Extraction of cyanobacteria from Palmyra Atoll for identification of biologically active compounds

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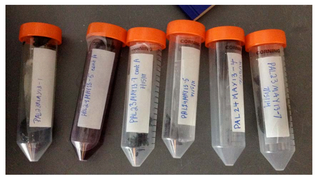
ABSTRACT

Within the last few decades, cyanobacteria and other marine organisms have come to light as potent producers of biologically active secondary metabolites. While these metabolites were first studied to identify toxic compounds, they are now being utilized in drug development to treat cancer, inflammation, viruses, hypertriglyceridemia, and other conditions. The purpose of this study was to identify biologically active natural products from three samples of unidentified cyanobacteria (probably *Moorea* species) collected in Palmyra Atoll. This was achieved through a series of extractions, liquid chromatography/mass spectrometry analysis, and molecular networking. It was determined that two of the of the three samples contain known potent natural products palmyramide A and that one of those two also contains the natural product curacin D. Through further comparison with standard compound databases, it may be found that these samples also contain novel compounds which harbor potential for future drug development.

EXTRACTION OF CYANOBACTERIA SAMPLES

1. Harvested 3 cyanobacteria from media
   - **PAL23MAY13-7 contaminant A (LR1)**
   - **PAL23MAY13-7 (LR4)**
   - **PAL24MAY13-4 (LR5)**
2. Freeze dried the samples
3. Ground up dried samples with mortar and pestle
4. Added DCM:MeOH=2:1 to powder and sonicated to break up cell membranes and release cell content
5. Filtered solution and collected DCM:MeOH extract
6. Repeated steps 3-5 until solid sample lost color
7. Dried the DCM:MeOH extract using the Rotary Evaporation then re-dissolved in DCM:MeOH and re-dried (weighed empty flask first)
8. Weighed dry extracts in vials to find extract weights



Freeze dried samples; only 3 were used in this experiment

Filtration of LR1

Rotary Evaporation

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC-MS)

9. Created one 1 mL of 1 mg/mL solution for each sample with filtration on C18 column
10. Ran samples through LC-MS (liquid chromatography-mass spectrometry)
11. Compared MS1 data with standards for previously analyzed and identified marine natural products
12. Showed the concentration of positive ions accepted by the compound being passed through the LC-MS HPLC column at a given point in time
13. Used molecular networking to compare relationships based on MS2 data with database standards
    i. MS2 data showed how different compounds (as identified by MS1 data) fragmented when hit by bursts of energy–measured the masses of the fragments and plotted similarities between the fragments
    ii. Fragment relationships were shown as models *(Fig. 1)* for each sample (LR1, LR4, and LR5) and overlaid with models of standard samples with similar characteristics
14. Matches between MS1 and MS2 sample data and that of database standards indicated identical or similar natural products within the samples
    i. Indicated Palmyramide A in LR4 and LR5 *(Fig. 2)*
    ii. Indicated Curacin D in LR4 *(Fig. 3)*

Molecular Network of LR4 and LR5

Yellow – LR5; blue – LR4; orange- both
square- Palmyramide A standards

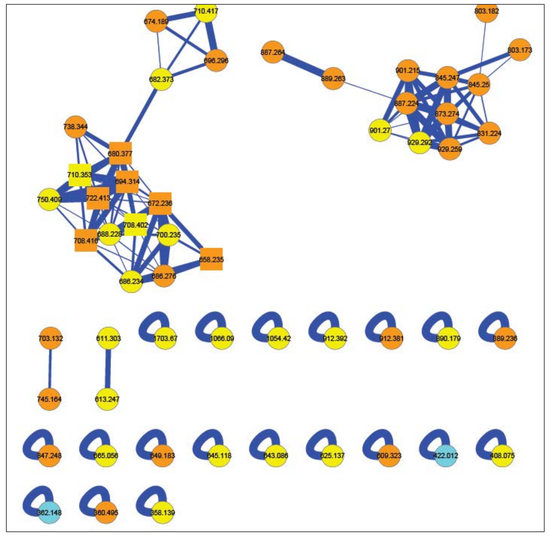


Fig. 1

LR5 LC-MS profile of [M+H]=672. Palmyramide A?

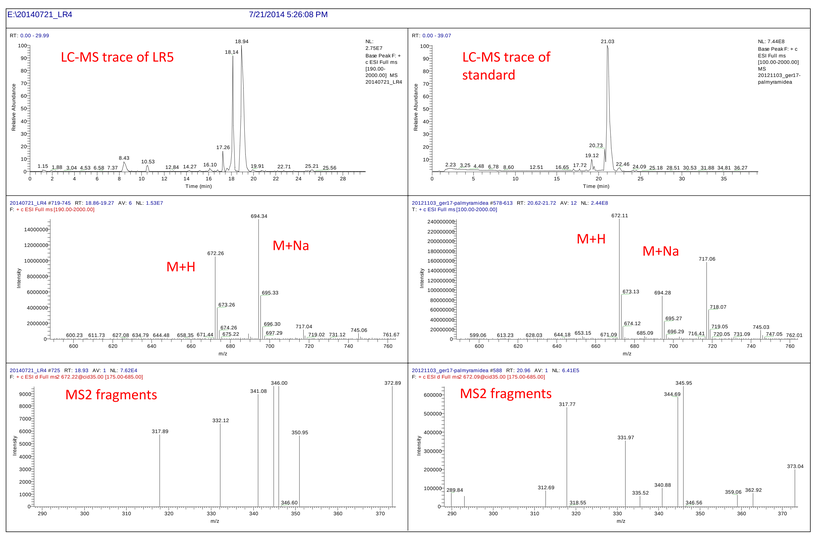
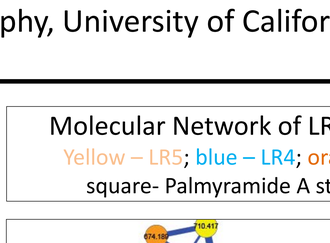
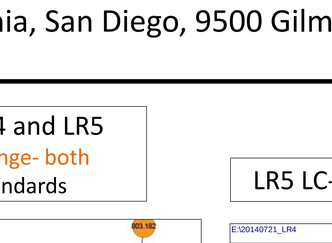


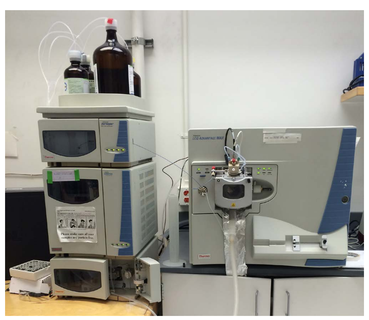
Fig. 2



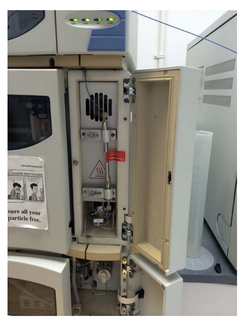
Microscopic LR4 (PAL23MAY13-7)



Microscopic LR5 (PAL24MAY13-4)



LC-MS



LC-MS HPLC column

Curacin D in LR4 (based on MS2 profiles)

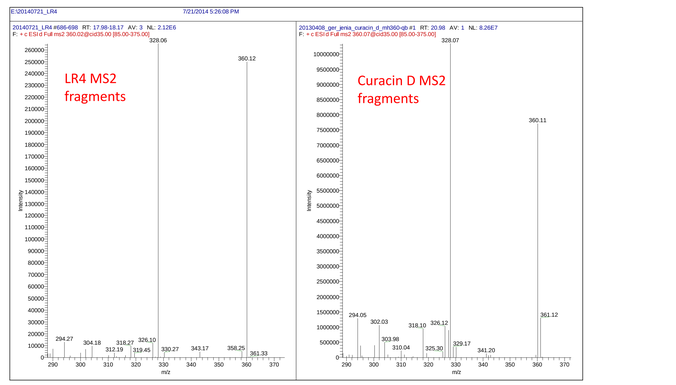


Fig. 3

CONCLUSIONS

- Samples PAL23MAY13-7 and PAL24MAY13-4 likely contain the natural product palmyramide A
- Sample PAL23MAY13-7 likely contains the natural product curacin D
- Both of these natural products have shown exciting potential to kill cancer cells, and have intriguing aspects of their biosynthesis

FUTURE DIRECTIONS

- Further analysis may show matches for other compounds in the three samples
- Novel compounds, which do not match any samples in gnps library (http://gnps.ucsd.edu/), may be identified
- Assays will show if novel products have potential to aid in drug development