

# Next generation sequencing of oomycete communities in nursery irrigation water

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Horticultural nurseries recapture and recycle irrigation water. We used the Illumina MiSeq platform to characterize the *Phytophthora* and *Pythium* communities in irrigation water to assess the effectiveness of water treatment methods.

## Sample collection and processing

Nursery irrigation water sampled to detect oomycetes

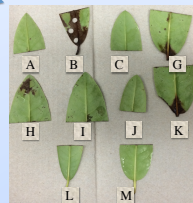


Water samples collected from 10 sites in a nursery every 2 weeks.



One liter water filtered through 75µm nylon mesh and 5 µm Millipore filters.

Filters bead-beaten before DNA extraction with chloroform/phenol.



One liter water baited with a *Rhododendron* leaf. Each leaf represents a different water sample.

DNA extracted from lesions with OPS Diagnostics Synergy™ kit.

## PCR and sequencing

PCR was performed and oomycete specific primers were adapted from Vannini et al. (2013) for use on the Illumina MiSeq platform with Nextera™ barcodes:

ITS6 + spacer + adapter: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG K GAAGGTGAAGTCGTAAACAAGG 3'  
ITS7 + adapter: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCGTCTTCATCGATGTGC 3'

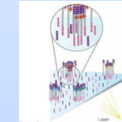


DNA extract was diluted to ≤ 25ng/µl from filtered water or baited leaves.

The template was amplified with Platinum™ taq using an annealing temperature of 60°C for 35 cycles.

Amplicon was submitted to the Oregon State University Center for Genome Research and Biocomputing Core lab for:

Amplicon PCR cleanup  
Index PCR + cleanup  
Normalization + pooling  
dsDNA fluorophore quantification  
Sequencing on the Illumina MiSeq on a 2 x 250 bp paired-end run.



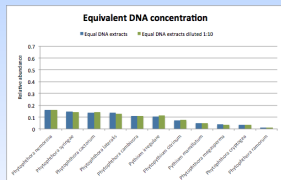
We submitted 96 samples in our test run - up to 384 samples can be sequenced at once.

## Bioinformatics

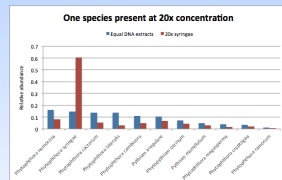
Sequence data processing was done using QIIME v. 1.8.0. Only forward reads were used for a preliminary analysis. Sequence bases with quality scores >25 were retained and chimera detection was conducted with "usearch61" method after primer sequences were removed. Curated sequences were grouped into operational taxonomic units (OTUs) at a similarity level of 99% by uclust algorithms. Oomycete reads were queried by BLAST algorithms against a reference dataset with *Phytophthora* sequences downloaded from Phytophthora-ID.org and other oomycete sequences used in Robideau et al. (2011). The R programming environment ([www.R-project.org](http://www.R-project.org)) was used for downstream analysis.

## Quality control: detection of individual species in mock communities

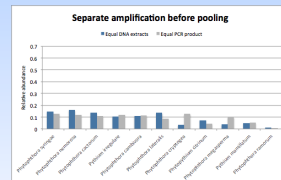
We created positive controls by extracting DNA from cultures of 11 oomycete species, which were diluted to 5 ng/µl before being combined to create mock communities.



5 ng/µl DNA extracts were combined in equal amounts and pooled. PCR was performed with the pooled samples and with a 1:10 dilution of the pooled samples.



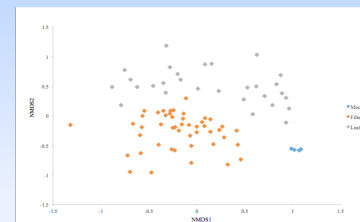
20x as much *P. syringae* was combined with the other species. The number of reads of *P. syringae* was approximately 16x the average number of reads for the other species.



Each species was amplified separately and the resulting amplicons were combined at 5 ng/µl each. When compared with extracts combined in equal amounts, PCR bias may be observed for some species.

## Comparison of detection methods: filters vs. baits

Leaf baited samples were only extracted if a lesion formed, while all water samples that were filtered were extracted.



OTU abundance-based ordination of samples (each dot represents the oomycete community sequenced from an individual sample).

Non-metric multidimensional scaling plot of oomycete communities resulting from the operational taxonomic unit (OTU) community profile of samples obtained using different detection methods (filtered water or baited leaves) and the mock community samples. The results show distinct community assemblages of oomycetes among the two different methods and the mock community.

## Summary and outlook

Illumina sequencing is proving to be a sensitive method to detect, identify, and determine the relative abundance of oomycetes from water samples comprised of mixed assemblages of species. Additional tests are in progress to determine limits of detection and to refine our data analysis.

## References

- Robideau, G.P. et al. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources*, 11: 1002–1011. doi: 10.1111/j.1755-0998.2011.03041.x
- Vannini, A. et al. 2013. Pyrosequencing of environmental soil samples reveals biodiversity of the *Phytophthora* resident community in chestnut forests. *FEMS Microbiol. Ecol.* 85:433–442.