, R. E., Tymiak, A. A., Weller, D. L., Carter, G. T., Munro, M. H. G., Hughes, R. G., Jr., Renia, H. E., Swynenberg, E. B., Stringfellow, D. A., Vava, J. J., Coats, J. H., Zurenko, G. E., Kuentzel, S. L., Li, L. H., Bakus, G. J., Brunsca, R. C., Craft, L. L., Young, D. N. & Conner, J. L. 1981. Marine natural products as sources of antiviral, antimicrobial, and antineoplastic agents. *Pure Appl. Chem.* 53:795–817.

Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H. & Boyd, M. R. 1989. New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. *J. Nat. Cancer Inst.* 81:577–86.