About Microbial Hazard and Risk in Spatial Habitats

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June 9, 2016

Case Study: Alarmist viral news concerning ISS

Sensationalism :

Filled with germs, Potentially harmful, Dangerous, Threat , Thrive at...

on the ISS - how dangerous are

the International Space Station? An article published in the
some potentially hazardous microbes floating around the

Science

International Space Station astronaut Karen Nyberg made a video showing how
her hair—and inevitably put her microbe-laden cells in the air.

The International S
potentiall

TAG NASA , International Space Station , bacteria , spacecraft , astronauts

Potentially Dangerous Bacteria Thrive At The International Space Station, NASA Warns

27 October 2015, 7:12 am EDT By Katrina Pascual Tech Times

TECH TIMES

The Telegraph

International Space Station filled with germs, Nasa warns

Bacterial pathogens mostly innocuous on Earth thrive in ISS as it is prime
breeding ground, study that could lead to stricter space cleaning regime finds

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Astronauts fa
International Sp

The ISS is home to potentially harmful bac

SPACE / 27 OCTOBER 15 / by BECCA CADDY

31 shares
0 comments

threat on
on - so what is diptheria?

Fashion Mums

Dear Bacteria,
I am happy to announce
Space Station too, and th
without any training or fa

Case Study: All was coming from this paper

RESEARCH | OPEN ACCESS

Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities

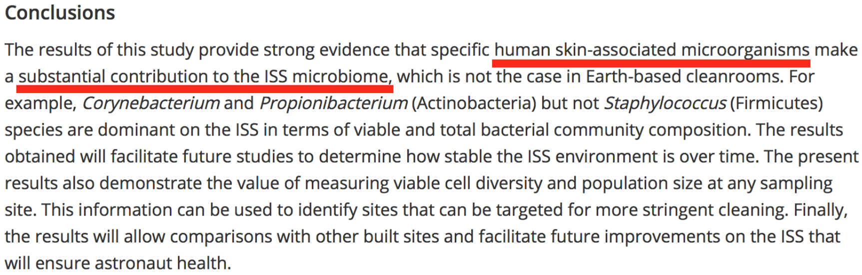
Aleksandra Checinska, Alexander J. Probst, Parag Vaishampayan, James R. White, Deepika Kumar, Victor G. Stepanov, George E. Fox, Henrik R. Nilsson, Duane L. Pierson, Jay Perry and Kasthuri Venkateswaran

Microbiome 2015 3:50 | DOI: 10.1186/s40168-015-0116-3 | © Checinska et al. 2015

Received: 28 July 2015 | Accepted: 28 September 2015 | Published: 27 October 2015

A very neutral title...

Case Study: But not in its factual conclusion



Conclusions

The results of this study provide strong evidence that specific human skin-associated microorganisms make a substantial contribution to the ISS microbiome, which is not the case in Earth-based cleanrooms. For example, *Corynebacterium* and *Propionibacterium* (Actinobacteria) but not *Staphylococcus* (Firmicutes) species are dominant on the ISS in terms of viable and total bacterial community composition. The results obtained will facilitate future studies to determine how stable the ISS environment is over time. The present results also demonstrate the value of measuring viable cell diversity and population size at any sampling site. This information can be used to identify sites that can be targeted for more stringent cleaning. Finally, the results will allow comparisons with other built sites and facilitate future improvements on the ISS that will ensure astronaut health.

And no alarm in the conclusions

Case Study: The alarm is in the discussion I

Alarm sentences in the publication:

Although the *Propionibacterium* species represent **natural skin commensals**, *P. acnes* is considered an **opportunistic pathogen** that leads to various infections. Similar concerns refer to *Corynebacterium* (**...**)

Translation:

P. acnes is **occasionnaly** responsible **very rarely** of infections **esssentially linked to prosthetic material** or impaired cardiac valves.

Case Study: The alarm is in the discussion II

In the publicaton:

Aspergillus niger was the predominant isolate, and although it does not have the potential to cause disease at the same rate as other *Aspergillus* species (*A. fumigatus, A. flavus*), it **was correlated** with pulmonary and ear infections

Translation:

No causality demonstrated

Case Study: confusion between HAZARD and RISK

Definitions (WHO)

- **Hazard:** something that is dangerous with the potential to cause damage
- **Risk:** the possibility of something bad happening (probability). High or low risk.

Case Study: Going back to the publication

- The paper was studying the ISS-associated microflora (aka **microbiome**)
    - It confirms that humans are the main source of bacteria.
    - **No surprise:** the crew is the main microbe-bearing entity
    - **ISS is an heavily anthropized ecosystem**
    - No conclusion can be drawn concerning the health of the crew.
    - Communication was inappropriate (except to get fundings).

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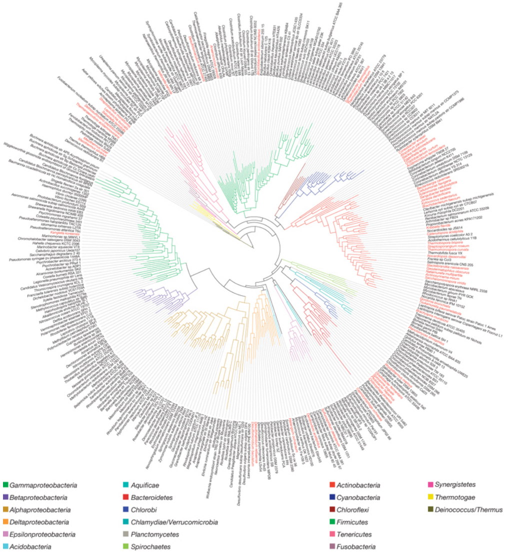
Case Study: Conclusion

This is exactly our problem

Facts and uncertainties concerning hazard and risk for humans in isolated heavily-anthropized environments

A quick panorama of Procaryotes I



- 2638 genera and 13539 known species
- Much more undescribed, mostly not cultivable
- Defined species: 1/10 1/100 or even less of the real diversity

The Human microflora: general constraints

- Excess of nutrients (glucose, peptides, lipids etc.)
- Water (except for the superficial skin, hair)
- Heat (from 32-37℃): mesophilic micro-organisms.
- Competition for iron and oxygen
- Bias toward Anaerobes or aero-anaerobes even on/inside the skin

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The Human microflora: Intestinal I

- The largest and most complex
- 10^{12} cells per gram of feces
- Mesophilic micro-organisms
- Anaerobes (*Bacteroidetes*) + Aero-anaerobes (*Firmicutes*) domining
- Skin/clothes contamination remaining after defecation

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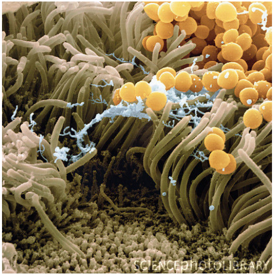
The Human microflora: Mouth and nose

- *Firmicutes* (*Staphylococcus*, *Streptococcus* etc.)
- *Actinobacteria*
- Mesophilic micro-organisms.
- Adherence to the epithelia
- Output during speech/breathe/sneeze



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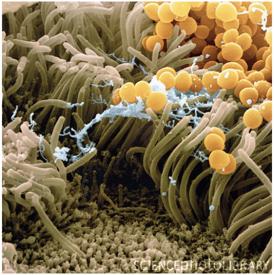

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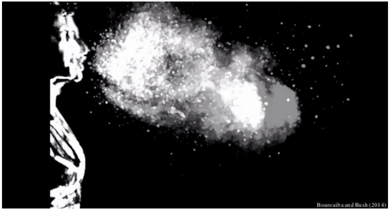

Bourouiba and Bush (2014)

The Human microflora: Hair and skin

- Constant dissemination power
- Lipidophilic
- *Actinobacteria* (*Corynebacterium*, *Propionibacterium*)
- *Malassezia* (fungus)
- Importance of hands and surfaces in exchanges

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Outside Humans, an harsh environment

- Lack of nutrients.
- **Lack of water** (except for water-production systems and condensation).
- Cleaning and Disinfection of the surfaces
- If in air : filtration or treatment (POTOK)

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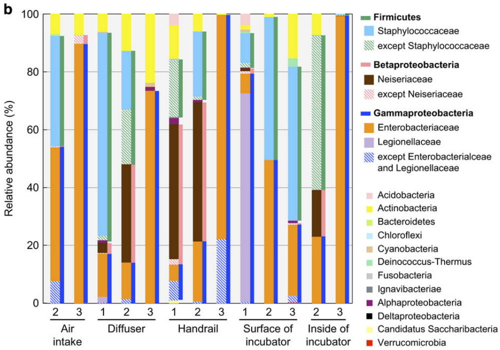
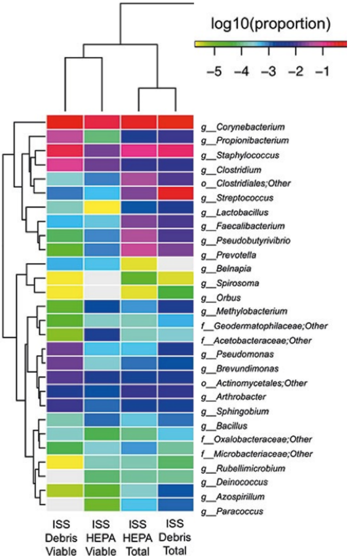
Microflora on the Surfaces: KIBO microflora



- *Staphylococcus* and other *Firmicutes*
- *Enterobacteria* and plenty of *Gamma-proteobacteria* and *Beta-proteobacteria*
- A few others (and uncertainties or errors)
- Top genera are from skin origin

Ichijo et al. Npj Microgravity 2016 doi.org/10.1038/npjmgrav.2016.7

Microflora on the Surfaces of the ISS I



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- Top genera are from skin origin
- Other main genera are from the gut
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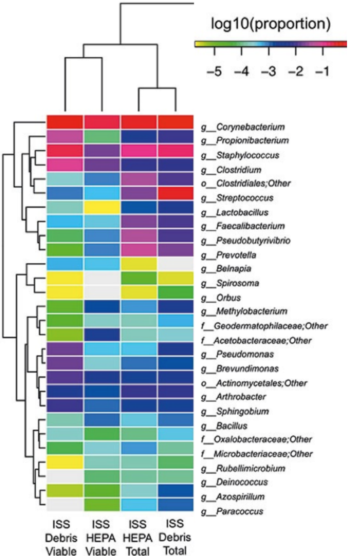

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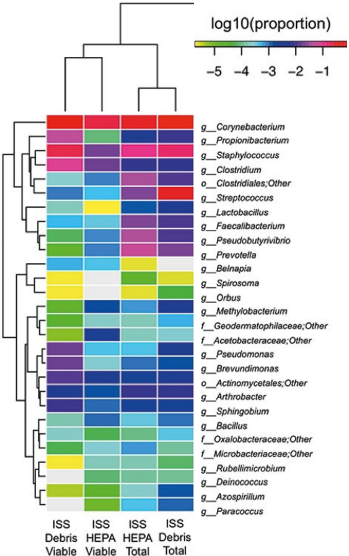

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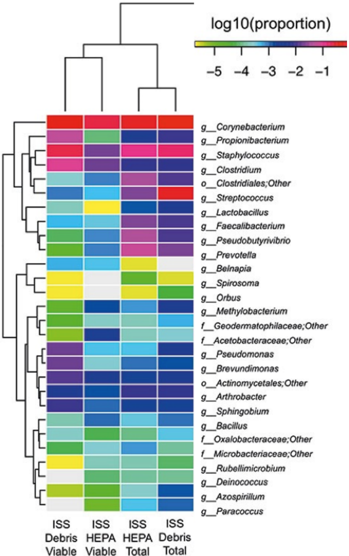

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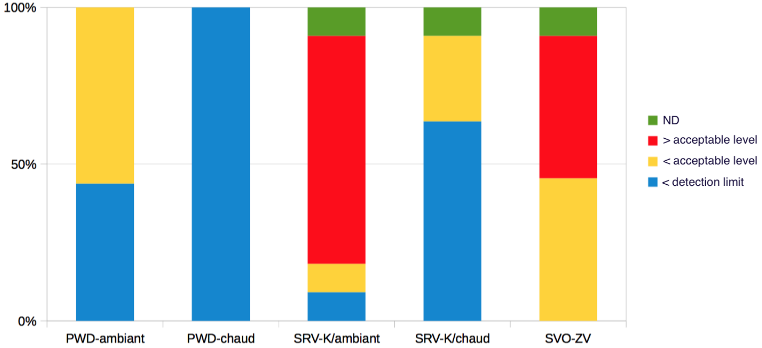
Microflora in the Air of ISS

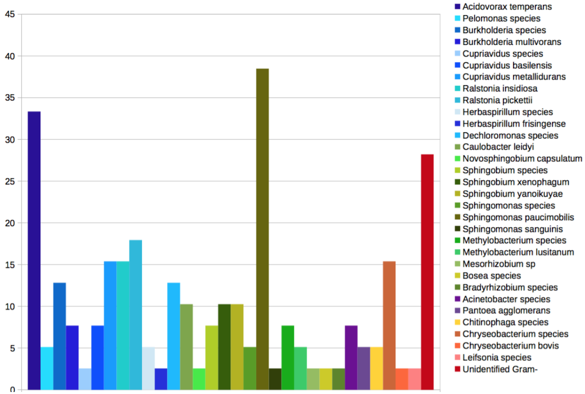
- Low bioburden
- Dryness resistant microbes (spores)
- Origin : Human skin and intestine (via the skin)

Microflora in the water of ISS I



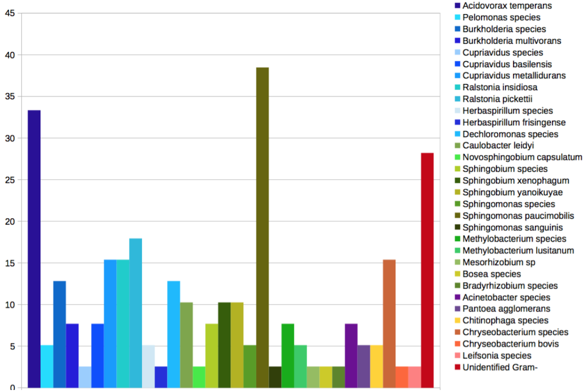
- Rather satisfying (the yellow and blue bars)
- Overall corresponding to the standards used on earth
- The less used sources of water are the most contaminated

Microflora in the water of ISS II



- *Alpha* 64% and *Beta proteobacteria* 86%
- ***Acidovorax temperans*** and ***Sphingomonas paucimobilis*** in 1/3 of the tests + *Methylobacterium*, *Burkholderia*, *Comamonnas* and *Cupriavidus*...

Microflora in the water of ISS II



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Microflora in the water of ISS III

Cosmona

Y.I Onufrienko with water canisters 2002

Environmental origin (from the Earth)

- Soil bacteria:
- Plant related (rhizosphere)
- Metal polluted areas
- Original contamination from a polluted water tank

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Microflora in the fresh food on board

Sciences. Les astronautes dégustent le premier légume de l’espace

PLANÈTE BUZZ 〉 SCIENCE & TECHNO 〉 **COURRIER INTERNATIONAL - PARIS**

Publié le 10/08/2015 - 11:10

La Station spatiale internationale va être témoin d’une première universelle ce lundi 10 août : ses occupants vont manger la première nourriture à avoir poussé ailleurs que sur Terre.

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- Minimal impact on the space-ship microbiome
- When eaten: buffering effect of the human microbiome

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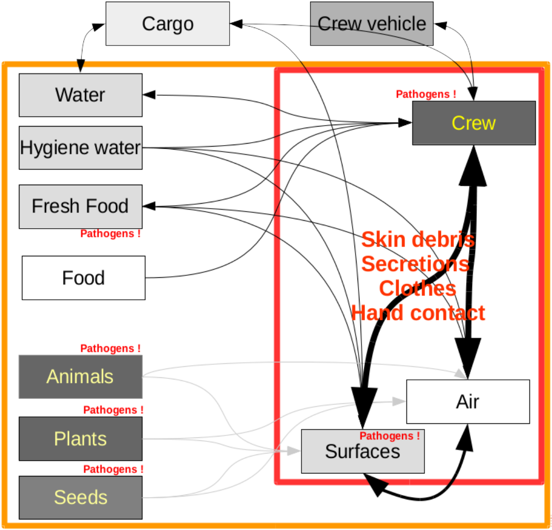
Microflora linked to Animal and other experiments

- The experiments are isolated from the cabin
- The microbes are contained inside the facilities.

General Microbial flow



Hidden Microbial Hot Spots

- **High amount of microbes in one place**
- If some water access :
- Microbes-build biofilm
- In dry zones: Flora exchanges

Typical cases

The MIR condensate water bubble, the fungus burst in ISS

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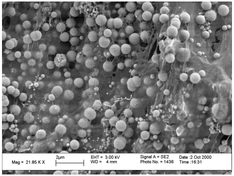
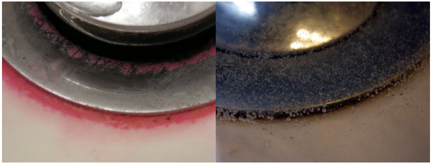
Hidden Microflora Hot Spots : biofilms





- Highly adherent microcolonies
- Multi-species *Bacteria/Archaea/Fungi*
- Leaching, pitting, clogging, long term contamination
- Extremely resistant to disinfection and stress
- Inside ISS water circuit: *Sphingomonas* and *Methylobacterium*
- One main contamination → stable microflora (Castro 2004)
- Sectors difficult to reach with moisture

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So what? Bacteria and fungi everywhere

Is it possible to evaluate the risk of infection ?

Risk analysis is used to develop an estimate of the risks to human health and safety, to identify and implement appropriate measures to control the risks, and to communicate with the society about the risks and measures applied.

WHO-FAO

Risk Analysis need usually five stages

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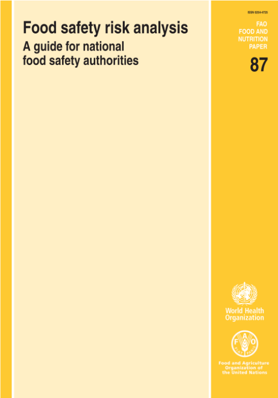

Food safety risk analysis
A guide for national
food safety authorities

ISSN 0254-4725
FAO
FOOD AND
NUTRITION
PAPER
87

World Health
Organization

Food and Agriculture
Organization of
the United Nations

First step: Hazard identification I

The pathogens are classified

- For Humans, WHO has set up a classification
- For animals and plants other lists exist
    - for animal health and zoonoses: *International Office of Epizootics*;
    - for plant health: the *Secretariat of the International Plant Protection Convention*.

First step: Hazard identification II

The pathogen are classified

- Class 4: may cause severe human disease; high risk of spread; no prophylaxis or treatment.
- Class 3: may cause severe human disease; risk of spread; prophylaxis or treatment usually available.

Hazard Identification

Pre-flight health survey: Obviously no such pathogen
No presence, no exposition = no probability of infection = no risk!

First step: Hazard identification III

The pathogen are classified

- Class 2: may cause human disease; unlikely to spread; prophylaxis and therapeutics available.
- Class 1: most *unlikely* to cause human disease

Hazard Identification

Only class 2 pathogens are to be considered.

The WHO list has been extended

- There are extensions at the US or European level, but also by countries (many lists)
- More than 2000 species (expanding regularly)

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First step: Hazard identification IV

What are these class 2 pathogens ?

- Most of them are opportunistic
- A majority comes from the Human microbiome
- Much less from environmental origin
- Number of potential pathogens increases

First step: Hazard identification V

Lists of pathogens

Are the pathogens lists really informative ?

- There are variations of the pathogenicity due to the presence or absence of clusters of pathogenicity genes.
- These pathogenicity islands that are either on the chromosome or on plasmids
- PAi may contain adhesins, secretion systems, toxins etc.

  invasins, modulins, effectors, superantigens, iron uptake systems, o-antigen synthesis, serum resistance,

  immunoglobulin A proteases, apoptosis, capsule synthesis and more
- Genetic exchanges occur inside or between species.
- Genetic exchanges may be increased in low gravity
- PAi are unstable, some genes may be missing or non functional.

First step: Hazard identification VI

Lists of pathogens

The name itself is NOT informative in term of adverse effects!

Look behind the name

Need to substitute the functions inside the microbe to the name of the microbe

The name worth for nothing?

Identification to the species level **is only informative** of the microbe potential

First step: Hazard identification VI

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First step: **Hazard identification**: Conclusion

Many Doubts

- We cannot rely only on pathogens lists
- The knowledge of the genome is only informative
- Most of the pathogens are members of the Human microbiome
- Lack of knowledge concerning environmental micro-organisms

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First step: **Hazard identification**: Conclusion

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Second step: Hazard characterization: Opportunist bacteria

- Most of the possible pathogens are classified as opportunists
- Opportunist micro-organisms give infection in the case of:
    - Accidental wounds
    - Surgical wounds
    - Presence of catheters and other prosthetic material (biofilm)
    - Major immunodepression
    - Infection promoted by unbalanced diabetic status

Note

These situations are not encountered for crew. Even if immunodepression exists the susceptibility to bacterial infection does not seems to be increased)

Third step: Risk estimation I

Target evaluation

- The target population is the crew
- Concerning infection, up to now no difference from a normal population

Third step: Risk estimation II

Exposure mode

- Up to now, the alimentary route through the water is the most evident
- Hand-Mouth contact possible
- The respiratory route seems excluded
- The cutaneous route is possible but with very limited

The endogenous route

The microbiome may be implied in infection (urinary tract essentially)

Third step: Risk estimation II

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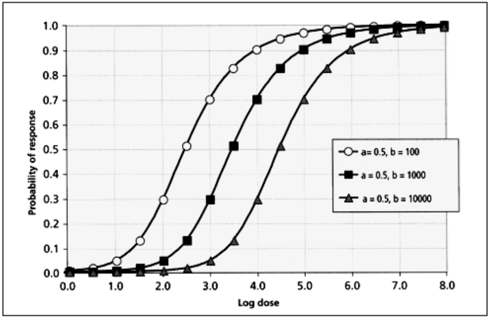
Exposure estimation

- Quantitative assessment is possible for contamination through the water
- The other routes can only give very small inoculum except if contact with a hot-spot

Third step: Risk estimation IV



Dose-effect modeling

- Relationship between the magnitude of exposure (dose) to a biological agent and the severity and/or frequency of illness (response)
- Modeling using data from well studied epidemics.

Risk estimation: Conclusion

Importance to fix Thresolds

- For a given bacteria, knowing the exposure level and Dose-effect curve we can fix safety thresholds
- Knowing safety thresholds we can set-up controls

In our case: no data available

- But the model is common to all pathogens: the smallest the dose, the lowest the probability
- Minimizing the contact/ingestion minimizes the risk

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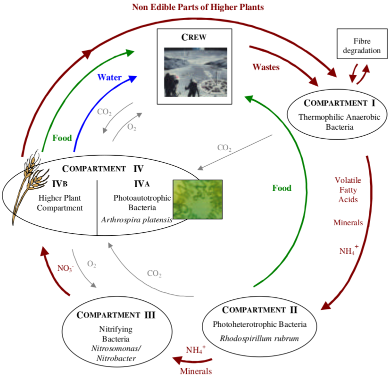
The MELISSA case: what will change



Non Edible Parts of Higher Plants

CREW

Fibre degradation

Wastes

Water

Food

CO_2

O_2

COMPARTMENT I
Thermophilic Anaerobic Bacteria

CO_2

COMPARTMENT IV

IVB
Higher Plant Compartment

IVA
Photoautotrophic Bacteria
Arthrospira platensis

Food

Volatile Fatty Acids

Minerals

NH_4^+

NO_3^-

O_2

CO_2

COMPARTMENT III
Nitrifying Bacteria
Nitrosomonas/ Nitrobacter

COMPARTMENT II
Photoheterotrophic Bacteria
Rhodospirillum rubrum

NH_4^+

Minerals

Complexification

- Global increase of the non-Human microbiome
    - Population size
    - Biodiversity: new **known** species and new **hidden** species (ex: vegetables and crops)
- More physical complexity
    - Complex bio-engineering and Technology
    - Potential new ecological niches and hot-spots

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Plant phytopathogenic bacteria

- *Pseudomonas, Xanthomonas*
- *Ralstonia* and many other...
- May be partially controlled before flight
- Can be transmitted by the seeds, coming from the microflora of fresh food, or even from the cabin or Humans
- A completely new risk assesment to do

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The MELISSA case: new hazards for the crew

Micro-organisms present in plants

- Some micro-organisms present on the roots (rhizosphere) are known pathogens
  - *Pseudomonas aeruginosa* (Wheat), *Burkholderia (ceno)cepacia*, *Stenotrophomonas maltophilia*...
  - *Escherichia coli* (O157:H7), *Listeria*...
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New micro-organisms in high concentration

Hazards and risk

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Fuzzy knowledge, fuzzy management

- Microbial hazard not well characterized - Anthropized environment
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Other situations:

- Clean rooms for immuno-compromised patients
- Safety in intensive care units and operating block
- Clean rooms in industry, including food industry
- Isolated life areas (submarines, polar stations)
- Planes microbial safety is also a concern

In many cases the safety thresholds are missing or determined arbitrarily

And the *technical limit of detection* is a simple and idiot way to fix a safety threshold

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We will improve our knowledge

We can create a database of adverse effects

- Link between micro-organisms and any negative effect
- Based on the evidence of the effect, frequency and gravity
- Potential of negative effects deduced from the genomes

Use the potential of genomics and bioinformatics

- 50.000 complete genomes avalaible in spring 2016, more than 1 million within 10 years
- Identification of pathogenicity island, understanding regulation and expression of the genes
- Networks of genes, proteins but also networks of interactions between micro-organisms (+ Human)

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Soon

Survey of the whole space-ship ecosystem

- Sampling and automated sample preparation exists (see the MIDASS project)
- In space sequencing of the extracted DNA validated
- Bioinformatics is quickly becoming rapid and reliable or even automated

Deep knowledge of the microbial status (adverse effects) and of the microbiome steady-state will be possible

Soon

Next step will be to anticipate the negative power of microbes

- Metagenomics analyse from the whole genome content of an environment
- Pathogenicity island can be identified
- Networks of genes will soon be computed to estimate the potential of a micro-organism
- Correlation with the adverse-effect database validated

Tomorrow

On this basis

- The survey of the microbial steady-state will enable the choice of effective but minimal counter-measures if any change occurs
- A scientifically oriented, safe, steady-state may be built
- The survey of global Human microbiome will be possible and we may imagine to maintain an safe steady-state of Human microbiome

Improving the life-support in space and on the Earth

Outside the technical differences due to the low gravity, **the solutions will also be used in our terrestrial life**

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Thank you for your attention

Thanks to

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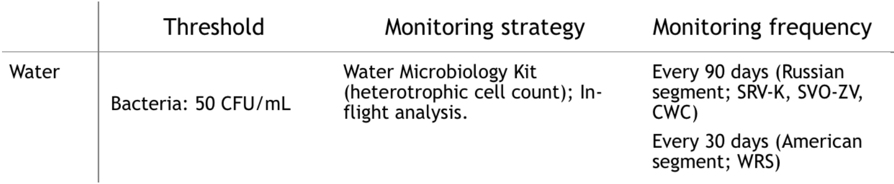
the THESEUS Cluster 4 colleagues
the BIOSIS expert team
the bioMérieux MIDASS Team
the LBBE team for helpful criticism
Stéphanie Raffestin and Christophe Lasseur

AIR MONITORING

	Threshold	Monitoring strategy	Monitoring frequency
Air	Bacteria: 1000 CFU/m^3 Fungi: 100 CFU/m^3	Russian segment: Ecosphera kit (heterotrophic cell count); In-flight analysis	Every 90 days (Analysis results after 7 days)
		American segment: Microbial Air Sampler Kit (heterotrophic cell count); In-flight analysis	Every 90 days (Analysis results after 5 days)

WATER MONITORING



	Threshold	Monitoring strategy	Monitoring frequency
Water	Bacteria: 50 CFU/mL	Water Microbiology Kit (heterotrophic cell count); In-flight analysis.	Every 90 days (Russian segment; SRV-K, SVO-ZV, CWC) Every 30 days (American segment; WRS)

SURFACE MONITORING

	Threshold	Monitoring strategy	Monitoring frequency
Surface	Bacteria: 10000 CFU/ 100 cm^2 Fungi: 100 CFU/100 cm^2	Russian segment: Test Tube Kit for Microbiological Sampling (swabbing a 10 cm by 10 cm surface area); Post-flight analysis (heterotrophic cell count).	Sampling is done 1 to 2 days before completion of each mission. Samples return simultaneous with crew return.
		American segment: American Surface Sampler Kit (heterotrophic cell count); In-fight analysis.	Every 90 days (2 sites)