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A comparative SEM morphological study of biogenic and abiogenic carbonates for the search for biostructures on Mars

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Abstract. Next space missions will investigate the possibility of extinct or extant life on Mars. In previous laboratory works by studying the infrared spectral modifications induced by thermal processing on different carbonate samples (recent shells and fossils of different ages), we developed a method able to discriminate biogenic carbonates from their abiogenic counterparts. The method has been successfully applied to microbialites, i.e. bio-induced carbonates deposits, and particularly to stromatolites, the laminated fabric of microbialites, some of which can be ascribed among the oldest traces of biological activity known on Earth. These results are of valuable importance since such carbonates are linked to primitive living organisms which can be considered as good analogues for putative Martian life forms. Due to the fact that the microstructures of biogenic carbonate may be different from those of abiogenic origin, we have recently investigated the microscopic morphology at different scales of our samples (shells, skeletal grains, microbialites and stromatolites) using a scanning electron microscope (SEM). In this paper we present some preliminary results that can be of valuable interest in view of the high resolution imaging systems that in the near future will explore the surface of Mars in the search for biological traces of life.

Key words. Exobiology – Mars – Microscopy

1. Introduction

The idea that Mars may be the first extraterrestrial body to yield evidence of life beyond Earth is nowadays widespread among planetary scientists. If life was once present on Mars, the so-called biomarkers, that can be preserved for billions of years under favourable conditions, may still exist. Biomarkers may be linked both with organic and inorganic compounds. In particular, minerals associated with fossils, may display distinctive morphologies, isotope signatures, chemical composition, or defect microstructures that can reveal their biological origin (Banfield et al. 2001).

Among the minerals, calcium carbonates ($CaCO_3$) are particularly interesting, because they can be produced either by abiotic processes or by biologically induced or controlled mineralization (Mann, 2001). Many living organisms on Earth, prokaryotes and eukary-

Table 1. List of the studied samples.

Sample	Description	Composition	Geologic period/epoch
Xenophora	Mollusca, Gastropoda	Aragonite	Pleistocene (1.8-0.1 Ma)
Ampullinopsis crassatina	Mollusca, Gastropoda	Aragonite	Oligocene (34-23 Ma)
GE	Stromatolite	Calcite, silicates (traces)	Upper Jurassic, Tithonian (151-146 Ma)
S/L	Skeletal organism (coral)	Calcite	Upper Triassic, Carnian (229-217 Ma)
S1A	Skeletal organism (sponge)	Calcite, Aragonite	Upper Triassic, Carnian (229-217 Ma)
U2	Skeletal organism (algae)	Calcite	Middle Triassic, Ladinian (237-229 Ma)
Calcite	Rock mineral	Calcite	—

otes, are able to biomineralize calcite or aragonite and the most primitive terrestrial evidence of life are biomineralized carbonates (Schopf, 1993; Westall et al., 2004). On the other hand, it is well known that carbonates are also produced by chemical precipitation following different processes not related to the presence of any life form (Wilkinson and Given, 1986).

The increasing evidence for the presence of carbonates on Mars (Pollack et al., 1990; Bandfield et al., 2003; Ehlmann et al., 2008; Boynton et al., 2009; Palomba et al., 2009; Michalski and Niles, 2010; Morris et al., 2010; Carter and Poulet, 2012; Michalski, et al. 2013), suggests that a number of locations may have existed where surface conditions would have been favourable for microbial habitability. Some of these sites may be good candidates for the exploration in search for signs of extinct or extant life both on the surface and in the near-subsurface.

The problem of discriminating between biominerals and their abiotic counterparts is far from trivial. Nevertheless by means of thermal processing, it is possible to distinguish, using differential thermal analysis (Cabane et al., 2004; Stalport et al., 2005, 2007) or infrared (IR) spectroscopy (Orofino et al., 2007), abiotic calcium carbonate minerals ($CaCO_3$, i.e. aragonite or calcite) from the corresponding biominerals. In a series of papers we have developed and applied our method (D'Elia et al., 2006; Orofino et al., 2007, 2009, 2010) to different carbonate samples in the form of fresh shells and fossils of different ages found in different places and easily recognizable as of biotic origin. The method has been then successfully applied to microbialites (Blanco et al., 2011, 2013, 2014), i.e. bio-induced carbonates deposits, and particularly to stromatolites, well known to be typical examples of very primitive forms of life on Earth (Westall et al., 2004). These samples of biocarbonates can be considered as good analogues of fossils of putative Martian life forms.

An alternative and ancillary approach to distinguish biotic from abiotic carbonates could be the study of their morphological aspect at different scales (Cady et al., 2003; Stolarski and Mazur, 2005; Bianciardi et al., 2014). The complexity of biomineralized structures gives an indication of the potential of organic constituents for controlling energetic factors during crystal synthesis. Many organisms mediate crystallization through the selective application of organic compounds that exert a detailed control over the structure (Belcher et al., 1996), orientation (Berman et al., 1988), growth kinetics (Mann et al., 1993), and nucleation sites of inorganic crystals (Winter and Seisser, 1994). Researchers have been studying biominerals for decades and have found that crystals growth in vitro in the presence of biomineral matrix have a

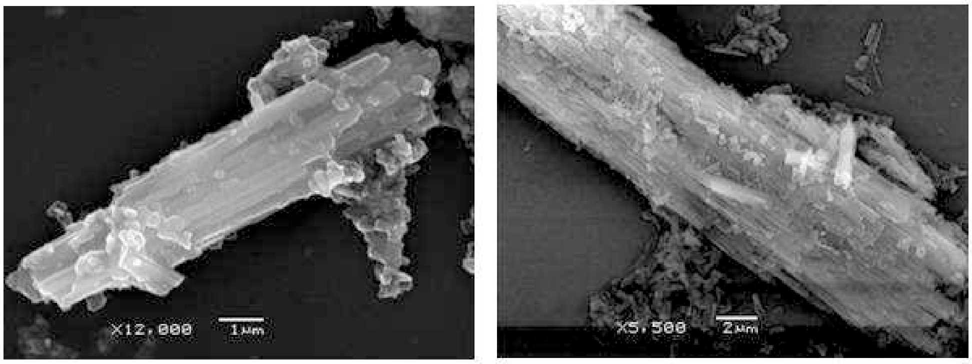

Fig. 1. SEM images of particles of the shell fossils Xenophora (left panel) and Ampullinopsis Crassatina (right panel).

distinct morphology from those grown without matrix. These findings generally support the hypothesis, suggested also by morphological observations, that matrix must limit crystal growth and may well influence the structure of biominerals.

D'Elia et al. (2006) examining with a Scanning Electron Microscope (SEM) at micrometer scale the morphology of two shell fossils composed of aragonite and calcite, showed that they exhibit a well organized crystal pattern compared with the compact structure of the mineral crystals imaged at the same scale. In this work we extend the morphological analysis to biocarbonates linked to primitive living organisms which can be considered as good analogues of remains of rock minerals linked to present or past life on Mars.

This research has been prompted by the need to provide images and data concerning mineral structures and textures useful to be compared to those that will be acquired by the high resolution imaging systems that will explore and characterize the near-sub surface of Mars studying the rock/regolith to search for the past or present life (e.g. CLUPI: the high performance Close-Up Camera System on board the 2018 ExoMars Rover; Josset et al., 2011, 2014). In the next section 2 the main characteristics of the analyzed samples are described. In section 3 we report the experimental results, together with some discussion and conclusions.

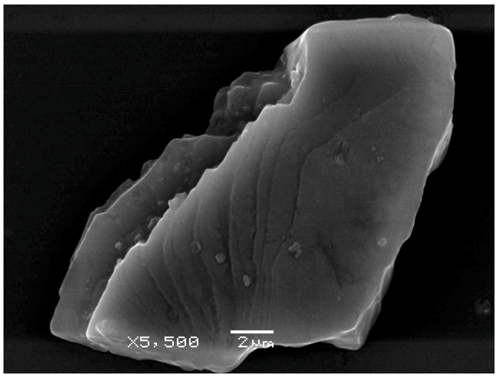

Fig. 2. SEM image of particles of the calcite mineral.

2. Sample description and preparation

The samples analyzed in this work are listed in Table 1. The first two are shell fossils composed of aragonite, a metastable phase of calcium carbonate ($CaCO_3$). The third is a fossil stromatolite, the laminated fabric of microbialites (bio-induced carbonates), while the others are skeletal fossils of very primitive organisms (coral, sponge and algae) embedded in three different microbialites. As it can be seen all of them are composed of calcium

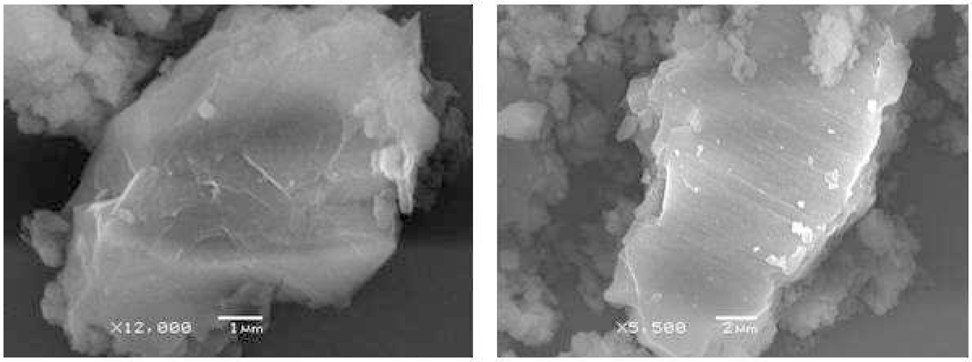

Fig. 3. SEM images of particles of fossil stromatolites (sample GE).

carbonate, calcite and aragonite, with some traces of silicates in the case of the stromatolites. A mineral rock sample of abiotic origin is also included for comparison. The estimated geological ages of the samples are also listed in Table 1. The beginning and the end of each period/epoch are those established by the International Commission on Stratigraphy (ICS) deputed to the terrestrial stratigraphy on a global scale (Ogg et al., 2008).

The two shell fossils, already studied by Orofino et al. (2010), have been collected in two different clay deposits located at two different sites which are about 30 km apart one from the other in the Salento Peninsula, Italy.

The stromatolitic sample GE, Upper Jurassic in age (Tithonian, 151-146 Ma) was collected from Thüste Quarries, south of Hanover, Germany. In this area stromatolites developed in stressed environments, probably represented by a lagoonal setting, with alternate deposition of oolitic limestone and evaporites (Jahnke and Ritzkowski, 1980).

The samples S/L, S1A and U2 are skeletal organisms (coral, sponge and algae respectively) embedded in microbial carbonates. They have been selected within two rock samples that developed, in time, in two distinct palaeoecological conditions characteristic of Alpe di Specie and Punta Grohmann carbonate outcrops in the Dolomites, Italy. In the S/L and S1A Alpe di Specie rock samples, skeletal organisms (Tubiphytes, skeletal cyanobacteria, sphinctozoan and inozoan sponges, etc.) represent a minor component of the rock (usually less than 40%). The composition is dominated by the micritic fraction (about 60%), mainly represented by autochthonous micrite (microbialite), with subordinate amounts of micrite interpreted as detrital (allochthonous micrite) (Russo et al., 1991; Tosti et al., 2011, 2012, 2014). The microbialites or autochthonous micrites, which may exhibit both dense microcrystalline (aphanitic) or peloidal microfabric, are sometimes organized in stromatolitic laminae or thrombolitic fabric.

The U2 was selected from a rock sample collected in the basinal section outcrop belonging to the Sasso Piatto Massif, in the province of Bolzano, Italy. The analyzed sample belongs to the Punta Grohmann buildups. These buildups represent the last records of the bioconstructions that first appeared in the Late Pennsylvanian. They are characterized by subcentimeter skeletons (mainly calcified microbes, Tubiphytes and other problematica, small sponges) intimately associated with microbialites and cements. The organic-induced nature of microbialite was supposed on the base of micromorphological and biogeochemical evidence (Russo et al., 1997; Tosti et al., 2011, 2012, 2014). Their biotic origin has been confirmed by Blanco et al. (2013, 2014) with independent methods.

In order to obtain fine particulate material for SEM analysis, all the samples, appropri-

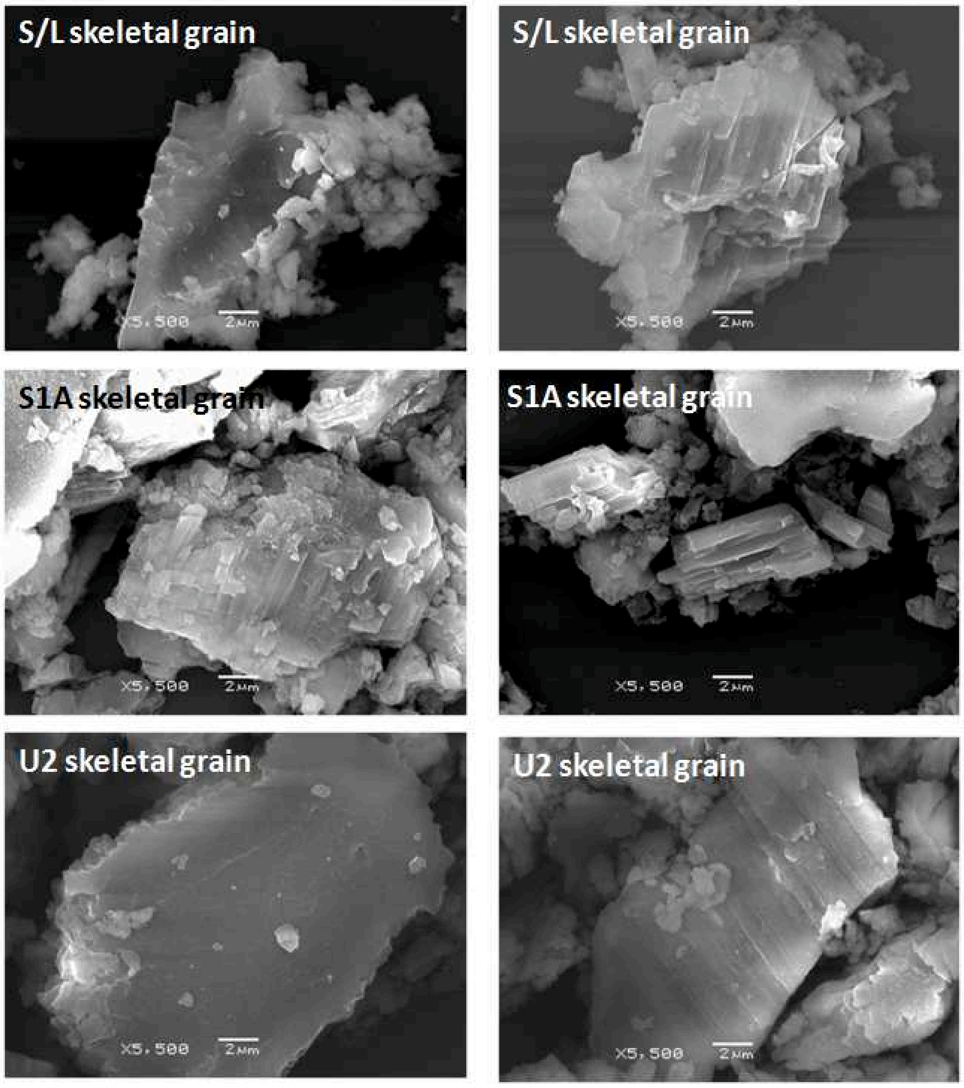

Fig. 4. SEM images of particles of fossil skeletal organisms (samples S/L, S1A and U2).

ately extracted from the bulk specimen, were ground with a mechanical mortar grinder for approximately 10 minutes and then the size fraction between 20 μm to 50 μm was sieve selected for our investigation. The mineral composition, reported in Table 1, has been determined using both the IR spectroscopy and the Energy Dispersive X-Ray (EDX) elemental analysis performed in our laboratory on all samples (for details see Blanco et al., 2014).

3. SEM analysis and conclusions

The morphological analysis has been done using a Scanning Electron Microscope (SEM, JEOL JSM - 6480LV), equipped with an

Table 2. Morphology of the studied samples (see text).

Sample	Compact structure	Crystal pattern
Xenophora	–	100%
Ampullinopsis crassatina	–	100%
GE	37%	63%
S/L	77%	23%
S1A	–	100%
U2	33%	67%
Calcite	100%	–

Energy Dispersive X-ray (EDX) spectrometer (iXRF Systems, EDS Sirius SD) for the elemental composition.

In Figures 1 and 2 are shown typical SEM images of particles of the shell fossils and of the calcite mineral crystals respectively. It is evident the laminar crystal pattern of the shells particles compared with the compact structure of the mineral crystals grains. Similar images of stromatolites and skeletal organisms are shown in Figures 3 and 4. Except for S1A, some particles exhibit a compact structure (left panels) while others show the crystal pattern similar to that of the shell fossils (right panels). The examination of more than 50 particles of each sample allowed us to get some quasi-statistical results reported in Table 2.

As it can be seen the results cannot be conclusive although they give indications that the crystal structure of biotic carbonate may be different from that of the abiotic mineral counterpart. This means that, in order to reach meaningful conclusions, we need to analyze the morphology of other samples as well as a number of statistically significant particles. We think that the effort toward this line of research may possibly provide a method, although not conclusive, to discriminate the origin of martian carbonates by the next generation of remote sensing instruments that will explore the surface and near-subsurce of the red planet in search for extraterrestrial life.

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