

Detection Limit Determination for

Phaeocollybia Species in Soil

Matt Gordon, M.S.

Molecular Solutions, LLC

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Introduction

Species-specific primers were developed for fifteen *Phaeocollybia* species so that the underground portion of these fungi could be detected in soil samples using PCR to reveal the presence of genetic markers. The ability to detect these species independently of their sporocarp production will allow direct monitoring of their status at known sites and will help determine the effectiveness of protection plans such as buffer zones. In this study, we determined the detection limit of *P. spadicea* and *P. radicata* in soil, using PCR with species-specific primers.

Materials and Methods

When testing soil for fungal DNA presence, the main target is the mycelial growth of the fungus within the soil matrix, so ideally a known amount of mycelium of the target species would be diluted with soil for detection limit testing. We did not have access to pure cultures of mycelium, but were able to collect fresh pseudorhiza of *P. spadicea* and *P. radicata*, and these were used in preparing soil dilutions. Although sporocarp tissue was available also, it was thought that the density of nuclei, and hence the DNA concentration, in pseudorhiza tissue would be closer to that in mycelium than would sporocarp tissue.

The soil used in the dilution studies was from a site (near Mary's Peak, Oregon) where *Phaeocollybias* were fruiting. The particular soil samples used were tested for the presence of *P. spadicea* and *P. radicata* and none was found. Stones were removed from the soil and 19.6 g of soil was added to a small blender with chunks of dry ice. This was blended until the soil had become fine and granular, then 0.2 g of each pseudorhiza was added and the mixture was blended for about 1 minute. Four subsamples were removed from this mixture for testing, then 3 g of the inoculated soil was mixed with 9 g of blank soil, along with dry ice as needed. This and subsequent dilutions (Table I) were done using a mortar and pestle rather than the blender because smaller volumes were used. Four subsamples were removed from each mixture, and 3 g of the mixture was diluted until all dilutions in Table I were done.

DNA was extracted from each subsample using a Chelex method, and cleaned using the UltraClean DNA purification kit (MoBio Laboratories, Carlsbad, CA). DNA was then tested for *P. spadicea* and *P. radicata* in PCR using the primers developed for this purpose. Quality control was addressed by using positive and negative controls, a lab instrument calibration program, and standard molecular biology precautions such as sterilization and use of disposable plastic-ware.

Results

Results are shown in the following table.

Table I Results of testing for *P. spadicea* (spa) and *P. radicata* (rad) in soil dilutions of pseudorhiza

Batch Number	Preparation	Dilution Ratio (soil to pseudorhiza)	Sub-sample	Sub-sample wt (g)	DNA Test Result
1	0.2g spa pseudorhiza	100:1	1-1	1.10	+
1	+ 0.2g rad pseudorhiza	100:1	1-2	1.03	+
1	+ 19.6 g soil	100:1	1-3	1.11	+
1		100:1	1-4	1.08	+
2	3.0 g Batch 1 + 9.0 g soil	400:1	2-1	1.14	+
2		400:1	2-2	1.11	+
2		400:1	2-3	1.02	+
2		400:1	2-4	1.19	+
3	3.0 g Batch 2 + 9.0 g soil	1600:1	3-1	1.12	+
3		1600:1	3-2	1.09	+
3		1600:1	3-3	1.20	+
3		1600:1	3-4	1.13	+
4	3.0 g Batch 3 + 9.0 g soil	6,400:1	4-1	1.03	+
4		6,400:1	4-2	1.06	+
4		6,400:1	4-3	1.18	+
4		6,400:1	4-4	1.10	+
5	3.0 g Batch 4 + 9.0 g soil	25,600:1	5-1	1.16	+
5		25,600:1	5-2	1.16	+
5		25,600:1	5-3	1.05	+
5		25,600:1	5-4	1.11	+
6	3.0 g Batch 5 + 9.0 g soil	102,400:1	6-1	1.08	-
6		102,400:1	6-2	1.14	+
6		102,400:1	6-3	1.13	+*
6		102,400:1	6-4	1.09	-
7	3.0 g Batch 6 + 9.0 g soil	409,600:1	7-1	1.10	-
7		409,600:1	7-2	1.01	-
7		409,600:1	7-3	1.04	-
7		409,600:1	7-4	1.17	-
8	3.0 g Batch 7 + 9.0 g soil	1,638,400:1	8-1	1.14	-
8		1,638,400:1	8-2	1.04	-
8		1,638,400:1	8-3	1.03	-
8		1,638,400:1	8-4	1.09	-
9	soil only	soil only	9-1	1.12	-
9		soil only	9-2	1.16	-
9		soil only	9-3	1.17	-
9		soil only	9-4	1.05	-
10	pseudorhiza only	no soil added	10-1	.03g spa + .03g rad	+
10		no soil added	10-2	.03g spa + .03g rad	+
10		no soil added	10-3	.03g spa + .04g rad	+
10		no soil added	10-4	.04g spa + .04g rad	+

*Positive for *P. spadicea*, negative for *P. radicata*

Discussion

Both species could be reliably detected to a dilution level of 25,600:1. This is 40 ppm, or 40 µg/ g of soil. This compares to a detection level, using essentially the same methods, of 10 ppm of *Albatrellus ellisii* in soil (Gordon, in press), and using somewhat different methods, of 11.4 ppm* of *Tuber melanosporum* in soil (Suz et al, 2007). The higher detection limit found for *Phaeocollybia* species in this study may have been due to our use of pseudorhiza tissue for dilution. The two referenced studies both used sporocarp material for dilution, and sporocarp tissue may be more highly nucleated than pseudorhiza tissue. If, as we assumed, the nucleation of pseudorhiza tissue is more similar to the mycelium than sporocarp tissue is, the results of the present study will be a more accurate estimate of the true detection limit for mycelium in soil.

Another possibility is that a compound was present in the soil that carried through the DNA extraction and clean-up process, and interfered with the PCR. Although all DNA samples were cleaned, no clean-up method is effective against all possible contaminants that may be found in soil. Bovine serum albumin (BSA) is commonly used in PCR to overcome the negative effects of various contaminants, and we did use BSA in all reactions to minimize contaminant effects. Since some of the samples at the 10 ppm level were positive, it is also possible that despite thorough mixing, the distribution of the fungal tissue was not even at this level, so that some samples had more fungal cells than others.

References

- Gordon M., Apple C. In Press. Field monitoring the seasonal variation in *Albatrellus ellisii* mycelium abundance with a species-specific genetic marker. *Mycologia*.
- Suz LM, Martin MP, Colinas C. 2006. Detection of *Tuber melanosporum* DNA in soil. *FEMS Microbiol Lett* 254:251-257

*Suz et al apparently used freeze-dried mushroom tissue, which would have the effect of concentrating the DNA five to nine fold, so that the equivalent detection level for fresh material in this study would be over 60 ppm.