



Draft Genome Sequences of *Aspergillus* and *Penicillium* Species Isolated from the International Space Station and Crew Resupply Vehicle Capsule

Adriana Blachowicz,^a Nitin Kumar Singh,^a Jason M. Wood,^a Marilyne Debieu,^b Niamh B. O'Hara,^b Christopher E. Mason,^{c,d} Kasthuri Venkateswaran^a

^aBiotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA

^bBiotia, New York, New York, USA

^cDepartment of Physiology and Biophysics, Weill Cornell Medicine, New York, New York, USA

^dWorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, New York, USA

ABSTRACT The draft whole-genome sequences (WGS) of 30 fungal strains isolated from the International Space Station and belonging to the *Penicillium* and *Aspergillus* genera were assembled. The WGS will allow for detailed genomic characterization to determine the possible applications and importance for space and biotechnological industries.

During an ongoing microbial tracking study of the International Space Station (ISS), 30 strains representing seven species belonging to the *Penicillium* ($n = 5$) and *Aspergillus* ($n = 2$) genera were isolated (1), and whole-genome sequences (WGS) were generated. The *Penicillium* genus encompasses more than 350 species found worldwide in soil, vegetation, air, indoor environments, and food (2). *Aspergillus* species are saprophytes found in a variety of environmental niches; however, some of them are also opportunistic human pathogens (3, 4). Members of the *Penicillium* and *Aspergillus* genera are of economic and industrial importance, including *Penicillium chrysogenum*, which is used to produce β -lactam antibiotics (5), *Penicillium camemberti*, which is used for maturation of soft cheeses such as Camembert, Brie, and Neufchatel (6), and *Aspergillus niger*, which is a known producer of citric acid (7, 8). Recently, *Penicillium polonicum* has been shown to tolerate and effectively remove lead (Pb) from polluted water, indicating a promising solution for new remediation strategies to purify contaminated water (9). Lastly, *Aspergillus unguis*, *Penicillium dipodomycola*, and *Penicillium griseoroseum* have been reported to produce novel bioactive compounds (10–13). Considering existing and yet-to-be uncovered, versatile applications of *Penicillium* and *Aspergillus* species, characterization of WGS of these fungi is critical. Additionally, since fungi are excellent models for studying evolution and adaptation, due to their experimental features (14), the ISS-isolated species are of unique significance for investigating how microgravity and irradiation affect them when compared to their ground counterparts.

The procedure for collecting samples and performing consecutive processing steps has been described elsewhere (1). Briefly, surface samples were collected with premoistened polyester wipes and then resuspended in 200 ml of sterile phosphate-buffered saline (PBS) with vigorous shaking. The extracted samples were concentrated using InnovaPrep (Drexel, MO) CP-150 concentrating pipettes. Aliquots (100 μ l) of each sample were plated onto potato dextrose agar (PDA) containing 100 μ g/ml chloramphenicol and incubated at 25°C for 7 days. A single colony was picked, restreaked onto PDA, and incubated at 25°C for 7 days. About 1 μ g of biomass grown overnight was collected and used for DNA extraction with the ZymoBIOMICS DNA MagBead kit (Zymo

Citation Blachowicz A, Singh NK, Wood JM, Debieu M, O'Hara NB, Mason CE, Venkateswaran K. 2021. Draft genome sequences of *Aspergillus* and *Penicillium* species isolated from the International Space Station and crew resupply vehicle capsule. *Microbiol Resour Announc* 10:e01398-20. <https://doi.org/10.1128/MRA.01398-20>.

Editor Antonis Rokas, Vanderbilt University

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply.

Address correspondence to Kasthuri Venkateswaran, kjvenkat@jpl.nasa.gov.

Received 9 December 2020

Accepted 7 March 2021

Published 1 April 2021

TABLE 1 Summary of the draft WGS of 30 *Aspergillus* and *Penicillium* strains isolated from the ISS

Strain ^a	Species identified based on ITS gene	Species identified based on calmodulin gene	Species identified based on β -tubulin gene	GenBank accession no.	SRA accession no.	Isolation location ^b	No. of scaffolds	Genome size (bp)	N_{50} (bp)	Median coverage (x)	G+C content (%)	No. of filtered reads used for assembly (million)
F3-1F3-F	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	JADBH10000000000	SRR12819683	Cupola	75	35,865,776	1,033,225	55	49.46	22.16
F3-4F1-F	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	JADBH10000000000	SRR12819679	Dining table	342	37,533,732	885,952	64	49.25	25.45
F3-4F2-F	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	JADBH10000000000	SRR12819669	Dining table	361	37,317,966	948,069	51	49.41	21.20
IIF6SW-F2	<i>Aspergillus unguis</i>	<i>Aspergillus unguis</i>	<i>Aspergillus unguis</i>	JADBGV10000000000	SRR12819688	PMM	20	25,892,012	2,741,393	93	50.3	26.47
IIF2SW-F1	<i>Penicillium camemberti</i>	<i>Penicillium camemberti</i>	<i>Penicillium biforme</i>	JACSPF000000000	SRR12825356	WHC	834	35,011,981	266,841	56	47.69	23.59
IIF8SW-F2	<i>Penicillium camemberti</i>	<i>Penicillium biforme</i>	<i>Penicillium biforme</i>	JACSPQ000000000	SRR12825359	Crew quarters	846	35,011,981	262,211	55	47.7	22.46
IIF8SW-F3	<i>Penicillium camemberti</i>	<i>Penicillium biforme</i>	<i>Penicillium biforme</i>	JACSOQ000000000	SRR12825358	Crew quarters	828	35,011,981	256,141	53	47.69	22.37
F3-3F1-F	<i>Penicillium camemberti</i>	<i>Penicillium biforme</i>	<i>Penicillium biforme</i>	JADBH10000000000	SRR12819582	ARED	1,098	36,654,083	303,871	56	47.69	22.97
IIF1SG-B2	<i>Penicillium camemberti</i>	<i>Penicillium biforme</i>	<i>Penicillium biforme</i>	JADBG50000000000	SRR12819681	Outside CRV	676	33,342,700	1,054,379	71	48.81	26.71
IIF1SW-F3	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSPG000000000	SRR12825368	Cupola	764	32,524,241	638,283	57	48.92	21.32
IIF2SG-B2	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBG10000000000	SRR12819680	Outside CRV	648	33,347,836	931,557	93	48.81	34.88
IIF2SW-F4	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSPPE000000000	SRR12825355	WHC	1,277	32,524,241	640,412	41	48.76	16.80
IIF2SW-F5	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSPD000000000	SRR12825354	WHC	445	33,524,241	718,663	82	48.96	28.82
IIF3SW-F3	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSPB000000000	SRR12825352	ARED	843	32,524,241	497,770	42	48.94	15.73
IIFASG-B1	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBGU000000000	SRR12819689	Inside CRV	651	33,345,064	939,642	76	48.81	28.76
IIF4SW-F1	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSPA000000000	SRR12825351	Dining table	481	32,524,241	947,115	54	48.96	18.77
IIF7SW-F1	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSOZ020000000	SRR12825350	LAB	485	32,524,241	782,807	72	48.96	25.44
IIF2TSW-F2	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBGW000000000	SRR12819687	WHC	460	32,073,134	709,694	64	48.99	22.76
IIF3TSW-F2	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSOU000000000	SRR12825364	ARED	1,276	32,524,241	643,620	48	48.76	19.83
IIF8SW-F4	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JACSON000000000	SRR12819587	Crew quarters	434	32,524,241	1,028,283	74	48.96	26.01
F3-2F3-F	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBGX000000000	SRR12819686	WHC	353	31,592,617	579,954	77	48.84	26.27
F3-2F4-F	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBGY100000000	SRR12819685	WHC	568	32,430,262	701,540	99	48.94	34.71
F3-2F5-F	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>	JADBGZ000000000	SRR12819684	WHC	486	32,607,577	1,028,360	66	48.84	23.62
IIF7SW-F3	<i>Penicillium dipodomyica</i>	<i>Penicillium dipodomyica</i>	<i>Penicillium chrysogenum</i>	JACSOY000000000	SRR12825349	LAB	1,372	32,524,241	578,872	45	48.81	17.99
IIF7SW-F2	<i>Penicillium dipodomyica</i>	<i>Penicillium dipodomyica</i>	<i>Penicillium chrysogenum</i>	JACSOZ000000000	SRR12825361	LAB	431	32,524,241	842,068	76	48.99	27.38
IIF3SW-F4	<i>Penicillium griseoroseum</i>	<i>Penicillium griseoroseum</i>	<i>Penicillium chrysogenum</i>	JACSPC000000000	SRR12825353	ARED	559	32,524,241	585,026	71	48.99	25.46
IIF7SW-F5	<i>Penicillium griseoroseum</i>	<i>Penicillium griseoroseum</i>	<i>Penicillium chrysogenum</i>	JACSW000000000	SRR12825366	LAB	361	32,524,241	577,257	61	48.99	24.14
IIF4SW-F4	<i>Penicillium griseoroseum</i>	<i>Penicillium griseoroseum</i>	<i>Penicillium chrysogenum</i>	JACSOQ000000000	SRR12825362	Dining table	348	32,524,241	492,361	39	48.85	17.39
IIF1SW-F3	<i>Penicillium polonicum</i>			JACSPV000000000	SRR12825365	Cupola	464	32,524,241	481,150	80	48.85	28.75

^aF1, flight 1; F2, flight 2; F3, flight 3; F4, flight 4; F5, flight 5; SG, surface wipe from ISS environment. F and B at the end are strain numbers.^bPMM, permanent multipurpose module; WHC, waste and hygiene compartment; ARED, advanced resistive exercise device; CRV, crew resupply vehicle; LAB, panel near portable water dispenser.

Corp., Irvine, CA). To acquire the WGS, shotgun libraries were prepared following the Illumina Nextera Flex protocol (15), and paired-end sequencing of 30 strains was performed on a NovaSeq 6000 S4 flow cell paired-end 2 × 150-bp platform. The quality of the raw reads obtained was confirmed with FastQC (v0.11.7) (16). Assessment of the quality filtering steps and adapter removal were performed using fastp (v0.20.0) (17). The cleaned sequences were assembled with SPAdes using the automatic coverage cutoff value (v3.11.1) (18). Assembly quality, number of contigs, N_{50} values, and genome size were calculated using QUAST (v5.0.2). Default parameters were used for all software. The species were identified based on the internal transcribed spacer (ITS), calmodulin, and β -tubulin sequences extracted from the assembled genomes. The details of the final assemblies and phylogenetic identification are summarized in Table 1.

Data availability. The WGS and raw data have been deposited in GenBank under the BioProject accession numbers PRJNA659567 and PRJNA667181. This project has also been deposited in the NASA GeneLab system (<https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-350/>). The versions described in this paper are the first versions.

ACKNOWLEDGMENTS

We thank astronauts Captain Terry Virts and Commander Jeffrey Williams for collecting samples aboard the ISS, the Implementation Team (Fathi Karouia) at NASA Ames Research Center for coordinating this effort for collecting samples from the ISS and CRV, and Aleksandra Chechinska-Sielaff for isolation of the strains. We thank Ryan Kemp (Zymo Corp.) for extracting the DNA and Dan Butler (Weill Cornell Medicine) for generating the shotgun sequencing. We also thank Anna Simpson for bioinformatic help. The Jet Propulsion Laboratory supercomputing facility staff, notably, Narendra J. (Jimmy) Patel and Edward Villanueva, is acknowledged for continuous support in providing the best possible infrastructure for BIG-DATA analysis.

Part of the research described was carried out at the Jet Propulsion Laboratory of the California Institute of Technology under a contract with NASA. This research was funded by 2012 Space Biology NNH12ZTT001N grant 19-12829-26 under task order NNN13D111T awarded to K.V., which also funded a postdoctoral fellowship for A.B.

REFERENCES

1. Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R, Karouia F, Fox GE, Venkateswaran K. 2019. Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* 7:50. <https://doi.org/10.1186/s40168-019-0666-x>.
2. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. 2014. Identification and nomenclature of the genus *Penicillium*. *Stud Mycol* 78:343–371. <https://doi.org/10.1016/j.simyco.2014.09.001>.
3. Mousavi B, Hedayati M, Hedayati N, Ilkit M, Syedmousavi S. 2016. *Aspergillus* species in indoor environments and their possible occupational and public health hazards. *Curr Med Mycol* 2:36–42. <https://doi.org/10.18869/acadpub.cmm.2.1.36>.
4. Pitt JI. 1994. The current role of *Aspergillus* and *Penicillium* in human and animal health. *Med Mycol* 32:17–32. <https://doi.org/10.1080/02681219480000701>.
5. Guzmán-Chávez F, Zwahlen RD, Bovenberg RAL, Driessens AJM. 2018. Engineering of the filamentous fungus *Penicillium chrysogenum* as cell factory for natural products. *Front Microbiol* 9:2768. <https://doi.org/10.3389/fmicb.2018.02768>.
6. Ropars J, Didiot E, de la Vega RCR, Bennetot B, Coton M, Poirier E, Coton E, Snirc A, Le Prieur S, Giraud T. 2020. Domestication of the emblematic white cheese-making fungus *Penicillium camemberti* and its diversification into two varieties. *Curr Biol* 30:P4441–4453. <https://doi.org/10.1016/j.cub.2020.08.082>.
7. Dhillon GS, Brar SK, Verma M, Tyagi RD. 2011. Utilization of different agro-industrial wastes for sustainable bioproduction of citric acid by *Aspergillus niger*. *Biochem Eng J* 54:83–92. <https://doi.org/10.1016/j.bej.2011.02.002>.
8. Max B, Salgado JM, Rodríguez N, Cortés S, Converti A, Domínguez JM. 2010. Biotechnological production of citric acid. *Braz J Microbiol* 41:862–875. <https://doi.org/10.1590/S1517-83822010000400005>.
9. Xu X, Hao R, Xu H, Lu A. 2020. Removal mechanism of Pb(II) by *Penicillium polonicum*: immobilization, adsorption, and bioaccumulation. *Sci Rep* 10:9079. <https://doi.org/10.1038/s41598-020-66025-6>.
10. Wang D, Bao Y-R, Yang X-X, Meng X-S, Chen G. 2015. A new alkaloid from *Penicillium dipodomyicola*. *Chem Nat Compd* 51:733–735. <https://doi.org/10.1007/s10600-015-1395-4>.
11. Hamed AA, Abdel-Aziz MS, Abd El Hady FK. 2018. Antimicrobial and anti-oxidant activities of different extracts from *Aspergillus unguis* SPMD-EGY grown on different media. *Bull Natl Res Cent* 42:29. <https://doi.org/10.1186/s42269-018-0027-0>.
12. Klaiklay S, Rukachaisirikul V, Aungphao W, Phongpaichit S, Sakayaroj J. 2016. Depsidone and phthalide derivatives from the soil-derived fungus *Aspergillus unguis* PSU-RSPG199. *Tetrahedron Lett* 57:4348–4351. <https://doi.org/10.1016/j.tetlet.2016.08.040>.
13. Banani H, Marcat-Houben M, Ballester A-R, Abbruscato P, González-Candela L, Gabaldón T, Spadaro D. 2016. Genome sequencing and secondary metabolism of the postharvest pathogen *Penicillium griseofulvum*. *BMC Genomics* 17:19. <https://doi.org/10.1186/s12864-015-2347-x>.
14. Gladieux P, Ropars J, Badouin H, Branca A, Aguilera G, de Vienne DM, Rodriguez de la Vega RC, Branco S, Giraud T. 2014. Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Mol Ecol* 23:753–773. <https://doi.org/10.1111/mec.12631>.
15. Singh NK, Bezdan D, Checinska Sielaff A, Wheeler K, Mason CE, Venkateswaran K. 2018. Multi-drug resistant *Enterobacter bugandensis* species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains. *BMC Microbiol* 18:175. <https://doi.org/10.1186/s12866-018-1325-2>.

16. Andrews S. 2019. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
17. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ pre-processor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
18. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotnik AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.