

Supplementary Materials: Rapid Detection and Identification of Mycotoxigenic Fungi and Mycotoxins in Stored Wheat Grain

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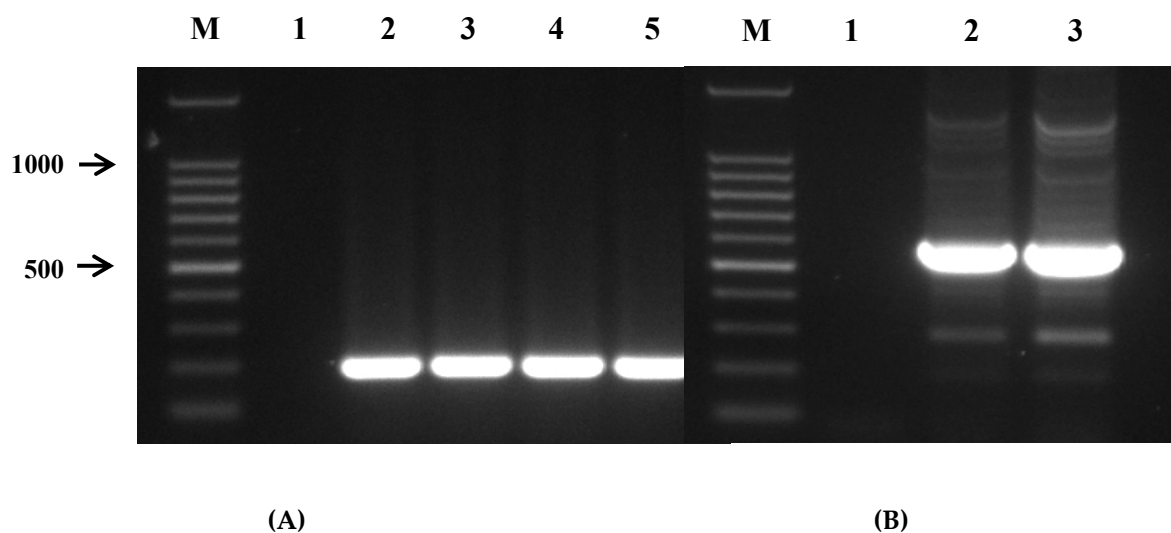


Figure S1. Species specific multiplex PCR assays using DNA isolated from stored wheat grain. (A) Primer set I (*Aspergillus* species-specific primers). Lanes: M – 100bp DNA ladder; 1 – Negative control; 2-5 – wheat grain samples naturally contaminated by *A. flavus*; (B) Primer set II (*Fusarium* species-specific primers). Lanes: 1 – Negative control; 2, 3 – wheat grain samples naturally contaminated by *F. culmorum*.

Table S1. Primer sets used in this study

Set no.	Species	Primer name	Sequence (5'-3')	Size (b/p)	Annealing temperature	References
I	<i>A. fumigatus</i>	PEX1	TATGTCTTCCCCTGCTCC	250	60 °C	Logotheti <i>et al.</i> , 2009 [53]
		PEX2	CTATGCCTGAGGGGCGAA			
	<i>A. flavus</i>	PepO1	CGACGTCTACAAGCCTTCTGGAAA	200		Logotheti <i>et al.</i> , 2009 [53]
		PepO2	CAGCAGACCGTCATTGTTCTTGTC			
	<i>A. parasiticus</i>	PAR1	GTCATGGCCGCCGGGGCGTC	430		Sardinas <i>et al.</i> , 2010 [31]
		PAR2	CCTGGAAAAAATGGTTGTTTTCG			
	<i>A. tubingensis</i>	TUB1	TCGACAGCTATTTCCCCCTT	505		Susca <i>et al.</i> , 2007 [54]
		TUB2	TAGCATGTCATATCACGGGCAT			
	<i>A. carbonarius</i>	CARBO1	AAGCGAATCGATAGTCCACAAGAATAC	371		Perrone <i>et al.</i> , 2004 [55]
		CARBO2	TCTGGCAGAAAGTTAATATCCGGTT			
II	<i>F. graminearum</i>	Fg16F	CTCCGGATATGTTGCGTCAA	400-500	55 °C	Nicholsons <i>et al.</i> , 1998 [56]
		Fg16R	GGTAGGTATCCGACATGGCAA			
	<i>F. culmorum</i>	Fc01F	ATGGTGAACCTCGTCGTGGC	570		Nicholsons <i>et al.</i> , 1998 [56]
		Fc01R	CCCTTCTTACGCCAATCTCG			
	<i>F. poae</i>	Fp82F	CAAGCAAACAGGCTCTTCACC	220		Parry and Nicholsons, 1996 [57]
		Fp82R	TGTTCACCTCAGTGACAGGT			
	<i>F. sporotrichioides</i>	AF330109CF	AAAAGCCCAAATTGCTGATG	332		Demeke <i>et al.</i> , 2005 [58]
		AF330109CR	TGGCATGTTTCATTGTCACCT			
	<i>F. verticillioides</i>	VER1	CTTCCTGCGATGTTTCTCC	578		Mule <i>et al.</i> , 2004 [59]
		VER2	AATTGGCCATTGGTATTATATAC			
III	<i>F. avenaceum</i>	FaF	CAAGCATTGTCGCCACTCTC	920	55 °C	Doohan <i>et al.</i> , 1998 [60]
		FaR	GTTTGGCTCTACCGGGACTG			
	<i>F. solani</i>	FS1	GCAGGTATGGCTTTTGGAA	175		Casasnovas <i>et al.</i> , 2013 [61]
		FS2	AGTAAACTCCGACAGGTGCAA			
	<i>A. niger</i>	An F	GATTTTCGACAGCATTTTCCAGAA	357		Palumbo <i>et al.</i> , 2015 [62]
		An R	GATAAAACCATTTGTTGTCGCGGTCG			
	<i>F. proliferatum</i>	PRO1	CTTTCCGCCAAGTTTCTTC	585		Mule <i>et al.</i> , 2004 [59]
		PRO2	TGTCAGTAACTCGACGTTG			
	<i>F. oxysporum</i>	FOF1	ACATAACCACTGTTGCCTCG	340		Mishra <i>et al.</i> , 2003 [63]
		FOR1	CGCCAATCAATTGAGGAACG			
IV	<i>P. expansum</i>	PE1	AATGTGTACTGACTGGTCGCAG	480	55°C	Dombrink-Kurtzman & McGovern, 2007 [64]
		PE2	CAACCAACATATTCGTGCCTGAC			
	<i>P. digitatum</i>	Pri 207	TAGTCCAAAAACAAATCGTCTGGC	250		Hamamoto <i>et al.</i> , 2000 [65]
		Pri 38c	CACCTGATCTGCCCTGTAAACA			
V	<i>P. paneum</i>	PP1	GAATACACACTGACTGGC	482	60°C	Dombrink-Kurtzman & McGovern, 2007 [64]
		PP2	TCAACCAACACATTCGTACCAGAC			

	<i>P. verrucosum</i>	otanpsF otanpsR	AGTCTTCGCTGGGTGCTTCC CAGCACTTTCCCTCCATCTATCC	750		Bogs <i>et al.</i> , 2006 [66]
	<i>P. roqueforti</i>	ITS183 ITS401	CTGTCTGAAGAATGCAGTCTGAGAAC CCATACGCTCGAGGACCGGAC	300		Pedersen <i>et al.</i> , 1997 [67]
	<i>A. terreus</i>	ATRF81 ATRR120	TACCTTCAAGCCTGACTACG ACCTGCTCGGCCAGTTTGCTG	386		Kanbe <i>et al.</i> , 2002 [68]
Set no.	Mycotoxin	Primer	Sequence (5'-3')	Size	Annealing temperature	References
VI	Aflatoxins	aflR1F	AACCGCATCCACAATCTCAT	798	58°C	Manonmani <i>et al.</i> , 2005 [69]
		aflR1R	AGTGCAGTTCGCTCAGAACA			
		NorF	ACCGCTACGCCGGCGCTCTCGGCAC	397		Priyanka <i>et al.</i> , 2014 [70]
		NorR	GTTGGCCGCCAGCTTCGACACTCCG			
		avf723F	ATGGTCACATACGCCCTCCTCGGG	950		Yu <i>et al.</i> , 2000 [71]
		avf1675R	GCCTCGCATTCTCTCGGCGACCGAA			
VII	Fumonisin/ Trichothecene/ Zearalenone	ver1	GCCGCAGGCCGCGGAGAAAGGTGGT	452	55°C	Skory <i>et al.</i> , 1992 [72]
		ver2	CCGCAGTCAATGGCCATGCAGCG			
		Fum1F	ATTATGGGCATCTTACCTGGAT	798		Ramana <i>et al.</i> , 2011 [11]
		Fum1R	ACGCAAGCTCCTGTGACAGA			
		Fum13F	AGTCGGGGTCAAGAGCTTGT	988		Ramana <i>et al.</i> , 2011 [11]
		Fum13R	TGCTGAGCCGACATCATAATC			
		tri5F	GAGAACTTTCCACCGAATAT	450		Ramana <i>et al.</i> , 2011 [11]
		tri5R	GATAAGGTTCAATGAGCAGAG			
		tri6F	GATCTAAACGACTATGAATCACC	546		Ramana <i>et al.</i> , 2011 [11]
		tri6R	GCCTATAGTGATCTCGCATGT			
VIII	Ochratoxin A	ZEA13F	CATTCTTGGTCTTGTGAGGA	351	58°C	Priyanka <i>et al.</i> , 2015 [8]
		ZEA13R	CCTTATGCTCATCGACATG			
		Aolc35F	GCCAGACCATCGACACTGCATGCTC	536		Priyanka <i>et al.</i> , 2015 [8]
		Aolc12R	CGACTGGCGTTCCAGTACCATGAGC			
		otanpsF	AGTCTTCGCTGGGTGCTTCC	750		Bogs <i>et al.</i> , 2006 [66]
		otanpsR	CAGCACTTTCCCTCCATCTATCC			

Table S2. Fungal isolates used in this study

Standard strains
<i>A. carbonarius</i> NRRL 368
<i>A. flavus</i> NRRL3518
<i>A. fumigatus</i> NRRL 62427
<i>A. niger</i> NRRL 328
<i>A. parasiticus</i> NRRL6111
<i>A. terreus</i> NRRL 269
<i>A. tubingensis</i> NRRL 66281
<i>A. ochraceus</i> NRRL 35018
<i>F. avenaceum</i> NRRL A-28073
<i>F. culmorum</i> NRRL 13320
<i>F. graminearum</i> NRRL 3376
<i>F. poae</i> NRRL 36300
<i>F. proliferatum</i> NRRL 31866
<i>F. roseum</i> NRRL 6469
<i>F. solani</i> NRRL 13416
<i>F. sporotrichioides</i> NRRL3299
<i>F. verticillioides</i> NRRL 25457
<i>P. digitatum</i> NRRL 1202
<i>P. expansum</i> NRRL 976
<i>P. roqueforti</i> NRRL 849
<i>P. verrucosum</i> NRRL 965
<i>P. viridicatum</i> NRRL 5571
Fungal strains isolated from wheat grains
<i>A. flavus</i> SS1
<i>A. flavus</i> SS2
<i>A. fumigatus</i> SS3
<i>F. verticillioides</i> SS4
<i>F. verticillioides</i> SS5
<i>F. culmorum</i> SS6
<i>P. viridicatum</i> SS7
<i>M. ruber</i> SS8