METHOD ARTICLE



REVISED Whole genome sequencing of colonies derived from cannabis flowers and the impact of media selection on benchmarking total yeast and mold detection tools [version 2; peer review: 2 approved]

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Abstract

Background:

Cannabis products are subjected to microbial testing for human pathogenic fungi and bacteria. These testing requirements often rely on non-specific colony forming unit (CFU/g) specifications without clarity on which medium, selection or growth times are required. We performed whole genome sequencing to assess the specificity of colony forming units (CFU) derived from three different plating media: Potato Dextrose Agar (PDA), PDA with chloramphenicol and Dichloran Rose Bengal with chloramphenicol (DRBC).

Methods:

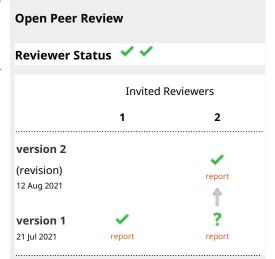
Colonies were isolated from each medium type and their whole genomes sequenced to identify the diversity of microbes present on each medium selection. Fungal Internal Transcribed Spacer (ITS3) and Bacterial 16S RNA(16S) quantitative polymerase chain reactions (qPCR) were performed, to correlate these CFUs with fungi- and bacterialspecific qPCR.

Results:

Each plating medium displayed a ten-fold difference in CFU counts. PDA with chloramphenicol showed the highest diversity and the highest concordance with whole genome sequencing. According to ITS3 and 16S qPCR confirmed with whole genome sequencing, DRBC under counted yeast and mold while PDA without chloramphenicol over counted CFUs due to bacterial growth without selection.

Conclusions:

Colony Forming Unit regulations lack specificity. Each medium



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Any reports and responses or comments on the article can be found at the end of the article.

produces significant differences in CFU counts. These are further dependent on subjective interpretation, failure to culture most microbes, and poor selection between bacteria and fungi. Given the most human pathogenic microbes found on cannabis are endophytes which culture fails to detect, molecular methods offer a solution to this long-standing quantification problem in the cannabis testing field.

Keywords

Cannabis, Total Yeast and Mold, Microbiome, Whole Genome Sequencing, qPCR

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Competing interests: The authors are employees of Medicinal Genomics which manufacture qPCR reagents for Cannabis testing.

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REVISED Amendments from Version 1

We have updated the manuscript to clarify human pathogens and plant pathogens. We have added references to the extensive prior art in the field scrutinizing ITS classification of cannabis microbes per Dr. Punja's suggestions. We have also expanded on the challenges assessing endophytes with culture based methods and how our study was restricted to only those colonies that could culture.

Any further responses from the reviewers can be found at the end of the article

Introduction

Total yeast and mold testing are required in many states to test the safety of cannabis, prior to the sale of cannabis flowers and cannabis-infused products. Cannabis is an inhaled product, and cases of cannabis-transmitted *Aspergillosis* have been reported in the clinical literature (Bal *et al.*, 2010; Gargani *et al.*, 2011; McKernan *et al.*, 2015, 2016; Remington *et al.*, 2015; Ruchlemer *et al.*, 2015). Cannabis is a unique matrix, in that antibiotic cannabinoids can make up to 20% of the flowers' weight, and many fungi infecting cannabis are endophytes. Endophytes are not easily cultured from the plant without lysing open plant cell walls. The conditions which lyse open plant cells walls also lyse open fungal cell walls, thus impacting the viability of the microbes in the lysis and homogenization processes required for testing. Cannabis flowers contain both bacteria and fungi, further complicating fungal quantification for colony forming units (CFU) that lack speciation. Antibiotic selections are often utilized to reduce background bacteria, but many of these antibiotics (e.g. chloramphenicol) inhibit the growth of the most human pathogenic fungi found on cannabis (*Fusarium*, *Pythium* and *Aspergillus*) (Smith & Marchant, 1968; Day *et al.*, 2009; Joseph *et al.*, 2015).

As part of an AOAC Emergency response validation (ERV) in the State of Michigan, we investigated the impact of medium selection on surveying total yeast and mold on cannabis. Cured cannabis flowers were homogenized and tested on 3 different plating media. These media's were chosen as they are referenced in the FDA Bacterial Analytical Manual (BAM) (https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam). These data were compared to ITS3- and 16S-based qPCR and whole genome sequencing. To further complement these cannabis flower samples, organisms were acquired from the American Tissue Culture Collection (ATCC) and plated as pure monocultures on different plating media to confirm the differential growth on each medium.

Methods

Plating

Samples originated from Steadfast Analytical Laboratories (Hazel Park, MI) and were tested independently at a laboratory within the Michigan Coalition of Independent Cannabis Testing Laboratories. Briefly, 10 grams of dried cannabis flowers were sampled from three lots of homogenized cannabis containing high, medium and low quantities of fungal and bacterial CFUs, as measured using culture-based techniques with chloramphenicol selection. 10 grams of homogenized flower were soaked with 90 ml of Tryptic Soy Broth (TSB, Medicinal Genomics #420205) in a filtered Nasco Whirl-Pak bag (#B01385). Samples were homogenized by hand, and then 0.1 mL of solution plated onto three media (DRBC, PDA with chloramphenicol, PDA, at 1:100 dilution). Two additional dilutions were prepared (10 mL into 90 mL) and the same plating protocol was followed. All plates were incubated for 5 days at 25°C.

qPCR

ITS3 qPCR was performed as described in McKernan *et al.* with two modifications. Briefly, 1ml of homogenate from a Whirl-Pak bag was collected and briefly micro-centrifuged to enrich for live organisms. This pellet was resuspended in 200 μ l ddH₂O and lysed with the addition of 12 μ l of Thaumatin-like protein (TLP) and incubated at 37°C for 30 minutes. This enzymatic lysis step (glucanase) ensures more complete lysis of fungal cell walls (Medicinal Genomics part #420206, McKernan *et al.*, 2015, 2016). 12.5 μ l of MGC Lysis buffer was added, vortexed and incubated for 5 minutes at 25°C. Lysed samples were micro-centrifuged and 200 μ l of supernatant was aspirated and added to 250 μ l of Medicinal Genomics binding buffer (MGC part# 420001) for magnetic bead isolation. The samples were incubated with the Medicinal Genomics magnetic bead mixture for 10 minutes, magnetically separated and washed two times with 70% ethanol. The beads were dried at 37°C for 5 minutes to remove excess ethanol and eluted with 25 μ l of ddH₂O. Quantitative PCR was performed using Medicinal Genomics PathoSEEK Total Yeast and Mold detection assay (MGC# 420103) and Medicinal Genomics PathoSEEK Total Aerobic Count Assay (MGC# 420106) according to the manufacturers' instructions on a BioRad CFX96 thermocycler.

DNA isolation from colonies for whole genome sequencing

A total of 45 colonies were picked with a pipette tip and introduced into 200 μ l of ddH₂O with 12.5 μ l of MGC TLP (MGC part #420206). TLP is a glucanase active at 37°C. Samples were digested for 30 minutes at 37°C and 12.5 μ l of

MGC Lysis buffer was added, vortexed and incubated for 5 minutes at 25°C. Lysed sample were micro-centrifuged and 200 μ l of supernatant was aspirated and added to 250 μ l of MGC binding buffer (MGC part # 420001) for magnetic bead isolation. The samples were incubated with the bead mixture for 10 minutes, magnetically separated and washed 2 times with 70% ethanol. The beads were dried at 37°C for 5 minutes to remove excess ethanol and eluted with 25 μ l of ddH₂O.

Library construction for whole genome sequencing. *Fragmentation*

Genomic DNA (gDNA) was quantified with a Qubit (Thermo Fisher Scientific) and normalized to reflect 4-8 ng/µl in 13 µl of TE buffer. Libraries were generated using enzymatic fragmentation with the NEB Ultra II kits (NEB part # E7103). Briefly, 3.5 µl of 5X NEB fragmentation buffer and 1 µl of Ultra II fragmentation enzyme mix are added to 13 µl of DNA. This reaction was tip-mixed 10 times, vortexed, and quickly centrifuged. Fragmentation was performed in a BioRad CFX96 thermocycler at 3.5 minutes at 37°C, 30 minutes at 65°C. The reaction was kept on ice until ready for adaptor ligation.

Adaptor ligation

Component	Volume (µl)
SureSelect Adaptor Oligo Mix (brown cap)	0.75
ddH ₂ O	0.5
Ultra II Ligation Master Mix	15
Ligation enhancer	0.5
Total Volume	16.75

The master mix for ligation was prepared on ice using 0.75 μ l of Agilent SureSelect Adaptor Oligo Mix, 0.5 μ l of ddH₂O, 15 μ l of NEB Ultra II Ligation Master Mix, 0.5 μ l of Ligation enchancer (New England Biolabs) for a total reaction volume of 16.75 μ l.

Ligation was performed by the addition of 16.75 µl of ligation master mix to the 17.5 µl Fragmentation/End Prep DNA reaction mixture, incubate for 15 minutes at 20°C. To purify excess adaptors and adaptor dimers, AMPure XP beads (Beckman Coulter #A63881) were vortexed at room temperature for resuspension and 16 µl (approximately 0.45X) of resuspended AMPure XP beads were added to the ligation reactions. This was well-mixed by pipetting up and down at

Colony Image	Assembly Coverag	e (Y) Large->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Km	ner Classification	TAC Ct TYM
RBC-D-2-1-B_001.fastq	.gz Epicoccum nigru	m Fungi	https://app.onecodex.com/analysis/public/802f842cd0b4427d	Name	Estimated Abundance	34.05 19
		DRBC-E-2-1-B	Assembly contigs	Epicocoum nigrum	Estimated Abundance	
	10000		# contigs (>= 25000 bp) 408	Pseudomonas oryzihabitans	0.87%	
	10000		# contigs (>= 50000 bp) 123	Pantoea aggiomerans	0.78%	
	1000 •	4***	Total length (>= 25000 bp) 19142192	Pantoea ananatis	0.46%	
		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Total length (>= 50000 bp) 9227130	Bacillus velezensis	0.38%	
	100	and a supplify	# contigs 2100	Pseudomonas coleopterorum	0.37%	
	and in the local division of the	and the second se		Pericillum citrinum Fusarium fulkuroi	0.11%	
State of the second state of the	10			Fusarum fujkurol Enterobacter cloacae	0.11%	
			Total length 31,609,610	Atemaria atemata	0.07%	
	1		GC (%) 46.53	= (Remaining)	0.13%	
	0 5	00 1000 1500 2000	N50 31465	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		
BC-D-2-1-C_001.fastq	.gz Penicillium citrin	ium Fungi	https://app.onecodex.com/analysis/public/95cffa015ae04bd3	Name	Estimated Abundance	32.87 15
		DRBC D-2-1-C	Assembly contigs	Penicilium citrinum	Estimated Abundance	
14		DNDC_0-2-1-C	# contigs (>= 25000 bp) 402	Penosuri otonum	0.66%	
	10000		# contigs (>= 50000 bp) 205	 Pseudomonas oryzihabitans 	0.58%	
and the second s	1000		Total length (>= 25000 bp) 24470385	Pantoea aggiomerans	0.38%	
AUVA	1000		Total length (>= 50000 bp) 17528385	Bacillus velezensis	0.34N	
	100	Singer: E. Samples		Pseudomonas coleopterorum	0.20%	
		Concernance and Concernance		Fusarium fujikuroi	0.09%	
ALL	10		Largest contig 237,982	Enterobacter cloacae	0.07%	
			Total length 31,648,269	Alternaria alternata	0.06%	
and the second sec	0	500 1000	GC (%) 46.49	(Remaining)	0.11%	
	U	500 1000	N50 55985			
BC-E-2-1-A_001.fastq.	gz Pseudomonas m	onteilii Bacteria	https://app.onecodex.com/analysis/public/98c255012f1a4611	Name	Estimated Abundance	17.90 N
			Assembly DRBC_E_2		95.84%	
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	10000		# contigs (>= 50000 bp) 35	Partoea ananatis Pseudomonas so. UBA6562	0.95%	
		1.	Total length (>= 0 bp) 6848402			
	1000		Total length (>= 1000 bp) 5899910	Delftia acidovorans	0.71%	
		1		Enterobacter cloacae	0.35%	
	100		Total length (>= 5000 bp) 5790136	Bacilius velezensis	0.23%	
41			# contigs 452	Enterobacteriaceae bacterium UBA		
	10	" or high	Largest contig 540,523	Pantoea aggiomerans	0.18%	
			Total length 6,101,403	Pseudomonas syringae group geno		
	1		GC (%) 61.70	Eusarium fujikuroi	0.07%	
	0	50 100 150	N50 161275	(Remaining)	0.31%	

Figure 1. DRBC with chloramphenicol. Colony Image (Left), Assembly sequence coverage (Y) compared with contig Length (X) where the contigs are sorted largest to smallest from left to right (Mid-Left). Assembly statistics calculated with Quast 5.0 (Mid Right). OneCodex speciated Kmer count (Right).

least 10 times. The mixture was incubated for 5 minutes at 25°C. The PCR plate was placed on an appropriate magnetic stand (Medicinal Genomics #420202) to separate the beads from the supernatant. After the solution was clear (about 5 minutes), the supernatant was carefully removed and discarded. We were careful not to disturb the beads containing target DNAmolecules. The magnetic beads were washed by adding 200 μ l of 70% ethanol to the PCR plate while on the magnetic stand. Followed incubation at room temperature for 30 seconds, and then careful removal and discarding of the supernatant. The ethanol wash was repeated once for a total of 2 washes. Trace amounts of ethanol were removed. The beads were air dried for ~ 7 minutes while the PCR plate was on the magnetic stand with the lid open. The PCR plate was then removed from the magnet and target DNA eluted from the beads into 10 μ l of H₂O, then 9 μ l of cleaned DNA was transferred to a fresh well.

PCR amplification

A volume of 12.5 μ l 2x NEBNext Q5 Hot Start Master Mix (New England Biolabs #M0492S) was added to 9 μ l ligated DNA, then 3.5 μ l of NEB 8bp index primer/universal primer were added to the mix. The reaction ran in a cycling program set at 98°C for 30 seconds as an initial denaturization step; six cycles of denaturation, annealing and extension were performed, cycling between 98°C for 10 seconds and 65°C for 75 seconds. A final 5-minute step at 65°C was performed, with a final 4°C forever step.

Colony Image Assembly Co RBC-E-2-1-B_001.fastq.gz Penicillium	overage (Y) Large->Small Contigs (X) citrinum Fungi	Assembly Statistics (Quast 5.0) https://app.onecodex.com/analysis/public/6f79f809b83048ba	OneCodex Kmer	classification	TAC Ct T 33.80
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100	m	Total length (>= 50000 bp) 9227130	Pseudomonas coleopterorum	0.21%	
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10		Largest contig 171,869	Pusarium fujikuroi	0.09%	
	• •	Total length 31,609,610	Alternaria alternata	0.07%	
1	F00 4000 4500	GC (%) 46.53	Pseudomonas sp. UBA6562 (Remaining)	0.06%	
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100000		# contigs (>= 25000 bp) 188 # contigs (>= 50000 bp) 156	Aspergillus sp. MA 6041 Alternaria arborescens	23.70%	
10000			Pantoea ananatis	2.41%	
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	and the same and the	GC (%) 52.00	Cadosporium Cadosporium	43862 (0.49%)	
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IBC-D-2-2-C 001.fastq.gz Fusarium fu	verage (Y) Large-Small Contigs (X) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 /Pantoea Mix	N50 1232928 Assembly Statistics (Quast 5.0) PMBC_D_2_2_C Assembly Statistics (PMB0450406407 Assembly Statistics (Quast 5.0) Max Jian Sensora contrastic (PMB0450406407 Assembly Statistics (Quast 5.0) # contigs (>= 25000 bp) D122 # contigs (>= 50000 bp) 92 Total length (>= 25000 bp) D1804622 Total length (>= 50000 bp) B347245 # contigs 616 Largest contig 942,550 GC (%) 53.84 N50 257012 Mmsc//issemescles.com/salve/s/s/s/s/s/s201623661 Assembly MscD_2_2_D # contigs (>= 25000 bp) # contigs (>= 50000 bp) 94 # contigs (>= 50000 bp) 94	Control Control Contro Contro Control Control Control Control Co	LEAST 3.000 LEAST 3.000 1227 4.00	32.14
IBC-D-2-2-C 001.fastq.gz Vertiillium	verage (Y) Large-Small Contige (A) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statistics (Quast 5.0) PMBC_D2_22_C Max Jians seconds conclusion (Add (COMB06-COMB06-COMB06-COMB06-COMB06-COMB06-COMB06-D) PDBC_D_2_2_C # contigs (>= 50000 bp) P2 Total length (>= 25000 bp) P3047245 # contigs (>= 50000 bp) P3047245 # contigs (>= 50000 bp) P3047245 # contigs (>= 50000 bp) P347245 # contigs (>= 50000 bp) P347245 # contigs (>= 53.844 N50 257012 Max Jians secolar com/main/main/main/main/main/main/main/mai	Control Control Contro Contro Control Control Control Control Co	EXAULT 2.07% Classification Introd doubles 1277 16788 1678 1678 1678 1	32.14
IBC-D-2-2-C 001.fastq.gz Vertiillium	verage (Y) Large-Small Contigs (X) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 /Pantoea Mix	N50 1232928 Assembly Statistics (Quast 5.0) Thus Unse assess conclusival with COMBING States Assembly DBRC_D_2_2_C # contigs (>= 25000 bp) # contigs (>= 50000 bp) Total length (>= 25000 bp) Total length (>= 50000 bp) # contigs	Conserved	EXAMPLOADS CLASSIFICATION 1274 4075 40	32.14
RBC-D-2-2-C_001.fastq.gz 10000 1000 100 100	verage (Y) Large-Small Contige (A) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statistics (Quast 5.0) Thus //assectax.com/ass/ass/ass/colspan="2">MRBC_D_Z_Z_C Assembly DNBC_D_Z_Z_C # contigs (>= 50000 bp) 112 # contigs (>= 50000 bp) 92 Total length (>= 50000 bp) 18347245 # contigs 616 Largest contig 942,560 Total length (>= 50000 bp) 18347245 # contigs 616 Largest contig 942,560 Total length 19,895,240 GC (%) 5.384 N50 257012 https://mansender.com/nabin/bal/bal/2t/tsi/BA209861 Assembly DREC_D_2_2_D # contigs (>= 25000 bp) 94 # contigs (>= 25000 bp) 88 Total length (>= 25000 bp) 88 Total length (>= 58000 bp) 88 Total length (>= 58000 bp) 88 33392362 Total length (>= 58000 bp) 33392362	Control	12403 3.079 22403 3.079 12403 4.004 1272 1406 140	32.14
10000 1000 1000 1000 1000 1000 1000 10	verage (Y) Large-Small Contige (A) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statistics (Quast 5.0) DRBC_D_22_C # contigs (>= 25000 bp) DI2 # contigs (>= 550000 bp) D92 Total length (>= 25000 bp) D94822 Total length (>= 25000 bp) D1094822 Total length (>= 59000 bp) B37245 # contigs (>= 50000 bp) B37245 # contigs (>= 50000 bp) B37245 # contigs (>= 50000 bp) B37245 MS0 257012 Mssebbb (bp) D8C_D_Z_D # contigs (>= 25000 bp) B8 Total length (>= 25000 bp) B4 Total length (>= 25000 bp) B332936 # contigs (>= 25000 bp) B33607324 Total length (>= 55000 bp) 3332936 # contigs (>= 4000 bp)	Conserved C	2003 3.000 2005 200 2005 200 200	32.14
RBC-D-2-2-C_001.fastq.gz 10000 1000 100 100 100 100 100	verage (Y) Large-Small Contige (A) jijkurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statistics (Quast 5.0) Mission Colspan="2">Mission Colspan="2">Mission Colspan="2">Mission Colspan="2" Assembly DRBC_D_Z_C # contigs (>= 50000 bp) 12 # contigs (>= 50000 bp) 12 Total length (>= 25000 bp) 10904822 Total length (>= 50000 bp) 18347245 # contigs 616 # contigs 942,560 Total length 19,895,240 GC (%) 53.84 N50 257012 Mms://ms:metodex.com/nat/sit/s/df/251920841 Assembly DBEC_D_2_2_D # contigs (>= 25000 bp) 94 # contigs (>= 50000 bp) 88 Total length (>= 50000 bp) 33607324 Total length (>= 50000 bp) 3332303 # contigs 403 Largest contig 1,666,949 Total length 34,991,419	Control	24403 (2009) Classification 1623 1623 1623 1623 16266 1626 1626 1626 1626 1626 1626	32.14
IBC-D-2-2-C 001.fastq.gz Fusarium fu	verage (Y) Large-Small Contige (X) jijikurol Fungl DRBC-D-2-2-C 9 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statics(Quast 5.0) Thus Unse sectors cont / washing/Addit/Controls(Controls) Assembly DBRC_D_Z_Z_C # contigs (>= 50000 bp) # contigs (>= 50000 bp) # contigs (>= 50000 bp) Total length (>= 50000 bp) Total length (>= 50000 bp) Iargest contig 942,560 Total length (>= 50000 bp) Total length (>= 50000 bp) 05/60 Contigs (>= 50000 bp) 942,560 Total length (>= 50000 bp) M50 Z57012 Thms://secondeck.com/ms/s/s/s/cl/?cl/Scl/Scl/Scl Assembly # contigs (>= 250000 bp) # contigs (>= 250000 bp) # contigs (>= 250000 bp) # contigs (>= 50000 bp) # contigs (>	Conserved	244313.0395 CLASSIFICATION 1227 443 454 454 454 454 454 454 45	32.14
BC-D-2-2-C 001.fastq.gz Versitian fu 10000 100 100 100 100 100 100	verage (Y) Large-Small Contige (X) jijikurol Fungl DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D	N50 1232928 Assembly Statistics (Quast 5.0) Mission Colspan="2">Mission Colspan="2">Mission Colspan="2">Mission Colspan="2" Assembly DRBC_D_Z_C # contigs (>= 50000 bp) 12 # contigs (>= 50000 bp) 12 Total length (>= 25000 bp) 10904822 Total length (>= 50000 bp) 18347245 # contigs 616 # contigs 942,560 Total length 19,895,240 GC (%) 53.84 N50 257012 Mms://ms:metodex.com/nat/sit/s/df/251920841 Assembly DBEC_D_2_2_D # contigs (>= 25000 bp) 94 # contigs (>= 50000 bp) 88 Total length (>= 50000 bp) 33607324 Total length (>= 50000 bp) 3332303 # contigs 403 Largest contig 1,666,949 Total length 34,991,419	Control	24403 (2009) Classification 1623 1623 1623 1623 16266 1626 1626 1626 1626 1626 1626	32.14
BC-D-2-2-C 001.fastq.qz Verticilium fu 10000 1000 100 100 100 100 100	Verage (*) Large-Small Contige (2) jijikuról Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantosa DRBC_D-2-2-D //Pantosa 50 300 150 200 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //macket.com/wasking/doc/OND004555640 Assembly DRBC_D_Z_Z_C # contigs (>= 25000 bp) # contigs (>= 25000 bp) Total length (>= 25000 bp) Idage for the state of	Conserved	2003 (2009)	32.31
IBC-D-2-2-C 001.fastq.gz Verticilium fu 10000 1000 100 100 100 100 100	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //assecodes.com/saylow/dbf/cdf00000305668 Assembly DBC_D_Z_C # contigs (>= 25000 bp) # contigs (>= 50000 bp) 92 Total length (>= 25000 bp) Total length (>= 50000 bp) 10347245 # contigs 616 Largest contig 942,550 Total length Total length 95,844 N50 257012 Thus //ssecretex.com/saylow/bdf/2/ct305035061 Assembly No Total length Total length # contigs (>= 25000 bp) 94 Contigs (>= 25000 bp) 94 # contigs (>= 25000 bp) 94 # contigs (>= 25000 bp) 94 # contigs (>= 25000 bp) 80 Total length (>= 25000 bp) 33607324 Total length (>= 25000 bp) 33203206 # contigs (> 600000 bp) 3360732	demongi	12403 (2009) Classification 127 628 629 7107 (174) 7102 (2014)	32.14
RBC-D-2-2-C_001.fastq.qz Fusarium fu 10000 1000 1001 1000 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 1000 100 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 100 10 <	Verage (*) Large-Small Contige (2) jijikuról Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantosa DRBC_D-2-2-D //Pantosa 50 300 150 200 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //ran seconds.com/analysin/dist/com/dist	Control	12003 (2003) 2128 (2004) 1218	32.31
RBC-D-2-2-C 001.fastq.qz 10000 100 100 100 100 100 100	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //ms excedex.com/us/pion/MSRC/000001355569 Assembly Statistics (Quast 5.0) #c contigs (>= 25000 bp) # contigs (>= 50000 bp) 92 Total length (>= 25000 bp) 101 # contigs (>= 50000 bp) 92 Total length (>= 52600 bp) 102 # contigs # contigs <t< td=""><td>Conception Conception Conception</td><td>LIAU 3.079) LIAU 3.079 LIAU 4.070 1.070</td><td>32.31</td></t<>	Conception	LIAU 3.079) LIAU 3.079 LIAU 4.070 1.070	32.31
RBC-D-2-2-C O01.fastq.qz Fusarium fu 10000 1000 1000 1000 100 1000 1000 100 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 100 1000 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100<	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //seasesdex.com/angload/shift(combined/c	denarous Anno control of the second	12433 (2009) Classification 1223 1243 1243 125 125 126 127 128	32.31
RBC-D-2-2-C O01.fastq.gz Fusarium fu 10000 1000 1000 1	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statics (Quast 5.0) Thus //assecode.com/saylow/add/cdf0000000000000000000000000000000000	Conception	12003 (2009) 12003 (2009)	32.31
RBC-D-2-2-C O01.fastq.qz Fusarium fu 10000 1000 1000 1000 100 1000 1000 100 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 1000 100 1000 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100<	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //seasesdex.com/angload/shift(combined/c	demandi Description Control Contro Control Control Control	2403 3.000 3 Classification 220 3 0 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	32.31
RBC-D-2-2-C O01.fastq.gz Fusarium fu 10000 1000 1000 1	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statics (Quast 5.0) Thus //assecode.com/saylow/add/cdf0000000000000000000000000000000000	Conception	12003 (2009) 12003 (2009)	32.31
IBC-D-2-2-C O01.fastq.gz Fusarium fx 10000 1000 1000 1000 10 1 0 10000 10000 1	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //macmacdax.com/us/us/mdk/cd00000555564 Assembly DBC_D_Z_C # contigs (>= 25000 bp) # contigs (>= 50000 bp) Total length (>= 25000 bp) Iargest contig # contigs #	Control	12403 (2.009) 12403 (2.009)	32.31
IBC-D-2-2-C O01.fastq.qz Fusarium fu 10000 1000 1000 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 10000 1000 1000 10000 1000 1000 10000 1000 1000 10000 1000 1000	Verage (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //seasesdes.com/earbing/def/COMDB0C100000100000000000000000000000000000	denancy Anno Alexandro	12433 (2003) 21285 (2010) 1213 1213 1213 1213 1213 1213 1213 1213 1214 1215 1217 <t< td=""><td>32.31</td></t<>	32.31
IBC-D-2-2-C O01.fastq.gz Fusarium fx 10000 1000 1000 1000 10 1 0 10000 10000 1	Verace (Y) Large-Small Contige (X) jijikurol Fungi DRBC-D-2-2-C 50 100 150 200 250 300 //Pantoea Mix DRBC_D-2-2-D 500 1000 1500 500 1000 1500	N50 1232928 Assembly Statistics (Quast 5.0) Thus //macsecks.com/us/us/md/statistics(CMB080535568 Assembly DBC_D_Z_C # contigs (>= 25000 bp) # contigs (>= 50000 bp) Base (>) # contigs (>= 50000 bp) C0 (%) 5.844 N50 Z57012 Mbs: (>= 50000 bp) Mbs: (>= 50000 bp) # contigs (>= 50000 bp) MS0 # c	Control	12403 (2.009) 1212 (2.000) 1212 (2.000) 1212 (2.000) 1212 (2.000) 1213 (2.000)	32.31

Figure 1. (continued)

Colony Image	Assembly Coverage (Y) Large	->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Kmer Cla	assification	TAC Ct	TYM C
RBC-D-2-2-F_001.fastq	.gz Alternaria alternata	Fungi	https://app.onecodex.com/analysis/public/a4efbcf564a04f36	Name Estima	led Abundance	33.15	18.6
and the second s		and the second se	Assembly contigs	Aitemaria alternata	97.93%		
ALC: NO.	DRBC-D	-2-2-F	# contigs (>= 25000 bp) 289	Pantosa ananatis	0.59%		
A CONTRACTOR OF CONTRACTOR	10000		# contigs (>= 50000 bp) 209	Pseudomonas oryzihabitans	0.09%		
State of Contract			Total length (>= 25000 bp) 32113182	Bacillus velezensis	0.26%		
	1000		Total length (>= 50000 bp) 29147302	Pantoea aggiomerans	0.23N		
	100	16. 10. 10 Same		Pseudomonas coleopterorum	0,19%		
	100	and a second sec	# contigs 645	Fusarium fujikuroi	0.08%		
All the second second second	10	1.6.1	Largest contig 602,840	Enterobacter cloacae	0.07%		
and state and states	Sec. 1	316	Total length 33,720,522	Penicilium obrinum Ustlago maydis	0.07%		
THE STREET	1		GC (%) 50.96	(Remained)	0.04%		
Annual Street of	0 100 200	300 400 500	N50 150893	- twinteng	0.00%		
BC-E-2-2-A_001.fastq		Fungi	https://app.onecodex.com/analysis/public/637a9d48029744da	Name Estima	ted Abundance	31.48	20.4
	DRBC-E-	2-2-A	Assembly DRBC_E_2_2_A	Fusarium fujikuroi	84.87%		
11	100000		# contigs (>= 25000 bp) 107	Fusarium proliferatum	5.96N		
			# contigs (>= 50000 bp) 98	Fusarium mangiferae	1.48%		
	10000		Total length (>= 25000 bp) 43700655	Eusarium oxysporum	1.09%		
	1000		Total length (>= 50000 bp) 43384462	Fusarium agapanthi	1.01%		
and the second se	100	Sec. 1	# contigs 2430	Pantoea aggiomerans Fusarium commune	0.90%		
And and the second second	100	Same Store	Largest contig 1,740,874	Fusarium commune Fusarium nygamai	0.69%		
	10	STATISTICS .	Total length 45,935,783	Pseudomonas montelli	0.63%		
			GC (%) 47.74	Pantoea ananatis	0.59%		
	0 100 20	0 300 400	N50 627582	III (Remaining)	1.91%		
BC-E-2-2-B_001.fastq	.gz Pantoea/Fusarium	Mix	https://app.onecodex.com/analysis/public/2b9786e853ca4b5c Assembly DRBC_E_2_2_B	Name Readcount (% of c	30689 (2.14%)	31.03	21.
	DRBC-E	2.2.B	# contigs (>= 25000 bp) 111	Penicilium citrinum	24098 (1.68%)		
A CONTRACTOR OF	10000		# contigs (>= 50000 bp) 95	 Ustilago maydis 	11310 (0.79%)		
	10000		Total length (>= 25000 bp) 40404941	Epicoccum nignum	10249 (0.72%)		
	1000		Total length (>= 50000 bp) 39815152	 Pseudomonas oryzihabitans 	9195 (0.64%)		
	100		# contigs 353	III Trichoderma harzianum	9181 (0.64%)		
State of the second sec	100	1111 C C C C C C C C C C C C C C C C C		Pantoea aggiomerans	9137 (0.64%)		
	10	all and the	Largest contig 2,203,702	Pseudomonas coleopteronum	8823 (0.62%)		
			Total length 40,669,557	Pseudomonas sp. UBA6562 Abrenaria abrenata	4474 (0.31%)		
	1 50	100 150	GC (%) 46.87	Aternaria alternata Bemaining1	4437 (0.31%) 36689 (2.56%)		
	0 50	100 120	N50 615580				
Colony Image	Assembly Coverage (Y) Large		Assembly Statistics (Quast 5.0)	OneCodex Kmer Cl	assification	TAC Ct	-
BC-E-2-2-C_001.fastq	.gz Fusarium fujikuroi	Fungi	https://app.onecodex.com/analysis/public/e0d96b04c56f4de9	Name Estimated	Abundance	32.14	16.
	DRBC-E	-2-2-C	Assembly DRBC_E_2_2_C	Fusarium fujikuroi	87.23%		
	10000		# contigs (>= 25000 bp) 102	E Fusarium proliferatum	6.02%		
			# contigs (>= 50000 bp) 98	Fusarium mangiferae	1.64%		
	1000	1 Cast	Total length (>= 25000 bp) 43604791	Fusarium oxysporum	0.85%		
			Total length (>= 50000 bp) 43437963	Fusarium agapanthi	0.79%		
The second	100	and the second	# contigs 410	Fusarium commune	0.70%		
10 marsh 1	10	South Milling	Largest contig 1,738,365	Fusarium nygamai Pantoea ananatis	0.60%		
	10	112	Total length 44,311,822	Eusatium verticilioides	0.57%		
	1		GC (%) 48.14	Pseudomonas oryzihabitans	0.46%		
	0 50 100	150 200 250	N50 630437	(Remaining)	0.88%		

Figure 1. (continued)

Step	Temp	Time	Cycle
Initial denaturation	98°C	30 sec	1
Denaturation Annealing/Extension	98°C 65°C	10 sec 75 sec	6
Final extension	65°C	5 min	1
Hold	4°C	forever	1

PCR reaction cleanup

AMPure XP beads were resuspended at room temperature with a brief vortex. A volume of 15 μ l of resuspended AMPure XP beads was added to the PCR reactions (~ 25 μ l). To mix well, we pipetted up and down at least 10 times. The mixture was incubated for 5 minutes at room temperature. The PCR plate was put on an appropriate magnetic stand to separate the beads from the supernatant. After the solution was clear (about 5 minutes), the supernatant was carefully removed and discarded. We were careful not to disturb the beads containing the target DNA. A volume of 200 μ l of 70% ethanol was added to the PCR plate while on the magnetic stand. The mix was incubated at room temperature for 30 seconds, and then the supernatant was carefully removed and discarded. The ethanol wash was repeated once more. The beads were air dried fof 7 minutes while the PCR plate was on the magnetic stand with the lid open. The target DNA molecules were eluted from the beads into 15 μ l of nuclease-free H₂O, and 15 μ l were transferred into a fresh well.

Sample quality control

Libraries were evaluated on an Agilent Tape Station prior to pooling for Illumina sequencing. Sequencing was performed by GeneWiz, Cambridge MA. A total of 473 million paired reads $(2 \times 150$ bp) were generated, averaging over 10 million read pairs per sample and a total sequence of 141Gb.

Analysis

Fastq files were uploaded to OneCodex) for Kmer analysis and Simpson's diversity index analysis for each genome (*Extended data:* Supplementary Table 1, sheet Summary https://doi.org/10.5281/zenodo.4759883). Reads were also

Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Kmer Classifica		TAC Ct TY
C-E-2-2-A_001.fastq.gz	Pseudomonas oryzihabitans Bacteria	https://app.onecodex.com/analysis/public/4215767e704f4341	Name	Estimated Abundance	18.27
	PC-E-2-2-A	Assembly PC_E_2_2_4		52,65%	
	10000	# contigs (>= 25000 bp) 18	Pseudomonas montelia	28.96%	
		# contigs (>= 50000 bp) 17	 Pseudomonas putida 	12.16%	
	1000 • • • •	Total length (>= 25000 bp) 4902243	Escherichia coli	3.45%	
	seture and an article	Total length (>= 50000 bp) 4864257	Pantoea ananatis	0.94%	
	100	# contigs 1892	Pseudomonas coleopterorum	0.42%	
	State State State	Largest contig 676,208	 Pantoea aggiomerans 	0.29%	
	10		Bacillus velezensis Pseudomonas syringae	0.28%	
		Total length 6,184,687	Fiscionicias synngse	0.12%	
	1	GC (%) 62.85	Remaining)	0.12%	
	0 50 100 150	N50 331094	in perang	0.43%	
-E-2-2-B_001.fastq.gz	Pseudomonas putida Bacteria	https://app.onecodex.com/analysis/public/a903ec0941a6466c	Name Pseudomonas putida	Estimated Abundance 38.10%	17.04 1
	PC-E-2-2-B	Assembly PC_E_2_2_E	Pseudomonas pubda Pseudomonas psychrotolerans	37.20%	
	10000	# contigs (>= 25000 bp) 26	Pseudomonas psychower ans Pseudomonas cryzihabitans	19.93%	
		# contigs (>= 50000 bp) 22	Pseudomonas coleopterorum	3.19%	
	1000	Total length (>= 25000 bp) 5144265	Pantoea azziomerans	0.54%	
		Total length (>= 50000 bp) 4994836	Pantoes aggiomerans Pseudomonas sp. UBA6562	0.42%	
	100	# contigs 193	Bacilus velezensis	0.42%	
	10	Largest contig 741,966	Bacinus venezensis	0.14%	
	* •	Total length 5,420,450	Pantoea ananatis	0.09%	
	1		Pantoea anavatis Kosakonia cowanii	0.09%	
	0 20 40 60		Rosakona cowanii	0.08%	
No. of Concession, Name of Conce	20 40 80	N50 296382	(stumming)	9.27%	
-E-2-2-C_001.fastq.gz	Pseudomonas coleopterorum Bacteria	https://app.onecodex.com/analysis/public/fd9dca39c4df4897	Name	Estimated Abundance	14.41
		Assembly PC_E_2_2_0		62.3%	14.41
	PC-E-2-2-C		Pseudomonas oryzihabitans	37.06%	
	10000	" contrago (." motor op/	Pseudomonas cannabina	0.17%	
		# contigs (>= 50000 bp) 17	Pseudomonas sp. UBA6562	0.16%	
	1000	Total length (>= 25000 bp) 4541770	Pantoea aggiomerans	0.15%	
	100	Total length (>= 50000 bp) 4453469	Enterobacter cloacae	0.03%	
	100	# contigs 152	Bacillus velezensis	0.03%	
	10	Largest contig 703,285	Eusarium fujikuroi	0.01%	
		Total length 4 688 673	Penicilium obrinum	0.01%	
	1	Total length 4,688,673	III Alternaria alternata	0.01%	
	1 0 10 20 30 40	GC (%) 62.04			
Coloru Imago	0 10 20 30 40	GC (%) 62.04 N50 304475	III Atemaria alternata IIII (Remaining)	0.01%	TACC
Colony Image	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X)	GC (%) 62.04 N50 304475 Assembly Statistics (Quast 5.0)	Atemaria alternaria OneCodex Kmer Classifica	0.07% 0.07%	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria	GC (%) 62,04 N50 304475 Assembly Statistics (Quast 5.0) https://apo.onecodex.com/ana/bii/Sobiic/Befb972204564eb	Atenaria atenata (Remaring) OneCodex Kmer Classifica Name	0.07% 0.07% Ition Estimated Abundance	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X)	GC (%) 62.04 N50 304475 Assembly Statistics (Quast 5.0) https://spo.onecodex.com/analysis/public/Rid97200868ecb Assembly PC_E_2_2_C	Atenaria altenata Demaining OneCodex Kmer Classifica Name Previornona florreicens	021% 021% ttion Estimated Abundance 87.50%	
Colony Image -E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria	$ \begin{array}{c} {\rm GC} (\sc sc s$	Atenura attenuta Denaring OneCodex Kmer Classifica Neme Pasdomnus fuencans Pasdomnus optikataus	0.07% 0.07% ttion Estimated Abundance 87.56%	
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	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria PC-E-2-2-D	GC (%) 62.04 NS0 304475 Assembly Statistics (Quast 5.0) htms://saconcode.com/ashoh/addir/kfb/220484ch Assembly Y PC_E_2_2_1 # contigs (>= 25000 bp) 13 # contigs (>= 50000 bp) 12 Total length (>= 25000 bp) 17/180	Atanara atemata Denairaig OneCodex Kmer Classifica Name Prodomosa gyabatas Prodomosa gyabatas Prodomosa gyabatas	00% 00% Estimated Abundance D55% 55% 6.5%	
	0 10 20 30 40 Assembly Coverage (Y) Large-small Contigs (X) Pseudomonas fluorescens Bacteria PC-E-2-20 10000 1000	GC (%) 62,04 NS0 304475 Assembly Statistics (Quast 5.0) Inter/Japa encode: con/analysiz/add/cft/37/2016484 Assembly (> 25000 bp) # contigs (>= 25000 bp) 13	Alemaia demaia (humaning (human	00% 00% Estimated Abundance 20% 0.5% 0.2%	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens PC-E-2-2-D 10000	GC (%) 62.04 NS0 304475 Assembly Statistics (Quast 5.0) htms://saconcode.com/ashoh/addir/kfb/220484ch Assembly Y PC_E_2_2_1 # contigs (>= 25000 bp) 13 # contigs (>= 50000 bp) 12 Total length (>= 25000 bp) 17/180	Annua Jannua Jenarna Jenarna Jenarna OneCodex Kmer Classifica Nate Prodomnas foreans Prodomnas Prodom	0.01% 0.01% Extinated Abundance 17.54% 6.25% 6.25% 6.05%	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria PC-E-2-20 10000 1000	$ \begin{array}{c} {\rm GC} \left({{\rm Y}_{0}} \right) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 304475} \\ \hline \\ {\rm Assembly Statistics} \left({{\rm Quast}\; 5.0} \right) \\ {\rm Intra //ana creations containst //and creations //ana creat$	Alemaia demaia (humaning (human	00% 00% Estimated Abundance 20% 0.5% 0.2%	
	0 10 20 30 40 Assembly Coverage (Y) Large-small Contigs (X) Pseudomonas fluorescens Bacteria PC-E-2-20 10000 1000	$ \begin{array}{c} {\rm GC} \left({{^{\circ}}{\rm{Ge}}} \right) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 394475} \\ \hline \\ {\rm Assembly Statistics} \left({\rm Quast 5.0} \right) \\ {\rm true //ass encodes cont analysized (child T2000 Her)} \\ {\rm Assembly } & {\rm PC}_{\rm E}, {\rm Z}, {\rm 20} \\ {\rm fcontigs} \left({\rm >= 25000 \ bp} \right) & {\rm 13} \\ {\rm fcontigs} \left({\rm >= 25000 \ bp} \right) & {\rm 13} \\ {\rm fcontigs} \left({\rm >= 25000 \ bp} \right) & {\rm 4877180} \\ {\rm Total length} \left({\rm >= 58000 \ bp} \right) & {\rm 487743} \\ {\rm fcontigs} & {\rm contigs} & {\rm 257, 974} \\ \\ {\rm Largest contigs} & {\rm 927, 974} \end{array} $	Annua Jannua (marka) Brenna (marka) Brenna (marka) Brenna (marka) Brenna (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Brenna (marka) Bre	02% 02% tion 23% 03% 02% 02% 02% 00%	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria PC-E-2-20 10000 1000	$ \begin{array}{c} {\rm GC} \ (\mbox{$\%$)$} & 62, 04 \\ {\rm NS0} & 304475 \\ \hline \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm Ittes} / Jean accodes cond analysis/addit/64/72048ets \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm Ittes} / Jean accodes cond analysis/addit/64/72048ets \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm Ittes} / Jean accodes conducted analysis (P_{\rm C}, Z_2, Z_1, Z_2, Z_2, Z_3, Z_3, Z_3, Z_3, Z_3, Z_3, Z_3, Z_3$	Annua Jamua (Jamua) Conceloadex Kmer Classifica Ever Podomora foreces Podomora (Solos) Podomora (Solos) Podomora (Solos) Podomora (Solos) Podomora (Solos) Podomora (Solos) Evendano (Solos) E	Drive Drive ttion Drivedawe Drivedaw	
	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria 00000 PC-E-2-2-0 00000 0 1000 • • • • • • • • • • • • • • • • • • •	$ \begin{array}{c} {\rm GC} \left({^{\circ}}_{0} \right) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 394475} \\ \hline \\ \hline \\ {\rm MSembly Statistics} \left({\rm Quast 5.0} \right) \\ {\rm true //seconds cont anisot / select / {\rm AS2} \\ {\rm Assembly Statistics} \left({\rm Quast 5.0} \right) \\ \hline \\ {\rm frue //seconds cont anisot / select / {\rm AS2} \\ {\rm frue //seconds cont anisot / select / {\rm AS2} \\ {\rm frue //seconds cont anisot / {\rm assembly PC} \\ {\rm frue //second$	Annua Jannua (marka) Brenna (marka) Brenna (marka) Brenna (marka) Brenna (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Prodomnas (marka) Brenna (marka) Bre	02% 02% tion 23% 03% 02% 02% 02% 02%	
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-E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large-Stmall Contrigs (X) Pseudomonas fluorescens Bacteria Bacteria 10000 PC-E-2-2-D 0 0 1000 • • • 10000 • • • • 1001 • • • • 100 • • • • 10 • • • • 10 • • • • 10 • • • • 10 • • • •	$ \begin{array}{c} {\rm GC} \left({\rm ^{(b)}} \right) & {\rm 62.04} \\ {\rm NS0} & {\rm 304475} \\ \hline \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm Htms://secondsr.cor/archiv/second$	Annual Jennual Perunang OncCodex Kmer Classifica Rea Prodomas functions Prodomas ophilosis Prodomas ophilo	01% 02% 51% 53% 63% 62% 62% 60% 60%	15.68
E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large-stmall Contigs (X) Pseudomonas fluorescens Bacteria 10000 - - - 10000 - - - 1000 - - - 100 - - - 1 0 100 200 300	$ \begin{array}{c} {\rm GC} \left({\rm 'b} \right) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 304475} \\ \hline \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm https://spacence/score/analyst/self-cfff2720345eb \\ {\rm Assembly } & {\rm PC}_{\rm C} \sum_{2,2} T \\ {\rm \# contigs} \left({\rm lse} 25080 \ {\rm bp} \right) & {\rm 13} \\ {\rm \# contigs} \left({\rm lse} 25080 \ {\rm bp} \right) & {\rm 13} \\ {\rm \# contigs} \left({\rm lse} 25080 \ {\rm bp} \right) & {\rm 4877180} \\ {\rm Total length} \left({\rm lse} 25080 \ {\rm bp} \right) & {\rm 4877180} \\ {\rm Total length} \left({\rm lse} 25080 \ {\rm bp} \right) & {\rm 487743} \\ {\rm \# contigs} & {\rm 157} \\ {\rm Largest contig} & {\rm 27, 974} \\ {\rm Total length} & {\rm 4, 967, 292} \\ {\rm GC} \left({\rm 'b} \right) & {\rm 65, 40} \\ {\rm NS0} & {\rm 524142} \\ \\ {\rm https://spacenceder.com/ashpit/belc/belcSt054belta} \\ \end{array} $	Annual Janual Money Janual Money January OneCodex Kmer Classifica Predmark Denses PredmarkDenses PredmarkDenses PredmarkDenses	0/% 0/% 1000 100	15.68
E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas fluorescens Bacteria 10000 - - 10000 - - 100 - - 100 - - 10 - - 10 - - 10 - - 10 - - 10 - - 10 - - 10 - - 10 - - 10 - - 100 200 300	$ \begin{array}{c} {\rm GC} \left({\rm \%} \right) & {\rm 62.04} \\ {\rm NS0} & {\rm 304475} \\ \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm Https://sea.concole.com/achain/s/addir/db/0720344esh \\ {\rm Assembly } & {\rm PC}_{\rm C} 2.2 L \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 13} \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 13} \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm \# \ contigs} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Total \ length} \ ({\rm sea.25000 \ bp}) & {\rm 4837473} \\ {\rm Https://spa.oncodes.com/ansign/spa.24142} \\ \\ {\rm Https://spa.oncodes.com/ansign/spa.24142} \\ \\ {\rm Https://spa.oncodes.com/ansign/spa.24142} \\ \\ {\rm Https://spa.oncodes.com/ansign/spa.24142} \\ \\ {\rm Https://spa.000000000000000000000000000000000000$	Arrenz Jamesa Brenzeg DoneCodex Kmer Classifica Real Predmars forease Predmars polations Predmars (States) Predmars (St	01% 01% 100 Extinued Alumbars 175 175 175 175 175 175 175 175	
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E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large-Small Contings (X) Bacteria Bacteria 10000 PC-E-2-D 0 0 1000 0 0 0 0 10 0 100 200 300 Pantoea agglomerans Bacteria Pc-E-2-E 10000 100 200 300 10000 100 200 300	$ \begin{array}{c} {\rm GC} \ (*) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 394475} \\ \hline \\ {\rm Assembly Statistics} \ ({\rm Quast 5.0}) \\ {\rm Inter./isso access conclusions/add/cl-dt/72044est} \\ {\rm Assembly Statistics} \ ({\rm Quast 5.0}) \\ {\rm Inter./isso access conclusions/add/cl-dt/72044est} \\ {\rm Assembly } & {\rm PC}_{\rm E}, {\rm Z}_{\sim}, {\rm D} \\ {\rm ff} \ contigs \ (>= 25000 \ {\rm bp}) & {\rm 13} \\ {\rm ff} \ contigs \ (>= 25000 \ {\rm bp}) & {\rm 4877180} \\ {\rm Total \ length} \ (>= 25000 \ {\rm bp}) & {\rm 487743} \\ {\rm fortal \ length} \ (>= 25000 \ {\rm bp}) & {\rm 487743} \\ {\rm fortal \ length} \ (>= 50000 \ {\rm bp}) & {\rm 487743} \\ {\rm Assembly \ Society \ S$	Annua Jannua Annua Jannua ConceCodex Kmer Classifica Nue Podomora (press) Podomora (press) Podomo	00% 00% 20% 20% 20% 20% 20% 20% 2	
E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large-Small Contings (X) Bacteria Bacteria Pc-E-2-2-D 0000 0 0 1000 0 0 0 0 100 0 10 200 300 10 0 100 200 300 Pance= agglomerans Bacteria Pc-E-2-2-E 10000 - - - - 100 0 0 300 300	$ \begin{array}{c} {\rm GC} \left({\rm 'b} \right) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 304475} \\ \hline \\ {\rm Assembly Statistics (Quast 5.0) \\ {\rm htms}/{\rm Jssa accodes.cor/anh/st/shd/ChST/2014ech} \\ {\rm Assembly } & {\rm PC}_{\rm E}_2, {\rm Z}_2 \\ {\rm \# contigs} \left({\rm lss2} {\rm S000 \ bp} \right) & {\rm 13} \\ {\rm \# contigs} \left({\rm lss2} {\rm S000 \ bp} \right) & {\rm 13} \\ {\rm \# contigs} \left({\rm lss2} {\rm S000 \ bp} \right) & {\rm 4877180} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 487743} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 487743} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 487743} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 487743} \\ {\rm Assembly } & {\rm (ss3} {\rm (ss2}, {\rm 7974} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 247442} \\ \\ {\rm htms}//{\rm asoaccodes.com/anh/sh/sh/ChSCHSCHMM} \\ {\rm Assembly } & {\rm PC}_{\rm E}_2, {\rm 2}_{\rm C} {\rm E} \\ {\rm \# contigs} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2} {\rm S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Total \ length} & {\rm (ss2) \ S000 \ bp} \right) & {\rm 24} \\ {\rm Largest \ contigs} & {\rm (ss2) \ S00 \ bp} \right) & {\rm 24} \\ {\rm Assembly} & {\rm 20} \\ {\rm Contigs} & {\rm (ss2) \ S00 \ bp} \right) & {\rm 24} \\ {\rm 20} \\$	Annua Jannua Humanga DoneCodex Kmer Classifica Fore Podomora (Instrument Podomora (Instrumen	00% 00% 00% 70% 70% 00% 00% 00% 00% 00%	
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-E-2-2-D_001.fastq.gz	0 10 20 30 40 Assembly Coverage (Y) Large-Small Contings (X) Bacteria Bacteria 10000 PC-E-2-2-D 0 0 100 0 100 200 300 100 0 100 200 300 Partice agglomerans Bacteria Bacteria PC-E-2-2-E 0 0 300 100 200 40 60 80 Pseudomonas sp. UBA6562 Bacteria PC-D-2-2-8 100000 10	$ \begin{array}{c} {\rm GC} \ (\%) & {\rm G2}, {\rm G4} \\ {\rm NS0} & {\rm 304475} \\ \hline \\ {\rm Assembly Statistics} \ ({\rm Quast 5.0}) \\ {\rm Inter./random code: contrainstrated (CM)^{12014640} \\ {\rm Assembly Statistics} \ ({\rm Quast 5.0}) \\ {\rm Inter./random code: contrainstrated (CM)^{12014640} \\ {\rm Assembly } & {\rm PC}_{\rm E}, {\rm Z}_{\sim}, {\rm DF} \\ {\rm \# \ contigs} \ (:= 25000 \ {\rm bp}) & {\rm 13} \\ {\rm \# \ contigs} \ (:= 25000 \ {\rm bp}) & {\rm 4877180} \\ {\rm Total \ length} \ (:= 25000 \ {\rm bp}) & {\rm 483743} \\ {\rm \# \ contigs} \ (:= 50000 \ {\rm bp}) & {\rm 4837473} \\ {\rm \# \ contigs} \ (:= 50000 \ {\rm bp}) & {\rm 4837473} \\ {\rm \# \ contigs} \ (:= 50000 \ {\rm bp}) & {\rm 4837473} \\ {\rm Hots} \ (:= 50000 \ {\rm bp}) & {\rm 4837473} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm \# \ contigs} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Hots} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Hots} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 550000 \ {\rm bp}) & {\rm 21} \\ {\rm Total \ length} \ (:= 550000 \ {\rm bp}) & {\rm 24} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 21} \\ {\rm Assembly} \ (:= 525000 \ {\rm bp}) & {\rm 2$	Annual Janna J Annual Janna J Polubinna (1998) Polubinna (19		
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Figure 2. PDA with chloramphenicol. Colony Image (Left), Assembly sequence coverage (Y) compared with contig Length (X) where the contigs are sorted largest to smallest from left to right (Mid-Left). Assembly statistics calculated with Quast 5.0 (Mid Right). OneCodex speciated Kmer count (Right).

assembled with MegaHit v.1.2.9 (Li *et al.*, 2015, 2016). The Nextflow mapping and assembly pipeline is published on GitHub. Quast 5.0 was used to calculate the assembly quality statistics (Gurevich *et al.*, 2013). Sequencing data is deposited in NCBI under Project ID PRJNA725256.

Results

Each colony which was imaged on plates and chosen for whole genome sequencing and OneCodex analysis is displayed in Figure 1 (DRBC), Figure 2 (PDA-chloramphenicol) and Figure 3 (PDA no chloramphenicol). A link to each OneCodex analysis and its respective NCBI submission ID is available in Supplementary Table 1 - Sheet Summary (*Extended data*, McKernan *et al.*, 2021). Some of the colonies from the plate merged with other colonies producing mixtures of genomes as evident in the OneCodex pie charts. These merged colonies were further evidenced by the display of bimodal sequence coverage (clusters of contigs at 1000X and 10X coverage) and compared with the plating images (Figure 4). A heatmap of sequencing read speciation and purity is seen in Figure 5. While merged colonies can be difficult to resolve visually, whole genome sequencing can resolve simple metagenomes and still extract additional diversity information from the samples. Colonies that were noticeably mixed according to sequence analysis and colony visual inspection were more prevalent with the PDA without selection colonies (Table 1).

Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Kmer Classification	
PC-D-2-2-D_001.fastq.gz	Penicillium citrinum Fungi	https://app.onecodex.com/analysis/public/f4f7a05888b54ca4	Name Estimated Abundance	28.78 16
	PC-D-2-2-D	Assembly PC_D_2_2_E	Periodium colinum 77.99%	
	10000	# contigs (>= 25000 bp) 402	Partoea agglomerans 5.22% Partoea ananatis 3.52%	
		# contigs (>= 50000 bp) 211	Pieudomonis montelii 3148	
	1000	Total length (>= 25000 bp) 26589484	Fisherichia coli	
	100	Total length (>= 50000 bp) 19786523	Pseudomonas colecoteronum 1.42%	
	100	# contigs 2324	Bacilus anyioliquefaciens 1.29%	
	10	Largest contig 355,055	Pseudomonas cryphabitans 1.10%	
	10	Total length 32,726,848	Bacilus velezensis 1.02%	
	1	GC (%) 46.46	Fusarum fujikuroi 0.78%	
	0 500 1000	N50 66412	(Remaining) 2.79%	
		N30 00412		
C-D-2-2-E_001.fastq.gz	Penicillium citrinum Fungi	https://app.onecodex.com/analysis/public/decbad79ad4f438c	Name Estimated Abundance	27.05 17
0-D-2-2-E_001.10310.92		Assembly PC_D_2_2_E		27.05 1
	PC-D-2-2-E	# contigs (>= 25000 bp) 436	Pantoea aggiomerans 8.27%	
	10000	# contigs (>= 2000 bp) 430 # contigs (>= 50000 bp) 217	Pseudomonas montelli 6.71%	
	1000		Pantoea ananatis 4.90%	
	1000	Total length (>= 25000 bp) 26450972	Bacillus velezensis 3.43%	
	100	Total length (>= 50000 bp) 18441260	Pseudomonas coleopterorum 3.17%	
	1 ALASSAL MAR BORGED	# contigs 7634	Escherichia coli 1.82%	
	10	Largest contig 268,477	Pseudomonas oryzihabitans 1.60%	1.
And and the second s		Total length 36,552,780	Pantoea vagans 1.45%	
	1	GC (%) 47.46	III Rusarium fujikurol 1.28%	
	0 500 1000 1500	N50 50555	= (Remaining) 6.98%	
C-D-2-2-F_001.fastq.gz	Penicillium citrinum Fungi	https://app.onecodex.com/analysis/public/36bb541f014c437f	Name Readcount (% of classified reads)	31.66 3
	Fusarium fujikuroi Mix	Assembly PC_D_2_2_F	Ustriago maydis 33661 (2.64%)	
and the second second	PC-D-2-2-F	# contigs (>= 25000 bp) 429	Penicilium citrinum 16863 (1.32%)	
and the second second	the second se	# contigs (>= 50000 bp) 297	 Fusarium fujikuroi 16724 (1.31%) 	
the states	10000	Total length (>= 25000 bp) 44070909	- Trichoderma harzianum 9119 (0.71%)	
St. Station -	1000	Total length (>= 50000 bp) 39227038	 Pseudomonas coleopterorum 8262 (0.65%) 	
A BOOM A		# contigs 2392	Epicoccum nigrum 7871 (5.62%)	
Manufa -	100		Pseudomonas oryzhabitans 7355 (0.58%)	
THERE .	10	Largest contig 709,629	Pantoea aggiomerans 5866 (0.46%)	
	1	Total length 47,801,658	Alternaria alternata 3718 (0.29%)	
	0 200 400 600 800	GC (%) 50.86	Aspergillus sp. MA 6041 2670 (0.21%)	
P MARKET	0 200 400 600 800	N50 128078	III (Remaining) 27272 (2,14%)	
Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	N50 128078 Assembly Statistics (Quast 5.0)		
		N50 128078 Assembly Statistics (Quast 5.0) https://app.onecodex.com/anahysis/public/02509/1971b94c1d	OneCodex Kmer Classification	
	Assembly Coverage (Y) Large->Small Contigs (X)	N50 128078 Assembly Statistics (Quast 5.0) https://app.onecodex.com/anahois/public/0250911971154c1d Assembly PC_D_22_c1	Persang 2222(2.14%) OneCodex Kmer Classification Kmer Admda stenda Codex Codex Codex	
	Assembly Coverage (Y) Large->Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G	N50 128078 Assembly Statistics (Quast 5.0) http://seo.excodes.com/sanbait/sabbit/02509f1921045cld Assembly PC_D_2_2_(4000000000000000000000000000000000		
	Assembly Coverage (Y) Large->Small Contigs (X) Alternaria alternata Mix Fungi	NS0 128078 Assembly Statistic (Quast 5.0) https://assembly.goad/cypose/symptomic (Quast 5.0) PC_D_2_2_ # contigs (>= 25000 bp) 464 # contigs (>= 56000 bp) 257		
	Assembly Coverage (Y) Large-S-mail Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 1000	N50 128078 Assembly Statistics (Quast 5.0) http://seo.excodes.com/sanbait/sabbit/02509f1921045cld Assembly PC_D_2_2_(4000000000000000000000000000000000		
	Assembly Coverage (Y) Large->Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G	NS0 128078 Assembly Statistic (Quast 5.0) https://assembly.goad/cypose/symptomic (Quast 5.0) PC_D_2_2_ # contigs (>= 25000 bp) 464 # contigs (>= 56000 bp) 257		
	Assembly Coverage (Y) Large-Ssmall Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 1000	NS0 120078 Assembly Statistics (Quast 5.0) https://sa.omcodex.com/unable/c02500f1971461d Assembly PC_D_2_2_(# contigs (>= 25000 bp) 464 # contigs (>= 50000 bp) 257 Total length (>= 25000 bp) 5802944		
	Assembly Coverage (Y) Large-S-mail Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 1000	$\begin{tabular}{ c c c c } \hline NS0 & 1.20078 \\\hline & Assembly Statistics (Quast 5.0) \\\hline & Massembly & PC_D_2_2_(\\ \# \ contigs \ (>= 25000 \ bp) & 464 \\ \# \ contigs \ (>= 25000 \ bp) & 257 \\\hline & Total \ length \ (>= 50000 \ bp) & 25697287 \\\hline & Total \ length \ (>= 50000 \ bp) & 387 \\\hline \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $		
	Assembly Coverage (Y) Large-Ssmall Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 1000	NS0 122078 Assembly Statistic (Quast 5.0) http://secondex.com/nubic/(SMS/0759719719461d Assembly PC_D_2_2_C # contigs (>= 25000 bp) 464 # contigs (>= 25000 bp) 256 # contigs (>= 25000 bp) 364/3642944 Total length (>= 25000 bp) 26097287 # contigs 384/610		
	Assembly Coverage (Y) Large-Ssmall Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 1000	NS0 128078 Assembly Statistics (Quast 5.0) MEMOVING CONTROLLANCE (Quast 5.0) MEMOVING CONTROLLANCE (Quast 5.0) # contigs (>= 25000 bp) PC_D_2_2_t # contigs (>= 50000 bp) 257 Total length (>= 25000 bp) 25607287 # contigs 1387 Total length (>= 50000 bp) 25607287 # contigs 1387 Largest contig 384,610 Total length 39,526,281		
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-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 100 10 10 0 2000 4000 6000	$\begin{tabular}{ c c c c } \hline NS0 & 128078 \\ \hline & Assembly Statistics (Quast 5.0) \\ \hline & Hts://secondex.com/analoi.(doi:0/25071971984d \\ \hline & Assembly & PC_D_2_2_C \\ \# & contigs (s= 25000 bp) & 464 \\ \# & contigs (s= 50000 bp) & 257 \\ \hline & total length (s= 25000 bp) & 25607287 \\ \hline & total length (s= 50000 bp) & 26097287 \\ \hline & total length (s= 50000 bp) & 387 \\ \hline & Largest contig & 384,610 \\ \hline & Total length & 39,526,281 \\ \hline & fot & 0 & 53.75 \\ \hline & NS0 & 77385 \\ \hline \end{tabular}$	2000 2000 2000 2000 2000 2000 2000 2000 20	25.06 2
-D-2-2-G_001.fastq.gz	Asembly Coverage (Y) Large-Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 100 100 100 2000 4000 6000 Pseudomonas oryzihabitan Bacteria	NS0 122078 Assembly Statistic/OXOFF V71VB4/d Assembly CONTRACT V7000	generating 2000000000000000000000000000000000000	25.06 2
-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 100 10 10 0 2000 4000 6000	$\begin{tabular}{ c c c c } \hline NS0 & 1220078 \\ \hline Assembly Statistics (Quast 5.0) \\ \hline Http://secondet.com/analoi.dok/02500719719461d \\ \hline Assembly & PC_D_2_2_C \\ \neq contigs (s= 50000 bp) & 464 \\ \# contigs (s= 50000 bp) & 257 \\ \hline Total length (s= 55000 bp) & 256097207 \\ \hline Total length (s= 55000 bp) & 25097207 \\ \hline Total length (s= 50000 bp) & 25097207 \\ \hline Total length (s= 50000 bp) & 35.75 \\ \hline NS0 & 77385 \\ \hline NS0 & 77385 \\ \hline \end{tabular}$	∎ 2000 2000 Bottom Encarded Monte ■ non-color Americano 2000 ■	25.06 2
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-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Simall Contigs (X) Alternaria alternata PC-D-2-2-G 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	$\begin{tabular}{ c c c c } \hline NS0 & 1.20078 \\ \hline Assembly Statistics (Quast 5.0) \\ \hline Mtss/Jeas accedes con/analot/dok/02500719719451d \\ \hline Assembly & PC_D_2_2_C \\ \# contigs (= 25000 bp) & 464 \\ \# contigs (= 50000 bp) & 257 \\ \hline Total length (== 25000 bp) & 26097287 \\ \hline Total length (== 50000 bp) & 26097287 \\ \hline Total length (== 50000 bp) & 3602944 \\ \hline Total length (== 50000 bp) & 350,75 \\ \hline NS0 & 77385 \\ \hline Mtss/Jeas accedes com/analot/s/dok/2405051ecf484 \\ \hline Assembly & PC_D_2_1J \\ \# contigs (== 50000 bp) & 18 \\ \# contigs (== 55000 bp) & 17 \\ \hline \end{tabular}$	2012 2012 2012	25.06 2
-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Simall Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	$\begin{tabular}{ c c c c c } \hline NS0 & 122078 \\ \hline 122078 & 122078 \\ \hline Assembly Statistics(Uaust 5.0) \\ \hline Mtss//seaseds.com/analot(Solid)(202078/37)184.1d \\ \hline Assembly & PC_D_2_2_C \\ \# contigs (s= 50000 bp) & 464 \\ \# contigs (s= 50000 bp) & 26097287 \\ \hline Total length (s= 55000 bp) & 26097287 \\ \# contigs (s= 50000 bp) & 26097287 \\ \hline Total length (s= 53.75 \\ NS0 & 77385 \\ \hline Mtss//seaseds.com/nsbuls/solid/safe/S53set088 \\ \hline Assembly & PC_D_2_1 \\ \# contigs (s= 25000 bp) & 18 \\ \# contigs (s= 25000 bp) & 17 \\ \hline Total length (s= 25000 bp) & 17 \\ \hline Total lengt (s= 50000 bp) & 17 \\ \hline Total lengt (s= 25000 bp) & 17 \\ \hline Total lengt (s= 57600 bp) & 471673 \\ \hline \end{tabular}$	2012 (1996) 2012 (1996) 2012 (1996) 2012 (1996) 2012 (1996) 2012 (1996) (19	25.06 2
-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Small Contigs (X) Alternaria alternata Mix Fungi PC-D-2-2-G 100 100 100 100 2000 4000 6000 Pseudomonas oryzihabitans Bacteria PC-D-2-1-A 10000 1000	$\begin{tabular}{l l l l l l l l l l l l l l l l l l l $	amount 2022 (3.14) Decodeda Marco Rassification A more general control of the second	25.06 2
-D-2-2-G_001.fastq.gz	Assembly Coverage (Y) Large-Simall Contigs (X) Alternaria alternata PC-D-2-2-G 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	NS0 122078 NS0 122078 Assembly Statistic (Quart 5.0) Mission Colspan="2">Mission Colspan="2">Mission Colspan="2">Mission Colspan="2" Assembly PC_D_2_2_C $\#$ contigs (>= 25000 bp) 464 4 contigs (>= 25000 bp) 26097287 $\#$ contigs (>= 50000 bp) 26097287 # contigs 1387 Largest contig 384,610 1387 1387 Total length (>= 25000 bp) 364,610 53,75 NS0 77385 Missing inserver com/inside/ide/ide/ide/ide/ide/ide/ide/ide/ide/	Temporal 2022 (2.14) Decolocide Mere Classification Enternational Name anoma classification Enternational Amma anoma classification Enternational Prema anoma Enternational Preman	25.06 2
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Figure 2. (continued)

A Simpson's diversity index analysis demonstrated PDA with CAMP provides the highest diversity score (Figure 6) While the DRBC had 100-fold lower CFU counts than PDA without selection, it predominantly displayed fungal colonies (80%) while PDA without selection was biased toward bacteria (22%). PDA with chloramphenicol displayed more fungi (55%) than bacteria and also produced a half log more fungal colonies than DRBC with chloramphenicol (Table 2).

One fungal sample (*Cladosporum*) presented delayed Ct (31.79) with PathoSEEK Total Yeast and Mold (ITS3-TYM) qPCR primers. Scrutiny of the primer sequences against the *Cladosporum* genome shows proper primer binding locations but missing probe sequences. This genome has low coverage (10X) and the repetitive ITS qPCR target regions are often poorly assembled in low coverage-genomes. This may explain the missing probe sequence in the low coverage fragmented assembly. Additionally, some significantly delayed PathoSEEK Total Aerobic Count (TAC) signal was observed in fungal colonies. This is the result of the use of the lytic enzyme (TLP) which is cloned and expressed in *E. coli* and contains some background *E. coli* DNA. This background TLP expression in *E. coli* produces signals that can be seen in blank preparations. In some cases, this signal is elevated due to mixed colonies observed in the sequencing data.

The qPCR method represents an increased selectivity in assessing fungal and bacterial CFU compared to DRBC, where only -92% of the colonies were fungal colonies. Quantitative PCR identified all fungi and never mistook one for bacteria. In a minority of cases we had visually mixed colonies. Even if we discount the mixed colonies and count, only the single bacterial colony out of 13 on DRBC, we obtain 92% (1/13) fungal colonies on DRBC where qPCR delivered perfect

Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	Assembly Statistics			mer Classification	TAC Ct TYN
C-D-2-1-C1_001.fastq.gz	Trichoderma harzianum Fungi	https://app.onecodex.com/analysis/public/3127		Name	Estimated Abundance	32.17 26
A REAL PROPERTY.	PC-D-2-1-C1	Assembly	PC_D_2_1_C1	Trichoderma		
	10000	# contigs (>= 25000 bp)	340	Trichoderma		
		# contigs (>= 50000 bp)	37	 Trichoderma 		
	1000 • •	Total length (>= 25000 bp)		Pantoea aggi		
		Total length (>= 50000 bp)		Pseudomona		
and the second second second	100			Rantoea anar		_
	and the state of the state of the	# contigs	5131	Bacillus velez		
	10		131,469	Pseudomona	s coleopterorum 0.70%	
and the second s		Total length	41,502,375	Pseudomona	s oryshabitans 0.37%	
A PROVIDE A	1	GC (%)	46.82	IIII Fusarium fuji	kuroi 0.31%	
CONTRACT OF	0 1000 2000 3000	N50	16662	III (Remaining)	1.98%	
C-D-2-1-C2_001.fastq.gz	Trichoderma harzianum Fungi	https://app.onecodex.com/analysis/public/fa69f		Name	Estimated Abundance	30.47 24
A A A A A A A A A A A A A A A A A A A	PC-D-2-1-C2	Assembly	PC_D_2_1_C2	Trichoderma	harzianum 75.59%	
and a state of the		# contigs (>= 25000 bp)	12	Trichoderma	atrobranneum 5.64%	*
A BUILD AND AND AND AND AND AND AND AND AND AN	10000	# contigs (>= 50000 bp)	0	Trichoderma	guithouense 5.48%	
As you want to make the second state	1000	Total length (>= 25000 bp)		Pantoea agg		
		Total length (>= 50000 bp)			as oryzihabitans 1.85%	
	100	# contigs	12671	Rentoea ana		
				 Bacilus vele 		
	10		34,730		as coleopterorum 1.09%	
	1		41,758,812		as psychrotolerans 1.02%	
and a second second	0 2000 4000 6000 8000 10000	GC (%)	47.09	= Pseudomon		
HARD AND I AND A	5 2000 4000 8000 8000 10000	N50	5262	Certaining)	3.09%	
0.0.0.000000000000000000000000000000000				1		ar in lar
C-D-2-1-C3_001.fastq.gz		https://app.onecodex.com/analysis/public/5877		Name	Estimated Abundance	32.18 23
	PC-D-2-1-C3	Assembly	PC_D_2_1_C3	 Trichoderma 		
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	10000	# contigs (>= 50000 bp)	19	 Trichoderma 		
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and the contract of the	1000	Total length (>= 50000 bp)	1137009	Pseudomon		
a second second	100	# contigs	6487	Pantoea ana Bacillus veles		
AL STREET, STR	- Andrew An		94,369			
A STORE STORE	10		41,771,792		as coleopterorum 1.24% as psychrotolerans 0.91%	
1 2 4 10 10	1		·*; · / 1; / 32	Pseudomony	is psychrocoleralls 0.91%	
			46 85	and the second	N 40 (194662)	
	0 2000 4000	GC (%)	46.85		es sp. UBA6562 0.51%	
12	0 2000 4000	N50	13238	= (Remaining)	2.71%	
Colony Image	0 2000 4000 Assembly Coverage (Y) Large->Small Contigs (X)	N50 Assembly Statistics (13238 Quast 5.0)	OneCodex K	2.71% mer Classification	
Colony Image C-E-2-1-B_001.fastq.gz	0 2000 4000 Assembly Coverage (Y) Large->Small Contigs (X) Pseudomonas oryzihabitans Bacteria	N50 Assembly Statistics (https://app.onecodex.com/analysis/public/beb70	13238 Quast 5.0) 0ca5c46431a	OneCodex K Name	2.71% mer Classification Estimated Abundance	
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Figure 2. (continued)

results. As a comparison, quantitative PCR demonstrated over 10 Cts (1024 fold) differences between the TYM and TAC signals on fungal colonies. The majority of the residual TAC signal being observed in fungi can be normalized and discounted with the background *E. coli* TLP DNA signal measured in blank preparations.

To confirm these observations several *Aspergillus* species and *Botrytis cinerea* were ordered from ATCC and plated on various plating medias in absence of background cannabis matrix (Table 3 and Figure 7). In all cases DRBC showed reduced CFU counts.

Discussion

Microbial media and their selection have a significant impact on the Simpson's diversity index of microbes observed with whole genome sequencing. This has been noted in prior microbiome surveys in cannabis, in which culturing the microbes changes the representation of the microbiome as measured by qPCR and sequencing performed directly off of the flower (McKernan *et al.*, 2015, 2016). Other cannabis microbiome studies also highlight discrepancies between plating and molecular methods (Winston *et al.*, 2014; Thompson *et al.*, 2017; Punja, 2018; Punja *et al.*, 2019; Comeau *et al.*, 2020; Taghinasab and Jabaji, 2020; Vujanovic *et al.*, 2020; Punja, 2021). Some of these discrepancies are a result of common cannabis plant pathogens (powdery mildew) that do not culture (Dryburgh *et al.*, 2018; Punja *et al.*, 2019; Jerushalmi *et al.*, 2020). It's important to recognize that each study is using different ITS primers and culturing

		y Statistics (Quast 5.0)		neCodex Kmer Classi	fication	TAC Ct TY
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			P_D_2_1_A Na		sated Abundance	
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*************			5670116	Pantoea alli Shigelia dysenteriae	4.42%	
100				Shigelia dysentenae Pseudomonas oryzihabitans	0.10%	
and the second se	# cont:		2129	Bacillus velezensis	0.05%	
10	 Largest 	t contig 40	0,518	Pseudomonas coleopterorum	0.06%	
	Total		,498,447		0.03%	
1				Fusarium fulkurol	0.03%	
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1000	Total	length (>= 25000 bp)	4810440	Citrobacter freundii	0.47%	
-			4722027	Pseudomonas oryzihabitans	0.25%	
100				Enterobacter doacae	0.23%	
	# cont:		886	Pseudomonas putida	0.19%	
10			4,181	Bacillus velezensis	0.18%	
	Total	length 5.	456,028		0.17%	
1	GC (%)		54.22 =	Delftia acidovorans	0.16%	
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D-2-1-C_001.fastq.gz Pantoea agglomera	ans Bacteria https://app.	onecodex.com/analysis/public/980e71	edf25942a0 Na	ame Estim	nated Abundance	17.77 N
And a second sec	Accomb		P_D_2_1_C		75.20%	
P-			26	Pantoea aggiomerans Pantoea ananatis	15.31%	
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		igs (>= 50000 bp)	20	Pantoea vagans		
1000.0	Total	length (>= 25000 bp)	4785028	Pseudomonas oryzihabitans	0.27%	
			4601583		0.23%	
100.0 100.0			285	Enterobacteriaceae bacterium UBA4		
	# cont:			Bacillus velezensis	0.21%	
10.0			57,633 🛛	Enterobacteriaceae bacterium UBA3	398 0.17%	
and the second se	Total	length 5,	026,841 =		0.16%	
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1.0			54.46 =	Pseudomonas psychrotolerans	0.14%	
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D-2-1-E_001.fastq.gz D-2-1-E_001.fastq.gz	200 300 400 500 NS6 V) Large-Semall Contigs (X) name. Assembly Bacteria Assembly # Cont. Assembly # Cont. P-D-2-D0 0 100 100 100 Contigs (X) # Cont. Assembly # Cont. 0 0 0 100 100 NS6 Nternata Mix Http://sec 4 Cont. Assembly # Cont. NS6 Mixed Bacteria Http://sec 4 Cont. Assembly # Cont. Assembly # Cont. PD-2-2-A # Cont. Total. # Cont. Total. PD-2-2-A # Cont. Total. # Cont. Total. Total. # Cont. # Cont. Total. # Cont. Total. # Cont. # Cont. # Cont. # Cont.	amendes com/analysis/addic/cle07 ly igs (>= 25000 bp) igs (>= 50000 bp) length (>= 50000 bp) length (>= 50000 bp) igs t igs (>= 25000 bp) length (>= 50000 bp) igs t igs (>= 25000 bp) igs (>= 25000 bp) igs (>= 25000 bp) length (>= 50000 bp) length (>= 50000 bp) length (>= 50000 bp) igs (>= 25000 bp) igs (>= 250000 bp) length (>= 50000 bp) length (>= 50000 bp) length (>= 50000 bp) length (>= 50000 bp) igs (= 50000 bp) length (>= 50000 bp) igs t t contig 2	306723 0 200723 0 200723194234 P_D_2_1_D 95 28 4277137 2243450 2843450 2933474 2943450 2933474 2943450 2933474 2943450 P_D_2_1_E 28 2869171 14877 28 2869171 2143023 27681 P_D_2_1_E 2143023 27681 P_D_2_1_E 28 3689171 92 2143023 27681 P_D_2_1_E 8 277453 2774555 2774555 2774555 2774555 2774555 2774555 27745555 277455557 277555755557 2775557555755575557557555755755755755755	Benutime Benutime Extent Second Karner Classifie Extent Image: Second Karner Classifie Extent Image: Second Karner Classifie Extent Second Karner Classifie Image: Second Karner Classifie Image: Second Karner Classifie Image: Second Karner Karne	0.5% fication	

Figure 3. PDA without chloramphenicol. Colony Image (Left), Assembly sequence coverage (Y) compared with contig Length (X) where the contigs are sorted largest to smallest from left to right (Mid-Left). Assembly statistics calculated with Quast 5.0 (Mid Right). OneCodex speciated Kmer count (Right).

techniques but ITS based methods can predict their inclusion and exclusion organisms *in-silico* and *a-priori*, where culture based methods cannot.

In this study the DRBC selection reduced bacterial growth more than PDA with chloramphenicol, but also reduced the fungal CFU 5-fold in the process. This has important implications for chloramphenicol-sensitive cannabis endophytes like *Aspergillus*, *Pythium* and *Fusarium*. Cannabis endophytes are an important consideration in this work as endophytes can colonize both the inside and outside of the plant and methods used to quantitatively access them need to lyse open plant cell walls. These conditions also lyse open pathogen cells walls and cell membranes, rendering the pathogens non-culturable. Many of the pathogens listed for cannabis testing are documented plant endophytes including *E. coli*, *Salmonella, Listeria* and *Aspergillus* (Li *et al.*, 2013; Wright *et al.*, 2013; Kljujev *et al.*, 2018a, 2018b).This presents challenges when attempting to benchmark molecular methods to culture-based platforms incapable of detecting endophytic pathogenic risk. This sequencing was performed only on colonies that were identified through culture and thus does not include the complete endophytic diversity of the cannabis samples.

Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Kmer Classification	TAC Ct TYN
P-D-2-2-B_001.fastq.gz	Bacillus velezensis Bacteria	https://app.onecodex.com/analysis/public/c7adba5be63646be	Name Estimated Abundance	21.59 N
	P-D-2-2-B	Assembly P_D_2_2_B	Bacillus velezensis 82.57%	
	10000	# contigs (>= 25000 bp) 16	Bacillus amyloliquefaciens 14.00% Bacillus eiseniae 2.22%	
		# contigs (>= 50000 bp) 15	Bacilus eisenae 222%	
	1000	Total length (>= 25000 bp) 4058977 Total length (>= 50000 bp) 4010903	Pantoea aggiomerans 0.21%	
	100	# contigs 309	Pseudomonas aeruginosa 0.19%	
		Largest contig 1,085,044	Pantoea ananatis 0.13%	
	10	Total length 4,404,170	Pseudomonas coleopterorum 0.04%	
	1	GC (%) 45.59	Bacilus atrophaeus 0.04% Pseudomonas oryzihabitans 0.03%	
	0 100 200 300 400	N50 462229	(Remaining) 0.22%	
P-D-2-2-C_001.fastq.gz	Pantoea agglomerans/Alternaria Mix P-D-2-2-C	https://app.onecodex.com/analysis/public/4bfc8477b18d4d00 Assembly P_D_2_2_C	Name Readcount (% of classified reads) Partoea aggiomerans 3178939 (26.58%)	20.70 25.
		# contigs (>= 25000 bp) 62	Alternaria alternata 390673 (3.27%)	
A REAL PROPERTY	10000	# contigs (>= 50000 bp) 27	 Pantoea sp. CFSAN033090 237909 (1.99%) 	
1	1000	Total length (>= 25000 bp) 3905762	Pseudomonas oryzihabitans 62383 (0.52%) Pantoea sp. a8 37064 (0.31%)	
	100	Total length (>= 50000 bp) 2679800	Pantoea sp. MBIJ3 36178 (0.3%)	
	10	# contigs 20418	Alternaria sp. MG1 34273 (0.29%)	
	"Philipping and a second se	Largest contig 268,520	Partoea sp. UBA3896 33750 (0.28%) Alternaria arborescens 24987 (0.21%)	
No. No. I	1 0 1000 2000 3000	Total length 21,304,064	Pantoea vagans 19289 (0.16%)	
	0 200 200 3000	GC (%) 52.23	III (Remaining) 37618 (0.32%)	
P-E-2-1-A_001.fastq.gz	Pantoea ananatis. Mixed Bacteria	https://app.onecodex.com/analysis/public/0044a7c2bd0d4295	Name Estimated Abundance	15.81 N
1	P-E-2-1-A	Assembly P_E_2_1_A	Pantoea ananatis 88.74%	
	100000	# contigs (>= 25000 bp) 23	Partoea aggiomerans 7.80% Partoea vagans 2.34%	
The second	10000	# contigs (>= 50000 bp) 19	Pantoea vagans 2.34% Enterobacteriaceae bacterium UBA5138 0.26%	
	1000	Total length (>= 25000 bp) 4758256	Enterobacteriaceae bacterium UBA3398 0.17%	
		Total length (>= 50000 bp) 4623610 # contigs 3340	Pseudomonas oryzihabitans 0.13%	
	100	# contigs 3340 Largest contig 1,009,934	Enterobacteriaceae bacterium UBA4753 0.13% Kosakonia cowanii 0.13%	
	10	Total length 7,358,440	Kosakonia cowanii 0.13% Bacillus velezensis 0.07%	
	1	GC (%) 53.86	Pseudomonas coleopterorum 0.06%	
and the second second	0 200 400 600	N50 180723	(Remaining) 0.14%	
Colony Image	Assembly Coverage (Y) Large->Small Contigs (X)	Assembly Statistics (Quast 5.0)	OneCodex Kmer Classification	TAC Ct TYN
-E-2-1-B_001.fastq.gz	Bacillus velezensis Bacteria	https://app.onecodex.com/analysis/public/05a94f22d73142ce Assembly PC E 2 1 B	Name Estimated Abundance	21.15 N
	P-D-2-1-B	Assembly PC_E_2_1_B # contigs (>= 25000 bp) 20	Bacilus velezensis Bo.22% Bacilus amyloiquefaciens 15.29%	
and the second second	10000	# contigs (>= 50000 bp) 19	Bacilus eiseniae 3.58%	
	1000	Total length (>= 25000 bp) 4933410	Pantoea aggiomerans 0.23%	
		Total length (>= 50000 bp) 4895124	Pantoea ananatis 0.13%	
			Preudomonas arrupinosa 0.13%	
	100	# contigs 102	Pseudomonas aeruginosa 0.13% Pseudomonas stutzeri 0.06%	
	100	# contigs 102 Largest contig 570,530	Pseudomonas stutzeri 0.06% Bacillus atrophaeus 0.06%	
•	100 0 0	# contigs 102 Largest contig 570,530 Total length 5,032,442	Pseudomonas stutzeri 0.06% Bacilius atrophaeus 0.06% Pseudomonas coleopterorum 0.06%	
	10	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69	Pseudomonas stutzeri 0.06% Bacillus atrophaeus 0.06%	
	10 1 0 20 40 60 80 100	# contigs 102 Largest contig 570,530 Total Length 5,032,442 GC (%) 65.69 N50 356575	Pudolmonas INUZIM 0.004 Becilia straphona 0.004 Pudolmonas delegatinum 0.004 Rushining 0.009 Quencing Quencing Quencing Quencing	
P-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69 NS9 336575 https://ispa.oncodex.com/analysis/addb/364059307b.14446	Pseudomonas stutzeri 0.06% Bacilius atrophanus 0.06% Pseudomonas colepterorum 0.06% Fusarium fujikuroi 0.03%	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mik P-E-2-1-C	# contigs 102 Largest contig 570,530 Total Length 5,032,442 GC (%) 65.69 N50 356575	Phydomena succeri 0.04 Berdina serphane 0.054 Phydomena relegatorum 0.044 Ramen (Hyteria 0.034 Ramen	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69 NS0 336575 http://wp.mecodex.com/amin/s/mkHc/16409307/14446 Assembly P_E_2_1_C # contigs (>= 25000 bp) 98 # contigs (>= 50000 bp) 52	Physioneas III.2em Bellis arrophenis Cospension Physioneas collegerarum Cospension Remaining Canama figliani Cospension Remaining Cospension Physioneas galgemerants 2017 Therefactures between USATS Therefactures between USATS	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix P-E-2-1-C 1000	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69 N50 356575 http://spomecodex.com/amb/ni/spdfic/364095930754446 Assembly P_E_2_1_C # contigs (>= 25000 bp) 98 # contigs (>= 25000 bp) 52 Total length (>= 25000 bp) 52 Total length (>= 25000 bp) 7299781	Phydomena succeri 0.04 Berdina serphane 0.054 Phydomena relegatorum 0.044 Ramen (Hyteria 0.034 Ramen	21.83 27.
2-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mik P-E-2-1-C	# contigs 102 Largest contig 570,530 Total length 5,832,442 GC (%) 65,69 NS9 336575 http://www.com/anahuk/makk//364093307b14446 Assembly P_E_2_1_C # contigs (>= 25000 bp) 98 # contigs (>= 25000 bp) 52 Total length (>= 25000 bp) 729781 Total length (>= 25000 bp) 752872	Phudomota Buzeri 0.04 Bellin asrophene 0.04 Phudomota relegatorum 0.04 Phudomota relegatorum 0.04 Phudomota relegatorum 0.024 Phudomota relegatorum 0.027 Phutos aggiorezna 0.227 Phutos aggiorezna 0.227 Phutos aggiorezna 0.217 Phutos aggiorez	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix P-E-2-1-C 1000	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69 NS0 336575 http://spi.orecoder.com/nalvis/subit	Polycimora student Bohla strephenis Costanti dilegtoni and December Decemb	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix P-E-2-1-C 1000 100	# contigs 102 Largest contig 570,530 Total length 5,032,442 GC (%) 65.69 NS9 336575 http://www.encodex.com/analysis/haddle/36093307b14446 Assembly P_E_2_1_C # contigs (>= 25000 bp) 98 # contigs (>= 25000 bp) 729781 Total length (>= 25000 bp) 729781 Total length (>= 25000 bp) 5822872 # contigs 7153 Largest contig 366,404	Phudomota Buzeri 0.04 Berdina angelphang 0.05 Phudomota relegatorum 0.04 Raam figliansi 0.029 Raam figliansi 0.029 Phudomota relegatorum 0.029 Phutos aggiorezensi 0.279 Photos aggiorezensi 0.279	21.83 27.
-E-2-1-C_001.fastq.gz	10 1 0 20 40 60 80 100 Enterobacter/Alternaria Mix P-E-2-1-C 1000 100	$\begin{array}{ccc} \# \mbox{ contigs } 102 \\ \mbox{Largest contig 570, 530 } \\ \mbox{Total length } 5, 032, 442 \\ \mbox{GC (%)} & 65, 69 \\ \mbox{NS9} & 356575 \\ \hline \\ \mbox{Intro://www.mexcew.com/ambrid/white/16409330734446} \\ \mbox{Assembly } P_E_2_1_C \\ \mbox{\# contigs (>= 25000 bp) } 98 \\ \mbox{\# contigs (>= 25000 bp) } 7299781 \\ \mbox{Total length (>= 25000 bp) } 7299781 \\ \mbox{Total length (>= 25000 bp) } 7299781 \\ \mbox{Total length (>= 25000 bp) } 7293781 \\ \mbox{Total length (>= 25000 bp) } 7153 \\ \mbox{Largest contig 306,404 \\ \mbox{Total length 14,692,225} \\ \end{array}$	Photomeras Incom Photomeras Incom Photomeras Incom Photomeras relegators Photomeras relegators Photomeras relegators Photomeras relegators Photomeras	21.83 27
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Figure 3. (continued)

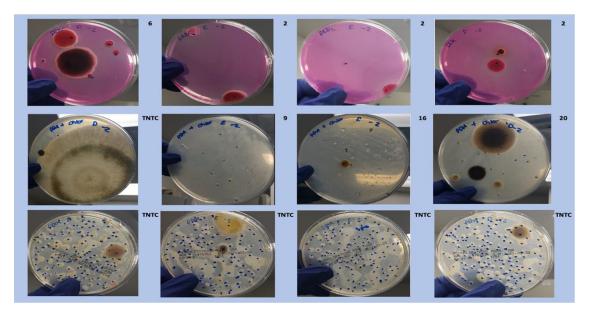


Figure 4. Plating images. Dichloran Rose Bengal (Top). Potato Dextrose Agar with chloramphenicol (PDA – CAMP, Middle). PDA without CAMP (Bottom).

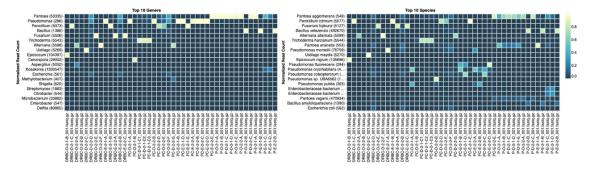


Figure 5. Summary heatmap of colony classification by whole genome sequencing. Sample nomenclature on the X axis describes the media the colonies were isolated from. DRBC prefix = DRBC. PC prefix = PDA with CAMP. P prefix = PDA without CAMP.

	Bacteria	Fungi	Mixed
DRBC CAMP	3	12	2
PDA CAMP	10	9	1
PDA No CAMP	14	4	4

Table 1. Summary of colony forming unit classification.

Both media types (PDA and DRBC) are referenced in the FDA Bacteriological Analytical Manual. States exclusively considering DRBC for ease of colony visualization should be aware of the species-specific sensitivities of using a single medium type, and consider species-specific testing for such human pathogenic organisms, to complement a partial yeast and mold test offered from a single selection-based medium. PCR-based techniques can identify more organisms than DRBC alone as no selection is occurring given thorough cell lysis is achieved for qPCR analysis. This is not a surprising result as Dichloran was developed as a media designed to suppress the growth of rapidly growing molds and bacteria (Henson, 1981).

Plating also suffers from having a very limited dynamic range. Since it is difficult to count colonies when more than 100 colonies are present on a plate, multiple dilutions are often required to understand the full range of CFU counts one

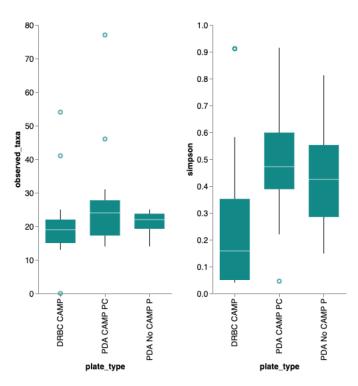


Figure 6. Simpson's Diversity Index. Simpson's diversity index (https://geographyfieldwork.com/Simpson%27sDiversityIndex.htm) is used to quantify the biodiversity of a habitat on a 0 to 1 scale. It takes into account the number of species present, as well as the relative abundance of each species. A diversity index of 1 represent infinite diversity where 0 reflects no diversity. Dichloran Rose Bengal (DRBC) plating demonstrates the lowest diversity. This is not surprising given DRBC contains 3 different selection agents. While this limits bacterial contamination it also limits yeast and mold growth.

Table 2. Cannabis samples plated on 3 different media. Plating on different media demonstrates a LOG scale difference in Colony Forming Units (CFU) with each plating medium. Sequencing can attribute only half of the colonies as bacteria on Potato Dextrose Agar (PDA) with chloramphenicol. This implies a 5-fold under counting of yeast and mold on Dichloran Rose Bengal (DRBC).

			DR	BC		
Sample	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g
Low A	0	4	1	1	0	0
Low B	0	3	1	1	1	0
Low C	2	1	0	0	0	0
Low D	2	5	2	0	2	0
Low E	2	0	2	0	0	0
			Average CFU/g			
			PDA with Chl	oramphenicol		
Sample	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g
Low A	8	16	12	1	3	2
Low B	8	12	8	1	0	3
Low C	13	19	13	1	2	1
Low D	3	12	21	2	0	1
Low E	9	7	4	0	3	3
			Average CFU/g			

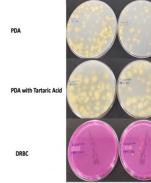
	PDA without Chloramphenicol							
Sample	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻² CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g	10 ⁻³ CFU/g		
Low A	127	133	124	32	32	21		
Low B	151	157	101	26	20	28		
Low C	TNTC	TNTC	TNC	41	45	37		
Low D	147	141	123	32	26	26		
Low E	138	102	119	23	15	24		
			Average CFU/g					

Table 2. Continued

Table 3. Mono-culture evaluations. Fungi species were ordered from American Tissue Culture Collection (ATCC) and plated on 2 different medias (PDA and DRBC) to assess growth performance of the organisms in absence of cannabis background bacteria and matrix.

Species	ATCC Number	qPCR	DRBC Plating	PDA
Aspergillus brasiliensis	16404	Amp	5 Day Growth	5 Day Growth
Aspergillus flavus	9643	Amp	Less growth in 7 Days	5 Day Growth
Aspergillus fumigatus	204305	Amp	Less growth in 7 Days	5 Day Growth
Aspergillus niger	16888	Amp	Less growth in 7 Days	5 Day Growth
Aspergillus terreus	1012	Amp	Less growth in 7 Days	5 Day Growth
Aspergillus tubigensis	1004	Amp	Less growth in 7 Days	5 Day Growth
Candida tropicalis	13803	Amp	5 Day Growth	5 Day Growth
Penicillium breviocompactum	9056	Amp	Less growth than PDA	5 Day Growth
Purpureocillium lilacinum	10114	Partial Amp	5 Day Growth	5 Day Growth
Rhizopus oryzae	52748	Partial Amp	Less growth than PDA	5 Day Growth

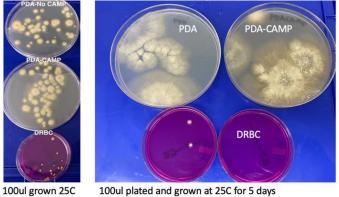
A.niger ATCC#1015 plated on PDA, PDA-TA, DRBC



100ul plated and grown at 25C for 7 days

A.niger on PDA, PDA-CAMP & DRBC

Botrytis cinerea ATCC#204446 plated on PDA, PDA-CAMP, DRBC (red)



100ul plated and grown at 25C for 5 days

Figure 7. Aspergillus niger and Botrytis cinerea monocultures plated on 3 different medias. Cultures were plated on Potato Dextrose Agar (PDA), PDA with selection (chloramphenicol or Tartaric acid), and Dichloran Rose Bengal (DRBC). Fewer colonies are consistently found on DRBC.

3 days

may encounter with a test which is attempting to quantify 10,000 CFUs/gram. This results in multiplying diluted CFUs 10, 100 and even a 1,000 fold to back-estimate the total CFU count. In this scenario a single colony can swing the CFU count from passing to failing (9 colonies x 1,000 fold dilution vs 10 colonies at 1,000 fold dilution). Quantitative PCR has a linear dynamic range over 5-6 orders of magnitude and no such multiplication is required. Thus, qPCR provides a more accurate itemization of actual CFUs counts.

In-vitro inclusion and exclusion testing with ITS3 qPCR on ATCC-sourced organisms demonstrated over 96% inclusion (50 yeast and mold) and zero bacterial cross reactivity (30 bacteria) (*Extended data:* Supplementary Table 1- Sheet TYM Inclusion & TYM Exclusion). *In-silico* analysis of ITS3 primer sequences, predicts over 1400 yeast and mold should amplify with the described ITS3 primer sequences. All plating media, even with three different forms of selection (DRBC), had bacterial contamination and each level of selection reduced fungal CFU counts.

Data availability

Underlying data

NCBI Bioproject: Under Counting of Total Yeast and Mold on Cannabis using DRBC, Accession number PRJNA725256, https://www.ncbi.nlm.nih.gov/bioproject?term=PRJNA725256.

Extended data

Zenodo: Whole genome sequencing of colonies derived from cannabis flowers and the impact of media selection on benchmarking total yeast and mold detection tools, https://doi.org/10.5281/zenodo.4759883 (McKernan *et al.*, 2021).

This project contains the following extended data:

Summary Table 1: OneCodex URLs and NCBI BioSample IDs for every sample.

TYM Inclusion: ATCC organisms tested for inclusion criteria

TYM Exclusion: ATCC organisms tested for exclusion criteria

Sequencing: Number of reads, Read Pairs and Total Gigabases sequenced for each sample.

Assembly: Complete Assembly statistics for each sample generated by QUAST

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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PubMed Abstract | Publisher Full Text

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PubMed Abstract | Publisher Full Text | Free Full Text

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Version 2

Reviewer Report 23 August 2021

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Zamir K. Punja

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

I approve the indexing of this revised paper with no reservations.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, plant pathology, plant biology, cannabis pathogens, postharvest quality

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 04 August 2021

https://doi.org/10.5256/f1000research.56852.r90093

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Zamir K. Punja

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

 Add "human pathogenic" where pathogenic is mentioned as the concern is with these particular microbes and not those potentially that could be affecting the plant as plant pathogens.

- The inclusion of the 3 selection media should be elaborated on. Why were these particular 3 media selected? Provide references to show where, or in what capacity, they may have been used in previously published work. There is abundant published work on the use of PDA with antibiotics to isolate fungi in the plant pathology literature. In fact, it is a standard medium used for isolation in labs worldwide. The addition of dichloran and rose bengal have also been used to restrict the growth of certain groups of fungi and bacteria as a semi-selective medium for isolation in particular from soil samples. Therefore, it would not be expected to provide a broad spectrum of recovery of fungi and yeasts. It is surprising that this medium would be used to assess total yeast and mold counts in cannabis.
- The comparison of the 3 media in this study sheds light on the differences in levels of recovery of fungi and yeasts. This is an important finding – not all media behave in the same manner. To observe a 10-fold difference in recovery between these media is quite significant as it illustrates the potential for under-representation in the recovery process.
- The use of whole genome sequencing to apply to the identification of colony-forming units is a definite plus for this work. It shows the ability to rapidly identify what is present on the culture media with regard to molds that originated from the samples.
- There are several prior reports of authors having recovered a range of fungi from cannabis buds and identified them using the ITS region. Please include these as a reference by which to compare the fungi and yeasts identified in the present study. It is important to build a body of knowledge on the exact identity of the general and species found on cannabis and how prevalent they are.
- The report of endophytes in cannabis should be accompanied by a reference citation. These
 particular microbes are more difficult to recover in culture media and therefore a molecular
 approach has merit.
- The inclusion of confirmed ATCC culture specimens to demonstrate differences in growth on the 3 media is a good confirmatory experiment.
- The cannabis samples that originated from Steadfast Analytical Laboratories would have had an analysis of total yeast and mold conducted on them. Is it possible to have these results compared to those from the present study to show how the commercial lab testing may differ from the current study? Or was that not an objective of the current study?
- During the preparation of samples for the ITS3 qPCR procedure, was there a subset of samples included that did not contain the TLP lysis step to show that it made a difference? Or is that included in prior published work?
- For the 45 colonies that were selected for whole genome sequencing, could the identified genus and species be presented in a separate table? Perhaps in accordance with the media from which they were derived from? These would be a summary of what is shown in Figures 1, 2, 3. This is in addition to the OneCodex analysis and the NCBI submission ID available in Supplementary Table 1 It also helps clarify the data shown in Figure 5.

- The Simpson's diversity index analysis shown in Figure 6 is extremely helpful to show the differences between the 3 media types in recovery.
- The results from qPCR of the homogenate that was collected from the Whirl-Pak bags and subjected to PathoSEEK. How did this compare with the colony identification of the same sample plated on the 3 different media with regards to the identification of the genus and species present? Can this be shown in a Table?
- The use of DRBC, if conducted by testing laboratories, is worrisome. It is known that the addition of dichloran and rose bengal is specifically used to discourage certain types of microbes from growing when used for recovery of specific types of fungi from soil samples. The inclusion of DRBC in a testing laboratory for cannabis TYM counts should be discouraged, as shown in the present work where total CFU's recovered were significantly lower compared to PDA with chloramphenicol. DRBC would significantly under-estimate the TYM counts as shown in Figure 4.
- In Table 2, the headings seem incorrect as there are two with "PDA with chloramphenicol" and one should be "PDA w/o chloramphenicol"
- Table 3 and Figure 7 clearly show how DRBC provides reduced growth compared to PDA.

Overall, this is an informative study and the results merit publication. Once the items identified by the reviewer are addressed, this study will be a good addition to the slowly expanding studies showing how complex the assessment of total yeast and mold levels in cannabis is. The information from these types of studies should guide government agencies on the pitfalls of certain methods used to assess TYMC.

Is the rationale for developing the new method (or application) clearly explained? $\ensuremath{\mathsf{Yes}}$

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, plant pathology, plant biology, cannabis pathogens, postharvest quality

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Aug 2021

Kevin McKernan, Medicinal Genomics, Beverly, USA

Thank you for this valuable feedback. I have interweaved our responses below and will update the manuscript accordingly:

- Add "human pathogenic" where pathogenic is mentioned as the concern is with these particular microbes and not those potentially that could be affecting the plant as plant pathogens.
- **Response:** Agree. Done
- The inclusion of the 3 selection media should be elaborated on. Why were these particular 3 media selected? Provide references to show where, or in what capacity, they may have been used in previously published work. There is abundant published work on the use of PDA with antibiotics to isolate fungi in the plant pathology literature. In fact, it is a standard medium used for isolation in labs worldwide. The addition of dichloran and rose bengal have also been used to restrict the growth of certain groups of fungi and bacteria as a semi-selective medium for isolation in particular from soil samples. Therefore, it would not be expected to provide a broad spectrum of recovery of fungi and yeasts. It is surprising that this medium would be used to assess total yeast and mold counts in cannabis.
- Response: We were not involved in the selection of these media types. The study was initiated on PDA in Michigan. After we completed the PDA study, the organizers informed us they were switching to DRBC based on Steadfast having used chloramphenicol based culture platforms to categorize the samples before shipping them to other labs in Michigan. We suspect the 3 media types were chosen due to their presence in the FDA BAM. We have added a sentence to clarify this.
- The comparison of the 3 media in this study sheds light on the differences in levels of recovery of fungi and yeasts. This is an important finding not all media behave in the same manner. To observe a 10-fold difference in recovery between these media is quite significant as it illustrates the potential for under-representation in the recovery process. The use of whole genome sequencing to apply to the identification of colony-forming units is a definite plus for this work. It shows the ability to rapidly identify what is present on the culture media with regard to molds that originated from the samples. There are several prior reports of authors having recovered a range of fungi from cannabis buds and identified them using the ITS region. Please include these as a reference by which to compare the fungi and yeasts identified in the present study. It is important to build a body of knowledge on the exact identity of the general and species found on cannabis and how prevalent they are.
- **Response:** Very good point. We have added a paragraph to describe the substantial

prior art here.

- The report of endophytes in cannabis should be accompanied by a reference citation. These particular microbes are more difficult to recover in culture media and therefore a molecular approach has merit.
- **Response:** Very good point. We have added references to emphasize Dichlorans inhibitory nature.
- The inclusion of confirmed ATCC culture specimens to demonstrate differences in growth on the 3 media is a good confirmatory experiment.
- Response: This is an important control but it should be known by people in the field that AOAC certification doesn't require the inclusion or exclusion testing to be performed in the presence of matrix and this inclusion and exclusion criteria can be obtained using a different media than DRBC. We believe this provides a false sense of safety. This is challenging to perform in the presence of matrix as there is no supplier of sterilized cannabis that ship across state lines and inclusion and exclusion testing can be impacted by background microbial content. Further legalization will improve this.
- The cannabis samples that originated from Steadfast Analytical Laboratories would have had an analysis of total yeast and mold conducted on them. Is it possible to have these results compared to those from the present study to show how the commercial lab testing may differ from the current study? Or was that not an objective of the current study?
- Response: As part of the AOAC study, we were blinded from these data. We do know that the high, medium and low categories were allocated according to culture-based methods that used CAMP and that quantitative PCR over estimated microbial burden on the low DRBC samples.
- During the preparation of samples for the ITS3 qPCR procedure, was there a subset of samples included that did not contain the TLP lysis step to show that it made a difference? Or is that included in prior published work?
- Response: We did not include TLP lysis versus no TLP as the study required we settle on a single method for evaluation. We are in the process of writing up that comparison for another publication.
- For the 45 colonies that were selected for whole genome sequencing, could the identified genus and species be presented in a separate table? Perhaps in accordance with the media from which they were derived from? These would be a summary of what is shown in Figures 1, 2, 3. This is in addition to the OneCodex analysis and the NCBI submission ID available in Supplementary Table 1 It also helps clarify the data shown in Figure 5.
- Response: This is an important point. This does exist in Figure 5 but we failed to clarify the sample nomenclature that clarifies this. We have added a sample key to describe which samples are DRBC, PDA-CAMP, PDA-no-CAMP.
- The Simpson's diversity index analysis shown in Figure 6 is extremely helpful to show the differences between the 3 media types in recovery. The results from qPCR of the homogenate that was collected from the Whirl-Pak bags and subjected to PathoSEEK. How

did this compare with the colony identification of the same sample plated on the 3 different media with regards to the identification of the genus and species present? Can this be shown in a Table? The use of DRBC, if conducted by testing laboratories, is worrisome. It is known that the addition of dichloran and rose bengal is specifically used to discourage certain types of microbes from growing when used for recovery of specific types of fungi from soil samples. The inclusion of DRBC in a testing laboratory for cannabis TYM counts should be discouraged, as shown in the present work where total CFU's recovered were significantly lower compared to PDA with chloramphenicol. DRBC would significantly under-estimate the TYM counts as shown in Figure 4.

Response: We were only allowed to ship cultures on plates across state lines. As a result, we have Cq scores for the colonies that were picked and isolated in Figures 1,2,3 under the TYM and TAC Cq columns on the right. This only informs on inclusion and exclusion capabilities of the primers for the colonies harvested but loses quantitative information. We were not allowed to ship homogenized matrix in the mail to assess the Cq prior to plating. Labs local to Michigan have performed this comparison and are free to publish those results. The summary of the results communicated to us were that the qPCR had better concordance with PDA with CAMP and over estimated CFUs on the DRBC Low samples. This significantly differs from Michigans stated intentions with the ERV where they voiced concerns about molecular methods undercounting risk

(https://help.medicinalgenomics.com/hubfs/Regulatory%20Info%20for%20Sales/Michigan%20MRA%2 The opposite turned out to be true. DRBC is undercounting risk compared to qPCR. The MRA was also led to believe that Klebsiella was not an appropriate validation organism as it was not commonly found on cannabis despite it having been published by Thompson et al previously. Candida albicans (which we have never seen documented on Cannabis) was prioritized as a CRM. We have added some language to address this to the best of our ability.

- In Table 2, the headings seem incorrect as there are two with "PDA with chloramphenicol" and one should be "PDA w/o chloramphenicol" Table 3 and Figure 7 clearly show how DRBC provides reduced growth compared to PDA.
- **Response:** Thank you!. Good catch. We have updated that.

Competing Interests: No competing interests were disclosed.

Reviewer Report 28 July 2021

https://doi.org/10.5256/f1000research.56852.r90094

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Cindy Orser

CLIP Laboratories, San Diego, CA, USA

A well-executed study of considerable breath and size to further solidify the argument against State regulatory bodies requiring agar plating to evaluate microbial load on cannabis flower in lieu of proven superiority of molecular assays. In addition, this study demonstrates confirmation of chloramphenicol's ability to knock down *Fusarium* and *Aspergillus* growth when plating on DRBC at relatively low chloramphenicol levels [~0.1 mg/mL], not sure of the concentration in PDA agar. Nonetheless, this study should finally put to rest the issue of which method is most accurate at representing microbial load on cured cannabis flower. With qPCR assays shown to be fundamentally superior to culturing and plating, the discussion should now move over to "sampling" and how inadequate the normal sample size per batch size is to give a glimpse into the microbiome of the cannabis flower and the intrigue of how cultivation methods influence the cannabis microbiome.

Is the rationale for developing the new method (or application) clearly explained? $\ensuremath{\mathsf{Yes}}$

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: plant microbiology; cannabis analytical testing; diagnostics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 10 Aug 2021

Kevin McKernan, Medicinal Genomics, Beverly, USA

Thank you for this feedback. The sampling comment is very pertinent. We were not in control of this aspect of the study so opted to save this topic for another manuscript. AOAC communicated to us that many different cannabis samples were mixed to normalize

chemotype affects on culture. This is a very good idea as we have seen chemotype specific effects on cannabis microbiology and its published to occur in *Trema orientalis* (https://pubmed.ncbi.nlm.nih.gov/34035994/).

One of the concerns with plating, is that the antibiotic cannabinoids and terpenes may get liberated from trichomes in the aggressive lab homogenization and media saturation process. This may influence the viability of some of the microbes. This aggressive homogenization and fluid saturation is not what a consumer experiences.

Many publications demonstrate the antibiotic nature of cannabinoids and how different cannabinoids exhibit different antibiotic properties thus we should expect different chemotypes to plate differently given there is no purification step prior to plating (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7105690/). Molecular methods lyse open cells and purify the DNA away from such potential growth inhibitors with Ethanol extractions.

To confirm these samples were indeed a mixture of different cannabis samples, we were sent DNA from these mixtures and performed 10Mb SureSelect capture and deep Illumina Sequencing on these samples to under stand how well they were mixed and how diverse they where. The read genotypes indeed suggested more than a single cannabis sample was present and likely more than 4 in each High, Medium and Low Categories.

We put these data public for anyone who is interested but felt it would bloat this manuscript with confirmatory data and distract from the core focus of the study.

https://www.kannapedia.net/strains/rsp11748/ https://www.kannapedia.net/strains/rsp11749/ https://www.kannapedia.net/strains/rsp11750/ https://www.kannapedia.net/strains/rsp11753/ https://www.kannapedia.net/strains/rsp11754/ https://www.kannapedia.net/strains/rsp11755/ https://www.kannapedia.net/strains/rsp11756/ https://www.kannapedia.net/strains/rsp11757/ https://www.kannapedia.net/strains/rsp11758/ https://www.kannapedia.net/strains/rsp11758/ https://www.kannapedia.net/strains/rsp11759/ https://www.kannapedia.net/strains/rsp11760/ https://www.kannapedia.net/strains/rsp11760/ https://www.kannapedia.net/strains/rsp11761/ https://www.kannapedia.net/strains/rsp11760/

Competing Interests: No additional conflicts than those already described in the manuscript.

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