PLANETARY PROTECTION

METAGENOMICS IN SPACEFLIGHT:

ESTABLISHING AN IMPLEMENTATION ROADMAP

NASA AMES | NOVEMBER 19-22

COSPAR
COMMITTEE ON SPACE RESEARCH

Metagenomics in Spaceflight

NASA AMES

November 19-22, 2025

esa NASA JAXA

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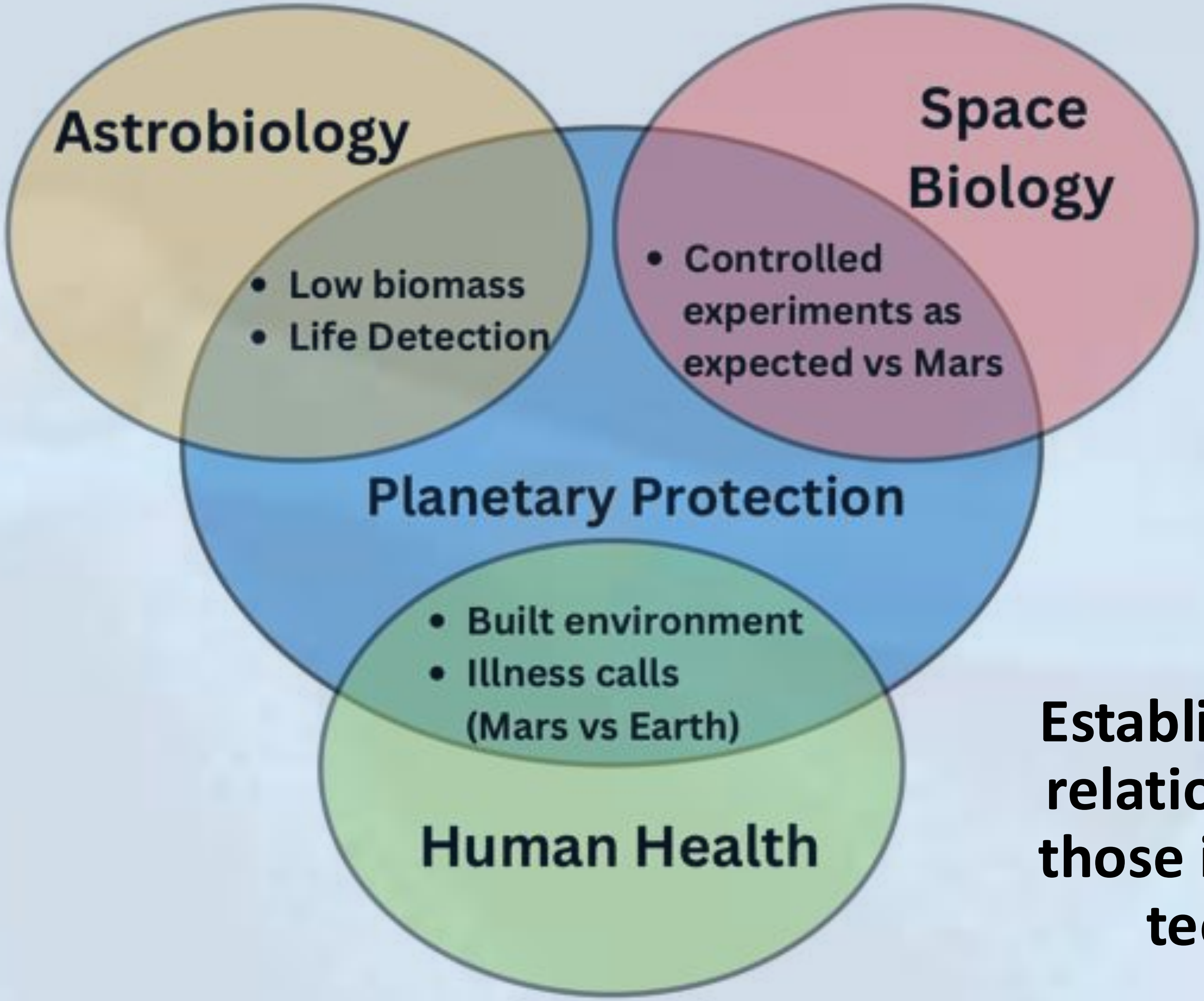
- NASA Science Mission Directorate (Astrobiology, Exobiology, Astromaterials and Curation, and Biological and Physical Science),
- NASA Exploration Systems Development Mission Directorate (Human Research Program),
- NASA, ESA and JAXA Offices of Planetary Protection
- ESA Life Science Support, and
- ESA Human and robotic explorations.

Objective

- Establish the path forward for what is needed to be able to leverage metagenomics (-omics, nucleic acid based) technologies for in-flight and safety critical decision making for space flight applications.
  - Planetary Protection - backward PP safety critical, forward PP for probability of contamination, harmful contamination definition and compliance
  - Astrobiology – science, life detection/safety critical
  - Space Biology – safety critical if sustainability, monitoring of experiments plant pathology etc., microbial performance in the space environment
  - Human health – safety critical, built environment, ECLSS and disease diagnostics

What is needed to use metagenomics on missions?



Establishing interdisciplinary relationships and leveraging those interfaces to move the technology forward!

Key Tracks / Breakout Group Themes

1. Safety Critical Decision Making
   - What does it take to apply performance-based objectives for metagenomics?
2. Microbial Dark Matter
   - Challenges of the unknown sequences – categorization and functionality.
3. Low Biomass
   - Limits of detection at ultra-low / picogram nucleic acid levels.
4. Bioinformatics and Databases
   - Addressing the need for collaborative data science repositories and bioinformatic noise reduction strategies.
5. Human Health / Built Environment
   - Exploring ISS, health care settings, built environment and the interpretation of metagenomics.
6. Technology Needs
   - Dive into challenges of ultra-low biomass sequencing and the applications of Oxford Nanopore
7. Roadmap to Implementation
   - Facilitated discussion to capture next steps / community actions needed to implement metagenomics.

Breakouts based on established process related gaps

Low Biomass (Gap #1)

What to do:

- sampling of extended areas (i.e. scraper system)
- additional sampling of "dirtier" areas in cleanrooms (i.e. changing rooms, airlocks), not only the main AIT (assembly integration and testing facilities)
- possibility of sampling non flight models (i.e. qualification, engineering, or structure thermal models), as these are generally not as clean as flight hardware
- sampling of ground support equipment (both electrical and mechanical)
- sampling of gloves and personnel microbiome
- spike-in (i.e. with lambda phage DNA)

Reagent contamination (Gap #2)

What to do:

- Procurement of ultrapure reagents
- Multiple displacement amplification – trade off

Bioinformatics (Gap# 3)

What to do:

- Give flexibility on selection of appropriate software (fast market changes)
- Transparent workflow tool, with relevant steps recorded/identified
- Standardised metagenomic pipelines, with users modifying defined set of parameters resulting in increased automatization

Genome catalogue (Gap# 4)

What to do:

- Extend current available databases
- Sequence all stored isolates (i.e. DSMZ)
- Introduction of metagenomic campaigns
- Collaboration with partners and creation of an international accessible

Quantifying contamination (Gap# 5)

What to do:

- Developing of alternative but complementary techniques
- qPCR, ddPCR

Credit: Silvio Sinibaldi

External Drivers For Metagenomics

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- SSB. 1992. Biological Contamination of Mars: Issues and Recommendations "The task group recommends that efforts be initiated immediately to adopt state-of-the-art methods for use in the determination of bioload"
- SSB. 2006. Preventing the Forward Contamination of Mars
  - "NASA should require the routine collection of phylogenetic data to a statistically appropriate level to ensure that the diversity of microbes in assembly, test, and launch operations (ATLO) environments, and in and on all NASA spacecraft to be sent to Mars, is reliably assessed"
  - NASA should take the following steps to transition toward a new approach to assessing the bioburden on spacecraft:
    - Transition from the use of spore counts to the use of molecular assay methods that provide rapid estimates of total bioburden (e.g., via limulus amebocyte lysate (LAL) analysis) and estimates of viable bioburden (e.g., via adenosine triphosphate (ATP) analysis). These determinations should be combined with the use of phylogenetic techniques to obtain estimates of the number of microbes present with physiologies that might permit them to grow in martian environments.
    - Develop a standard certification process to transition the new bioassay and bioburden assessment and reduction techniques to standard methods.
    - Complete the transition and fully employ molecular assay methods for missions to be launched in 2016 and beyond."
- Planetary Protection Independent Review Board (PPIRB), 2019, NASA Planetary Protection Independent Review Board (PPIRB): Report to NASA/SMD: Final Report
  - Major Finding – "encouraging the use of modern molecular biological approaches to PP, such as metagenomic analyses of cleanroom samples"
- NASEM 2021. Report Series: Committee on Planetary Protection: Evaluation of Bioburden Requirements for Mars Missions
  - The present requirements also do not consider the types of microorganisms sampled. Tests using genetic assays could better characterize microbial populations, including the presence of extremophiles. This genetic information could inform both risk assessments and mitigation techniques that can reduce the risk of harmful contamination.

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Background and Motivations

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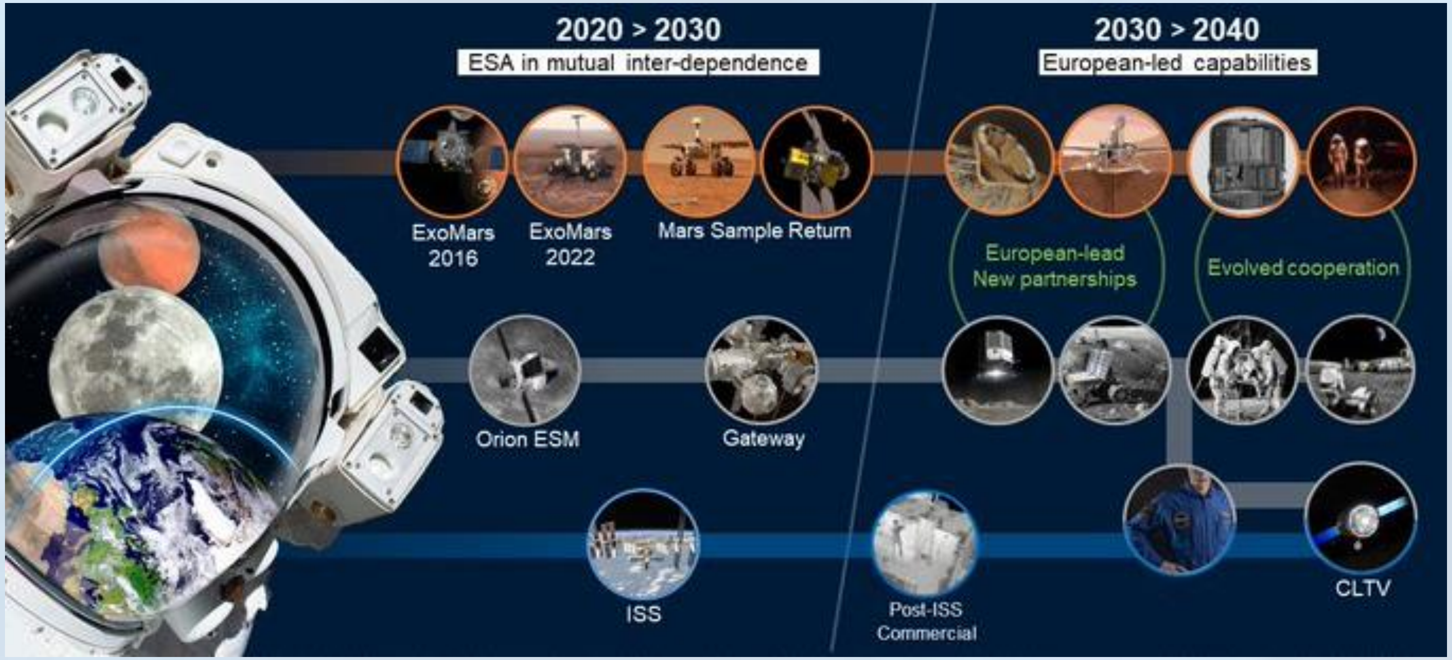
Planetary protection is driven by objectives for specific missions and target bodies. Complex mission, complex objectives

Need: Modernise PP tools for facing new space era challenges



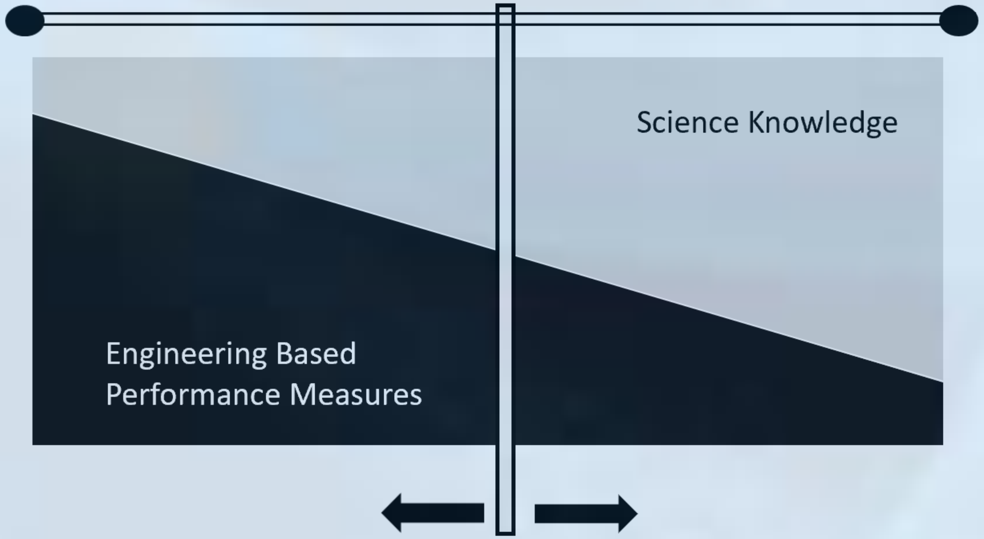
Extract from ESA Terrae Novae 2040+

Background and Motivations

The bigger picture: why metagenomic?

Shift from prescriptive requirements (i.e. spore assay) to performance based and risk informed decision framework

PP Contamination Risk = Engineering Performance Measures + Science Knowledge



- Science parameters less defined
- More onus on missions to absorb the risk
- Increased uncertainty due to bounding/probability-based modeling

- Science parameters well understood
- Less onus on missions to absorb the risk
- Optimal position for assessing risk

Background and Motivations

The bigger picture: why metagenomic?

Information about the function, i.e. not only the “who”, but the “what” and the “how”

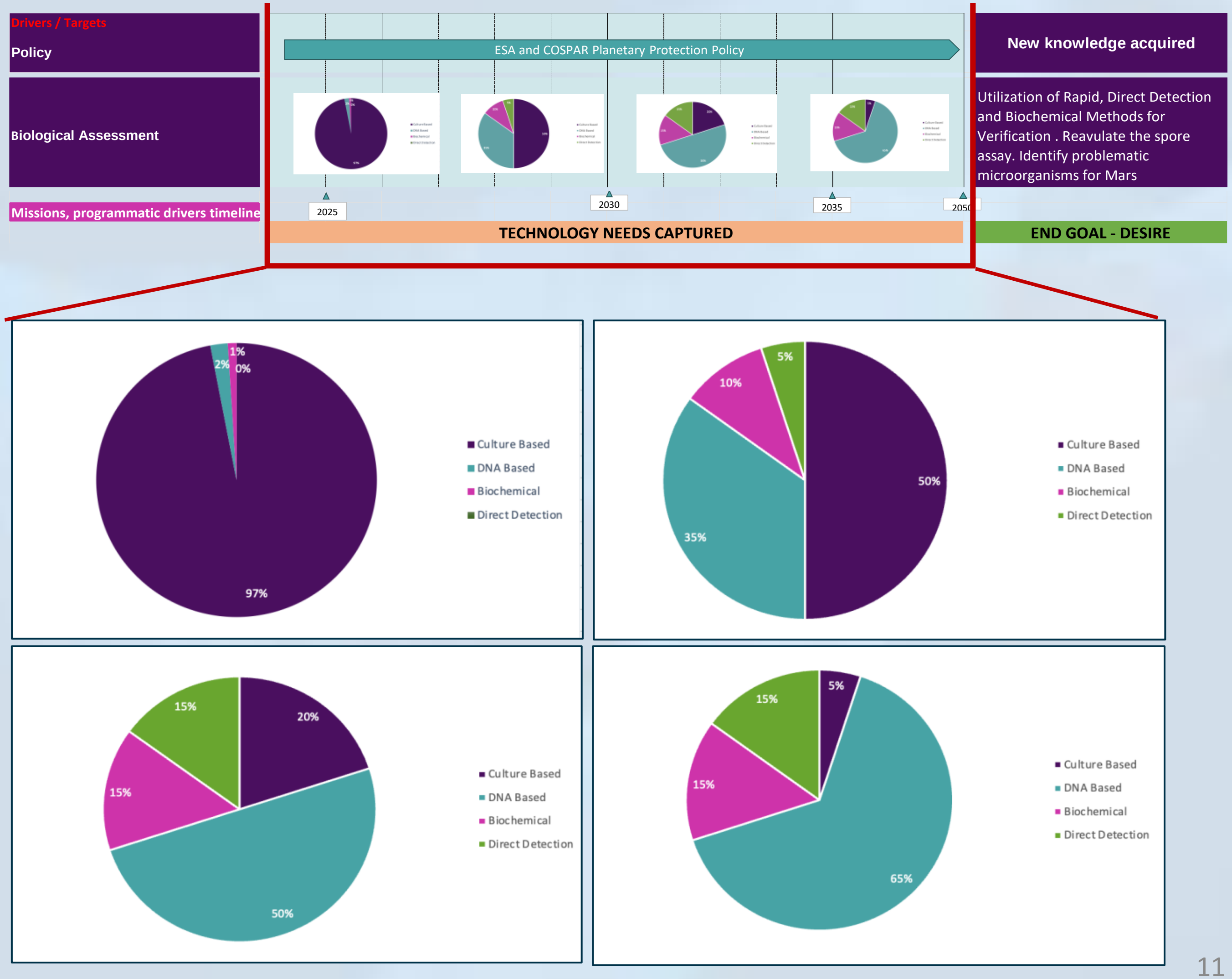
Potential powerful tool(s) to help:

1. assessing harmful contamination even beyond pre-launch
2. Influence system design and operations of a spacecrafts
3. Re-thinking of PP approaches, so far until “pre-launch”

Background and Motivations

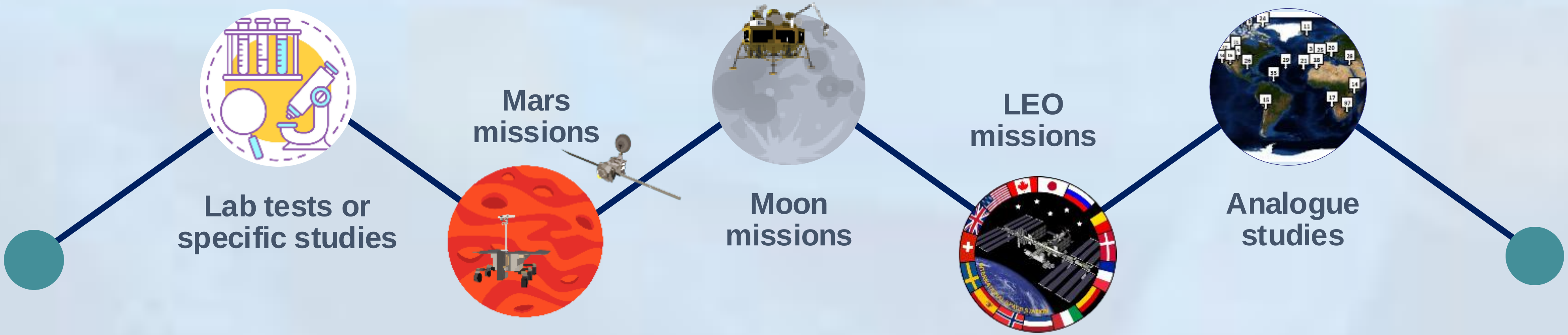
Planetary Protection international roadmap (Mars)

- Switch from culture-based assay to culture independent/DNA based
- Currently it is estimated that 97% of methods used rely on culturing
- Future activities should consider investing in molecular biology and dropping culture based down to 50% by 2030 and 5% to 2050.



Way forward

Working across disciplines, with support from international stakeholders, involved throughout the process



Envisioned Resources

1 Astrobiology
2 Space biology
3 Human health
4 Bult environment

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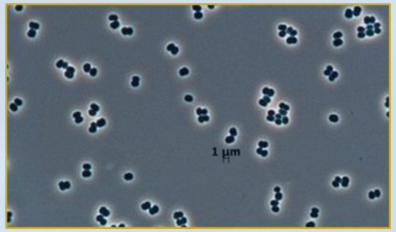
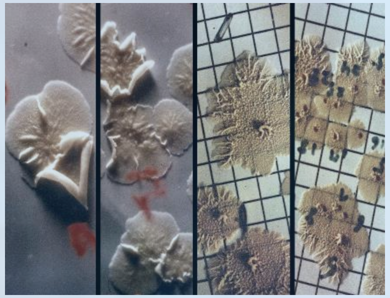
Backup

Standard Spore Assay as the Gold Standard...

- Used on robotic spacecraft dating back to Viking.
- Current requirements based on spore biological management.
- Only approved technique on spacecraft to assess biological cleanliness.
- Spores have proven to be the most difficult form of life to eradicate
  - UV, space vacuum, radiation resistant
  - tolerance to spacecraft microbial reduction modalities (e.g., solvent cleaning and heat).



Bacterial species found in ESA and NASA cleanrooms; Credit: ESA



Bacillus subtillus grown aboard Skylab; Credit: NASA

What is Planetary Protection?

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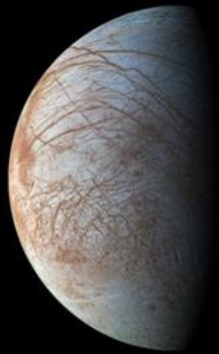
What is Planetary Protection?

Planetary Protection (PP) is the practice of protecting solar system bodies from contamination by terrestrial life and preventing harm to Earth's environment from the return of samples to Earth containing possible extraterrestrial life forms.

Planetary protection is focused on limiting biological contamination of other solar systems bodies from Earth's terrestrial organisms and preventing the return of potentially harmful extraterrestrial organisms and organic materials to Earth.

The overarching goal of Planetary Protection is to support safe and sustainable exploration of chemical evolution and origin/s of life in the solar system



Europa
(Radius 1561 km)

Earth
(Radius 6378 km)

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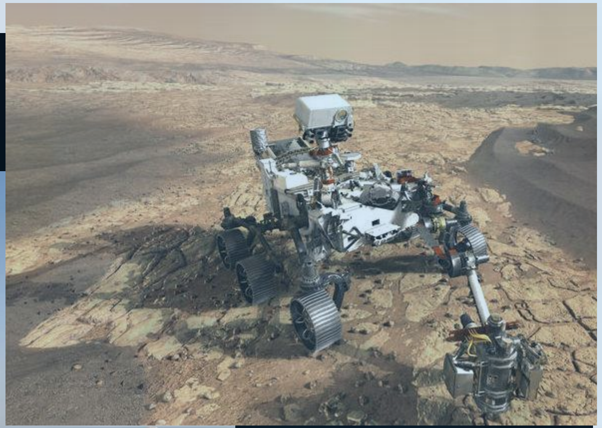
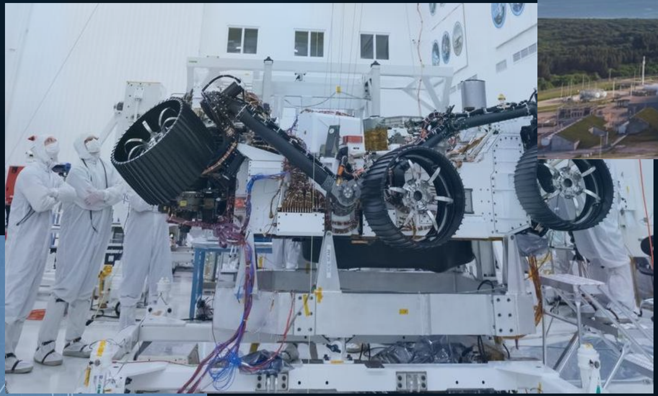
What is Planetary Protection?

Implementing planetary protection begins with a mission proposal which identifies where the spacecraft will travel and what activities and operations will occur during exploration of the destination.

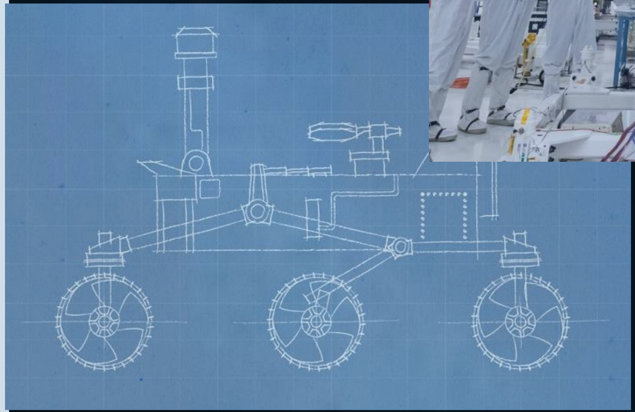


Perseverance on Mars

Mars 2020 launch



Mars 2020 at JPL



Rover Concept

At each step of a mission's timeline, the Office of Planetary Protection supports planning, monitors performance, reviews activities, and independently verifies biological cleanliness.

Hardware Design (Systems Engineering)

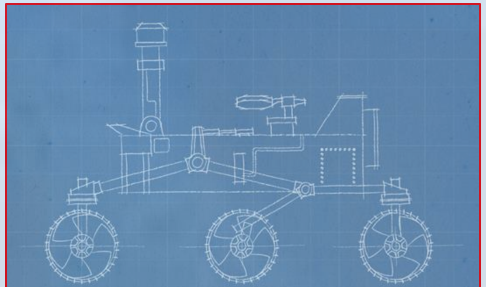
- Establish Driving Requirements
  - 5×10^5 spores total
  - 3×10^5 spores landed
  - 300 spores / m^2
- Bioburden Allocation
- Organic Inventory
- Implementation Planning

Hardware Implementation (Clean)

- Microbial Reduction (e.g., heat microbial reduction, etc.)
- Verification of cleanliness (e.g., NASA Standard Assay for spores)

Recontamination Prevention (Keeping it Clean)

- Cleanroom assembly and test
- Hardware cleaning and covering
- Late critical system installation
- Launch environment cleanliness
- Pre-launch report
  - Bioburden, organic reporting



Credit: NASA / JPL

Planetary Protection starts in early mission phases

Credit: ESA

Verification – Swab of the ExoMars camera

Credit: NASA / JPL

Late integration of the Mars 2020 Sample Tubes

NASA & ESA Spore Assay

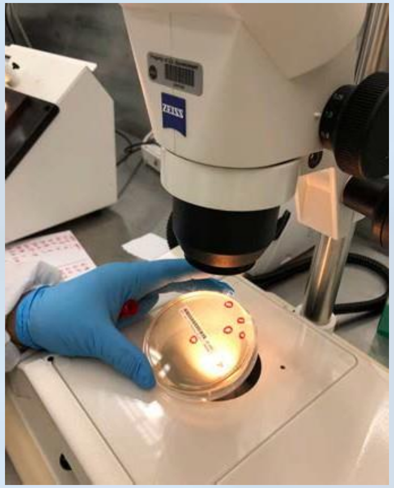
Sterilization of glassware and growth media in autoclave

Heat shock of the extracted samples at 80°C for 15 minutes

Quenching shocked samples in ice bath

Quantitative aliquots plated

Visual enumeration of colony forming units after incubation at 32°C for 24, 48, and 72 h

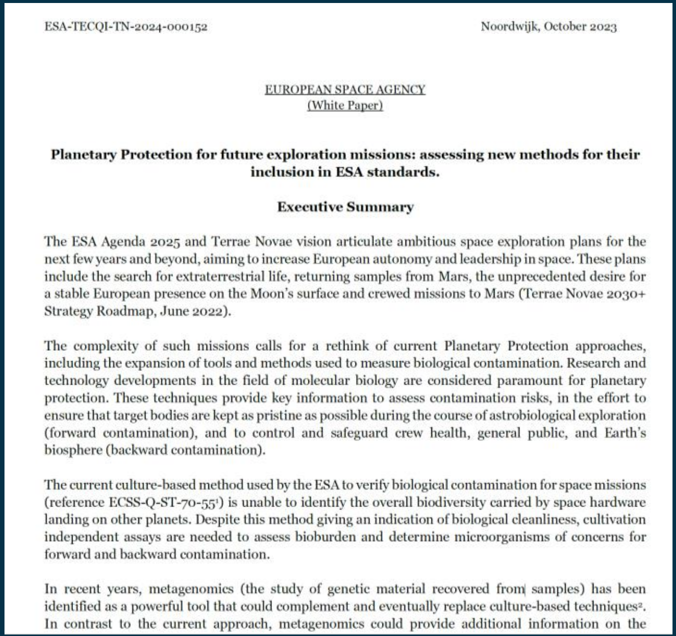


Bar coding enables reliable tracking of bioburden data

Summary of the ESA Metagenomic workshop (2023)

Silvio Sinibaldi
Planetary Protection Officer
European Space Agency

Objectives of the workshop


ESA-TECQI-TN-2024-000152 Noordwijk, October 2023

EUROPEAN SPACE AGENCY
(White Paper)

Planetary Protection for future exploration missions: assessing new methods for their inclusion in ESA standards.

Executive Summary

The ESA Agenda 2025 and Terrae Novae vision articulate ambitious space exploration plans for the next few years and beyond, aiming to increase European autonomy and leadership in space. These plans include the search for extraterrestrial life, returning samples from Mars, the unprecedented desire for a stable European presence on the Moon's surface and crewed missions to Mars (Terrae Novae 2030+ Strategy Roadmap, June 2022).

The complexity of such missions calls for a rethink of current Planetary Protection approaches, including the expansion of tools and methods used to measure biological contamination. Research and technology developments in the field of molecular biology are considered paramount for planetary protection. These techniques provide key information to assess contamination risks, in the effort to ensure that target bodies are kept as pristine as possible during the course of astrobiological exploration (forward contamination), and to control and safeguard crew health, general public, and Earth's biosphere (backward contamination).

The current culture-based method used by the ESA to verify biological contamination for space missions (reference ECSS-Q-ST-70-55[1]) is unable to identify the overall biodiversity carried by space hardware landing on other planets. Despite this method giving an indication of biological cleanliness, cultivation independent assays are needed to assess bioburden and determine microorganisms of concerns for forward and backward contamination.

In recent years, metagenomics (the study of genetic material recovered from samples) has been identified as a powerful tool that could complement and eventually replace culture-based techniques[2]. In contrast to the current approach, metagenomics could provide additional information on the

<u>Workshop chairs:</u>

Silvio Sinibaldi – ESA Independent Safety Office
Sandra Ortega Ugalde – ESA Life Support & Physical Sciences

Workshop Attendance

Speaker	Affiliation
Dietmar Pilz	European Space Agency (ESA)
Britta Schade	European Space Agency (ESA)
Silvio Sinibaldi Robert Lindner Sandra Ortega Ugalde	European Space Agency (ESA)
Nick Benardini	National Aeronautics and Space Administration
Alexander Mahnert	Medical University of Graz
Karen Olsson-Francis	UKSA
Michael Macey	The Open University
Laia Closa Gil	Blood and Tissue Bank
Silas Kieser	Kieser Metagenomics

Panel:

ESA 2023 metagenomic workshop in a nutshell

21 international experts

representing space and non-space industries, private sector, national agencies

10 oral presentations

given by panellists to share knowledge and recent advances in the field

3 hands-on work cases

1. Current status, limitations and knowledge/technology gaps
2. Future work/R&D required to reach adequate maturity level
3. Requirements for metagenomic methods

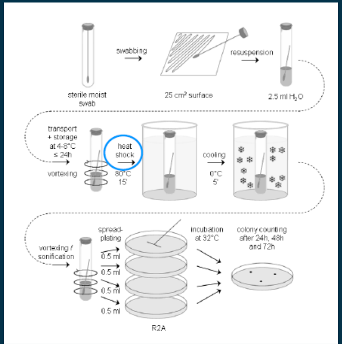
1 Outcome injected in the creation of a preliminary PP roadmap to close knowledge gaps and develop new toolkits for planetary protection

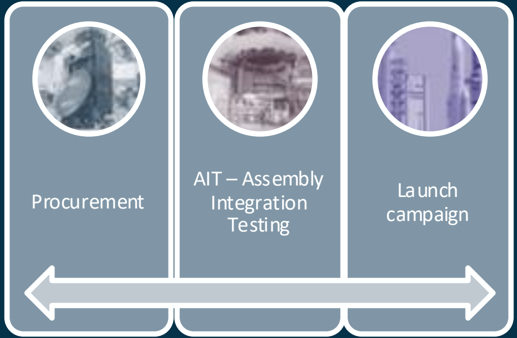
2 Planetary protection benefits largely from international consensus. Such workshops are excellent examples of inclusive collaboration for sustainable and responsible space exploration, and enabling mission teams to explore the Solar System

3 A white paper produced to push the topic inside and outside the agency, and define concrete next steps to modernise planetary protection standards

Motivations

ECSS-Q-ST-70-55 –Microbial examination of flight hardware and cleanrooms, or the "ESA & NASA spore assay"



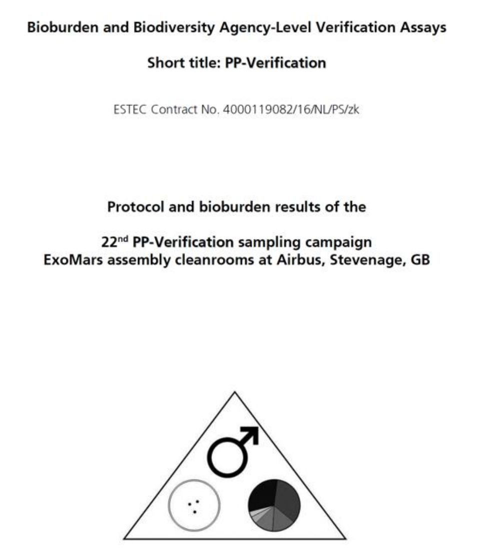


Tens of thousands of assays performed in a typical Cat IV mission to Mars

1. Not representative of all biodiversity in cleanrooms and space hardware
2. Only <0.1% of bioburden contaminant are identified
3. Not able to identify and quantify problematic species for planetary protection (see PPOSS report, statement #4)
4. Lack of evidence is not evidence of lack (VBNC)

Motivations

Independent verification and cleanliness knowledge, what do we actually do with those data for planetary protection?



Bioburden and Biodiversity Agency-Level Verification Assays

Short title: PP-Verification

ESTEC Contract No. 4000119082/16/NL/PS/zk

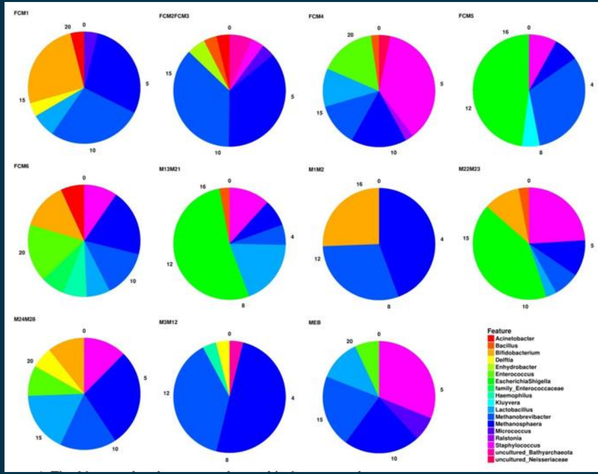
Protocol and bioburden results of the

22nd PP-Verification sampling campaign
ExoMars assembly cleanrooms at Airbus, Stevenage, GB

Campaign No.	Date	Country / City	Company / institution	Mission
1	05. – 06.09.2011	D, Friedrichshafen	EADS	CR
2	12 + 26.11.2012	D, Köln	DLR	Pretests ExoMars2016
3	09. – 10.01.2013	I, Aprilia	Aerosecur	ExoMars2016
4	18. – 19.04.2013	NL, Noordwijk	ESA-ESTEC	ExoMars2016
5	24. – 26.04.2013	NL, Noordwijk	ESA-ESTEC	ExoMars2016
6	17. – 19.09.2013	I, Torino	TAS-I	ExoMars2016
7	29. – 30.10.2013	KZ, Baikonur	Cosmodrome	CR
8	26. – 27.05.2014	I, Torino	TAS-I	ExoMars2016
9	03. – 04.09.2014	D, Göttingen	MPI	ExoMars2022
10	30.09.2014	F, Paris	LISA	ExoMars2022
11	09. – 10.12.2014	I, Torino	TAS-I	ExoMars2016
12	18.02.2015	I, Torino	TAS-I	ExoMars2016
13	31.03.2015	I, Padua	TAS-I	ExoMars2016
14	22. – 23.05.2015	F, Cannes	TAS-F	ExoMars2016
15	10.11.2015	GB, Stevenage	Airbus	ExoMars2016
16	-	KZ, Baikonur	Cosmodrome	ExoMars2016
17	03.05.2016	I, Torino	TAS-I	ExoMars2022
18	15.06.2016	GB, Stevenage	Airbus	ExoMars2022
19	22.11.2016	I, Torino	TAS-I	ExoMars2022
20	22.05.2017	CH, Sachseln	Maxon	Mars2020, Exomars2022
21	13.03.2018	I, Torino	TAS-I	ExoMars2022
22	15.05.2018	GB, Stevenage	Airbus	ExoMars2022
23	25.06.2018	NOR, Horten Kjeller	FFI	Mars2020
24	23.10.2018-24.10.2018	I, Torino	TAS-I	ExoMars2022
25	03.03.2019-05.03.2019	D, Friedrichshafen	Airbus	JUICE
26	09.04.2019	I, Torino	TAS-I	ExoMars2022
27	29.07. – 30.07.2019	UK, Stevenage	Airbus	ExoMars2022
28	27.08.2019	I, Torino	TAS-I	ExoMars2022
29	10.12.2019	F, Cannes	TAS-F	ExoMars2022
30	22.01.2020	F, Toulouse	TAS-F	ExoMars2022
31	11. – 12.10.2021	GF, Kourou	CSG	JUICE
32	09.11.2021	F, Toulouse	Airbus	JUICE
33	10.12.2021	I, Torino	TAS-I	ExoMars2022

Only for knowledge, **not certification**, ref. ECSS-Q-ST 70-55:

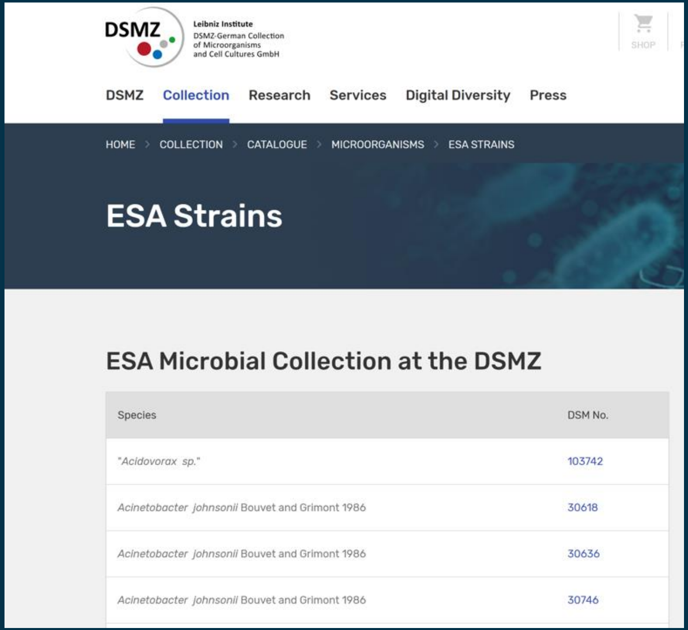
- Biodiversity: ESA wipe assay for cultivation of oligotrophic, alkaliphilic, vegetative, anaerobic microorganisms and fungi;
- Molecular analysis, 16S rRNA gene



Most abundant genera in cleanrooms

Motivations

ESA strain collection, what do we actually do with it for planetary protection?



https://www.dsmz.de/collection/catalogue/microorganisms/special-groups-of-organisms/esa-strains

The ESA's Planetary Protection Culture Collection includes around 883 isolates at the time being. Example below from JUICE

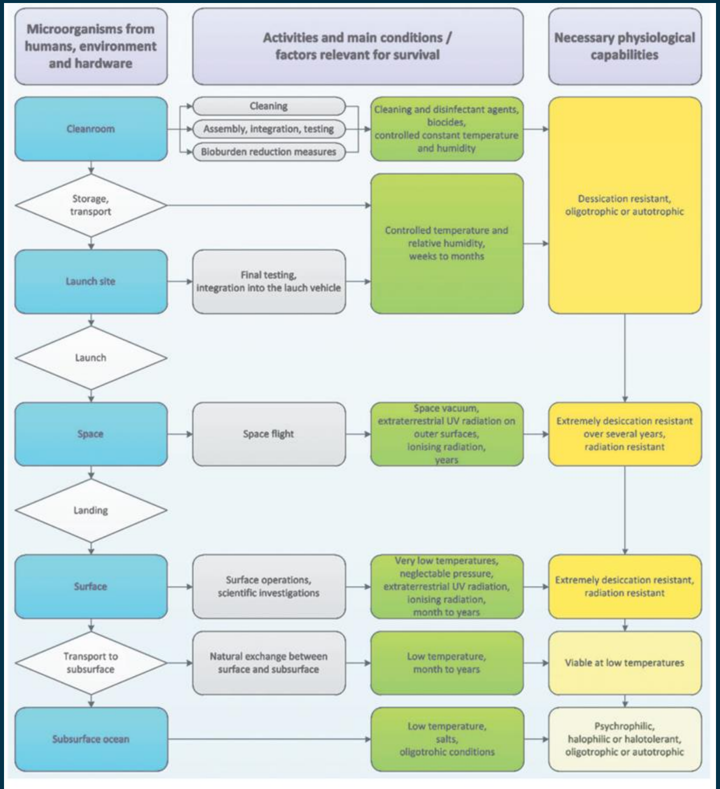
Sample No. For DSMZ	Sample no. / name Campaign	Nearest neighbor	DSM number assigned
4	A7_2	Cytobacillus oceanisediminis, C. firmus	114021
5	A9_1	Kocuria rhizophila	114052
7	A8_1	Deinococcus sp.	114022
8	A8_2	Phycicoccus sp.	114065
10	A9_2	Gordonia sp. (terrae)	114053
11	W8	Bacillus amyloliquefaciens	114027
12	W13	Bacillus subtilis group	114028
13	W12	Bacillus subtilis group	114029
14	W23_1	Priestia megaterium	114023
17	W27_1	Cytobacillus firmus, C. oceanisediminis	114024
19	W27_3	Bacillus pumilus group	114000
20	W27_4	Bacillus cereus group	-
21	W29_1	Bacillus cereus group	-
23	W33_1	Fictibacillus nanhaiensis, F. phosphorivorans	114025
25	W33_3	Bacillus cereus group	-
26	W33_4	Fictibacillus nanhaiensis, F. phosphorivorans	114026
27	W33_5	Fictibacillus nanhaiensis, F. phosphorivorans	114099
28	W33_6	Bacillus cereus group	-
29	W34_1	Fictibacillus nanhaiensis, F. phosphorivorans	114043
30	W34_2	Bacillus pumilus group	114038
31	W34_3	Bacillus cereus group	-
33	W34_5	Bacillus pumilus group	113998
35	W36_1	Bacillus pumilus group (stratosphaericus)	114039
37	W36_3	Lysinibacillus fusiformis	114040
38	W36_4	Lysinibacillus fusiformis	114044
39	W38	Paenibacillus sp. (chitinolyticus)	114046
40	W43 1	Fictibacillus nanhaiensis, F. phosphorivorans	114045
41	W43 2	Brevibacillus sp.	114031
43	W43 4	Priestia megaterium	113997
44	W43 5	Paenibacillus sp. (chitinolyticus)	114041
45	W43 6	Fictibacillus nanhaiensis, F. phosphorivorans	114066
46	W43 7	Lysibacillus boronitolerans	114032
47	W43 8	Fictibacillus nanhaiensis, F. phosphorivorans	114067
48	W43 9	Fictibacillus nanhaiensis, F. phosphorivorans	114068
50	W43 11	Cytobacillus sp. (horneckia)	114011
51	W43 12	Aneurinibacillus humi	114012

Motivations

Metagenomics for future space missions

Metagenomic / molecular biology role in:

- Identifying sources of contamination
- Designing tailored sterilisation processes
- Establishing survivability of problematic species for planetary protection
- Helping risk assessment in probabilities to contaminate other Worlds and protect Earth



Credit: Rettberg et al, 2019 - PPOSS

Objectives of the workshop

Overall: trigger the discussion in Europe on how metagenomic / molecular biology can aid future space missions. Coordinate inputs to create a long-term strategy, roadmap (with all stakeholders involved) for inclusion to future ESA planetary protection policies

Specific objectives:

1 Assess "the good, the bad and the ugly":
User perspective, highlighting limitations, i.e. low biomass, cross contamination of reagents, efficiency to discriminate live/dead cells, cost, time elapse between sampling and analysis, cleanroom vs flight

2 Future work/R&D required to fill the gaps:
Road to a standardised the method, from collection of samples, type of consumables, DNA extraction, sequencing, bioinformatic
Coordinate the effort internationally to avoid duplication. Transparent process to inform the decision-making process
Database, for e.g. similar to ESA DSMZ

3 Validation and requirement:
Needs to reach an adequate maturity level to start validation;
Requirements to be placed, looking at the bigger picture of risk informed decision framework

Outcome

ESA strain collection / genome library	• Extending the scope for existing databases to account for metagenomics • Assess sampling data from past biodiversity campaigns • Sequence all isolates present in the DSMZ database (more than 800) • Include the use of phenotype prediction into databases
Metagenomic sequencing	• Introduction of metagenomic campaigns on ESA missions in addition to 16s rRNA gene sequencing (current) methods to understand microbial "who" and "what" • Collaboration with international partners (i.e. NASA) to do not duplicate community effort and pool a larger amount of data • Testing to tackle knowledge gaps, i.e. low biomass, reagent contamination, bioinformatic, socialising with private sectors • Organisation of additional workshops to involve scientific community from different sectors
Quantitative methods	• Invest on comparative test to study quantitative PP methods, i.e. q-PCR, dd-PCR, etc. to get rapid assessments (beneficial for European industry) • trials on the field with (essential) involvement of European industries
Experimental testing to train contamination models with real data	• Select and test planetary protection relevant microorganisms under simulated space conditions • Feed the data onto representative model/AI tool
Develop an internationally agreed model for contamination	• Build/develop statistic expertise to modify/tailor existing planetary protection models • Trial on real cases, like past missions with relevance to planetary protection • Shift from prescriptive to risk informed based assessments and tools
Planetary protection documentation	• Update relevant PP standards to allow more flexibility for mission teams

TERRAE NOVAE VISION

LEO

Sustained presence in, and use of LEO
Gateway

Moon

Argonauts, humans by 2030

Mars

Crewed missions
Robotic mission (MSR, ExoMars)
Studies (i.e. Mars Ice Access)

Space Transportation

Cargo / crew transportation
Space rider