Searching for Megaviruses in Iceland

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Presence of Megaviruses from Diverse Icelandic Environments

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ABSTRACT

The proposed Megavirales order comprises members of the previously known nucleocytoplasmic large DNA viruses (NCLDV). NCLDV viruses in the Megaviridae family are generally known for their nucleic acid packaging machinery such as transcription and replication. A new family of megaviruses infecting free-living amoebae such as Acanthamoeba, Mieramphastica, and Pandoravirus has been isolated from water and soil samples from Chile, France, India, and the United States. We chose to study the occurrence of megaviruses in Iceland because of the diverse habitats at within one island. No research has been carried out on the presence of megaviruses in Iceland. Samples of water and soil were collected from lava fields, moss/lichen mountain fields and mixed forestry plantations. Acanthamoeba castellanii (ATCC #30010) at a 5x10^7 cell/mL concentration was used in an amoebal co-culture. A 1:10 dilution of soil or water sample preparation was added to each well. Cell lysis was determined by measuring absorbance. The number of cells/ml was determined by a microbial counting method. Megaviruses were identified by metagenomic analysis. The results show that megaviruses are present in Iceland. Additionally, we would like to thank Ohio Wesleyan University’s Theory Practice Grant Program for funding travel to Iceland and funding the metagenomic analysis by Phase Genomics. Lastly, I would like to thank Phase Genomics for working with myself and the university. I would also like to thank Iceland for giving me the opportunity to participate in the ASM Undergraduate Research Fellowship. Lastly, I would like to thank Phase Genomics for sending washing equipment and the university.

BACKGROUND

Megaviruses are a new family of viruses that replicate in amoebal cells and encode several unique proteins like proteins involved in transcription and replication. Megaviruses have a median genome size of 1.25Mb. They are known for their large genome size and unique viral proteins. Megaviruses are known to cause cellular lysis in amoebal cells. Megaviruses are found in both natural and artificial environments. Megaviruses have been found in water and soil samples from Chile, France, India, and the United States. Megaviruses are also known to infect free-living amoebae such as Acanthamoeba, Mieramphastica, and Pandoravirus.

METHODS

Sampling

Figure 1. Electron micrographs of (a) Mimiviruses and (b) Mimivirus viral factories (Aherfi et al. 2016). Megaviruses viruses have a diameter of 150-750nm and genome lengths of 17-1.25Mb. Relative to other viruses, they replicate rather independently of the host cell in viral factories. Megaviruses have been found in water and soil samples from Iceland.

Culture

Figure 2. A 2016 map of locations in which Megaviruses have been found. Megaviruses have been found in natural and manmade environments around the globe. No research has been done on their presence in Iceland (black arrow).

Figure 3. Iceland was chosen as a sampling location due to the wide variety of soil types and water sources on a relatively small area of land. Samples were collected from lava field soils, heath soils, glacial fed rivers, man-made forests, black sand beaches, and blackish waters.

Sampling Location

Figure 4. The top of soil was collected with a sterile scoop. 2-3 replicates were taken on a 5m transect. Soil was held at 4 ⁰C until time of analysis. (b) Icelandic lava field shrub healths, (b) moss and lichen mountain fields

Figure 5. Co-culture flow chart

(a) A 1:10 dilution of soil or water was added to a fresh culture of Acanthamoeba castellanii (5x10^7 cells/mL) and cultured onto a fresh culture of A. castellanii plus antibiotics at a 7:10 dilution. (b) Cells exhibiting lysis were sub-cultured onto a fresh culture of A. castellanii plus antibiotics at a 7:10 dilution. (c) Wells exhibiting lysis were sub-cultured onto a fresh culture of A. castellanii plus antibiotics at a 1:10 dilution. (d) Plates were incubated at 28⁰C for 3-5 days and were observed for lysis.

Figure 6. The moss and lichen mountain field and mixed forestry plantation samples were sent to Phase Genomics for metagenomic sequencing.

Figure 7. Percent lysis of A. castellanii co-cultures was taken by visual estimation of cell confluence at 3-5 days post inoculation compared to 0 hours post inoculation. R-studio was used to generate box plots showing the percent lysis for three samples treated with (+) and without (-) an antibiotic cocktail. In each figure, a reduction in lysis is seen in co-cultures treated with antibiotics. (a) A amoebal cultures inoculated with soil from Icelandic lava field shrub healths, a range of 13-33% lysis with a 20% median is seen for cultures treated with antibiotics, while a range of 12-78% lysis with a median of 44% is seen for untreated cultures. (b) In amoebal cultures inoculated with soil from moss and lichen mountain fields, a range of 0-50% lysis with a 37% median is seen for cultures treated with antibiotics, while a range of 28-95% lysis with a median of 61% is seen for untreated cultures. (c) In amoebal cultures inoculated with soil from a mixed forestry plantation, a range of 16-75% lysis with a median of 27% is seen for cultures treated with antibiotics while a range of 0-85% lysis with a 58% median is seen for untreated cultures.

RESULTS

Table 1: Percent lysis of amoeba in various Icelandic soils

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Percent Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lava Fields</td>
<td>27%</td>
</tr>
<tr>
<td>Moss/Lichen Fields</td>
<td>44%</td>
</tr>
<tr>
<td>Mixed Forestry</td>
<td>58%</td>
</tr>
</tbody>
</table>

Figure 8. Percent lysis of amoeba in various Icelandic soils

(a) lava Fields % Lysis
(b) Moss/Lichen Mountain Fields % Lysis
(c) Mixed Forestry Plantation % Lysis

REFERENCES CITED


CONCLUSIONS

- Ameoba lysing microorganisms exist in Icelandic soils
- Non-bacterial and non-fungal amoeba lysing microorganisms exist in Icelandic soils
- PCR on soil samples using Polymerase B primers for different Megaviridae species to confirm megaviruses presence
- Phase Genomics Metagenomic analysis to determine soil microbiome and megavirus presence

FUTURE DIRECTIONS

- Ameoba lysing microorganisms exist in Icelandic soils
- Non-bacterial and non-fungal amoeba lysing microorganisms exist in Icelandic soils
- PCR on soil samples using Polymerase B primers for different Megaviridae species to confirm megaviruses presence
- Phase Genomics Metagenomic analysis to determine soil microbiome and megavirus presence

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