Janez Kosel
Institute for the Protection of Cultural Heritage of Slovenia
janez.kosel@zvkds.si

Milica Ljaljević Grbić
University of Belgrade, Faculty of Biology

Ivica Dimkić
University of Belgrade, Faculty of Biology

Tamara Janakiev
University of Belgrade, Faculty of Biology

Črtomir Tavzes
Institute for the Protection of Cultural Heritage of Slovenia

Slađana Popović
University of Belgrade, Faculty of Biology

Aleksandar Knežević
University of Belgrade, Faculty of Biology

Lea Legan
Institute for the Protection of Cultural Heritage of Slovenia

Klara Retko
Institute for the Protection of Cultural Heritage of Slovenia

Polonca Ropret
Institute for the Protection of Cultural Heritage of Slovenia

Nina Žbona
Institute for the Protection of Cultural Heritage of Slovenia

Nikola Unković
University of Belgrade, Faculty of Biology

Uncovering Autochthonous Deteriogenic Biofilm and Developing Beneficial Bacteria-Based Bioformulations from Indigenous Isolates for Biocontrol and Biocleaning of the Rožanec Mithraeum Limestone Monument

The conservation of the Rožanec Mithraeum limestone monument in Slovenia demands an integrated understanding of the fungal and bacterial communities driving surface alteration and deterioration. High-throughput mycobiome sequencing (metagenomic) combined with light, FTIR, and Raman microscopy identified the epilithic lichen Gyalecta jenensis and its carotenoid-rich photobiont Trentepohlia aurea as the source of the salmon-hued biofilm on the monument. Biopitting – the principal deterioration symptom – was attributed to endolithic thalli and ascomata of Verrucaria sp., whose oxalic-acid secretion dissolves carbonate substrates. FUNGuild analysis further confirmed a high relative abundance of lichenised and symbiotrophic fungi as key deteriogens.

In the next step, the monument's indigenous bacterial community – isolated and identified during the initial metagenomic phase – was analysed as a basis for developing a synergistic bacterial consortium with the potential to be reintroduced for biocontrol and biocleaning treatment of the same Mithraeum monument. Parallel 16S rRNA gene metabarcoding revealed that Proteobacteria, Actinobacteriota, and Cyanobacteria dominate the indigenous bacterial community, while culturable isolates were chiefly Bacillus and Paenibacillus. Enzymatic profiling highlighted Bacillus mycoides MIT8.7 and Paenibacillus amylolyticus/taichungensis/tylopili/tundrae MIT8.18 as potent producers of amylase and protease, with subsidiary lipase, cellulase, mannanase, and xylanase activities essential for biocleaning.

Plate-growth inhibition assays (PGI%) pinpointed Bacillus velezensis MIT7.8 and Pseudomonas chlororaphis subsp. aurantiaca MIT4.11 as the most effective antagonists against seven autochthonous biodeteriogenic fungi. Additionally, full cultures of Streptomyces anulatus 1–3 TSA and Streptomyces sp. 11-11MM matched the efficacy of commercial biocides (0.3% Preventol RI80, 100% Keim, 100% BFA) without inducing changes in surface roughness (Rq, Ra) over three months on locally sourced limestone models.

By elucidating indigenous bacteria capable of targeted biocontrol and biocleaning, this study lays the groundwork for an eco-friendly, in situ bacterial consortium. Such a formulation promises an effective alternative to traditional chemical biocides for the long-term preservation of stone cultural heritage.

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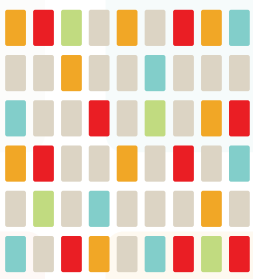

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