





# Draft Genome Sequences of Heat Shock-Tolerant Microbes Isolated from a Spacecraft Assembly Facility

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**ABSTRACT** Heat shock-tolerant microorganisms belonging to the orders *Bacillales* and *Micrococcales* were isolated from the Spacecraft Assembly Facility at the Jet Propulsion Laboratory, and 63 draft genome sequences were assembled and identified. Further analyses of these genomes can provide insight into methods for preventing forward contamination.

Heat shock-tolerant *Bacillales* and *Micrococcales* ( $n = 63$  strains) were isolated from a spacecraft assembly facility (SAF) at the Jet Propulsion Laboratory (JPL). These microbes have previously been found in SAFs (1–3) and are relevant to NASA planetary protection missions. These robust microorganisms were still viable after undergoing heat shock at 80°C for 15 min and have scientific interest for NASA with reference to forward contamination of explored planets such as Mars and beyond. *Bacillales* are Gram stain-positive, obligate to facultative anaerobes that are ubiquitous in nature, often found in soils, sediments, fresh water, marine environments, air, skin, and food products (4). Many species within *Bacillales* are able to produce spores that are highly resistant to various physicochemical conditions and may also provide resistance to some of the extreme environmental conditions of space (5–7). Most *Bacillales* strains isolated from SAF have survived UVC irradiation (200 to 280 nm) and are more resistant than a standard domestic strain, *Bacillus subtilis* (8). Several medical, pharmaceutical, and agricultural industries have widely used *Bacillus* species for the production of various compounds such as enzymes, antibiotics, etc. (9). *Micrococcales* are also Gram stain-positive, but non-spore-forming, obligate aerobes often found on skin and food products (10). *Bacillales* and *Micrococcales* have been implicated as food spoilage agents (10) and as normal microorganisms of the skin microbiome that can be problematic during surgery, causing nosocomial infections, including bacteremia (11–13).

Samples were collected from the floor of the SAF at JPL between 2 February and 28 July 2016 as detailed by Hendrickson et al. (14). The samples were collected from 1 m<sup>2</sup> of the floor using a 23- by 23-cm<sup>2</sup> premoistened polyester wipe, placed in a glass bottle containing phosphate-buffered saline, thoroughly mixed for 30 s, and concentrated using a 0.45- $\mu$ m concentrating pipette tip (InnovaPrep, Drexel, MO, USA). An aliquot of 425  $\mu$ l was heat shocked (80°C, 15 min) and grown on tryptic soy agar incubated at 32°C as outlined by the NASA standard assay for microbiological contamination of space hardware (15). A single purified colony was picked, reinoculated into tryptic soy broth, and incubated at 32°C for 2 days under aerobic conditions before extracting DNA. DNA was extracted using the ZymoBIOMICS DNA MagBead kit with the provided instructions. Whole-genome sequence (WGS) libraries were prepared using the Illumina Nextera DNA Flex kit and sequenced using a NovaSeq 6000 S4 flow cell (paired-end, 2  $\times$  150-bp format). The sequences were quality controlled using FastQC v.0.11.7 (16) and fastp v.0.20.0 (17) to remove poor-quality and adapter sequences. The WGS was assembled using SPAdes v.3.11.1 (18), and QUAST v.5.0.2 (19) was used to

**Citation** Wood JM, Peres JB, Bateh J, Singh NK, Aronson HS, Hendrickson R, Mason CE, Venkateswaran K. 2021. Draft genome sequences of heat shock-tolerant microbes isolated from a spacecraft assembly facility. *Microbiol Resour Announc* 10:e00653-21. <https://doi.org/10.1128/MRA.00653-21>.

**Editor** Steven R. Gill, University of Rochester School of Medicine and Dentistry

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**Received** 25 June 2021

**Accepted** 13 August 2021

**Published** 9 September 2021

**TABLE 1** Accession numbers, sampling locations, and assembly details for the bacterial strains isolated from the JPL spacecraft assembly facility

Sample name	Bacterial species <sup>a</sup>	GenBank accession no.	SRA accession no.	Location <sup>b</sup>	Collection date (yr-mo-day)	No. of contigs	Genome size (bp)	N <sub>50</sub> (bp)	Depth of coverage (x)	G+C content (%)	No. of filtered reads
179-F 581 HS	<i>Alkalinobacillus clausii</i>	JAHHYG000000000	SRR14491914	Location 5	2016-04-12	69	4,503,474	158,227	531.41	44.61	15,247,468
179-C2.2 HS	<i>Alkalithalobacillus gibsonii</i>	JAHHD800000000	SRR14491909	Location 2	2016-03-01	14	4,145,563	1,041,195	781.96	43.45	21,374,150
179-D 10A.1 HS	<i>Bacillus altitudinis</i>	JAHHYO00000000	SRR14491921	Location 10	2016-03-15	27	3,754,669	388,342	774.51	41.18	19,337,472
179-I 9D2.1 HS	<i>Bacillus altitudinis</i>	JAHHYO00000000	SRR14491894	Location 11	2016-06-14	32	3,703,660	387,656	366.56	41.18	9,152,004
179-K 3C3 HS	<i>Bacillus altitudinis</i>	JAHHYO00000000	SRR14491875	Location 7	2016-07-12	23	3,703,577	381,543	784.60	41.25	19,589,554
179-K 1C1 HS	<i>Bacillus glycerofermentans</i>	JAHHXD000000000	SRR14491883	Location 1	2016-07-12	65	4,371,489	191,398	965.49	46.37	29,656,364
179-D 1D1 HS	<i>Bacillus haynesii</i>	JAHHYO00000000	SRR14491872	Location 1	2016-06-15	13	4,241,859	1,027,801	652.58	45.74	18,427,806
179-I 3A1 HS	<i>Bacillus licheniformis</i>	JAHHYO00000000	SRR14491905	Location 3	2016-03-14	31	4,142,746	493,971	585.01	46.17	16,519,698
179-I 10B1 HS	<i>Bacillus licheniformis</i>	JAHHYO00000000	SRR14491893	Location 10	2016-06-14	29	4,337,844	560,056	475.88	45.75	13,938,094
179-J 6A2 HS	<i>Bacillus licheniformis</i>	JAHHYO00000000	SRR14491893	Location 5	2016-06-28	15	4,251,134	2,232,824	321.97	45.83	9,091,934
179-M 8A1 HS	<i>Bacillus licheniformis</i>	JAHHYO00000000	SRR14491873	Location 9	2016-07-28	21	4,203,797	697,462	693.19	46.20	19,574,558
179-I 1C1 HS	<i>Bacillus paralicheniformis</i>	JAHHYO00000000	SRR14491910	Location 1	2016-06-14	21	4,305,720	912,478	475.68	45.83	13,432,394
179-K 9C1 HS	<i>Bacillus paralicheniformis</i>	JAHHYO00000000	SRR14491882	After GL	2016-07-12	14	4,356,537	2,302,671	669.96	45.88	18,918,642
179-K 2B1 HS	<i>Bacillus paralicheniformis</i>	JAHHYO00000000	SRR14491880	Location 3	2016-07-12	15	4,339,137	2,317,658	864.81	45.85	24,420,740
179-K 3C1 HS	<i>Bacillus paralicheniformis</i>	JAHHYO00000000	SRR14491879	Location 7	2016-07-12	29	4,308,761	379,887	684.40	45.86	19,326,278
179-D 9B5 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491924	Location 12	2016-03-15	43	3,659,344	178,902	512.81	41.22	12,665,158
179-E 5D1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491922	Location 5	2016-03-30	39	3,672,488	219,630	511.92	41.19	12,643,140
179-E 7A1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491932	Location 12	2016-03-30	38	3,897,521	265,128	689.37	41.19	17,025,912
179-F 3A2 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491915	Location 4	2016-04-12	32	3,652,963	389,228	453.50	41.19	11,200,274
179-F 6C1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491913	Location 9	2016-04-12	40	4,175,106	255,886	426.41	41.05	10,531,224
179-F 7A1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491912	Location 13	2016-04-12	31	3,652,268	389,228	522.66	41.19	12,908,512
179-K 5D5 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491891	Location 11	2016-07-12	32	3,652,119	389,206	801.91	41.19	19,805,280
179-K 7B4 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491885	AS	2016-07-12	32	3,652,327	389,228	836.33	41.19	20,655,254
179-K 2D1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491884	Location 3	2016-07-12	31	3,652,555	389,228	834.47	41.19	20,609,504
179-K 3C2 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491878	Location 7	2016-07-12	49	3,679,526	156,853	782.33	41.24	19,321,676
179-M 4C1 HS	<i>Bacillus pumilus</i>	JAHHYO00000000	SRR14491876	Location 9	2016-07-28	79	3,828,439	153,422	972.97	41.03	24,030,106
179-I 2B1 HS	<i>Bacillus safensis</i>	JAHHYO00000000	SRR14491908	Location 6	2016-06-14	13	3,671,652	957,957	534.95	41.60	13,504,738
179-I 2B2 HS	<i>Bacillus safensis</i>	JAHHYO00000000	SRR14491907	Location 6	2016-06-14	13	3,671,552	891,192	471.74	41.60	11,909,140
179-I 2B3 HS	<i>Bacillus safensis</i>	JAHHYO00000000	SRR14491906	Location 6	2016-06-14	12	3,669,435	957,957	714.24	41.59	18,031,006
179-A4 HS	<i>Bacillus subtilis</i>	JAHHZ000000000	SRR14491932	Location 7	2016-02-02	12	4,047,850	704,147	587.95	43.70	16,523,758
179-C6.1 HS	<i>Bacillus subtilis</i>	JAHHZ000000000	SRR14491887	Location 6	2016-03-01	14	4,211,076	1,080,553	567.37	43.36	15,945,254
179-D 3C1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491871	Location 3	2016-03-15	10	4,005,781	2,065,731	615.41	43.77	16,541,050
179-D 8B1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491929	Location 13	2016-06-14	13	3,989,520	1,035,505	621.60	43.78	17,469,598
179-F 1A1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491917	Location 1	2016-04-12	11	4,094,361	2,112,290	468.34	43.66	13,162,240
179-F 10A1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491911	Location 10	2016-04-12	15	4,025,424	1,024,710	534.93	43.68	15,033,732
179-I 4D1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491902	Location 4	2016-06-14	27	3,996,990	284,852	248.04	43.73	6,970,900
179-I 6A2 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491904	Location 9	2016-06-14	25	3,997,572	284,852	364.42	43.73	10,241,546
179-I 7B1 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491899	Location 13	2016-06-14	12	4,101,024	1,070,568	414.08	43.53	11,637,316
179-J 10A2 HS	<i>Bacillus subtilis</i>	JAHHYO00000000	SRR14491897	Location 10	2016-06-28	12	3,995,942	1,033,674	388.36	43.76	10,914,612
179-C7.2 HS	<i>Brevibacillus parabrevis</i>	JAHYZ000000000	SRR14491874	Location 7	2016-03-01	35	6,094,361	664,847	502.48	52.24	20,266,642
179-I 5A1 HS	<i>Brevibacterium luteolum</i>	JAHHXO00000000	SRR14491901	Location 5	2016-06-14	37	3,236,987	471,910	482.39	66.87	10,225,434
179-K 6B1 HS	<i>Cyrobacillus oceanisediminis</i>	JAHHXO00000000	SRR14491888	After AS	2016-07-12	33	5,507,177	652,861	551.87	41.12	20,094,618
179-M 9A2 HS	<i>Cyrobacillus oceanisediminis</i>	JAHHYO00000000	SRR14491877	Location 12	2016-07-28	57	5,471,699	210,281	654.96	41.14	23,847,998
179-D 9B4 HS	<i>Micrococcus luteus</i>	JAHHYO00000000	SRR14491925	Location 12	2016-03-15	76	2,552,648	68,435	917.59	72.92	25,299,916
179-K 6B2 HS	<i>Micrococcus luteus</i>	JAHHYO00000000	SRR14491890	After AS	2016-07-12	68	2,666,484	105,095	1,486.77	71.55	24,790,452
179-K 6B5 HS	<i>Micrococcus luteus</i>	JAHHYO00000000	SRR14491889	After AS	2016-07-12	81	2,419,133	72,889	923.41	73.04	15,396,854
179-K 7B3 HS	<i>Micrococcus luteus</i>	JAHHYO00000000	SRR14491886	AS	2016-07-12	31	2,444,982	140,230	1,149.61	73.05	19,168,576
179-K 2A2 HS	<i>Oceanobacillus carei</i>	JAHHZD000000000	SRR14491893	Location 3	2016-07-12	89	3,569,995	80,335	368.44	35.81	8,791,318
179-B9C HS	<i>Priestia megaterium</i>	JAHHYO00000000	SRR14491931	After AS	2016-02-16	69	5,616,472	358,972	455.15	37.57	16,212,722
179-F 3A1 HS	<i>Priestia megaterium</i>	JAHHYO00000000	SRR14491916	Location 4	2016-04-12	67	6,163,057	969,567	37.34	37.34	7,070,512
179-K 10A1 HS	<i>Priestia megaterium</i>	JAHHYO00000000	SRR14491881	Before GL	2016-07-12	52	5,855,780	691,570	493.83	37.44	17,590,672
179-B10A HS	<i>Staphylococcus saprophyticus</i>	JAHYZ000000000	SRR14491919	After AS	2016-02-16	29	2,617,135	365,510	834.61	32.96	14,002,352
179-C4.1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHZB000000000	SRR14491898	Location 4	2016-03-01	66	4,854,421	159,566	469.91	37.16	14,909,374
179-D 5C1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491870	Location 8	2016-03-15	65	4,854,547	147,152	411.88	37.16	13,068,112
179-D 6C1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491928	Location 5	2016-03-15	66	4,852,470	147,102	329.26	37.16	10,446,788
179-D 7D1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491927	Location 9	2016-03-15	65	4,853,002	147,593	702.12	37.16	22,277,174
179-D 9B1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491926	Location 12	2016-03-15	72	4,852,401	124,509	452.42	37.16	14,354,478
179-D 9B3 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491930	Location 12	2016-03-15	70	4,853,959	147,152	503.46	37.16	15,974,018
179-E 4C1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491892	Location 8	2016-03-30	65	4,854,833	149,458	559.06	37.17	17,738,122
179-E 7A2 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491918	Location 2	2016-03-30	66	4,850,779	124,521	327.71	37.16	10,397,580
179-I 5A2 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491903	Location 5	2016-06-14	66	4,853,958	162,753	336.15	37.16	10,665,362
179-I 6A1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491900	Location 9	2016-06-14	64	4,853,800	160,072	356.16	37.16	11,300,502
179-I 8A1 HS	<i>Vibriobacillus pantothenicus</i>	JAHHYO00000000	SRR14491895	Location 12	2016-06-14	72	4,849,727	139,539	399.56	37.15	12,677,332

<sup>a</sup> Species identity was determined using OrthoANIu, with a minimum average nucleotide identity of 95%.

<sup>b</sup> Locations as described by Hendrickson et al. (14); GL, gowning line; AS, air shower.

determine the genome size, number of contigs, and  $N_{50}$  value. The genome completeness and contamination were determined using CheckM (20), and all genomes were >99% complete and <0.5% contaminated. Default settings were used for all programs, excluding fastp (which used 512 adapters for screening). The species identity (average nucleotide identity, >95%; Table 1) was determined using OrthoANlu (21). The genomes were annotated using NCBI Prokaryotic Genome Annotation Pipeline v.5.2 (22).

**Data availability.** This WGS project has been deposited at NCBI GenBank (accession numbers provided in Table 1) under BioProject accession number [PRJNA728748](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA728748) and at NASA GeneLab (data set [GLDS-391](https://www.nasa.gov/data/GLDS-391)). The versions described in this paper are the first versions.

## ACKNOWLEDGMENTS

Part of the research described in this publication was carried out at the Jet Propulsion Laboratory (JPL), California Institute of Technology, under a contract with NASA. This research was funded by project M20M, administered at JPL in 2018. This project also funded a postdoctoral fellowship for J.M.W. and JPL student fellowships for J.B. and J.B.P.

We thank the members of the Biotechnology and Planetary Protection Group, JPL, for collecting the samples, Ryan Kemp (Zymo Corp.) for extracting the DNA, and Dan Butler (Weill Cornell Medicine) for generating the shotgun sequencing. We acknowledge the JPL supercomputing facility staff, notably Narendra J. Patel (Jimmy) and Edward Villanueva, for continuous support in providing the best possible infrastructure for BIG-DATA analysis.

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