

JVDI: Supplementary material

Becker et al. Identification of fungal isolates by MALDI-TOF mass spectrometry in veterinary practice: validation of a web application

Supplementary Table 1. Details on the PCR assays used for DNA sequencing, and assays used for the identification of each species.

Name of PCR assay	Locus	Primers	Reference
ACT	Actin	ACT-512F/ACT-783R	2
ACT-2	Actin	ACT1F-Dh/ACT1R-Dh	7
BT	Beta-tubulin	BT2a/BT2b	4
BT-2	Beta-tubulin	Ben2f/BT2b	5
CAL	Calmodulin	CL1/CL2A	8
CAL-2	Calmodulin	CF1L/CF4	9
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	GPD1/GPD2	1
ITS	Internal transcribed spacer	ITS4/ITS5	11
LSU	26S rDNA	LR0R/LR5	10
LSU-2	23S rDNA	No name published	3
RPB2	RNA polymerase II subunit	RPB2-5F/RPB2-7cR	6
TEF	Translation elongation factor 1 alpha	EF1-728F/EF1-986R	2
Species	PCR assay for identification of species		
<i>Alternaria hordeiaustralica</i>	ITS, BT, TEF, GAPDH		
<i>Arthrimum marii</i>	ITS, BT, TEF		
<i>Aspergillus flavus</i>	ITS, BT		
<i>Aspergillus fumigatus</i>	ITS, BT		
<i>Aspergillus nidulans</i>	ITS, BT, CAL		
<i>Aspergillus tubingensis</i>	ITS, BT-2, CAL-2		

Species	PCR assay for identification of species
<i>Aspergillus welwitschiae</i>	ITS, BT-2, CAL-2
<i>Beauveria bassiana</i>	ITS, BT
<i>Candida albicans</i>	ITS
<i>Candida ethanolica</i>	ITS
<i>Candida glabrata</i>	ITS
<i>Candida inconspicua</i>	ITS
<i>Candida tropicalis</i>	ITS
<i>Chaetomium cochlioides</i>	ITS, BT
<i>Cladosporium allicinum</i>	ITS, TEF, ACT
<i>Clavispora lusitaniae</i>	ITS
<i>Cutaneotrichosporon smithiae</i>	ITS
<i>Cyberlindnera fabianii</i>	ITS
<i>Cyberlindnera mississippiensis</i>	ITS
<i>Debaryomyces hansenii</i>	ITS, ACT-2
<i>Debaryomyces</i> sp.	ITS, ACT-2
<i>Didymella pomorum</i>	ITS, BT, ACT
<i>Diutina catenulata</i>	ITS
<i>Diutina rugosa</i>	ITS
<i>Donkioporia expansa</i>	ITS
<i>Epicoccum nigrum</i>	ITS, BT
<i>Fusarium cerealis</i>	ITS, TEF
<i>Fusarium incarnatum-equiseti</i>	ITS, TEF
<i>Galactomyces candidus</i>	ITS
<i>Geosmithia</i> sp.	ITS, BT
<i>Gymnoascus reesii</i>	ITS
<i>Holtermanniella</i> sp.	ITS
<i>Kazachstania bovina</i>	ITS
<i>Kazachstania telluris</i>	ITS

Species	PCR assay for identification of species
<i>Kluyveromyces marxianus</i>	ITS
<i>Lichtheimia corymbifera</i>	ITS
<i>Lichtheimia ramosa</i>	ITS
<i>Magnusiomyces capitatus</i>	ITS
<i>Meyerozyma guilliermondii</i>	ITS
<i>Microsporium canis</i>	ITS, BT
<i>Mortierella alpina</i>	ITS
<i>Mucor circinelloides</i>	ITS
<i>Mucor hiemalis</i>	ITS, BT
<i>Nannizzia gypsea</i>	ITS, BT
<i>Mortierella alpina</i>	ITS
<i>Mucor circinelloides</i>	ITS
<i>Mucor hiemalis</i>	ITS, BT
<i>Nannizzia gypsea</i>	ITS, BT
<i>Paecilomyces saturatus</i>	ITS, BT
<i>Paecilomyces variotii</i>	ITS, BT, CAL
<i>Penicillium fellutanum</i>	ITS, BT
<i>Phoma</i> sp.	ITS, BT
<i>Pichia deserticola</i>	LSU
<i>Pichia fermentans</i>	ITS
<i>Pichia kudriavzevii</i>	ITS
<i>Prototheca zopfii</i>	LSU-2
<i>Pseudogymnoascus pannorum</i>	ITS
<i>Pseudogymnoascus</i> sp.	ITS, BT, RPB2
<i>Purpureocillium lilacinum</i>	ITS, BT
<i>Rhizopus microsporus</i>	ITS
<i>Rhizopus stolonifer</i>	LSU
<i>Saccharomyces cerevisiae</i>	ITS

Species	PCR assay for identification of species
<i>Scedosporium boydii</i>	ITS, BT
<i>Sordaria fimicola</i>	ITS, BT
<i>Sordaria lappae</i>	ITS, BT
<i>Talaromyces piceus</i>	ITS, BT
<i>Trichoderma capillare</i>	ITS, TEF
<i>Trichoderma hamatum</i>	ITS, TEF
<i>Trichophyton benhamiae</i>	ITS, TEF
<i>Trichophyton equinum</i>	ITS, TEF
<i>Trichophyton</i> sp.	ITS, TEF, LSU
<i>Wickerhamomyces anomalus</i>	ITS
<i>Yamadazyma mexicana</i>	ITS

Supplementary Table 2. Identification by species of the 290 isolates using either MALDI-TOF MS or classical methods.

Species	MALDI-TOF MS identification				Classical identification			
	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID
<i>Alternaria hordeiaustralica</i> (1), NA		1				1		
<i>Arthrimum marii</i> (1)	1					1		
<i>Aspergillus flavus</i> (1)	1							1
<i>Aspergillus fumigatus</i> (36)	36				35			1
<i>Aspergillus nidulans</i> (1)	1							1
<i>Aspergillus tubingensis</i> (1)	1				1*			
<i>Aspergillus welwitschiae</i> (1)	1				1*			
<i>Beauveria bassiana</i> (3)	3				1		2	

Identification of veterinary fungal isolates by MALDI-TOF MS

Species	MALDI-TOF MS identification				Classical identification			
	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID
<i>Candida albicans</i> (16)	16				14	1		1
<i>Candida ethanolica</i> (4), NA				4		2	1	1
<i>Candida glabrata</i> (10)	10				7			3
<i>Candida inconspicua</i> (4)	4				4			
<i>Candida tropicalis</i> (1)	1							1
<i>Chaetomium cochlioides</i> (2)	2					1	1	
<i>Cladosporium allicinum</i> (1)	1						1	
<i>Clavispora lusitaniae</i> (1)	1						1	
<i>Cutaneotrichosporon smithiae</i> (1), NA				1			1	
<i>Cyberlindnera fabianii</i> (1)	1							1
<i>Cyberlindnera mississippiensis</i> (1), NA				1				1
<i>Debaryomyces hansenii</i> (2)	2				1		1	
<i>Debaryomyces</i> sp. (1), NA				1			1	
<i>Didymella pomorum</i> (1), NA			1				1	
<i>Diutina catenulata</i> (14)	14				12			2
<i>Diutina rugosa</i> (17)	17				14			3
<i>Donkioporia expansa</i> (2), NA				2			2	
<i>Epicoccum nigrum</i> (3)	3				2		1	
<i>Fusarium cerealis</i> (1), NA				1			1	
<i>Fusarium incarnatum-equiseti</i> (1)	1						1	
<i>Galactomyces candidus</i> (11)	11				2			9
<i>Geosmithia</i> sp. (1), NA		1					1	
<i>Gymnoascus reesii</i> (1)	1						1	
<i>Holtermanniella</i> sp. (2), NA			1	1			2	
<i>Kazachstania bovina</i> (1)	1					1		

Identification of veterinary fungal isolates by MALDI-TOF MS

Species	MALDI-TOF MS identification				Classical identification			
	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID
<i>Kazachstania telluris</i> (3)	3				2			1
<i>Kluyveromyces marxianus</i> (2)	2				1			1
<i>Lichtheimia corymbifera</i> (3)	3				2	1		
<i>Lichtheimia ramosa</i> (3), NA				3		2		1
<i>Magnusiomyces capitatus</i> (1)	1				1			
<i>Meyerozyma guilliermondii</i> (2)	2				2			
<i>Microsporium canis</i> (7)	7				3	3	1	
<i>Mortierella alpina</i> (1), NA				1			1	
<i>Mucor circinelloides</i> (12)	11		1			2	2	8
<i>Mucor hiemalis</i> (1)				1		1		
<i>Nannizzia gypsea</i> (1)	1				1			
<i>Paecilomyces saturatus</i> (5)	5							5
<i>Paecilomyces variotii</i> (1)	1				1			
<i>Penicillium fellutanum</i> (1)	1					1		
<i>Phoma</i> sp. (1), NA				1			1	
<i>Pichia deserticola</i> (1), NA				1			1	
<i>Pichia fermentans</i> (45)	45				31			14
<i>Pichia kudriavzevii</i> (26)	26				25			1
<i>Prototheca zopfii</i> (1), NA				1				1
<i>Pseudogymnoascus pannorum</i> (1)	1						1	
<i>Pseudogymnoascus</i> sp. (1), NA		1					1	
<i>Purpureocillium lilacinum</i> (4)	4				1		3	
<i>Rhizopus microsporus</i> (1)	1					1		
<i>Rhizopus stolonifer</i> (1)	1				1			
<i>Saccharomyces cerevisiae</i> (2)	2				2			

Species	MALDI-TOF MS identification				Classical identification			
	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID	Correct at species level	Correct at genus level	Mis-ID at genus level	No ID
<i>Scedosporium boydii</i> (1)	1				1*			
<i>Sordaria fimicola</i> (1)	1						1	
<i>Sordaria lappae</i> (1), NA		1				1		
<i>Talaromyces piceus</i> (2), NA				2				2
<i>Trichoderma capillare</i> (2), NA		1		1				2
<i>Trichoderma hamatum</i> (1)	1					1		
<i>Trichophyton benhamiae</i> (1)	1					1		
<i>Trichophyton equinum</i> (2)	2				2			
<i>Trichophyton</i> sp. (1), NA				1		1		
<i>Wickerhamomyces anomalus</i> (5)	5				4			1
<i>Yamadazyma mexicana</i> (1), NA				1				1
Total	258	5	3	24	174	22	31	63

ID = identification; NA = species not available in MALDI-TOF MS database. Number of isolates in parentheses.

* Morphologic identification at the species complex level.

References

- Berbee ML, et al. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 1999;91:964–977.

2. Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 1999;91:553–556.
3. Ewing A, et al. 16S and 23S plastid rDNA phylogenies of *Prototheca* species and their auxanographic phenotypes. *J Phycol* 2014;50:765–769.
4. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 1995;61:1323–1330.
5. Hubka V, Kolarik M. β -tubulin paralogue *tubC* is frequently misidentified as the *benA* gene in *Aspergillus* section *Nigri* taxonomy: primer specificity testing and taxonomic consequences. *Persoonia* 2012;29:1–10.
6. Liu YJ, et al. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 1999;16:1799–1808.
7. Lopandic K, et al. Molecular characterization of the closely related *Debaryomyces* species: proposition of *D. vindobonensis* sp. nov. from a municipal wastewater treatment plant. *J Gen Appl Microbiol* 2013;59:49–58.
8. O'Donnell K, et al. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* 2000;41:61–78.
9. Peterson SW. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* 2008;100:205–226.
10. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 1990;172:4238–4246.

11. White TJ, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, et al., eds. PCR Protocols: A Guide to Methods and Applications. New York, NY: Academic Press, 1990:315–322.