



Draft Genome Sequences of Fungi Isolated from Mars 2020 Spacecraft Assembly Facilities

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ABSTRACT During the Mars 2020 mission, several fungal strains were isolated from surfaces where spacecraft components were assembled. Draft genome sequencing and characterization will help identify the genes responsible for radiation resistance, supporting the development of countermeasures to prevent fungal contamination of extraterrestrial environments.

NASA planetary protection (PP) assays traditionally focus on the detection of endospore-forming bacteria (1, 2) because these can potentially survive on spacecraft launched to other planets. However, fungi and their conidia are other likely candidates for enduring spaceflight and other extreme space environmental conditions (3, 4). Identification and characterization of fungal isolates associated with spacecraft environments are essential to developing countermeasures for preventing fungal contamination on future exoplanet missions. As part of this effort, during microbial monitoring of the Mars 2020 cleanrooms, 26 fungal strains were isolated, and whole-genome sequencing (WGS) was carried out.

Sample collection, fungal isolation, and identification steps were performed as previously described (5). Briefly, samples were collected from cleanroom surfaces using moist polyester wipes and were agitated in sterile phosphate-buffered saline (PBS). The resulting PBS suspension was concentrated using an InnovaPrep CP150 concentrating pipette and spread onto plates containing different media (dichloran rose bengal agar, potato dextrose agar [PDA], and dichloran glycerol agar). Plates were incubated at 25°C for 7 to 14 days, and fungal strains were isolated, purified, and stored at –80°C for further identification. Under the same growth conditions, fungal isolates were revived on PDA plates to obtain pure culture and produce biomass for DNA isolation followed by internal transcribed spacer (ITS) region gene sequencing. The PDA plate-grown culture was scraped, and genomic DNA extraction was performed using the ZymoBIOMICS MagBead DNA kit (catalog no. D4302; Zymo Research, Irvine, CA). The DNA WGS libraries were prepared with an Illumina Nextera DNA Flex library preparation kit (6) and sequenced on the NovaSeq 6000 paired-end 2 × 150-bp platform using an S4 flow cell. FastQC v.0.11.7 and Fastp v.0.20.0 were used to filter and trim the reads, and the genomes were assembled using SPAdes v.3.11.1 (7, 8).

Amplicons of the ITS region were amplified from the DNA for all strains and sequenced, except for the strain FJI-L2-BK-P3 (5). Genus-level identification was made via BLAST searches against the NCBI ITS database (9). Subsequently, four additional secondary marker genes (*GAPDH*, *CaM*, *Tef1a*, and *BenA*) were extracted from WGS for species-level identification (10). Species-level identification was performed using a specific secondary marker gene suitable for each genus type as per recent recommendations and literature (Table 1) (10–15). The extracted marker genes were BLAST searched against NCBI nucleotide database (sequences from type material filter applied) to obtain the best percentage of identity matches, based on which the species names were assigned. Marker gene-based analysis of the 26 fungal

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TABLE 1 Accession numbers, sampling locations, and assembly details for fungal strains isolated from the NASA spacecraft assembly facilities

Sample/strain name	Fungal species	Secondary marker gene used for identification ^a	% identity with secondary marker gene	Accession no.		SRA	Medium used	Facility ^b	No. of contigs	Genome size (bp)	N ₅₀ (bp)	Depth of coverage	G+C content (%)	No. of filtered reads
				ITS	WGS									
FKIL-L2-CM-DRAB1	<i>Alternaria alternata</i>	<i>GAPDH</i>	100.0	MT704920	JAKLNU0000000000	SRRI8739383	Dichloran rose bengal agar	KSC-PHSF	137	34,215,686	1,397,744	115	51.05	2,054,427
FKIL-L8-BK-P2B	<i>Alternaria alternata</i>	<i>GAPDH</i>	100.0	MT704925	JAKLNU0000000000	SRRI8739377	Potato dextrose agar	KSC-PHSF	105	33,359,331	1,380,845	55	51.3	2,650,675
FKIL-L7-BK-DRAB2	<i>Aspergillus calidoustus</i>	<i>CalM</i>	99.8	MT704900	JAKLMU0000000000	SRRI8739397	Dichloran rose bengal agar	KSC-PHSF	619	61,805,297	569,410	86	52.38	3,275,987
FKIL-L2-CM-DR1	<i>Aspergillus calidoustus</i>	<i>CalM</i>	99.5	MT704921	JAKLMU0000000000	SRRI8739382	Dichloran rose bengal agar	KSC-PHSF	313	39,874,905	569,221	154	51.11	6,174,864
FKIL-L3-BK-PAB1	<i>Aspergillus calidoustus</i>	<i>CalM</i>	99.5	MT704923	JAKLNM0000000000	SRRI8739379	Potato dextrose agar	KSC-PHSF	371	40,055,042	622,573	131	51.11	5,891,335
FKIL-L3-BK-PAB1	<i>Aspergillus calidoustus</i>	<i>CalM</i>	99.8	MT704928	JAKLNR0000000000	SRRI8739374	Potato dextrose agar	KSC-PHSF	385	40,340,333	676,118	123	51.19	7,181,415
FKIL-L3-BK-DRAB4	<i>Aspergillus calidoustus</i>	<i>CalM</i>	99.8	MT704929	JAKLNS0000000000	SRRI8739373	Dichloran rose bengal agar	KSC-PHSF	301	40,773,058	742,271	149	51.02	8,633,370
FKIL-L6-BK-DRAB1	<i>Aspergillus tubingensis</i>	<i>CalM</i>	99.6	MT704898	JAKLMT0000000000	SRRI8739398	Dichloran rose bengal agar	KSC-PHSF	429	40,391,628	505,550	186	51.06	10,486,873
FJIL-L3-CM-P3	<i>Aureobasidium pullulans</i>	<i>ITS</i>	100.0	MT704926	JAKLNP0000000000	SRRI8739376	Potato dextrose agar	KSC-PHSF	88	35,008,407	662,607	168	49.13	5,784,450
FKIL-L8-BK-P1	<i>Exophiala oligosperma</i>	<i>ITS</i>	99.5	MT704887	JAKLML0000000000	SRRI8739407	Potato dextrose agar	JPL-SAF	258	28,497,861	345,423	45	50.30	8,272,240
FJIL-L2-BK-P3	<i>Naganishia albidia</i>	<i>Tef1a</i>	96.5	MT704904	JAKLMV0000000000	SRRI8739396	Potato dextrose agar	KSC-PHSF	373	42,391,843	426,265	202	52.39	11,716,730
FJIL-L4-SW-DR1	<i>Penicillium bifforme</i>	<i>Bena</i>	98.6	MT704880	JAKLLX0000000000	SRRI8739403	Dichloran rose bengal agar	JPL-SAF	478	27,861,060	190,774	323	53.93	2,221,225
FJIL-L5-SW-P3	<i>Penicillium bifforme</i>	<i>Bena</i>	98.6	MT704892	JAKLMO0000000000	SRRI8739404	Potato dextrose agar	JPL-SAF	1,188	35,656,037	154,974	221	48.01	5,288,969
FKIL-L9-SW-P1	<i>Penicillium brevicompactum</i>	<i>Bena</i>	98.4	MT704911	JAKLNP0000000000	SRRI8739391	Potato dextrose agar	KSC-PHSF	824	35,881,231	203,931	234	47.96	11,713,998
FJIL-L10-BK-P2	<i>Penicillium cerasidense</i>	<i>Bena</i>	94.5	MT704893	JAKLMO0000000000	SRRI8739402	Potato dextrose agar	JPL-SAF	510	31,228,461	280,015	308	49.50	5,806,819
FJIL-L3-BK-DG1	<i>Penicillium cerasidense</i>	<i>Bena</i>	94.5	MT704870	JAKLLZ0000000000	SRRI8739381	Potato dextrose agar	JPL-SAF	339	34,882,906	572,968	222	46.89	10,657,997
FJIL-L3-CM-PAB4	<i>Penicillium decumbens</i>	<i>Bena</i>	97.8	MT704897	JAKLMA0000000000	SRRI8739371	Dichloran glycerol agar	JPL-SAF	326	34,674,451	561,144	209	47.07	4,351,551
FKIL-L3-CM-DRAB1	<i>Penicillium decumbens</i>	<i>Bena</i>	99.2	MT704917	JAKLMS0000000000	SRRI8739399	Potato dextrose agar	JPL-SAF	216	24,008,368	508,196	327	50.43	4,033,252
FKIL-L2-CM-P1	<i>Talaromyces louisianensis</i>	<i>Bena</i>	99.8	MT704919	JAKLNU0000000000	SRRI8739385	Dichloran rose bengal agar	KSC-PHSF	281	26,798,112	500,337	217	50.02	6,316,652
FKIL-L2-CM-DRAB3	<i>Talaromyces louisianensis</i>	<i>Bena</i>	99.8	MT704922	JAKLNU0000000000	SRRI8739384	Potato dextrose agar	KSC-PHSF	364	35,494,196	689,550	114	46.07	4,017,393
FKIL-L3-BK-DRAB3	<i>Talaromyces ruber</i>	<i>Bena</i>	100.0	MT704905	JAKLMW0000000000	SRRI8739380	Dichloran rose bengal agar	KSC-PHSF	385	35,499,968	735,697	108	46.07	6,621,100
FJIL-L7-BK-P1	<i>Trichoderma rugosum</i>	<i>Tef1a</i>	96.4	MT704867	JAKLLV0000000000	SRRI8739395	Dichloran rose bengal agar	KSC-PHSF	346	30,034,193	296,592	357	46.42	10,773,815
FJIL-L10-BK-P1	<i>Trichoderma rugosum</i>	<i>Tef1a</i>	96.4	MT704875	JAKLMD0000000000	SRRI8739415	Potato dextrose agar	JPL-SAF	341	32,491,321	269,895	316	53.23	3,744,657
FJIL-L10-BK-P3	<i>Trichoderma rugosum</i>	<i>Tef1a</i>	96.4	MT704876	JAKLME0000000000	SRRI8739368	Potato dextrose agar	JPL-SAF	291	32,566,608	265,352	223	53.15	4,289,739
						SRRI8739367	Potato dextrose agar	JPL-SAF	240	32,648,028	288,145	238	53.06	3,683,568

^a Identification was carried out as per recent literature and Houbraken et al. (10–15).

^b KSC-PHSF, Kennedy Space Center-Payload Hazardous Servicing Facility; JPL-SAF, Jet Propulsion Laboratory-Spacecraft Assembly Facility.

genomes revealed the presence of nine genera and 13 fungal species. The majority of the species identified belonged to the genera *Penicillium* (4 species; 8 strains) and *Aspergillus* (2 species; 7 strains); the other seven genera each had a single species representative.

In addition to radiation resistance and producing conidia that can withstand harsh environmental conditions, the fungi isolated in this study are also of clinical relevance (16–19). *Alternaria alternata* is highly UVC resistant compared to other common environmental fungi, such as *Aspergillus* and *Penicillium* species (20). The effects of UV on *Aspergillus calidoustus* are not yet reported; however, *Aspergillus niger* exhibits UV resistance (21). Similarly, members of *Exophiala* have high resistance to UVC, UVB, and environmental UV radiation (22). *Aureobasidium pullulans* belongs to a class of black yeasts that are resistant to UV and gamma irradiation (23, 24). Some strains of *Naganishia albida* are also resistant to UVA, UVB, and UVC (15).

UV resistance in the genus *Penicillium* varies, depending on the species and strain (25, 26). The members of *Talaromyces* are also resistant to UV radiation (27, 28). Some species of *Trichoderma* are resistant to UVC, and UV-inactivated spores show photoreactivation (29). The phenomenon of photoreactivation in such species is relevant to planetary protection and needs further attention.

Data availability. The WGSs and raw data have been deposited in GenBank under BioProject accession no. [PRJNA800051](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA800051). This project has also been deposited in the NASA GeneLab system (30) under project no. GLDS-497. The version described in this article is the first version.

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