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BACTERIA CONSORTIA AND DETERIORATION OF ARCHAEOLOGICAL WATERLOGGED WOOD:

IDENTIFICATION BY MOLECULAR AND MICROSCOPY TECHNIQUES

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In this study molecular tools were applied to reveal and identify bacterial colonization in samples from archaeological waterlogged wood. The results obtained by observation of wooden sections, shown the presence of black and dark-brown areas and mineral concretions. The SEM analysis revealed a specific cell walls alteration, attributable to bacterial activity, other than abundant pyrite framboids (FeS_2). Molecular methods allow us to extract microbial genomic DNA from wood samples and *in vitro* amplify (PCR) bacteria DNA target sequences (16S, ITS-rRNA). Through sequences analysis of PCR products cellulosolytic and ligninolytic bacteria, such as *Pseudomonas*, *Cellulomonas*, *Xanthomonas* and *Bacillus* genera, have been revealed. Moreover the presence of *Marinobacter* spp. and *Desulforudis audaxviator*, respectively iron-oxidizing and sulfate-reducing bacteria, were identify.

OM OBSERVATIONS and SEM ANALYSIS

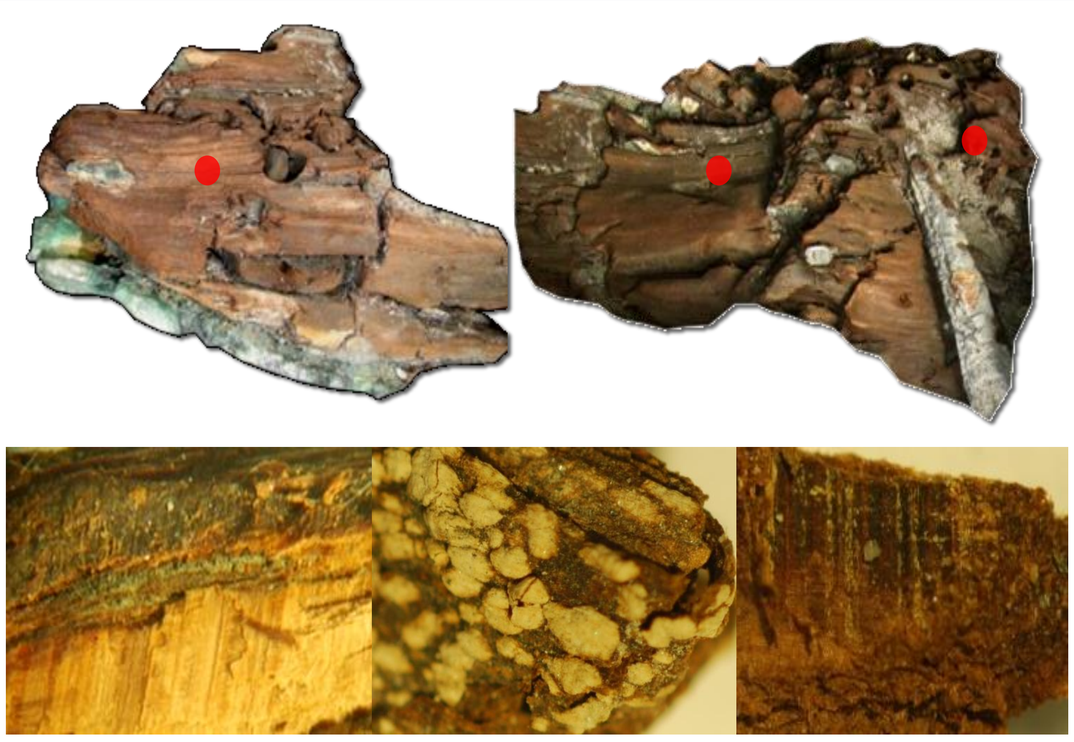

Fig.1 Small fragments (*Pinus* sp.) sampled from different areas (pointed in red), of wooden structure (upper figure). OM observations at different magnifications show chromatic alterations of wood fragments (dark areas) and mineral concretions/deposits, must probably due to sulphur compounds. Data show common alterations revealed in waterlogged wood, indicating both long-term anoxic burial.

DETERIORATION OF WOOD STRUCTURE

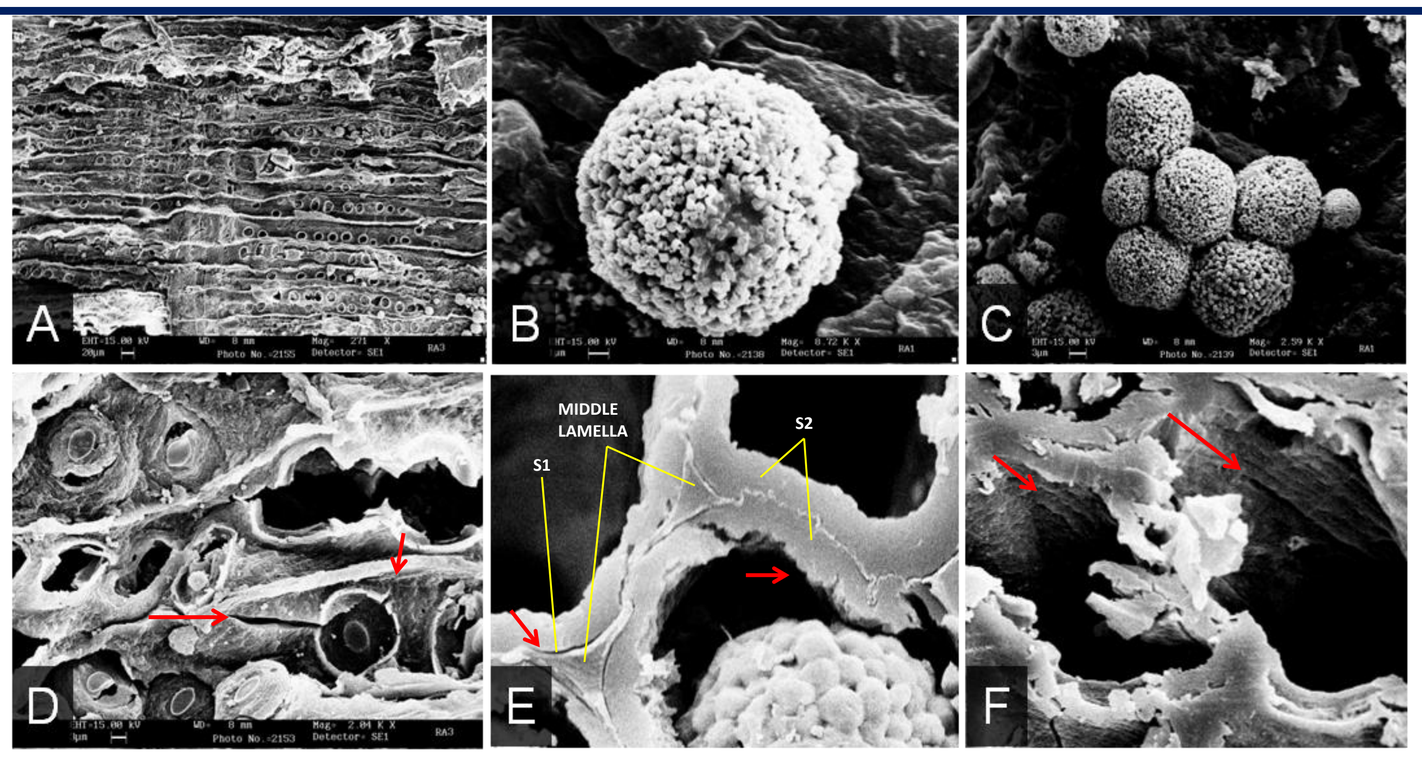

Fig.2 SEM micrographs. A-D) radial view: cracks and fractures in cell walls (arrows) probably due to mechanical deterioration; B-C) single or cluster of pyrite framboids; E) transverse section: framboid is inside the wall cell; primary and secondary walls (S1, S2) are shown, S1 is degradated, while S2 is divided from middle lamella (arrow); cell walls profile appears degraded by erosion bacteria (arrow); F) presence of channels (arrows) and the aspect of secondary wall is typical of the bacterial decay. The identification of state of conservation indicate both long-term burial in anoxic environment and colonization by sulphate-reducing bacteria.

MOLECULAR ANALYSIS

PRIMERS	**ITS1*f***: 5'- TCC GTA GGT GAA CCT GCG G- 3'(*forward*) **ITS1*r***: 5'- GCT GCG TTC TTC ATC GAT GC-3'(*reverse*)
	16S rRNA specific gene for: *Pseudomonas* (*PSEUB1-PSEUB2*), *Cellulomonas* (*CEL1-CEL3*), *Bacillus* (*BFP1-BFP2*)
REACTION MIXTURE	Genomic DNA, 1X Reaction Buffer, 10 µM primer *f* 10 µM primer *r*, 2 Mm dNTP mix, 2 mM MgCl2, 5 u/µl Taq DNA polimerase
REACTION CYCLES	1 cycle: 95 ° C for 5 minutes 30 cycles: 94 ° C for 1 min; 50°C - 58 ° C for 1 min; 72 ° C for 2 min final extension step: 72 °C for 7 min

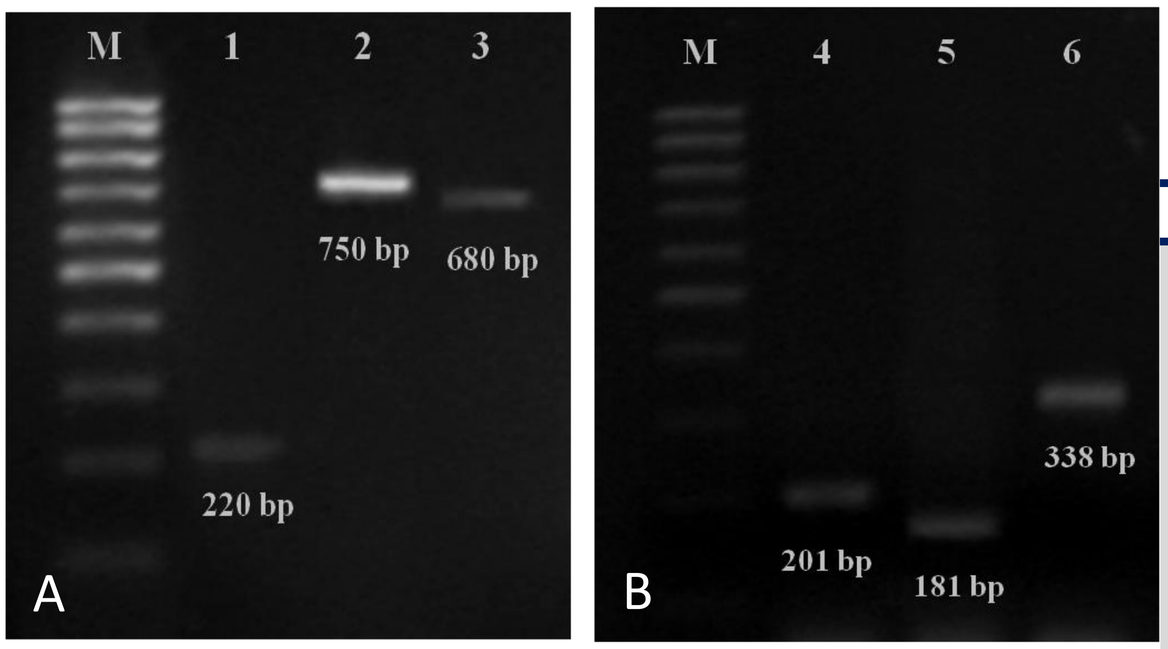

Fig.3 Gel electrophoresis of PCR products (agarose gel, 2%). A) Amplification products of ITS bacterial region; molecular investigation results confirm the presence of bacteria such as *Desulforudis audaxviator* (220 bp), *Marinobacter* spp. (750 bp) and *Xanthomonas* spp. (680 bp); B) amplification products of 16S-rRNA bacterial gene, specific primers for (lane 4) *Cellulomonas* spp. (201 bp); (lane 5) *Bacillus* spp. (181 bp); (lane 6) *Pseudomonas* spp. (338 bp). Marker Sharpmass (M).

IDENTIFICATION OF BACTERIAL CONSORTIA

ITS	***Desulforudis audaxviator*** ***Marinobacter*** ***Xanthomonas***	Sulphate- reducing bacteria Iron-oxiding bacteria Ligninolytic bacteria
16S	***Cellulomonas*** ***Bacillus*** ***Pseudomonas***	Cellulosolytic bacteria commonly recovered in degradeted wood

Total bacterial DNA was extracted directly from wood fragments by Stool mini Kit (*Quiagen*), partially modified. Genomic DNA was utilized as template in PCR reactions. Specific ribosomal DNA sequences, 16S gene or ITS, were respectively amplified by specific or universal primers.

CONCLUSION

The results of this study allowed:

✓ to evaluate alterations of waterlogged wood fragments through microscopy techniques

✓ to develop a suitable protocol for extraction of microbial DNA directly from wood samples

✓ identification of bacteria with ligninolytic and cellulosolytic activity through molecular techniques

REFERENCES

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