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RESEARCH ARTICLE

Investigation of antimicrobial and protease-inhibitory activity from cultured cyanobacteria

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Abstract

A culture collection of cyanobacteria has been established at the University of Illinois at Chicago. This collection includes marine, terrestrial, and freshwater strains and contains representatives of the five orders of cyanobacteria: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales. In this study, extracts from a subset of 61 strains, 16 marine and 45 freshwater/terrestrial, were evaluated against three current protease targets, i.e. 20S proteasome and two SARS viral proteases, two important bacterial targets, i.e. *Mycobacterium tuberculosis* and *Bacillus anthracis*, and in the *Artemia salina* toxicity assay. In total, extracts of 12 strains possessed significant levels of activity in one or more targets. The overwhelming majority of active extracts (11 of 12) were from either freshwater or terrestrial forms of cyanobacteria, with the greater part of these (9 of 12) being heterocyst-forming strains. These results further support the use of cultured cyanobacteria as a source of biologically active natural products.

Keywords: *Anthrax; antimicrobial; brine shrimp; cyanobacteria; protease inhibition; proteasome; SARS; tuberculosis*

Introduction

Cyanobacteria have been shown to be a rich source of biologically active secondary metabolites (Davies-Coleman et al., 2003; Han et al., 2003; Kaya et al., 2002; Nogle & Gerwick, 2003; Williams et al., 2004). Frequently, the cyanobacterial material is collected directly from the field; however, this biomass is often an assemblage of multiple organisms, which casts doubt on the true origin of an isolated natural product (Macmillan & Molinski, 2005; Nogle & Gerwick, 2002). This also makes the recollection and re-isolation of a particular natural product difficult. In addition, field collections heavily favor cyanobacteria that naturally have dense growth.

In contrast, the use of cultured cyanobacteria allows for the investigation of species that may not grow to sufficient density in the wild, as well as the ability to rapidly obtain additional biomass for the re-isolation of natural products of interest. Controlled culture conditions also ensure a greater degree of purity of the biological material since unialgal or even axenic (i.e. lacking epiphytic microorganisms) strains are utilized to produce the needed biomass. Our goal is to establish a culture collection of cyanobacteria that can be utilized in natural product drug discovery efforts. Herein, we present the results of the biological evaluation of material obtained from our collection.

The six biological targets in this study were chosen based on previous reports of biological activities from cyanobacteria. The three protease targets, 20S proteasome and two viral proteases (SARS PLpro and 3CLpro), were selected based on reports of protease inhibitors isolated from cyanobacteria (Ploutno et al., 2002; Yamaki et al., 2005). The importance of the proteasome, as part

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of the ubiquitin-proteasome pathway, in regulation of transcription and the cell cycle has made inhibition of the proteasome a key target for the treatment of cancer (Burger & Seth, 2004; Ciechanover, 1994; Ciechanover & Iwai, 2004; Khan et al., 2006; King et al., 1996; Ohta & Fukuda, 2004). Drug discovery efforts targeting this enzyme have led to the development of Bortezomib (Velcade™, Millennium Pharmaceuticals, Inc.), which was approved by the US FDA in May 2003 for the treatment of multiple myeloma (Burger & Seth, 2004). In addition, salinosporamide A, a proteasome inhibitor obtained from a marine actinomycete, is currently in phase I clinical trials for the treatment of multiple myeloma (Feling et al., 2003).

In addition, we evaluated our collection of cyanobacterial extracts for the potential to inhibit two viral proteases from the coronavirus (CoV) responsible for severe acute respiratory syndrome (SARS) (Liang, 2006). This virus gained public recognition in 2003 during the SARS epidemic that originated in southern China. Two proteases, the papain like protease (PLpro) and the chymotrypsin like protease (3CLpro), have been shown to be important in viral replication, and inhibition of these proteases interrupts the virus's life cycle and decrease viral loads (Chen et al., 2005; Harcourt et al., 2004).

Cyanobacteria have also been shown to be a source of antimicrobial compounds (Harvey, 2000; Jaki et al., 2000). For this project we selected two current microbial targets, *Mycobacterium tuberculosis* and *Bacillus anthracis*. *Mycobacterium tuberculosis*, which affects an estimated 1.76 billion people worldwide, is naturally resistant to many antibiotics (Cole et al., 1998; Morens et al., 2004). In addition, the emergence of HIV-TB coinfection, multi-drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis strains only further complicates the treatment of this disease. Thus, novel treatments for TB are needed. Natural products continue to provide useful leads for the development of new antimycobacterial agents (El Sayed et al., 2000; Okunade et al., 2004).

On the other hand, *Bacillus anthracis* is not a traditional public health concern. During the twentieth century in the USA, there were only 18 reported cases of inhalation anthrax, the most lethal form of anthrax (Quintiliani & Quintiliani, 2003). In 2001, the threat of anthrax garnered increased attention after the terrorist attacks in the USA (Knight, 2001). Naturally occurring resistance and, in particular, engineered resistance in *B. anthracis* add to the threat to public health and safety. Consequently, there is focus on the search for new treatments for anthrax to reduce the renewed threat of this disease.

In addition to the five assays listed above, the brine shrimp, *Artemia salina*, assay was utilized to measure toxicity of the extracts (Meyer et al., 1982).

Materials and methods

Collection

Cyanobacterial strain acquisition

Strains of cyanobacteria were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX) (Starr & Zeikus, 1993) and the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) (Andersen et al., 1997). In addition, unique strains of cyanobacteria were isolated in our laboratory from collections made in the Great Lakes region of the USA. Unialgal strains were obtained through a combination of algal isolation techniques. The three techniques used in this study were isolation by streak plate, micropipette and serial dilution plate (Andersen & Kawachi, 2005; Hoshaw & Rosowski, 1973). Taxonomic identification was completed via microscopic observation and aided with the taxonomic guides written by Komarek (2003), Komarek et al. (2003), and Komarek and Anagnostidis (2005). For this study, strains from UTEX are designated with a U preceding the strain number, C for CCMP strains, and I for strains isolated by the authors.

Three media were employed for cyanobacterial isolation; Z and modified versions of BG-11 and BG-11_0, which we have designated BG-12 and BG-12_0, respectively. The composition of Z medium has been previously described by Falch et al. (1995). The preparation of BG-11 and BG-11_0 has been described by Andersen et al. (2005). We modified BG-11 and BG11_0 by substituting citric acid and ferric ammonium citrate with 5.0 mL of a FeEDTA solution per L of media. The FeEDTA solution, also used in the Z medium, was prepared according to Schlösser (1994).

Cultivation

Media

Culture media for freshwater strains were Allen, Z, Z45, and DY-V. These media were selected based upon media previously used in the cultivation of acquired strains. For strains isolated at UIC, Z medium was designated as the preferred media due to a moderate concentration of the major nutrients and a nitrogen:phosphorus ratio most like the Redfield ratio (Wetzel, 2001). Media recipes for Allen and DY-V have been detailed by Andersen et al. (2005). The composition of the Z45 medium has been described by Mian et al. (2003). For the culture of marine strains, ES and f/2 were utilized (Andersen et al., 2005). For all of the media, silica (Na_2SiO_3) a nutrient needed for the growth of diatoms, was not included, if listed in the protocol. The concentration of the major nutrients in these media is detailed in Table 1.

Table 1. Concentration (mM) of major nutrients in media.

Nutrient	Allen	BG-12	DY-V	ES	f/2	Z	Z45
C (CO_3)	0.189	0.189	-	-	-	0.189	-
N (NO_3)	17.6	17.6*	0.235	0.199	0.883	5.74	5.74
N (NH_4)	-	-	-	0.060	-	0.002	0.002
P (PO_4)	0.215	0.175	0.071	0.022	0.036	0.399	5.74

*Nutrient absent in BG-12_0 modification of this medium.

Culture conditions

For each strain, stock cultures were grown using 150 mL of liquid media in a 250 mL Erlenmeyer flask. From the stock cultures, 5–10 mL was used to inoculate 1.0 L of liquid media in a 2.8 L Fernback flask. The 1.0 L culture was cultivated at 20°C under a mean illuminance of 1.93 klx. An automated timer system provided a 24 h light/dark cycle that consisted of 18 h of illumination and 6 h of darkness.

Extraction

The 1.0 L cultures were allowed to grow for 6–8 weeks prior to harvest. The cell material was harvested by centrifugation then freeze-dried. The lyophilized biomass was extracted via maceration with methanol:dichloromethane (1:1). Three rounds of maceration were used to afford the final extract. The resulting extract was dried *in vacuo*. For each extract, library solutions in dimethyl sulfoxide (DMSO) were prepared at concentrations of 4 and 10 mg/mL. These library solutions were stored at –80°C and were used to supply material needed for evaluation in the different assay systems.

Protease bioassays

All extracts were initially evaluated for percentage inhibition at a concentration of 100 µg/mL. Extracts demonstrating inhibition that was 70% or greater were then evaluated in a dose-dependent fashion to determine the IC_{50} of the extract. The 4 mg/mL extract solutions were utilized as the source of cyanobacterial extracts for the protease assays.

20S Proteasome

The assay was performed according to the protocol provided with the BIOMOL 20S Proteasome Assay Kit for Drug Discovery (BIOMOL International LP, Plymouth Meeting, PA, USA catalog number AK740-0001). The protocol was modified such that the 10 min incubation period was performed at 37°C. Enzyme was acquired from BostonBiochem (20S proteasome, human, BostonBiochem, USA catalog number E-360) and substrate from BIOMOL (Suc-LLVY-AMC, catalog number P802-0005). This substrate is specific for the chymotrypsin-like activity of the 20S proteasome. Fluorescence was measured using either a Tecan Genios Pro microplate reader or a Hewlett Packard model AF10000 fluorimeter with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

SARS-CoV PLpro

SARS-CoV PLpro protein (polyprotein residues 1544-1825) was purified as previously described by Barretto et al. (2005). Inhibition assays were performed in duplicate in black 96-well plates containing the following components: 50 mM HEPES pH 7.5, 0.1 mg/mL BSA, 0.5 mM DTT, 50 nM PLpro, 5% DMSO, and 100 µg/mL cyanobacterial extract. The peptidic substrate RLRGG-AMC (Bachem, King of Prussia, PA) was added to a final concentration of 50 µM to initiate the reaction, and product fluorescence was measured using a Tecan Genios Pro microplate reader (excitation wavelength: 360 nm, emission wavelength: 460 nm).

SARS-CoV 3CLpro

SARS-CoV 3CLpro assay was performed in a 96-well microtiter plate as described by Grum-Tokars et al. (2008). Briefly, the assay contained the following components: 50 mM HEPES, 50 mM NaCl, 1 mM DTT, and 500 nM 3CLpro, 5% DMSO, and 100 µg/mL cyanobacterial extract. The FRET-based substrate utilized in this assay was 1 µM Alexafluor 488 and the resulting fluoresence was measured using a Tecan Genios Pro microplate reader (excitation wavelength: 490 nm, emission wavelength: 535 nm).

Antimicrobial bioassays

For the antimicrobial assays, extracts displaying minimum inhibitor concentration (MIC) values less than or equal to 100 µg/ml were considered active.

Mycobacterium tuberculosis

Evaluation of cyanobacterial extracts was performed using the microplate alamar blue assay (MABA) as described by Collins and Franzblau (1997). Cyanobacterial extracts were provided for this assay using the 10 mg/mL stock solutions.

Bacillus anthracis

Extracts were evaluated at a range of concentrations from 100 µg/mL to 48.8 ng/mL using a 4 mg/mL stock solution in DMSO. Evaluation of the extracts was performed as previously described by Athamna et al. (2004).

Brine shrimp toxicity assay (Artemia salina)

Evaluation of cyanobacterial extracts was performed at a concentration of 100 µg/mL, using the assay described by Metcalf et al. (2002). Metcalf's method was modified by omitting the second 24-hour incubation of *A. salina*

cysts and including only one observation after 16–18 hours of incubation of the nauplii in the test solutions.

Results

Culture collection

A collection of 61 strains were cultured and evaluated in the assays described. A complete list of all strains evaluated is given in Table 2. This group of 61 includes members of all five orders of cyanobacteria and represents all six media currently used by the University of Illinois at Chicago culture collection (Table 3). Those strains isolated from field collections during this project are listed in Table 4.

Protease inhibition assays

Extracts were considered active when exhibiting inhibition greater than or equal to 70% at 100 μg/mL. For the 20S proteasome assay, it was found that 12 extracts were active. In the SARS-CoV assays, one extract displayed activity in the PLpro assay and three were active in the 3CLpro assay. The only overlap of activity among the protease inhibition assays was the extract of strain *Tolypothrix* sp. (C 1185), which displayed activity against both SARS proteases.

All active extracts were evaluated to determine the IC_{50} values (Table 2). The IC_{50} values obtained for the extracts active against the SARS 3CLpro were at or below 10 μg/mL. Similarly, 6 of the 12 proteasome active extracts displayed significant levels of activity with IC_{50} values less than 10 μg/mL. However, the extract active in the PLpro assay, *Tolypothrix* sp. (C 1185), displayed an IC_{50} of 80 μg/mL in the PLpro assay and the dose response was linear. Due to the linear response displayed, the extract from *Tolypothrix* sp. (C 1185) was further investigated. It was shown that the majority of the activity could be explained by the shielding of the 4-amino-7-methyl-coumarin (AMC) fluorophore, and not by specific inhibition of the PLpro enzyme. On the other hand, the activity of this extract in the 3CLpro assay was still considered significant since the IC_{50} value was ten-fold lower, 6.0 μg/mL, and the dose-dependent response could be described by an idealized IC_{50} dose response curve. In addition, the flourophore used and the excitation and emission wavelength pairs were different for the two SARS-CoV protease assays.

Antimicrobial assays

The active threshold for the antimicrobial assays was defined at an MIC value less than or equal to 100 μg/mL. In the *B. anthracis* assay, five extracts displayed activity at MIC values ranging from 3.1 to 100 μg/mL. The extracts of two strains, *Nostoc muscorum* Ag. (U 2301) and *Fischerella ambiguia* (Näg) Gom. (U 1903), which showed the greatest activity in the anthrax assay (MIC values of 6.3 and 3.1 μg/mL respectively), were the only extracts to display a significant level of activity in the TB assay.

Brine shrimp toxicity assay

Three extracts, *F. ambigua* (U 1903), *Tolypothrix* sp. (C 2147), and *Lyngbya aestuarii* (Mert.) Liebmann (U 2515), displayed significant activity at 100 μg/mL. The toxicity of these extracts was greater than 90% mortality at 100 μg/mL. All other extracts displayed mortality rates less than or equal to 10% at this dose.

Discussion

Our data revealed that several cyanobacterial extracts possess significant levels of activity in two of the three proteases evaluated, SARS-CoV 3CLpro and the 20S proteasome. It should be noted that both 3CLpro and the targeted proteolytic activity of the 20S proteasome are chymotrypsin-like, whereas the PLpro enzyme is a papain-like protease. For these designations, chymotrypsin-like and papain-like, the model proteases are a digestive protease in animals (chymotrypsin) and a plant protease isolated from the papaya fruit (papain). The chymotrypsin-like activity is not limited to digestion in higher animals but, has been reported in arthropods, e.g., *Daphnia magna*, which often graze on cyanobacteria (von Elert et al., 2004). Based on this information, one could speculate that the disparity of activity between the chymotrypsin-like and papain-like proteases could be due to an adaptation of cyanobacteria to grazing. However, further research is needed to substantiate this theory.

Analysis of the antibacterial results showed a correlation of very strong antianthrax activity and antimycobacterial activity. These results would support the idea that *M. tuberculosis* is more resistant to the antibacterial effects of the extracts than *B. anthracis*. This idea is also supported by the fact that *M. tuberculosis* is naturally resistant to many antibiotics due to the presence of several drug efflux systems, a highly hydrophobic cell envelope, and various drug modifying enzymes (Cole et al., 1998). In the case of either target, significant activity was found among the extracts evaluated. Further chemical analysis of the extract from *F. ambigua* (U 1903) has lead to the isolation of several antibacterial ambiguine isonitriles. Details of the isolation and characterization of these compounds will be presented in a subsequent publication.

Three extracts displayed significant activity in the brine shrimp toxicity assay, with only one extract, *Lyngbya aestuarii* (U 2515), not displaying activity against any other targets evaluated. In addition, this was

Table 2. Full list of strains investigated and associated biological activity.

Scientific Name	Source[1]	Medium	Assay[2]					
			PR[3]	PL[3]	CL[3]	AX[4]	TB[4]	BS[5]
Order Chroococcales								
Aphanocapsa sp.	I 10009	Z	-	-	-	-	-	-
Aphanothece sp.	C 2529	f/2	-	-	-	-	-	-
Chamaesiphon sp.	U 2347	Allen	1.0	-	-	-	-	-
Chroococcus sp.	C 2128	DY-V	15.4	-	-	-	-	-
Enthophysalis cf. sp.	C 2136	DY-V	-	-	-	-	-	-
Eucapsis sp.	U 1519	Z45	30.0	-	-	-	-	-
Gloeocapsa sp.	U EE 3	Allen	-	-	-	-	-	-
Synechococcus cf. *elongates*	C 1629	f/2	-	-	-	-	-	-
Synechocystis nigrescens (nom. prov. R.A. Lewin)	U 2587	ES	-	-	-	-	-	-
Synechocystis sp.	I 10006	Z	-	-	-	-	-	-
Synechocystis sp.	I 10010	Z	28.3	-	-	-	-	-
Order Pleurocapsales								
Dermocarpa violacea Crouan	U 1635	ES		-	-	-	-	-
Pleurocapsa fuliginosa Thuret in Hauck	U 2555	ES		-	-	-	-	-
Order Oscillatoriales								
Arthrospira fusiformis Vouk	U 2720	Allen	-	-	-	-	-	-
Arthrospira platensis (Oerst.) Geitler	C 1295	f/2	-	-	-	-	-	-
Geitlerinema carotinosum (Geitler) Anagnostidis	C 2534	f/2	-	-	-	-	-	-
Lyngbya aestuarii (Mert.) Liebmann	U 2515	ES	-	-	-	-	-	100 %
Lyngbya aestuarii Gomont	C 473	f/2	-	-	-	-	-	-
Lyngbya lagerheimii (Möb.) Gom.	U 2574	Allen	-	-	-	-	-	-
Lyngbya sp.	U 2516	ES	-	-	-	-	-	-
Lyngbya sp.	C 2520	f/2	-	-	-	-	-	-
Lyngbya spiralis Geitler	U 1831	Z45	-	-	-	-	-	-
Microcoleus vaginatus var. *cyano-viridis* Baker & Bold	U 1815	Z45	68.2	-	-	-	-	-
Microcoleus cf. sp.	C 1184	1184	-	-	-	-	-	-
Oscillatoria amoena (Kütz.) Gom.	U 1306	Z45	46.3	-	-	-	-	-
Oscillatoria brevis (Kütz.) Gom.	U 1567	Z45	0.1	-	-	-	-	-
Oscillatoria sp.	C 1519	f/2	-	-	-	-	-	-
Oscillatoria tenuis Ag.	U 1566	Allen	-	-	-	-	-	-
Phormidium tenue (Agardh ex Gomont) Anagnostidis & Komarek	C 1231	f/2	-	-	-	-	-	-
Phormidium autumnale (Ag.) Gom.	U 1580	ES	-	-	-	-	-	-
Planktothrix agardhii (Gomont) Anagnostidis & Komarek	C 600	DY-V	-	-	-	-	-	-
Planktothrix agardhii (Gomont) Anagnostidis & Komarek	C 601	DY-V	-	-	-	-	-	-
Plectonema boryanum Gom.	U 596	Z45	-	-	-	-	-	-
Plectonema sp.	U 1541	Allen	-	-	-	-	-	-
Schizothrix calcicola (Ag.) Gom.	U 1817	Allen	-	-	-	-	-	-
Schizothrix calcicola var. radiata Baker & Bold	U 1819	DY-V	-	-	-	-	-	-
Schizothrix calcicola (Ag.) Gom.	U 2245	DY-V	-	-	-	-	-	-
Symploca sp.	I 10001	Z	-	-	-	-	-	-
Order Nostocales								
Anabaena sp.	C 2066	DY-V	-	-	-	-	-	-
Aphanizomenon flos-aquae (L.) Ralfs	U 2384	Z45	-	-	-	-	-	-
Calothrix anomala Mitra	U 1319	Z45	0.1	-	-	-	-	-
Calothrix parietina Thur.	U 1952	Z45	5.9	-	-	-	-	-

Table 2 continued on next page

Table 2. Continued

Scientific Name	Source[1]	Medium	Assay[2] PR[3]	PL[3]	CL[3]	AX[4]	TB[4]	BS[5]
Cylindorspermopsis raciborskii (Wolosz.)	C 1973	DY-V	-	-	-	-	-	-
Cylindrospermum licheniforme Kütz.	U 2014	Allen	-	-	-	-	-	-
Fremyella diplosiphon (Gom.) Drouet	U 481	Allen	-	-	-	-	-	-
Gloeotrichia ghosi Singh	U 1920	Z45	-	-	-	-	-	-
Gloeotrichia sp.	U 583	Z	-	-	-	-	-	-
Nodularia harveyana (Thw.) Thuret	U 2093	Z45	-	-	-	-	-	-
Nodularia spumigena (Mertens in Juergens) Born.& Flah.	U 2092	Allen	-	-	-	-	-	-
Nostoc commune Vaucher	U 1621	Z45	0.3	-	-	-	-	-
Nostoc edaphicum Kondrat'eva	U 2492	Z45	-	-	-	-	-	-
Nostoc foliaceum Moug.	U 1624	Z45	-	-	-	-	-	-
Nostoc muscorum Ag.	U 2301	Allen	-	-	10.0	3.1	< 100	-
Nostoc muscorum Ag.	U 1933	ES	-	-	-	-	-	-
Scytonema hofmanni Ag.	U 1834	Z45	15.1	-	-	25	-	-
Scytonema sp.	U 2588	ES	-	-	-	-	-	-
Spririrestis rafaelensis (Flechtner nom. nud.)	U 2660	Z45	-	-	-	-	-	-
Tolypothrix sp.	C 1185	DY-V	-	80	6.0	100	-	-
Tolypothrix sp.	C 2147	DY-V	0.2	-	-	-	-	90 %
Order Stigonematales								
Fischerella ambigua (Näg.) Gom.	U 1903	Allen	-	-	10.0	6.3	2.7	90 %
Hapalosiphon welwitschii W. & G.S. West	U 1830	Z45	-	-	-	50	-	-

[1] Source abbreviations: C, Provasoli-Guillard National Center for Culture of Marine Phytoplankton; U, Culture Collection of Algae at the University of Texas at Austin; I, Isolated by authors at University of Illinois at Chicago.
[2] Assay abbreviations: PR, 20S Proteasome; AX, *Bacillus anthracis*; TB, *Mycobacterium tuberculosis*; PL, SARS-CoV PLpro; CL, SARS-CoV 3CLpro; BS, Brine shrimp.
[3] IC_{50} value reported in μg/ml, minus sign (–) denotes IC_{50} values greater than 100 μg/ml,values in bold denote significant activity ≤ 10 μg/ml.
[4] MIC value reported in μg/ml, minus sign (–) denotes MIC values greater than 100 μg/ml, values in bold denote significant activity ≤ 50 μg/ml.
[5] Lethality rate at 100 μg/ml, minus sign (–) denotes lethality ≤ 10%.

Table 3. Summary of strains investigated.

Order	Freshwater/terrestrial	Marine	Total
Chroococcales	8	3	11
Pleurocapsales	0	2	2
Oscillatoriales	16	9	25
Nostocales	19	2	21
Stigonematales	2	0	2
Total	45	16	61

Table 4. Strains isolated by authors.

Strain ID	Genus	Collection Site
10001	*Symploca*	Forest Ave., Downers Grove, IL
10006	*Synechocystis*	Wood St., Chicago, IL
10009	*Aphanocapsa*	Harbor at Menominee, MI
10010	*Synechocystis*	UIC Field Station, Downers Grove, IL

the only extract from marine cyanobacteria to display significant activity in any of our assay systems.

In total, extracts of 12 different strains possessed significant levels of activity in one or more targets. When the data were grouped by taxonomic order, the highest density of significant active extracts was found among the orders Nostocales and Stigonematales (9 out of 12). These orders, combined, constitute all of the heterocyst-forming cyanobacteria. This cohort was the only group to have representative extracts, which showed significant activity in both the antimicrobial and SARS protease bioassays. Whereas extracts displaying activity in the 20S proteasome and brine shrimp assay were not limited to the cohort of heterocystic cyanobacteria. Further analysis of the data, when organized by growth medium, showed the cyanobacteria cultivated in freshwater conditions exhibited the most significant activity (11 out of 12). These results further support the use of cyanobacteria, in particular freshwater, heterocystic strains, in drug discovery efforts for antiviral, anticancer, and antibiotic lead compounds.

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