Impact of the microbial community on mycotoxin production during grain storage in soil-dug silos

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Archaeology & Biology:

Impact of the microbial community on mycotoxin production during grain storage in soil-dug silos

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Experimental archaeology: Recreating underground silos to rediscover ancestral practices and evaluate the risks of stock deteriorations in these pre-industrial grain storage facilities commonly found in archaeological excavations [SilArchaeoBio]

Experimental underground silos were dug using ancestral tools by a team of archaeologists from Inrap and CNRS and biologists from INRAE, across two sites in the Perpignan region, and used to store different cereals (einkorn, barley and wheat). In 2022, a straw lining was added in some pits to absorb infiltrated water and create a barrier to protect the grain. This technique was tested as a way to preserve the quality of the grains recovered after 8 months of underground storage (Figure 1).

Where straw lining was present, the straw was showing obvious signs of fungal rot, but the grain located directly next to it looked well preserved. On the contrary, when grain was stored directly against the soil, a clear layer of moldy grain (2 to 7 cm) appeared, especially in the shoulder area below the chimney (Figure 2).

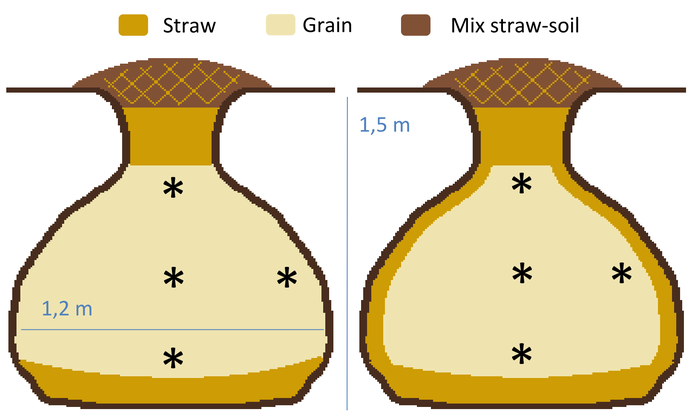

Figure 1: Experimental silos set-up, with different straw linings

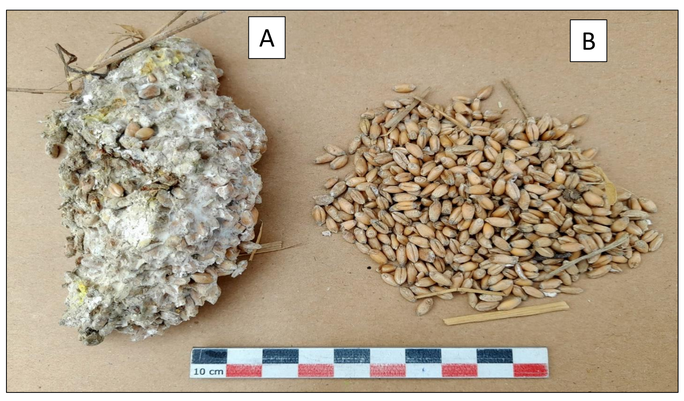

Figure 2: Wheat stored in silo without straw lining; (A) grain located directly against the soil wall; (B) grain from the centre of the pit

Grain located in different areas of the silo was sampled (*), DNA of the epiphyte microbial community was extracted and qPCR analysis were processed. Apart from one particularly damaged sample, all 16S results were similar to the initial grain stock. Bacterial community did not take over in the underground silo storing conditions. On the other hand, the ITS qPCR results (Figure 3) confirm the presence of fungi in the moldiest grain samples, especially located on the edges and bottom of the pits. Metabarcoding analyses of these samples are being processed.

Aflatoxins and Ochratoxin A were quantified in some samples, to evaluate the contamination of the grain by mycotoxins. An example is presented in Figure 4, focusing on Silo 8, where the difference between the sample locations in the pit impacted greatly on mycotoxins production. The most humid regions of the silo (edges and bottom) favoured the accumulation of mycotoxins. The centre of the underground silo was also contaminated by both Aflatoxins and Ochratoxin A, but at concentrations lower than 5.0 µg/Kg.

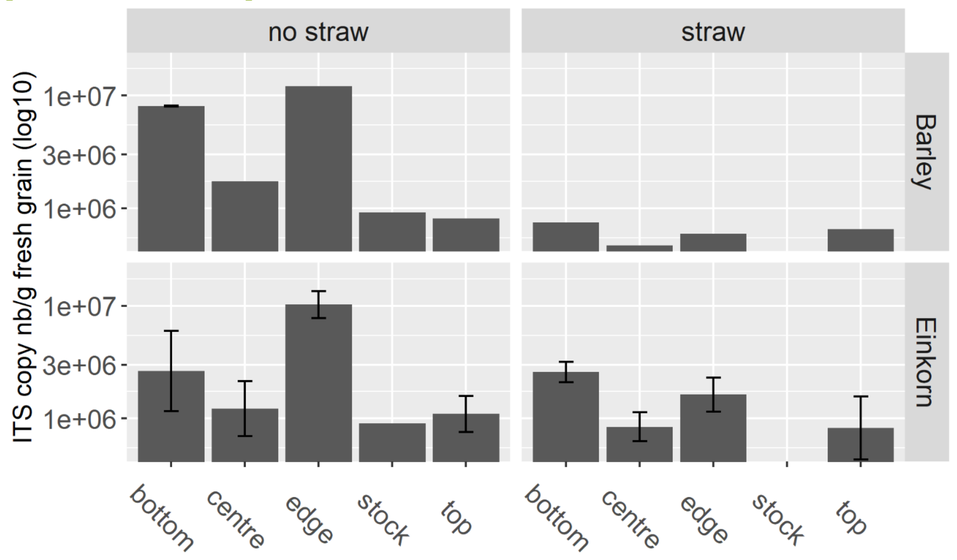

Figure 3: ITS copy number per gram of fresh grain, across the different silos, in samples located in distinct regions of the silo

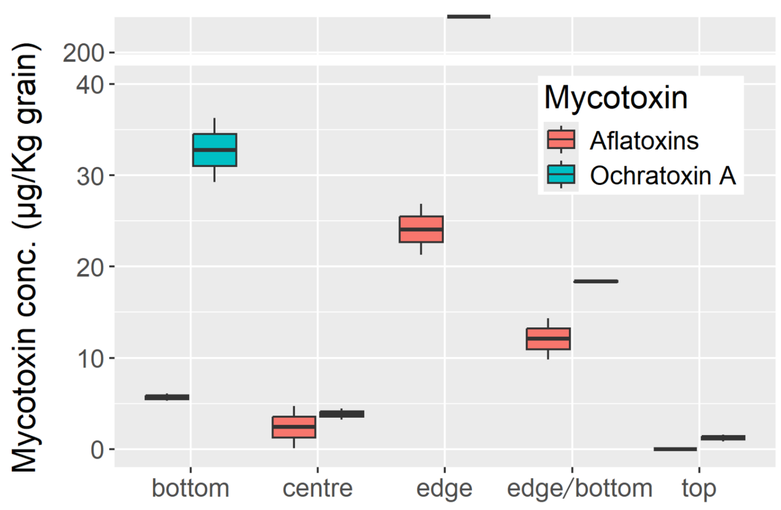

Figure 4: Mycotoxins concentrations in Silo 8, quantified with Ridascreen kits specific to Ochratoxin A and Aflatoxins.

Laboratory microcosms: storage conditions and kinetics of microbial colonisation; mycotoxin contamination of grains in underground silos [SilArchaeoBio]

The underground silos were monitored with temperature and relative humidity probes across 8 months storage. From this data, two temperatures and two humidity conditions were chosen (1st and 3rd quartiles) to conduct controlled-environment experiments with three cereal species. These different cereals were incubated in sealed containers with a set temperature of 15°C or 23°C and an initial volume of added water (6 or 12 ml), and kept up to 1.5 months. By using sacrificial samples, we can follow the proliferation of microbial communities as the grain accumulates water content, see Figure 5.

During these microcosm experiments, culturable bacterial and fungal isolates were grown on PDA to assess a non-exhaustive microbial diversity. A phylogenetic tree of the microbes isolated so far (23°C only) is displayed in Figure 6. Further qPCR analysis will allow a precise evaluation of the bacteria and fungi communities, and mycotoxins quantification will provide essential information as to which humidity / temperature conditions trigger the production of these metabolites in different cereal species.

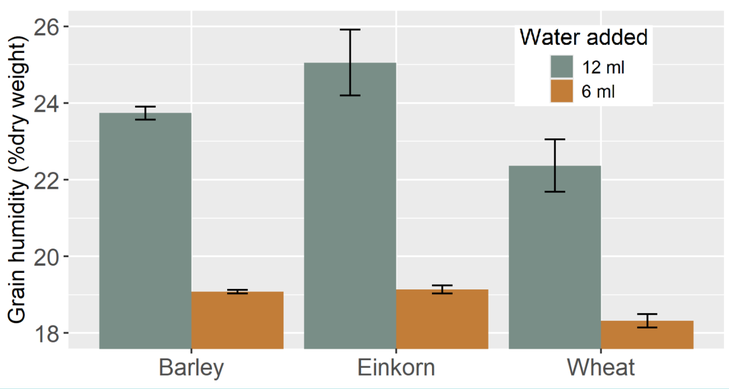

Figure 5: Grain water content after 14 days incubation in microcosms

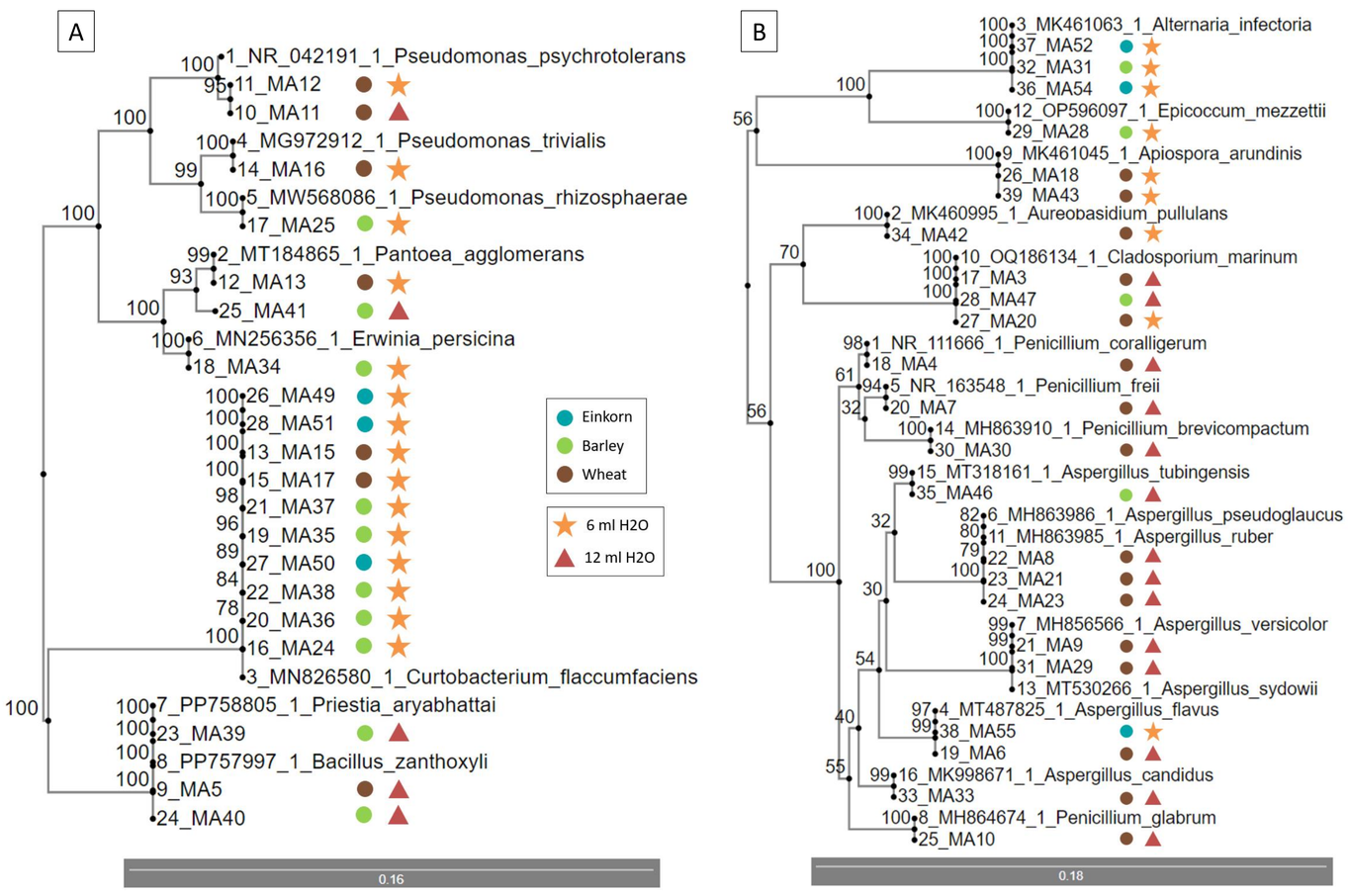

Figure 6: Phylogenetic trees of the bacterial (A) and fungal (B) isolates from the 23°C experiments and their isolation origin. MAFFT alignment, NJ method, Jukes-Cantor model and 1000 bootstraps tree.

Perspectives: How to use this knowledge to improve grain storage nowadays while reducing its carbon footprint [LocaStock]

→ The multidisciplinary research project SilArchaeoBio produces (1) know-how and data helping archaeologists to understand how the underground silos were used by ancient populations and their putative role in local economy, and (2) improved knowledge on the cereal grain storage ecosystem for ancient cereal varieties used in organic agriculture.
→ Capitalizing on this knowledge and expertise, we aim to adapt them to current technologies to develop contemporary underground silos as a resilient alternative to energy-dependant storage facilities. Organic farmers, citizens and scientists work together in the LocaStock project to imagine, experiment and disseminate innovative solutions for storing grains. This participative science project challenges the development of a local, safe, and environment-friendly way to preserve harvests.

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Project SilArchaeoBio and experimental archaeology in a video

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