

Supplementary Material

Mycobiome Sequencing and Analysis Applied to Fungal Community Profiling of the Lower Respiratory Tract During Fungal Pathogenesis

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1 Supplementary Figures and Tables

Supplementary Table 1. Proportional abundance of fungal species in 3 mock communities detected using ITS1 mycobiome analysis. Mock communities were prepared as a mixture of equal quantities (50ng) of genomic DNA of each species. Values in bold represent >2-fold differences from the expected proportional abundances. Primer scores >0 indicate the potential for forward or reverse primer mismatch to negatively affect PCR product generation.

Phylum	Species	ITS1 Mycobiome Pipeline Result	Expected Proportional Abundance	Mycobiome Proportional Abundance	Forward/Reverse Primer Score
<u>Mock Community #1</u>					
(Total DNA concentration: 2.76 ng/μl)					
<i>Ascomycota</i>	<i>Aspergillus pseudoglaucus</i>	<i>Aspergillus</i> sec <i>Aspergillus</i>	5.0%	2.4%	0/0
	<i>Aspergillus clavatonanicus</i>	<i>Aspergillus</i> sec <i>Clavati</i>	5.0%	7.5%	0/0
	<i>Aspergillus lentulus</i>	<i>Aspergillus</i> sec <i>Fumigati</i>	5.0%	4.9%	0/0
	<i>Aspergillus niger</i>	<i>Aspergillus</i> sec <i>Nigri</i>	5.0%	7.8%	0/0
	<i>Aspergillus terreus</i>	<i>Aspergillus</i> sec <i>Terrei</i>	5.0%	6.3%	0/0
	<i>Aspergillus pseudodeflectus</i>	<i>Aspergillus</i> sec <i>Usti</i>	5.0%	3.9%	0/0
	<i>Aspergillus versicolor</i>	<i>Aspergillus</i> sec <i>Versicolores</i>	5.0%	2.2%	0/0
	<i>Blastomyces dermatitidis/gilchristii</i>	<i>Blastomyces dermatitidis/gilchristii</i>	5.0%	2.2%	0/0

	<i>Coccidioides immitis/posadasii</i>	<i>Coccidioides</i>	5.0%	3.2%	0/0
	<i>Exophiala dermatitidis</i>	<i>Exophiala dermatitidis</i>	5.0%	1.8%	1/1.4
	<i>Histoplasma capsulatum</i>	<i>Histoplasma capsulatum</i>	5.0%	0.9%	0/0
	<i>Penicillium chrysogenum</i>	<i>Penicillium</i> sp.	5.0%	1.5%	0/0
	<i>Penicillium corylophilum</i>	<i>Penicillium</i> sec <i>Exilicaulis</i>	5.0%	6.8%	0/0
	<i>Purpureocilium lilacinum</i>	<i>Purpureocilium lilacinum</i>	5.0%	0.3%	1/0
	<i>Sarocladium strictum</i>	<i>Sarocladium strictum</i>	5.0%	1.5%	1/0
	<i>Scedosporium prolificans</i>	<i>Scedosporium prolificans</i>	5.0%	8.0%	1/0
	<i>Talaromyces columbinus</i>	<i>Talaromyces</i> sec <i>Islandici</i>	5.0%	2.5%	0/0
Basidiomycota	<i>Cryptococcus neoformans</i>	<i>Cryptococcus</i>	5.0%	3.2%	0/0
	<i>Trichosporon asahii</i>	<i>Trichosporon asahii</i>	5.0%	15.4%	0/0
Zygomycota	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	5.0%	15.2%	0/0
Other		Other	0%	2.4%	
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Mock Community #2					
(Total DNA concentration: 2.60 ng/μl)					
Ascomycota	<i>Eurotium chevalieri</i>	<i>Aspergillus</i> sec <i>Aspergillus</i>	5.0%	3.5%	0/0
	<i>Aspergillus ochraceus</i>	<i>Aspergillus</i> sec <i>Circumdati</i>	5.0%	10.5%	0/0
	<i>Aspergillus clavatus</i>	<i>Aspergillus</i> sec <i>Clavati</i>	5.0%	10.8%	0/0
	<i>Aspergillus flavus</i>	<i>Aspergillus</i> sec <i>Flavi</i>	5.0%	8.2%	0/0
	<i>Aspergillus fumigatus</i>	<i>Aspergillus</i> sec <i>Fumigati</i>	5.0%	5.4%	0/0
	<i>Emericella nidulans</i>	<i>Aspergillus</i> sec <i>Nidulantes</i>	5.0%	4.3%	0/0
	<i>Aspergillus calidoustus</i>	<i>Aspergillus</i> sec <i>Usti</i>	5.0%	5.0%	0/0
	<i>Aspergillus sydowii</i>	<i>Aspergillus</i> sec <i>Versicolores</i>	5.0%	6.5%	0/0
	<i>Fusarium solani</i>	<i>Fusarium/Gibberella</i> sp.	5.0%	6.6%	1/0
	<i>Paecilomyces variotii</i>	<i>Paecilomyces variotii</i>	5.0%	2.0%	0/0
	<i>Rasamsonia argillacea</i>	<i>Rasamsonia argillacea</i>	5.0%	6.5%	0/0
	<i>Scedosporium apiospermum</i>	<i>Scedosporium apiospermum</i>	5.0%	7.5%	1/0
	<i>Sporothrix schenckii</i>	<i>Sporothrix schenckii</i>	5.0%	0.0%	1/1.4
Basidiomycota	<i>Cryptococcus gattii</i>	<i>Cryptococcus</i> <i>neoformans/gattii</i>	5.0%	2.9%	0/0
Zygomycota	<i>Apophysomyces variabilis</i>	<i>Apophysomyces variabilis</i>	5.0%	5.0%	0/0
	<i>Cunninghamella elegans</i>	<i>Cunninghamella elegans</i>	5.0%	0.3%	0/0.4
	<i>Lichtheimia corymbifera</i>	<i>Lichtheimia corymbifera</i>	5.0%	1.0%	0/0.8

	<i>Mucor circinelloides</i>	<i>Mucor circinelloides</i>	5.0%	9.6%	0/0
	<i>Rhizomucor pusillus</i>	<i>Rhizomucor pusillus</i>	5.0%	0.0%	0/2.2
	<i>Rhizopus microsporus</i>	<i>Rhizopus microsporus</i>	5.0%	2.5%	0/0
Other		Other	0%	2.0%	
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Mock Community #3					
(Total DNA concentration: 5ng/μl)					
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<i>Ascomycota</i>	<i>Alternaria alternata</i>	<i>Alternaria alternata</i>	4.76%	5.2%	1/0
	<i>Aspergillus fumigatus</i>	<i>Aspergillus sec Fumigati</i>	4.76%	7.7%	0/0
	<i>Aspergillus flavus</i>	<i>Aspergillus sec Flavi</i>	4.76%	7.2%	0/0
	<i>Aspergillus niger</i>	<i>Aspergillus sec Nigri</i>	4.76%	9.6%	0/0
	<i>Aspergillus terreus</i>	<i>Aspergillus sec_Terrei</i>	4.76%	5.9%	0/0
	<i>Aspergillus versicolor</i>	<i>Aspergillus sec_Versicolores</i>	4.76%	4.7%	0/0
	<i>Candida albicans</i>	<i>Candida albicans</i>	4.76%	2.7%	0/0
	<i>Candida dubliniensis</i>	<i>Candida dubliniensis</i>	4.76%	1.3%	0/0
	<i>Candida glabrata</i>	<i>Candida glabrata</i>	4.76%	10.5%	0.4/0
	<i>Candida parapsilosis</i>	<i>Candida parapsilosis cplx</i>	4.76%	<0.1%	0.4/0
	<i>Candida tropicalis</i>	<i>Candida tropicalis</i>	4.76%	1.9%	0/0
	<i>Cladosporium cladosporioides</i>	<i>Cladosporium sp.</i>	4.76%	3.7%	1/0
	<i>Candida lusitaniae</i>	<i>Clavispora lusitaniae</i>	4.76%	0.4%	0/0
	<i>Fusarium oxysporum</i>	<i>Fusarium/Gibberella sp.</i>	4.76%	5.1%	1/0
	<i>Candida guilliermondii</i>	<i>Meyerozyma guilliermondii</i>	4.76%	2.2%	0/0
	<i>Penicillium chrysogenum</i>	<i>Penicillium sp.</i>	4.76%	2.3%	0/0
	<i>Candida krusei</i>	<i>Pichia kudriavzevii</i>	4.76%	11.2%	0/0.8
	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	4.76%	1.6%	0.4/0
<i>Basidiomycota</i>	<i>Sporidiobolus salmonicolor</i>	<i>Sporidiobolus salmonicolor</i>	4.76%	3.4%	0/0
	<i>Trichosporon asahii</i>	<i>Trichosporon asahii</i>	4.76%	11.7%	0/0
<i>Zygomycota</i>	<i>Mucor racemosus</i>	<i>Mucor racemosus</i>	4.76%	1.4%	0/0
Other		Other	0%	0.3%	
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Supplementary Table 2. List of primers

Target	Purpose	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
ITS1-5.8S-ITS2	Identification	ITS5: GGAAGTAAAAGTCGTAA CAAGG	ITS4: TCCTCCGCTTATTGATA TGC	White et al., 1990
ITS1	Mycobiome generation	ITSF: GTAAAAGTCGTAACAAG GTTTC	ITSR: GTTCAAAGAYTCGATG ATTAC	Findley et al., 2013
ITS2	Mycobiome generation	ITS3: GCATCGATGAAGAACGC AGC	ITS4: TCCTCCGCTTATTGATA TGC	White et al., 1990
ITS1-5.8S-ITS2	Mycobiome generation	ITSF: GTAAAAGTCGTAACAAG GTTTC	ITS4: TCCTCCGCTTATTGATA TGC	White et al., 1990; Findley et al., 2013
18S-28S	Primer binding analysis	ITS-BMB-CR: GTACACACCGCCCGTCG	ITS-LR1: GGTTGGTTTCTTTTCCT	Lane et al., 1985; Vilgalys and Hester 1990

White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics., p 315-322. *In* MA Innis, DH Gelfand, JJ Sninsky and TJ White (ed), PCR protocols: a guide to methods and applications. Academic Press, Cambridge.

Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., et al. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature* 498,367-370.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., and Pace, N.R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* 82,6955-6959.

Vilgalys, R. and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172,4238-4246.

Supplementary Table 3. Characteristics of *Blastomyces* M+/C+ BAL specimens, *Blastomyces* M-/C+ BAL specimens, BAL specimens culture-positive for other fungi, fungal-culture-negative BAL specimens, and negative controls. Semi-quantitative fungal culture results provide detail on the filamentous fungi and yeast cultured from each sample on IMA and/or BCCG (+++ heavy growth, ++ moderate growth, + little growth). The DNA quantitation criteria (see Materials and Methods) were used to calculate the normalized ITS abundances expressed in arbitrary units (a.u.) for each sample. Samples that failed to generate ITS1 amplicons or <5000 fungal sequence reads are not listed.

Category	Sample	Culture results	Vol. DNA input (μl)	No. PCR cycles	ITS amplicon conc. (ng/μl)	Fungal read proportion	Total normalized ITS abundance (a.u.)
<i>Blastomyces</i> M+/C+							
	MC001	<i>Blastomyces dermatitidis/gilchristii</i> ++	4.0	35	45.5	92.5%	7.66
	MC002	<i>Blastomyces dermatitidis/gilchristii</i> ++ / yeast+++	4.0	35	18	72.6%	2.38
	MC003	<i>Blastomyces dermatitidis/gilchristii</i> +++/ <i>C. albicans/dubliniensis</i> +	4.0	30	24.4	99.9%	141.94
	MC004	<i>Blastomyces dermatitidis/gilchristii</i> ++/ <i>C. glabrata</i> ++	4.0	30	53.5	99.9%	311.20
	MC006	<i>Blastomyces dermatitidis/gilchristii</i> +++	9.5	35	8.13	44.1%	0.27
	MC008	<i>Blastomyces dermatitidis/gilchristii</i> +++	1.0	28	20.3	99.9%	1889.35
	MC013	<i>Blastomyces dermatitidis/gilchristii</i> +/- yeast +++	4.0	30	21.9	99.7%	127.11

MC014	<i>Blastomyces dermatitidis/gilchristii</i> +/- yeast+++	4.0	30	10.7	99.8%	62.13
MC016	<i>Blastomyces dermatitidis/gilchristii</i> ++	9.5	35	6.06	81.9%	0.38
MC113	<i>Blastomyces dermatitidis/gilchristii</i> ++/ yeast +	4.0	35	11.1	94.6%	1.91
MC114	<i>Blastomyces dermatitidis/gilchristii</i> +++	1.0	25	28.8	83.1%	17829.20
MC115	<i>Blastomyces dermatitidis/gilchristii</i> +++/ yeast +	1.0	28	6.44	98.7%	592.02
MC116	<i>Blastomyces dermatitidis/gilchristii</i> +++/ yeast+	1.0	28	9.67	97.5%	877.87
MC117	<i>Blastomyces dermatitidis/gilchristii</i> ++/ yeast +	4.0	35	7.66	94.3%	1.31
MC119	<i>Blastomyces dermatitidis/gilchristii</i> +	1.0	25	8.85	86.5%	5704.89
MC120	<i>Blastomyces dermatitidis/gilchristii</i> ++ / yeast++	1.0	25	23.1	96.2%	16562.03
MC121	<i>Blastomyces dermatitidis/gilchristii</i> +++	4.0	33	7.61	93.9%	5.20
MC122	<i>Blastomyces dermatitidis/gilchristii</i> +++	2.0	30	12.7	91.4%	135.21

MC123	<i>Blastomyces dermatitidis/gilchristii</i> ++/ yeast ++	2.0	30	17.8	99.5%	206.24
MC124	<i>Blastomyces dermatitidis/gilchristii</i> ++/ yeast+	4.0	35	4.17	74.2%	0.56
<i>Blastomyces</i> M-/C+						
XC017	<i>Blastomyces dermatitidis/gilchristii</i> ++	4.0	35	4.97	83.9%	0.76
XC018b	<i>Blastomyces dermatitidis/gilchristii</i> +	9.5	35	13.9	80.3%	0.85
XC019b	<i>Blastomyces dermatitidis/gilchristii</i> +	2.0	30	7.04	77.9%	63.87
XC021	<i>Blastomyces dermatitidis/gilchristii</i> ++	4.0	35	12.5	92.8%	2.11
XC032	<i>Blastomyces dermatitidis/gilchristii</i> ++	1.0	25	38.7	99.9%	28810.86
XC126	<i>Blastomyces dermatitidis/gilchristii</i> +	4.0	33	14.5	94.6%	9.98
XC127	<i>Blastomyces dermatitidis/gilchristii</i> ++/ yeast +	2.0	30	12.1	98.7%	139.01
XC128	<i>Blastomyces dermatitidis/gilchristii</i> +/ yeast +/bacteria +++	2.0	30	19.3	99.9%	224.39
XC129	<i>Blastomyces dermatitidis/gilchristii</i> ++ / yeast +	4.0	35	14	76.3%	1.94

XC131	<i>Blastomyces dermatitidis/gilchristii</i> +	9.5	35	8.02	99.6%	0.61
XC132	<i>Blastomyces dermatitidis/gilchristii</i> +	2.0	30	5.58	95.5%	62.05
XC130	<i>Blastomyces dermatitidis/gilchristii</i> ++	9.5	35	3.1	93.1%	0.22
XC134	<i>Blastomyces dermatitidis/gilchristii</i> +/- yeast +++	4.0	33	11.1	85.6%	6.91
XC135	<i>Blastomyces dermatitidis/gilchristii</i> +/- yeast +/- bacteria +++	4.0	33	14.8	71.7%	7.72
XC136	<i>Blastomyces dermatitidis/gilchristii</i> +	2.0	30	13	99.7%	150.83
Other fungi, culture-positive						
MC009	<i>Histoplasma capsulatum</i> ++/ yeast ++	9.5	35	10.8	50.6%	0.42
XC025	<i>Histoplasma capsulatum</i> ++	1.0	28	10.8	99.6%	1002.01
XC026	<i>Histoplasma capsulatum</i> ++	4.0	35	9.56	52.7%	0.92
XC027	<i>Histoplasma capsulatum</i> +/- yeast +	4.0	30	40	99.9%	232.66
XC028	<i>Histoplasma capsulatum</i> +++ / yeast +++	4.0	30	25.7	98.9%	147.90
XC029	<i>Histoplasma capsulatum</i> +/- yeast +	4.0	35	5.26	59.9%	0.57
XC023	<i>Coccidioides immitis/posadasii</i> +	4.0	35	13	73.6%	1.74

XC024	<i>Coccidioides immitis/posadasii</i> ++	9.5	35	3.64	99.6%	0.28
XC030	<i>Coccidioides immitis/posadasii</i> ++ / Basidiomycota-like sp. +	4.0	35	27.1	73.5%	3.62
MC010	<i>Phaeoacremonium sp.</i> +++	4.0	30	12.3	54.2%	38.79
MC011	<i>Aspergillus fumigatus</i> ++/ <i>Phaeoacremonium sp.</i> +	4.0	30	28.4	98.6%	163.02
XC031	<i>Scedosporium apiospermum</i> +	4.0	35	8.71	25.2%	0.40
Culture-negative						
XX036	bacteria +	9.5	35	3.8	85.6%	0.25
XX039	-	9.5	35	2.32	59.5%	0.11
XX144	-	9.5	35	10.4	80.0%	0.64
XX148	-	9.5	35	5.28	89.2%	0.36
XX155	-	9.5	35	5.07	76.4%	0.30
XX158	-	9.5	35	4.26	97.5%	0.32
XX159	-	9.5	35	4.86	90.4%	0.34
XX160	-	4.0	35	12.3	61.2%	1.37
XX167	-	9.5	35	4.97	94.6%	0.36
XX169	-	4.0	35	19.3	80.1%	2.81
XX165	-	9.5	35	6.06	87.4%	0.41
XX170	-	4.0	35	4.75	88.3%	0.76
XX171	-	4.0	35	7.2	57.8%	0.76
XX178	-	9.5	35	4.86	91.6%	0.34
XX179	-	4.0	35	7.88	67.5%	0.97

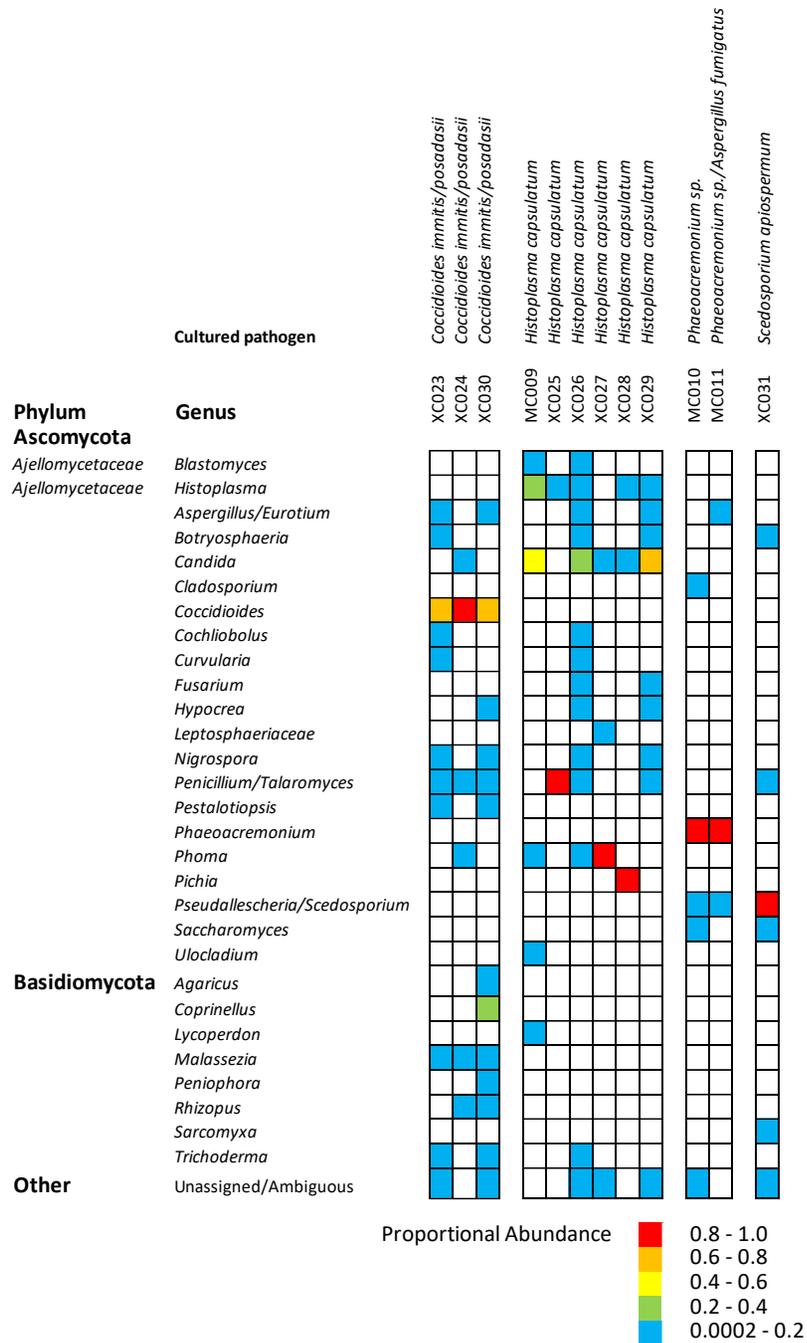
Negative controls

NTC-35	NTC	9.5	35	1	72.6%	0.06
NTC-40	NTC	9.5	40	13	98.1%	0.03
PCRneg-35	PCR negative	9.5	35	0.1	99.8%	0.01
PCRneg-40	PCR negative	9.5	40	2	16.7%	0.0008

Supplementary Table 4. PERMANOVA of Bray-Curtis dissimilarities between fungal communities represented by genus-level OTU abundances in *Blastomyces* M+/C+ BAL specimens, *Blastomyces* M-/C+ BAL specimens, culture-negative BAL specimens and negative controls. *denotes statistical significance.

	df	SS	MS	F	R ²	P
Category	3	3.1748	1.05828	3.8276	0.1868	0.001*
Residuals	50	13.8244	0.27649		0.8132	
Total	53	16.9992			1.0000	

<u>Post-hoc</u>	F	R ²	p	p-adj.
<i>Blastomyces</i> M+/C+ vs. <i>Blastomyces</i> M-/C+	26.132	0.442	0.001	0.006*
<i>Blastomyces</i> M+/C+ vs. Culture-negative	8.306	0.201	0.007	0.042*
<i>Blastomyces</i> M+/C+ vs. Negative controls	10.069	0.314	0.006	0.036*
<i>Blastomyces</i> M-/C+ vs. Culture-negative	2.199	0.073	0.123	0.738
<i>Blastomyces</i> M-/C+ vs. Negative controls	0.270	0.016	0.810	1.000
Culture-negative vs. Negative controls	0.850	0.048	0.388	1.000



Supplementary Figure 1. Relative abundance of fungal genera in BAL specimens that were culture positive for fungi other than *Blastomyces*.