Microbial Communities From Biofuels & Ornamental Plants

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Biofuel Microbiome Exploration

Problem

An unknown substance is building up in biofuel tanks in Oklahoma. The contaminated tanks needs to be cleaned and the substance identified.

Objective and Justification

- To determine the biological composition of the plugging substance.
- To prevent the spreading of this substance due to its unknown effects on the biofuel.
- To conclude the most effective treatment to cleanse the biofuel.

Methods

- 3 DNA extraction kits were assessed
  - ZymoBIOMICS DNA/RNA miniprep kit
  - Qiagen DNeasy powersoil kit
  - Omega Bio-Tek E.Z.N.A. soil DNA kit
- The extracted DNA was sent for High Throughput Sequencing (HTS).

Results

- The Qiagen DNeasy powersoil kit was determined to produce the highest concentration of DNA from extraction.

GENOME FRACTION ALIGNED TO REFERENCE GENOMES

BLASTnr, SPADES and KRAKEN2 software used

species found:

- Galactomyces
- Ogataea-Candida clade
- Barnettozyma-Candida clade
- Cyberlindnera-Candida clade
- Hafniaceae
- Hafnia-Obesumbacterium
- Trabulsiella
- Micrococcus
- Desulfovibrio_fructosivorans
- Megasphaera cerevisiae
- Microvirgula aerodenitrificans
- Ochrobactrum_anthropi

Chrysanthemum Virome Exploration

Problem

A farm in Tulsa, Oklahoma, distributes chrysanthemums nationally. The owner noticed some varieties showed signs of disease. If the plants are infected, they will spread viruses nationwide. Also, the remaining healthy plants cannot be sold.

Objective and Justification

- There is a need to verify which chrysanthemum varieties are infected with viruses & viroids. This information will allow to identify pathogens present and control.
- This research will prevent the spreading of viruses & viroids.

Methods

- Agdia DAS- ELISA to test plant infection by 15 targeted viruses
- The presence of two viroids was examined:
  - ❖ Agdia tested by CchMVD hybridization assay kit
  - ❖ Agdia tested by CSVD hybridization assay kit
  - ❖ RT-PCR and Recombinase Polymerase Amplification (RPA)

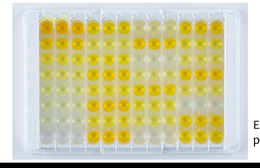
Results

Virus Infection Results

Most prevalent virus:	CVB, TMV, CMV, TEV	4/15
Less prevalent virus:	CymRSV, INSV, SMV	3/15
Not found:	ZyMV	1/15
Healthy varieties:		8/165
St. Tropez, Kimie, Homoromo, Saba, Lavander Pixie, Kokka bunni clean, Kelvin tattoo, Judith Baker		
Varieties single infection:		26
Single virus infections:		6/15
	CVB, CymRSV, CMV,TMV,TSV,TSWV	

Multiple infections:

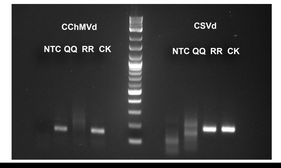
2 viruses	37 varieties
3 viruses	43 varieties
4 viruses	19 varieties
5 viruses	18 varieties
6 viruses	5 varieties
7 viruses	1 varieties



ELISA plate showing positive reaction in yellow

Viroid Infection Results

- Varieties infected with CChMVd: 56
- Varieties infected with CSVd: 19
- Varieties with Viroid mix infection: 9
- Viroid free varieties: 81
- Most prevalent viroid: CChMVd
- Less prevalent viroid: CSVd
- Varieties Virus & Viroid free: 4



RPA viroid products after the incubation

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