Identification of Microorganisms Using 16S rRNA Gene Sequencing

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Abstract

TimberFish is a startup fish farming company that uses non-agricultural plant materials as well as food processor waste as feed and nutrition sources for the fish. Determining the microorganism profile of the tanks could help provide insight into the technology's effectiveness. The 16S rRNA gene is a useful biomarker and was used in this project as a means for identifying microorganisms in a water tank sample. Three unique 16S sequences were sent out for DNA sequencing. The sequences were then analyzed to determine the types of microorganisms present in the water tank at TimberFish. The sequence analysis showed that two of the organisms sent out for sequencing were bacteria and one of the organisms was a fungus.

Background

Fig. 1: TimberFish Technologies, a local and sustainable fish farm in Westfield, NY. The farm uses wastewater from an affiliated distillery as its water source. The wastewater is filtered with woodchip biofilters containing invertebrates that consume microbes prior to being incorporated into the growth tanks, in which the invertebrates also act as a food source for the fish by falling into the growth tanks from the biofilters. The discharged water is clean and meets quality standards. (Fig. 1 taken from "Governor Cuomo Announces Opening of TimberFish Technologies in Western New York". governor.ny.gov)

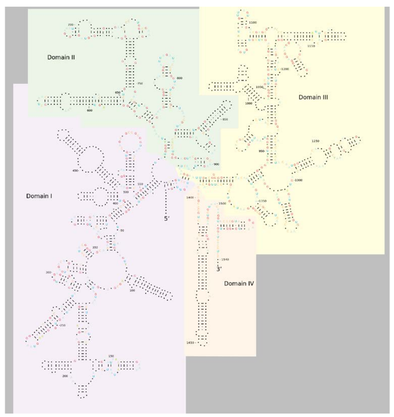

Fig. 2: Secondary structure of the 16S rRNA gene. The 16S rRNA gene is a common genetic marker to identify bacteria. The 16S rRNA gene is present in all bacteria. It is also functionally conserved, meaning that changes in the gene sequence are a result of evolutionary differences. The 16S rRNA gene is also large enough (~1,500 base pairs) for sequence analysis and species identification. (Fig. 2 taken from Public Domain. Author: Squidonius.)

Methods

DNA Isolation: A water sample from the W11 growth tank at TimberFish was filtered using vacuum filtration and gDNA was isolated using the DNeasy PowerWater Kit by Qiagen. This yielded a sample of genomic DNA from the organisms present in the W11 growth tank.

PCR Amplification of 16S rRNA Gene: The 16S rRNA gene was amplified from the gDNA sample using Bead PCR tubes and 16S primers to yield a heterogenous sample of amplified 16S genes (Fig 3).

Ligation of PCR Product to Plasmid: Using the Zero Blunt TOPO PCR Cloning Kit by Invitrogen, the 16S PCR products were ligated to pCR4Blunt-TOPO plasmid vectors.

Transformation of Plasmid into *E. coli*: pCR4Blunt-TOPO plasmids were transformed into competent *E. coli* cells. The recombinant plasmids were kanamycin resistant, so the use of kanamycin-containing plates selected for growth of recombinant plasmids containing the 16S PCR product.

Plasmid Preparation: Recombinant plasmids from the transformed cells were isolated and purified using the QIAprep Spin Miniprep Kit.

Restriction Digest of Plasmid: A restriction digest and subsequent gel electrophoresis of the plasmid preparation product at the EcoRI sites was performed in order to identify which purified plasmid samples were recombinant with the 16S PCR product. (Fig 4)

16S rRNA Sequence Analysis: Plasmid products that were identified as recombinant with a 16S gene insert were sent out for Sanger sequencing (Fig 5). Sequence results were analyzed using the ApE software and ribosomal database website to determine which microorganisms have 16S sequences most similar to the one's obtain in this experiment. Inferences are then able to be made about the identity of bacteria present in the W11 growth tank at TimberFish (Fig 6).

Results

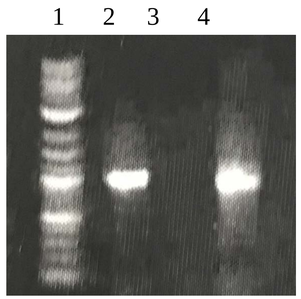

Fig. 3: Agarose gel electrophoresis of PCR amplification of the 16S rRNA gene. PCR was performed to amplify the 16S gene from the gDNA sample. Lane 1 was the hyperladder. Lane 2 was the positive control amplification of the 16S gene from *E. coli*. Lane 3 was the negative control. Lane 4 was the amplification of the 16S gene from a gDNA sample isolated from a growth tank at TimberFish. The sharp, bright bands from Lane 2 and Lane 4 show that the amplification of the 16S gene was successful for the gDNA sample.

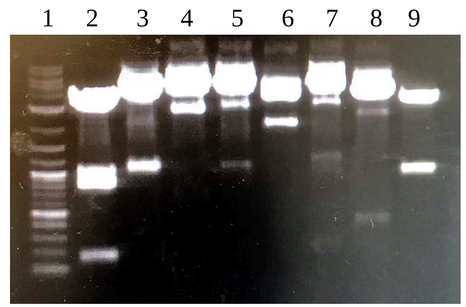

Fig. 4: Agarose gel electrophoresis of the restriction digest of isolated and purified plasmids. Lane 1 is the hyperladder. Lanes 2-9 are isolated plasmids from the plasmid preparation. A second, smaller band indicates a 16S gene insert that was cut out by the ECO RI restriction enzyme. The plasmids in these lanes were recombinant.

Fig. 5: Section of an electropherogram from a 16S gene insert sent out for sequencing to GeneWiz. A red peak corresponds to a T nucleotide, a black peak corresponds to a G nucleotide, a blue beak corresponds to an C nucleotide, and a green peak corresponds to an A nucleotide.

Fig. 6: Ribosomal database search results. As shown, the microorganism was identified as similar to bacteria of two different phylum. The first bacteria, *Curvibacter*, a gram negative, rod-shaped, aerobic. These bacteria were originally isolated from well water in Japan. The unknown microorganism is also taxonomically similar to *Spartobacteria*, an anaerobic, gram negative, rod bacteria that has been isolated from rice paddy fields.

The second sample sent out for sequencing was most taxonomically similar to bacteria from the genus *Spartobacteria*, which is described in figure 6. The third sample was identified as being fungi from one of three possible phylum, *Ascomycota*, *Chytridiomycota*, a fungus that has not been classified.

Conclusions

Three microorganisms from a growth tank at TimberFish were characterized, two being bacteria and one being fungi. Future goals of this project could be to sequence and identify more microorganisms for this particular growth tank, eventually identifying microorganisms present in biofilters and growth tanks throughout the filtration process at TimberFish.

References

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