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SEM AND FTIR ANALYSES COMBINED WITH SEQUENCE-BASED DIRECT IDENTIFICATION OF DETERIORATION-ASSOSIATED FUNGI ENHANCES IDENTIFICATION OF EXCAVATED 5TH C. BC PALAIOTEXTILE SAMPLES

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Introduction

Past analyses by German archaeologists (1969) of a 5th c. BC, excavated archaeotextile find, from grave 35 HTR 73, at the Kerameikos cemetery in Athens, Greece had indicated the presence of silk fibres.

AIMS

The aim of this study was to re-test remains of the same conserved textile find by environmental scanning electron microscopy (ESEM) and Fourier transform infrared (FTIR) analyses and to identify by PCR any possible implication of fungi, which may have caused misidentification of the textile fibre. Material evidence on the presence of silk in 5th c. BC Athens could thus be established or denied.

Methods

Microscopy

For the analyses the following equipment was used: an ESEM PHILIPS XL 30, where no sample preparation was necessary; an SEM JEOL JSM-5310, where the samples were coated with a gold-palladium alloy; an FTIR Perkin-Elmer attached to an Autoimage optical microscope, where all scans were run at reflection mode and no sample preparation was necessary; and an optical Nikon Eclipse 800 microscope.

PCR-sequencing

DNA was automatically extracted and the 28S region was partially amplified with the general primers NL-1 and 260R.[1] The PCR products were sequenced and the derived sequences were blasted to the GenBank.

Inoculation of textiles with fungal cultures

Multiple sterile silk, linen and cotton swatches were inoculated with *A. terreus* (strain UOA/HCPFEnv49; Genbank accession ITS no. JF509458; tubulin JF509463; calmodulin JF927640) recovered from an adjoining swatch of the same find. The same swatches were inoculated with common soil fungal saprophyte in our region such as *Scopulariopsis brevicaulis* and

Results

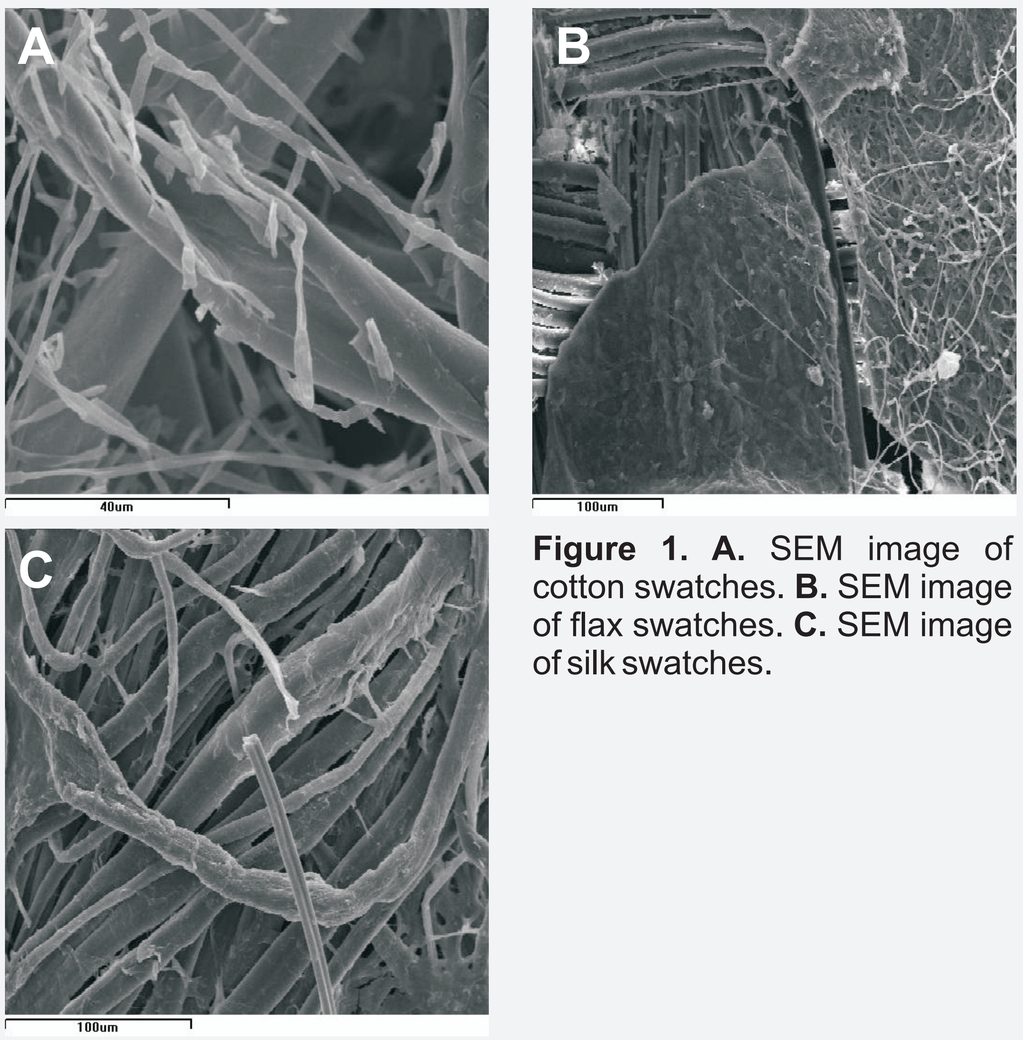

Figure 1. A. SEM image of cotton swatches. **B.** SEM image of flax swatches. **C.** SEM image of silk swatches.

References

1. T Vollmer, M Stormer, K Kleesiek, J Dreier. Evaluation of novel broad-range real-time PCR assay for rapid Detection of human pathogenic fungi in various clinical specimens. J Clin Microbiol 2008; 46:1919–1926.

Results

ESEM and FTIR analyses did not detect silk, while cellulosic bast fibres (eg flax) were positively detected. The presence of cotton was also suggested but the poor condition of the fibres denied definite identification. Moreover, ESEM analyses showed evidence of deterioration by microorgamisns, possibly degrading the morphology of the fibres thus affecting fibre identification. All cultures for bacterial cells or spores were negative. ESEM images showed profuse 1.5-2 micrometers sub-globose partially dehydrated fungal conidia-like structures covering the excavated find. Minute find swatches were subjected to ITS-based standard automated forensic PCR. Sequencing of the PCR products identified the cellulolytic fungus Aspergillus terreus. Subsequently, multiple sterile silk, linen and cotton swatches were inoculated with A. terreus (strain UOA/HCPFEnv49; Genbank accession ITS no. JF509458; tubulin JF509463; calmodulin JF927640) recovered from an adjoining swatch of the same find. The same swatches were inoculated with common soil fungal saprophyte in our region such as Scopulariopsis brevicaulis and A. fumigatus. Each inoculated swatch was incubated at 25oC for 3 weeks, dried and subjected to ESEM. Differences in lytic pattern and intensity among different textiles were observed; allowing match of the excavated sample's pattern exclusively to the A. terreus inoculated cotton swatches.

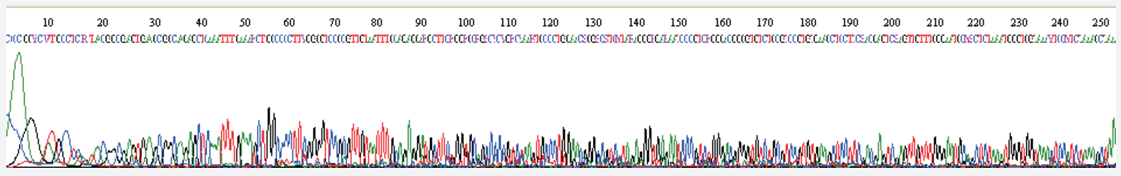

Figure 2.
Sequencing of the PCR product identified the cellulolytic fungus *Aspergillus terreus*.

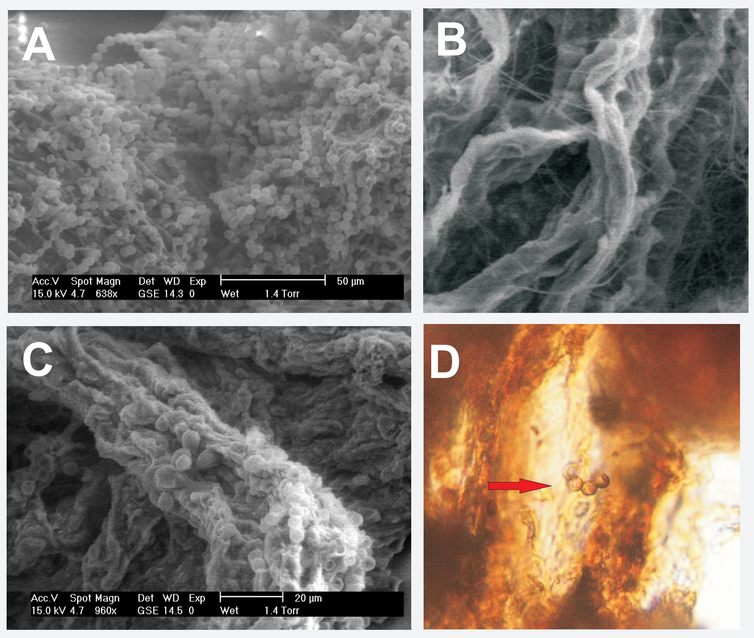

Figure 3.
A. & B. ESEM images showing profuse 1.5-2 micrometers sub-globose partially dehydrated fungal conidia-like structures covering the excavated find. **C.** ESEM image of samples from the find. Filamentous microorganisms can be observed. **D.** Conidia like structures (arrows) in optical microscopy of a direct preparation at x400 magnification.

Conclusions

Molecular fungal deterioration analysis enhances fibre identification.

Direct fungal non-culture detection/identification enables the selection of the appropriate treatment and storage methods. These further protect the find making it accessible for future study.

Finally, although bibliographically supported, the presence of silk in the 5th c. BC, grave 35 HTR 73 in Athens has no material evidence to date.