Time traveling through the International Space Station Dust Microbiomes using Amplicon and Metagenomic Nanopore Sequencing

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Introduction

The International Space Station (ISS) is a testament to human innovation, offering valuable insights into space exploration. Through the analysis of dust samples collected over the past two decades of ISS operation, we aim to identify how the microbial communities have evolved and adapted to the space environment over time. This temporal dimension adds an important layer of understanding, allowing us to discern patterns of microbial succession, respond to shifts in relative abundance, and discover genomic adaptations of terrestrial microbes within the extreme environment of the ISS. The temporal analysis of the dust microbiome enriches our knowledge of the dynamic interactions between microorganisms and the ISS habitat, providing valuable insights for future space exploration endeavors and enhancing our comprehension of life's resilience in extreme conditions.

Analytical workflow

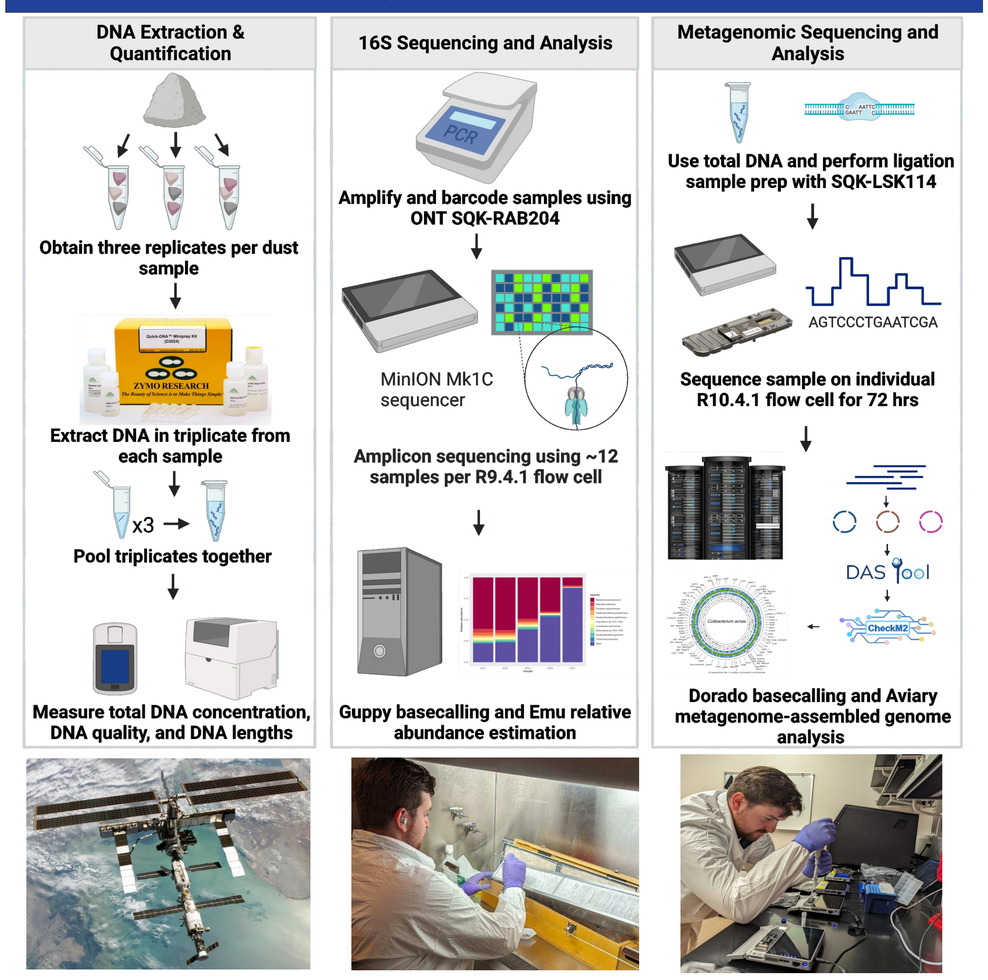


Figure 1: Project workflow from processing stored dust samples to nanopore sequencing and analysis

16S rRNA Amplicon Sequencing

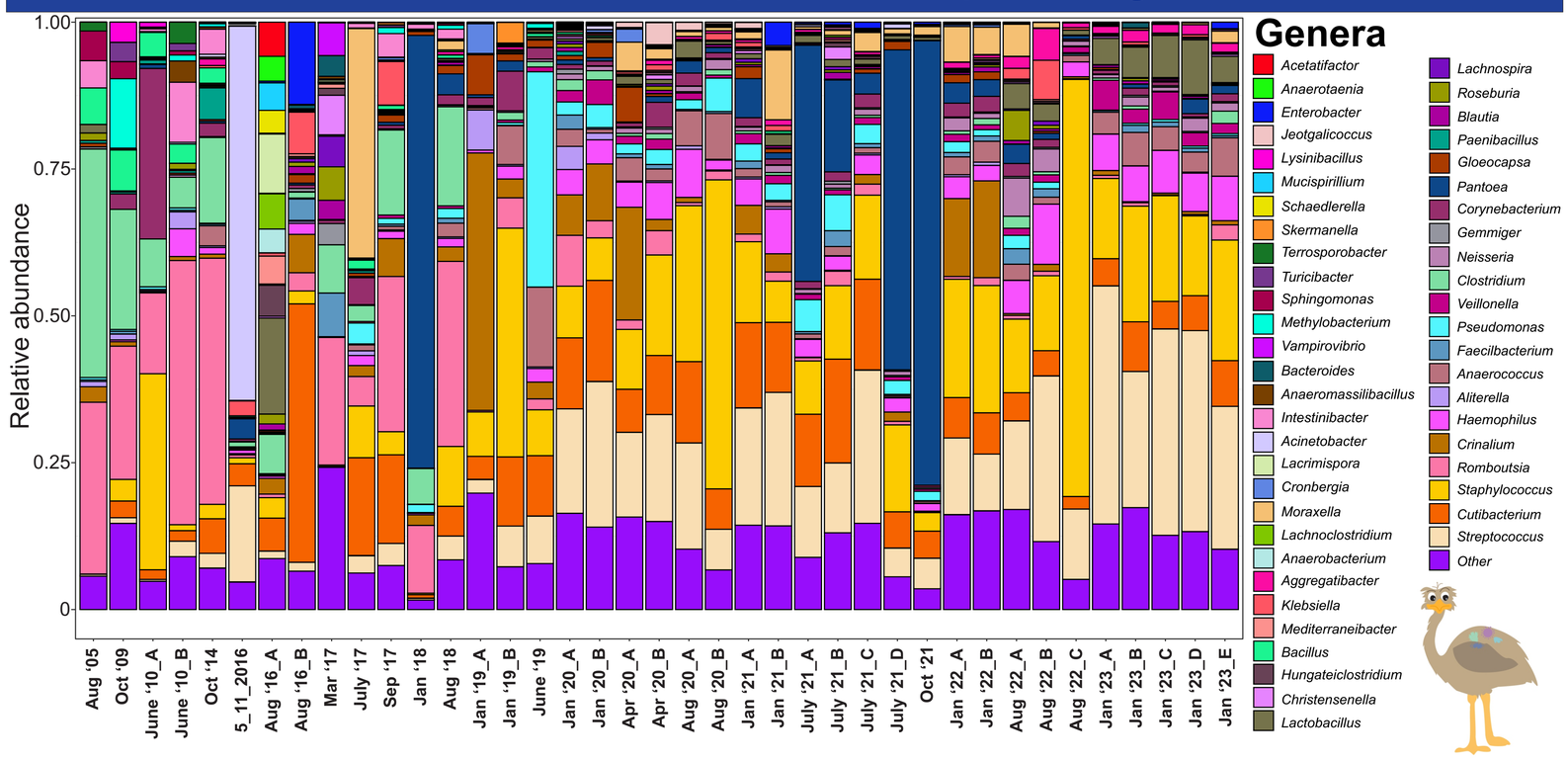


Figure 2: Normalized 16S relative abundance plot of ISS dust data classified using Emu. 3% min. abundance

Individual Diversity Statistics

Chronological Clustering

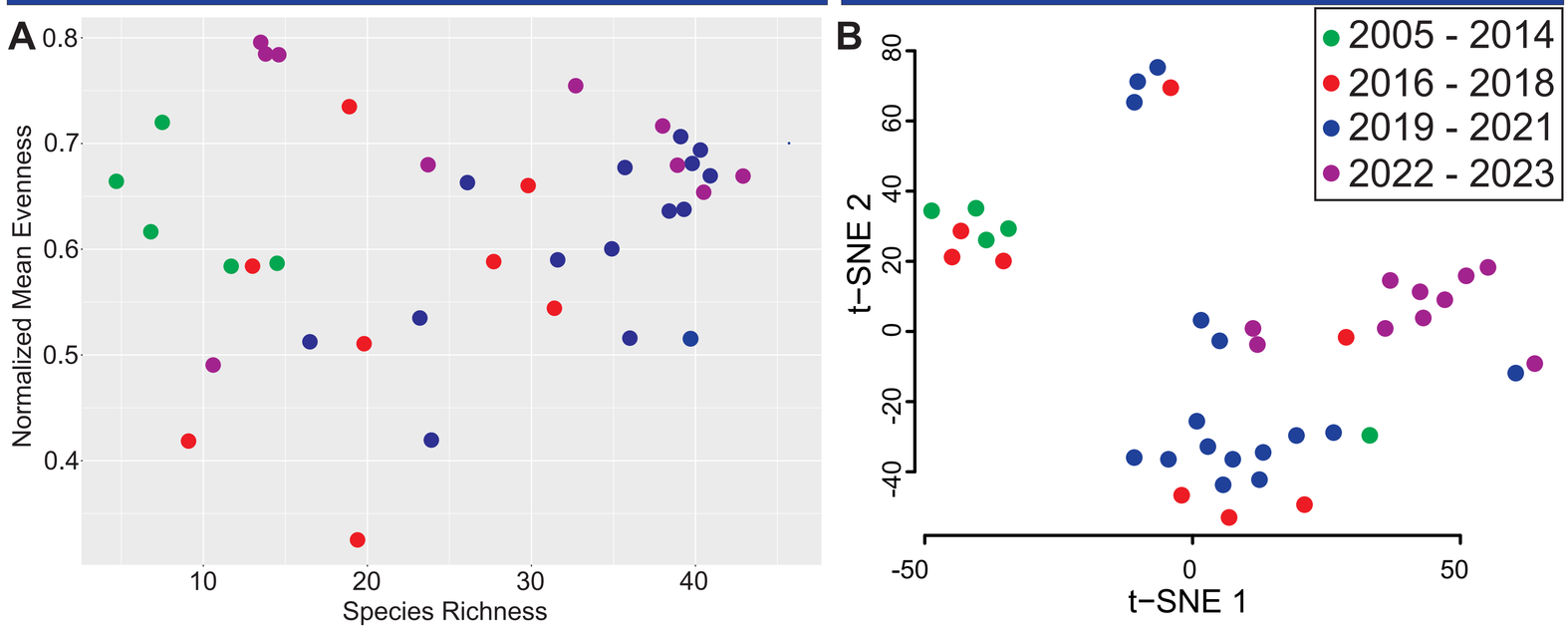
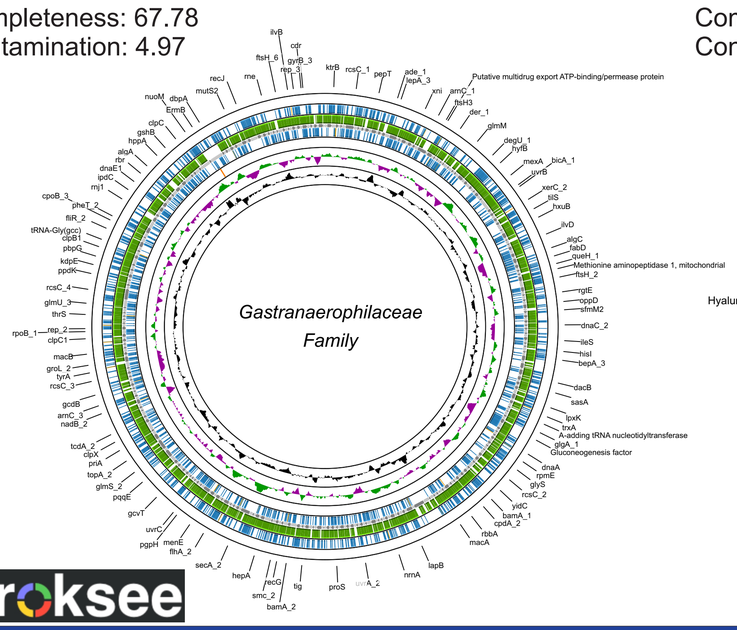


Figure 3: **A** Normalized mean evenness vs. species richness plot of 16S counts produced by Emu from ISS dust data **B** t-SNE plot of normalized 16S count data from the ISS dust dataset

Metagenomic Sequencing of ISS Dust Samples

A **March 2017**

Completeness: 67.78
Contamination: 4.97



B **August 2020**

Completeness: 96.85
Contamination: 8.26

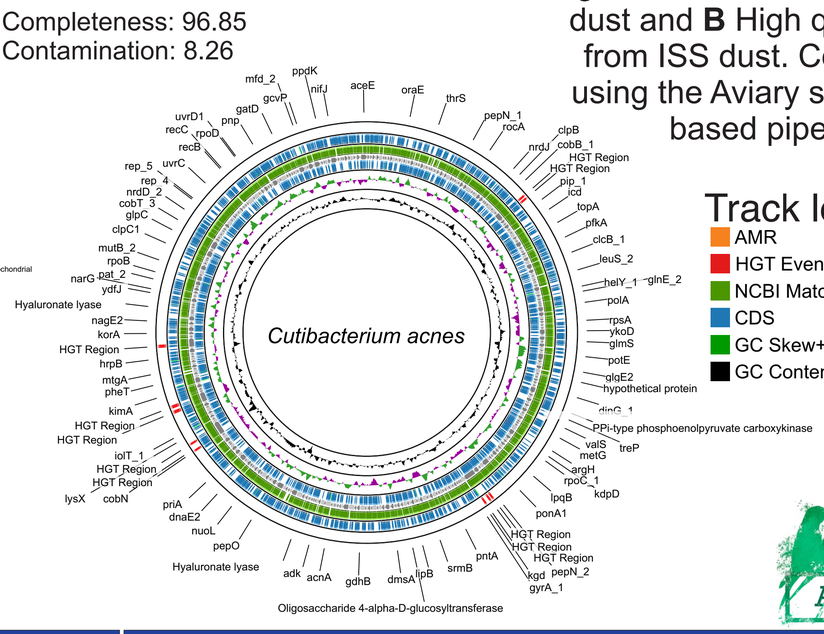


Figure 4: **A** Draft MAG from ISS dust and **B** High quality MAG from ISS dust. Constructed using the Aviary snakemake-based pipeline.

Conclusions

Nanopore sequencing allowed for the identification of bacteria within dust samples from the ISS using 16S rRNA amplicon sequencing. Metagenomic sequencing made it possible to complete bacterial metagenomes while detecting horizontal gene transfers and antimicrobial resistance genes. Our study has laid the groundwork for bacterial MAG analysis from ISS samples, preparing us for the future “spacework”!

Future Directions

- Perform human DNA depletion prior to SQK-LSK114 library preparation to obtain more complete bacterial genomes
- Utilize this pipeline completely onboard for analysis of microbes, implementing Apple silicon hardware
- Investigate other microbial matrices originating from the International Space Station

Detection of Lateral Gene Transfer

Table 1: Top Lateral Gene Tranfser events from metagenomic sequencing of ISS dust. LGT’s identified using WAAFLE filtered by read length >= 2 kb

SAMPLE	MAX SCORE	CLADE A	CLADE B
Oct. ‘09	0.9703	*Campylobacter jejuni*	*Streptococcus agalactiae*
	0.9684	*Campylobacter jejuni*	*Cutibacterium acnes*
	0.9658	*Campylobacter jejuni*	*Chlamydia psittaci*
May ‘16	0.9928	*Eubacterium ramulus*	*Campylobacter jejuni*
	0.9834	*Turicella otitidis*	*Cyanothece sp. CCY0110*
	0.9791	*Vibrio vulnificus*	*Streptococcus agalactiae*
March ‘17	0.9871	*Eubacterium hallii*	*Alistipes shahii*
	0.9861	*Roseburia intestinalis*	*Cyanothece sp. CCY0110*
	0.984	*Porphyromonas somerae*	*Alistipes onderdonkii*
Aug. ‘20	0.9926	*Cutibacterium sp. KPL2008*	*Cutibacterium sp. 409 Hc1*
	0.988	*Lactobacillus crispatus*	*Limnohabitans sp. Rim47*
	0.986	*Actinomyces sp. oral taxon 448*	*Staphylococcus sp. E463*
July ‘21	0.9886	*Corynebacterium glucuronolyticum*	*Corynebacterium pyruviciproducens*
	0.9875	*Cyanothece sp. CCY0110*	*Klebsiella pneumoniae*
	0.984	*Klebsiella pneumoniae*	*Campylobacter jejuni*
Jan. ‘23	0.9956	*Lactobacillus crispatus*	*Limnohabitans sp. Rim47*
	0.9952	*Eubacterium ramulus*	*Eubacterium cylindroides*
	0.9941	*Cutibacterium sp KPL2009*	*Cutibacterium acnes*

Detection of Antimicrobial Resistance

Table 2: Antimicrobial resistance (AMR) genes found with metagenomic sequencing of ISS dust using ABRicate with NCBI AMR database.

SAMPLE	% Identity	Gene Name	Resistance
Oct. ‘09	93.62	*(MLS)erm(CX)*	MACROLIDE-LINCOSAMIDE
	N/A	N/A	N/A
	N/A	N/A	N/A
May ‘16	99.11	*aac(3)-XI*	AMINOGLYCOSIDE
	98.00	*blaZ*	BETA-LACTAM
	97.53	*aph(3')-Ia*	KANAMYCIN
March ‘17	99.80	*dfrF*	TRIMETHOPRIM
	99.72	*blaI_of_z*	BETA-LACTAM
	99.56	*lnu(A)*	LINCOSAMIDE
Aug. ‘20	99.85	*msr(A)*	ERYTHROMYCIN, STREPTOGRAMIN B
	99.74	*blaI_of_z*	BETA-LACTAM
	99.59	*erm(A)*	AZITHROMYCIN, Broad MYCINS
July ‘21	99.07	*fosB-Sepi*	FOSFOMYCIN
	99.07	*fusB*	FUSIDIC ACID
	99.06	*lsa(C)*	LINCOSAMIDE, STREPTOGRAMIN
Jan. ‘23	100.00	*mph(C)*	ERYTHROMYCIN, Broad MYCINS
	99.89	*cfxA3*	CEPHALOSPORIN
	99.75	*tet(Q)*	TETRACYCLINE

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