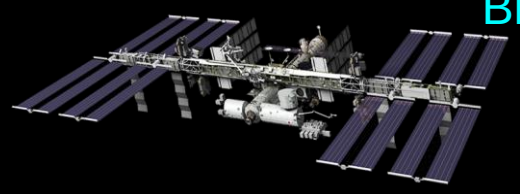
Microbes in Space

Kasthuri Venkateswaran



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Outline of the presentation

- Microbiology in NASA
  - Astrobiology
  - Planetary Protection
- Microbial observatory of International Space Station
  - Traditional standard methods
  - State-of-the art molecular methods
  - Extremophiles and “problematic” opportunistic pathogens
  - Novel species characterization
- International Space Station as a testbed for future human missions

Principles of Planetary Protection

- The second principles of the astrobiology:
  - Astrobiology encourages planetary stewardship through an emphasis on protection against forward and back biological contamination and recognition of ethical issues associated with exploration.

Basic Planetary Protection Policy
(Paraphrased)

- Preserve planetary conditions for future biological and organic constituent exploration
  - *avoid forward contamination; preserve our investment in scientific exploration*
- To protect Earth and its biosphere from potential extraterrestrial sources of contamination
  - *avoid backward contamination; provide for safe solar-system exploration*

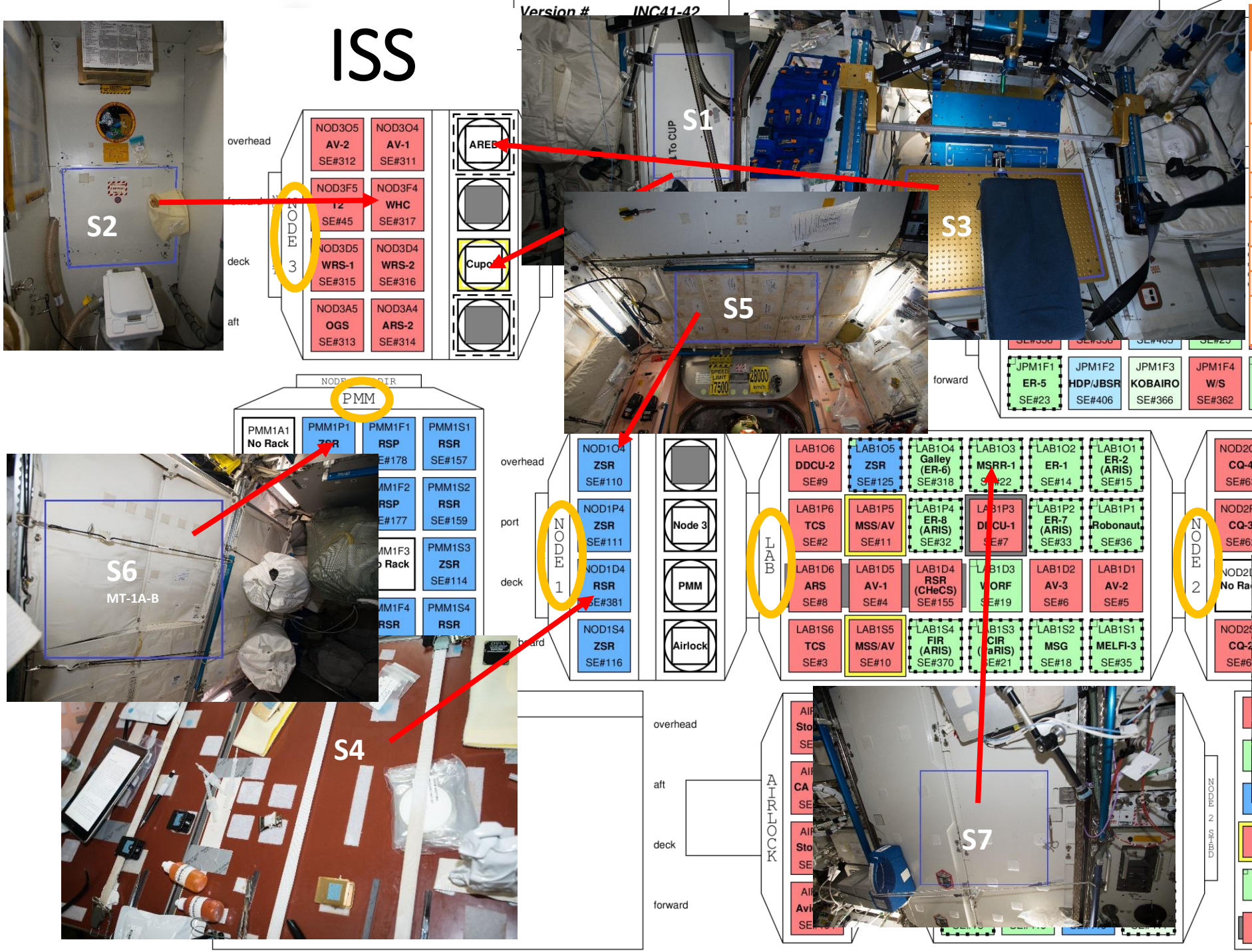
Complies with Article IX of the United Nations Outer Space Treaty of 1967

Microbial Observatory Experiment MT1B sampling crew; iss043e198419

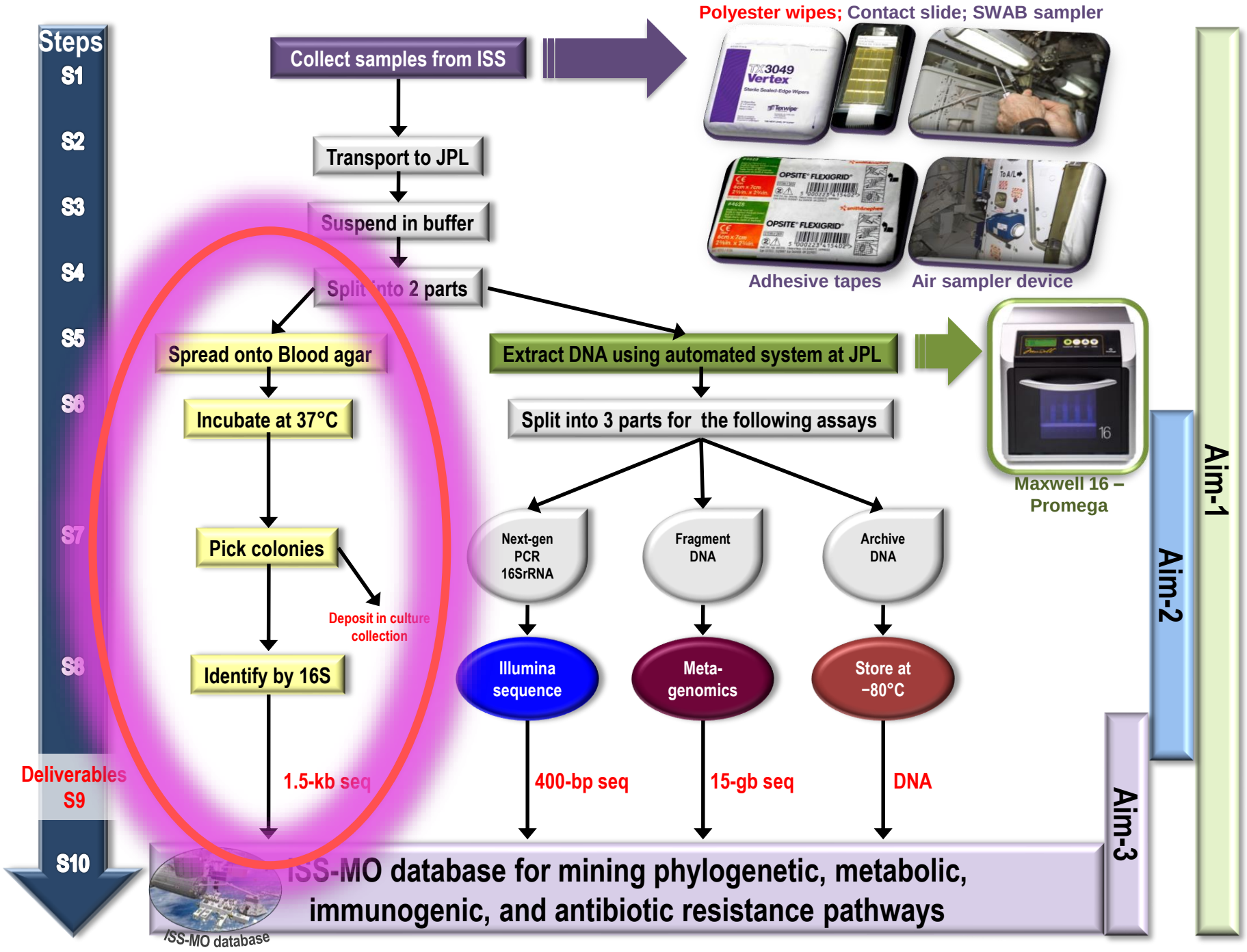
NASA astronaut Scott Kelly

NASA astronaut Terry Virts, Commander collected the Microbial Observatory Experiment samples for MT1A and MT1B

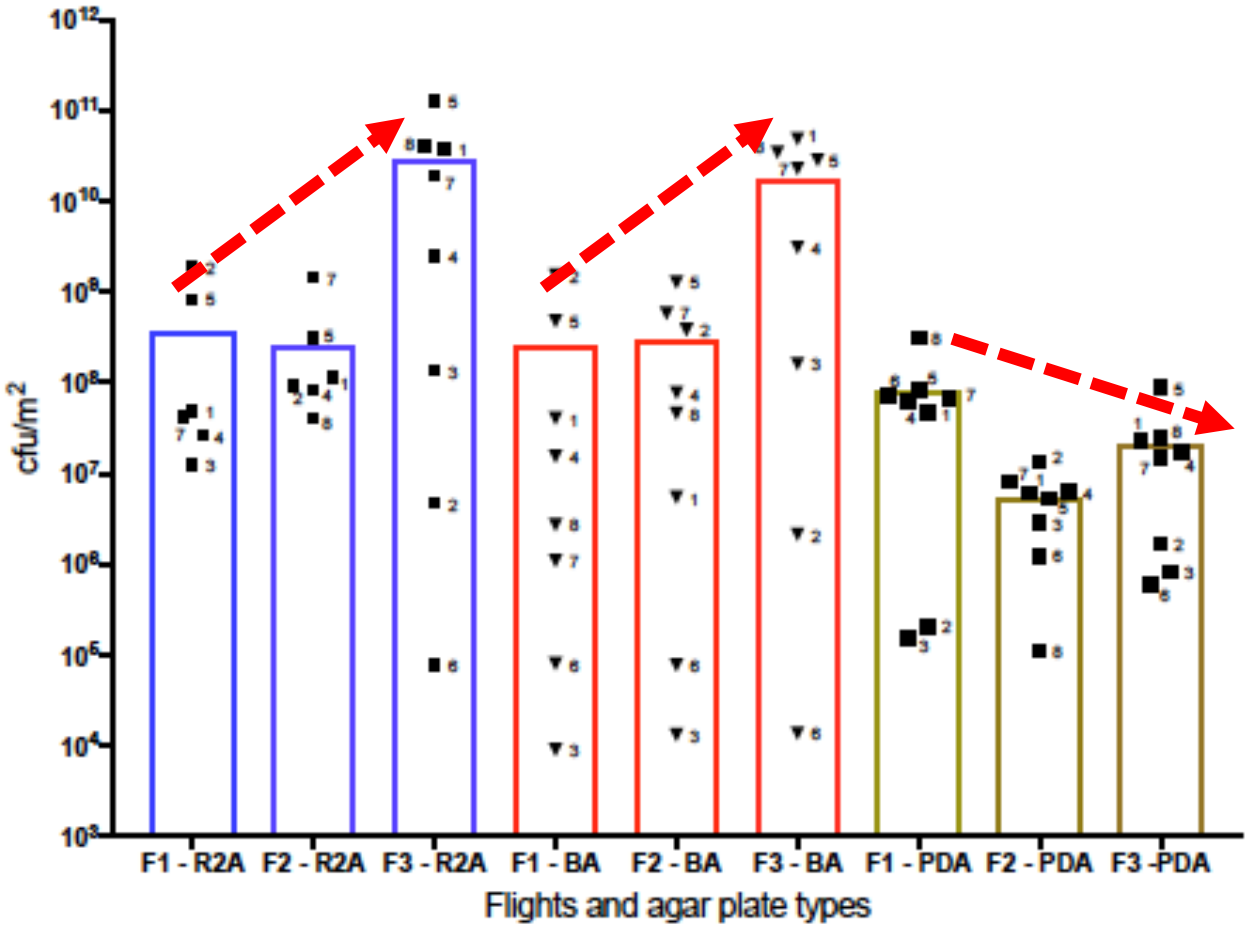
ISS



Sampling #	ISS Module	Surface Sampling Location
S1	Port panel next to cupola	In Node 3 next to the cupola, on the way down into the cupola on the port panel.
S2	N3_F4	Node 3 "F4" location right in front of the WHC
S3	Node 3	ARED Foot platform
S4	Node 1	Dining Table
S5	N1_O4	Node 1 Overhead 4
S6	PMM_P1	PMM Port 1
S7	LAB_O3	Lab Overhead 3
S8	Node 2	Port crew quarters Bump-Out exterior aft wall

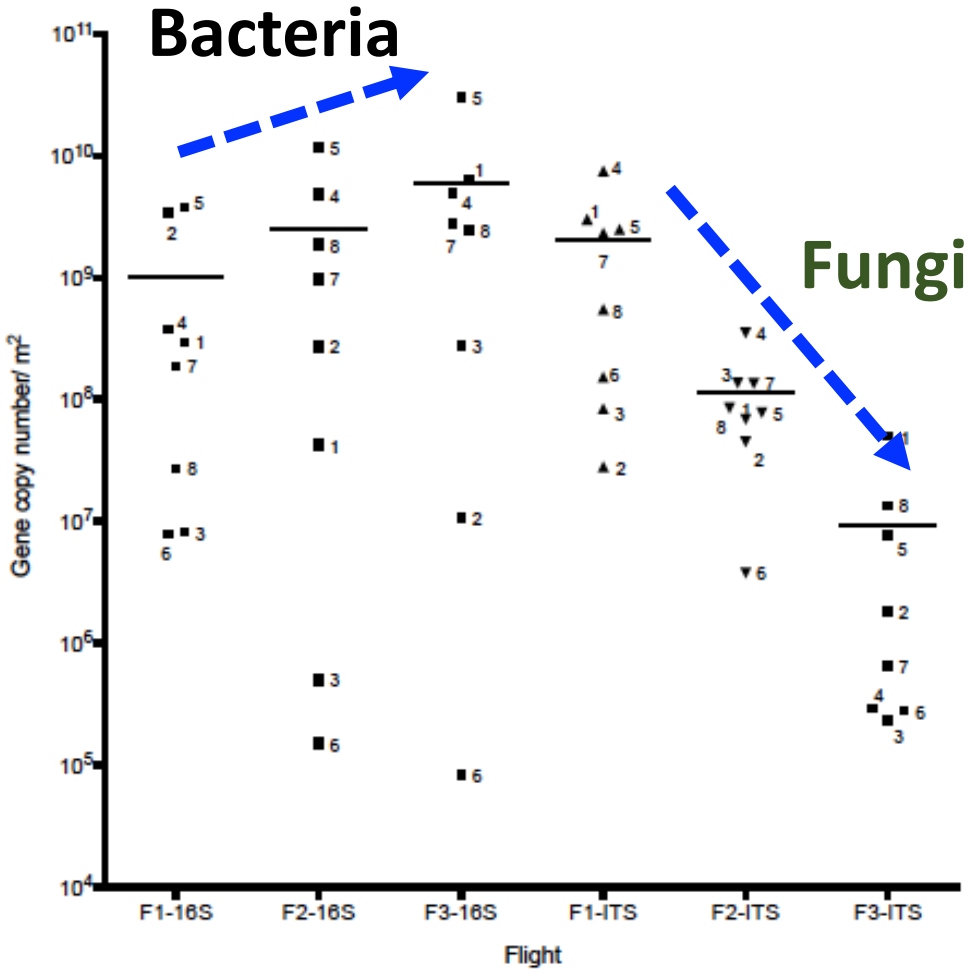

Steps
S1
S2
S3
S4
S5
S6
S7
S8
Deliverables
S9
S10
Collect samples from ISS
Polyester wipes; Contact slide; SWAB sampler
Adhesive tapes
Air sampler device
Transport to JPL
Suspend in buffer
Split into 2 parts
Spread onto Blood agar
Incubate at 37°C
Pick colonies
Deposit in culture collection
Identify by 16S
1.5-kb seq
Extract DNA using automated system at JPL
Maxwell 16 – Promega
Split into 3 parts for the following assays
Next-gen PCR 16SrRNA
Fragment DNA
Archive DNA
Illumina sequence
Meta-genomics
Store at −80°C
400-bp seq
15-gb seq
DNA
ISS-MO database for mining phylogenetic, metabolic, immunogenic, and antibiotic resistance pathways
ISS-MO database
Aim-1
Aim-2
Aim-3

Cultivable microbes

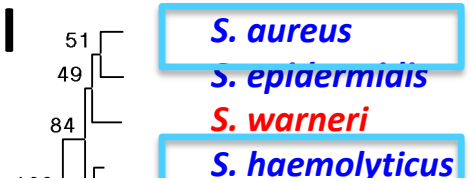


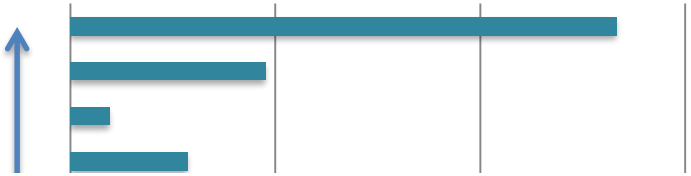
R2A/BA: Bacteria **Fungi**

PMA – qPCR Viable bacteria



Cultivable Bacterial Diversity





AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

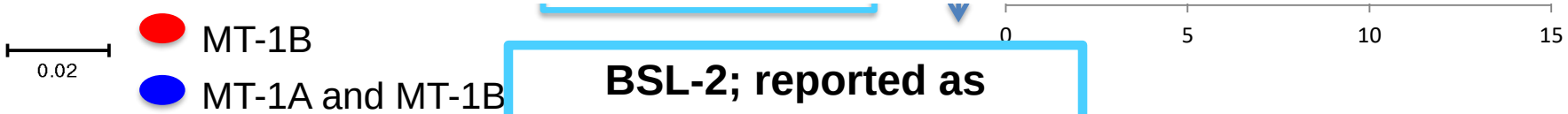
CrossMark click for updates

Draft Genome Sequences of Biosafety Level 2 Opportunistic Pathogens Isolated from the Environmental Surfaces of the International Space Station

Aleksandra Checinska Sielaff,[a] Nitin K. Singh,[a] Jonathan E. Allen,[b] James Thissen,[b] Crystal Jaing,[b] Kasthuri Venkateswaran[a]

Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA[a]; Lawrence Livermore National Laboratory, Livermore, California, USA[b]

The draft genome sequences of 20 biosafety level 2 (BSL-2) opportunistic pathogens isolated from the environmental surfaces of the International Space Station (ISS) were presented. These genomic sequences will help in understanding the influence of microgravity on the pathogenicity and virulence of these strains when compared with Earth strains.



BSL-2; reported as opportunistic pathogen

N=105

SNP phylogeny

1. The phenotypic traits such as motile rods, enterotoxin production, lack of capsule, and resistance to gamma phage/penicillin observed in ISS isolates were not characteristics of *B. anthracis*.
2. Whole-genome sequence characterizations showed that ISS strains had the *plc*R non-*B. anthracis* ancestral "C" allele and lacked anthrax toxin-encoding plasmids pXO1 and pXO2, excluding their identification as *B. anthracis*.
3. The genetic identities of all 11 ISS isolates characterized via *gyr*B analyses arbitrarily identified them as members of the *B. cereus* group, but traditional DNA-DNA hybridization (DDH) showed that the ISS isolates are similar to *B. anthracis* (88% to 90%) but distant from the *B. cereus* (42%) and *B. thuringiensis* (48%) type strains.
4. The DDH results were supported by average nucleotide identity (98.5%) and digital DDH (86%) analyses. However, the collective phenotypic traits and genomic evidence were the reasons to exclude the ISS isolates from *B. anthracis*.
5. Nevertheless, MLST and WG-SNP analyses placed these isolates in a clade that is distinct from previously described members of the *B. cereus* sensu lato group but closely related to *B. anthracis*.

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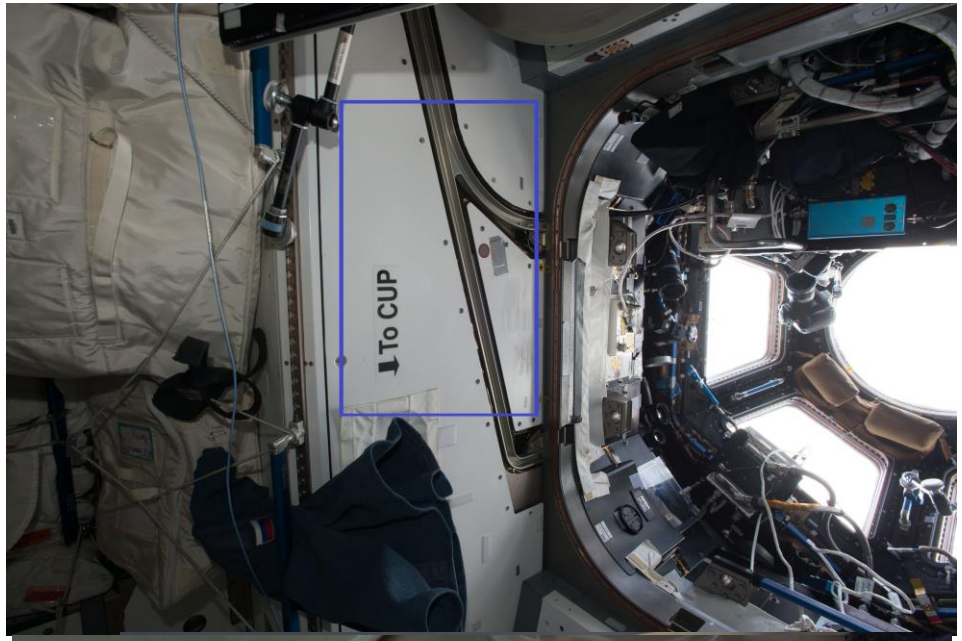
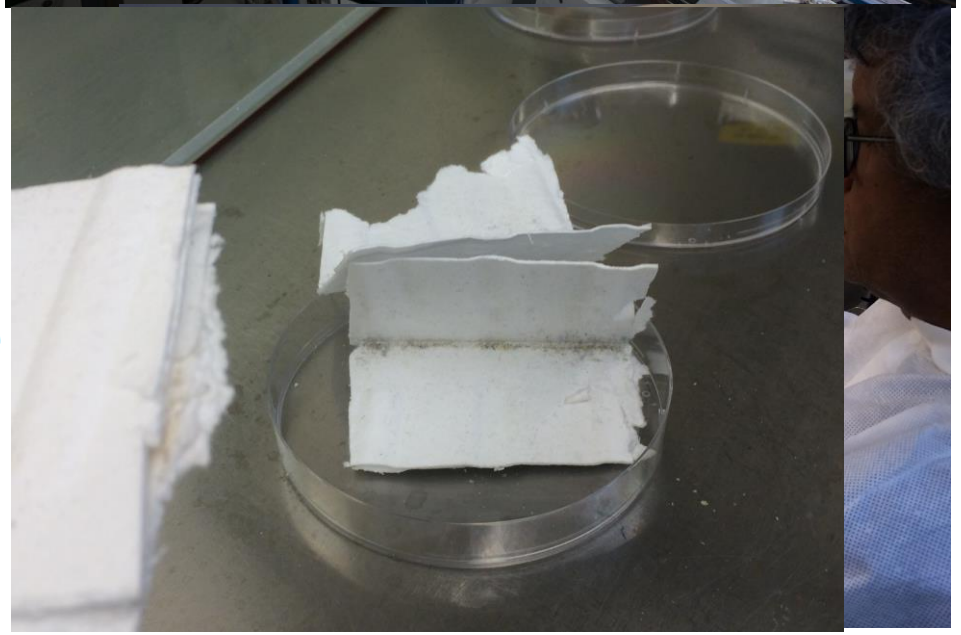
RESEARCH ARTICLE
Clinical Science and Epidemiology



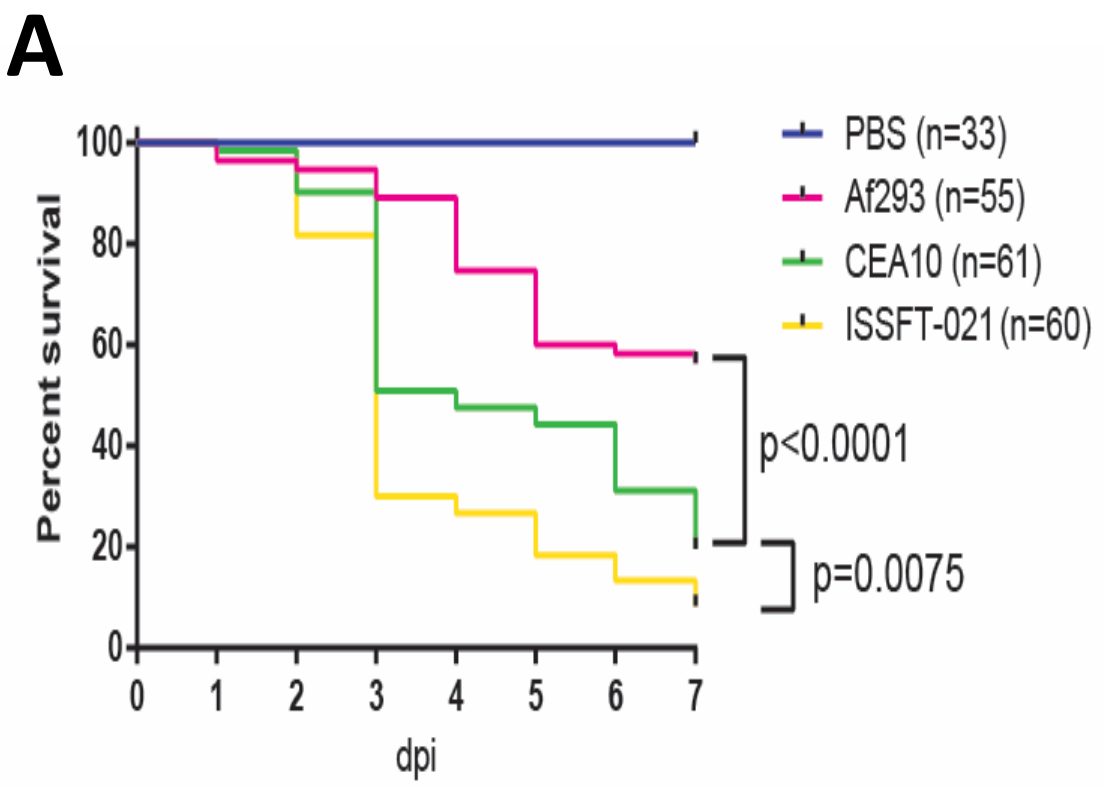
Characterization of *Aspergillus fumigatus* Isolates from Air and Surfaces of the International Space Station

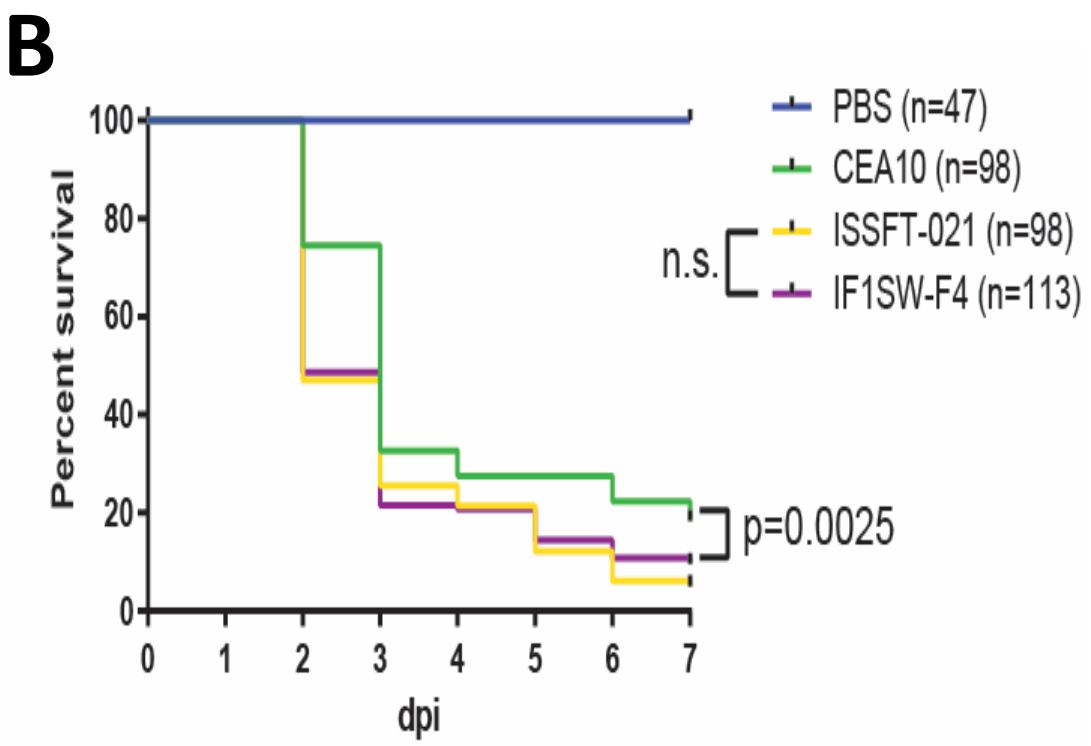


Benjamin P. Knox,[a,b] Adriana Blachowicz,[c,d] Jonathan M. Palmer,[e] Jillian Romsdahl,[d] Anna Huttenlocher,[b,f] Clay C. C. Wang,[d,g] Nancy P. Keller,[b,h] Kasthuri Venkateswaran[c]

Microbiology Doctoral Training Program, University of Wisconsin—Madison, Madison, Wisconsin, USA[a]; Department of Medical Microbiology and Immunology, University of Wisconsin—Madison, Madison, Wisconsin, USA[b]; Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA[c]; Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, California, USA[d]; Center for Forest Mycology Research, U.S. Forest Service, Madison, Wisconsin, USA[e]; Department of Pediatrics, University of Wisconsin—Madison, Madison, Wisconsin, USA[f]; Department of Chemistry, Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, California, USA[g]; Department of Bacteriology, University of Wisconsin—Madison, Madison, Wisconsin, USA[h]

Virulence assessment in a larval zebrafish model of invasive aspergillosis





A) Survival outcome through 7 days post infection (dpi) of neutrophil-deficient *mpx*:*mCherry*-2A-*Rac2D57N* larvae, where neutrophils specifically are unable to reach the site of infection. B) Survival outcome of the second ISS isolate, IF1SW-F4, compared to CEA10 and ISSFT-021 in *mpx*:*mCherry*-2A-*Rac2D57N* larvae. Shown are data pooled from three (panel A) or four (panel B) independent experimental replicates. Statistical analyses were performed using Cox Proportional-Hazard regression analysis.

International Space Station Projects

- Are they all the same strain?
- Is there a contamination problem on the ISS?
- Are projects related to microbes, for whatever reason, not being contained from crew living environment

Purpose:

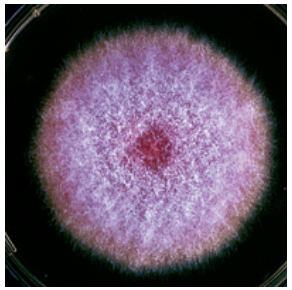
- Need to improve habitability on long duration space missions
- Veggie- The Vegetable

e

ss

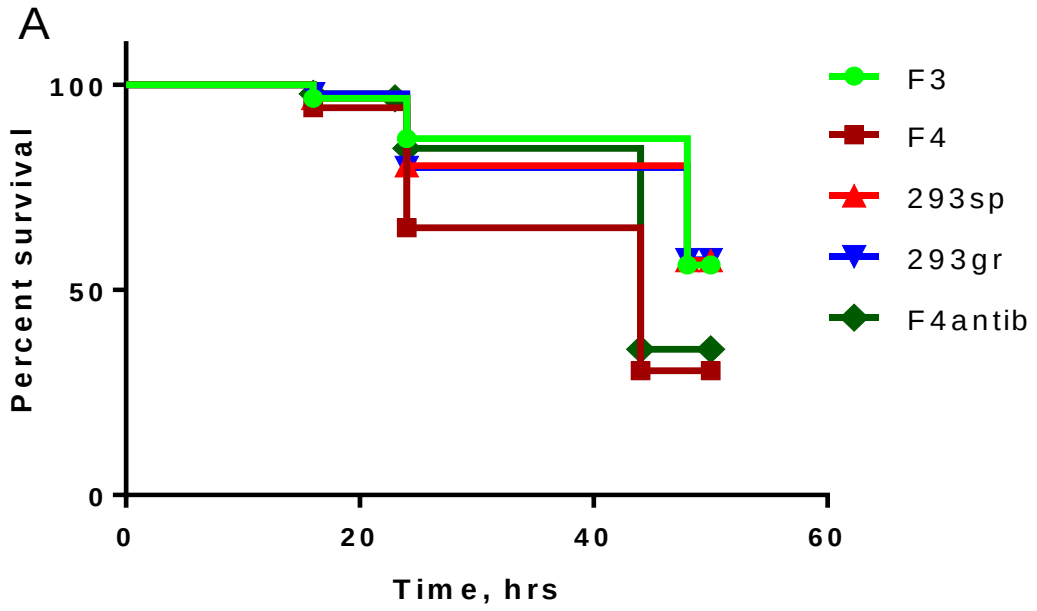
Fungus

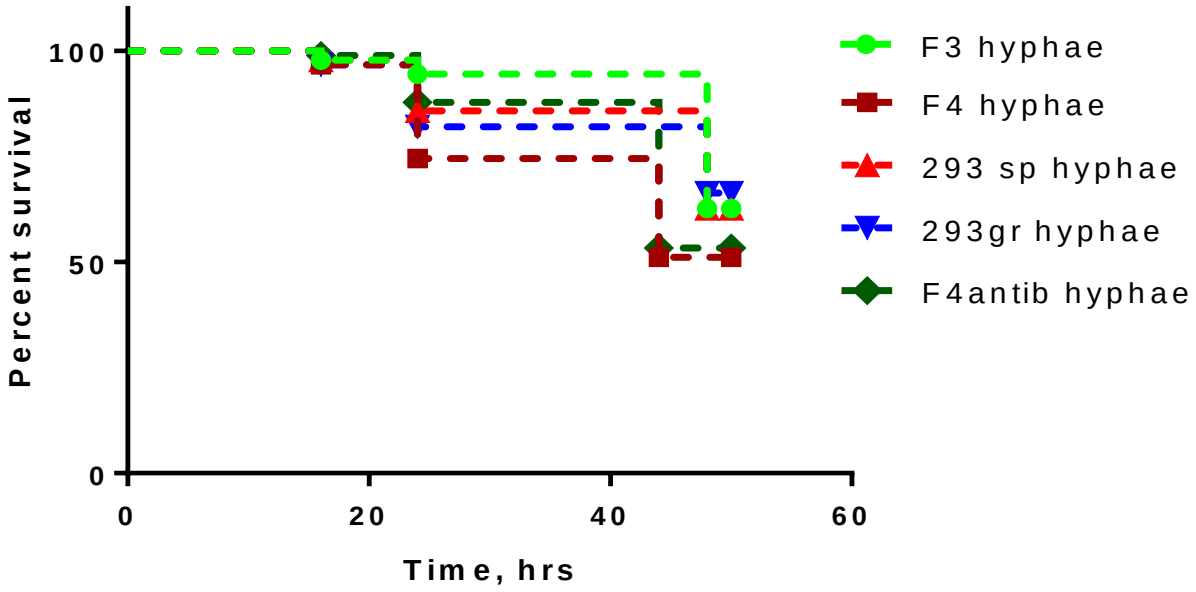
Fusarium oxysporum

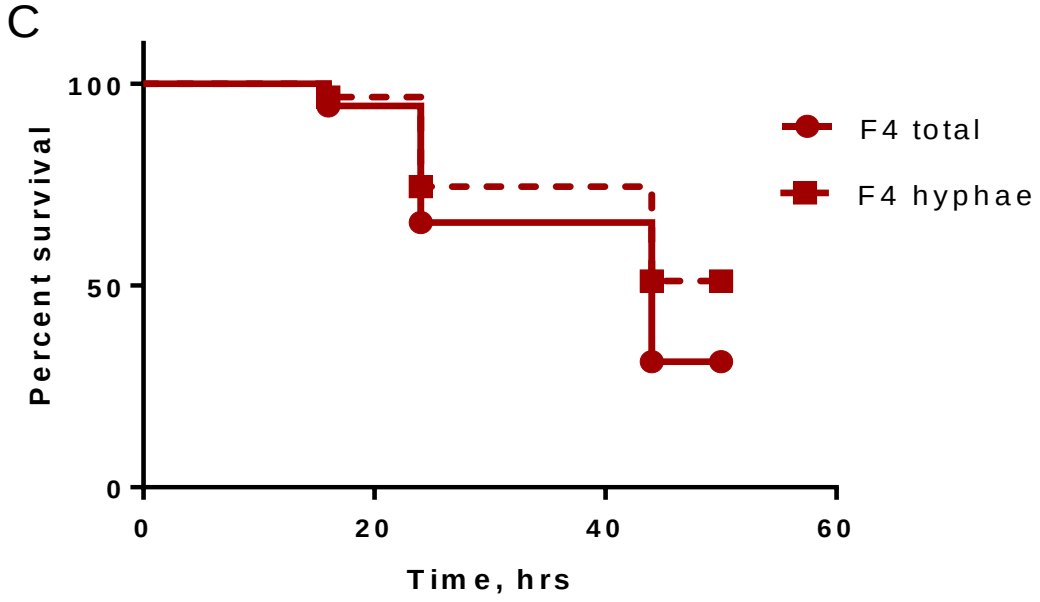


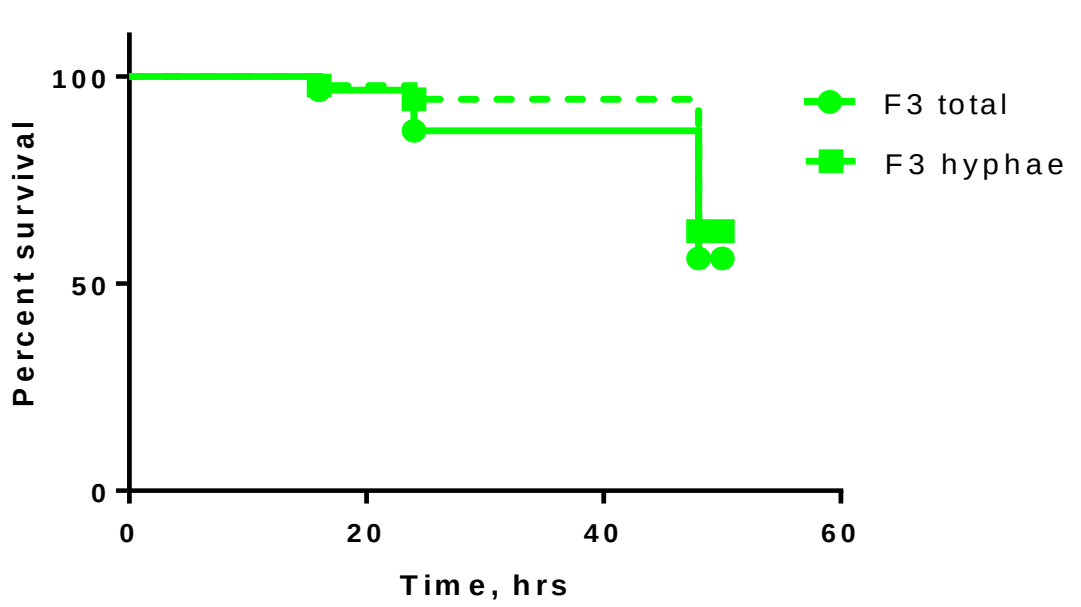
Purpose:

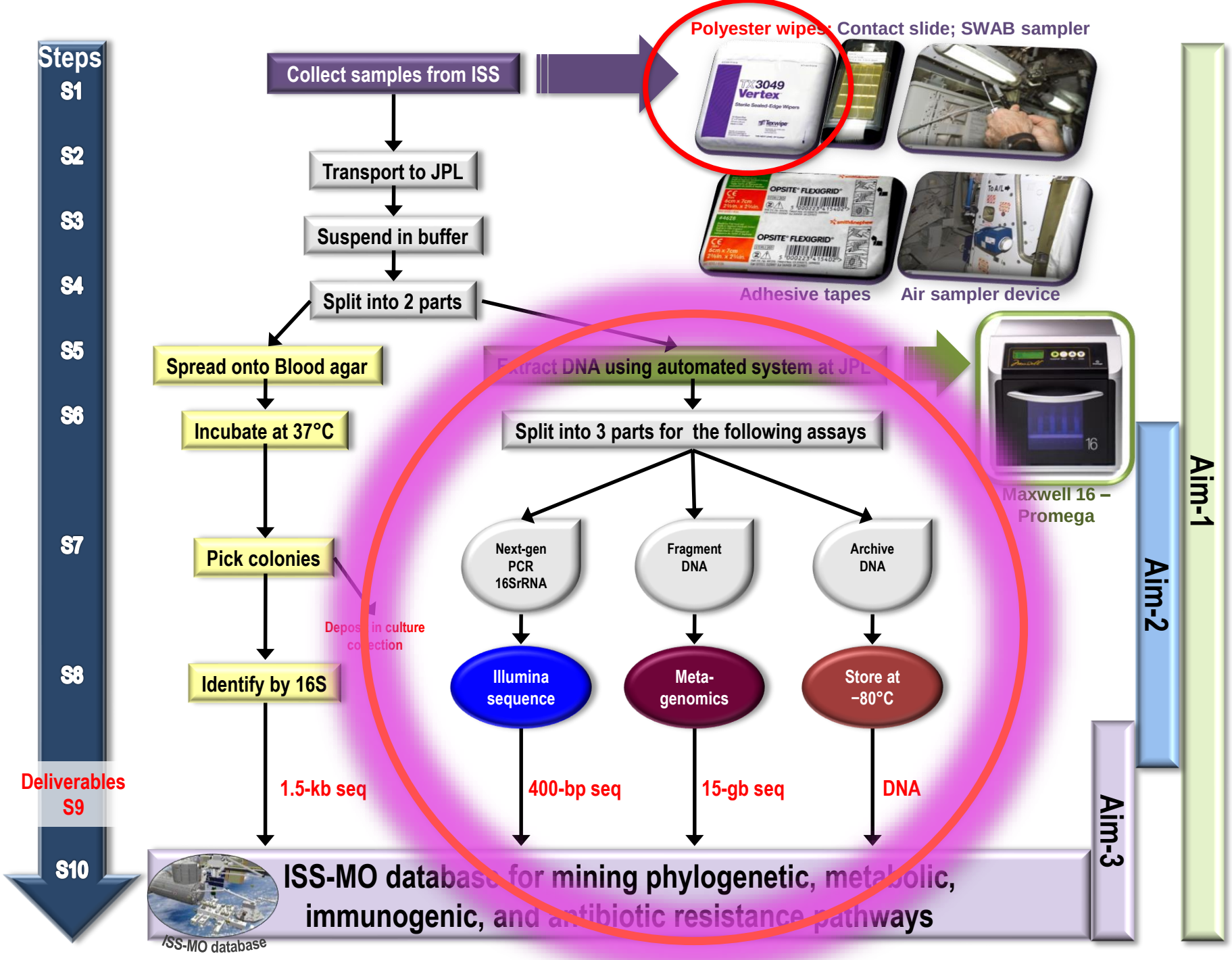
- Characterize airborne and surface-associated microbes


A
Percent survival
100
50
0
0
20
40
60
Time, hrs
F3
F4
293sp
293gr
F4antib

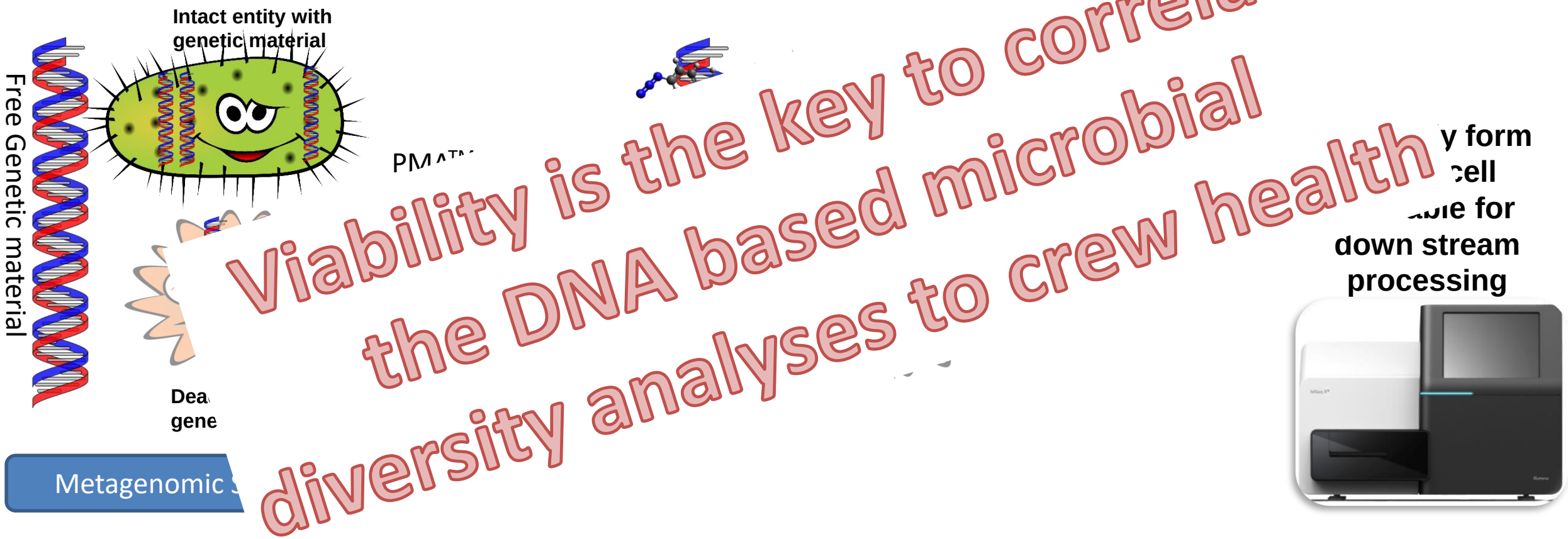

B
Percent survival
100
50
0
0
20
40
60
Time, hrs
F3 hyphae
F4 hyphae
293 sp hyphae
293gr hyphae
F4antib hyphae


C
Percent survival
100
50
0
0
20
40
60
Time, hrs
F4 total
F4 hyphae


D
Percent survival
100
50
0
0
20
40
60
Time, hrs
F3 total
F3 hyphae


Steps
S1
S2
S3
S4
S5
S6
S7
S8
Deliverables
S9
S10
Collect samples from ISS
Transport to JPL
Suspend in buffer
Split into 2 parts
Spread onto Blood agar
Incubate at 37°C
Pick colonies
Deposit in culture collection
Identify by 16S
Extract DNA using automated system at JPL
Split into 3 parts for the following assays
Next-gen PCR 16SrRNA
Fragment DNA
Archive DNA
Illumina sequence
Meta-genomics
Store at −80°C
1.5-kb seq
400-bp seq
15-gb seq
DNA
ISS-MO database for mining phylogenetic, metabolic, immunogenic, and antibiotic resistance pathways
ISS-MO database
Polyester wipes; Contact slide; SWAB sampler
Adhesive tapes
Air sampler device
Maxwell 16 – Promega
Aim-1
Aim-2
Aim-3

Can we differentiate live cells from dead ones when sequencing is employed?



Viability is the key to correlate the DNA based microbial diversity analyses to crew health

Vaishampayan P, Probst AJ, La Duc MT, Bargoma E, Benardini JN, Andersen GL, Venkateswaran K. 2013. New perspectives on viable microbial communities in low-biomass cleanroom environments. ISME J **7:**312-324.

Shotgun metagenome resolved microbial diversity of ISS environmental surfaces

A

- *Rhodotorula* sp. JG-1b - 35%
- *Penicillium rubens* - 17%
- *Staphylococcus saprophyticus* - 4%
- *Pantoea conspicua* - 4%
- *Klebsiella pneumoniae* - 4%
- *Sphingomonas sanguinis* - 3%
- *Penicillium chrysogenum* - 2%
- *Staphylococcus aureus* - 2%
- *Paenibacillus polymyxa* - 2%
- *Pantoea dispersa* - 2%
- *Methylobacterium* sp. yr596 - 1%
- *Pantoea ananatis* - 1%

B

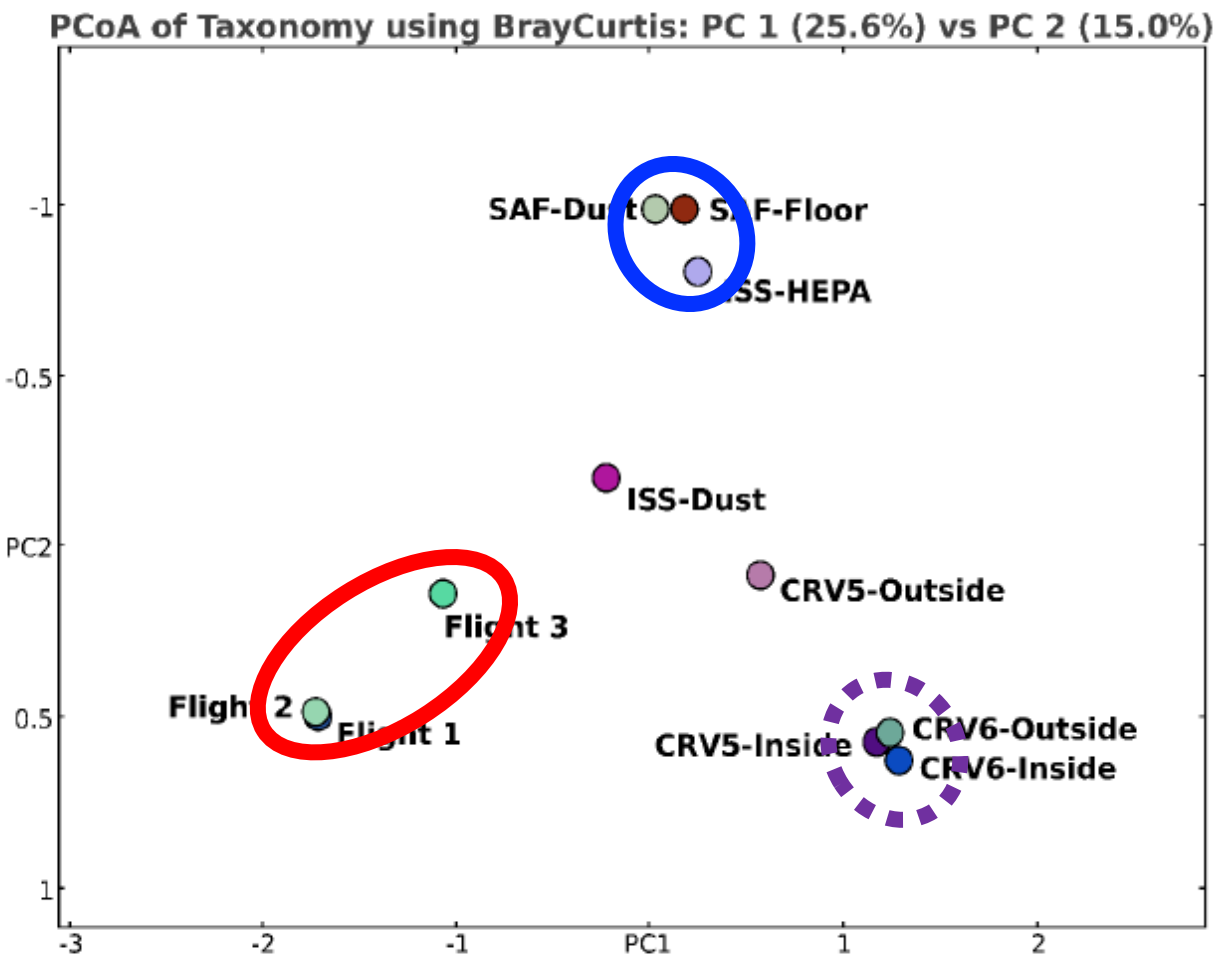
- *Methylobacterium aquaticum* - 1%
- *Enterobacter aerogenes* - 1%
- *Pantoea* sp. FF5 - 1%
- *Rhodotorula toruloides* - 1%
- *Pantoea vagans* - 1%
- *Enterobacter cloacae* - 1%
- *Pantoea* sp. NGS-ED-1003 - 1%
- *Penicillium camemberti* - 1%
- *Sphingomonas melonis* - 1%
- *Penicillium nordicum* - 1%
- *Escherichia coli* - 1%

Pantoea ananatis - 1%

-2 -1 0 1 2 3

NMDS1

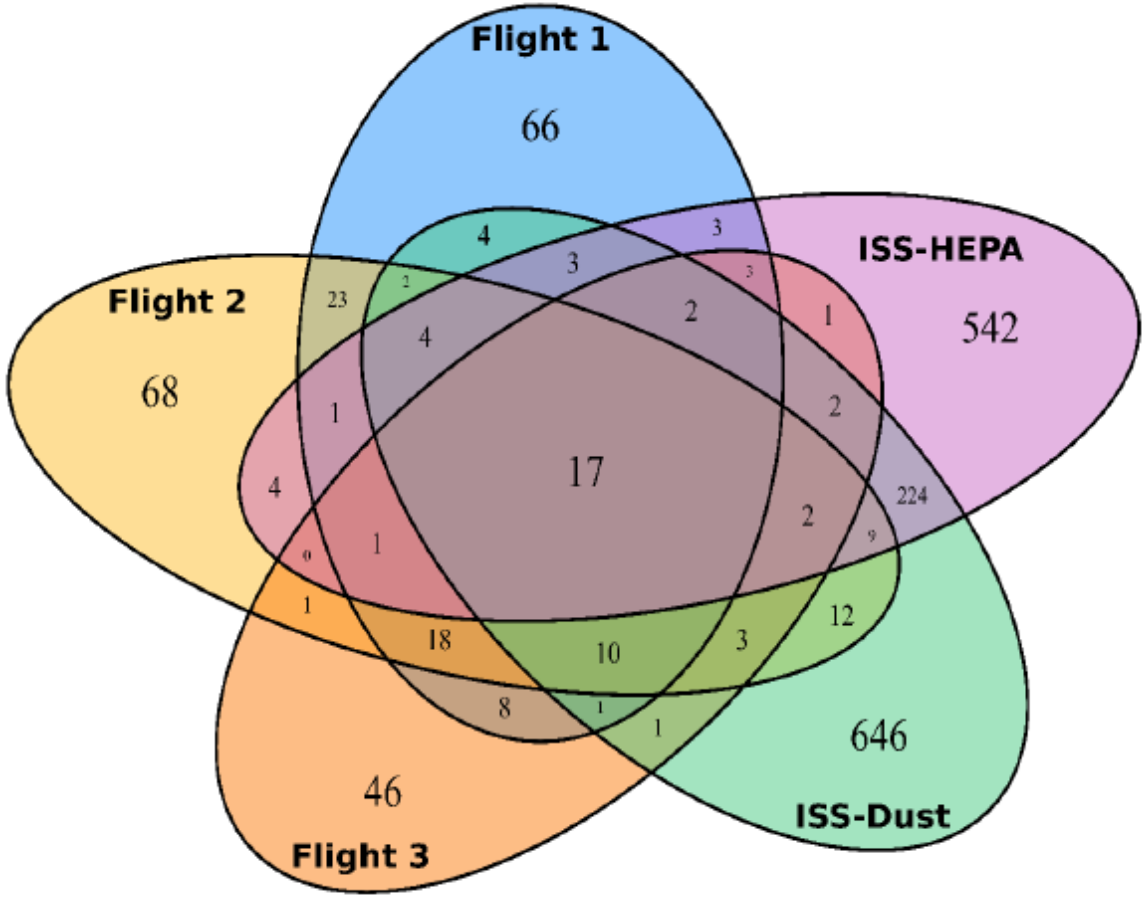
Source of ISS microbial populations (shotgun)



- Metagenome sequences compared
  - MT-1 ISS surfaces: F1 (March 2015), F2 (May 2015), and F3 (May 2016)
  - ISS dust (collected 1-day using vacuum cleaner; 2012)
  - ISS HEPA (particulates accumulated for >40-months; 2011),
  - Crew Resupply Service (CRS; that took cargo to the ISS in 2015 to 2016),
  - Spacecraft assembly facility (SAF) cleanroom dust (2014) and SAF dust (2016).

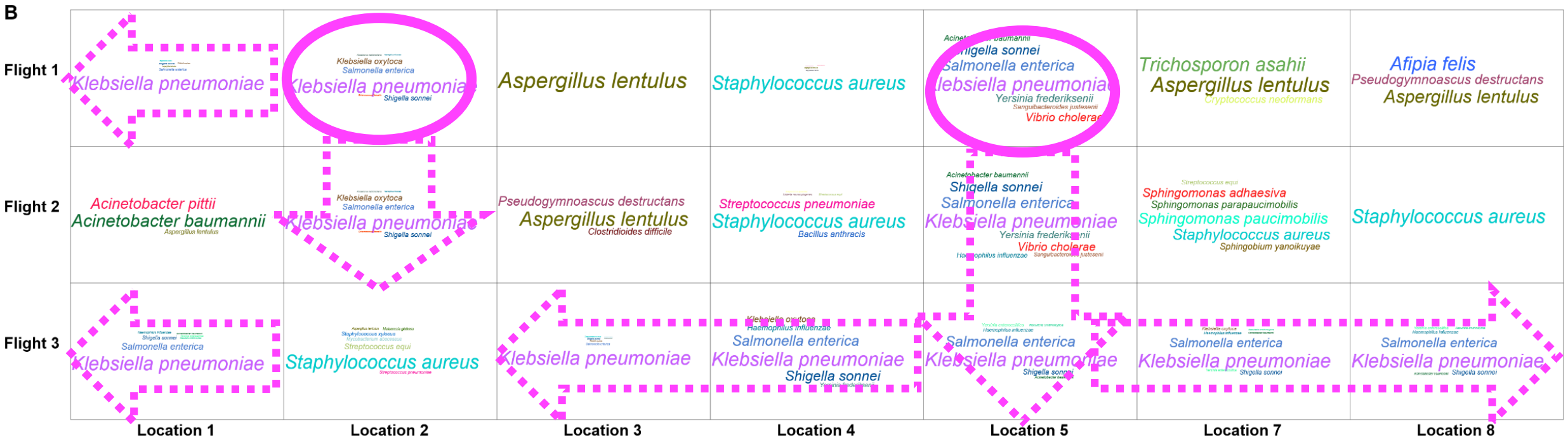
- The MT-1 ISS surfaces tend to align together
- Separate from other samples including ISS-dust, ISS-HEPA, SAF, and CRV suggesting its distinctive microbial composition.
- This might be due to the sample collection period of the ISS where microorganisms could have been replenished at various time points.
- CARGO, human and other subsystems such as plant, rodents, etc. should be explored for its source.

Core microbiome of ISS (metagenome sequencing)



- **The ISS microbiome is unique to a particular subsystem but also some microbial species are common (core microbiome; 17 species).**
- **The ISS core microbiome was dominated by**
  - **Fungi (*P. brasilianum, P. chrysogenum, P. digitatum, P. expansum, P. freii, P. griseofulvum, P. roqueforti, P. rubens, A. calidoustus,* and *A. niger*).**
  - **Bacteria (*Cutibacterium acnes, E. cloacae, E. coli, P. ananatis, S. enterica, S. aureus,* and *S. epidermidis*).**
- **Compare with other microbiome studies:**
  - **The ISS microbiome represents a "minimal core" model, hypothesized based on the large sets of human microbiome data, in which all the human subjects shared few microbial species, large overlaps were found in subsets but a very little was common between all the sets.**
  - **This was not the case for the built-in Earth indoor microbiome studies where it was reported that the normal range of indoor environmental conditions might not be large enough to impact microbial communities.**

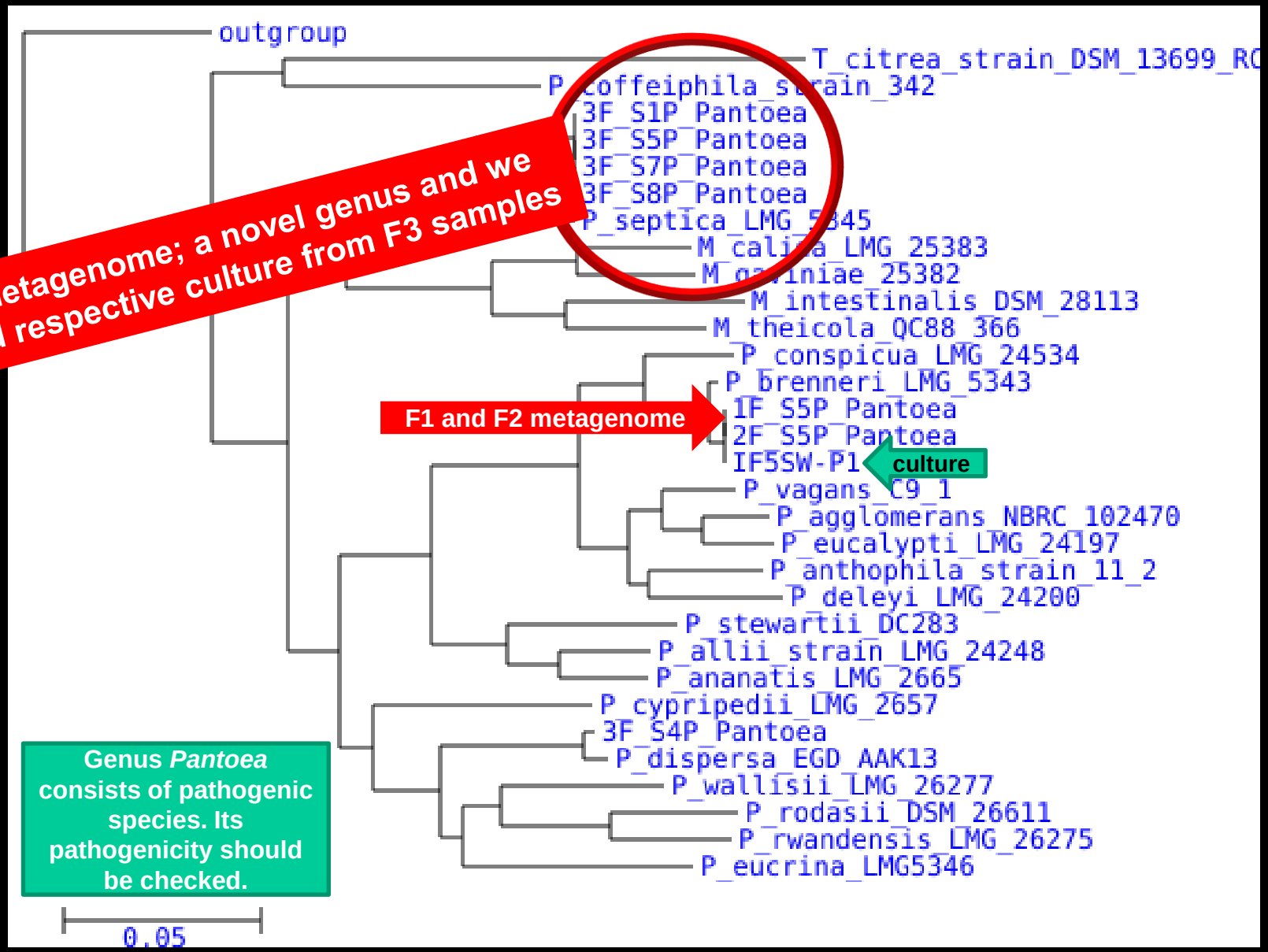
Spread of BSL-2 microorganism in ISS



- **Is the spread of *K. pneumoniae* species (an opportunistic pathogen) coming from one strain?**
- **If so, how to find out?**
  - **Metagenome analyses can be carried out to source track the strain specific traits of the *K. pneumoniae* species**
  - **GeneLab data can be mined using future NASA solicitations (no MT-1 resources)**
- **Is this by one single *K. pneumoniae* strain causing issues?**
- **If so, how to eradiate?**
  - **Need more resources and can be proposed in future about sanitizing to eradicate them.**

Genome to Life

1. Singh et al. 2018 **Description of *Kalamiella piersonii* gen. nov., one of the 20 full bacterial genomes retrieved from the ISS environmental metagenomic sequences.** In preparation



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Survival of *Bacillus pumilus* Spores for a Prolonged Period of Time in Real Space Conditions

Parag A. Vaishampayan,[1] Elke Rabbow,[2] Gerda Horneck,[2] and Kasthuri J. Venkateswaran[1]

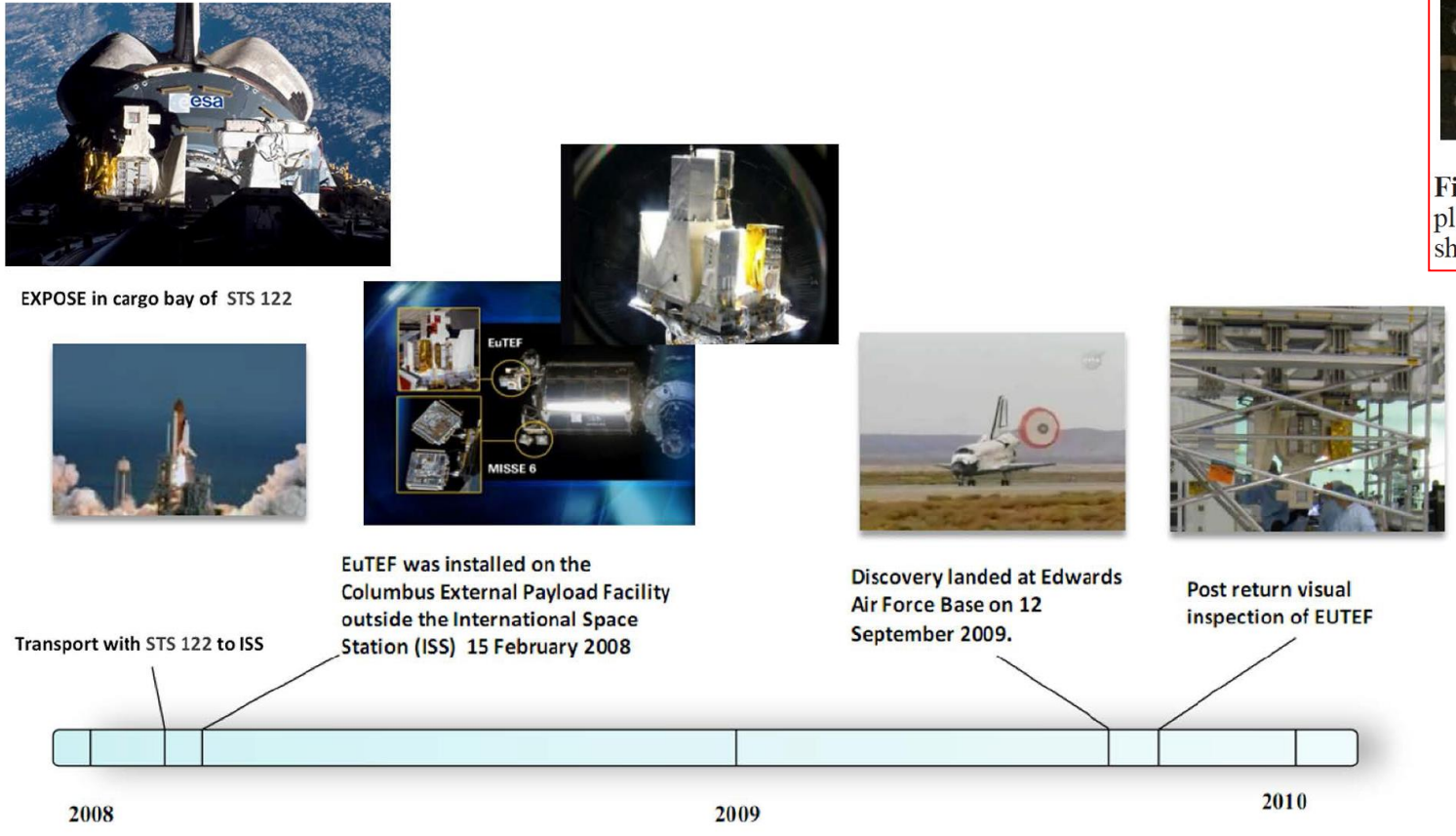


Fig. 2. Time line of the EXPOSE-E mission from launch to landing/retrieval.

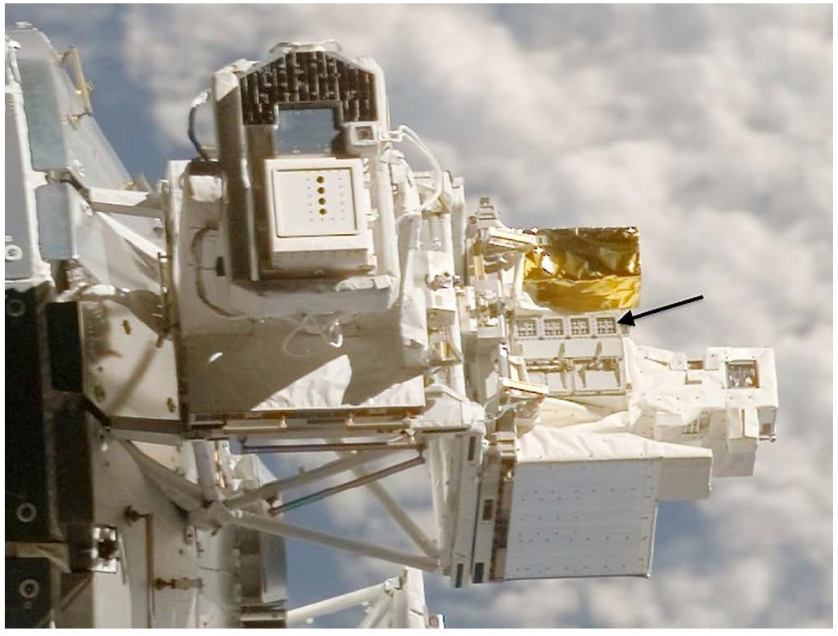


Fig. 1. The EXPOSE facility as part of EuTEF attached to the outer platform (balcony) of the Columbus module of the ISS. The arrow shows the EXPOSE facility.

Why *B. pumilus* spores are persistent in clean rooms?

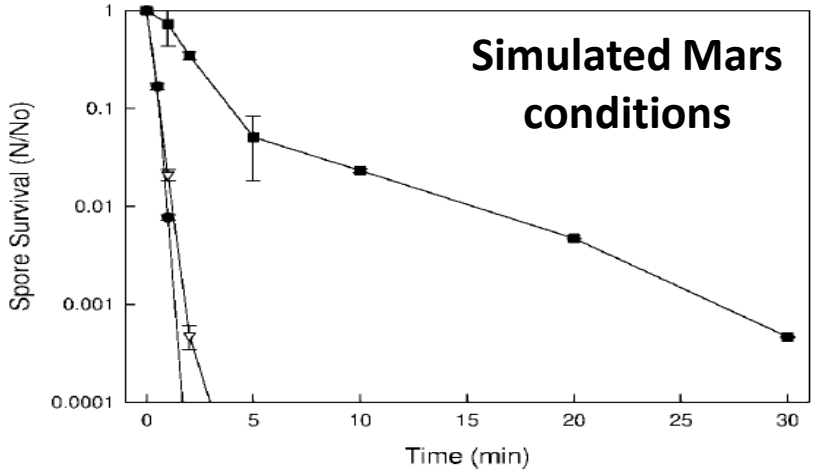
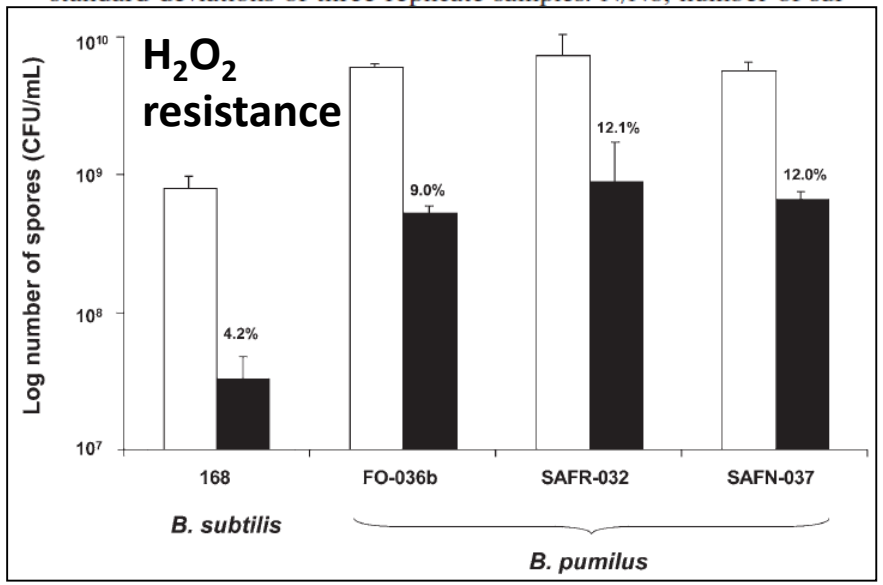


FIG. 3. Effect of Mars UV irradiation (JPL simulator) on hydrated spores of select *Bacillus* species. ■, *B. pumilus* SAFR-032; ●, *B. megaterium* ATCC 14581; ▽, *B. subtilis* 168. The error bars indicate the standard deviations of three replicate samples. N/No, number of sur-



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2005, p. 8147–8156 Vol. 71, No. 12
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.12.8147–8156.2005
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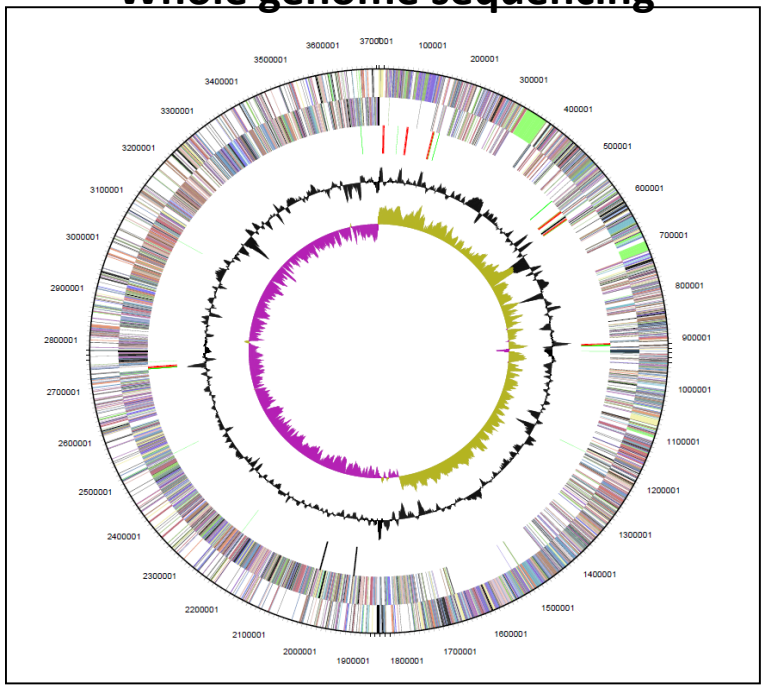
Survival of Spacecraft-Associated Microorganisms under Simulated Martian UV Irradiation

David A. Newcombe,[1] Andrew C. Schuerger,[2] James N. Benardini,[1] Danielle Dickinson,[1] Roger Tanner,[3] and Kasthuri Venkateswaran[1]*

Whole genome sequencing



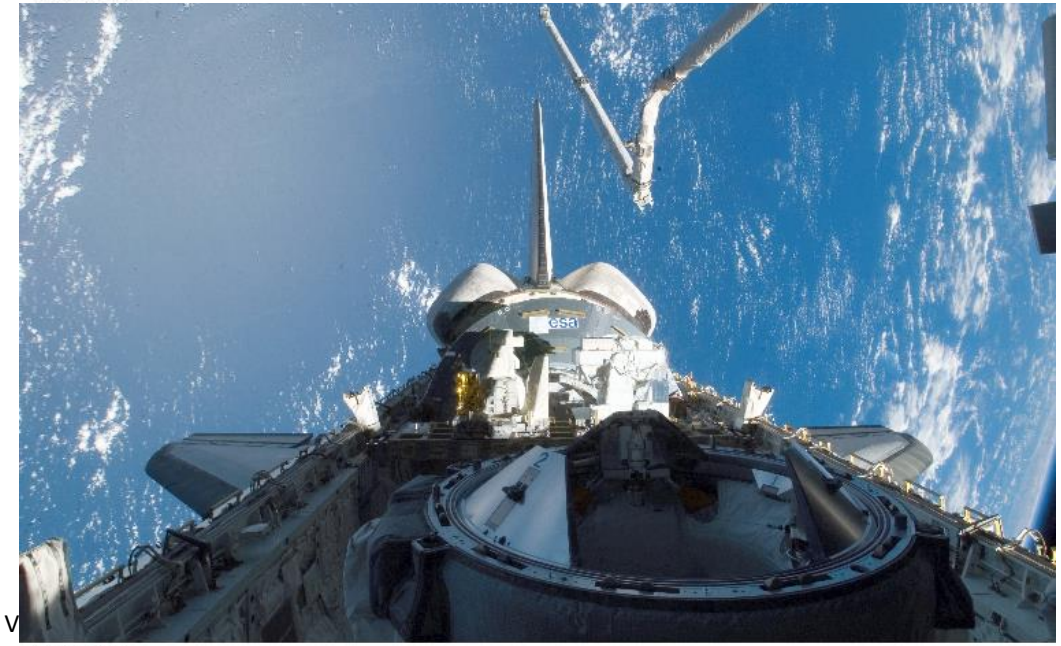
OPEN ACCESS Freely available online PLoS one

Paradoxical DNA Repair and Peroxide Resistance Gene Conservation in *Bacillus pumilus* SAFR-032

PLoS ONE (2007) 2:e928.

EXPOSE-EuTEF
Columbus-ESA

S122E009992

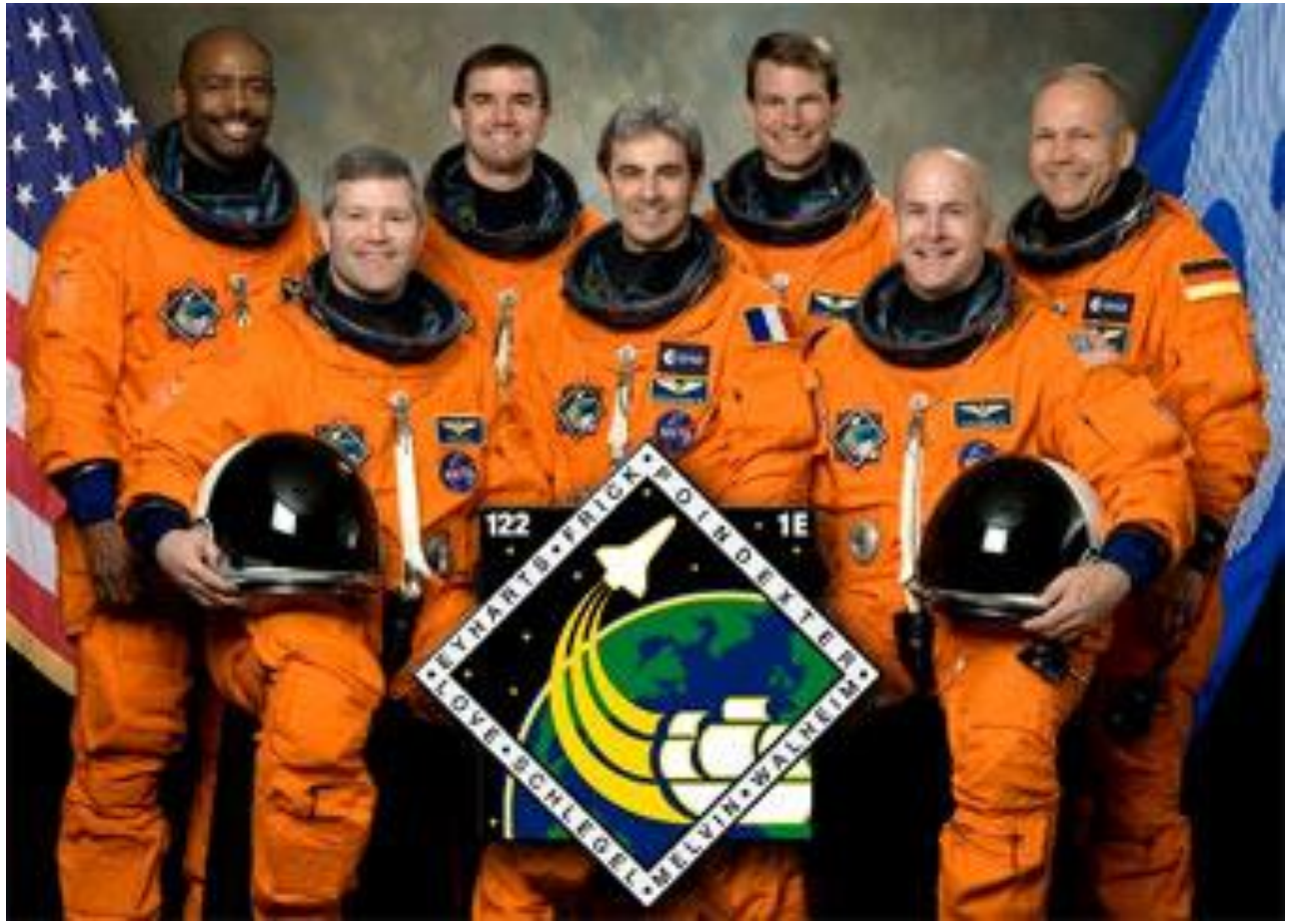


S122E006316

S122E006274

Space exposed and ground control spores were analyzed

STS-122 crew; Feb 2008

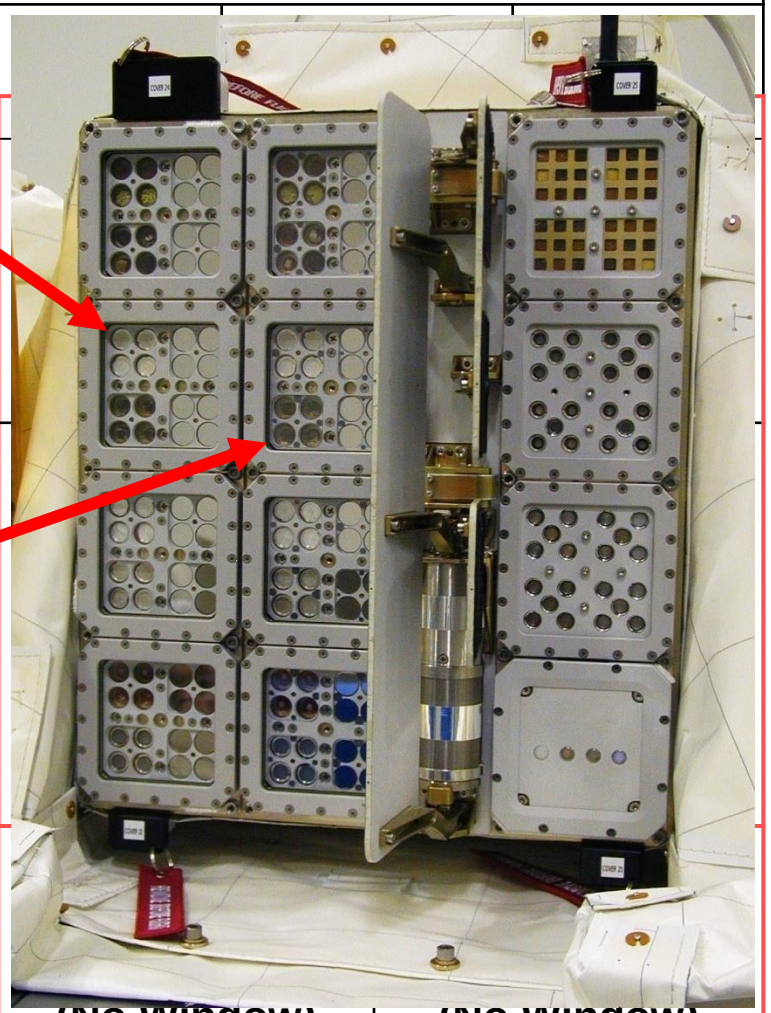


The 24th shuttle flight to the International Space Station, STS-122, delivered and installed Columbus, the European Space Agency's new laboratory.

EXPOSE-EuTEF – Top Windows – Atmosphere in Flight

Tray # 1, 2 + 3

Tray 1 „Space“	ADAPT > 110 nm (MgF_2) Nitrogen	PROTECT > 110 nm (MgF_2) Nitrogen		
Tray 2 „Mars“	ADAPT > 200 nm (Suprasil) Mars CO_2	PROTECT > 200 nm (Suprasil) Mars CO_2		
Tray 3 „Space“	R3D-E (No Window) Nitrogen	SEEDS > 110 nm (No Window) Nitrogen	(No Window) Nitrogen	(No Window) Nitrogen



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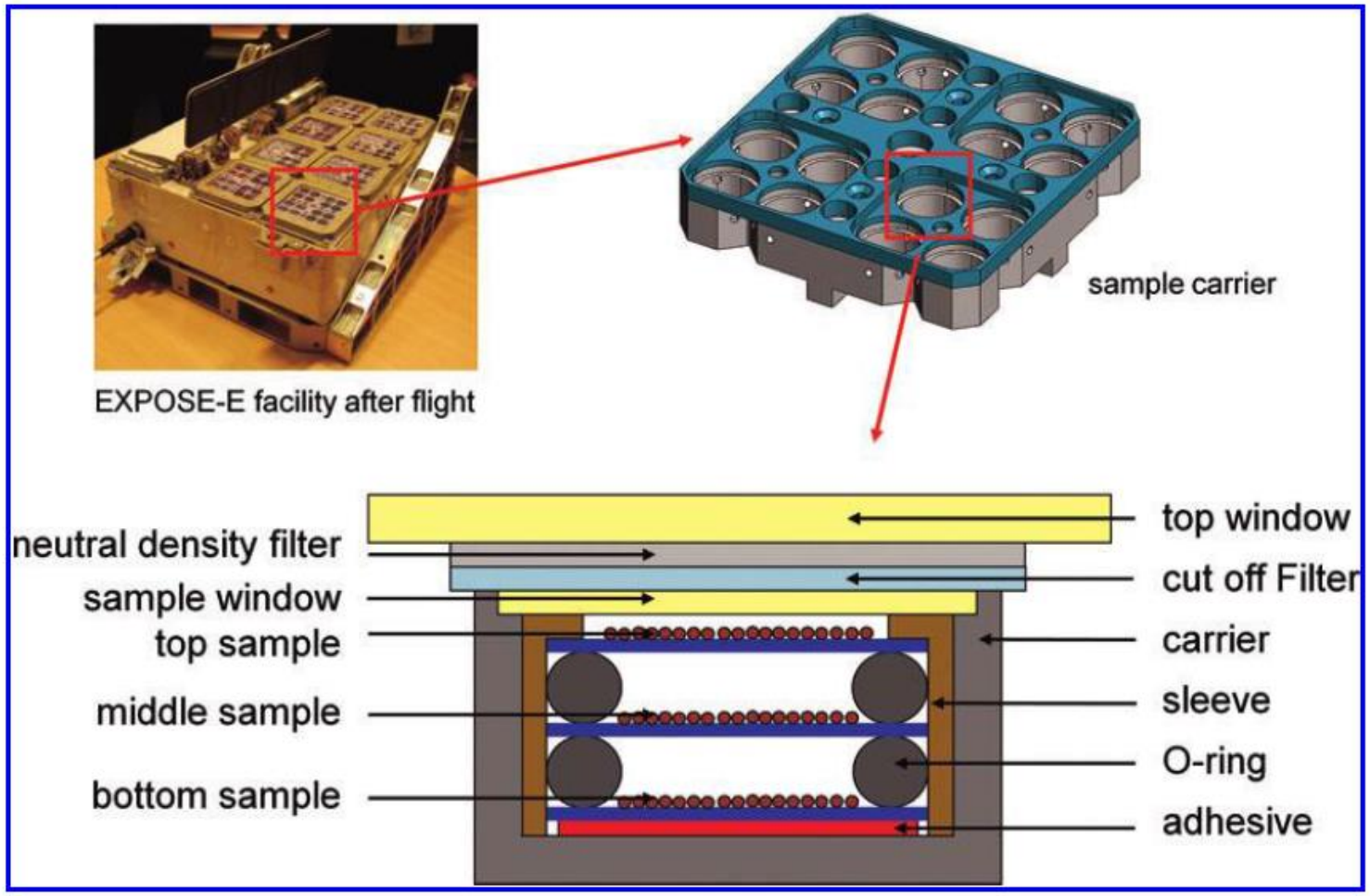

FIG. 2. EXPOSE-E facility after the mission, sample carrier, and scheme of a sample stack (air-dried layers of spores on aluminum coupons) (credit ESA, RUAG, KT, and Marko Wassmann, DLR). Color images available online at www.liebertonline.com/ast

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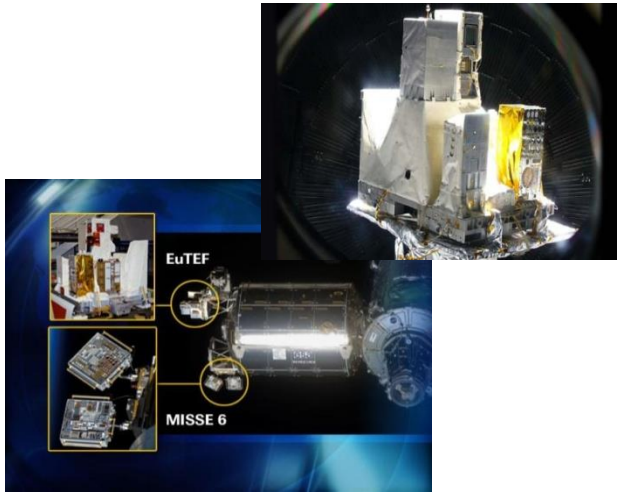
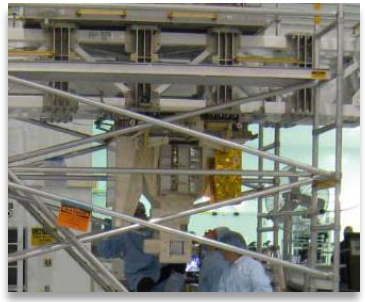
How do *B. pumilus* spores behave in "actual" space conditions?

Launch with STS-122
7 February 2008

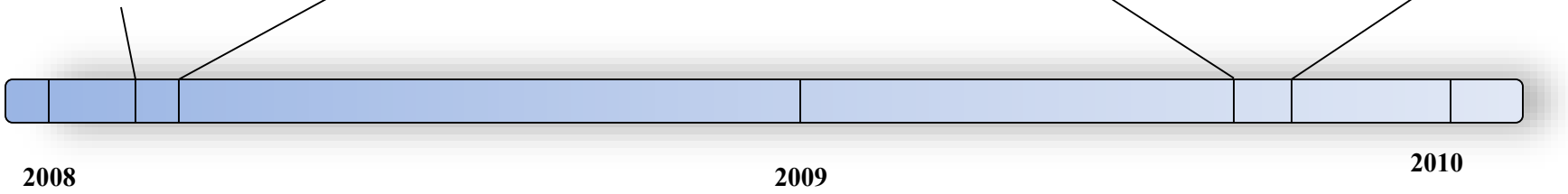


EuTEF was installed on the Columbus External Payload Facility outside the International Space Station (ISS) 15 February 2008

Discovery landed at Edwards Air Force Base on 12 September 2009.



Post return visual inspection of EUTEF



~18 months exposure in "real space" conditions

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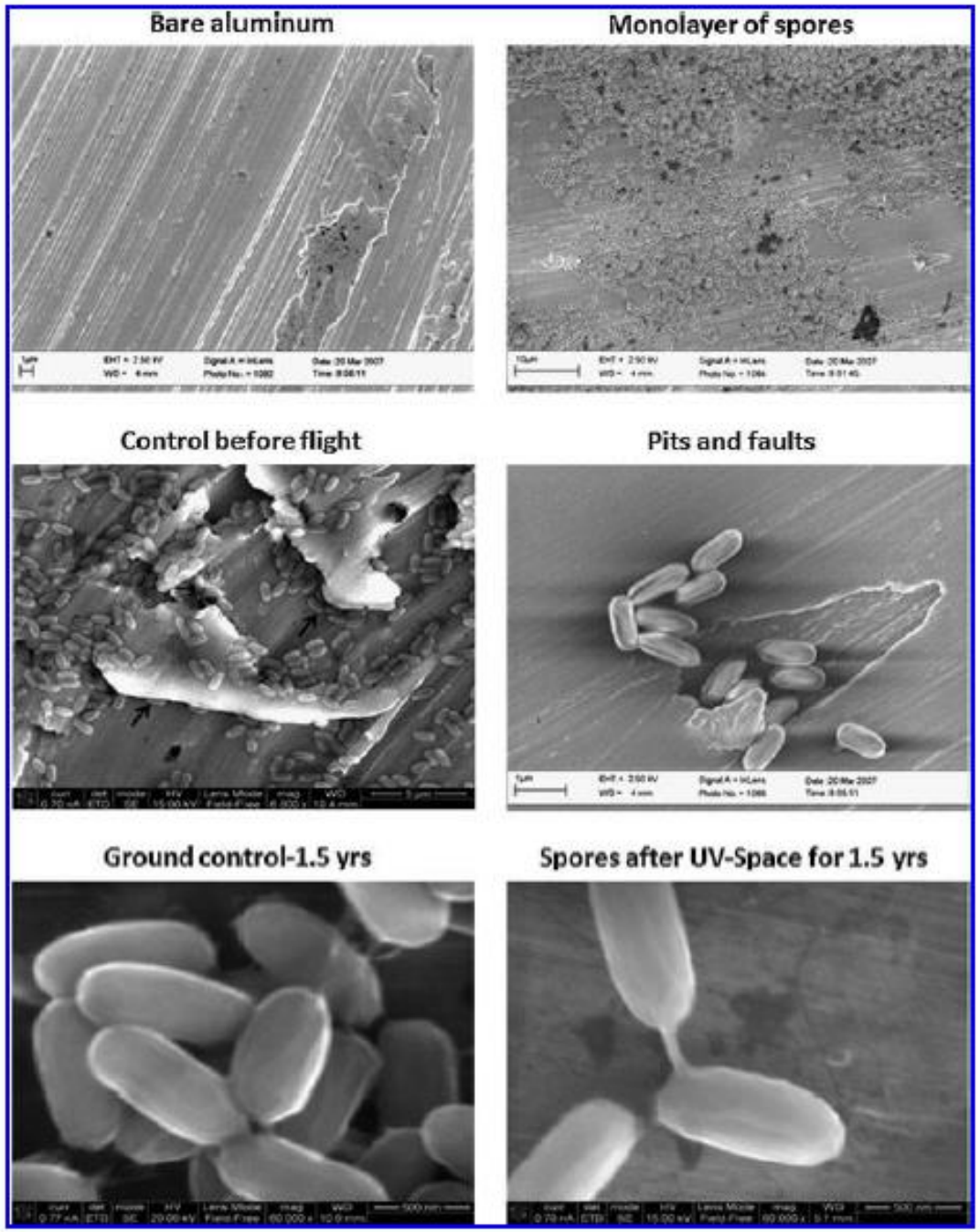
Morphology of "space-surviving" spores. The surface properties of the aluminum metal coupons and the purity, as well as the monolayer configuration of spores exposed to various space and simulated Mars conditions are shown here. Even though the aluminum coupons are mirror polished (ASTM 2002), the FE-SEM micrographs revealed the presence of pits and faults. These locations are potential spots for spores to hide such that the UV rays would be rendered ineffective at penetrating the micrometer-layer–thick aluminum flakes and the biological specimens would ultimately be protected. The *B. pumilus* SAFR-032 spores prepared exhibited monolayers when 10^7 spores were seeded per 13 mm–diameter coupons. The control spores before and after 1.5 years of exposure to space conditions did not show any deformation in their morphology. Although fewer spores were visible in the 1.5-year UV-Space–exposed specimen, the morphology of the remaining intact spores was not altered.



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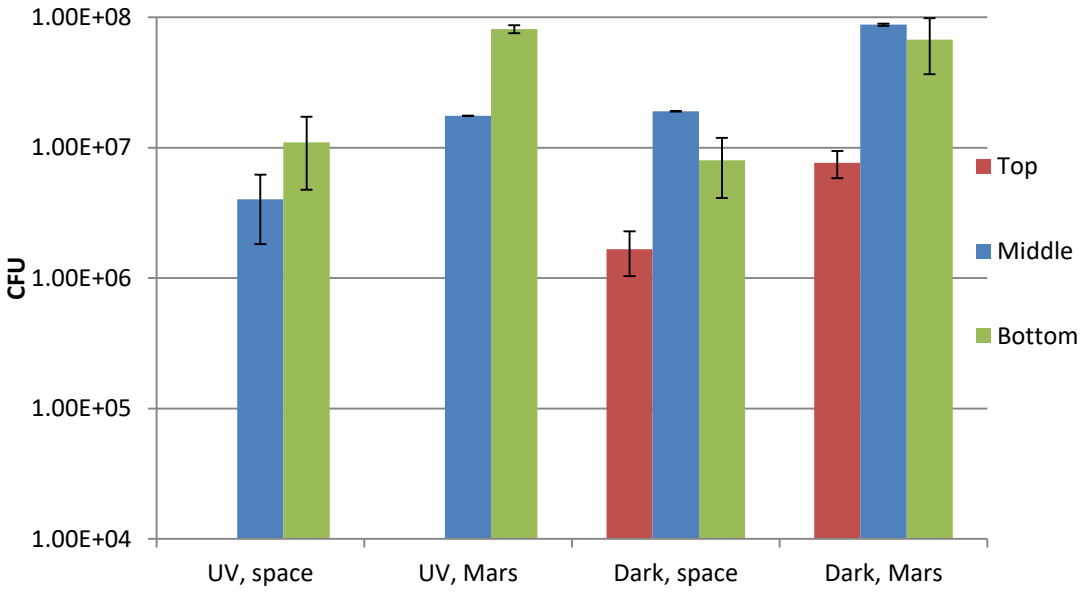
Survival of spores after space exposure



- **UV exposure has the strongest effect on the viability**
  - **Directly exposed spores (top layer) are dead → except 19 survivors**
- **Almost no reduction of the viability under Dark-Mars conditions**
- **Space conditions are harsher than Mars conditions**

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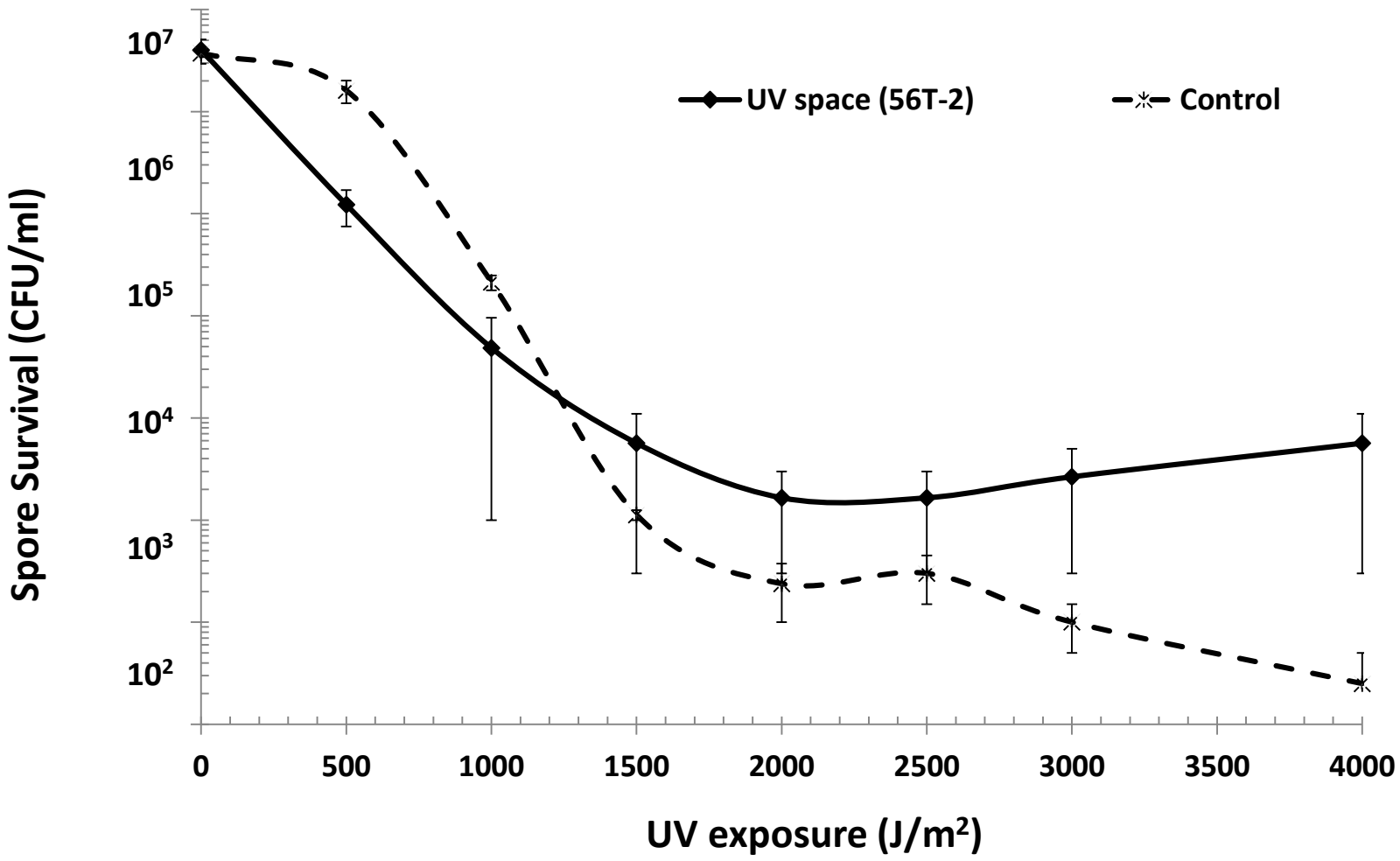
UVC resistance of "space-surviving" *Bacillus pumilus* SAFR-032 spores. After Earth return, spores of surviving population from each conditions were purified from first generation and exposed to various cumulative doses of UVC



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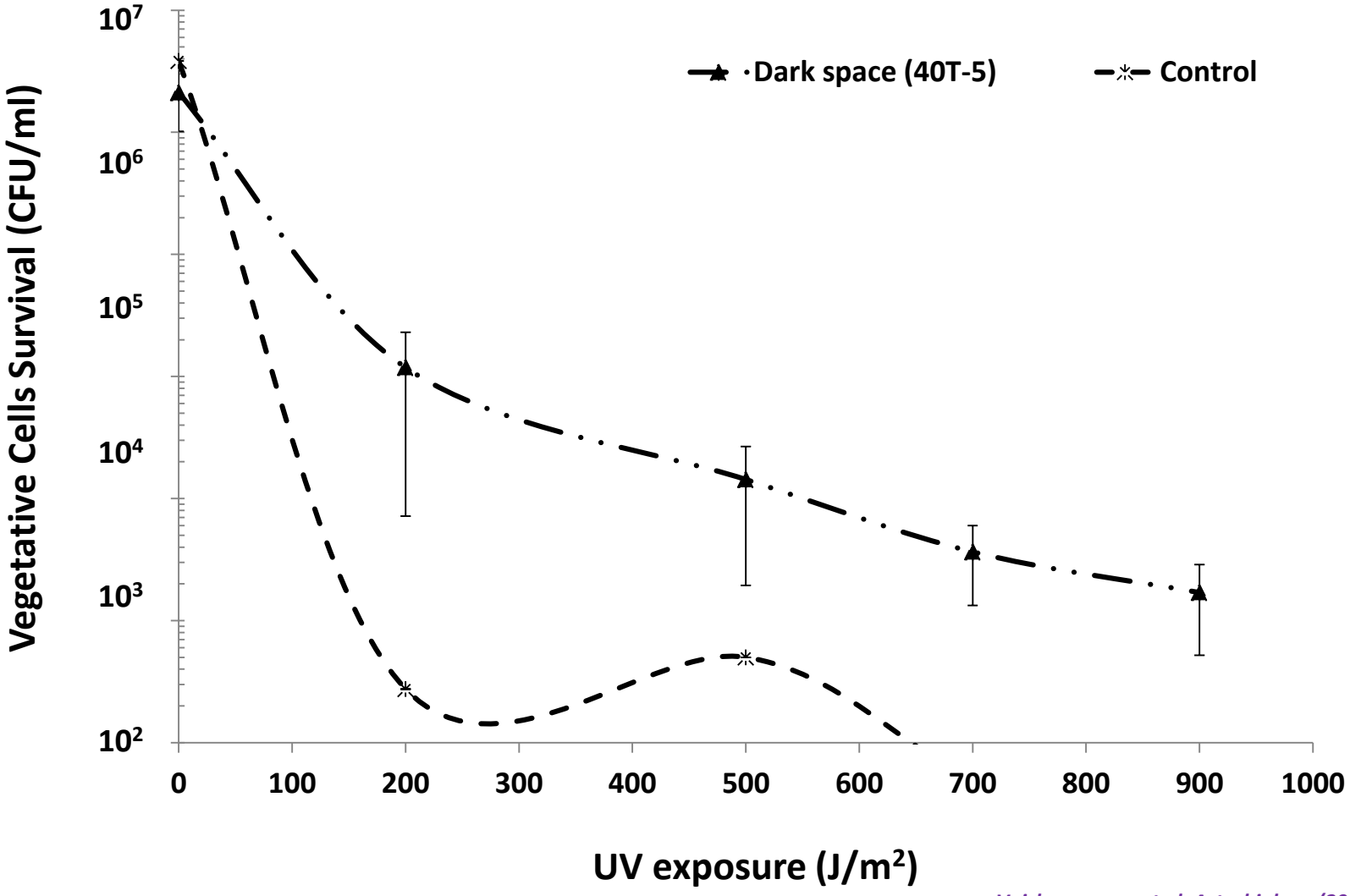
UVC resistance of "space surviving" *Bacillus pumilus* SAFR-032 vegetative cells. After Earth return, first generation of vegetative cells isolated from each conditions were exposed to various cumulative doses of UVC (254 nm).



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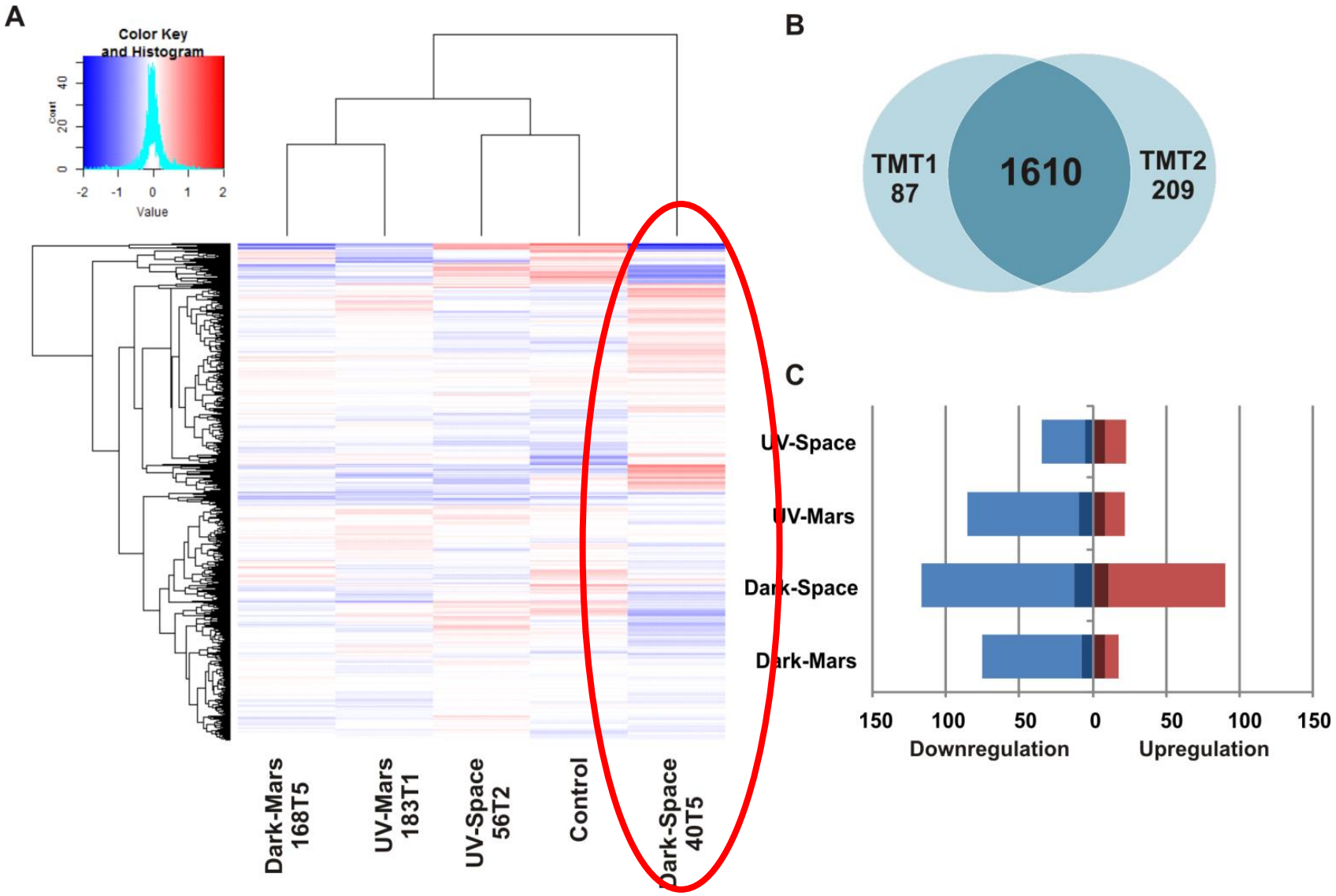
Proteomics characterization of space-exposed *B. pumilus* SAFR-032



(A) Heat map hierarchical clustering of proteins that have been quantified in each strain (B) Venn diagram representing the overlap of proteins between biological duplicates (C) Number of proteins differentially expressed in each strain compared to the control (Fold change ≥ ±1.5). The dark blue/red colors represent the differentially expressed proteins with p-value ≤ 0.05 (one-way ANOVA).