Extracting and Sequencing the 16S rRNA Gene of a Panamanian Cyanobacterium

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ABSTRACT

Cyanobacteria, a type of photosynthetic bacteria found mainly in aquatic environments, has yielded many natural product chemicals with anti-inflammatory and anticancer properties. Scientist Dr. Bill Gerwick collected an unknown specimen of cyanobacteria given the code number “VQR 16S” from off the coast of Panama. Dr.Bill Gerwick and his coworkers want to discover if this is a new species of cyanobacteria, or if it has already been catalogued in the scientific literature. Therefore, the goal of this work was to extract the 16S rRNA gene (the universal barcode gene of prokaryotes), obtain its sequence, and then compare this with a large database of other 16S rRNA cyanobacterial gene sequences. To extract the 16s rRNA gene a PCR was run with primers for the cyanobacterial 16S gene. The PCR product was then inserted into plasmids, which were then transformed into *E. coli* to grow. After growing in *E. coli* the plasmid was purified from the *E. coli* and a polymerase chain reaction using plasmid primers to verify the size of the insert before sending off for sequencing. The compounds, polycavernosides, has already been isolated from this unknown cyanobacterium. Polycavernosides are potential lead compound against cancer.

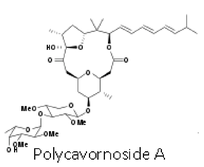
Procedure

1. 7/16/14 - Ran DNA through multiple displacement amplification. (REF 1)
2. 7/16/14 - Ran the DNA through a gel with the multiple displacement amplification products to make sure that DNA was amplified.
   A. 7/16/14 - No bands showed up from the gel, therefore the DNA was diluted with H20 and run again.
3. 7/18/14 - DNA was run through steps one and two again, and this time the DNA was amplified.
4. 7/18/14 – The amplified DNA was used in a PCR reaction using 16srRNA primers. The products were taken and put through a ligation reaction and put into plasmids.
5. 7/18/14 - Plasmids were transformed into competent *E. coli* cells
6. 7/18/14 - The *E. coli* were plated onto 3 different antibiotic plates containing different amounts of the culture.
7. 7/21/14 - Only two colonies grew on one plate out of all the three plates due to a temperature mishap in the incubator.
8. 7/21/14 - The two growing colonies were transferred into different falcon tubes and put through a process which extracted the 16S gene from the DNA in the plasmids from the colonies.
9. 7/21/14 - Steps 5 and 6 were repeated to grow more colonies.
10. 7/22/14 - The plasmids containing the 16S gene from the first two colonies were put through polymerase chain reaction to verify that the 16S gene was collected.
11. 7/23/14 - More colonies grew from the replated *E. coli* and they were put through steps 8 and 10.
12. 7/24/14 - All of the colonies were sent to be sequenced.

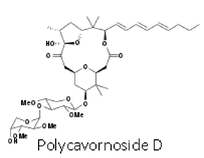
Picture of Cyanobacteria

This picture shows some of the many cultures of cyanobacteria which the Gerwick Lab has collected. Each one of them has different characteristics and qualities to further advance the goal of drug discovery.

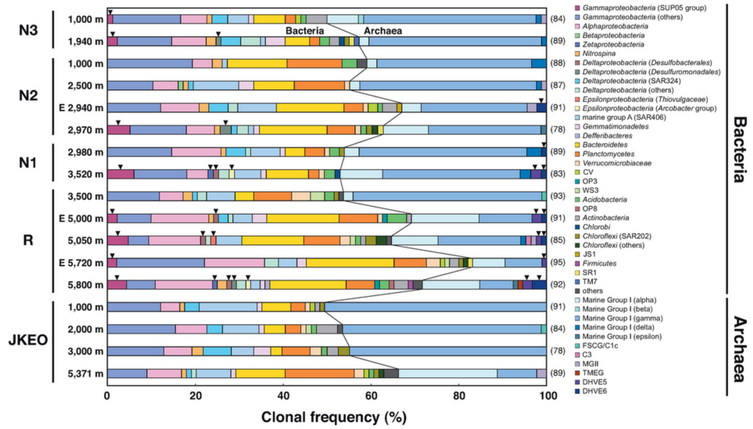
Structures of Polycavernosides



Structures courtesy of Dr. Gabriel Navarro

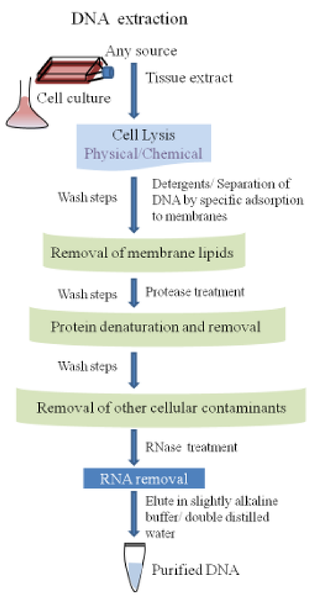


16S rRNA Gene



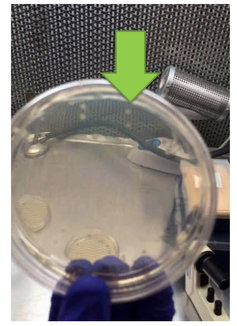
The 16S rRNA Gene is a specific part of prokaryotic DNA found in all bacteria and archaea. It is used for sequencing due to the fact that every bacteria has it; therefore the specific 16S rRNA of an unknown species can be deciphered based on comparisons with other bacteria’s 16S genes. The 16S gene is also convenient for sequencing because it is only 1.5 kb long. The function of the 16S gene has never changed over time, unlike other genes, but has remained the same. This makes it reliable to use for sequencing. Lastly, DNA is more dependable in taxonomic classification than the phenotype. Above is a diagram of the whole prokaryotic 16S rRNA gene phylotype compositions (REF 2).

Extracting the 16S Gene from Plasmids



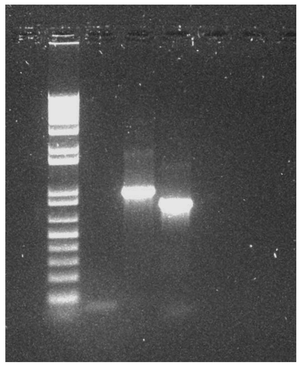
To extract the plasmid containing the 16S rRNA gene from *E. coli* is an extensive process. First the colonies are scraped from the plates and put into Falcon tubes containing media and incubated overnight. Next the Falcon tubes are put into a centrifuge and are centrifuged until the cells are pelleted out. Then the liquid is removed. The products are put into a buffer P-1 from the Qiagen extraction kit. Next, 350 microliters of Buffer 3 are added and mixed. This is put into a centrifuge for 10 minutes. Afterwards, the centrifuge supernatant is applied to the QIAprep spin column through pipetting. This is centrifuged for 30-60 seconds and then the flow through liquids are discarded. Next the QIAprep column is cleaned by spinning through 2 x 375 mL buffer PE. Then the product is centrifuged again for 30-60 seconds. Next the QIAprep spin column in transferred to a collection tube and 20 microliters of sterile water are added to the spin column. It sits for one minute and then is centrifuged for one minute. This whole process essentially breaks open the cells and only collects the plasmids while everything else is washed out. (REF 3)

Collinies



This picture shows many colonies of *E.coli* that took up plasmids on a plate with antibiotics. These colonies represent cells which are alive and growing. This particular plate is the re-plated #2 plate. We collected two of these colonies for further evaluation.

Gel Scan



This gel scan shows the 16S Gene of the VQR next to a 16S Gene which has already been sequnced and verified as a 16S Gene. This is a gel of DNA products after they were run through polymerase chain reactions. This step is necessary to show that it is in fact a 16S Gene that I extracted, and not another gene. This is proven because both bands are of nearly the same size.

CONCLUSIONS

In conclusion the 16S rRNA gene is still in the process of being sequenced, and therefore I am not yet able to determine if the species is a new one or if it has previously been discovered. When the 16S gene sequence is back from sequencing, the Gerwick lab will be evaluating its results and hopefully using these to further their investigation of drug discovery from this organism.

References

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