Direct PCR Detection, Cloning, and Characterization of Mold Populations from Soils and Compost

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Abstract

Molds are somewhat difficult to isolate from environmental samples. Furthermore, identification using phenotypic analysis is inaccurate, slow, and extremely subjective. A procedure was developed to detect and identify mold populations from a New Jersey soil, New York soil, and compost samples. Fungal internal transcribed space sequences (ITS) were amplified by PCR using primers ITS1 and ITS4. Microbial DNA was extracted using the Zymo Microbe DNA MiniPrep protocol. DNA fragments of approximately 640 base pair were detected in all positive samples. Clone libraries were constructed with the amplified DNA fragments by ligating the detected fragments with vector pCR®4-TOPO. Transformations were performed using competent Mix and Go *Escherichia coli* cells. Plasmids were isolated from each clone using the Zyppy Plasmid Miniprep and inserts were screened by PCR using M13 DNA primers. All clones with at least an 800 bp insert were DNA sequenced followed by BLAST analysis to determine the identity of the cloned fragments. DNA sequencing of clone libraries showed that compost samples were predominantly comprised of *Thermomyces lanuginosus*. Mold clones from the NJ soil were mostly unidentified species while the most abundant sequences in the NY soil were related to *Cortinarius flexipes*.

Objectives

- To extract microbial DNA from soil and compost.
- To amplify Mold ITS sequences from the extracted DNA using PCR.
- To clone the ITS sequences to develop clone libraries.
- To extract the plasmids and sequence the clones to determine the names of the different mold populations.

Materials and Methods

DNA extractions

DNA was extracted from 300 mg of soils and compost using the ZR Soil Microbe DNA MiniPrep protocol (Zymo Research, Irvine, CA).

Materials and Methods

PCR reactions

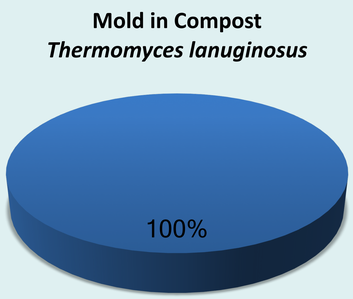
The reactions were targeting a fragment of approximately 640 base pair (bp) of fungal internal transcribed space sequences (ITS) using degenerate primers ITS1 and ITS4. The reaction conditions were 95ºC for 3 minutes, and 30 cycles of 95ºC /30 sec., 65ºC/30 sec., 72ºC/30 sec. with a final extension of 5 minutes at 72ºC. Ready-To-Go (RTG) PCR beads (GE Healthcare, Buckinghamshire, UK) were used for each PCR reaction. Amplified fragments were detected using the Lonza Flash Gel System.

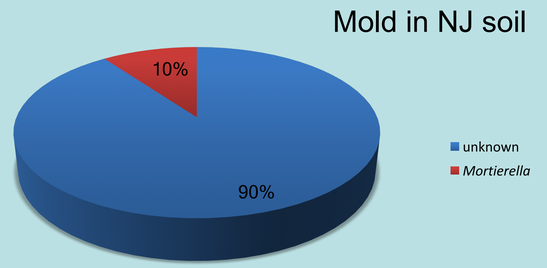
Cloning libraries

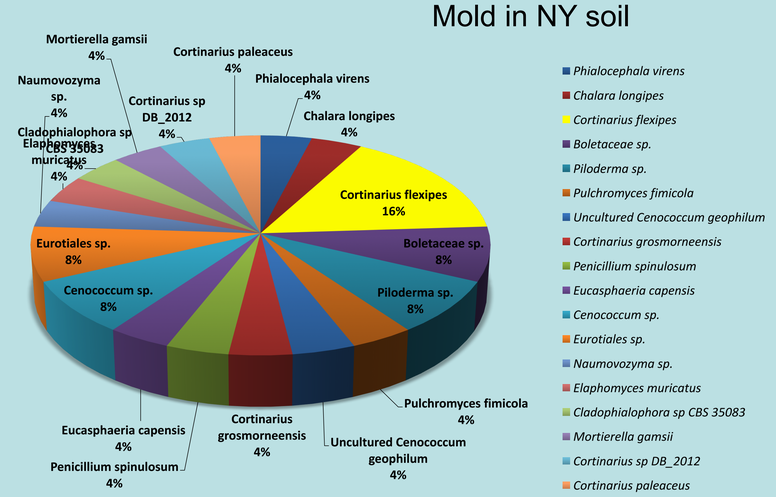
The DNA fragments from the PCR reactions of Mold ITS genes were cloned using plasmid pCR®4-TOPO (Life Technologies, Thermo Fisher Scientific, Grand Island, NY) according to the manufacturer's instructions. Transformations were performed using Mix and Go Competent *E. coli* strains (Zymo Research, Irvine, CA). White colonies grown on Luria Bertani (LB) Agar with ampicillin (50 ug/ml) were transferred to LB broth containing ampicillin (50 ug/ml). Samples were incubated overnight at 37°C.

Plasmids were isolated from each mold clone using the Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA). Cloned inserts were reamplified using M13 DNA primers. DNA sequencing reactions of the amplified PCR fragments was performed by Genewiz, Inc. (South Plainfield, New Jersey). Homology searches were performed using the UNITE fungal ITS trainset 07-04-2014 database on the Classifier application from the Ribosomal Database Project (http://rdp.cme.msu.edu). Homology values equal or greater than 97% were considered accurate for species level identification.

Results







Conclusions

- Microbial DNA was extracted from soils and compost.
- Mold ITS sequences were detected in soils using PCR.
- Clone libraries were constructed to determine the identity of the different mold populations.
- Compost samples were predominantly comprised of *T. lanuginosus* while most species in the NJ soil were unidentified. The NY soil showed *Cortinarius flexipes* as the most abundant species.

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