Annotated Bibliography on Oomycete Primers

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November 30, 2020

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Ecology & Research

Background

This bibliography summarizes some of the most recent advances in the design of oomycete primers. This is by no means an exhaustive review of molecular methods for identifying oomycetes. Our primary objective was to identify the promising methods of preparing short amplicons (<500 bp) for paired-end reads on the MiSeq platform, particularly for environmental DNA extracted from soils or plant tissue. For our study, we settled on the **oom18s** forward primer (Legeay et al., 2019) and the **ITS7-ae** reverse primer (Esmaeili Taheri et al., 2017) for targeting the ITS1 region. Please note: to the best of our knowledge, this particular primer set has not been fully validated with environmental DNA.

Annotations

Towards a universal barcode of oomycetes – a comparison of the cox1 and cox2 loci

(Choi et al., 2015)

Methods – The cytochrome c oxidase subunit 2 gene (cox2 locus) is suggested as a universal barcode region for oomycetes. Sequencing performed on Applied Biosystems 3730 DNA Analyzer.

Results – Cox2 successfully amplified all oomycete genera tested while cox1 failed for 3 genera. Cox2 had higher PCR efficiency and had higher species identification success due to higher interspecific and lower intraspecific divergences than cox1. Cox2 recommended as a partner DNA barcode along with ITS rDNA.

A Molecular Phylogeny of Phytophthora and Related Oomycetes

(Cooke et al., 2000)

Methods – Semi-nested PCR using a round of DC6/ITS4 primers followed by a round using the ITS6/ITS4 primes. ITS6 is similar to ITS5 forward primer (White et al., 1990) but modified to better match *Phytophthora megasperma.* Sequencing performed on ABI373 automated sequencer (Applied Biosystems)

Degenerate ITS7 primer enhances oomycete community coverage and PCR sensitivity to Aphanomyces species, economically important plant pathogens

(Esmaeili Taheri et al., 2017)

Background – Metagenomic analysis of oomycetes with amplicon sequencing is typically done using the ITS6-ITS7 primers targeting the ITS1 region. ITS7 is a perfect match for most oomycete taxa, however it contains 3 mismatches to plant pathogens within the genus Aphanomyces

Methods – Degenerate primers were used by replacing mismatch sites to create primer ITS7-a.e. for sequencing on Illumina MiSeq

Results – New primer set resulted in decreased abundance of *Pythium* spp. and increased abundance *Aphanomyces* spp.

Comparison and validation of Oomycetes metabarcoding primers for Phytophthora high throughput sequencing

(Legeay et al., 2019)

Background – ITS region presents low polymorphism in Phytophthora so RAS-Ypt region may be more appropriate. RAS-Ypt is a single copy marker and so may yield more accurate relative abundances than multi-copy regions like ITS. Most RAS-Ypt genes in 24 Phytophthora species are less than 460bp long, although 3/24 species had genes longer than 600bp

Methods – A mock community comprised of DNA from 24 phytophthora species was used to compare 3 primer sets targeting ITS1 or RAS region. Nested PCR amplification and Illumina MiSeq. RAS-Ypt barcode may have limited database accessions; custom database used instead of NCBI.

Results – 95% of species detected. oom18S/ITS7 > 18ph2f/5.8S-1R primer pair was able to detect 7 phytophthora species in environmental DNA sample. DC6/ITS7 > oom18S/ITS7 primer pair only detected 1 species. However, DC6/ITS7 > oom18S/ITS7 covers Peronosporaceans (downy mildews)

Oomycete-specific ITS primers for identification and metabarcoding

(Riit et al., 2016)

Methods – New oomycete forward primers developed: ITS1oo and ITS3oo, both paired with ITS4 reverse primer. ITS1oo is a modification of the ITS-O primer. It binds to the 18s region for full ITS coverage.

ITS3oo binds to the 5.8s region for amplifying the ITS2 region only. Sequencing performed using Illumina Miseq 2x300 bp

Characterisation and phylogeny of repeated elements giving rise to exceptional length of ITS2 in several downy mildew genera (Peronosporaceae)

(Thines, 2007)

Background – Some oomycete taxa, particularly the downy mildews (Peronosporaceae) have ITS sequences as large as 1121 and 2587kb, largely due to repeated elements in ITS2. Primers used: ITS-1o

References

Choi, Y.-J., Beakes, G., Glockling, S., Kruse, J., Nam, B., Nigrelli, L., Ploch, S., Shin, H.-D., Shivas, R.G., Telle, S., Voglmayr, H., Thines, M., 2015. Towards a universal barcode of oomycetes – a comparison of the cox1 and cox2 loci. Mol Ecol Resour 15, 1275–1288. https://doi.org/10.1111/1755-0998.12398

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G., Brasier, C.M., 2000. A Molecular Phylogeny of Phytophthora and Related Oomycetes. Fungal Genetics and Biology 30, 17–32. https://doi.org/10.1006/fgbi.2000.1202

Esmaeili Taheri, A., Chatterton, S., Gossen, B.D., McLaren, D.L., 2017. Degenerate ITS7 primer enhances oomycete community coverage and PCR sensitivity to Aphanomyces species, economically important plant pathogens. Can. J. Microbiol. 63, 769–779. https://doi.org/10.1139/cjm-2017-0100

Legeay, J., Husson, C., Cordier, T., Vacher, C., Marcais, B., Buée, M., 2019. Comparison and validation of Oomycetes metabarcoding primers for Phytophthora high throughput sequencing. J Plant Pathol 101, 743–748. https://doi.org/10.1007/s42161-019-00276-9

Riit, T., Tedersoo, L., Drenkhan, R., Runno-Paurson, E., Kokko, H., Anslan, S., 2016. Oomycete-specific ITS primers for identification and metabarcoding. MycoKeys 14, 17–30. https://doi.org/10.3897/mycokeys.14.9244

Thines, M., 2007. Characterisation and phylogeny of repeated elements giving rise to exceptional length of ITS2 in several downy mildew genera (Peronosporaceae). Fungal Genet. Biol. 44, 199–207. https://doi.org/10.1016/j.fgb.2006.08.002

White, T. J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, Thomas J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1