Basics of microbiology

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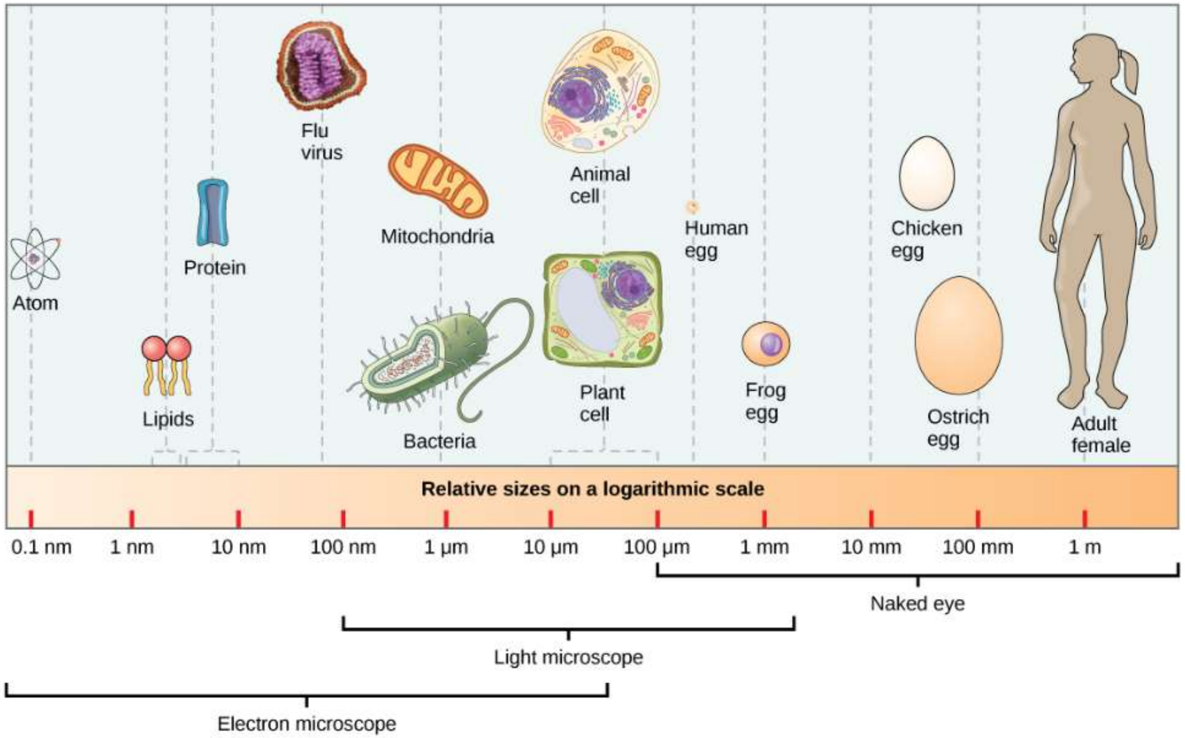
What is microbiology?

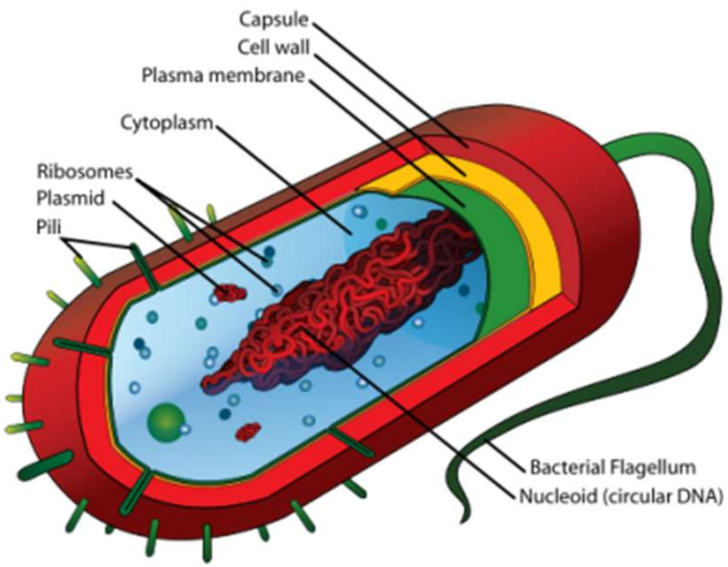
- Study of “micro-organisms” (very small organisms)
- Bacteria, archaea, some eukaryotes, including most protists, some fungi, as well as some micro-animals and plants - viruses



From: https://openstax.org/books/concepts-biology/pages/3-2-comparing-prokaryotic-and-eukaryotic-cells

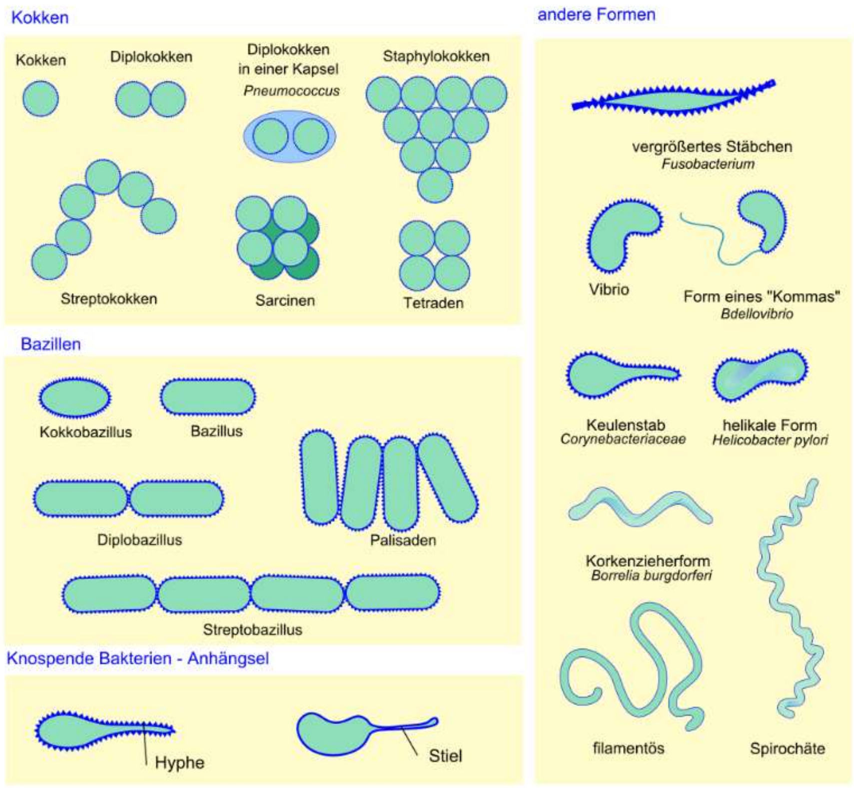
Bacteria and archaea

- Bacteria and Archaea are Prokaryotes
- All functions are located within one cell.
- DNA is not separated by a membrane.
- Average size 1 to 5 µm



Bacteria and archaea

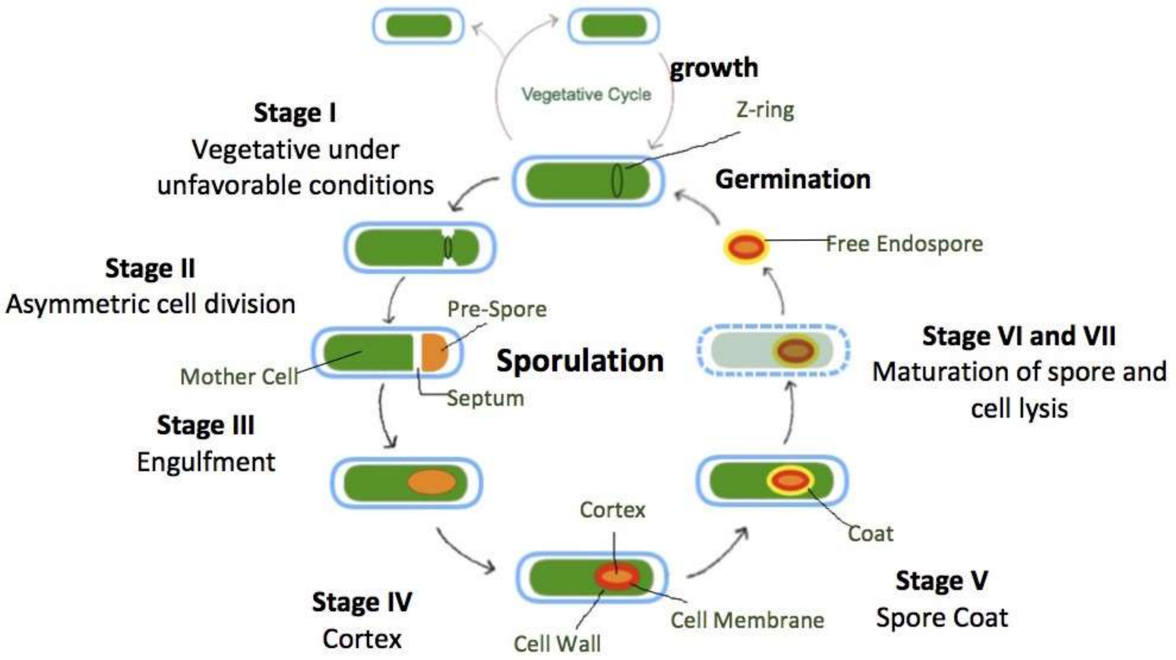
- Different shapes



From Wikipedia

Bacterial spores

- Spore formation of some microbes as a survival strategy to escape temporarily and / or spatially from unfavorable conditions

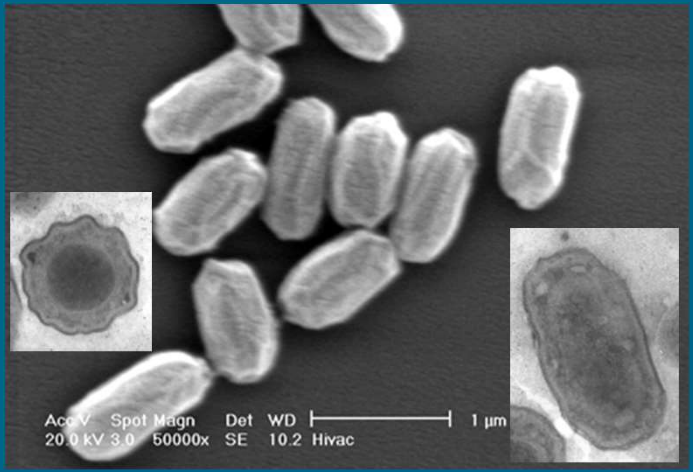


By Daniellemaclean144 - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=54141303

Bacterial spores

- Trigger of spore formation: starvation, changes in the environment
- Metabolically inactive spores are more resistant against challenging environmental conditions than the corresponding vegetative cells.

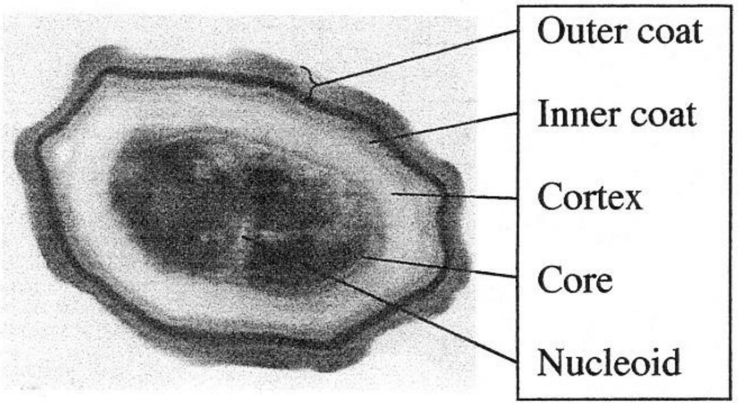
  - Desiccation
  - UV radiation
  - Ionising radiation
  - Heat
  - High salt concentrations
  - Oxidising compounds
  - Aggressive chemical agents (acids, acetone, alcohols,...)



Bacillus pumilus

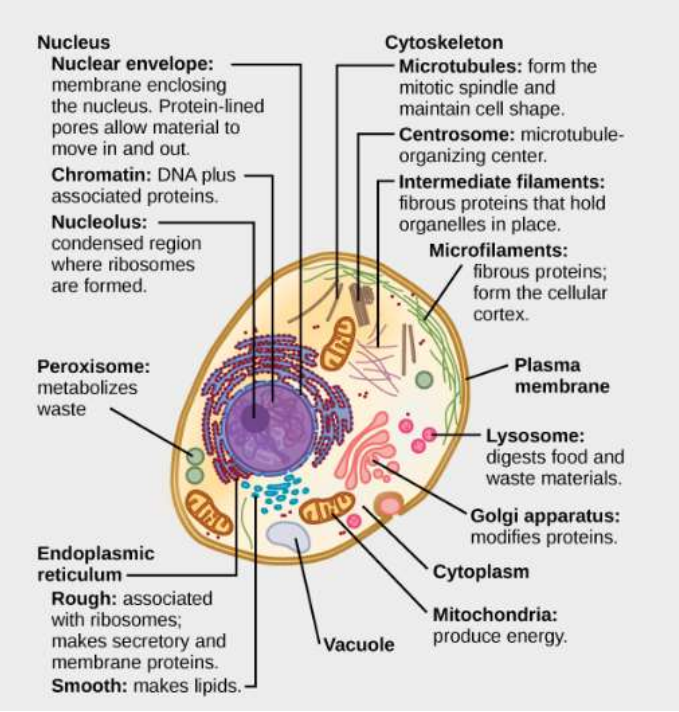
Parameters contributing to spore resistance

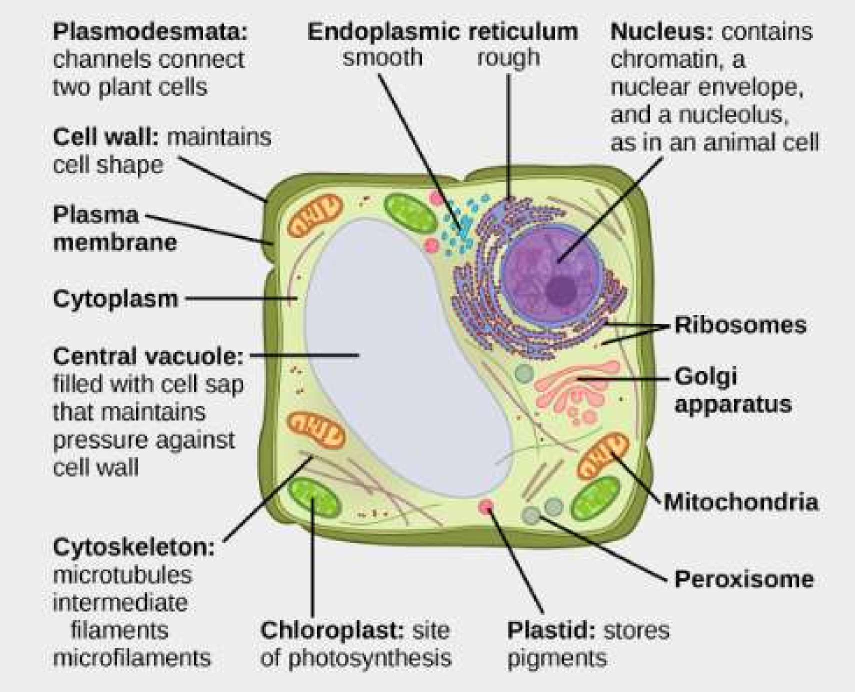
- Protective spore coats
- Thick cortex
- Low core water content
- High mineral content (Ca^{2+})
- Small acid soluble proteins at DNA



Eukaryotic cells (all plants, fungi and animals)

- Eukaryotic cells have a nucleus.
- Cells size up to 20 µm

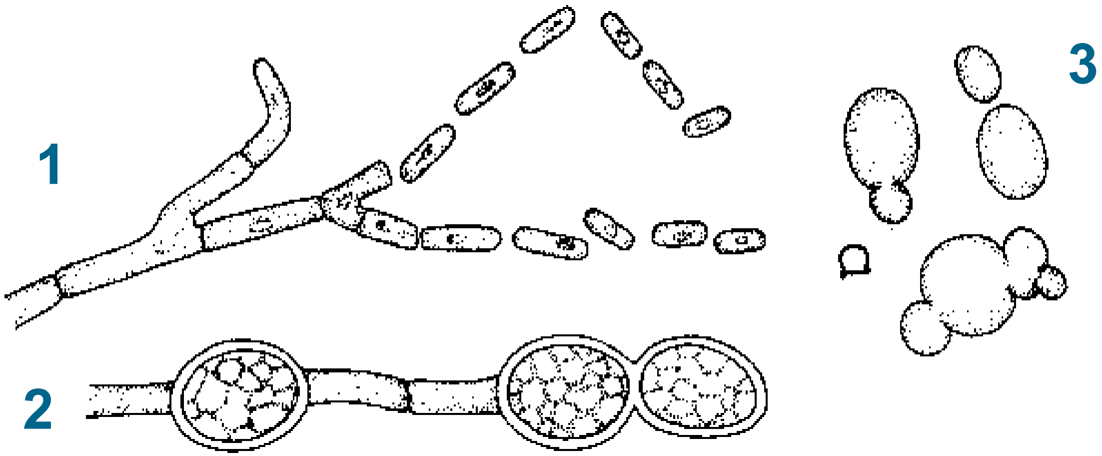




From: https://openstax.org/books/concepts-biology/pages/3-3-eukaryotic-cells

Fungal cells

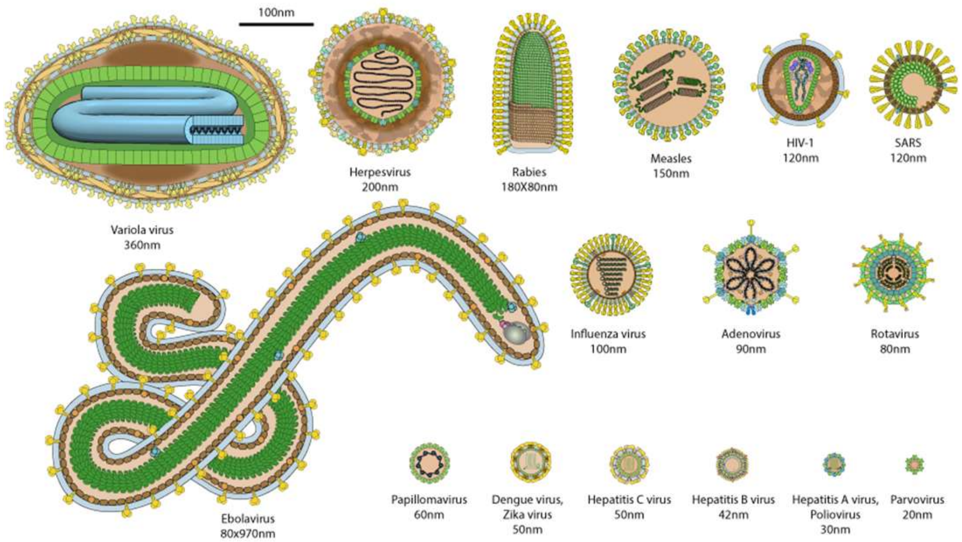
- Fungal cells have a nucleus.
- Average size of fungal cells and spores: 5-20 µm

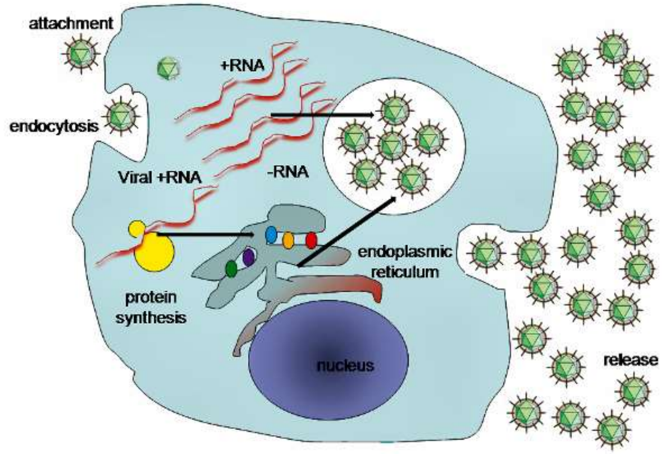


1: Hyphae break into pieces
2: Spores formed in special containments (Ascospores)
3: Sprouting of yeast cells

Viruses

- Average size of viruses: 0.02-0.3 μm
- Need host cell for replication





From Wikipedia

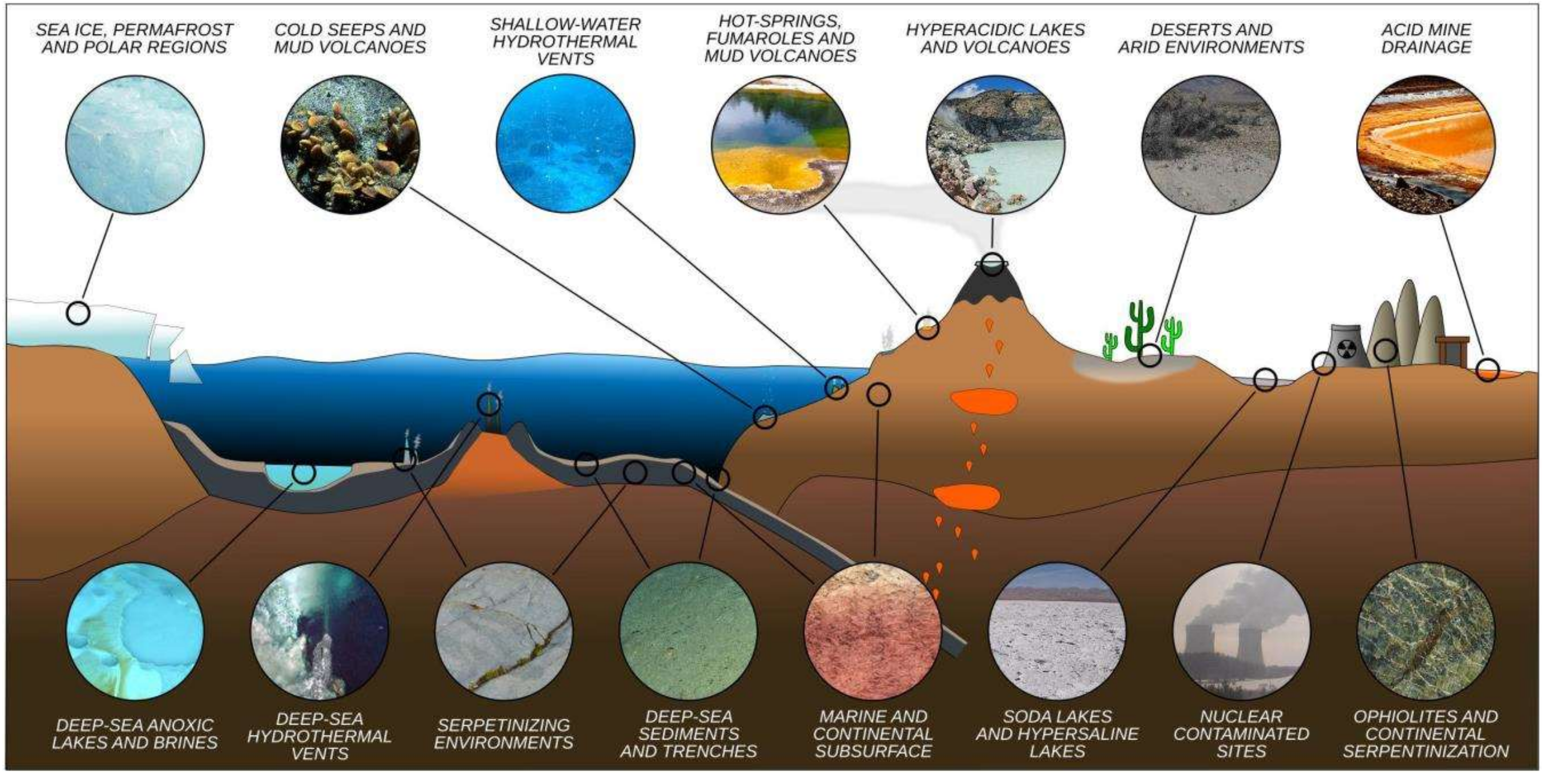
Life on Earth

During life‘s evolution on Earth microorganisms

- have existed since the emergence of life about 3.5 to 3.8 billion years ago,
- are ubiquitous,
- inhabit “extreme“ niches.

→ Microrganisms are the most successful forms of life on Earth.

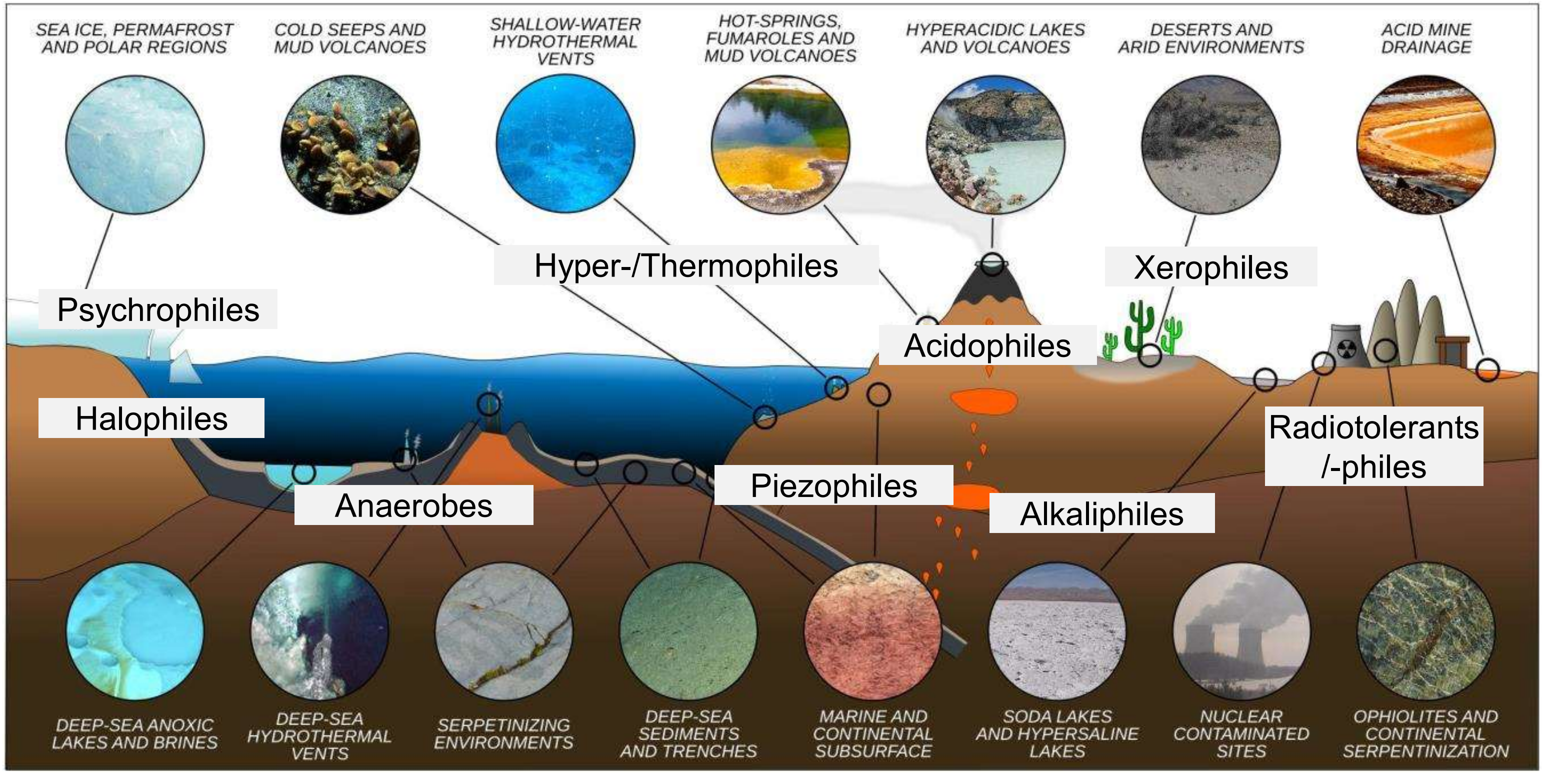
Extreme environments on Earth



Representative idealized cross section of Earth's crust showing the diversity of extreme environments and their approximate location

Merino et al., 2019, doi: 10.3389/fmicb.2019.00780

Extreme environments on Earth



… are inhabited by extremophilic microrganisms, many of them are polyextremophilic.

Merino et al., 2019, doi: 10.3389/fmicb.2019.00780

Important functions of microorganisms

- Only very few microorganisms make us sick.
- All others are necessary for the ecosystem ‚Earth' (and us!).
- They produce half of the oxygen we breathe.
- They are the basis of the food chain all life on Earth depends on.
- There is no life on Earth without microorganisms.

Credit: ESA

The human microbiome

- More bacteria and archaea in and on our body than own human cell
- 150x more genes
- Most microbes are essential.
- Up to 10,000 species
- Up to 1 kg pure microbial material in the gut
- Skin: as many microbes than humans on Earth

The human microbiome fulfills complex tasks.

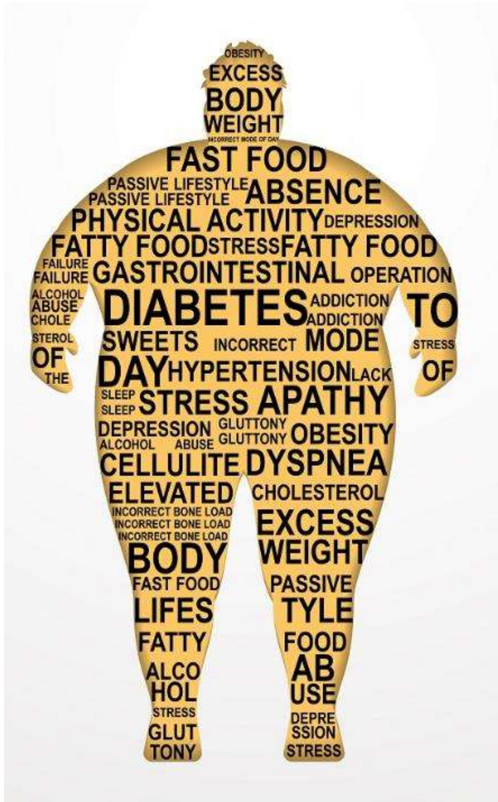
- Digestion / energy gain
- Production of co-factors / vitamins
- „Cross-talk“ with human cells, brain
- Immune system regulation
- Removal of toxins, protection
- …

Joint goal: Living in balance

The human microbiome

Microbiome composition correlates with diseases

- obesity
- auto immune diseases
- diabetes
- inflammatory bowel diseases
- autism, depression
- colorectal cancer
- diarrhea
- …

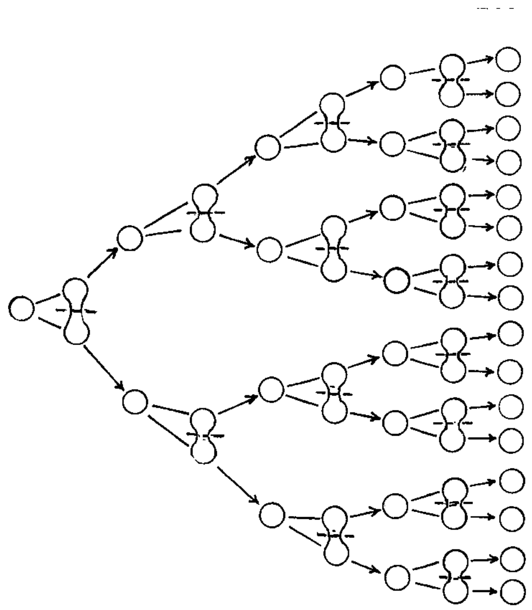


From: [Facts About Obesity - Causes & Rates – Weight Loss Surgery Info (yourbariatricsurgeryguide.com)]

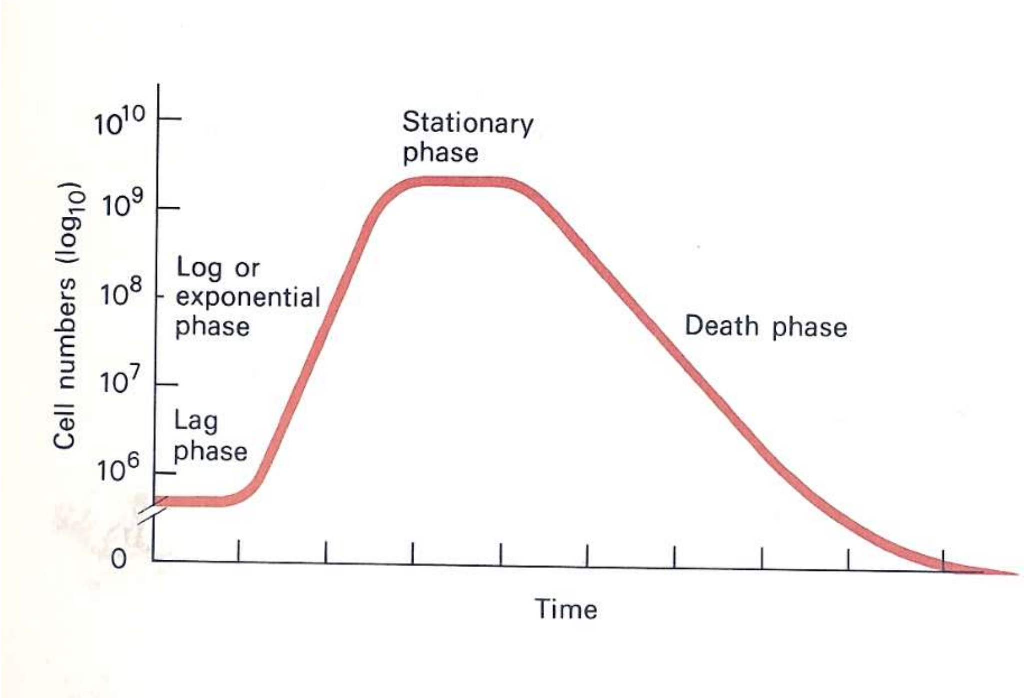
The human microbiome

- Humans distribute microorganisms.

Microbial growth and cultivation

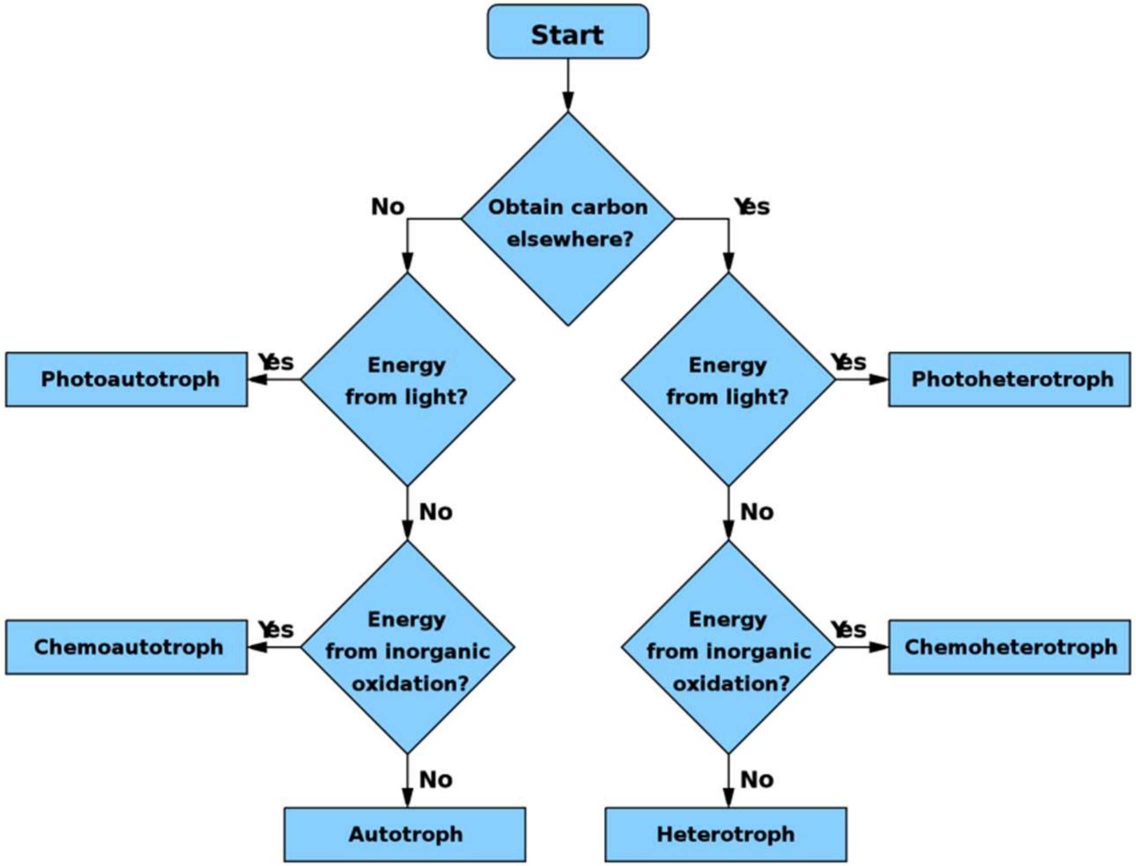


Exponential growth by cell division



Growth curve

Microbial growth requirements



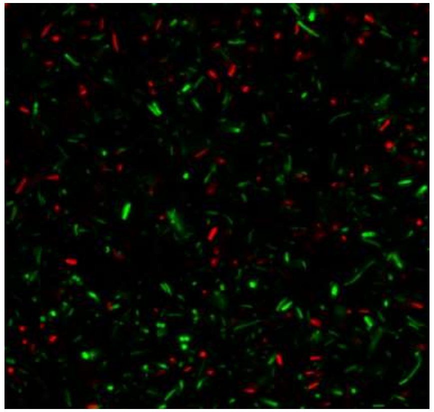
Microbial growth

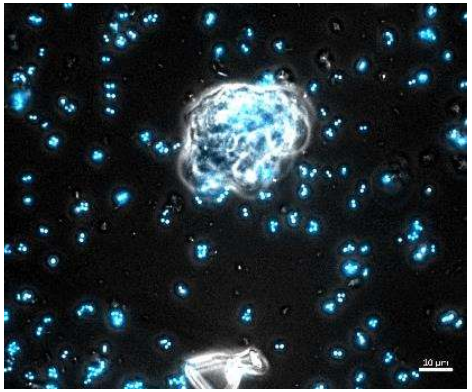
- Every microorganism has different requirements wrt temperature, oxygen, carbon source, energy source, minerals, vitamins...
- Some might need specific partners for growth.
- Only maximal 1% of the microorganisms is culturable under standard laboratory conditions.

Uncultivated microorganisms

Different and complementary technologies are necessary to try to understand the diversity, the physiology and the capacity of the microbes *in situ.*

- Microscopy techniques (visualize)
- Nano-SIMS
- Analyze genomic information
- ...





Detection of microorganisms with molecular methods

- Without cultivation → detection of uncultivable species
- Very powerful high-throughput methods
- Fast development in sequencing technologies, bioinformatics tools and data bases
- Quantification and standardization difficult
- Very low biomass samples difficult
- No information with respect to physiological status (alive / dead)

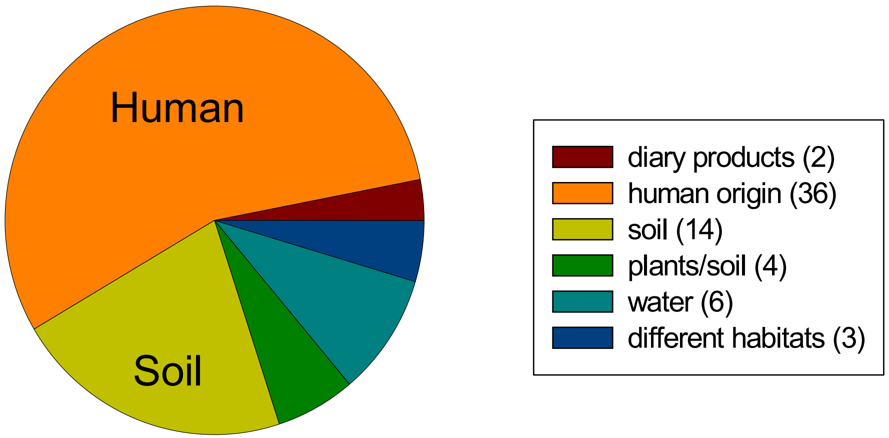
- Amplicon sequencing and metagenomics → Identification → Who is there?
- Metatranscriptomics, metaproteomics, metabolomics → Functional profiling → What do they do?

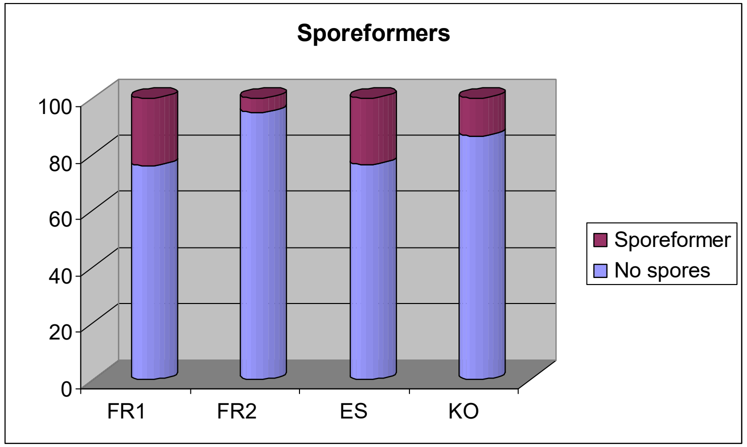
The clean room environment - an extreme niche

- Rigorous maintenance procedures
  - Cleaning and sterilization, HEPA air filtration, constant control of humidity and temperature
- Extreme “biotope”
  - Low nutrient levels, desiccation, harsh conditions
- Selective for resistant microbes (= survival specialists)?

Microorganisms in clean rooms

- Majority of clean room isolates are human associated microorganisms.
- Others are from the environment.
- Many spore formers
- Contamination not homogeniously distributed

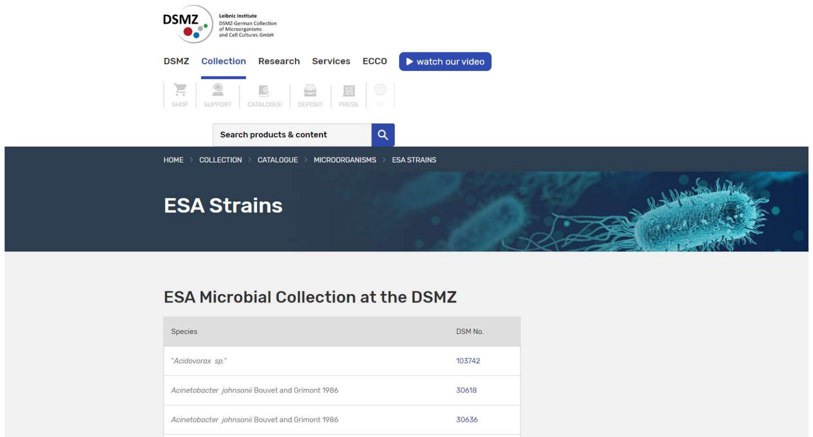




Microbial isolates from spacecraft and cleanrooms

DSMZ

Leibniz Institute
DSMZ-German Collection
of Microorganisms
and Cell Cultures GmbH



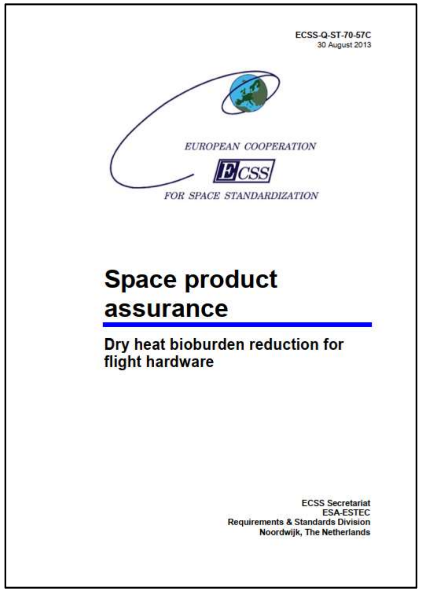
Biological resource centers are instrumental for the future advancement of science, public health and bioeconomy. The Leibniz Institute DSMZ meets the challenges of the limited knowledge of microbial biodiversity and its functional implications, the demand for appropriate model systems for basic research, and the shortage of innovative novel bioproducts. With its comprehensive collections of biomaterials and its unique expertise in the areas of cultivation, identification, taxonomy/phylogeny and conservation, the DSMZ also plays a key role for the translation from basic research to applications of biodiversity.
The supply, investigation and utilization of biodiversity are the guidelines of the DSMZ.

Fully automated storage system for microorganisms

Things to remember

- A single microbial cell is not visible with the naked eye.
- Microorganisms are everywhere.
- Several microorganisms are adapted to extreme conditions.
- Some microorganisms can form spores.
- Bacterial spores are resistant to many physical and chemical stressors.
- Each microorganism has ist specific growth requirements.
- The majority of microorganisms present in many natural environments are not readily cultivable.
- The majority of microorganisms from spacecraft and SAF originates from humans.
- A large amount of isolates from spacecraft and SAF are spore formers and are present as spores.
- The microbial contamination on spacecraft and in SAF is inhomogenously distributed.
- For the detection and quantification of microorganisms cultivation-dependent and molecular methods are necessary.

How to reduce the bioburden?



ECSS-Q-ST-70-57C
30 August 2013

EUROPEAN COOPERATION
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Space product assurance

Dry heat bioburden reduction for flight hardware

ECSS Secretariat
ESA-ESTEC
Requirements & Standards Division
Noordwijk, The Netherlands

ECSS-Q-ST-70-57C

This standard defines procedures for the reduction of microbiological contamination of flight hardware using **dry heat.**

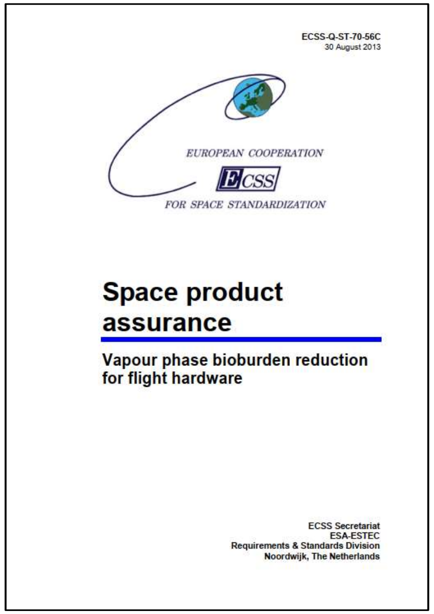
The procedures specified in this standard cover:

Reduction of microbiological contamination on exposed surfaces, mated surfaces and encapsulated material

Reduction of microbiological contamination in dry, ambient and uncontrolled humidity environments.

This standard also specifies requirements for the conditioning of the flight hardware, bioburden reduction cycle development, and equipment to be used for applying a bioburden reduction procedure.

How to reduce the bioburden?


ECSS-Q-ST-70-56C
30 August 2013

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Space product assurance

Vapour phase bioburden reduction for flight hardware

ECSS Secretariat
ESA-ESTEC
Requirements & Standards Division
Noordwijk, The Netherlands

ECSS-Q-ST-70-56C

This standard specifies procedures for the reduction of microbiological contamination of flight hardware using **hydrogen peroxide** vapour.

The procedures specified in this standard cover:

Reduction of microbiological contamination on exposed surfaces

Reduction of microbiological contamination in controlled ambient and vacuum environments.

This standard also specifies requirements for the conditioning of the flight hardware, bioburden reduction cycle development, and equipment to be used for applying a bioburden reduction procedure.

How to measure the bioburden?


ECSS-Q-ST-70-55C
15 November 2008

EUROPEAN COOPERATION
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Space product assurance

Microbial examination of flight hardware and cleanrooms

ECSS Secretariat
ESA-ESTEC
Requirements & Standards Division
Noordwijk, The Netherlands

ECSS-Q-ST-70-55C

This standard defines test procedures for **quantitative and/or qualitative microbiological examination** of surfaces of flight hardware and in microbiologically controlled environments (e.g. cleanroom surfaces, cleanroom air, isolator systems).

The test methods described in this standard apply to controlling the microbiological contamination on all manned and unmanned spacecraft, launchers, payloads, experiments, ground support equipment, and cleanrooms with planetary protection constraints.

ECSS-Q-ST-70-55C

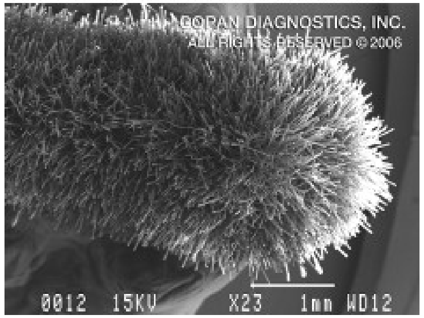
The following test methods are described:

Surface and **air** sampling and detection of biological contaminants with swabs, wipes, contact plates and air samplers, followed by **cultivation** for bioburden determination.

Sampling of biological contaminants by **DNA analysis** from swabs and wipes.

Surface sampling with swabs

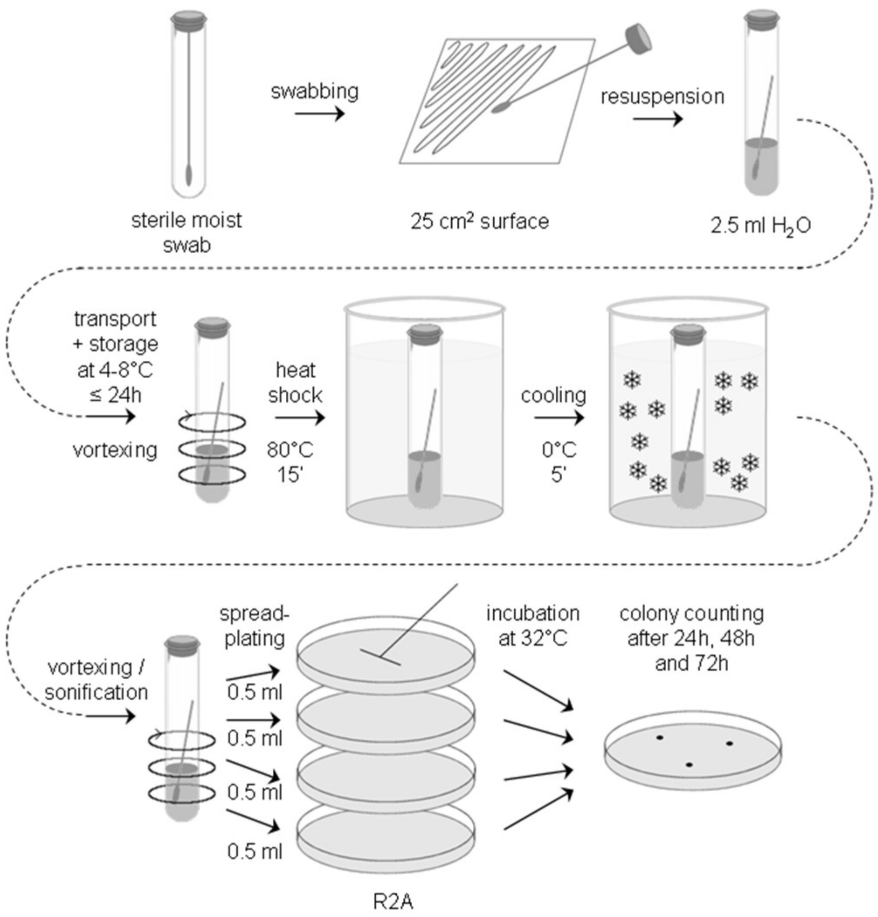
Electron Microscope photograph of a nylon flocked swab.



For areas up to 25 cm^2

Bioburden analysis with the planetary protection standard assay

for the enumeration of aerobic mesophilic heat tolerant spores and vegetative bacteria



Results after 72h of incubation

Surface sampling with wipes

For areas up to 1 m^2

Analysis in analogy to the swab assays

Sampling in different cleanroom classes

ISO 5

ISO 8

Planetary protection requirements

- have to be taken into consideration from the very beginning,
- limit the allowed bioburden of a spacecraft for category III, IV and V (restricted) missions,
- have a major impact on mission design,
- have a major impact on spacecraft hardware and payload
  - material selection
  - spacecraft design
  - sterilisation strategies
  - assembly, integration and testing,
- necessiates documentation, reviews, management structures,

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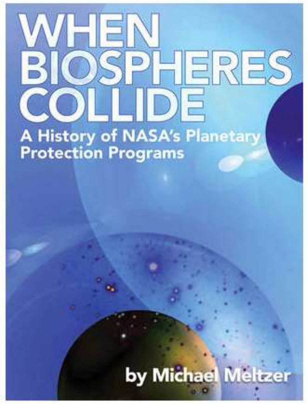
- necessiates documentation, reviews, management structures,
- **are implemented since the Viking missions (1976).**



The history of planetary protection is presented by tracing the responses to interplanetary concerns on NASA's missions.
Credits: NASA

The International Planetary Protection Handbook

Description of the state of the art and good practice for implementing planetary protection requirements

→ Introduction and general informations
→ Case studies for different PP categories
→ Lessons learned from past missions
→ Implementation in different space agencies

Free download from the COSPAR webpage:
https://cosparhq.cnes.fr/assets/uploads/2021/02/PPOSS_International-Planetary-Protection-Handbook_2019_Space-Research-Today.pdf

EC Horizon 2020, grant agreement 687373,
Planetary Protection of Outer Solar System (2016 – 2018)


Thank your for your attention!