Sanger Sequencing Analysis of Potexvirus PCR Products (Potex-5 and Potex-2RC) collected during West Florida Coastal Surveys of Seagrass from Feb 2022 to Oct 2023

Website: https://www.bco-dmo.org/dataset/931469
Data Type: Other Field Results
Version: 1
Version Date: 2024-07-02

Project
» Collaborative Research: VIDA Seagrass: Viral Infection Dynamics Among Seagrass (VIDA Seagrass)

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Abstract
This dataset includes Sanger sequence references for 40 Thalassia testudinum samples collected during systematic seagrass surveys from multiple Florida sites, including Terra Ceia Aquatic Preserve, Tampa Bay seagrass sites S1T5 and S3T8 (Lassing Park), Panacea located in the Florida Panhandle, and Florida Keys sites including Bush Key, Garden Key, Marquesas Key, and Key West. We investigated potexvirus distribution in seagrasses using a degenerate reverse transcription polymerase chain reaction (RT-PCR) assay originally designed to capture potexvirus diversity in terrestrial plants. The assay, which implements Potex-5 and Potex-2RC primers, successfully amplified a 584 nt RNA-dependent RNA polymerase (RdRp) fragment from TVX-infected seagrasses. Following validation, we screened 74 opportunistically collected, apparently healthy seagrass samples for potexviruses using this RT-PCR assay. Potexvirus PCR products were successfully generated from Thalassia testudinum samples. Sequences from these products are deposited in NCBI GenBank under the accession numbers OR827692-OR827705, OR854648, OR863396, OR879052-OR879056, and PP430548-PP430571.

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Coverage

Location: Western Florida, USA

Spatial Extent: **N**:29.99911 **E**:-81.799295 **S**:24.560981 **W**:-84.35056
Temporal Extent: 2022-02-04 - 2023-10-03

Methods & Sampling

Sample collection

A systematic seagrass survey was conducted on 133 defined sites ~10m apart within a ~9000 m2 seagrass meadow in Terra Ceia Aquatic Preserve. These sites are routinely monitored for turtlegrass Virus X (TVX). This dataset includes sequence references for 40 *Thalassia testudinum* samples from multiple Florida sites, including Terra Ceia Aquatic Preserve, Tampa Bay seagrass sites S1T5 and S3T8 (Lassing Park), Panacea located in the Florida Panhandle, and Florida Keys sites including Bush Key, Garden Key, Marquesas Key, and Key West.

The survey examined the host species *Thalassia testudinum*, *Halodule wrightii*, *Halophila stipulacea*, *Syringodium filiforme*, *Ruppia maritima*, and *Zostera marina*. Potexvirus PCR products were successfully generated only from *Thalassia testudinum* samples. *Thalassia testudinum* specimens successfully amplified with the Potex-5/Potex-2RC primer pair were collected from:

1. A systematic seagrass survey at Terra Ceia Aquatic Preserve on August 1st, 2022
2. Tampa Bay seagrass site S3T8 (Lassing Park) on October 3rd, 2023
3. Panacea located in the Florida Panhandle on May 10th, 2023
4. Florida Keys and Dry Tortugas National Park, Florida, USA collected between May 16th-20th, 2022

RNA extraction and cDNA synthesis

Total RNA extraction was performed on 30-100 mg of leaves from multiple shoots pooled by seagrass species and collection site using Zymo Research's (ZR) Quick-RNA™ Plant Miniprep kit. Each pooled seagrass sample was homogenized in a BashingBead Lysis Tube containing 2 mm ceramic beads and 800 µL RNA lysis buffer (provided in the kit) for 5 minutes at maximum speed using a FisherbrandTM Bead Mill 4 Homogenizer (Fisher Scientific, Waltham, MA, USA). Tissue homogenates were centrifuged at maximum speed (21,130 g) for 1 minute and RNA was extracted from the total volume (~800 µL) of the supernatant. To ensure successful RNA extraction, after each round of extraction, a random subset of RNA samples was quantified using the QubitTM RNA high sensitivity (HS) assay (Invitrogen™). From each sample, cDNA was synthesized from 8 µL RNA using the SuperScriptTM IV First-Strand Synthesis System (Invitrogen™) and following manufacturer's instructions for random hexamers.

PCR amplification and sequencing

PCR was performed under the following conditions: initial denaturation at 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 30 seconds, annealing at 51.5°C (as published in van der Vlugt and Berendsen, 2002) for 30 seconds, extension at 72°C for 1 minute, followed by elongation at 72°C for 10 minutes and cooling at 11°C. The PCR product was visualized following gel electrophoresis on a 1% (wt/vol) agarose gel stained with ethidium bromide. All PCR reactions, except for the no template control, yielded visible bands. All PCR products were purified using the Zymoclean Gel DNA Recovery Kit (Irvine, CA, USA), quantified using the QubitTM DNA high sensitivity (HS) assay (Invitrogen™, Waltham, MA, USA), and Sanger sequenced bidirectionally by Eurofins Genomics (Louisville, KY, USA).

Data Processing Description

Sequence reads from each sample were mapped to the Potex-5/Potex-2RC amplicon region (with forward and reverse primer sequences removed) in the TVX genome (NCBI accession: NC_040644:van der Vlugt (2002)) using the "Map Sanger Reads to Reference" function implemented in Unipro UGENE v48.1 (Okonechnikov, 2012), with a trimming quality threshold of 20 and mapping minimum similarity of 70%. Electropherograms of mapped reads were manually inspected to remove primer sequences and low-quality bases and to resolve ambiguous bases.

BCO-DMO Curation Notes

- Imported original file "Potexvirus_NCBI_accessions.xlsx" into the BCO-DMO system.
- Created "Aphia_id" field for Aphia reference identifier.
- Convert date from %m-%d-%y to %Y-%m-%d format

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Data Files

File
931469_v1_potexvirus_seagrass.csv (Comma Separated Values (.csv), 4.41 KB) MD5:b72ae662854ffa2278a09127fa4db674 Primary data file for dataset ID 931469, version 1

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Related Publications

Lim, S. J., Rosario, K., Kernbach, M. E., Gross, A. J., Furman, B. T., & Breitbart, M. (2023). Limited potexvirus diversity in eastern Gulf of Mexico seagrass meadows. https://doi.org/10.1101/2023.12.11.571111
Results

Okonechnikov, K., Golosova, O., & Fursov, M. (2012). Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics, 28(8), 1166–1167. https://doi.org/10.1093/bioinformatics/bts091
Software

van der Vlugt, R.A., Berendsen, M. Development of a General Potexvirus Detection Method. European Journal of Plant Pathology 108, 367–371 (2002). https://doi.org/10.1023/A:1015644409484
Methods

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Related Datasets

References

University of South Florida. Thalassia testudinum, Limited potexvirus diversity in eastern Gulf of Mexico seagrass meadows. 2024/07. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1130510. NCBI:BioProject: PRJNA1130510.

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Parameters

Parameter	Description	Units
sample	Sample ID	unitless
region	Region of the sampling site in format (State, Country)	unitless
site	Sampling site description	unitless
date	Date of sample collection based on local time, both US Eastern and US Central timezones, in ISO 8601 format	unitless
latitude	Latitude where sample was collected	decimal degrees
longitude	Longitude where sample was collected	decimal degrees
sample_host	Species name of the host organism from which the genetic material was extracted	unitless
Aphia_id	Persistent identifier for taxon of the host organism from which the genetic material was extracted	unitless
Genbank_accession	NCBI GenBank accession number	unitless

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Instruments

Dataset-specific Instrument Name	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Tissue homogenates were centrifuged at maximum speed (21,130 g) for 1 minute and RNA was extracted from the total volume (~800 µL) of the supernatant.
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	FisherbrandTM Bead Mill 4 Homogenizer (Fisher Scientific, Catalog No.15-340-164)
Generic Instrument Name	Homogenizer
Dataset-specific Description	Each pooled seagrass sample was homogenized in a BashingBead Lysis Tube containing 2 mm ceramic beads and 800 µL RNA lysis buffer (provided in the kit) for 5 minutes at maximum speed using a FisherbrandTM Bead Mill 4 Homogenizer (Fisher Scientific, Waltham, MA, USA).
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

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Project Information

Collaborative Research: VIDA Seagrass: Viral Infection Dynamics Among Seagrass (VIDA Seagrass)

Coverage: Tampa Bay, Florida

NSF Award Abstract:
Seagrasses are marine flowering plants (or angiosperms) that create expansive underwater meadows that form the basis of highly productive and valuable ecosystems in coastal oceans. Unlike terrestrial systems where angiosperms dominate plant diversity, seagrasses are the only flowering plants in marine environments. Based on the profound impacts of viral infections on terrestrial plants, viruses are expected to influence seagrass ecology. However, no prior work has investigated viral infection dynamics in seagrasses or the impact of viruses on seagrass health. This project provides fundamental knowledge about seagrass-virus interactions through field and laboratory studies of Thalassia testudinum (i.e., turtlegrass, a climax species and key ecosystem engineer), and turtlegrass virus X (TVX), the only seagrass virus currently reported from experimental research. The lack of a seagrass-virus study system has kept the scientific community from learning which factors drive viral infection in marine angiosperms. By establishing the first seagrass-virus study system, a novel virus-host pathosystem for which virtually nothing is known, this project contributes to a more comprehensive understanding of seagrass ecology and serves as a model for investigating the growing number of seagrass viruses discovered through sequencing efforts. This multifaceted project trains one postdoctoral researcher, two graduate students, and six undergraduate students. Dissemination of results and data through open access channels informs the broader community and provides scientists with data for their own research to propel the field of seagrass virology. This project also engages educators and students participating in programs that strive to increase participation from underrepresented groups in STEM fields. Teachers from the Jacksonville Teacher Residency Program are getting involved through development of lessons that dive into seagrass biology. Students from Girls Incorporated, Girl Scouts, and the University of South Florida's Oceanography Camp for Girls are participating as citizen scientists by photographing Tampa Bay's seagrass ecosystems and contributing their observations to the Seagrass Spotter website. This project also increases awareness of seagrass ecosystems and challenges the public perception that all viruses are pathogenic through hands-on activities at the annual St. Petersburg Science Festival.

Seagrass-virus interactions are being investigated through a two-tiered approach involving field studies in Tampa Bay, Florida and microcosm experiments. Field surveys focus on elucidating the nature of turtlegrass-TVX interactions (positive, neutral or negative) and the relationship between turtlegrass genotypic diversity and virus distribution in a natural population where TVX has persisted for at least five years. TVX load is monitored bimonthly over two years to assess how viral load relates to turtlegrass genotype and performance (growth, health, reproductive effort), and abiotic parameters. The investigated turtlegrass meadow contains TVX-positive and negative specimens, thus providing a perfect natural laboratory with homogenous environmental characteristics that allow exploration of the drivers of viral infection. Given that environmental changes may alter host-microbe interactions, complementary microcosm experiments are evaluating turtlegrass responses

to TVX infection at the physiological (survival, photochemical capacity, cellular responses) and molecular (transcriptomic) levels in a controlled environment under normal conditions and in the context of salinity changes, an important seagrass stressor. Microcosm experiments also provide the first profiles of seagrass gene expression and measurement of cellular metabolites in response to viral infection. Expected results have direct implications for understanding seagrass production and resilience in the face of global climate change and anthropogenic stress.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2219547
NSF Division of Ocean Sciences (NSF OCE)	OCE-2219548

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