Identification of Positive Coding Sequences for Proteins Essential to the Pathogenic Behavior of *Naegleria fowleri*

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Abstract

It is not known why *Naegleria fowleri* can become pathogenic since under normal circumstances *N. fowleri* is a free living ameboflagellate that does not require a host to perform its life cycle. Genes that are significant to its virulence as a pathogen may also be related to why *N. fowleri* can become pathogenic when exposed to the right environments, particularly that of the mammalian central nervous system. The goal of this study is to use 454 sequencing technology to sequence and analyze the DNA of *N. fowleri* and attempt to locate sequences coding for proteins that are essential to the pathogenic behavior of *N. fowleri*.

Introduction

Naegleria fowleri (ATCC # 30894) is an Ameboflagellate that can cause severe acute PAM, found in both soil and freshwater habitats. PAM is a CNS (central nervous system) infection: primary amebic meningoencephalitis. PAM generally occurs in previously healthy children or young adults after diving or swimming in fresh water. *N. fowleri* can produce pore forming proteins that are capable of lysing brain tissue. The parent strain used in this study: Lee, was isolated from a case of PAM in a young adult from Richmond VA in the 1960's. Lee mp was created when the Lee strain was

passaged through mouse brains at monthly intervals and is highly pathogenic. Sequences of genomic DNA from Lee mp could help identify virulence factors present in *N. fowleri*

Under normal circumstances *N. fowleri* is a free living ameboflagellate that does not require a host to perform its life cycle. Genes that are significant to its virulence as a pathogen may also be related to why *N. fowleri* can become pathogenic when exposed to the right environments, particularly that of the mammalian central nervous system.

The parent strain used in making the cDNA library Lee, was isolated from a case of PAM in a young adult from Richmond VA in the 1960's. Lee mp (mouse passaged) was used to make a cDNA library. Lee mp was created when the Lee strain was passaged through mouse brains at monthly intervals and is highly pathogenic. The cDNA library created using mouse-passaged amebae (Lee mp). In the past this cDNA library has been effective in locating protein sequences produced by *N. fowleri*. Genomic sequencing was thought to be a logical next step in continued search for protein sequences necessary to the pathogenicity of *N. fowleri*.

Hypothesis: Genomic DNA was isolated and sequenced using 454 sequencing technology in order to identify unique coding sequences that permit identification of virulence factors in *N. fowleri*.

Materials and Methods

Lee strain amebae was cultured for four days before being harvested by centrifugation. Centrifugation was 2000 rpms for twenty minutes. Pellets gathered after centrifugation were washed with PBS. The genomic DNA was isolated from the strain of *N. fowleri* designated Lee using a Qiagen kit. The pellets were treated with a lysis buffer

and Protienase K overnight. Next an ethanol precipitation was preformed before vortexing and adding to a spin column. The DNA was then eluted from the column in two washes. DNA was placed into TE buffer before with a concentration of at least 5 micro grams per 100 micro liters. Gel electrophoresis was run to ensure that the DNA was in fragments shorter than 500 base pairs or longer than 1.5 kilobase pairs. 260:280 ratios were also taken for the DNA as well as 260:230 ratios to check for purity of the DNA, target values for purity were 1.8 and 2.0 respectively.

DNA that was obtained from the purification was sent to a core sequencing facility. The genomic information received from the 454 sequencing was taken subjected to BLASTX searches. The sequences were subjected to more specific searches should the reveal any genes, especially genes possibly related pathogenicity.

Results

After several trials it was found that the DNA was not ligating to the beads in the 454 sequencing machine. After some modifications to the original protocol intended to eliminate possible inhibitors in growing and harvesting processes for the amebae; DNA was sent to be sequenced, DNA again did not ligate to the beads in this trial. Results from further modification and adjustments of the protocol are still pending.

Disscussion

It was originally thought that the an inhibitor was present in the growth medium for the amebae that could be preventing the DNA from ligating to beads in the 454 machine. Hemin was thought to be the original inhibitor. After eliminating hemin from

the growth medium it was found that the problem continued to persist in that the DNA was still not ligating to the beads. It is thought that the continued trouble with ligation of the DNA to the Beads in the 454 sequencing machine is probably due to some sort of DNA inhibitor that is getting through the purification process.

The next course of action is to locate the possible inhibitor or inhibitors that are most likely causing the problem. Once the inhibitors are located it may be possible to eliminate them and complete the sequencing. If every course of action along this path fails use of the cDNA library will resume with immunoscreening and western blotting for proteins.

Significance and Potential Ramifications

Proteins significant to pathogenicity are potential drug targets for treating PAM. Any proteins that are significant to pathogenic virulence may also be significant to why *N. fowleri* can become pathogenic under the appropriate conditions. By sequencing genomic DNA of *N. fowleri* it may be possible to identify genes potentially coding for proteins that contribute to the pathogenicity of the organism.

Citations and Sources

Hawley. 1981. The Condensed Chemical Dictionary.10ed. Van Nostrand Reinhold Company, Inc.

http://www.dpd.cdc.gpv/dpdx

http://www.promega.com/profiles/1001/ProfilesinDNA_1001_09.pdf

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