R&TD Innovative Spontaneous Concepts 2018

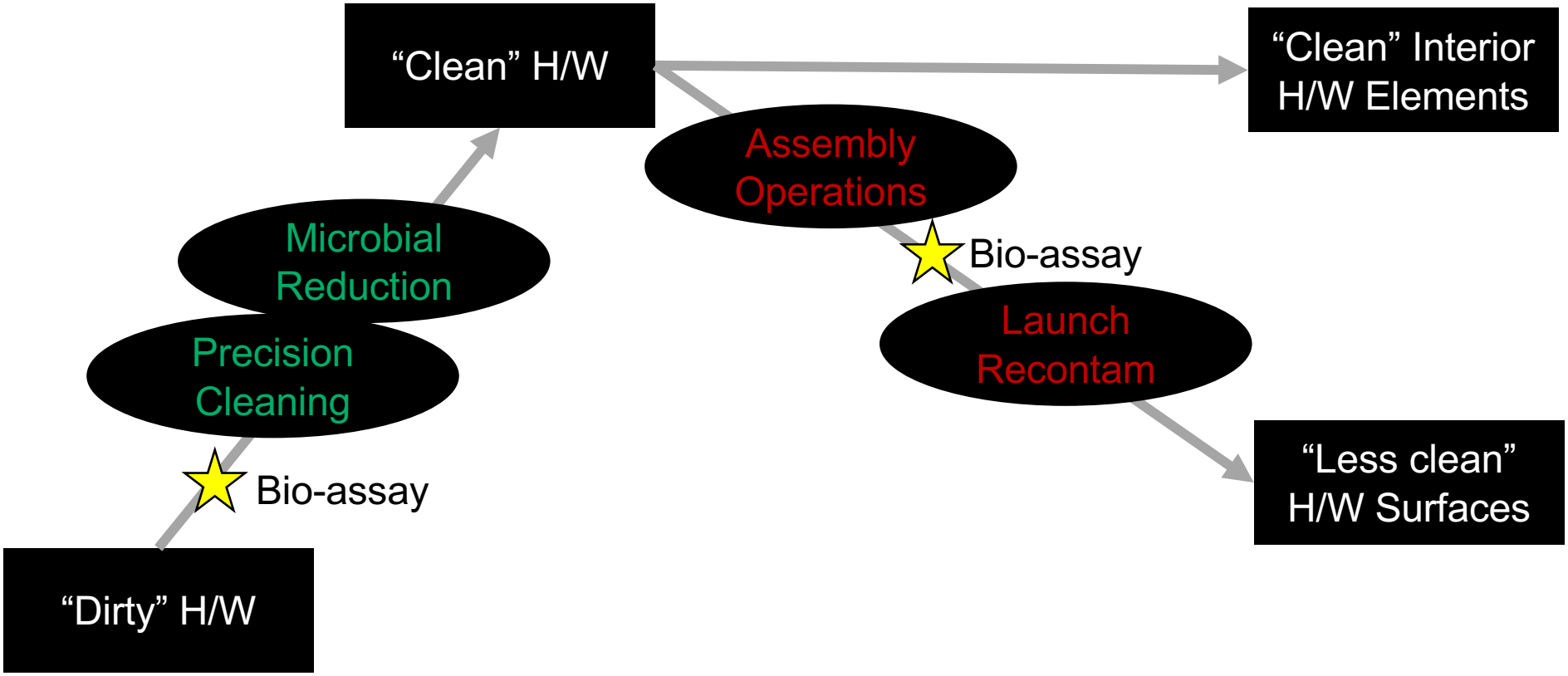
Far-UVC application to spacecraft microbial reduction

Arman Seuylemezian (352N)
Lisa Guan (352N)
David Welch (Columbia University Irving Medical Center)
Manuela Buonnano (Columbia University Irving Medical Center)

Jet Propulsion Laboratory
California Institute of Technology

Problem Statement

- Current methods for microbial reduction do not mitigate on-going microbial contamination associated with assembly activities, launch

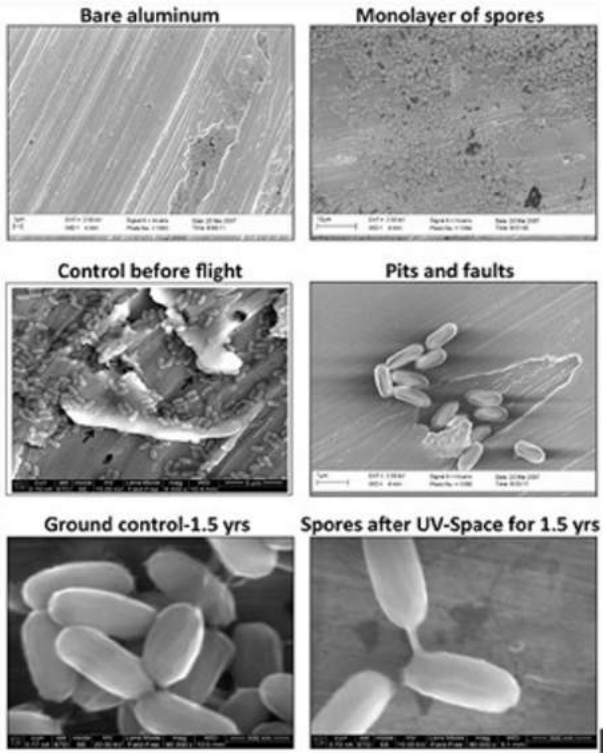


Project Objective

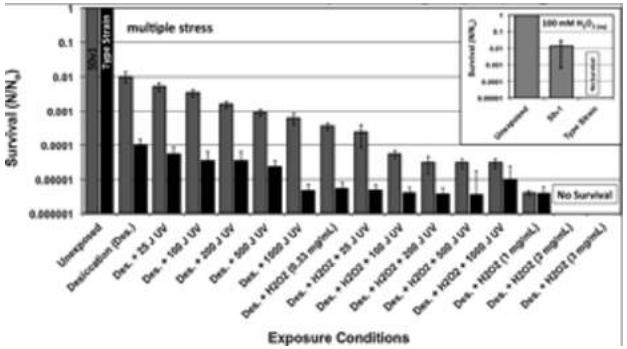
- Far-UVC is a recently developed technique which utilizes wavelengths at or below 222 nm to inactivate microbes
- The novelty in this wavelength of light is that it's not harmful to humans yet is effective in inactivating microbes.

Goals:

- Test the efficacy of far-UVC light on inactivating spores of *Bacillus pumilus* SAFR-032 and *Acinetobacter radioresistens* 50v1
- Characterize the effects of varying dose rate on the efficacy of far-UVC
- To investigate far-UVC light as a surface microbial reduction tool for planetary protection purposes

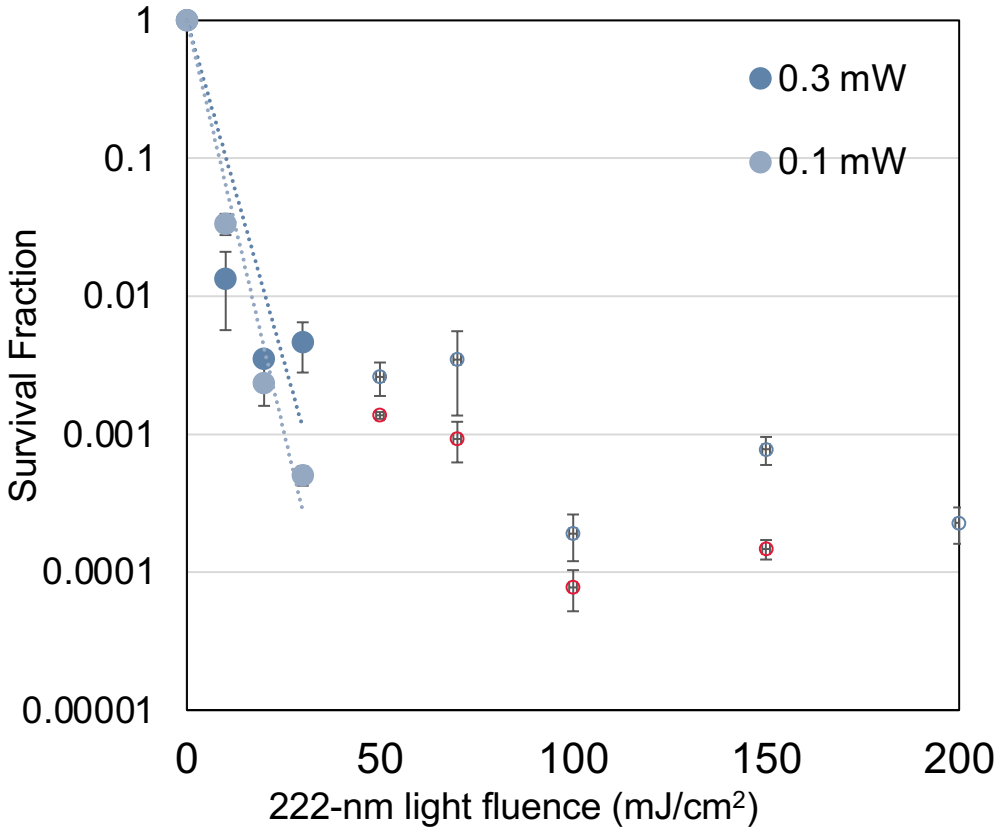


P. Vaishampayan et al., Survival of Bacillus pumilus Spores for a Prolonged Period of Time in Real Space Conditions. Astrobiology Vol 12, No 5, 2012

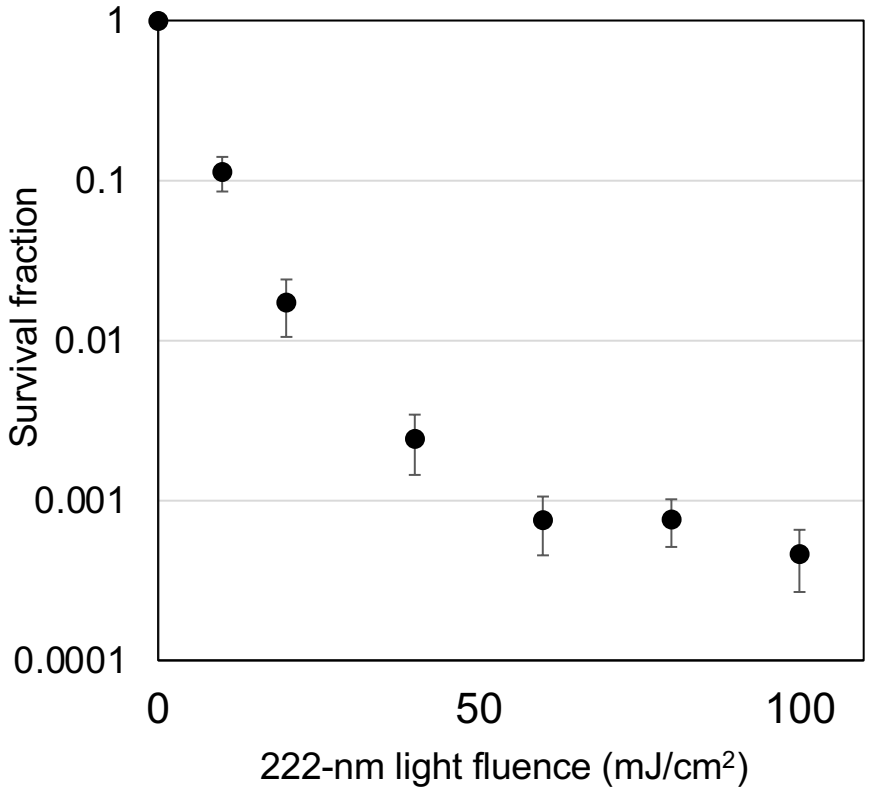


K.B. McCoy, I. Derecho, T. Wong, H.M. Tran, T.D. Huynh, M.T. La Duc, K. Venkateswaran, and R. Mogul.Astrobiology.Sep 2012.854-862.http://doi.org/10.1089/ast.2012.0835

Bacillus pumilus SAFR-032



Acinetobacter radioresistens 50v1



Methods and Approach

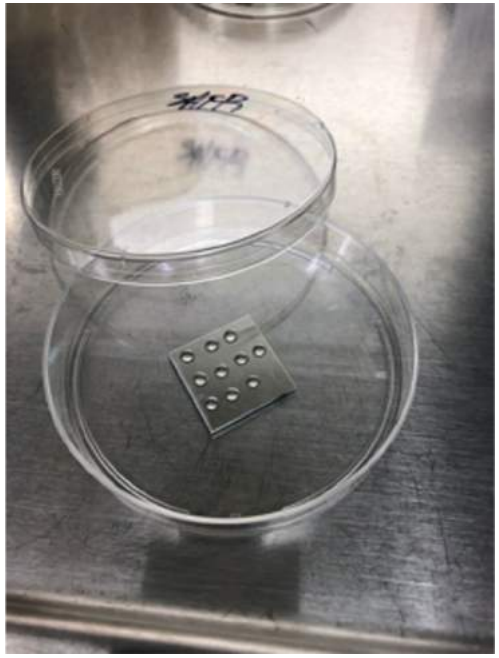


Figure 3. Aluminum 6061 coupons were used to seed the bacterial cells/spores into 10 individual spots of 10 ul each containing ~10^5 colony forming units (CFU). Spores/cells were recovered by first depositing 200 ul of polyvinyl alcohol and letting it air dry for 1 hour at room temperature then peeling the PVA film and dissolving it into 2 ml of sterile water/PBS respectively.

818-UV/DB low power UV enhanced silicon photodetector with an 843-R optical power meter (Newport, Irvine, Ca)

Preliminary Data (not from this study)

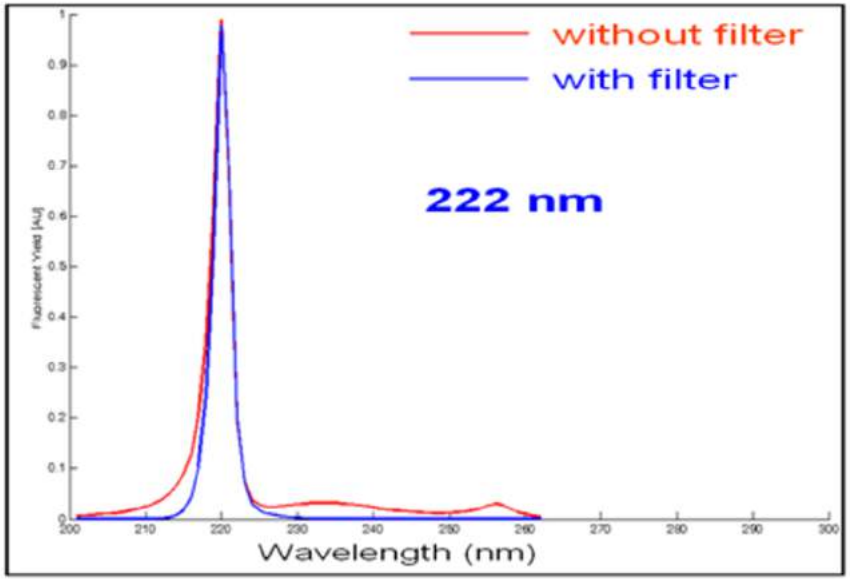


Figure 1. Measured non-filtered (red) and filtered (blue) UV spectra from our 222 nm KrCl excimer lamp.

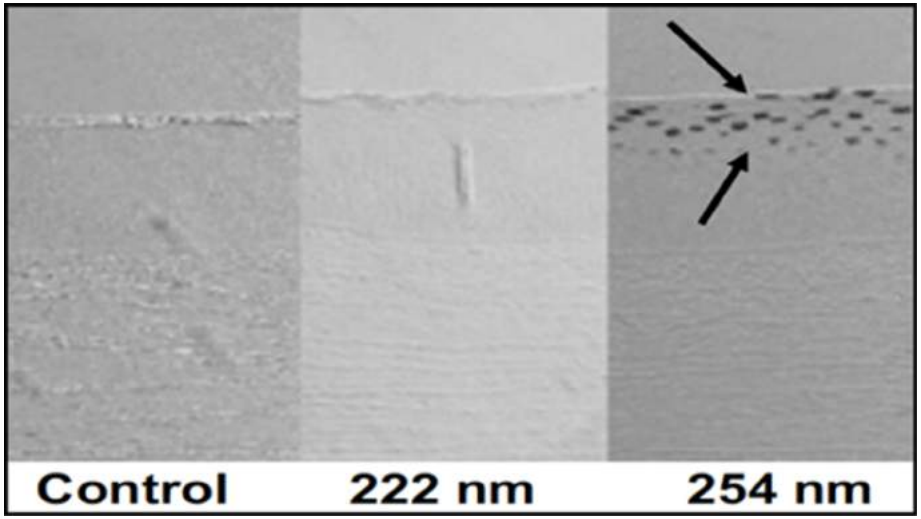


Figure 2. Bovine corneal tissue exposed to 300 mJ/cm2 of 222 nm far-UVC light or 254 nm germicida light. Darkly stained CPD (cyclobutane pyrimidine dimers) positive nuclei are seen in the 254 nm exposed tissue, but the 222 nm exposed tissue show no increase in CPD over zero-dose controls

Article | Open Access | Published: 24 June 2020

Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses

Manuela Buonanno, David Welch, Igor Shuryak & David J. Brenner

Scientific Reports **10**, Article number: 10285 (2020) | Cite this article

72k Accesses | **1815** Altmetric | Metrics

Effects of varying dose rate

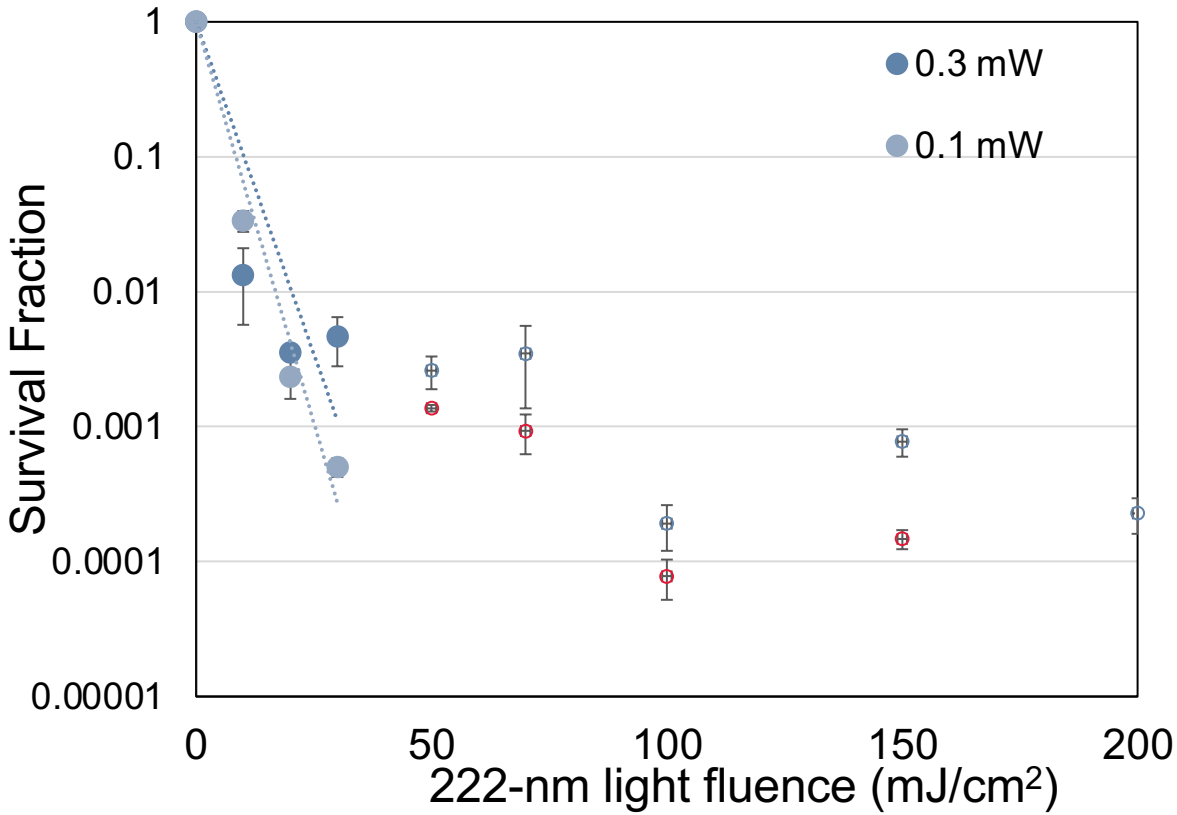
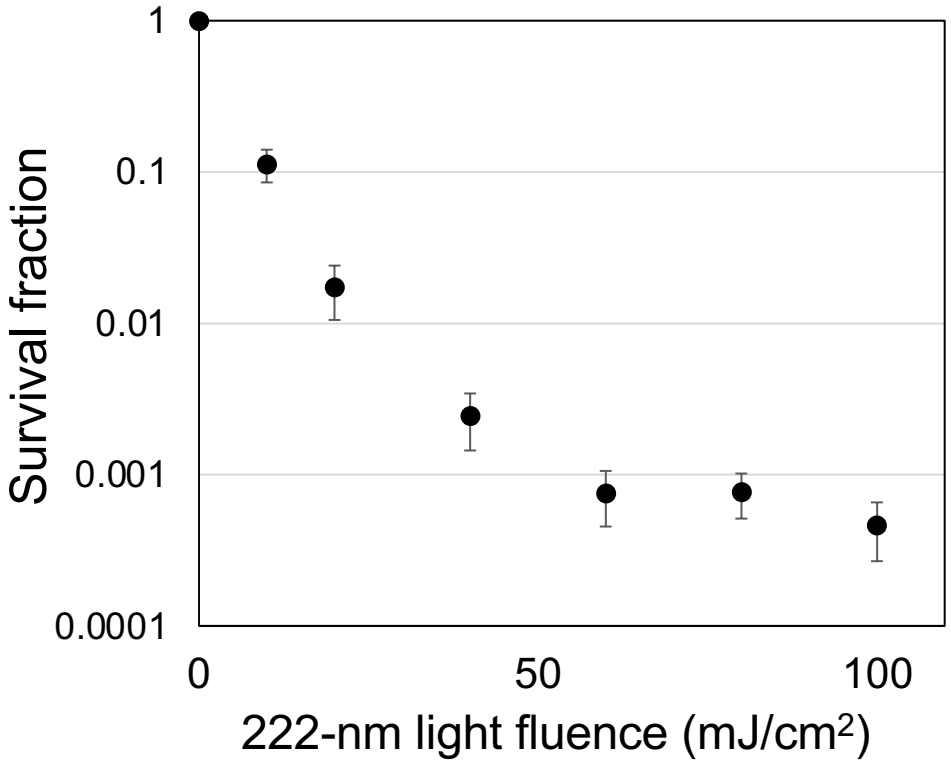


Figure 4. Survival of *B. pumilus* SAFR-032 spores exposed to different fluences of 222-nm light delivered at 0.1 or 0.3 mW/cm^2 (average ± SEM).

- Starting population was ~10^5 – 10^6 spores per coupon roughly equivalent to M2020 quota
- Demonstrated 3 logs of kill, flattening due to clumping/shielding
- The effects of varying fluence rate are insignificant based on the D_{90} required to inactive spores of B. *pumilus* SAFR-032.

Efficacy of far-UVC in inactivating Acinetobacter radioresistens 50v1



- Acinetobacter is a non-spore forming microbe, that is hardy and capable of surviving harsh extremes.
- Testing conditions were similar to that of B.*pumilus*, fluence rate was at 0.5 mW/cm^2
- There was a significant difference in the dose required to inactivate spores of *B. pumilus* at 0.1 mW/cm^2 and *A. radioresistens* 50v1 at 0.5 mW/cm^2 ($p<0.005$).

Advantages of far-UVC vs microbial reduction modalities

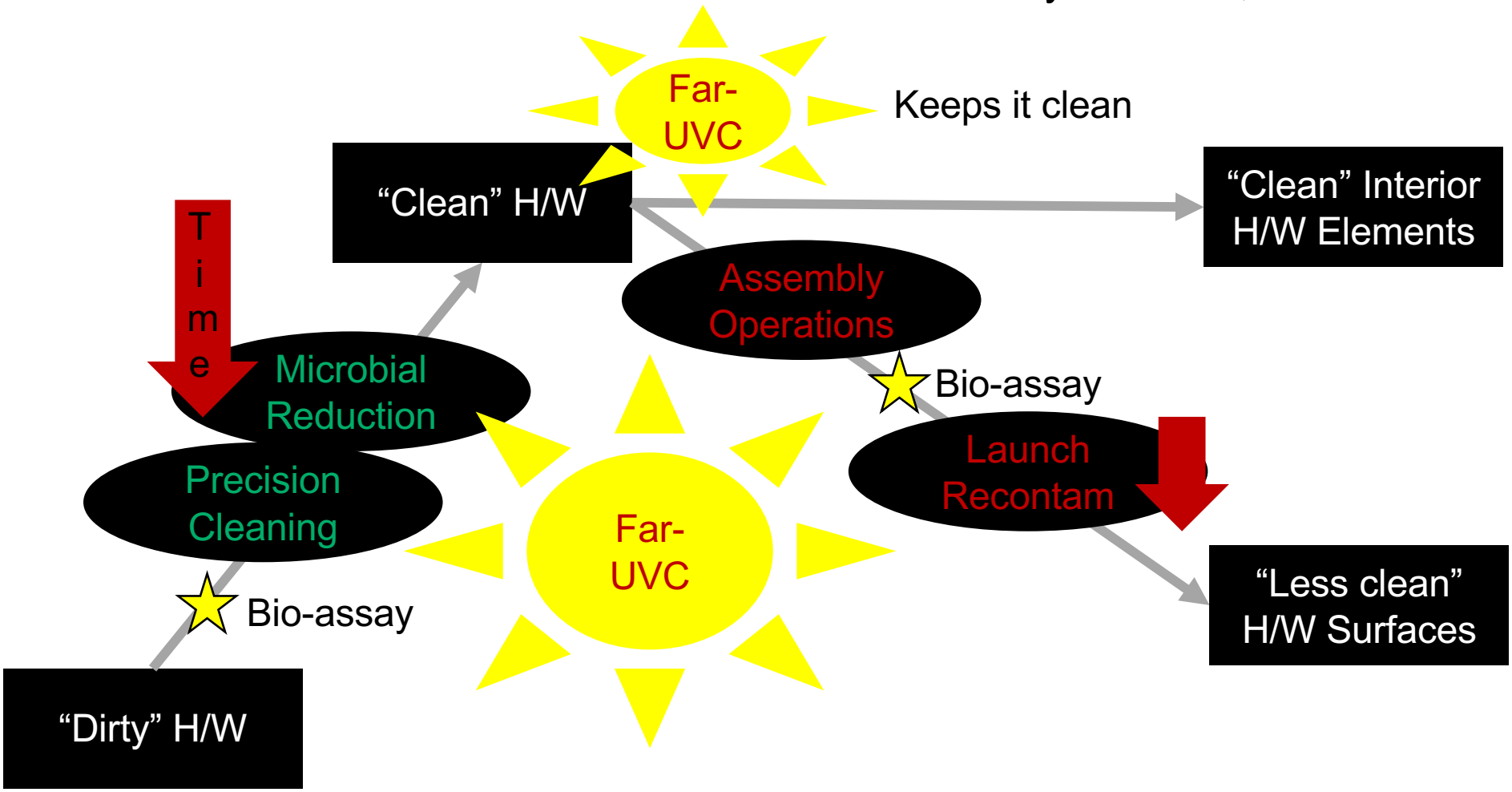
- Requires minimal intervention by engineers and would therefore have minimal impact on scope of work
- Can be implemented within the assembly environment to continually mitigate microbial bioburden.
- Can be used as a recontamination prevention strategy
  - Launch Pad
  - Payload Fairing during launch
  - Environmental Testing
  - Transport

Technical Path Forward

1. Link industry standard 254 nm treatment to 222 nm(far-UVC) treatment to ensure acceptance
2. Determine compatibility for spacecraft and facilities materials to establish the potential applications of this technology.
3. Test a wider range of model organisms to demonstrate efficacy across multiple species previously isolated from spacecraft.
4. Establish human- and hardware-safe application protocol
    1. Identify cleanroom friendly lamps
    2. Characterize UV spectrum of candidate lamps
    3. Propose facilities specific implementation plans
5. Perform proof of concept study in an active cleanroom

Value Added

- Current methods for microbial reduction do not mitigate on-going microbial contamination associated with assembly activities, launch



Publications

Advances in Space Research
Available online 10 September 2020
In Press, Corrected Proof

Far-UVC light as a new tool to reduce microbial burden during spacecraft assembly

Arman Seuylemezian [a], Manuela Buonanno [b], Lisa Guan [a], David J. Brenner [b], David Welch [b]

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https://doi.org/10.1016/j.asr.2020.08.037

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Abstract

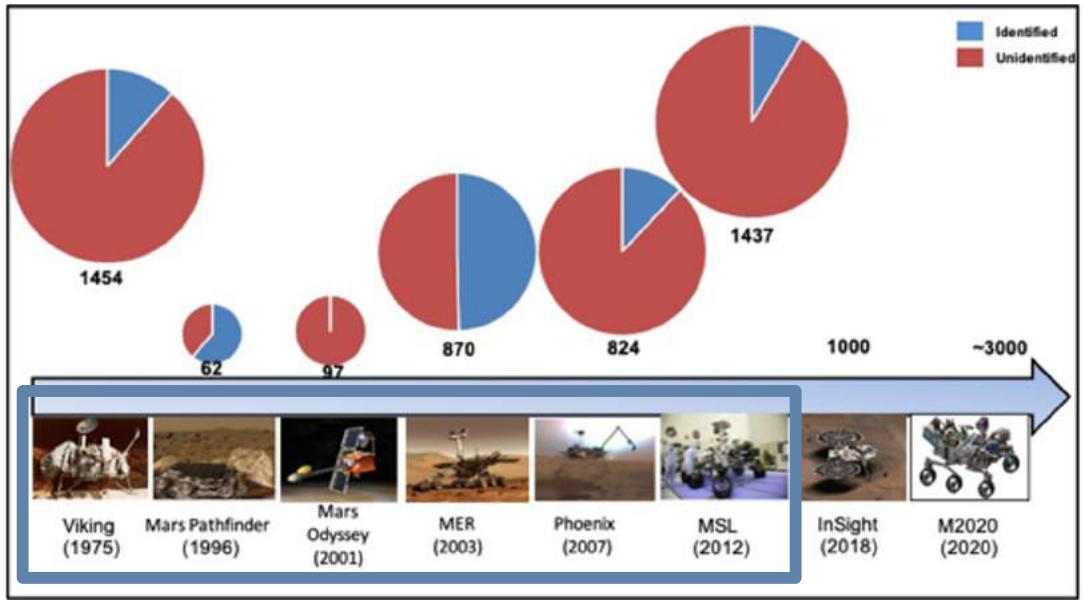
This work aims to investigate far-UVC light at 222 nm as a new microbial reduction tool for planetary protection purposes which could potentially be integrated into the spacecraft assembly process. The major advantage of far-UVC (222 nm) compared to traditional germicidal UVC (254 nm) is the potential for application throughout the spacecraft assembly process in the presence of humans without adverse health effects due to the limited penetration of far-UVC light into biological materials. Testing the efficacy of 222-nm light at inactivating hardy bacterial cells and spores isolated from spacecraft and associated surfaces is a necessary step to evaluate this technology. We assessed survival of *Bacillus pumilus* SAFR-032 and *Acinetobacter radioresistens* 50v1 exposed to 222-nm light on proxy spacecraft surfaces simulated by drying the bacteria on aluminum coupons. The survival fraction of both bacteria followed a single stage decay function up to 60 mJ/cm^2, revealing similar susceptibility of both species to 222-nm light, which was independent of the exposure rate. Irradiation with far-UVC light at 222 nm is an effective method to decontaminate the proxy spacecraft materials tested in this study.

Jet Propulsion Laboratory
California Institute of Technology

jpl.nasa.gov

Problem Statement

- Our current understanding of the culture based microbial diversity of spacecraft and associated surfaces is based upon 16S rRNA sequences which are limited in resolution.
- The goal of this proposal is to
  - perform taxonomic identification of all the bacterial isolates from the JPL microbial archive collected from spacecraft surfaces from NASA missions and functional genomics using the Whole Genome Sequencing (WGS) data from the most dominant, reoccurring and novel isolates.
- Whole Genome Sequencing will provide
  - More accurate species identifications
  - Functional characterization
  - Subspecies profiling/source tracking
  - A reference database for all future missions utilizing metagenomics



Genome Encyclopedia of Spacecraft Associated Microbes (GESAM)

Jet Propulsion Laboratory
California Institute of Technology

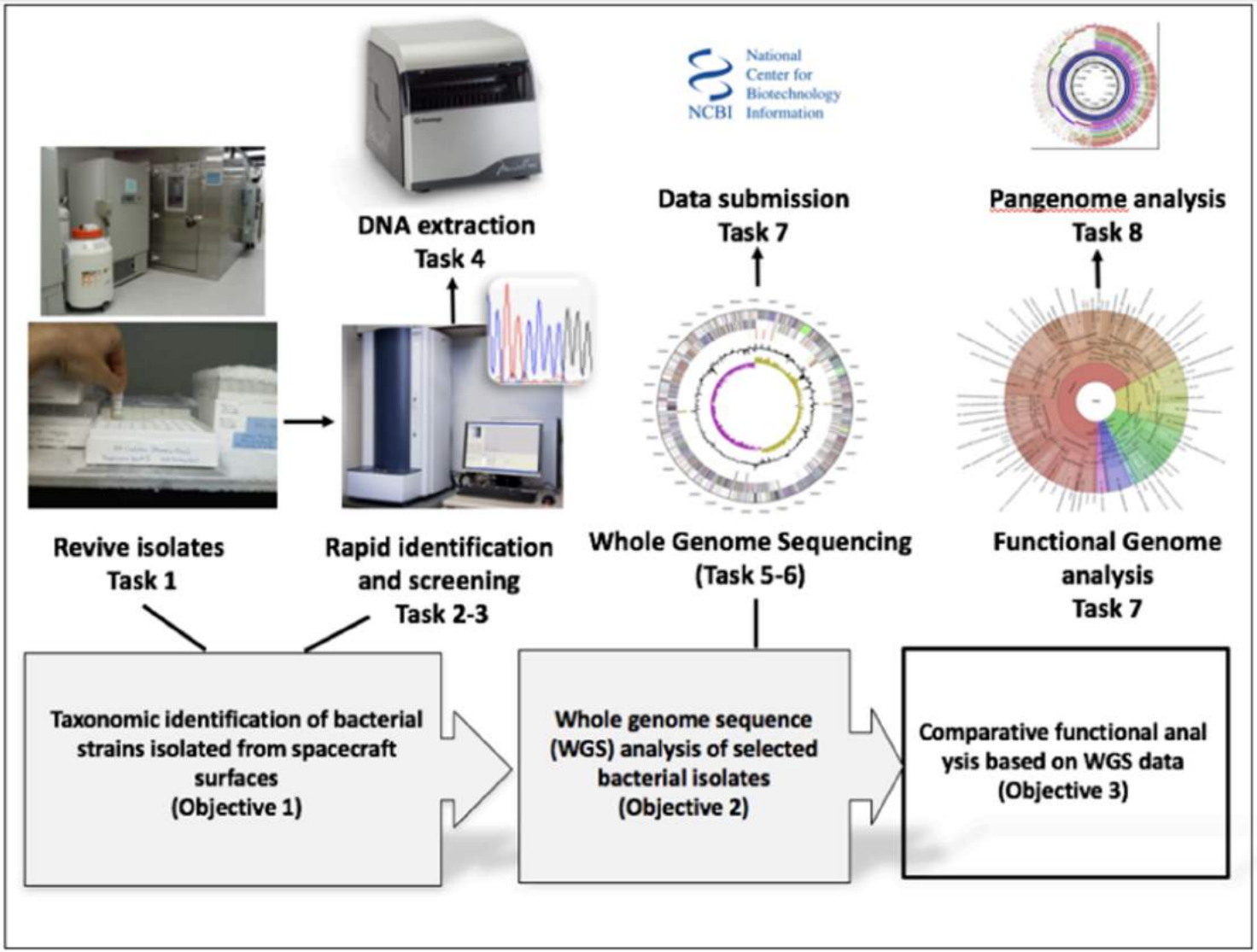
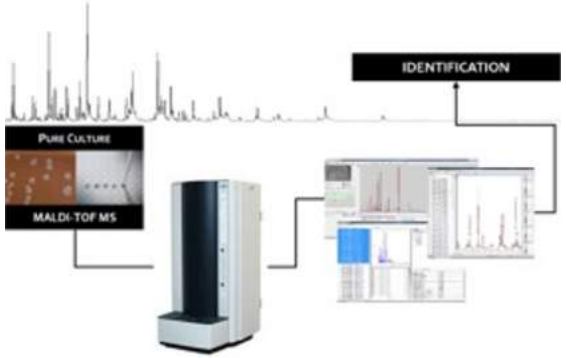
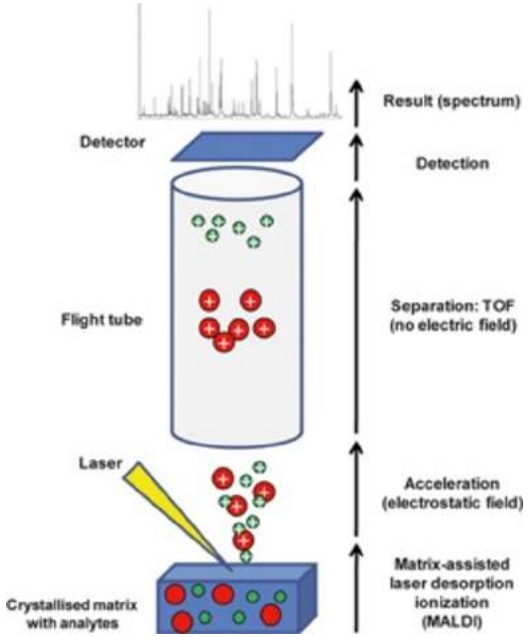


Figure 2. A comprehensive flow chart outlining the GESAM project objectives and tasks.

What is MALDI-TOF MS

- Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
- Generates ions from large molecules while minimizing fragmentation
- Identification based on a protein profile





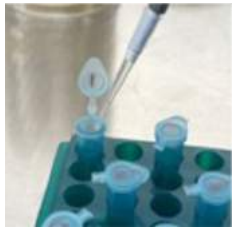
Identification Techniques (MALDI-TOF MS)

Formic Acid Tube Extraction

Inoculate two loops of biomass into 1 ml of 75% ethanol

Vortex for 15 minutes

Pellet isolate and decant ethanol



Add 100 µL 70% formic acid and vortex again for 15 minutes

Add 100 µL 100% acetonitrile and centrifuge x2 minutes

Add 1 µL supernatant to target and overlay with matrix

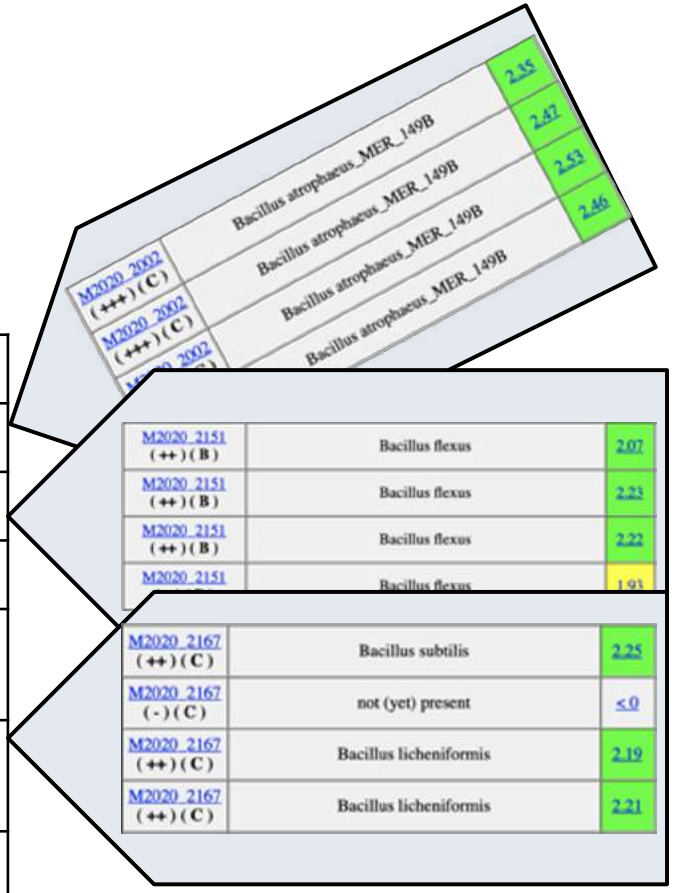
MALDI-TOF Background

Goal : Rapid species- level identification of bacterial colonies

Slide provided by: Akemi Hinzer

MALDI-TOF Scoring Strategy

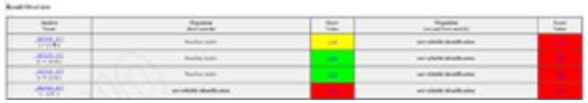
Match Type	Score Met	3 Scores	Interpretation
Perfect ID	2.2	✓	Confident in identification
Redo Species	2.2		Identified without confidence
Genus Match	2.0	✓	Confident in genus identification
Redo Genus	2.0		Genus identified without confidence
Taxonomic Group	2.0	✓	Genus identified with mismatched species
Not Reliable	-		All scores were below 2.0
No Peaks			Experiment produced no peaks to match

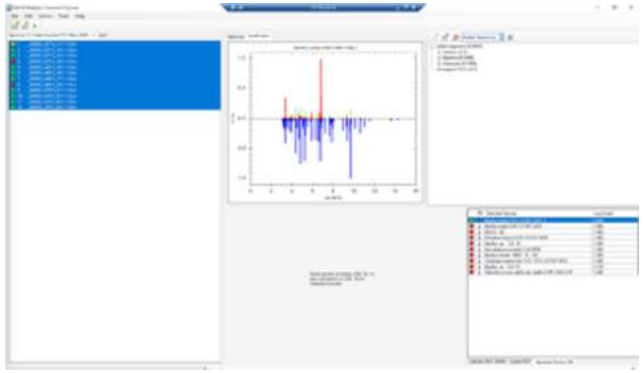


Slide provided by: Akemi Hinzer

Data Analysis

- Mass spectra of unknown isolates are compared against
  - The Bruker reference database containing:
    - 8540 bacterial strains
  - The JPL in-house curated database containing:
    - 509 bacterial strains





16S rRNA Sanger Sequencing

- Three primer sanger sequencing
  - 27F, 1492R, and 512F
  - More robust, yields higher overlap region than using 2 primers
- Species level identification
  - Although some species are indiscernible using only 16S rRNA
- Still the gold standard for characterizing potentially novel organisms

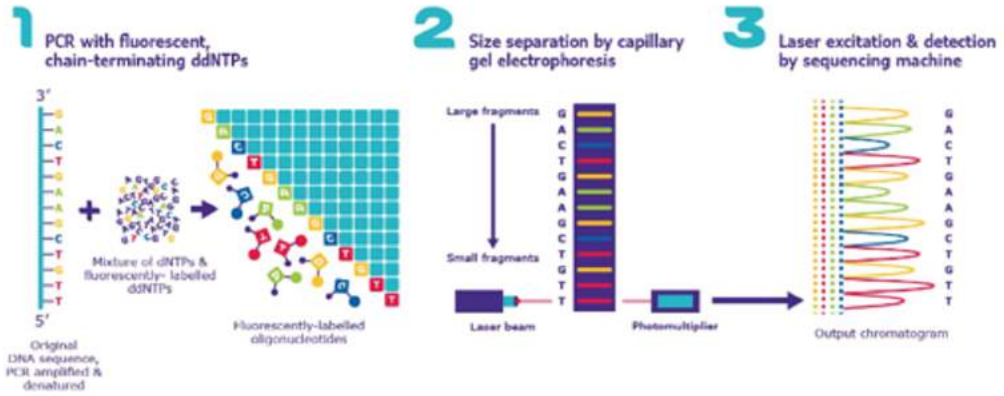
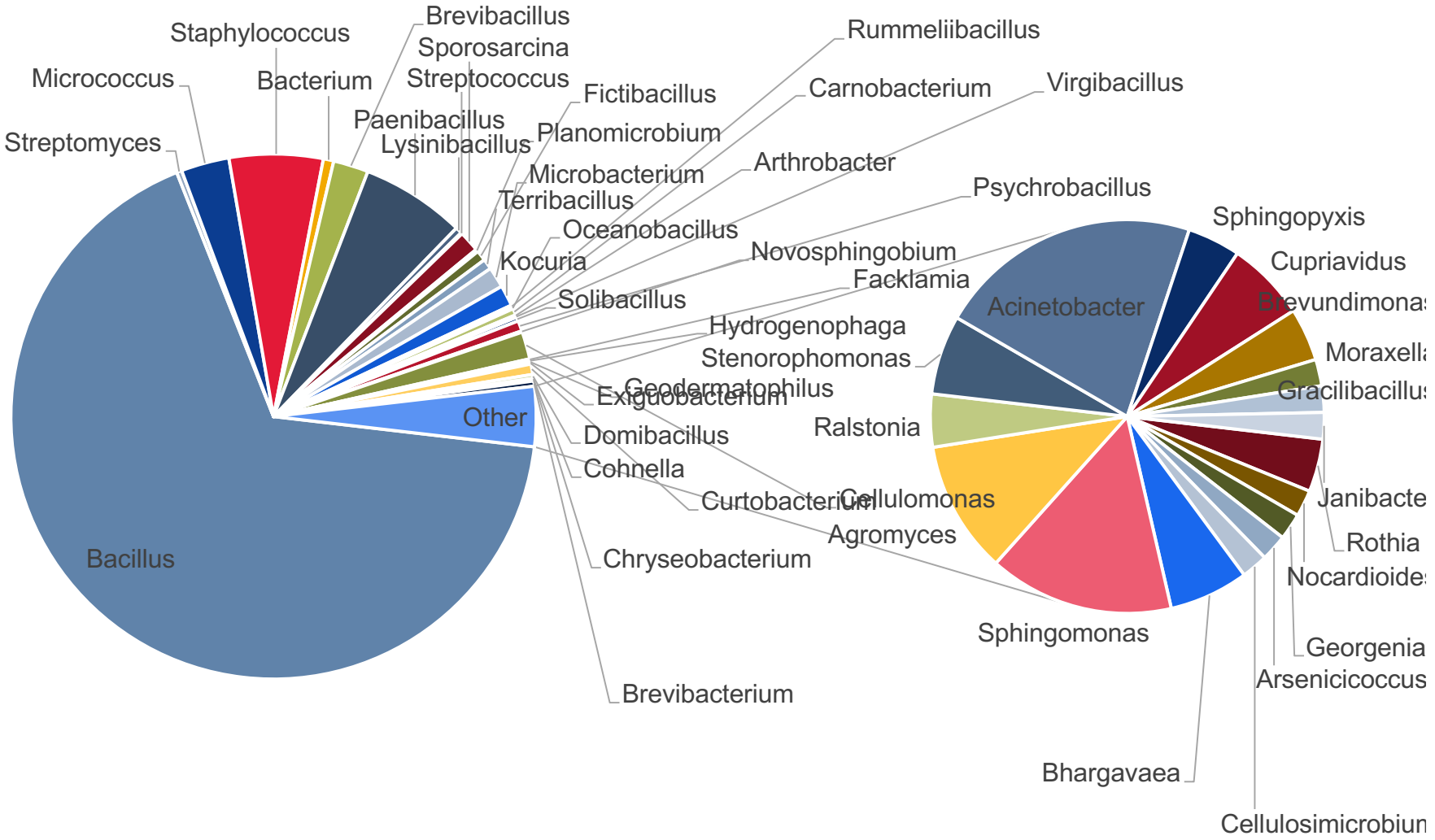
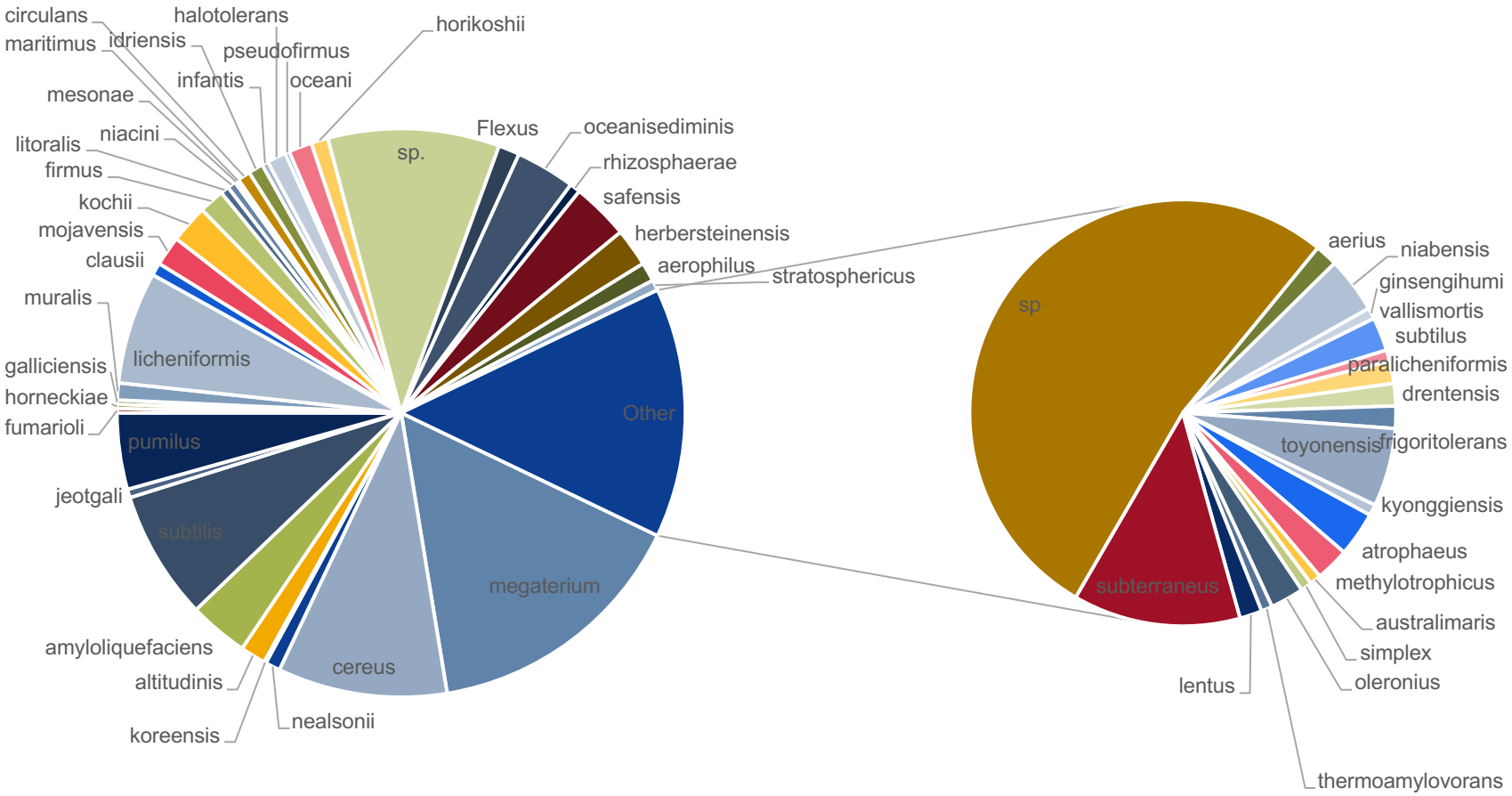


Figure 1. Three Basic Steps of Automated Sanger Sequencing.

Genus Level Diversity ~1,200 isolates from spacecraft and associated surfaces



Species Diversity of 838 strains belonging to the Bacillus Genus

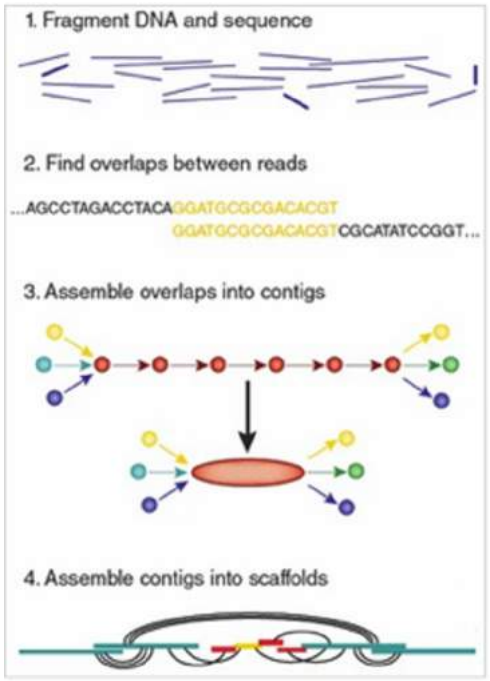


- DNA Extraction
  - Manual
    - Filter based extraction kits
  - Automated
    - Magnetic bead based extraction (Maxwell 16 System)
- Quality Check
  - >1 ng/ul is sufficient for sequencing however if extracting from pure cultures >30 ng/ul is reasonable.
  - High molecular weight DNA, running a gel to see the general fragment size is a good idea.

DNA Extraction, Whole Genome Sequencing and Assembly

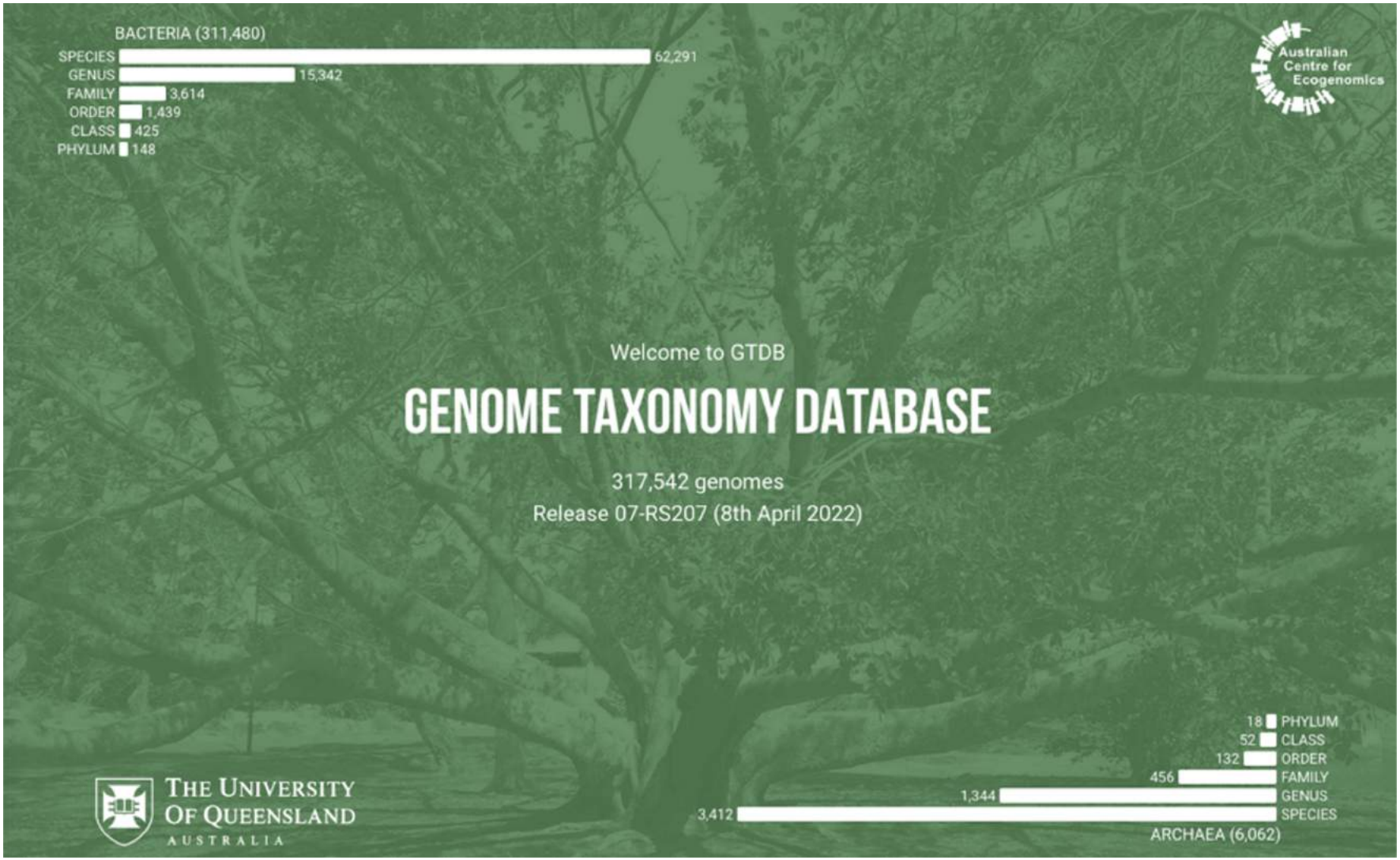
Whole Genome Sequencing

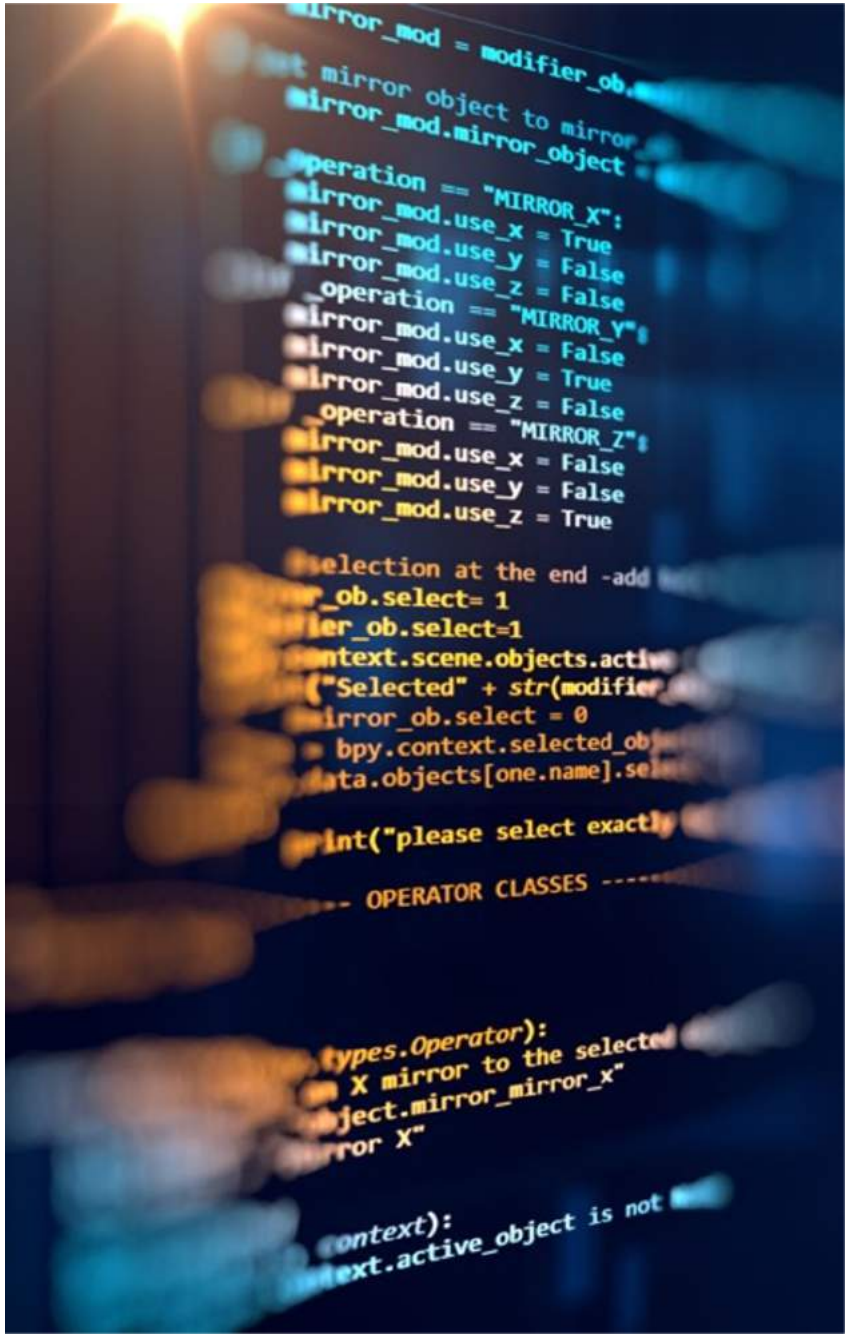
- Illumina paired-end platform (250 bp reads)
- Average of 30-50X coverage (~250 mB per genome raw data)



Data Analysis

- Quality/length filtering: 0.01% accuracy, >200 bp reads, trim for adapter sequences
- Genome Assembly:
  - <100 contigs
  - L50 (number of contigs needed to reach 50% of the genome): good indication of assembly quality

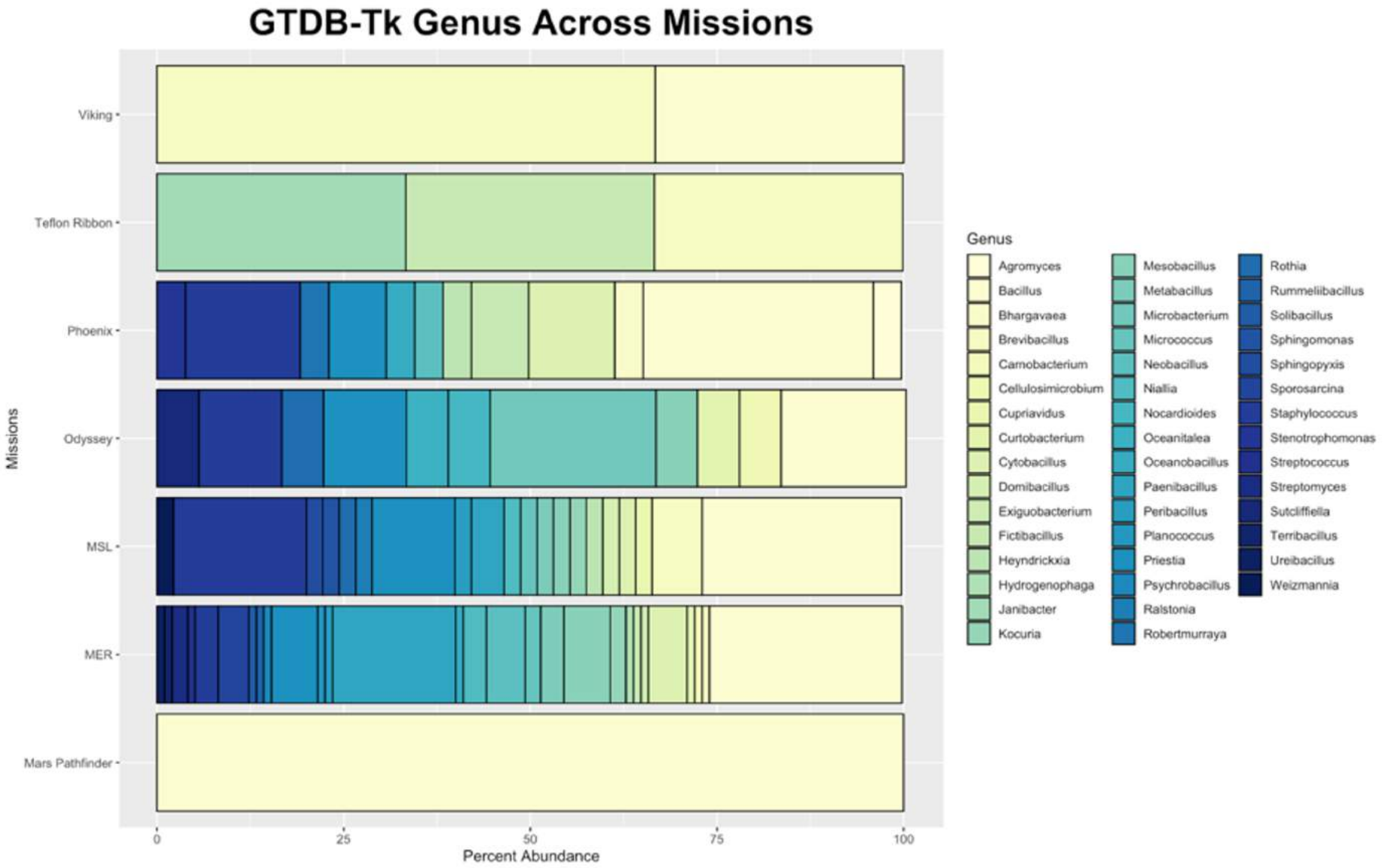

BACTERIA (311,480)
SPECIES 62,291
GENUS 15,342
FAMILY 3,614
ORDER 1,439
CLASS 425
PHYLUM 148
Australian Centre for Ecogenomics
Welcome to GTDB
GENOME TAXONOMY DATABASE
317,542 genomes
Release 07-RS207 (8th April 2022)
THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
18 PHYLUM
52 CLASS
132 ORDER
456 FAMILY
1,344 GENUS
3,412 SPECIES
ARCHAEA (6,062)



How does GTDB-Tk work?

- GTDB-Tk stands for **Genome Taxonomy Database Toolkit** and its workflow consists of 3 steps:
  - Identify
  - Align
  - Classify
- What does this mean for use of the GTDB-Tk Identification database?
  - A more robust method of classification than 16S
  - More parameters used
  - More information including functional genes and applications for Pangenome analysis of isolates

Taxonomic Identification of 200 whole genome sequenced isolates



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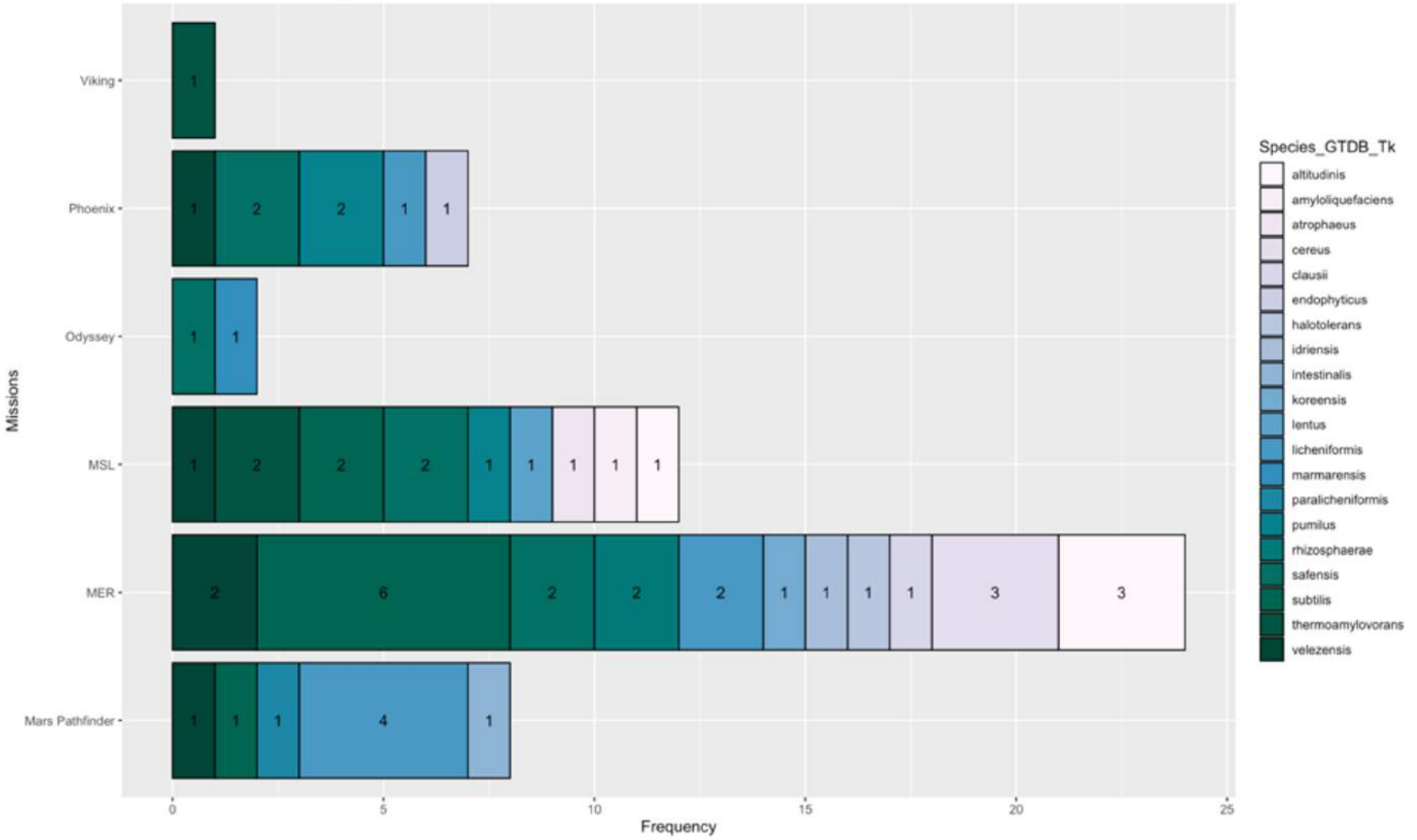
GTDB-Tk Species within the Bacillus Genera Across Missions



Slide provided by Michelle Tran

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Next Steps/On-going projects:

- Perform genomic comparisons of strain level variants to identify patterns between species consistently isolated across multiple missions
- Characterize potentially novel organisms
- Identify unique genes present in spacecraft isolated strains in comparison with those from other environments
- Perform metagenomic read mapping against these genomes to identify relative abundance of particular culturable strains across metagenomic datasets.