=== MINI-REVIEW ===

# DIVERSITY OF ENDOLITHIC PROKARYOTES LIVING IN STONE MONUMENTS

## HORIA LEONARD BANCIU[1,2,✉]

**SUMMARY.** Stone monuments (statues, fountains, tombs, memorials, buildings, etc.) are apparently indestructible artefacts that were built to last. Depending on the chemical composition and structure, stones may be friendly or hostile to colonization by eukaryotic and/or prokaryotic communities. Among various lithotypes, porous rocks (limestone, sandstone) have highest bioreceptivity. Abiotic factors have a strong impact on the deterioration rate of stone monuments. Biological activity of living colonizers may intensify the weathering of such substrates. Moreover, human activity could enhance the bioerosion by providing more nutrients to epilithic and endolithic microbial communities. Highly diverse lithic communities have been detected in stone monuments, both by culture-dependent and molecular approaches. Epilithic and endolithic prokaryotes are well represented by cyanobacteria that are among the pioneers of colonizing the stone surfaces in partnership with fungi and algae. Active boring algae and cyanobacteria are euendoliths *par excellence*. Chemoheterotrophic actinobacteria and gammaproteobacteria are dominant bacterial species found in biofilm as well as in endolithic niches of stone sampled from various types of monuments. To date, there is little information on endolithic archaea and chemolithoautotrophic bacteria. Further in-depth investigations on the diversity and metabolic roles of lithic communities are needed with respect to the conservation strategies.

**Keywords:** actinobacteria, biodeterioration, bioreceptivity, cyanobacteria, halotolerant bacteria, lithotypes.

## Introduction

Stone monuments from impressive megaliths of Neolithic Age and delicate limestone sculptures of Antiquity to modern marble and concrete buildings are expressions of human sensitivity and need for everlasting proofs of existence. The

---

[1,✉] ***Correspondence address:*** *Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, 5-7 Clinicilor Str., 400006 Cluj-Napoca, Romania. E-mail: Horia.Banciu@ubbcluj.ro*

[2] *Institute for Interdisciplinary Research in Bio-Nano Sciences, Babeş-Bolyai University, 42 A. Treboniu Laurian Str., 400271 Cluj-Napoca, Romania.*

basic materials used for most of stone monuments are either light rocks, such as sandstone and limestone, or hard materials (e.g. granite, dolomite). Various mixtures of basic soft or hard materials could be used to prepare mortar, ceramic or concrete. Porous or light rocks were used by ancient civilizations for their ease to extract and shape. Monuments such as Greek or Roman statues, temples, thombstones or Egyptian pyramids and obelisks were made of such materials. Granite, dolomite, and marble were preferred when long lasting masterpieces were constructed. Weathering by a combination of water, wind, heat and cold contrast is the main destructive cause of such solid man-made structures. Besides these abiotic factors, the biological activity due to the growth of macroscopic or microscopic organisms on the surface or inside the stone is another reason for an even faster deterioration of the stone monuments. When stone degradation is catalyzed by living organisms, the process is called bioerosion or biodeterioration. Bioerosion affects stone artifacts at different rates depending on the type of rock material (lithotype) used for building, on the abiotic factors and not at least, on the degree of environmental pollution as result of human activities. The present short review aims to summarize recent knowledge on the diversity of prokaryotes living inside building stones, their metabolic strategies to overcome the limitation of life in the rock and their involvement in biodeterioration of monuments made of stone.

## Ecological niches hosted by rocks and stone artifacts

There are two sorts of habitats that can be found in relation with a solid mineral material: on the surface and inside. Depending on the environmental conditions and chemical composition of the substrate, the surface of rock could be colonized by macroscopic eukaryotes, mostly represented by mosses (class Bryophyta), fungi and lichens (as a symbiotic consortium of fungi, green algae and/or cyanobacteria) that seem to be the first colonizers of stone surfaces (Lisci *et al.*, 2003). When humidity and light reach appropriate values, biofilms form. Biofilms appearing on rock and, correspondingly, on stone monuments surfaces are complex consortia of algae and bacteria that are oftenly grazed by microscopic eukaryotes (protozoa). All forms of life prone to living on surfaces of stones are categorized as *epilths* (a.k.a. *epilithobionts*). *Hypoliths* are organisms that develop just below the surface of rocks. Cyanobacteria seem to form the dominant group (46-90%) of hypolithic prokaryotes in natural environments followed by heterotrophic bacteria (Chan *et al.*, 2012). When surface is broken into crevices or fissures, as well as when porosity of stone material is large enough, water and organic compounds difuse within the body of stone. These are, however, the minimal requirements to support life and rock become colonized by inside. *Endoliths* (a.k.a. *endolithobionts*) are all microscopic eukaryotes (fungi and protozoa) and prokaryotes (bacteria and archaea) inhabiting pores or crevices of hard materials such as rocks, corals or even shells. In these minute ecological niches, light and organic carbon are hardly present, while water become accessible only intermitantly.

Thus, no wonder that many endolithic taxa are considered as extremophiles – organisms that thrive at extreme environmental conditions (Pikuta *et al.*, 2007). In this category several examples of archaea and bacteria that survive within an extremely narrow space in micropores of deep sediments (e.g. igneous rocks), away from water and organic compounds, both at high temperature and pressure, in acidic or alkaline rocks, are known (Jiménez, 1990; Newberry *et al.*, 2004; Fry *et al.*, 2008). The diversity of endolithic prokaryotes in deep sediments is surprisingly high and representatives of Alpha-, Beta- and Gammaproteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, as well as archaea from Euryarchaeota and Crenarcaheota classes have been identified both by culture-dependent and molecular approaches. Several aerobic and anaerobic deep-subsurface endoliths have been successfuly cultured (e.g. species belonging to *Carnobacterium* spp., *Clostridium* spp., *Marinilactibacillus* spp., *Pseudomonas* spp., *Acetobacterium* spp., *Deinococcus* spp., *Arthrobacter* spp., *Micrococcus* spp., etc.). It seems that the uncultured species are actually the dominant ones (Zlatkin *et al.*, 1996; Parkes *et al.*, 2009). Interesting observations point that the majority of endolithic prokaryotes adopted an anaerobic or microaerophilic heterotrophic way of life, in an environment where the organic substrates could also be generated by thermal activation of organic matter buried in rocks (Balkwill *et al.*, 1989; Fredrickson *et al.*, 1991; Fry *et al.*, 2008). On the other hand, chemolithoautotrophic microbes inhabiting deep sediments gain energy by the reduction of oxidized sulfur, manganese or iron minerals. These prokaryotes belonging to „intraterrestrial" biosphere (or subsurface biome) as well as members of recently characterized microbial communities of extremely arid zones (e.g. Atacama desert) are good models for astrobiologists to imagine similar forms of life that might be found on extraterrestrial grounds (Wierzchos *et al.*, 2011). Moreover, the deep-subsurface biosphere could represent the largest microbial habitat yet to be explored (Edwards *et al.*, 2012).

Stone matrix itself accomodates several types of lithobiontic ecological niches. In this regard, Golubic and his colleagues (1981) have divided endoliths by their strategy of cavity colonization into three categories. The true endoliths or *euendoliths* are actively boring the interior of rock forming tunnels or microcrevices. After vacation by euendoliths, these small cavities could be secondary colonized by *cryptoendoliths*. Cryptoendoliths also dwell the structural pores of rock. *Chasmoendoliths* are rather opportunistic invaders of cracks (or large cavities) formed in stone. The precise characterization of the composition of endolithic communities and their interaction with the mineral surroundings is the key of understanding the role of such organisms in the biodeterioration of stone monuments (de los Rios and Ascaso, 2005). Therefore, a multitude of classic and novel approaches have been employed to study the endolithic prokaryotes inhabiting stone monuments, from microscopic studies (light and scanning electron microscopy) to molecular techniques.

**Phototrophic endoliths**

Active boring endoliths mainly comprise the phototrophic microflora including algae and cyanobacteria. Phototrophic euendoliths have been found to perforate calcareous substrata and they are responsible for the biological erosion of coastal limestone, calcareous shells, coral skeletons, limestone oolites, and sand grains (Shachak *et al.*, 1987; Salvadori, 2000; Aline, 2008; Zardi *et al.*, 2009). Among euendolithic chlorophyta, *Ostreobium quekettii* is particularly abundant in coral skeletons along with the cyanobacterium *Plectonema terebrans* (Aline, 2008; Verbruggen and Tribollet, 2011). On stone monuments, green algae (Division Chlorophyta) and diatoms (Class Bacillariophyceae) have been frequently identified and studied as inhabitants of epilithic biofilms. Species of genera *Chlorella*, *Stichococcus*, and *Chlorococcum* are found on the surface of many outdoor stone monuments of various lithotypes (limestone, granite, marble, travertine and sandstone) around Mediterranean Basin (Macedo *et al.*, 2009) or in tropical areas (Kumara and Kumar, 1999). Epilithic algae in association with fungi and bacteria form patinas or sheaths on stone surfaces wherever moisture, light, and inorganic nutrients are available at optimal values. However, little is known about endolithic algae living in stone artifacts. Species of *Trentepohlia*, *Chlorella*, *Klebsormidium*, and *Stichococcu*s were observed as colonizers of cryptoendolithic niches on churches in Portugal and Spain (Macedo *et al.*, 2009).

Active burrowing cyanobacteria are found within several morphogenera of uncertain phylogeny: *Hyella*, *Solentia*, *Plectonema*, and *Mastigocoleus*. Boring cyanobacteria possess a unique mechanism of calcium carbonate dissolution while excavating the solid carbonate substrate. Garcia-Pichel *et al.* (2010) have proposed a mechanism by which calcium is extracted from the matrix at the front (apical) pole of the trichome, and carried intracellularly by $Ca^{2+}$-channels. Calcium ions are further transported along the multicellular cyanobacterial filaments, passing from one cell to another by membrane $Ca^{2+}$-ATPase. At the distal pole of boring filament, a P-type $H^{+}/Ca^{2+}$-ATPase actively excrete calcium ions to external milieu at the exchange with protons ($2H^{+}/Ca^{2+}$). Simultaneously, at burrowing pole, the bicarbonate ($HCO_3^{-}$) results from calcium carbonate ($CO_3^{2-}$) dissolution under the presence of protons exported by cyanobacterial cells. Bicarbonate is further taken up and used for inorganic carbon fixation during photosynthesis. Besides their damaging effects on natural or anthropogenic carbonate substrate, euendolithic phototrophs (green and red algae, cyanobacteria) are thought to play important role in natural recycling of calcium and carbonate ions.

Cyanobacterium *Hyella fontana* have been identified as member of endolithic community on marble and limestone monuments in Spain (Fountain from Patio de la Lindaraja in Alhambra, Granada) (Bolivar and Sánchez-Castillo, 1997) and marble statues in Rome, Italy (Macedo *et al.*, 2009). *Plectonema battersii* and strains of *Plectonema* spp. have been described in biofilms covering fountains (Trevi Fountain, Rome) (Nugari and Pietrini, 1997) and walls and statues (Medici Fortress and statues in

Boboli Garden, Florence) (Tomaselli *et al.*, 2000). As a result of their boring activity, euendolithic cyanobacteria may form tunnels with diameters ranging from 3 to 25 µm, depending on the size of endolithic filaments (Salvadori, 2000). In this manner, mechanical destruction of monuments at microscopic scale is achieved and niches for other colonizing microbes are created.

Some of the most widespread cryptoendolithic phototrophs are the cyanobacterial species from genera *Gloeocaps*a, *Chroococcus*, *Chroococcidiopsis* (Order Chroococcales), and *Scytonema* (Order Nostocales). They were found to form biofilms on surfaces as well as within the matrix of stone artifacts and natural rocks (Friedmann, 1980; Banerjee *et al.*, 2000; Tomaselli *et al.*, 2000; Pointing and Belnap, 2012). Cryptoendolithic cyanobacteria have a preference for porous transparent rocks (such as marble, limestone, travertine, or sandstone) and do not grow within dark and dense volcanic stone (granite, dolomite) (Macedo *et al.*, 2009). *Chroococcidiopsis* strains are versatile and face harsh environmental conditions including high or low temperatures, high salinity, high UV irradiation and desiccation (Billi *et al.*, 2011; Stivaletta *et al.*, 2012; Baqué *et al.*, 2013). *Gloeocapsa* spp. includes highly adaptable species to changing environmental conditions. It was observed that *Gloeocapsa* strains synthesize intracellular and water-soluble mycosporine-like amino acids (MAAs) that have high UV absorbance, therefore acting as UV sunscreens (Garcia-Pichel *et al.*, 1993). Moreover, scytonemin is another photoprotective compund that is excreted by some cyanobacterial strains (e.g. *Scytonema* spp.) populating biofilms of stone monuments (Keshari and Adhikary, 2013). When dessication impedes the photoprotective mechanisms, cyanobacteria retreat inside rock and form colonies as endolithic biofilms. Because of light limitation, phototrophic endoliths do not grow deep in stone, preferring microniches down to only few milimeters below rock surface (Warscheid and Braams, 2000). Salvadori (2000) reported the abundant presence of cyanobacterial endoliths at a depth of 0.11-0.25 mm below surface of Carrara marble and the colonization of filamentous boring cyanobacteria at a maximum depth of 1.9 mm in limestone scales taken from Neptune fountain (Trento, Italy).

## Endolithic Bacteria and Archaea on Stone Monuments

Acting as pioneers in stone colonization, phototrophic epi- and endolithic organisms provide nutrients and assure humidity and light (UV) shelter for heterotrophic communities of bacteria and archaea (Scheerer *et al.*, 2009). Autotrophic bacteria inhabiting natural rocks or stone monuments rely mainly on the water and mineral nutrients brought by external factors (wind, rainfall, or direct human activity) and/or having endogenous origin.

Heterotrophic bacteria that invade and populate the interior of stone-made monuments are highly diverse. Chemotrophic prokaryotes may contaminate stones down to 5 cm deep or even deeper (Waarscheid and Braams, 2000). Considered as one of the first colonizers of rock habitats together with phototrophic microorganisms,

Gram-positive actinomycetes (Class Actinobacteria) are well represented in endolithic communities around the world (Walker and Pace, 2007). In stone artifacts, actinobacteria are consistently represented by species from genera *Arthrobacter* (identified as hypoliths of mural paintings in indoor stone monuments from Spain and Austria) (Heyrman *et al.*, 2005). Several strains of *Streptomyces* have been isolated from ancient Egyptian mural paintings and stone supports (Abdel-Haliem *et al.*, 2013). *Streptomyces* spp. was pointed as one of main agents causing colour change of tomb paintings by producing a range of acids (oxalic, citric, and sulfuric acid), biopigments (melanins) and hydrogen sulfide. Molecular approaches have revealed that the endolithic microbiota of Mayan stone monuments of limestone origin has a high abundance of Actinobacteria, Acidobacteria, and low G+C Firmicutes (McNamara *et al.*, 2006). Full genome sequence of *Blastococcus saxobsidens*, an actinobacterium isolated from calcareous stone in Sardinia (Italy), was recently unveiled (Chouaia *et al.*, 2012).

Chemoheterotrophic bacteria from Phylum Firmicutes are frequently identified on surface as well as inside the stone artifacts. Strains of *Bacillus* spp. with its spore-forming strategy of survival and broad range of nutrients utilized, are some of most oftenly found bacteria within various lithotypes (see review of Scheerer *et al.*, 2009). Surface samples (3 mm depth) samples from wall paintings and building materials allowed Piñar et al (2001) to isolate several strains related to moderately halophilic Gram-positive *Halobacillus litoralis* by enrichment procedure. Interestingly, it was found that *Halobacillus* spp. is able to precipitate carbonate in a process called biomineralization (Rivandeyra *et al.*, 2004). The saline ecological niches related to stone materials seem to be important in several situations such as salt efflorescences that form during weathering of stone. Locally, carbonate, chloride, nitrate, and sulfate salts accumulate and extremely saline microniches appear. In such endolithic saline microhabitats, a predominance of halotolerant actinobacteria (*Kocuria* spp., *Micrococcus* spp., *Arthrobacter* spp.) and bacilli communities (e.g. *Bacillus* spp., *Staphylococcus* spp., *Paenibacillus* spp.) is remarkable (Laiz *et al.*, 2000). Other chemoheterotrophic bacteria detected by means of molecular biology from various monuments belong to Gammaproteobacteria (e.g. *Halomonas* spp., *Pseudomonas* spp.) (Rölleke *et al.*, 1996; Suihko *et al.*, 2007).

The oligotrophic lifestyle is also present in low nutrient stones and it is carried out by methanotrophs and methanogens. Isolates belonging to *Methylobacterium* spp., *Methylocystis* spp. (both from Class Alphaproteobacteria), and to facultative methylotrophic *Methylosinus* spp. (Class Gammaproteobacteria) have been described in stone samples from historical buildings in Germany and Italy. These methane-oxidizing bacteria belong to type II methanotrophs and their presence was well correlated with sandstone and limestone materials. From the deteriorated marble samples of a Kremlin crypt (Moscow, Russia), Doronina *et al.* (2005) have isolated an aerobic and moderately haloalkaliphilic methylotroph, *Methylophaga muralis* (formely *M. murata*). It was suggested that the low atmospheric methane concentration

alone cannot support the presence of methane-utilizing bacteria on stone samples. Anthropogenic emissions of methane and methanol as well as traces of biological methanogenesis present in stone substrate could enhance methanotrophic growth in these ecological niches (Kussmaul *et al.*, 1989). Same author, however has demonstrated the presence of bacterial mini-methane producers (*Clostridium* spp.) and of methanogenic archaea in several stone samples. Their presence would have been possible in the anoxic microsites of stone material and, in this regards, they are endoliths.

Chemolithoautotrophic bacteria present as epi-, hypo- or endolithic microbiota use $CO_2$ (or $HCO_3^-$) as carbon source and inorganic compounds as electron donors. Inorganic sources of energy mainly consist of reduced sulfur compounds ($S_2O_3^{-2}$, $S^0$, $H_2S$), reduced nitrogen compounds ($NO_2^-$, $NH_3$). As a result of their activity, inorganic acids ($HNO_3$, $HNO_2$, $H_2SO_4$) are produced with dissolving effects on hosting non-alkaline material (Waarscheid and Braams, 2000; Scheerer *et al.*, 2009). Populations of chemolithoautotrophic sulfur-oxidizing (*Thiobacillus* spp.) and nitrogen-cycling bacteria (*Nitrobacter* spp., *Nitrosomonas* spp., *Nitrospira* spp.) have been detected in samples of stones originated from various monuments (see review of Scheerer *et al.*, 2009) .

## Conclusions

Various types of stone matrices have the potential to accommodate a variety of ecological microniches. When appropriate physical and chemical conditions meet, rock is firstly colonized by mixed populations of phototrophic microbes forming biofilms. Additionally, fungi and macrophytes may find favorable conditions to develop on stone surfaces. Biological activity of epilithobionts overlaps the effects of the abiotic factors, providing further deep microhabitats for cyanobacteria, chemotrophic bacteria and archaea. The endolithic prokaryotic communities inhabiting various stone monuments were found to be surprisingly diverse, many members of these ecological group showing high adaptative versatility. Altough the description of molecular and culturable diversity of endolithobionts is well established, their metabolic interactions and overall effects on the rate of deterioration of stone monuments is less understood.

# REFERENCES

Abdel-Haliem M.E., Sakr A.A., Ali M.F., Ghaly M.F., Sohlenkamp C. (2013) Characterization of *Streptomyces* isolates causing colour changes of mural paintings in ancient Egyptian tombs. *Microbiol. Res.* **168**(7): 428-437.

Aline T. (2008) Dissolution of dead corals by euendolithic microorganisms across the northern Great Barrier Reef (Australia) *Microb. Ecol.* **55**: 569–580.

Balkwill D.L., Fredrickson J.K., Thomas J.M. (1989) Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep southeast coastal plain subsurface sediments. *Appl. Environ. Microbiol.* **55**(5): 1058–1065.

Banerjee M., Whitton B.A., Wynn-Williams D.D., Phosphatase activities of endolithic communities in rocks of the Antarctic Dry Valleys. *Microbial Ecology* **39**(1): 80-91, 2000.

Baqué M., Viaggiu E., Scalzi G., Billi D. (2013) Endurance of the endolithic desert cyanobacterium Chroococcidiopsis under UVC radiation. *Extremophiles.* **17**(1):161-169.

Billi D., Viaggiu E., Cockell C.S., Rabbow E., Horneck G., Onofri S. (2011) Damage escape and repair in dried Chroococcidiopsis spp. from hot and cold deserts exposed to simulated space and martian conditions. *Astrobiology.* **11**(1):65-73.

Bolivar, F.C., Sánchez-Castillo, P.M. (1997) Biomineralization processes in the fountains of the Alambra, Ganada, Spain. *Int. Biodeterior. Biodegrad.* **40**, 205–215.

Chan Y., Lacap D.C., Lau M.C., Ha K.Y., Warren-Rhodes K.A., Cockell C.S., Cowan D.A., McKay C.P., Pointing S.B. (2012) Hypolithic microbial communities: between a rock and a hard place. *Environ. Microbiol.* **14**(9):2272-2282.

Chouaia B., Crotti E., Brusetti L., Daffonchio D., Essoussi I., Nouioui I., Sbissi I., Ghodhbane-Gtari F., Gtari M., Vacherie B., Barbe V., Médigue C., Gury J., Pujic P., Normand P. (2012) Genome sequence of *Blastococcus saxobsidens* DD2, a stone-inhabiting bacterium. *J Bacteriol.*, **194**(10):2752-2753.

Cuzman O.A., Ventura S., Frediani, P. (2011) Biodiversity on Stone Artifacts, In: "The Importance of Biological Interactions in the Study of Biodiversity", López-Pujol, J. (Ed.), InTech, Rijeka, Croatia.

Cuzman O.A., Ventura S., Sili C., Mascalchi C., Turchetti T., D'Acqui L.P., Tiano P. (2010) Biodiversity of phototrophic biofilms dwelling on monumental fountains. *Microb. Ecol.* **60**(1):81-95.

de Los Ríos A., Ascaso C. (2005) Contributions of *in situ* microscopy to the current understanding of stone biodeterioration. *Int Microbiol.* **8**(3):181-188.

Doronina N.V., Li Ts.D., Ivanova E.G., Trotsenko Iu.A. (2005) *Methylophaga murat*a sp. nov.: a haloalkaliphilic aerobic methylotroph from deteriorating marble. *Microbiology* (Moscow), **74**, 440-447.

Edwards K.J., Fisher A.T., Wheat C.G. (2012) The deep subsurface biosphere in igneous ocean crust: frontier habitats for microbiological exploration. *Front Microbiol.*, **3**:8.

Fredrickson J.K., Balkwill D.L., Zachara J.M., Li S.M.W., Brockman F.J., Simmons M.A. (1991) Physiological diversity and distributions of heterotrophic bacteria in deep cretaceous sediments of the Atlantic coastal plain. *Appl. Environ. Microbiol.* **57**, 402–411.

Friedmann, E.I. (1980) Endolithic microbial life in hot and cold deserts. *Orig. Life,* **10**, 223-235.

Fry J.C., Parkes R.J., Cragg B.A., Weightman A.J., Webster G. (2008) Prokaryotic biodiversity and activity in the deep subseafloor biosphere. *FEMS Microbiol. Ecol.* **66**(2):181-196.

Garcia-Pichel F., Wingard C.E. and Castenholz R.W. (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium Gloeocapsa sp. *Appl. Environ. Microbiol.,* **59**: 170-176.

Garcia-Pichel F., Ramirez-Reinat E., Gao Q.J. (2010) Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca2+ transport. *Proc. Natl. Acad. Sci. USA*, **107** (2010), 21749–21754.

Golubic S., Friedmann I., Schneider J. (1981) The lithobiontic ecological niche, with special reference to microorganisms. *J. Sediment. Res*. **51**:475-478.

Heyrman J., Verbeeren J., Schumann P., Swings J., De Vos P. (2005). Six novel *Arthrobacter* species isolated from deteriorated mural paintings. *Int. J. Syst. Evol. Microbiol*. **55**, 1457-64.

Jiménez L. (1990) Molecular analysis of deep-subsurface bacteria. *Appl. Environ. Microbiol.*, **56**(7): 2108-2113.

Keshari N., Adhikary S.P. (2013) Characterization of cyanobacteria isolated from biofilms on stone monuments at Santiniketan, India. *Biofouling*. 2013 May; **29**(5):525-536.

Kumar R., Kumar A.V. (1999) Biodeterioration of stone in tropical environments: an overview. Getty Publications, Los Angeles, pp. 85.

Kussmaul M., Wilimzig M. and Bock E. (1998) Methanotrophs and methanogens in masonry. *Appl. Environ. Microbiol.* **64**, 4530-4532.

Laiz L., Recio D., Hermosin B., Saiz-Jimenez C. (2000). Microbial communities in salt efflorescences. In Ciferrio O., Tiene P., Mastromei G. (eds). "On microbes and art. Proceedings of an international conference on microbiology and conservation". New York: Kluwer Academic/Plenum Publishers, pp.77-88.

Lisci M., Monte M., Pacini E. (2003) Lichens and higher plants on stone: a review. *Int. Biodet. Biodeg*. **51**:1-17.

Macedo M.F., Miller A.Z., Dionísio A., Saiz-Jimenez C. (2009) Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview. *Microbiology*. **155**(Pt 11):3476-3490.

May E., Papida S., Hesham A., Tayler S., Dewedar A. (2000) Comparative studies of microbial communities on stone monuments in temperate and semi-arid climates. In "Of microbes and art. The role of microbial communities in the degradation and protection of cultural heritage" (O. Ciferri, P. Tiano and G. Mastromei, Eds.), pp. 49–62. Kluwer Academic/ Plenum Publisher, New York.

McNamara C.J., Perry T.D.[4th], Bearce K.A, Hernandez-Duque G., Mitchell R. (2006) Epilithic and endolithic bacterial communities in limestone from a Maya archaeological site. *Microb. Ecol*. **51**(1):51-64.

Newberry C.J., Webster G., Cragg B.A., Parkes R.J., Weightman A.J., Fry J.C. (2004) Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ. Microbiol.*, **6**(3):274-287.

Nugari M.P., Pietrini A.M. (1997) Trevi Fountain: an evaluation of inhibition effect of water-repellents on cyanobacteria and algae. *Int. Biodet. Biodegrad.* **40**:247-253.

Parkes R.J., Sellek G., Webster G., Martin D., Anders E., Weightman A.J., Sass H. (2009) Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). *Environ. Microbiol.* **11**(12):3140-3153.

Pikuta E.V., Hoover R.B., Tang J. (2007) Microbial extremophiles at the limits of life. *Crit. Rev. Microbiol.*, **33**, 183-209.

Piñar G., Ramos C., Rölleke S., Schabereiter-Gurtner C., Vybiral D., Lubitz W., Denner E.B. (2001) Detection of indigenous *Halobacillus* populations in damaged ancient wall paintings and building materials: molecular monitoring and cultivation. *Appl. Environ. Microbiol.* **67**(10):4891-4895.

Pointing S.B., Belnap J. (2012) Microbial colonization and controls in dryland systems. *Nat. Rev. Microbiol.* **10** (8):551-62.

Rivadeneyra M.A., Párraga J., Delgado R., Ramos-Cormenzana A., Delgado G. (2004) Biomineralization of carbonates by *Halobacillus trueperi* in solid and liquid media with different salinities. *FEMS Microbiol. Ecol.* **48**, 39-46.

Rölleke S., Muyzer G., Wawer C., Wanner G., Lubitz W. (1996) Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl. Environ. Microbiol.* **62**(6):2059-2065.

Saiz-Jimenez C., Laiz L. (2000). Occurrence of halotolerant/halophilic bacterial communities in deteriorated monuments. *Int. Biodeterior. Biodegrad.* **46**, 319-326.

Salvadori O. (2000) Characterisation of endolithic communities of stone monuments and natural outcrops. In Ciferri O., Tiani P., Mastromei G. *et al.* (eds.), "Of Microbes and Art: The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage", Springer Science and Business Media New York, pp. 89-101.

Scheerer S., Ortega-Morales O., Gaylarde C. (2009) Microbial deterioration of stone monuments-an updated overview. *Adv. Appl. Microbiol.* **66**:97-139.

Shachak M., Jones C.G., Granot Y. (1987) Herbivory in rocks and the weathering of a desert. *Science* 236:1098-1099.

Stivaletta N., Barbieri R., Billi D. (2012) Microbial colonization of the salt deposits in the driest place of the Atacama Desert (Chile). *Orig. Life Evol. Biosph*. **42**(2-3):187-200.

Suihko M.L., Alakomi H.L., Gorbushina A., Fortune I., Marquardt J., Saarela M. (2007) Characterization of aerobic bacterial and fungal microbiota on surfaces of historic Scottish monuments. *Syst Appl Microbiol.*, **30**(6):494-508.

Tomaselli L., Lamenti G., Bosco M., Tiano P. (2000). Biodiversity of photosynthetic microorganisms dwelling on stone monuments. *Int. Biodeterior. Biodegrad.* **46**, 251-258.

Urzì C., Salamone P., Schumann P., Rohde M., Stackebrandt E. (2004) *Blastococcus saxobsidens* sp. nov., and emended descriptions of the genus Blastococcus Ahrens and Moll 1970 and Blastococcus aggregatus Ahrens and Moll 1970. *Int. J. Syst. Evol. Microbiol.* **54**(Pt 1): 253-259.

Verbruggen H., Tribollet A. (2011) Boring algae. *Curr Biol.* **21** (21):R876-7.

Walker J.J., Pace N.R. (2007) Endolithic microbial ecosystems. *Annu Rev Microbiol.* **61**: 331-47.

Warscheid T., Braams J. (2000) Biodeterioration of stone: a review. *Int. Biodeterior. Biodegrad.* **46**:343-368.

Wierzchos J., Camara B., De Los Rios A., Davila A.F., Sanchaz Almazo M., Artieda O., Wierzchos K., Gomez-Silva B., McKay C., Ascaso C. (2011). Microbial colonization of Ca-sulfate crusts in the hyperarid core of the Atacama Desert: Implications for the search for life on *Mars. Geobiology*, **9**, 44-60.

Zardi G.I., Nicastro K.R., McQuaid C.D., Gektidis M. (2009) Effects of endolithic parasitism on invasive and indigenous mussels in a variable physical environment. *PLoS ONE* **4**:e6560.

Zlatkin I.V., Schneider M., de Bruijn F.J., Forney L.J. (1996) Diversity among bacteria isolated from the deep subsurface, *J. Ind. Microbiol.*, **17**, 219-227.