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Prevalence of *Bacillus* species in the lytic cultural heritage of Santa Lucia alle Malve Rupestrian Church

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Keywords

cultural heritage, biodeterioration, microbiota, *Bacillus*, Bacillota, lithic monuments

Abstract

Santa Lucia alle Malve (SLM) is a unique rupestrian heritage site, entirely carved into limestone. This monument, which was a church in the ancient settlement of Benedictine nuns over a millennium ago in southern Italy, holds exceptional value not only from an architectural and cultural perspective but also in terms of its microbial ecology. Until now, the specific microbiota of this site had remained unexplored. In this study, the bacterial community inhabiting the interior walls of Santa Lucia alle Malve was investigated using a metagenomic approach, alongside the isolation and comprehensive characterization of cultivable strains from various sampling sites. Both methodologies consistently revealed a dominance of spore-forming bacteria from the phylum Bacillota, particularly the genus *Bacillus*. Notably, most of the cultivable strains belonged to the *Bacillus cereus sensu lato* group and the *Bacillus. licheniformis* clade. Despite the high genetic similarity among these microorganisms, each strain exhibited a unique set of phenotypic traits, highlighting the potential complexity of the SLM metabolome. Additionally, two isolates were identified as *Bacillus thuringiensis*, entomopathogenic bacteria with possible applications in biological pest management. Finally,

Staphylococcus warneri, a human skin commensal found in the church, suggests human influence on the microbial landscape.

Introduction

Historical monuments such as buildings, statues, or archaeological sites are important and remarkable parts of human cultural heritage. As reminders of human civilization's long and complex history, these objects are often used for recreation and serve as important tourism destinations in many regions, thus generating substantial income. The historical lithic monuments are created from various stone formations, including marble, granite, plaster, limestone, sandstone, and others. Despite the usual first impression that these structures can last eternally, they are gradually but inevitably degraded.[1-4]

Various living organisms, such as bacteria, algae, fungi, and lichens, can colonize cultural heritage.[5] All these life forms may be responsible for biodeterioration (or bioweathering) [1]. However, the detection of organisms on cultural monuments does not necessarily prove that they are modifying the structure or aesthetic properties of the materials. However, estimating the microbial community on lytic heritage is a rational first step and a necessary starting point. The bioreceptivity (the susceptibility of a structure to colonization by living organisms) of various materials used in the creation of architectural structures is unequal and depends on their physicochemical properties and environmental conditions.[5,6] Limestone is believed to be one of the most vulnerable lithic materials to bacterial colonization and degradation processes caused by microorganisms,[7-9] Yet this material is characteristic of numerous remarkable cultural heritage sites worldwide. Therefore, innumerable attempts have been made to assess the biotic composition of limestone-made lytic heritage, including archaeological sites, temples, cathedrals, cemetery gravestones, and other similar structures.[3,10–18] The limestone structures studied to date are free-standing and made of quarried limestone blocks; moreover, the samples were usually taken from outer walls. However, some of the most characteristic and unique limestone cultural heritage monuments – the rock-excavated Rupestrian dwellings – have not yet been assessed for their bacterial community composition. Such rare and outstanding structures are abundant in the European city of Matera, located in southern Italy, and include thousands of buildings that once served as churches, houses, shops, or workshops.

The city of Matera is situated on one side of a gorge within the extensive hilly system known as 'Murgia Materana', specifically in the upper part of the right flank of the Gravina canyon, intersected by the river Gravina. This region is characterized geologically by a rocky block of upper Cretaceous limestones arranged in horizontal layers and a surface covered with tuff. The abundance of bioclastic and lithoclastic calcarenites deposited throughout Murgia Materana facilitated human settlements, given the ease with which this lithic material could be worked. Rupestrian settlements, dating back to the Paleolithic era, have evolved, sometimes undergoing numerous significant transformations.[19,20]

The *Sassi di Matera* ("sassi" meaning "stones" in Italian) is a Rupestrian heritage site divided into three districts: Sasso Barisano, Sasso Caveoso, and Civita. Renowned for their historical and architectural significance, the Sassi's Rupestrian structures have been designated as a UNESCO World Heritage Site since 1993, and in 2019, Matera was named the European Capital of Culture [19,20]. Two main categories of Rupestrian churches emerged in Matera, distinguishable by building techniques: those entirely carved into the rocky wall and those excavated in caves with added architectural elements such as facades and bell towers.

An eighth-century, fully carved-in-stone Rupestrian SLM church in the “Sasso Caveoso” area was selected for this research due to its cultural value and architectural features, which are highly representative of the local area. This UNESCO heritage site served Benedictine nuns from the 8th to the 16th century, first as a church and then as a monastery. Later, this monument was transformed into a house, then a store, and eventually became a deconsecrated church. Today, it attracts tourists, with an annual visitation of 34,000 (the number of visitors was provided by the Tourist Cooperative Society of Matera “*Oltre L'Arte*” - https://www.oltrelartematera.it/). Some of the most valuable features attracting visitors to SLM are the intricate frescoes covering the inner surfaces of the Rupestrian building. However, despite its historical and cultural richness, the internal surfaces of the structure exhibit noticeable deterioration, particularly in terms of biodeterioration (see Fig. 5). Autotrophic organisms, including algae, lichens, bryophytes, and mosses (e.g., *Grimmia pulvinata* and *Tortula muralis* identified in the vicinity of a water leak from the ceiling), colonize the internal walls, confirming the high humidity present in the internal environment. Evident limestone discolourations suggest the presence of microorganisms, such as fungi or bacteria, likely thriving due to water infiltration and limited exposure to daylight.

This paper describes the variability of bacterial communities inhabiting the inner walls of the Santa Lucia alle Malve (SLM), an ancient church located in the heart of Matera.[21] This structural pearl in Matera exemplifies Rupestrian architecture with its limestone excavation, frescoed interiors, and notable deterioration, highlighting the importance of researching SLM microbiota.

We employed two alternative approaches to identify and characterize the bacteria inhabiting the monument: a metagenomic study of the entire microbial community and the phenotypic/genotypic identification of cultivable bacteria from the sampling sites. Additionally, we devoted considerable attention to revealing the abundance of phenotypic traits exhibited by cultivable bacteria that overgrow the SLM walls. Finally, the presence of entomopathogenic *B. thuringiensis* strains in SLM is discussed. This study aims to emphasize the importance of understanding microbial colonization for the conservation of cultural heritage sites and highlight the potential ecological roles of bacteria in biodeterioration and bioprotection processes.

Results

Metagenomic analysis

Metagenomic analysis was performed on material collected from four sampling sites within the inner walls of the SLM. In total, 3,355,934 high-quality reads were included in downstream analyses. The mean number of reads per sample was 838,984, whereas the median number of reads per sample was 794,003. Rarefaction curves with 95% confidence intervals indicated that the sequencing depth was sufficient to recover the full community structure (Supplementary Figure S0).

The presented metagenomic analysis is the result of sequencing and analysis of the 16S rRNA V4 region. Among marked bacteria, more than 99% belonged to the phylum Bacillota (Fig. 2a). The most predominant genus within this group is *Bacillus*, followed by *Viridibacillus*, *Paenibacillus*, and *Lysinibacillus* (Fig. 2b). Apart from Bacillota, the marginal representation

(0.2%) of other phyla was also noted, namely Pseudomonadota, Actinomycetota, Bacteroidota, and Cyanobacteriota.

Phenotypic characterization of cultivable bacterial strains

Seven bacterial strains exhibiting diverse colony morphologies were isolated from the sampling sites (Fig. 3) from the inner stone walls of SLM, including: a) sampling site 1 – strains SLM1.1, SLM1.2, SLM1.4, SLM1.5; b) sampling site 2 – SLM2.9; c) sampling site 3 – strain SLM3.12; sampling site 4 – strain 4.15. These isolates underwent comprehensive phenotypic characterization. All examined strains were found to be Gram-positive. Notably, isolate SLM1.1 was the only staphylococcus-shaped strain, while the remaining isolates were rod-shaped, with vegetative cells varying in size (2.0–4.0 µm in length and 0.7–1.5 µm in width). Scanning electron microscopy (SEM) images of vegetative cells and light microscopy images of Gram-stained bacteria are presented in Supplementary Fig. S1.

All the tested strains, except SLM1.1, formed endospores when cultivated on the sporulation medium (Fig. 2). Moreover, strains SLM2.9 and SLM3.12 produced characteristic crystals associated with the sporulation process, which is characteristic of most *Bacillus thuringiensis* strains. These crystals are typically composed of insecticidal Cry and/or Cyt proteins. After amido black staining, these parasporal crystals were visible in the light microscope (Fig. 3, g and i) as dark purple to black objects. These crystals are visible in the corresponding SEM pictures as spherical/irregular (Fig. 2 h and j) structures accompanying bacterial endospores.

In the next part of this study, the biochemical and physiological traits of the tested isolates have been determined. The strains were oxidase-negative, catalase-positive, and did not produce sulfides when cultivated in a semisolid SIM medium for 24 hours at 30°C. All except SLM1.1 and SLM2.9 were motile. Strains SLM1.2, SLM1.4, SLM1.5, SLM2.9, and SLM4.15 reduced nitrates. However, strain SLM2.9 showed a weak positive reaction. These results are summarised in Supplementary Table S1. Four API kits (BIOMÉRIEUX) were used to assess the biochemical properties of the isolates comprehensively. The coccus-shaped strain SLM1.1 was tested using the API Staph. A positive reaction was observed in tests assessing the utilization of D-glucose, D-fructose, D-mannose, D-maltose, D-trehalose, D-mannitol, and D-saccharose, as well as the production of acetyl-methyl-carbinol, arginine dihydrolase, and urease. After comparison with the Apiweb database (API Staph v5.0), these results suggest that the SLM1.1 is *S. warneri* (ID = 99.2%). The remaining Gram-positive rod-shaped strains were tested using the API 50 CH with CHB Medium, API 20 NE, and API ZYM. These strains were assessed for almost 90 biochemical, physiological, and enzymatic traits. These results are reported in Supplementary Table S2 (API 50 CHB with CHB/E Medium), Supplementary Table S3 (API 20 NE), and Supplementary Table S4 (API ZYM). The strains studied displayed substantial differences in phenotypic traits. The most similar were SLM2.9 and SLM3.12, with differences only in motility and a few enzymatic activity tests. Strain SLM1.2 exhibited significantly different assimilation and enzymatic behaviors compared to the other strains, as indicated by the results of 14 tests. The test results were analyzed using the Apiweb database (API Staph v5.0 and API CHB50 v4.1). Strains SLM1.4, SLM1.5, and SLM4.15 were identified as *Bacillus cereus* with a probability ranging from 80.8% to 96.6%, while isolates SLM2.9 and SLM3.12 were identified as *Bacillus mycoides* with a probability of 89.1% to 95.5%. Strain SLM1.2 was designated as *Brevibacillus non-reactive* by the Apiweb application; however, many contradictory results were observed, suggesting that the correct species may not yet be included in the Apiweb database. The results of phenotypic identification using Apiweb are summarized in Table 1.

It should be noted that in most cases, the identification of cultured strains using the apiweb™ tool is approximate at best. For example, the two strains identified as *Bacillus mycoides* are, in fact, *Bacillus thuringiensis,* as determined by the formation of parasporal crystals (Fig. 4) and the absence of rhizoid-type growth on the medium (data not shown). Also, other identification results (such as "*Brevibacillus* non-reactive" and "*Bacillus cereus* 2") are somewhat vague and not precise. Therefore, the method, including the usage of the apiweb™ tool, is not recommended for the identification of *Bacillus* species. The reason for this may be limited or outdated data on the phenotypic reactions of Bacillus strains in the commercial database, which is primarily used for clinical strains.

16S rRNA gene sequencing

To identify the cultivable isolates by genotyping, the bacterial 16S rRNA genes were amplified and sequenced (Supplementary File S1). The alignment of seven sequences shows that 16S rRNA genes are identical in SLM1.5 and SLM4.15 strains and 2.9 and 3.12 isolates (Fig. 4). Isolates SLM1.2 and SLM1.4 cluster together, while SLM1.1 is genetically most distant to all the others.

The obtained 16S rRNA sequences were also aligned with data deposited in the GenBank database,[22] using the BLAST tool. Strain SLM1.1 was identified as *Staphylococcus warneri*, which aligns with the result obtained using phenotypic identification. The remaining strains were assigned to the genus *Bacillus;* however, the exact species identification is not unequivocal because of identical 16S rRNA gene sequences in closely related *Bacillus* species (Table 1). In the case of SLM 1.5, 2.9, 3.12, and 4.15, the genotypic approach indicates equal similarity to *B. cereus sensu lato*, described as "a group of bacteria displaying close phylogenetic relationships but a high ecological diversity".[23] These results align with the phenotypic approach, which identifies these four isolates as "Bacillus cereus" or "Bacillus mycoides" - also members of the *B. cereus* sensu lato group. In the case of SLM 1.2 and 1.4, the comparison of 16S rRNA shows their similarity to three species within the *B. licheniformis* clade: *B. haynesii*, *B. licheniformis,* and *B. sonorensis*.

Discussion

The surface of all lithic cultural architecture is covered by organisms representing various taxonomic and ecological groups.

Literature research has highlighted how the biodiversity of species populating lithic heritage contributes to the biodeterioration process of stone.[24–26] However, microorganisms, despite their potential role as agents of biodeterioration, can also be used positively for the conservation and restoration of cultural heritage, thanks to their "dual activity." Recent studies have reported that some microorganisms are also capable of producing biocalcite, which can help consolidate stone surfaces.[13,27–29]

It should not be underestimated that the abundance of specific microbes associated with lithic heritage can threaten the health of tourists (who are usually continuous in the city of Matera, with peaks in influx during the months of June-August and the Christmas and Easter holidays) and the personnel responsible for maintaining the artifacts. It is therefore clear that human activity (including improper management of the asset) and microclimatic conditions favorable to proliferation (high humidity, temperature, lack of ventilation) are factors that should not be underestimated.

To accurately assess the potential impact of the biocoenosis of lithic monuments on surface biodeterioration, bioconsolidation, or human health, the primary task is to identify the organisms that inhabit a cultural monument. Considering the above, this work aimed to detect and characterize the prokaryotic communities inhabiting the interior walls of SLM, a rock-hewn church designated a UNESCO World Heritage Site.

The results of this study demonstrate the dominance of bacteria from the phylum Bacillota on the internal walls of SLM, a Rupestrian church designated as a World Heritage site. This dominance drew our attention to this group. The metagenomic approach and the culture-dependent part of the study suggest that most of the microbiota are represented by the genus *Bacillus*. Additionally, one *S. warneri* strain has been isolated. The prevalence of *Bacillus* species may be due to the specific environmental conditions (which are very similar across all sampling sites within the church) and the ability of the bacteria to produce endospores, which are dormant forms that allow bacteria to survive unfavourable conditions, such as low nutrition. This finding is in line with studies suggesting that the carbonate stone (like the one constituting the SLM walls) is favourable for the settlement of spore-forming bacteria.[14,30]

The genotypic approach showed that the *Bacillus* species isolated in SLM are closely related. Strains SLM1.5, 2.9, 3.12, and 4.15 form a separate clade (Fig. 4), and their 16S rRNA gene sequences are more than 99.9% identical. Moreover, the sequences are similar between strains SLM1.5 and 4.15 and between strains SLM2.9 and SLM3.12. Also, the isolates SLM1.2 and SLM1.4 are genetically identical (>99.8% identity of their 16S rRNA). This observation suggests a low diversity of *Bacillus strains* inhabiting the internal walls of SLM. However, despite the high similarities depicted in the genotypic approach of the study, the phenotypic characterization revealed the unique nature of the isolates, i.e., each one has a distinct phenotypic profile (Supplementary Tables S1, S2, S3, and S4). This diversity of physiological and morphological traits reflects the potential complexity of metabolomes in the bacterial communities inhabiting the internal walls of SLM.

Two isolates obtained in this study (SLM2.9 and SLM3.12) were identified as *B. thuringiensi*s species based on their ability to produce parasporal crystals during sporulation. The crystal formation is a distinctive and discriminating feature upon which *B. thuringiensis* can be distinguished from other closely related, genetically indistinguishable bacteria within the *B. cereus* group (also known as *Bacillus cereus sensu lato*), namely: *B. anthracis*, *B. bingmayongensis B. cereus* (*sensu stricto*), *B. cereus* biovar *anthracis, B. cytotoxicus*, *B. gaemokensis, B. manliponensis, B. mycoides*, *B. pseudomycoides, B. toyonensis, B. weihenstephanensis* and *B. wiedmannii.*[23,31] The identification of SLM2.9 and SLM3.12 as *B. thuringiensis* is supported by comparing their phenotypic traits with the Apiweb™ database and 16S rRNA sequencing. Both approaches indicate species within the *B. cereus sensu lato* group (Table 1). *B. thuringiensis* is commonly considered pathogenic to different invertebrate groups, such as insects or nematodes. The toxicity of *B. thuringiensis* towards invertebrates is attributed to the biocidal activity of Cry and Cyt proteins present in the parasporal crystals.[32] This bacterium has been isolated from various sources, including soil, phylloplane, dead insects, food products, and mammalian guts, among others. To the best of our knowledge, this is the first isolation of *B. thuringiensis* from such a characteristic environment as the internal limestone surfaces of a Rupestrian church. Previously, various *Bacillus spp.* bacteria have been reported from stone buildings.[29] Noteworthy is that *B. thuringiensis* constituted a significant percentage of isolates obtained from the samples (two out of seven). Crystals with specific shapes display biological activity against certain groups of invertebrates. For example, bipyramidal crystals are typically found in lepidopteran-active *B. thuringiensis* strains, whereas spherical (ovoid) crystals contain proteins that are active against dipteran insects.[32–34] Also, it is worth considering why *B. thuringiensis* entomopathogens can be observed in SLM, though it is an environment where insects are scarcely found (LS personal communication). Although seemingly peculiar, our observation aligns with the work of Martin and Travers,[35] who found that the abundance of insects at a sampling site does not predict the presence of *B. thuringiensis*, and vice versa. The bacterium has been discovered in invertebrate-scarce environments. Additionally, *B. thuringiensis* has been observed to cause epizootic outbreaks very rarely. Therefore, the results presented in this paper contribute to the discussion by proposing an alternative, "insect-independent" life cycle of *B. thuringiensis*, for example, a plant symbiont role.[31,37] The coexistence of *B. thuringiensis* and the abundance of autotrophic organisms, such as mosses identified as *G. pulvinata* and *T. muralis* in SLM sampling sites, could be a trail worth following to test the theory mentioned above.

Identifying unequivocal species was impossible for the remaining *Bacillus* strains obtained from SLM samples. However, two isolates (SLM1.5 and SLM4.15) are likely also members of *B. cereus sensu lato*, based on their phenotypic and genotypic characterization. As summarised previously, certain bacteria within this group (namely some strains of *Bacillus cereus sensu stricto*) can be pathogenic towards mammals (including humans), causing diarrheal and emetic syndromes,[31] they are mostly considered opportunistic pathogens.[38] Also, many avirulent strains of *B. cereus* are noted - these microbes may act as plant symbionts (similar to closely related *B. thuringiensis* discussed above), i.e., promoting plant growth. Furthermore, the role of *B. cereus* as an animal symbiont cannot be excluded.[31] The remaining two of the *Bacillus* strains characterized in this study (SLM1.2 and SLM1.4) show a high degree of genetic similarity with *B. licheniformis, B. haynesii,* and *B. sonorensis* – closely related, non-pathogenic species within the *B. licheniformis* clade [39]. However, comparing the phenotypic traits between the reference strains[39–41] and SLM1.2/SLM1.4 isolates suggest that the latter may be different biotypes or even novel species within the *B. licheniformis* clade.

Apart from the six *Bacillus* isolates characterized in this work, the *S. warneri* strain (SLM1.1) was also detected in SLM. *S. warneri* usually occurs as a harmless human skin commensal.[42,43] This species cannot produce endospores; thus, it is much less resistant to adverse environmental conditions than the *Bacillus* species. Since *S. warneri*'s growth outside the human body is rather unlikely, its presence on stone surfaces may be transient, and we can speculate that it may be linked to people visiting the SLM church. The fact is that during visits, tourists can unintentionally bring with them and diffuse many microorganisms, such as algae, fungi, or bacteria.[44,45].

In conclusion, the bacterial community inhabiting the internal walls of the Rupestrian church, known as SLM, has been unveiled through metagenomic analysis. The predominance of Bacillota, primarily the genus Bacillus, has been observed in this environment. The culture-dependent part of the study has fully confirmed the substantial overrepresentation of *Bacillus* spp. - the isolated strains belong to the closely related *B. cereus sensu lato* group or *B.*

licheniformis clade. However, despite the high genetic similarity of the isolates, they show significant differences in their phenotypic features, suggesting a rich metabolome in the SLM lytic architecture. Interestingly, entomopathogenic strains of *B. thuringiensis* are also present among the obtained isolates. The study also identifies *S. warneri*, a human skin commensal, likely introduced by visitors, underscoring the impact of human activity on the microbial landscape of cultural heritage sites. In any case, the composition of the SLM bacterial community does not indicate any severe microbiological hazards to visitors. Overall, the research underscores the importance of understanding microbial colonization and its implications for preserving and managing historical monuments. Future studies should explore the potential biocidal and bioprotective roles of these microbial communities and their interactions with other biotic and abiotic factors to develop effective strategies for preserving such invaluable cultural heritage.

Conclusion

This study reveals the microbial diversity inhabiting the internal limestone surfaces of the Santa Lucia alle Malve Rupestrian Church. The dominance of *Bacillus* species, particularly those within the *B. cereus* sensu lato group and *B. licheniformis* clade, highlights the potential role of spore-forming bacteria in biodeterioration and bioprotection. The presence of *B. thuringiensis* and *S. warneri* highlights both the ecological complexity and the human influence on the microbial landscape. These findings underscore the importance of microbial characterization for effective conservation strategies and suggest future research directions to investigate microbial interactions and their implications for the preservation of cultural heritage.

Materials and methods

Sampling

The sampling was conducted in May, a period with a moderate influx of tourists. This choice was motivated by Matera's touristic importance and the uniqueness of its Sassi, which attract large numbers of visitors.

The month of May was characterized by a mild and pleasant climate, typical of the spring period, with mild temperatures and a relatively low number of rainy days, as reported in Fig. 1.

Samples were collected from the surfaces of inner limestone according to the Italian Cultural Heritage Ministry's Recommendation No. 3/1980 by scraping off the material into sterile tubes using a scalpel and touching the surfaces with sterile, dry swabs. Collected samples were marked as SLM1/2/3/4, SLM indicating their origin from SLM, and numbers indicating the sampling site inside the church, as shown in Fig. 6.

It should be noted that the church (*lat/lon 40°39′47.69″N / 16°36′44.79″E) consists of a single space divided into multiple areas only by natural limestone beams.

Samples were stored in laboratory-controlled conditions ,without additives until they were used for analysis.

Bacterial strain isolation

The material obtained from four sampling sites (SLM1-4) was used to isolate viable, cultivable bacteria. Bacterial strains were isolated by suspending collected sample material in 500 µL of sterile Ringer's solution (MERCK, Italy), vertexing for 10 minutes, and pipetting a 100 µL aliquot into 5 mL of Plate Count Broth (PCB; 0.5% tryptone (SIGMA-ALDRICH, (MERCK), Germany), 0.25% yeast extract MICROBIOL S.R.L, Italy), 0.1% glucose (SIGMA-ALDRICH (MERCK) Germany), pH 7.0). After 24 h of incubation at 30°C, the bacteria were streaked onto Plate Count Agar (PCA; PCB supplemented with 1.5% agar (MICROBIOL S.R.L., Italy)) and further incubated at 30°C for 7 days. The isolation procedure was designed to recover a qualitative representation of the cultivable bacterial microbiota, rather than to provide an exhaustive quantitative assessment of isolate abundance at each site and thus, from the total pool of colonies obtained across all four sites, seven single bacterial colonies, representing various morphologies (shape, size, margin, and appearance), were transferred to sterile PCA plates to obtain pure cultures. Isolated strains were named according to their sampling sites (e.g., SLM1.1 and SLM1.2, indicating two strains isolated from sampling site 1). The strains were stored in laboratory-controlled conditions at -80°C in 20% glycerol (SIGMA-ALDRICH (MERCK), Germany), until they were used for analysis.

16S rRNA gene sequencing

According to the manufacturer's instructions, the bacterial DNA was extracted from the pure culture stains using the Bacterial & Yeast Genomic DNA Purification Kit (EURx, Poland) and subsequently quantified spectrophotometrically at 260 nm. The 16S rRNA gene was amplified in a PCR reaction using fD1 (AGAGTTTGATCCTGGCTCAG)[46] and rD1 (GACGGGCGGTGTGTACA)[47,48] primers. PCR mixtures contained ~60 ng of bacterial DNA, 0.25 µM of each primer, and OptiTaq PCR Master Mix (EURx, Poland) diluted according to the manufacturer's instructions. After an initial 3-minute denaturation at 94°C, 28 amplification cycles were carried out, consisting of 25 seconds denaturation at 94°C, 30 seconds annealing at 54°C, and 1 minute elongation at 72°C, followed by a final elongation step of 7 minutes at 72 °C, PCR products were separated by electrophoresis in a 1.5% agarose gel. Amplicons were purified using EPPiC Fast (A&A BIOTECHNOLOGY, Poland) and subjected to Sanger sequencing with BigDye 3 chemistry and a Genetic Analyzer 3130xl (APPLIED BIOSYSTEMS, USA) according to the manufacturer's protocols. The phylogenetic tree was inferred using MEGA 7.0.20 software[49] with the Neighbour-Joining method, and the evolutionary distances were computed using the Maximum Composition Likelihood method. A search set based on BLAST[50] with the "rRNA/ITS database" was used, and the obtained sequences were aligned to determine the closest known relative species.

Metagenomic analysis

The hypervariable V4 region of the 16S ribosomal RNA (rRNA) gene was amplified from samples collected in SLM to assess bacterial community composition. PCRs were carried out using double-indexed V4F (CGATCAGCAGCCGCGGTAATA) and V4R (ATGGACTACCAGGGTATCTAA) primers[51] fused with dual-indexed adapters for sequencing in the Ion Torrent system (THERMO FISHER SCIENTIFIC, USA). All PCRs were performed in duplicate, each with a final volume of 10 µl containing Hot FIREPol DNA polymerase (SOLIS BIODYNE, Tartu, Estonia), 0.25 µM of each primer, and 1 µl of template DNA. The following thermal program was used: 95°C (12 min), then 30 cycles of 95°C (15 s), 50°C (1 min), 72°C (30 s), and final incubation at 72°C (5 min). The reaction without a DNA template and with blank DNA extraction was used as a negative control. Sequencing was performed using the Ion PGM Hi-Q View Sequencing Kit (THERMO FISHER SCIENTIFIC, USA) and Ion 318 chip (THERMO FISHER SCIENTIFIc, USA) on the Ion Torrent PGM system according to the manufacturer's protocol.

Bioinformatic analyses were performed as described elsewhere.[52] Briefly, raw sequence data were pre-filtered using Ion Torrent Suite 5.10.1 to remove polyclonal and low-quality reads. Sequences <200 bp were excluded in Geneious R11.1.5 (BIOMATTERS LTD., Auckland, New Zealand). Low-quality bases were trimmed using Trimmomatic[53] v0.39 and FASTX-Toolkit.[54] Reads were then demultiplexed and primer-trimmed in Geneious R11.1.5. Operational taxonomic units (OTUs) were clustered from 4,214,541 reads at 97% similarity using USEARCH[55] v11.0.667 and denoised into zero-radius operational taxonomic units (ZOTUs). Singletons (<10 reads) and chimeras were removed using the default settings in UCHIME2[56] v4.2.40. The remaining ZOTUs were taxonomically assigned against the SILVA SSU rRNA database (https://www.arb-silva.de/) using ARB for small subunit ribosomal RNAs[57] v138. The UNCROSS2[58] algorithm was used to remove ZOTUs detected in control samples from the dataset.

Phenotypic characterization

Following the manufacturer's instructions, the API galleries (BIOMÉRIEUX, France) were used to determine the majority of bacterial phenotypic features. The following kits were used in this project: API Staph (standardized test for the identification of the genera *Staphylococcus*, *Micrococcus*, and *Kocuria*), API 50 CH with CHB/E Medium (standardized test for the identification of genus *Bacillus* and related genera), API ZYM (standardized test for semiquantitative analysis of bacterial enzymatic activities) and API 20 NE (standardized test for the identification of non-fastidious, non-enteric Gram-negative rods). The results were compared with those of an Apiweb database (BIOMÉRIEUx, France) to determine the species of each isolate. According to the manufacturer's recommendation, Microbact Oxidase Detection strips (OXOID, UK) were used to investigate cytochrome C oxidase activity. Catalase activity tests, Gram stain reaction, and nitrate and nitrite reduction tests were performed as elsewhere described,[59] except that liquid media were used instead of semisolid media in the latter procedure. The motility of the bacteria and hydrogen sulfide production were checked in a semisolid SIM medium (DIFCO, USA) after 24 h of incubation at 30°C. Sporulation was assessed using methods previously described.[60] Briefly, colonies were grown for 24 h at 30°C on BHI medium (OXOID, UK). Subsequently, bacteria were plated on the sporulation medium[61] and incubated for an additional 4 to 5 days. The collected microbiological material was stained with amido black (POL-AURA, Poland) for 60 s and carbolfuchsin (POL-AURA, Poland) for 20 s, and examined using a light microscope. Additionally, both vegetative cells and biological material obtained through the sporulation procedure were analyzed using a Zeiss Evo 40 Scanning Electron Microscope (SEM). Samples were fixed in 2% glutaraldehyde (Polysciences, USA), buffered with 0.1 M sodium cacodylate (POLYSCIENCES, USA), pH 7.2, air-dried, attached to stubs with double-sided sticky tape, and coated with gold before being observed in the SEM.

References

1. Negi, A. & Sarethy, I. P. Microbial Biodeterioration of Cultural Heritage: Events, Colonization, and Analyses. *Microb Ecol* **78**, 1014–1029 (2019).
2. Boniek, D., de Abreu, C. S., dos Santos, A. F. B. & de Resende Stoianoff, M. A. Evaluation of microbiological air parameters and the fungal community involved in the potential risks of biodeterioration in a cultural heritage of humanity, Ouro Preto, Brazil—Folia *Microbiol (Praha)* **66**, 797–807 (2021).
3. Suchy, H., Zalar, P., & Macedo, M. F. Microbial Diversity of Biodeteriorated Limestone Cultural Heritage Assets Identified Using Molecular Approaches—A Literature Review. *Applied Sciences*, **14**(16), 7429 (2024). https://doi.org/10.3390/app14167429).
4. Masi, M., Petraretti, M., De Natale, A., Pollio, A. & Evidente, A. Fungal Metabolites with Antagonistic Activity against Fungi of Lithic Substrata. *Biomolecules* **11**, 295 (2021).
5. Pinna, D. Microbial Growth and its Effects on Inorganic Heritage Materials. in *Microorganisms in the Deterioration and Preservation of Cultural Heritage* (ed. Joseph, E.) 3–35 (Springer International Publishing, Cham, 2021). doi:10.1007/978-3-030-69411-1_1.
6. Komar, M., Nowicka-Krawczyk, P., Ruman, T., Nizioł, J., Dudek, M., & Gutarowska, B. . Biodeterioration potential of algae on building materials - Model study. International *Biodeterioration & Biodegradation*, **180**, 105593 (2023). https://doi.org/10.1016/j.ibiod.2023.105593.
7. Favero-Longo, S. E. & Viles, H. A. A review of the nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection. *World J Microbiol Biotechnol* **36**, 100 (2020).
8. Mazzoli, R., Giuffrida, M. G. & Pessione, E. Back to the past: "find the guilty bug—microorganisms involved in the biodeterioration of archeological and historical artifacts." *Appl Microbiol Biotechnol* **102**, 6393–6407 (2018).
9. Li, Q, Zhang, B, Yang, X, Ge, Q. Deterioration-Associated Microbiome of Stone Monuments: Structure, Variation, and Assembly. *Appl Environ Microbiol*. **84**:e02680-17 (2018). https://doi.org/10.1128/AEM.02680-17.
10. Coelho, C. *et al.* Bacterial and Archaeal Structural Diversity in Several Biodeterioration Patterns on the Limestone Walls of the Old Cathedral of Coimbra. *Microorganisms* **9**, 709 (2021).
11. Ortega-Morales, B. O., Camacho-Chab, J. C., Ortega-Cano, M. E., Camacho-Chab, P. A., Pereañez-Sacarías, J. E., Ortiz-Alcántara, J. M., Arena-Ortíz, M. L., Montero-Muñoz, J., Reyes-Estebanez, M. M., Alvarado-López, C. J., & Bartolo-Pérez, P. Bacterial bioconsolidation of deteriorated limestone exposed to tropical conditions.

Int. Biodeterior. Biodegrad. **204**, 106141 (2025). https://doi.org/10.1016/j.ibiod.2025.106141.

12. Kip, N., & Van Veen, J. A. The dual role of microbes in corrosion, *ISME J.* **9**(3), 542-551 (2015). https://doi.org/10.1038/ismej.2014.169

13. Jroundi, F., Schiro, M., Elert, K., & Teresa, M. Protection and consolidation of stone heritage by self-inoculation with indigenous carbonatogenic bacterial communities. *Nat. Commun.* **8**, 279 (2017). https://doi.org/10.1038/s41467-017-00372-3

14. Trovão, J., & Portugal, A. The impact of stone position and location on the microbiome of a marble statue. *The Microbe*, **2**, 100040 (2024). https://doi.org/10.1016/j.microb.2024.100040

15. Olapade, O.A. Biogeography and Community Diversity of Epilithic Bacterial Assemblages on Monuments: Comparisons between Maya Sites. *J. Pollut. Eff. Cont.* **4** (4), 176 (2016). https://doi.org/10.4176/2375-4397.1000176

16. Skipper, P. J. A., Skipper, L. K., & Dixon, R. A. . A metagenomic analysis of the bacterial microbiome of limestone, and the role of associated biofilms in the biodeterioration of heritage stone surfaces. *Sci. Rep*. **12**, 4877 (2022). https://doi.org/10.1038/s41598-022-08851-4

17 Villa, F., Vasanthakumar, A., Mitchell, R. & Cappitelli, F. RNA-based molecular survey of biodiversity of limestone tombstone microbiota in response to atmospheric sulphur pollution. *Lett Appl Microbiol* **60**, 92–102 (2015).

18. Rampazzi, L. *et al.* An interdisciplinary approach to a knowledge-based restoration: The dark alteration on Matera Cathedral (Italy). *Appl Surf Sci* **458**, 529–539 (2018).

19. De Giorgi, L., Lazzari, M., Leucci, G. & Persico, R. Geomorphological and non-destructive GPR survey for the conservation of frescos in the rupestrian churches of Matera (Basilicata, southern Italy). *Archaeol Prospect* **30**, 47–55 (2023).

20. Chiarella, D., Festa, V., Sabato, L. & Tropeano, M. The city of Matera and its 'Sassi' (Italy): an opportunity to broadcast geology in the European Capital of Culture 2019. *Geology Today* **35**, 174–178 (2019).

21 Mang, S. M., Scrano, L. & Camele, I. Preliminary Studies on Fungal Contamination of Two Rupestrian Churches from Matera (Southern Italy). *Sustainability* **12**, 6988 (2020).

22. Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. & Sayers, E. W. GenBank. *Nucleic Acids Res* **44**, D67–D72 (2016).

23. Fayad, N., Kallassy Awad, M. & Mahillon, J. Diversity of *Bacillus cereus* sensu lato mobilome. *BMC Genomics* **20**, 436 (2019).

24. Ilieş, D. C., Apopei, A.-I., Mircea, C., Ilieş, A., Caciora, T., Zharas, B., Barbu-Tudoran, L., Hodor, N., Turza, A., Pereş, A. C., Hassan, T. H., Safarov, B., & Noje, I.-C. Investigating the Role of Microclimate and Microorganisms in the

Deterioration of Stone Heritage: The Case of Rupestrian Church from Jac, Romania. *Appl. Sci.* **14**(18), 8136 (2024). https://doi.org/10.3390/app14188136.

25. Marvasi, M., Mastromei, G., & Perito, B. Bacterial Calcium Carbonate Mineralization in situ Strategies for Conservation of Stone Artworks: From Cell Components to Microbial Community. *Front Microbiol*, **11**, 530815 (2020). https://doi.org/10.3389/fmicb.2020.01386.
26. Coutinho, M. L. *et al.* Microbial communities on deteriorated artistic tiles from Pena National Palace (Sintra, Portugal). *Int Biodeterior Biodegradation* **84**, 322–332 (2013).
27. Nazel, T. Bioconsolidation of Stone Monuments. An Overview. *Restoration of Buildings and Monuments* **22**, 37–45 (2016).
28. Marvasi, M., Mastromei, G. & Perito, B. Bacterial Calcium Carbonate Mineralization in situ Strategies for Conservation of Stone Artworks: From Cell Components to Microbial Community. *Front Microbiol* **11**, 1386 (2020).
29. Ortega-Morales, B. O. & Gaylarde, C. C. Bioconservation of Historic Stone Buildings—An Updated Review. *Applied Sciences* **11**, 5695 (2021).
30. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. & Setlow, P. Resistance of *Bacillus* Endospores to Extreme Terrestrial and Extraterrestrial Environments. *Microbiology and Molecular Biology Reviews* **64**, 548–572 (2000).
31. Ehling-Schulz. M, Lereclus. D, Koehler. T.M. The Bacillus cereus Group: Bacillus Species with Pathogenic Potential. *Microbiol Spectr.* **7,** 3-0032 (2019). https://doi.org/10.1128/microbiolspec.gpp3-0032-2018
32. Azizoglu, U., Salehi Jouzani, G., Sansinenea, E. et al. Biotechnological advances in Bacillus thuringiensis and its toxins: Recent updates. *Rev Environ Sci Biotechnol* **22**, 319–348 (2023). https://doi.org/10.1007/s11157-023-09652-5
33. Osman, G. H. *et al.* Bioinsecticide *Bacillus thuringiensis* a Comprehensive Review. *Egypt J Biol Pest Control* **25**, 271–288 (2015).
34. Singh, T., Tiwari, Y., & Awasthi, G.. Understanding the impact of Bacillus thuringiensis proteins on non-target organisms. *IJSRBS,* **6**(2), 169–176 (2019). Retrieved from https://ijsrbs.isroset.org/index.php/j/article/view/215.
35. Martin, P. A. & Travers, R. S. Worldwide Abundance and Distribution of *Bacillus thuringiensis* Isolates. *Appl Environ Microbiol* **55**, 2437–42 (1989).
36. Belousova, M. E., Malovichko, Y. V., Shikov, A. E., Nizhnikov, A. A., & Antonets, K. S.). Dissecting the Environmental Consequences of Bacillus thuringiensis Application for Natural Ecosystems. *Toxins*, **13**(5), 355 (2021). https://doi.org/10.3390/toxins13050355.
37. Rousset, R, Gallet, A. Unintended effects of Bacillus thuringiensis spores and Cry toxins used as microbial insecticides on non-target organisms. *Curr Opin Environ*

Sci Health, **43**, 100598 (2025). https://doi.org/10.1016/j.coesh.2025.100598. hal-05176477.

38. Zhao, X., Van Overbeek, L., Burgess, C. M., Holden, N., Brennan, F., Johannessen, G. S. Allende, A., Höfte, M., Cottyn, B., Pothier, J. F., Schikora, A., & Uyttendaele, M. Human Pathogenic Microorganisms in Fresh Produce Production: Lessons Learned When Plant Science Meets Food Safety. *J. Food Prot.* **88**(7), 100551 (2025).. https://doi.org/10.1016/j.jfp.2025.100551.
39. Dunlap, C. A. *et al. Bacillus swezeyi* sp. nov. and *Bacillus haynesii* sp. nov., isolated from desert soil. *Int J Syst Evol Microbiol* **67**, 2720–2725 (2017).
40. Vos, P., Garrity, G., Jones, D., Krieg, N.l R., *et al.* Bergey's Manual of Systematic Bacteriology - Vol 3: The Firmicutes. (Springer, 2009). https://link.springer.com/book/10.1007/978-0-387-68489-5.
41. Palmisano, M. M., Nakamura, L. K., Duncan, K. E., Istock, C. A. & Cohan, F. M. *Bacillus sonorensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert Arizona. *Int J Syst Evol Microbiol* **51**, 1671–1679 (2001).
42. Becker, K., Skov, R. L. & von Eiff, C. *Staphylococcus*, *Micrococcus*, and Other Catalase-Positive Cocci. in *Manual of Clinical Microbiology* (eds. Jorgensen, J. H. et al.) 354–382 (ASM Press, Washington, DC, USA, 2015). doi:10.1128/9781555817381.ch21.
43. Kini, G. D., Patel, K., Parris, A. R. & Tang, J. S. An Unusual Presentation of Endocarditis Caused by *Staphylococcus warneri*. *Open Microbiol J* **4**, 103–105 (2015).
44. Wang, W. *et al.* Seasonal variations of airborne bacteria in the Mogao Grottoes, Dunhuang, China. *Int Biodeterior Biodegradation* **64**, 309–315 (2010).
45. Wang, W. *et al.* Seasonal dynamics of airborne fungi in different caves of the Mogao Grottoes, Dunhuang, China. *Int Biodeterior Biodegradation* **64**, 461–466 (2010).
46. Miller, C. S. *et al.* Short-Read Assembly of Full-Length 16S Amplicons Reveals Bacterial Diversity in Subsurface Sediments. *PLoS One* **8**, e56018 (2013).
47. Widmer, F., Seidler, R. J., Gillevet, P. M., Watrud, L. S. & Di Giovanni, G. D. A Highly Selective PCR Protocol for Detecting 16S rRNA Genes of the Genus *Pseudomonas* (Sensu Stricto) in Environmental Samples. *Appl Environ Microbiol* **64**, 2545–2553 (1998).
48. Gürtler, V. Typing of *Clostridium difficile* strains by PCR-amplification of variable length 16S-23S rDNA spacer regions. *J Gen Microbiol* **139**, 3089–97 (1993).
49. Kumar, S., Stecher, G. & Tamura, K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* **33**, 1870–1874 (2016).
50. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J Mol Biol* **215**, 403–10 (1990).

51. Makowska, N. *et al.* Metagenomic analysis of β-lactamase and carbapenemase genes in the wastewater resistome. *Water Res* **170**, 115277 (2020).

52. Trzebny, A., Slodkowicz-Kowalska, A., Björkroth, J. & Dabert, M. Microsporidian Infection in Mosquitoes (Culicidae) Is Associated with Gut Microbiome Composition and Predicted Gut Microbiome Functional Content. *Microb Ecol* **85**, 247–263 (2023).

53 Bolger, A. M., Lohse, M., Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data, *Bioinformatics* **30** (15), 2114–2120 (2014), https://doi.org/10.1093/bioinformatics/btu170

54 Hannon, G.J. FASTX-Toolkit. 2010. Available online: https://bio.tools/fastx-toolkit (accessed on 1 September 2022).

55 Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010). https://doi.org/10.1093/bioinformatics/btq461

56 Edgar, R. C. UCHIME2: improved chimera prediction for amplicon sequencing. *bioRxiv* 074252 (2016). https:// doi. org/ 10. 1101/074252

57 Yilmaz, P., Parfrey, L. W., Yarza, P. et al. The SILVA and "all-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res* **42**, 643–648 (2014). https://doi.org/10.1093/nar/gkt1209

58 Edgar, R. C. UNCROSS2: Identification of cross-talk in 16S rRNA OTU tables. *bioRxiv* 400762 (2018). https:// doi. org/ 10. 1101/400762

593.Tindall, B. J., Sikorski, J., Smibert, R. A. & Krieg, N. R. Phenotypic Characterization and the Principles of Comparative Systematics. in *Methods for General and Molecular Microbiology* (eds. Beveridge, T. J., Breznak, J. A., Marzluf, G. A., Schmidt, T. M. & Snyder, L. R.) 330–393 (ASM Press, Washington DC, 2007). doi:10.1128/9781555817497.ch15.

60. Baranek, J., Jakubowska, M. & Gabała, E. Insecticidal activity of *Bacillus thuringiensis* towards *Agrotis exclamationis* larvae–A widespread and underestimated pest of the Palearctic zone. *PLoS One* **18**, e0283077 (2023).

61. Lecadet, M. M., Blondel, M. O. & Ribier, J. Generalized transduction in *Bacillus thuringiensis* var. *berliner* 1715 using bacteriophage CP-54Ber. *J. Gen. Microbiol.* **121**, 203–212 (1980).

Figure legends

Fig.1. Climate parameters for Matera in May 2020

Fig. 2. The percentage of phyla constituting the microbial community within samples collected in Santa Lucia alle Malve church.

Fig. 3. Light microscopic (left side) and scanning electron microscopic (right side) observations of the strains post sporulation. a, b) SLM1.2; c, d) SLM1.4; e, f) SLM1.5; g, h) SLM2.9; i, j) SLM3.12; k, l) SLM4.15. Red arrows indicate parasporal crystals characteristic for *B. thuringiensis* strains.

Fig. 4. Phylogenetic tree based on partial 16S rRNA sequences of six *Bacillus* strains obtained during this study. *S. warneri* was used as an outgroup. Bootstrap values (based on 1000 replicates) are indicated at branch points. Bar - 0.01 nucleotide substitutions per site.

Fig. 5. Macroscopic observation of Santa Lucia alle Malve church: a, b) frontal facade; c-f) internal view showing moss and algae growth as well as deterioration of frescoes.

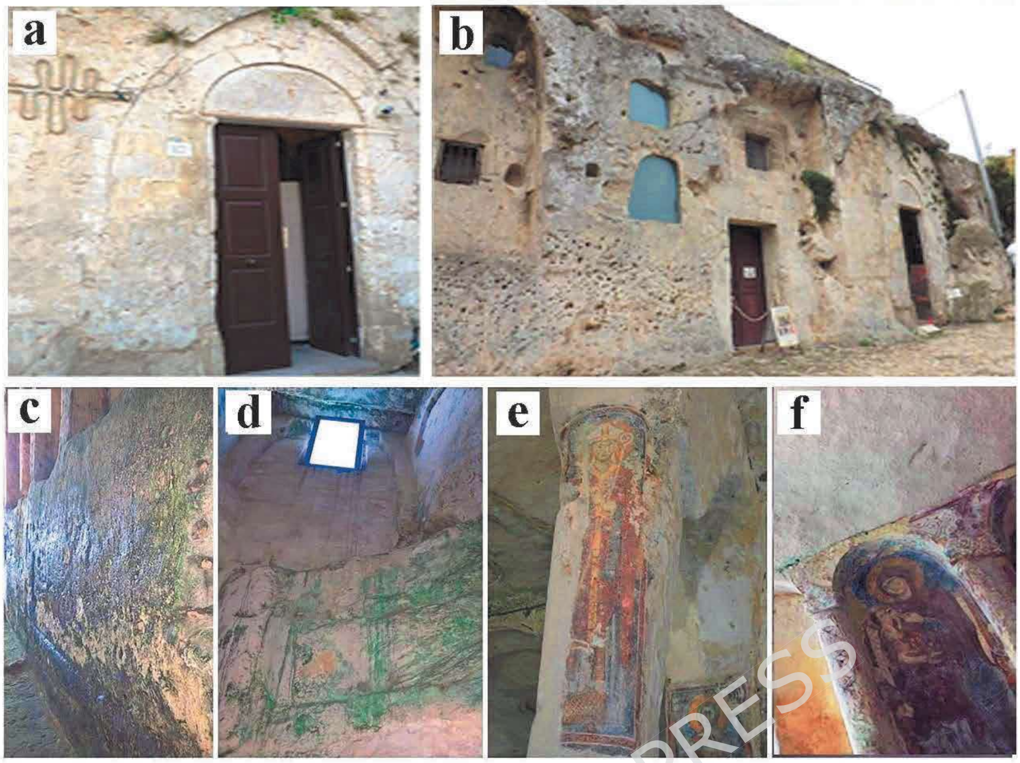
Fig. 6. Axonometry of Santa Lucia Alle Malve with sampling sites (SLM1-4) indicated. Arrow shows main entrance to the structure.

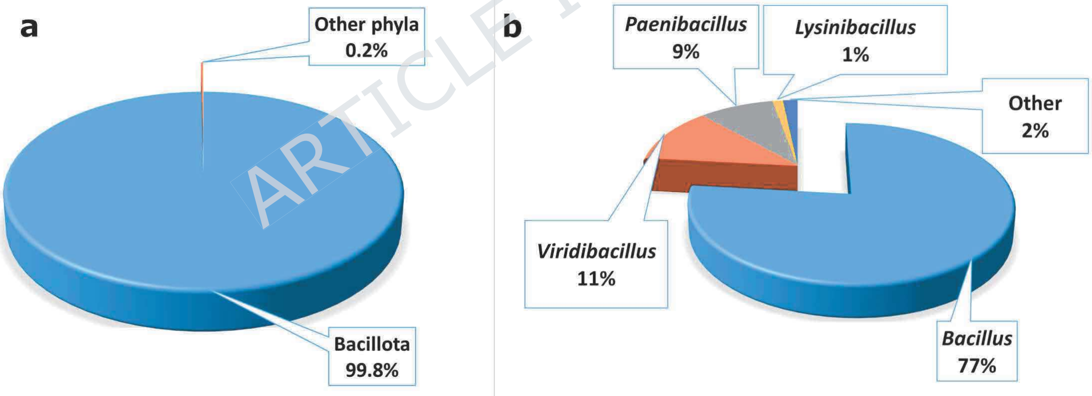
Table 1: Genotypic and phenotypic identification of strains isolated in Santa Lucia alle Malve rupestrian church.

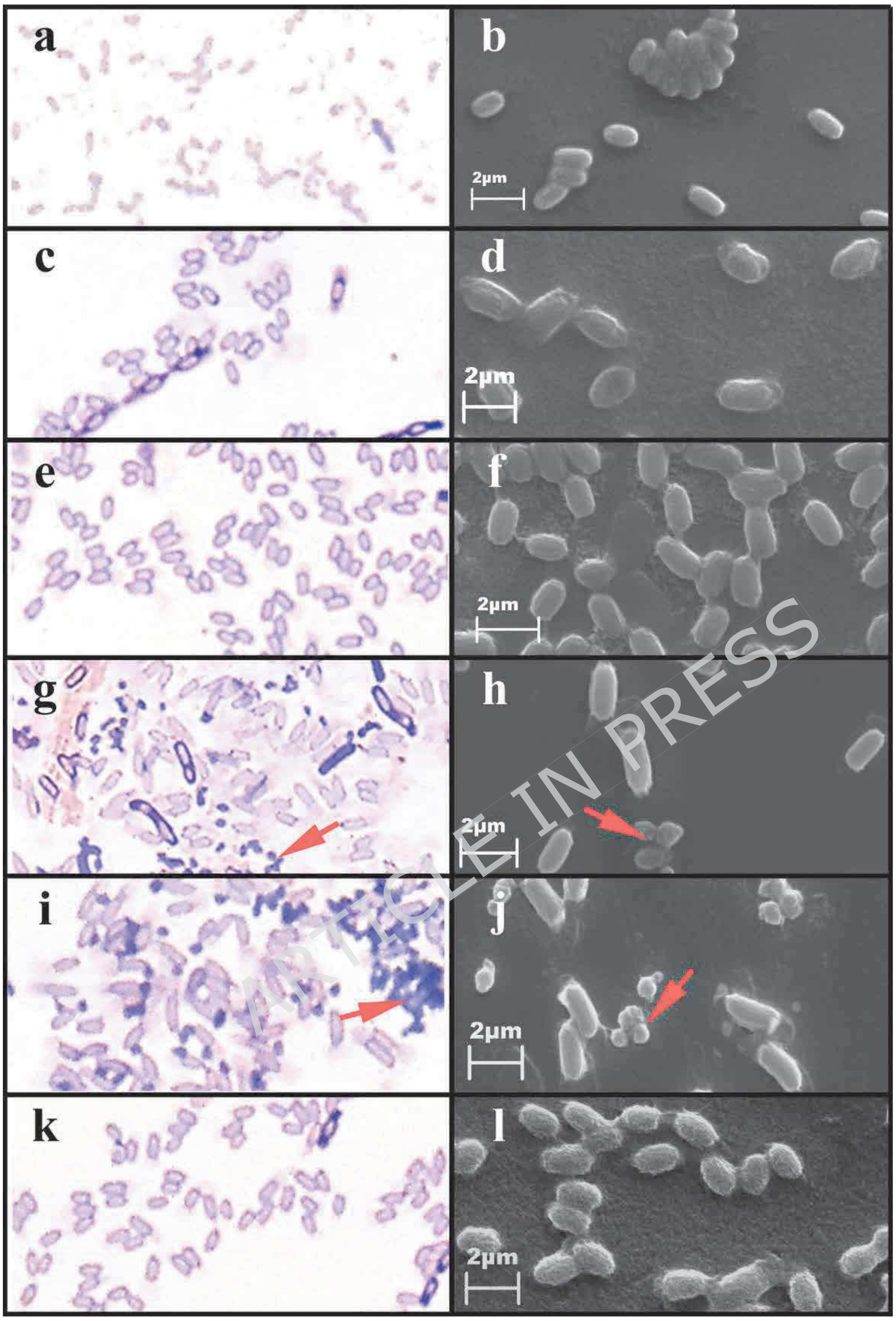
Strain ID	Phenotypic identification		Genotypic identification (closest identified phylogenetic relatives)	
	Species	Probability (%)[a]	Species	16S sequence identity (%)[b]
SLM1.1	*Staphylococcus warneri*	99.2	*Staphylococcus warneri*	100
SLM1.2	*Brevibacillus* non-reactive	97.9	*Bacillus licheniformis* clade	>99.61
SLM1.4	*Bacillus cereus* 2	96.6	*Bacillus licheniformis* clade	>99.67
SLM1.5	*Bacillus cereus* 2	96.3	*Bacillus cereus sensu lato*	100
SLM2.9	*Bacillus mycoides*	95.5	*Bacillus cereus sensu lato*	>99.79
SLM3.12	*Bacillus mycoides*	92.3	*Bacillus cereus sensu lato*	>99.79
SLM4.15	*Bacillus cereus* 2	80.8	*Bacillus cereus sensu lato*	100

[a] - the probability of correct identification according to the apiweb™ database

[b] - the percent identity between the 16S rRNA partial gene sequence of each tested strain and the most closely related strains shown in the BLAST alignment results


a
b
c
d
e
f


a
Other phyla 0.2%
Bacillota 99.8%
b
Paenibacillus 9%
Lysinibacillus 1%
Other 2%
Viridibacillus 11%
Bacillus 77%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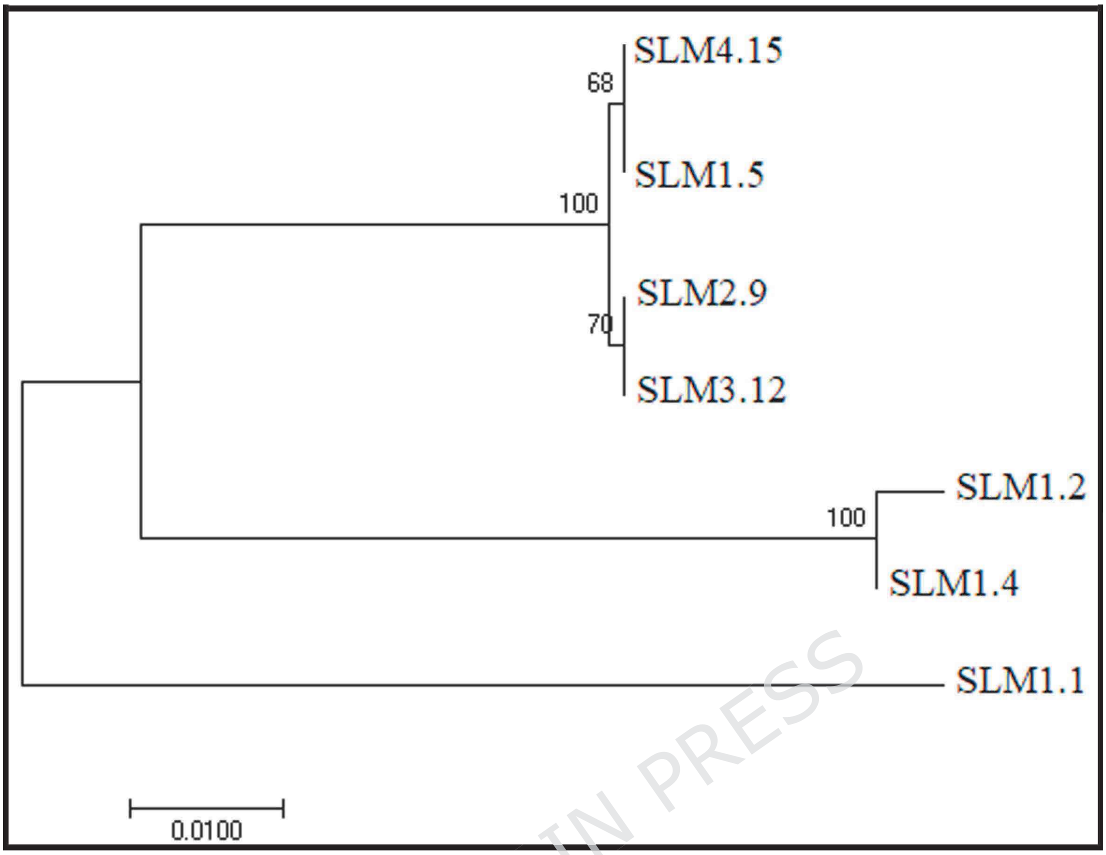
SLM4.15
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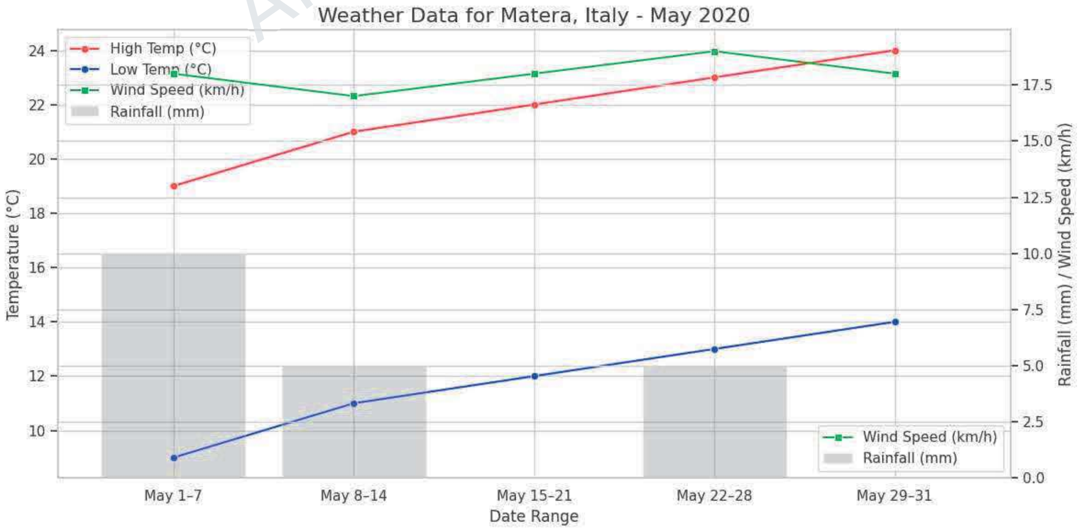
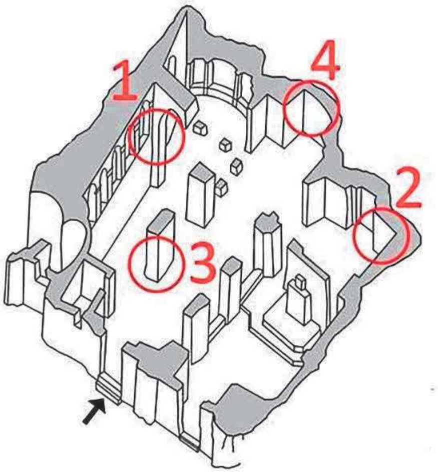
SLM1.5
100
SLM2.9
70
SLM3.12
SLM1.2
100
SLM1.4
SLM1.1
0.0100


Weather Data for Matera, Italy - May 2020
High Temp (°C)
Low Temp (°C)
Wind Speed (km/h)
Rainfall (mm)
Temperature (°C)
24
22
20
18
16
14
12
10
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Rainfall (mm) / Wind Speed (km/h)
Wind Speed (km/h)
Rainfall (mm)
May 1–7
May 8–14
May 15–21
May 22–28
May 29–31
Date Range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