The Effect of Organic and Conventional Agricultural Production on Fungal Community Assemblage in Ethiopian Green Coffee Beans

Iman Sylvain and Tim James. Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109

Abstract We are developing coffee production as a model to understand the transport of fungi through the global food trade and to evaluate the ways in which organic and conventional agricultural production influences fungal community assemblage on harvested crops. We used culture-based and culture-independent methods along with molecular techniques to identify fungal communities on Ethiopian green coffee beans. The coffee was either produced by conventional or organic methods. Our findings suggest that farming practices have significant effects on fungal community assemblage in harvested coffee. USDA Certified Organic coffee hosts a lower diversity and abundance of fungal species than conventional coffee. Methods of sampling strongly affect the recovery of species. Culture-based and culture-independent methods resulted in distinct views of fungal community assemblage in green coffee beans.

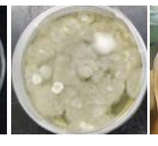
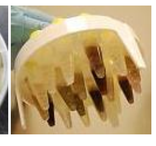
Introduction

As the fourth largest agricultural commodity, the importance of the coffee trade cannot be overstated for the economies of many tropical countries. Development of shade-grown, organic, and fair trade coffee practices may have numerous economic and environmental benefits, but little is known about how these different methods of production affect fungal communities on coffee. Adequately accessing fungal communities in coffee may have human health implications by reducing the risk of mycotoxins. Mycotoxins such as ochratoxin and aflatoxin are mutagenic and carcinogenic secondary metabolites produced by a variety of *Aspergillus, Fusarium,* and *Peniciilium* species (Edwards, et al. 2002). These contaminants are found in coffee at various stages of production, including cherry ripening, drying and storage (Taniwaki, et al. 2003). USDA Organic certification prevents the use of specific fungicides, pesticides, and chemical fertilizers during the growth and processing of agricultural productions. In this study we use both culture-based and culture-independent methods to determine how organic and conventional production methods affect fungal communities on green coffee beans.

Methodology

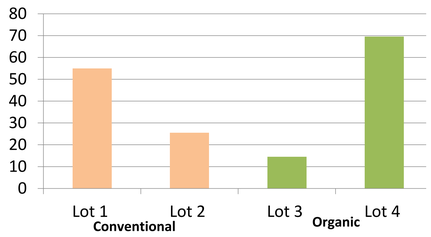
Four lots of Ethiopian coffee were purchased. All samples were shade grown and wet-processed. Lots 1 and 2 were produced by conventional methods, and Lots 3 and 4 were certified organic. DNA-barcoding techniques using the ITS rRNA region allowed us to identify fungal species present in the coffee beans. Culture-based methods involved surface-sterilization using a 2% bleach solution, plating 200 beans per lot on MEA++ and DG18 media, isolating pure cultures of fungi, and extracting fungal DNA for PCR and Sanger sequencing. Culture-independent methods involved drying 20 beans per lot in a 60°C oven for 7 days before freezing with liquid nitrogen to grind the beans into fine powder. DNA extraction was then carried out on the coffee powder, and PCR was performed with fungal-specific ITS primers, cloned into a plasmid, and 24 subclones were sequenced per lot. All DNA sequences were analyzed using Sequencher 4.1. Sequences were compared to GenBank using BLAST to determine the closest matched OTUs. Community analyses based on OTU data were conducted using MOTHUR. Significance differences in communities were estimated using Libshuff tests and diversities calculated using Chao and Shannon estimators.



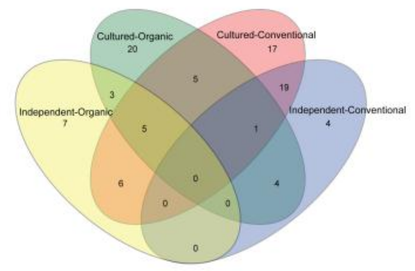




Group	No. Sequences	O.T.U.s	Chao	Shannon
Lot 1 Cultured	94	20	32 (22.66-74.20)	2.05 (1.75-2.35)
Lot 2 Cultured	44	12	22.5 (14.03-66.19)	1.79 (1.44-2.15)
Lot 3 Cultured	27	10	13.3 (10.50-32.07)	1.95 (1.61-2.29)
Lot 4 Cultured	154	24	62.25 (35.59-150.21)	1.15 (0.86-1.43)
Lot 1 Independent	18	8	9.5 (8.18-20.47)	1.73 (1.29-2.18)
Lot 2 Independent	9	8	18.5 (10.03-62.19)	2.04 (1.59-2.49)
Lot 3 Independent	8	4	4.5 (4.030-12.261)	1.21 (0.71-1.71)
Lot 4 Independent	14	9	19.5 (11.03-63.19)	1.96 (1.49-2.44)
Cultured-Conventional	139	53	143 (88.78-279.36)	3.02 (2.74-3.30)
Cultured-Organic	183	38	125.75 (67.88-295.73)	1.88 (1.60-2.16)
Independent-Conventional	52	28	53.5 (36.05-108.79)	3.08 (2.84-3.32)
Independent-Organic	44	21	51.33 (29.18-133.43)	2.68 (2.39-2.98)

Percentage of Total Bean Infection for All Cultured Samples



Venn Diagram Accessing Fungal Sampling and Coffee Production Method at distance 0.01



Top Blast Match O.T.U.	Occurrence in Conventional n = 191
Aspergillus niger	55
Wickerhamomyces anomalus	30
Aspergillus tubingensis	17
Cladosporium sp.	15
Eurotium rubrum	11

Top Blast Match O.T.U.	Occurrence in Organic n = 227
Aspergillus niger	107
Aspergillus tubingensis	46
Eurotium rubrum	11
Eurotium amstelodami	10
Dipodascaceae sp.	8

Top Blast Match O.T.U.	Occurrence in All Cultured Samples n = 319
Aspergillus niger	163
Aspergillus tubingensis	65
Eurotium rubrum	23
Wickerhamomyces anomalus	12
Aspergillus fumigatus	9
Chaetomium reflexum	7
Candida orthopsilosis	5
Cystofilobasidium feraegula	4
Cladosporium sp.	4
Fusarium sp.	4

Top Blast Match O.T.U.	Occurrence in All Culture-Independent Samples n = 96
Wickerhamomyces anomalus	18
Cladosporium sp.	16
Pichia pijperi	11
Eurotium amstelodami	10
Dipodascaceae sp.	9
Debaryomyces hansenii	6
Epicoccum sp.	4
Uncultured Basidiomycete	3
Alternaria alternata	2
Hanseniaspora uvarum	2

★ At least five species: *Aspergillus fumgatus*, *A. niger*, *A. phoenicis*, *Penicillium brevicompatum*, and *P. citrinum*, overlapped with endophytic species found by Vega, et al. (2010) in coffee leaves, seeds, and fruits.

Discussion

Both production and sampling methods seem to affect which fungal species are recovered from dried green coffee beans. Overall, culture-dependent methods recovered communities dominated by *Aspergillus*, particularly *A. niger*, as has been recorded in other studies (Perrone, et al. 2007). In contrast, culture-independent investigations found a much lower frequency of *Aspergillus,* the fungus largely suspected of causing mycotoxins in coffee. Conventional and organic coffee production results in fungal community assemblages that are significantly different (Libshuff, results not shown), with organic samples hosting lower diversity and abundance of OTUs. The lot to lot (farm to farm) variation in bean infection percentage suggests that farming practices, such as the use of fungicides, fertilizers, or pesticides, may create distinct chemical environments for microorganisms in agroecosystems, resulting in differentiation in habitat suitability. Organic shade coffee farms are often cited to be reservoirs for biodiversity, but this may not extend to the fungi found as contaminants on the harvest, as observed in this study. Using various sampling methods strongly affected the species recovered. Culture-based methods result in a greater number of species being identified, though species recovered through culture-independent methods are non-overlapping and distinct. This might be because culture-independent methods are unbiased to preferential spore germination on media plates, alleviating competition between fungi.

Future Research

We plan to study the ways in which various methods of processing coffee (dry or wet) affects fungal communities on green coffee beans, as well as the effect of sun and shade farming, which enlist different methods of agroforestry. Future projects also involve population genetic analysis of mycotoxigenic *Aspergillus* species on green coffee beans from various countries and coffee-producing geographical regions. Preliminary data from Mexican and Kenyan lots show very different fungal communities than the ones found in Ethiopian beans.

Acknowledgements

I would like to acknowledge all of the members of the James Mycology lab, especially Courtney Frye. As well, thank you to the John Vandermeer/Ivette Perfecto Coffee Agroecology Labs. Thank you Frontiers Masters in Ecology program and the Department of Ecology and Evolutionary Biology and Rackham Graduate School at U. Michigan.

References

Edwards, S., et al. 2002. PCR-based detection and quantification of mycotoxigenic fungi. Mycological Research 106.

Perrone, G., et al. 2007. Biodiversity of Aspergillus species in some important agricultural products. Studies in Mycology 59.

Taniwaki, M., et al. 2003. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. International Journal of Food Microbiology 82.

Vega, F., et al. 2010. Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico, and Puerto Rico. Fungal Ecology 3.