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Lessons from the human microbiome field: Pre-analytical variables for reliable non-human biobanking and research

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Introduction & Problem

Microbiome research increasingly relies on the availability of high-quality biobanked microbiome samples and metadata, as well on the reuse of samples, metadata and analysis data.
The pre-analytical phase is a vulnerable and decisive part of microbiome research, strongly affecting sample, data reliability and comparability, as well as sample and data reuse.

In the human microbiome field, the European "quality standard" CEN/TS 17626:2021 developed with contributions from SPIDIA4P and Medical University of Graz/BBMRI.at) provides established guidance for pre-analytical processes.
However, pre-analytical aspects in non-human microbiome biobanking and research (e.g. plant, soil, marine) remain often insufficiently addressed or underestimated.

Aim

The aim was to identify the pre-analytical workflows and define potential sample-type-specific pre-analytical variables and metadata for non-human sample types by transferring concepts from human microbiome standardization to environmental microbiome research.

Material & Methods

Within the EU-funded project MICROBE and its dedicated work package on "Standardization & Quality Control", we used CEN/TS 17626:2021 as a reference framework. A structured literature review and workshops involving non-human microbiome experts from the European Plant Science Organisation (EPSO) and/or MICROBE (for soil, plant, marine) were performed.

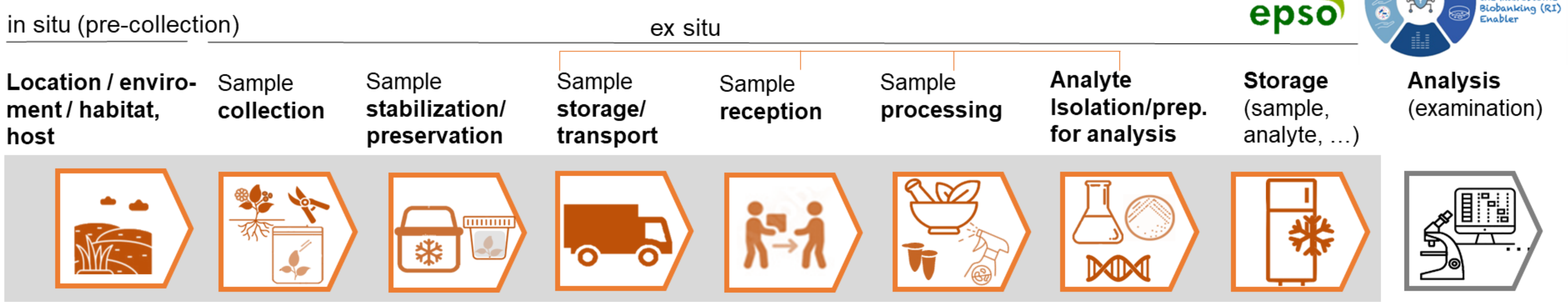
Results

- Pre-analytical workflows for plant, soil and marine microbiome samples were defined covering multiple steps from the in situ location to long-term storage.

- Across these steps, key variables were identified that can influence microbiome composition and biomolecule (e.g. DNA) profile and quality.

- Pre-analytics can profoundly distort microbiome data through microbial shifts, degradation, contamination, extraction bias, inhibition, and host DNA interference.

Examples of pre-analytical variables for plant, soil, marine water microbiome analyses



Major pre-analytical factors that can have a **dramatic effect on the analysis result**

Location/environment/habitat/host
- Geography (GPS)
- Climate
- Plant/field, water management
- Type of environment
- Former weather
- Plant genetics
- Plant developm. stage
- Plant health/disease
- Soil/water type
- Soil/water properties
- (Legal/regulatory), …

Collection
- C. site heterogeneity
- C. procedure/method/devices
- C. date/time
- C. container
- Labelling
- Plant compartments/soil/water depth -> heterogeneity
- Weather conditions during c.
- Water filtering (volume, µm)
- Water fraction separation
- …

Preservation
- Time-point (in field or lab)
- Method
- Reagents
- Temp.
- …

Storage/ transport
- Duration
- Condition (Temp., …)

Processing
- Sieving
- Homogenization
- Surface sterilization
- Filtration
- Aliquotting
- Pooling
- …

DNA extraction
- Method
- Kit/reagents
- Host cell lysis
- Microbiome lysis
- Inhibitors in sample
- Host DNA
- ..

- (Cross-)contamination

Color.code:
plant, soil, marine water-specific; for all,

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Major pre-analytic effects on microbiome data:
- Unintended microbial growth or loss
- Biomolecule degradation (e.g. DNA)
- Contamination
- Extraction bias (differential (lysis
- Inhibition of downstream reactions
- Reduced sequencing efficiency (host DNA)

Discussion & Conclusion

Systematic mapping of pre-analytical workflows and variables provides a foundation for harmonized procedures and standardized metadata collection in non-human microbiome research..

These are essential to reduce variability, improve reproducibility, and enable interoperable microbiome biobanking and research in a One Health context.

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