



Draft Genome Sequences of *Tremellomycetes* Strains Isolated from the International Space Station

Swati Bijlani,^a Nitin K. Singh,^b Christopher E. Mason,^c Clay C. C. Wang,^a ^(b)Kasthuri Venkateswaran^b

^aUniversity of Southern California, Los Angeles, California, USA ^bJet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA ^cThe WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, New York, USA

ABSTRACT The draft genome sequences of six eukaryotic microbial strains belonging to the class *Tremellomycetes* isolated from the International Space Station were assembled. Further characterization of these sequences will aid in the understanding of the influence of microgravity conditions on these organisms' potential pathogenicity.

n an ongoing microbial observatory experiment on the International Space Station (ISS), species belonging to the class *Tremellomycetes* were identified (1). This class comprises yeasts, dimorphic fungi, and organisms that form hyphae or complex fruiting bodies (2). In this class, the genera *Naganishia* and *Papiliotrema* were amended to accommodate the *Cryptococcus albidus* clade and a few other *Cryptococcus* species, respectively (3). Among the *Cryptococcus* species, *Cryptococcus neoformans* and *Cryptococcus gattii* are the most common human pathogens; however, recently, there has been an increase in infections caused by non-*neoformans Cryptococcus* species (4–8). This report presents the draft genome assemblies of four such non-*neoformans Cryptococcus* species, enabling the identification of genetic determinants responsible for their potential pathogenicity under the influence of microgravity compared to their ground controls.

In this study, the draft genome sequences of six strains belonging to the class Tremellomycetes isolated from the ISS were determined (1). Descriptions of the sample collection, processing, and presumptive identification of these isolates were published elsewhere (1). Briefly, samples collected from the ISS were processed, and 100 μ l of each dilution was plated onto potato dextrose agar (PDA) with 100 μ g/ml chloramphenicol. The plates were incubated at 25°C for 7 days. The single colony obtained was restreaked onto PDA plates and incubated at 25°C for 3 days, and a biomass of approximately 1 μ g wet weight was collected and pooled for DNA extraction. Total nucleic acid extraction was carried out using a ZymoBIOMICS 96 MagBead DNA kit (Lysis tubes) (Zymo Research, USA) after bead beating using a Bertin Precellys homogenizer. This was followed by library preparation using the Illumina Nextera Flex protocol as per Illumina document number 100000025416 v07. The initial amount of DNA for library preparation was quantified, and depending on the input DNA concentration, 5 to 12 cycles of PCR were carried out to normalize the output. The amplified genomic DNA fragments were indexed and pooled in a 384-plex configuration. Whole-genome shotgun sequencing was performed on a NovaSeq 6000 S4 flow cell paired-end (PE) 2 imes 150-bp platform with a paired-end module. The data were filtered with the NGS QC Toolkit v2.3 (9) for high-quality (HQ) vectorand adaptor-free reads for genome assembly (cutoff read length for HQ, 80%; cutoff quality score, 20). The numbers of filtered reads obtained are listed in Table 1, and they were used for assembly with the SPAdes v3.14.1 (10) genome assembler (k-mer

Citation Bijlani S, Singh NK, Mason CE, Wang CCC, Venkateswaran K. 2020. Draft genome sequences of *Tremellomycetes* strains isolated from the International Space Station. Microbiol Resour Announc 9:e00504-20. https://doi.org/10.1128/MRA.00504-20.

Editor Vincent Bruno, University of Maryland School of Medicine

Copyright © 2020 Bijlani et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

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

Received 1 May 2020 Accepted 2 June 2020 Published 25 June 2020

Species and strain	NCBI accession no.	Isolation location	No. of scaffolds	Genome size (bp)	N ₅₀ (bp)	Median coverage (×)	G+C content (%)	No. of filtered reads used for assembly (million)
Naganishia adeliensis IF1SW-F1	JAAZPZ00000000.1	Port panel next	149	19,403,212	506,784	134	53.37	14.71
		to cupola						
Naganishia albida IF6SW-B1	JAAZPV000000000.1	PMM port 1 ^a	128	19,422,953	506,783	303	53.37	34.76
Naganishia sp. IF7SW-B1	JAAZQA00000000.1	Lab overhead 3	123	19,429,219	511,828	286	53.37	32.79
Naganishia albida IIF5SW-F1	JAAZPY00000000.1	Node 1 overhead 4	136	19,428,531	506,790	258	53.37	30.81
Papiliotrema laurentii IF7SW-B5	JAAZPW000000000.1	Lab overhead 3	155	19,200,339	389,502	234	56.19	27.19
Papiliotrema laurentii IF7SW-F4	JAAZPX00000000.1	Lab overhead 3	128	19,020,389	484,400	270	56.22	30.21

TABLE 1 Summary of the draft whole-genome sequences of six strains belonging to the class Tremellomycetes isolated from the ISS

^a PMM port 1, permanent multipurpose module.

size, 32 to 72 bases). Default parameters were used for all software. The details of the final assembly are summarized in Table 1.

The species were identified based on the internal transcribed spacer (ITS) sequences extracted from the assembled genomes. The ITS sequence of *Naganishia* sp. strain IF7SW-B1 retrieved from the assembled genome did not show \geq 98% identity to any *Naganishia* species and, therefore, requires taxonomic characterization. Isolation of *Naganishia* and *Papiliotrema* species from the ISS is significant, and their persistence during space flight needs to be further studied.

Data availability. The whole-genome sequences and raw data have been deposited in GenBank under the BioProject accession number PRJNA623412. This project has also been deposited in the NASA GeneLab system (GLDS-290; https://genelab-data.ndc..nasa.gov/genelab/accession/GLDS-290). The version described in this paper is the first version.

ACKNOWLEDGMENTS

Part of the research described in this publication was carried out at the Jet Propulsion Laboratory of the California Institute of Technology under a contract with NASA. We thank astronaut Captain Terry Virts for collecting samples aboard the ISS, the implementation team at NASA Ames Research Center (Fathi Karouia) for coordinating this effort, and Aleksandra Chechinska-Sielaff for the isolation of the strains.

Government sponsorship is acknowledged. This research was funded by 2012 Space Biology NNH12ZTT001N grant number 19-12829-26 under task order NNN13D111T awarded to K.V., which was also subcontracted to C.C.C.W. and funded the postdoctoral fellowship for S.B.

We thank Ryan Kemp, Zymo Corporation, for extracting the DNA and Dan Butler, Cornell Medicine, for generating the shotgun sequencing. The JPL supercomputing facility staff is acknowledged, notably, Narendra J. Patel (Jimmy) and Edward Villanueva, for their continuous support in providing the best possible infrastructure for BIG-DATA analysis.

REFERENCES

- 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.
- Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson K-H, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD,

Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss W, White M, Winka K, Yao Y-J, Zhang N. 2007. A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547. https://doi.org/10.1016/j .mycres.2007.03.004.

- Liu XZ, Wang QM, Goker M, Groenewald M, Kachalkin AV, Lumbsch HT, Millanes AM, Wedin M, Yurkov AM, Boekhout T, Bai FY. 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. Stud Mycol 81:85–147. https://doi.org/10.1016/j.simyco.2015.12.001.
- Aghaei Gharehbolagh S, Nasimi M, Agha Kuchak Afshari S, Ghasemi Z, Rezaie S. 2017. First case of superficial infection due to *Naganishia albida* (formerly *Cryptococcus albidus*) in Iran: a review of the literature. Curr Med Mycol 3:33–37.
- 5. Londero MR, Zanrosso CD, Corso LL, Michelin L, Soldera J. 2019.

Catheter-related infection due to *Papiliotrema laurentii* in an oncologic patient: case report and systematic review. Braz J Infect Dis 23:451–461. https://doi.org/10.1016/j.bjid.2019.10.005.

- Tintelnot K, Losert H. 2005. Isolation of *Cryptococcus adeliensis* from clinical samples and the environment in Germany. J Clin Microbiol 43:1007. https://doi.org/10.1128/JCM.43.2.1007.2005.
- Burnik C, Altintas ND, Ozkaya G, Serter T, Selcuk ZT, Firat P, Arikan S, Cuenca-Estrella M, Topeli A. 2007. Acute respiratory distress syndrome due to *Cryptococcus albidus* pneumonia: case report and review of the literature. Med Mycol 45:469–473. https://doi.org/10.1080/13693780 701386015.
- Khawcharoenporn T, Apisarnthanarak A, Mundy LM. 2007. Nonneoformans cryptococcal infections: a systematic review. Infection 35: 51–58. https://doi.org/10.1007/s15010-007-6142-8.
- Patel RK, Jain M. 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS One 7:e30619. https://doi.org/ 10.1371/journal.pone.0030619.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin 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.