Detection of Plant Pathogens in Plant, Soil and Water Samples via DNA Metabarcoding

Presenter contact:
ypei01@uoguelph.ca

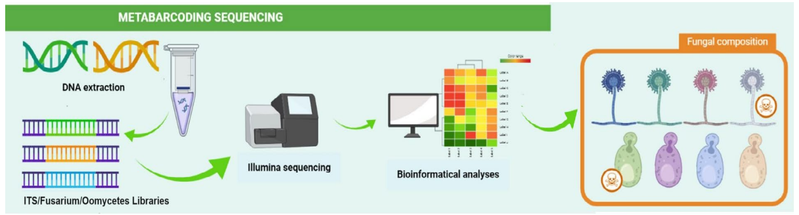
Yuan Pei, Nicola Linton, Xuechan Shan and Shu Chen
Agriculture and Food Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON, Canada

Introduction

- Plants can be infected by a wide range of pathogens. Current diagnostic methods are limited to detect one or a few organisms at a time or only pre-determined pathogens.
- DNA metabarcoding has emerged as a promising solution for detecting multiple pathogens simultaneously with high accuracy.
- The objective of this research is to establish and evaluate a rapid and reliable high throughput sequencing method to simultaneously detect known fungal and oomycete plant pathogens in one test.

Metabarcoding

Metabarcoding involves PCR with universal primers to amplify a gene fragment (i.e. DNA barcode) that can be used to detect a defined group of organisms simultaneously in a complex sample. The PCR products are then indexed and sequenced. The resulting sequences are compared with a database to make taxonomic assignments (Inacio, 2021).



Methods

DNA was extracted using the DNeasy® Powersoil Pro Kit (Qiagen). Amplicon libraries were constructed using two rounds of PCR and sequenced per illumina protocol (Illumina 2013). The sequences were filtered to obtain high quantity reads (>30 Q score). Microbial species were assigned using the Basespace and Geneious analysis pipelines with custom databases that contain a total of 279 sequences covering 169 species.

Table 1. Microbial species used to create Mock Communities (MC)

MC 1 (Fungal assay)	MC 2 (Oomycetes assay)	MC 3 (*Fusarium* assay)
Rhizoctonia fragariae/solani	*Phytophthora cactorum*	*F. arthrosporioid*
Verticillium albo/atrum	*Phytophthora capsici*	*F. equiseti*
Verticillium dahliae	*Pythium irregulare*	*F. oxysporum*
Verticillium longisporum	*Pythium sylvaticum*	*F. salami*
Penicillium brevicompactum	*Aphanomyces cochlioides*	*F. fujikuroi*
Macrophomina phaseolina	*Acetobacter aceti*	*F. acuminatum*
Cylindrocarpon destructans	*Bacillus thuringiensis*	*F. graminearum*
Colletotrichum trichum	*Escherichia coli*	*F. brachygibbosum*
Colletotrichum acutatum	*Saccharomyces cerevisiae*	*F. proliferatum*
Colletotrichum coccodes	*Candida albicans*	*F. citri*
Gluconobacter ceriunus		*Penicillium oxalicum*
Pseudomonas aeruginosa		*Trichoderma atroviride*

Results

PCR primer evaluation and database establishment

Three sets of primers targeting fungi, oomycete and *Fusarium* species were evaluated by aligning the primer sequences to the published sequences of representative strains for their suitability and inclusivity using BLASTn and Geneious software. Three databases were established that contained 113 sequences of 65 fungal species, 37 sequences of 25 oomycete species and 133 sequences of 79 Fusarium species respectively.

Method setup & specificity testing using pure cultures

Three metabarcoding assays were first benchmarked using mock communities of pure cultures of plant pathogens and control organisms (Table 1).The mock communities, each of which contained 10 -12 target and non-target species, were tested using the metabarcoding assays. All the target species were successfully detected in its corresponding sample (Figure 1), confirming specificity of the method.

Limit of detection (LOD) determination

Four samples containing four representative species with DNA concentrations of 1,10,10^2 and10^3 pg/µl were prepared and tested respectively. All of the target species were detected at 1 pg/µl, indicating the LOD is 1 pg/µl (or possibly lower) of a target DNA (Figure 2).

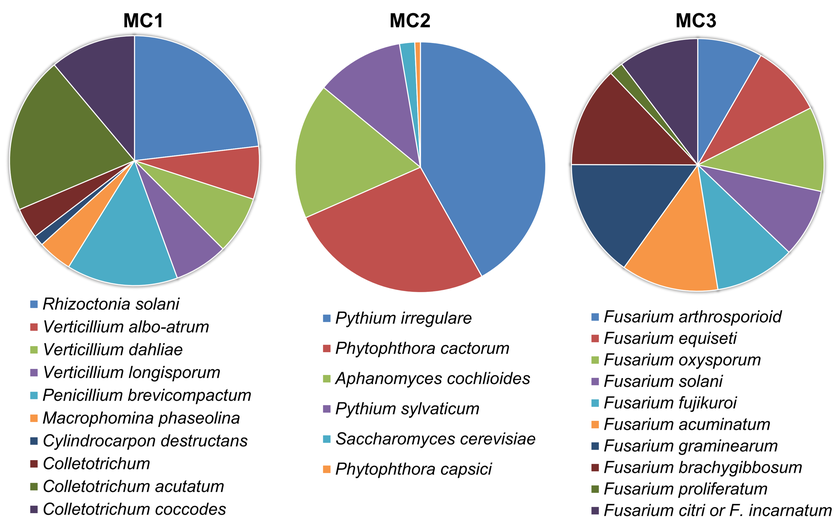
Matrix effect study

Three field samples, including soil, tomato leaf, and water, were artificially contaminated with *S. cerevisiae* cells at 1x10^3, 1x10^4, 1x10^5 cfu/g or L and tested using the fungal ITS assay. *S. cerevisiae* was detected at 1x10^3 cfu/L in water, and between 1x10^3 - 1x10^4 cfu/g in soil and leaf samples (Table 2), indicating a limited matrix effect.

Comparative study against established method

The method was compared against the existing DNA Multiscan® method (De Ceuster, Sint-Katelijne-Waver, Belgium) (https://dnamultiscan.com/en/) that allows for the detection of over 30 pathogens in one test. The preliminary comparative study included a total of 90 samples of water (n=30), plants (n=30), and soil (n=30). Overall, the metabarcoding method was more sensitive than the Multiscan® method; the frequently-detected species showed a >93% average match rate and 1% average no match rate, suggesting comparable results between the two methods. Using elongation factor 1-α gene as the target for *Fusarium* detection, the metabarcoding method allowed for more accurate detection and differentiation among *Fusarium* species.

Figure 1. Species Detected in Mock Communities - Specificity Test



Reference:
Cobo-Díaz, J. F. *et al.* (2019). FEMS Microbiology Ecology, 95(7), fiz084.
Inacio JDL. *et al.*(2021). Food research international, 146
Illumina (2013).16S Metagenomic Sequencing Library Preparation
Karlsson, I. *et al.* (2016). Applied and Environmental Microbiology, 82(2), 491–501.

Figure 2. Samples and species detected in LOD testing

	DNA Level (pg/µL)			
DNA source species	LOD-1	LOD-2	LOD-3	LOD-4
F. fujikuroi	10	100	1000	1
P. cactorum	1	10	100	1000
S. cerevisiae	1000	1	10	100
P. brevicompactum	100	1000	1	10

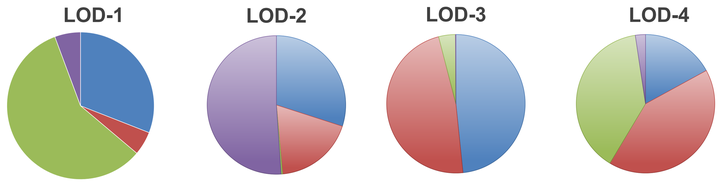


Table 2. Detection of *S. cerevisiae* (number of *S. cerevisiae* sequence reads) in spiked soil, water and leaf samples - Matrix effect study

Sample	*S. cerevisiae* added cfu/g or L	Number of sequence reads
Soil	1x10^5	8,051
	1x10^4	143
	1x10^3	4
Water	1x10^5	35,478
	1x10^4	3,603
	1x10^3	84
Leaf	1x10^5	356
	1x10^4	7
	1x10^3	0

Table 3. Metabarcoding vs. Multiscan® -- Comparative Study Results

	Match	No Match	Match Rate	No Match Rate
Botrytis cinerea	15	0	100%	0%
Fusarium oxysporum	45	6	88%	7%
Fusarium solani	29	6	83%	7%
Fusarium sp.	73	0	100%	0%
Pythium aphanidermatum	3	1	75%	1%
Pythium dissotocum	5	2	71%	2%
Pythium irregulare	15	2	88%	2%
Pythium myriotylum/zingiberis	2	0	100%	0%
Pythium polymastum	0	0	100%	0%
Pythium sulcatum	1	0	100%	0%
Pythium sylvaticum	17	0	100%	0%
Pythium ultimum	7	0	100%	0%
Pythium uncinulatum	0	1	0%	1%
Pythium sp.	53	1	98%	1%
Phytophthora cactorum	11	0	100%	0%
Phytophthora capsici	0	0	100%	0%
Phytophthora cinnamomi	0	0	100%	0%
Phytophthora cryptogea	0	0	100%	0%
Phytophthora drechsleri	0	3	0%	3%
Phytophthora fragariae	0	0	100%	0%
Phytophthora infestans	0	0	100%	0%
Phytophthora nicotianae	0	1	0%	1%
Phytophthora sp.	13	0	100%	0%
Rhizoctonia solani	3	0	100%	0%
Rhizoctonia sp. (binucleate)	12	2	86%	2%
Sclerotinia sp.	2	0	100%	0%
Thielaviopsis basicola	2	0	100%	0%
Verticillium albo-atrum	1	0	100%	0%
Verticillium dahliae	2	0	100%	0%
Verticillium sp.	2	0	100%	0%

Conclusion

The metabarcoding method was an effective and promising tool for detecting multiple plant fungal and oomycete pathogens in soil, water and plant samples. The databases need to be improved to allow for accurate genus/species assignments before the method can be used in a diagnostic lab.

Acknowledgement:
We would like to express gratitude to Nicole Tabujara and Honghe Cao from Plant Disease Clinic team of the AFL for providing samples and DNA Multiscan testing results.