

Supplementary Material

Diversity and Biomineralization Potential of the Epilithic Bacterial Communities Inhabiting the Oldest Public Stone Monument of Cluj-Napoca (Transylvania, Romania)

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Supplementary tables

Supplementary Table S1. Primers used in PCR amplification.

| Primer name | Target Group | Primer sequence (5' to 3') | Reference |
|--------------------|---------------------|-----------------------------------|-----------------------|
| 1492R | Universal | GGTACCTTGTTACGACTT | (Lane, 1991) |
| 27F | Bacteria | AGAGTTTGATCCTGGCTCAG | (Lane, 1991) |
| 338F | Bacteria | ACTCCTACGGGAGGCAGCAG | (Lane, 1991) |
| 518R | Bacteria | ATTACCGCGGCTGCTGG | (Muyzer et al., 1993) |
| 341-GC | Bacteria | ACGGGGGGCCTACGGGAGGCAGCAG | (Muyzer et al., 1993) |

Bacterial Communities Inhabiting Limestone Monuments

Supplementary Table S2. The number of distinct PCR-DGGE bands and the Margalef index for the analysed microbial communities. The samples names reflect their location on Saint Donatus statue.

| Samples | Number of bands | Margalef species richness |
|----------------|------------------------|----------------------------------|
| North | 28 | 3.989 |
| South | 31 | 4.539 |
| East | 20 | 2.973 |
| West | 31 | 4.317 |
| Upper | 34 | 4.813 |
| Lower | 32 | 4.488 |

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Supplementary Table S3. 16S rRNA gene-based taxonomy of the bacterial strains isolated from the Saint Donatus statue and their Gram coloration. The table contains the closest GenBank sequence match, together with its accession number and sequence identity.

| Sample | BLASTN-based taxonomy | Identity | Gram staining |
|--------------|-------------------------------------------------|----------|---------------|
| North_I_1A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| North_I_2A | <i>Pseudomonas synxantha</i> (KC834326.1) | 100% | Negative |
| North_I_4A | <i>Pseudomonas synxantha</i> (KC834326.1) | 100% | Negative |
| North_II_1A | <i>Pseudomonas grimontii</i> (KR054989.1) | 100% | Negative |
| North_II_2A | <i>Pseudomonas synxantha</i> (NR_113583.1) | 100% | Negative |
| North_II_3A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| North_II_4A | <i>Pseudomonas synxantha</i> (KC834326.1) | 99% | Negative |
| North_I_3B | <i>Klebsiella sp.</i> OS-B4 (FN178363.1) | 99% | Negative |
| North_I_4B* | <i>Pseudomonas fluorescens</i> (KU291443.1) | 99% | Negative |
| North_I_5B* | <i>Bacillus pumilus</i> (KJ476724.1) | 99% | Positive |
| North_II_1B | <i>Klebsiella sp.</i> OS-B4 (FN178363.1) | 99% | Negative |
| North_II_2B | <i>Pseudomonas lurida</i> (KM891560.1) | 99% | Negative |
| North_II_4B | <i>Pseudomonas sp.</i> SGb394 (HQ224639.1) | 99% | Negative |
| South_I_1A | <i>Exiguobacterium undae</i> (FN870071.1) | 99% | Positive |
| South_I_2A | <i>Arthrobacter luteolus</i> (DQ486130.1) | 100% | Positive |
| South_I_3A | <i>Arthrobacter luteolus</i> (DQ486130.1) | 99% | Positive |
| South_I_4A | <i>Exiguobacterium undae</i> (AB669472.1) | 99% | Positive |
| South_I_5A* | <i>Bacillus pumilus</i> (KJ476724.1) | 99% | Positive |
| South_II_2A | <i>Pantoea agglomerans</i> (AF130947.2) | 99% | Negative |
| South_II_3A | <i>Exiguobacterium undae</i> (AB669472.1) | 99% | Positive |
| South_II_4A | <i>Pantoea agglomerans</i> (KR265469.1) | 99% | Negative |
| South_II_5A* | <i>Bacillus pumilus</i> (KJ476724.1) | 100% | Positive |
| South_I_1B | <i>Pseudomonas putida</i> (KF640247.1) | 98% | Negative |
| South_I_4B* | <i>Pseudomonas putida</i> (KF640247.1) | 99% | Negative |
| South_I_5B* | <i>Brevibacterium iodinium</i> (KM259941.1) | 99% | Positive |

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| | | | |
|-------------|----------------------------------------------------|------|----------|
| East_I_1A | <i>Sanguibacter keddieii</i> (KF911337.1) | 100% | Positive |
| East_I_2A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| East_I_4A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| East_II_2A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| East II 4 A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| East_I_3B | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| East_I_5B* | <i>Bacillus pumilus</i> (KJ476724.1) | 99% | Positive |
| East_II_1B | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 95% | Negative |
| East_II_2B | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| East_II_3B* | <i>Bacillus safensis</i> (KJ672329.1) | 99% | Positive |
| East_II_4B* | <i>Bacillus pumilus</i> (KJ476724.1) | 99% | Positive |
| East_II_5B* | <i>Bacillus licheniformis</i> (KU363978.1) | 99% | Positive |
| West_I_1B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_I_2B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_I_3B | <i>Serratia marcescens</i> (KC790279.2) | 99% | Negative |
| West_I_4B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_II_1B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_II_2B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_II_3B | <i>Serratia sp. M401</i> (KJ944090.1) | 100% | Negative |
| West_II_4B | <i>Serratia marcescens</i> (KC790279.2) | 100% | Negative |
| West_II_5B* | <i>Streptomyces albidoflavus</i> (KT758342.1) | 100% | Positive |
| AWest_II_1 | <i>Pantoea sp. CH-N10</i> (HQ396801.1) | 99% | Negative |
| AWest_II_2 | <i>Pantoea sp. CH-N10</i> (HQ396801.1) | 99% | Negative |
| AWest_I_2B* | <i>Bacillus pumilus</i> (KJ476724.1) | 100% | Positive |
| AWest_I_3B | <i>Pantoea sp. CH-N10</i> (HQ396801.1) | 99% | Negative |
| AWest_II_3 | <i>Pantoea sp. CH-N10</i> (HQ396801.1) | 99% | Negative |
| AWest_II_4* | <i>Bacillus pumilus</i> (KJ476724.1) | 97% | Positive |
| Upper_I_1A | <i>Pseudomonas sp. UT1</i> (JX133179.1) | 99% | Negative |
| Upper_I_2A* | <i>Stenotrophomonas chelatiphaga</i> (NR_116366.1) | 99% | Negative |
| Upper_I_3A* | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 100% | Negative |
| Upper_II_1A | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 100% | Negative |
| Upper_II_2A | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 99% | Negative |
| Upper_II_3A | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 100% | Negative |

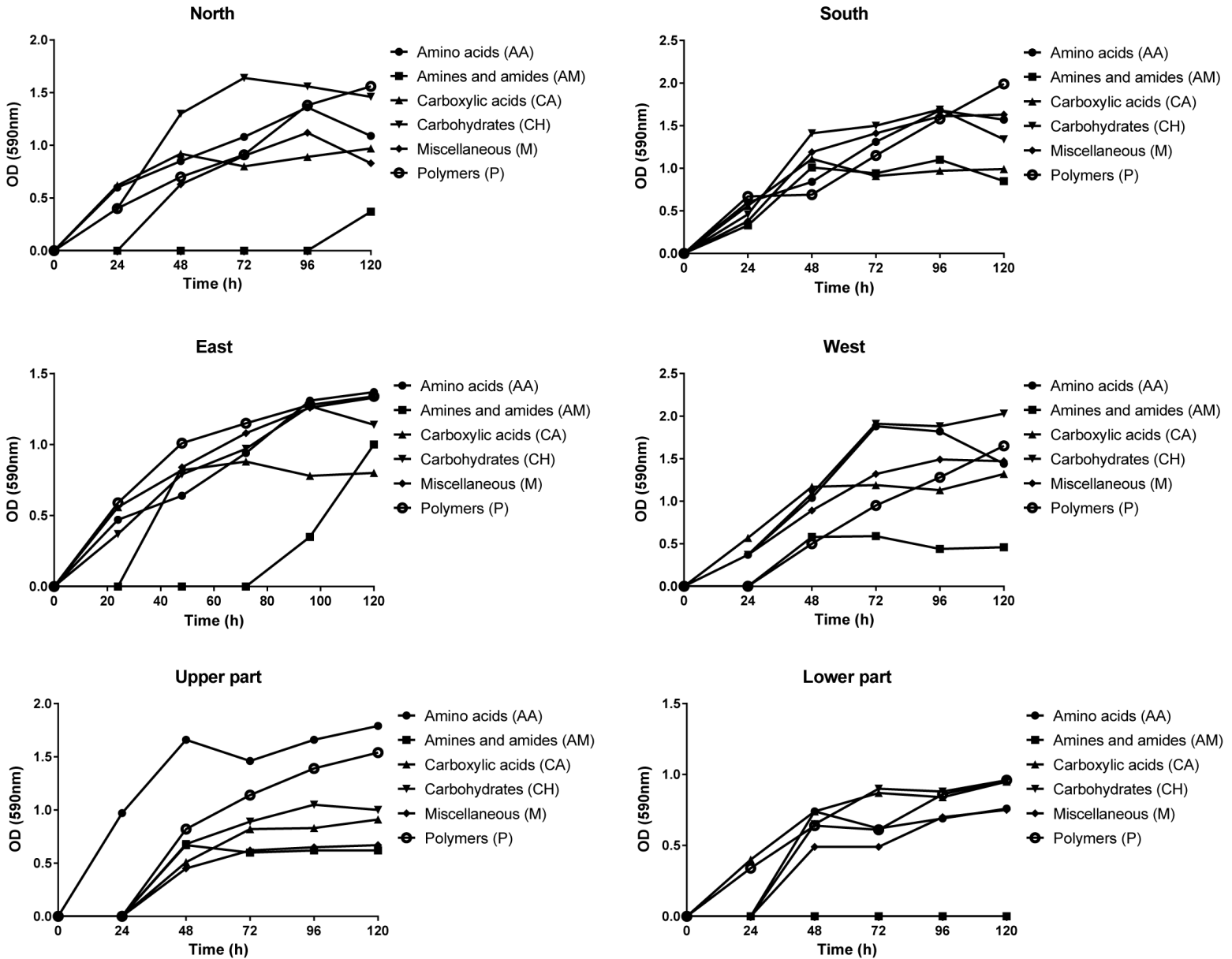
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| | | | |
|--------------|-------------------------------------------------|------|----------|
| Upper_II_4A | <i>Exiguobacterium sibiricum</i> (LK391534.1) | 99% | Positive |
| Upper_I_1B* | <i>Pseudomonas synxantha</i> (KC834326.1) | 100% | Negative |
| Upper_I_2B | <i>Pseudomonas putida</i> (KT767824.1) | 98% | Negative |
| Upper_I_3B | <i>Pseudomonas synxantha</i> (KC834326.1) | 100% | Negative |
| Upper_I_4B* | <i>Pseudomonas synxantha</i> (NR_113583.1) | 99% | Negative |
| Upper_II_1B* | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 99% | Negative |
| Upper_II_2B* | <i>Pseudomonas oryzihabitans</i> (KJ401059.1) | 100% | Negative |
| Upper_II_3B | <i>Pseudomonas synxantha</i> (KC834326.1) | 100% | Negative |
| Upper_II_4B | <i>Pseudomonas synxantha</i> (KC834326.1) | 99% | Negative |
| Lower_I_1A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| Lower_I_2A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| Lower_I_4A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| Lower_I_5A* | <i>Bacillus altitudinis</i> (KJ826579.1) | 99% | Positive |
| Lower_II_1A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| Lower_II_2A | <i>Microbacterium oxydans</i> (LN890028.1) | 100% | Positive |
| Lower_II_3A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 100% | Negative |
| Lower_II_4A | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| Lower_I_1B | <i>Pseudomonas rhizosphaerae</i> (KF147111.1) | 99% | Negative |
| Lower_II_2B | <i>Stenotrophomonas rhizophila</i> (KR055001.1) | 99% | Negative |
| Lower_II_4B* | <i>Pseudomonas graminis</i> (KF147075.1) | 99% | Negative |
| Lower_II_5B* | <i>Bacillus pumilus</i> (KJ476724.1) | 99% | Positive |

* Bacterial strains showed to be carbonatogenic

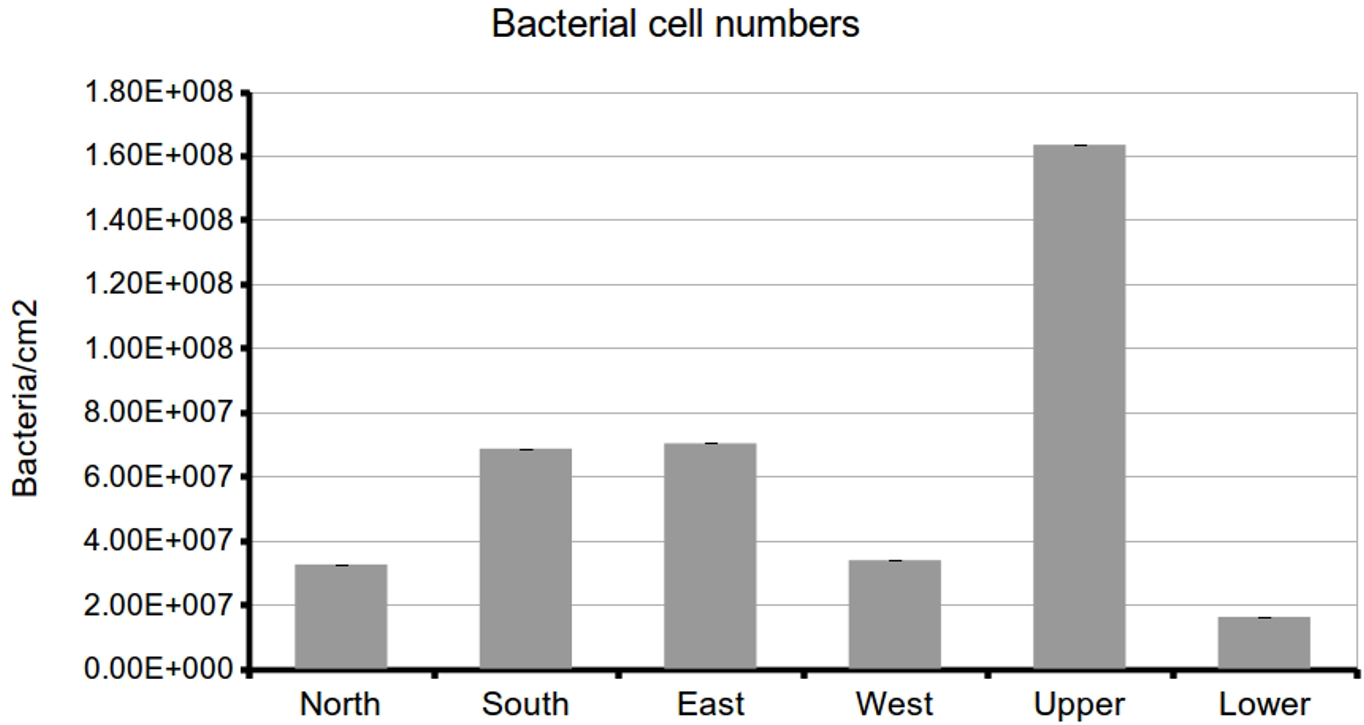
Supplementary figures

Supplementary Figure S1



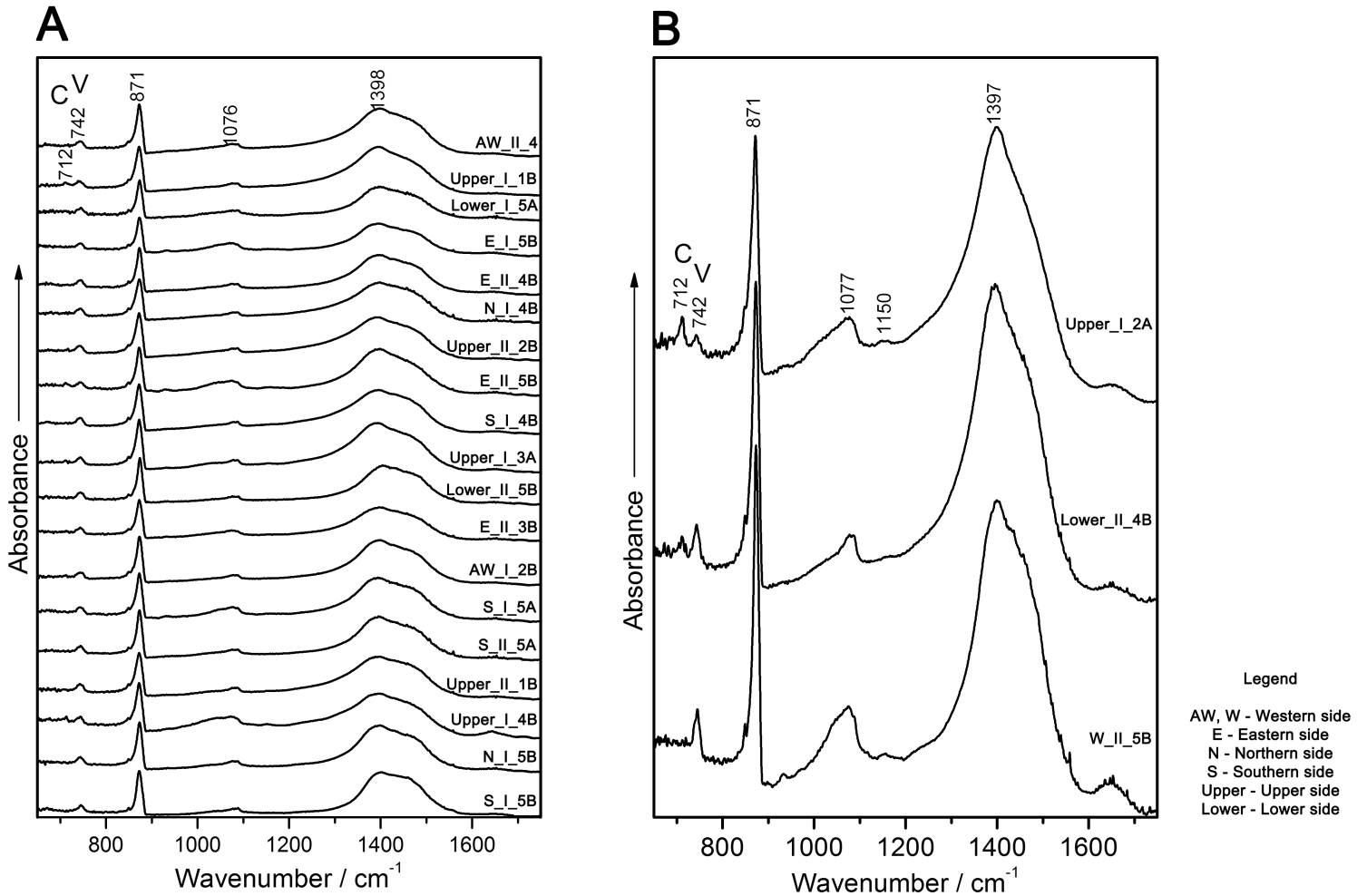
Time variation of the average well colour development in the BIOLOG EcoPates resulted from organic carbon substrate utilization by the microbial communities present on Saint Donatus statue.

Supplementary Figure S2



Bacterial cell numbers/cm² as estimated by quantitative real-time PCR. The sampling sites are represented on the abscissa. The columns represent means and the error bars (black colour) represent s.d. values.

Supplementary Figure S3



FTIR spectra of the crystals recovered from the M-3 agar media recorded on a conventional 4100 Jasco FTIR spectrometer using an ATR sampling device (ZnSe crystal, one reflection). The spectral resolution was 4 cm^{-1} and the number of scans was 32. The samples in the A image were mostly vaterite (V), while the ones from the B image contained predominantly calcite (C).