

Genome Sequences of Bacteria Isolated from the International Space Station Water Systems

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Running Title: Water Processor Derived Bacteria from Space Station

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Abstract

We report draft genomes of five bacteria recovered from the United States and Russian water systems onboard the International Space Station: bacteria of the genera *Ralstonia*, *Burkholderia*, *Cupriavidus*, *Methylobacterium*, and *Pseudomonas*. These sequences will help further the understanding of water reclamation and environmental control and life support systems in space.

Announcement

Water associated bacteria capable of biofilm formation onboard the International Space Station (ISS) have represented a challenge for the operation of environmental control and life support system (ECLSS) (1). Bacteria isolated from the Water Recovery System, which recycles urine, humidity condensate, and other non-potable water to water for crew consumption and hygiene, have been used in multiple ground studies that pertain to ECLSS biofouling (2-5). Similarly, isolates from the Russian SRV-K, which recovers water from humidity condensate, and the SVO-ZV, which supplies ground water delivered to the ISS, have been and continue to be investigated (6). Several NASA-affiliated studies conceived of the idea of using a defined consortium of bacteria that are most frequently found in the ISS water to evaluate different means of biofouling control (7, 8). A subsequent microbial control report suggested using five specific spaceflight isolates of *Ralstonia insidiosa*, *Burkholderia multivorans*, *Cupriavidus metallidurans*, *Methylobacterium fujisawaense*, and *Pseudomonas aeruginosa* based on available 16S rRNA identifications (3). All but *P. aeruginosa* have been recovered on multiple occasions from ISS water systems since 2002. These five specific organisms have been provided to other investigators assessing methods

of microbial control within spaceflight water systems. Therefore, in this study, we established their complete genomes to facilitate further investigation.

Following sample collection onboard the ISS, water was returned to Earth in a Teflon collection bag and processed in the Microbiology Laboratory at the Johnson Space Center (JSC). For all isolates, bacteria were cultured on Reasoner's 2A plates for 48 hours at 35 °C. Colonies obtained were subcultured and glycerol stocks were archived at -80 °C. Initial identifications were completed through 16S rRNA sequencing following the JSC Sanger sequencing standard operating procedures with primers 5F and 531R (9, 10). To obtain the full genome sequences, samples were cultured in Tryptic Soy Broth for 24 hours at 35 °C. High molecular weight gDNA was obtained with the Circulomics Nanobind CCB Big DNA kit (PacBio) without shearing or size selection and assessed with a Qubit fluorometer (ThermoFisher) and TapeStation (Agilent). To obtain long reads, libraries were prepared with the Oxford Nanopore Technologies (ONT) SQK-RBK004 kit. Libraries were loaded into a R9.4.1 flow cell and run on a MinION Mk1C for 72 hours. Raw reads were basecalled using the high accuracy configuration of Guppy v.4.3.4, adapters were removed with Porechop v.0.2.4, and reads shorter than 2kb were filtered using filtlong v.0.2.0 with flags "--min_length 2000 --keep_percent 80 --trim --split 750." Corresponding paired-end 300 bp short reads were obtained from libraries prepared with the Illumina DNA Prep kit with consecutive sequencing with V3 reagents on an Illumina MiSeq. These reads were filtered using BBduk (BBTools v.38.54) and quality filtered with "minlen=50 qtrim=rl trimq=25 hdist=1 tpe tbo." Hybrid *de-novo* genome assembly was carried out using Unicycler v.0.5.0 (11). Default parameters were used for all software unless otherwise

70 specified. The genome assembly metrics for each isolate are listed in Table 1. Annotations
71 were performed using the NCBI Prokaryotic Genome Annotation Pipeline v.6.4 (12). The
72 obtained genomes have greater than 99.6% completeness as assessed using CheckM
73 v.1.0.18 (13).

74 **Table 1. Summary of draft genome sequences belonging to the five bacteria isolated from ISS water.**

Bacterial Identification	NASA Sample Name	Source	GenBank Accession Number	Genome Size (bp)	No. of Nanopore Reads	Nanopore N ₅₀ (bp)	No. of Illumina Reads	No. of Contigs	G+C Content (%)	Genome N ₅₀ (bp)	No. of CDSs
<i>Ralstonia insidiosa</i>	171870003-1	ISS WPA wastewater	JAQPZM000000000	6,271,672	166,983	28,078	1,374,153	5	63.33	3,668,357	5,878
<i>Burkholderia contaminans</i>	172630038-1	ISS WPA wastewater	JAQPZL000000000	8,711,463	42,546	23,803	1,902,557	6	66.23	3,246,788	7,851
<i>Cupriavidus metallidurans</i>	162430002-4	ISS WPA wastewater	JAQPZK000000000	7,278,803	79,657	21,481	926,800	5	63.47	3,954,298	6,809
<i>Methylobacterium</i> species	092160098-2	ISS SVO-ZV	JAQPZJ000000000	7,859,068	13,825	21,791	778,677	10	69.23	5,338,800	7,450
<i>Pseudomonas aeruginosa</i>	0201761-1	ISS SRV-K	CP117300	6,901,248	80,489	29,628	695,299	1	65.94	6,901,248	6,443

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Data availability statement

The complete genome sequences have been deposited in GenBank under the BioProject accession number [PRJNA929559](#). The raw reads were deposited into the SRA database under BioProject accession number [PRJNA929559](#). The versions described in this paper are the first version.

Acknowledgments

This research was carried out at the National Aeronautics and Space Administration Lyndon B. Johnson Space Center. We thank the ISS crew members for sample collection and the Microbiology Laboratory for isolating and archiving the bacteria. This work was supported in part by research funds from NASA Polaris grant number 663323.08.62.01. The JSC contract team acknowledges funding through NASA contract NNJ15HK11B made possible by the NASA Polaris grant and the JSC's Office of the Chief Technologist R. Clayton and C. Westhelle.

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