

The microbiome of a XIV century medieval codex: are microbes part of cultural heritage objects?



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We present a comprehensive study of the microbiome of a medieval codex of the XIV century held at the Leipzig University Library (UBL), Germany. The overarching research hypothesis proposes microbes as integral parts of cultural heritage objects and potential biographical elements, challenging the current conservation practice narrative which labels microbes as detrimental to the objects. Initial research outcomes and next steps are presented.

The Project: MIKROBIB

Contamination & Legibility of the World: Articulating Microbes in Collections
An Interdisciplinary Project across Life Sciences and Humanities



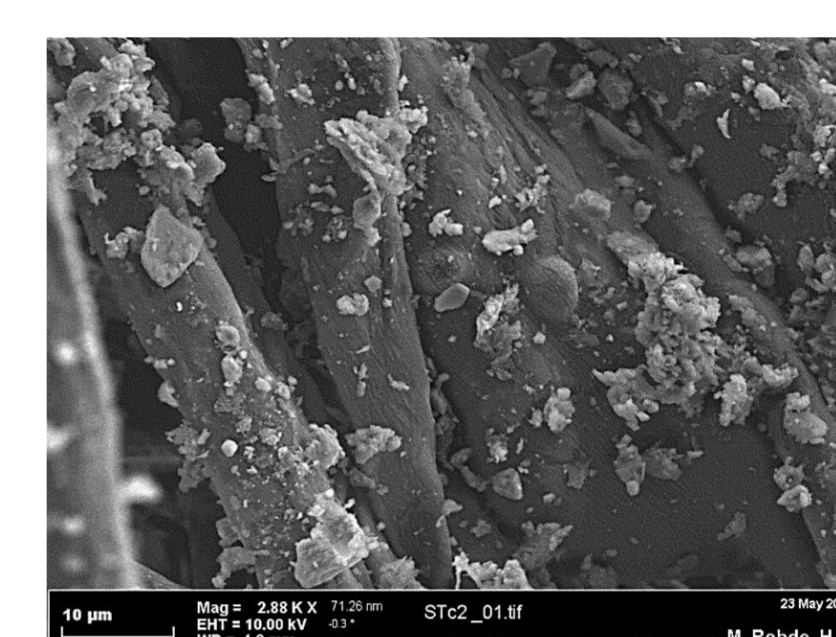
The Object Under Study : UBL MS 12



- Ms 12, XIV century Bible, parchment, wood.
- Chained book (public use), not digitized or studied
- Monasteries Leipzig area (Altzelle or Pegau)
- Custody at UB Leipzig after Reformation

The Challenge: Sampling Heritage Objects -touching the (almost) untouchable-

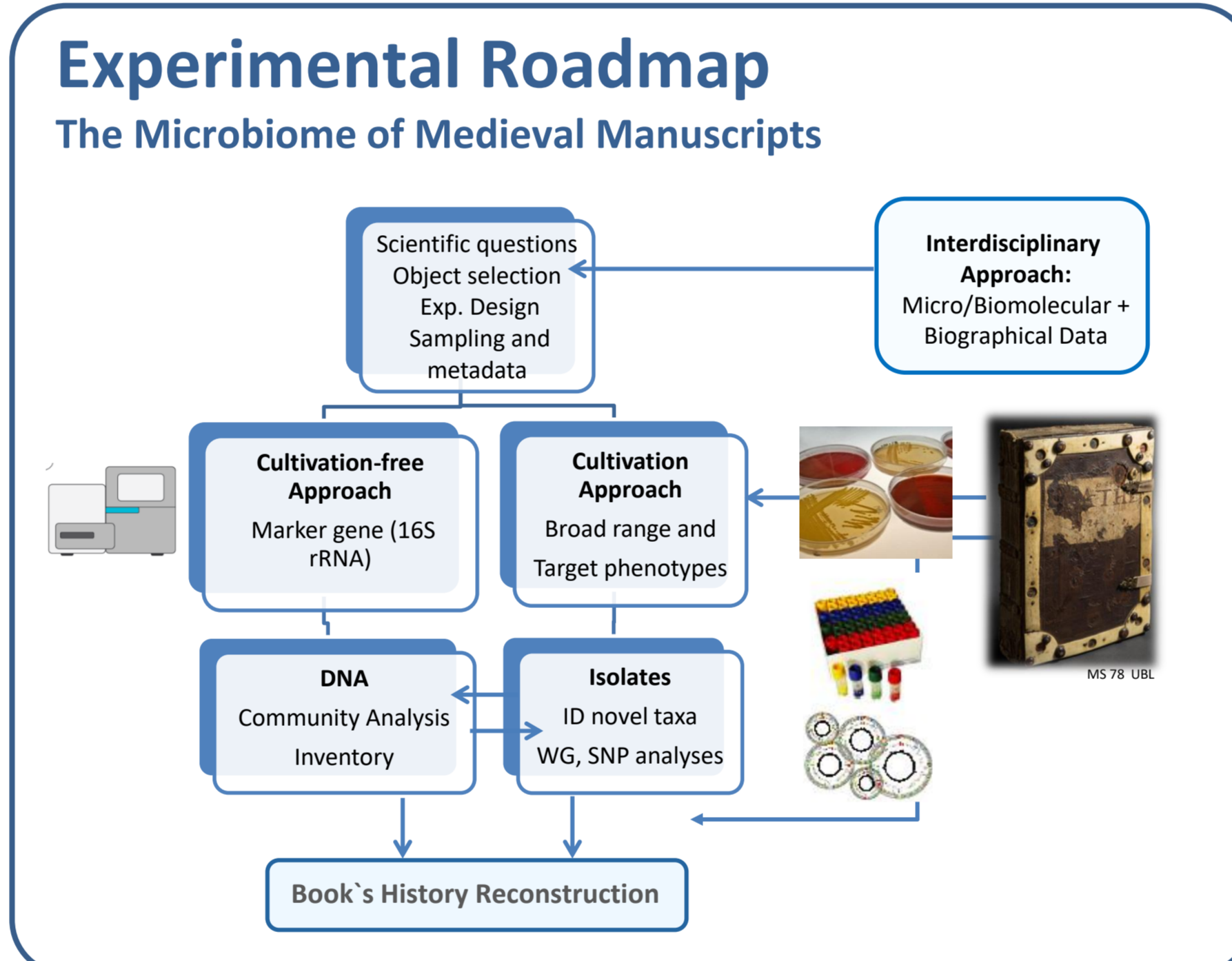
- sampling on site, easy implementation
- non destructive, minimally invasive
- Limited, restricted access
- unicates
- no re-sampling
- presence of inhibitors, interferences
- degraded biomolecules



Cultivation Independent Approach

Ancient books' DNA: low yield, mixed sources and quality

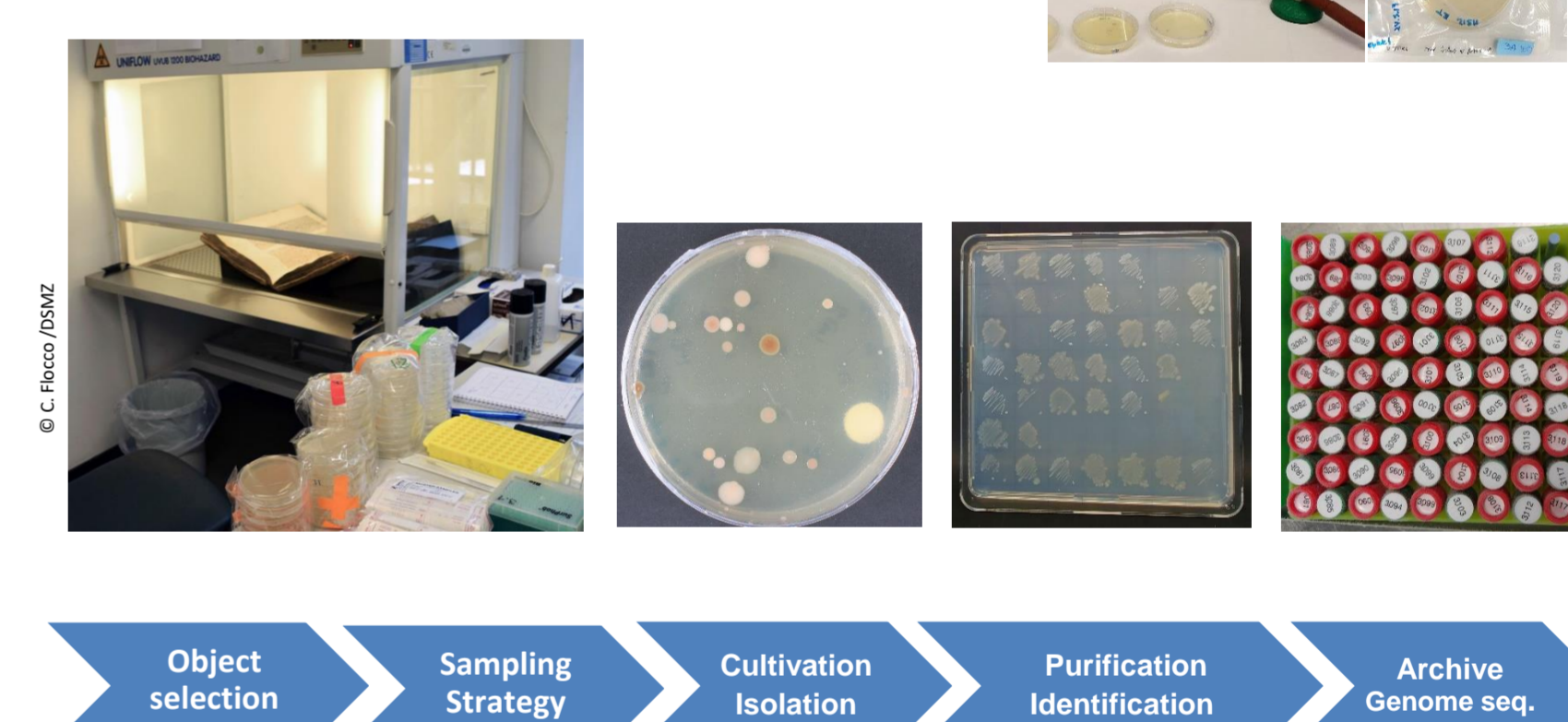
- **Source**
 - Endogenous
 - animal sources (parchment)
 - plant sources (paper, wood)
 - Exogenous
 - microbial
 - human (+ assoc. microbiome)
 - insect
- **Compartment**
 - intracellular (iDNA)
 - extracellular (eDNA)
- **Cells status (microbial)**
 - viable (incl. endospores)
 - dead
- **Age**
 - contemporaneous
 - ancient DNA (aDNA)



Culturomics Approach

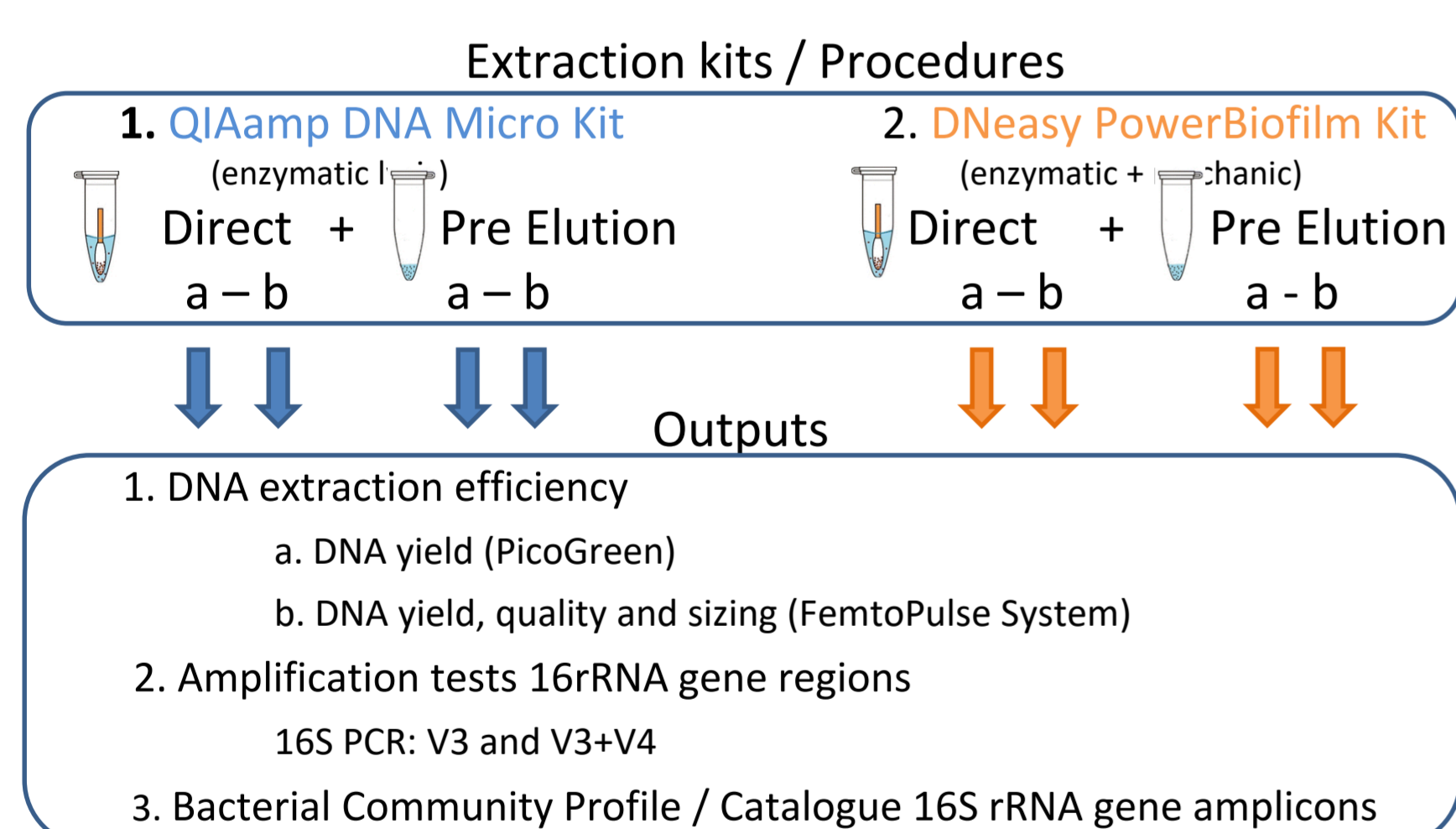
Large simultaneous cultivation set up

- Combination of cultivation media composition, atmosphere, cultivation devices, book areas and time
- Expanded to extreme conditions resembling parchment



WetLab Workflow Optimization

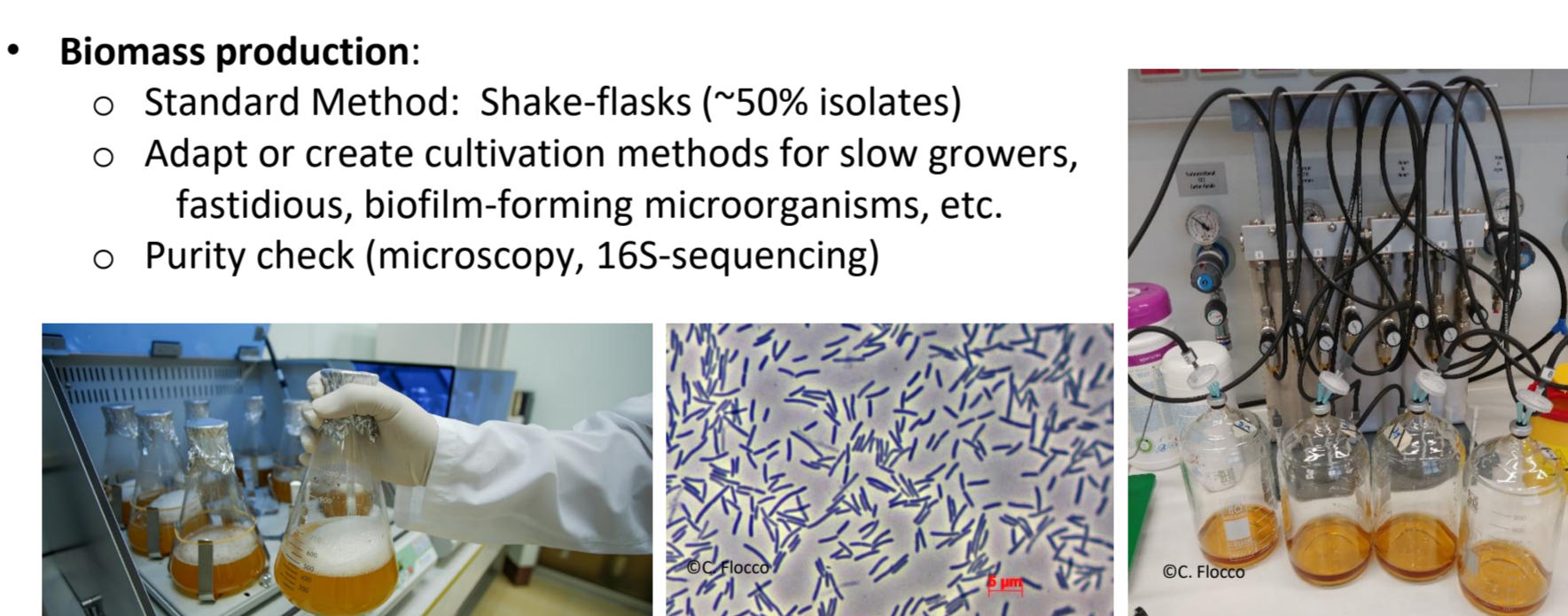
Microbial community analysis: 16S rRNA gene profiling



Whole Genome Sequencing

Isolation and genome analysis of selected bacteria from books

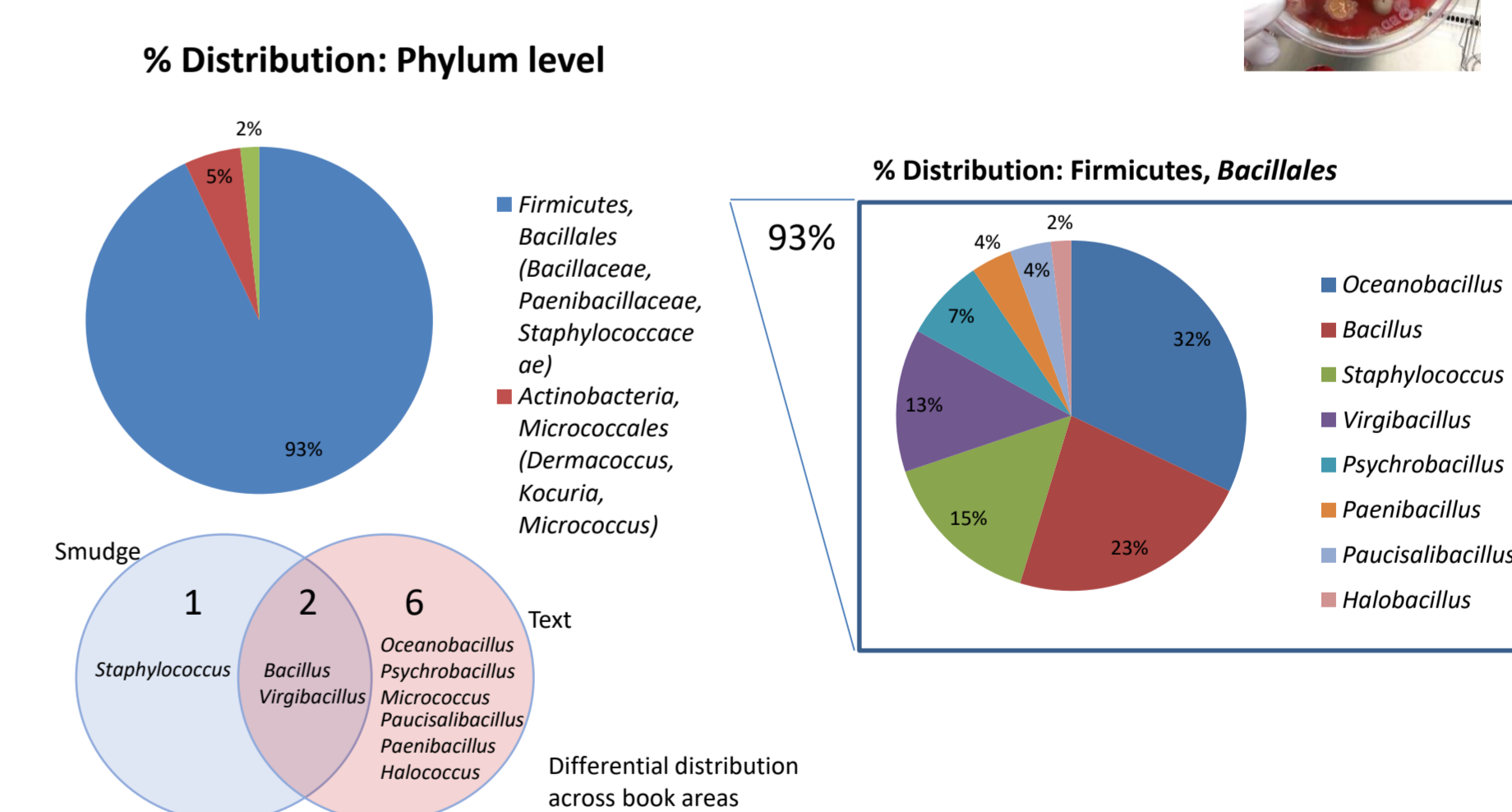
- **Selection:** ~30 representative isolates/novel species/medical-historical interest: Genera: *Bacillus*, *Virgibacillus*, *Staphylococcus*, *Paenibacillus*, *Halobacillus*, *Oceanobacillus*, *Sporosarcina*, *Streptomyces*, *Cutibacterium*, *Dermacoccus*



➔ Bioinformatics: phylogenomic and in depth genome analysis

Ms12 Isolates classification

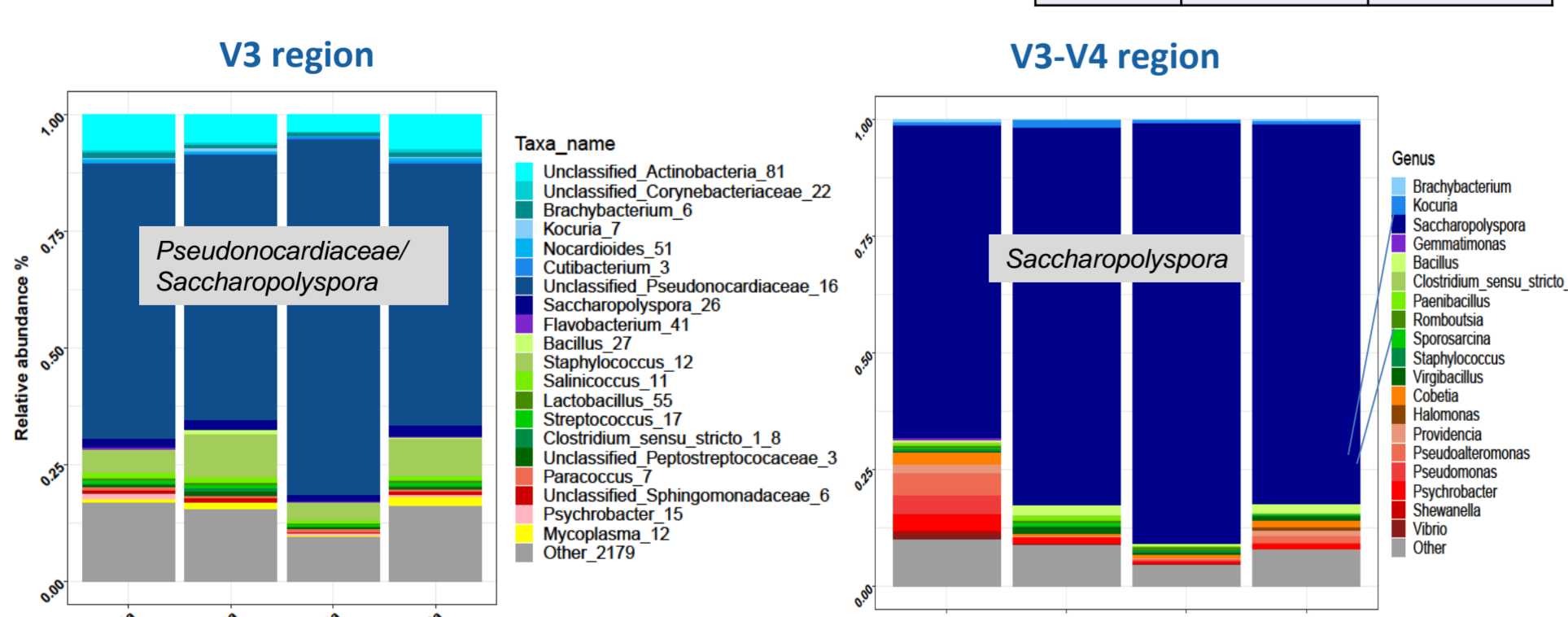
16s rRNA gene-based – representative subset



Microbial Community

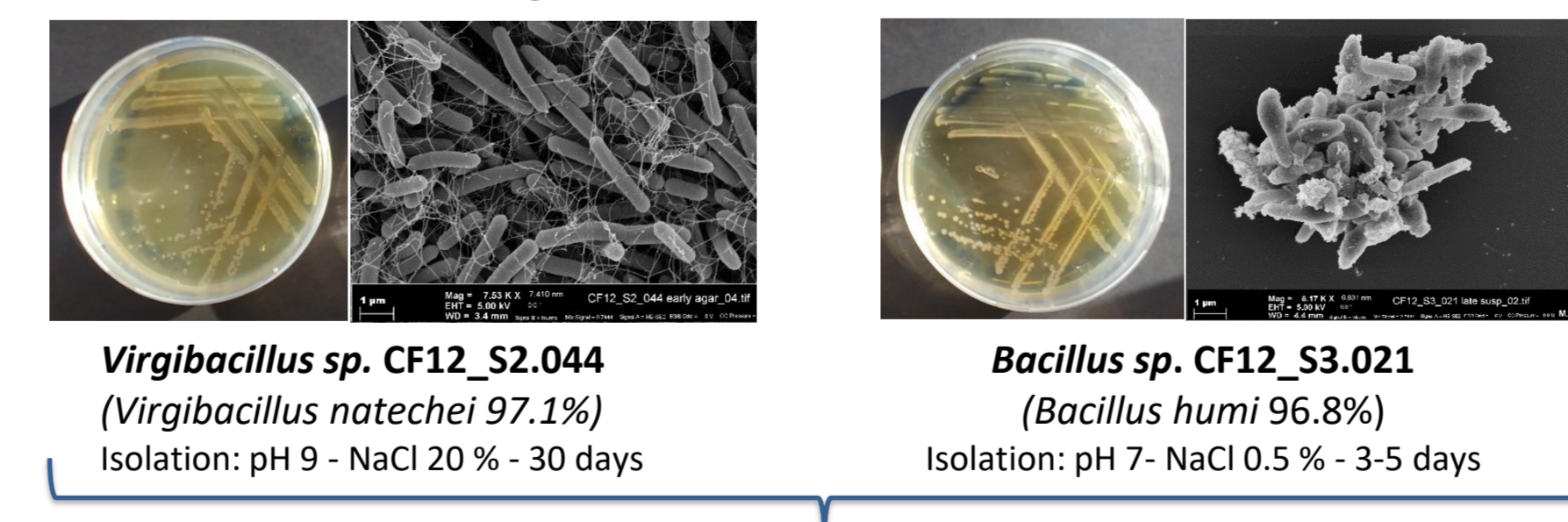
Comparison of 16S rRNA gene profiles

	MS12	QIAamp DNA Micro	DNeasy PowerBiofilm
PreElution		2. M1a	4. B2a
Direct		1. M13a	3. B13a



- Similar profile: extraction methods or previous steps not affecting
- Relatively low number of taxa
- Dominance of *Saccharopolyspora*, both HTS methods (16S rRNA copies: 5-16, most 12)

Habitat Adaptation and Novel Taxa



Virgibacillus sp. CF12_S2.044
(*Virgibacillus natechei* 97.1%)
Isolation: pH 9 - NaCl 20% - 30 days

Bacillus sp. CF12_S3.021
(*Bacillus humi* 96.8%)
Isolation: pH 7 - NaCl 0.5% - 3-5 days

Polyphasic approach

Macro: colony 2-3 mm, cream, round, raised, margin entire

Microscopy: G+ rods (single+chains), end. ST

Growth: 25°C (22-32) pH 8.6 (7-9) NaCl% 7.5 (5-15), aerobic, alkaliphilic halophile

Chemotax: PG: mesoDAPdir; MK:MK7 >> MK8

Genome: 3.9 Mbp -1chr -39.1 GC%, 3832 pcg

Macro: colony 2-3 mm, cream, round, raised, margin entire

Microscopy: G+ rods (single+chains), end.ST/T

Growth: 32°C (25-40) pH 7.5 (7-8.5) NaCl% 2 (1.5-2.5), aerobic, halotolerant

Chemotax: PG: A4B L-Orn (-D-Asp), MK: MK7

Genome: 4.4 Mbp, 1chr, 37.2 GC%, 4326 pcg

Summary and Next Steps

- Microbial community adapted to parchment habitat
 - Gram+, endospore/biofilm forming bacteria
 - Dry, low a_w environments
 - Survival on surfaces
 - Skin microbiome (animal, human) associated
- Cultivation approach
 - predominantly *Bacillaceae*
- Cultivation independent approach
 - predominantly Actinobacteria (*Pseudonocardia* / *Saccharopolyspora*).
- Novel taxa
 - potential biographical and biotechnol. value.
- Phylogenomics: (in progress)
 - molecular dating
 - evolutionary relationships
- New case study: Ms 11 (bound to Ms12)
 - Provenance revised, UBL (Altzelle / Buch?);
 - Benediktine Kloster Pegau
 - Abbatat Albert von Langendorf (1311-48)
 - Illustration style: hints to early period
- Ms12 + Ms11
 - 2 volume-Bible, early XIV. Cent.
 - Comparative Biomolecular Analyses
 - Expand to other Mss (materiality, use)
 - Work in progress...