

Article

Detection of Mycotoxigenic Fungi and Residual Mycotoxins in Cannabis Buds Following Gamma Irradiation

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1. Supplementary

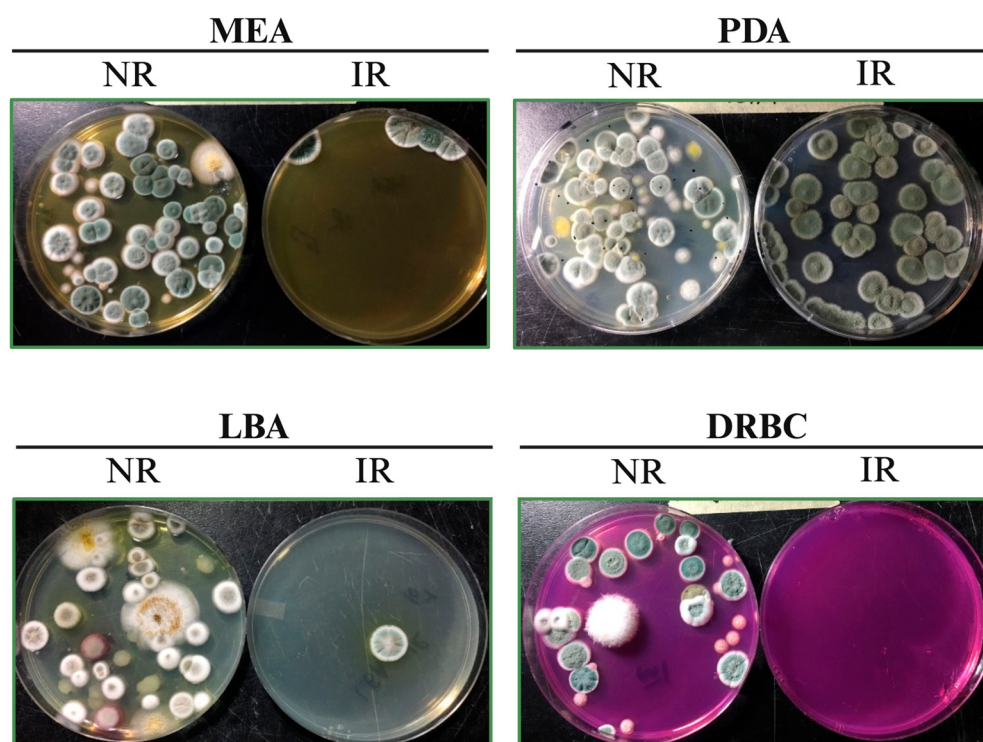


Figure S1. Representative images of fungal growth of irradiated (IR) and non-irradiated (NR) cannabis samples on different media (10^{-3} dilution). MEA = malt extract agar; PDA = potato dextrose agar; LBA = Luria–Bertani agar; DRBC = dichloran–rose Bengal–chloramphenicol agar.

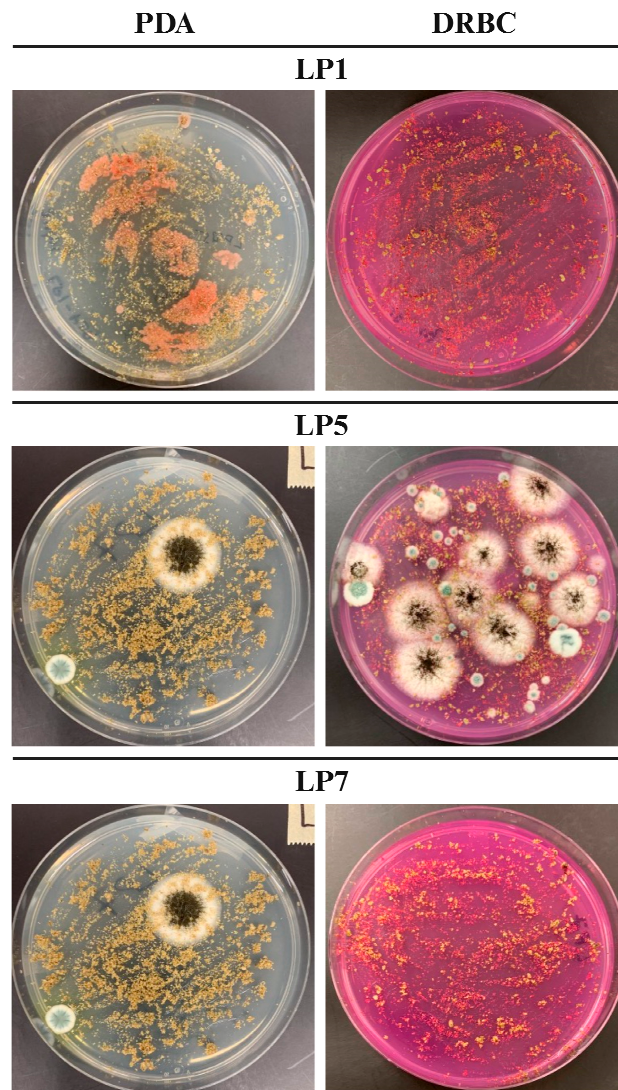


Figure S2. Representative images of fungal growth of licensed produced cannabis samples (LP1, 5, 7) on PDA and DRBC media (direct plating). PDA = potato dextrose agar; DRBC = dichloran–rose Bengal–chloramphenicol agar.

Table S1. CFU counts and microbial taxa isolated from NR, IR, and LP1-10 cannabis samples on selective and non-selective media.

Sample ¹	Media ²	Bacteria CFU ³	Bacteria Species	Fungi CFU ³	Fungi Species
NR	MEA	0	-	5x10 ⁴	<i>A. flavus</i> , <i>A. japonicus</i> , <i>A. ochraceus</i> , <i>A. sclerotiorum</i>
	LBA	2x10 ³	<i>E. hormaechei</i> , <i>P. aeruginosa</i>	2x10 ⁴	<i>A. japonicus</i> , <i>C. oxysporum</i> , <i>A. tamarii</i> , <i>F. proliferatum</i> , <i>P. copticola</i>
	PDA	7x10 ³	<i>E. hormaechei</i> , <i>P. aeruginosa</i>	5x10 ⁴	<i>Penicillium</i> sp., <i>P. citrinum</i>
	DRBC	0	-	3x10 ⁴	<i>P. simplicissimum</i> , <i>A. flavus</i> , <i>A. japonicus</i> , <i>A. ochraceus</i>
	TSA	0	-	0	-
IR	MEA	0	-	4x10 ³	<i>A. flavus</i> , <i>C. tenuissimum</i> , <i>P. commune</i>
	LBA	0	-	1x10 ³	<i>P. copticola</i> , <i>P. citrinum</i> , <i>P. griseofulvum</i>
	PDA	0	-	3x10 ⁴	<i>A. flavus</i> , <i>P. commune</i>
	DRBC	0	-	0	-
	TSA	0	-	0	-
LP1	PDA	2 x10 ³	<i>M. tardum</i> , <i>P. aueruginosa</i>	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP2	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP3	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP4	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP5	PDA	2 x10 ³	<i>Pseudomonas</i> sp.	1.1x10 ⁴	<i>A. niger</i> , <i>A. tamarii</i> , <i>A. tubingensis</i> , <i>A. piperis</i> , <i>A. sydowii</i> , <i>A. awamori</i> , <i>A. chevalieri</i> , <i>C. globosum</i> , <i>P. citrinum</i> , <i>P. commune</i>
	LBA	2 x10 ³	<i>P. aueruginosabacteri</i>	1.2x10 ⁴	<i>A. niger</i> , <i>A. tamarii</i> , <i>A. tubingensis</i> , <i>A. piperis</i> , <i>A. sydowii</i> , <i>A. awamori</i> , <i>A. chevalieri</i> , <i>C. globosum</i> , <i>P. citrinum</i> , <i>P. commune</i>
	DRBC	0	-	8x10 ³	<i>A. niger</i> , <i>P. citrinum</i>
LP6	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP7	PDA	0	-	2 x10 ³	<i>Penicillium citrinum</i> , <i>Aspergillus niger</i>
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP8	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP9	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-
LP10	PDA	0	-	0	-
	LBA	0	-	0	-
	DRBC	0	-	0	-

¹ NR = non-irradiated sample; IR = irradiated sample; LP = licensed producer sample; ² CFU = Colony forming units (CFU.g⁻¹); ³ MEA = Malt extract agar; LBA = Luria-Bertani Agar, PDA = Potato dextrose agar, TSA = Tryptic Soy Agar, DRBC = Dichloran-rose Bengal-chloramphenicol agar.

Table S2. Relative abundance of fungal genera detected by NGS in cannabis samples.

Genus	NR	IR
<i>Penicillium copticola</i>	0.00284	8.99E-05
<i>Penicillium simplicissimum</i>	0.00122	0.00025
<i>Diaporthe caulivora</i>	0.00063	2.40E-05
<i>Conocybe nigrescens</i>	0	0.00032
<i>Penicillium steckii</i>	8.99E-05	0.00019
<i>Penicillium olsonii</i>	8.39E-05	0.0001
<i>Sarocladium strictum</i>	1.20E-05	8.99E-05
<i>Cladosporium sphaerospermum</i>	7.79E-05	3.60E-05
<i>Aspergillus ochraceus</i>	6.59E-05	1.20E-05
<i>Trichoderma hamatum</i>	5.99E-06	6.59E-05
Others	0.99573	0.99883

Table S3. PCR primers designed and used in this study.

Target	Primer	Sequence	Amplicon	References
Aflatoxin	Nor1-F	5'-ACCGCTACGCCGGCACTCTCGG-3'	396	[56]
	Nor1-R	5'-GGCCGCCAGCTTCGACACTCCG-3'		
Trichothecene family	Tri6-F	5'-GATCTAAACGACTATGAATCACC-3'	541	[57]
	Tri6-R	5'-GCCTATAGTGATCTCGCATGT-3'		
Trichodiene	Tri5-F	5'-TCTTAACACTAGCGTGCGCCTTCT-3'	193	[58]
	Tri5-F	5'-CATGCCAACGATGTGTTGGAGGGA-3'		
Deoxynivalenol	Tri13-F	5'-CATCATGAGACTTGTCRAGTTTGGG-3'	282	[59]
	Tri13-F	5'-GCTAGATCGATTGTGCATTGAG-3'		
Ochratoxin	Pks-F	5'-AGTGATGACTGGAGGGAGGTGAAT-3'	199	[58]
	Pks-R	5'-ACGAGCATGCGGTATCAATGGTCA-3'		