Discovery, prevalence and biogeographical distribution of a novel RNA virus in the seagrass Thalassia testudinum

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Introduction

Seagrass meadows fulfill numerous essential ecosystem functions. Unfortunately, seagrasses are currently under threat due to various stressors such as eutrophication, coastal development, dredging activities, global climate change and disease. Given global declines in coverage and increasing urbanization of coastal estuaries, it is critical to better understand the impact of pathogens on the ecology of seagrasses. Although studies have investigated bacteria, fungi and protists in seagrasses, virtually nothing is known about viral infection in these ecologically-important plants.

Methods

Virus detection

Ten individual seagrass leaves were sampled in December 2016 in the Terra Cea Aquatic Preserve in Tampa Bay, FL, USA (at sampling location N01, Fig. 3) and pooled together for screening.

- Sampling, epiphyte removal & rinsing of seagrass blades
- Virus-like Particle (VLP) purification & nucleic treatment
- RNA/DNA extraction
- Sequence-independent Single Primer Amplification (SISPA)
- Cloning & amplicon sequencing
- SISPA → Primers with random sequences are used to amplify unknown viral sequences
  1. Reverse transcription with primer K-AN
  2. Kinase reaction
  3. PCR with primer K

DNA

RNA

sisDNA

VLP

RNA-dependent RNA polymerase (RdRp)

Triple gene block (TGB)

Capsid

TPV genome organization

Development of a qPCR-assay for individual testing

A qPCR assay targeting a segment of the RdRp coding region of TtPV was used to test a total of 40 individual seagrass blades collected at two time points (February & August) in 2017 from four locations (N01, N02, N03 and N04) in the Tampa Bay area (Fig. 3).

Results

Discovery of a new potexvirus infecting T. testudinum

- A new virus was discovered in the pooled sample of December 2016 (Fig. 3, location N01) with a filamentous morphology (Fig. 4)
- Proposed name: Thalassia testudinum potexvirus (TtPV)
- The TtPV genome, 6252 nt long, was completed through primer walking and RACE
- TtPV shares 66% genome-wide pairwise identity with the potexvirus Foxtail mosaic virus (FoMV) that infects terrestrial plants, mainly grasses (Poaceae)

TPV genome organization

Viral methyltransferase (pfam01660) aa positions 38-179
Viral helicase 1 (pfam01443) aa positions 624-854
RdRp_2 (pfam00978) aa positions 1051-1330

Prevalence, viral load and morphology of TtPV

<table>
<thead>
<tr>
<th>Site</th>
<th>Viral load (VLPs/mg)</th>
<th>Prevalence (n = 10, %)</th>
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<tbody>
<tr>
<td></td>
<td>Feb</td>
<td>Aug</td>
</tr>
<tr>
<td>N01</td>
<td>2.27*10⁸</td>
<td>1.53*10⁸</td>
</tr>
<tr>
<td>N02</td>
<td>2.33*10⁹</td>
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<tr>
<td>N03-N04</td>
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</tbody>
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Viral load and prevalence for sites N01-N04 in Tampa Bay, FL

TPV was not found in T. testudinum samples obtained from 7 different sites in Florida Bay sampled in May 2017.

References