Cold Spring Harbor Laboratory
DNA Learning Center

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DNA barcoding for identification of consumer-relevant fungi sold in New York

URBAN BARCODE RESEARCH PROGRAM

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Project Summary

Aim: *Investigating dried fungi (mis)labeling in New York City.*

Intended Outcomes: Many foods in the US have been mislabeled in order to gain a greater profit for cheaper products replacing expensive ones (Moyer et *al*., 2017). We are interested to see if we can identify mislabeled species of fungi sold in New York, including a commercial packet of dried fungi.

Description:

Reliably identifying wild collected foods can be a challenge, especially when they belong to poorly known groups like Fungi (UNITE). Although significant progress has been made in our understanding of fungi diversity, identification based on phenotype can be difficult for even trained experts. Fungi typically have a cryptic nature and can have a similar appearance to distantly related species (Yahr *et al.,* 2016). What contributes to the mislabeling of fungi may be the phenotypical appearance of the dried fungi being unclear. Our goal was to collect and analyze sequences and data from dried and fresh fungi sold for commercial uses. We used DNA technology for species identification of 10 samples that were collected from local supermarkets in two New York City boroughs. Although there were no cases of mislabeling, we found that our single-locus ITS DNA barcoding approach allowed to identify only 44% of all samples at the species level. This highlights the need for a curated, centralized ITS reference database that allows third-party annotations.

Materials and Methods

Dried and fresh fungi samples were collected from local supermarkets in two New York City boroughs. All dried samples were obtained from a single bag of dried of edible "Mixed Mushrooms". Samples were visually sorted by phenotype, documented and vouchered. While fresh samples were easy to identify, many of the dried samples were broken down into smaller pieces and lacked key morphological characteristics that could be used for identification. The DNA was extracted from approximately 0.1 g of specimen using the standard silica DNA extraction method (www.dnabarcoding101.org), with one minor modification: for each sample, a small piece was removed and pre-soaked in dH_20 before being ground in lysis buffer with a sterile pestle. The extracted DNA was amplified using fungi-specific ITS primers (ITS1F/ITS4 as described by White *et al.*, 1990) and NEB Taq 2X master mix (New England Biolabs). Then, the amplified DNA was stained with SYBR green (Invitrogen) and confirmed using gel electrophoresis. Positive amplicons were sent to GENEWIZ Inc. for sequencing in both directions. After the DNA was sequenced, DNA Subway software (http://www.cyverse.org/dna-subway) was used to analyze the DNA sequences. To verify species identifications through DNA Subway sample consensus sequences were downloaded and submitted directly to Basic Local Alignment Search Tool (BLAST) in NCBI-GenBank (Altschul *et al.*, 1990) and UNITE Ver. 7.1, an open-access, curated fungi-specific database (https://unite.ut.ee). Finally a phylogenetic tree was constructed based on DNA Subway's MUSCLE and PHYLIP maximum likelihood (ML) methods.

The DNA Barcoding Workflow

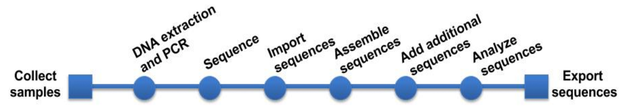


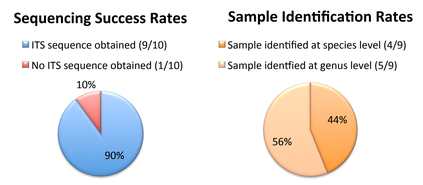
Figure 1: How DNA barcoding works.

Results

Sample	Date Purchased	Location of purchase	Labeled on Package	Picture	Identified using UNITE serial BLAST search
S 1	February 2nd, 2017	Supermarket, LIC, NY	Cloud-ear (dried)		*Auricularia heimuer* [Cloud-ear]
S 2	February 2nd, 2017	Supermarket, LIC, NY	Shiitake (dried)		*Lentinula edodes* [Shiitake]
S 3	February 2nd, 2017	Supermarket, LIC, NY	Wood ear (dried)		No sequence
S 4	February 2nd, 2017	Supermarket, LIC, NY	Oyster (dried)		*Pleurotus* [Oyster]
S 5	February 2nd, 2017	Supermarket, LIC, NY	White Button (dried)		*Agaricus bisporus* [White button]
S 6	February 2nd, 2017	Supermarket, LIC, NY	Unknown (dried)		*Pleurotus ostreatus* [Oyster]
S 7	February 2nd, 2017	Supermarket, LIC, NY	White Button (fresh)		*Agaricus bisporus* [White button]
S 8	February 2nd, 2017	Supermarket, LIC, NY	Korean Black (dried)		*Auricularia auricula-judae* [Wood-ear]
S 9	February 24, 2017	Supermarket, Manhattan, NY	Yellow foot (fresh)		*Craterellus* [Chanterelle]
S 10	February 24, 2017	Supermarket, Manhattan, NY	Yellow foot (fresh)		*Craterellus* [Chanterelle]

Table 1 (above): List of fungi sampled in this study for ITS barcoding.

Figures 2 and 3 (right): ITS sequencing and sample identification success rates.



Acknowledgements

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Sample ID	Location	Fungi sp. Per Label	Common Name	DNA Subway		BLAST, GenBank		UNITE	
				Fungi Barcode ID	% Match	Fungi Barcode ID	% Match	Fungi Barcode ID	% Match
S 1	Supermarket, LIC, NY	Cloud-ear (dried)	Cloud Ear	*Auricularia heimuer*	100	*Auricularia heimuer*	100	*Auricularia heimuer*	100
S 2	Supermarket, LIC, NY	Shiitake (dried)	Shiitake	*Lentinula edodes*	86	*Lentinula edodes*	99	*Lentinula edodes*	99.9
S 3	Supermarket, LIC, NY	Wood ear (dried)	Wood ear	*N/A*		*N/A*		*N/A*	
S 4	Supermarket, LIC, NY	Oyster (dried)	Oyster	*Pleurotus geesterani*	95	*Pleurotus geesterani*	94	*Pleurotus sp.*	94
S 5	Supermarket, LIC, NY	White Button (dried)	White Button	*Agaricus bisporus*	100	*Agaricus bisporus*	100	*Agaricus bisporus*	100
S 6	Supermarket, LIC, NY	Oyster (dried)	Oyster	*Pleurotus sp.*	90	*Pleurotus ostreatus*	91	*Pleurotus ostreatus*	91
S 7	Supermarket, LIC, NY	White Button (fresh)	White Button	*Agaricus bisporus*	100	*Agaricus bisporus*	100	*Agaricus bisporus*	100
S 8	Supermarket, LIC, NY	Korean Black (dried)	Korean Black	*Auricularia heimuer*	100	*Auricularia heimuer*	100	*Auricularia auricula-judae*	100
S 9	Supermarket, NYC, NY	Yellow foot (fresh)	Yellow foot	*Craterellus tubaeformis*	100	*Craterellus tubaeformis*	100	*Craterellus sp.*	100
S 10	Supermarket, NYC, NY	Yellow foot (fresh)	Yellow foot	*Craterellus tubaeformis*	100	*Craterellus tubaeformis*	100	*Craterellus sp.*	100

Table 2: Species identification based on ITS DNA barcoding from all samples analyzed (n=10). Each consensus sequence of samples S1 – S10 was subjected to three search tools to verify identity: 1) DNA Subway; 2) direct submission to BLAST search in NCBI-GenBank; and 3) a serial BLAST search in the curated database termed UNITE.

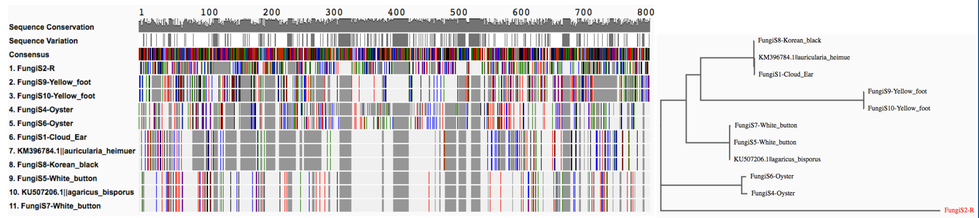

Figure 4: Sample sequences (S1 - S10) aligned with corresponding BLAST sequences hits using the MUSCLE multiple alignment software. The same data set is depicted as gene tree using PHYLIP ML to generate a phylogenetic tree using the maximum likelihood (ML) method. Sample S2 (Shiitake) was chosen as out-group as only one sequencing read was available for species identification.

Discussion

There were no cases of mislabeling. For one sample (sample S3) we were not able to obtain a DNA sequence because of unsuccessful PCR amplification in spite of several attempts (Table 1). This may be due to potentially high degradation of the sample's DNA due to heat treatment prior packaging - sample S3 was very dry. Once DNA is broken into short fragments, generating a ITS barcodes can be difficult, as primer binding sites may be degraded. Moreover, secondary metabolites can inhibit PCR by decreasing the Taq-polymerase's activity. Failure to generate a ITS barcode may also be because the primers used were not suited for amplifications of all species.

Sample S2 only yielded a single sequencing read (reverse direction). Samples S1, S2, S5, S6, and S7 were labeled correctly in both the species and genus level. Samples S4, S8, S9, and S10 were labeled at the correct genus level but we were unable to resolve them down to the species level as there were discrepancies between databases (see Figures 2 and 3). Species with highest % match/max score/bit score were considered the taxonomic identity (Table 2). UNITE Ver. 7.1 serial BLAST search was utilized in addition to GenBank because it has been found that 27% of fungal ITS sequences entered into GenBank were inadequately identified and 20% were incorrectly labeled (Raja and Oberlies *et al*., 2016). Future studies would benefit highly from a curated, centralized ITS reference database that allows rapid third-party annotations.