16s rRNA Phylogeny of Moorea Bouillonii

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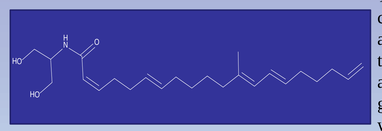
Marine Cyanobacterial secondary metabolites present important opportunities for pharmaceutical research. However, in order to effectively utilize these opportunities, taxonomic identification is necessary. Such identification is done through analysis of a ribosomal RNA unit known as 16s rRNA gene. This segment of the prokaryotic genome changes very little over time and thus allows for precise identification of very closely related organisms.

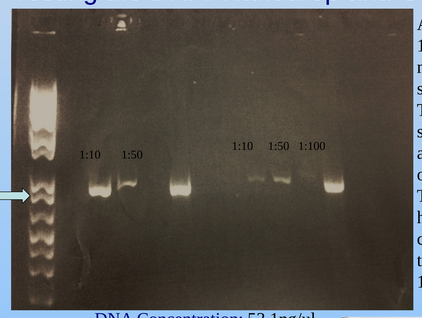
Useful Compounds of M.Bouillonii



The amino-glycerol compound

The Gerwick chemistry lab isolated two compounds from the M.Bouillonii specimen; apratoxin A, which is known to be cytotoxic to pancreatic and lung cancer cells, and another previously unidentified amino glycerol compound. This second compound was found to have cannabinoid receptor modulatory effects, indicating its potential medical use as an appetite regulator, as well as a chemotherapy relief and anti-cancer agent.

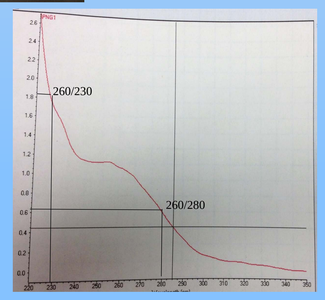
Testing the DNA: Nanodrop and Gel Electrophoresis



After DNA dilutions of 1:1, 1:10, 1:50, and 1:100 were made, PCR proved to be successful for 1:10 and 1:50. This means that the correct segment of DNA was amplified, followed by cloning of the PCR products using the TOPO cloning (Invitrogen), hence, allowing the project to continue to transformation of the resulting plasmid into TOP 10 cells.

DNA Concentration: 53.1ng/ul
260/280: 1.81
260/230: 0.66

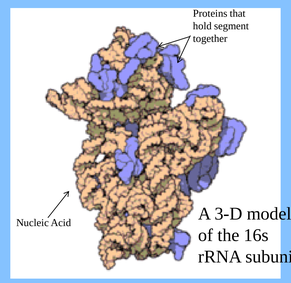
These Nanodrop results show a rather low concentration of DNA, however this is acceptable because large amounts of DNA are not needed in order to amplify the 16s rRNA gene. The 260/230 level, which indicates the level of DNA purity, is also very low, which is why the DNA dilutions were necessary. Impure DNA can result in PCR failure due to the other organic compounds that compromise the process. The 260/280 level is adequate indicating not too many impurities from proteins.



The 16s rRNA Sequence

GCTCGGGTCGATACAAGCTACACCTAGTATCCATCGTTTACAG
CTAGGACTACTGGGGTATCTAATCCCATTCGCTCCCC
TAGCTTTCGTCCCTCAGTGTCAGGTTCAGTTCCAGTAGAGCGC
CTTCGCCACCGATGTTCTTCCCGAAATCTACGCATTC
ACCGCTACACCGGGAATTCCCTCTACCCCTACTGCCCTCTAGT
TCCTCAGTTTCCACTGCCTGCCCAGAGTTAAGCCCG
GTCTTTAACAGCCGACTTGAGGTACCACCTACGGACGCTTTA
CGCCCAATAATTCCGGATAACGCTTGCATCCTCCGTT
TACCGCGGCTGCTGGCACGGAGTTAGCCGATGCTTTTTCCTC
AGGTACCGTCACTTTCTTCCCTCGATAAAAGAGGT
TACAACCCAAAAGCCTTCCTCCCTCACGCGGTATTGCTCCGT
CAGGCTTTCGCCCATTGCGGAAAATTCCCCACTGCGCCTCCC
GTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGCTCA
TCCTCTCAGACCAGCTACTGATCGTTGCCTTGGTAGGCTTTTA
CCCTACCAACTAGCTAATCAGACGCGGAGCTCCTCTTATGGCAA
TAAATCTTTCACCTCTCGGCACATCCGGTATTAGCCACTGTTT
CCGGTGGTTGTCCCCGACCCTAAGGCAGANTCTCACGCGTTA
CTCACCCGTCCGCCACTCCCCCGAAGGGGCGTTCGACTTGCA
TGTGTTAAGCATACCGCCAGCGTTCATCCTGAGCCANGATCA
AACTCTCACTGAGGGACGAAAGCTAGGGGAGCGAATGGGAT
TAGATACCCCAGTAGTCCTAGCTGTAAACGATGGATACTAGGT
GTAGCTTGTATCGACCCAAGCTGTGCCGAAGCCAACGCGTTA
AGTATCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCA
AAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGG
TTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACA
TGTCGCGAATCCCGGTGAAAGCTGGGAGTGCCTTAGGGGGC
GCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTTT
TAGTTGCCAGCACGTGAAGGTGGGCACTCTAGAGAGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAG
CATGCCCCTTACGCCCTGGGCGACACACGTACTACAATGGTC
AGGACAATGGGCAGCCAACTCGCAAGGGGGAGCTAATCTCA
TCAAACCTGGCCTCAGTTCAGATTGCCGGCTGCAACTCGCCG
GCATGAAGGAGGAATCGCTAGTAATCGCCGGTCAGAATACGG
CGGTGAATCCGTTCCCGGGCCTTGTACACACCGCCCGTCACA
CCATGGAAGCTGGCCACGCCCGAAGTCGTTACCCTAACCCTT
TTGGGAGGGGGATGCCGAANGCAGGGTTGGTGACTGGGGGT
GAAGTCGTAACAAGGTANCCGTAA

This 1500 base pair sequence is used to identify the Cyanobacteria utilizing the very low rate of change from species to species. Only about 5 base pairs of this sequence are different than other closely related Cyanobacteria species[1]. By identifying which base pairs are different, and the frequency of these alterations, a program can distinguish this organism from its relatives in a matter of seconds.



A 3-D model of the 16s rRNA subunit

Protocol Outline

DNA Isolation and PCR → Cloning, Transformation, and Plasmid Extraction → Analyzing the Sequences

Transformation of E.coli

In order to mass-replicate the vector containing the gene of interest, the vectors were placed in chemically competent E.coli cells, which are capable of such mass replication without altering the DNA sequence. Since two sets of primers were used (27 forward, 809 reverse; and 740 forward, 1494 reverse), each of the samples were separately replicated in order to complete the entire gene. This split in the gene of interest is fixed when the sequences are obtained. Figure A represents the successful transformation of the E.coli containing the 27 to 809 base pair vector. Figure B represents the successful transformation of the 740 to 1494 base pair vector. Figure C represents negative control using bacteria not containing the vector, which also allows for ampicillin resistance, was used to verify the functionality of the ampicillin antibiotic on the plates. Five of the colonies on each successful plates were cultured in individual tubes, and then treated with PCR to further replicate the vector. Lastly, the vector was isolated and cleaned to be sent to the Retrogen© lab for sequencing. Sequences were received the next morning and analyzed as seen below.

Figure A:

Figure B:

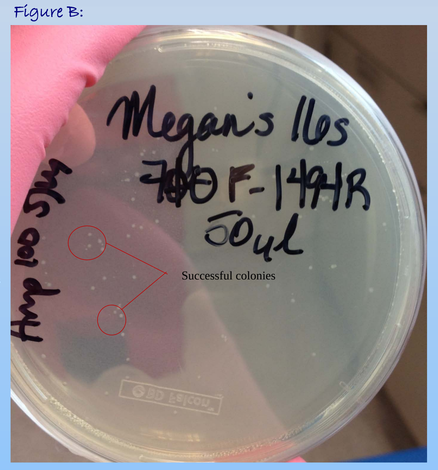
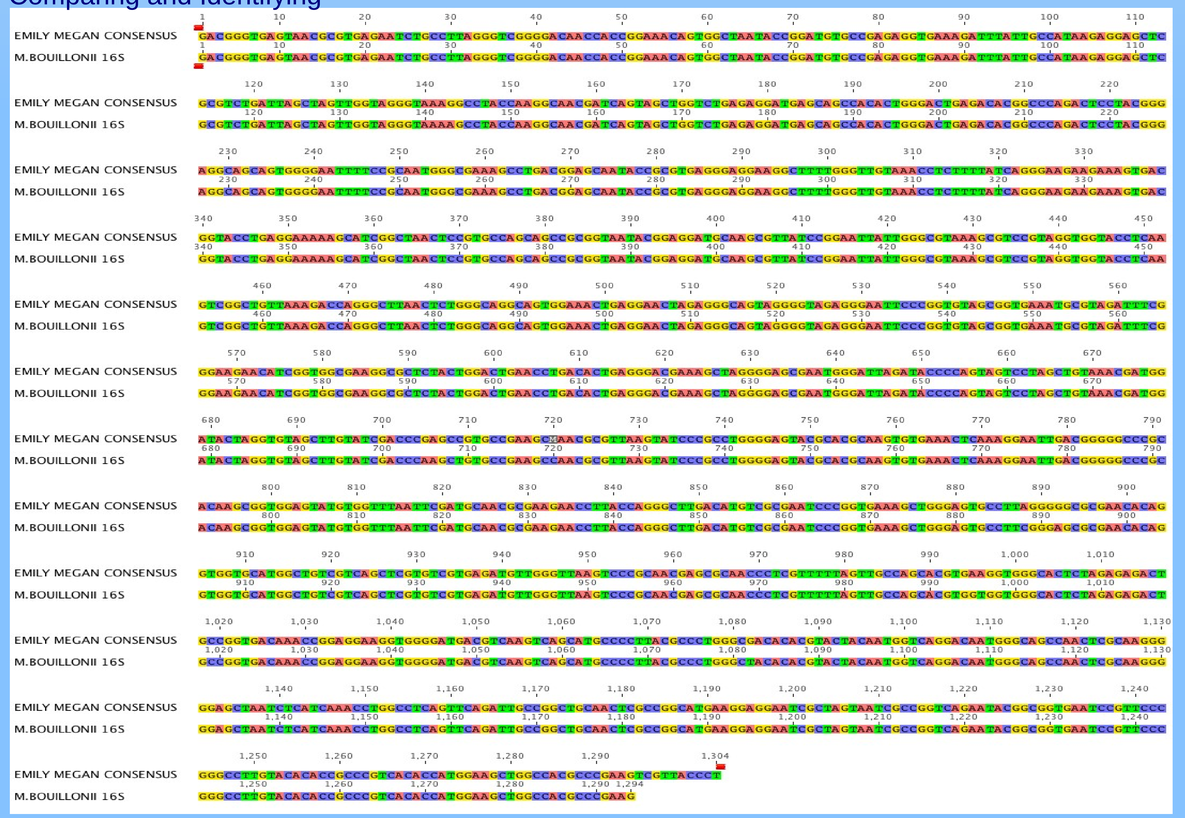


Figure C:

Comparing and Identifying



The upper strand shown is the 16s rRNA sequence for the Papua New Guinea unidentified cyanobacteria. The lower strand is the general 16s rRNA sequence for the Moorea Bouillonii species, as provided by the NCBI database. If the two strands were followed base-pair by base-pair, no discrepancy between the two sequences would be found. From this it can be concluded that the unidentified cyanobacteria is, in fact, a M.bouillonii. This fact was already suspected as a result of the richness of its secondary metabolite production; a morphological identification at the time of collection. M.bouillonii is a tropical cyanobacterium that is filamentous and often form large, mat-like colonies[1]. This finding is valuable to continued research on this useful organism.

Underwater image of a large tuft of Moorea Bouilonii

Acknowledgements

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References

1. Engene, N. et. al. and Gerwick, WH (2011) Phylogeny-Guided Isolation of Ethyl Tumonoate A from the Marine Cyanobacterium cf. Oscillatoria margaritifera. Journal of Natural Products 74, 1737-1743.