Genomic Enumeration of Antibiotic Resistance in Space through Whole Genome Nanopore Sequencing

Christian Mena[1], Jordan McKaig[2], Sarah Stahl-Rommel[1], Hang N. Nguyen[1], Christian L. Castro,[1] G. Marie Sharp[3], Kathleen Ryan[4], Noelle C. Bryan[5], Michael Gilmore[6,7], Ralf Moeller[8], Elisabeth Grohmann[9], Aaron S. Burton[10], Sarah L. Castro-Wallace[11], and Christopher E. Carr[2,4]

[1]JES Tech, Houston, TX, USA, [2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA, [3]KBR, Houston, TX, USA, [4]Daniel Guggenheim School of Aerospace Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [5]Department of Atmospheric Sciences, Colorado State University, Fort Collins, CO, USA, [6]Massachusetts Eye and Ear, Boston, MA, USA, [7]Broad Institute of MIT and Harvard, Cambridge, MA, USA, [8]Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany, [9]Faculty of Life Sciences and Technology, Berlin University of Applied Sciences, Berlin, Germany, [10]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, Houston, TX, USA, [11]Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, TX, USA

Introduction

The growing antibiotic resistance crisis is significantly costly both financially and in terms of human life. Unfortunately, resistance to antibiotics is becoming more widespread and cannot be contained by the bounds of Earth. Previous research has revealed that isolates from the International Space Station (ISS) demonstrate antibiotic resistance[1]. Through the use of culture- and molecular-based capabilities that are well-established onboard the ISS, the Genomic Enumeration of Antibiotic Resistance in Space (GEARS) payload will characterize the frequency and genomic identity of antibiotic resistant organisms. While selective media aims to define resistance frequency on ISS surfaces, full genome characterization is required to identify antibiotic resistance genes, as well as their frequency and relatedness across genomes. Nanopore sequencing has been continually performed onboard since 2016 but has not included sample preparation and sequencing of an organism's full genome[2, 3, 4]. The work described here details a spaceflight-compatible method for *in situ* whole genome nanopore sequencing of culture isolates. Specifically, optimizations toward DNA extraction, library preparation, and the implementation of new sequencing chemistries beyond those of current ISS protocols have been validated with antibiotic-resistant organisms returned from the ISS. Beyond advancing spaceflight sequencing, establishing antibiotic-resistance profiles of ISS organisms will enhance NASA's crew health risk assessments.

Figure 1: Astronaut Kayla Barron holding components of the Surface Sampler Kit (SSK) used for culture-based microbial monitoring of ISS surfaces.

Figure 2: Astronaut Dr. Serena Auñón-Chancellor holding the MinION Mk1B onboard the ISS. The MinION is being used by NASA for both culture-dependent and culture-independent microbial identifications.

Methods

An optimal DNA extraction window from cultures was determined by plating *Enterococcus faecalis* (ISS isolate) onto Tryptic Soy Agar (TSA) plates containing 8 µg/ml of gentamicin followed by incubation at ambient temperature for 3 – 10 days. DNA extraction followed previous spaceflight methods[4] with a modified 0.4X bead-based purification. DNA quantity and quality was determined using a Qubit 4.0 fluorometer with the dsDNA high sensitivity kit (ThermoFisher) and a TapeStation 4200 (Agilent Technologies) with genomic screen tapes. Library preparation was completed using Rapid Barcoding Kit (Oxford Nanopore Technologies, SQK-RBK114) with R10.4.1 flow cells with modifications for spaceflight (**Fig. 3**). *Ralstonia pickettii* cells served as a positive control to validate the workflow.

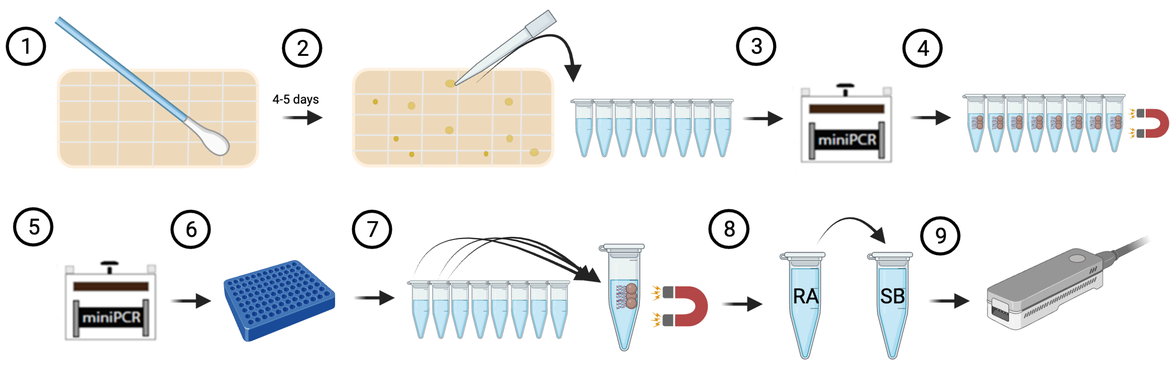
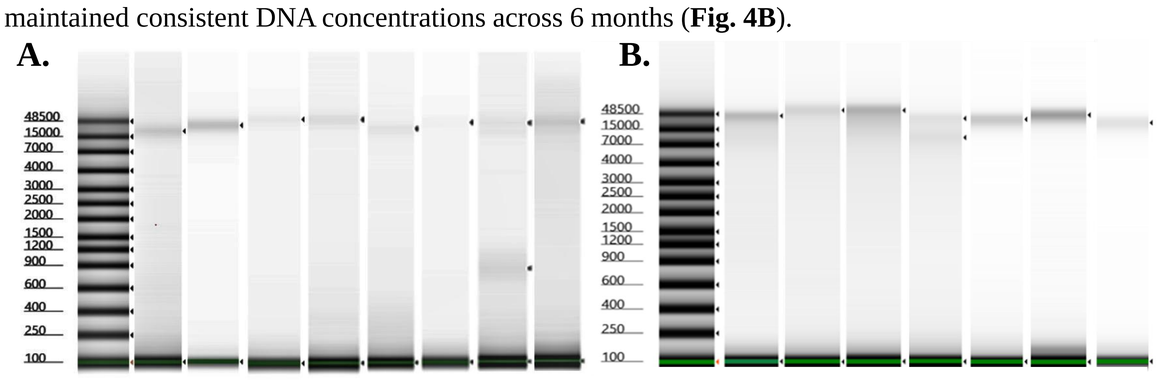


Figure 3: Concept of Operations for GEARS Payload. 1) Swab a surface of the ISS and apply the swab to slide containing antibiotic; 2) Following 4 – 5 days of incubation at ambient temperature, using a pipette tip, transfer part of a colony to DNA extraction buffer; 3) Lyse cells within miniPCR; 4) Using magnetic particles, purify DNA from the cellular debris; 5) Fragment and barcode the gDNA within miniPCR using RBK114; 6) Stop fragmentation reaction by rapid cooling; 7) Pool samples into a single tube for magnetic purification and size select gDNA; 8) Add sequencing adaptors (RA) to fragmented gDNA, then transfer the now prepared sequencing libraries to the sequencing buffer (SB); 9) Load the sample into a prepped R10.4.1 flow cell on the MinION Mk1B and initiate sequencing with MinKNOW 22.10.10 on a Space Station Computer for 48 hours. *Figure 3 and 6 made with BioRender.*

Results

To determine the spaceflight operations timeline, the DNA concentration across multiple days of growth was evaluated. DNA extracted from colonies between 4 – 5 days after growth initiation at ambient temperature yielded the best results (**Fig. 4A**). Additionally, frozen positive control cells maintained consistent DNA concentrations across 6 months (**Fig. 4B**).



E. faecalis Quantification (ng/µL)	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
	1.97	5.43	1.31	0.88	0.65	0.55	0.84	0.71

R. pickettii Control Quantification (ng/µL)	Day 0	1 Week	2 Mos	3 Mos	4 Mos	5 Mos	6 Mos
	1.71	1.09	.586	1.05	1.56	1.06	1.37

Figure 4: Quantitative and qualitative results from genomic DNA extracted with a rapid, ISS-compatible method. **A)** *E. faecalis* following incubation over a 10-day period. **B)** positive control cells, *R. pickettii* over the course of 6 months at -80 °C.

Results Continued

High quality draft genome assemblies with 99% completeness and < 1.4% contamination for *E. faecalis* and *R. pickettii* from a single colony were created using the described spaceflight method, **Fig. 5** and **Table 1.** The *E. faecalis* genome displayed 155 pseudogenes and 29 antimicrobial resistance (AMR) genes. *R. pickettii* displayed 133 pseudogenes and 99 AMR genes (**Fig. 5**). These two annotated genomes represent the beginning of whole genome sequencing and analysis capabilities onboard the ISS.

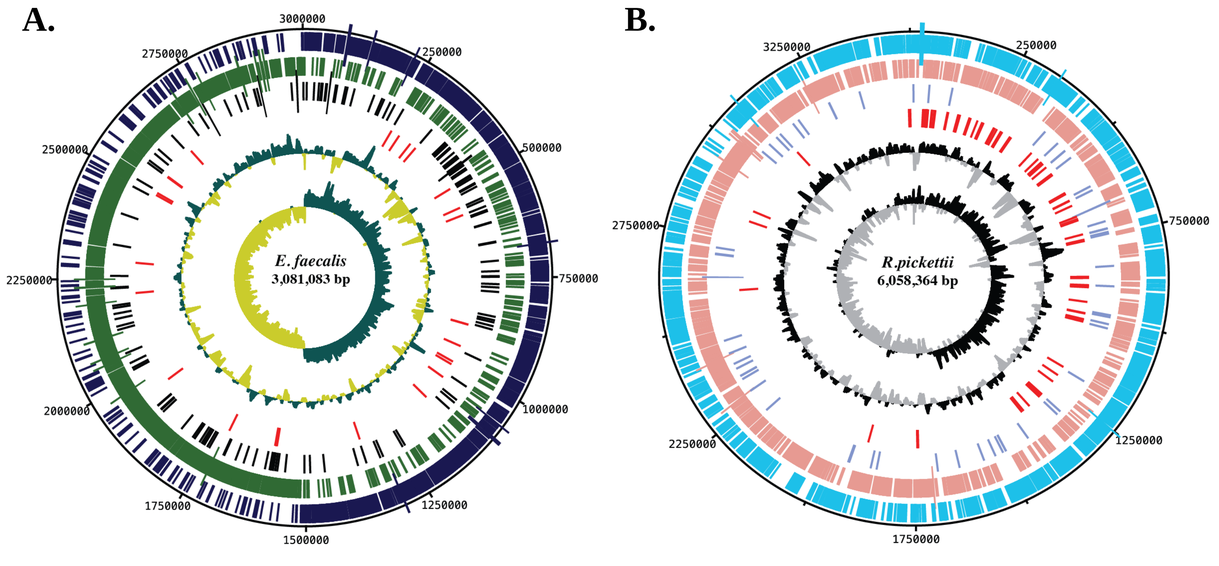


Figure 5: Circular map draft genomes of two bacteria, A) *E. faecalis* and B) *R. pickettii*, isolated from the ISS and sequenced on the ground to validate the developed spaceflight-compatible whole genome sequencing protocol for the GEARS payload. From the inner to the outer circle, genomic features are presented as follows: ring 1 – GC skew, ring 2 – GC percentage plot, ring 3 – genes associated with antimicrobial resistance, ring 4 – pseudogenes, ring 5 – reverse strand CDS, ring 6 – forward strand CDS, ring 7 – genomic position in bp. Genome assembled with Unicycler v.0.5.0, annotated with PGAP v.6.4, and figures prepared with DNAplotter integrated in Artemis v. 18.02.

Table 1: Draft genome metrics

Organism	Source	No. of Reads	N_{50} (bp)	Assembly Length	No. of contigs	GC (%)	No. of CDSs[a]	Genome N_{50} (bp)	Depth of Coverage
E. faecalis	ISS Node3 WHC[b]	197,858	10,575	3,081,083	2	37.28	2,994	3,004,305	366.45
R. pickettii	ISS PWD[c]	103,462	15,029	6,058,364	8	63.36	5,804	3,508,846	157.62

[a] CDSs, Number of coding sequences
[b] WHC, Waste and Hygiene Compartment
[c] PWD, Potable Water Dispenser

Conclusions & Future Work

The method developed for the GEARS payload has produced nearly complete genomes that are comparable in completeness and quality to other ground-based methods and previously-published work[1]. This method holds the potential to allow for a deeper *in situ* assessment of bacterial isolates cultured onboard the ISS, which could increase understanding of the adaptations to the spaceflight environment and mechanisms of resistance. The GEARS payload is scheduled to launch to the ISS in 2024 with 4 sampling sessions to be completed over the course of a year (**Fig. 6**).

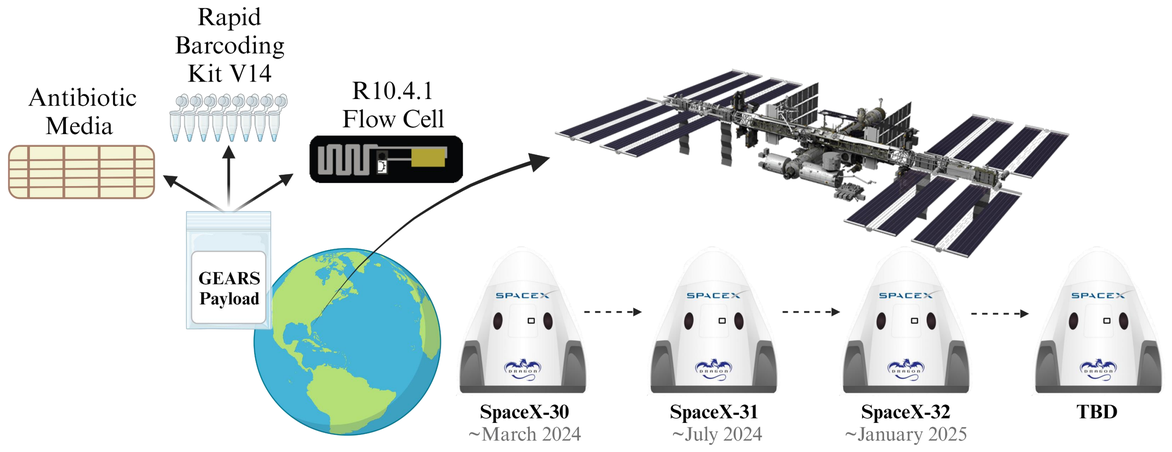


Figure 6: The GEARS payload launching in 2024 will bring new ONT chemistry and flow cells to the ISS. GEARS will be the first investigation to demonstrate whole genome sequencing off Earth.

Acknowledgements

This work was funded by the National Aeronautics and Space Administration under Grant No. 80NSSC21K0234. The team thanks Rachel Moore, Gwo-shing Sun, Parag A. Vaishampayan, and Elizabeth M. Pane. Christian Mena, Sarah Stahl-Rommel, Hang N. Nguyen, G. Marie Sharp, and Christian L. Castro acknowledge funding through NASA contract NNJ15HK11B.

References: (1) Bryan, Noelle C., *et al.* "Genomic and Functional Characterization of *Enterococcus faecalis* Isolates Recovered From the International Space Station and Their Potential for Pathogenicity." *Front Microbiol* (2021) **11**:515319. (2) Castro-Wallace, Sarah L., *et al.* "Nanopore DNA Sequencing and Genome Assembly on the International Space Station." *Sci. Rep.* (2017) **7**,18022. (3) Burton, Aaron S., *et al.* "Off Earth Identification of Bacterial Populations Using 16S rDNA Nanopore Sequencing." (2020) *Genes* **11**:76. (4) Stahl-Rommel, Sarah, *et al.*, "Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing." (2021) *Genes* **12**:106.