High bacterial diversity in pioneer biofilms colonizing ceramic roof tiles

Mattea Romani, Claire Carrion, Frédéric Fernandez, Laurent Intertaglia, David Pecqueur, Philippe Lebaron, Raphaël Lami

High bacterial diversity in pioneer biofilms colonizing ceramic roof tiles

Mattea Romani[1], Claire Carrion[2], Frédéric Fernandez[3], Laurent Intertaglia[1,4], David Pecqueur[5], Philippe Lebaron[1], Raphaël Lami[1*]

[1] Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique de Banyuls sur Mer, Avenue Pierre Fabre, 66650 Banyuls-sur-Mer, France.

[2] Université de Limoges, Biologie Intégrative Santé Chimie Environnement (BISCEm), Rue du Docteur Marcland, 87025 Limoges, France.

[3] Université de Montpellier, Microscopie Électronique Analytique (MEA), Place E. Bataillon, 34095 Montpellier, France.

[4] Sorbonne Université, CNRS, Bio2MAR, Observatoire Océanologique de Banyuls sur Mer, Avenue Pierre Fabre, 66650 Banyuls-sur-Mer, France.

[5] Sorbonne Université, CNRS, BioPIC, Observatoire Océanologique de Banyuls sur Mer, Avenue Pierre Fabre, 66650 Banyuls-sur-Mer, France.

Revision: 2 Submission: 19th December 2018

Corresponding author:

* raphael.lami@obs-banyuls.fr

Running title: Bacterial colonization of ceramic roof tiles

To be submitted to International Biodeterioration & Biodegradation

25

26 Declarations of interest: ‘none’

ABSTRACT

Ceramic roof tiles are extremely common building materials that are subjected to the natural phenomenon of biodeterioration, which initially modifies the tile surface and ultimately causes its destruction. The bacterial diversity of the visible biofilm responsible for biodeterioration has been previously examined. In contrast, the early stages of tile colonization and pioneer biofilm growth on these surfaces have been poorly explored. To investigate these pioneering stages of bacterial tile colonization, we combined imagery and conventional culture-based approaches, as well as Illumina-based high-throughput sequencing methods to examine samples collected from unexposed new tiles and tiles that were subjected to few-months outdoor exposure. In all the samples, we observed a pioneering biofilm including a significant bacterial diversity, on both new materials and those subjected to slight exposure, with a total of 279 and 411 different OTUs detected, respectively. This pioneer diversity was dominated by Proteobacteria (more than 50% of the total bacterial diversity) and, at the genus level, by *Sphingomonas* and the genus 1174-901-12 related to the Beijerinckiaceae. Interestingly, the major patterns of the observed bacterial diversity remained similar between samples collected from unexposed and exposed tiles. Collectively, these data clearly indicate the need to focus on the pioneer colonizing bacteria that form the initial biofilm on building materials, which can subsequently lead to mature biofilm formation and visible biodeterioration.

Keywords: Ceramic roof tiles; Biodeterioration; Pioneer biofilms; Bacterial diversity; Illumina-based High-throughput sequencing.

1 INTRODUCTION

Ceramic tiles are traditionally used as building materials. The first tiles were hand molded with dried earth and had a canal-like shape (semicylindrical) (Viollet-le-Duc, 1854). Currently, ceramic tiles are produced industrially, but the traditional canal-like shape has been retained. The use of these tiles remains extremely widespread. For example, clay tiles cover 74% of French roofs (FFTB, 2015). Tiles are commercially available in a wide range of colors and shapes, presenting very different chemical compositions and being produced by very diverse manufacturing processes (Fassier, 2009). Similar to many other types of building materials (bricks, glazed tiles, stones, and concrete) (Saiz-Jimenez, 2001; Warscheid and Braams, 2000), ceramic tiles are exposed to rain, wind, hail, snow, frost, substantial temperature variations, pollution, and other extreme environmental conditions (Coutinho et al., 2015). Therefore, ceramic tiles undergo natural degradation, including biodedeterioration (Guiamet et al., 2013; Warscheid and Braams, 2000). The latter corresponds to vital activities that occur on tiles, such as cell proliferation (i.e., colonization by microorganisms and biofilm formation) and production of acids by diverse types of microorganisms, and cause degradation of the material (Guiamet et al., 2013). Tiles are particularly prone to biodeterioration because of their high porosity (between 19% and 30%, with pores ranging in size from 6 to 7 μm) that makes them one of the most porous building materials (Fassier, 2009; Gazulla et al., 2011). This susceptibility of tiles to colonization by organisms is also called bioreceptivity (Miller et al., 2012).

The major disadvantage of roof tile biocolonization is the resulting unsightly appearance, as the biofilm can be pigmented and can change the color of the roof (Di Martino, 2016). For example, dark or green spots may appear, and the roof may turn darker. In the long term, other adverse effects are observed, as biodeterioration weakens ceramic tiles by forming

deep cracks and increasing the porosity of the tiles (Berdahl et al., 2008; Coutinho et al., 2015). This weakness promotes erosion and wear on the roof due to bad weather and favors colonization by larger organisms such as lichens, plants or bryophytes. Tile damage can also lead to loss of structural and thermal insulation, loss of drainage capacity or solar reflectance (Berdahl et al., 2008; Pena-Poza et al., 2018). Biodeterioration of tiles is therefore the result of biocolonization by a wide variety of micro- and macroorganisms, such as bacteria, fungi, microalgae, plants, bryophytes and lichens (Barberousse et al., 2006; Coutinho et al., 2015; Fassier, 2009; Ortega-Calvo et al., 1995; Warscheid and Braams, 2000). Only the "green part" of the bacterial diversity (i.e. cyanobacteria) present on roof tiles have been extensively studied.

Cyanobacteria were described as primary colonizers of clay tiles (Gazzano et al., 2013) in studies that relied on culture-based diversity inventories (Barberousse et al., 2006; Coutinho et al., 2013; Crispim et al., 2003; Hauer et al., 2015; Macedo et al., 2009; Nowicka-Krawczyk et al., 2014). Among the most frequent cyanobacterial species identified on building materials were *Aphanocapsa* sp., *Calothrix* sp., *Chroococcus* sp. and *Synechococcus* sp. (Hauer et al., 2015; Macedo et al., 2009). In contrast, the diversity of heterotrophic bacteria on building materials remains very poorly studied. To date, only a few works have focused on the cultivable diversity of these bacteria (Krakova et al., 2015; Urzì et al., 2010), revealing the potential importance of *Arthrobacter agilis*, *Bacillus* sp., *Paenibacillus* sp., *Pseudomonas* sp. and *Rhodococcus* sp. (Krakova et al., 2015; Urzì et al., 2010) as pioneer organisms.

All these organisms colonize tiles by forming biofilms, which allow bacteria to grow under extreme conditions, such as those that occur on the tops of roofs (Flemming et al., 2016; Flemming and Wingender, 2010; Mayer et al., 1999). The classic pattern of biofilm development is as follows: biofilm growth is initiated from planktonic (i.e., free-living)

bacteria (Siboni et al., 2007), which adhere to a surface and form microcolonies before the development of a mature biofilm that is embedded in extracellular matrix (ECM), composed notably of polysaccharides, proteins and extracellular DNA (Flemming and Wingender, 2010; Molin and Tolker-nielsen, 2003). The environment created by the ECM allows not only improved tolerance and resistance to stress factors, such as the presence of antibiotics, heavy metals or biocides in the environment, but also improved resistance to desiccation. Moreover, the ECM improves the supply and storage of nutrients via the capture of resources by sorption and degradation of these resources by extracellular enzymes in the surrounding environment. Therefore, in biofilms, organisms have access to nutrients that they could not use outside these biofilms and to microenvironments that are very favorable for their growth (Flemming and Wingender, 2010). Thus, the formation of a mature biofilm from a pioneering biofilm leads to colonization by macro-organisms and is a crucial step in biodeterioration processes.

However, there is a distinct lack of studies that focus on the crucial initial steps of pioneer biocolonization, which determines the conditions for further steps of biofilm development. Indeed, a large majority of current studies focus on advanced cases of biodeterioration, with visible colonization and numerous green or dark spots. Thus, mostly mature biofilms have been studied on building materials, with a focus on the algal diversity present on these surfaces (Coutinho et al., 2013; Di Martino, 2016; Hallmann et al., 2013; Krakova et al., 2015; Ma et al., 2015; Maresca et al., 2017; Tomaselli et al., 2000; Urzì et al., 2010; Warscheid and Braams, 2000). Even though some studies have focused on the diversity of pioneer cyanobacteria present on building materials (Barberousse et al., 2006; Hallmann et al., 2013), the overall diversity of bacteria has hardly been studied (Krakova et al., 2015; Urzì et al., 2010) by either culture-based or culture-independent approaches.

To fill this gap, this study aims to characterize the existence of a pioneering biofilm on new and few exposed ceramic roof tiles (i.e. clay tiles), with a focus on heterotrophic bacterial colonization during the early colonization stages. These stages occur before the appearance of green or dark spots, before the biofilms become visible on the tiles and cause cracking, and before the increase in porosity promotes increased colonization by other organisms. To fully depict the pioneering bacterial diversity of clay tile biofilms, we combined imagery and culture-based investigations with state-of-the-art Illumina-based high-throughput sequencing methods based on non-exposure and 5 or 8 months of exposure.

2 MATERIALS AND METHODS

2.1 Sampling strategy.

Pioneer biofilms were sampled on 3 different types of commercial ceramic roof tiles that are commonly available in the French market and were named T1, T2 and T3 in this study. These tiles were sampled without exposure (new commercial products) and after slight outdoor exposure for 5 or 8 months at two commercial and industrial exhibition sites located in France. The site of exposure (site n°1) was located in Aquitaine. At this location, tiles were exposed with a north orientation, for 8 months. The second site of exposure (site n°2) was located in Burgundy. At this location, tiles were exposed with a south/southwest orientation, for 5 months. The environmental data to characterize both sites were also collected (Table 1) The T1 tiles had a black engobe with water-repellent coating; the T2 tiles had a red engobe with a water-repellent coating; and the T3 tiles had a pale red engobe but no water-repellent coating. These three different types of tiles were chosen to know (i) if the chemical

composition and the color of the tile could affect the bacterial colonization. For example, the color could influence the temperature of the tile, as a black tile will heat up more in summer than a lighter tile. and (ii) if the water-repellent coating protects tiles against biological colonization. This coating is designed to reduce the water permeability of the tile and therefore, by reducing the water available to microorganisms, might slow down biological colonization (Urzì and De Leo, 2007).

Biofilms from all the types of tiles (either new or after slight exposure to the outdoors) were collected in triplicate (i.e. tree tiles of each type were sampled), and for each tile, 4 15 cm x 10 cm areas were sampled. First, the tiles were gently washed with distilled water before sampling. Then, the tiles were sampled using 2 different methods. (i) Two areas on each tile were scraped with a sterile scalpel (Urzì et al., 2010), producing a biofilm and tile powder that were transferred into a sterile tube containing 10 ml of saline water (9 $g.L^{-1}$ NaCl). This suspension was then mixed for 15 s at maximum speed. (ii) Two other areas on each tile were sampled using a sterile swab previously soaked with a buffer solution (saline water (9 $g.L^{-1}$ NaCl) + 1% Triton X-100), a methodology adapted from Grice et al., 2009. This swab was rubbed for 30 s on the tile and placed in a sterile tube with 5 mL of saline water (9 $g.L^{-1}$ NaCl). The samples were then vortexed for 30 s at a maximum speed and stored at 4°C until use.

2.2 Negative controls preparation

For 2.3 to 2.7 experiments, negative controls were prepared with sterile tiles. These ones were autoclaved and systematically included in the experimental procedures. For all fluorescent microscopic approaches, an unstained sample was systematically observed for each type of tile to confirm the specificity of the selected fluorescent probes.

169

170 **2.3 Scanning Electron Microscopy and Energy Dispersive X-ray spectrometry.**

171 SEM and EDS were conducted to characterize the presence of a pioneering biofilm on the
172 studied tiles by direct observations and analyses of elementary composition (carbon in
173 particular). For each type of tile, a small sample (the same as used for fluorescence
174 microscopy) was studied, at the University of Montpellier, Platform MEA, using a SEM (FEI
175 Quanta FEG 200) instrument at an operational acceleration voltage of 15kV and under low
176 vacuum ($3.76e^{-1}$ Torr). Images were obtained using the backscattered electron detector
177 (BSED). Chemical analysis was done using Energy Dispersive X-ray spectrometry (EDS),
178 aluminium (Al) was labelling in yellow, silicon (Si) in red (the two major components of tile)
179 and carbon (C) was labelled in green.

180

181 **2.4 Samples staining**

182 Samples staining was conducted to detect the presence of microbial DNA and biofilm matrix
183 in the studied pioneering biofilms using fluorescent macroscopy, confocal laser scanning
184 microscopy (CLSM) and two-photon microscopy. For each type of tile, a small sample
185 (around 2 cm x 2 cm) was labeled with SYTO 9™ (DNA intercalant, green, Invitrogen™) and
186 FilmTracer™ SYPRO™ Ruby Biofilm Matrix Stain (Polysaccharide labelling, red, Invitrogen™).
187 Tile samples were immersed in the FilmTracer™ SYPRO™ and incubated for 30 minutes, in
188 the dark at room temperature. After incubation, tile samples were rinse 3 times with ultra-
189 pure water. Then, tile samples were incubated in the dark for 15 min with SYTO 9 (10µM) at
190 room temperature and rinse 3 times with TBS (Domenech et al., 2016).

191

2.5 Fluorescence microscopy and macroscopy

All samples were preliminary observed under a CLSM (Confocal SP8-X Leica) with 10X or 20X magnification (Neu and Lawrence, 2014; Schlafer and Meyer, 2017) (Data not shown). Samples were then observed under a CLSM (confocal LSM880 Zeiss) with a 20X air objective, a fluorescent macroscope (AZ100 Nikon) with a 4X air objective and a zoom 8X, and a two-photon microscope (MVX10 FV1000, Olympus) using a 25X water immersion objective. Moreover, CLSM was associated with surface imaging of the tile by using the reflected light on the ceramic. Images were analyzed with Fiji software (Rueden et al., 2017; Schindelin et al., 2019).

2.6 Evaluation of bacterial density by flow cytometry.

Flow cytometry was used to measure bacterial cells abundances in the sampled pioneering biofilms. For each type of tile, one 15 cm x 10 cm area was scraped with a sterile scalpel into a sterile tube containing 1mL of saline water (9 $g.L^{-1}$ NaCl) filtered at 0.2µm. Samples were filtered at 20µm (to remove the larger particles that could clog the cytometer). 200µL of bacterial cells were stained with 1µL of SYBR® Green I (1:100 dilution in DMSO, Lonza) and were incubated at least 15 min in the dark at room temperature (Hammes et al., 2008). Briefly, cells were counted by flow cytometry (CytoFlex, Beckman Coulter) for 60 sec at a flow rate of 60 $\mu L.min^{-1}$, at the Oceanological Observatory of Banyuls sur Mer, Platform BIOPIC. Polycarbonate beads (1.002 µm, Polyscience Europe) were used to normalize cell fluorescence and light scattering values. Cell density was calculated by removing the blank (saline water filtered at 0.2µm).

2.7 In-situ analysis of dehydrogenase activity.

This technique aims to characterize the presence of viable cell in the sampled pioneering biofilms. These assays were performed only on T2 and T3 tiles, due to the black engobe of T1. Briefly, analysis of dehydrogenase activity (revealing cell viability (Gong, 1997) was performed on unexposed tiles following the protocol described by Warscheid et al., 1990. Tile samples were covered by PBS (Thundyil et al., 2013) containing 0.2% of Tetrazolium Red (2,3,5-triphenyl tetrazolium chloride) and incubated in the dark for 24h at 25°C. After incubation, samples were dried 1h at 60°C and pictures were taken (Warscheid et al., 1990). Another negative control was done, tile samples were incubated in PBS only for 24h in the dark at 25°C.

2.8 Isolation of bacterial strains by culture-based techniques.

To depict the bacterial diversity colonizing clay tiles, we conducted isolation and characterization of bacterial strains. The isolation of bacterial strains on tiles followed routine and previously described protocols used to isolate strain on building materials (Puškárová et al., 2016; Urzì et al., 2016). Briefly, for each sample, 100 µL of the solution collected previously was inoculated on Petri dishes containing R2A culture medium (BD Difco) (Puškárová et al., 2016; Urzì et al., 2016) with 100 $mg.L^{-1}$ cycloheximide (Urzì et al., 2016) and 4 $mg.L^{-1}$ benomyl (Lage and Bondoso, 2012) or solid BG11 culture medium (BG11 50× (Sigma Aldrich) supplemented with 15 $g.L^{-1}$ agar) (Rippka et al., 1979; Urzì et al., 2016) with 100 $mg.L^{-1}$ cycloheximide (Urzì et al., 2016). Both culture media were supplemented with sterile red ceramic roof tile powder (15 $g.L^{-1}$) or left untreated. Cultures were maintained at 25°C in the dark for cultures on R2A and under continuous light for cultures on solid BG11 until isolation and identification.

2.9 Identification of isolated microbial strains.

After, bacterial strains were isolated on solid medium, a single colony was transferred to a liquid culture medium (same media as the solid culture without tile powder) and identified using routine protocols based on sequencing of the 16s rRNA genes (Blanchet et al., 2017). Molecular identification of heterotrophic bacteria started with the preparation of a cellular pellet that was obtained from 1 mL of liquid culture after centrifugation (10 000×g, 3 min). Then, the genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions for gram-positive bacteria.

Molecular identification of cyanobacterial strains started with the preparation of a cellular pellet, which was obtained from 1 mL of liquid culture and resuspended in 10 µL of ultra-purified water. The genomic DNA was then extracted using the WB120028 CloneSaver card in 96-well format (FTA card technology, Whatman). After incubation of the CloneSaver card for 1 hour at room temperature, a small disc was punched out and washed 3 times for 5 min with TE buffer (Whatman).

PCR amplification of (i) bacterial 16s rRNA gene fragments was performed using the primer pair 27F (5'-AGRGTTTGATCMTGGCCTCA-3'; 10 µM) and 1492R (5'-TACGGYTACCTTGTTAYGACTT-3'; 10 µM) and (ii) cyanobacterial 16s rRNA gene fragments was performed using the primer pair 106F (5'-CGGACGGGTGAGTAACGCGTGA-3'; 10 µM) and 1313R (5'-CTTCACGTAGGCGAGTTGCAGC-3'; 10 µM). Briefly, the PCR protocol consisted of a denaturation step of 5 min at 95°C; 30 cycles of denaturation (95°C for 15 s), annealing (55°for bacterial 16S and 60°C cyanobacterial 16S for 15 s), and elongation (72°C for 15 s); and a terminal elongation step of 10 min at 72°C. All details of this protocol were previously published in Leboulanger et al., 2017.

All DNA amplification reactions were checked by migration of a genomic DNA aliquot on a 1% agarose gel. Then, the PCR products were purified with the Agencourt AMPure XP Kit following the manufacturer's instructions. The sequencing reaction was performed using the BigDye Terminator technology (Nimagen) with the following protocol: 30 cycles of denaturation (95°C for 10 s), annealing (50 or 60°C for 5 s depending on the previous PCR), and elongation (55°C for 2 min 30 s). The PCR products obtained were cleaned using the Agencourt CleanSeq Kit following the manufacturer's instructions. Sanger sequencing was carried out with the AB3130xl 16 capillary Sanger sequencer (Applied Biosystems) on the Bio2Mar platform (http://bio2mar.obs-banyuls.fr/fr/index.html).

The 16s rRNA gene sequences were cleaned on Snap Gene Viewer 3.3.3 software, and phylogenetic affiliation was assessed on EZBioCloud (http://ezbiocloud.net/). A phylogenetic tree was constructed in MEGA7 by following the K2+G+I model (Kumar et al., 2016). The phylogenetic tree was constructed using FigTree1.4.3 software (http://tree.bio.ed.ac.uk/software/figtree/). All sequences of the identified strains have been submitted to GenBank and have accession numbers MK207390 to MK207433 (Table S1) and maintained in the Banyuls Bacterial Culture Collection (BBCC).

2.10 Evaluation of the biofilm-forming capacity of isolated and identified strains.

A pre-culture of each identified strain was performed in 6mL of R2B. When the cultures reached an optical density (O.D.) of 0.8. Each strain was deposited in a 96-well microplate in triplicate, diluted 1/100 in R2B; as well as a negative control containing only R2B. The 96-well microplate was incubated for 48 hours at 25°C and in the dark. After 48 hours of incubation, the O.D. at 640nm was measured to control bacterial growth. A crystal violet assay was performed to identify strains able to form biofilm, following protocols described in

Blanchet et al., 2017. To achieve this goal, the culture medium was gently removed from the 96-well microplate to avoid damaging the biofilm, and the wells were rinsed with PBS. Then, crystal violet (0.2%) was added to the wells and the microplate was incubated for 15 minutes in the dark. Afterwards, the wells were rinsed 4 times with ultra-pure water, before adding a discoloration solution (10% acetic acid, 50% ethanol). Finally, the O.D. was measured at 540nm. Strains with O.D. > 1.5 were considered to form a high level of biofilm (++), those with 1.5 ≥ O.D. ≥ 0.5 were considered to form biofilm (+), and those with O.D. < 0.5 were considered as non-biofilm forming strains (-).

2.11 Extraction of total DNA for Illumina MiSeq-based high-throughput sequencing.

Tubes containing samples (scraped or swabbed) were centrifuged (15 min, 10 000×g, 25°C), and then, the supernatant was removed. The pellet was resuspended in the lysis solution of the Quick DNA Fungal/Bacterial Miniprep Kit (Zymo Research) and transferred into the BashingBead lysis tubes from the kit. The kit was used in accordance with the manufacturer's instructions with the addition of beta-mercaptoethanol to the genomic lysis buffer for a final dilution of 0.5% (v/v) as recommended. The manufacturer's instructions were followed with the exception of the first step, where vortexing was replaced with the use of the FastPrep-24 5G homogenizer (2 times, 45 s, 6 $m.s^{-1}$) (MP Biomedicals) (Coutinho et al., 2013; Jroundi et al., 2015). Finally, the extracted DNA was quantified with a Quantus fluorometer (Promega) using the Picogreen Low program.

2.12 Identification of bacterial communities with Illumina-based high-throughput sequencing.

Illumina-based high-throughput sequencing of 16s rRNA gene fragments was performed using MR DNA (Shallowater, TX, USA; http://www.mrdnalab.com). Briefly, the V4 variable region of the 16s rRNA gene was amplified with the PCR primers 515R/806F, which included a barcode on the forward primer. A 30-cycle PCR was conducted using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. After amplification, PCR products were checked by 2% agarose gel electrophoresis to determine whether amplification had been successful and measure the relative intensities of the bands. Multiple samples were pooled together in equal proportions based on their molecular weights and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then, the pooled and purified PCR products were used to prepare the DNA library by following the Illumina TruSeq DNA library preparation protocol. Sequencing was performed on a MiSeq following the manufacturer's guidelines.

The obtained FASTQ files (BioProject ID: PRJNA506643, accession numbers: SAMN10465566 to SAMN10465585) were analyzed with QIIME (1.9.1) (Caporaso et al., 2010), USEARCH v11 (Edgar, 2018, 2010) and the Brazilian Microbiome Project scripts (Pylro et al., 2014). Using QIIME, the mapping files were validated to ensure correct formatting. The barcodes were extracted from the FASTQ files, and those files were demultiplexed. Using USEARCH v11 and the scripts published by the Brazilian Microbiome Project, quality filtering based on the characteristics of each sequence was performed to remove any low-quality or ambiguous reads. Then, the files were concatenated and dereplicated. Chimeras

were identified and removed; the sequences were denoised, and the zero-radius operational taxonomic units (ZOTUs) were defined with the unoise3 command from USEARCH v11 software (Edgar, 2016). Then, a representative sequence of each ZOTU based on 97% similarity of the 16s rRNA gene sequence, named operational taxonomic units (OTU), was selected and taxonomically assigned based on the Silva 132 database (Quast et al., 2013; Yilmaz et al., 2014). The representative sequences were aligned and filtered before the production of a table of OTUs, and sequences affiliated with chloroplasts and mitochondria were removed from the table according to previously published studies (Bokulich et al., 2017). Bacterial communities were analyzed for taxonomic composition using the command summarize_taxa_trough_plots.py. Alpha diversity indexes (observed richness, Chao 1, and Shannon diversity index) were calculated and statistically analyzed with the Phyloseq R package (1.26.0) on RStudio (1.1.463) with R 3.5.0. Venn diagrams were created from the table.biom (obtained during bioinformatic data processing) corresponding to each category of interest (tile type and exposure) and using the following website: http://bioinformatics.psb.ugent.be/webtools/Venn/. Samples were processed according to tile type and weathering step.

In addition, in order to extract sub-specific information from our dataset, the FASTQ files were also analyzed with the R package DADA2 (Divisive Amplicon Denoising Algorithm) (1.10.0) (Callahan et al., 2016). This methodology rely on a statistical modeling approach to denoise raw sequencing reads and to infer the abundance of “biological variants”. First, the set of Illumina-sequenced paired-end FASTQ files from which the barcodes/adapters, were removed by the sequencing platform were demultiplexed and split by sample with QIIME (1.9.1) before being analyzed using the online pipeline published by the authors of the DADA2 R package (https://benjjneb.github.io/dada2/tutorial.html). The end product of this

pipeline is an amplicon sequence variant (ASV) table, a higher-resolution table compared to the OTU table previously obtained, and which records the number of times each amplicon sequence variant was observed in each sample. In this approach, the taxonomic assignation process was based on the Silva 132 database. Finally, the obtained data were represented with the phyloseq (1.26.0) and ggplot2 (3.1.0) R packages on RStudio (1.1.463). All data from the DADA2 analysis are presented as supplementary data and supplementary figures S3 to S6.

3 RESULTS

3.1 Evaluation of the presence of a pioneering biofilm

The presence of a pioneering biofilm was evidenced combining different imaging techniques on unexposed tiles (Figure 1). First, under SEM, dark spots could be seen (white circle and arrows, Figures 1A, 1B, SEM). They appeared blurred, like a matrix (Figure 1B, SEM). With retro-diffused imaging, these dark spots appeared richer in light elements than the rest of the tile, probably due to the presence of carbon and organic matter. EDS was achieved to confirm that dark spots were effectively richer in carbon than the rest of the tile (Figures 1C, 1D, EDS). In this sense, these dark spots appeared to be the first evidence of the presence of a pioneering biofilm on all sampled tiles. Then, several fluorescent microscopy approaches were combined (CLSM and two-photon microscopy) to observe the presence of cells and matrix on those tiles (Figure 1) and to confirm the existence of such pioneering biofilms . On tiles, clusters of cells were shown up in green (SYTO9) (Figure 1E, fluorescent macroscopy). The observed biofilms were very thin and sparse. Nevertheless, CLSM of the fluorescent dye associated with reflection imaging of the tile allowed to show more precisely the location in

a 3D-scale of the cell clusters in green (SYTO9) (Figures 1F, CLSM). Cells were also frequently observed in the anfractuosity of the tile (Figures 1F, CLSM). Finally, two-photon microscopy combining DNA dye in green (SYTO9) and matrix dye in red (FilmTracer™ SYPRO™) evidenced that clusters of cells were surrounded and embedded in a matrix (Figures 1G, 1H, two-photon microscopy). Also, the plot Z-axis profiles of SYTO 9™ staining (Figure 1I) and FilmTracer™ SYPRO™ staining (Figure 1J) showed that the maximum of fluorescence was located at 46µm from the surface of the tile for the SYTO9 and that the thickness of the matrix ranged between 10 and 126µm. Those profiles reinforced the images evidencing that cells were embedded in a biofilm matrix. These techniques were completed by TTC assays and flow cytometry measures (Supp. Data).

3.2 Evaluation of biofilm growth

For each type of tile, total extracted DNA was quantified (Figure 2). Total extracted DNA corresponded all DNA present on the tiles and therefore to the biofilm as a whole, including heterotrophic bacteria but also all other colonizing microorganisms. A significant increase in the amount of DNA collected was systematically observed after the exposition of tiles. For T1 tiles, an average of 97 $pg.cm^{-2}$ (±88) and of 688 $pg.cm^{-2}$ (±378) of DNA were extracted before and after 8 months exposure, respectively. Similarly, for T2 tiles and T3 tiles, respectively 7.5 $pg.cm^{-2}$ (±4.6) and 5.7 $pg.cm^{-2}$ (±6) of DNA were extracted before exposure, while 149.8 $pg.cm^{-2}$ (±3.3) and 143.6 $pg.cm^{-2}$ (±33.1) of DNA were extracted after 5 months exposure. In addition, the capacity of isolated strains to form biofilm was evaluated using a classical crystal violet-based assay (Table 2). It revealed that 12 of the 41 isolated and tested strains were able to form a large biofilm. Two of these strains were isolated on unexposed

tiles; 2 strains were isolated on tiles exposed 5 months and 8 strains isolated on tiles exposed 8 months (Table 2).

3.3 Bacterial diversity among cultivated and isolated strains from unexposed tiles (new tiles).

3.3.1 Culturable bacterial diversity from unexposed tiles (new tiles).

A total of 9 strains were isolated from new and unexposed tiles and identified based on their 16s rRNA gene sequences (Table 3). Only bacteria present on the T1 tiles could be isolated, and all of these bacteria were affiliated with the phylum Firmicutes under the genus *Bacillus*. The potential species identified were *B. safensis* (100.00% and 99.88% 16s rRNA similarity with the closest known isolate), *B. circulans* (100.00%) and *B. simplex* (99.88%).

3.3.2 Culturable bacterial diversity from exposed tiles.

More bacterial strains (37 strains) were isolated, cultured and identified from the exposed tiles than from the unexposed ones (Table 3). These strains were assigned to 5 different phyla, including Firmicutes (8 strains), Cyanobacteria (2 strains), Bacteroidetes (1 strain), Proteobacteria (8 strains) and Actinobacteria (18 strains). In addition, there were some common potentially identified species between the different types of tiles, including *Bacillus simplex* (2 strains), *Rhodococcus corynebacterioides* (4 strains), *Arthrobacter agilis* (2 strains) and *Mesorhizobium alhagi* (4 strains). All these strains presented at least 98.58% 16s rRNA gene similarity with the closest known isolate. As noted above, 2 strains of phototrophic microorganisms (cyanobacteria) were isolated on T1 tiles and identified as being closely

related to the cyanobacterial clone 3GSG3R_K29 (accession number: JX127188, 99% 16s rRNA gene similarity) (Hallmann et al., 2013).

3.4 Diversity of bacterial phyla and species identified by Illumina-based high-throughput sequencing on unexposed tiles.

3.4.1 Diversity of the bacterial phyla identified on unexposed tiles.

The bacterial diversity observed on all the unexposed tiles was very similar regardless of the type of sampled tile (Figure 3). Indeed, the 3 types of tiles were largely colonized by Proteobacteria, which represented between 40 and 70% of the total observed OTU (97% of 16s rRNA gene similarity) diversity (Figure 3). The Alphaproteobacteria class was dominant and represented more than 50% of the Proteobacteria colonizing tiles (Supplementary Figure S1). The OTUs affiliated with Actinobacteria represented between 8 and 15% (on average 11.3 +/- 3.7); Firmicutes were between 9 and 13% (on average 11.5 +/- 2.2); and Bacteroidetes were between 3 and 12% (on average 7.2 +/- 4.3) (Figure 3). Among the slight differences that were observed between the different types of collected tiles, notably, it was possible to identify some sequences related to Deinococcus-Thermus (also named [Thermi]) from both T2 and T3 tiles (8% of the OTUs). In contrast, only 0.5% of the OTUs were identified as Deinococcus-Thermus from T1 tiles.

3.4.2 Diversity of bacterial species identified on new (unexposed) tiles.

Our results show that the unexposed new tiles were colonized by large consortia of phylogenetically diverse bacteria, with 97% 16s rRNA gene similarity. A total of 145 OTUs (97% of 16s rRNA gene similarity) were identified on T1 tiles, 294 OTUs on T2 tiles and 290

OTUs on T3 tiles. Very few OTUs represented more than 1% of the total number of sequences. In fact, only 19 clustered OTU sequences represented more than 1% of the sequences for T1 tiles, 17 OTUs for T2 tiles and 20 OTUs for T3 tiles (Figure 4). Nevertheless, these few OTUs represented a large proportion of the bacterial diversity present on unexposed tiles: 70% of the bacteria identified on T1 tiles, 55% on T2 tiles and 68% on T3 tiles.

Only 8 OTUs belonging to 3 different phyla were common to the 3 types of tiles. These OTUs were affiliated with *Corynebacterium* sp. (Actinobacteria), *Staphylococcus* sp. (Firmicutes), *Streptococcus* sp. (Firmicutes), the genus 1174-901-12 related to the Beijerinckiaceae (Alphaproteobacteria), *Methylobacterium* sp. (Alphaproteobacteria), *Sphingomonas* sp. (Alphaproteobacteria), *Acidiphilum* sp. (Alphaproteobacteria), and *Acinetobacter* sp. (Gammaproteobacteria). Notably, these 8 OTUs represented a large proportion of the bacterial diversity on the colonized tiles, constituting between 26% and 41% of the bacterial diversity identified on the 3 types of tiles.

3.5 Diversity of bacterial phyla and species identified by Illumina-based high-throughput sequencing on exposed tiles.

3.5.1 Diversity of the bacterial phyla identified on tiles exposed to the outdoors.

The exposed tiles also showed colonization by similar major bacterial phyla (Figure 3) despite exposures at different sites and under different climatic conditions (Table 1). Site n°1 is located in an area of oceanic climate with reduce marine influences. This site is very humid. Site n°2 is located in an area of semi-continental climate, a less humid area but with a lot of fog in winter and heavy rainfall in spring (the seasons during which the tiles were

474 exposed) (Table 1). All the exposed tiles were mainly colonized by Proteobacteria (between
475 45 and 57% of all OTUs (97% of 16s rRNA gene similarity) (on average 51 +/- 5.8),
476 Actinobacteria (between 6 and 14%, on average 11 +/- 4.3) and Bacteroidetes (between 8
477 and 13%, on average 8.7 +/- 3.4). After exposure, the T1 tiles showed a much lower
478 percentage of Firmicutes than the unexposed tiles (9% versus 0.2%). The T1 tiles also
479 exhibited significant colonization by cyanobacteria (7.5% of total OTUs), whereas these
480 bacteria constituted only between 0.3 and 1.1% of all the OTUs observed on the T2 and T3
481 tiles. Despite differences in exposure conditions, compositions and colors, the tiles appear to
482 be colonized by very similar bacterial phyla. Alphaproteobacteria was present in abundance
483 (approximately 50% of the Proteobacteria) on all the types of tiles. However, this proportion
484 varied slightly from tile to tile (on average 30% +/- 7). The abundance of
485 Gammaproteobacteria did not vary much (on average 21% +/- 6). Deltaproteobacteria were
486 present at a very low percentage (between 0.2 and 0.7%, on average 0.4% +/- 0.3).
487
488 **3.5.2 Diversity of bacterial species identified on tiles exposed to the outdoors.**
489 Similar to unexposed tiles, the short-term exposed tiles were also colonized by large
490 bacterial consortia. On T1 tiles, 201 OTUs (97% of 16s rRNA gene similarity) were identified,
491 323 OTUs on T2 tiles and 364 OTUs on T3 tiles. Of all these OTUs identified, only a few
492 constituted more than 1% of the total sequences: 17 OTUs on T1 tiles, 17 on T2 tiles, and 20
493 on T3 tiles (Figure 5). However, as seen before exposure to the outdoors, these few OTUs
494 represented a large proportion of the diversity, constituting 82% of the total diversity
495 present on T1 tiles, 52% on T2 tiles and 48% on T3 tiles. Among these abundant OTUs,
496 *Hymenobacter* sp. (Bacteroidetes) and *Sphingomonas* sp. (Alphaproteobacteria) were
497 common to all the types of tiles. Furthermore, a substantial proportion of Cyanobacteria on

498 T1 tiles was reported (Figure 5A), comprising exclusively OTUs affiliated with
499 Chroococcidiopsaceae (also known as Chroococcidiopsidaceae).
500
501 **3.6 Comparison of bacterial diversity between unexposed and exposed tiles.**
502
503 Alpha diversity indexes (Chao1, Shannon and observed richness) were calculated for the
504 different types of tiles and exposure conditions studied (Table 4). Bacterial diversity was
505 confirmed to be high on all the types of tiles studied and under all exposure conditions. The
506 total observed richness was between 150 and 353 (on average 261 +/- 80); the Chao1 index
507 was between 265 and 423 (on average 343 +/- 68); and the Shannon index (H) was between
508 5.4 and 7.2 (average: 6.1 +/-1.0) (Table 4). However, no significant difference could be
509 shown between any type of diversity index and any type of tile or exposure condition
510 (nonparametric *t*-test using Monte Carlo permutations to calculate the *p*-value; *p*-value >
511 0.05). However, Bray-Curtis based nMDS (Non-metric multidimensional scaling) analysis of
512 our different samples showed two distinct groups: tiles exposed 0 and 5 months on one side
513 and tiles exposed 8 months on the other (Supplementary Figure S3). In the long term, the
514 bacterial diversity on tiles could therefore change.
515 When considering the total observed diversity in our samples, we identified 452 different
516 OTUs (97% of 16s rRNA gene similarity). A large fraction of this diversity did not vary
517 substantially from sample to sample. In fact, a total of 181 OTUs were common to all sample
518 types examined in this study, representing 40% of all the observed OTUs (Figure 6). New
519 unexposed tiles shared 92% of their OTUs with exposed tiles (after 5 months and 8 months).
520 Unexposed T1 tiles presented 145 identifiable OTUs, while a total of 201 OTUs were
521 identified on exposed tiles (Figure 7A). Among these OTUs, 121 were shared between

522 unexposed and exposed tiles, representing a large proportion of the total diversity. In fact,
523 unexposed T1 tiles shared 83% of their diversity with exposed T1 tiles. The exposed T1 tiles
524 therefore shared 60% of their diversity with the unexposed T1 tiles. After exposure,
525 Firmicutes, Acidobacteria and Armatimonadetes presented decreased proportions
526 compared to the whole community, while Bacteroidetes, Cyanobacteria and Deinococcus-
527 Thermus exhibited increased abundances, as described previously.
528 The unexposed T2 tiles had 294 identifiable OTUs, while 323 OTUs were identified on the
529 exposed tiles (Figure 7B). Of these OTUs, 230 were shared between the exposed and
530 unexposed tiles. Indeed, the unexposed T2 tiles shared 78% of their diversity with the
531 exposed T2, and the exposed T2 tiles shared 71% of their diversity with the unexposed T2
532 tiles. After exposure, Acidobacteria, Actinobacteria, Bacteroidetes and Deinococcus-Thermus
533 represented a decreased proportion of the community as a whole, while the proportion of
534 Verrucomicrobia increased, as described above.
535 On unexposed T3 tiles, 290 OTUs were identified, while 364 OTUs were identified on
536 exposed T3 tiles (Figure 7C). Of these OTUs, 248 were common to both exposed and
537 unexposed tiles, representing a large proportion of the total diversity. In fact, the unexposed
538 T3 tiles shared 86% of their diversity with the exposed T3 tiles. After exposure,
539 Betaproteobacteria, Bacteroidetes and Deinococcus-Thermus represented a decreased
540 proportion of the community as a whole, while the proportion of Actinobacteria increased.
541
542
543 # 4 DISCUSSION
544 Biological colonization of clay roof tiles is a problem for both building owners and tile
545 manufacturers. Disruption of these biofilms constitutes an important scientific challenge and

546 has economic value. Biocides such as quaternary ammonium compounds or isothiazolinones
547 as well as water repellents are currently used to combat this colonization (Urzì and De Leo,
548 2007; Zhang et al., 2015). However, these solutions are costly, and the impact of these
549 materials on human health and the environment remains poorly understood (Zhang et al.,
550 2015). In addition, after these treatments, all types of building materials are rapidly
551 recolonized by microorganisms, which can be resistant to these treatments (Urzì and De Leo,
552 2007). Such recolonization is rapid, and a few months is frequently sufficient to induce
553 visible growth (Coutinho et al., 2015; Pozo-Antonio et al., 2016; Urzì et al., 2016; Urzì and De
554 Leo, 2007).

555 To date, most studies have focused on the "green" portions of the biofilms present
556 on ceramic tiles or other types of building materials, i.e., cyanobacteria and algae
557 (Barberousse et al., 2006; Crispim and Gaylarde, 2005; Hauer et al., 2015; Macedo et al.,
558 2009). Many algal and cyanobacterial species have been identified in these studies, such as
559 *Chlorella* sp. (Coutinho et al., 2015), *Klebsormidium* sp. (Macedo et al., 2009; Vázquez-Nion
560 et al., 2016), *Chroococcus* sp. and *Synechococcus* sp. (Hauer et al., 2015; Macedo et al.,
561 2009). In contrast, only a few studies examined the presence of heterotrophic bacteria or
562 fungi, and these studies were not focused on ceramic roof tiles. Such studies usually rely on
563 culture-based approaches to examine prokaryotic diversity (Krakova et al., 2015; Urzì et al.,
564 2010; Vázquez-Nion et al., 2016). The use of high-throughput molecular approaches provides
565 new and interesting insights into bacterial diversity, and we also focused on early steps of
566 colonization, at which stage bacterial diversity is more likely to play a key role in biofilm
567 settlement (Siboni et al., 2007).

568 Our study showed that a pioneering biofilm was present on new and few exposed
569 tiles. Thus, even before outdoor exposure, microorganisms colonize the tiles in more or less

570 developed and voluminous patches. Combining different and complementary technical
571 approaches, (CLSM, fluorescent macroscopy, two-photon microscopy, SEM, EDS, TTC assays,
572 flow cytometry) we revealed that this pioneering biofilm was present and composed of
573 viable cells. Indeed, we observed dark spots using SEM composed of carbon, as revealed by
574 an EDS analysis. Complementary observations based on fluorescent macroscopy, confocal
575 microscopy and two-photon microscopy highlighted the presence of DNA and of a thick
576 matrix as dyed using green and red fluorescent compounds. An heterogeneity (in terms of
577 fluorescence and aspect) of the observed matrix was also found. Such heterogeneity could
578 be explained by the high diversity of colonizing bacteria and the diversity of biofilm
579 producers, which was confirmed by testing the capacity of isolated strains to produce (or
580 not) biofilm using a classical crystal violet assay. Nevertheless, all our data collectively depict
581 the presence of a pioneering biofilm on new and few exposed tiles.

582 Our study revealed that the ceramic roof tiles were colonized by a wide diversity of
583 heterotrophic bacteria. From a total of 452 different OTUs identified (97% of 16s rRNA gene
584 similarity), 379 OTUs were detected and identified on unexposed tiles, and 411 OTUs were
585 detected on tiles exposed to the outdoors for 5 or 8 months. All the major phyla were found
586 to be colonizers of ceramic roof tiles. In addition, a high subspecific diversity was detected
587 with a total of 3 943 ASVs or "biological variants". Moreover, the diversity indexes (Chao1,
588 Shannon (H), observed OTUs) were quite high, even on new tiles, also revealing the
589 importance of bacterial diversity during the first steps of clay tile biological colonization.

590 Interestingly, this significant bacterial diversity was detected on all the tiles studied.
591 Our study clearly indicates that *Sphingomonas* sp. and the genus 1174-901-12 related to the
592 Beijerinckiaceae (assigned by BLAST as uncultured bacterial RNA by 16S rRNA partial
593 sequencing; clone: B0610R003_F24 (accession number: AB657476)) were the most common

OTUs found on all the types of studied tiles during the early steps of tile colonization. Interestingly at the subspecific-level, the ASVs assigned to the genus 1174-901-12 (Beijerinckiaceae) was the most commonly found ASVs on all the types of tiles. To the best of our knowledge, the family Beijerinckiaceae has never been previously identified on tiles via culture-based methods, which was not the case for the bacterial genus *Sphingomonas* depicted in Coutinho et al. (2015). We propose here the use of these microorganisms as prokaryotic models of pioneering bacteria for the study of biofilm development on building materials. Our result provides new insights into the importance of bacterial diversity in the development of biofilms responsible for tile biodeterioration.

Interestingly, the observed total bacterial specific and subspecific diversity was relatively similar between tiles despite differences in exposure conditions (as revealed by Table 1) and tile composition, as shown by the calculated diversity indexes. Indeed, the same phyla were detected on tiles at rather similar percentages, with a predominance of Proteobacteria, which represented approximately 50% of the bacterial diversity on each type of tile. Taxonomic analysis at the OTU level (97% of 16s rRNA gene similarity) revealed that the tiles were mainly colonized by Alphaproteobacteria, representing approximately 30% of the bacterial diversity on each tile. Also, an important pattern of tile colonization is that heterotrophic bacteria predominate over Cyanobacteria. However, cyanobacterial colonization was slightly different between tiles. Indeed, this phylum represented less than 2% of the bacterial diversity on most of the tiles studied, except on T1 tiles, where cyanobacteria represented approximately 7.5% of the total bacterial diversity. Interestingly, T1 tiles were exposed from summer (extreme conditions: high temperatures and high UV intensity, no rainfall) to winter (low UV intensity, frost), with more unfavorable growth conditions compared to T2 and T3 tiles, which were exposed from winter to spring (higher

618 temperature, medium UV intensity, rainfall but no extreme conditions). Such observations
619 indicated that the “green” portion of tile biodeterioration should not lead to the exclusion of
620 the very significant diversity of non-green organisms when modeling pioneering biofilms.
621 Our study also provided new insights into the bacterial diversity of colonized clay tiles
622 after only a few months of outdoor exposure. Interestingly, the T2 and T3 tiles presented
623 almost identical colonization profiles either in OTUs and ASVs based analyses, comprising 45-
624 55% Proteobacteria, 13-19% Firmicutes, 6-13% Actinobacteria and 6-8% Bacteroidetes. The
625 T1 tiles, which were subjected to different exposure conditions compared to T2 and T3 tiles,
626 presented a slightly different type of colonization profile. Even though the T1 tiles were
627 largely colonized by Proteobacteria, similar to the T2 and T3 tiles, these tiles were colonized
628 by Cyanobacteria and hardly colonized by Firmicutes. Interestingly, the T2 and T3 tiles were
629 distinguished by differences in coating, i.e., with the presence and the absence, respectively,
630 of a water-repellent cover and two different types of engobes. However, the T2 and T3 tiles
631 were subjected to exactly the same type of outdoor exposure. Our results show that these 2
632 types of tiles shared 276 OTUs from a total of 323 OTUs and 364 OTUs, respectively. This
633 result indicates that these tiles shared approximately 80% of the colonizing bacterial
634 diversity. Additionally, the diversity indexes were not significantly different between these 2
635 types of tiles. Thus, the chemical composition of ceramic tiles did not appear to have an
636 important impact on the colonizing bacterial diversity. Further sampling and comparison will
637 have to be conducted to confirm this important observation. In addition, after exposure, the
638 T2 and T3 tiles showed colonization by original bacteria such as SAR11 and *Vibrio* sp. which
639 are aquatic bacteria. Previous studies have shown that this type of bacteria has already been
640 found in the atmosphere, fog or rainwater (Dueker et al., 2012; Gandolfi et al., 2013; Hu et
641 al., 2018; Woo et al., 2013). The presence of these original bacteria could therefore be

642 explained by the heavy rainfall in April and May on site 2. Similarly, the presence of SAR11
643 on unexposed T1 and T3 tiles could be explained by its storage in a wet or rainy area.
644 Our cultivation approaches revealed the presence of bacteria that were previously
645 identified in various studies. Indeed, the strains *Arthrobacter agilis*, *Bacillus simplex*,
646 *Rhodococcus corynebactroides* and *Rhodococcus facians*, which were identified based on
647 their 16s rRNA gene sequences, were previously isolated from the walls of Paleolithic caves
648 (Urzì et al., 2010), catacombs (Krakova et al., 2015; Urzì et al., 2010), ceramic tiles (Coutinho
649 et al., 2015), and bricks (Coutinho et al., 2015). Such observations provide support and
650 strength to our sampling methods. From a wider perspective, this knowledge of the
651 pioneering bacterial diversity of colonized tiles provides insights for identification of new and
652 innovative targets to combat the early biofilms that develop on clay roof tiles. The most
653 abundant bacteria identified in this study, such as *Sphingomonas* sp. or members related to
654 the Beijerinckiaceae, are known to exhibit very diverse physiological activities that could
655 affect tile deterioration process. These bacteria are able to grow under a wide range of
656 conditions, exhibiting growth between 15 to 55°C and at pH values between 3 and 14
657 (Rosenberg et al., 2014). Many *Sphingomonas* species are known to produce
658 exopolysaccharide compounds that are involved in biofilm formation (Pollock, 1993) and are
659 also known to degrade xenobiotic and recalcitrant compounds (Bending et al., 2003), leading
660 to potential resistance against some treatments (Rosenberg et al., 2014). *Sphingomonas*
661 species are also known to be closely associated to filamentous cyanobacteria, as they could
662 play a role in nitrogen assimilation (Urzì et al., 2010). Moreover, Beijerinckiaceae-related
663 genera are known to produce acids that could potentially play a key role in the
664 biodeterioration of tiles (Rosenberg et al., 2014; Urzì et al., 2010). In addition,
665 Staphylococcus, Streptococcus and Corynebacterium were identified among the dominant

OTUs. The presence of these bacteria might be due to human manipulations (manufacture, storage, installation of tiles on roofs). Such observation suggests that human manipulation might play an important role in the formation of pioneering biofilms on tiles. Indeed, this type of bacteria has already been found on tiles with much longer exposures (Jardak et al., 2017).

Collectively, our results highlight the presence of a pioneer biofilm developing on new and few outdoor exposed tiles. We depict a significant diversity of the bacteria that colonize roof tiles as pioneer organisms. These results provide new insights into the biofilm settlement dynamics that are responsible for roof tile biodeterioration.

5 ACKNOWLEDGMENTS

We thank the imagery core facility of BISCEm (Université de Limoges) and Platinum (Xlim institute) for CLSM imagery, fluorescent macroscopy and the two-photon microscopy. We also thank the imagery core facility of MEA (Université de Montpellier) for SEM imagery and EDS analyses. Also, we acknowledge the BIOPIC platform (Sorbonne Université, Observatoire Océanologique de Banyuls-sur-Mer), for their help in flow cytometry and for the preliminary CLSM imagery assays. The authors also thank Dr. Nyree West and the Bio2MAR platform (Sorbonne Université, Observatoire Océanologique de Banyuls-sur-Mer), for their support in Illumina-based high-throughput sequencing data analysis. This work was funded by the Sorbonne University Grant n°C17/2072-P16/0888

6 REFERENCES

Barberousse, H., Lombardo, R.J., Tell, G., Couté, A., 2006. Factors involved in the colonisation of building façades by algae and cyanobacteria in France. Biofouling 22, 69–77.

https://doi.org/10.1080/08927010600564712

Bending, G.D., Lincoln, S.D., Sørensen, S.R., Morgan, J.A.W., Aamand, J., Walker, A., 2003. In-field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative Sphingomonas spp. and soil pH. Appl. Environ. Microbiol. 69, 827–834. https://doi.org/10.1128/AEM.69.2.827

Berdahl, P., Akbari, H., Levinson, R., Miller, W.A., 2008. Weathering of roofing materials – An overview. Constr. Build. Mater. 22, 423–433. https://doi.org/10.1016/j.conbuildmat.2006.10.015

Blanchet, E., Prado, S., Stien, D., Oliveira da Silva, J., Ferandin, Y., Batailler, N., Intertaglia, L., Escargueil, A., Lami, R., 2017. Quorum sensing and quorum quenching in the mediterranean seagrass Posidonia oceanica microbiota. Front. Mar. Sci. 4, 1–10. https://doi.org/10.3389/fmars.2017.00218

Bokulich, N.A., Chung, J., Battaglia, T., Henderson, N., Li, H., Lieber, A., Wu, F., Perez-perez, G.I., Chen, Y., Zheng, X., Contreras, M., Dominguez-bello, M.G., 2017. Antibiotics, birth mode, and diet shape microbiome maturation during early life. HHS Public Access 8. https://doi.org/10.1126/scitranslmed.aad7121.Antibiotics

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2 : High-resolution sample inference from Illumina amplicon data. Nat. Methods 13. https://doi.org/10.1038/nmeth.3869

Caporaso, J.G., Kuczynski, J., Stombaugh, J., William, A.W., González, A., Knight, R., 2010. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr Protoc Bioinforma. 1–28. https://doi.org/10.1002/0471250953.bi1007s36.Using

Coutinho, M.L., Miller, A.Z., Gutierrez-Patricio, S., Hernandez-Marine, M., Gomez-Bolea, A., Rogerio-Candelera, M.A., Philips, A.J.L., Jurado, V., Saiz-Jimenez, C., Macedo, M.F.,

2013. Microbial communities on deteriorated artistic tiles from Pena National Palace (Sintra, Portugal). Int. Biodeterior. Biodegrad. 84, 322–332. https://doi.org/10.1016/j.ibiod.2012.05.028

Coutinho, M.L., Miller, A.Z.Z., Macedo, M.F., 2015. Biological colonization and biodeterioration of architectural ceramic materials: An overview. J. Cult. Herit. 16, 759–777. https://doi.org/10.1016/j.culher.2015.01.006

Crispim, C.A., Gaylarde, C.C., 2005. Cyanobacteria and biodeterioration of cultural heritage: A review. Microb. Ecol. https://doi.org/10.1007/s00248-003-1052-5

Crispim, C.A., Gaylarde, P.M., Gaylarde, C.C., 2003. Algal and cyanobacterial biofilms on calcareous historic buildings. Curr. Microbiol. 46, 79–82. https://doi.org/10.1007/s00284-002-3815-5|10.1007/s00284-002-3815-z

Di Martino, P., 2016. What about biofilms on the surface of stone monuments. Open Conf. Proc. J. 7, 14–28. https://doi.org/10.2174/2210289201607022001 4

Domenech, M., Pedrero-vega, E., Prieto, A., García, E., 2016. Evidence of the presence of nucleic acids and β -glucan in the matrix of non-typeable Haemophilus influenzae in vitro biofilms. Sci. Rep. 1–13. https://doi.org/10.1038/srep36424

Dueker, M.E., Weathers, K.C., Juhl, A.R., Uriarte, M., Observatory, D.E., 2012. Coupling of fog and marine microbial content in the near-shore coastal environment. Biogeosciences 9, 803–813. https://doi.org/10.5194/bg-9-803-2012

Edgar, R.C., 2018. Accuracy of taxonomy prediction for 16S rRNA and fungal ITS sequences. PeerJ 1, 1–29. https://doi.org/10.7717/peerj.4652

Edgar, R.C., 2016. UNOISE2 : improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST.

738 Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461
739 Fassier, M., 2009. Interactions entre l'environnement et la terre cuite. Université de
740 Limoges.
741 FFTB, 2015. Fédération Française Tuiles et Briques [WWW Document]. URL
742 http://www.fftb.org/wp-
743 content/uploads/2017/04/FFTB_COLLECTIVITES_2015_WEB.pdf (accessed 7.22.18).
744 Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633.
745 https://doi.org/10.1038/nrmicro2415
746 Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016.
747 Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575.
748 https://doi.org/10.1038/nrmicro.2016.94
749 Gandolfi, I., Bertolini, V., Ambrosini, R., Bestetti, G., Franzetti, A., 2013. Unravelling the
750 bacterial diversity in the atmosphere. App Microbiol Biotechnol 4727–4736.
751 https://doi.org/10.1007/s00253-013-4901-2
752 Gazulla, M.F., Sanchez, E., Gonzalez, J.M., Portillo, M.C., Orduna, M., 2011. Relationship
753 between certain ceramic roofing tile characteristics and biodeterioration. J. Eur. Ceram.
754 Soc. 31, 2753–2761. https://doi.org/10.1016/j.jeurceramsoc.2011.07.023
755 Gazzano, C., Favero-Longo, S.E., Iacomussi, P., Piervittori, R., 2013. Biocidal effect of lichen
756 secondary metabolites against rock-dwelling microcolonial fungi, cyanobacteria and
757 green algae. Int. Biodeterior. Biodegrad. 84, 300–306.
758 https://doi.org/10.1016/j.ibiod.2012.05.033
759 Gong, P., 1997. Short Communication Dehydrogenase Activity in Soil : a Comparison
760 betweeb the TTC and INT Assay Under Their Optimum Conditions. Science (80-.). 29,
761 211–214. https://doi.org/10.1016/S0038-0717(96)00290-8

762 Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., Program, N.C.S.,
763 Bouffard, G.G., Blakesley, R.W., Murray, P.R., Green, E.D., Turner, M.L., Segre, J.A.,
764 2009. Topographical and temporal diversity of the human skin. Science (80-.). 324,
765 1190–1192. https://doi.org/10.1126/science.1171700.Topographical
766 Guiamet, P., Crespo, M., Lavin, P., Ponce, B., Gaylarde, C., de Saravia, S.G., 2013.
767 Biodeterioration of funeral sculptures in La Recoleta Cemetery, Buenos Aires,
768 Argentina: Pre- and post-intervention studies. Colloids Surfaces B Biointerfaces 101,
769 337–342. https://doi.org/10.1016/j.colsurfb.2012.06.025
770 Hallmann, C., Stannek, L., Fritzlar, D., Hause-Reitner, D., Friedl, T., Hoppert, M., 2013.
771 Molecular diversity of phototrophic biofilms on building stone. FEMS Microbiol. Ecol.
772 84, 355–372. https://doi.org/10.1111/1574-6941.12065
773 Hammes, F., Berney, M., Wang, Y., Vital, M., Egli, T., 2008. Flow-cytometric total bacterial
774 cell counts as a descriptive microbiological parameter for drinking water treatment
775 processes. Water Res. 42, 269–277. https://doi.org/10.1016/j.watres.2007.07.009
776 Hauer, T., Mühlsteinová, R., Bohunická, M., Kaštovský, J., Mareš, J., 2015. Diversity of
777 cyanobacteria on rock surfaces. Biodivers Conserv 24, 759–779.
778 https://doi.org/10.1007/s10531-015-0890-z
779 Hu, W., Niu, H., Murata, K., Zhang, D., Wu, Z., Hu, M., Kojima, T., 2018. Bacteria in
780 atmospheric waters : Detection , characteristics and implications. Atmos. Environ. 179,
781 201–221. https://doi.org/10.1016/j.atmosenv.2018.02.026
782 Jardak, M., Abdelli, F., Laadhar, R., Lami, R., Stien, D., Aifa, S., Mnif, S., 2017. Evaluation of
783 biofilm-forming ability of bacterial strains isolated from the roof of an old house. J. Gen.
784 Appl. Microbiol. 186–194. https://doi.org/10.2323/jgam.2016.10.005
785 Jroundi, F., Gonzalez-Muñoz, M.T., Sterflinger, K., Piñar, G., 2015. Molecular Tools for

786 Monitoring the Ecological Sustainability of a Stone Bio-Consolidation Treatment at the
787 Royal Chapel, Granada. PLoS One 10, e0132465.
788 https://doi.org/10.1371/journal.pone.0132465
789 Krakova, L., De Leo, F., Bruno, L., Pangallo, D., Urzì, C., 2015. Complex bacterial diversity in
790 the white biofilms of the Catacombs of St. Callixtus in Rome evidenced by different
791 investigation strategies: Bacterial diversity in white biofilms. Environ. Microbiol. 17,
792 1738–1752. https://doi.org/10.1111/1462-2920.12626
793 Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis
794 Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–1874.
795 https://doi.org/10.1093/molbev/msw054
796 Lage, O.M., Bondoso, J., 2012. Bringing Planctomycetes into pure culture. Front. Microbiol.
797 3, 1–6. https://doi.org/10.3389/fmicb.2012.00405
798 Leboulanger, C., Agogué, H., Bernard, C., Bouvy, M., Carré, C., Cellamare, M., Duval, C.,
799 Fouilland, E., Got, P., Intertaglia, L., Lavergne, C., Floc'H, E. Le, Roques, C., Sarazin, G.,
800 2017. Microbial diversity and cyanobacterial production in dziani dzaha crater lake, a
801 unique tropical thalassohaline environment. PLoS One 12, 1–28.
802 https://doi.org/10.1371/journal.pone.0168879
803 Ma, Y., Zhang, H., Du, Y., Tian, T., Xiang, T., Liu, X., Wu, F., An, L., Wang, W., Gu, J.-D., Feng,
804 H., 2015. The community distribution of bacteria and fungi on ancient wall paintings of
805 the Mogao Grottoes. Sci. Rep. 5, 1–9. https://doi.org/10.1038/srep07752
806 Macedo, M.F., Miller, A.Z., Dionísio, A., Saiz-Jimenez, C., 2009. Biodiversity of cyanobacteria
807 and green algae on monuments in the Mediterranean Basin: An overview. Microbiology
808 155, 3476–3490. https://doi.org/10.1099/mic.0.032508-0
809 Maresca, J.A., Moser, P., Schumacher, T., 2017. Analysis of bacterial communities in and on

concrete. Mater. Struct. 50, 1–13. https://doi.org/10.1617/s11527-016-0929-y

Mayer, C., Moritz, R., Kirschner, C., Borchard, W., Maibaum, R., Wingender, J., Flemming, H.C., 1999. The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int. J. Biol. Macromol. 26, 3–16. https://doi.org/10.1016/S0141-8130(99)00057-4

Miller, A.Z., Sanmartín, P., Pereira-Pardo, L., Dionísio, A., Saiz-Jimenez, C., Macedo, M.F., Prieto, B., 2012. Bioreceptivity of building stones: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2012.03.026

Molin, S., Tolker-nielsen, T., 2003. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. Curr. Opin. 255–261. https://doi.org/10.1016/S0958-1669(03)00036-3

Neu, T.R., Lawrence, J.R., 2014. Investigation of Microbial Biofilm Structure by Laser Scanning Microscopy. Adv Biochem Eng Biotechnol 146, 1–51. https://doi.org/10.1007/10_2014_272

Nowicka-Krawczyk, P., Zelazna-Wieczorek, J., Otlewska, A., Koziróg, A., Rajkowska, K., Piotrowska, M., Gutarowska, B., Zydzik-Białek, A., 2014. Diversity of an aerial phototrophic coating of historic buildings in the former Auschwitz II-Birkenau concentration camp. Sci. Total Environ. 493, 116–123. https://doi.org/10.1016/j.scitotenv.2014.05.113

Ortega-Calvo, J.J., Ariño, X., Hernandez-Marine, M., Saiz-Jimenez, C., 1995. Factors affecting the weathering and colonization of monuments by phototrophic microorganisms. Sci. Total Environ. 167, 329–341. https://doi.org/10.1016/0048-9697(95)04593-P

Pena-Poza, J., Ascaso, C., Sanz, M., Pérez-Ortega, S., Oujja, M., Wierzchos, J., Souza-Egipsy, V., Cañamares, M. V., Urizal, M., Castillejo, M., García-Heras, M., 2018. Effect of

biological colonization on ceramic roofing tiles by lichens and a combined laser and biocide procedure for its removal. Int. Biodeterior. Biodegrad. 126, 86–94. https://doi.org/10.1016/j.ibiod.2017.10.003

Pollock, T.J., 1993. Gellan-related polysaccharides and the genus Sphingomonas. ournal Gen. Microbiol. 139, 1939–1945. https://doi.org/10.1099/00221287-139-8-1939

Pozo-Antonio, J.S., Rivas, T., LÃ³pez, A.J., Fiorucci, M.P., Ramil, A., 2016. Effectiveness of granite cleaning procedures in cultural heritage: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.07.090

Puškárová, A., Bučková, M., Habalová, B., Kraková, L., Maková, A., Pangallo, D., 2016. Microbial communities affecting albumen photography heritage: a methodological survey. Sci. Rep. 6. https://doi.org/10.1038/srep20810

Pylro, V.S., Fernando, L., Roesch, W., Morais, D.K., Clark, I.M., Hirsch, P.R., Tótola, M.R., 2014. Data analysis for 16S microbial pro fi ling from different benchtop sequencing platforms. J. Microbiol. Methods 107, 30–37. https://doi.org/10.1016/j.mimet.2014.08.018

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen 111, 1–61.

Rosenberg, E., Delong, E.F., Lory, S., Stackebrandt, E., Thompson, F., 2014. The Prokaryotes - Alphaproteobacteria and Betaproteobacteria, Springer R. ed. https://doi.org/10.1007/978-3-642-30197-1

858 Rueden, C.T., Schindelin, J., Hiner, M.C., Dezonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W.,
859 2017. ImageJ2 : ImageJ for the next generation of scientific image data. BMC
860 Bioinformatics 18, 1–26. https://doi.org/10.1186/s12859-017-1934-z
861 Saiz-Jimenez, C., 2001. Biodeterioration: An Overview of the State-of-the-Art and
862 Assessment of Future Directions. Biodegrad. Cult. Herit. 10–16, 520.
863 Schindelin, J., Arganda-carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S.,
864 Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D.J., Hartenstein, V., Eliceiri, K.,
865 Tomancak, P., Cardona, A., 2019. Fiji : an open-source platform for biological-image
866 analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019
867 Schlafer, S., Meyer, R.L., 2017. Confocal microscopy imaging of the bio fi lm matrix ☆. J.
868 ofMicrobiological Methods 138, 50–59. https://doi.org/10.1016/j.mimet.2016.03.002
869 Siboni, N., Lidor, M., Kramarsky-Winter, E., Kushmaro, A., 2007. Conditioning film and initial
870 biofilm formation on ceramics tiles in the marine environment. Fems Microbiol. Lett.
871 274, 24–29. https://doi.org/10.1111/j.1574-6968.2007.00809.x
872 Thundyil, J., Manzanero, S., Pavlovski, D., Cully, T.R., Lok, K., Widiapradja, A., Chunduri, P., Jo,
873 D., Naruse, C., Asano, M., Launikonis, B.S., Sobey, C.G., Coulthard, M.G., Arumugam, T.
874 V, 2013. Evidence That the EphA2 Receptor Exacerbates Ischemic Brain Injury. PLoS One
875 8, 2–9. https://doi.org/10.1371/journal.pone.0053528
876 Tomaselli, L., Lamenti, G., Bosco, M., Tiano, P., 2000. Biodiversity of photosynthetic micro-
877 organisms dwelling on stone monuments. Int. Biodeterior. Biodegrad. 46, 251–258.
878 Urzì, C., De Leo, F., 2007. Evaluation of the efficiency of water-repellent and biocide
879 compounds against microbial colonization of mortars. Int. Biodeterior. Biodegrad. 60,
880 25–34. https://doi.org/10.1016/j.ibiod.2006.11.003
881 Urzì, C., De Leo, F., Bruno, L., Albertano, P., 2010. Microbial Diversity in Paleolithic Caves: A

882 Study Case on the Phototrophic Biofilms of the Cave of Bats (Zuheros, Spain). Environ.
883 Microbiol. 60, 116–129. https://doi.org/10.1007/s00248-010-9710-x
884 Urzì, C., De Leo, F., Krakova, L., Pangallo, D., Bruno, L., 2016. Effects of biocide treatments on
885 the biofilm community in Domitilla's catacombs in Rome. Open Conf. Proc. J. 572, 252–
886 262. https://doi.org/10.1016/j.scitotenv.2016.07.195
887 Vázquez-Nion, D., Rodríguez-Castro, J., López-Rodríguez, M.C., Fernández-Silva, I., Prieto, B.,
888 2016. Subaerial biofilms on granitic historic buildings: microbial diversity and
889 development of phototrophic multi-species cultures. Biofouling 32, 657–669.
890 https://doi.org/10.1080/08927014.2016.1183121
891 Viollet-le-Duc, E.E., 1854. Dictionnaire raisonné de l'architecture française du XIe au XVIe
892 siècle, A. Morel. ed.
893 Warscheid, T., Braams, J., 2000. Biodeterioration of stone: a review. Int. Biodeterior.
894 Biodegrad. 46, 343–368.
895 Warscheid, T., Petersen, K., Krumbein, W.E., 1990. A rapid method to demonstrate and
896 evaluate microbial activity on decaying sandstone. Stud. Conserv. 35, 137–147.
897 https://doi.org/10.1179/sic.1990.35.3.137
898 Woo, A.C., Brar, M.S., Chan, Y., Lau, M.C.Y., Leung, F.C.C., Scott, J.A., Vrijmoed, L.L.P., Zawar-
899 reza, P., Pointing, S.B., 2013. Temporal variation in airborne microbial populations and
900 microbially- derived allergens in a tropical urban landscape. Atmos. Environ. 74, 291–
901 300. https://doi.org/10.1016/j.atmosenv.2013.03.047
902 Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J.,
903 Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species Living Tree Project (LTP)"
904 taxonomic frameworks. Nucleic Acids Res. 42, 643–648.
905 https://doi.org/10.1093/nar/gkt1209

Zhang, C., Cui, F., Zeng, G.M., Jiang, M., Yang, Z.Z., Yu, Z.G., Zhu, M.Y., Shen, L.Q., 2015. Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. Sci. Total Environ. 518, 352–362. https://doi.org/10.1016/j.scitotenv.2015.03.007

7 TABLES (LEGENDS)

Table 1: Environmental data collected at the sites of tiles exposition.

Table 2: Biofilm-forming capacity of isolated strains based on a crystal violet assay. (++) high biofilm-forming (+) biofilm-forming.

Table 3: Cultured bacterial strains with their identification (based on 16SrRNA gene analysis), isolation medium and origin.

Table 4: Alpha diversity estimates for the 3 types of ceramic roof tiles studied (mean ± standard deviation; number of sequences per sample rarefied to 13 618).

8 FIGURES (LEGENDS)

Figure 1: Evaluation of the presence of a pioneering biofilm on unexposed tiles: (A, B) SEM images, white arrows and circle: dark spots rich in light elements; (C, D) EDS analysis – red: Si, yellow: Al, green: C, white arrows and circle corresponded to the dark spots of 1A and 1B; (E) Fluorescent macroscope image – green: SYTO9; (F) CLSM image – green: SYTO9; (G, H) Two-photon microscopy images – green: SYTO9 / red: FilmTracer™ SYPRO™ (G) max

928 intensity, (H) 3D-view of the biofilm slice; (H,I) Plot Z-axis profile of the staining with SYTO9
929 (H) and FilmTracer™ SYPRO™ (I) of the 3D-view (H).
930 Figure 2: Quantity of extracted DNA on each tile and for each type of exposure, in picograms
931 per square centimeter of tile.
932 Figure 3: Bacterial diversity at the phylum level observed on the 3 types of ceramic roof tiles
933 and determined by Illumina-based high-throughput sequencing. Twenty-one different phyla
934 colonize the 3 types of tiles. Only the phyla representing more than 0.5% of total sequences
935 on at least one type of tile were considered.
936 Figure 4: Bacterial diversity at the OTU level (97% of 16s rRNA gene similarity) determined by
937 Illumina-based high-throughput sequencing on unexposed roof tiles. Only OTUs identified as
938 being present at greater than 1% are named with their family in parentheses. (A) Tiles T1. (B)
939 Tiles T2. (C) Tiles T3.
940 Figure 5: Bacterial diversity on exposed roof tiles at the OTU level (97% of 16s rRNA gene
941 similarity) determined by Illumina-based high-throughput sequencing. Only OTUs identified
942 as being present at greater than 1% are named with their family in parentheses. (A) Tiles T1.
943 (B) Tiles T2. (C) Tiles T3.
944 Figure 6: Venn diagram representing shared OTUs (97% of 16s rRNA gene similarity) as a
945 function of the exposure undergone by the tiles; all types of tiles are combined in this
946 analysis.
947 Figure 7: Venn diagrams representing shared OTUs and rings representing bacterial diversity
948 at the OTU level (97% of 16s rRNA gene similarity) on Tiles T1 (A), Tiles T2 (B) and Tiles T3 (C)
949 tiles. Rings color code represents the different phyla of the OTUs representing more than 1%
950 of total sequences (corresponds to Figures 4 and 5).
951

9 SUPPLEMENTARY MATERIALS (LEGENDS)

Table S1: Accession number and closest relative species corresponded to isolated and identified bacterial strains maintained in Banyuls Bacterial Culture Collection.

Supplementary Figure S1: (A,B) TTC assays based observations : the red coloration induced by the positive TTC test was difficult to observe due to the red color of the tiles and to the presence of colored engobe. This is why this test was achieved to complete flow cytometry data, (C, D) flow cytometry.

Supplementary Figure S2: Diversity of Proteobacteria on the 3 types of ceramic roof tiles as determined by Illumina-based high-throughput sequencing.

Supplementary Figure S3: Bray-Curtis based non-metric multidimensional scaling (NMDS) plot of all samples analyzed in this study.

Supplementary Figure S4: Bacterial diversity at the phylum level on the 3 types of ceramic roof tiles determined by Illumina-based high-throughput sequencing and DADA2 package analysis.

Supplementary Figure S5: Venn diagram representing shared ASVs as a function of the exposure undergone by the tiles; all types of tiles are combined in this analysis.

Supplementary Figure S6: Bray-Curtis based non-metric multidimensional scaling (NMDS) plot of all samples analyzed in this study using the DADA2 package analysis.

Supplementary Figure S7: ASVs representing more than 1% of total sequences at least in one sample, splitted by phylum and type of tile.