Moniliophthora roreri: Molecular identification

M. Catherine Aime & Jorge Díaz-Valderrama, unpublished. May 2022.

DNA extraction

Any type of extraction method for fungi can also be used, such as CTAB. However, we use the Wizard® Genomic Purification Kit for DNA extractions, because it is economical and works well:

https://www.promega.com/products/nucleic-acid-extraction/genomic-dna/wizard-genomic-dna-purification-kit/?catNum=A1120

Starting from tissue in DNA buffer (this tissue can be directly harvested from cacao pods, as explained in the field sampling sheet, or from cultures by removing a portion of the culture with sterile forceps and placing in buffer), follow the manufacturer's procedures. Optional: To speed up the initial grinding step, we used 2mm Zirconia beads (BioSpec Products, Bartlesville, OK) in a Mini-Beadbeater-24 (BioSpec Products) for 5 minutes.

Quantification and assessment of purity of DNA can be performed by measuring the ratios of absorbance of ultraviolet light at 260 nm over 280 nm (A260/280) and 230 nm (A260/230), widely used indicators of purity of nucleic acids (Gallagher and Desjardins 2006; Teare et al. 1997), in a NanoDrop™ One spectrophotometer (ThermoFisher Scientific, Waltham, MA). Most proteins have the strongest absorbances at 280 nm while other impurities like phenols and salts, at 230 nm (Teare et al. 1997); therefore, A260/280 and A260/230 ratio values lower than the thresholds of 1.8 and 2.0, respectively, indicate contamination of protein and/or other impurities (Teare et al. 1997; Thermo Fisher Scientific n.d.; Wilfinger, Mackey, and Chomczynski 1997). If a NanoDrop is not available, concentration can be estimated from an agarose gel using an calibrated ladder or DNA marker of known quantity. For best results, adjust concentration of DNA for PCR's to between 0.2 to 4.0 ng/µl.

PURDUE UNIVERSITY

M. Catherine Aime, Dept. Botany & Plant Pathology, West Lafayette, IN 47907, USA

UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS

Jorge R. Díaz-Valderrama, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Chachapoyas, Peru

Primers for confirming Moniliophthora roreri

Moniliophthora roreri is a basidiomycete fungus. Therefore, for best results, use primers designed for basidiomycetes. This is not as important if you are analyzing pure cultures, but it is very important if you are trying to amplify stroma taken from the surface of desiccated pods, as these will have lots of environmental fungi on them.

The internal transcribed spacer region (ITS) is the fungal barcode, and can be used to confirm *M. roreri.* Primers (ITS1-F and ITS4-B), and protocols for amplification, are in the paper Aime & Phillips-Mora 2005.

ITS1-F	CTT GGT CAT TTA GAG GAA GTA A	Gardes & Bruns 1993
ITS4-B	CAG GAG ACT TGT ACA CGG TCC AG	Gardes & Bruns 1993

Primer sequences published in: Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes: application to identification of mycorrhizae and rusts. Mol Ecol 2:113-118.

Diagnostic test for confirming MAT type of Moniliophthora roreri

To determine the specific mating type of the fungus, we have developed specific primers that can be used as a diagnostic test that can be performed on an agarose gel (no need to sequence). Our data show that there are two invasive genotypes of *M. roreri.* The *A1B1* mating type is the more invasive genotype, is the only genotype present in Central America, and the genotype that recently invaded Jamaica. The *A2B2* mating type is more common in South America and has recently invaded Peru (Díaz-Valderrama and Aime 2016). Isolation from pure cultures is recommended to use the diagnostic test.

The primers were designed in such a way that diagnosis of mating type alleles is based on the presence or absence of the amplicon band in the agarose gel. These primers will effectively discriminate between all *A* and *B* mating alleles found in this study without the need of Sanger

PURDUE UNIVERSITY
M. Catherine Aime, Dept. Botany & Plant Pathology, West Lafayette, IN 47907, USA

UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS
Jorge R. Díaz-Valderrama, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Chachapoyas, Peru

sequencing (Figure 4 and 5). They will allow the rapid detection of a *M. roreri* mating type in areas where the disease has not been previously observed.

Table 1. Specific primers to diagnose and identify the mating locus alleles in *M. roreri*

Gene	Mating locus allele	Primer names and sequences (5' to 3')		PCR product size (bp)	Thermo-cycling conditions
Mr_HD1	*A1*	Mr_HD1_both_R	GGAAGAGTGATGGGCACAGA	1192	95ºC, 2 min; 35 cycles of 95ºC, 30 s 57ºC, 30 s 72ºC, 1min 45 s; 72 ºC, 5 min
		Mr_HD1_A1_F	AGTCTGCGGTGGACAATTTCA		
Mr_HD1	*A2*	Mr_HD1_both_R	GGAAGAGTGATGGGCACAGA	1166	
		Mr_HD1_A2_F	TATGAAGACCCAGCGCAAGT		
Mr_HD2	*A1*	Mr_HD2_Int_R	CTCTTCGTTCCTGCCTCGTT	1263	
		Mr_HD2_A1_R2	ATGGGTATTCCAACGGCCTCT		
Mr_HD2	*A2*	Mr_HD2_Int_R	CTCTTCGTTCCTGCCTCGTT	1275	
		Mr_HD2_A2_R2	ATGGGTATTCCGACGCTTCC		
STE3_Mr4	*B1*	Mr_Rec4_F2	CCCTCTGGAACCAAAGATTCTG	572	95ºC, 2 min; 35 cycles of 95ºC, 30 s 57ºC, 45 s 72ºC, 1min; 72 ºC, 5 min
		Mr_Rec4_R2	TGCACAGTCTGAGTAACGAGT		
STE3_Mr4	*B2*	Mr_R4_A2_F	ACATTGCGGTTCATCCCCAT	989	
		Mr_R4_A2_R	TAGATGAGCAAGCGTAGGCG		
Mr_Ph4	*B1*	Mr_Ph4_A1_F	CTTGCACGAAAGGCGAACAA	786	
		Mr_Ph4_A1_R	TTTATGTCGGAGGTGTGGGC		
Mr_Ph4	*B2*	Mr_Ph4_A2_F2	GGTGGACAAAAACTGGCGAC	622	

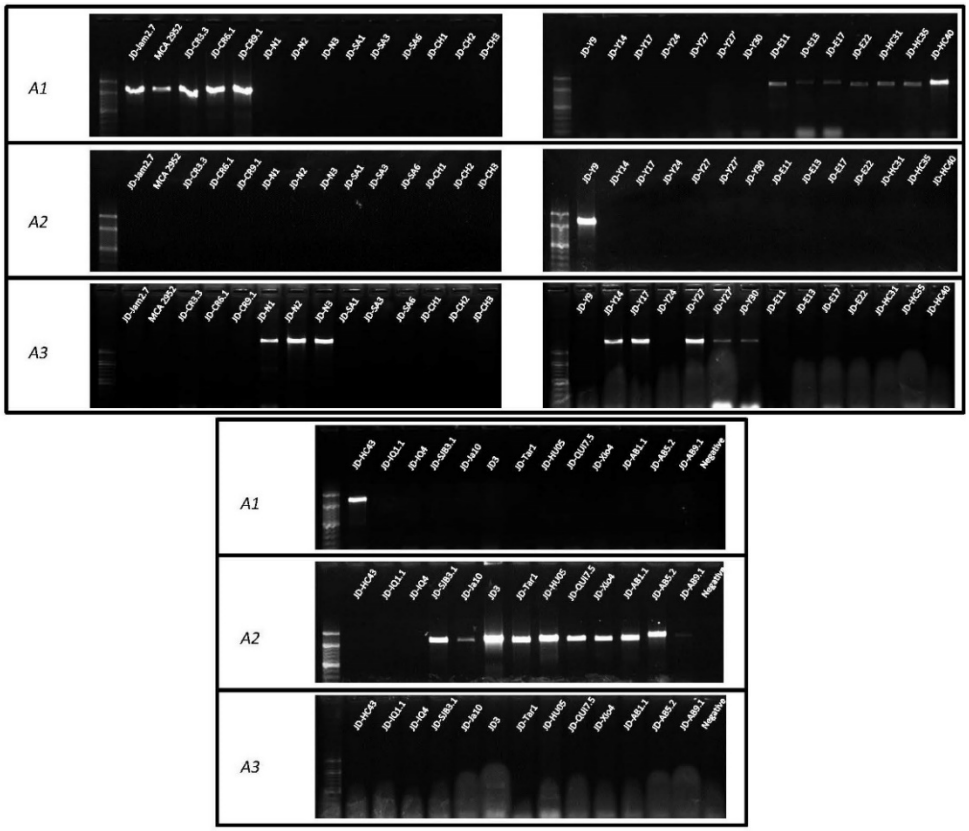
Primer sequences published in: Diaz-Valderrama JR, Aime MC. 2016. The cacao pathogen Moniliophthora roreri (Marasmiaceae) possesses a tetrapolar mating system but reproduces clonally. Heredity 116:491-501. doi: 10.1038/hdy.2016.5

PURDUE UNIVERSITY

M. Catherine Aime, Dept. Botany & Plant Pathology, West Lafayette, IN 47907, USA

UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS

Jorge R. Díaz-Valderrama, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Chachapoyas, Peru



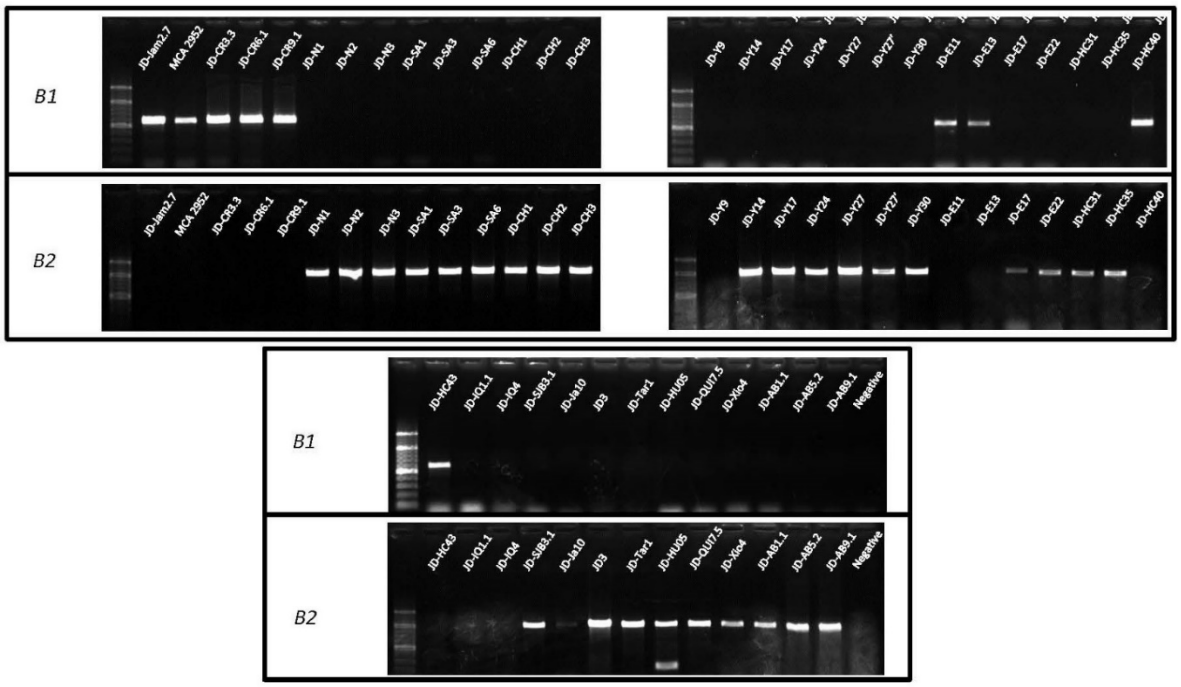
A locus genotyping. Gel photographs of PCR products amplified with primers designed for diagnostics of *A* mating alleles of *M. roreri*. Primers used in here were specific for the *Mr_HD1* gene (Díaz-Valderrama and Aime 2016). Samples are arranged vertically while *A* allele photographs, horizontally. The molecular ladder used was 100 bp O'RangeRuler™ (Thermo Fisher Scientific); the conspicuous bands in ladders are 1500, 1000 and 500 bp.

PURDUE UNIVERSITY

M. Catherine Aime, Dept. Botany & Plant Pathology, West Lafayette, IN 47907, USA

UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS

Jorge R. Díaz-Valderrama, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Chachapoyas, Peru



B locus genotyping. Gel photographs of PCR products amplified with primers designed for diagnostics of *B* mating alleles of *M. roreri*. Primers used in here were specific for the *STE3-_Mr4* gene (Díaz-Valderrama and Aime 2016). Samples are arranged vertically while *B* allele photographs, horizontally. The molecular ladder used was 100 bp O'RangeRuler™ (Thermo Fisher Scientific); the conspicuous bands in ladders are 1500, 1000 and 500 bp.

PURDUE UNIVERSITY

M. Catherine Aime, Dept. Botany & Plant Pathology, West Lafayette, IN 47907, USA

UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS

Jorge R. Díaz-Valderrama, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Chachapoyas, Peru