Culture collections on the intersection of microbiome and material research: *What can be achieved?*

Dennis Enning

Berliner Hochschule für Technik

Anna A. Gorbushina

Bundesanstalt für Materialforschung und –prüfung & Freie Universität Berlin

Let’s start with a curious question…

The entire *biosphere* on Earth is how much bigger (by mass) than the planet’s *technosphere*?

A) 10.000 times bigger

B) 1.000 times bigger

C) 100 times bigger

D) 10 times bigger

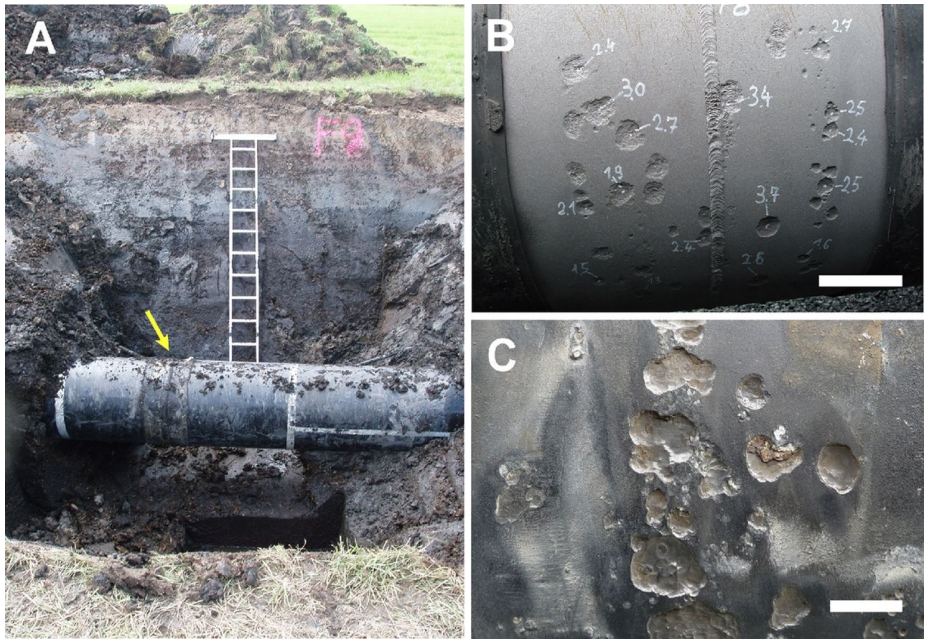
E) They are about the same

* Elhacham *et al.* (2020). *Nature* 588, 442 - 444


© 123RF

Microbial impact on human-made materials (examples)

Oil and gas pipelines (corrosion)



Enning and Garrelfs (2014). *AEM* 80, 4

3.5 million kilometers

MIC: >$10B/yr in economic loss

Solar panels (fouling / soiling)

About 5.000 km^2

Up to 10% loss in efficiency

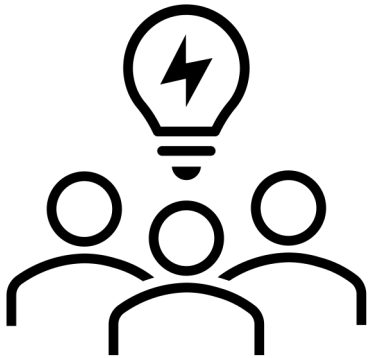
Goals of this talk

Propose concept and merits of **reference organisms**

- Two examples with relevance to energy infrastructure

Spark discussion on a **culture collection**

- Organizational considerations

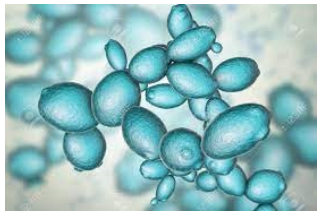


Reference (micro)organisms ≠ Model (micro)organisms

Model organisms

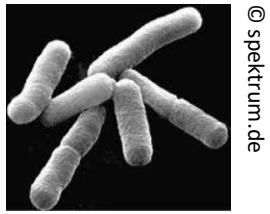
Mus musculus

D. melanogaster



© 123RF

S. cerevisiae



© spektrum.de

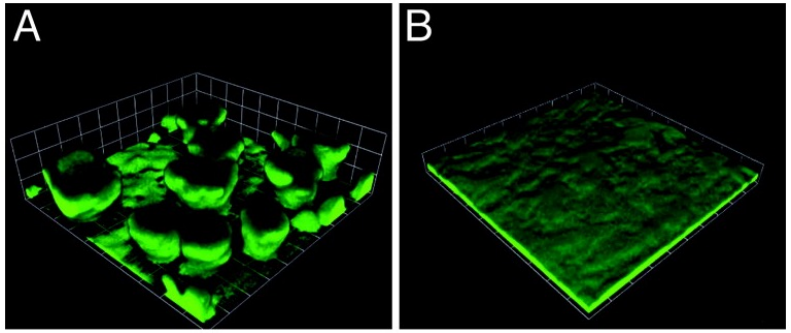
E. coli

Reference organisms

- Terminology is adapted from material science (‘reference materials’)
- Organisms with proven and **relevant impact on materials**
- **Consolidated research focus** on few relevant microorganisms (less ‘scatter’)

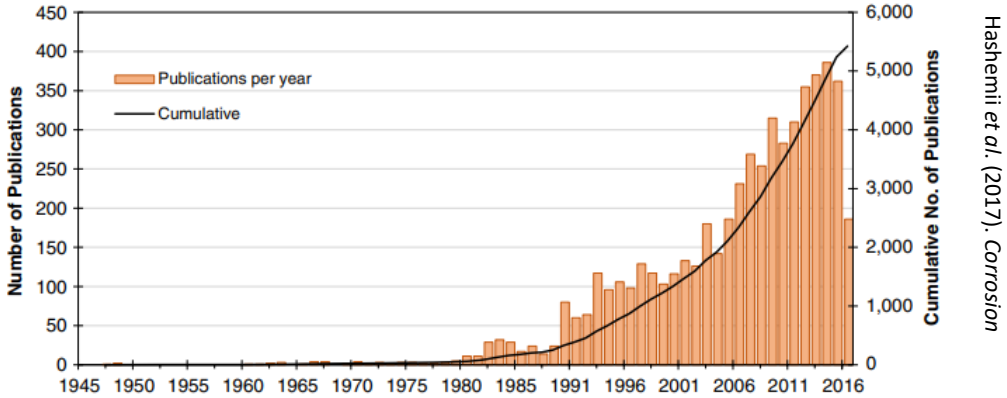
Step back: *In the study of material-microbe interactions, more focus on fewer organisms is needed*

Pseudomonas aeruginosa biofilm



Banin *et al.* (2005). *PNAS*

Publications on microbial corrosion



Hashemii *et al.* (2017). *Corrosion*

Identified issues

- Many of the pure cultures studied are irrelevant from an applied perspective (not representative, do not cause technically relevant damage,…)
- Large majority of studies are purely descriptive (and repetitive)
- Hundreds of organisms studied, rather than focus on a few relevant ones (breadth instead of depth)

Step back: *Why not study the biodeterioration and biodegradation entirely through meta-omics approaches?*

Meta(x)omics approaches

- Study process in actual or closely simulated environment
- Determine (potentially) all microorganisms present, along with their metabolic potential and actual activity

Advantage of pure culture work

- Reproducible laboratory simulation and testing
    - Quantification of process rates, test material susceptibility, develop mitigation technologies
- Gain mechanistic understanding
    - Develop monitoring technologies

Step back: *Why not study the biodeterioration and biodegradation entirely through meta-omics approaches?*

Meta(x)omics approaches

- Study process in actual or closely simulated environment
- Determine (potentially) all microorganisms present, along with their metabolic potential and actual activity

Advantage of pure culture work

- Reproducible laboratory simulation and testing
  - Quantification of process rates, test material susceptibility, develop mitigation technologies
- Gain mechanistic understanding
  - Develop monitoring technologies

Which microorganisms would be ‘ennobled’ as refence organisms?

Selection criteria (similar to *Koch’s postulates*)

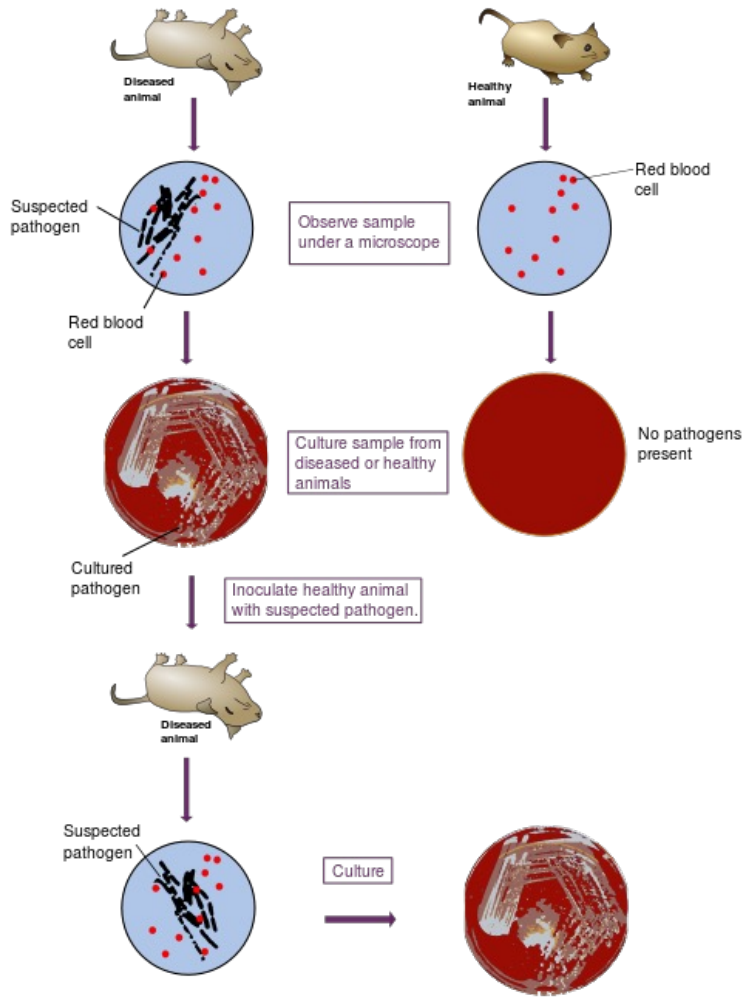
- Present at the site of damage
- Capable of causing damage of the material to a technically relevant extent

Selection mechanism

- Formal process of granting status (more later)

Benefits (reminder)

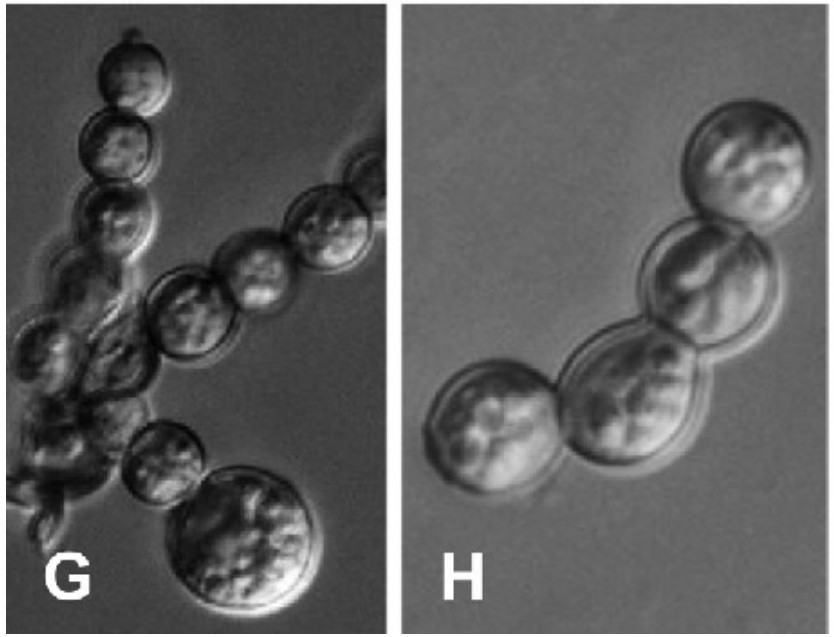
- Focus research on fewer (and relevant!) organisms
- Accelerate generation of <u>applicable</u> discoveries (and technologies)
- Guide to industry (and academia) for strain selection in applied research



Wikimedia.org

Two (proposed) reference organisms

Weathering and soiling of silicon

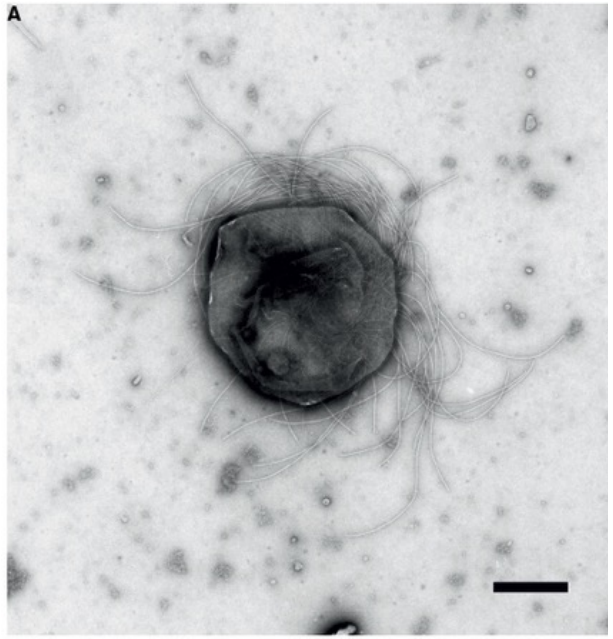


Nai *et al.* (2013). *Fungal Genetics and Biology*

Knufia petricula

Strain A95

Corrosion of carbon steel

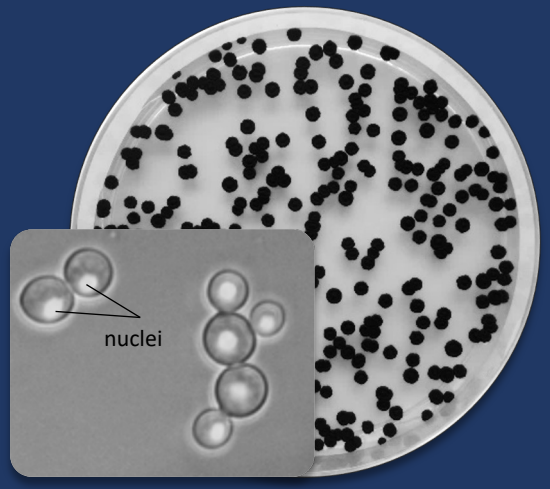


Albers and Jarrell (2015). *Frontiers in Microbiology*

Methanococcus maripaludis

Strain OS7

Weathering & soiling of silicon



Nai *et al.* (2013). *Fungal Genetics and Biology*

Knufia petricola

strain A95

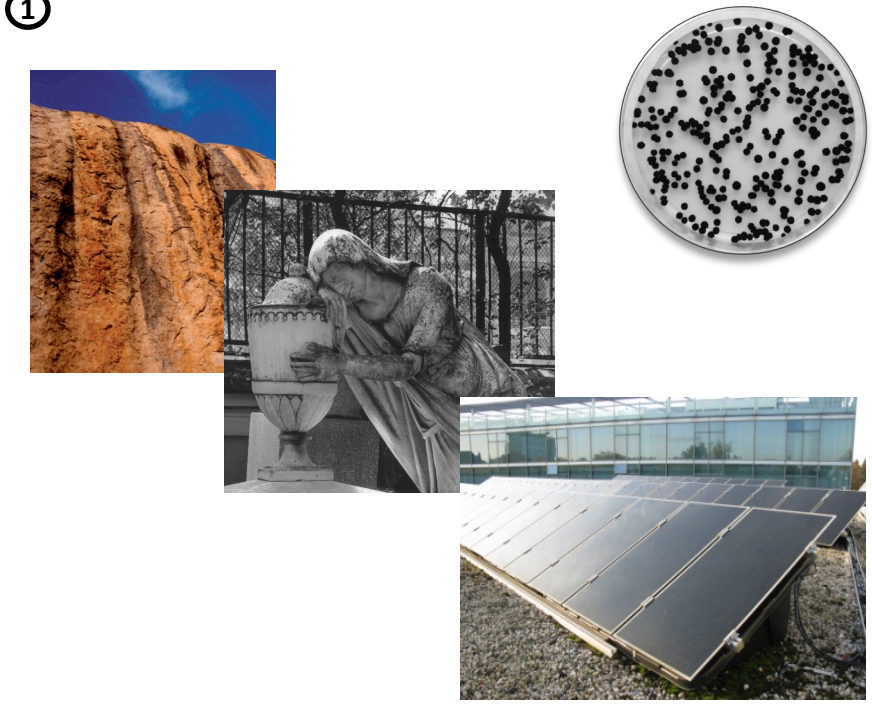
K. petricola (strain A95) – A ‘reference organisms’ in the weathering and soiling of silicates

Selection criteria (similar to *Koch’s postulates*)

① Present at the site of damage

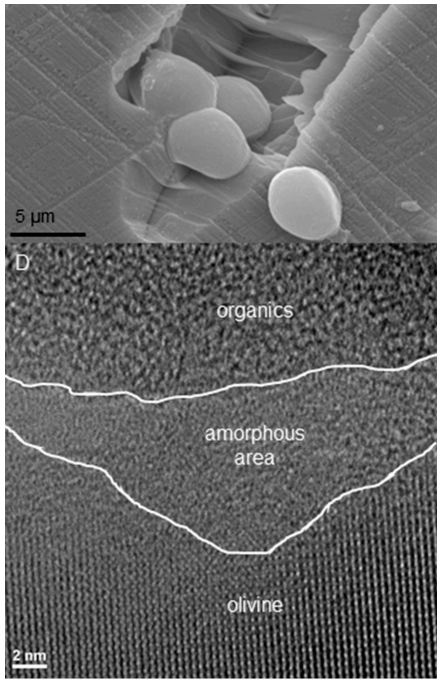
② Capable of causing damage of the material to a technically relevant extent

①

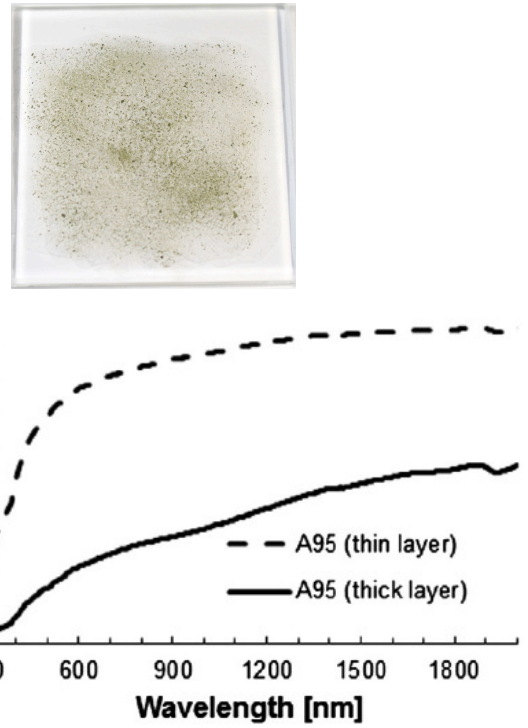


Gorbushina & Broughton (2009). *Ann. Review Microbiology,*
Noack-Schönmann *et al.* (2014) *Int. Biodeterioration & Biodegradation*

②



Gerrits *et al.* (2021). *Chemical Geology*



Noack-Schönmann *et al.* (2014)
Int. Biodeterioration & Biodegradation

K. petricola (strain A95) – A ‘reference organisms’ in the weathering and soiling of silicates

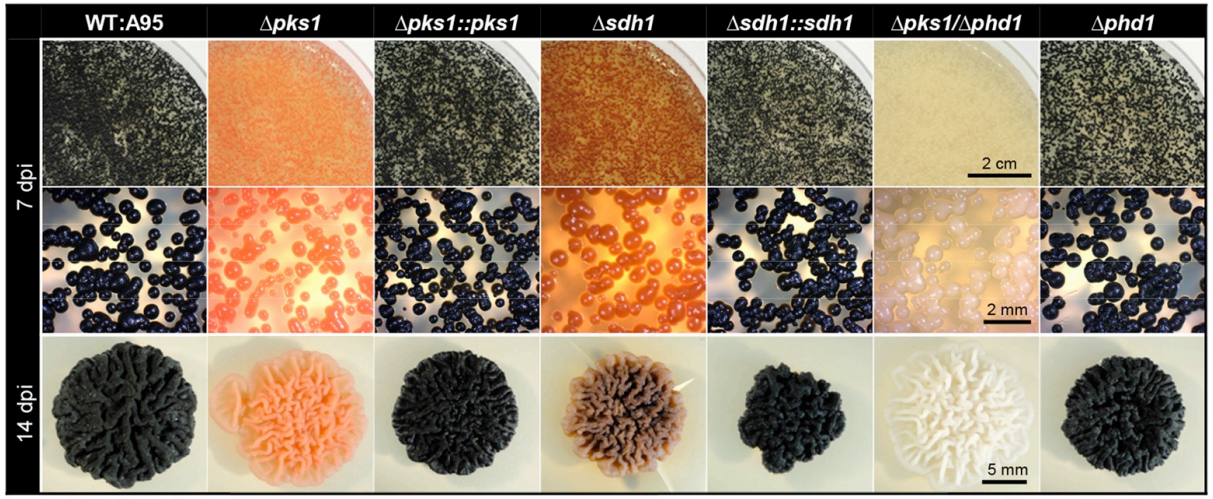
Special traits of the organism:

Comparatively decent **growth rates** in axenic culture and **genetic amenability**

Insights and achievements through a focus on <u>one</u> organism (10+ years)

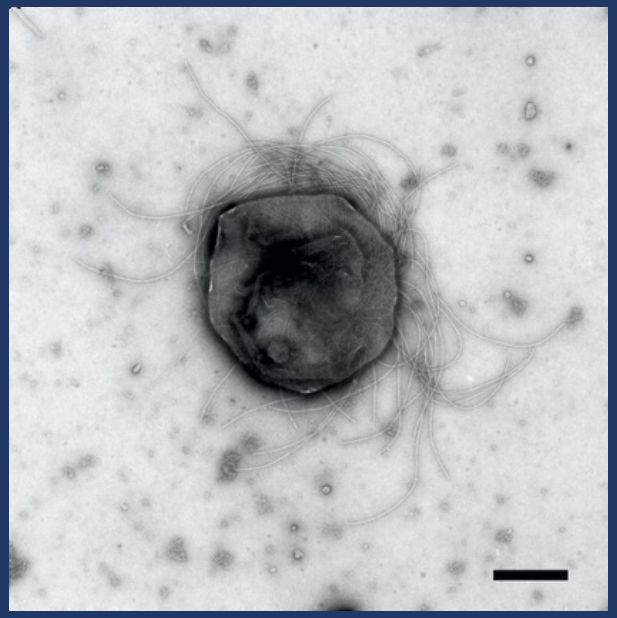
- Relevance demonstrated and quantified
- Genetic system established:
  - High quality genome sequence
  - 2 genomic loci for neutral insertion
  - 3 genomic loci for insertion w/ color-based screening
  - CRISPR/Cas9 (genome editing)
  - …
- In-depth mechanistic studies possible

Genome-editing of *K. petricola* (e.g. pigment synthesis)



Noack-Schönmann et al. 2014, AMB Express
Voigt, Knabe et al. 2020, Sci Rep
Erdmann et al. 2022, *Front Fungal Biol*

Corrosion of carbon steel



Albers and Jarrell (2015). *Frontiers in Microbiology*

Methanococcus maripaludis

(strain OS7)

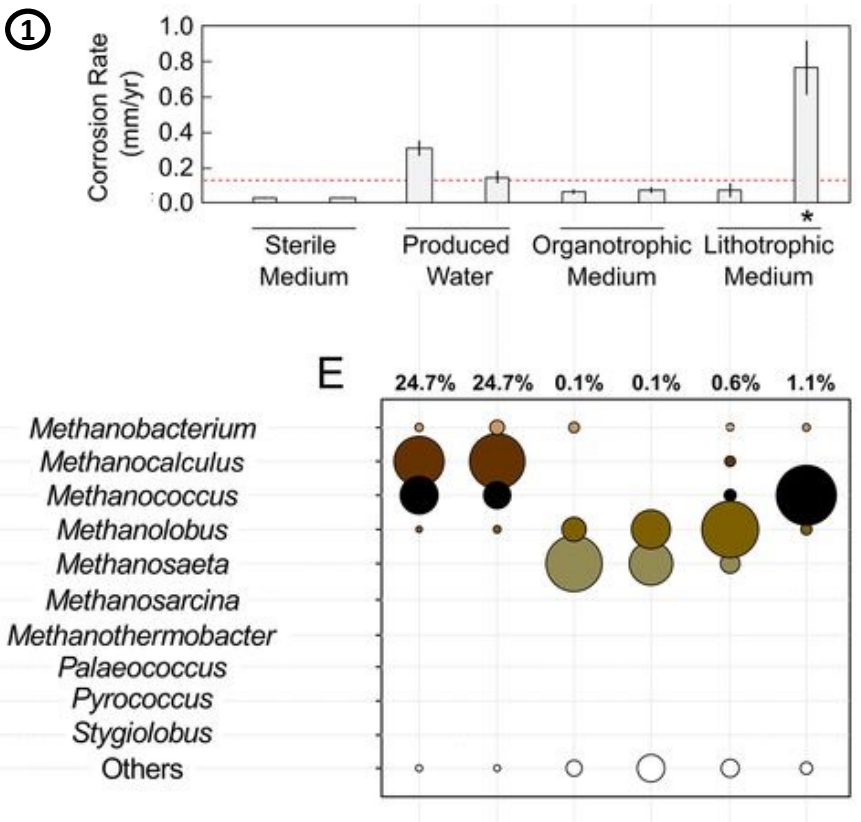
M. maripaludis (strain OS7) – A ‘reference organism’ in the corrosion of steel

Selection criteria (similar to *Koch’s postulates*)

① Present at the site of damage

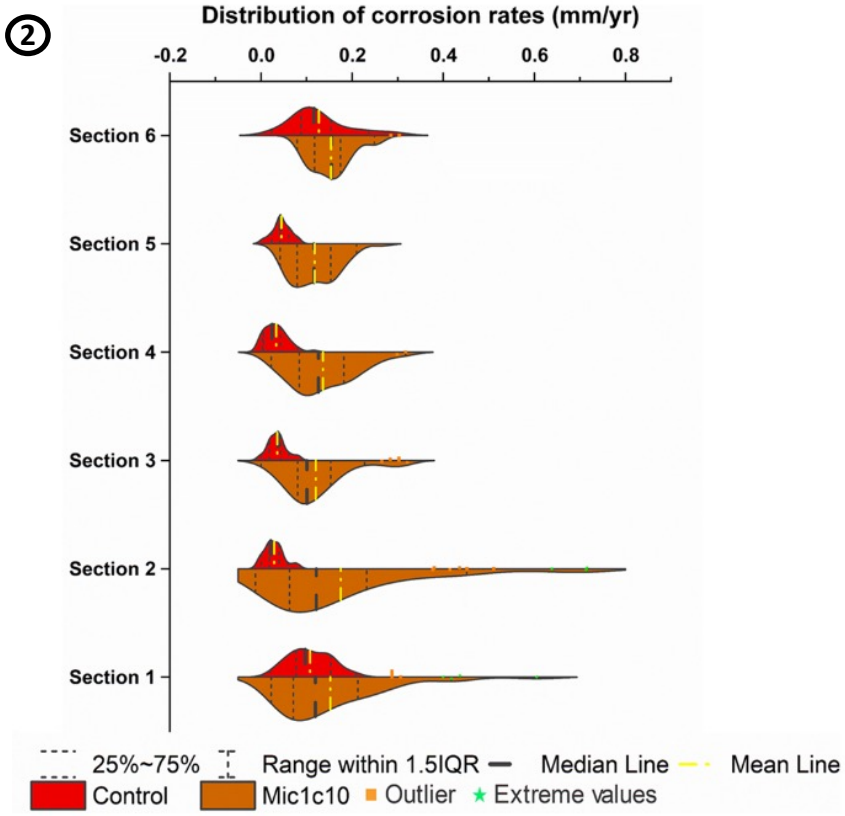
② Capable of causing damage of the material to a technically relevant extent

①



Lahme *et al.* (2021). *AEM*

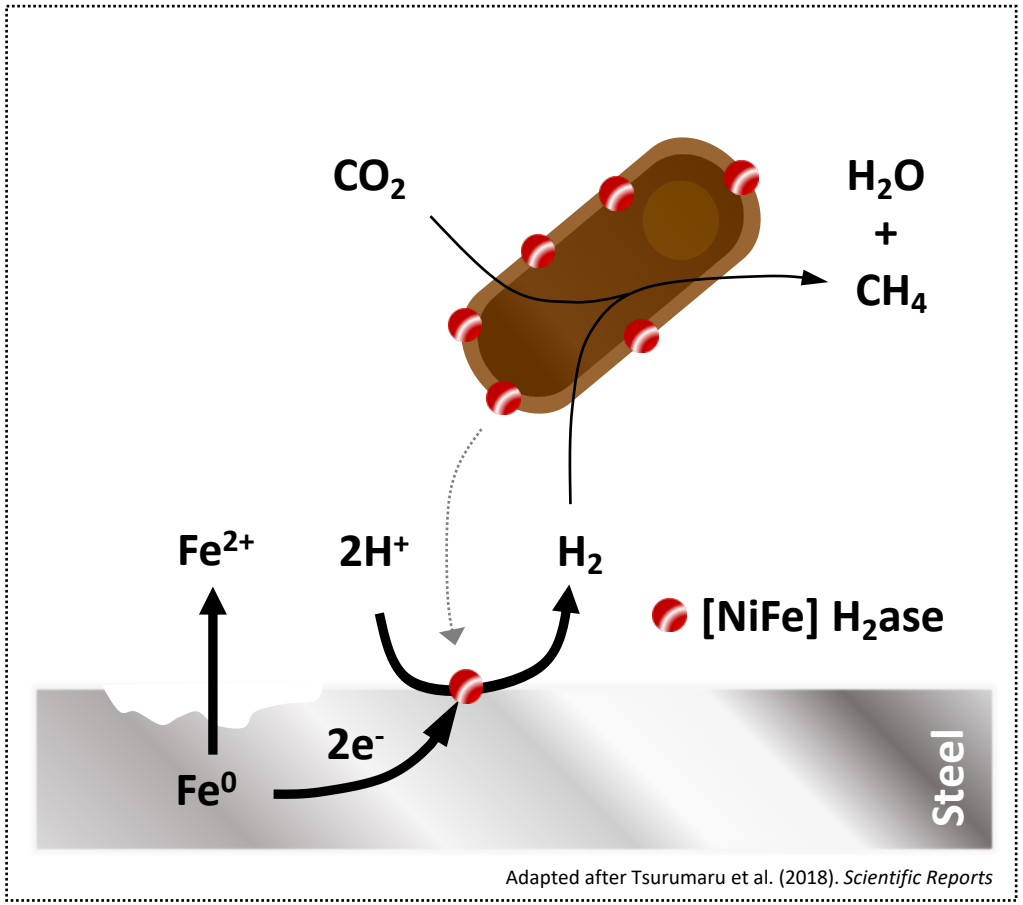
②



An *et al.* (2021). *Corrosion Science*

M. maripaludis (strain OS7) – A ‘reference organism’ in the corrosion of steel

Molecular mechanisms of corrosion resolved



Adapted after Tsurumaru et al. (2018). *Scientific Reports*

Insights through a focus on <u>one</u> organism

- Relevance demonstrated and quantified
- Mechanistic understanding of microbe-metal interaction gained

Real world merits (for industry)**:** Development of a diagnostic tool (qPCR) to detect steel-degrading methanogens in pipelines

qPCR assay for *micH:*

vs.

Corrosive methanogens

Harmless methanogens

see Lahme *et al.* (2021). *AEM*

Companies commercially offering the *micH* assay

The two first candidates for the proposed culture collection?

RO Name: *Knufia petricola* isolate A95

Is the cause of the following biodeterioration phenomenon/process: dissolution of minerals, fouling of air-exposed materials

Relevant to the following material: rocks, solar panels, material surfaces, exposed to the atmosphere

Can biodeterioration effect/colonisation of the material surface by this RO be quantified: yes (CFU and qPCR)

If yes, please, name the method(s): colonization/biomass can be measured with qPCR and biodeterioration can be analyzed with (i) leachate chemistry via ICP-OES/MS (inductively coupled plasma optical emission spectrometry/mass spectrometry) and via SEM/TEM-EDX (scanning electron microscopy/transmission electron microscopy-energy dispersive X-ray spectrometry)

Most prominent under following climatic conditions: TBD

Biofilm former: forms SABs in association with algae, cyanobacteria, other fungi and heterotrophic bacteria

Pure culture available: yes

Frequency of occurrence: Sub-aerial biofilms (SAB) are ubiquitous, found on air-exposed surfaces at all altitudes and latitudes

How many isolates are represented by this particular strain: a rock-inhabiting clade in *Chaetothyriales*

How high is the growth rate/generation time: 17h in MEB

Genetically amenable: yes

Full genome sequence available: yes

Date of discovery (since when is known to science as the cause of biodeterioration): 1996

Original references: ….

…

Knufia petricola strain A95

M. maripaludis strain OS7

A collection of reference organisms: *What can be achieved?*

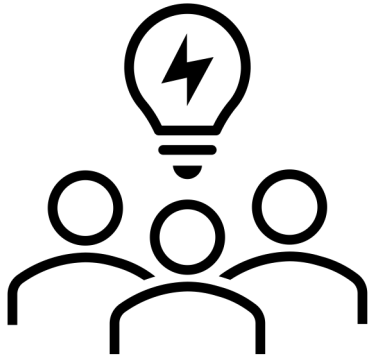
Small and specialized collection

- An organism's inclusion into the collection grants a 'special status' to the organism
  (at least in the context of material science, biodeterioration and biodegradation)

Mechanism for strain inclusion (the 'gateway')

- Generally, cause and effect must be demonstrated, along with technical relevance
- Specific selection mechanism to be determined, e.g.
  - Demonstration of presence in the affected environment (published in peer-reviewed journal)
  - Demonstration of ability to cause technically relevant damage (corrosion, fouling, …) under field-simulating conditions (published in peer-reviewed journal)
  - Three or more publications in total on the organisms from at least two different labs
  - International (and distinguished) selection committee

Merits and limits of such a strain collection?



Discussion (very) welcome!

Thank you for your attention

BHT Berliner Hochschule für Technik

BAM Bundesanstalt für Materialforschung und -prüfung

Back-up

Optimized protocols for
PEG/Ca^{2+}-mediated transformation of protoplasts

Transient CRISPR/Cas9
e.g. AMA plasmid-based with
ribozyme- and tRNA-sgRNA cassettes

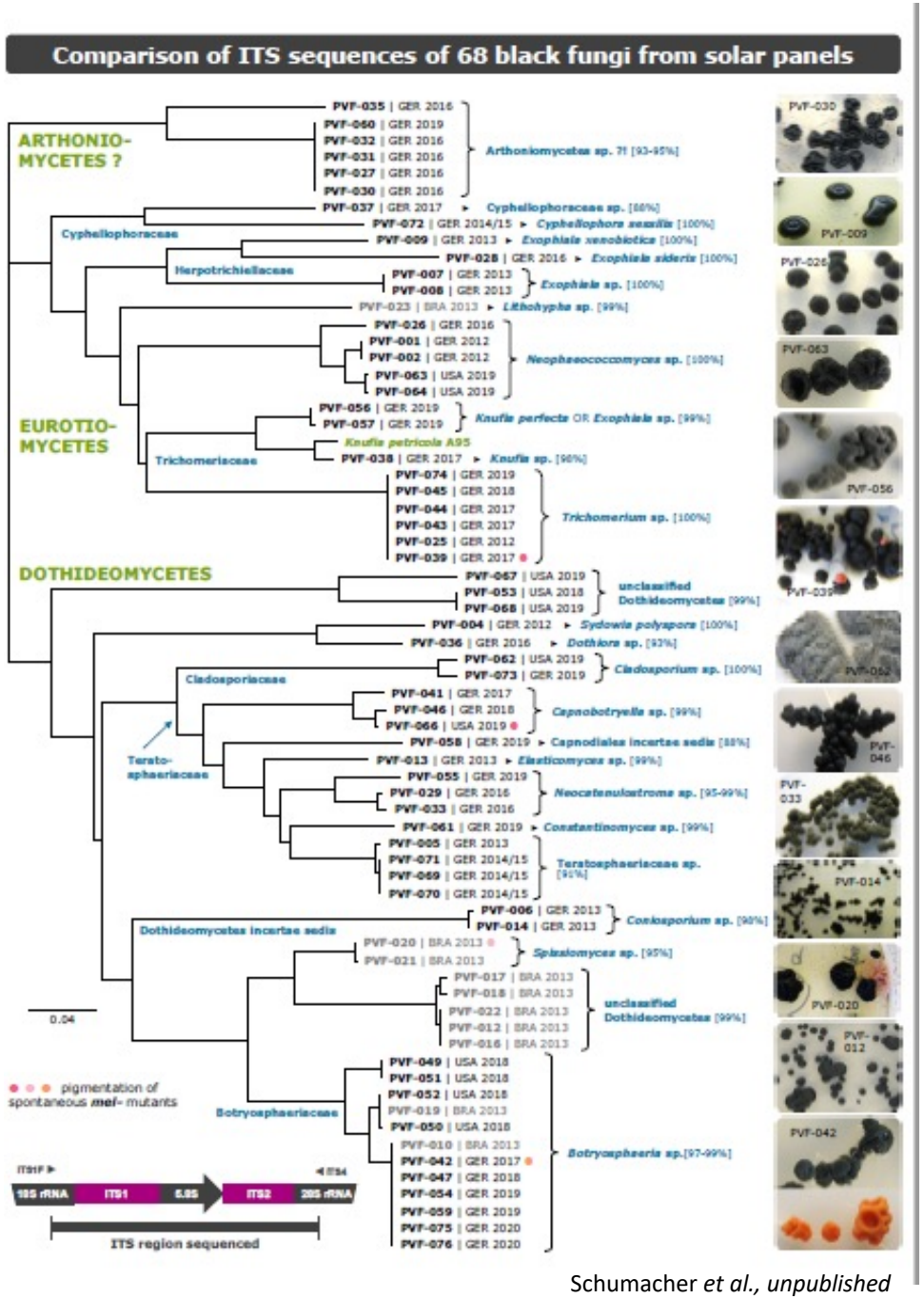
3 genomic loci for insertion
w/ **color-based screening**

2 genomic loci for neutral insertion (*intergenic region 1/2*)

5 selection marker systems
(hygR, natR, genR, baR, suR)

Modular cloning vectors compatible
with yeast recombination cloning &
NEBuilder HiFi DNA assembly

Genome sequence of A95
13 contigs (8 chromosomes + mtDNA)
28.1 Mb ▶ ~9.800 genes



Schumacher *et al., unpublished*