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Finding RNA-DNA Hybrid Viruses

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Abstract

Until extremely recently it was thought that recombination between DNA and RNA viruses was practically nonexistent. The discovery of the RNA-DNA Hybrid Virus (RDHV) genome in a metavirome from a high-temperature acidic lake changed this view(Diemer and Stedman, 2012). We and others, have discovered multiple examples of this recombination hiding in plain sight in multiple published and unpublished metagenomes from many different environments and recent publications (Rosario et al., 2012, Tae Woong Whon et al., 2012, Mitsuhiro Yoshida et al., 2013, and others through personal communication. Comparing the proteins within these hybrid viruses against each other will reveal conserved regions. This will also reveal insight into the evolutionary relationships between the various viruses. Locating conserved sequences will allow for the creation of detection tools such as degenerate primers which can be employed on environmental samples to detect the presence of similar viruses. Finding more Hybrid RNA-DNA Viruses may eventually help further the understanding of viral evolution.

Methods

Sequences of capsid proteins with homology to BSL RDHV capsid proteins were collected from metagenomes and other research groups. The bioinformatics software program Geneious was used to make translation alignments. From these alignments degenerate primers were designed. Some of these primers were used on virus-size particle concentrate samples from reclaimed water from Florida and amplified with PCR. The amplicon was sequenced and primers were designed for long inverse PCR, using Dream Taq (Thermo Scientific) to amplify the complete genome. The resulting amplicon was inserted into the Topo TA vector using the TOPO/TA cloning kit (Invitrogen) and transformed into chemically competent Nova Blue *E.coli*. Primers were designed to sequence the entire genome by genome walking using BDT(Life Technologies) sequencing. Geneious was used to compare and investigate phylogenic relationships of the replication initation (rep) protein and capsid proteins with other RNA/DNA hybrid viruses and metagenomes.

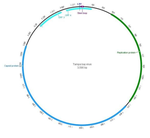
Background

RNA-DNA hybrid viruses

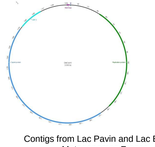
Capsid Protein
Replication Initiation Protein
Open Reading Frame
Stem Loop

BSL RDHV
(Diemer, Stedman., 2012)

Tampa Bay Virus
(Lauren Mcdowell)

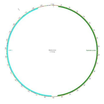


DMClaHV
(Ian Hewson)



DfcycV
(Rosario, et al., 2012)

Uncultured Marine Virus
(Mitsuhiro Yoshida et al., 2013)



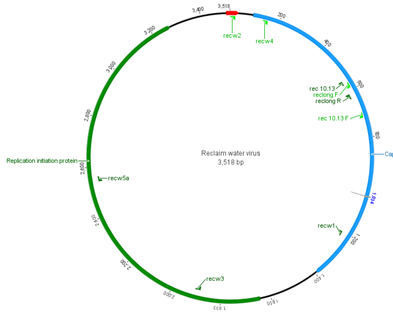
Contigs from Lac Pavin and Lac Bourget Metagenomes France
(Simon Roux 2012)

•RDHV and similar viruses contain a capsid protein similar to that found in RNA viruses and a replication initiation protein similar to that found in ssDNA circovirdiae.

Capsid protein amino acid sequence alignment with degenerate primers designed to amplify entire genome

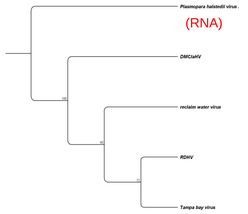
Results

Reclaimed Water Virus

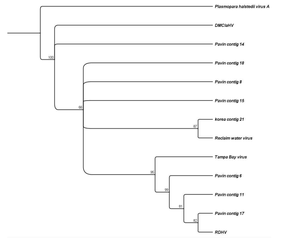


•Red indicates unfinished section of genome 50-100bp.
•Rec 10,13 are initial primers used to locate virus in sample.
•Reclong primers used for long inverse PCR.
• Recw primers used to complete BDT sequencing.
• Stem loop has not been located yet probably in missing section of genome.

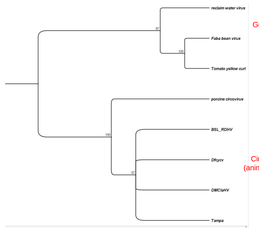
Capsid protein phylogenies from RNA-DNA hybrid viruses with known whole genomes



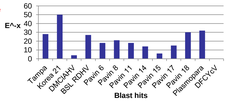
Capsid protein phylogenies from RNA-DNA hybrid viruses



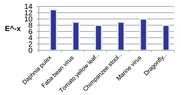
Replication initiation protein phylogenies from known complete genomes.



Blast search Reclaim Capsid Protein



Blast search of Reclaim replication initiation protein



Discussion

RNA-DNA hybrid viruses are extremely diverse in both the replication initiation (rep) protein and the capsid proteins. The newly discovered reclaimed water virus capsid protein is homologous to BSL RDHV, Tampa Bay virus, and DMClaHV but its replication initiation protein is very different. The rep protein from BSL RDHV is homologous to the *Circoviridae* family of animal ssDNA viruses but the rep protein from the reclaimed water virus is homologous to the plant *Geminiviridae* ssDNA viruses. The inverse is true when comparing BSL RDHV with Dfcycv. Here the rep protein is homologous but the capsid proteins are very different. It is also interesting that geography appears to play little role in the phylogeny of RNA-DNA hybrid viruses. The capsid protein from BSL RDHV, from Boiling Springs Lake in California, is most similar to capsid proteins from Lac Pavin in France. As for the reclaimed water virus, from Florida, the other virus which shared the most homology within the capsid protein came from an air around Seoul, South Korea. Much more can be learned about this new group of viruses as more metagenomes are collected and more genomes are sequenced. It is also still unclear how exactly these hybrid viruses came to be and answering that may help further our understanding of viral evolution.

References

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