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# Biosynthesis of silver-based nanoparticles using supernatants of microbial cultures for Cultural Heritage preservation

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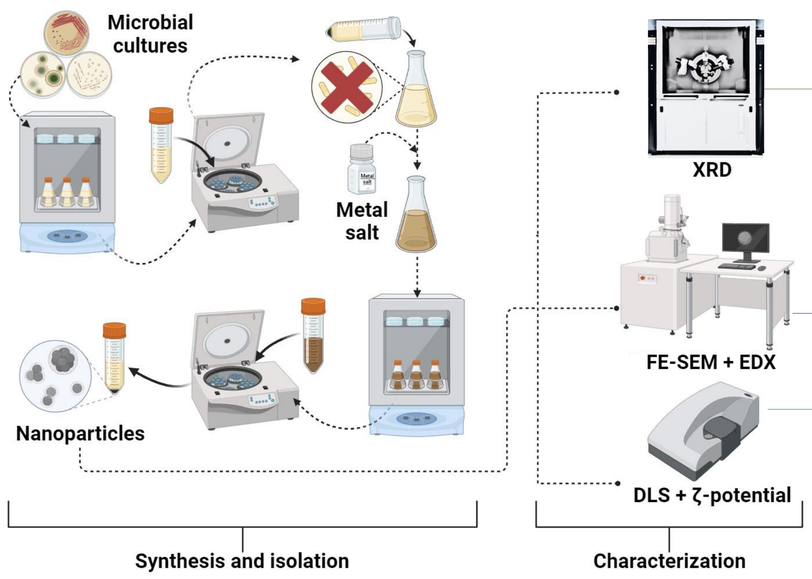
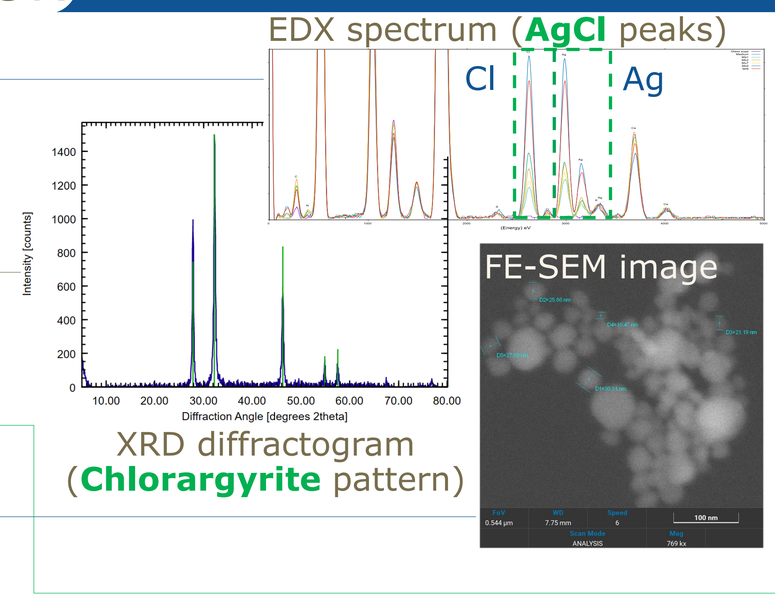
## CONTEXT

Microbial contamination of cultural heritage materials is one of its most prevalent and impacting preservation issues that can lead to visual, structural, and chemical changes[1]. Currently, different approaches try to address this issue by employing products and techniques that can lead to structural or chemical alteration of materials[2] and pose several environmental risks[3]. For these reasons, nanotechnological solutions can be new greener alternatives. Studies on metal-based nanoparticles have described their efficient antimicrobial properties and long-term effects at low concentrations[4]. However, their stability is highly dependent on surface functionalization, and their chemical synthesis methods may also raise environmental issues. The use of microbial cultures lowers the environmental impact of metal-based NPs' synthesis and stabilizes them with probable antimicrobial potentiating molecules secreted by these microorganisms[5].

## SYNTHESIS & CHARACTERIZATION



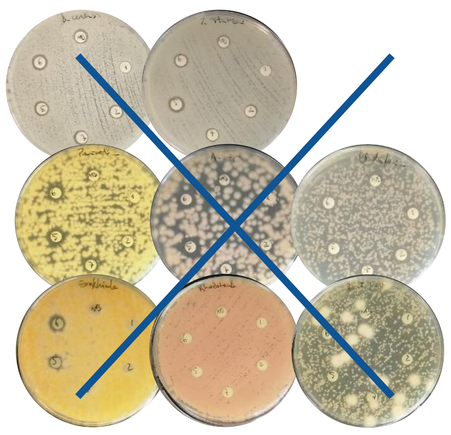




| Sample (NPs) | Size FE-SEM (nm) | Size DLS (nm) | Zeta (mV) |
|---|---|---|---|
| **M1** | 22±5 | 70 | -21±5 |
| **M1Ma1** | 15±3 | 25 | -26±11 |
| **M1Mb2** | 38±18 | 43 | -31±5 |
| **M1Ms5** | 55.5* | 61 | -17±6 |
| **M1Mr6** | 67±24 | 80 | NA |

*Calculated from fitted curve (DLS vs. FE-SEM)

## ANTIMICROBIAL ACTIVITY

### Disc-diffusion assay



Inhibition zones in most samples.

Inverse log relation between inhibition diameter and diffusion rate of NPs (i.e., **low NPs diffusion when NPs size ~ agar pore**).

### Minimum inhibitory concentration (MIC) ppm

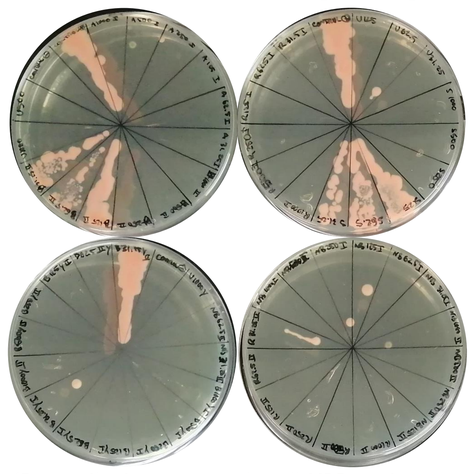
| Sample (NPs) | *Microbial strain (isolated from archaeological materials)* | | | | | |
|---|---|---|---|---|---|---|
| | *Pseudomonas stutzeri* | Orange yeast (UY1)* | Black yeast (UY2)* | *Rhodotorula mucilaginosa* | *Aspergillus niger* | *Penicillium citrinum* |
| **M1** | 19.1 | 19.1 | 19.1 | 19.1 | 19.1 | 38.2 |
| **M1Ma1** | 12.4 | 37.3 | 12.4 | 12.4 | 31.1 | 18.7 |
| **M1Mb2** | 17.9 | 17.9 | 17.9 | 17.9 | 17.9 | 17.9 |
| **M1Ms5** | 24.7 | 24.7 | 24.7 | 24.7 | 24.7 | 24.7 |
| **M1Mr6** | 27.0 | 27.0 | 27.0 | 27.0 | 27.0 | 27.0 |

Results in ppm after correction of the initial 2000 ppm uniformization to the approximated NPs concentration from EDX quantification analysis (n=2). *Waiting sequencing.

**Low MICs** from all samples against tested cultures (< 40 ppm)

Variability between samples ⟶ effect of microbial-secreted molecules

### Minimum lethal concentration (MLC)



(*R. mucilaginosa* example; all MIC strains tested)

Minimum lethal concentration (**MLC**) is, generally, **10X higher than MIC** (116 ppm to 593 ppm). Related with the antimicrobial mechanism of the NPs[5]?

## FINDINGS

Silver-based nanoparticles can be successfully synthesized using microbial culture supernatants.

The composition of the culture media highly influences nanoparticles' molecular composition (data not shown).

**Biosynthesized silver nanoparticles show high *in vitro* antimicrobial activity** against microorganisms isolated from archaeological materials.

Silver nanoparticles have both microbiostatic (at lower ppm) and microbiocidal (at higher ppm) activity.

Despite being usually tested, standard **disk diffusion assays are not suitable for assessing nanoparticles' antimicrobial potential**.

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FCT projects UIDB/04449/2020 and UIDP/04449/2020 and grant UI/BD/153583/2022.

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## REFERENCES

[1] Branysova, T. et al. J. Cult. Herit. 55, 245–260 (2022)
[2] Cappitelli, F. et al. Microorganisms 8, 1542 (2020)
[3] Kakakhel, M. A. et al. Int. Biodet. Biodeg. 143, 104721 (2019)
[4] Rudramurthy, G. et al. Molecules 21, 836 (2016)
[5] Carrapiço, A. et al. Microorganisms 11 (2), 378, (2023)