INVESTIGATION OF ANTIMICROBIAL ACTIVITIES OF *CAULERPA MEXICANA* AND *UDOTEA LOOENSIS* AGAINST SURFACE ASSOCIATED STRAINS OF *VIBRIO* SPP. AND *BACILLUS* SP.

Zipporah Phillips[1], Kaitlin Ferguson[1], Bobby Owens[1], Jordan Ekhoff[1], Elizabeth Philip[1], Theodore Weyna[2], Ian Miller[2], Jason Kwan[2], Tomasz Jurga[1], and Melany P. Puglisi[1]

[1]Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628

[2]University of Wisconsin-Madison, School of Pharmacy, 777 Highland Avenue, Madison, WI 53705

Abstract

In a broad survey of extracts of common marine algae from the Florida Keys against an extensive panel of environmental bacteria, extracts from the green alga *Udotea looensis* exhibited significant activity against two strains of *Vibrio* sp. and one strain of *Bacillus* sp. To continue the investigation of the antibacterial extract from *U. looensis*, the crude extract was subjected to bioassay-guided fractionation. *U. looensis* were collected by snorkeling (Florida Bay, Florida Keys) at depths of 1-3 meters in July 2012 and 2013, immediately frozen at -20 degrees Celsius and transported to CSU. The sample was extracted in 1:1 MeOH:EtOAc over 72 hours followed by partitioning between dH_2O and EtOAc. The EtOAc partition was further partitioned in 9:3:7 $CHCl_3$:dH_2O:MeOH. The $CHCl_3$ partition was subjected to repeated Si gel chromatography using a Biotage Isolera One, yielding multiple active fractions. Fractions were analyzed by NMR on a JOEL 400 MHz NMR. Extracts and column fractions were assayed against three strains of bacteria maintained in A1 media: *Vibrio* sp. CM, *Vibrio* sp. UL and *Bacillus* sp. in a 96-well plate format using standard methods with DMSO as the carrier solvent. Controls included streptomycin, tetracycline, and DMSO. Initial and final readings were measured using a Biotech plate reader (625nm) at 24-72 hours, strain dependent. Crystalline fractions were analyzed on a Bruker Avance III 600I with 5 mm proton (13C/15N/31P) Cryogenic Probe. Mass spectra were obtained on an ESI ultra high resolution QTOF MS (MaXis 4G) by a 1 in 1000 dilution of 95 to 5 mixture of acetonitrile to water of a working stock 1 mg/ml solution.

Introduction

It is well documented that increasing ocean temperatures, changes in pH and salinity, and high nutrient levels have had devastating effects on coral reefs.[1-5] These changes can result in major disease outbreaks by opportunistic or emerging pathogens that can have devastating effects resulting in the mass mortality of individual populations over short periods of time.[1,7] Studies have demonstrated that algal extracts exhibit antimicrobial activities against a broad range of ecologically relevant organisms.[8-10] Subsequent bioassay-guided fractionation of algae surveyed in these studies resulted in the isolation of active metabolites that inhibit the growth of marine fungi and bacteria at concentrations well below the natural concentration.[11-13] Identification of metabolites active against environmental strains may provide new lead compounds for the development of new antibiotics.[14]

Members in the family *Vibrionaceae* are associated with the surface of algae and have been identified as opportunistic pathogens that cause disease in algae and marine animals.[15] In our broad survey of common macroalgal extracts from the seagrass beds of the Florida Keys, the crude extracts from *Caulerpa mexicana* and *Udotea looensis* exhibited potent activity against three strains of bacteria, *Vibrio* sp. CM isolated from the surface of *C. mexicana*, *Vibrio* sp. UV from the surface of *Ulva lactuca* and *Bacillus* sp. RS from Red Sea sediment. *Caulerpa* spp., order Caulerpaceales, produce high concentrations of sesquiterpenes that inhibit the growth of *Vibrio kiggathi* and *V. phisoboreum*.[16] *Udotea*, order Bryopsidales, is a ubiquitous green marine alga that has been known to produce antiproliferative, antifungal and antimicrobial compounds.[17,18]

The objective of this study is to use bioassay-guided fractionation to isolate and identify the antimicrobial compounds from *Caulerpa mexicana* and *Udotea looensis*.

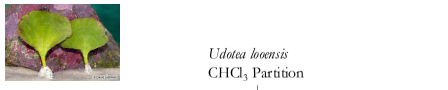
Methods

Caulerpa mexicana and *Udotea looensis* were collected by snorkeling from the Florida Bay in the Florida Keys at depths of 1-3 meters during July 2012 and 2013, and were immediately frozen at -20 °C and transported to CSU college of pharmacy and stored at -80 °C. Algae were extracted in 1:1 MeOH:EtOAc over 72 hours and dried. *C. mexicana* was partitioned between dH_2O and EtOAc. The EtOAc partition was subjected to repeated Si gel chromatography using a medium pressure liquid chromatography apparatus Biotage Isolera One to yield multiple active fractions. *U. looensis* was partitioned between dH_2O and EtOAc. The EtOAc partition was further partitioned in 9:3:7 $CHCl_3$:dH_2O:MeOH. The $CHCl_3$ partition was subjected to repeated Si gel chromatography using a Biotage Isolera One to yield multiple active fractions. Active fractions were analyzed by ^1H NMR on a JOEL 400 MHz NMR.

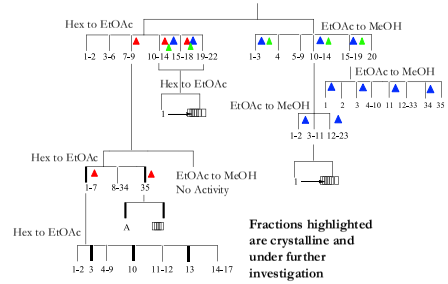
Extracts and column fractions were assayed against three environmental strains of bacteria: *Vibrio* sp. CM isolated from the surface of *C. mexicana*, *Vibrio* sp. UL isolated from the surface of *Ulva lactuca* and *Bacillus* sp. RS from Red Sea sediment. 50µL of A1 media, 50µL of diluted bacteria grown in A1 liquid media, and 0.6µL of DMSO-extract were added to each well of a 96-well plate. The controls were: streptomycin, tetracycline, and DMSO. The initial and final readings were made by using a Biotech plate reader at 625nm and read after 24-72 hours, depending on the strain of bacteria.

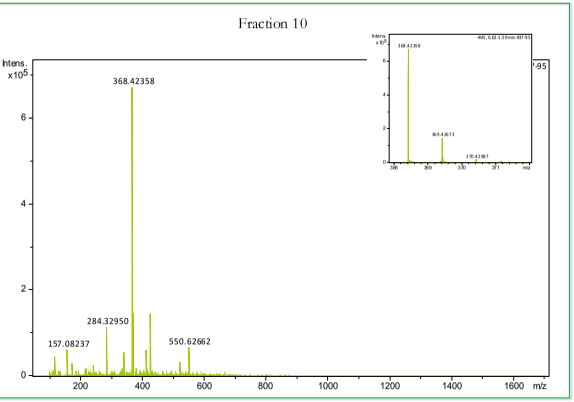
Crystalline fractions were tested on a Bruker Avance III 600I with 5 mm 1H(13C/15N/31P) Cryogenic Probe at the National Magnetic Resonance Facility at Madison in deuterated methanol. Both a proton spectra and a COSY were obtained. Mass spectra were obtained on a ESI ultra high resolution QTOF MS (MaXis 4G) by a 1 in 1000 dilution of 95 to 5 mixture of acetonitrile to water of a working stock 1 mg/ml solution.

Results



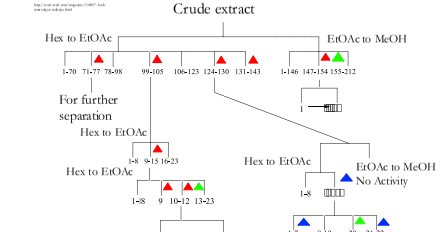
Udotea looensis
$CHCl_3$ Partition





Vibrio sp. UL
Vibrio sp. CM
Bacillus sp. RS

Caulerpa Mexicana
Crude extract



Discussion and Conclusion

Bioassay-guided fractionation of extracts from *Caulerpa mexicana* and *Udotea looensis* demonstrates that these algae are rich with metabolites that inhibit the growth of *Vibrio* sp. UL, *Vibrio* sp. CM and *Bacillus* sp. RS. Activity is mainly seen in the non-polar fractions of *C. mexicana* against *Vibrio* sp. UL. We also see activity against *Vibrio* sp. UL in the non-polar fractions of *U. looensis*, while the more polar fractions contain metabolites that inhibit the growth of *Vibrio* sp. CM and *Bacillus* sp. RS. Fractionation of *U. looensis* yielded one fraction with crystals. The MS data shows that this metabolite could be potentially be a novel antimicrobial compound.

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